

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

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

3
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5
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12
13
14 **Abstract:**

15 Maintenance is the energy that living organisms are bound to use to maintain their
16 structure in a viable state. It includes all the metabolic and physiological costs that are not
17 directly associated to the production of biomass (growth and reproduction) or to
18 development (maturation). In the framework of the DEB theory, somatic maintenance rate
19 can either be proportional to organism structural volume V or, more marginally, to
20 structural surface $V^{2/3}$. Being mostly associated to similar metabolic processes, volume-
21 specific maintenance costs are not expected to vary substantially at both intra- and inter-
22 specific levels. In the DEB theory, the volume-specific maintenance rate $[\dot{p}_M]$ is therefore
23 supposed to keep constant from birth to death and to remain approximately constant
24 between species. However, a recent meta-analysis of DEB parameters estimated using the
25 Add-my-Pet collection (Kooijman, 2014) reveals troubling patterns apparently violating this
26 inter-specific scaling rule and challenging the DEB theory. It is indeed shown in this study
27 that empirically-derived volume-specific maintenance rates scale approximately with
28 $L_m^{-0.4}$ and display a very high variability around this trend. Overall, estimated maintenance
29 rates in Add-my-Pet span over three to four orders of magnitude, thus invalidating the
30 assumption of constant maintenance rate between species, which underpins the covariation
31 rules for parameter values of the DEB theory. In an attempt to address this major problem
32 for the DEB theory, we propose a simple physiological mechanism that would

33 simultaneously explain the apparent decrease of volume-specific maintenance rate with
34 ultimate size and its apparent variability for a given range of maximum size. Our
35 proposition consists in making protein (and more generally structure) turnover explicit in
36 maintenance and linking protein damage rate to aerobic metabolism and the production of
37 ROS, which are decreasing with both structural volume and maximum structural volume.
38 We show that this implies that the actual volume specific maintenance rate varies both at
39 the intra- and inter-specific levels in a range very similar to what is observed in the Add-my-
40 Pet data estimations. If true, this implies that the apparent decrease of volume-specific
41 maintenance rate with ultimate size is an artefact and it requires modifications of the
42 standard DEB theory in order to capture empirical inter-specific scaling patterns of DEB-
43 parameters while keeping the consistency of the theory at both intra- and inter-specific
44 levels.

45

46 **Introduction**

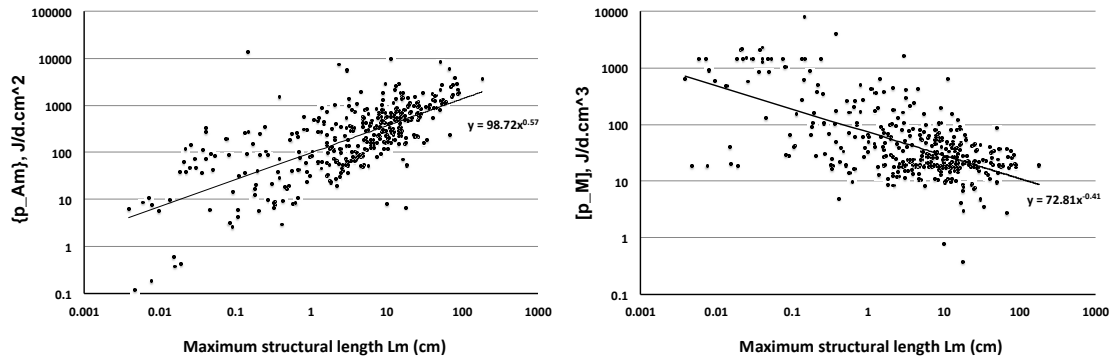
47 The DEB theory (e.g. Kooijman, 2010) is the most comprehensive metabolic theory of life
48 existing to date (van der Meer, 2006; Jusup et al., 2017). It is also the best tested empirically,
49 thanks to its ability to generate a variety of distinct testable predictions, both at the intra-
50 and the inter-specific levels (Kooijman, 2010; Jusup et al., 2017). Recently, for the first time,
51 empirical estimates of DEB parameters have been collected for an increasingly large
52 number of species and gathered in the Add-my-Pet collection
53 (http://www.bio.vu.nl/thb/deb/deblab/add_my_pet/index.html). Add-my-Pet provides a
54 unique opportunity to look at the way DEB parameter values are distributed among species,
55 hoping that deviations from the generic theoretical expectation would reveal evolutionary
56 adaptations to specific environments and characterize particular life history strategies. Add-
57 my-Pet also offers a chance to test the validity of the interspecific scaling rules, and in
58 particular the fundamental assumption that the volume-specific somatic maintenance rate
59 $[\dot{p}_M]$ remains approximately constant between species and that, as a corollary, the
60 maximum surface-specific assimilation rate $\{\dot{p}_{A_m}\}$ scales with maximum structural size.

61 Maintenance is the energy that living organisms are bound to use to maintain their
62 structure in a viable state. Maintenance includes all the metabolic and physiological costs
63 that are not directly associated to the production of biomass (growth and reproduction) or
64 to development (maturation). These comprehend the costs of removing and replacing

65 damaged proteins, maintaining chemical and electrical gradients through cellular
66 membranes, maintaining the immune system functional, forming products (scales, hair,
67 nails, etc), maintaining muscular tonicity, circulating body fluids (blood, lymph, etc),
68 moving, maintaining a constant body temperature for endotherms or a constant osmotic
69 pressure for aquatic organisms, etc. In the framework of the DEB theory, somatic
70 maintenance rate can either be proportional to organism structural volume V or to
71 structural surface $V^{2/3}$ (e.g. Kooijman, 2000). Being mostly associated to similar metabolic
72 processes, volume-specific maintenance costs have no obvious reason to vary substantially
73 at both intra- and inter-specific levels. In the DEB theory, the volume-specific maintenance
74 rate $[\dot{p}_M]$ is therefore supposed to remain approximately constant between species.
75 Consequently, since the maximum length that a given species can reach is proportional to
76 the ratio of its maximum surface-specific assimilation rate divided by the volume-specific
77 maintenance rate ($L_m = \frac{\kappa\{\dot{p}_{Am}\}}{[\dot{p}_M]}$), maximum surface-specific assimilation rate is expected to
78 scale with the maximum organism length L_m (Kooijman, 2006). The inter-specific scaling of
79 the maximum surface-specific assimilation rate is fundamental to the DEB theory. It is at the
80 core of the covariation rules for parameter values that explain why a small set of “extensive”
81 parameters scale with maximum structural length while “intensive” parameters are
82 independent from it. This provides mechanistic explanations to well-established empirical
83 body-size scaling relationships of important life-history traits such as respiration rate,
84 gestation time, incubation time or growth rate for instance, amongst many other (Kooijman,
85 2010). Furthermore, body-size scaling relationships can be used as a solid basis to derive
86 models of ecological communities that integrate the diversity of life-history traits from
87 small to large species (Maury and Poggiale, 2013).

88 However, the examination of estimated somatic maintenance rate and maximum surface-
89 specific assimilation rate as a function of the species maximum size in the Add-my-Pet
90 collection (Kooijman, 2014) reveals troubling patterns apparently violating the covariation
91 rules for parameter values and challenging the DEB theory. Kooijman (2014) indeed shows
92 that empirically-derived maximum surface-specific assimilation rates scale approximately
93 with $L_m^{0.6}$ (instead of scaling with L_m as predicted by the DEB theory) while volume-
94 specific maintenance rates scale approximately with $L_m^{-0.4}$ (instead of remaining constant
95 as assumed by the DEB theory). Further to these trends, both rates exhibit a very high and
96 unexpected variability around their tendency (Fig. 1, see also

97 http://www.bio.vu.nl/thb/deb/deblab/add_my_pet/patterns.html for the most recent
 98 figure with more species included).



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

104

105 The large number of species included in the Add-my-Pet collection provides robustness to
 106 the trends identified for both parameters and to the important and systematic variability of
 107 the estimates around these trends. Overall, estimated maintenance rates span over three to
 108 four orders of magnitude, with maintenance of the smallest species ($L_m \approx 10^{-2} cm$) being in
 109 average three orders of magnitude higher than maintenance of the largest species
 110 considered ($L_m \approx 10^2 cm$). In average, the dispersion around this trend varies from 1 to
 111 more than 2 orders of magnitude for a given maximum size. These patterns clearly deviate
 112 from the theoretical DEB expectations. Kooijman (2014) proposes the “waste to hurry”
 113 hypothesis to explain them. The rationale is evolutionary. It assumes that species in variable
 114 environments would have increased their assimilation rate and simultaneously evolved
 115 means to waste their energy by increasing their maintenance for remaining small, growing
 116 fast and reproducing early. This would speed-up their life cycle and allow these species to
 117 adapt to environments where the availability of resources undergoes large and high
 118 frequency changes. The mechanism proposed by Kooijman (2014) involves the use of futile
 119 cycles that appear when two biochemical reactions run simultaneously in opposite
 120 directions and compensate each other, thus dissipating energy with no net production of
 121 one compound and therefore no obvious purpose.

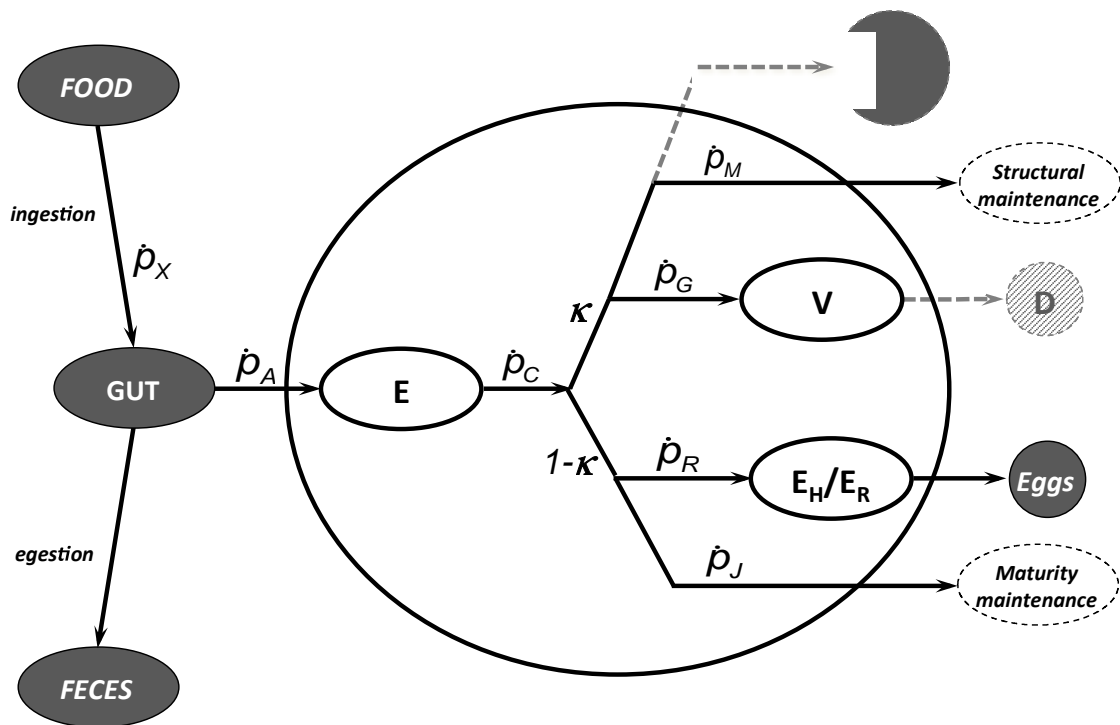
122 We believe that the empirical patterns of maintenance revealed in Add-my-Pet have much
123 profound impacts on the DEB theory. They are indeed too systematic to be considered as
124 simple deviations from the theoretical expectations: the volume-specific maintenance rate
125 can obviously not anymore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

140 In an attempt to address this major problem for the DEB theory, we propose a simple
141 physiological mechanism that would simultaneously explain the apparent decrease of
142 volume-specific maintenance rate with ultimate size and its apparent variability for a given
143 range of maximum size. Our proposition rests on the idea that protein (and more generally
144 structure) turnover constitutes an important component of maintenance (e.g. Bouma et al.,
145 1994; Kooijman, 2010; Waterlow, 1984), which varies with aerobic metabolism (e.g.
146 Cabiscol et al., 2000; Pikosky et al., 2006; Waterlow, 1984, 2006), and hence decreases with
147 size at both intra- and inter-specific levels. If true, it implies that the apparent decrease of
148 volume-specific maintenance rate with ultimate size and its variability are artefacts and it
149 requires modifications of the standard DEB theory in order to capture empirical inter-
150 specific scaling patterns of DEB-parameters while keeping the consistency of the theory at
151 the intra-specific level. As a corollary, it also implies that the DEB parameters estimated
152 using the standard DEB model are not valid with the modified DEB model and need to be re-
153 estimated.

154

155 **Standard Dynamic Energy Budget (DEB) model**

156 The Dynamic Energy Budget (DEB) theory (e.g. Kooijman, 2000, 2010) describes
 157 mechanistically the processes involved in the acquisition and use of energy by individual
 158 organisms. The energetics of individuals is represented using three state variables: energy
 159 stored in the reserve compartment E (J), structural volume V (cm³) (with the associated
 160 structural length L (cm) defined as $L = V^{1/3}$), and energy stored in the reproductive buffer
 161 E_R (J). Energy fluxes between those compartments are made explicit through the use of
 162 powers \dot{p} (J.s⁻¹) (see Fig. 2 and Table 1). For every individual organism, energy in food is
 163 ingested (\dot{p}_X) and assimilated (\dot{p}_A) before being stored into reserves. Reserves are
 164 mobilized (\dot{p}_C) and a fixed fraction κ of the energy utilized from reserves is allocated to
 165 growth of structural material (\dot{p}_G) and somatic maintenance (\dot{p}_M), the remaining fraction
 166 $1 - \kappa$ being devoted to maturity maintenance (\dot{p}_J) and development or reproduction (\dot{p}_R).
 167 Only a fraction κ_R of the energy in E_r is turned into eggs reserve.
 168 The five DEB core parameters used in this study and their value given in Kooijman (2010)
 169 for a $L_m=1$ cm organism is provided Table 2. By convention, [] stands for volumetric
 170 concentrations and { } for surface-specific concentrations so that $[E] = E/V$ and $\{\dot{p}_{x_m}\} =$
 171 $\dot{p}_{x_m}/V^{2/3}$ for instance (Kooijman, 2000). All the rates have a dot like \dot{p}_X to indicate the
 172 dimension « per time ».



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

178
 179
 180 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 | $\dot{p}_X = \{\dot{p}_{X_m}\}fV^{2/3}$ |
| Assimilation | $\dot{p}_A = \kappa_X \dot{p}_X = \{\dot{p}_{A_m}\}fV^{2/3}$ |
| 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 | $\dot{p}_J = \frac{1 - \kappa}{\kappa} [\dot{p}_M] \min(V, V_p)$ |
| { Maturation ($V < V_p$) { Reproduction ($V \geq V_p$) | $\dot{p}_R = (1 - \kappa) \dot{p}_C - \dot{p}_J$ $= (1 - \kappa) \left[\frac{[E]}{[E_G] + \kappa[E]} \left([E_G] \dot{v} V^{2/3} + [\dot{p}_M] V \right) - \frac{[\dot{p}_M] \min(V, V_p)}{\kappa} \right]$ |

181

182 Table 2: main DEB parameters used in this study and their value given in Kooijman (2010) for a

183

$L_m=1\text{cm}$ organism.

| Parameters | Symbol | Value an unit |
|---|-------------------------------------|--|
| Maximum surface-specific assimilation rate | $\{\dot{p}_{A_m}\}$ | 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 | $\dot{v} = \{\dot{p}_{A_m}\}/[E_m]$ | 0.02 cm.d ⁻¹ |
| Scaled functional response | f | 1 / |

185 **Somatic maintenance and the protein turnover rate**

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

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

$$215 \quad \kappa \dot{p}_c = \dot{p}_M + \dot{p}_{P_r} + [E_G] \left(\frac{dV}{dt} + \dot{D} \right) \quad (1.)$$

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

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

$$223 \quad \kappa\dot{p}_c = \dot{p}_M + ([\gamma] + [E_G])o\dot{p}_c + [E_G]\frac{dV}{dt} = \dot{p}_M + \rho\dot{p}_c + [E_G]\frac{dV}{dt} \quad (2.)$$

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

226 Equation (2) can be reorganized as

$$227 \quad \dot{p}_c = \frac{\dot{p}_M + [E_G]\frac{dV}{dt}}{\kappa - \rho} \quad (3.)$$

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

$$229 \quad \dot{p}_c = [E] \left(\frac{\{\dot{P}_{Am}\}}{[E_m]} V^{2/3} - \frac{dV}{dt} \right) \quad (4.)$$

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

$$232 \quad \dot{p}_c = [E] \left(\frac{\{\dot{P}_{Am}\}}{[E_m]} V^{2/3} - \frac{dV}{dt} \right) = \frac{[E]([E_G]\dot{V}V^{2/3} + \dot{p}_M)}{[E_G] + [E](\kappa - \rho)} = \frac{[E]([E_G]\dot{V}V^{2/3} + [\dot{P}_M]V)}{[E_G] + [E](\kappa - \rho)} \quad (5.)$$

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

$$235 \quad [\dot{p}_p] = \rho[\dot{p}_c] = \frac{\rho[E]([E_G]\dot{V}V^{-1/3} + [\dot{P}_M])}{[E_G] + [E](\kappa - \rho)} \quad (6.)$$

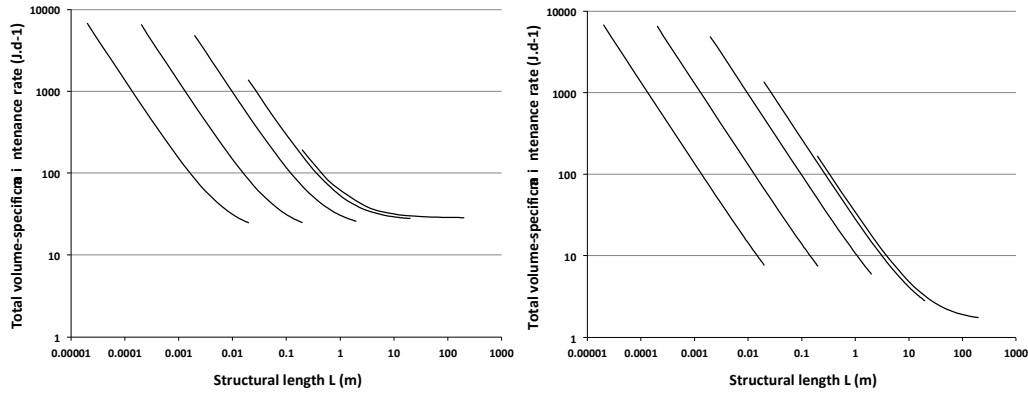
236 The total volume-specific maintenance rate is therefore not a constant as would be expected
 237 ignoring protein turnover and surface-specific costs. On the contrary, it is expected to vary
 238 at the intraspecific level with structural volume V as

$$239 \quad [\dot{p}_p] + [\dot{p}_M] = \frac{\rho[E]([E_G]\dot{V}V^{-1/3} + [\dot{P}_M])}{[E_G] + [E](\kappa - \rho)} + [\dot{p}_M] \quad (7.)$$

240 At the inter-specific level, the total volume-specific maintenance rate is expected to vary
 241 with the zoom factor $z = \frac{L_m}{L_m^1}$ as:

$$242 \quad [\dot{p}_p] + [\dot{p}_M] = \frac{\rho[E_m^1]z([E_G]\dot{V}V^{-1/3} + [\dot{P}_M])}{[E_G] + [E_m^1]z(\kappa - \rho)} + [\dot{p}_M] \quad (8.)$$

243 The total volume-specific maintenance rate is therefore highly dependant on organism size.
 244 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
 245 (Fig. 3).

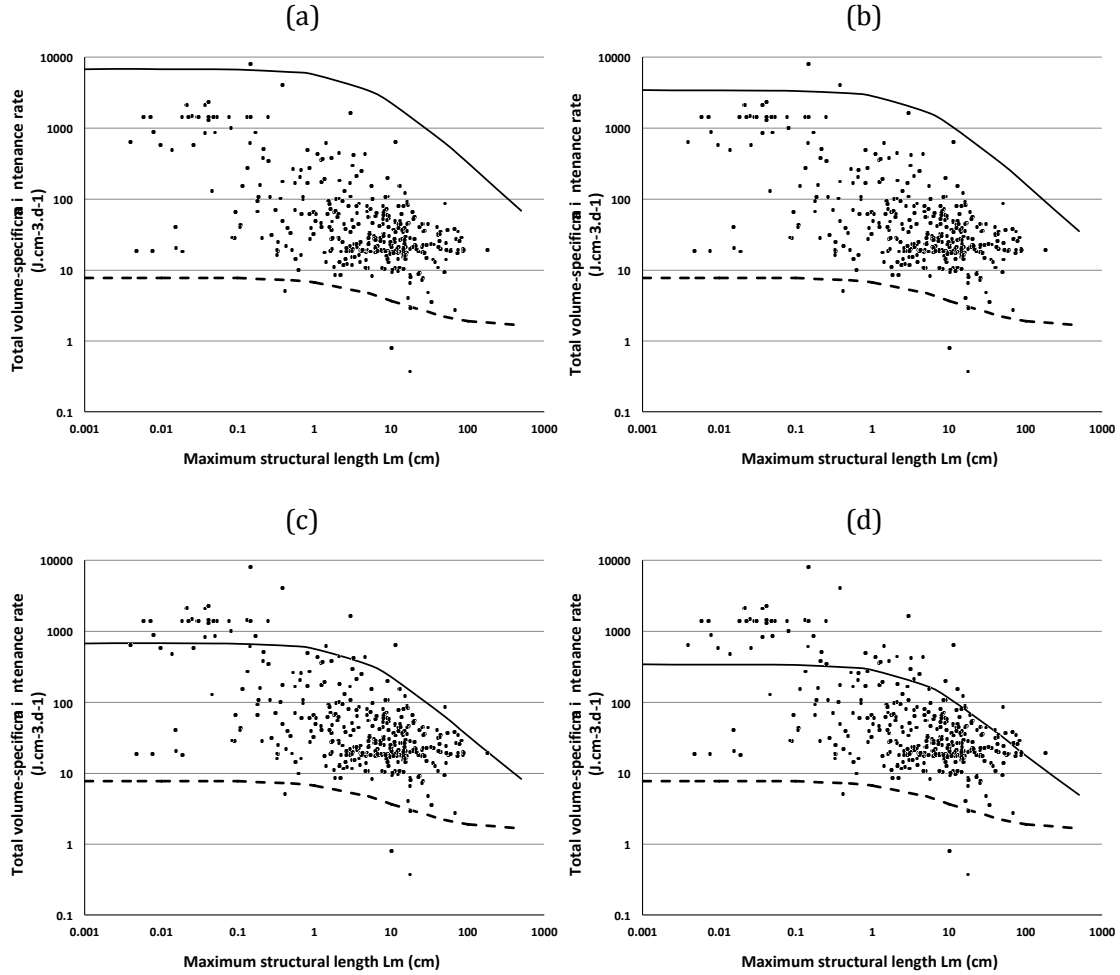


246 *Fig. 3: Total volume-specific maintenance rate $[\dot{p}_p] + [\dot{p}_M]$ for five animal species with $L_m = 0.02$ cm,*
 247 *$L_m = 0.2$ cm, $L_m = 2$ cm, $L_m = 20$ cm, $L_m = 200$ cm. Parameters' values given in Table 2 are used. For*
 248 *the sake of drawing the figure, we assume that $k_M = k_J$. Given the covariation rules for parameter*
 249 *values, this implies that the Length at birth is proportional to maximal length ($L_b = 10^{-3}L_m$)*
 250 *(Kooijman, 2010). The fraction of aerobic metabolism allocated to protein turnover is fixed to $\rho =$*
 251 *0.3 according to empirical observations showing tat protein turnover represents between 10% and*
 252 *50% of total resting metabolism (e.g. Waterlow, 2006). Left $[\dot{p}_M] = 18$ J. cm⁻³. d⁻¹ and right:*
 253 $[\dot{p}_M] = 1$ J. cm⁻³. d⁻¹
 254

255 Empirical patterns in the add-my-pet database

256 Protein turnover is responsible for a significant proportion of maintenance. We have shown
 257 above that it is likely to be size-dependant at both the intra and inter-specific levels. The
 258 parameter estimation procedure in the Add-my-Pet database is based on the equations of
 259 the standard DEB model that don't account explicitly for the cost of protein turnover.
 260 Estimated maintenance rates are therefore likely to be biased and to reflect both the inter-
 261 and intra-specific scaling of protein turnover rate that are not made explicit in the equations
 262 of the standard DEB model. For a given species (a given maximum structural length L_m), we
 263 can therefore expect the estimated maintenance per unit of structural volume to be
 264 somewhere in between the minimum and the maximum total volume-specific maintenance
 265 rates predicted by equation (8) (Fig. 4). If the data available for estimating the parameters
 266 were dominated by small individuals, the estimated maintenance is likely to have been

267 pulled toward the upper predicted bound (at V_b) while we expect it to be closer to the lower
 268 bound (at V_m) if the data used were coming from large individuals.



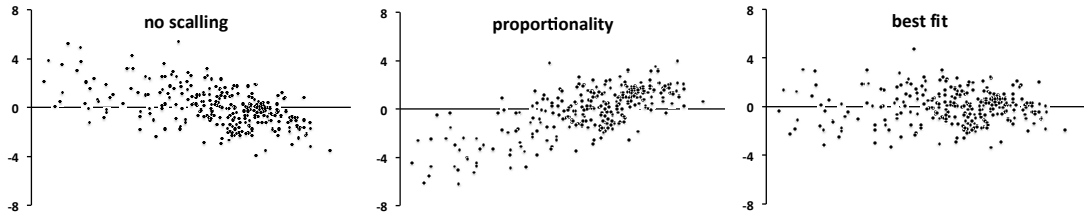
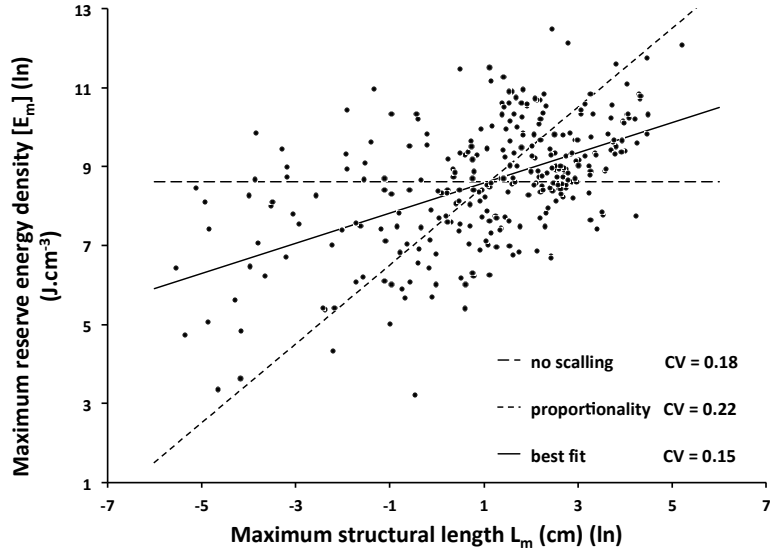
269 Fig. 4: Predicted value of the total volume-specific maintenance rate $[\dot{p}_p] + [\dot{p}_M]$ at birth (continuous
 270 line) and at maximum structural size (dashed line), as well as estimated volume-specific
 271 maintenance rate for the 389 entries of the Add-my-Pet database (downloaded the 25/10/2014) as
 272 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)
 273 $L_b = 10^{-2}L_m$, (d) $L_b = 2.10^{-2}L_m$. Parameters values given in in Table 2 are used except for $[\dot{p}_M] =$
 274 $1 \text{ J} \cdot \text{cm}^{-3} \cdot \text{d}^{-1}$. For the sake of drawing the figure, we assume that $k_M = k_J$. Given the covariation
 275 rules for parameter values, this implies that the Length at birth is proportional to maximal length
 276 ($L_b = 10^{-3}L_m$) (Kooijman, 2010). The fraction of aerobic metabolism allocated to protein turnover
 277 is fixed at $\rho = 0.3$. The maximum reserve energy density scales with maximum structural length as
 278 $[E_m] = 1125 L_m \text{ (J} \cdot \text{cm}^{-3})$ according to Kooijman (2010).

279

280 Figure 4 clearly shows that most volume-specific maintenance rate values empirically
281 derived from the Add-my-Pet database are comprised between the expected curves, despite
282 the fact that they were estimated using the standard DEB model. If a re-estimation of these
283 parameters is done with the changes proposed in this paper, it is likely that most parameter
284 estimates will change as well (see the discussion section).
285

286 **Influence of the scaling of $[E_m]$**

287 In the framework of the DEB theory, the maximum surface-specific assimilation rate $\{\dot{p}_{A_m}\}$
288 is an extensive parameter (proportional to L_m) and the energy conductance $\dot{v} = \{\dot{p}_{A_m}\}/[E_m]$
289 is an intensive parameter (independent from L_m). The maximum reserve density $[E_m]$ is
290 therefore an extensive parameter, which is proportional to the maximum structural size L_m .
291 However, empirical patterns in the Add-my-Pet database show that this proportionality is
292 not supported empirically (Fig. 5) and that the scaling of $[E_m]$ with species maximum length
293 might actually be weaker than expected (the linear regression gives $[E_m] = 3612.5 L_m^{0.3819}$)
294 while the size-independent inter-specific variability dominates.



295 Fig. 5: First line: estimated maximum reserve energy density $[E_m]$ from the Add-my-Pet database as a
 296 function of maximum length L_m (each dot corresponds to one of the 389 entries of the database as
 297 downloaded the 25/10/2014). The en dashed line is the theoretically expected relationship $[E_m] \propto$
 298 L_m fitted to the dots; the em dashed line corresponds to $[E_m] \propto L_m^0$ (absence of relationship between
 299 $[E_m]$ and L_m) fitted to the dots; the continuous line corresponds to the least-square linear regression
 300 of $\ln([E_m])$ versus $\ln(L_m)$, which yields $[E_m] \propto L_m^{0.3819}$. The coefficients of variations (CV) are
 301 provided on the figure for the three regressions. The smaller the CV the better the fit and the larger
 302 the CV the worst the fit. The residuals (predicted values minus observed values) as a function of L_m
 303 (cm) are shown on the second line. They clearly show that both the absence of scaling and the
 304 proportionality hypotheses are unsupported by the Add-my-Pet estimates.

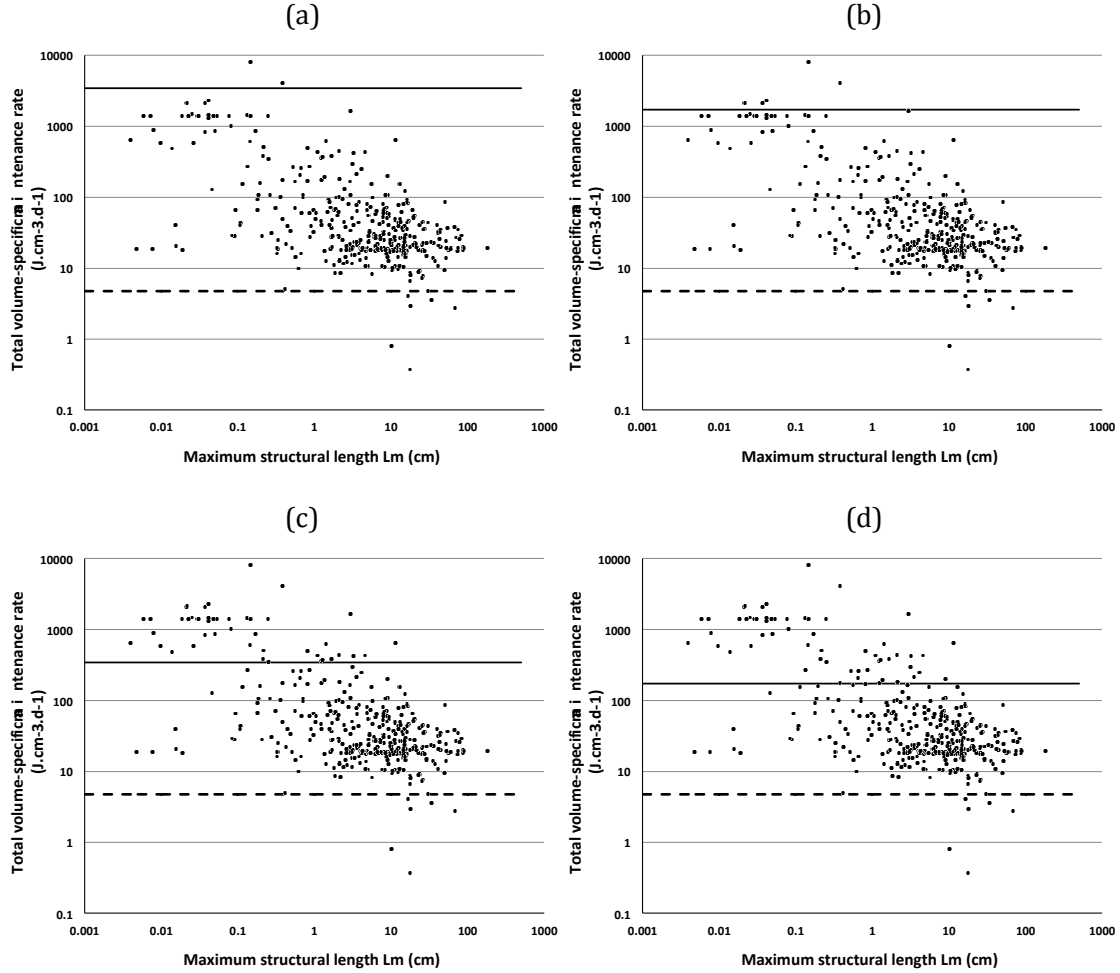
305

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

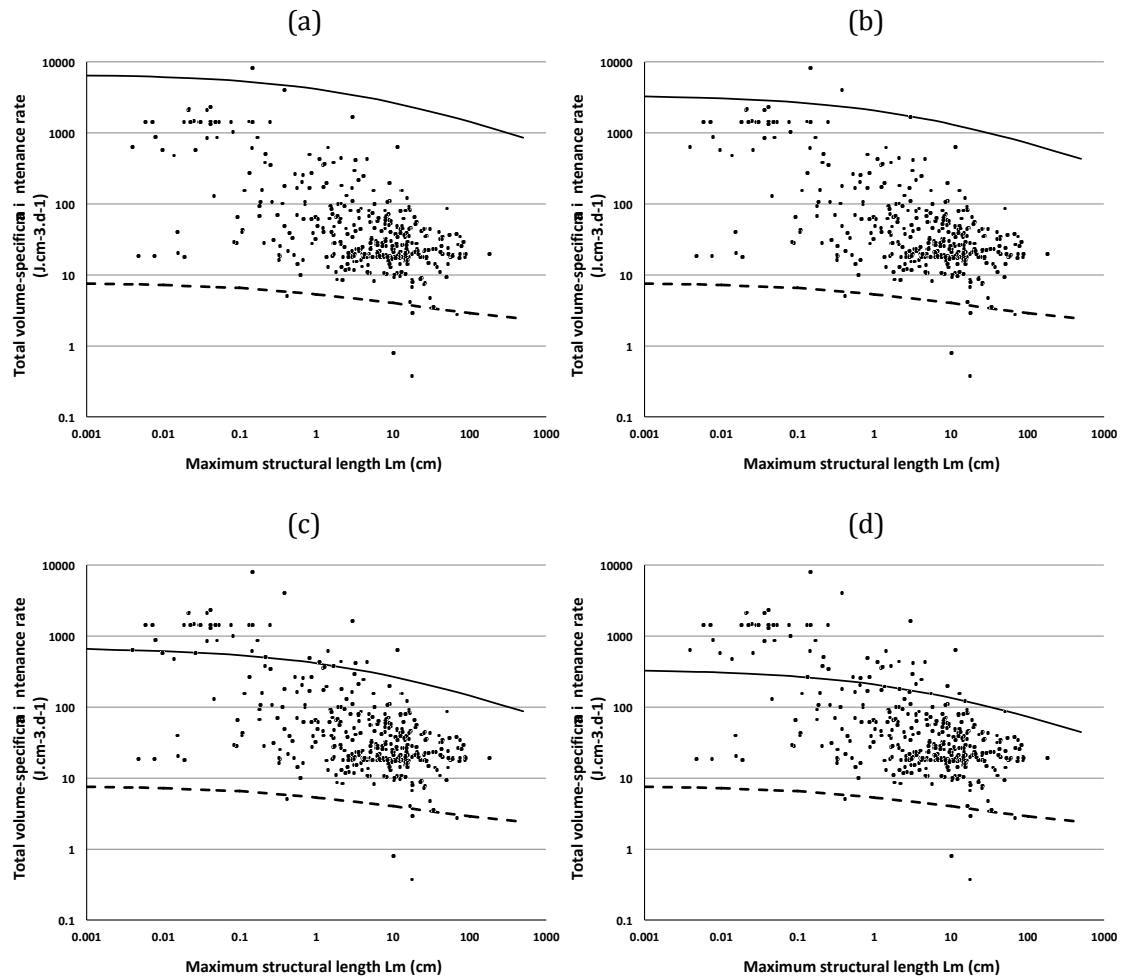
$$309 \quad [\dot{p}_p] + [\dot{p}_M] = \frac{\rho[E_m^1]z^\alpha([E_G]vz^{1-\alpha}V^{-1/3} + [\dot{p}_M])}{[E_G] + [E_m^1]z^\alpha(\kappa - \rho)} + [\dot{p}_M] \quad (9.)$$

310

311 The comparison of Fig. 4 drawn assuming that $[E_m] \propto L_m$, Fig. 6 drawn assuming that
 312 $[E_m] \propto L_m^0$ and Fig. 7 drawn assuming that $[E_m] \propto L_m^{0.3819}$ demonstrates the importance that
 313 the inter-specific scaling of $[E_m]$ has on the scaling of both maximum and minimum volume-
 314 specific maintenance rates.



315 *Fig. 6: Predicted value of the total volume-specific maintenance rate $[\dot{p}_p] + [\dot{p}_M]$ at birth (continuous*
 316 *line) and at maximum structural size (dashed line), as well as estimated volume-specific*
 317 *maintenance rate for the 389 entries of the Add-my-Pet database (downloaded the 25/10/2014) as*
 318 *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)*
 319 *$L_b = 10^{-2}L_m$, (d) $L_b = 2.10^{-2}L_m$. Parameters values given in in Table 1 are used except for $[\dot{p}_M] =$*
 320 *$1 \text{ J} \cdot \text{cm}^{-3} \cdot \text{d}^{-1}$. For the sake of drawing the figure, we assume that $k_M = k_J$. Given the covariation*
 321 *rules for parameter values, this implies that the Length at birth is proportional to maximal length*
 322 *($L_b = 10^{-3}L_m$) (Kooijman, 2010). The fraction of aerobic metabolism allocated to protein turnover*
 323 *is fixed at $\rho = 0.3$. The maximum reserve energy is independent from maximum structural length*
 324 *and equal to $[E_m] = 5510 \text{ J} \cdot \text{cm}^{-3}$ according to Figure 6.*



326 Fig. 7: Predicted value of the total volume-specific maintenance rate $[\dot{p}_p] + [\dot{p}_M]$ at birth (continuous
 327 line) and at maximum structural size (dashed line), as well as estimated volume-specific
 328 maintenance rate for the 389 entries of the Add-my-Pet database (downloaded the 25/10/2014) as
 329 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)
 330 $L_b = 10^{-2}L_m$, (d) $L_b = 2.10^{-2}L_m$. Parameters values given in in Table 2 are used except for $[\dot{p}_M] =$
 331 $1 \text{ J} \cdot \text{cm}^{-3} \cdot \text{d}^{-1}$. For the sake of drawing the figure, we assume that $k_M = k_J$. Given the covariation
 332 rules for parameter values, this implies that the Length at birth is proportional to maximal length
 333 ($L_b = 10^{-3}L_m$) (Kooijman, 2010). The fraction of aerobic metabolism allocated to protein turnover
 334 is fixed at $\rho = 0.3$. The maximum reserve energy density scales with maximum structural length as

$$[E_m] = 3612.5 L_m^{0.3819} \text{ (J} \cdot \text{cm}^{-3}\text{)} \text{ according to Figure 6.}$$

336

337 **Consequences on growth, development and reproduction**

338 ***Consequences on growth***

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

341
$$\left. \begin{aligned} \frac{dV}{dt} &= \frac{\dot{v}V^{2/3}[E](\kappa-\rho)-[\dot{P}_M]V}{[E_G]+[E](\kappa-\rho)} \\ f &= \text{constant} \end{aligned} \right\} \Rightarrow \frac{dV}{dt} = \frac{(\kappa-\rho)f\{\dot{P}_{Am}\}V^{2/3}-[\dot{P}_M]V}{[E_G]+(\kappa-\rho)f[E_m]} \quad (10.)$$

342

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

345
$$L_t = \frac{(\kappa-\rho)f\{\dot{P}_{Am}\}}{[\dot{P}_M]} \left(1 - e^{\frac{-[\dot{P}_M]t}{3([E_G]+(\kappa-\rho)f[E_m])}} \right) \quad (11.)$$

346 With the maximal structural length:

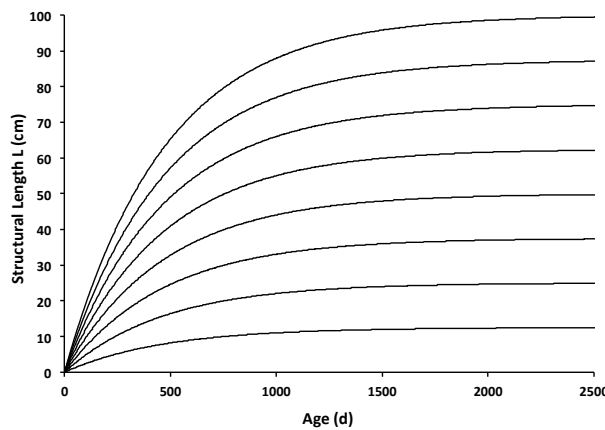
347
$$V_m^{1/3} = L_m = \frac{(\kappa-\rho)\{\dot{P}_{Am}\}}{[\dot{P}_M]} \quad (12.)$$

348 And the growth rate of structure

349
$$\dot{r}_B = \frac{-[\dot{P}_M]}{3([E_G]+(\kappa-\rho)f[E_m])} \quad (13.)$$

350

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



355 *Fig. 8: Von Bertalanffy growth curve at $f=1$ with the fraction of aerobic metabolism allocated to*
356 *protein turnover ρ varying from 0 (upper curve) to 0.7 (lower curve) with a 0.1 increment. The*
357 *maximum structural length is arbitrarily taken to be equal to $L_m = 100\text{cm}$ for $\rho = 0$ and the*

358 parameters given in Table 2 are used except for the maximum reserve energy density, which scales
 359 with maximum structural length as $[E_m] = 3612.5 L_m^{0.3819} \text{ (J.cm}^{-3}\text{)}$ according to Figure 6.

360

361 **Consequences on reproduction and development**

362 From equation (3) we can write:

$$363 \quad (1 - \kappa)\dot{p}_c = \frac{1-\kappa}{\kappa-\rho} \left(\dot{p}_M + [E_G] \frac{dV}{dt} \right) \quad (14.)$$

364 We derive the maturity maintenance flux:

$$365 \quad \dot{p}_J = \frac{1-\kappa}{\kappa-\rho} [\dot{p}_M] \min(V, V_p) \quad (15.)$$

366

367 The development/reproduction flux then reads:

$$368 \quad \dot{p}_R = \frac{1-\kappa}{\kappa-\rho} [E_G] \frac{dV}{dt} \quad (16.)$$

369

370 From equation (5), we can express this flux as:

$$371 \quad \dot{p}_R = (1 - \kappa) \left[\frac{[E]([E_G]\dot{V}V^{2/3} + [\dot{p}_M]V)}{[E_G] + [E](\kappa - \rho)} - \frac{[\dot{p}_M]}{\kappa - \rho} \min(V, V_p) \right] \quad (17.)$$

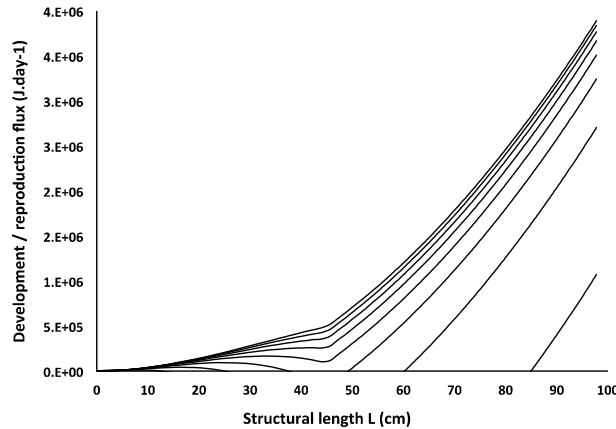
372

373 At constant food we get:

$$374 \quad \dot{p}_R = (1 - \kappa) \left[\frac{f[E_m]([E_G]\dot{V}V^{2/3} + [\dot{p}_M]V)}{[E_G] + f[E_m](\kappa - \rho)} - \frac{[\dot{p}_M]}{\kappa - \rho} \min(V, V_p) \right] \quad (18.)$$

375

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



380 *Fig. 9: Development / reproduction flux at $f=1$ with the fraction of aerobic metabolism allocated to*
 381 *protein turnover ρ varying from 0 (upper curve) to 0.7 (lower curve) with a 0.1 increment. The*
 382 *maximum structural length is arbitrarily taken to be equal to $L_m = 100\text{cm}$ for $\rho = 0$ and the*
 383 *parameters given in Table 2 are used except for the maximum reserve energy density, which scales*
 384 *with maximum structural length as $[E_m] = 3612.5 L_m^{0.3819} \text{ (J. cm}^{-3}\text{)}$ according to Figure 5. The*
 385 *length at puberty is arbitrarily fixed at 45cm.*
 386

387 Discussion

388 *Linking protein turnover to oxidative stress could explain maintenance patterns*

389 Protein turnover constitutes the bulk of maintenance

390 Protein turnover includes the degradation of damaged proteins (catabolism) and the
 391 synthesis of new proteins (anabolism). It allows non-functional, damaged, or even toxic
 392 proteins to be destroyed and replaced by functional ones. Protein breakdown is generally
 393 due to lysosomal proteases, which digest endocytosed proteins or to cytoplasmic
 394 complexes, called proteasomes, which digest old or abnormal proteins that have been
 395 tagged with ubiquitin for destruction. Protein synthesis involves the process of translation
 396 on ribosomes. It is a well-known fact that the costs associated to protein turnover represent
 397 a large fraction of aerobic metabolism and by far the largest part of maintenance (80 to 90%
 398 according to Kooijman, 2010). For instance in vegetal species, Quigg and Beardall (2003)
 399 estimate that 30% and 36% of respiratory demand for two marine microalgae species are
 400 due to protein turnover; Scheurwater et al. (2000) estimate that between 22-30% of daily
 401 ATP production for two grass plant species is spent in protein turnover; Bouma et al. (1994)
 402 estimate that protein turnover in bean's leaves requires 17-35% of total dark respiration

403 while De Visser et al. (1992) estimate that it requires 30-60% of dark leaves respiration. In
404 the animal realm, Gill et al. (1989) estimate that protein turnover requires 19% of whole
405 body ATP expenditure for growing lambs; White et al. (1988) estimate that it costs only 7-
406 8% for three species of wallabies but they also report that protein synthesis accounted for
407 approximately 21% of the heat production in young growing pigs and 17% of total heat
408 production in finishing beef steers. MacRae and Lobley (1986) derived higher values (25%
409 of heat production) from data on lean and obese adult humans as well as Davis et al. (1981)
410 who report 42% of heat production for growing lambs. Rabbits studied by Nicholas et al.
411 (1977) spent 22% of total heat production for protein turnover, which is in agreement with
412 other findings for eutherian mammals. Waterlow (1984) indeed reports values in the range
413 of 15-20% of total resting metabolism for 6 mammal species (mouse, rat, rabbit, sheep,
414 man, cow). Overall, the ratio between protein turnover and the energy spent in the
415 metabolism varies in a strikingly narrow range (roughly around 30% +/- 20%) in the
416 studies shown above, despite the diversity of animal and vegetal species considered and the
417 variety of methods used to estimate it.

418 Protein turnover is linked to aerobic metabolism

419 Aerobic organisms use di-oxygen to oxidize organic nutrients and produce ATP. But aerobic
420 metabolism continuously generates toxic reactive by-products (generically named ROS for
421 reactive oxygen species), such as superoxide anion radical, hydrogen peroxide, and the
422 highly reactive hydroxyl radicals (Cabiscol et al., 2000). ROS continuously damage proteins
423 as well as DNA, RNA and lipids such as polyunsaturated fatty acids in cell membranes
424 (Birnie-Gauvin et al., 2017; Cabiscol et al., 2000). This continuous degradation of structural
425 molecules is highly detrimental to the functionality of cells and it would ultimately lead to
426 cellular death if costly repair mechanisms were not permanently deployed. The link
427 between aerobic metabolism and protein turnover is also well established at the organism
428 level. Empirical studies show for instance that aerobic exercise increases skeletal muscle
429 protein turnover (e.g. Picosky et al., 2006). At the intra-specific level again, Waterlow
430 (1984) reports that immature animals have higher rates of protein turnover per unit of
431 body weight than adults of the same species, even when net synthesis due to growth has
432 been deducted. In premature infants, the net rate of protein turnover was for instance found
433 to be twice as high as in the 1-year-old child and 3-4 times as high as in the adult (Pencharz,
434 Farri & Papageorgiou, 1983). This suggests that protein turnover varies with body size, just
435 as aerobic metabolism does. At the inter-specific level, protein turnover has been found to

436 scale approximately with body mass at a power 0.72 (Waterlow, 2006), while the total RNA
437 content of the liver, representing the capacity for protein synthesis, scales as body mass at a
438 power 0.75 (Munro and Downie, 1964). This variability matches exactly the Kleiber rule
439 (Kleiber, 1947), namely the observation that for the vast majority of animals, metabolic rate
440 scales approximately to the $\frac{3}{4}$ power of the animal's mass, as does the respiration rate.

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

443 The DEB theory recognizes the importance of ROS in degrading DNA and RNA. The ageing
444 mortality is assumed to be proportional to the amount of cellular damages that accumulate
445 at a rate proportional to the amount of DNA lesions, which increases at a rate proportional
446 to the intra-cellular concentration of ROS. Finally, the rate of ROS formation is assumed to
447 be proportional to the catabolic power \dot{p}_C , which is a good proxy for the respiration rate -
448 excluding the consumption of oxygen due to assimilation- (Kooijman, 2000, 2010; van
449 Leeuwen et al., 2010). It is surprising that the link between aerobic metabolism, protein and
450 more generally structure turnover is not explicit in the DEB theory. What we propose here
451 is to make this link explicit and to consider that the oxidation rate of structural molecules
452 (mostly proteins but also structural lipids, DNA and RNA) is proportional to the catabolic
453 power \dot{p}_C , as it is assumed in the DEB theory for DNA and RNA to derive ageing mortality
454 (Kooijman, 2010). Linking aerobic metabolism to maintenance as we propose would
455 improve the consistency of the DEB theory by treating the oxidation of structural molecules
456 exactly as it is done to derive ageing mortality (Kooijman, 2010) and by making the
457 turnover of structure explicit in the maintenance rate. Doing so, we have shown that the
458 volume-specific maintenance rate becomes linked to metabolism and displays both intra-
459 specific (changes with the structural volume V) and inter-specific (changes with the
460 maximum structural volume V_m) variability patterns that are compatible in their magnitude
461 with what is observed in Add-my-Pet (Fig. 4, 6, 7 and 10). In particular, Fig. 10 drawn using
462 the empirical trends of $\{\dot{P}_{Am}\}$ and $[E_m]$ in the Add-my-Pet estimates ($\{\dot{P}_{Am}\} = 98.79 L_m^{0.5662}$
463 and $[E_m] = 1125 L_m^{0.3819}$, cf. Fig. 1 and 5) demonstrates that accounting for protein turnover
464 enables to explain both the estimated trend and the variability of maintenance. Our
465 proposition would therefore simultaneously restore the covariation rules for parameter
466 values implied by the standard DEB model (the volume-specific somatic maintenance rate
467 $[\dot{p}_M]$ would keep approximately constant between species -as would ρ , the fraction of
468 aerobic metabolism allocated to protein turnover- and the maximum surface-specific

469 assimilation rate $\{\dot{p}_{A_m}\}$ would scales with maximum structural size) by explaining a
470 substantial part of the intra- and inter-specific variability of estimated maintenance while
471 accounting for major processes of the metabolism (the link between aerobic metabolism,
472 the production of ROS and maintenance costs) that were previously overlooked in the DEB
473 theory.

474

475 ***The « waste to hurry » hypothesis***

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

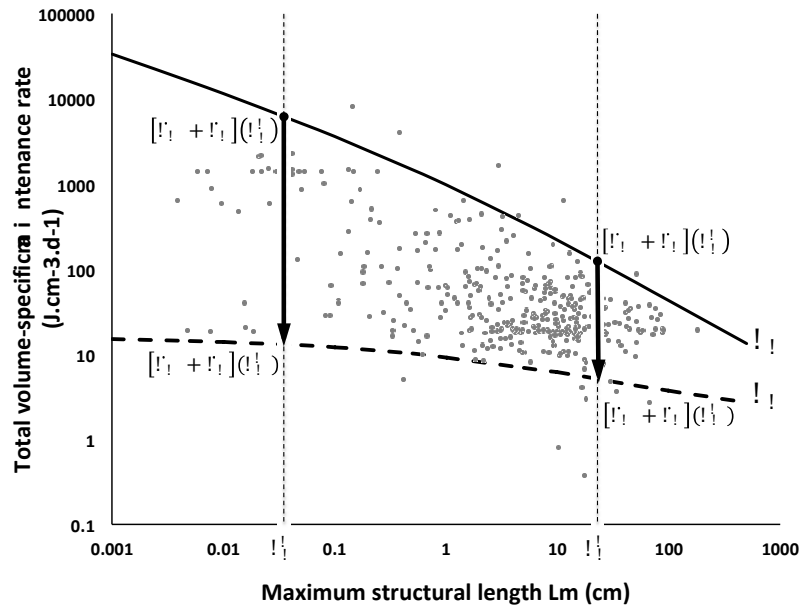
486 Kooijman (2014) proposes that futile cycles could underlie the “waste to hurry” hypothesis
487 and explain the high maintenance of small species. We are however not aware of
488 observations that would corroborate this proposition. Another possible explanation for the
489 existence of futile cycle is that metabolic pathways that are not activated continuously must
490 be maintained in activity to be able to restart immediately when needed, just by
491 deactivating the negative part of the futile cycle. Otherwise cells would need to re-
492 synthesize the oxidized enzymes involved and the intermediary products each time they
493 would need to start producing the final product. If one needs to drive 0 to 100km/h in 5
494 seconds when the traffic light turns green, it is better to keep the engine running and just
495 put into gear and accelerate rather than rebuilding the engine, refilling the oil and gas tanks,
496 restarting the engine and accelerate to keep up to the needs....

497

498 ***Re-estimating the DEB parameters?***

499 *The variability of maintenance in Add-my-Pet is consistent with the predictions made*
500 *considering the turnover of structure explicitly*

501 If we admit that a substantial fraction of maintenance varies with aerobic metabolism,
 502 equation (8) shows that the importance of maintenance has to change dramatically within
 503 species (with V) and between species (with V_m). For any individual of a given species, the
 504 total volume-specific maintenance rate decreases from fecundation to maximum structural
 505 volume over several orders of magnitude. Assuming for the sake of drawing the figure that
 506 the structural length at birth L_b is proportional to the maximum structural length L_m , Figure
 507 10 illustrates this phenomenon from birth (at the onset of feeding, between the embryo
 508 stage and the juvenile stage) to maximal size. For a given species of maximal size L_m^1 , the
 509 total volume-specific maintenance rate decreases along the arrow from $[\dot{p}_M + \dot{p}_p](L_b^1)$ at
 510 birth to $[\dot{p}_M + \dot{p}_p](L_m^1)$ for a fully grown individuals. The DEB theory presently overlooks
 511 this important intraspecific variation of maintenance and assumes that the volume-specific
 512 maintenance rate keeps constant from fecundation to death. Estimated values of $[\dot{p}_M]$ in
 513 Add-my-Pet are therefore likely to fall somewhere in between the minimum and maximum
 514 expected values, reflecting a sort of average value of total volume-specific maintenance rate
 515 $([\dot{p}_M + \dot{p}_p])$ over the size range of the data used for parameter estimation, and destabilizing
 516 the parameter estimation process when the data used correspond to very different size
 517 ranges. Figure 10 shows that most estimated $[\dot{p}_M]$ values indeed fall in between the
 518 expected minimum and maximum values for the total volume specific maintenance rate. A
 519 few data points are however higher than the expected value at V_b , despite the fact that the
 520 size at birth used for drawing the figure is already quite small ($L_b = 8.10^{-3}L_m$). This could
 521 be due to the use of data collected during the embryonic stage for parameter estimation.
 522 Embryos have indeed a structural volume potentially much smaller than the structural
 523 volume at birth and therefore a total volume-specific maintenance rate much higher than its
 524 expected value at birth. Finally, the good match of predictions with Add-My-Pets estimates
 525 in Fig. 10 also suggests that part of the intra and inter-specific maintenance trends due to
 526 protein turnover has been erroneously attributed to $[\dot{p}_M]$, $\{\dot{P}_{Am}\}$ and $[E_m]$ by the Add-my-
 527 Pet parameter estimation procedure, to compensate for the fact that the standard DEB
 528 model considers the volume-specific maintenance rate to keep constant at the intra-specific
 529 level.



530 *Fig. 10: Predicted value of the total volume-specific maintenance rate $[\dot{p}_p] + [\dot{p}_M]$ at birth*
 531 *(continuous line) and at maximum structural size (dashed line), as well as estimated volume-specific*
 532 *maintenance rate (grey dots) for the 389 entries of the Add-my-Pet database (downloaded the*
 533 *25/10/2014) as a function of maximum length L_m . For the sake of drawing the figure, we assume*
 534 *that $k_M = k_j$. Given the covariation rules for parameter values, this implies that the Length at birth*
 535 *is proportional to maximal length (Kooijman, 2010). It is arbitrary fixed at $L_b = 8.10^{-3}L_m$. The*
 536 *fraction of aerobic metabolism allocated to protein turnover is fixed at $\rho = 0.15$. According to the*
 537 *empirical trends in the Add-my-Pet estimates, the maximum volume-specific assimilation rate is*
 538 *supposed to scale with maximum structural length as $\{\dot{P}_{Am}\} = 98.79 L_m^{0.5662}$ (cf. Fig. 1) and the*
 539 *maximum reserve energy density is assumed to scale with maximum structural length as $[E_m] =$*
 540 *$1125 L_m^{0.3819}$ (cf. Fig. 5). All the other parameters' values given in in Table 2 are used except for*
 541 *$[\dot{p}_M] = 1 J.cm^{-3}.d^{-1}$.*

542
 543 Modifying the DEB model implies that parameters have to be re-estimated

544 The numerical values given in the present paper to the fraction of aerobic metabolism
 545 allocated to protein turnover ($\rho = 0.3$) and to the volume-specific structural maintenance
 546 rate ($[\dot{p}_M] = 4 J.cm^{-3}.d^{-1}$) were chosen arbitrarily according to empirical observations
 547 showing that protein turnover represents around 30% +/- 20% of total resting metabolism
 548 (e.g. Waterlow, 2006, cf. the 1st paragraph of the discussion section) and 80-90% of total
 549 maintenance costs (Kooijman, 2010). All the other parameter values used here (Table 2)
 550 were those given in Kooijman (2010) to represent a generic organism. Figure 4 shows that

551 with these parameters' values, the costs of structure turnover of large species represents
552 roughly from 50% to 95% of total maintenance costs for large and small individuals
553 respectively and for small species it accounts from 90% to 99.95% of total maintenance.
554 However, if our proposition is true, the DEB core equations have to be modified (equation
555 5) and their parameters re-estimated. Even if they don't change qualitatively, testable
556 predictions such as growth, reproduction or respiration curves that are used to estimate the
557 parameters change quantitatively when introducing the cost of structure turnover in
558 maintenance (Fig. 8 and 9), and the relative importance of the underlying energy fluxes also
559 changes. Consequently, fitting the modified DEB equations to observations will change the
560 parameters' values that have previously been estimated. This is a serious consequence of
561 our proposition. It implies that parameter's values estimated with the current version of the
562 DEB model, such as those in the Add-my-Pet collection, are not valid for use with the
563 modified DEB model. If we admit that the effects of aerobic metabolism on the turnover of
564 structure have to be included in the DEB equations, then parameters have to be re-
565 estimated for every species considered in the Add-my-Pet collection.

566

567 *Parsimony has to be regarded at the inter-specific level*

568 The 14 primary parameters of the standard DEB model (including the 5 core parameters
569 $\{\dot{p}_{A_m}\}$, $[\dot{p}_M]$, \dot{v} , $[E_G]$, and κ presented Table 2) are usually difficult to estimate as they have
570 confounded effects on the model's predictions that can be compared to data (e.g. Marques et
571 al 2018). The information content of the data is furthermore often too weak to identify all
572 the parameters simultaneously (Marques et al. present issue). One strategy to overcome
573 this over-parameterization issue is to use observations of different nature simultaneously
574 (for instance growth data with length-weight observations, reproduction and respiration
575 data), in an integrated statistical estimation framework (e.g. Lika et al., 2011). Adding one
576 extra core-parameter to the standard model (the fraction ρ of aerobic metabolism allocated
577 to protein turnover as we propose here) can be seen as a non-parsimonious extension of the
578 model in a situation where over-parameterization is already an issue. We believe that this is
579 a superficial view however, which omits to consider the problem in its broader inter-
580 specific dimension. If the model is kept in its present form with a constant volume-specific
581 somatic maintenance rate $[\dot{p}_M]$, the empirical falsification of the inter-specific scaling of
582 maintenance and its evolutionary justification (waste to hurry) imply that $[\dot{p}_M]$ becomes a
583 free parameter that has to be re-estimated for every species considered. The number of

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

593

594 *Re-estimating the DEB parameters: toward an integrated intra- inter-specific estimation*
595 *strategy?*

596 The new formulation of the DEB model proposed here requires that the model's parameters
597 be re-estimated. Re-estimating simultaneously the standard DEB parameters and the new
598 parameter ρ might be challenging, in a situation where over-parameterization and
599 parameter confounding is already a difficult issue for the standard DEB model (Lika et al.,
600 2011; Marques et al., 2018). This is especially true considering that κ and ρ often appear
601 together in the modified equations (as in the new catabolic power equation 5, or the new
602 growth equation 11), and are therefore likely to be difficult to estimate simultaneously. This
603 is not the case in the new development/reproduction flux equation (equation 17) however,
604 as the energy allocated to reproduction, development and its maintenance keeps
605 proportional to $(1 - \kappa)$. This might enable the simultaneous estimation of κ and ρ when
606 data constraining \dot{p}_G and data constraining \dot{p}_R are available and can be used simultaneously.
607 When such complementary data are not available, a possible strategy would be to take
608 advantage of the considerable amount of information held in the inter-specific variability of
609 maintenance regarding the value of the new parameter ρ (Fig. 4, 6, 7 and 10). The modified
610 DEB parameters could indeed be estimated for several species simultaneously, ideally
611 covering a wide range of maximum length, and assuming that $[\dot{p}_M]$ and ρ keep constant
612 between species, or at least between species of the same taxa. This approach could certainly
613 be tested using a selection of species in the Add-my-Pet collection.

614

615 ***Scaling of the maximum reserve energy density***

616 In the framework of the DEB theory, the maximum reserve energy density $[E_m]$ is an
617 extensive compound parameter (supposed to be proportional to maximum structural length
618 L_m and equal to $\{\dot{p}_{A_m}\}/\dot{v}$). With its usual value ($[E_m]_{ref} = 1125 J.cm^{-3}$ for $L_m = 1cm$), and
619 assuming for simplicity that the energy content of reserve and structure is the same and
620 equal to $4 J.cm^{-3}$ (Kooijman, 2010), the scaling of $[E_m]$ with L_m implies that the reserve
621 compartment of a $L_m = 10\mu m$ microorganism would account for approximately 22% of
622 body weight and 58% for a $L_m = 50\mu m$ organism. This corresponds to the range of values
623 measured for planktonic organisms for which reserves constitute from 30% to 60% of body
624 weight (e.g. Granum et al., 2002; Laws and Bannister, 1980; Lopez et al., 2016). However,
625 assuming that $[E_m]$ is proportional to maximum structural length L_m also implies that
626 larger animals would be composed of an unrealistic amount of reserve (96.56%, 99.64%,
627 99.96% and 99.99% for organisms of structural length $L_m = 1mm, 1cm, 10cm$ and $1m$
628 respectively). This unrealistic implication of the theoretical scaling of maximum reserve
629 density is corroborated by the empirical pattern of $[E_m]$ versus L_m in Add-my-Pet, which
630 doesn't match the theoretical expectation either. In Add-my-Pet, estimated $[E_m]$ are indeed
631 scaling approximately with $L_m^{0.4}$, and they display an important variability around this trend
632 (Fig. 5). This absence of clear scaling of $[E_m]$ with L_m is also observed at the taxa level, with
633 some taxa displaying no scaling of maximum reserve capacity (e.g. *actinopterygii*) and other
634 that seem to display some weak positive relationship between maximum length and
635 maximum energy density (e.g. *chondrichthyes*) (Kooijman and Lika, 2014).

636 What the scaling of maximum reserve density estimated in Add-my-Pet would become with
637 the modified DEB equations is not known however, as all the parameters including $[E_m]$ (or
638 \dot{v}) would have to be re-estimated if the DEB model is modified (see above). In the absence of
639 a non-ambiguous theoretical argument and no empirical indication in favour of a scaling of
640 $[E_m]$ with maximum structural length L_m , we suggest that $[E_m]$ (or alternately \dot{v}) be re-
641 estimated as a free parameter with the modified DEB equations for every species
642 considered so that the scaling of the maximum reserve capacity with maximum length can
643 be re-evaluated empirically. The reserve compartment allows covering the metabolic needs
644 between two feeding events. When reserves are not sufficient, growth ceases and mild
645 starvation starts. Maximum reserve energy density is therefore a critical parameter that is
646 controlling the time to starvation in the absence of food. It is logical to assume that
647 evolution has optimized its value according to the variability of the environment in which

648 the considered species is living. We are therefore expecting an important inter-specific
649 variability of $[E_m]$, but not necessarily a strong relationship with L_m .
650 The scaling of $[E_m]$ with maximum structural length L_m has a strong influence on the scaling
651 of the total volume-specific maintenance rate including the cost of structure turnover (Fig.
652 4, 6 and 7). Empirical patterns of maintenance rate in Add-my-Pet are fully compatible with
653 maximum reserve energy density $[E_m]$ varying less than proportionally to L_m (Fig. 10).

654

655 **Conclusion**

656 The inter-specific variability of estimated maintenance rates in the Add-my-Pet collection
657 (Kooijman, 2014) reveals troubling patterns apparently violating the covariation rules for
658 parameter values implied by the standard DEB model and challenging the DEB theory.
659 Protein (and more generally structure) turnover rate constitutes an important component
660 of maintenance, which varies with aerobic metabolism. We propose that this dependence on
661 metabolism could explain the apparent decrease of volume-specific maintenance rate with
662 species maximum structural size and its variability. If true, this would require modifications
663 of the standard DEB theory in order to capture inter-specific scaling patterns of DEB-
664 parameters while keeping the consistency of the theory at the intra-specific level.

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

675 The modifications that we propose to the DEB theory would not change the qualitative
676 nature of standard DEB predictions (e.g. growth or reproduction curves). However, the core
677 DEB parameters would need to be re-estimated along with the new parameter ρ , the
678 fraction of aerobic metabolism allocated to protein turnover. We believe that adding one
679 extra intensive parameter as we suggest is actually more parsimonious and therefore

680 preferable than re-estimating $[\dot{p}_M]$ for every species, as required by the current formulation
681 of the DEB model that cannot rest on interspecific scaling rules anymore.
682 Finally, we suggest that parameter estimation for selected species should be conducted with
683 the modified DEB equations to test our proposition.
684

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