Confirmation Report

Applications of metabolic theory: the scaling of physiological traits with body-size in insects

James Maino

Supervisors: Dr. Michael Kearney; Prof. Bas Kooijman

Abstract:
My PhD research addresses two issues in metabolic theory that will help remediate some of the incoherence of body-size scaling relationships (BSSR) and facilitate some novel predictive applications in biology. Firstly, two competing metabolic theories that propose different mechanisms for BSSR will be evaluated by testing divergent predictions against empirical data. Secondly, metabolic theory will be used to develop a mechanistic, bio-energetic model that describes the full lifecycle of holometabolous insect development. Finally, these theoretical frameworks and modelling tools will be used in one of two suggested applications, which include 1) testing the mechanistic basis of life history trade-offs; 2) predicting effects of human impacts on BSSR and the subsequent implications for ecosystems.

Department of Zoology
University of Melbourne

Department of Theoretical Biology
Vrije Universiteit
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1. *Introduction*

The following outlines my PhD research on applications of metabolic theory. This research will help develop a theoretical framework for the scaling of physiological traits with body size, particularly metabolic rate, which simultaneously explains the scaling of a number of other related life history traits, such as growth rate, development rate, reproduction rate, or mortality rate. This topic could be introduced via a number of avenues, as the fundamentality of metabolic rate sees its relevance at all scales of biology – from DNA replication rates to biomass turnover in ecosystems (Brown et al. 2004; Peters 1986; Schmidt-Nielsen 1984; Calder 1996). However, I would like to begin by placing this research within a broad context and to draw attention to the value of physicochemical constraints in the study of complex phenomena.

In complex biological systems, such as cells, organs, animals, or ecosystems, understanding universal constraints on the constituent processes is indispensible to developing a robust theoretical understanding of the system itself. Without constraints, there is nothing to limit the range of possible processes or number of outcomes to be considered. For example, if the laws of motion did not apply, something as simple as predicting the path of a baseball in flight would be a futile task. Conversely, the more constraints that apply in a given system, the fewer alternative outcomes we must consider, and the more simple our descriptions and accurate our predictions become. Conservation of energy and matter is one such universal constraint that lends its utility to countless fields of science. Biology is no exception and has made good use of this law to further our understandings at all levels of biological organisation. At micro scales, conservation of energy and matter help us to understand the complex enzymatic reactions occurring in mitochondria that transform energy and materials during cellular respiration (Chance & Williams 2006). At the macro scale, ecological stoichiometry explores how balances of energy and matter interact with organisms to influence ecosystem structure and provides important insights into the processes of population growth, resource competition, and nutrient cycling (Kooijman et al. 1999; Sterner & Elser 2002).

My PhD research focuses on another potentially important set of constraints in biological systems: These are the restrictions imposed by an organism's size. The consequences of body size are far reaching and important, and can be likened to the structural constraints that must be considered in civil engineering (Bonner 2006). It is a simple fact to any engineer that a bridge designed to cross a small creek is not simply a scaled down version of one designed to cross a large river. They will each be made from very different materials and, importantly, consist of very different designs. These

design differences do not simply reflect discrepancies in artistic tastes; rather, they are strict requirements of design set by certain physical constraints. Consider, for example, a support beam: the strength of the beam is proportional to its cross section or volume$^{2/3}$, but because its weight is proportional to volume a beam's relative strength scales with volume$^{-1/3}$. This simple notion allows us to foresee problems in the structural soundness of the Sydney Harbour Bridge were it constructed from wood using an upsized design of a small footbridge. Similarly, body-size in organisms imposes strict constraints on physiology, reducing the range, number or optimality of solutions to the problems with which they are faced. As such, body size has been employed as an effective heuristic in the study of a range of physiological variables (Peters 1986; Calder 1996; Schmidt-Nielsen 1984; West et al. 1997).

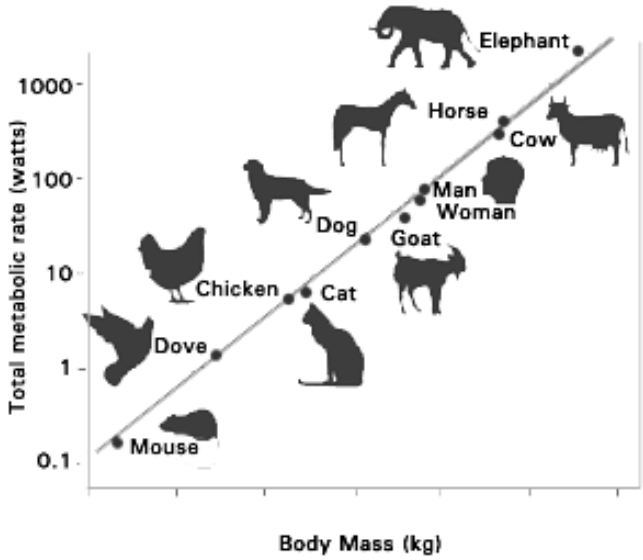

Figure 1. The scaling of mammalian basal metabolic rate (BMR) with body mass is estimated well using the relationship $BMR = aM^{\frac{3}{4}}$ where a is some constant. (Original figure from Schmidt-Nielson (1984))

The constraint of body-size is perhaps most familiar, as well as apparent, when it is coupled to metabolic rate in a famous plot spanning organisms from mice to elephants (see Figure 1) that has come be known as Kleiber's law (Kleiber 1932). Kleiber's 'law' holds that basal metabolic rate scales with mass$^{3/4}$ but it should be noted that the word 'law' is used somewhat loosely here as recent studies have shown (White & Seymour 2005; Glazier 2005; Kolokotrones et al. 2010). Regardless of whether or not the pattern observed by Kleiber deserves the status of 'law', there is little doubt that the scaling of metabolic rate with mass represents one of the strongest quantitative relationships in biology - a science that is subject to very few, if any, universal rules. This simple relationship has

been used intensively in comparative physiology as a null model for identifying deviations from the norm, and more recently, has been extended and applied with considerable success at macroecological scales (Brown et al. 2004; Damuth 2007; Allen & Gilooly 2009; Price et al. 2010). As metabolic rate is the rate at which organisms take up, transform, and expend energy and materials it provides a window into a fundamental constraint on ecological processes. In Brown et al.'s seminal 2004 paper 'Toward a Metabolic Theory of Ecology' it is shown that an ecosystem's 'average metabolic rate' (as predicted by the mean body size of component organisms and the mean ambient temperature) can explain considerable variation in many ecological phenomena. These phenomena span diverse scales from rates of molecular evolution and species diversity, to rates of nutrient cycling in ecosystems.

In addition to metabolic rate, body size is tied to a number of important life history traits. These include rates of reproduction, mortality, growth, development and ingestion, locomotion speed, gut capacity, range size, and the capacity to buffer variations in food or climate (Peters 1986; Calder 1996; Schmidt-Nielsen 1984; Bonner 2006). Recently there has been much interest in using these intercorrelated traits to build simple bio-energetic models that predict ontogenetic growth patterns and life history traits (Kooijman 2010; Gillooly et al. 2002; Makarieva 2004; West et al. 2001; Hou et al. 2008). For example, the scaling of metabolic rate through ontogeny has been used to predict the scaling of growth rate, which can be then used to infer changes in ribosomal RNA concentration (Allen & Gilooly 2009). As ribosomal RNA accounts for much of an organism's phosphorus content (Elser et al. 2003) further predictions about changes in stoichiometry can be made. Taken together, the power of body size to determine so many physiological features strongly suggests that body size imposes universal constraints on the evolution of life histories.

2. The Issue

In spite of the ubiquity and strength of these relationships there are still many holes in our understanding of just how body-size is restricting the evolution of physiological traits. Allometry – which is the study of how biological traits vary with mass - largely consists of a disjointed collection of scaling patterns with little coherence. 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 process 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 null theory, these patterns are likely to be considered as independent phenomena despite possible underlying connections (Harte 2004). In general terms, the goal of metabolic theory is to provide a single coherent set of answers to many questions. As mentioned previously, the simple empirical relationship between body size and metabolic rate can be explicitly connected to the scaling of particular stoichiometries, such as ontogenetic changes in phosphorus content. Metabolic theory builds on this line of thought by using mechanism to explicitly link a suite of biological processes together, forming a well grounded platform for the exploration of biological patterns. From this perspective we can use our knowledge of one process to inform our understanding of another in a way that would be unachievable through their independent consideration.

That body size and metabolic rate are important considerations in many areas of biology is not a contentious claim. However, the particular causal mechanism accounting for these body size scaling relationships has long been a source of great controversy (Lasiewski & Dawson 1967; Isaac & Carbone 2010; White et al. 2007). In attempting to make sense of these strong patterns researchers have proposed a range of explanations; some building on simple Euclidean scaling relationships of surface areas and volumes, such as limits on heat dissipation (Speakman 2010), with others hypothesising the importance of more complicated processes, such as the scaling of molecular oscillators embedded in biomembranes (Demetrius & J. Tuszynski 2010). Of all proposed metabolic theories, none have received so much attention as West, Brown and Enquist's nutrient supply network model (WBE from here on), from which it was claimed Kleiber's ¾ metabolic scaling law could be derived using simple physical principles (West et al. 1999). The WBE model was even applied interspecifically to make a range of predictions on ontogenetic processes (West et al. 2001). But like other explanations the WBE model is not an entirely satisfying explanation failing to account for a number of observations, such as curvilinearity in metabolic scaling or deviations from the hypothesised ¾ scaling exponent (Kolokotrones et al. 2010). The WBE model has also since been criticised for the generality of its proposed mechanism (discussed in more detail below). 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. Today, the incredible variation in explanations of metabolic scaling has led to widespread disagreement and vociferous debate, fuelling much scepticism as to whether a general explanation for allometric scaling relationships is even attainable.

3. *The contribution of my research*

My PhD research contributes to this field by addressing two gaps in metabolic theory (a theoretical gap and a taxonomical gap), which will help remediate at least some of the incoherence of scaling relationships in biology. The practical value of ideas and tools developed will then be shown in a novel predictive ecological application. As such, my PhD research can be broken into three distinct stages:

The first part of my research addresses a gap in metabolic theory concerning the evaluation and testing of theories. West Brown and Enquist's's nutrient supply network model previously mentioned and Kooijman's Dynamic Energy Budget (DEB) theory are two well-known and competing explanations for metabolic scaling. Together they are two of the most widely used theoretical frameworks in bioenergetics but typically occupy different biological domains. While the two theories propose principles that are similarly general in scope the differing interests and expertise of the proponents of the two respective theories has seen DEB most often applied in individual based studies (Kooijman 2010), as well as ecotoxicology (Kooijman et al. 2009), with WBE principally applied at broader biological scales and coarser resolutions (Brown et al. 2004). As a result there is still much confusion in the field regarding qualitative differences between competing theories. This lack of communication is an impediment to the development of metabolic theory which grows the more they continue independently.

Recently, WBE has been increasingly applied at the individual level (Chen Hou et al. 2008; Wenyun Zuo, Melanie E Moses, et al. 2011; C Hou et al. 2011), but DEB is yet to contribute to the debate surrounding the cause of universal scaling relationships in biology. The lack of literature on DEB with regard to metabolic scaling will be addressed in one publication that will outline the potential of DEB's reserve dynamics to be a more parsimonious explanation to metabolic scaling relationship, as well as its current limitations. A second publication will build on this theme by contrasting explanations and predictions for ontogenetic growth patterns. Despite the increasing domain of application of WBE, there has been little justification as to why one theory should be used over the other, with some experts going as far to say it is simply a "matter of taste" (Brown et al. 2004). This notion will be tested by contrasting the ability of these theories to explain other scaling relationships such as the scaling of growth rate, or assimilation and respiration through ontogeny. Contrasting theories and highlighting where predictions overlap and diverge makes model differences explicit, and provides a benchmark to assess consistency with reality, usefulness, and parsimony. In addition,

if competing theories are not entirely mutually exclusive, direct comparison can aid in the separation of components most amenable to synthesis from those that are fundamentally incompatible.

The second part of my research aims to use metabolic theory to develop a general bio-energetic model that describes the full lifecycle of insect development. Such a contribution is important as insects account for the overwhelming majority of terrestrial animal diversity but have so far been largely neglected in the development of metabolic theories. As a result, the study of insects has received only minor benefits from metabolic theory while contributing very little to its theoretical development. The general model I will develop will help open up a wealth of data so that the mechanistic basis of scaling relationships can be explored using a taxonomical group particularly suited for experimentation. The model will also be useful in building understandings of the ecophysiology of invertebrates in the same way bio-energetics has benefitted the study of vertebrate species (e.g. Kearney 2012).

Finally, these theoretical frameworks and modelling tools will be applied to one of two areas of biology. I present potential applications of DEB covariation rules to contribute to 1) mechanistic interpretations of the evolution of life histories and trait covariation, and 2) the investigation of human impacts on changing body-size distribution, population structures, and subsequent ecological implications.

In the following section I provide more detail on each of these projects. At the end of each section I will provide a table highlighting some future aims for each of the three parts of my research. For convenience these three tables will be also be compiled into one single table that can be found in Appendix A. For a timeline of the key stages of my candidature see Appendix B. A list of presentations made during my candidature can be found in Appendix C.

Please be aware that the first and second project description is more detailed as it is anticipated much of it will be included in publications. It is my hope to gain helpful comments or criticisms on the ideas elaborated in the following sections, but its inclusion will also be helpful to the confirmation committee members as it elaborates some core components of DEB theory, which are applied across all three parts of my research.

4. Applications of metabolic theory - Part 1: Contrasting metabolic theories

There is instant appeal in the West, Brown and Enquist Nutrient Supply Network (WBE hereafter) model as an explanation for metabolic scaling as it uses simple physical principles about the scaling of vascular supply networks to make a *priori* predictions about the complex biological phenomenon of metabolic scaling. The (roughly) quarter-power scaling relationship of metabolic rate with mass is observed over body sizes that span some 21 orders of magnitude and has fascinated biologists for over a century. While other proposed models have received considerable attention (Darveau et al. 2002; Kolokotrones et al. 2010), West and Brown (2005) correctly point out that "many 'competing' models make no *a priori* predictions about the scaling of metabolic rate."

There is, however, another idea that has so far been virtually overlooked in the metabolic scaling debate, which also makes specific *a priori* predictions. It may also have the potential to fill in many of the gaps left by the WBE model without totally excluding the important insights it provides. The idea is that metabolic scaling arises from constraints on the mobilisation of stored metabolites and has been a core conceptual component in Kooijman's Dynamic Energy Budget theory (DEB hereafter) for almost 30 years. Although most known for its application at individual scales, the DEB framework can use its simple physicochemical principles to makes *a priori* predications about broad scaling patterns of life history traits, which includes the scaling of metabolic rate. Importantly, DEB's explanation for metabolic scaling is potentially more fundamental than that offered by WBE as it encompasses processes that are more general to organisms than the branching vascular nutrient supply networks proposed by WBE as being the universal constraint of metabolism.

While not all organism posses vascular supply networks, all organisms mobilise internal energy and material stores before metabolites are transported to fuel metabolism. This occurs under certain physicochemical constraints. In DEB theory, these constraints on the mobilisation of stored metabolites are formalised into what is called reserve dynamics. One way to look at the core difference in DEB and WBE, is that DEB explains how the metabolism might be constrained by the supply process of storable metabolites (e.g. stored proteins, lipids, carbohydrates), while WBE explains how the constraint may be on the delivery process of non-storable metabolites, (e.g oxygenated blood at the capillaries in mammals). Interestingly, in both theories metabolic scaling is explained by the scaling of the relevant metabolite interface (exposed periphery of reserve pools in DEB, and the terminal unit in the supply network in WBE), but neither of these surfaces scale as $mass^{2/3}$, or what one might expect from simple Euclidean geometry.

Here I aim to explain how reserve dynamics - a foundational component of DEB theory - can contribute to the debate on metabolic scaling by showing how simple, biologically realistic constraints on the use of stored metabolites can restrict fluxes of energy and materials through

organisms and constrain metabolism. The DEB based equation that is derived for the scaling of metabolic rate is numerically identical to that of the WBE, though it rests on a set of profoundly different, though perhaps complimentary, assumptions.

4.1 Does the delivery of nutrients constrain metabolism?

There are a number of empirical results that may be suggestive of a constraint on nutrient transport and delivery. One of these is the observation that blood flow in vertebrates is connected with metabolic rate (Coulson & Hernandez 1977). Oxygen is highly reactive and represents a non-storable nutrient that is crucial for aerobic metabolism. Because of this, it must be continually replenished to ensure catabolic processes can be maintained. In vertebrates ranging from shrews to whales, arterial blood is comprised of approximately the same materials and, importantly, contains roughly the same oxygen content of around 200mls of oxygen per litre. As this blood circulates, the same amount of oxygen (roughly 40% of that contained in arterial blood) is extracted at each pass (Coulson & Hernandez 1977). Thus, the key variable that is changing across organisms of different sizes is not the composition of blood or oxygen extraction, but rather, the rate of blood flow that seems to determine the oxygen supply rate.

In addition to this, other studies have supported the hypothesis that oxygen limitation caused by reduced blood flow is indeed constraining the metabolic rate of cells in living tissue. Several studies have shown that cultured cells removed from supply constraints affecting *in vivo* cells have higher metabolic rates (Brown et al. 2007; Gauthier et al. 1990). Taken together, these findings suggest blood flow (or more generally nutrient delivery to tissues) is, for some reason, constrained in larger organisms, which limits the rate at which cells can utilise energy and materials for metabolism.

The WBE model proposed that if evolution rearranges supply networks such that energy losses in nutrient transport are minimised, the rate of substrate delivery per mass can be shown to decrease as organism size increases. Their prediction is based on an idealised tubular nutrient supply network that is self-similar, space filling, and possesses invariant terminal units. West and Brown (2005) predict that blood flow in large mammals is slower and cellular metabolic rates are downregulated to obey the constraints of reduced resource supply. However, as we will show, the same observed pattern of decreasing supply could be explained equally well by a more fundamental constraint acting at the sites of stored metabolites. Consequently, it may be premature to conclude that the nutrient transport optimality is the most important consideration. Kooijman (S.A.L.M. Kooijman 1993) argues that the mobilisation of stored nutrients set the ultimate limit on the rate at which

supply can occur. This explanation is potentially more fundamental as a direct result of the generality of the underlying mechanism and simplicity of constraints.

4.2 Constraints on the use of stored nutrients

All organisms take up food and store nutrients. If this were not the case, as soon as food became unavailable for assimilation, organisms would be unable to fuel metabolism and would perish. As soon as nutrients are assimilated from the gut (or transported into the cytoplasm in the case of unicellulars) a problem arises of where they should be stored. Strict limits are placed on the concentration of any substrate in solution because osmotic pressures must be maintained at relatively constant levels. An instantaneous ten-fold increase in blood glucose levels would raise the osmotic pressure by approximately 15 percent, which would pose a serious problem. An alligator can eat as much as 15g of protein per kilo of body weight at one time. For a 70kg alligator this would equate to slightly more than 1 kg of protein or roughly 8500 mmoles of amino acids (Coulson & Hernandez 1977). If all the amino acids were absorbed in the 48 hours it would take for digestion , and they were present in the body fluids at the same, the osmotic pressure would increase by approximately 59% (Coulson & Hernandez 1977). Despite the absorption of this massive amount of substrate over a relatively short period measured levels of amino acids in plasma, remain approximately unaltered (Coulson & Hernandez 1970). Moreover, the alligator would only need 18g or approximately 2% of the total amino acids absorbed to meet its daily metabolic requirements (Hernandez & Coulson 1952). The majority of these amino acids are used at a later date and so must be stored.

Organisms must simultaneously cope with variable feeding conditions as well 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, this polymer is a protein, though 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 apply.

DEB formalises this notion by partitioning organisms into two biomass 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, development and maintenance of

structure, as well as reproduction (not considered here). For simplicity it is assumed that all assimilated materials first enter the reserve compartment, and that the maintenance of these storage pools contributes very little (if any) to overall maintenance costs. It is also assumed that reserve varies with food density such that well (poorly) fed animals have more (less) reserve per mass, but that animals under constant feeding conditions have a constant reserve per mass. The exposed periphery of these packets of reserve sets the rate at which they can be mobilised for metabolism. Under DEB, this explains why the ontogenetic growth of an organism stops at ultimate size; maintenance scales linearly with body mass, but under constant feeding conditions the exposed surface area of reserve scales with mass$^{2/3}$ until eventually there is only enough mobilised reserve to cover maintenance costs. Figure 2 represents this schematically.

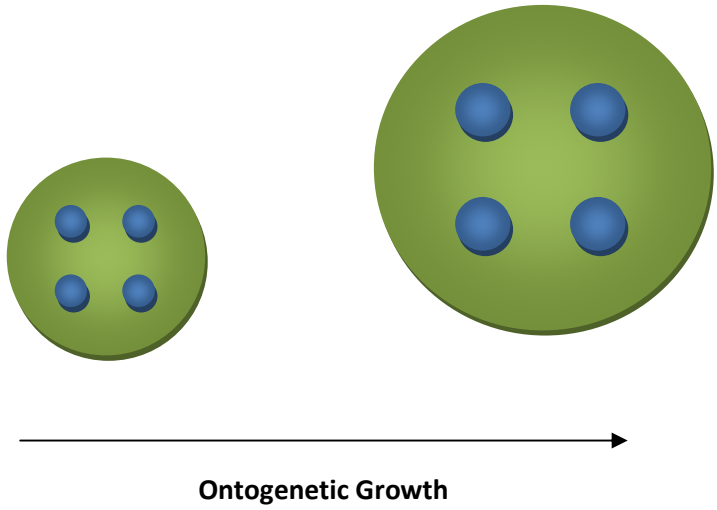

Figure 2. Reserve dynamics imply structural isomorphy, that is, the size of the green circle representing structure is proportional to the size of one of the blue circles representing a reserve pool. The surface area interface of reserve scales with Reserve/Structure1/3. In regular ontogeny, growth ceases at ultimate size because all the energy mobilised from reserve is being allocated to maintenance.

Because of this limitation a two-fold increase in adult structure would require a more than two fold increase in reserve. This dilution of structure by reserve decreases mass specific maintenance, and thus basal metabolic rate decreases with size. We derive the specific relationship here:

If structure (V) incurs a maintenance cost of $[p_M]$ per unit of structure the cost of maintenance per time p_M is:

$$p_M = V[p_M]$$

From figure 2 we can see the interface of stored reserve scales with $E/V^{\frac{1}{3}}$. The conductance, v, sets the shape of the exposed surface area of reserve and the rate at which each unit of reserve interface can be mobilised. Thus, the total mobilisation flux (p_C) from reserve (at ultimate size) is:

$$p_C = \frac{vE}{V^{\frac{1}{3}}}$$

and the maintenance of structure equals the mobilisation rate of reserve:

$$p_M = p_C$$

or

$$V[p_M] = \frac{vE}{V^{\frac{1}{3}}}$$

Rearranging this equation shows that ultimate reserve scales with ultimate structure as:

$$E = \frac{V^{\frac{4}{3}}[p_M]}{v}$$

Organism mass is the sum of the weight the two biomass compartments, structure and reserve, which have mass densities, d_V and d_E respectively:

$$M = d_V V + d_E E$$

Substituting E in this equation we obtain:

$$M = d_V V + \frac{d_E V^{\frac{4}{3}}[p_M]}{v}$$

If basal metabolic rate, B, of post-absorptive organisms at ultimate size is the energy flux required to pay maintenance costs we have:

$$B = V\,[p_M]$$

Substituting this into the previous equation we arrive at an equation relating metabolic rate to mass:

$$M = \frac{d_V B}{[p_M]} + \frac{d_E B^{\frac{4}{3}}}{v\,[p_M]^{\frac{4}{3}}}$$

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

$$M = C_0 B + C_1 B^{4/3}$$

This is the precise equation that that can be derived from the WBE model (see Savage et al. 2008). As table 1 below shows, these two coefficients are comprised of very different parameters under the two theories.

	DEB	**WBE**
$\boldsymbol{C_0}$	$\frac{d_V}{[p_M]}$	$C_2 \frac{V_{cap}\left(\bar{N} - \frac{1}{n^{1/3} - 1}\right)}{B_{cap}}$
$\boldsymbol{C_1}$	$\frac{d_E E_m^{ref}}{\mu_E (V_m^{ref})^{1/3} [p_M]^{4/3}}$	$C_2 \frac{V_{cap} n^{(1-\bar{N})/3}}{B_{cap}^{4/3}(n^{1/3} - 1)}$

Table 1. Both DEB and WBE can be used to derive the equation $M = C_0 B + C_1 B^{4/3}$ where $C_0$and C_1 are constants defined in the above

Despite the different interpretations of the two constants, the equation can easily be shown to converge on Kleiber's law (Kleiber 1932) at the limit of mass:

$$\lim_{M \to \infty} \; B \propto M^{\frac{3}{4}}$$

It is important to emphasize that this relationship only emerges when mass is very large and, contrary to many common perceptions, BMR does not depend on mass as a power function under either of these frameworks. In other words, this function would predict exponents that diverge from ¾ for finite masses when C_0 is positive.

As the constants C_0 and C_1 relate to different physical parameters under each respective framework this equation has profoundly different interpretations. Interestingly, something that has been pointed out in metabolic theory (Isaac & Carbone 2010), but not investigated in any detail, is that different explanations may be simultaneously valid. In physics, for example, water can be modelled as a fluid using Navier-Strokes equations, or as collections of particles using statistical mechanics (del Rio 2008). Similarly, taking these two frameworks together shows how the theoretical scaling of the interface of stored metabolites can match the theoretical scaling of the nutrient delivery interface (terminal network units) in an optimal supply network. DEB's limits on reserve mobilisation do not preclude the notion that evolution may act to optimise nutrient supply networks. DEB predicts that

organisms require less energy per mass with size while WBE shows how this can coincide with reduced energy losses in transport for species with idealised vascular networks. 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).

The key point is that DEB proposes the mobilisation of stored substrates as a more fundamental constraint on metabolic organisation. It is a more fundamental constraint as the mechanisms can be seen to apply to all organisms, not just those possessing closed circulatory systems. In addition, no criteria for optimality are invoked, only simple reserve dynamics are needed to make *a priori* predictions for metabolic scaling. Finally, the constraint on the mobilisation of stored metabolites is effective much earlier in the nutrient supply chain of metabolism than WBE's proposed delivery constraint.

4.3 Related tasks and expected completion dates

Project	Completion date (Expected date)
Manuscript on reconciling metabolic theories to functional ecology	
- Scrutinise assumptions of WBE and DEB models by deriving metabolic scaling predictions from first principles	*Completed*
- Use literature to find appropriate data set on metabolic scaling to test models (found data from a meta-study on 637 mammals)	*Completed*
- Evaluate models for weaknesses in terms of consistency of assumptions with reality and goodness of fits to data	*Completed*
- Complete first draft of manuscript and receive feedback from both supervisors	*Completed*
- Submit manuscript to Animal Ecology	March-2012
Manuscript on contrasting ontogenetic growth models	
- Research the link between metabolic scaling and ontogenetic growth in WBE and DEB models	*Completed*
- Understand how similarities in prediction arise and find divergences in prediction to be tested	*Completed*
- Find appropriate data to allow the empirical testing of divergent predictions (found data on ontogenetic assimilation (23 mammals), respiration (307 fish) and growth rates (approx 260 animals from 10 classes))	*Completed*
- Conclude key deficiencies in the models ability to predict data	*Completed*
- Complete first draft of manuscript and receive feedback from both supervisors	April-2012
- Submit manuscript to Trends in Ecology and Evolution	June-2012
Manuscript on testing whether metabolic scaling relationships are causal to	

other scaling relationships (if time permits)	
- Find detailed data on a number of species so that the covariation of traits can be studied within individuals rather than between groups (using body size as a proxy)	April-2012
- Fit model to predict traits using body size and metabolic rate as independent variables and compare against null expectations of competing theories	June-2012
- Conclude key deficiencies in each model's ability to predict data	June-2012
- Complete first draft of manuscript and receive feedback from both supervisors	August-2012
- Submit manuscript to Proceedings of the Royal Society B	September-2012

5. *Applications of metabolic theory - Part 2: A bio-energetic model for the holometabolous life cycle*

The above showed how DEB theory can mechanistically explain the metabolic scaling relationship observed across a wide range of different sized species using the constraint of reserve dynamics. In its common application in ontogenetic growth modelling DEB theory invokes more constraints on the fluxes of energy and materials, such as the allocation of energy and material to reproduction, in order for the bioenergetics of the entire life cycle of organisms to be specified. DEB based growth models have been applied with great success to organisms ranging from nematodes (T Jager et al. 2005) to the Pacific Blue Fin tuna (Jusup et al. 2011). Surprisingly, one important taxonomic group accounting for the majority of terrestrial animal diversity has so far been overlooked in applications of DEB theory. These are the holometabola (Stork 2007) – also known as endopterygota or insects that go through distinctive larval, pupal, and adult stages. Holometabolous insects pose several problem for ontogenetic growth modelling, which include discontinuous growth due to moulting (H Frederik Nijhout et al. 2010), non-standard growth curves (growth in insects is almost exponential (Tammaru & Esperk 2007)), as well as the energetics of metamorphosis, such as an explanation of the U-shaped curve for metabolic rate during pupation (Merkey et al. 2011). Here we build on the standard model structure (Kooijman 2010) such that the DEB framework can be applied to holometabolous insects. The proposed augmented DEB model offers a new interpretation of these unique patterns, creates a platform from which insects may contribute to the development of metabolic theory, and provides a tool that can be incorporated into predictive biological models. The

following develops a conceptual model for the full life cycle of holometabolous insects and provides some examples of its application to growth patterns in some well studied insects.

5.1 Further constraints on metabolic organisation

In the previous section on metabolic scaling it was assumed that the total energy and material flux mobilised from stored reserves was directed to growth and somatic maintenance. Of course, in reality energy budgets are more complicated than this simple abstraction and the standard DEB model accommodates at least some of this complexity by considering processes of development, maturity and reproduction.

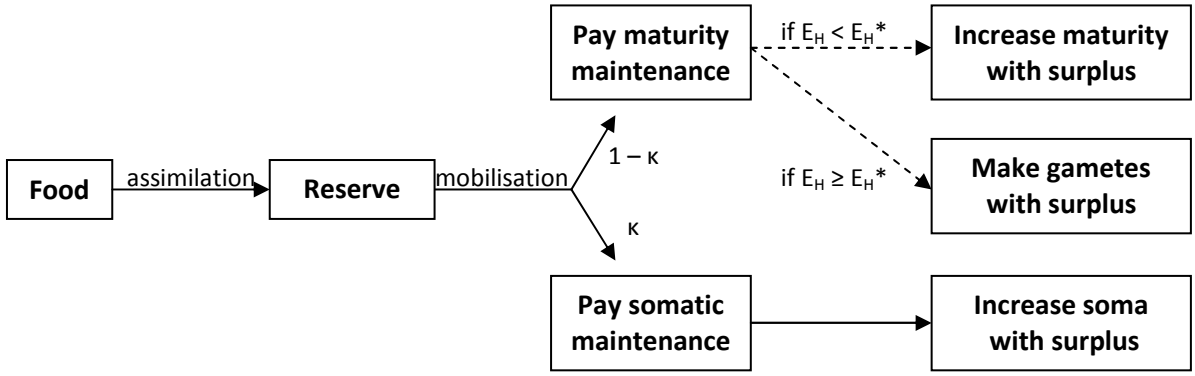

Figure 3. A schematic representation of the metabolic allocation scheme in the standard DEB model, where κ is the proportion of mobilised resources allocated to growth and maintenance, E_H is total amount of energy spent on maturation, and E_H^* is the level of maturity required to become reproductively mature.

Another cornerstone of DEB theory is the so called κ rule, which assumes a constant proportion of the mobilised flux from reserve (this proportion is denoted as κ) is directed to growth and maintenance while the proportion of the remaining flux (1 – κ) is directed to maturation and reproduction (Kooijman 2010). Kappa (κ) is assumed to be a fixed constant during ontogeny so somatic processes and reproductive processes do not compete directly for resources. A state variable, E_H, is assigned to keep track of the level of development at a given time called maturity. Maturity is an abstract quantity that does not contribute to biomass; rather, investments in maturity may be likened to an investment in learning information. The (1 – κ) reserve flux is used to increase

the level of E_H but just as learned information incurs a revision cost, acquired maturity incurs a maintenance cost – aptly termed maturity maintenance. As a result, maturity only increases if there is sufficient energy surplus remaining after costs of maturity maintenance have been subtracted from the (1 – κ) reserve flux. This explains why some organisms will mature at different sizes and ages, or fail to mature completely, when feeding conditions are poor (Sousa et al. 2010). Once E_H reaches some predetermined threshold level, E_H^*, the organism switches to reproductive maturity where the energy surplus remaining from the (1 – κ) reserve flux is now directed to producing gametes instead of increasing E_H. This is represented schematically in figure 3.

While the standard DEB model is sufficient for describing the bioenergetics of the life cycles of a range of organisms, including as reptiles (Kearney 2012), fish (Pecquerie et al. 2009 but see Jusup et al. 2011 and Augustine et al. 2011) and bivalves (Bacher & Gangnery 2006), there are key features in life cycles of the holometabola that the standard model captures poorly (or not at all). First, there is the issue of discontinuous growth and moulting. The standard model has only two maturity thresholds (birth and reproductive maturity) – much less than would be needed to specify a typical number of larval moult thresholds. Second, there is the issue of near exponential growth in the larval stages of many insects (Tammaru & Esperk 2007). This is a problem for the standard DEB model, which is identical to the von Bertalanfy growth equation (von Bertalanffy & Pirozynski 1952) under constant food and quite distinct from an exponential function. As it turns out, these two issues can be resolved simultaneously with a simple, biologically justifiable modification. Furthermore most of the additional parameters are not freely varying and can be obtained from direct measurement.

5.2 Larval development

While the elastic bodies of larvae can house significant growth, the head parts of larvae are made of a hard chitinous material, which can only increase in size through the process of moulting. After measuring these discontinuous size increases of head parts in the 1890s, Dyar found that the increase in head width between two consecutive instars is approximately a constant proportion (Dyar 1890). Interesting as it was, Dyar offered no explanation for the cause of this pattern. But in (1997) Hutchinson et al. contended that, within an instar, larval head parts would eventually limit rates of uptake and growth such that time spent moulting pays off in the end. Hutchinson et al. used this idea to show how an optimum moulting strategy to maximise growth rate could yield Dyar's law. These results are important for the following derivation.

In DEB theory the parameter relevant to assimilation rates is the maximum surface area specific assimilation rate, $\{p_{Am}\}$, which has dimensions energy per unit structure$^{2/3}$ per time (Kooijman 2010).

In the standard model $\{p_{Am}\}$ is fixed for the entire lifecycle and the maximum total assimilation flux, p_{Am} , is given by $\{p_{Am}\}$ x structure$^{2/3}$. Importantly, $\{p_{Am}\}$ sets the rate at which energy and materials enter the reserve compartment and so influences the reserve density. As explained in the previous section, larger organisms have higher reserve densities and this is achieved through a higher $\{p_{Am}\}$. As it is reasonable to assume that the hard head parts of larvae will eventually limit uptake rates in the absence of moulting, it is also reasonable to assume that upon moulting the mass specific assimilation rate increases (Nijhout 1981). For example, uptake rates shortly after a moult are likely to be higher than shortly before the moult despite very little change in weight. This can be represented by a change in $\{p_{Am}\}$ where the precise change is specified using Dyar's law. It can be shown (Kooijman 2010) that $\{p_{Am}\}$ is proportional to maximum structural length, $V_m^{\frac{1}{3}}$ which is proportional to some physical length measure, such as a head width. Assuming, in the absence of moulting, that each instar has a maximum structural length proportional to the head width, we can increase $\{p_{Am}\}$ by the same factor with which head width increases between instars. As per Dyar's law, the change in $\{p_{Am}\}$ with each instar is simply a constant factor, and more importantly, as this factor is the same as the change in head widths, it is readily measurable. Thus for instar i we have $\{p_{Am}^i\} = \{p_{Am}^1\}\gamma^{i-1}$ where γ is the proportional increase in head width each instar. This is shown visually in figure 4.

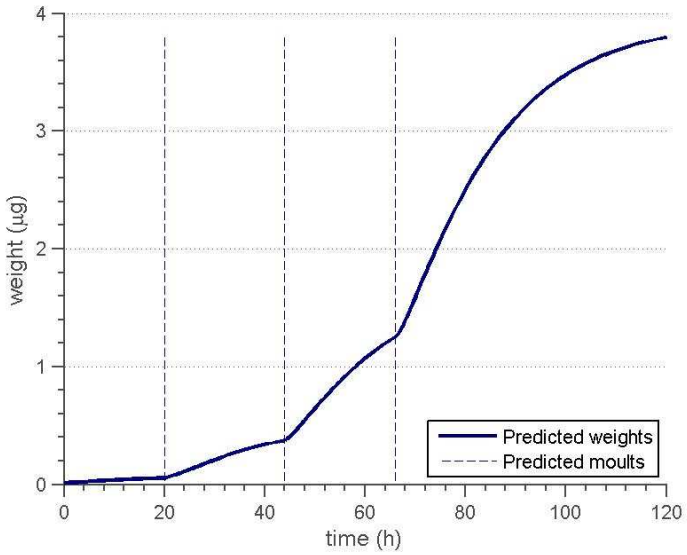

Figure 4. Here equations for growth are estimated for each of four instars. The mass specific assimilation rate increase with each moult. This approach for modelling larval development is similar to approaches taken in modelling the larval development of fish with DEB theory, which displays distinct growth patterns when compared with growth later in ontogeny (Augustine et al. 2011; Jusup et al. 2011). The key difference here is that the present approach is biologically justifiable and easily estimable from direct observation.

In figure 4 the moult times are assumed as given but one of the useful features of DEB theory is its scaling relationships or covariation of parameter values. These scaling relationships were demonstrated in part 1 with regard to reserve density and metabolic rate, but they also allow us to predict how the moult thresholds of maturity, E_{Hmoult}^{i}, will scale with $\{p_{Am}^{i}\}$ for each i^{th} instar. This is important as the covariation of parameter values means that we do not have to independently estimate the maturity threshold required for each moult and model simplicity is maintained. In can be shown that maturity thresholds, E_H , scale with ultimate structure, V_m. As V_m is directly proportional to $\{p_{Am}\}^3$, and $\{p_{Am}^{i}\} = \{p_{Am}^{1}\}\gamma^{i-1}$ for instar i , the moult thresholds are given by the equation $E_{Hmoult}^{i} = E_{Hmoult}^{1}\gamma^{3(i-1)}$. Using these modifications, an augmented DEB model was used to predict the larval growth of two well studied holometabola: *Aedes aegypti* and *Drosophila melanogaster*. Predicted growth curves are plotted against real data in figure 5.

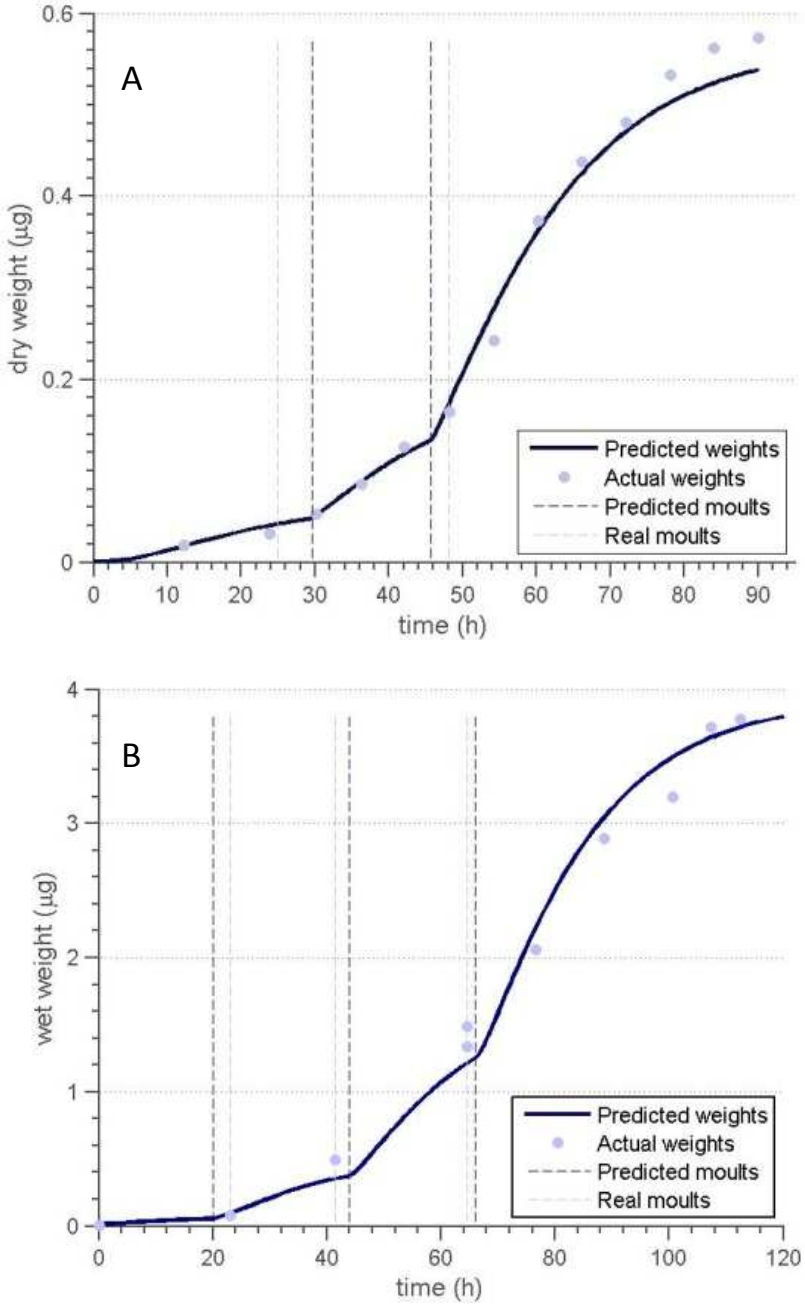

Figure 5. Fitted growth curves to dry weights of *D. melanogaster* (A) and wet weights of *A. aegypti* (B). *D. melanogaster* data from Bakker (1959) and *A. aegyti* data from Telang et al. (2007).

5.3 Metamorphosis

A third issue the standard DEB model faces when modelling the holometabolic life cycle is the metamorphosis that occurs during pupation. As is turns out, however, the DEB module for

embryonic development (Kooijman 2010) offers some important insights, which, with slight modification, can be applied to model pupation.

In DEB theory an undeveloped fertilised egg is considered to be almost entirely reserve with only a negligible amount of structure. This explains why freshly laid eggs do not respire. From this store of building materials, structure is gradually constructed which increases respiration until some point where it begins to decrease until egg hatch. This decrease in respiration is explained by the depletion of stored reserves required to fuel metabolism (See figure 6 where some DEB based respiration curves fitted to embryonic development data are provided). Pupation is similar to embryonic development in the sense that there is no feeding taking place so that energetic demands must be paid from stored resources. A key feature in pupation that does not occur in embryos is the conversion of larval structure into metabolites to fuel the growth of imaginal structures. This can be easily modelled using DEB principles by assuming larval structure are converted to reserve.

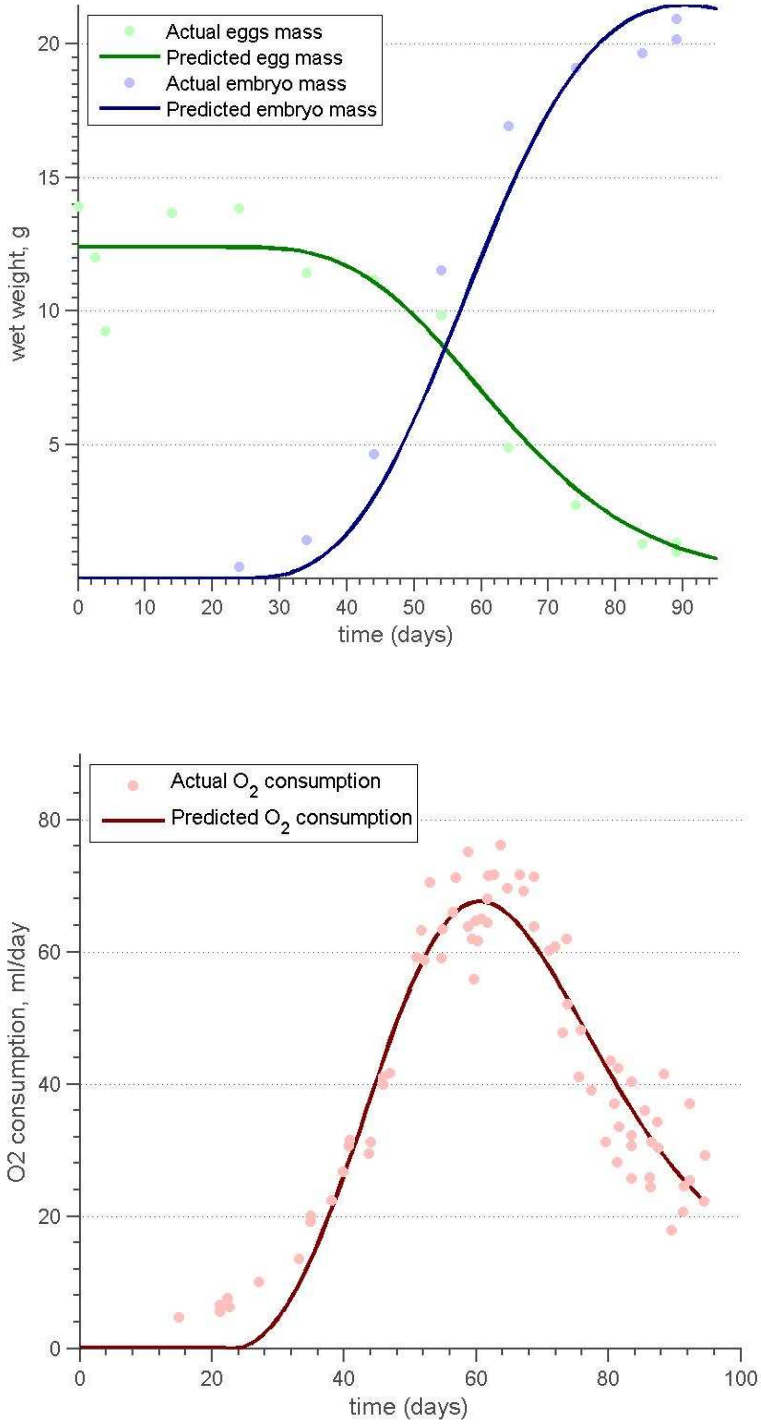

Figure 6. DEB based equation modelling embryonic respiration in the New Guinea soft-shelled turtle *Carettochelys insculpta* (adapted from Kooijman 2010). Respiration increases as embryonic structure accumulates, but decreases prior to hatching due to the depletion of stored reserves.

To model pupation we need to estimate the proportion of the larval structure that comprises the imaginal disks, δ. The growth of imaginal structure during pupation continues in the same way as

embryonic structure in an egg until eclosion. Another key feature in pupation is the conversion of structure to reserve – the rate of this influx of reserve (as well as larval reserves) determining the resources available for the growth of imaginal disks. The conversion of larval structure to pupal reserves is modelled simply as the reverse process of synthesising structure. One further parameter is required to set the maturity threshold for eclosion. Together, the maturity threshold, the proportion of larval structure comprised of imaginal structure, and the values for larval structure and reserve at time of pupation (given by the larval development module) specify the entire pupation module. The evolution of the variables for larval structure, imaginal structure, and reserves can be seen in figure 7.

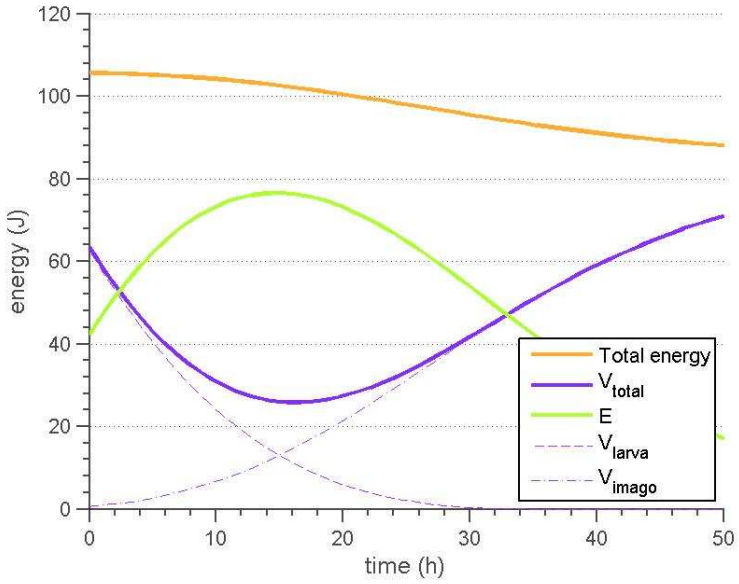

Figure 7. A generic example of the development of larval structure, imaginal structure, and stored reserves during pupation. Larval structure decreases as it is degraded into reserve such that total stored reserve initially increases. This reserve is used to fuel the development of imaginal structures.

This metamorphosis module is used to specify the bio-energetics of pupation, and can be used to predict related processes, such as O_2 consumption. In figure 8 an application to *D. melanogaster* pupation is demonstrated. This model offers a novel explanation for the U-shaped curve of pupal respiration by proposing that it is the changing quantities of structure and reserve throughout metamorphosis accounting for the pattern. Importantly, because there is overlap between the parameters used to estimate growth and metamorphosis in DEB we need fewer parameters to estimate these processes than what would be needed if they were considered independently.

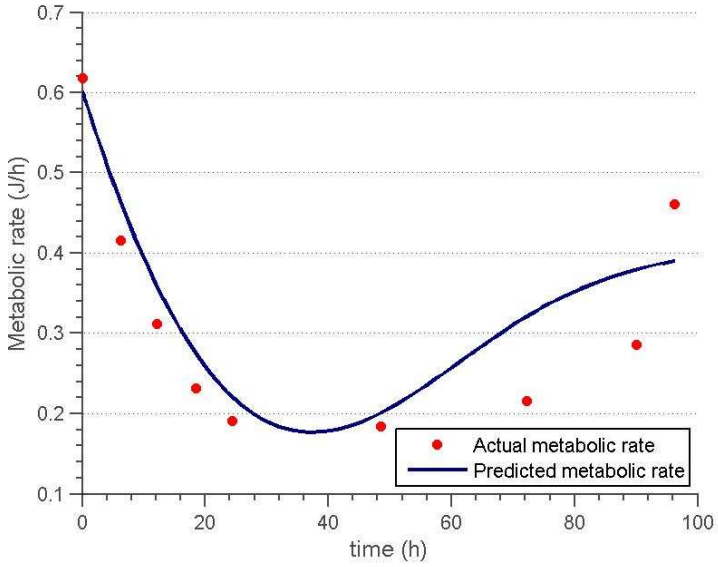

Figure 8. Metabolic rate as predicted by a DEB based model for the holometabolous lifecycle. Data from Merkey et al. (2011). Original study reports CO_2 consumption from which metabolic rate is calculated based on the energy content of lost nutrients during pupation (as reported in the same study).

5.4 The imago

The adult module differed very little from that in the standard DEB model. Adult structure is simply set by the size of the insect at eclosion and reproductive parameters are estimated using data in the usual way. The plot appearing in figure 9 shows the state variables of reproductive adult through time in a generic adult insect. The biggest fluctuations in the state variables are the losses in weight associated with oviposition. In DEB theory, while eggs are still housed in the ovaries before oviposition they are considered part of the 'reproduction buffer'.

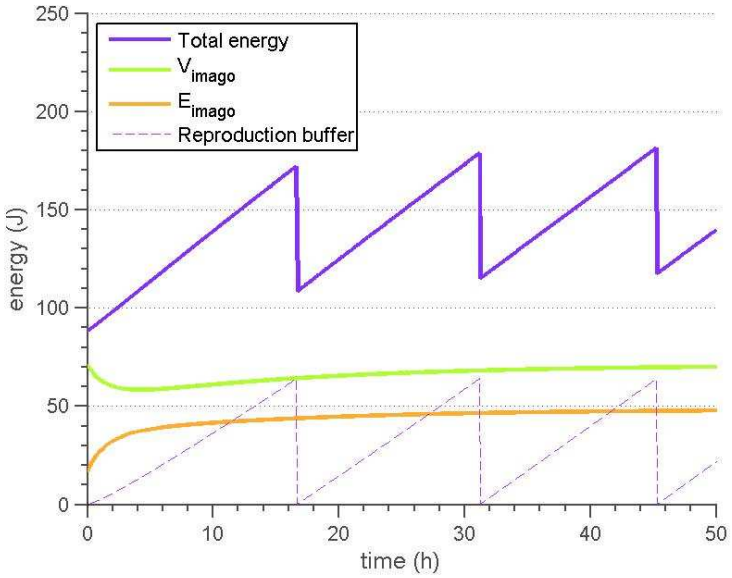

Figure 9. An idealised example of the state variables of a generic insect through time since eclosion. Weight increases until enough resources are accumulated to lay eggs. After eggs are laid there is a subsequent decrease in weight.

This extended DEB model has only recently been constructed so has seen limited application. But the success with which the model has predicted data so far, holds a lot of promise for this work to make a meaningful contribution to the field. While the theoretical value of these contributions is hopefully clear from the above, in the following I aim to show the practical value of such models. The applications I will propose should be regarded as the result of a preliminary 'brain storm' that will guide me in the remainder of my candidature.

5.5 Related tasks and expected completion dates

Project	**Completion date (Expected date)**
Manuscript on a bio-energetic model for holometabolous insects	
- Review literature for key features of holometabola	*Completed*
- Understand limitations in standard DEB model	*Completed*
- Trial different modifications until happy with model structure	*Completed*
- Find data on the full lifecycle for a generic insect that can be used to test model (found hatch times, larval growth, and pupal respiration data on *Drosophila melanogaster* and *Aedes aegypti*)	*Completed*
- Write draft of manuscript specifying model, justifying modifications and presenting predictions of data	*Completed*
- Receive feedback from supervisors	April-2012

- Submit to The American Naturalist	June-2012
Manuscript on the scaling of physiological traits with body-size among holometabola	
- Assemble a collection of data on a variety of holometabolous insects	July-2012
- Estimate model parameters for the set of insects	August-2012
- Compare parameters and explain differences in light of physiological differences between species	August-2012
- After controlling for body size, search for features (such as particular life history strategies) that can help to predict the parameter set specifying ontogenetic development	August-2012
- Complete first draft of manuscript and receive feedback from both supervisors	September-2012
- Submit manuscript to Journal of Experimental Biology	October-2012

6. *Applications of metabolic theory – Part 3: The scaling of life history traits with body size*

The scaling relationships deriving from DEB theory could be used to the benefit of two broad areas of research that are currently being considered as possible future directions. The first area is in quantitative genetics and evolutionary developmental biology, and will likely involve the collection of primary experimental data; the second is in macro-ecology where meta-analyses of secondary resources are likely to play a more important role. It is still to be decided which of these directions will best serve my research outcomes and it is hoped the committee will be able to share their thoughts on this issue.

6.1 The mechanistic basis of life history trade-offs

Understanding how a genotype translates into a phenotype is one of the most fundamental problems in biology (Flatt & Heyland 2011) but our knowledge on the mechanistic basis of this mapping is still very limited (Stearns 2000; Flatt et al. 2005; Roff & Fairbairn 2007). Due to a whole host of complex genetic interactions, pleiotropy, and the confounding effects of environment on gene expression, inferring an organism's phenotype from its genotype is no simple task. For evolutionary biologists trying to understand the evolution of life histories, the concept of 'life history trade-offs' as imposed by energetic limitations, such as a negative relationship between fertility and life-span, has been a useful conceptual framework. But despite the numerous and seemingly obvious

tradeoffs between life history traits in a wide range of taxa, most reported trade-off relationships essentially describe no more than a statistically inferred negative correlation (Flatt & Heyland 2011). And while our understanding of the molecular and genetic basis of life history traits is progressing at a rapid rate, a major challenge is the integration of these mechanistic insights into the evolutionary framework (Roff & Fairbairn 2007).

In DEB theory the co-variation rules for the scaling of model parameters provide more than a description of observed trait correlations and life-history trade-offs. Rather, this co-variation of parameters follows *a priori* from a set of mechanistically-based, first principles. The parameters have direct interpretations relating to the metabolic architecture of an organism and define *a priori* expectations for a number of life history traits (including metabolic rate as discussed above, maturity rates, reproduction rates, and mortality rates). As a result, DEB theory may be a useful framework in linking genetic architectures with metabolic architectures to better understand the mechanistic basis of the evolution of life histories.

One possible experimental study might want to ask whether the constraints on bio-energetic organisation, as proposed by DEB theory, are restricting the evolution of genotypes or whether the mechanistic basis of life history trade-offs is independent of available energetic resources. This could be investigated through a selection experiment on *Drosophila melanogaster* where body size is manipulated to observe how the genetic basis of the covariation of traits aligns with changes in DEB parameters. This would be a convenient study in light of the development of the holometabolous DEB model previously described.

6.2 Exploring impacts on ecosystems using metabolic theory

There is no doubt that the Earth's warming climate will unleash a suite of new pressures on biological systems that will bring about significant changes to the biosphere (Solomon et al. 2007). As discussed at the outset of this report, biological systems are highly complex, making the precise nature of the biological responses to climatic pressures difficult to identify, let alone quantify. The holometabolous model previously described will be helpful for exploring implications of pressures on the life histories of insects. The mechanistic approach taken can increase the coherence of available data, improve the robustness of predictions and, perhaps, yield some novel forecasts that have not been previously considered.

One area to which this model could be applied is in the prediction of dyssynchronous physiological responses to climatic change. The significance of dysynchronous responses can be made clear at both the individual level and the ecosystem level. At the individual level, the dyssynchronous responses of rates of growth and maturation to temperature can explain the pattern of decreasing body size with increasing temperature that is known to occur in many organisms (this is called the temperature-size rule or TSR). For instance, if maturation rate is more sensitive to temperature than growth rate, at high temperatures a small amount of growth will have occurred at the time of maturity relative to the larger amount of growth that can occur at colder temperatures (Zuo et al. 2011).

At the ecosystem level, this paradigm of dyssynchronous responses to temperature may also be used to predict the effects of changing size distributions of interdependent organisms. A question we may want to ask, for example, is how climate change increases or decreases the predation pressure of a species. If a predator species is more sensitive to temperature than its prey, holding the number of individuals in both population constant, a mismatch between the energetic demands of a predator population and the energetic supply of its prey will develop. The developed DEB model can be used to explore whether this phenomenon is likely to occur in real populations by testing the scaling of body-size in a predator and prey species in experimental conditions. In combination with a robust theoretical framework, like DEB theory, such data can be used to provide important insights about the new arrangements of populations that are likely to be brought about by climate change. These predictions may be used, for example, by agronomists to inform precautionary measures and minimise potential economic losses.

In addition to climate change other significant impacts altering ecosystems may be explored using DEB based models. Of all threats to the Earth's biodiversity, habitat destruction is the most significant, and is regarded as the leading cause of species extinctions. Increased fragmentation reduces possible range sizes, which have been found to scale predictably with body size (Damuth 1991; Damuth 1981; Damuth 2007), possibly in relation to the scaling of energetic demands implied by metabolic scaling. If the pressures of habitat destruction are sufficiently gradual so as to allow evolutionary adaptation, it is feasible that species will experience a selective pressure towards smaller body sizes, and consequently smaller range sizes, to deal with increased habitat fragmentation. DEB based models can be used to make predictions about what body sizes will be required to deal with a particular reduction in habitat size or quality, and whether or not the required size decreases are attainable at the rate at which evolutions process.

Also impacting the distribution of body-sizes is overexploitation of biological resources, such as the overharvesting of global fish stocks which is causing a shift towards smaller sizes (Genner et al. 2010). The ecological consequences of the new species size distributions resulting from human pressures can be explored using DEB based models, which make specific predictions about the covariation of life-history traits with body size (Kooijman 2010). Although selection can work independently on physiological traits, under DEB, the null expectation is that certain traits will be 'dragged along' with body size as it changes. This allows us to make predictions, for example, about the way human imposed selective pressures on smaller body size is causing reduced development times, which is in turn causing a seasonal misalignment between interdependent species. This seasonal misalignment may be further compounded by dyssychronous responses to climate change mentioned above.

6.3 Related tasks and expected completion dates

Project	**Completion date (Expected date)**
Practical application of the holometabolous model to ecophysiology/genetics	
- Understand the benefits of mechanistic approaches to predictive ecology	*Completed*
- Propose some novel applications of the developed models to enhance predictions of human impacts on biological systems	*Completed*
- Explore the potential benefit of DEB covariation rules to understanding how the genetic architecture influences evolutionary trajectories	February-2012
- Receive feedback from supervisors	
- Decide on area of focus	February-2012
- Decide on particular research question	March-2012
- Plan required resources	April-2012 May-2012

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Appendices

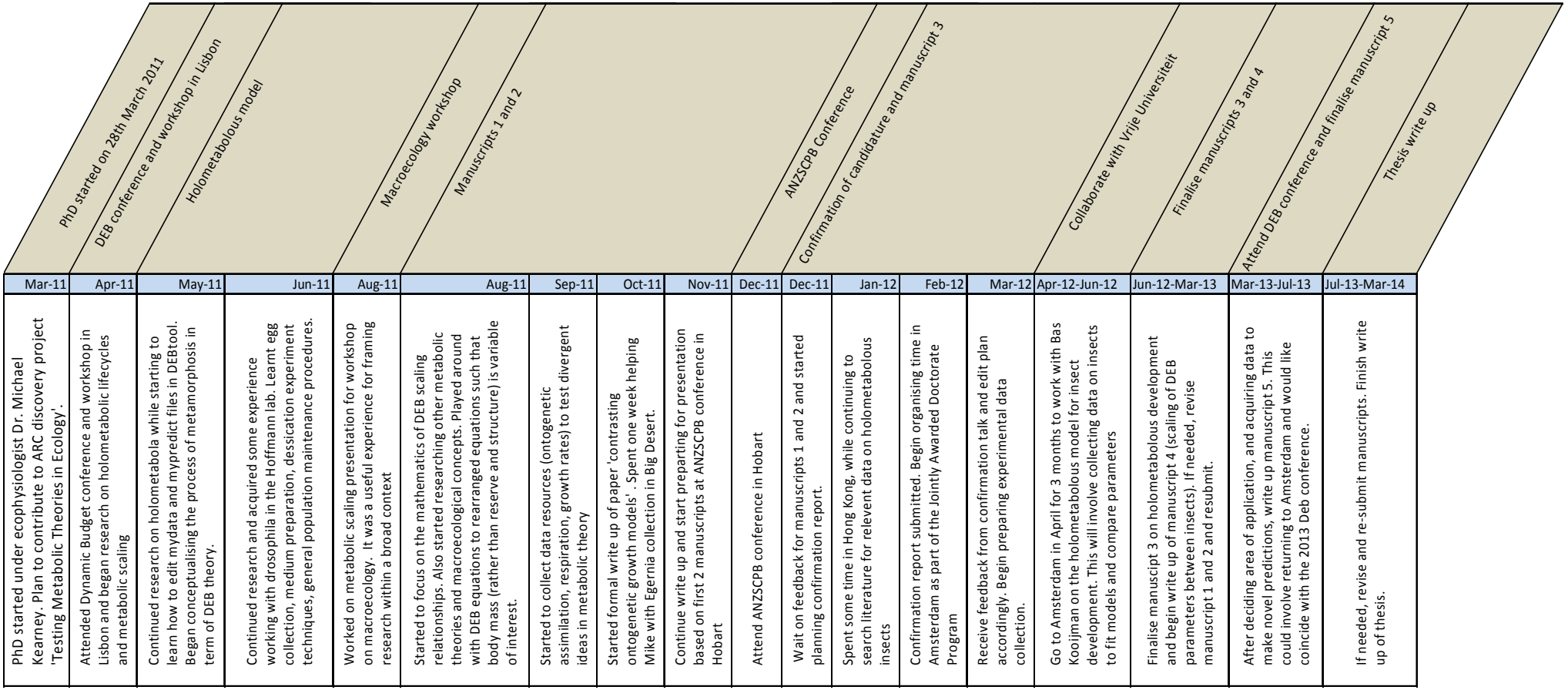
Appendix A – List of completed and to-be-completed tasks for publications throughout candidature

Part 1: Contrasting metabolic theories	**Completion date (Expected date)**
Manuscript on reconciling metabolic theories to functional ecology	
- Scrutinise assumptions of WBE and DEB models by deriving metabolic scaling predictions from first principles	Completed
- Use literature to find appropriate data set on metabolic scaling to test models (found data from a meta-study on 695 mammals)	Completed
- Evaluate models for weaknesses in terms of consistency of assumptions with reality and goodness of fits to data	Completed
- Complete first draft of manuscript and receive feedback from both supervisors	Completed
- Submit manuscript to Animal Ecology	March-2012
Manuscript on contrasting ontogenetic growth models	
- Research the link between metabolic scaling and ontogenetic growth in WBE and DEB models	Completed
- Understand how similarities in prediction arise and find divergences in prediction to be tested	Completed
- Find appropriate data to allow the empirical testing of divergent predictions (found data on ontogenetic assimilation (23 mammals), respiration (307 fish) and growth rates (approx 260 animals from 10 classes))	Completed
- Conclude key deficiencies in the models ability to predict data	Completed
- Complete first draft of manuscript and receive feedback from both supervisors	April-2012
- Submit manuscript to Trends in Ecology and Evolution	June-2012
Manuscript on testing whether metabolic scaling relationships are causal to other scaling relationships (if time permits)	
- Find detailed data on a number of species so that the covariation of traits can be studied within individuals rather than between groups (using body size as a proxy)	April-2012
- Fit model to predict traits using body size and metabolic rate as independent variables and compare against null expectations of competing theories	June-2012
- Conclude key deficiencies in each model's ability to predict data	June-2012
- Complete first draft of manuscript and receive feedback from both supervisors	August-2012
- Submit manuscript to Proceedings of the Royal Society B	September-2012
Part 2: A bioenergetic model for the holometabolous life cycle	
Manuscript on a bio-energetic model for holometabolous insects	
- Review literature for key features of holometabola	Completed
- Understand limitations in standard DEB model	Completed
- Trial different modifications until happy with model structure	Completed

- Find data on the full lifecycle for a generic insect that can be used to test model (found hatch times, larval growth, and pupal respiration data on *Drosophila melanogaster* and *Aedes aegypti*)	Completed
- Write draft of manuscript specifying model, justifying modifications and presenting predictions of data	Completed
- Receive feedback from supervisors	April-2012
- Submit to The American Naturalist	June-2012
Manuscript on the scaling of physiological traits with body-size among holometabola	
- Assemble a collection of data on a variety of holometabolous insects	July-2012
- Estimate model parameters for the set of insects	August-2012
- Compare parameters and explain differences in light of physiological differences between species	August-2012
- After controlling for body size, search for features (such as particular life history strategies) that can help to predict the parameter set specifying ontogenetic development	August-2012
- Complete first draft of manuscript and receive feedback from both supervisors	September-2012
- Submit manuscript to Journal of Experimental Biology	October-2012
Part 3: Changing environments, changing body size	
Practical application of the holometabolous model	
- Understand the benefits of mechanistic approaches to predictive ecology	Completed
- Propose some novel applications of the developed models to enhance predictions of human impacts on biological systems	Completed
- Receive feedback from supervisors	February-2012
- Decide on area of focus	March-2012
- Decide on particular research question	April-2012
- Plan required resources	May-2012

Appendix B – Summary of key events throughout candidature

Timeline



Appendix C - List of presentations made during probationary candidature

Date	Presentation given
April-11	DEB 2011 Workshop – Lisboa, Portugal: 'Metabolic Theory of Ecology vs. Dynamic Energy Budgets'
August-11	Macroecology and Climate Change – Brisbane, Australia: 'The importance of body size scaling: what can Dynamic Energy Budget theory offer? '
December-11	Australia and New Zealand Comparative Physiology and Biochemistry 2011 – Hobart, Australia: 'Contrasting Metabolic Theories: parallel predictions and divergent explanations'