



Damage-related protein turnover explains inter-specific patterns of maintenance rate and suggests modifications of the DEB theory



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ABSTRACT

Maintenance is the energy that living organisms are bound to use to maintain their structure in a viable state. It includes all the metabolic and physiological costs that are not directly associated to the production of biomass (growth and reproduction) or to development (maturation). In the framework of the DEB theory, the somatic maintenance rate can either be proportional to organism structural volume V or, more marginally, to structural surface $V^{2/3}$. Being mostly associated to similar metabolic processes, volume-specific maintenance costs are not expected to vary substantially at both intra- and inter-specific levels. In the DEB theory, the volume-specific maintenance rate $[\dot{p}_M]$ is therefore supposed to keep constant from birth to death and to remain approximately constant between species. However, a recent meta-analysis of DEB parameters estimated using the Add-my-Pet collection (Kooijman, 2014) reveals troubling patterns apparently violating this inter-specific scaling rule and challenging the DEB theory. It is indeed shown in this study that empirically-derived volume-specific maintenance rates scale approximately with $L_m^{-0.4}$ and display a very high variability around this trend. Overall, estimated maintenance rates in Add-my-Pet span over three to four orders of magnitude, thus invalidating the assumption of constant maintenance rate between species, which underpins the covariation rules for parameter values of the DEB theory. In an attempt to address this major problem for the DEB theory, we propose a simple physiological mechanism that would simultaneously explain the apparent decrease of volume-specific maintenance rate with ultimate size and its apparent variability for a given range of maximum size. Our proposition consists in making protein (and more generally structure) turnover explicit in maintenance and linking protein damage rate to aerobic metabolism and the production of ROS, which are decreasing with both structural volume and maximum structural volume. We show that this implies that the actual volume specific maintenance rate varies both at the intra- and inter-specific levels in a range very similar to what is observed in the Add-my-Pet data estimations. If true, this implies that the apparent decrease of volume-specific maintenance rate with ultimate size is an artefact and it requires modifications of the standard DEB theory in order to capture empirical inter-specific scaling patterns of DEB-parameters while keeping the consistency of the theory at both intra- and inter-specific levels.

1. Introduction

The DEB theory (e.g. Kooijman, 2010) is the most comprehensive metabolic theory of life existing to date (van der Meer, 2006; Jusup et al., 2017). It is also the best tested empirically, thanks to its ability to generate a variety of distinct testable predictions, both at the intra- and the inter-specific levels (Kooijman, 2010; Jusup et al., 2017). Recently, for the first time, empirical estimates of DEB parameters have been collected for an increasingly large number of species and gathered in the Add-my-Pet collection (http://www.bio.vu.nl/thb/deb/deblab/add_my_pet/index.html). Add-my-Pet provides a unique opportunity to look at the way DEB parameter values are distributed amongst species, hoping that deviations from the generic theoretical expectation would reveal evolutionary adaptations to specific environments and characterize particular life history strategies. Add-my-Pet also offers a chance to test the validity of the interspecific scaling rules, and in particular the fundamental assumption that the volume-specific somatic

maintenance rate $[\dot{p}_M]$ remains approximately constant between species and that, as a corollary, the maximum surface-specific assimilation rate $\{\dot{p}_{A_m}\}$ scales with maximum structural size.

Maintenance is the energy that living organisms are bound to use to maintain their structure in a viable state. Maintenance includes all the metabolic and physiological costs that are not directly associated to the production of biomass (growth and reproduction) or to development (maturation). These comprehend the costs of removing and replacing damaged proteins, maintaining chemical and electrical gradients through cellular membranes, maintaining the immune system functional, forming products (scales, hair, nails, etc), maintaining muscular tonicity, circulating body fluids (blood, lymph, etc), moving, maintaining a constant body temperature for endotherms or a constant osmotic pressure for aquatic organisms, etc. In the framework of the DEB theory, somatic maintenance rate can either be proportional to organism structural volume V or to structural surface $V^{2/3}$ (e.g. Kooijman, 2000). Being mostly associated to similar metabolic processes, volume-

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specific maintenance costs have no obvious reason to vary substantially at both intra- and inter-specific levels. In the DEB theory, the volume-specific maintenance rate $[p_M]$ is therefore supposed to remain approximately constant between species. Consequently, since the maximum length that a given species can reach is proportional to the ratio of its maximum surface-specific assimilation rate divided by the volume-specific maintenance rate ($L_m = \frac{\kappa[p_{Am}]}{[p_M]}$), maximum surface-specific assimilation rate is expected to scale with the maximum organism length L_m (Kooijman, 2006). The inter-specific scaling of the maximum surface-specific assimilation rate is fundamental to the DEB theory. It is at the core of the covariation rules for parameter values that explain why a small set of “extensive” parameters scale with maximum structural length while “intensive” parameters are independent from it. This provides mechanistic explanations to well-established empirical body-size scaling relationships of important life-history traits such as respiration rate, gestation time, incubation time or growth rate for instance, amongst many other (Kooijman, 2010). Furthermore, body-size scaling relationships can be used as a solid basis to derive models of ecological communities that integrate the diversity of life-history traits from small to large species (Maury and Poggiale, 2013).

However, the examination of estimated somatic maintenance rate and maximum surface-specific assimilation rate as a function of the species maximum size in the Add-my-Pet collection (Kooijman, 2014) reveals troubling patterns apparently violating the covariation rules for parameter values and challenging the DEB theory. Kooijman (2014) indeed shows that empirically-derived maximum surface-specific assimilation rates scale approximately with $L_m^{0.6}$ (instead of scaling with L_m as predicted by the DEB theory) while volume-specific maintenance rates scale approximately with $L_m^{-0.4}$ (instead of remaining constant as assumed by the DEB theory). Further to these trends, both rates exhibit a very high and unexpected variability around their tendency (Fig. 1, see also http://www.bio.vu.nl/thb/deb/deblab/add_my_pet/patterns.html for the most recent figure with more species included).

The large number of species included in the Add-my-Pet collection provides robustness to the trends identified for both parameters and to the important and systematic variability of the estimates around these trends. Overall, estimated maintenance rates span over three to four orders of magnitude, with maintenance of the smallest species ($L_m \approx 10^{-2}cm$) being in average three orders of magnitude higher than maintenance of the largest species considered ($L_m \approx 10^2cm$). In average, the dispersion around this trend varies from 1 to more than 2 orders of magnitude for a given maximum size. These patterns clearly deviate from the theoretical DEB expectations. Kooijman (2014) proposes the “waste to hurry” hypothesis to explain them. The rationale is evolutionary. It assumes that species in variable environments would

have increased their assimilation rate and simultaneously evolved means to waste their energy by increasing their maintenance for remaining small, growing fast and reproducing early. This would speed-up their life cycle and allow these species to adapt to environments where the availability of resources undergoes large and high frequency changes. The mechanism proposed by Kooijman (2014) involves the use of futile cycles that appear when two biochemical reactions run simultaneously in opposite directions and compensate each other, thus dissipating energy with no net production of one compound and therefore no obvious purpose.

We believe that the empirical patterns of maintenance revealed in Add-my-Pet have much profound impacts on the DEB theory. They are indeed too systematic to be considered as simple deviations from the theoretical expectations: the volume-specific maintenance rate can obviously not anymore be considered to keep approximately constant between species when it varies over almost four orders of magnitude amongst species and displays such a clear decreasing tendency with species maximum structural size. We believe that this pattern simultaneously invalidates the covariation rules for parameter values, which constitute a major part of the DEB theory, and suggests that we are missing something that would explain the systematic trend of maintenance observed with maximum size. There is therefore here a major problem. While the “waste to hurry” hypothesis helps to understand the general evolutionary interest of being a small species with high maintenance in variable environments, it doesn't provide us with a clear and formal mechanism that would explain the magnitude of the observed decrease of maintenance with species size, its systematic nature, and the regular pattern of variability observed around this trend. At the moment, we are left with the idea that the covariation rules for parameter values implied by the standard DEB model doesn't work anymore, that the predictive capacity of the DEB theory has to be abandoned at the inter-specific level and restricted to the intra-specific level, and that we are missing an explanation for the inter-specific patterns observed.

In an attempt to address this major problem for the DEB theory, we propose a simple physiological mechanism that would simultaneously explain the apparent decrease of volume-specific maintenance rate with ultimate size and its apparent variability for a given range of maximum size. Our proposition rests on the idea that protein (and more generally structure) turnover constitutes an important component of maintenance (e.g. Bouma et al., 1994; Kooijman, 2010; Waterlow, 1984), which varies with aerobic metabolism (e.g. Cabiscol et al., 2000; Pikosky et al., 2006; Waterlow, 1984, 2006), and hence decreases with size at both intra- and inter-specific levels. If true, it implies that the apparent decrease of volume-specific maintenance rate with ultimate size and its variability are artefacts and it requires modifications of the standard

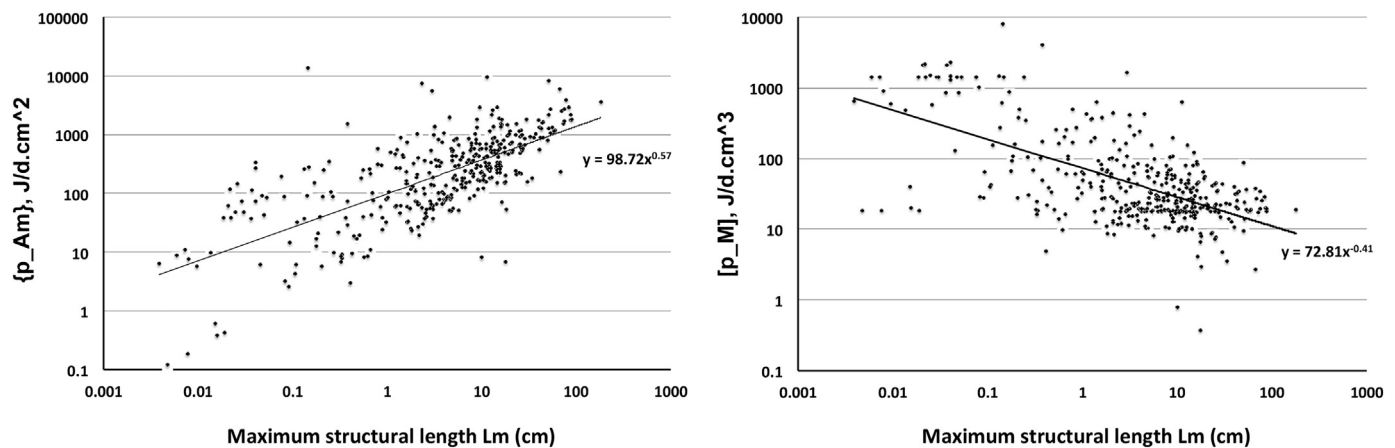


Fig. 1. Empirical scaling of maximum surface-specific assimilation rate $\{p_{Am}\}$ (left) and volume-specific maintenance rate $[p_M]$ (right) with maximum length L_m from the Add-my-Pet database (downloaded the 25/10/2014 from http://www.bio.vu.nl/thb/deb/deblab/add_my_pet/index.html). Parameters estimated empirically for 389 species seem to violate the DEB expectation that the inter-specific level $\{p_{Am}\}$ is proportional to L_m while $[p_M]$ is independent from L_m .

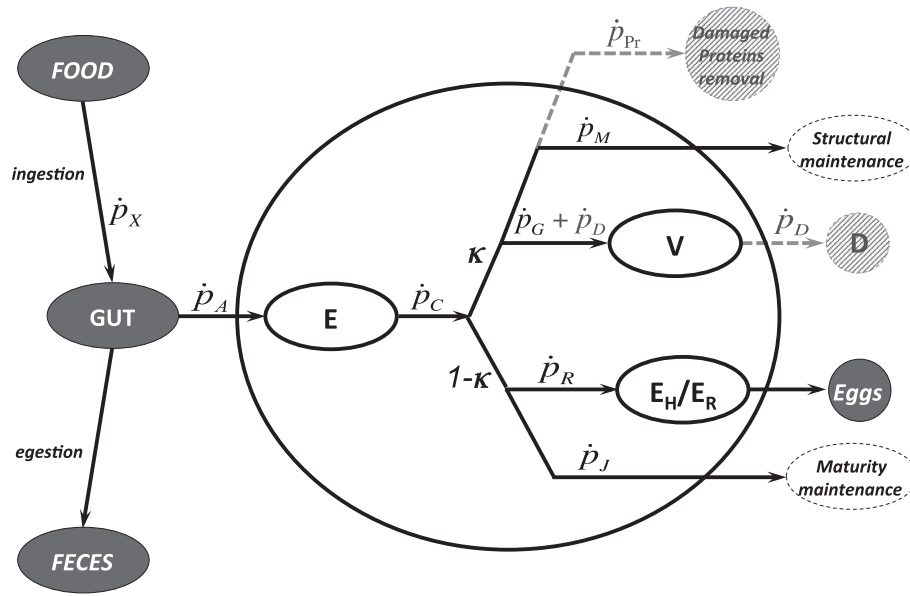


Fig. 2. State variables (E, V, E_H/E_R) and energy fluxes ($\dot{p}_X, \dot{p}_A, \dot{p}_C, \dot{p}_M, \dot{p}_G, \dot{p}_R$ and \dot{p}_J) involved in the energetics of individual organisms in the framework of the standard DEB theory (see section “Standard Dynamic Energy Budget”). The additional energy fluxes proposed in the present study (\dot{p}_{Pr} and \dot{p}_D) are represented with dashed grey arrows (see section “Somatic maintenance and the protein turnover rate”)

DEB theory in order to capture empirical inter-specific scaling patterns of DEB-parameters while keeping the consistency of the theory at the intra-specific level. As a corollary, it also implies that the DEB parameters estimated using the standard DEB model are not valid with the modified DEB model and need to be re-estimated.

2. Standard dynamic energy budget (DEB) model

The Dynamic Energy Budget (DEB) theory (e.g. Kooijman, 2000, 2010) describes mechanistically the processes involved in the acquisition and use of energy by individual organisms. The energetics of individuals is represented using three state variables: energy stored in the reserve compartment E (J), structural volume V (cm³) (with the associated structural length L (cm) defined as $L = V^{1/3}$), and energy stored in the reproductive buffer E_R (J). Energy fluxes between those compartments are made explicit through the use of powers \dot{p} (J.s⁻¹) (see Fig. 2 and Table 1). For every individual organism, energy in food is ingested (\dot{p}_X) and assimilated (\dot{p}_A) before being stored into reserves. Reserves are mobilized (\dot{p}_C) and a fixed fraction κ of the energy utilized from reserves is allocated to growth of structural material (\dot{p}_G) and somatic maintenance (\dot{p}_M), the remaining fraction $1 - \kappa$ being devoted to maturity maintenance (\dot{p}_J) and development or reproduction (\dot{p}_R). Only a fraction κ_R of the energy in E_r is turned into eggs reserve.

The five DEB core parameters used in this study and their values

Table 2

Main DEB parameters used in this study and their value given in Kooijman (2010) for a L_m = 1 cm organism.

Parameters	Symbol	Value a unit
Maximum surface-specific assimilation rate	$\{\dot{p}_{Am}\}$	22.5 J.cm ⁻² .d ⁻¹
Volume-specific maintenance rate	$[\dot{p}_M]$	18 J.cm ⁻³ .d ⁻¹
Volume-specific cost of growth	$[E_G]$	2800 J.cm ⁻³
Maximum reserve energy density	$[E_m]$	1125 J.cm ⁻³
Fraction of energy allocated to structural growth and maintenance	κ	0.8/
Energy conductance	$\dot{v} = \{\dot{p}_{Am}\}/[E_m]$	0.02 cm.d ⁻¹
Scaled functional response	f	1/

given in Kooijman (2010) for a L_m = 1 cm organism are provided Table 2. By convention, [] stands for volumetric concentrations and { } for surface-specific concentrations so that $[E] = E/V$ and $\{\dot{p}_{X_m}\} = \dot{p}_{X_m}/V^{2/3}$ for instance (Kooijman, 2000). All the rates have a dot like \dot{p}_X to indicate the dimension « per time ».

3. Somatic maintenance and the protein turnover rate

The DEB theory assumes that maintenance can be partitioned into volume-specific and surface-specific maintenance costs. Surface-specific

Table 1

Basic DEB powers as a function of the state variables E and V (as in Kooijman, 2000).

Fluxes (J.d ⁻¹)	Formulation
Ingestion	$\dot{p}_X = \{\dot{p}_{X_m}\}V^{2/3}$
Assimilation	$\dot{p}_A = \kappa_X \dot{p}_X = \{\dot{p}_{A_m}\}V^{2/3}$
Catabolic	$\dot{p}_C = \frac{[E]}{[E_G] + \kappa[E]} \left([E_G] \dot{v} V^{2/3} + [\dot{p}_M] V \right)$
Structural maintenance	$\dot{p}_M = [\dot{p}_M] V$
Structural growth	$\dot{p}_G = \kappa \dot{p}_C - \dot{p}_M$
Maturity maintenance	$\dot{p}_J = \frac{1-\kappa}{\kappa} [\dot{p}_M] \min(V, V_p)$
$\left\{ \begin{array}{l} \text{Maturation } (V < V_p) \\ \text{Reproduction } (V \geq V_p) \end{array} \right.$	$\dot{p}_R = (1 - \kappa) \dot{p}_C - \dot{p}_J = (1 - \kappa) \left[\frac{[E]}{[E_G] + \kappa[E]} \left([E_G] \dot{v} V^{2/3} + [\dot{p}_M] V \right) - \frac{[\dot{p}_M] \min(V, V_p)}{\kappa} \right]$

maintenance costs such as heat regulation are supposed to be relatively marginal in the energy balance of most organisms while volume-specific costs constitute the bulk of maintenance (Kooijman, 2010). Amongst those volume-specific costs, protein turnover and cell repair in general are usually regarded as the most important components of maintenance (e.g. Bouma et al., 1994; Kooijman, 2010; Waterlow, 1984), at least in aerobic organisms that oxidize organic molecules to produce ATP. In addition to ATP, aerobic metabolism in mitochondria is indeed producing reactive oxygen species (ROS) that continuously damage DNA, RNA, and oxidize amino acids in proteins. ROS are a normal product of cellular metabolism. To avoid being lethally damaged, organisms have to continuously spend energy to counteract the oxidative effects of ROS, both in producing anti-oxidative enzymes and in degrading and resynthesizing damaged structural proteins to maintain cells and tissues functional (e.g. Birnie-Gauvin et al., 2017; Cabiscol et al., 2000; Pikosky et al., 2006; Waterlow, 1984, 2006). Protein turnover rate has therefore to be linked to the aerobic metabolism. Disregarding the variability of protein turnover rates between the various structural tissues of the body, we postulate that at the organism level, protein turnover rate and associated maintenance costs are proportional to protein damaging rate that is in turn assumed to be proportional to the rate at which damage-inducing compounds are produced by aerobic metabolism. This allows linking explicitly maintenance costs to aerobic metabolism. All powers (\dot{p}_A , \dot{p}_G , \dot{p}_M , \dot{p}_r and \dot{p}_R) are actually contributing to respiration. However, neglecting the contribution of assimilation (\dot{p}_A) to respiration, aerobic metabolism can be considered to be approximately proportional to the catabolic power \dot{p}_c (Kooijman, 2010). Since at constant food supply the reserve density is stationary (and therefore $\dot{p}_A = \dot{p}_c$), the assumption that respiration is approximately proportional to \dot{p}_c keeps valid even if the contribution of assimilation to respiration is considered, when food availability is not changing substantially.

Considering all maintenance components including protein turnover costs and other volume-specific maintenance costs (Fig. 2), the catabolic power \dot{p}_c can be expressed as follows:

$$\kappa \dot{p}_c = \dot{p}_M + \dot{p}_r + [E_G] \left(\frac{dV}{dt} + \dot{D} \right) \quad (1)$$

with \dot{p}_r ($J.s^{-1}$) being the cost of removing damaged structural proteins and \dot{D} ($cm^3.s^{-1}$) being the volume of structural proteins damaged per unit of time (and \dot{p}_D the corresponding energy flux cf. Fig. 2).

According to the above hypothesis, $\dot{D} = \sigma \dot{p}_c$ with σ ($cm^3.J^{-1}$) being the volume of structural proteins indirectly damaged by one joule spent in the aerobic metabolism. We can also write that $\dot{p}_r = [\gamma] \dot{D}$ with $[\gamma]$ ($J.cm^{-3}$) being the cost of removing a fixed volume of damaged structure. Finally, Eq. (1) can be rewritten:

$$\kappa \dot{p}_c = \dot{p}_M + ([\gamma] + [E_G]) \sigma \dot{p}_c + [E_G] \frac{dV}{dt} = \dot{p}_M + \rho \dot{p}_c + [E_G] \frac{dV}{dt} \quad (2)$$

with $\rho = ([\gamma] + [E_G]) \sigma$ being the fraction of the reserve energy mobilized that is allocated to protein turnover.

Eq. (2) can be reorganized as

$$\dot{p}_c = \frac{\dot{p}_M + [E_G] \frac{dV}{dt}}{\kappa - \rho} \quad (3)$$

and combined to the \dot{p}_c expression demonstrated in Kooijman, 2010:

$$\dot{p}_c = [E] \left(\frac{\{\dot{p}_{Am}\}}{[E_m]} V^{2/3} - \frac{dV}{dt} \right) \quad (4)$$

After trivial calculations this provides us with a new expression of the catabolic power that includes explicitly protein turnover maintenance costs:

$$\begin{aligned} \dot{p}_c &= [E] \left(\frac{\{\dot{p}_{Am}\}}{[E_m]} V^{2/3} - \frac{dV}{dt} \right) = \frac{[E] \left([E_G] \dot{V} V^{2/3} + \dot{p}_M \right)}{[E_G] + [E] (\kappa - \rho)} \\ &= \frac{[E] \left([E_G] \dot{V} V^{2/3} + [\dot{p}_M] V \right)}{[E_G] + [E] (\kappa - \rho)} \end{aligned} \quad (5)$$

This implies that the volume-specific maintenance rate associated to protein turnover (including removal of damaged proteins and new protein synthesis) $[\dot{p}_p]$ is equal to:

$$[\dot{p}_p] = \rho [\dot{p}_c] = \frac{\rho [E] \left([E_G] \dot{V} V^{-1/3} + [\dot{p}_M] \right)}{[E_G] + [E] (\kappa - \rho)} \quad (6)$$

The total volume-specific maintenance rate is therefore not a constant as would be expected ignoring protein turnover and surface-specific costs. On the contrary, it is expected to vary at the intraspecific level with structural volume V as

$$[\dot{p}_p] + [\dot{p}_M] = \frac{\rho [E] \left([E_G] \dot{V} V^{-1/3} + [\dot{p}_M] \right)}{[E_G] + [E] (\kappa - \rho)} + [\dot{p}_M] \quad (7)$$

At the inter-specific level, the total volume-specific maintenance rate is expected to vary with the zoom factor $z = \frac{L_m}{L_m}$ as:

$$[\dot{p}_p] + [\dot{p}_M] = \frac{\rho [E_m^1] z \left([E_G] \dot{V} V^{-1/3} + [\dot{p}_M] \right)}{[E_G] + [E_m^1] z (\kappa - \rho)} + [\dot{p}_M] \quad (8)$$

The total volume-specific maintenance rate is therefore highly dependant on organism size. At the intra-specific level it varies as $c + \frac{1}{L}$ whereas it varies as $\frac{c+L}{d+L}$ at the inter-specific level (Fig. 3).

4. Empirical patterns in the Add-my-Pet database

Protein turnover is responsible for a significant proportion of maintenance. We have shown above that it is likely to be size-dependent at both the intra and inter-specific levels. The parameter estimation procedure in the Add-my-Pet database is based on the equations of the standard DEB model that don't account explicitly for the size-dependent cost of protein turnover. Estimated maintenance rates are therefore likely to be biased and to reflect both the inter- and intra-specific scaling of protein turnover rate that are not made explicit in the equations of the standard DEB model. For a given species (a given maximum structural length L_m), we can therefore expect the estimated maintenance per unit of structural volume to be somewhere in between the minimum and the maximum total volume-specific maintenance rates predicted by Eq. (8) (Fig. 4). If the data available for estimating the parameters were dominated by small individuals, the estimated maintenance is likely to have been pulled toward the upper predicted bound (at V_b) while we expect it to be closer to the lower bound (at V_m) if the data used were coming from large individuals.

Fig. 4 clearly shows that most volume-specific maintenance rate values empirically derived from the Add-my-Pet database are comprised between the expected curves, despite the fact that they were estimated using the standard DEB model. If a re-estimation of these parameters is done with the changes proposed in this paper, it is likely that most parameter estimates will change as well (see the discussion section).

5. Influence of the scaling of $[E_m]$

In the framework of the DEB theory, the maximum surface-specific assimilation rate $\{\dot{p}_{Am}\}$ is an extensive parameter (proportional to L_m) and the energy conductance $\dot{V} = \{\dot{p}_{Am}\}/[E_m]$ is an intensive parameter (independent from L_m). The maximum reserve density $[E_m]$ is therefore an extensive parameter, which is proportional to the maximum structural size L_m . However, empirical patterns in the Add-my-Pet database

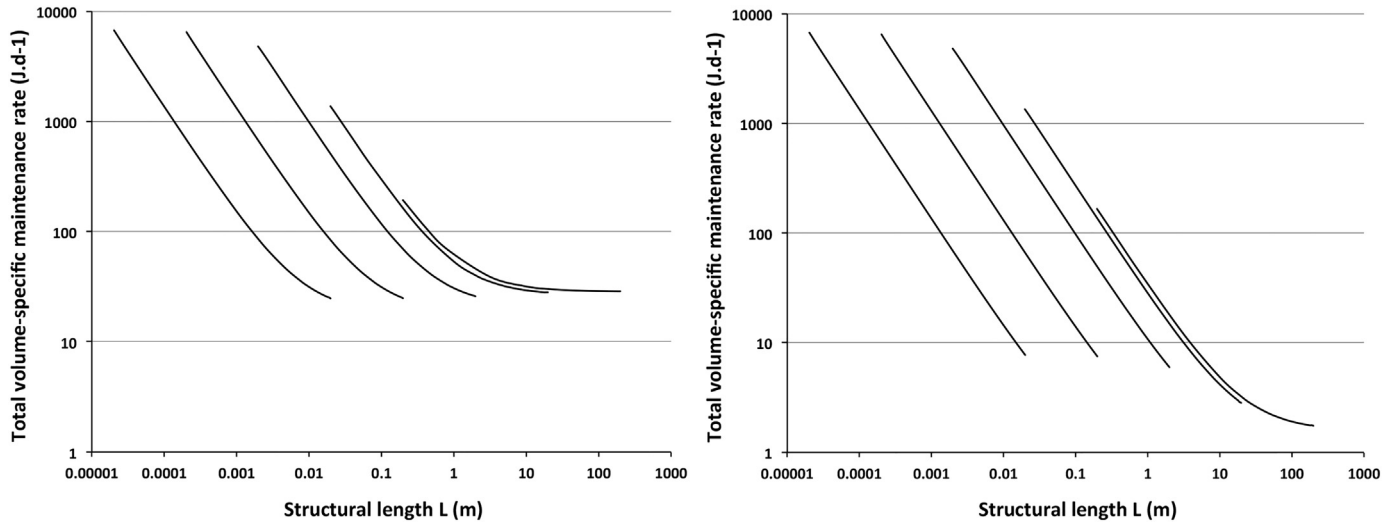


Fig. 3. Total volume-specific maintenance rate $[\dot{p}_p] + [\dot{p}_M]$ for five animal species with $L_m = 0.02 \text{ cm}$, $L_m = 0.2 \text{ cm}$, $L_m = 2 \text{ cm}$, $L_m = 20 \text{ cm}$, $L_m = 200 \text{ cm}$. Parameters' values given in Table 2 are used. For the sake of drawing the figure, we assume that $k_M = k_J$. Given the covariation rules for parameter values, this implies that the length at birth is proportional to maximal length ($L_b = 10^{-3}L_m$) (Kooijman, 2010). The fraction of aerobic metabolism allocated to protein turnover is fixed to $\rho = 0.3$ according to empirical observations showing that protein turnover represents between 10% and 50% of total resting metabolism (e.g. Waterlow, 2006). Left: $[\dot{p}_M] = 18 \text{ J.cm}^{-3}.\text{d}^{-1}$ and right: $[\dot{p}_M] = 1 \text{ J.cm}^{-3}.\text{d}^{-1}$.

show that this proportionality is not supported empirically (Fig. 5) and that the scaling of $[E_m]$ with species maximum length might actually be weaker than expected (the linear regression gives $[E_m] = 3612.5 L_m^{0.3819}$) while the size-independent inter-specific variability dominates.

From Eq. (7) we can derive an expression for the total volume-specific maintenance rate when the maximum reserve energy density scales with an arbitrary power α of the zoom factor:

$$[\dot{p}_p] + [\dot{p}_M] = \frac{\rho [E_m^1] z^\alpha ([E_G] \dot{v} z^{1-\alpha} V^{-1/3} + [\dot{P}_M])}{[E_G] + [E_m^1] z^\alpha (\kappa - \rho)} + [\dot{P}_M] \quad (9)$$

The comparison of Fig. 4 drawn assuming that $[E_m] \propto L_m$, Fig. 6 drawn assuming that $[E_m] \propto L_m^0$ and Fig. 7 drawn assuming that $[E_m] \propto L_m^{0.3819}$ demonstrates the importance that the inter-specific scaling of $[E_m]$ has on the scaling of both maximum and minimum volume-specific maintenance rates.

6. Consequences on growth, development and reproduction

6.1. Consequences on growth

Using a quasi-steady state assumption and Eqs. (4) and (5), we can derive an expression for the structural growth:

$$\left. \frac{dV}{dt} = \frac{\dot{v} V^{2/3} [E](\kappa - \rho) - [\dot{P}_M] V}{[E_G] + [E](\kappa - \rho)} \right\} \Rightarrow \frac{dV}{dt} = \frac{(\kappa - \rho) f \{ \dot{P}_{Am} \} V^{2/3} - [\dot{P}_M] V}{[E_G] + (\kappa - \rho) f [E_m]} \quad (10)$$

$f = \text{constant}$

Which, after integration between 0 and t provides us with the age-dependent expression of structural length (the growth curve):

$$L_t = \frac{(\kappa - \rho) f \{ \dot{P}_{Am} \}}{[\dot{P}_M]} \left(1 - e^{-\frac{[\dot{P}_M] t}{3([E_G] + (\kappa - \rho) f [E_m])}} \right) \quad (11)$$

With the maximal structural length:

$$V_m^{1/3} = L_m = \frac{(\kappa - \rho) \{ \dot{P}_{Am} \}}{[\dot{P}_M]} \quad (12)$$

And the growth rate of structural length

$$\dot{v}_B = \frac{-[\dot{P}_M]}{3([E_G] + (\kappa - \rho) f [E_m])} \quad (13)$$

Both maximal structural length and growth rate depend on the fraction of aerobic metabolism allocated to protein turnover ρ . Fig. 8 shows how the cost of protein turnover affects quantitatively growth but doesn't modify qualitatively its von Bertalanffy nature.

6.2. Consequences on reproduction and development

From Eq. (3) we can write:

$$(1 - \kappa) \dot{p}_c = \frac{1 - \kappa}{\kappa - \rho} \left(\dot{p}_M + [E_G] \frac{dV}{dt} \right) \quad (14)$$

We derive the maturity maintenance flux:

$$\dot{p}_j = \frac{1 - \kappa}{\kappa - \rho} [\dot{P}_M] \min(V, V_p) \quad (15)$$

The development/reproduction flux then reads:

$$\dot{p}_R = \frac{1 - \kappa}{\kappa - \rho} [E_G] \frac{dV}{dt} \quad (16)$$

From Eq. (5), we can express this flux as:

$$\dot{p}_R = (1 - \kappa) \left[\frac{[E] ([E_G] \dot{v} V^{2/3} + [\dot{P}_M] V)}{[E_G] + [E](\kappa - \rho)} - \frac{[\dot{P}_M]}{\kappa - \rho} \min(V, V_p) \right] \quad (17)$$

At constant food we get:

$$\dot{p}_R = (1 - \kappa) \left[\frac{f [E_m] ([E_G] \dot{v} V^{2/3} + [\dot{P}_M] V)}{[E_G] + f [E_m](\kappa - \rho)} - \frac{[\dot{P}_M]}{\kappa - \rho} \min(V, V_p) \right] \quad (18)$$

The development/reproduction flux depends on the fraction of aerobic metabolism allocated to protein turnover ρ . Fig. 9 shows how the cost of protein turnover affects quantitatively the development/reproduction flux but doesn't modify qualitatively its shape.

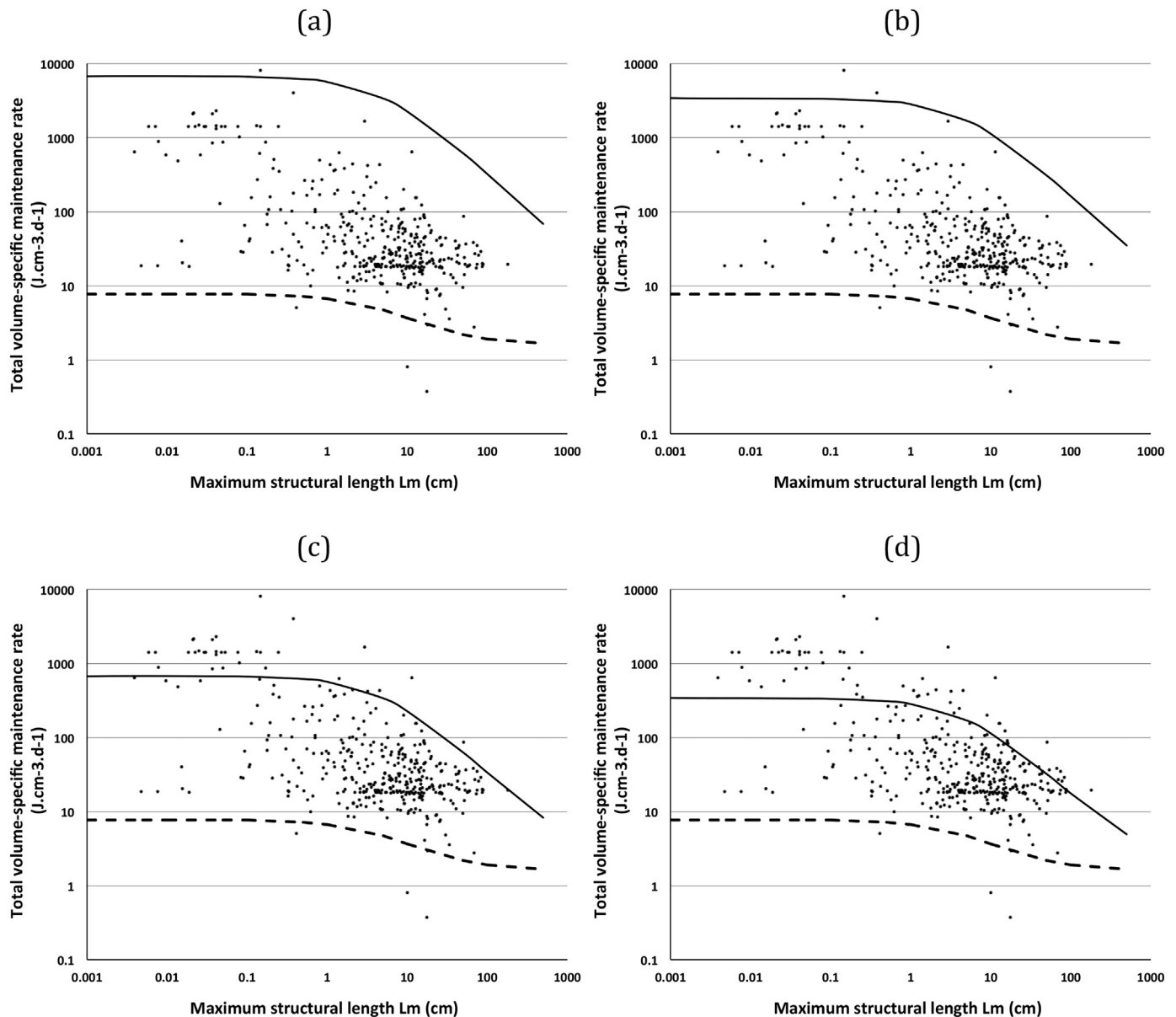


Fig. 4. Predicted value of the total volume-specific maintenance rate $[\dot{p}_p] + [\dot{p}_M]$ at birth (continuous line) and at maximum structural size (dashed line), as well as estimated volume-specific maintenance rate for the 389 entries of the Add-my-Pet database (downloaded the 25/10/2014) as a function of maximum length L_m . (a) the length at birth $L_b = 10^{-3}L_m$. (b) $L_b = 2.10^{-3}L_m$. (c) $L_b = 10^{-2}L_m$. (d) $L_b = 2.10^{-2}L_m$. Parameters values given in Table 2 are used except for $[\dot{p}_M] = 1 J \cdot cm^{-3} \cdot d^{-1}$. For the sake of drawing the figure, we assume that $k_M = k_p$. Given the covariation rules for parameter values, this implies that the length at birth is proportional to maximal length ($L_b = 10^{-3}L_m$) (Kooijman, 2010). The fraction of aerobic metabolism allocated to protein turnover is fixed at $\rho = 0.3$. The maximum reserve energy density scales with maximum structural length as $[E_m] = 1125 L_m (J \cdot cm^{-3})$ according to Kooijman (2010).

7. Discussion

7.1. Linking protein turnover to oxidative stress explains maintenance patterns

7.1.1. Protein turnover constitutes the bulk of maintenance

Protein turnover includes the degradation of damaged proteins (catabolism) and the synthesis of new proteins (anabolism). It allows non-functional, damaged, or even toxic proteins to be destroyed and replaced by functional ones. Protein breakdown is generally due to lysosomal proteases, which digest endocytosed proteins or to cytoplasmic complexes, called proteasomes, which digest old or abnormal proteins that have been tagged with ubiquitin for destruction. Protein synthesis involves the process of translation on ribosomes. It is a well-known fact that the costs associated to protein turnover represent a

large fraction of aerobic metabolism and by far the largest part of maintenance (80 to 90% according to Kooijman, 2010). For instance in vegetal species, Quigg and Beardall (2003) estimate that 30% and 36% of respiratory demand for two marine microalgae species are due to protein turnover; Scheurwater et al. (2000) estimate that between 22 and 30% of daily ATP production for two grass plant species is spent in protein turnover; Bouma et al. (1994) estimate that protein turnover in bean's leaves requires 17–35% of total dark respiration while De Visser et al. (1992) estimate that it requires 30–60% of dark leaves respiration. In the animal realm, Gill et al. (1989) estimate that protein turnover requires 19% of whole body ATP expenditure for growing lambs; White et al. (1988) estimate that it costs only 7–8% for three species of wallabies but they also report that protein synthesis accounted for approximately 21% of the heat production in young growing pigs and 17% of total heat production in finishing beef steers. MacRae and

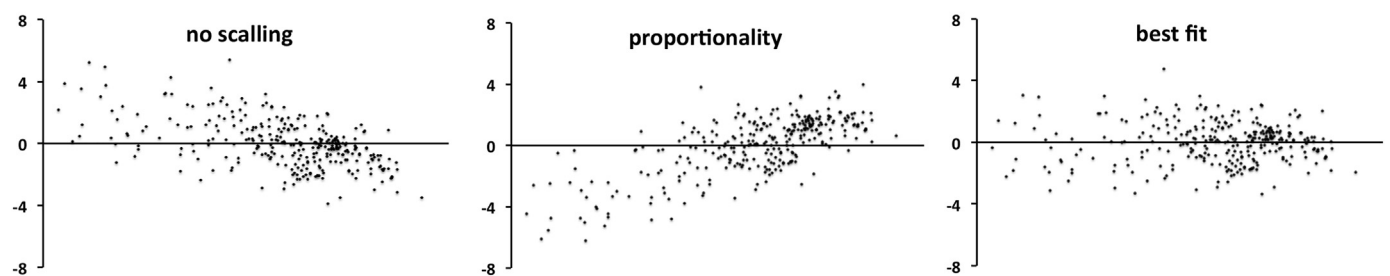
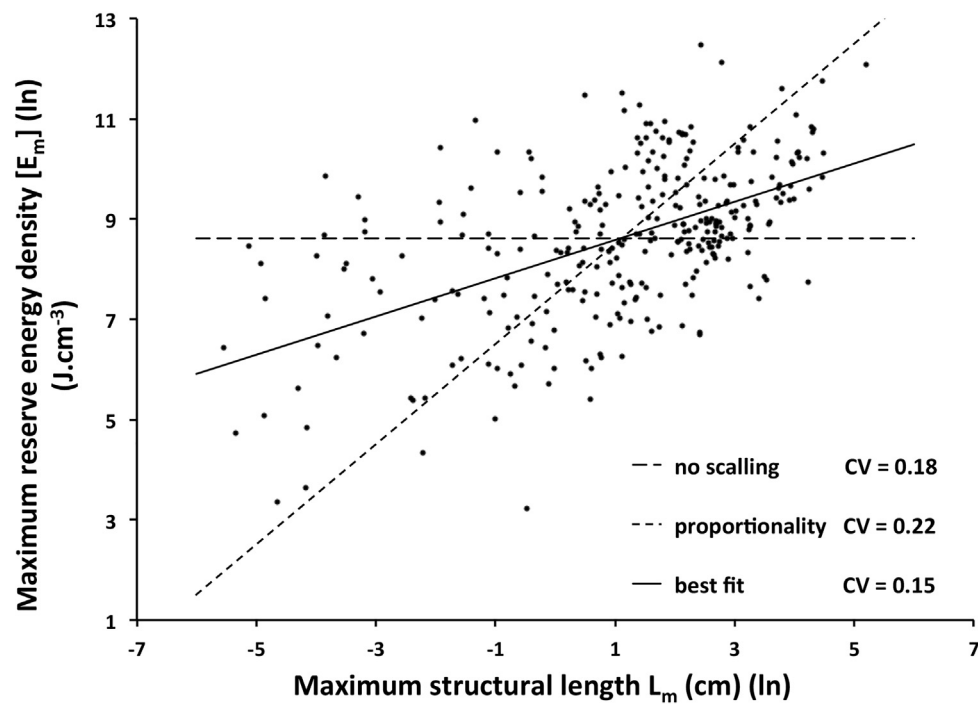


Fig. 5. First line: estimated maximum reserve energy density $[E_m]$ from the Add-my-Pet database as a function of maximum length L_m (each dot corresponds to one of the 389 entries of the database as downloaded the 25/10/2014). The en dashed line is the theoretically expected relationship $[E_m] \propto L_m$ fitted to the dots; the em dashed line corresponds to $[E_m] \propto L_m^0$ (absence of relationship between $[E_m]$ and L_m) fitted to the dots; the continuous line corresponds to the least-square linear regression of $\ln([E_m])$ versus $\ln(L_m)$, which yields $[E_m] \propto L_m^{0.3819}$. The coefficients of variations (CV) are provided on the figure for the three regressions. The smaller the CV the better the fit and the larger the CV the worst the fit. The residuals (predicted values minus observed values) as a function of L_m (cm) are shown on the second line. They clearly show that both the absence of scaling and the proportionality hypotheses are unsupported by the Add-my-Pet estimates.

Lobley (1986) derived higher values (25% of heat production) from data on lean and obese adult humans as well as Davis et al. (1981) who report 42% of heat production for growing lambs. Rabbits studied by Nicholas et al. (1977) spent 22% of total heat production for protein turnover, which is in agreement with other findings for eutherian mammals. Waterlow (1984) indeed reports values in the range of 15–20% of total resting metabolism for 6 mammal species (mouse, rat, rabbit, sheep, man, cow). Overall, the ratio between protein turnover and the energy spent in the metabolism varies in a strikingly narrow range (roughly around $30\% \pm 20\%$) in the studies shown above, despite the diversity of animal and vegetal species considered and the variety of methods used to estimate it.

7.1.2. Protein turnover is linked to aerobic metabolism

Aerobic organisms use di-oxygen to oxidize organic nutrients and produce ATP. But aerobic metabolism continuously generates toxic reactive by-products (generically named ROS for reactive oxygen species), such as superoxide anion radical, hydrogen peroxide, and the highly reactive hydroxyl radicals (Cabiscol et al., 2000). ROS continuously damage proteins as well as DNA, RNA and lipids such as polyunsaturated fatty acids in cell membranes (Birnie-Gauvin et al.,

2017; Cabiscol et al., 2000). This continuous degradation of structural molecules is highly detrimental to the functionality of cells and it would ultimately lead to cellular death if costly reparation mechanisms were not permanently deployed. The link between aerobic metabolism and protein turnover is also well established at the organism level. Empirical studies show for instance that aerobic exercise increases skeletal muscle protein turnover (e.g. Pikosky et al., 2006). At the intra-specific level again, Waterlow (1984) reports that immature animals have higher rates of protein turnover per unit of body weight than adults of the same species, even when net synthesis due to growth has been deducted. In premature infants, the net rate of protein turnover was for instance found to be twice as high as in the 1-year-old child and 3–4 times as high as in the adult (Pencharz et al., 1983). This suggests that protein turnover varies with body size, just as aerobic metabolism does. At the inter-specific level, protein turnover has been found to scale approximately with body mass at a power 0.72 (Waterlow, 2006), while the total RNA content of the liver, representing the capacity for protein synthesis, scales as body mass at a power 0.75 (Munro and Downie, 1964). This variability matches exactly the Kleiber rule (Kleiber, 1947), namely the observation that for the vast majority of animals, metabolic rate scales approximately to the $\frac{3}{4}$ power of the animal's mass, as does

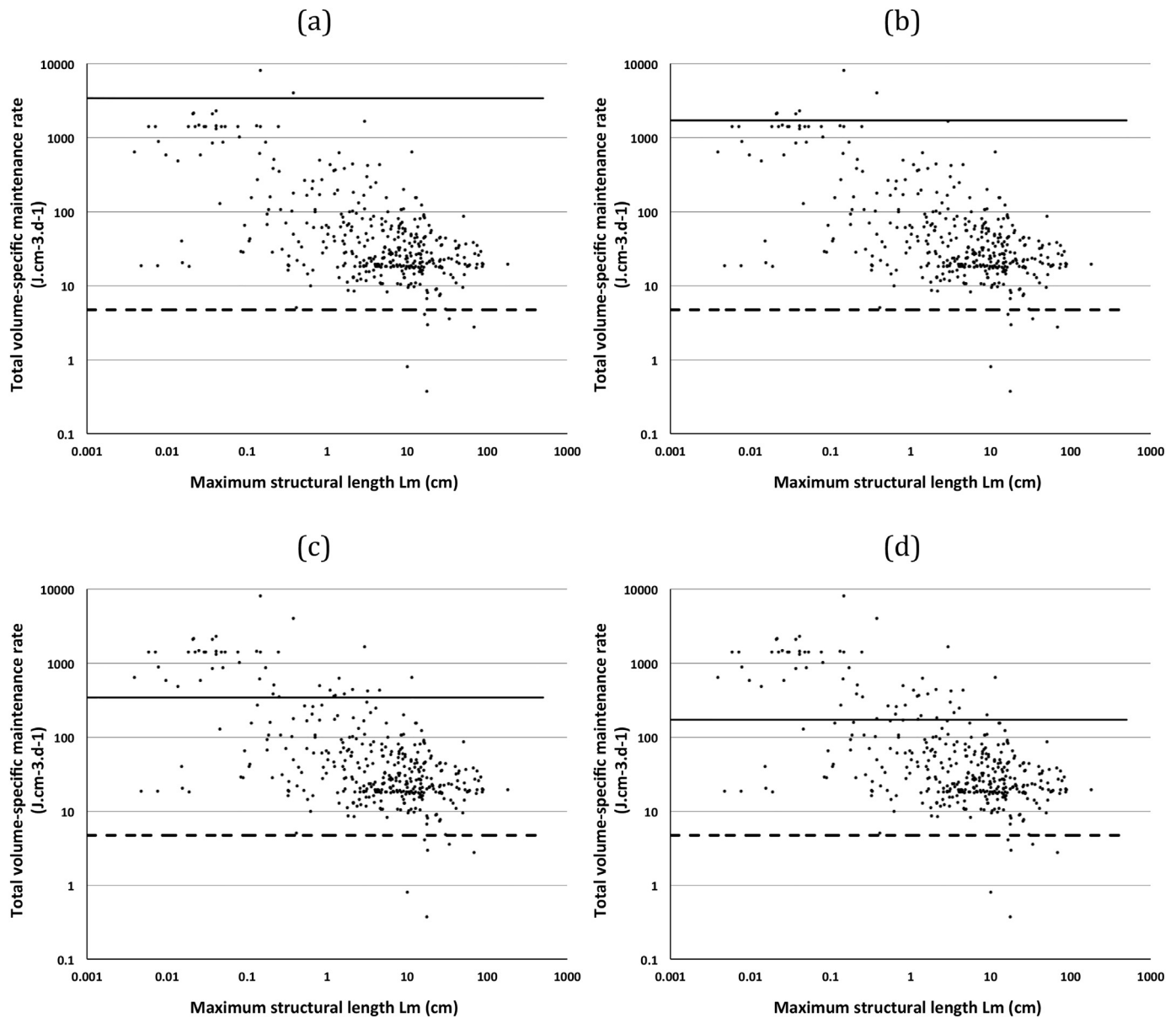


Fig. 6. Predicted value of the total volume-specific maintenance rate $[\dot{p}_p] + [\dot{p}_M]$ at birth (continuous line) and at maximum structural size (dashed line), as well as estimated volume-specific maintenance rate for the 389 entries of the Add-my-Pet database (downloaded the 25/10/2014) as a function of maximum length L_m . (a) the length at birth $L_b = 10^{-3}L_m$. (b) $L_b = 2.10^{-3}L_m$. (c) $L_b = 10^{-2}L_m$. (d) $L_b = 2.10^{-2}L_m$. Parameters values given in Table 1 are used except for $[\dot{p}_M] = 1 J \cdot cm^{-3} \cdot d^{-1}$. For the sake of drawing the figure, we assume that $k_M = k_p$. Given the covariation rules for parameter values, this implies that the length at birth is proportional to maximal length ($L_b = 10^{-3}L_m$) (Kooijman, 2010). The fraction of aerobic metabolism allocated to protein turnover is fixed at $\rho = 0.3$. The maximum reserve energy is independent from maximum structural length and equal to $[E_m] = 5510 J \cdot cm^{-3}$ according to Fig 5.

the respiration rate.

7.1.3. Linking aerobic metabolism to maintenance improves the consistency of the DEB theory and might explain the patterns in Add-my-Pet

The DEB theory recognizes the importance of ROS in degrading DNA and RNA. The ageing mortality is assumed to be proportional to the amount of cellular damages that accumulate at a rate proportional to the amount of DNA lesions, which increases at a rate proportional to the intra-cellular concentration of ROS. Finally, the rate of ROS formation is assumed to be proportional to the catabolic power \dot{p}_C , which is a good proxy for the respiration rate -excluding the consumption of oxygen due to assimilation- (Kooijman, 2000, 2010; van Leeuwen et al., 2010). It is surprising that the link between aerobic metabolism, protein and more generally structure turnover is not explicit in the DEB theory. What we propose here is to make this link explicit and to

consider that the oxidation rate of structural molecules (mostly proteins but also structural lipids, DNA and RNA) is proportional to the catabolic power \dot{p}_C , as it is assumed in the DEB theory for DNA and RNA to derive ageing mortality (Kooijman, 2010). Linking aerobic metabolism to maintenance as we propose would improve the consistency of the DEB theory by treating the oxidation of structural molecules exactly as it is done to derive ageing mortality (Kooijman, 2010) and by making the turnover of structure explicit in the maintenance rate. Doing so, we have shown that the volume-specific maintenance rate becomes linked to metabolism and displays both intra-specific (changes with the structural volume V) and inter-specific (changes with the maximum structural volume V_m) variability patterns that are compatible in their magnitude with what is observed in Add-my-Pet (Figs. 4, 6, 7 and 10). In particular, Fig. 10 drawn using the empirical trends of $\{P_{Am}\}$ and $[E_m]$ in the Add-my-Pet estimates ($\{P_{Am}\} = 98.79 L_m^{0.5662}$ and $[E_m] = 1125$

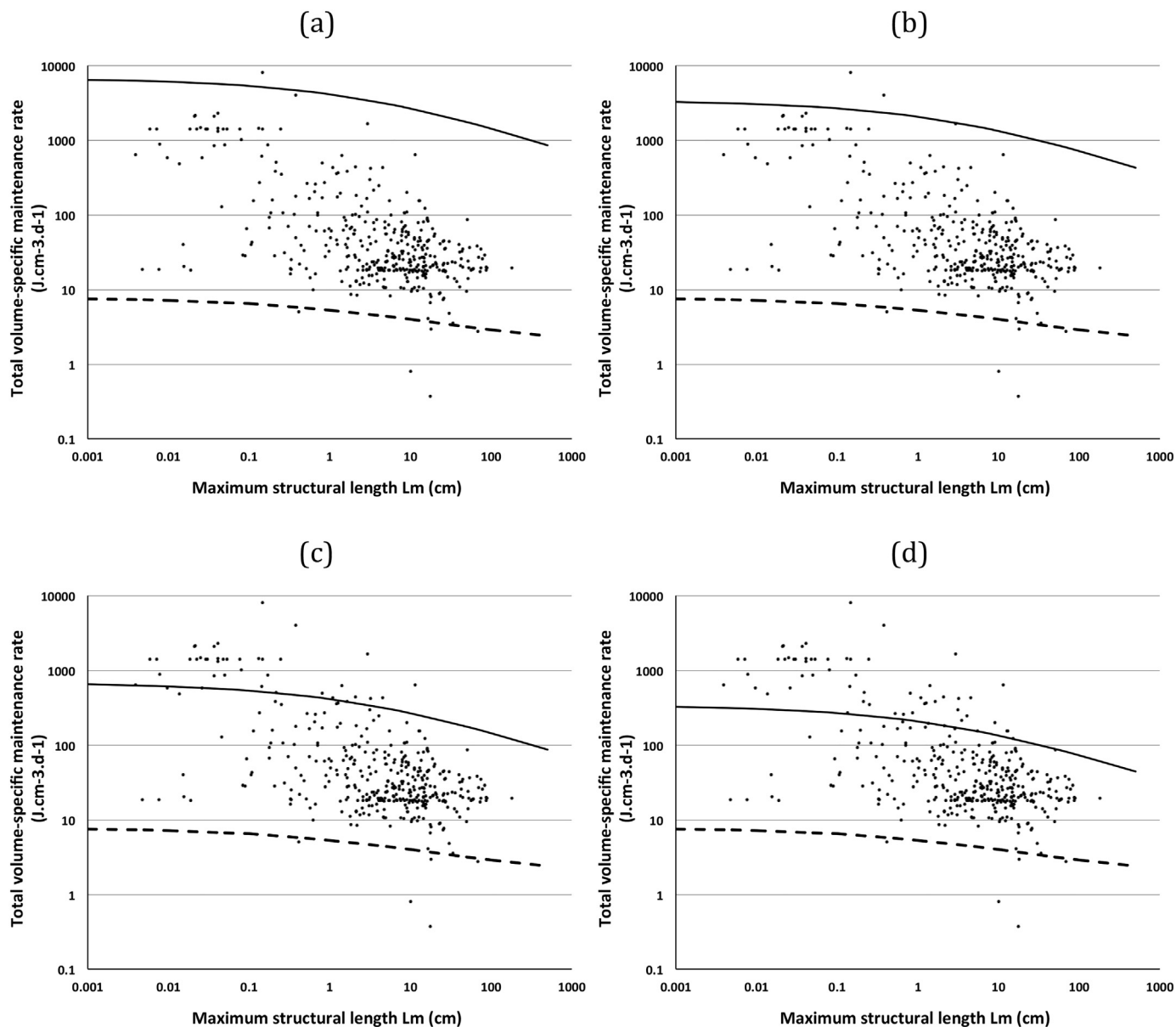


Fig. 7. Predicted value of the total volume-specific maintenance rate $[\dot{p}_p] + [\dot{p}_M]$ at birth (continuous line) and at maximum structural size (dashed line), as well as estimated volume-specific maintenance rate for the 389 entries of the Add-my-Pet database (downloaded the 25/10/2014) as a function of maximum length L_m . (a) the length at birth $L_b = 10^{-3}L_m$. (b) $L_b = 2.10^{-3}L_m$. (c) $L_b = 10^{-2}L_m$. (d) $L_b = 2.10^{-2}L_m$. Parameters values given in Table 2 are used except for $[\dot{p}_M] = 1 J \cdot cm^{-3} \cdot d^{-1}$. For the sake of drawing the figure, we assume that $k_M = k_r$. Given the covariation rules for parameter values, this implies that the length at birth is proportional to maximal length ($L_b = 10^{-3}L_m$) (Kooijman, 2010). The fraction of aerobic metabolism allocated to protein turnover is fixed at $\rho = 0.3$. The maximum reserve energy density scales with maximum structural length as $[E_m] = 3612.5 L_m^{0.3819} (J \cdot cm^{-3})$ according to Fig 5.

$L_m^{0.3819}$, cf. Figs. 1 and 5) demonstrates that accounting for protein turnover enables to explain both the estimated trend and the variability of maintenance. Our proposition would therefore simultaneously restore the covariation rules for parameter values implied by the standard DEB model (the volume-specific somatic maintenance rate $[\dot{p}_M]$ would keep approximately constant between species -as would ρ , the fraction of aerobic metabolism allocated to protein turnover- and the maximum surface-specific assimilation rate $\{\dot{p}_{Am}\}$ would scales with maximum structural size) by explaining a substantial part of the intra- and inter-specific variability of estimated maintenance while accounting for major processes of the metabolism (the link between aerobic metabolism, the production of ROS and maintenance costs) that were previously overlooked in the DEB theory.

7.2. The « waste to hurry » hypothesis

Kooijman (2014) proposes the “Waste to Hurry” hypothesis to explain the decreasing trend of volume-specific maintenance rate with maximum length. The “Waste to Hurry” is an evolutionary argument. It states that high maintenance is a way to speed-up metabolism to track efficiently high frequency changes in environmental conditions. High maintenance would therefore be an adaptation to variable environments. The hypothesis proposed here doesn't contradict the “Waste to Hurry”. On the contrary, it provides clear mechanisms for it. In our framework, if a species “needs” its maintenance to be high to hurry, it just needs to be small (namely have a small maximal volume-specific assimilation rate $\{\dot{P}_{Am}\}$), have a small structural volume at birth V_b and die long before reaching its maximum structural volume V_m .

Kooijman (2014) proposes that futile cycles could underlie the

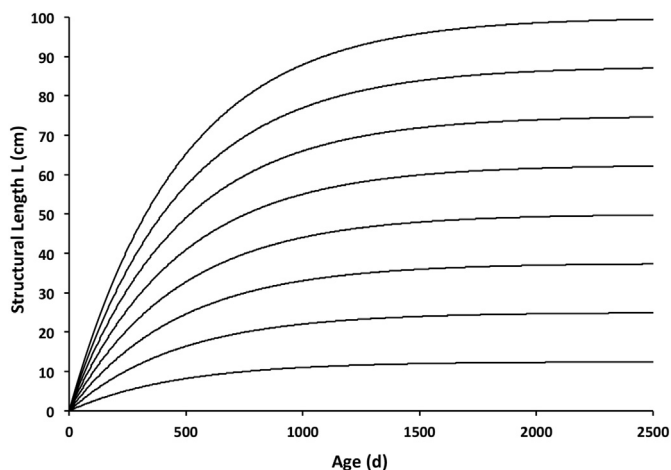


Fig. 8. Von Bertalanffy growth curve at $f = 1$ with the fraction of aerobic metabolism allocated to protein turnover ρ varying from 0 (upper curve) to 0.7 (lower curve) with a 0.1 increment. The maximum structural length is arbitrarily taken to be equal to $L_m = 100\text{cm}$ for $\rho = 0$ and the parameters given in Table 2 are used except for the maximum reserve energy density, which scales with maximum structural length as $[E_m] = 3612.5 L_m^{0.3819} (J \cdot \text{cm}^{-3})$ according to Fig 5.

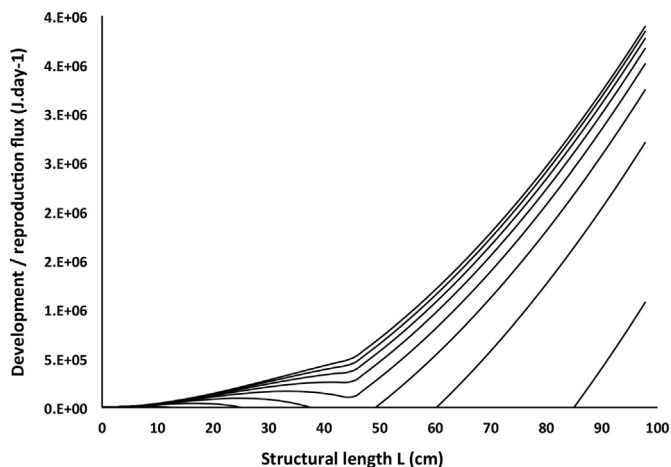


Fig. 9. Development/reproduction flux at $f = 1$ with the fraction of aerobic metabolism allocated to protein turnover ρ varying from 0 (upper curve) to 0.7 (lower curve) with a 0.1 increment. The maximum structural length is arbitrarily taken to be equal to $L_m = 100\text{cm}$ for $\rho = 0$ and the parameters given in Table 2 are used except for the maximum reserve energy density, which scales with maximum structural length as $[E_m] = 3612.5 L_m^{0.3819} (J \cdot \text{cm}^{-3})$ according to Fig. 5. The length at puberty is arbitrarily fixed at 45 cm.

“waste to hurry” hypothesis and explain the high maintenance of small species. We are however not aware of observations that would corroborate this proposition. Another possible explanation for the existence of futile cycle is that metabolic pathways that are not activated continuously must be maintained in activity to be able to restart immediately when needed, just by deactivating the negative part of the futile cycle. Otherwise cells would need to re-synthesize the oxidized enzymes involved and the intermediary products each time they would need to start producing the final product. If one needs to drive 0 to 100 km/h in 5 s when the traffic light turns green, it is better to keep the engine running and just put into gear and accelerate rather than rebuilding the engine, refilling the oil and gas tanks, restarting the engine and accelerate to keep up to the needs....

7.3. Re-estimating the DEB parameters?

7.3.1. The variability of maintenance in Add-my-Pet is consistent with the predictions made considering the turnover of structure explicitly

If we admit that a substantial fraction of maintenance varies with aerobic metabolism, Eq. (8) shows that the importance of maintenance has to change dramatically within species (with V) and between species (with V_m). For any individual of a given species, the total volume-specific maintenance rate decreases from fecundation to maximum structural volume over several orders of magnitude. Assuming for the sake of drawing the figure that the structural length at birth L_b is proportional to the maximum structural length L_m , Fig. 10 illustrates this phenomenon from birth (at the onset of feeding, between the embryo stage and the juvenile stage) to maximal size. For a given species of maximal size L_m^1 , the total volume-specific maintenance rate decreases along the arrow from $[\dot{p}_M + \dot{p}_p](L_b^1)$ at birth to $[\dot{p}_M + \dot{p}_p](L_m^1)$ for a fully grown individuals. The DEB theory presently overlooks this important intraspecific variation of maintenance and assumes that the volume-specific maintenance rate keeps constant from fecundation to death. Estimated values of $[\dot{p}_M]$ in Add-my-Pet are therefore likely to fall somewhere in between the minimum and maximum expected values, reflecting a sort of average value of total volume-specific maintenance rate ($[\dot{p}_M + \dot{p}_p]$) over the size range of the data used for parameter estimation, and destabilizing the parameter estimation process when the data used correspond to very different size ranges. Fig. 10 shows that most estimated $[\dot{p}_M]$ values indeed fall in between the expected minimum and maximum values for the total volume specific maintenance rate. A few data points are however higher than the expected value at V_b , despite the fact that the size at birth used for drawing the figure is already quite small ($L_b = 8.10^{-3} L_m$). This could be due to the use of data collected during the embryonic stage for parameter estimation. Embryos have indeed a structural volume potentially much smaller than the structural volume at birth and therefore a total volume-specific maintenance rate much higher than its expected value at birth. Finally, the good match of predictions with Add-My-Pets estimates in Fig. 10 also suggests that part of the intra and inter-specific maintenance trends due to protein turnover has been erroneously attributed to $[\dot{p}_M]$, $\{\dot{P}_{Am}\}$ and $[E_m]$ by the Add-my-Pet parameter estimation procedure, to compensate for the fact that the standard DEB model considers the volume-specific maintenance rate to keep constant at the intra-specific level.

7.3.2. Modifying the DEB model implies that parameters have to be re-estimated

The numerical values given in the present paper to the fraction of aerobic metabolism allocated to protein turnover ($\rho = 0.3$) and to the volume-specific structural maintenance rate ($[\dot{p}_M] = 4 J \cdot \text{cm}^{-3} \cdot \text{d}^{-1}$) were chosen arbitrarily according to empirical observations showing that protein turnover represents around $30\% \pm 20\%$ of total resting metabolism (e.g. Waterlow, 2006, cf. the 1st paragraph of the discussion section) and 80–90% of total maintenance costs (Kooijman, 2010). All the other parameter values used here (Table 2) were those given in Kooijman (2010) to represent a generic organism. Fig. 4 shows that with these parameters' values, the costs of structure turnover of large species represents roughly from 50% to 95% of total maintenance costs for large and small individuals respectively and for small species it accounts from 90% to 99.95% of total maintenance.

However, if our proposition is true, the DEB core equations have to be modified (Eq. 5) and their parameters re-estimated. Even if they don't change qualitatively, testable predictions such as growth, reproduction or respiration curves that are used to estimate the parameters change quantitatively when introducing the cost of structure turnover in maintenance (Figs. 8 and 9), and the relative importance of the underlying energy fluxes also changes. Consequently, fitting the modified DEB equations to observations will change the parameters' values that have previously been estimated. This is a serious

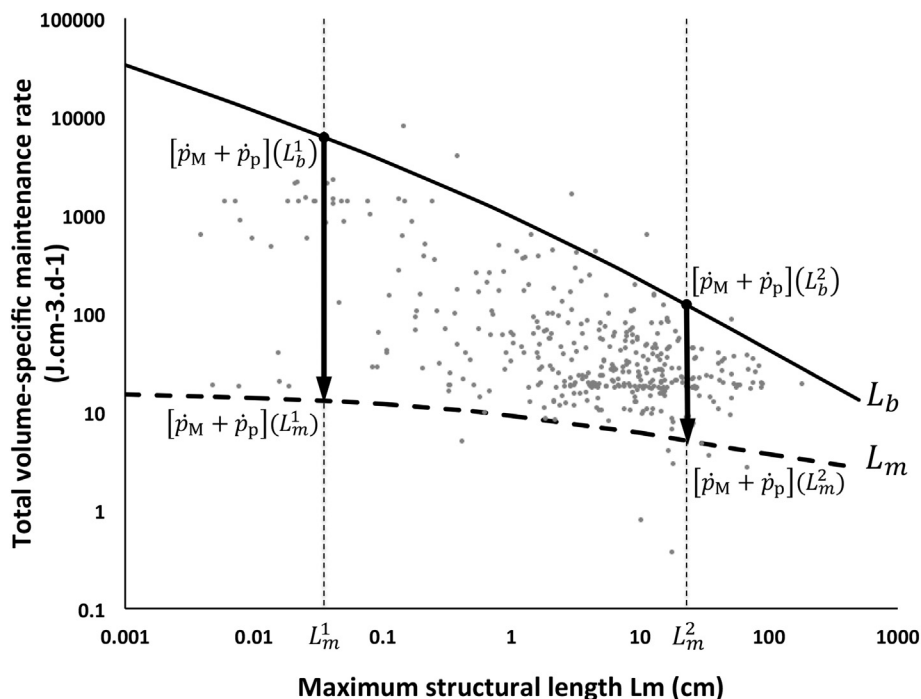


Fig. 10. Predicted value of the total volume-specific maintenance rate $[\dot{p}_p] + [\dot{p}_M]$ at birth (continuous line) and at maximum structural size (dashed line), as well as estimated volume-specific maintenance rate (grey dots) for the 389 entries of the Add-my-Pet database (downloaded the 25/10/2014) as a function of maximum length L_m . For the sake of drawing the figure, we assume that $k_M = k_J$. Given the covariation rules for parameter values, this implies that the length at birth is proportional to maximal length (Kooijman, 2010) and we fix it arbitrarily at $L_b = 8.10^{-3}L_m$. The fraction of aerobic metabolism allocated to protein turnover is fixed at $\rho = 0.15$. According to the empirical trends in the Add-my-Pet estimates, the maximum volume-specific assimilation rate is supposed to scale with maximum structural length as $[\dot{p}_{Am}] = 98.79 L_m^{0.5662}$ (cf. Fig. 1) and the maximum reserve energy density is assumed to scale with maximum structural length as $[E_m] = 1125 L_m^{0.3819}$ (cf. Fig. 5). All the other parameters' values given in in Table 2 are used except for $[\dot{p}_M] = 1 J \cdot cm^{-3} \cdot d^{-1}$

consequence of our proposition. It implies that parameter's values estimated with the current version of the DEB model, such as those in the Add-my-Pet collection, are not valid for use with the modified DEB model. If we admit that the effects of aerobic metabolism on the turnover of structure have to be included in the DEB equations, then parameters have to be re-estimated for every species considered in the Add-my-Pet collection.

7.3.3. Parsimony has to be regarded at the inter-specific level

The 14 primary parameters of the standard DEB model (including the 5 core parameters $\{\dot{p}_{Am}\}, [\dot{p}_M], \psi, [E_G],$ and κ presented Table 2) are usually difficult to estimate as they have confounded effects on the model's predictions that can be compared to data (e.g. Marques et al., 2018a). The information content of the data is furthermore often too weak to identify all the parameters simultaneously (Marques et al. 2018b). One strategy to overcome this over-parameterization issue is to use observations of different nature simultaneously (for instance growth data with length-weight observations, reproduction and respiration data), in an integrated statistical estimation framework (e.g. Lika et al., 2011). Adding one extra core-parameter to the standard model (the fraction ρ of aerobic metabolism allocated to protein turnover as we propose here) can be seen as a non-parsimonious extension of the model in a situation where over-parameterization is already an issue. We believe that this is a superficial view however, which omits to consider the problem in its broader inter-specific dimension. If the model is kept in its present form with a constant volume-specific somatic maintenance rate $[\dot{p}_M]$, the empirical falsification of the inter-specific scaling of maintenance and its evolutionary justification (waste to hurry) imply that $[\dot{p}_M]$ becomes a free parameter that has to be re-estimated for every species considered. The number of degrees of freedom of the DEB model is therefore increasing dramatically with the number of species considered, at the expense of parsimony. On the

contrary, we have shown that considering the aerobic roots of structure turnover explicitly would restore the inter-specific scaling rules and thus dramatically reduce the number of degrees of freedom of the model since $[\dot{p}_M]$ and ρ would keep constant between species, at least in a given taxa. In this case, individual bioenergetics would be captured for any species using the 14 primary parameters of the model plus the new parameter (ρ). Our proposition would therefore considerably improve the parsimony of the DEB theory, considered simultaneously at the intra- and inter-specific levels.

7.3.4. Re-estimating the DEB parameters: toward an integrated intra- inter-specific estimation strategy?

The new formulation of the DEB model proposed here requires that the model's parameters be re-estimated. Re-estimating simultaneously the standard DEB parameters and the new parameter ρ might be challenging, in a situation where over-parameterization and parameter confounding is already a difficult issue for the standard DEB model (Lika et al., 2011; Marques et al., 2018a, 2018b). This is especially true considering that κ and ρ often appear together in the modified equations (as in the new catabolic power Eq. 5, or the new growth Eq. 11), and are therefore likely to be difficult to estimate simultaneously. This is not the case in the new development/reproduction flux equation (Eq. 17) however, as the energy allocated to reproduction, development and its maintenance keeps proportional to $(1 - \kappa)$. This might enable the simultaneous estimation of κ and ρ when data constraining \dot{p}_G and data constraining \dot{p}_R are available and can be used simultaneously.

When such complementary data are not available, a possible strategy would be to take advantage of the considerable amount of information held in the inter-specific variability of maintenance regarding the value of the new parameter ρ (Figs. 4, 6, 7 and 10). The modified DEB parameters could indeed be estimated for several species simultaneously, ideally covering a wide range of maximum length, and

assuming that $[\dot{p}_M]$ and ρ keep constant between species, or at least between species of the same taxa. This approach could certainly be tested using a selection of species in the Add-my-Pet collection.

7.4. Scaling of the maximum reserve energy density

In the framework of the DEB theory, the maximum reserve energy density $[E_m]$ is an extensive compound parameter (supposed to be proportional to maximum structural length L_m and equal to $\{\dot{p}_{A_m}\}/\dot{v}$). With its usual value ($[E_m]_{ref} = 1125 \text{ J. cm}^{-3}$ for $L_m = 1 \text{ cm}$), and assuming for simplicity that the energy content of reserve and structure is the same and equal to 4 J. cm^{-3} (Kooijman, 2010), the scaling of $[E_m]$ with L_m implies that the reserve compartment of a $L_m = 10 \mu\text{m}$ micro-organism would account for approximately 22% of body weight and 58% for a $L_m = 50 \mu\text{m}$ organism. This corresponds to the range of values measured for planktonic organisms for which reserves constitute from 30% to 60% of body weight (e.g. Granum et al., 2002; Laws and Bannister, 1980; Lopez et al., 2016). However, assuming that $[E_m]$ is proportional to maximum structural length L_m also implies that larger animals would be composed of an unrealistic amount of reserve (96.56%, 99.64%, 99.96% and 99.99% for organisms of structural length $L_m = 1 \text{ mm}$, 1 cm , 10 cm and 1 m respectively). This unrealistic implication of the theoretical scaling of maximum reserve density is corroborated by the empirical pattern of $[E_m]$ versus L_m in Add-my-Pet, which doesn't match the theoretical expectation either. In Add-my-Pet, estimated $[E_m]$ are indeed scaling approximately with $L_m^{0.4}$, and they display an important variability around this trend (Fig. 5). This absence of clear scaling of $[E_m]$ with L_m is also observed at the taxa level, with some taxa displaying no scaling of maximum reserve capacity (e.g. *actinopterygii*) and other that seem to display some weak positive relationship between maximum length and maximum energy density (e.g. *chondrichthyes*) (Kooijman and Lika, 2014).

What the scaling of maximum reserve density estimated in Add-my-Pet would become with the modified DEB equations is not known however, as all the parameters including $[E_m]$ (or \dot{v}) would have to be re-estimated if the DEB model is modified (see above). In the absence of a non-ambiguous theoretical argument and no empirical indication in favour of a scaling of $[E_m]$ with maximum structural length L_m , we suggest that $[E_m]$ (or alternately \dot{v}) be re-estimated as a free parameter with the modified DEB equations for every species considered so that the scaling of the maximum reserve capacity with maximum length can be re-evaluated empirically. The reserve compartment allows covering the metabolic needs between two feeding events. When reserves are not sufficient, growth ceases and mild starvation starts. Maximum reserve energy density is therefore a critical parameter that is controlling the time to starvation in the absence of food. It is logical to assume that evolution has optimized its value according to the variability of the environment in which the considered species is living. We are therefore expecting an important inter-specific variability of $[E_m]$, but not necessarily a strong relationship with L_m .

The scaling of $[E_m]$ with maximum structural length L_m has a strong influence on the scaling of the total volume-specific maintenance rate including the cost of structure turnover (Figs. 4, 6 and 7). Empirical patterns of maintenance rate in Add-my-Pet are fully compatible with maximum reserve energy density $[E_m]$ varying less than proportionally to L_m (Fig. 10).

8. Conclusion

The inter-specific variability of estimated maintenance rates in the Add-my-Pet collection (Kooijman, 2014) reveals troubling patterns apparently violating the covariation rules for parameter values implied by the standard DEB model and challenging the DEB theory. Protein (and more generally structure) turnover rate constitutes an important component of maintenance, which varies with aerobic metabolism. We propose that this dependence on metabolism could explain the apparent

decrease of volume-specific maintenance rate with species maximum structural size and its variability. If true, this would require modifications of the standard DEB theory in order to capture inter-specific scaling patterns of DEB-parameters while keeping the consistency of the theory at the intra-specific level.

We believe that our proposition would strengthen the consistency of the DEB theory. It would indeed relate the maintenance of structure to aerobic metabolism in a way that is supported by current knowledge regarding protein turnover and that is fully consistent with the treatment of ageing in the DEB theory. Our proposition would restore the DEB covariation rules for parameter values, which state that the volume-specific somatic maintenance rate $[\dot{p}_M]$ remains approximately constant between species and the maximum surface-specific assimilation rate $\{\dot{p}_{A_m}\}$ scales with maximum structural size. It would explain mechanistically the trends and most of the variability of these parameters in Add-my-Pet. The inter-specific variability that would remain would be a good candidate for evolutionary interpretations and characterization of specific life history strategies.

The modifications that we propose to the DEB theory would not change the qualitative nature of standard DEB predictions (e.g. growth or reproduction curves). However, the core DEB parameters would need to be re-estimated along with the new parameter ρ , the fraction of aerobic metabolism allocated to protein turnover. We believe that adding one extra intensive parameter as we suggest is actually more parsimonious and therefore preferable than re-estimating $[\dot{p}_M]$ for every species, as required by the current formulation of the DEB model that cannot rest on interspecific scaling rules anymore.

Finally, we suggest that parameter estimation for selected species should be conducted with the modified DEB equations to test our proposition.

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