The importance of body size: scaling of physiological traits in insects

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Summary

Biological phenomena occur across wide scales in space, time, and organisational complexity. Molecules, which are small, quickly transforming units, exhibit new emergent properties when they are arranged into ecosystems. These properties of ecosystems, such as species diversity, distribution, standing biomass, or rates of nutrient turnover involve large spatial and temporal scales, as well as many underlying processes that make their study inherently complex. Integration across disciplines and across levels of biological organisation is one of the grand challenges in biology. Towards this end, novel methods are required so that cross-disciplinary phenomena can be quantified using a common metric. Energy and mass are two universal currencies that are able to cut through the hierarchy of biology, which must be both conserved irrespective to the scale of inquiry.

Dynamic Energy Budget (DEB) theory builds upon the laws of energy and mass conservation by identifying other universal constraints on the metabolic organisation of diverse species. While DEB theory is commonly perceived to be relevant at fine biological resolutions, particularly the individual level, it has received little recognition from population, community, and ecosystem biologists despite its application to many supra-individual topics. In this thesis, I bring principles of DEB theory to bear against several current problems in biology that each span multiple organisational levels. As pointed out by renowned mathematical ecologist, Richard Levins, different models may take a different emphasis on precision, generality, or realism and do so antagonistically (at the expense of the other qualities). In thesis I take an emphasis on generality by developing simple, parameter-sparse DEB-based models that are able to yield predictive synthesis on cross-disciplinary issues,

demonstrating the parsimony of DEB approaches. This departs from previous DEB studies on macro-ecological patterns, which take more of an emphasis on precision. I also focus on the taxonomical group of the insects – a group which is comparatively understudied in the DEB literature.

The first of these problems surrounds a theoretical underpinning to the famous pattern of metabolic scaling. Metabolic scaling is the observation that as organisms increase in size, the energy turnover in a fixed unit of biomass decreases. This pattern has great biological importance and now forms the basis of the emerging field of metabolic ecology. Much of the current interest and controversy in metabolic scaling relates to recent ideas about the role of supply networks in constraining energy supply to cells. I show that an alternative explanation for physicochemical constraints on individual metabolism, as formalised by DEB theory, can contribute to the theoretical underpinning of metabolic ecology, while increasing coherence in the topic of metabolic scaling. In particular, I emphasise how DEB theory considers constraints on the storage and use of assimilated nutrients, and illustrate how this explains the frequently observed quarter-power scaling of many biological rates without relying on optimisation arguments or implying cellular nutrient supply limitation. Because the DEB theory mechanism for metabolic scaling is based on the universal process of acquiring and using pools of stored metabolites, it applies to all organisms irrespective of the nature of metabolic transport to the cells, but without necessarily excluding insights from transport-based models.

Design constraints imposed by increasing size cause metabolic rate in animals of different species to increase more slowly than mass. However, mechanistic explanations for interspecific metabolic scaling do not apply for ontogenetic size changes within a species implying different mechanisms for these scaling phenomena. Next, I show that the DEB theory approach of compartmentalizing biomass into reserve and structural components

provides a unified framework for understanding both ontogenetic and interspecific metabolic scaling. I formulate the theory for the insects and show that it can account for ontogenetic metabolic scaling during the embryonic and larval phases, as well as the U-shaped respiration curve during pupation. After correcting for the predicted ontogenetic scaling effects, which I show to follow universal curves, the scaling of respiration between species is approximated by a ¾ power law, supporting past empirical studies on insect metabolic scaling and my theoretical predictions. The ability to explain ontogenetic and interspecific metabolic scaling effects under one consistent framework suggests that the partitioning of biomass into reserve and structure is a necessary foundation to a general metabolic theory.

The uptake of resources from the environment is a basic feature of all life. Consumption rate has been found to scale with body size with an exponent close to unity across diverse organisms. However, like metabolic rate, past analyses have ignored the important distinction between ontogenetic and interspecific size comparisons. I present a mechanistic model, based on DEB theory, for the body mass scaling of consumption, which separates interspecific size effects from ontogenetic size effects. The model predicts uptake to scale with surface-area ($mass^{2/3}$) during ontogenetic growth but more quickly (between $mass^{3/4}$ and $mass^{1}$) for interspecific comparisons. Available data for 41 insect species on consumption and assimilation during ontogeny provides strong empirical support for the theoretical predictions. In particular, consumption rate scaled interspecifically with an exponent close to unity (0.89) but during ontogenetic growth scaled more slowly with an exponent of 0.70. Assimilation rate (consumption minus defecation) through ontogeny scaled more slowly than consumption due to a decrease in assimilation efficiency as insects grow. Again, these results highlight how body size imposes different constraints on metabolism depending on whether the size comparison is ontogenetic or inter-specific.

Finally, I use the principles of DEB theory to explore the universality of growth patterns in insects. Insects are typified by their small size, large numbers, impressive reproductive output, and rapid growth. However, insect growth is not simply rapid; rather, insects follow a qualitatively distinct trajectory to many other animals. I present a mechanistic growth model for insects and show that the up-regulation of assimilation during the growth phase can explain the near-exponential growth trajectory of insects. The presented model is tested against growth data on 50 insects, and compared against other mechanistic growth models. Unlike other mechanistic models, the presented growth model predicts energy reserves per biomass to increase with age, which implies a higher production efficiency and energy density of biomass in later instars. These predictions are tested against data compiled from the literature whereby it is confirmed that insects increase their production efficiency (by 24 percentage points) and energy density (by 4 J/mg) between hatching and the attainment of full size. The model suggests that insects achieve greater production efficiencies and enhanced growth rates by up-regulating assimilation and increasing energy reserves per biomass, which are less costly to maintain than structural biomass. My findings illustrate how the explanatory and predictive power of mechanistic growth models comes from their grounding in underlying biological processes.

These applications of DEB theory highlight novel insights on some well-studied, but unresolved issues in biology. More importantly, the theoretical basis of these insights demonstrates the value of a quantitative framework for metabolic organisation to the study of macro-physiological patterns, and how simplified DEB models can contribute to the emerging field of metabolic ecology. While the grand challenge of unification across scales still remains, the results of this thesis hold much promise for metabolic theory as a platform for synthesis in biology.

Samenvatting

Biologische verschijnselen vinden plaats op een grote schaal in ruimte, tijd en organisatorische complexiteit. Moleculen, die klein zijn en snel transformeren, laten nieuwe emergente eigenschappen zien als zijn onderdeel uitmaken van een ecosysteem. Deze eigenschappen van ecosystemen, zoals biodiversiteit, biomassa, of snelheden van nutriënten gebruik vinden plaats op grote tijd en ruimte schalen, evenals de vele onderliggende processen die de studie van dit alles bemoeilijken. Integratie van disciplines en van niveaus van biologische organisatie is een van de grote uitdagingen in de biologie. Hiertoe zijn nieuwe methoden nodig zodat verschijnselen die verschillende disciplines betreffen gekwantificeerd kunnen worden met gebruikmaking van eenzelfde metriek. Energie en massa zijn twee universele grootheden die dwars door alle hiërarchieën van de biologie gebruikt kunnen worden en altijd en op alle niveaus behouden zijn.

Dynamische Energie Budget (DEB) theorie is gebouwd op energie en massa behoudwetten, rekening houdend met algemene randvoorwaarden van metabole organisatie van de verschillende soorten. Hoewel DEB theorie algemeen erkend wordt relevant te zijn voor de fijnere schaal van organisatie, speciaal die op het niveaus van het individu, is van zo'n erkenning nauwelijks sprake bij populatie-, levensgemeenschap- en ecosysteem-biologen, ondanks de vele toepassingen op supra-individu niveau. In dit proefschrift pas ik DEB principes toe op verschillende hedendaagse biologische problemen, die elk een veelvoud van organisatorische niveaus omvatten. Zoals de beroemde mathematische ecoloog Richard Levins naar voren heeft gebracht, leggen verschillende modellen op antagonistische wijze verschillende nadruk op nauwkeurigheid, algemeenheid en realisme, ten koste van andere kwaliteiten. In dit proefschrift leg ik de nadruk op algemeenheid door eenvoudige,

parameter-arme modellen te ontwikkelen die op DEB gebaseerd zijn en een voorspellende syntheses maken over onderwerpen die verschillende disciplines betreffen om zodoende de toereikendheid van DEB benaderingen te laten zien. Het neemt afstand van vroegere DEB studies over macro-ecologische patronen, die meer nadruk legden op nauwkeurigheid. Ik beperk mij tot de taxonomische groep van insecten, een groep die nog niet zoveel bestudeerd is in de DEB literatuur.

Het eerste probleem betreft de theoretische onderbouwing van het beroemde patroon van metabole schaling. Metabole schaling is de waarnemingen dat, wanneer organismen toenemen in grootte, het energieverbruik per eenheid biomassa afneemt. Dit patroon is van enorme biologische betekenis en vormt nu de basis van het opkomende gebied van metabole ecologie. Veel van de bestaande interesse en controversie binnen metabole schaling houdt verband met de beperkende rol van netwerken in de energievoorziening van het cellulaire metabolisme. Ik laat zien dat een alternatieve verklaring voor fysisch-chemische randvoorwaarden op het metabolisme van het individu, zoals geformaliseerd door DEB theorie, een bijdrage kan leveren aan de theoretische onderbouwing van metabole ecologie, terwijl bovendien de samenhang in metabole schaling wordt vergroot. Meer in bijzonder benadruk ik hoe DEB theorie de randvoorwaarden van opslag en gebruik van opgenomen nutriënten behandelt, en illustreer hoe dit de vaak waargenomen 3/4-machts-schaling van vele biologische snelheden verklaart zonder gebruikmaking van optimalisatie argumenten of beperkingen van nutriënten voorziening van cellen. Omdat het DEB mechanisme voor metabole schaling gebaseerd is op het universele proces van opname en opslag van metabolieten, is het op alle organismen van toepassing, ongeacht de manier waarop ze metabolieten naar cellen transporteren, zonder overigens transport modellen uit te sluiten.

Randvoorwaarden voor het ontwerp, voortkomend uit toenemende grootte, veroorzaken dat het metabolisme van verschillende soorten minder snel toeneemt dan hun

massa. Mechanistische verklaringen voor de inter-specifieke schaling van metabolisme zijn niet van toepassing op een groeiend individu, zodat hiervoor andere mechanismen verantwoordelijk moeten zijn. Vervolgens laat ik zien dat de DEB benadering om biomassa op te splitsen in reserve en structuur een samenbindend raamwerk levert om zowel de ontogenetische als de interspecifieke metabole schaling te verklaren. Ik formuleer de theorie voor insecten en laat zien dat het de ontogenetische metabole schaling gedurende de embryonale en larvale stadia verklaart, alsmede de U-vormige respiratie curve gedurende het pop-stadium. Na correctie van de voorspelde ontogenetische schaling volgt respiratie een universele curve tussen soorten die benaderd wordt door de 3/4-machts-relatie, en ondersteunen daarmee empirische schalings-studies van insecten en mijn theoretische beschouwingen. Het vermogen om ontogenetische en interspecifieke metabole schalings-effecten te verklaren binnen een consistent raamwerk suggereert dat de opsplitsing van biomassa in reserve en structuur een noodzakelijke basis vormt van een metabole theorie.

De opname van grondstoffen uit het milieu ligt ten grondslag aan al het leven. De opnamesnelheid door verschillende organismen schaalt met een exponent die dicht bij 1 ligt. Net als bij metabole snelheid hebben vroegere analyses echter ten onrechte verzuimd verschil te maken tussen ontogenetische en inter-specifieke schaling. Ik presenteer een mechanistische model, gebaseerd op DEB theorie, voor de biomassa-schaling van opname, dat verschil maakt tussen ontogenische en inter-specifieke effecten van grootte. Het model voorspelt dat opname evenredig toeneemt met oppervlak (massa tot de macht 2/3) gedurende ontogenetische groei, maar sneller (massa tot de macht tussen 3/4 en 1) voor inter-specifieke vergelijkingen. Beschikbare data van opname en assimilatie voor 41 soorten insecten gedurende hun ontogenie levert sterke ondersteuning van de theoretische voorspellingen. Meer in het bijzonder schaalde opname interspecifiek met een exponent dicht bij 1 (namelijk 0.89), maar gedurende ontogenetische groei met een exponent van 0.70. Assimilatie (opname

minus uitscheiding) gedurende ontogenie schaalde langzamer dan opname vanwege de verminderde assimilatie efficiëntie wanneer insecten groeien. Opnieuw belichten deze resultaten hoe lichaamsgrootte verschillende randvoorwaarden oplegt aan het metabolisme, afhankelijk of de grootte ontogenetisch of inter-specifiek vergeleken wordt.

Tenslotte gebruik in principes van DEB theorie om de universaliteit van groei patronen bij insecten te onderzoeken. Insecten worden gekenmerkt door hun geringe grootte, grote aantallen en hun indrukwekkende reproductie en groei snelheden. Insecten groeien echter niet zomaar snel: zij volgen een kwalitatief verschillende traject ten opzichte van andere dieren. Ik presenteer een mechanistisch groei model voor insecten en laat zien dat versnelling van assimilatie gedurende de groei-fase de bijna-exponentiële groei van insecten kan verklaren. Het model is getest voor groei-data van 50 soorten insecten en vergeleken met andere mechanistische groei modellen. In tegenstelling tot deze modellen laat mijn model reserve per biomassa toenemen met de leeftijd, met als gevolg dat de produktie-efficiëntie toeneemt en de energie per biomassa hoger is voor latere vervellings-stadia. Deze voorspellingen werden getest tegen data uit de literatuur en bevestigen dat insecten hun productie-efficiëntie verhogen (met 24 procent) alsmede hun energie dichtheid (met 4 J/mg) tussen het uit-het-ei-kruipen en volledig uitgegroeid zijn. Het model suggereert dat insecten hun toenemende productie efficiëntie en toenemende groei en reserve dichtheid bereiken via versnelling van hun assimilatie; reserve kost minder onderhoud dan structuur. Mijn bevindingen illustreren de verklarende kracht van mechanistische groei modellen die aandacht geven aan de onderliggende biologische processen.

Deze toepassingen van DEB theorie belichten nieuwe inzichten betreffende goed-bestudeerde, maar slecht begrepen, problemen in de biologie. Nog belangrijker, echter, demonstreert de theoretische achtergrond van deze inzichten de waarde van een kwantitatief raamwerk voor de metabole organisatie voor macro-fysiologische patronen, en hoe

vereenvoudigde DEB modellen kunnen bijdragen aan het opkomende veld van metabole ecologie. Terwijl de enorme uitdaging van unificatie over schalen nog steeds bestaat, houden de resultaten van dit proefschift veel beloften in voor metabole theorie als een platvorm voor synthese in de biologie.

Declaration

This is to certify that:

i. the thesis comprises only my original work towards the PhD except where indicated in the Preface,

ii. due acknowledgement has been made in the text to all other material used,

iii. the thesis is fewer than 100 000 words in length, exclusive of tables, maps, bibliographies and appendices

James Maino

Preface

Much of Chapter 2 was developed in Amsterdam while I was visiting Vrije Universiteit as part of my Double Doctorate (Jointly Awarded Doctorate), in collaboration with Roger Nisbet, Michael Kearney, and Bas Kooijman, who each helped design the study and contributed to the writing. I also designed the study but, in addition, conducted the analysis, wrote more than 75% of the chapter, and responded to reviewers during peer review.

Chapters 2, 3, 4 and 5 have been written in journal format so there is some unavoidable repetition. In each case I am the primary author, conceived the main ideas, compiled and analysed the data, and wrote the chapters.

Acknowledgements

To my eyes, I see that science is a many-splendored thing,
Both accretionary advancements, and those revolutionising.
But for all the glamour that goes along with renowned discovery,
There is a secret side to science that few people get to see.
Those lengthy hours isolated, watching progress turn to waste
Would, indeed, be sizably worse if all my friends had been misplaced
Ashley, Angelos, Jackson, Sean, would only name few
Of those that made recovery swift, when my efforts would fall through
As a student one can feel quite small, among scholars of great stature
But on top of supervisorial shoulders, my thesis grew with each chapter
Mike's boundless passion and enthusiasm were invaluable to me
His cleverness spread into his puns, as most his students would agree
Bas' fearsome knowledge of the astounding natural world
Led him to a grand idea, from which a grand theory unfurled
My lab included Elia and Candice, who made the workplace great
I also thank my family and Bob, who I cannot under-rate.
These past few years I've watched the time as it hurriedly flew along.
I'll forever be thankful to those who said that in science I belong.

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Chapter 1: Introduction

'Behind the mere smashing of aggregates into smaller pieces lies a deeper agenda that also takes the name of reductionism: to fold the laws and principles of each level of organization into those at more general, hence more fundamental levels. Its strong form is total consilience, which holds that nature is organized by simple universal laws of physics to which all other laws and principles can eventually be reduced. This transcendental world view is the light and way for many scientific materialists (I admit to being among them), but it could be wrong. At the least, it is surely an oversimplification. At each level of organization, especially at the living cell and above, phenomena exist that require new laws and principles, which still cannot be predicted from those at more general levels. Perhaps some of them will remain forever beyond our grasp. Perhaps prediction of the most complex systems from more general levels is impossible. That would not be all bad. I will confess with pleasure: The challenge and the crackling of thin ice are what give science its metaphysical excitement.'

–E.O. Wilson, 1999

The study of scaling is an attempt to understand why bigger is not only bigger. A big cello produces a lower pitched sound than a small violin. A large cup of hot water will cool more slowly than a small one. When the size of a bridge is increased, the design must change to compensate for its diminishing strength. Size imposes physical constraints on design. In biology, these physical constraints are relevant to understanding the way in which organisms are designed. Like the cello and the violin, acoustic principles explain the pitch difference between a lion's 'roar' and cat's 'meow' (Hast 1989). Euclidean geometry helps us understand why a small cup cools quickly and how a polar bear's large thermal inertia facilitates surviving the harsh conditions of the arctic (Schmidt-Nielsen 1984). The same physics used by engineers to ensure bridges do not collapse explains why an ant can support 5000 times their body weight (Nguyen et al. 2014).

The physical principles underlying other scaling relationships are less transparent. One relationship of particular interest is the scaling of metabolic rate. Metabolic rate is the rate at which organisms process energy and materials to fuel life processes such as growth and reproduction. With energy and mass conservation being one of the few laws that penetrates all spatial and temporal scales of biology, metabolic rate is a fundamental rate of life. Organism metabolism constrains life processes spanning molecules to ecosystems, including DNA mutation rates (Gillooly et al. 2005b), individual reproduction rates (Hamilton et al. 2011), population growth rates (Brown et al. 2004), and ecosystem carbon cycling (Brown et al. 2004). Curiously, when metabolic rate (y) is expressed as an allometric function ($y = aM^b$) of body mass (M), where a is the normalisation constant, the estimated exponent b does not indicate volumetric scaling ($b = 1$) or surface-area scaling ($b = 2/3$) but, for diverse organisms, tends to take an intermediary value close to a ¾ (Isaac and Carbone 2010).

While much intellectual effort has been directed at uncovering the possible principles driving metabolic scaling, there is still much debate on the topic (White et al. 2007, Kolokotrones et al. 2010, Isaac and Carbone 2010). Mechanistic explanations of the peculiar scaling of metabolic rate are diverse. Some studies have used simple Euclidean geometry to propose limits on heat dissipation (Speakman 2010), while others have hypothesised the importance of more complicated processes, such as the consequences of elastic criteria on power output (McMahon 1973), or the scaling of molecular oscillators embedded in biomembranes (Demetrius and Tuszynski 2010). Of all explanations, none have received so much attention as West, Brown and Enquist's nutrient supply network model (WBE model hereafter), from which it was claimed that the ¾ power scaling of metabolic rate was able to be predicted from simple physical principles constraining nutrient delivery through a network, such as the circulatory system in vertebrates (West et al. 1997, 1999). The WBE

model has since been criticised for the generality of its proposed mechanism (Chapter 2) and the many exceptions deviating from the predicted ¾ power scaling of metabolism (Kolokotrones et al. 2010, Isaac and Carbone 2010). Indeed, many factors have been shown to affect the precise scaling exponent including measurement temperature (Glazier 2005, Killen et al. 2010), metabolic level (Glazier 2014), organism complexity (DeLong et al. 2010), the mode of foraging (Glazier 2006, Pawar et al. 2012), and the degree of shape shifting during growth (Hirst et al. 2014, Glazier et al. 2015). Despite these issues, there is no doubt that the WBE model has profoundly shaped the current state of the field through directing and stimulating research.

Despite numerous proposed explanations, a broadly accepted mechanistic underpinning to the important scaling pattern of metabolic rate remains elusive. While there is much prospect for unifying theory in biology based on metabolism (Brown et al. 2004), theoretical progress is lagging behind the accumulation of experimental work. Incoherence in the field of metabolic scaling is problematic as our ability to predict variation in biological processes is currently limited by the ability of our theory to mechanistically explain and link these patterns under the one consistent conceptual framework (Enquist et al. 2003). Without a well formulated theory, patterns are likely to be considered as independent phenomena despite possible underlying connections (Harte 2004). In general terms, the goal of a metabolic theory is to provide a single coherent set of answers to many questions. A metabolic theory should explicitly link life processes such that knowledge of one process can be used to inform our understanding of another in a way that would be unachievable through their independent consideration.

One under-applied theory of metabolic organisation that holds much promise for unification in biology is the Dynamic Energy Budget (DEB) theory (Kooijman 2010). Developed by Kooijman and colleagues over several decades, DEB theory is a framework

grounded in simple physicochemical principles, whereby a generic metabolic architecture can capture energetic features of diverse organisms spanning unicellular organisms to large vertebrates. In this way, DEB theory has served as a useful tool in a wide range of comparative biological research (Mueller et al. 2012, Kooijman 2013, Lika et al. 2014). The 'dynamic' aspect of DEB theory relates to its explicit characterisation of energetic processes, such as growth, uptake, or reproduction, which depend heavily on an organism's stage of development. The evolution of energetic processes through ontogeny is specified with a set of ordinary differential equations. Thus, a key feature of DEB theory is that it captures differences in energy budgets between the life stages of an individual as well as differences between species. Importantly, DEB theory considers the relationship between surface-area mediated processes (e.g. nutrient uptake) and volumetric processes (e.g. somatic maintenance) and is consequently well-positioned to predict the consequences of body-size on metabolism.

Although the principles of DEB theory concern metabolic organisation at the individual level, the use of the universal currencies – energy and mass – allow core concepts to be naturally extended to higher levels of biological organisation. Indeed, there exists a rich literature using DEB approaches for population level modelling (Lorena et al. 2010, Martin et al. 2012, 2013). While there has recently been much interest in the development a metabolic theory of ecology, the ability of DEB theory to penetrate ecological spheres of study has been questioned (Marquet et al. 2014). A recent synthesis of research relating to metabolic ecology included only a cursory reference to DEB theory, claiming that "this approach had limited impact because Kooijman's models are very complex, with too many parameters and functions for most applications" (Brown and Sibly 2012).

This thesis explores whether the principles of dynamic energy budgeting can contribute to a unifying metabolic theory in biology by building on its application to broad

scales of analysis with an emphasis of parameter-sparse models. This departs from previous DEB studies on macro-ecological phenomena (Kooijman 2013, Lika et al. 2014), which place a greater emphasis on precision at the expense of generality (Levins 1966) by allowing more parameters to vary between species. In Chapter 2, I demonstrate that DEB theory does indeed have relevance in the ecological domain by using its core principles to derive the famous ¾ scaling of metabolic rate that now underpins the emerging field of metabolic ecology (Brown et al. 2004). The proposed mechanistic underpinning the phenomenon of metabolic scaling does not suffer from a lack of generality, as do some competing theories, because the principles of DEB theory are grounded in processes basic to life. Furthermore, I demonstrate that 'competing theories' may arrive at the same predictions but may not necessarily be mutually exclusive despite their different underpinnings.

For the remainder of this thesis I focus on testing novel DEB predictions against data from a range of insects. Insects are understudied in the DEB literature but constitute the majority of multicellular diversity. Following the broad metabolic scaling discussion of Chapter 1, I explore whether the size constraints apparent between species are equivalent to the size constraints imposed within a species for ontogenetic comparisons (i.e. as an organism grows in size). I demonstrate that DEB theory uniquely predicts divergent patterns for ontogenetic and interspecific comparisons. Using insects as a case study, I explore differences in the scaling of metabolic rate (as measured by oxygen consumption) across ontogenetic development (for each embryonic, immature, and pupal stages) for a range of species of various sizes (Chapter 3). I also test for predicted differences in the scaling of rates of food consumption and assimilation for ontogenetic and interspecific comparisons (Chapter 4). By elaborating the important distinction between ontogenetic and interspecific effects, I demonstrate how simpler, but more frequently applied models of metabolic scaling are inadequate.

Finally, in Chapter 5, I explore the exponential shaped growth trajectory of insects within the context of the standard DEB model, which is commonly applied to heterotrophic organisms but does not predict exponential growth rates. I propose a simple modification to the standard DEB model that explains exponential growth in insects. The resulting model yields novel predictions of insect growth efficiency and biomass energy density which are tested against a data set compiled from the literature.

Chapter 2: Reconciling theories for metabolic scaling

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Abstract

Metabolic theory specifies constraints on the metabolic organisation of individual organisms. These constraints have important implications for biological processes ranging from the scale of molecules all the way to the level of populations, communities, and ecosystems, with their application to the latter emerging as the field of metabolic ecology. While ecologists continue to use individual metabolism to identify constraints in ecological processes, the topic of metabolic scaling remains controversial. Much of the current interest and controversy in metabolic theory relates to recent ideas about the role of supply networks in constraining energy supply to cells. We show that an alternative explanation for physicochemical constraints on individual metabolism, as formalised by Dynamic Energy Budget (DEB) theory, can contribute to the theoretical underpinning of metabolic ecology, while increasing coherence between intra- and inter-specific scaling relationships. In particular, we emphasize how the DEB theory considers constraints on the storage and use of assimilated nutrients, and derive an equation for the scaling of metabolic rate for adult heterotrophs without relying on optimisation arguments or implying cellular nutrient supply limitation. Using realistic data on growth and reproduction from the literature we parameterise the curve for respiration and compare the *a priori* prediction against a mammalian dataset for respiration. Because the DEB theory mechanism for metabolic scaling is based on the universal process of

acquiring and using pools of stored metabolites (a basal feature of life), it applies to all organisms irrespective of the nature of metabolic transport to cells. Although the DEB mechanism does not necessarily contradict insight from transport based models, the mechanism offers an explanation for differences between the intra- and inter-specific scaling of biological rates with mass, suggesting novel tests of the respective hypotheses.

Introduction

The controversial topic of metabolic scaling has seen a revival in recent years as ecologists have begun to more strongly relate ecological phenomena to constraints on individual metabolism (Brown et al. 2012). Renewed interest was sparked by West, Brown and Enquist's (1997) nutrient supply network model (WBE hereafter). This model was subsequently used as a theoretical justification for the widespread application of the empirically observed ¾ power scaling of metabolic rate with individual size to understand ecological patterns more generally – a research agenda now known as the "The Metabolic Theory of Ecology" (Brown et al. 2004). Other models based on nutrient supply have since emerged (Banavar et al. 1999; Banavar et al. 2002; Banavar et al. 2010), which also aim to understand how design constraints on the transport of metabolites may be restricting the metabolic organisation of organisms. These 'transport models' have instant appeal as they use simple physical principles about the scaling of vascular supply networks to make *a priori* predictions about the general pattern of metabolic scaling that is observed over some 20 orders of magnitude of body size. Other proposed models have also received considerable attention (Darveau et al. 2002; Kolokotrones et al. 2010) but as West et al. (2003; 2005) correctly point out, "many 'competing' models make no *a priori* predictions about the scaling of metabolic rate."

While metabolic scaling is ubiquitous in biology, only a small minority of life's diversity is known to possess the equivalent of closed vascular supply systems. In contrast, all organisms must take up, store, and mobilise energy and materials as part of their basic metabolism. Emphasizing the significance of physicochemical constraints on the build-up and use of stored metabolites, Dynamic Energy Budget (DEB) theory (Kooijman 1986; Kooijman 2000; Kooijman 2010) offers a competing explanation for metabolic scaling that has yet to

contribute substantially to the debate (but see van der Meer 2006a; Kearney & White 2012). Like the WBE explanation, the DEB theory explanation also makes *a priori* predictions about metabolic scaling using very simple mechanistic principles, and does so without necessarily running contrary to the important insights that physical transport models provide about theoretical properties of vascular supply networks.

The dynamic use of stored metabolites (also known as reserve dynamics (Kooijman 2010)) has been a core conceptual component in DEB theory for almost 30 years (Kooijman 1986). The significance placed on reserve dynamics in DEB theory is motivated by the observation that metabolism depends more on nutritional history than on present feeding conditions – a phenomenon dramatically demonstrated by the Humpback whale, which can travel halfway around the world nursing a 2000 pound pup without feeding. Although best known for its application at the scale of the developing individual, the DEB framework can use its simple physicochemical principles to make *a priori* predictions about broad scaling patterns of many life history traits between species, including the scaling of metabolic rate (Kooijman 2010).

This paper aims to emphasise and explain how the DEB concept of reserve dynamics can contribute to the debate on metabolic scaling by invoking simple and realistic constraints on the use of stored metabolites, which restrict fluxes of energy and materials through organisms, and consequently constrains metabolism. As expressed by Brown and Sibly in a review of recent work in metabolic ecology, 'biological metabolism includes the uptake of resources from the environment, transformation of these substances within the body, allocation of these products to maintenance, growth and reproduction, and excretion of wastes into the environment. So, to a first approximation, the metabolic rate sets the pace of life, and the rates of all biologically mediated ecological processes' (2012, p. 22). For this reason, understanding constraints on metabolic organisation is important for understanding almost all life processes.

Our broad goal is to encourage further empirical and theoretical comparison between the WBE and DEB theories, something which is practically non-existent in the literature (but see van der Meer 2006a; Kearney & White 2012). Brown et al. (2004) stated that they 'view the DEB and MTE [Metabolic Theory of Ecology] approaches as complementary. They make different trade-offs between specificity and generality, and consequently have different strengths, weaknesses, and applications'. We too regard the theories as potentially complementary, but for different reasons. We do not believe the level of specificity (or generality) to be an inherent feature of each theory, but rather a practical consideration that is dependent on the context of the theory's application. To make it clear that DEB theory can be readily applied generally, and at broad scales, we derive an equation for the metabolic scaling of adult heterotrophs that is numerically identical to that of the WBE nutrient supply model, but which rests on a set of profoundly different assumptions. Using realistic values on growth and reproduction from the literature we estimated the parameters of this equation to make an *a priori* prediction of the scaling of metabolic rate, which is compared against a large mammalian data set.

We show that the DEB explanation does not rely on any arguments of evolutionary optimality. Moreover, the DEB approach clearly distinguishes mechanisms associated with intra- and inter-specific variation in rates of respiration, uptake, and reproduction, and also describes the metabolism of embryos without making any further assumptions. This is an important point of departure from transport-based models and suggests how the respective hypotheses may be tested experimentally (Kearney and White 2012). We also derive other DEB scaling relationships and summarize them as a table of scaling predictions. These results illustrate how DEB theory can contribute to the theoretical underpinning of the emerging field of metabolic ecology.

Constraints on the use of stored nutrients

All organisms take up and store nutrients, either directly or from food. Without nutrient storage, organisms would perish upon the cessation of feeding, unable to cover the basic costs of metabolism. But, as soon as assimilated nutrients make their way from the gut into the blood, the task of storage poses immediate problems. Strict limits are placed on the concentration of any substrate in solution. One particularly important example is the maintenance of osmotic pressures; an instantaneous ten-fold increase in blood glucose levels would raise osmotic pressure by approximately 15 per cent (Coulson et al. 1977), posing serious physiological risks, familiar to any person suffering from diabetes.

Despite the risks to osmotic balances posed by the simple act of feeding, organisms can process foods at astounding rates. An alligator eats as much as 15 g of protein per kilogram of body weight in one meal which, for a 70 kg individual, would equate to slightly more than 1 kg of protein or roughly 8.5 moles of amino acids (Coulson et al. 1977). Assuming all the protein was absorbed as amino acids in the 48 hours it would take for digestion and that they were present in the body fluids at the same time, the osmotic pressure would increase by approximately 59% (Coulson et al. 1977). Despite the absorption of this massive amount of substrate over this relatively short period, measured levels of amino acids in plasma and osmotic pressures remain approximately unaltered (Coulson & Hernandez 1970). Moreover, the alligator would only need 18 g or approximately 2 per cent of the total amino acids absorbed to meet its daily metabolic requirements (Hernandez & Coulson 1952). The majority of the ingested amino acids are used at a later date and so must be stored.

Organisms must simultaneously cope with variable feeding conditions as well as with the problem of maintaining internal osmotic pressures and they do this by storing absorbed substrates as pools of polymers, which do not affect osmotic pressures. In the case of amino

acids, these polymers are proteins, although the same story could be told for carbohydrates as well as lipids. The key point is that, regardless of the organism, most assimilated substrates are best stored as macromolecules. But, because these macromolecules are not well mixed in solution across the body, the periphery of these storage sites becomes more relevant to reaction rates in place of the overall concentration. As a result, simple enzyme kinetics no longer applies.

For animals, DEB theory formalises this notion by partitioning biomass into two compartments: reserve and structure. Reserve represents the sum of all pools of polymers (lipids, carbohydrates, proteins etc.) from which energy and materials are mobilised for the growth and maintenance of structure, and reproductive processes. It is assumed that all assimilated materials first enter the reserve compartment (for details see Lika & Kooijman 2011), and that these storage pools do not contribute to overall maintenance costs. For convenience, reserve is typically expressed in units of energy, while structure is expressed as a volume (this is so structure can be easily related to suitable physical measures of size, such as carapace widths in insects, or femur length in mammals). However, reserve and structure can just as easily be expressed in terms of mass. Reserve and structure are each assumed to have a constant composition (the strong homeostasis assumption), which implies stoichiometric constraints on their growth in amounts.

Fig. 2-1 shows how access to reserve through its surface area interface with structure is expected to scale with size. Mobilisation of reserve for metabolism is via enzymes that travel around in the metabolically active structural matrix and do not actually penetrate the pools of reserve, but operate at the interface. The surface area of the pool (such as the membrane of a vacuole) determines how fast a cell can mobilise the enclosed reserve substrate. This property is used to derive the equation for metabolic scaling below. The scaling of the surface area interface of reserve rests on the assumption of ‘structural isomorphy’: that the dimensions of

each reserve component, which are sub-cellular pools of polymers suspended in a matrix of structure, are a fixed proportion of the total amount of structure. The assumption is useful for providing an intuitive mechanism behind DEB theory's reserve dynamics, but can be relaxed in place of the assumption of 'weak homeostasis' at the cost of increased abstraction (Kooijman 2010).

'Weak homeostasis' is the assumption that the composition of the individual as a whole does not change during growth in constant food environments. Given that an organism's biomass can be partitioned into compartments of constant, but potentially unique, composition (e.g. structure and reserve), a constant biomass stoichiometry is achieved through maintaining a constant proportionality between the amounts of these compartments. This assumption is motivated by the observation that stoichiometric homeostasis is a key life process (Sterner and Elser 2002) and is useful for any metabolic theory that works with metabolic pools of constant composition; else it is not possible to access the amounts and composition of pools in a developing individual. This inability would affect the testability and applicability of such theories substantially. Empirical evidence for weak homeostasis is voluminous (see e.g. Król *et al.* 2005; Chilliard, Delavaud, & Bonnet 2005; Fink, Peters, & Von Elert 2006; Ingenbleek 2006; Steenbergen *et al.* 2006), and can considered to be a stylised fact (Sousa et al. 2008). The composition of reserve and structure is determined in practice by mapping observed composition of biomass at different constant food levels to their expected relative amounts at these food levels. Metabolic theories that refrain from the delineation of pools need to follow specific metabolites and suffer from the necessity to distinguish the 'important metabolites', where only a few 'important metabolites' can be quantified.

The implication of DEB's reserve dynamics (Fig. 2-1) is that across species the amount of reserves must increase relative to the amount of structure to compensate for the sublinear scaling of the reserve surface area and mobilisation rate. If the amount of reserves increases

sufficiently, the release of reserves will keep pace with the maintenance demands of increasing structure. But because the relative proportion of structure decreases with size to make room for the reserve, mass specific maintenance will decrease and the metabolic rate will scale sublinearly with mass inter-specifically. Although not strictly reserve, body fat, for example, scales inter-specifically as mass$^{1.19}$ in mammals (Pitts and Bullard 1968, Calder 1984) and has a very low maintenance costs (Elia 1992).

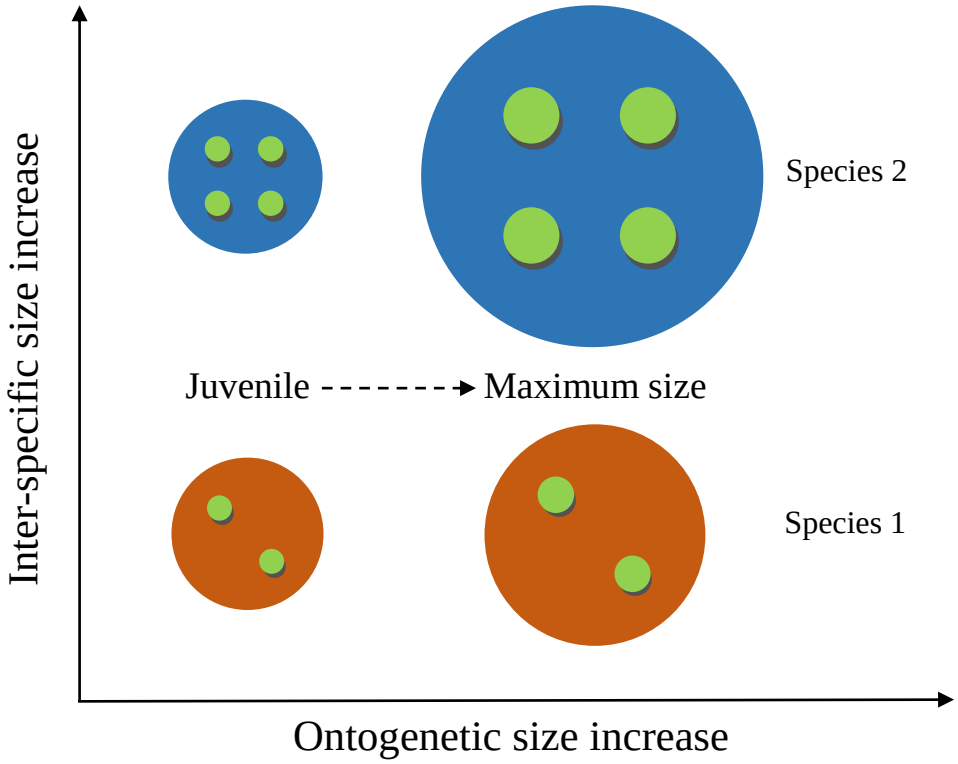

Figure 2-1. The body-size scaling of the reserve periphery able to be mobilised for metabolism is determined by assuming a constant proportionality (structural isomorphy) between each reserve pool (small inner circles) to total structure (large outer circles) both within and between species. If the number of reserve pools within a species is constant, ontogenetic size eventually reaches some maximum limit. This is due to the mismatch in scaling between the supply of reserve being mobilised and the demand of structural maintenance. While maintenance scales with structural volume, reserve mobilisation scales with the surface area of reserve sites, and thus scales more slowly within species. Larger adult sizes can be attained by increasing the number of reserve pools, which increases the total surface-area and mobilisation rate of reserve per unit structure and hence the amount of adult structure that can be sustained. Total reserve (E) is equal to the number of reserve

pools (n) times the pool size (r), or $E = nr$. The diagram shows that among species total reserve increases both through changes in the size and number (n and r) of reserve sites, while within species, only pool size (r) changes. The reserve interface (I) scales with $nr^{\frac{2}{3}}$. Using the assumption of constant proportionality of reserve pools to structure ($r \propto V$), the interface can also be expressed as, $I \propto E/V^{\frac{1}{3}}$. This argument assumes the individual pools comprising reserve to have identical size, composition, and shape, but these are not necessary requirements, and the argument can be extended to cover unequal pools.

We now consider the relationship between reserve mobilisation, metabolic rate and body mass under DEB theory (reproduction processes are not considered here but do not change the result). If structure (V) incurs a maintenance cost of $[\dot{p}_M]$ per unit of structure, the cost of maintenance in energy per time is:

$$\dot{p}_M = V[\dot{p}_M] \tag{2.1}$$

From Fig. 2-1 we can see that the interface or periphery of stored reserve scales with $E/V^{\frac{1}{3}}$ where E is expressed in units of energy and V is expressed in units of volume. The reserve mobilisation flux in energy per time is taken to be proportional to this interface:

$$\dot{p}_C = \frac{\dot{v}E}{V^{\frac{1}{3}}} \tag{2.2}$$

where the proportionality constant $\dot{v}$ has the dimension length per time and thus has the interpretation of a conductance. For non-growing organisms at ultimate size (V_m), $E = E_m$and all mobilised energy is being consumed by maintenance:

$$\dot{p}_M = \dot{p}_C \tag{2.3}$$

or, after substituting $\dot{p}_M$and $\dot{p}_C$:

$$V_m[\dot{p}_M] = \frac{\dot{v}E_m}{V_m^{\frac{1}{3}}} \tag{2.4}$$

Rearrangement of this equation shows that ultimate reserve scales inter-specifically with ultimate structure as:

$$E_m = \frac{V_m^{\frac{4}{3}}[\dot{p}_M]}{\dot{v}} \tag{2.5}$$

Adult mass is the sum of the weight of the two biomass compartments, structure and reserve at maximum size, and can be converted to mass using the respective mass density constants, d_V (wet-mass per volume) and d_E (wet-mass per energy):

$$M = d_V V_m + d_E E_m \tag{2.6}$$

Substituting E in this equation we obtain:

$$M = d_V V_m + \frac{d_E V_m^{\frac{4}{3}}[\dot{p}_M]}{\dot{v}} \tag{2.7}$$

If basal metabolic rate ($\dot{B}$) of post-absorptive organisms is the rate at which reserve is mobilised for metabolism (and completely consumed by maintenance costs at ultimate size) we have:

$$\dot{B} = V_m\,[\dot{p}_M] \tag{2.8}$$

This expression for metabolic rate is a special case and it should be stressed that metabolic rate cannot always be equated to the rate of respiration contributed by maintenance. In general, respiration would also need to include overheads of growth, assimilation and reproduction, while metabolic rate would also include the energy allocated to product formation (Kooijman 2013). Nevertheless, as we are dealing with post-absorptive animals at ultimate size we can substitute $\dot{B}$ into the previous equation to arrive at an equation relating metabolic rate to mass:

$$M = \frac{d_V \dot{B}}{[\dot{p}_M]} + \frac{d_E \dot{B}^{\frac{4}{3}}}{\dot{v}[\dot{p}_M]^{\frac{1}{3}}} \tag{2.9}$$

Remembering that d_V, d_E, $[p_M]$ and $\dot{v}$ are constants, we can simplify this relationship to:

$$M = C_0 \dot{B} + C_1 \dot{B}^{4/3} \tag{2.10}$$

This is precisely the equation that can be derived from the WBE model (see Savage et al. 2008). As Table 2-1 below shows, these two coefficients are comprised of very different parameter combinations under the two theories.

TABLE 2-1.

COMPARISON OF THE COEFFICIENTS IN DEB AND WBE MODELS SHOWS THAT THE SAME EQUATION FOR METABOLIC SCALING CAN EMERGE FROM TWO THEORIES BASED ON DIFFERENT ASSUMPTIONS, LEADING TO A PROFOUNDLY DIFFERENT INTERPRETATION OF THE METABOLIC SCALING RELATIONSHIP.

	DEB	**WBE**
C_0	$\frac{d_V}{[\dot{p}_M]}$	$C_2 \frac{V_{cap}\left(\bar{N} - \frac{1}{n^{1/3} - 1}\right)}{B_{cap}}$
C_1	$\frac{d_E}{\dot{v}\,[\dot{p}_M]^{1/3}}$	$C_2 \frac{V_{cap} n^{(1-\bar{N})/3}}{B_{cap}^{4/3}(n^{1/3} - 1)}$
	d_V: density of structure	C_2 : yield of mass on blood volume
	$[\dot{p}_M]$: maintenance rate of structure	V_{cap}: volume of capillary
	d_E: density of reserve	$\bar{N}$: transition level to area-preserving branching
	$\dot{v}$: conductance	n: branching ratio
		B_{cap}: metabolic rate of capillary

Despite the different interpretations of the two constants, the equation converges on Kleiber's law (Kleiber 1932) at the limit of large mass:

$$\lim_{M\to\infty} \dot{B} \propto M^{\frac{3}{4}} \tag{2.11}$$

It is important to emphasize that this relationship only holds at the infinite limit of mass and, contrary to common perceptions, metabolic rate does not depend on mass as a power function under either of these frameworks. In other words, this function predicts exponents that diverge from ¾ for finite masses when C_0 is positive. Nevertheless, the function approximates the ¾ power relationship well, particularly for small values of C_0. As the constants C_0 and C_1relate to different physical parameters under each respective framework, this equation has profoundly different interpretations when explaining metabolic scaling. In other words, the two theories lead to equations that are quantitatively identical, but diverge qualitatively. Interestingly, something that has been pointed out in metabolic theory (Isaac and Carbone 2010), but not investigated in any detail, is that different models may be simultaneously valid. Indeed, it is likely that these two frameworks are simultaneously offering useful insight. Without any optimality arguments, DEB's reserve dynamics explains why organisms require less energy per mass with increasing size while, for organisms with vascular supply networks, WBE shows how this decreased metabolic demand coincides with a network arrangement that reduces energy losses in transport. Thus, many of the predictions relating to variables of the cardiovascular system are still likely to be relevant approximations, including predicted blood volume, heart rate, stroke volume, blood pressure, radius of the aorta, volume of tissue served by a capillary, number and density of capillaries, dimensions of capillaries and oxygen affinity of haemoglobin (West, Brown, & Enquist, 1997, Savage et al. 2008). However, such transport constraints may not be the causal determinant of the scaling of respiration.

Organisms adapt to and are constrained by physical principles. Without recourse to optimality arguments, predictions can be made of how modes of transport must change as organisms grow larger and cohesion forces becomes less dependable, inertial forces enter the fore, and gravity becomes an increasing concern. Similarly, using only physical principles we have shown that larger organisms necessitate proportionally more reserve biomass to overcome the mismatched scaling of somatic maintenance and energy mobilisation. The proposed mechanism of reserve dynamics is feasibly more evolutionarily basal (Kooijman and Troost 2007) than other explanations based on network supply constraints, but does not preclude network design optimality where they occur. In this way, the scope of the DEB theory mechanism can be seen to apply to all species, not only those possessing branching vascular supply systems. The dynamics of the use of stored nutrients is a cornerstone of DEB theory and has been used widely and with great success in a variety of applications. This constraint on metabolic organisation can also readily make *a priori* predictions of metabolic scaling, and has implications for many life history traits (incubation times, juvenile periods, life span, reproduction rates, etc.).

An *a priori* respiration calculation

Mathematical descriptions of processes force us to be explicit about all underlying assumptions and provide an objective method for establishing the level of our understanding of a process (Nijhout et al. 2006). We have shown how mechanistic theories based on completely different principles can be used to derive the same equations for metabolic scaling, making it impossible to empirically distinguish between models based only on the quality of fits to data on metabolic scaling. However, the coefficients C_0 and C_1 are derived from

assumptions about other processes, which are, in principle, measurable and provide an important point of departure.

TABLE 2-2.

THE PARAMETERS REQUIRED TO SPECIFY THE METABOLIC SCALING RELATIONSHIP FOR MAMMALS WERE ESTIMATED SEPARATELY (SEE SUPPLEMENTARY DATA) FROM THE LITERATURE AND 'PLUGGED IN' TO THE DERIVED DEB EQUATION. THE RATE PARAMETERS ARE GIVEN FOR 20°C AND ARE OBTAINED FROM AVERAGES FOR THE MAMMAL ENTRIES OF 'ADD MY PET'.

Parameter	*Units*	*Description*	*Value*
d_V	g (wet)/cm^3	Density of structure	1.0
d_E	g(wet)/J	Energy-to-mass coefficient of reserve	1.45 x 10^{-4}
$\dot{v}$	cm/d	Conductance	0.043
$[\dot{p}_M]$	J/cm^3	Somatic maintenance	90.4

DEB parameters have been estimated for a large number of animal species from most large phyla and all 13 classes of chordates (this collection is called `add_my_pet' and is freely available online (see supplementary data)). At the time of writing this database included entries from 12 mammals, including the eastern grey kangaroo, African elephant, common dolphin, and brown rat. For each animal in the collection the 'covariation method' (Lika et al. 2011a) was applied to simple life-history data to estimate the set of 12 core DEB parameters, which include $\dot{v}$ and $[\dot{p}_M]$. These core parameters specify the unique bioenergetic lifecycles of organisms. The intuition behind the estimation procedure is that, although the parameters cannot themselves be measured directly, observational data can be used to restrict the value that these parameters can take. For example, even the trivial observation that all animals dissipate heat at ultimate size restricts $\dot{v}$ and $[\dot{p}_M]$ to values greater than zero. In a similar way, much more comprehensive data allows the core parameters to be systematically specified with great precision (Lika et al. 2011b). Estimated values for the constants d_V and d_E are typical

values for mammals that were taken from the literature (Kooijman, 2010), while $\dot{v}$ and $[\dot{p}_M]$ are averaged from the 12 mammalian entries in the 'add_my_pet' collection (see Table 2-2). These separately determined values were used in combination with the derived equation to predict the metabolic scaling relationship for mammals.

This *a priori* equation is compared against a recently compiled, temperature-corrected mammalian data-set (McNab 2008, Kolokotrones et al. 2010) in Fig. 2-2. Although the fit to the data is by no means perfect, the result of the predicted relationship is striking, particularly as no respiration data were used in determining any of the parameter values (except for the tammar wallaby). This approach stands in bold contrast to letting the parameters vary freely and allowing the "best fit" to decide what value they should take. Letting our knowledge of the physical parameters determine the fit provides a good test of the robustness of model assumptions.

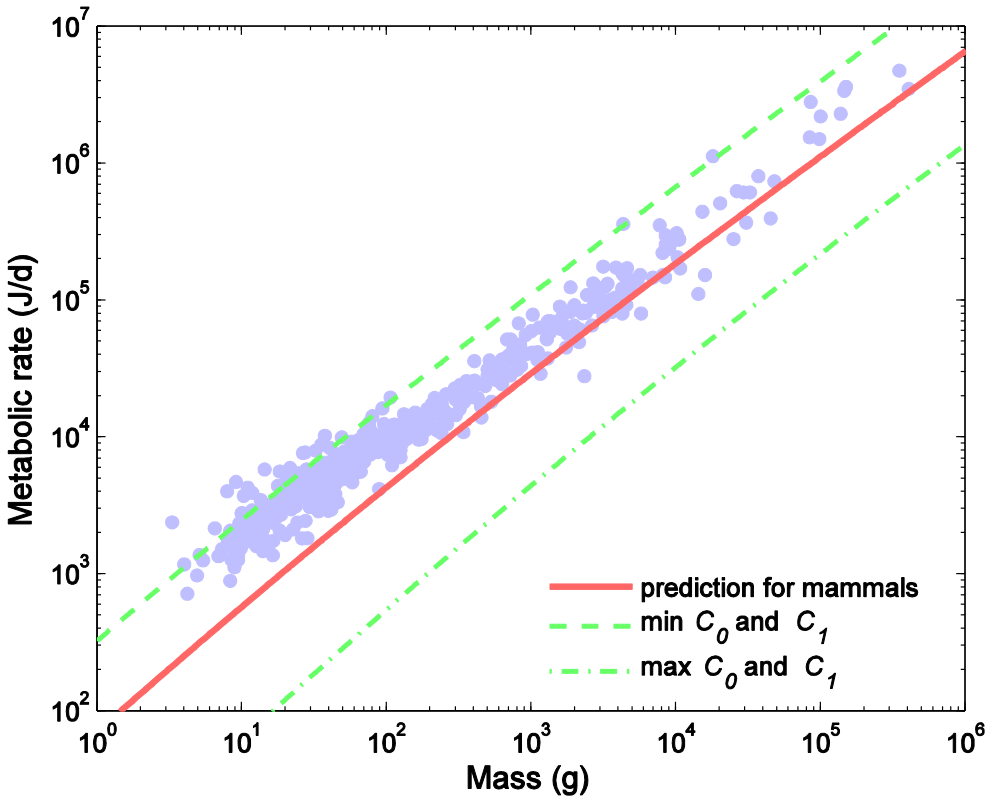

Figure 2-2. Rather than letting the 'best fit' determine the parameters, DEB theory was used to specify an a priori prediction of the metabolic scaling relationship for

mammals using the mean DEB parameters of 12 mammals in the 'add_my_pet' collection, and typical values for the caloric content of mammalian biomass. Metabolic rates are temperature corrected using an Arrhenius function with the Arrhenius temperature estimated to be 8627 Kelvin. The effect of variation in the coefficients C_0 and C_1 are represented by upper and lower predictions based on the lowest and highest values of $\dot{v}$ and $[\dot{p}_M]$ that were observed for mammals in the 'add my pet' collection (see supplementary data). Variation in the mass, density and energy density coefficients (d_V and d_E) of mammalian biomass was assumed to be insignificant compared to the observed variation in the DEB parameters $\dot{v}$ and $[\dot{p}_M]$, which can differ by some two orders of magnitude for different mammals. The non-allometric DEB function predicts metabolic rate to scale with mass$^{0.767}$ for the largest observed masses in the data set, and with mass$^{0.915}$ for the smallest. Data from McNab (2008) and Kolokotrones et al. (2010).

Although the predicted equation follows from simple physicochemical design constraints, consideration of biological and ecological constraints of organisms quickly renders the assumption of constant model parameters as unrealistic (as evidenced by the difference in upper and lower predictions). Population density and trophic level are potentially other important factors not considered here that may be able to account for deviations around the broad pattern of metabolic scaling ((Hechinger et al. 2011, DeLong et al. 2014). In addition to the direct effect of temperature on metabolism through biochemical kinetics (Gillooly et al. 2001), environmental temperature may also exert indirect effects on metabolism through the modification of competitive outcomes that produce interactions with body size (Reuman et al. 2014). Indeed, the evolution of life-histories optimised to a wide range of selective environments must be considered to make any sense of deviations from our simple prediction. Smaller mammals, for example, are likely to be more frequently exposed to conditions below their thermal-neutral zone and may thus have higher resting metabolic rates as a correlated response to higher heat-generating capacity overall (Rezende et al. 2004). Indeed, the previously mentioned add_my_pet collection of eco-physiological data and DEB parameters revealed a deviation from the expected pattern in parameter values that extends outside mammals: small-bodied species that live off blooming resources have a much higher somatic

maintenance than expected (Kooijman 2013). This eco-physiological adaptation was hypothesised to result from the wasting of resources in order to boost production (growth and reproduction), while keeping adult body size small.

DeLong *et al.* (2010) recently tested Kleiber's law across a size range inclusive of unicellular eukaryotes and prokaryotes and supported deviations from Kleiber's law at the extreme of small sizes. The linear scaling of unicellular eukaryotes was argued to be a response of the linear increase in the membrane-bound sites of ATP synthesis located in organelles, concordant with the linear scaling of structure with mass predicted by DEB theory at very small sizes. This steeper linear scaling of smaller organisms is also supported by Huete-Ortega *et al.* (2012) in a study on the metabolic scaling of unicellular autotrophic protists where they highlight the importance of changing surface-to-volume ratios. Indeed, changing surface-to-volume ratios slow growth down during the cell cycle in a way that is well-captured by DEB theory, which successfully describes microbial growth, respiration and product formation (Kooijman 1986a, 2010, Evers 1991, Hanegraaf and Muller 2001, Brandt et al. 2003, 2004, Eichinger et al. 2010). The DEB explanation has particular, and experimentally confirmed, implications for population growth (Ratsak 1995; Ratsak, Maarsen, & Kooijman 1996 on ciliates, Kooijman & Kooi 1996 on myxamoebas, Hanegraaf, Stouthamer, & Kooijman 2000; Muller 2011; Muller *et al.* 2011 on yeasts, Lorena *et al.* 2010 on microalgae) and readily explains the difference between flocculated growth and growth in cell suspension (Brandt and Kooijman 2000) and the effect of genome size on population growth (Stouthamer and Kooijman 1993).

Implications, extensions, and limitations

DEB theory considers the inter-specific relationship between metabolic rate and mass as being mediated by two compartments (structure and reserve), each with separate dynamics. But, just as metabolic rate can be expressed as a function of reserve and structure, so can many other life history traits. In contrast to the WBE approach, which uses the theoretical scaling of oxygen delivery to derive other life-history scaling relationships, DEB theory views reserve dynamics as fundamental to these relationships, including how the rate of oxygen consumption scales with body size. Knowledge of how the relative contribution of reserve to biomass varies with size allows the calculation of simple scaling relationships of life-history traits.

Table 2-3 provides DEB theory equations for some other important inter-specific scaling relationships in terms of mass (see supplementary information for derivations). These equations are not all strict power functions but, at the infinite limit of mass, many converge on the quarter-power scaling relationships frequently observed in biology. However, even in finite mass ranges these non-allometric functions can approximate quarter-power scaling.

TABLE 2-3.

DEB THEORY WAS USED TO DERIVE EQUATIONS FOR THE SCALING OF A NUMBER OF IMPORTANT LIFE-HISTORY TRAITS. WHEN EXPRESSED IN TERMS OF TOTAL MASS, RATHER THAN STRUCTURE AND RESERVE, THE WELL-KNOWN QUARTER-POWER SCALING RELATIONSHIPS EMERGE AT THE INFINITE LIMIT OF MASS. FOR DERIVATIONS OF THE RELATIONSHIPS, SEE SUPPLEMENTARY INFORMATION.

	Inter-specific scaling of traits	
Trait	In terms of V_m and E_m	limit as mass $(M) \to \infty$
Body mass	$M = d_V V_m + d_E E_m$	∞
Metabolic rate	$\dot{B} = [p_M] V_m$	$\dot{B} \propto M^{\frac{3}{4}}$
*Growth rate**	$\dot{r}_B = \frac{[\dot{p}_M]}{3[E_G] + 3E_m/V_m}$	$\dot{r}_B \propto M^{-\frac{1}{4}}$
*Food uptake rate***	$\dot{p}_A = [\dot{p}_A] V_m$	$\dot{p}_A \propto M^{\frac{3}{4}}$
Starvation time	$t_S = \frac{E_m}{V_m [\dot{p}_M]}$	$t_S \propto M^{\frac{1}{4}}$
*Mass at birth****	$M_b = \frac{V_b}{V_m}(d_V V_m + d_E E_m)$	$M_b \propto M^1$
Development time to V_p	$t_P = \frac{1}{\dot{r}_B} ln \frac{V_m^{\frac{1}{3}} - V_b^{\frac{1}{3}}}{V_m^{\frac{1}{3}} - V_p^{\frac{1}{3}}}$	$t_P \propto M^{\frac{1}{4}}$
Egg mass	$M_e \simeq d_E \left(\frac{V_b}{V_m}\right)^{\frac{1}{3}} E_m \left(1 - \frac{1}{4}\left(\frac{V_b}{V_m}\right)^{\frac{1}{3}} \right)^{-3}$	$M_e \propto M^1$
Reproductive rate#	$\dot{R} = \frac{(1-\kappa) d_E \dot{v} E_m}{V_m^{\frac{1}{3}} M_e}$	$\dot{R} \propto M^{-\frac{1}{4}}$

* $[E_G]$ is the cost per unit of structure. This growth rate co-efficient is identical to the von Bertalanffy growth rate.

**$[\dot{p}_A]$ is volume specific uptake rate.
***V_b is structure at birth and must be less than some arbitrary structural volume, V_p.
#$1 - \kappa$ is the proportion of the mobilisation flux allocated to reproduction, assuming the remaining proportion κ is being allocated to maintenance at full size. Prior to reproductive age this energy is assumed to be dissipated as a cost of development.

There are theoretically sound reasons for the expression of biomass in terms of reserve and structural mass, not least of all because this distinction helps to increase the coherence of intra- versus inter-specific scaling (Kooijman 2010, Sousa et al. 2010). Zeuthen (1947) was the first to point to the fundamental difference between these scaling relationships, an important warning that went almost lost in recent discussions. Reproductive rate, for example, increases with mass intra-specifically but decreases inter-specifically; the reason being that the cost per neonate is expected to be constant within species, but varying between species. Under food restriction, adult reserve can decrease intra-specifically, so the amount of resources available for reproduction decreases, and reproductive rate declines with size. This reduction in adult reserve also occurs for inter-specific size decreases (see Fig. 2-1), but is accompanied by a greater decrease in offspring size (see Table 2-3), which has the net effect of increasing reproductive rate.

This distinction also leads to different expectations of the scaling of uptake rates. Table 2-3 shows that assimilation is expected to scale inter-specifically with mass$^{3/4}$ at the infinite limit of mass (higher exponents for finite masses). During ontogeny, however, assimilated energy needs to match the rate of reserve mobilisation, and so only scales with mass$^{2/3}$ (Kooijman 1986a, 2010). Indeed, uptake rates have been found to scale inter-specifically with an exponent significantly larger than 2/3 and 3/4 (Pawar et al. 2012), which is the DEB expectation for finite sizes.

Perhaps most relevant to the topic at hand, thinking of biomass in terms of structure and reserve also leads to a very different interpretation of inter- vs. intra-specific scaling of respiration – an area of metabolic theory that has attracted much criticism in the past. Studies investigating the intraspecific scaling of respiration rate frequently find the relationship is best approximated by a mass exponent significantly different from ¾ (Glazier 2005, 2006, Caruso et al. 2010), while others question the appropriateness of fitting a simple power law altogether (Glazier 2005, 2006, Sears et al. 2012). Intra-specifically and under constant food, DEB theory predicts that maintenance costs would scale proportionally to mass. The decrease in mass-specific respiration that is frequently observed through ontogenetic development is explained by the decreasing contribution of growth overheads to respiration. Under DEB theory, the changing relative contributions from growth, assimilation, and maintenance to intraspecific respiration explains variation in the estimated exponent. This important distinction between intra- and inter-specific cases has been used to successfully predict the ontogenetic respiration of bryozoans, which scaled with $mass^{1/2}$ (White et al. 2011). The reserve concept also captures the time course of respiration rates during embryonic development. At the beginning of embryonic development the egg consists almost entirely of reserve and hardly respires but, as the embryo grows, somatic maintenance and growth overheads contribute more and more to respiration until hatching. This frequently observed pattern is shown in Fig. 2-3.

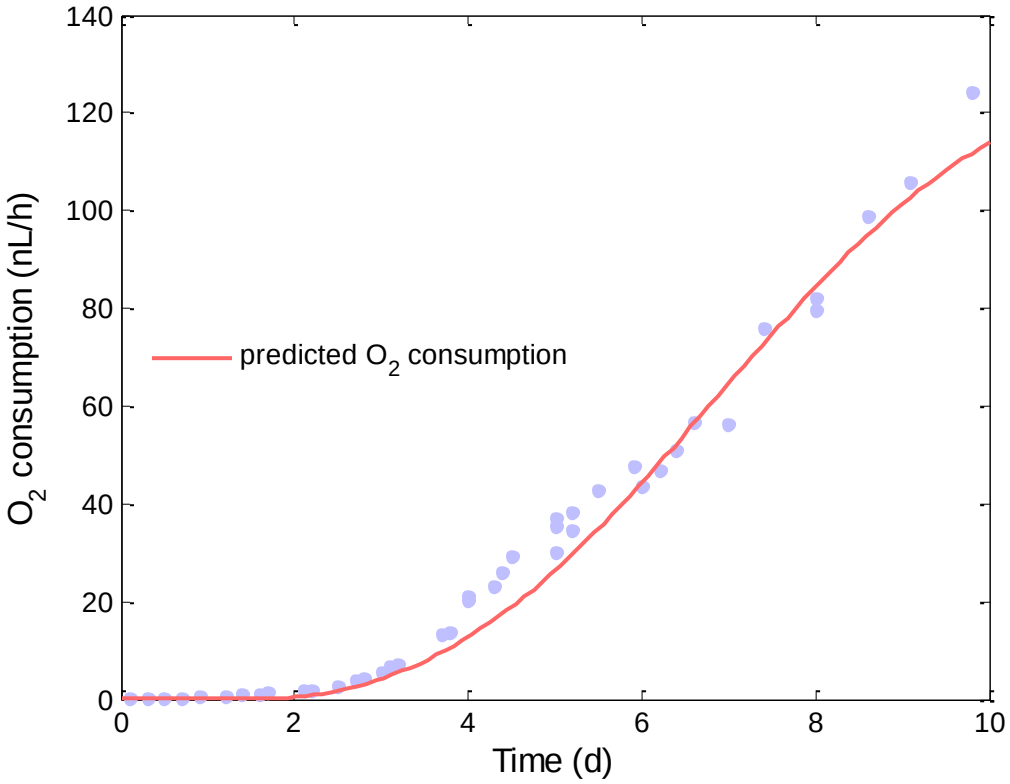

Figure 2-3. DEB based equation modelling embryonic respiration in the pond snail *Lymnaea stagnalis* (adapted from Kooijman 2010). Respiration increases until hatching as embryonic structure accumulates.

The previous examples illustrate how the biological quantities of reserve and structure can be more informative than the total body mass of an organism. Unlike body mass, however, these abstract quantities can be difficult to measure in practice. If reserve was defined as something easily measurable, such as fat storage, an elephant weighing roughly 200,000 times more than a mouse would have approximately $200{,}000^{1/4} \sim 21$ times more fat storage per mass than a mouse. As such a simplistic interpretation would lead to incongruities with empirical knowledge, constituents of reserve and structure are best defined by their dynamics: reserve consists of those elements of biomass that have a finite turnover, while those elements that are maintained indefinitely comprise structure. Both reserve and structure are treated as generalized compounds: mixtures mainly consisting of carbohydrates, protein and lipids, with

potentially different weight coefficients. The utility of the concepts of reserve and structure should not be seen in the ease of their measurement but in their theoretical implications. Allele frequencies in a population, for example, were initially very difficult to measure but have conceptually revolutionised evolutionary biology. In a similar vein, Houston and McNamara (2014) take the theoretical currencies of animal condition and reserve, in addition to energy and time, to create a more nuanced model for foraging behaviour, suggesting ways in which the DEB state variables of reserve and structure could be further extended.

Discussion

The discussed theories for metabolic scaling each offer idealised 'canonical' models. The similarity in the predicted inter-specific scaling of metabolic rate with body mass can be interpreted intuitively as design constraints on the rate of energy and material flows within organisms. In 'transport models', nutrients are delivered through a network to 'terminal units' (WBE) or 'service volumes' (Banavar et al. 2002), each with an invariant metabolic rate (i.e. maintenance cost). Network design constraints imply that the volume of animal associated with each terminal unit increases with body mass, and that there is a matching between supply and demand. Since terminal units have fixed metabolic rates, metabolic rate per unit mass decreases with body mass. In DEB theory, metabolic rate is constrained by the utilisation process of storable metabolites (reserve) and the regulation of their concentration within an organism. Assimilate is eventually transformed into structural biomass with an invariant specific maintenance cost, where the chemical intermediate of this process is defined as reserve. Constraints on reserve utilisation require that the ratio of structure to reserve decreases inter-specifically with body mass. Since structure has a fixed maintenance requirement, metabolic rate per unit mass for a non-growing animal decreases with body mass.

Interestingly, in both the DEB and WBE theory, metabolic scaling is explained by the scaling of the relevant metabolite interface (exposed periphery of reserve pools in DEB theory, and the terminal unit in the supply network in the WBE), but neither of these metabolite interfaces scale with $mass^{2/3}$, as one might naively expect from simple Euclidean geometry. If these important interfaces really are scaling in unison then, taken together, these theories show how optimal nutrient transport designs can coincide with the decreased metabolic demands resulting from simple reserve dynamics. There are, however, some clear areas of divergence, particularly regarding the relationship between inter- and intra-specific scaling of biological rates, which suggest fruitful tests to further refine the current theories of metabolism.

We end by noting that mechanistic models are built from a small set of core assumptions or first principles that are causally linked and capture something fundamental about a physical reality. The explanatory capacity of mechanistic models is based on the fundamental processes they consider. This provides robust predictive power, especially under novel circumstances. However, because these models only focus on a small number of processes, deviations from their predictions by specific organisms may be as instructive as congruence. When a particular species deviates from the patterns that were predicted, we can look to that organism's life history for explanations. With DEB theory, this is typically done by looking for the minimum set of modifications to the standard DEB model, such that a 'phylogeny' of model variants emerge (van der Meer 2006b); there is no need to start from scratch with each new focal organism. With the growing body of data on DEB parameters, the DEB theory of inter-specific scaling is thus not only of fundamental scientific interest, but also of considerable practical value.

Chapter 3: Ontogenetic and interspecific metabolic scaling in insects

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Online resources:

Data available from the Dryad Digital Repository: http://doi.org/10.5061/dryad.3qv3p

Abstract

Design constraints imposed by increasing size cause metabolic rate in animals to increase more slowly than mass. This ubiquitous biological phenomenon is referred to as metabolic scaling. However, mechanistic explanations for interspecific metabolic scaling do not apply for ontogenetic size changes within a species implying different mechanisms for scaling phenomena. Here we show that the Dynamic Energy Budget theory approach of compartmentalizing biomass into reserve and structural components provides a unified framework for understanding ontogenetic and interspecific metabolic scaling. We formulate the theory for the insects and show that it can account for ontogenetic metabolic scaling during the embryonic and larval phases, as well as the U-shaped respiration curve during pupation. After correcting for the predicted ontogenetic scaling effects, which we show to follow universal curves, the scaling of respiration between species is approximated by a ¾ power law, supporting past empirical studies on insect metabolic scaling and our theoretical predictions. The ability to explain ontogenetic and interspecific metabolic scaling effects

under one consistent framework suggests that the partitioning of biomass into reserve and structure is a necessary foundation to a general metabolic theory.

Introduction

In biology, metabolic rate is a fundamental property of organisms that governs the flow of energy and materials at all levels of biological organization (Schmidt-Nielsen 1984, Brown et al. 2004). Since, Kleiber (1932), metabolic rate in most plants and animals has been widely found to scale interspecifically with mass (M) as approximately $M^{3/4}$ when large body-size ranges are considered (Hemmingsen 1960, Savage et al. 2004, Chown et al. 2007a, Ehnes et al. 2011). A major biological challenge is to find a general theory of metabolism to account for such scaling relationships (Kearney and White 2012). In the most prominent explanations of metabolic scaling it is often implicitly assumed that the physical constraints responsible for interspecific metabolic scaling are also responsible for ontogenetic patterns (West et al. 1997, Banavar et al. 1999, Darveau et al. 2002, Kozłowski et al. 2003, Hou et al. 2008b, Glazier 2010, Kolokotrones et al. 2010). However, ontogenetic metabolic scaling is often found to deviate qualitatively and quantitatively from interspecific patterns (Wieser 1984, Glazier 2006, Chown et al. 2007a, Moran and Wells 2007, Caruso et al. 2010, Yagi et al. 2010, Sears et al. 2012). This not only questions the universal power-law scaling of metabolic rate, but also suggests that a completely different mechanism may underlie ontogenetic metabolic scaling. Here, using insects as a case study, we develop a framework that captures the diverse ontogenetic scaling of metabolism (Fig. 3-1) as well as interspecific effects. As insects dominate the known diversity of animal life, any metabolic theory claiming to be universally general to life must also account for this important taxonomical group. We test our predictions against a data set we compiled on the respiratory metabolism of insects during

various life-history stages.

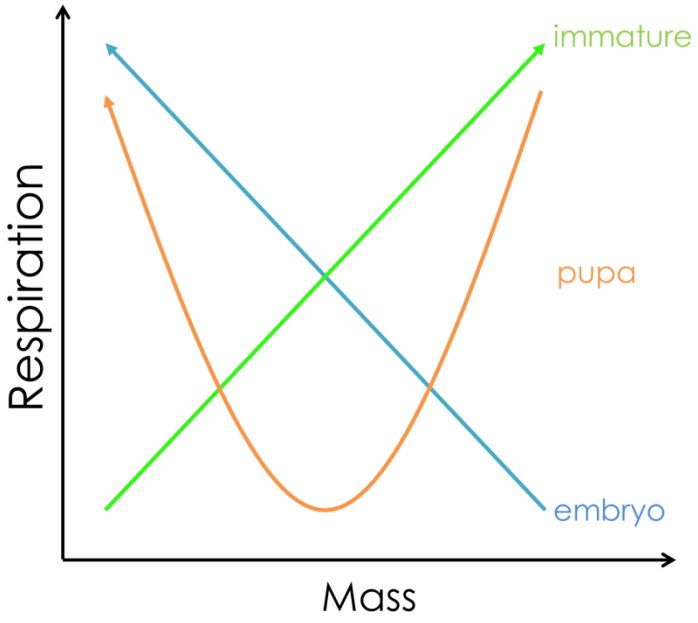

Figure 3-1. Throughout ontogeny insects exhibit a range of metabolic scaling patterns. This schematic illustrates the variety of scaling patterns for key life history stages with the time course of development indicated by the arrowhead. Time moves from right to left for non-feeding embryos and pupae, which lose mass as they develop.

A mechanistic model of metabolism

The variety of respiration patterns during insect ontogeny cannot be captured using a single allometric function of the form $y = aM^b$. As allometric functions are monotonic, the U-shaped scaling of metabolic rate with mass during the pupation of holometabolous insects precludes their use and shows that mass is an unreliable indicator of metabolic state. Moreover, for insects in general (including non-holometabolous insects) embryonic

respiration can vary by orders of magnitude while mass remains comparatively unchanged (Fink 1925, Rakshpal 1962), suggesting mass-independent effects are an important, overlooked factor in metabolic scaling (Glazier 2005). A general framework for metabolic organization that can capture all of these patterns under a simple set of assumptions is Dynamic Energy Budget (DEB) theory (Kooijman 2010). In addition, as most prominent theories are either only applicable to organisms with closed circulatory systems (West et al. 1997, Banavar et al. 1999, Kolokotrones et al. 2010), or do not make a priori predictions (Darveau et al. 2002, Glazier 2006, Kozłowski et al. 2010), DEB theory represents a general predictive framework in which to interpret metabolism for all organisms.

DEB departs from many prominent theories that consider mass to be the most important determinant of metabolism, by viewing body mass as a function of more fundamental quantities. In DEB theory, biomass is comprised of 'reserve' and 'structure'. Reserve is defined as the sum of all intermediary materials between the uptake of food and the payment of costs incurred by general metabolism, which include the growth and maintenance of structure, and reproduction (not considered here but see Kooijman (2010)). In this way, both the composition of biomass (proportion of reserve to structure) and the size of the organism (structure) can affect metabolism. This captures, for example, why two organisms of identical mass but different feeding histories are not equivalent in terms of their metabolism, or why organisms may continue to grow and reproduce under starvation.

Besides food availability, ontogeny can cause the ratio of 'reserve' and 'structure' to vary dynamically with time, which explains differences in respiration patterns. In a DEB framework, it is convenient to think of a fresh egg as almost entirely reserve, with an initially small amount of structure that increases with development. Under this view, due to the costs associated with the growing amount of structure, respiration is expected to increase as embryonic development proceeds in spite of any increase in total mass (Kooijman 1986b).

On the other hand, for growing larvae and nymphs (hereafter jointly referred to as immatures) respiration is expected to increase with mass. Given that food is unrestricted, immatures are assumed to be constrained by pressures of stoichiometric homeostasis (Sterner and Elser 2002) and thus maintain a constant composition of reserve and structure (Kooijman 2010). Structure, and therefore maintenance metabolism, is then proportional to mass and increases with immature development. During pupation in holometabola, there is no feeding and metabolism is fueled via the depletion of reserves. Larval structure is histolysized and energy and materials are recuperated for the growth of adult structure. Pupal respiration would thus be expected to first decrease during the histolysis of larval structures and to then increase as adult structure is formed.

Allometric functions may adequately capture metabolic scaling for interspecific comparisons, but for reasons discussed above, they would not be able to capture these diverse ontogenetic patterns. We propose that a simple way to capture ontogenetic effects is to multiply the expected metabolic rate of a species by a dimensionless polynomial equation that adjusts the metabolic rate at ultimate size to the particular developmental stage.

$$B = paM^b \qquad (3.1)$$

In this way the expression for metabolic rate (B) now consists of an interspecific component (aM^b) and an ontogenetic component (p). Three polynomials are presented to adjust for the stage of development during the embryonic, immature and pupal phase (Table 3-2). Developmental stage is represented by scaled time for eggs and pupae (time divided by emergence time) and scaled mass for immatures (mass divided by ultimate mass). We use scaled time for embryonic and pupal stages because, compared to the immature phase, mass changes are small and are consequently prone to measurement error. Polynomial functions

are well suited to capturing ontogenetic changes becuase they are simple, non-monotonic functions with desirable statistical properties. As polynomials reduce to a linear function, their parameters are easily estimated from data. More importantly, under special circumstances, DEB theory predicts ontogenetic respiration to follow a polynomial function of developmental stage (Appendix B), which offers a mechanistic interpretation and method to estimate polynomial coefficients from underlying biological processes.

Some simplifying assumptions are required to derive polynomial correction factors from DEB theory. We take respiration as proportional to somatic maintenance, which ignores the contributions from growth and feeding usually considered in a DEB framework. We also assume that the amount of reserve is not limiting development rate during the embryonic and pupal phase (see Appendix B). The result of these simplifying assumptions is that the effect of ontogeny p is separate from interspecific effects aM^b, and respiration can be divided by interspecific effects and plotted on universal curves.

$$\frac{B}{aM^b} = p \tag{3.2}$$

Or conversely, the interspecific scaling of metabolism can be observed by adjusting respiration by the predicted ontogenetic effects shown in table 3-2 and plotting the result against species mass.

$$\frac{B}{p} = aM^b \tag{3.3}$$

Theoretically derived polynomial equations are compared with the polynomial of the same degree estimated by least squares regression in terms of the variance explained and the Akaike Information Criterion (AIC) (Burnham and Anderson 2002). Interspecific scaling

exponents are estimated by applying an ordinary least squared regression to log-transformed data. DEB theory predicts the interspecific component of respiration aM^b to vary with species' mass raised to an exponent between ¾ and 1. DEB theory predicts Kleiber's ¾ scaling rule at the limit of large mass, but at small masses the exponent will be closer to 1 (Maino et al. 2014). This is because, at very small masses, reserve makes up a very small amount of biomass and structure can be approximated by total mass. Structure, and thus maintenance metabolism, increase proportional to mass at very small sizes. At very large sizes reserve contributes significantly to mass, meaning structure, and thus maintenance metabolism, scale sublinearly with size or with an exponent less than 1. Readers are directed to Maino et al. (2014) for a detailed discussion and derivation of predictions for interspecific metabolic scaling.

Data set

To demonstrate the unique ontogenetic and interspecific effects on respiration we compiled ontogenetic respiration data for embryonic, immature and pupal developmental stages. Data was retrieved from a comprehensive literature search of insect respiration through ontogeny, which resulted in 64 studies on insects from nine orders being included in the present analysis (See table 3-1 for a summary of the data set). Where possible, data was extracted from tables or requested from the original authors of the study, otherwise figures were digitized so that data points could be extracted. All respiration data was standardized to a common temperature of 20°C using the Arrhenius equation and an Arrhenius temperature of 8000K (Gillooly et al. 2001). Most respiration measurements were reported in μL O_2 consumption, which were converted to joules assuming a conversion coefficient of 48.9 μL/J.

TABLE 3-1.

SUMMARY OF INSECTS COMPRISING DATA SET FOR EACH DEVELOPMENTAL STAGE

	Coverage of data over lifecycle*		
Insect species	Egg	Immature	Pupa
Blattodea		•	
Blaberus discoidalis		•	NA
Blatella germanica		•	NA
Periplaneta americana		•	NA
Coleoptera			
Aphodius rufipes		•	
Callosobruchus analis		•	
Crioceris asparagi	•		
Cryptolestes ferrugineus		•	•
Hippodamia convergens			•
Leptinotarsa decemlineata	•		•
Paropsis charybsis			•
Popillia japonica	•		•
Rhyzopertha dominica		•	•
Sitophilus granarius		•	
Tenebrio molitor	•		•
Tribolium confusum	•		
Diptera			
Chironomus riparius		•	
Delia platura			•
Drosophila melanogaster			•
Glossina morsitans			•
Lucilia illustris		•	
Musca domestica			•
Sarcophaga argyrostoma			•
Tipula abdominalis		•	
Hemiptera			
Anasa tristis	•		NA
Lygaeus kalmia		•	NA
Oncopeltus fasciatus	•	•	NA
Philaenus spumarius		•	NA
Rhodnius prolixus	•		NA
Trigonotylus coelestialium		•	NA
Hymenoptera			
Apis mellifera		•	•
Macrocentrus ancylivora			•
Solenopsis invicta		•	
Lepidoptera			
Actias luna	•		
Ancylis comptana			•

Bombyx mori	•	•	•
Galleria mellonella			•
Hyalophora cecropia	•		
Manduca sexta			•
Ostrinia obumbratalis	•		
Pachysphinx modesta		•	
Orthoptera			
Acheta domesticus		•	NA
Allonemobius socius	•		NA
Encoptolophus sordidus		•	NA
Gryllus pennsylvanicus	•		NA
Gryllus veletis	•		NA
Melanoplus differentialis	•		NA
Melanoplus sanguinipes		•	NA
Phasmatodea			
Phyllium crurifolium		•	NA
Tricoptera			
Potamophylax cingulatus		•	
Sericostoma personatum		•	

*see supporting data set for data sources; NA = Not Applicable

To make respiration of holometabolous larvae comparable with non-holometabolous nymphs, we excluded the prepupal period after the cessation of feeding. As we do not consider reproduction here we also excluded data from reproductive stages. For all sources, when multiple data were given for one time point, the mean was taken. In addition, where females and males were separated, female data was used. For embryonic and pupal data that only listed mass-specific rates, absolute rates were recovered by assuming eggs and pupae lose a negligible amount of dry weight before eclosion. This holds approximately for dry-weights, which are used in all analyses. When only egg dimensions were given, weight was calculated from volume. Our full data set with references and comments is available in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.3qv3p (Maino and Kearney 2014).

Results

Our results confirm that, egg, immature and pupal respiration follow generic patterns for a diverse range of insects (Fig. 3-2). During embryonic development respiration rate increases despite the lack of feeding or mass gain. In immatures, respiration increases with development, while during pupation respiration exhibits both decreasing and increasing phases as emergence is approached.

TABLE 3-2.

ONTOGENETIC EFFECT ON RESPIRATION PREDICTED BY DEB THEORY COMPARED AGAINST BEST-FIT POLYNOMIALS

Stage	Parameters determined by DEB theory	r^2	AIC	Parameters determined by best-fit	r^2	AIC
Egg	$p_e = 0.12\tau^3 + 0.37\tau^2 + 0.38\tau + 0.13$	0.85	102.6	$p_e = 1.01\tau^3 - 0.94\tau^2 + 0.80\tau + 0.14$	0.86	95.3
Larva/ Nymph	$\log_{10} p_l = \log_{10} \mu^* + 0$	0.90	2463	$\log_{10} p_l = 0.81 \log_{10} \mu^* - 0.15$	0.94	2083
Pupa	$p_p = 2.75\tau^2 - 2.87\tau + 1$	0.40	458.3	$p_p = 2.14\tau^2 - 2.08\tau + 0.84$	0.50	425.5

* immature data, which spanned several order of magnitude, were log transformed to ensure errors were normally distributed

Dimensionless polynomials derived from a simplified DEB framework were able to capture these broad ontogenetic patterns (Fig. 3-2a-c), explaining 40-90% of the variation in the ontogenetic component of respiration (Table 3-2). Furthermore, after controlling for ontogenetic effects on respiration, the interspecific metabolic scaling exponents were found to be within the expected range of ¾ and 1 (Fig. 3-2e-f).

Best-fit polynomials determined by least squares regression were able to explain 50-94% of the variation in the ontogenetic component of respiration (Table 3-2). Comparing least squares polynomials with the theoretically derived polynomials found that the least squares polynomial consistently explained more variance and had lower AIC scores, even

after accounting for the fewer free parameters in the DEB polynomials. However the theoretically derived polynomial explained comparable levels of variance (1%, 4%, and 10% less variance than the best-fit polynomial for egg, immature and pupal stages respectively).

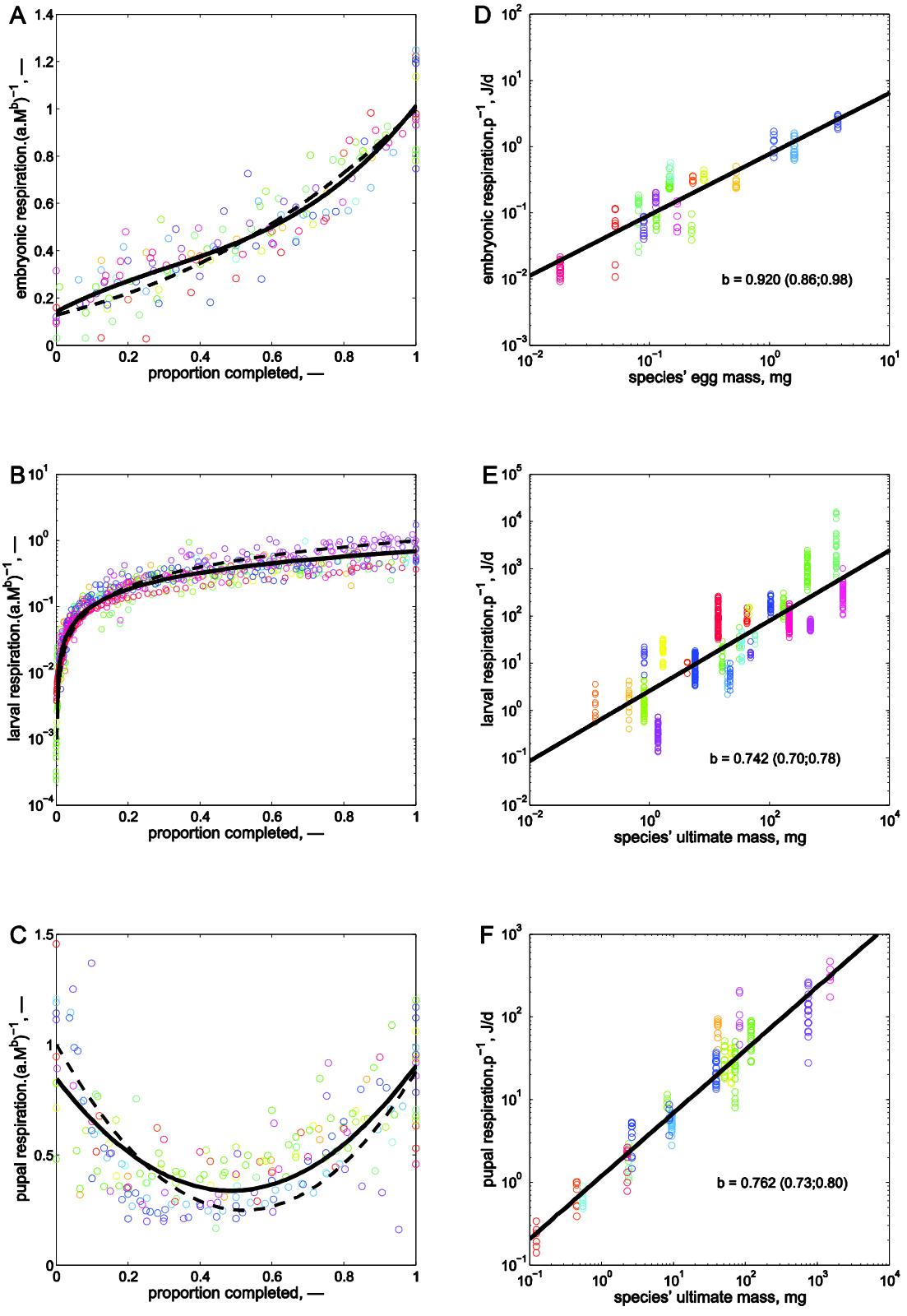

Figure 3-2. After normalizing interspecific effects, ontogenetic respiration for (a) eggs, (b) immatures, and (c) pupae is predicted to follow a dimensionless polynomial function of the proportion of the developmental stage completed (dashed line) (see Appendix B). The best-fit (least squares) polynomial of the same degree is also shown with a solid line. After accounting for these ontogenetic effects, respiration for (d) eggs, (e) immatures, and (f) pupae scaled interspecifically with mass raised to an exponent between 0.74-0.92 (parentheses contain the 95% confidence interval). Unique shades represent unique species with the legend given in supporting data set.

Discussion

Our presented framework is the first to explain not only the interspecific scaling of insect metabolism but also the distinct ontogenetic scaling, as most dramatically illustrated by the U-shaped time-course of respiration during pupation. Interspecific scaling exponents recovered from the data after controlling for ontogenetic effects supported our theoretically predicted exponents for insect metabolic scaling. In addition to our theoretical predictions, our findings support past empirical studies. Addol-Bediako et al. (2002) found insect respiration to scale with an exponent of 0.77, but noted that the scaling exponent changed when flying and non-flying insects were separated. Studies conducted independently by Chown et al. (2007) and Ehnes et al. (2011), found that respiration data on ~400 insects scaled with interspecific mass with an exponent indistinguishable from 0.75. Interestingly, Ehnes et al. found that estimated exponents for other non-insect invertebrate groups were significantly lower than 0.75, highlighting groups where simple models for metabolic scaling may need to be extended.

We have shown that dimensionless polynomials estimated from both DEB theory and by regression were able to correctly predict the diverse patterns in respiration that occur throughout insect ontogeny. Our theoretical approach departs from the prevalent view that the same mechanisms underlie both ontogenetic and interspecific patterns (West et al. 1997,

Banavar et al. 1999, Darveau et al. 2002, Kozłowski et al. 2003, Hou et al. 2008b, Glazier 2010, Kolokotrones et al. 2010). In explaining diverse scaling patterns in one consistent framework, we highlight the usefulness of a theoretical approach based on compartmentalizing biomass to capture mass-independent effects.

Although our theoretical curves lay close to the best-fit polynomials, it is important to emphasize the difference in approaches. The DEB polynomials were derived from underlying biological processes and have a mechanistic interpretation based on energetic implications of the changing proportions of reserve and structure through ontogeny. In comparison, the best-fit polynomials are simple descriptors of data. Unlike the best-fit polynomials, whose parameters are constrained by the data, the DEB polynomials are constrained by our simple assumptions of the processes underpinning metabolic rate. Deviations from simple mechanistic models highlight when other processes may need to be considered. For example, the best-fit polynomial for immature respiration estimated an ontogenetic scaling exponent of less than one. This is likely due to the significant contribution to respiration of surface area mediated processes such as nutrient absorption, which were not considered in the current study.

To capture these deviations our simplified approach can be replaced by a more nuanced approach whereby differences between individuals can be represented though the addition of species-specific parameter values. This is the standard approach in typical studies implementing DEB models. Once parameters for different species have been determined, patterns in parameter values can then be tested for systematic variation. This approach has been used to explain divergent life history traits in groups of related frogs (Mueller et al. 2012) and fish (Perciformes) (Lika et al. 2014), as well as in more broad-scale analysis that include several different phyla (Kooijman 2013). Estimated DEB parameters have physical dimensions, which allow natural interpretation and straightforward comparison. The

downside of such approaches is that a large amount of data, including various energetic and developmental data, is required to estimate species parameters with confidence. Our present results show that in addition to more detailed studies, DEB theory can also be used to explore broad-scale interspecific patterns in only one type of data – respiration in this case.

Although here we focused on insects, the implications are far reaching and apply to animals in general. Size imposes constraints on metabolism that depend on whether the observed mass increase is ontogenetic or interspecific. In DEB theory these constraints are reflected by changing proportions of reserve and structure, of which the relative quantities are predicted to vary in specific ways under different circumstances. The partitioning of biomass into reserve and structure predicts metabolic properties of biomass to change even when mass is (approximately) constant, and is thus a necessary abstraction to capture the metabolic scaling of diverse organisms throughout various developmental stages.

Chapter 4: Ontogenetic and interspecific scaling of consumption in insects

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Online resources:

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.35n9f/1

Abstract

The uptake of resources from the environment is a basic feature of all life. Consumption rate has been found to scale with body size with an exponent close to unity across diverse organisms. However, past analyses have ignored the important distinction between ontogenetic and interspecific size comparisons. Using dynamic energy budget theory, we present a mechanistic model for the body mass scaling of consumption, which separates interspecific size effects from ontogenetic size effects. Our model predicts uptake to scale with surface-area ($mass^{2/3}$) during ontogenetic growth but more quickly (between $mass^{3/4}$ and $mass^{1}$) for interspecific comparisons. Available data for 41 insect species on consumption and assimilation during ontogeny provides strong empirical support for our theoretical predictions. Specifically, consumption rate scaled interspecifically with an exponent close to unity (0.89) but during ontogenetic growth scaled more slowly with an exponent of 0.70. Assimilation rate (consumption minus defecation) through ontogeny scaled more slowly than consumption due to a decrease in assimilation efficiency as insects grow.

Our results highlight how body size imposes different constraints on metabolism depending on whether the size comparison is ontogenetic or inter-specific.

Introduction

Attention to the physico-chemical constraints on metabolic organisation has increasingly aided the interpretation of seemingly distinct biological phenomena (Brown et al. 2004). The consideration of cells, individuals, populations, and ecosystems as simple 'energy processors' increases the comparability of biological units that span vast spatio-temporal scales. This approach is the hallmark of the emerging field of metabolic ecology (Humphries and McCann 2013). Diverse species implement a startling array of unique strategies when faced with the problem of resource acquisition but, importantly, are constrained by their shared need to fuel growth, somatic maintenance, and reproduction in the context of a body comprised of cells. Thus, understanding constraints on the resource consumption rates of individuals should elucidate constraints on overall ecosystem functioning.

Surface areas often mediate the passage of food from the environment into an organism, for example, a spider's web, the mouth of a filter feeder, the gut of caterpillar, or the plasma membrane of a prokaryote. This has led some to argue that, all other things being equal, consumption should scale in proportion to a relevant surface area and hence with $mass^{2/3}$ (von Bertalanffy 1957, Kooijman 2010). Others have claimed a ¾ power scaling of consumption on the basis of the ¾ power scaling of metabolic rate, arguing that supply matches demand (Calder 1984, Peters 1986, West et al. 2001). Recently it has been found that consumption scales higher than ¾ across diverse organisms (Pawar et al. 2012). This claim was made on the basis of a large data-set including 376 species of juveniles and adults, with body masses ranging from 5.24×10^{-14} kg to 800 kg. However, as the scaling exponent and underlying mechanism may differ between ontogenetic and interspecific comparisons,

pooling juveniles and adults in allometric analyses may be inappropriate (Maino and Kearney 2014).

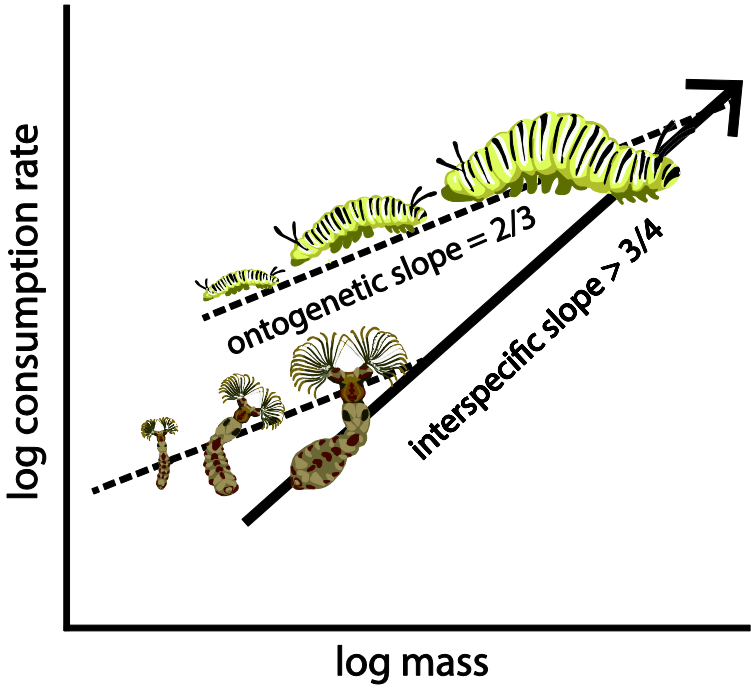

Figure 4-1. Theoretical predictions of the body mass scaling exponent of consumption for ontogenetic and interspecific size comparisons. Interspecific scaling of consumption at maximum size is predicted to be steeper than the ontogenetic scaling of consumption.

Here, we quantify the difference between the ontogenetic and interspecific scaling of consumption among insects (Figure 4-1). We explain these differences using a parameter-sparse, generic dynamic energy budgeting approach (Kooijman 2010), which predicts different scaling exponents depending on whether the analysis is ontogenetic or interspecific. A single mechanistic equation for the scaling of consumption with size that partitions these distinct ontogenetic and interspecific effects is presented and tested against a newly compiled data set on ontogenetic consumption rates for over 41 insects (data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.35n9f/1). Insects are an important case study as they dominate the known diversity of animal life; any metabolic theory claiming to

be universally general to life must also account for this important taxonomical group. In addition, as many models make the simplifying assumption that consumption is proportional to assimilation (consumption minus defecation), we also compare the scaling of assimilation.

Model formulation and methods

Following Kooijman (2010) biomass is taken as being comprised of ‘reserve’ and ‘structure’ components where reserve consists of intermediate chemical substrates between the transformation of food to production (structural biomass and reproductive outputs), and dissipation (payment of structural overhead and maintenance costs). The reserve concept is motivated by the observation that nutritional history affects the quality (composition) of biomass, which in turn has metabolic consequences. The presence of reserve in biomass explains why organisms continue to function when their guts are empty, and may continue growth and reproduction under starvation (via the depletion of reserve).

In DEB theory the concept of ‘reserve’ differs from more classical use in bioenergetics studies where reserve typically relate to storage compounds. Likewise, ‘structure’ does not relate only to ‘support structures’ in organisms such as chitin or cellulose. Molecules are assigned to reserve and structure on the basis of their dynamics. Molecules that can be considered as having a constant turnover, irrespective of nutritional history, are assigned to structural compartment, while molecules that turnover at different rates should be considered reserve. Thus, structural lipids in cell walls would be considered structure, while lipids comprising the fat body are reserve. Of course, not all biomass components can be classed easily into structure or reserve compartments. Indeed, DEB models can be extended to include more compartments as necessary (Kooijman 2010). Taken to the extreme each molecules could be modelled as a separate compartment with its own dynamics. The key

point is that allowing components of biomass to exhibit two dynamics (reserve and structure) is closer to reality than treating mass as one homogenous compartment as in the case in most allometric studies.

Within a species, DEB theory assumes that the rates of maximum consumption and assimilation are each proportional to structural mass$^{2/3}$. This is supported by the observation that surface areas almost always mediate consumption and absorption processes and that, during growth, shape remains approximately constant. The relevant surface is taken proportional to structure$^{2/3}$ rather than mass$^{2/3}$, as mass also includes reserve, which can decrease under food restriction. The higher structure to reserve ratio that is predicted after a period of starvation captures compensatory feeding responses (Carvalho et al. 2005). Motivated by the importance of stoichiometric homeostasis in life processes (Sterner and Elser 2002), the chemical constituents of reserve are assumed proportional to those of structure under constant food levels (e.g. *ad libitum* feeding) known as the assumption of 'weak homeostasis'. This results in the expression for ontogenetic consumption rate $\dot{p}_X$ in terms of structure V:

$$\dot{p}_X = \{\dot{p}_{Xm}\}V^c \qquad (4.1)$$

where $\{\dot{p}_{Xm}\}$ is the maximum surface-specific consumption rate and $c = 2/3$. Under constant feeding conditions, structure is proportional to mass (due the weak homeostasis assumption) so we can substitute V for μV_m where μ is the ratio of mass to ultimate mass and V_m is ultimate structure ($V \propto M$ so $\mu = V/V_m = M/M_m$ during ontogeny, where $M_m$is ultimate mass).

The surface-specific consumption rate $\{\dot{p}_{Xm}\}$ is constant during the growth of an individual, but varies between individuals of different size. The reason for this is because if

consumption scales as $V^{\frac{2}{3}}$, with maintenance scaling more quickly as V^1, size with eventually reach an asymptotic limit of V_m. This asymptotic size can be increased by decreasing volume specific maintenance or increasing surface-specific consumption. DEB theory assumes the latter on the basis that density-based (or 'intensive') parameters are approximately invariant (Kooijman 2010), such as the energetic requirement of cells in vitro (West et al. 2002). Thus, uptake per volume of structure $[p_{Xm}] = \{\dot{p}_{Xm}\}/V_m^{\frac{1}{3}}$ is taken to be constant between species. Importantly, this does not imply maintenance metabolism or uptake scale as mass[1] between species due to the contribution of reserve to mass (Maino et al. 2014). We now substitute $\{\dot{p}_{Xm}\}$ for $[\dot{p}_{Xm}]V_m^{\frac{1}{3}}$, which yields:

$$\dot{p}_X = [\dot{p}_{Xm}]V_m\mu^c \quad (4.2)$$

The expression consists of two components: the rate of consumption at ultimate size $[\dot{p}_{Xm}]V_m$ (interspecific size effect), which is scaled by the dimensionless component for developmental stage μ^c (ontogenetic size effect). As μ scales proportional to ontogenetic mass, any scaling exponent calculated using the quantity μ will be the same as that calculated for ontogenetic mass. However, the intercept with be different due to the (hypothesised) interspecific effect of $[\dot{p}_{Xm}]V_m$. Consumption rate at ultimate size $[\dot{p}_{Xm}]V_m$ can be substituted by a more usual allometric function of body mass aM^b.

$$\dot{p}_X = aM^b\mu^c \quad (4.3)$$

Ultimate size is now reflected by ultimate body mass M. Ultimate body mass is not proportional to ultimate structure V_m because larger organisms have less structure and more reserve (Kooijman 2010, Maino et al. 2014). The scaling exponent b reflects this non-proportionality and is expected to take values between to ¾ to 1 (Maino et al. 2014) whereas the ontogenetic exponent c is predicted to be close to 2/3. Thus, ontogenetic consumption is expected to scale more slowly than interspecific size comparisons (Figure 4-1).

The effect of temperature can be introduced by using the Arrhenius-Boltzman correction factor $\exp(-\mathrm{E}/\mathrm{RT})$, where R is the gas constant, E is the effective activation energy and T is body temperature in Kelvin (Gillooly et al. 2001). Including this temperature correction factor into the previous equation gives:

$$\dot{p}_X = e^{-E/RT} a M^b \mu^c \qquad (4.4)$$

To test these predictions, we compiled a data set of consumption and assimilation rates through ontogeny for different insects. Data was retrieved from a comprehensive literature search of insect consumption and assimilation through ontogeny, which resulted in data for 41 insects from 6 orders being included in the present analysis (Coleoptera (n = 14), Lepidoptera (n =14), Hemiptera (n = 6), Orthoptera (n =5), Diptera (n =1), and Neuroptera (n = 1)). Where possible, data was extracted from tables or requested from the original authors of the study, otherwise figures were digitized so that data points could be extracted.

Assimilation was calculated either as consumption minus defecation, or biomass production plus energy dissipation (usually measured as oxygen consumption) as per Klekowski *et al.* (1967). All weight measurements were standardised to dry weight milligrams. Rates were standardised to milligrams per day. Units of mass was chosen for the analysis rather than energy as they were most commonly used in the studies comprising the compiled data set. This minimised the amount of conversion required to standardise units and thus minimised the error introduced into the analysis. Of the data on 40 insects used in this study, 26 sources were reported in units of mass, 9 used a combination of mass and energy units, 3 used only energy units, while only 2 used respiration and growth measurement to recover assimilation (assimilation = respiration + growth). In majority of the studies using energy units, energy densities of food and insect biomass were given. When not given, we

assumed the energy content of biomass (food and insect) to be 25 J/mg. As this latter assumption was employed in only the small minority of studies using energy units the analysis is not sensitive to small changes around this value. Weights associated with a given consumption or uptake rate correspond to the end of the measured feeding period. Data always spanned multiple instars and did not include feeding in the adult phase. This was done to minimise the effect of senescence, and also because diets often change markedly upon sexual maturity (Grimaldi and Engel 2005). Terminal mass is taken as the maximum larval weight for holometabola, or adult weight otherwise. When data on multiple diets or temperatures were given we used the most optimal conditions, i.e. largest body sizes. Where sexes were separated, female values were used..

We use this data to test the hypotheses that interspecific mass exerts a larger effect on the scaling of uptake (observed as higher exponent) compared to ontogenetic mass. Taking the logarithm of equation 4.4 multiplied by the temperature correction factor yields:

$$\log_{10} \dot{p}_X = \log_{10} a + b \log_{10} M + c \log_{10} \mu + d/\mathrm{T} \qquad (4.5)$$

where $d = \mathrm{E}/\mathrm{R} \log_{10} e$. The result is simple linear equation that allows interspecific and ontogenetic scaling effects to be estimated simultaneously. For comparison, we test this model against the null model which does not separate the effect of ontogenetic mass from interspecific mass and uses a single scaling exponent:

$$\log_{10} \dot{p}_X = \log_{10} a + B \log_{10} m + d/\mathrm{T} \qquad (4.6)$$

where m is the mass of the insect at the time of measurement, and B is the scaling exponent of mass (pooled for all species despite different ontogenetic stage). Models are compared in terms of the explained variance (R^2) and AIC values (Burnham and Anderson 2002). Unlike R^2 values, AIC values take into account the number of model parameters and can be used quantify the probability of competing models, based on the ratio of their likelihoods (Akaike

weights). We also test the frequently made assumption that consumption rate scales proportional to assimilation rate by repeating the analysis for assimilation rate $\dot{p}_A$ where $\dot{p}_A = [\dot{p}_{Am}]V_m\mu^c$.

Results

The results of the multiple linear regression analysis (Table 4-1) confirm that the full model (equation 4.5) performed better than the null model (equation 4.6) in terms of explained variance and Akaike weights. For consumption rate, the proportion of variance explained by the full model was 0.82 compared to 0.81 explained by the null model. Likewise, for assimilation rate, the proportion of variance explained by the full model was, again, higher at 0.83 compared to 0.81 explained by the null model. While the explained variance may seem comparable, the small Akaike weights of the null model for consumption and assimilation show a very low relative likelihood of the null model and strongly support the full model. The AIC results suggest that the weight of evidence for the full model is almost one (= 1.00 at two significant figures) compared to a weighting of nearly 0 for the null model, which did not separate ontogenetic mass from interspecific mass.

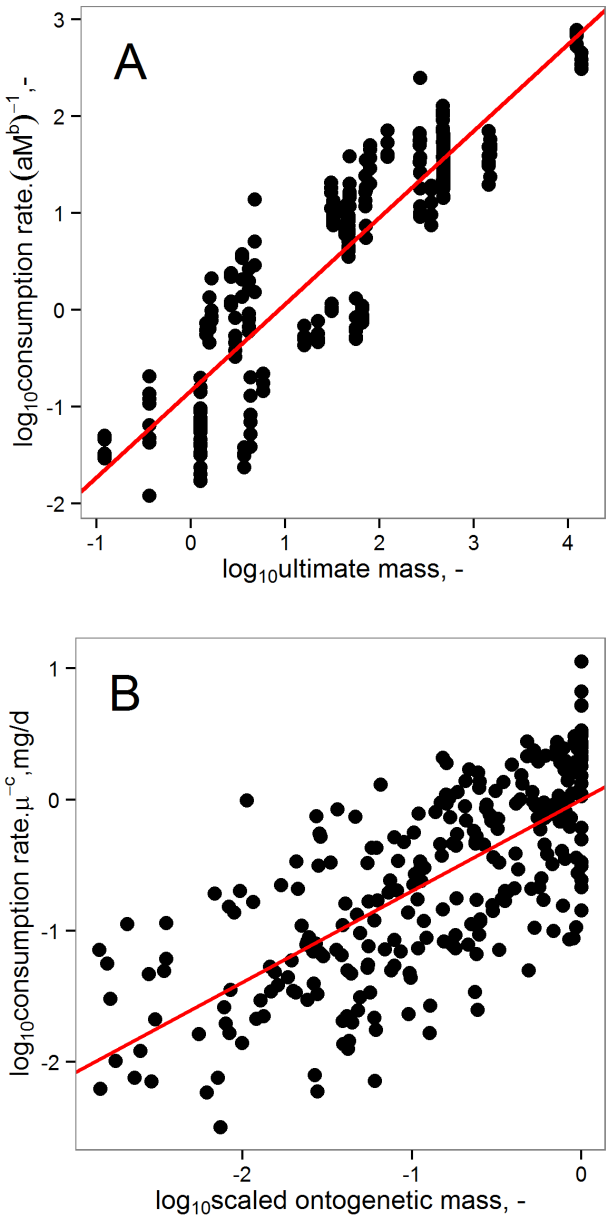

Figure 4-2. Partial residual plots showing the temperature corrected effects of interspecific mass and ontogenetic mass on consumption rate. **(A)** After normalising for ontogenetic effects, consumption scales with the species' ultimate mass with an estimated exponent of 0.894. This is significantly higher (95% confidence level) than the ontogenetic exponent of 0.695. **(B)** After normalising for interspecific effects, consumption as a function of scaled ontogenetic mass μ (mass divided by ultimate mass) is predicted to scale with $\mu^{2/3}$. The estimated ontogenetic scaling exponent includes 2/3 at the 95% confidence level. Axes units given as a dash indicate dimensionless variables.

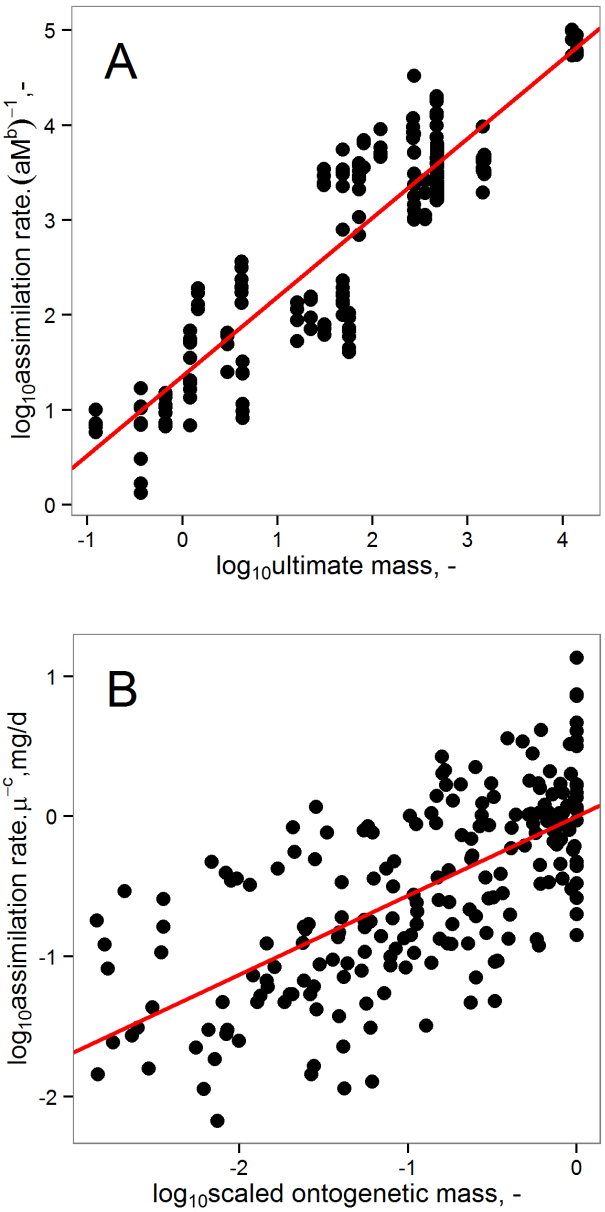

Figure 4-3. Partial residual plots showing the temperature corrected effects of interspecific mass and ontogenetic mass on assimilation rate. **(A)** After normalising for ontogenetic effects, assimilation scales with the species' ultimate mass with an estimated exponent of 0.834. This is significantly higher (95% confidence level) than the ontogenetic exponent of 0.564. **(B)** After normalising for interspecific effects, assimilation as a function of scaled ontogenetic mass μ (mass divided by ultimate mass) is predicted to scale with $\mu^{2/3}$. The estimated ontogenetic scaling exponent excludes 2/3 at the 95% confidence level. Axes units given as a dash indicate dimensionless variables.

TABLE 4-1. MULTIPLE LINEAR REGRESSION PARAMETER ESTIMATES FOR CONSUMPTION AND ASSIMILATION MODELS

	Parameter estimates								
	$\log_{10}(a)$	b	B	c	d	R^2	AIC	Δ_i	w_i
Consum. rate									
Null model	-0.635 (-4.92, 3.65)		0.842 (0.794, 0.89)		236 (-1046, 1518)	0.812	441.3	16.76	0.00
Full model	-0.836 (-4.99, 3.31)	0.894 (0.842, 0.945)		0.695 (0.615, 0.776)	233 (-1010, 1475)	0.824	424.5	0.00	1.00
Assim. rate									
Null model	0.184 (-5.72, 6.09)		0.762 (0.711, 0.813)		-74.8 (-1850, 1701)	0.806	333.4	28.16	0.00
Full model	1.36 (-4.18, 6.9)	0.834 (0.781, 0.888)		0.564 (0.48, 0.648)	-522 (-2189, 1146)	0.831	305.2	0.00	1.00

Notes: The full and null model refers to equation 4.5 and 4.6 respectively. Parentheses contain 95% confidence intervals of estimates. Δ_i is the change in AIC values of the competing models, while w_i signifies Akaike Weights (Burnham and Anderson 2002).

More importantly, the full model strongly suggests that the phenomenon of ontogenetic scaling is distinct from interspecific scaling. As predicted by theory the interspecific exponent (b) for the full model was higher than the ontogenetic exponent (c) for both consumption and assimilation (Figure 4-2 and 4-3). This result is statistically robust as the 95% confidence intervals of the ontogenetic and interspecific exponents do not overlap (Table 4-1). Interspecific scaling exponents for both consumption and assimilation were within the theoretical bounds of ¾ to 1. Consumption rate scaled interspecifically with an exponent of 0.894, but scaled ontogenetically with an exponent of 0.695. Likewise, assimilation rate scaled interspecifically with an exponent of 0.834, but scaled ontogenetically with an exponent of 0.564. As predicted, the ontogenetic exponent for consumption could not be distinguished from 2/3 at the 95% confidence level. However, the lower ontogenetic exponent for assimilation excluded 2/3 at the 95% confidence level.

The lower ontogenetic scaling exponent of assimilation compared with consumption was explained by the decrease in assimilation efficiency (assimilation/consumption x 100%) that occurred during development. A pairwise student *t*-test of the change in assimilation

efficiency from early to late in the growth period (for those insects where consumption and assimilation data was available) revealed an average decrease of 29% (n = 26, t = 5.42, p = 1.25e-05). These results are summarised in Figure 4-4.

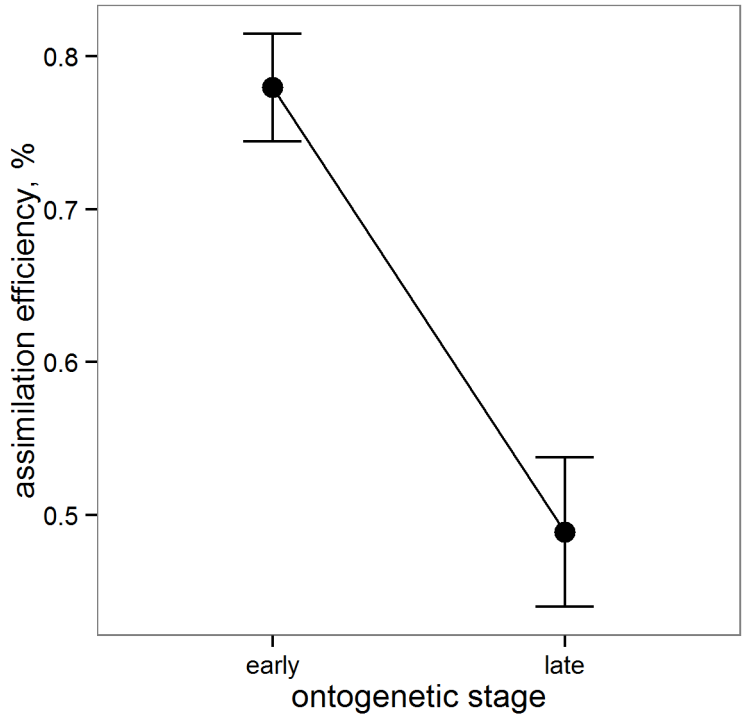

Figure 4-4. Change in assimilation efficiency for growing insects. There is typically a decrease assimilation efficiency (as measured as the ratio of assimilation to consumption x 100%). The declining efficiency explains why ontogenetic consumption scales more quickly than assimilation in insects. Error bars denote standard errors (n = 26).

Consumption rate was estimated to scale with an exponent indistinguishable from 2/3 through ontogeny, however, this estimate was based on pooled data for a number of species. Estimating the scaling exponent for individual insects reveals that, within species, consumption scales with exponents that exclude 2/3 at the 95% confidence level (Figure 4-5). However, 2/3 still adequately describes the central tendency of all exponents, with a mean exponent of 0.676±0.076 (95% CI).

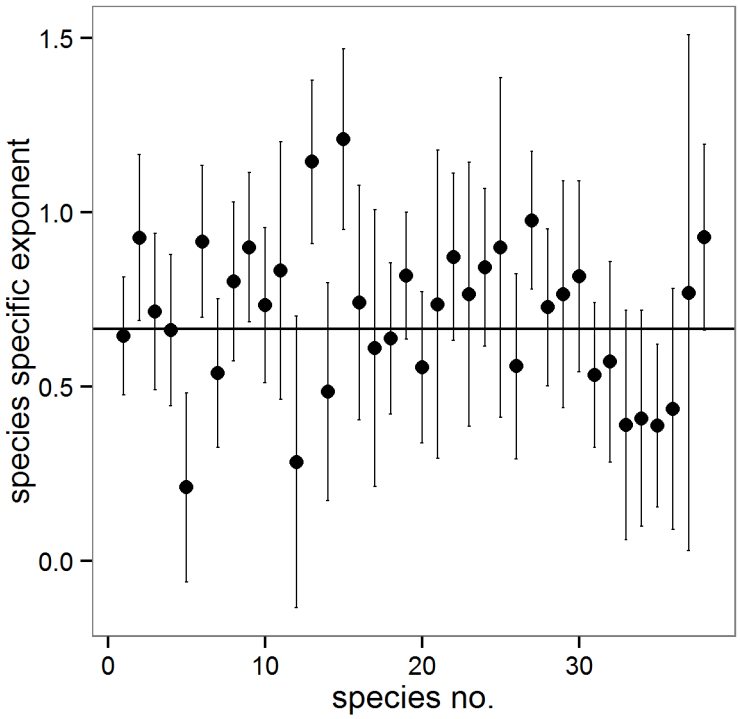

Figure 4-5. Estimates of the ontogenetic scaling of consumption for individual species shows that some species reject a surface-area rule. However, a surface scaling law (horizontal line) still describes the central tendency; the mean of all individual exponents is equal to 0.676± 0.076 (95% confidence interval). The error bars denote 95% confidence intervals.

Discussion

The mechanistic basis for the scaling of consumption with size differs depending on whether the comparison is ontogenetic or interspecific, as supported by the superior fit of the full model and the different ontogenetic and interspecific scaling exponents. The interspecific scaling exponent was closer to that found by Pawar et al. (2012) in their larger study that extended outside the insects, than 2/3 or the canonical ¾ power law. Pawar et al. further divided their analysis based on the dimensionality of the interaction between the organism and its food resources. They found (at abundant resources) consumption for 2D interacting organisms (e.g. in benthic habitats) scaled with a mass exponent of 0.85±0.5 (95%

confidence interval) and for 3D interacting organisms (e.g. in pelagic habitats) scaled with an exponent of 1.06±0.6 (95% confidence interval). While dimensionality did effect the interspecific scaling of consumption as they predicted, all estimated exponents were close to the range of ¾ to 1, as we predict for interspecific scaling, and were significantly higher than the canonical 3/4.

In contrast, the ontogenetic scaling of consumption was better approximated by a less steep, surface area or mass$^{2/3}$ scaling. DEB theory explains the scaling as a result of organisms being more similar in shape to a larger conspecific than to one of a different species. If maintenance scales with volume and uptake scales with a surface area, organisms will reach an asymptotic size where energy uptake matches maintenance demand with nothing remaining for growth. To overcome this limit in ontogenetic size, uptake is expected to scale greater than mass$^{2/3}$ between species (Maino, Kearney, Nisbet, & Kooijman, 2014). It turns out that between species, maintenance is expected to scale with a mass exponent between ¾ and 1, from which it follows that interspecific consumption must scale with a similar exponent.

Given the wide interest in determining metabolic scaling exponents from data (Isaac and Carbone 2010) it will be important for future studies to acknowledge the distinct effect of ontogeny, as failing to make this distinction will bias estimates of exponents. For example, when the effect of ontogeny was ignored for the body mass scaling of consumption and assimilation rates (null model), the interspecific scaling exponent was lower than the case where ontogenetic effects were considered (full model).

In insects, metabolic rate has been shown to scale with exponents higher than 2/3. Addol-Bediako et al. (2002) found insect respiration to scale with interspecific mass with an exponent of 0.77, while more recent and larger studies (~400 insects) conducted

independently by Chown et al. (2007) and Ehnes et al. (2011), found metabolic rate scaled with an exponent between 0.75 and 0.81. This range overlaps with the 95% confidence interval of our estimate for the interspecific scaling of assimilation rate and supports the expectation based on the energetic balancing of supply and demand in insects. As assimilated energy is the energy available for metabolic processes, an organism's energetic supply corresponds more closely to assimilation than to consumption. This highlights when it may be problematic to assume that consumption rate scales in proportion to assimilation rate. For example, Pawar et al. (2012) based their analyses on consumption rather than assimilation, which may help to explain why their estimated exponents were higher than that expected on the basis of simple supply and demand arguments.

During ontogeny, assimilation was found to scale more slowly than consumption. The lower ontogenetic scaling of assimilation compared with consumption can be explained by the decline in assimilation efficiency that was found to occur in insects in our data set. Indeed, past studies have also found this to be a general phenomenon in insect nutrition (Scriber 1977). In insects, a number of intrinsic and extrinsic factors may account for this decrease in assimilation efficiency. First, as an organism grows, its mouthparts also increase in size, which decreases food selectivity (Hochuli 2006). This can lead to an increased consumption of lower quality foods, such as the ingestion of older and larger leaves, which contain less nitrogen and water and more indigestible cellulose. Second, as an insect ages so too may its food source, which in turn may be associated with a decrease in food quality (Scriber and Slansky 1981). This can involve decreases in nitrogen and water content, and increases in defensive allelochemicals of plants – all of which have been shown to negatively impact assimilation efficiency (Scriber and Slansky 1981, Muthukrishnan and Pandian 1987). Third, the nutrient demands of an insect may change with age rendering the food source lower quality relative to the new nutritional target. For example, insects preparing for

reproduction require greater energy reserves and specific fatty acids (Stockhoff 1993). Fourth, as holometabolic insects prepare for pupation, decreased production of hormones from brain neurosecretory cells, the corpora cardiaca and corpora allata causes a decrease in digestive enzyme production and, as a consequence, assimilation efficiency drops (Sindhu and Nair 2004).

Our results support the assumption of a surface area scaling of consumption made by some prominent mechanistic growth models (von Bertalanffy 1957, Kooijman 2010). However, we highlight that the simplifying assumption that consumption is proportional to assimilation should be used with caution.

Interestingly, insects often exhibit exponential or near-exponential growth (von Bertalanffy 1951, Tammaru and Esperk 2007), which is a faster growth rate than predicted by popular mechanistic models for growth (von Bertalanffy 1957, West et al. 2001, Hou et al. 2008a, Kooijman 2010). If the assumption of uptake scaling with a surface area broadly holds, this suggests that the other core assumption of somatic maintenance (or rate of catabolism) scaling with mass^1 may not be valid in general. Indeed, as insects develop, a greater proportion of biomass consists of lipids with low maintenance costs (Elia 1992, Hayes et al. 1992). This lower cost of maintaining biomass in later instars may also explain higher biomass production efficiencies in later instars (Scriber and Slansky 1981).

Although the broad scaling pattern of ontogenetic consumption can be well approximated by a surface area rule, there exists substantial unexplained variation in the data. In addition, while the central tendency of individual species' exponents does not reject a 2/3 power scaling of consumption within species, many exhibit a scaling that is significantly different from 2/3 (Figure 4-5). It is not surprising that such deviations exist as the implemented model is simple and focuses only on a small number of processes. However,

such deviations from broad trends may be as instructive as congruence, and demonstrate that we must look to an organism's life history to explain departures from simple bio-physical expectations. According to DEB theory, relaxing the invariance of parameter values can capture deviations from simple biophysical expectation, such that each species (or even individual) has a unique parameter set specifying its metabolic architecture. With enough data, parameters of diverse species can then be explored for systematic variation (Kooijman 2013). A recent study that applied a full life-cycle DEB models to nine species of Perciformes captured differences in the evolution of metabolic rate for growing larval by relaxing the parameters held constant in the present study (Lika et al. 2014). The authors suggested that differences in parameter values could be largely explained by differences in the spawning season and food availability. Demersal fish spawning in the autumn and winter experience higher temperatures and lower food availability and benefit from lower metabolic rates and delayed development.

Alternatively, deviations from the constant shape assumption (isometric growth) of DEB theory can be measured empirically and related to changes in metabolic parameters. A recent study by Hirst et al. (2014) found that the degree of shape flattening exhibited by growing marine invertebrates explained deviations from simple Euclidean expectations for perfectly isometric organisms. In the same way, shape changes that are known to occur in growing insects (Shingleton et al. 2007) may account for some of the large interspecific variation in the scaling of consumption (Figure 4-5).

Previous work has shown that dynamic energy budget theory offers a consistent framework to explore differences in the ontogenetic and interspecific scaling of a number of physiological attributes (Maino et al. 2014, Maino and Kearney 2014). We have shown here that this explanatory power extends to the scaling of consumption and assimilation, which will help refine assumptions currently used in models ranging from individual growth (Hou et

al. 2008a, Kooijman 2010) to ecosystem dynamics (Yodzis and Innes 1992, Brown et al. 2004).

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Chapter 5: Testing mechanistic models of growth in insects

James L. Maino and Michael R. Kearney

Online resources:
Supplementary data available from http://1drv.ms/1BwVdcz

Abstract

Insects are typified by their small size, large numbers, impressive reproductive output, and rapid growth. However, insect growth is not simply rapid; rather, insects follow a qualitatively distinct trajectory to many other animals. Here we present a mechanistic growth model for insects and show that the up-regulation of assimilation during the growth phase can explain the near-exponential growth trajectory of insects. The presented model is tested against growth data on 50 insects, and compared against other mechanistic growth models. Unlike the other mechanistic models, our growth model predicts energy reserves per biomass to increase with age, which implies a higher production efficiency and energy density of biomass in later instars. These predictions are tested against data compiled from the literature whereby it is confirmed that insects increase their production efficiency (by 24 percentage points) and energy density (by 4 J/mg) between hatching and the attainment of full size. The model suggests that insects achieve greater production efficiencies and enhanced growth rates by up-regulating assimilation and increasing energy reserves per biomass, which are less costly to maintain than structural biomass. Our findings illustrate how the explanatory and

predictive power of mechanistic growth models comes from their grounding in underlying biological processes.

Introduction

Mechanistic growth models formalise knowledge of underlying bioenergetic processes of uptake, development and maintenance to derive net production. The resulting functions are constrained not only by the data they must fit, but by the knowledge of physiological processes they incorporate in their assumptions. They are consequently seen as more robust than purely descriptive, phenomenological approaches (Helmuth et al. 2005, Denny and Benedetti-Cecchi 2012).

Several generic mechanistic models for animal growth exist (West et al. 2001, van der Meer 2006b, Hou et al. 2008b) that are based on the simple differential equation

$$\frac{dm}{dt} = am^c - bm^d \tag{5.2.1}$$

where a and b are coefficients and c and d are the scaling exponents of body mass m. The catabolism (maintenance) exponent d is typically taken as 1 while the anabolism (assimilation) exponent is assumed to take values of 2/3 and 3/4 on the basis of surface area or metabolic scaling exponents respectively. When the assimilation exponent is taken as 2/3, the von Bertalanffy function for mass m through time t emerges as

$$m = m_\infty \left(1 - \left(1 - m_b^{\frac{1}{3}}/m_\infty^{\frac{1}{3}}\right) e^{-C_v t}\right)^3 \tag{5.3.2}$$

where $m_b$is mass at birth, $m_\infty$is asymptotic mass, and $C_v = b/3$ is the von Bertalanffy growth rate. This function can be reduced to a universal function of dimensionless time and mass $\mu = 1 - e^{-\tau}$ where $\mu = (m/m_\infty)^{\frac{1}{3}}$ and $\tau = C_v t - ln(1 - (m_b/m_\infty)^{\frac{1}{3}})$. For insects, such a curve tends to over-predict growth rates early in development and under-predict them closer to full size (Figure 5-1).

It has long been suggested that insect growth is more closely characterized by exponential growth, with von Bertalanffy himself noting that insects could be classed as a unique metabolic and growth type that is closer to exponential (von Bertalanffy 1951). Taking the assimilation exponent as 1 results in an exponential function for mass through time

$$m = m_b e^{C_e t} \tag{5.4.3}$$

where $C_e = a - b$ is the exponential growth rate. Similarly, the function can be reduced to a function of dimensionless time and mass $\epsilon = e^{\delta}$ where $\epsilon = m/m_b$ and $\delta = C_e t$. By inspection, an exponential function better describes insect growth compared to the von Bertalanffy function, but the concave pattern of the residual variation suggests insects grow more slowly than an exponential function (Figure 5-1). Indeed, Esperk and Tammaru (Tammaru and Esperk 2007) concluded recently that insects grow slower than exponentially.

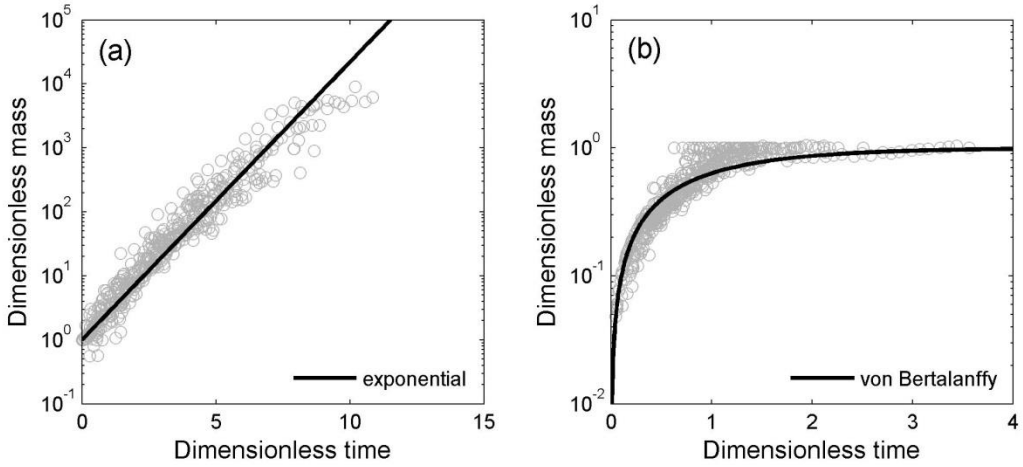

Figure 5-1. Universal growth curves can be derived from the exponential growth curve (a) and the von Bertalanffy growth curve (b). The universal von Bertalanffy curve $\mu = 1 - e^{-\tau}$ is expressed in terms of dimensional mass $\mu = (m/m_\infty)^{\frac{1}{3}}$ and dimensionless time $\tau = C_v t - ln(1 - (m_b/m_\infty)^{\frac{1}{3}})$. The universal exponential growth curves $\epsilon = e^{\delta}$ is expressed in terms of dimensionless mass $\epsilon = m/m_b$ and dimensionless time $\delta = C_e t$. Growth data plotted for 50 insects suggest insect growth is typically faster than von Bertalanffy but slower than exponential.

Here we show that a simple modification to a well-known mechanistic model for growth accounts for the unique trajectory of insect growth. The modification also produces novel predictions that are tested against insect growth data compiled from the literature.

Theoretical framework

The standard dynamic energy budget (DEB) model (Kooijman 1986a, 2010) represents a quest for the simplest model that can describe the full life-cycle bioenergetics of all living organisms. A unique feature of DEB models is the partitioning of biomass into 'reserve' and 'structure' (Figure 5-2). Reserve is defined as the intermediate chemical substrates between the transformation of food and the growth and maintenance of structure (and reproduction, which is dealt with elsewhere (Kooijman 2010)). The reserve concept is motivated by the observation that nutritional history qualitatively affects biomass, which in turn has metabolic consequences. The variable content of the amount of reserve per mass adds a qualitative aspect to biomass, which is otherwise assumed to be homogenous in most growth models. Metabolism in a DEB framework is thus seen to be dictated not by immediate feeding conditions but the amount of reserve and structure. In the absence of feeding, sufficient reserve will fuel the continuation of structural growth and reproduction as is frequently observed in starved animals.

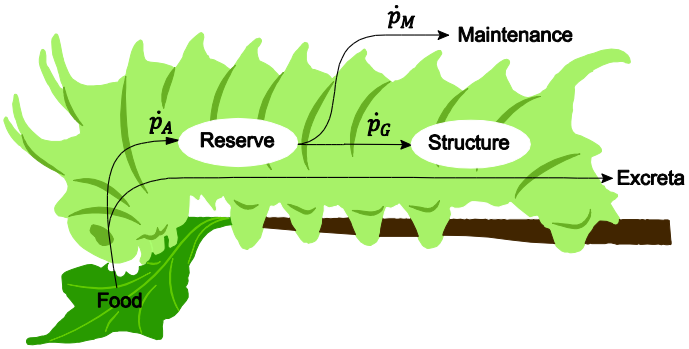

Figure 5-2. A simplified schematic of a standard DEB model without maturation or reproduction. DEB theory uniquely partitions organism biomass into reserve and structure compartments. For the sake of simplicity we do not consider allocation to maturation or reproduction, which would usually be treated as an extra branch before allocation to growth and maintenance.

Under constant environmental conditions, the von Bertalanffy curve emerges as a special case of the standard DEB model. Because DEB theory is based on first principles it provides the von Bertalanffy growth curve, and its parameters, a more precise mechanistic interpretation. This is true given four key assumptions of the standard DEB model.

Assumption 1: No changes in shape occur during growth, which implies that surface area is proportional to volume$^{2/3}$.

Assumption 2: Energy assimilation $\dot{p}_A$ is proportional to structural surface area or $\dot{p}_A = f\{\dot{p}_{Am}\}V^{\frac{2}{3}}$ where $\{\dot{p}_{Am}\}$ is the maximum specific assimilation rate and f is a scaled Type II functional response of the food level (Holling 1959) taking values between 0 and 1.

Assumption 3: Maintenance $\dot{p}_M$ is proportional to the amount of structure or $\dot{p}_M = [\dot{p}_M]V$ where $[\dot{p}_M]$ is the specific maintenance rate of structure.

Assumption 4: Under constant food the ratio of reserve to structure (reserve density) $\frac{\mathrm{E}}{\mathrm{V}} = [\mathrm{E}]$ is constant.

Given the energetic cost per unit of structure $[E_G]$ and the energy flux to growth $\dot{p}_G$, the change in structural volume is

$$\frac{dV}{dt} = \frac{\dot{p}_G}{[E_G]} \tag{5.2.1}$$

The change in reserve energy E can be expressed as the energy assimilation rate, minus the maintenance rate, minus the energy flux to growth $\dot{p}_G$:

$$\frac{dE}{dt} = f\{\dot{p}_{Am}\}V^{\frac{2}{3}} - [\dot{p}_M]V - \dot{p}_G \tag{5.2.2}$$

Using the chain rule for differentiation, the change in the reserve density $[E]$ is

$$\frac{d[E]}{dt} = \frac{dE}{dt}V^{-1} - \frac{dV}{dt}EV^{-2} \tag{5.2.3}$$

Substituting equation (5.2.2) and (5.2.3) into (5.2.1) gives

$$\frac{\mathrm{dV}}{\mathrm{dt}} = V\frac{f\{\dot{p}_{Am}\}/V^{\frac{1}{3}} - [\dot{p}_M] - \frac{d[E]}{dt}}{[E_G] + [E]} \tag{5.2.4}$$

For constant food levels the ratio of reserve to structure is constant so (2.4) simplifies to

$$\frac{\mathrm{dV}}{\mathrm{dt}} = \frac{f\{\dot{p}_{Am}\}V^{\frac{2}{3}} - [\dot{p}_M]V}{[E_G] + [E]^*} \tag{5.2.5}$$

where the ratio of reserve to structure $[E]^*$ is now constant. This equation is identical to the von Bertalanffy growth curve but parameters can now be interpreted in terms of their underlying processes.

To extend the formulation to non-steady states where food level may vary, we use a result from Kooijman (2010) that restricts how the reserve density may vary:

$$\frac{\mathrm{d}[\mathrm{E}]}{\mathrm{dt}} = \frac{f\{\dot{p}_{Am}\} - [E]\dot{v}}{V^{\frac{1}{3}}} \tag{5.2.6}$$

The simple result of equation (5.2.6) relies on no further assumptions but requires an involved derivation that is not covered here (see Kooijman, 2010; Maino et al., 2014). The interpretation is that in the absence of assimilation the reserve density decreases according to first-order dynamics. The introduced parameter $\dot{v}$ has dimensions length per time and is called energy conductance.

We may now solve reserve and structure at non-steady states at which the food level f and reserve density is not constant. Rearranging the previous equations give:

$$\frac{dE}{dt} = f\{\dot{p}_{Am}\}V^{\frac{2}{3}} - [E]\frac{[\dot{p}_M]V + [E_G]\dot{v}V^{\frac{2}{3}}}{[E_G] + [E]} \tag{5.2.7}$$

$$\frac{dV}{dt} = \frac{[E]\dot{v}V^{2/3} - [\dot{p}_M]V}{[E_G] + [E]} \tag{5.2.8}$$

A mechanistic growth model for insects

To improve the fit of the DEB model to insects, which do not follow a von Bertalanffy shaped growth trajectory, we introduce a simple modification that incurs no additional free parameters: assimilation is taken as proportional to structure[1] rather than structure[2/3]. Taking assimilation to scale volumetrically has been used to improve the fit of

DEB models to range of species including some fish, echinoderms, crustaceans, molluscs, and jellyfish (Kooijman 2014). These modifications are often accompanied by a change in conductance, which is not considered here. This assumption of volumetrically scaling assimilation may seem arbitrary but it is analogous to the assumption of the exponential growth model, which takes assimilation proportional to mass, except that DEB theory takes structure as the quantity relevant to uptake. A side-effect of the volumetric scaling of assimilation is the accumulation of reserve, which causes growth to be slower than exponential but faster than von Bertalanffy. This is because assimilation scales with mass (structure and reserve) more slowly than with structure.

To capture this up-regulation of assimilation, we replace the surface scaling assimilation term $f\{\dot{p}_{Am}\}V^{\frac{2}{3}}$ in equation (5.2.7) with one that scales with structural volume $f[\dot{p}_{Am}]V$. This results in

$$\frac{dE}{dt} = f[\dot{p}_{Am}]V - [E]\frac{[\dot{p}_M]V + [E_G]\dot{v}V^{\frac{2}{3}}}{[E_G] + [E]} \tag{5.2.9}$$

where $[\dot{p}_{Am}]$ is the volume-specific assimilation rate. This does not change the dependence of structure on reserve, only the amount of reserve. Structure (equation 5.2.7) and reserve (equation 5.2.8), which are in units of volume and energy respectively, can be converted to mass (m) using the appropriate conversion coefficients.

$$\frac{dm}{dt} = d_V\frac{dV}{dt} + e_E^{-1}\frac{dE}{dt} \tag{5.2.10}$$

where d_V is the dry mass density of structure (dry mass/volume), e_E is the energy density of reserve (energy/dry mass).

In the following we show that this modification can capture insect growth trajectories more effectively than von Bertalanffy, WBE, and exponential curves. In addition, we show

that the model makes predictions about insect biomass production efficiency and energy density. These predictions are tested against a new data set compiled from the literature (Dataset S1).

Methods and Materials

Assessing the model for insect growth

To assess the generality of our growth model for insects it is tested against a newly compiled data set on insect growth and compared against von Bertalanffy and exponential growth functions.

Growth data was retrieved from a comprehensive literature search of insect growth from hatching to terminal size (maximum larval size for holometabola). This resulted in data for 50 insects from 6 orders being included in the present analysis (Coleoptera (n = 8), Lepidoptera (n = 15), Hemiptera (n = 9), Hymenoptera (n = 2), Orthoptera (n = 8), Diptera (n = 7), and Neuroptera (n = 1)). Where possible, data was extracted from tables or requested from the original authors of the study, otherwise figures were digitized so that data points could be extracted. Mass was standardized to milligrams (dry weight) and time was standardised to days and temperature corrected to 20ºC using an Arrhenius-Boltzmann correction factor with an Arrhenius temperature of 8000 Kelvin (Gillooly et al. 2001). Data with comments and sources can be accessed at http://1drv.ms/1BwVdcz

Least squares fitting of all growth functions was performed using the ‘fit’ package in the numerical computing environment, MATLAB. Quality of model fits to data were assessed using Akaike Information Criteria (AIC) values (Burnham and Anderson 2002) and the proportion of variance explained by the model.

The von Bertalanffy, WBE and exponential growth functions each have one free parameter (C_v, C_{WBE}, C_e respectively). Mass at birth m_b and ultimate mass m_∞ are taken from the literature. For the DEB function, we set specific assimilation $\{\dot{p}_{Am}\}$ as the free parameter; standard values for all other parameters are taken from Kooijman (Kooijman 2010) and are given in Table 5-1. These values are estimates of typical parameters for a generalised animal and are the usual starting point before fine tuning the parameters in a full DEB models for individual species (Lika et al. 2011a). We take $\{\dot{p}_{Am}\}$ as the free parameter because theory predicts it to vary as a consequence of body size, while other parameters do not (but can be changed as a result of other selective pressures). Unlike the other growth functions, which can be solved analytically, the DEB function must be integrated numerically across the growth period. As in the standard DEB model, the ratio of reserve to structure is set as the ratio $\{\dot{p}_{Am}\}/\dot{v}$ which, in combination with the value for mass at hatching, provides the initial values.

TABLE 5-1.
PARAMETER LIST FOR INSECT DEB MODEL

Description	Parameter	Value	Units
Free parameters			
Specific assimilation	$\{\dot{p}_{Am}\}$	-	J mm^{-2} d^{-1}
Fixed parameters*			
Specific maintenance	$[\dot{p}_M]$	0.18	J mm^{-3}d^{-1}
Cost of structure	$[E_G]$	2.8	J mm^{-3}
Conductance	$\dot{v}$	0.2	mm d^{-1}
Structural volume to dry mass	d_V	0.2	mg mm^{-3}
Reserve energy to dry mass	e_E	23	J mg^{-1}

*Fixed parameters are set at the default values given in (Kooijman 2010)

Novel model predictions

Unlike other growth models, which do not separate biomass into structure and reserve, the insect DEB model predicts the composition of biomass to change with

development. In the standard model, the ratio of reserve to structure is constant. However, increasing specific assimilation to a volumetric scaling will cause this ratio to increase as shown by equation (5.2.6). The intuitive interpretation is that if specific assimilation increases, reserve use increases more slowly than reserve accumulation.

As growth proceeds, the increasing amount of reserve per biomass implies that the mass-specific cost of biomass maintenance decreases and production efficiency increases in later instars (Figure 5-3). The increasing amount of reserve also predicts an increasing energy density (J/mg dry weight) of biomass. This is because reserve is typically comprised of a larger proportion of energetically rich substances, such as lipids, in order to fuel metabolism in the absence of food (Lika et al. 2011a).

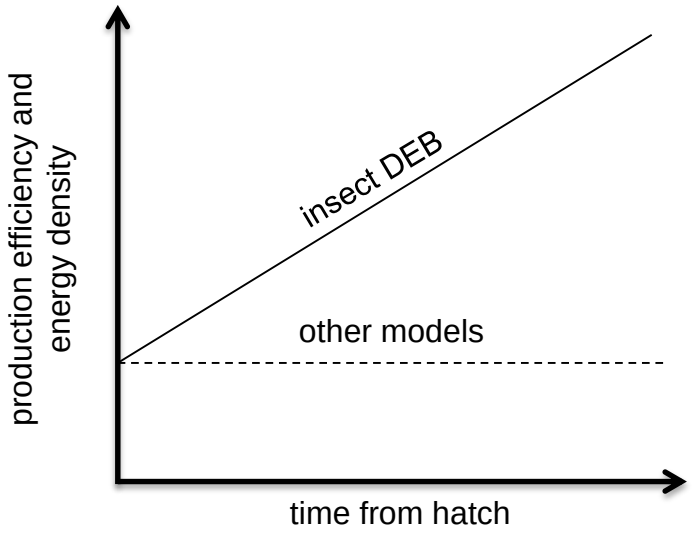

Figure 5-3. In contrast to most mechanistic growth models, the presented DEB model for insect growth implies the amount of reserve per structure will increase during the growth period. The consequences for production efficiency and biomass energy density are summarized in the diagram.

To test these predictions we compiled production efficiency and biomass energy density data from the literature, as with the growth data. The data set includes production efficiency data on 24 insects from five orders and energy density data on 15 insects from four orders. Production efficiency was measured as growth divided by assimilation (consumption minus excretion). The reproductive phase was excluded as we did not consider the implications of reproduction here. When assimilation was not reported, it was able to be derived on the basis of energy and mass conservation by either subtracting measured excretion from consumption or summing heat dissipation (respiration) with growth. Where respiration data was used was reported in μL O_2 consumption they were converted to joules assuming a conversion coefficient of 48.9 μL/J. Where sex was separated, female values were used. Data with comments and sources can be accessed at http://1drv.ms/1BwVdcz

Because there are multiple measures of each species throughout the growth phase, a linear mixed effect model was fitted to production efficiency and energy density, with a fixed effect of development stage (instar/total instars) and a random effect for each species.

Results

Assessing growth models

When assimilation is assumed to scale with the lowest and highest mass exponents ($m^{2/3}$ vs. m^{1}) we arrive at von Bertalanffy and exponential growth curves respectively. These two extremes are shown in figure 5-1 in terms of dimensionless variables. From inspection, the von Bertalanffy curve does not capture the rapid growth of insects, while the concave down residuals in the exponential plot suggest that insects may exhibit slower than exponential growth.

This is confirmed in our analysis of model fits to insect data (Table 2). While the exponential model does much better than the von Bertalanffy function (mean Akaike weight of 0.373 vs 0.019), the presented DEB model is consistently the best model in terms of the variance explained and AIC weights. The only exception was for the Lepidoptera, where the exponential model out-performed the DEB model. The WBE model does only slightly better than the von Bertalanffy model (Akaike weight of 0.033 vs 0.019).

TABLE 5-2.
COMPARISON OF MODEL FIT IN TERMS OF EXPLAINED VARIANCE AND AIC WEIGHTS

	Model							
	exponential		von Bertalanffy		WBE		Insect DEB	
	r^2	w_i	r^2	w_i	r^2	w_i	r^2	w_i
Coleoptera	0.979	0.465	0.931	0.006	0.937	0.008	**0.982**	**0.520**
Diptera	0.888	0.032	0.900	0.049	0.913	0.069	**0.969**	**0.851**
Hemiptera	0.971	0.246	0.954	0.022	0.959	0.043	**0.989**	**0.689**
Hymenoptera	0.949	0.227	0.872	0.006	0.888	0.010	**0.961**	**0.756**
Lepidoptera	**0.974**	**0.642**	0.891	0.001	0.907	0.003	0.962	0.354
Neuroptera*	0.978	0.211	0.909	0.000	0.921	0.001	**0.984**	**0.788**
Orthoptera	0.988	0.273	0.981	0.041	0.984	0.084	**0.996**	**0.602**
All	0.964	0.373	0.924	0.019	0.934	0.033	**0.977**	**0.575**

Notes: r^2 denotes the average proportion or variation explained by each model for each insect group, while w_i denotes the mean Akaike Weights of each model for each insect group, i.e. the likelihood it is the best model (Burnham and Anderson 2002). The best model in terms of r^2 and w_i values are highlighted in bold.*n=1

Novel model predictions

The quality of fits to data is only one aspect of determining the appropriateness of a model. A good fit for the wrong reasons can occur when models imply unrealistic properties. The DEB model departs from the other models in it separation of reserve and structure and predictions relating to production efficiency and the energy density of biomass through ontogeny.

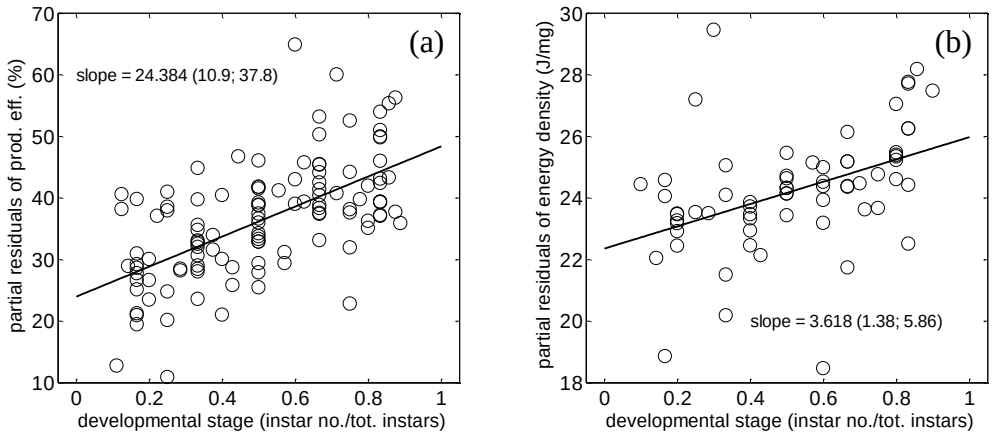

Figure 5-4. The partial residuals of production efficiency **(a)** and energy density **(b)** are plotted against developmental stage, while controlling for a random effect for each species. The slopes are both positive with their 95% confidence intervals (in parentheses) excluding zero. The value used for total instars includes the adult instar, for hemimetabolous insects and the pupa for holometabolous insects, i.e. when developmental stage is equal to one, the insect is an adult or pupa.

Controlling for random species effects, figure 5-4a shows the fixed effect of developmental stage (instar/total instars) on production efficiency (production per instar/consumption minus excretion per instar). In our data set, production efficiency is found to significantly increase (95% confidence level) by an estimated 24.4 percentage points from the beginning to the end of the growth period. Given that the estimated starting production efficiency is only 24.0%, this approximately represents a two-fold increase in efficiency.

The energetic content of biomass increases with a significant positive relationship (at the 95% confidence level) as development progresses. Figure 5-4b shows the effect of developmental stage on biomass energy density while controlling species random effects. Energy content per biomass was found to increase by an estimated 3.6 J/mg between the start and end of the growth period, with an estimated starting value of 22.4 J/mg.

Discussion

The up-regulation of assimilation occurs in many animals for various reasons, including hibernation, pregnancy or migration whereby greater uptake capacity is attained by temporarily increasing organ size (McLandress and Raveling 1981, Piersma and Lindström 1997, Hume et al. 2002). Indeed, it has been found in the lepidopterans *Bombyx mori* (Blossman-Myer and Burggren 2010) and *Manduca sexta* (Yeoh et al. 2012), that the midgut mass per body weight increases with each instar.

The upregulation of assimilation during the growth phase may have an adaptive significance for insects. The majority of insects are holometabolous and do not eat during pupation (Grimaldi and Engel 2005), with many even lacking functional mouthparts during the adult phase (Grimaldi and Engel 2005). For these insects, nutrients acquired during the immature stages strongly determine reproductive effort (Rivero et al. 2001, Boggs and Freeman 2005). But even insects that do not metamorphose may be more nutrient limited as they age. Herbivorous insects, for example, commonly experience a decreasing quality in the nutrient content of food as they age as their host plants develop (in terms of water and nitrogen) (Scriber and Slansky 1981). In general, adult insects do not have access to the same diet available to immature stages, not least of all due to their different morphology (Truman and Riddiford 1999, Chown and Nicolson 2004). This explains why insect reproduction is constrained by resources accumulated during the immature phase and cannot be offset by compensatory feeding in the adult stage (Rivero et al. 2001, Boggs and Freeman 2005).

The presented mechanistic growth model for insects, which results from a simple modification to the standard DEB model, successfully accounts for deviations from von Bertalanffy growth under constant conditions. Unlike the exponential model, which assumes assimilation to scale with mass^1, the presented DEB model takes assimilation to scale with

the more relevant quantity of structural mass, which ignores accumulated reserve mass. This results in a scaling exponent of mass of less than one as the proportion of reserve increases with development. The average mass-scaling of assimilation in the DEB model can be calculated as 0.78 from the fitted models. Interestingly, this is not significantly different to ¾ ($t = 1.978$, $df = 49$, $p = 0.053$), which was the scaling exponent in the WBE model. The reason why the DEB model outperforms the WBE is because the maintenance exponent is less than in the WBE model due to the increasing proportion of reserve.

Recent work conducted by Llandres et al. (2014, *in press*) made alternative modifications to the standard model that could explain energetic patterns in the context of a parasitic wasp. These modifications incurred 10 additional free parameters compared with the standard DEB model. Such a model will be difficult to test on the basis of growth data alone and will require more detailed energetic data across the whole lifecycle of a variety of insects. The model presented here is much simpler (adding no free parameters to the standard model) but still explains many features of insect growth.

Here we have only considered insects, which are ecologically dominant and speciose among terrestrial invertebrates, but in a recent study by Hirst and Forster it was found that growth in 73% of 58 marine invertebrates was best modelled by an exponential function (Hirst and Forster 2013). The study did not consider a mechanistic interpretation of the broadly evident exponential growth pattern, but, as in the case of insects, up-regulated assimilation in these species may also explain faster growth rates in some case. In insects, we proposed that the up-regulation of assimilation may be adaptive in cases where the adult feeding environment and available nutrition is distinct from immature phases. This is certainly the case in metamorphosing holometabola, which represent the majority of insects. Interestingly, most marine invertebrates also undergo a larval phase before metamorphosing into an adult, and, like insect metamorphosis, this has important nutritional consequences for

their growth and development(Ciemior et al. 1979). Indeed, those marine invertebrates best described by non-exponential functions did not undergo metamorphosis (Amphipoda and Ctenophora) or did not feed in the larval phase (Gastropoda) (Hadfield 2001). However, some groups that were best described by an exponential function did not exhibit metamorphosis or a non-feeding larval phase (e.g. Chaetognatha, Cephalopoda, Appendicularia). This provides some support to the idea that distinct immature and adult phases affect growth trajectories. To further uncover the mechanisms driving these different patterns, more physiological studies exploring ontogenetic growth allometries of organs relevant to digestion are required.

Unlike the von Bertalanffy and WBE growth models, the presented DEB model does not have an asymptotic size, meaning that growth must be terminated by some other mechanism. Indeed, while von Bertalanffy argued it was the mismatched scaling of anabolism and catabolism that caused growth to asymptote in vertebrates, he noted that this mechanism did not apply to insects (von Bertalanffy 1951). Rather, he supposed that in insects, growth was interrupted by some developmental cue. The absence of a physical limit that determines size in insects has been supported experimentally. Caterpillars chemically induced to enter an extra instar before pupation have been observed to continue their rapid growth trajectory beyond their usual terminal weight (Ciemior et al. 1979, Sindhu and Nair 2004).

It has long been known that body-size is a good predictor of insect molting and metamorphosis (Nijhout and Williams 1974). This has led to the concept of the ‘critical weight’, which is defined as the weight threshold that must be passed in order to trigger commitment to molting. The mechanism responsible for size detection in insects has been well elaborated in only a small number of species. Among these species it has been variously found that commitment to molting is triggered by abdominal stretch receptors (Wigglesworth

1934), the exhaustion of a pre-packaged food supply (Shafiei et al. 2001), or size-imposed oxygen limitation (Callier and Nijhout 2011). More recently it has been found that nutritional condition may better describe the process of molting (Chambers and Klowden 1990, Telang et al. 2007, Layalle et al. 2008). As the DEB model considers both size (structure) and nutritional condition (reserve), it offers natural handles to both of these quantities, which could be used to explore developmental triggers in insects.

DEB models are increasingly being used to explain broad patterns across species using simple physicochemical principles. One exciting area of application is in body size scaling relationships, which form the basis of the emerging field of metabolic ecology (Brown et al. 2004). DEB predicts that the famous sublinear scaling of metabolic rate with body mass between species occurs as a result of the sublinear scaling of structure with mass (assuming, simplistically, that metabolic rate at terminal size is equivalent to the maintenance cost of structure). This predicted sublinear scaling of structure occurs because larger organisms require greater amounts of reserve per mass (Maino et al. 2014). Structure at terminal size for insects included in the growth analysis can be estimated using the fitted DEB models (equation 5.2.8). As shown in figure 5-5, terminal structure scales sublinearly with body mass, which is consistent with the DEB expectation based on past studies of insect metabolic scaling (Chown et al. 2007b, Ehnes et al. 2011).

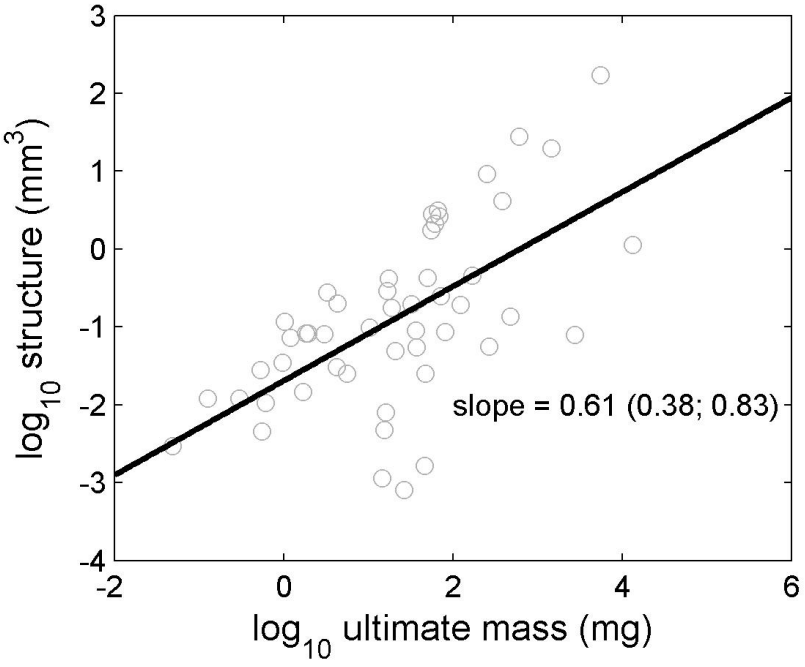

Figure 5-5. Ultimate structure is determined from the fitted DEB models for each species (equation 5.2.8) and is plotted against species ultimate mass. The 95% confidence interval of the regression slope (in parentheses) excludes 1, supporting the prediction that structure scales sublinearly with the mass of a species.

Mechanistic models are frequently cited as having more robust predictive power than their phenomenological counterparts (Helmuth et al. 2005, Denny and Benedetti-Cecchi 2012), but a more understated advantage is that their explanatory power is based on underlying processes. Models may fit data well, but for the wrong reasons. Indeed, all tested models can be interpreted mechanistically and explained high levels of variance (even the worst model explained roughly 92% of the variation in the growth data). However, novel predictions of the change in insect production efficiency and energy density with age highlight the usefulness of a DEB approach. Typical growth models assume biomass to be homogenous, rather that separating mass into quantities of structure of reserve, which makes it difficult to capture these patterns. As is becoming increasingly apparent, the concepts of

reserve and structure are useful for explaining a broad variety of biological patterns, from metabolic scaling, to the diversity of growth patterns among organisms.

Chapter 6: General conclusions

Summary

In this thesis I have approached the long-standing issue of body-size scaling in biology using the physicochemical principles of DEB theory. In order to explore the effectiveness of such an approach, novel predictions have been brought to bear against new data sets to test whether a DEB approach can offer new insights to old questions. In summary, I have assessed the position of DEB theory as a mechanistic underpinning to metabolic scaling theory in ecology, explored differences in metabolic scaling patterns for interspecific and ontogenetic comparisons, and explained the universal growth trajectory of insects within the context of the standard DEB model.

A key strength of a DEB approach, in contrast to other metabolic theories, is that its foundational principles are general to all of life. Other theories have attempted to explain metabolic scaling in terms of taxon-specific processes, such as vascular network supply constraints (West et al. 1997), or heat dissipation requirements of endotherms (Speakman 2010), but as metabolic scaling is present in a wide range of taxa, including terrestrial invertebrates, birds, reptiles, and mammals (Isaac and Carbone 2010), any successful theory of metabolism must not be taxon specific. The price of generalisability is abstraction, but there is much to gain from a general and unifying metabolic theory. Abstracting individuals into simple energy processors would seem to overlook many other important aspects of their biology, such as their unique phylogeny, physiology, or ethology, but this strategy has facilitated great advances in one of the grand challenges in biology: making sense of

interacting phenomena occurring across wide scales in space, time, and organisational complexity. The study of cells, individuals, communities and ecosystems have benefitted from such a regime (Brown et al. 2004, Humphries and McCann 2013). Similarly, the simple abstraction of partitioning individual organisms into compartments of reserve biomass and structural biomass allows us to account for an astounding variety of energetic transformations that occur between species and as an organism develops.

Realistic constraints on the mobilisation of reserve for metabolism are able to explain the famous 3/4 power scaling of metabolic rate (Chapter 2). Interestingly, the DEB formulation was shown to converge on prominent mechanistic equations, but with a different physical interpretation of parameters. It was further demonstrated in this thesis that a DEB approach not only allows the *a priori* estimation of the metabolic scaling exponent for mammals, but also that the intercept of the relationship could be determined by finding realistic estimates of some core DEB parameters. This contrasts to the common approach of fitting power functions to data and analysing the resulting parameter estimates. Such applications showcase the power of mechanistic models in biology.

The implications of body size are typically studied at the interspecific level. It is often implicitly assumed that the same restrictions of body size affect growing organisms as they increase in biomass, but this assumption had not been tested in detail. The next goal of the thesis was to determine whether, as predicted by theory, there exist differences in the body size scaling of energetic quantities between interspecific and ontogenetic comparisons (Chapter 3 and 4). For my compiled data set on insects, I found that mass changes through ontogeny correspond to a variety of scaling patterns in oxygen consumption (often used as a proxy for metabolic rate). The nature of the particular scaling pattern depended on where in ontogenetic development the analysis was conducted. The presented framework allowed the partitioning of ontogenetic and interspecific effects on metabolism and, after controlling for

ontogenetic effects, was used to recover the negative allometric scaling of interspecific metabolic rate. The estimated scaling exponents supported theoretical predictions derived from DEB theory and adhered to past empirical studies.

The idea of testing differences in ontogenetic and interspecific energetic constraints was extended to the scaling of uptake processes in insects; specifically, the consumption and digestion (assimilation) of food (Chapter 4). The presented framework again allowed the partitioning of ontogenetic effects from interspecific effects and revealed distinct signals in the scaling exponents depending on the level of comparison.

Finally, I tested whether these same principles could explain the related metabolic process of growth in insects (In Chapter 5). While the baseline model required modification to capture insect growth, the consequences of this modification implied universal characteristics of growth that were tested and confirmed. Here I discuss three important results of this thesis and place them in a broad context.

The role of DEB theory in ecology

Ecology typically relies on the collection of data that is coarser compared with lower organisational levels of study. The analysis of this coarse data requires coarse grained models that must necessarily ignore or abstract certain components of an ecosystem for the sake of tractability. For example, it is common practice to represent trophic relationships in an ecosystem as nodes (species) and edges (interactions) in a network rather than modelling the population numbers, and the specific exchanges of energy, nutrients and chemicals that occurs as part of these interactions (Montoya et al. 2006). There are two main reasons for this abstraction. Firstly, methodological practicalities are a significant barrier to the collection of detailed ecosystem level data. Accurate measurements for the complex energy and nutrient

exchanges taking place between interacting individuals are difficult to obtain even in simplified lab settings (Klekowski et al. 1967). To achieve this level of detail at ecologically meaningful scales of space and time is exceedingly difficult, and consequently, detailed energetic data of whole ecosystems is rare. Secondly, the abstraction of ecosystems into networks facilitates the study of emergent phenomena. For example, network theory has served as a useful conceptual tool in understanding the relationship between ecosystem complexity and stability with important implications for conservation biology (Montoya et al. 2006). This example illustrates that coarse ecological models are used for pragmatic reasons associated with data paucity, but also aid in the study of higher level processes.

It is almost a truism to state that individuals comprise populations, which form communities, and constitute the biological component of ecosystems. Less obvious is how is this acknowledgement is useful to ecologists, given the astounding variety of species, each with their own unique interactions and life-histories. Rather than taking a focus on species differences, it is the commonality between individuals (particularly of different species) that we can look to for useful heuristics in the development of ecosystem models. The principles of DEB theory aim to distil common features of life under one generic framework in order to make this goal tenable. For the standard DEB model, 14 parameters are required to describe the energy transformations associated with uptake, maintenance, development, growth and reproduction through the entire life-cycle of the organism (Kooijman 2010). Small deviations in parameter values can capture big difference in the lifecycle energetics of diverse organisms.

Although the standard DEB model has relatively few parameters for the number of processes explained (~1.5 per process), the standard model captures biological processes at a level of detail that has been criticised as excessive when, for example, compared with other mechanistic models of growth (Hou et al. 2008a, Zuo et al. 2009). However, the comparative

simplicity of these other models comes at the cost of being able to explain other related energetic processes, such as embryonic development or reproduction. Indeed, by not considering reproduction, the number of free parameters required to model energetic processes is substantially reduced (Chapters 2-5). More recently, DEB theory has been labelled as an 'inefficient theory' in ecology for the number of parameters in the standard DEB model (Marquet et al. 2014). Such criticisms highlight an ongoing misrepresentation of the DEB theory as a specific model rather than the theoretical principles that underlie its emergent models. A more useful view is that DEB theory rests on set of core axiomatic principles that can be used to study a range of problems in biology, which results in models of differing complexity depending on the nature of the phenomenon under study.

Much of the motivation of this thesis has been to contribute to growing awareness that the principles of DEB theory are relevant to higher levels of biological organisation. In doing so, it is apparent that parameter sparse models based on DEB theory are useful for explaining a range of biological processes. Application to the study of metabolic scaling of adult interspecifics resulted in a model with 2 free parameters (Chapter 2). Application to the scaling of consumption between and within species at different temperatures resulted in a model with 4 free parameters (Chapter 4). The number of processes considered immediately relates to the complexity of the model and the number free parameters required for their description.

Metabolic ecology is a research agenda that has gained much traction in recent years (Humphries and McCann 2013). The quantitative underpinning to this field is a central equation relating the rate of biological processes (I) to body size (M) and temperature (T):

$$I = i_0 M^{3/4} e^{-E/kT} \qquad (6.1)$$

where i_0 is a normalisation constant, E is the activation energy and k is Boltzmann's constant. This equation has just two free parameters and has been used with great success to study phenomena at all scales of biology organisation (Brown et al. 2004). Key proponents of metabolic ecology have reduced the free number of parameters in this equation on the basis of the mechanistic explanation proposed by West, Brown, and Enquist (1997) for the strong heuristic power of a ¾ mass exponent (Isaac and Carbone 2010). Chapter 2 of this thesis demonstrated that the principles of DEB theory can also account for the effectiveness of the ¾ exponent in metabolic ecology. The exponential temperature dependence of biological rates is assumed on the basis of principles of molecular kinetics in biochemical reactions (Boltzmann 1872, Arrhenius 1889, Gillooly et al. 2002). This same temperature dependence relationship underpins the method of temperature correction in DEB theory (Kooijman 2010). In this way, the principles of DEB theory can form an alternate mechanistic basis of the central equation of metabolic ecology.

Although DEB theory is commonly perceived to have most relevance to individual level processes, its underlying principles of body-size scaling, homeostasis, and energy conservation are universal to all natural processes, such that they are of use in understanding higher level biological processes. DEB models have been applied to a range of higher level processes ranging through population dynamics (Kooijman and Metz 1984), symbioses (Muller et al. 2009), prey-predator systems (Kooi et al. 1999, Troost et al. 2007), food chains (Kooi et al. 1997), ecosystems (Nisbet and Muller 2000, Bruggeman and Kooijman 2007), carbon cycling (Omta et al. 2007), and adaptive dynamics (Troost et al. 2007). Indeed, the criteria of Marquet et al. (2014) specifying an efficient theory (used erroneously as a basis by which to critique DEB theory) are, on closer consideration, a set of criteria that are met by DEB theory. DEB theory is based on first principles, relies on few assumptions, is expressed in the language of mathematics, serves as a standard reference point for interpreting

biological phenomena, and is parameter sparse. Importantly, the presented models derived from DEB theory in Chapters 2-5 were not so over-parameterised that they become simple descriptors of data, but yielded testable novel predictions that were brought to bear against empirical data. DEB theory is thus well-positioned as an efficient theory that will continue to foster progress in ecology.

Mass-independent effects on metabolic scaling

Another key outcome of this thesis was to highlight situations in the study of scaling phenomena where allometric functions are inappropriate, and the subsequent proposal of alternative methods to study mass-independent effects. This is a significant development because the study of metabolic scaling is dominated by the use of allometric functions. Typically, mass-independent effects have been captured by fitting one allometric function to the baseline group, and another to the study group (e.g. metabolic rate of animals during activity vs. resting (Weibel et al. 2004), hibernating vs. non-hibernating (Geiser 2004), or multicellular vs. unicellular species (DeLong et al. 2010)). Mass-independent effects can be tested for by analysing changes in the estimated exponent and coefficient of fitted allometric functions. For example, DeLong et al. (2010) presented empirical evidence suggesting that between major evolutionary transitions (prokaryotes to eukaryotes to metazoans) the scaling relationship changes from superlinearity to sublinearity. While this illustrates how the separate fitting of allometric functions can account for discrete mass independent effects, this is not a viable approach for capturing continuous effects on metabolic scaling relationships.

The time course of pupal respiration (Chapter 3) is one such relationship, indicating a smooth transition from decreasing to increasing phases that render the use of monotonic allometric functions unsuitable. I proposed the use of dimensionless polynomials to correct

for continuous mass-independent effects on metabolic scaling. The same approach successfully accounted for the effects of development on respiration across the whole lifecycle of insects. More generally, such an approach could be extended to explore the effect of other continuous variables on metabolic rate, such as activity levels (Taylor et al. 1970). These approaches help to disentangle the effect of interspecific variation in body mass from that of ontogenetic variation.

The separation between ontogenetic and interspecific mass changes is important for understanding variation in the allometric scaling exponent but may also shed light on the less-studied allometric intercept or normalisation constant (Apol et al. 2008). On reason for this is that few theories are able to predict variation in this intercept, which is instead treated as a free parameter to be fitted from data. This thesis confirmed that there are differences in ontogenetic and interspecific scaling exponents (Chapter 2-4), but a more subtle point is that this systematic variation in exponents predicts systematic variation in the normalisation constant of different species. Consumption rate, for example, was correctly predicted to exhibit an ontogenetic exponent smaller than the interspecific exponent, from which it follows that the normalisation constant for each species should also increase with interspecific body size (Chapter 3, Figure 3-1). Estimating the relationship between the intercept and body size for consumption rate finds a positive, but statistically non-significant, slope ($p = 0.18$). For assimilation rate the slope is also positive, but is significantly greater than zero ($p = 0.04$). Systematic variation in the fitted allometric intercept is weak in the compiled data, but detectable. Here I only considered insects, but future studies could pursue this line of reasoning by increasing the variety and number of species considered.

Universality of the von Bertalanffy growth curve

The von Bertalanffy growth curve, or similar growth curves such as the WBE growth model, have been upheld as universal growth curves for animals (West et al. 2001, Hou et al. 2008a, Sousa et al. 2010). But as with other topics in biology, a bias toward the study of vertebrates may have resulted in a premature claim to generality. In Chapter 5 it was demonstrated that for invertebrates (particularly insects), growth is poorly captured by a von Bertalanffy growth function. The finding also holds for marine invertebrates (Hirst and Forster 2013). For a growth model to be labelled as universal across animals, it must account for insects, which make up the majority of animal diversity. Consequently, it would seem that there is little hope for the attainment of a simple universal growth model.

However, as has been argued, DEB theory is broader than a particular growth model, and offers a metabolic framework that can be applied universally to the study of animal growth. In the DEB literature, there has been an increasing acknowledgement that von Bertalanffy shaped growth may not be as ubiquitous as once believed. Kooijman (2014) poses four scenarios within the context of DEB theory that may account for such deviations in von Bertalanffy growth, and other correlated energetic features. Such deviations in growth – termed 'metabolic acceleration' – are becoming increasingly apparent when DEB theory is applied systematically to a large number of diverse organisms (Kooijman 2013, Lika et al. 2014). In addition to the insects, taxonomical groups exhibiting these patterns include Crustacea, Arachnida, Mollusca, Actinopterygii, Echinodermata, Cnidaria, and Ctenophora (Kooijman 2014).

To capture the growth of insects, a simple modification to the standard DEB model is presented in Chapter 5. This deviation is closest to the acceleration mode that Kooijman (2014) terms 'type A' acceleration, in which the specific assimilation rate of structure changes with size. Interestingly, Kooijman finds that the predominant deviation to the standard DEB model in over 500 species studied is a scenario called 'type M' acceleration.

During growth, type M acceleration causes specific assimilation and conductance to increase proportional to the increase in structural length. This scenario is convenient because the weak homeostasis assumption remains valid under constant conditions (unlike in other modes of acceleration), which has useful theoretical implications for determining chemical compositions of different biomass pools (Kooijman 2014). Type M acceleration predicts exponential growth, constant chemical composition and biomass growth efficiency under constant conditions. However, in Chapter 5 it was demonstrated that insect biomass growth efficiency and energy density increase with development. In addition, the growth rate of insects was slower than exponential but faster than von Bertalanffy growth (Chapter 5, Figure 5-1).

The modification in Chapter 5 added no free parameters to the standard model and was able to explain these patterns. Another proposed method to account for these patterns is to apply 'type M' acceleration to the standard model but to make additional modifications that bring predictions more in line with empirical patterns. Recent work conducted by Llandres et al. (2014, *in press*) pursued this line of thought and found that allocation to reproductive reserves during larval growth could explain these patterns in the context of a parasitic wasp. As the larvae grows, an increased proportion of biomass was predicted to consist of resources set aside for reproduction, which has similar effects to the modification described in Chapter 5. The downside of such an approach is the increased model complexity and introduced parameters associated with new energy allocation schemes and additional biomass compartments. These changes incurred 10 additional free parameters compared with the standard DEB model, but included extra processes such as silk production, a feeding embryonic phase, and a pupal phase. Such a model will be difficult to test on the basis of growth data alone and will require more detailed energetic data across the whole lifecycle of a variety of insects.

Reasons for deviations from the standard DEB model (or von Bertalanffy growth) are diverse, with some studies suggesting acceleration may be caused by changes in body shape (Pecquerie et al. 2009, Jusup et al. 2011). More commonly it is thought that physiological constraints on food assimilation may be important drivers. Jager et al. (2005) found that food limitation in juvenile nematodes could explain deviations from the standard model and, more recently, food limitation has been implicated in the metabolic acceleration of a pond snail (Zimmer et al. 2012, 2014). The taxonomical groups exhibiting metabolic acceleration are evolutionarily distinct, which suggests convergence on metabolic types (Kooijman 2014). Kooijman suggests that these distinct groups may face the shared problem of establishing gut flora in early juveniles, with the consequence of delayed metabolism during early growth while gut flora are not fully functional. The converse of this are juveniles of species that are inoculated with parental gut flora via saliva, as in birds and mammals. Indeed, birds and mammals were found to obey the standard DEB model very closely (Kooijman 2014). Whether this explanation is general remains to be seen, particularly as there exist many examples of species outside of birds and mammals that seem to fit the standard DEB model (Kooijman 2014). One example of particular interest is the absence and presence of metabolic acceleration in Perciformes (Lika et al. 2014), which can be attributed to differences in feeding conditions during spawning. Food availability thus seems to play a crucial role in metabolic acceleration in a number of contexts. However, such explanations do not justify the quantitative form of the acceleration, which, as mentioned has typically been taken as conductance and specific assimilation scaling with structural length.

Practical limitations and future developments

Although the usefulness of Dynamic Energy Budget theory has been demonstrated in a number of current theoretical problems in biology, it is important to note that the development of DEB theory is ongoing, and that there presently exists a number of practical issues that can be significantly improved.

The key quantities of reserve and structure in DEB theory are difficult measure in practice, both in amount and composition, which makes the widespread testing of these concepts difficult. This is not a problem unique only to DEB models. As pointed out in Chapter 2, population allele frequencies in quantitative genetics are difficult to measure in practice but are an important theoretical construct. However, statistical sampling can be used to infer population characteristics to some degree of confidence, which partially circumvents this problem faced by population geneticists. DEB theory currently lacks such a straightforward solution to the problem of measuring reserve and structure. DEB theory uses the concepts of reserve and structure to keep track of more complicated metabolic processes. Insofar as they keep track of the state of the organism, they serve the same function as mass in allometric models. But, as was discussed in Chapter 2 and 3, mass is not a reliable indicator of metabolic state during ontogeny, which necessitate the partitioning of biomass. The trade-off is that, unlike mass, reserve and structure can only be measured indirectly, and require detailed stoichiometric information at a variety of controlled feeding levels to infer their precise composition and quantity (Kooijman 2010). In order for the core DEB concepts to be tested more widely, the process of quantifying reserve and structure will need to be streamlined, such as by finding reliable and easily measureable correlates of reserve and structure. For example, it was hypothesised that ribosomal RNA should correlate with reserve (Kooijman 2010) and so should increase with size (Chapter 2), however, the opposite result has been found (Gillooly et al. 2005a).

The difficultly in testing core concepts is also hampered by the difficulty in implementing DEB models. Although the core model assumptions are simple, the resulting equations are not. Despite the availability of prewritten code, implementing the programs requires a serious amount of time investment, with even more time required to understand the code (Jager et al. 2013). This burden on the user could be lessened through the development of simplified frameworks based on DEB theory as has been proposed by Jager et al. (2013).

Concluding remarks

If the underlying principles of a model are sound, deviations from expectations do not prove a model wrong, only insufficient. Misalignment between predictions and data serves the significant purpose of highlighting important processes that have been omitted in a model, and fuels the iterative process that leads to its improvement. The only criteria, then, by which this iterative process ends, is that of sufficient usefulness. In this thesis, I have tested the usefulness of DEB models to shed light on long standing problems in biology from metabolic scaling to the universality of animal growth patterns. Indeed, proposed modifications to existing DEB models were shown to bring data into better alignment with expectations, while yielding novel predictions that were empirically confirmed. These applications highlight the usefulness of DEB theory as an interpretive framework for a wide range of biological processes, and the usefulness, in general, of mechanistic models based on sound theory that are able to cut across vastly different organisational levels. Further research, particularly on the application of DEB theory to ecological level processes, will likely reinforce the role of metabolic theory as a unifying framework in biology.

References

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Llandres, A.L, Marques, G.M., Maino, J.L., Kooijman, S.A.L.M., Kearney, M.R. & Casas, J

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Addol-Bediako, A., S. Chown, and K. Gaston. 2002. Metabolic cold adaptation in insects: a large-scale perspective. Functional Ecology 16:332–338.

Apol, M. E. F., R. S. Etienne, and H. Olff. 2008. Revisiting the evolutionary origin of allometric metabolic scaling in biology. Functional Ecology 22:1070–1080.

Arrhenius, S. 1889. Uber die Reaktionsgeschwindigkeit bei der Inversion von Rohrzucker durcj Sauren. Zeitschrift fur Physik Chemique 4:226–248.

Babel, W. 1985. Correlation between cell composition and carbon conversion efficiency in microbial growth: a theoretical study. Applied microbiology and biotechnology:201–207.

Banavar, J. R., J. Damuth, A. Maritan, and A. Rinaldo. 2002. Supply-demand balance and metabolic scaling. Proceedings of the National Academy of Sciences of the United States of America 99:10506–9.

Banavar, J. R., A. Maritan, and A. Rinaldo. 1999. Size and form in efficient transportation networks. Nature 399:130–2.

Banavar, J. R., M. E. Moses, J. H. Brown, J. Damuth, A. Rinaldo, and R. M. Sibly. 2010. A general basis for quarter-power scaling in animals. Proceedings of the National Academy of Sciences of the United States of America 107:15816–15820.

Barrow, G. M. 1974. Physical chemistry for the life sciences. McGraw-Hill.

Battley, E. H. 1991. An alternate method of calculating the heat of growth in Escherichia coli K-12 on succinic acid. Biotechnology and Bioengineering 38:480–492.

Von Bertalanffy, L. 1951. Metabolic types and growth types. The American Naturalist 85:111–117.

Von Bertalanffy, L. 1957. Quantitative Laws in Metabolism and Growth. The Quarterly Review of Biology 32:217–231.

Blossman-Myer, B. L., and W. W. Burggren. 2010. Metabolic allometry during development and metamorphosis of the silkworm Bombyx mori: analyses, patterns, and mechanisms. Physiological and biochemical zoology 83:215–31.

Boggs, C. L., and K. D. Freeman. 2005. Larval food limitation in butterflies: effects on adult resource allocation and fitness. Oecologia 144:353–61.

Boltzmann, L. 1872. Weitere Studien uber das Warmegleich- gewicht unter Gasmolekulen. Sitzungsberichte der mathematisch-naturwissenschlaftlichen Classe der kaiserlichen Akademic der Wissenschaften Wien 66:275–370.

Bownes, M. 1975. A photographic study of development in the living embryo of Drosophila melanogaster. Journal of Embryology and Experimental Morphology 33:789–801.

Brandt, B. W., F. D. L. Kelpin, I. M. M. van Leeuwen, and S. A. L. M. Kooijman. 2004. Modelling microbial adaptation to changing availability of substrates. Water research 38:1003–13.

Brandt, B. W., and S. A. L. M. Kooijman. 2000. Two parameters account for the flocculated growth of microbes in biodegradation assays. Biotechnology and bioengineering 70:677–84.

Brandt, B. W., I. M. M. van Leeuwen, and S. A. L. M. Kooijman. 2003. A general model for multiple substrate biodegradation. Application to co-metabolism of structurally non-analogous compounds. Water research 37:4843–54.

Brown, J. H., J. F. Gillooly, A. P. Allen, V. M. Savage, and G. B. West. 2004. Toward a metabolic theory of ecology. Ecology 96:858–73.

Brown, J. H., and R. M. Sibly. 2012. The Metabolic Theory of Ecology and Its Central Equation. Pages 21–33 *in* R. M. Sibly, J. H. Brown, and A. Kodric-Brown, editors. Metabolic Ecology: A Scaling Approach. John Wiley & Sons, Ltd.

Brown, J. H., R. M. Sibly, and A. Kodric-Brown. 2012. Introduction: Metabolism as the Basis for a Theoretical Unification of Ecology. Pages 1–6 *in* R. M. Sibly, J. H. Brown, and A. Kodric-Brown, editors. Metabolic Ecology: A Scaling Approach. John Wiley & Sons, Ltd.

Bruggeman, J., and S. A. L. M. Kooijman. 2007. A biodiversity-inspired approach to aquatic ecosystem modeling. Limnology and Oceanography 52:1533–1544.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference: a practical information-theoretic approach. 2nd edition. Springer-Verlag, Berlin.

Calder, W. A. 1984. Size, function, and life history. Courier Dover Publications, N. Chemsford, MA.

Callier, V., and H. F. Nijhout. 2011. Control of body size by oxygen supply reveals size-dependent and size-independent mechanisms of molting and metamorphosis. Proceedings of the National Academy of Sciences of the United States of America 108:14664–9.

Caruso, T., D. Garlaschelli, R. Bargagli, and P. Convey. 2010. Testing metabolic scaling theory using intraspecific allometries in Antarctic microarthropods. Oikos 119:935–945.

Carvalho, G. B., P. Kapahi, and S. Benzer. 2005. Compensatory ingestion upon dietary restriction in Drosophila melanogaster. Nature Methods 2:813–815.

Chambers, G. M., and M. J. Klowden. 1990. Correlation of nutritional reserves with a critical weight for pupation in larval Aedes aegypti mosquitoes. Journal of the American Mosquito Control Association 6:394–9.

Chilliard, Y., C. Delavaud, and M. Bonnet. 2005. Leptin expression in ruminants: nutritional and physiological regulations in relation with energy metabolism. Domestic animal endocrinology 29:3–22.

Chown, S. L., E. Marais, J. S. Terblanche, C. J. Klok, J. R. B. Lighton, and T. M. Blackburn. 2007a. Scaling of insect metabolic rate is inconsistent with the nutrient supply network model. Functional Ecology 21:282–290.

Chown, S. L., E. Marais, J. S. Terblanche, C. J. Klok, J. R. B. Lighton, and T. M. Blackburn. 2007b. Scaling of insect metabolic rate is inconsistent with the nutrient supply network model. Functional Ecology 21:282–290.

Chown, S. L., and S. Nicolson. 2004. Insect Physiological Ecology: Mechanisms and Patterns. Oxford University Press, New York.

Ciemior, K. E., F. Sehnal, and H. A. Scheinderman. 1979. Moulting, growth and survival of Galleria mellonella L. (Lep., Pyralidae) treated with juvenoids. Zeitschrift für Angewandte Entomologie 88:414–425.

Coulson, R. A., and T. Hernandez. 1970. Protein digestion and amino acid absorption in the cayman. The Journal of nutrition 100:810–26.

Coulson, R. A., T. Hernandez, and J. D. Herbert. 1977. Metabolic rate, enzyme kinetics in vivo. Comparative Biochemistry and Physiology 56A:251–262.

Darveau, C. A., R. K. Suarez, R. M. Andrews, and P. W. Hochachka. 2002. Allometric cascade as a unifying principle of body mass effects on metabolism. Nature 417:166–70.

DeLong, J. P., T. C. Hanley, and D. a Vasseur. 2014. Competition and the density dependence of metabolic rates. The Journal of Animal Ecology 83:51–8.

DeLong, J. P., J. G. Okie, M. E. Moses, R. M. Sibly, and J. H. Brown. 2010. Shifts in metabolic scaling, production, and efficiency across major evolutionary transitions of life. Proceedings of the National Academy of Sciences of the United States of America 107:12941–5.

Demetrius, L., and J. Tuszynski. 2010. Quantum metabolism explains the allometric scaling of metabolic rates. Journal of the Royal Society Interface 7:507–514.

Denny, M., and L. Benedetti-Cecchi. 2012. Scaling Up in Ecology: Mechanistic Approaches. Annual Review of Ecology, Evolution, and Systematics 43:1–22.

Ehnes, R. B., B. C. Rall, and U. Brose. 2011. Phylogenetic grouping, curvature and metabolic scaling in terrestrial invertebrates. Ecology Letters 14:1–8.

Eichinger, M., R. Sempéré, G. Grégori, B. Charrière, J. C. Poggiale, and D. Lefèvre. 2010. Increased bacterial growth efficiency with environmental variability: results from DOC degradation by bacteria in pure culture experiments. Biogeosciences 7:1861–1876.

Elia, M. 1992. Organ and tissue contributions to metabolic rate. Pages 61–79 *in* M. J. Kinney and N. H. Tucker, editors. Energy metabolism: Tissue determinants and cellular corollaries. Raven, New York.

Enquist, B. J., E. P. Economo, T. E. Huxman, A. P. Allen, D. D. Ignace, and J. F. Gillooly. 2003. Scaling metabolism from organisms to ecosystems. Nature 423:639–42.

Evers, E. 1991. A model for light- • limited continuous cultures: Growth, shading, and maintenance. Biotechnology and bioengineering 38:254–259.

Fink, D. 1925. Metabolism during embryonic and metamorphic development of insects. The Journal of General Physiology 7:527–543.

Fink, P., L. Peters, and E. Von Elert. 2006. Stoichiometric mismatch between littoral invertebrates and their periphyton food. Archiv für Hydrobiologie 165:145–165.

Geiser, F. 2004. Metabolic rate and body temperature reduction during hibernation and daily torpor. Annual review of physiology 66:239–74.

Gillooly, J. F., A. P. Allen, J. H. Brown, J. J. Elser, C. Martinez del Rio, V. M. Savage, G. B. West, W. H. Woodruff, and H. A. Woods. 2005a. The metabolic basis of whole-organism RNA and phosphorus content. Proceedings of the National Academy of Sciences of the United States of America 102:11923–7.

Gillooly, J. F., A. P. Allen, G. B. West, and J. H. Brown. 2005b. The rate of DNA evolution: effects of body size and temperature on the molecular clock. Proceedings of the National Academy of Sciences of the United States of America 102:140–5.

Gillooly, J. F., E. L. Charnov, G. B. West, V. M. Savage, and J. H. Brown. 2002. Effects of size and temperature on developmental time. Nature 417:70–73.

Gillooly, J. F., E. L. Charnov, G. B. West, V. M. Savage, J. H. Brown, G. B. West, V. M. Savage, and E. L. Charnov. 2001. Effects of size and temperature on metabolic rate. Science 293:2248–51.

Glazier, D. S. 2005. Beyond the "3/4-power law": variation in the intra- and interspecific scaling of metabolic rate in animals. Biological reviews of the Cambridge Philosophical Society 80:611–62.

Glazier, D. S. 2006. The 3/4-Power Law is not universal : evolution of isometric, ontogenetic metabolic scaling in pelagic animals. BioScience 56:325–332.

Glazier, D. S. 2010. A unifying explanation for diverse metabolic scaling in animals and plants. Biological reviews of the Cambridge Philosophical Society 85:111–38.

Glazier, D. S. 2014. Scaling of Metabolic Scaling within Physical Limits. Systems 2:425–450.

Glazier, D. S., A. G. Hirst, D. Atkinson, and D. S. Glazier. 2015. Shape shifting predicts ontogenetic changes in metabolic scaling in diverse aquatic invertebrates. Proceedings of the Royal Society B: Biological Sciences 282:1–9.

Grimaldi, D., and M. S. Engel. 2005. Evolution of the Insects. Cambridge University Press.

Hadfield, M. 2001. Metamorphic competence, a major adaptive convergence in marine invertebrate larvae. American Zoologist 41:1123–1131.

Hamilton, M. J., A. D. Davidson, R. M. Sibly, and J. H. Brown. 2011. Universal scaling of production rates across mammalian lineages. Proceedings. Biological sciences / The Royal Society 278:560–6.

Hanegraaf, P. P., and E. B. Muller. 2001. The dynamics of the macromolecular composition of biomass. Journal of theoretical biology 212:237–51.

Hanegraaf, P. P., a H. Stouthamer, and S. A. L. M. Kooijman. 2000. A mathematical model for yeast respiro-fermentative physiology. Yeast (Chichester, England) 16:423–37.

Harte, J. 2004. The value of null theories in ecology. Ecology 85:1792–1794.

Harvey, J., I. Harvey, and D. Thompson. 1994. Flexible larval growth allows use of a range of host sizes by a parasitoid wasp. Ecology 75:1420–1428.

Hast, M. H. 1989. The larynx of roaring and non-roaring cats. Journal of Anatomy 163:117–121.

Hayes, S., J. Mcclintock, C. Watson, and R. Douglaswatson. 1992. Growth, energetics and food conversion efficiency during the last larval stadium of the tobacco hornworm (Manduca sexta). Comparative Biochemistry and Physiology Part A: Physiology 102:395–399.

Hechinger, R. F., K. D. Lafferty, A. P. Dobson, J. H. Brown, and A. M. Kuris. 2011. A common scaling rule for abundance, energetics, and production of parasitic and free-living species. Science 333:445–448.

Helmuth, B., J. G. Kingsolver, and E. Carrington. 2005. Biophysics, physiological ecology, and climate change: does mechanism matter? Annual review of physiology 67:177–201.

Hemmingsen, A. . 1960. Energy metabolism as related to body size and respiratory surfaces, and its evolution. Rep. Steno Memorial Hospital Nordisk Insulin Laboratorium 9:1–110.

Hernandez, T., and R. A. Coulson. 1952. Hibernation in the alligator. Experimental Biology and Medecine 79:145–149.

Hirst, A. G., and J. Forster. 2013. When growth models are not universal: evidence from marine invertebrates. Proceedings. Biological sciences / The Royal Society 280:20131546.

Hirst, A. G., D. S. Glazier, and D. Atkinson. 2014. Body shape shifting during growth permits tests that distinguish between competing geometric theories of metabolic scaling. Ecology Letters 17:1274–81.

Hochuli, D. F. 2006. Insect herbivory and ontogeny : How do growth and development influence feeding behaviour , morphology and host use ?563–570.

Holling, C. 1959. Some characteristics of simple types of predation and parasitism. The Canadian Entomologist XCI:385–398.

Hou, C., W. Zuo, M. E. Moses, W. W. H. W. W. H. Woodruff, J. H. Brown, and G. B. West. 2008a. Energy uptake and allocation during ontogeny. Science 322:736–9.

Hou, C., W. Zuo, M. Moses, and W. Woodruff. 2008b. Energy uptake and allocation during ontogeny. Science 322:736–739.

Houston, A. I., and J. M. McNamara. 2014. Foraging currencies, metabolism and behavioural routines. The Journal of Animal Ecology 83:30–40.

Huete-Ortega, M., P. Cermeño, A. Calvo-Díaz, and E. Marañón. 2012. Isometric size-scaling of metabolic rate and the size abundance distribution of phytoplankton. Proceedings. Biological sciences / The Royal Society 279:1815–23.

Hume, D., C. Beiglböck, T. Ruf, F. Frey-Roos, U. Bruns, and W. Arnold. 2002. Seasonal changes in morphology and function of the gastrointestinal tract of free-living alpine marmots (Marmota marmota). Journal of comparative physiology. B, Biochemical, systemic, and environmental physiology 172:197–207.

Humphries, M. M., and K. S. McCann. 2013. Metabolic ecology. The Journal of Animal Ecology 83:7–19.

Ingenbleek, Y. 2006. 5th Amino Acid Assessment Workshop The Nutritional Relationship Linking Sulfur to Nitrogen in Living Organisms 1:1641–1651.

Isaac, N. J. B., and C. Carbone. 2010. Why are metabolic scaling exponents so controversial? Quantifying variance and testing hypotheses. Ecology letters 13:728–35.

Jager, T., O. A. Álvarez, J. E. Kammenga, and S. A. L. M. Kooijman. 2005. Modelling nematode life cycles using dynamic energy budgets. Functional Ecology 19:136–144.

Jager, T., B. T. Martin, and E. I. Zimmer. 2013. DEBkiss or the quest for the simplest generic model of animal life history. Journal of Theoretical Biology 328:9–18.

Jusup, M., T. Klanjscek, H. Matsuda, and S. A. L. M. Kooijman. 2011. A Full Lifecycle Bioenergetic Model for Bluefin Tuna. PLoS ONE 6:e21903.

Kearney, M. R., and C. R. White. 2012. Testing metabolic theories. The American Naturalist 180:546–65.

Killen, S. S., D. Atkinson, and D. S. Glazier. 2010. The intraspecific scaling of metabolic rate with body mass in fishes depends on lifestyle and temperature. Ecology letters 13:184–93.

Kleiber, M. 1932. Body size and metabolism. Hilgardia 6:315–353.

Klekowski, R. Z., T. Prus, and H. Zyromska-Rudzka. 1967. Elements of energy budget of Tribolium castaneum (Hbst) in its developmental cycle. Pages 859–881 *in* K. Petrusewicz, editor. Secondary productivity of terrestrial ecosystems (Principles and Methods) Vol. II. Warszawa, Krakow.

Kolokotrones, T., Van Savage, E. J. Deeds, W. Fontana, and V. M. Savage. 2010. Curvature in metabolic scaling. Nature 464:753–6.

Kooi, B. W., M. Boer, and S. A. L. M. Kooijman. 1997. Complex dynamic behaviour of autonomous microbial food chains. Journal of Mathematical Biology 36:24–40.

Kooi, B. W., M. P. Boer, and S. A. L. M. Kooijman. 1999. Resistance of a food chain to invasion by a top predator. Mathematical Biosciences 157:217–236.

Kooijman, S. A. L. M. 1986a. Energy budgets can explain body size relations. Journal of Theoretical Biology:269–282.

Kooijman, S. A. L. M. 1986b. What the hen can tell about her eggs: egg development on the basis of energy budgets. Journal of mathematical biology 23:163–85.

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

Kooijman, S. A. L. M. 2010. Dynamic energy budget theory for metabolic organisation. Cambridge University Press, Cambridge.

Kooijman, S. A. L. M. 2013. Waste to hurry: dynamic energy budgets explain the need of wasting to fully exploit blooming resources. Oikos 122:348–357.

Kooijman, S. A. L. M. 2014. Metabolic acceleration in animal ontogeny: An evolutionary perspective. Journal of Sea Research.

Kooijman, S. A. L. M., and B. W. Kooi. 1996. Catastrophic behavior of myxamoebae. Nonlinear World 3:77–84.

Kooijman, S. A. L. M., and J. Metz. 1984. On the dynamics of chemically stressed populations: the deduction of population consequences from effects on individuals. Ecotoxicology and Environmental Safety 8:254–274.

Kooijman, S. A. L. M., and T. A. Troost. 2007. Quantitative steps in the evolution of metabolic organisation as specified by the Dynamic Energy Budget theory. Biological reviews of the Cambridge Philosophical Society 82:113–42.

Kozłowski, J., M. Czarnoleski, A. François-Krassowska, S. Maciak, and T. Pis. 2010. Cell size is positively correlated between different tissues in passerine birds and amphibians, but not necessarily in mammals. Biology Letters 6:792–6.

Kozłowski, J., M. Konarzewski, and A. T. Gawelczyk. 2003. Cell size as a link between noncoding DNA and metabolic rate scaling. Proceedings of the National Academy of Sciences 100:14080–14085.

Król, E., P. Redman, P. J. Thomson, R. Williams, C. Mayer, J. G. Mercer, and J. R. Speakman. 2005. Effect of photoperiod on body mass, food intake and body composition in the field vole, Microtus agrestis. The Journal of experimental biology 208:571–84.

Layalle, S., N. Arquier, and P. Léopold. 2008. The TOR pathway couples nutrition and developmental timing in Drosophila. Developmental cell 15:568–77.

Levins, R. 1966. The strategy of model building in population biology. American Scientist 54:421–431.

Lika, K., M. R. Kearney, V. Freitas, H. W. van der Veer, J. van der Meer, J. W. M. Wijsman, L. Pecquerie, and S. A. L. M. Kooijman. 2011a. The “covariation method” for estimating the parameters of the standard Dynamic Energy Budget model I: Philosophy and approach. Journal of Sea Research 66:270–277.

Lika, K., M. R. Kearney, and S. A. L. M. Kooijman. 2011b. The “covariation method” for estimating the parameters of the standard Dynamic Energy Budget model II: Properties and preliminary patterns. Journal of Sea Research 66:278–288.

Lika, K., and S. A. L. M. Kooijman. 2011. The comparative topology of energy allocation in budget models. Journal of Sea Research 66:381–391.

Lika, K., S. A. L. M. Kooijman, and N. Papandroulakis. 2014. Metabolic acceleration in Mediterranean Perciformes. Journal of Sea Research (In Press).

Lorena, A., G. M. Marques, S. A. L. M. Kooijman, and T. Sousa. 2010. Stylized facts in microalgal growth: interpretation in a dynamic energy budget context. Philosophical transactions of the Royal Society of London. Series B, Biological sciences 365:3509–21.

Maino, J. L., and M. R. Kearney. 2014. Ontogenetic and Interspecific Metabolic Scaling in Insects. The American Naturalist 184:695–701.

Maino, J. L., and M. R. Kearney. 2015. Ontogenetic and interspecific scaling of consumption in insects. Oikos 124.

Maino, J. L., M. R. Kearney, R. M. Nisbet, and S. A. L. M. Kooijman. 2014. Reconciling theories for metabolic scaling. Journal of Animal Ecology 83:20–9.

Marquet, P. A., A. P. Allen, J. H. Brown, J. A. Dunne, B. J. Enquist, J. F. Gillooly, P. A. Gowaty, J. L. Green, J. Harte, S. P. Hubbell, J. O'Dwyer, J. G. Okie, A. Ostling, M. Ritchie, D. Storch, and G. B. West. 2014. On Theory in Ecology. BioScience XX:1–10.

Martin, B. T., T. Jager, R. M. Nisbet, T. G. Preuss, and V. Grimm. 2013. Predicting population dynamics from the properties of individuals: a cross-level test of dynamic energy budget theory. The American naturalist 181:506–19.

Martin, B. T., E. I. Zimmer, V. Grimm, and T. Jager. 2012. Dynamic Energy Budget theory meets individual-based modelling: a generic and accessible implementation. Methods in Ecology and Evolution 3:445–449.

McLandress, M., and D. Raveling. 1981. Changes in diet and body composition of Canada geese before spring migration. The Auk 98:65–79.

McMahon, T. 1973. Size and Shape in Biology 179:1201–1204.

McNab, B. K. 2008. An analysis of the factors that influence the level and scaling of mammalian BMR. Comparative biochemistry and physiology. Part A, Molecular & integrative physiology 151:5–28.

Van der Meer, J. 2006a. Metabolic theories in ecology. Trends in ecology & evolution 21:136–40.

Van der Meer, J. 2006b. An introduction to Dynamic Energy Budget (DEB) models with special emphasis on parameter estimation. Journal of Sea Research 56:85–102.

Montoya, J. M., S. L. Pimm, and R. V Solé. 2006. Ecological networks and their fragility. Nature 442:259–64.

Moran, D., and R. M. G. Wells. 2007. Ontogenetic scaling of fish metabolism in the mouse-to-elephant mass magnitude range. Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology 148:611–20.

Mueller, C. A., S. Augustine, S. A. L. M. Kooijman, M. R. Kearney, and R. S. Seymour. 2012. The trade-off between maturation and growth during accelerated development in

frogs. Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology 163:95–102.

Muller, E. B. 2011. Synthesizing units as modeling tool for photosynthesizing organisms with photoinhibition and nutrient limitation. Ecological Modelling 222:637–644.

Muller, E. B., B. Ananthasubramaniam, T. Klanjšček, and R. M. Nisbet. 2011. Entrainment of cell division in phytoplankton with dynamic energy budgets. Journal of Sea Research 66:447–455.

Muller, E. B., S. A. L. M. Kooijman, P. J. Edmunds, F. J. Doyle, and R. M. Nisbet. 2009. Dynamic energy budgets in syntrophic symbiotic relationships between heterotrophic hosts and photoautotrophic symbionts. Journal of Theoretical Biology 259:44–57.

Muthukrishnan, J., and T. Pandian. 1987. Insecta. Pages 373–511 *in* T. J. Pandian and F. J. Vernberg, editors. Animal Energetics Vol 1. Academic Press Inc., San Diego, California.

Nguyen, V., B. Lilly, and C. Castro. 2014. The exoskeletal structure and tensile loading behavior of an ant neck joint. Journal of Biomechanics 47:497–504.

Nijhout, H. F., G. Davidowitz, and D. A. Roff. 2006. A quantitative analysis of the mechanism that controls body size in Manduca sexta. Journal of Biology 5.

Nijhout, H. F., and C. M. Williams. 1974. Control of moulting and metamorphosis in the tobacco hornworm, Manduca sexta (L.): growth of the last-instar larva and the decision to pupate. The Journal of experimental biology 61:481–91.

Nisbet, R., and E. B. Muller. 2000. From molecules to ecosystems through dynamic energy budget models. Journal of Animal Ecology 69:913–926.

Odell, J. P. 1998. Energetics of metamorphosis in two holometabolous insect species: Manduca sexta (Lepidoptera: Sphingidae) and Tenebrio molitor (Coleoptera: Tenebrionidae). The Journal of Experimental Zoology 280:344–353.

Omta, A. W., S. A. L. M. Kooijman, and H. Dijkstra. 2007. Influence of (sub)mesoscale eddies on the soft-tissue carbon pump. Journal of Geophysical Research 112:1–14.

Pawar, S., A. I. Dell, and V. M. Savage. 2012. Dimensionality of consumer search space drives trophic interaction strengths. Nature 486:485–9.

Pecquerie, L., P. Petitgas, and S. A. L. M. Kooijman. 2009. Modeling fish growth and reproduction in the context of the Dynamic Energy Budget theory to predict environmental impact on anchovy spawning duration. Journal of Sea Research 62:93–105.

Peters, R. H. 1986. The ecological implications of body size. Cambridge University Press.

Piersma, T., and Å. Lindström. 1997. Rapid reversible changes in organ size as a component of adaptive behaviour. Trends in Ecology & Evolution.

Pitts, G. C., and T. R. Bullard. 1968. Some interspecific aspects of body composition in mammals. *in* J. T. Reid, A. Bensadoun, and L. S. Bull, editors. Body composition in animals and man. National Academy of Science, Washington, DC.

Rakshpal, R. 1962. Respiratory metabolism during embryogenesis of Gryllus veletis (Alexander and Bigelow)(Orthoptera: Gryllidae). Physiological Zoology 35:47–51.

Ratsak, C. H. 1995. Modeling the individual growth of Tetrahymena sp. and its population consequences. Journal of Eukaryotic Microbiology 42:268–276.

Ratsak, C. H., K. Maarsen, and S. A. L. M. Kooijman. 1996. Effects of protozoa on carbon mineralization in activated sludge. Water Research 30:1–12.

Reuman, D. C., R. D. Holt, and G. Yvon-Durocher. 2014. A metabolic perspective on competition and body size reductions with warming. The Journal of Animal Ecology 83:59–69.

Rezende, E. L., F. Bozinovic, and T. Garland. 2004. Climatic adaptation and the evolution of basal and maximum rates of metabolism in rodents. Evolution; international journal of organic evolution 58:1361–74.

Rivero, a, D. Giron, and J. Casas. 2001. Lifetime allocation of juvenile and adult nutritional resources to egg production in a holometabolous insect. Proceedings. Biological sciences / The Royal Society 268:1231–7.

Sander, K. 1990. The Insect Oocyte: Fertilization, Activation and Cytoplasmic Dynamics. Pages 605–624 *in* B. Dale, editor. Mechanism of Fertilization: Plants to Humans SE - 41. Springer Berlin Heidelberg.

Savage, V. M., E. J. Deeds, and W. Fontana. 2008. Sizing up allometric scaling theory. PLoS computational biology 4:e1000171.

Savage, V. M., J. F. Gillooly, W. H. Woodruff, G. B. West, A. P. Allen, B. J. Enquist, and J. H. Brown. 2004. The predominance of quarter-power scaling in biology. Functional Ecology 18:257 – 282.

Schmidt-Nielsen, K. 1984. Scaling, why is animal size so important? Cambridge University Press.

Scriber, J. M. 1977. Limiting effects of low leaf-water content on the nitrogen utilization, energy budget, and larval growth ofHyalophora cecropia (Lepidoptera: Saturniidae). Oecologia 28:269–287.

Scriber, J. M., and F. Slansky. 1981. The Nutritional Ecology of Immature Insects. Annual Review of Entomology 26:183–211.

Sears, K. E., A. J. Kerkhoff, A. Messerman, and H. Itagaki. 2012. Ontogenetic Scaling of Metabolism, Growth, and Assimilation: Testing Metabolic Scaling Theory with Manduca sexta Larvae. Physiological and biochemical zoology : PBZ 85:159–73.

Shafiei, M., A. P. Moczek, and H. F. Nijhout. 2001. Food availability controls the onset of metamorphosis in the dung beetle Onthophagus taurus (Coleoptera: Scarabaeidae). Physiological Entomology 26:173–180.

Shingleton, A. W., W. A. Frankino, T. Flatt, H. F. Nijhout, and D. J. Emlen. 2007. Size and shape: the developmental regulation of static allometry in insects. BioEssays 29:536–48.

Sindhu, A., and V. Nair. 2004. Influence of juvenile hormone analogue on food consumption and digestive enzyme activities in Spodoptera mauritia Boisd. Indian Journal of Experimental Biology 42:491–494.

Sousa, T., T. Domingos, and S. A. L. M. Kooijman. 2008. From empirical patterns to theory: a formal metabolic theory of life. Philosophical transactions of the Royal Society of London. Series B, Biological sciences 363:2453–64.

Sousa, T., T. Domingos, J.-C. Poggiale, and S. A. L. M. Kooijman. 2010. Dynamic energy budget theory restores coherence in biology. Philosophical transactions of the Royal Society of London. Series B, Biological sciences 365:3413–28.

Speakman, J. R. 2010. The Heat Dissipation Limit Theory and Evolution of Life Histories in Endotherms — Time to Dispose of the Disposable Soma Theory ? Comparative and General Pharmacology 50:793–807.

Steenbergen, R., T. S. Nanowski, R. Nelson, S. G. Young, and J. E. Vance. 2006. Phospholipid homeostasis in phosphatidylserine synthase-2-deficient mice. Biochimica et biophysica acta 1761:313–23.

Sterner, R. W., and J. J. Elser. 2002. Ecological stoichiometry: the biology of elements from molecules to the biosphere. Princeton University Press, New Jersey.

Von Stockar, U., L. Gustafsson, C. Larsson, I. Marison, P. Tissot, and E. Gnaiger. 1993. Thermodynamic considerations in constructing energy balances for cullular growth. Biochemica et Biophysica Acta 1183:221–240.

Stockhoff, B. A. 1993. Ontogenetic change in dietary selection for protein and lipid by gypsy moth larvae. Journal of Insect Physiology 39:677–686.

Stouthamer, a H., and S. A. L. M. Kooijman. 1993. Why it pays for bacteria to delete disused DNA and to maintain megaplasmids. Antonie van Leeuwenhoek 63:39–43.

Tammaru, T., and T. Esperk. 2007. Growth allometry of immature insects: larvae do not grow exponentially. Functional Ecology 21:1099–1105.

Taylor, C. R., Knut Scmidt-Nielsen, and L. Raab. 1970. Scaling of energetic cost of running to body size in mammals. American Journal of Physiology 219:1104–1107.

Telang, A., L. Frame, and M. R. Brown. 2007. Larval feeding duration affects ecdysteroid levels and nutritional reserves regulating pupal commitment in the yellow fever mosquito Aedes aegypti (Diptera: Culicidae). Journal of Experimental Biology 210:854–864.

Troost, T. a., B. W. Kooi, and U. Dieckmann. 2007. Joint evolution of predator body size and prey-size preference. Evolutionary Ecology.

Truman, J. W., and L. M. Riddiford. 1999. The origins of insect metamorphosis. Nature:447–452.

Weibel, E. R., L. D. Bacigalupe, B. Schmitt, and H. Hoppeler. 2004. Allometric scaling of maximal metabolic rate in mammals: muscle aerobic capacity as determinant factor. Respiratory physiology & neurobiology 140:115–32.

West, G. B., and J. H. Brown. 2005. The origin of allometric scaling laws in biology from genomes to ecosystems : towards a quantitative unifying theory of biological structure and organization. Journal of Experimental Biology:1575–1592.

West, G. B., J. H. Brown, and B. J. Enquist. 1997. A general model for the origin of allometric scaling laws in biology. Science 276:122.

West, G. B., J. H. Brown, and B. J. Enquist. 1999. The Fourth Dimension of Life: Fractal Geometry and Allometric Scaling of Organisms. Science 284:1677–1679.

West, G. B., J. H. Brown, and B. J. Enquist. 2001. A general model for ontogenetic growth. Nature 413:628–31.

West, G. B., V. M. Savage, J. F. Gillooly, B. J. Enquist, W. H. Woodruff, and J. H. Brown. 2003. Why does metabolic rate scale with body size? Nature 421.

West, G. B., W. H. Woodruff, and J. H. Brown. 2002. Allometric scaling of metabolic rate from molecules and mitochondria to cells and mammals. Proceedings of the National Academy of Sciences of the United States of America 99 Suppl 1:2473–8.

White, C. R., P. Cassey, and T. Blackburn. 2007. Allometric exponents do not support a universal metabolic allometry. Ecology 88:315–23.

White, C. R., M. R. Kearney, P. G. D. Matthews, S. A. L. M. Kooijman, and D. J. Marshall. 2011. A manipulative test of competing theories for metabolic scaling. The American naturalist 178:746–54.

Wieser, W. 1984. A distinction must be made between the ontogeny and the phylogeny of metabolism in order to understand the mass exponent of energy metabolism. Respiration Physiology 55:1–9.

Wigglesworth, V. 1934. The physiology of ecdysis in Rhodnius prolixus (Hemiptera). II. Factors controlling moulting and "metamorphosis." Quarterly Journal of Microscopical Science 2:191–222.

Wightman, J. 1978. The ecology of Callosobruchus analis (Coleoptera: Bruchidae): Morphometrics and energetics of the immature stages. The Journal of Animal Ecology 47:117–129.

Wilson, E. O. 1999. Consilience: The Unity of Knowledge. Vintage Books.

Yagi, M., T. Kanda, T. Takeda, A. Ishimatsu, and S. Oikawa. 2010. Ontogenetic phase shifts in metabolism: links to development and anti-predator adaptation. Proceedings of the Royal Society B: Biological Sciences 277:2793–2801.

Yeoh, A. J., K. Davis, A. V Vela-Mendoza, B. a Hartlaub, and C. M. Gillen. 2012. Effect of body size on expression of Manduca sexta midgut genes. Journal of experimental zoology. Part A, Ecological genetics and physiology 317:141–51.

Yodzis, P., and S. Innes. 1992. Body size and consumer-resource dynamics. American Naturalist 139:1151–1175.

Zeuthen, E. 1947. Body size and metabolic rate in the animal kingdom, with special regard to the marine micro-fauna. H. Hagerup, Copenhagen.

Zimmer, E. I., V. Ducrot, T. Jager, J. Koene, L. Lagadic, and S. A. L. M. Kooijman. 2014. Metabolic acceleration in the pond snail Lymnaea stagnalis? Journal of Sea Research.

Zimmer, E. I., T. Jager, V. Ducrot, L. Lagadic, and S. A. L. M. Kooijman. 2012. Juvenile food limitation in standardized tests: a warning to ecotoxicologists. Ecotoxicology (London, England) 21:2195–204.

Zuo, W., M. E. Moses, C. Hou, W. H. Woodruff, G. B. West, and J. H. Brown. 2009. Response to Comments on “Energy Uptake and Allocation During Ontogeny.” Science 325:1206–1206.

Appendices

Appendix A: Supplementary information for 'Reconciling theories for metabolic scaling'

A priori prediction for mammalian scaling

After arriving at the equation relating body mass to the metabolic rate of heterotrophs at ultimate size (equation 2.9) we needed to estimate each of the parameters d_V, d_E, $[\dot{p}_M]$ and $\dot{v}$. The following details how these parameters estimates were obtained.

As mammals are mostly comprised of water, the density of structure, d_V, was simply assumed to be 1 g/cm^3. The energy-to-mass coefficient of reserve, d_E, was calculated by assuming that the contents of reserve can be written as a generalised macro-molecule. Following Kooijman (2010) and known compositions and enthalpies of key macro-molecules such as proteins, carbohydrates and lipids (Barrow 1974, Babel 1985, Battley 1991, von Stockar et al. 1993), the generalised molecular composition of reserve is taken to be $C_1H_{1.80}O_{0.50}N_{0.15}$ relative to carbon (ignoring other elements that make only minor contributions to dry-mass), with a molar weight of 23.9 g/mol and a chemical potential of 550 kJ/mol. With the additional assumption that 70% of mammalian biomass is comprised of water we calculate d_E to be 1.45 x 10^{-4}.

The remaining parameters $[p_M]$ and $\dot{v}$ were taken as the average of those estimated for the 12 mammalian entries that appear in the freely available 'add my pet' library (available online at http://www.bio.vu.nl/thb/deb/deblab/debtool/). These were estimated to be 90.3 J/cm^3/d and 0.043 cm/d respectively and can be found in the supplementary spreadsheet that

accompanies this document. To place the variance of these estimates in context, predictions based on observed extreme values for each of these parameters were also given in the main text. As the collection is rapidly growing, the supplementary spreadsheet is a copy of the library as it was at the time of writing, complete with hyperlinks to specific entries with references to the data sources used in the estimation process. The original code used to implement both the DEB theory (Kooijman 2010) and the 'covariation method' for parameter estimation (Lika et al. 2011a, 2011b) is called DEBtool and freely available at http://www.bio.vu.nl/thb/deb/deblab/debtool/. The code can be called in Matlab or the open-source equivalent, Octave. Data used to estimate the core DEB parameters that specify each mammals' unique bio-energetic life histories typically include simple measurements such as:

- age and body size at key life history events such as birth, puberty, death
- weight and/or length measures through time
- reproductive rate
- the effect of different feeding regimes on the above measurements

Readers interested in applying DEBtool to their own study organisms are encouraged make use of these tools and to add their 'pet' to the online collection.

Derivations of inter-specific scaling relationships of life-history parameters

Scaling of growth rate with mass

The reserve mobilisation flux was taken to be proportional to the periphery or interface of reserve and was shown in the main text to be:

$$\dot{p}_C = \frac{\dot{v}E}{V^{\frac{1}{3}}} \qquad \text{(A1)}$$

It was also shown that when $V = V_m$ for non-growing adults the mobilised energy flux is entirely consumed by the costs of maintenance:

$$\dot{p}_C - \dot{p}_M = 0 \text{ at ultimate size} \quad \text{(A2)}$$

or

$$\frac{\dot{v}E}{V_m^{\frac{1}{3}}} - [\dot{p}_M]V_m = 0 \quad \text{(A3)}$$

When the organism is still growing, however, maintenance is not consuming all of this flux but neither is the remaining surplus utilised entirely by growth, $\dot{p}_G$. This strange situation arises due to the requirement of structural isomorphy; to ensure the ratio of reserve to structure remains constant while structure is growing, some of this surplus flux, $\dot{p}_{rej}$, is fed back to the reserve compartment. This can be written as:

$$\frac{\dot{v}E}{V^{\frac{1}{3}}} - [\dot{p}_M]V = \dot{p}_G + \dot{p}_{rej} \quad \text{(A4)}$$

To offset the dilution of reserve by structural growth it turns out that the fraction of $\frac{\dot{v}E}{V^{\frac{1}{3}}} - [\dot{p}_M]V$ required to be rejected and allocated back to reserve is $\frac{\mathrm{E/V}}{\mathrm{E/V} + [\mathrm{E_G}]}$ and the fraction used for growth is $\frac{[\mathrm{E_G}]}{\mathrm{E/V} + [\mathrm{E_G}]}$. We can then write the flux allocated to growth as:

$$\dot{p}_G = \frac{[E_G]\left(\dot{v}E/V^{\frac{1}{3}} - [\dot{p}_M]V\right)}{E/V + [E_G]} \quad \text{(A5)}$$

Dividing this energy flux by the cost of each unit structure we arrive at the equation for structural growth:

$$\frac{dV}{dt} = \frac{\dot{v}E/V^{\frac{1}{3}} - [\dot{p}_M]V}{E/V + [E_G]} \quad \text{(A6)}$$

Remembering that the ratio E/V is constant when food is constant and $\frac{dV}{dt} = 0$ for V_{m} we can integrate equation A6 to obtain:

$$t(V) = \frac{1}{\dot{r}_B} ln \frac{V_m^{\frac{1}{3}} - V_b^{\frac{1}{3}}}{V_m^{\frac{1}{3}} - V^{\frac{1}{3}}} \tag{A7}$$

where V_m is ultimate structure, V_b is the structure at birth, and the von Bertalanffy growth rate is:

$$\dot{r}_B = \frac{[\dot{p}_M]}{3[E_G] + 3E_m/V_m} \tag{A8}$$

While the ratio E/V is constant within species (under constant food), this ratio at maximum size varies between species as we saw in the main text, such that:

$$E_m = \frac{V_m{}^{\frac{4}{3}}[\dot{p}_M]}{\dot{v}} \tag{A9}$$

Mass can be written as:

$$M = d_V V_m + d_E E_m \tag{A10}$$

and using equations A8 ,A9, and A10 we have:

$$M = \left(\frac{\dot{v}}{3\dot{r}_B} - \frac{[E_G]\dot{v}}{[\dot{p}_M]}\right)^3 + \frac{d_E[\dot{p}_M]}{\dot{v}}\left(\frac{\dot{v}}{3\dot{r}_B} - \frac{[E_G]\dot{v}}{[\dot{p}_M]}\right)^4 \tag{A11}$$

where $\dot{r}_B$ is proportional to $M^{-\frac{1}{4}}$ at the infinite limit of mass.

Food uptake rate

The assimilation flux is the energy entering the reserve compartment and for adult organisms can be written as:

$$\dot{p}_A = [\dot{p}_A]V_m \tag{A12}$$

where $[\dot{p}_A]$ is the volume specific assimilation rate.

Using equations A9, A10 and A12 we have the relationship between adult mass and uptake rate:

$$M = \frac{d_V \dot{p}_A}{[\dot{p}_A]} + \frac{d_E \dot{p}_A^{\frac{4}{3}} [\dot{p}_M]}{[\dot{p}_A]^{\frac{4}{3}} \dot{v}} \tag{A13}$$

where $\dot{p}_A$ is proportional to $M^{\frac{3}{4}}$ at the infinite limit of mass.

Starvation time

If starvation time, t_S, is the time it takes for adult reserve to be depleted by the maintenance costs of adult structure we have the function:

$$t_S = \frac{E_m}{V_m [\dot{p}_M]} \tag{A14}$$

Using equations A9, A10 and A12 we have the relationship between adult mass and starvation time:

$$M = d_V \left(\frac{t_S}{\dot{v}}\right)^3 + \frac{d_E t_S^4 [p_M]}{\dot{v}^5} \tag{A15}$$

where t_S is proportional to $M^{\frac{1}{4}}$ at the infinite limit of mass.

Mass at birth

As the ratio of structure at birth to adult structure $\frac{V_b}{V_m}$ is constant we can write mass at birth as:

$$M_b = \frac{V_b}{V_m}(d_V V_m + d_E E_m) \tag{A16}$$

or in terms of adult mass:

$$M_b = \frac{V_b}{V_m} M \tag{A17}$$

For more a more general scheme where size at birth is not fixed (or variable size at other life-history events, such as reproductive size) see Kooijman (2010).

Development time

From equation A7 we have that the time to reach some abitrary structural size, V_p, is:

$$t_P = \frac{1}{\dot{r}_B} ln \frac{V_m^{\frac{1}{3}} - V_b^{\frac{1}{3}}}{V_m^{\frac{1}{3}} - V^{\frac{1}{3}}} \tag{A18}$$

Using equations A9, A10 and A18 we have the relationship between adult mass and development time:

$$M = \left(\frac{\dot{v}\, t_p}{3 \ln \frac{V_m^{\frac{1}{3}} - V_b^{\frac{1}{3}}}{V_m^{\frac{1}{3}} - V^{\frac{1}{3}}}} - \frac{[E_G]\dot{v}}{[\dot{p}_M]} \right)^3 + \frac{d_E[\dot{p}_M]}{\dot{v}} \left(\frac{\dot{v}\, t_p}{3 \ln \frac{V_m^{\frac{1}{3}} - V_b^{\frac{1}{3}}}{V_m^{\frac{1}{3}} - V^{\frac{1}{3}}}} - \frac{[E_G]\dot{v}}{[\dot{p}_M]} \right)^4 \tag{A19}$$

where t_p is proportional to $M^{\frac{1}{4}}$ at the infinite limit of mass.

Egg mass

For the purposes of this document, the rather technical derivation of egg mass from DEB parameters was considered unnecesary, but interested readers are directed to Kooijman (1986; 2000). The important result is that, using no extra parameters, the mass of an egg can be specified. The resulting formula can be approximated by a two-term Taylor expansion:

$$M_e \simeq d_E \left(\frac{V_b}{V_m}\right)^{\frac{1}{3}} E_m \left(1 - \frac{1}{4}\left(\frac{V_b}{V_m}\right)^{\frac{1}{3}}\right)^{-3} \quad \text{(A20)}$$

Using equation A9, A10 and A20 we obtain:

$$M = d_V \left(\frac{\dot{v} M_e}{[\dot{p}_M] d_E k_b \left(1 - \frac{1}{4} {k_b}^{\frac{1}{3}}\right)^{-3}}\right)^{\frac{3}{4}} + \frac{M_e}{k_b \left(1 - \frac{1}{4} {k_b}^{\frac{1}{3}}\right)^{-3}} \quad \text{(A21)}$$

where $\mathrm{M_e}$ is proportional to M^1 at the infinite limit of mass.

Reproductive rate

First, we assume that some proportion $(1 - \kappa)$ of the reserve mobilisation flux at ultimate size (equation A1) is allocated to reproduction while the remaining flux (κ) is allocated to growth and maintenance. Prior to reproductive maturity, energy from the $1 - \kappa$ reproduction flux is assumed to be dissipated as a 'maturity' cost, or installation and regulation of regulatory systems required for reproduction (but also includes the immune system). When reproductive maturity is reached, this flux is used for the production of neonates. Dividing the reproduction flux $\frac{(1-\kappa)\dot{v} E_m}{{V_m}^{\frac{1}{3}}}$ by the energetic cost of a neonate $\frac{\mathrm{M_e}}{\mathrm{d_E}}$ we obtain the reproductive rate:

$$\dot{R} = \frac{(1-\kappa) d_E \dot{v} E_m}{V_m^{\frac{1}{3}} M_e} \tag{A22}$$

This is a special case where maturity maintenance costs are assumed to be zero, but see Kooijman 2010 for more general case. Using equation A9, A10 and A20 we obtain:

$$M = d_V \left(\frac{(1-\kappa)\dot{v}\, d_E \left(\frac{V_b}{V_m}\right)\left(1 - \frac{1}{4}\left(\frac{V_b}{V_m}\right)^{\frac{1}{3}}\right)^3}{\dot{R}} \right)^3 + d_E \left(\frac{(1-\kappa)\dot{v}\, d_E \left(\frac{V_b}{V_m}\right)\left(1 - \frac{1}{4}\left(\frac{V_b}{V_m}\right)^{\frac{1}{3}}\right)^3}{\dot{R}\dot{v}^{\frac{1}{4}}} \right)^4 [p_M] \tag{A23}$$

where $\dot{R}$ is proportional to $M^{-\frac{1}{4}}$ at the infinite limit of mass.

Appendix B: Derivation of polynomial coefficients

Following Kooijman (2010), we express the total energy mobilization rate for metabolism $\dot{p}_C$ (energy per time) as a function of reserve E and structure V:

$$\dot{p}_C = E\frac{[E_G]\dot{v}/V^{\frac{1}{3}} + [\dot{p}_M]}{E/V + [E_G]} \tag{B5}$$

where $[E_G]$ is the specific cost of building structural biomass (energy per volume), $[\dot{p}_M]$ is the specific maintenance cost of structure (energy per volume per time), and $\dot{v}$ is a proportionality coefficient controlling the residence time of molecules in reserve (length per time). For convenience, reserve is typically expressed with dimensions of energy, while structure is expressed as a volume. This allows structure to be easily related to suitable physical measures of size that do not vary with nutritional condition. The total maintenance energy flux $\dot{p}_M$ (energy per time) is proportional to the amount of structure or $\dot{p}_M = V[\dot{p}_M]$ and is subtracted from $\dot{p}_C$ and divided by the cost per unit structure to find the rate of structural growth:

$$\frac{dV}{dt} = (\dot{p}_C - V[\dot{p}_M])/[E_G]. \tag{B6}$$

Change in reserve can be expressed as the inflow of assimilated nutrients (taken as proportional to structural surface or $\dot{p}_A = \{\dot{p}_{Am}\}V^{\frac{2}{3}}$ when feeding is *ad libitum*) minus the rate of total energy mobilization, $\dot{p}_C$. In the following we take respiration proportional to the amount of maintenance, i.e. we collapse maturation and growth overheads into maintenance costs, which are normally separately modeled in DEB theory. This does not strictly ignore growth and maturation costs but assumes they scale proportionally to maintenance. We make

this assumption for a purely pragmatic purpose: for ontogenetic scaling effects and interspecific effects to be separated, these costs must scale proportionally or else the expression for metabolic rate is a mixed power function of structure that cannot be factorized. We believe this position is justified for our broad scaled analysis.

Embryonic respiration

Embryonic development, in a DEB framework, is considered identical to post-embryonic development with the exception that an egg does not feed and initially consists almost entirely of reserve. Some further simplifying assumptions are necessary in order to derive a simple prediction for respiration. First, we reduce the terms comprising equation B2 by assuming that a developing embryo is not limited by reserve, i.e., $E \gg V$. This simplifying assumption is supported by the embryonic respiration data, which show no sharp decline in respiration prior to hatching (Fig. 3-2) (see Kooijman (2010) for other cases). Non-zero respiration values for all initial egg measurements we obtained indicate that structure is non-zero at oviposition. This may be due either to some development having already taken place inside the adult prior to egg lay (Sander 1990), or, more likely, the rapid speed at which many insect eggs develop, which poses practical problems for attaining respiration measurements of completely undeveloped eggs (Bownes 1975). We therefore express structure at oviposition V_e^o as proportional to structure at egg-hatch or $V_e^o = \kappa_e V_e^h$ where $0 \leq \kappa_e < 1$.

Assuming a starting structure of V_e^o and that during embryonic development E ≫ V, Equation B2 can be solved to find structure as a function of time t:

$$V(t) = \left(\frac{\dot{v}(t + 3{V_e^o}^{\frac{1}{3}}/\dot{v})}{3} \right)^3 \qquad \text{(B3)}$$

As structure at oviposition is proportional to structure at hatch or $V_e^o = \kappa_e V_e^h$, we can solve hatch time as:

$$t_h = 3(V_e^{h\frac{1}{3}} - (\kappa_e V_e^h)^{\frac{1}{3}})/\dot{v} \quad \text{(B4)}$$

Substituting $\mathrm{t} = \tau t_h$ and (B4) into (B3) and multiplying by the structural specific maintenance rate $[\dot{p}_M]$ results in a simple expression for embryonic respiration $\dot{B}_e$, which can be expressed in terms of the proportion of embryonic development completed τ:

$$\dot{B}_e(V_e^h, \tau) = [\dot{p}_M] V_e^h \left(\tau\left(1 - \kappa_e^{\frac{1}{3}}\right) + \kappa_e^{\frac{1}{3}}\right)^3 \quad \text{(B5)}$$

Immature respiration

For immatures, structural volume V scales with total biomass m under constant food conditions due to stoichiometric pressures that maintain a fixed ratio of structure to reserve (Kooijman 2010). Structure scaled by ultimate structure V/V_m is equivalent to mass scaled by ultimate mass m/M and is given by the scaled value μ. Immature respiration ($\dot{B}_l$) can now be expressed as:

$$\dot{B}_l(V_m, \mu) = [\dot{p}_M] V_m \mu \quad \text{(B6)}$$

where V_m is the amount of structure at maximum size.

Pupal respiration

For holometabolous insects, we modeled pupal respiration as following the same dynamics as embryonic development, with the exception that larval structure is converted to reserve while adult structures grow from imaginal disks (taken to be some negligible amount of structure).

The histolysis of larval structure is, for simplicity, assumed to occur at the negative rate of structural growth. Pupal development is thus modeled as the degeneration of larval structure V_1 summed with the growth of adult structure V_2:

$$V(t) = V_1 + V_2 = \left(\frac{\dot{v}(-t + 3V_m^{\frac{1}{3}}/\dot{v})}{3} \right)^3 + \left(\frac{\dot{v}t}{3} \right)^3 \quad \text{(B7)}$$

Expansion of (B7) gives:

$$V(t) = \frac{1}{3}\dot{v}^2 t^2 V_m^{\frac{1}{3}} - \dot{v}tV_m^{\frac{2}{3}} + V_m \quad \text{(B8)}$$

Adult size is strongly correlated with terminal larval size (Chown and Nicolson 2004) so structure at adult eclosion (V_p) is taken as proportional to terminal larval structure (V_m) or $V_p = \kappa_p V_m$ where $\kappa_p > 0$. As structure at adult emergence V_p is proportional to structure at the initiation of pupation V_m or $V_p = \kappa_p V_m$, we can solve the quadratic equation (B8) for emergence time as:

$$t_h = 3V_m^{\frac{1}{3}} \left(1 + \sqrt{\frac{4\kappa_p - 1}{3}} \right) / 2\dot{v} \quad \text{(B9)}$$

The square root term in (B9) is taken to be positive because a negative value causes eclosion to be triggered on the decreasing part of the pupal respiration curve.

Substituting $\mathrm{t} = \tau t_h$ and (B9) into (B8) and multiplying by the structural specific maintenance rate $[\dot{p}_M]$ gives a simple equation for pupal respiration ($\dot{B}_p$) as a function of the proportion of pupation completed τ:

$$\dot{B}_p(V_m, \tau) = [\dot{p}_M] V_m ((C_1\tau)^2/3 - C_1\tau + 1) \quad \text{(B10)}$$

where $C_1 = (3 + 3\sqrt{(4\kappa_p - 1)/3})/2$.

Equations B5, B6, and B10 can be partitioned into interspecific (the first two terms) and ontogenetic effects (the remaining terms). The interspecific dependent quantities $[\dot{p}_M]V_e^h$ and $[\dot{p}_M]V_m$ represent maintenance metabolism at hatching and at terminal size respectively, which are expected to scale with mass raised to an exponent between ¾ and one (Kooijman 2010, Maino et al. 2014), and can be substituted by the usual allometric expression for metabolic rate aM^b. The parameters $\kappa_p$and $\kappa_e$can estimated by least squares after normalizing data by the interspecific component and are found to be 0.13 and 0.88 respectively. Expanding the ontogenetic component of equations B5, B6, and B10 and using estimated values for $\kappa_p$and κ_e results in the polynomials found in table 2.

For comparison, the weight ratio (adult mass: initial pupal mass) has been found to be 0.86 for the mealworm beetle (Odell 1998), 0.80 for the bean weevil (Wightman 1978), 0.53 in a parasitic wasp (Harvey et al. 1994), and 0.5 for the tobacco hornworm (Odell 1998). These weight ratios are lower than the estimated structure ratio κ_p of 0.88 (adult structure: initial pupal structure) because they include the contribution of reserve to weight, while κ_p considers only structural mass. During pupation, significant reserve is depleted to fuel the growth of adult structures, including structures required for flight and reproduction. The significant reduction in reserve can explain why the weight ratio is typically less than the structure ratio.