

2

3 **Shape shifting predicts ontogenetic changes in**
4 **metabolic scaling in diverse aquatic invertebrates**

5

6

7 Douglas S. Glazier¹, Andrew G. Hirst^{2,3} and David Atkinson⁴

8

9

10 ¹Department of Biology, Juniata College, Huntingdon, Pennsylvania, 16652, USA

11 ²School of Biological and Chemical Sciences, Queen Mary, University of London, E1 4NS, UK

12 ³Centre for Ocean Life, National Institute for Aquatic Resources, Technical University of
13 Denmark, Kavalergarden 6, Charlottenlund, 2920, Denmark

14 ⁴Institute of Integrative Biology, University of Liverpool, Biosciences Building, Crown Street,
15 L69 72B, UK

16

17

18 Metabolism fuels all biological activities, and thus understanding its variation is fundamentally
19 important. Much of this variation is related to body size, which is commonly believed to follow
20 a 3/4-power scaling law. However, during ontogeny many kinds of animals and plants show
21 marked shifts in metabolic scaling that deviate from 3/4-power scaling predicted by general
22 models. Here we show that in diverse aquatic invertebrates, ontogenetic shifts in the scaling of
23 routine metabolic rate from near isometry ($b_R =$ scaling exponent ~ 1) to negative allometry ($b_R <$
24 1), or the reverse, are associated with significant changes in body shape (indexed by $b_L =$ the
25 scaling exponent for body mass in relation to body length). The observed inverse correlations
26 between b_R and b_L are predicted by metabolic scaling theory that emphasizes resource/waste
27 fluxes across external body surfaces, but contradict theory that emphasizes outward-directed
28 transport of resources through internal networks. Geometric estimates of the scaling of surface
29 area with body mass (b_A) further show that ontogenetic shifts in b_R and b_A are positively

30 correlated. These results support new metabolic scaling theory based on surface-area influences
31 that may be applied to ontogenetic shifts in b_R shown by many kinds of animals and plants.

32
33

34 **Subject Areas:**

35 developmental biology, ecology, physiology, theoretical biology

36

37 **Keywords:**

38 aquatic invertebrates, body shape, metabolic scaling, ontogeny, resource-transport networks,
39 surface area

40

41

42

43 **1. Introduction**

44 All living activities depend on metabolism for energy and materials. Therefore, understanding
45 variation in metabolic rate is of fundamental importance in biology. Much of this variation is
46 related to body size, but how and why these relationships occur remain vexing questions.

47 General models assume that metabolic rate scales monotonically with body size according to the
48 simple power function

49
$$R = aM^b, \tag{1.1}$$

50 where R is metabolic rate, a is the scaling coefficient (antilog of the intercept in a log-log plot),
51 M is body mass, and b (henceforth b_R) is the scaling exponent (linear slope of a log-log plot that
52 frequently approximates 3/4) [1, 2]. However, metabolic scaling often shows marked shifts
53 during ontogeny in animals and plants (b_R varying mostly between 2/3 and 1, but also showing
54 values outside this range) [3-7] that are not well understood. These metabolic shifts are

55 important because they appear to be fundamentally linked to other ontogenetic changes in the
56 physiology, growth rate, cell size, body composition, behavior and ecology of a species [3-7].

57 Here we show that ontogenetic changes in body shape and associated surface-area-related
58 resource supply predict frequently observed shifts from near isometric ($b_R \sim 1$) to negatively
59 allometric ($b_R < 1$) intraspecific metabolic scaling (= type III scaling [4]) in diverse aquatic
60 invertebrates. Crucially, we also show that shape shifting predicts more rarely observed changes
61 in metabolic scaling that occur in the opposite direction (from shallow to steep scaling). Our
62 results demonstrate that the prediction of metabolic scaling from the body-shape related scaling
63 of surface area applies more widely than that recently described by Hirst et al. [8]. We show that
64 this predictive power applies to marked variation in metabolic scaling seen not only among
65 diverse pelagic (open-water) animal taxa [8], but also during the intraspecific ontogeny of both
66 pelagic invertebrates and those that exhibit developmental shifts from pelagic to benthic (bottom-
67 dwelling) lifestyles. A critical assumption of our shape-shifting model is that the supply of
68 oxygen and (or) nutrients scales with external body surface, thus implying that resource uptake is
69 distributed over the body surface, which we evaluate herein. We also discuss potential
70 implications of our findings for ontogenetic shifts in metabolic scaling observed in many other
71 kinds of animals and plants.

72

73 2. Theoretical background

74 Metabolic rate may be controlled by the supply of, or demand for, resources [9, 10]. Both should
75 be considered in order to attain a comprehensive understanding of the scaling of metabolic rate
76 with body size [4, 11, 12]. Here we test opposing predictions from influential metabolic scaling

77 theories that focus on transport of resources and waste products between the external
78 environment and metabolizing cells. This transport may be influenced by two major steps: the
79 exchange of materials across body surfaces, and the transport of materials through internal
80 networks. These steps are the foci for two prominent theoretical approaches to understanding
81 and predicting biological scaling: surface area (SA) theory [4, 8, 13, 14] and resource-transport
82 network (RTN) theory [1, 2, 15, 16], respectively.

83 Although both surface area and internal transport networks may be important in influencing
84 metabolic rate and its scaling with body size, SA theory predicts that body-shape changes should
85 have diametrically opposite effects on the scaling exponent b (see equation 1.1) than those
86 predicted by existing RTN theory [8]. If an organism shows isomorphic growth (i.e., it grows
87 with equal proportions in all three dimensions, so as to maintain a constant shape), SA theory
88 (based on simple Euclidean geometry) predicts that $b_R = 2/3$, whereas RTN theory typically
89 predicts that $b_R = 2/3$ [2, 16] or $3/4$ [1, 2, 15], depending on the physical properties of the
90 transport network [17, 18]. These predicted scaling exponents of $2/3$ and $3/4$ have received the
91 most attention by biologists since the seminal studies of Rubner [13] over a century ago.
92 However, if an organism displays nearly 2D growth (e.g., it grows in length and width without
93 any significant change in depth, thus appearing increasingly flat), SA theory predicts that $b_R \sim 1$
94 [8, 14], whereas RTN models predict that $b_R \sim 1/2$ [16], $5/8$ [2, 8] or $2/3$ [15], depending on
95 network geometry and dynamics. In addition, if an organism exhibits nearly 1D growth (e.g., it
96 grows in length without any significant change in width or depth, thus showing an increasingly
97 elongated shape), SA theory predicts that $b_R \sim 1$ [8, 14], whereas RTN theory predicts that $b_R \sim 0$
98 [16], $1/4$ [2, 8] or $1/2$ [15], again depending on network properties. Therefore, increased
99 elongation or flattening during ontogeny (trends toward 1D or 2D growth) should lead to an

100 increase in b_R , according to SA theory, whereas RTN theory predicts a decrease in b_R .
101 Conversely, increased thickening during ontogeny (specifically trends away from increasingly
102 elongated 1D or flattened 2D growth, but toward isomorphic 3D growth) reverses the changes in
103 b_R predicted by the two theories: SA theory predicts decreasing, and RTN theory increasing b_R .
104 Studying the effects of ontogenetic shape-shifting on metabolic scaling thus provides an
105 excellent opportunity to test the relative validity of models based on two major competing
106 theories of metabolic scaling, which is much needed for the field to advance [19, 20].

107

108 3. Testing theory using animals with mixed ontogenetic metabolic scaling

109 Several kinds of aquatic animals with complex life cycles exhibit ontogenetic shifts in metabolic
110 scaling, with scaling exponents (b_R) most often changing from near 1 in larvae or young
111 juveniles to < 1 in older juveniles or adults [3, 4, 21], but also more rarely showing reverse shifts
112 [22, 23]. From the literature we collected data on aquatic invertebrates that have complete or
113 partial pelagic (open water) life histories to test whether and how these ontogenetic shifts in
114 metabolic scaling are related to changes in body shape.

115 Scaling exponents (b_L) of least squares regressions (LSR) of \log_{10} body mass in relation to
116 \log_{10} major body length, separately calculated for larvae, juveniles and adults, were used to
117 quantify differences in shape-related growth between these life-history stages [24]. Logarithmic
118 transformation was used to permit easy detection of proportional changes [25]. If growth is
119 occurring proportionally in three dimensions without any change in mass density, b_L should be 3,
120 whereas if growth involves pure elongation in only one dimension (along the major length axis)
121 or pure flattening because of size increases in only two dimensions, b_L should be 1 or 2,
122 respectively [8]. For intermediate patterns of body-shape change, involving disproportionate

123 growth in one or two of the longest dimensions, $1 \leq b_L \leq 3$. Values of b_L may even be > 3 , if
 124 growth in width and (or) depth is proportionately greater than that for length (i.e., the animal is
 125 becoming thicker and/or broader) [8].

126 Unfortunately, actual measurements of body surface area during ontogeny are rare [8].
 127 Therefore, as a first-order approximation, we used Euclidean geometry for smooth surfaces to
 128 deduce scaling exponents (b_A) of \log_{10} body surface area in relation to \log_{10} body mass from b_L
 129 values. Values of b_A were inferred from b_L values that are ≤ 3 by using formulae for the extreme
 130 possibilities of different degrees of elongation (1D growth) and flattening (2D growth) [8]. The
 131 formula for different degrees of elongation is:

$$132 \quad b_A = \frac{1}{2} (1 + 1/b_L), \quad (3.1)$$

133 whereas the formula for different degrees of flattening is:

$$134 \quad b_A = 2(1/b_L). \quad (3.2)$$

135 Equation 3.1 applies for $1 \leq b_L \leq 3$; and equation 3.2 applies for $2 \leq b_L \leq 3$. Ranges of potential
 136 b_A values were also inferred from b_L values that are > 3 by using formulae quantifying
 137 disproportionate thickening in one or two dimensions. The formula for thickening of just the
 138 shortest dimension is the same as equation 3.2, whereas the formula for thickening of just the
 139 two shortest dimensions is the same as equation 3.1. When data on the scaling of body width
 140 with body length were available, it was possible to predict a single value of b_A rather than a range
 141 (see Supplementary Information).

142 The empirical b_L values and inferred b_A values were then compared to scaling exponents (b_R)
 143 for regressions of \log_{10} routine metabolic (oxygen consumption) rate (RMR) in relation to \log_{10}

144 body mass of different ontogenetic stages both within and among several species of aquatic
145 invertebrates.

146 Both b_L and b_R values were based on LSR, the method used for all of the literature scaling
147 analyses included in our study. An alternative, often used method, reduced major axis (RMA)
148 analysis, gave similar b values ($b_{RMA} = b_{LSR}/r$) to those from LSR, because reported correlation
149 coefficients (r) were always high (≥ 0.8). The r values for b_L averaged 0.97 ± 0.01 (\pm one
150 standard error, $n = 15$), and those for b_R averaged 0.96 ± 0.01 ($n = 19$) (calculated from r^2 values
151 in the Supplementary Information for Table 1). As a result, b_L and b_R values based on RMA
152 analyses averaged only ~ 3 -4% higher than those based on LSR.

153

154 4. Results

155 We compared conspecific b_L , b_R and inferred b_A values for different ontogenetic phases of a
156 ctenophore, a scyphozoan, two bivalves, two crustaceans, and two thaliaceans (table 1), as well
157 as mean heterospecific values for the nauplii, copepodites and adults of several copepod
158 crustaceans (table 2).

159 For seven of the eight species sampled, ontogenetic shifts in the metabolic scaling exponent
160 (b_R) are accompanied by inverse shifts in the scaling exponent of body mass in relation to length
161 (b_L) (table 1, figure 1a). The only exception is the pelagic tunicate *Salpa thompsoni*, which
162 showed no significant difference in b_R or b_L between the solitary (oozoid) and aggregate
163 (blastozoid) life-cycle stages (table 1, figure 1a). Remarkably, even this exception supports a
164 link between b_R and b_L , because both life stages of *S. thompsoni* have relatively high b_R values
165 associated with relatively low b_L values, as compared to the exponents exhibited by the other

166 species sampled (figure 1a). Furthermore, inverse shifts in b_R and b_L occurred in the other seven
167 species sampled regardless of whether b_R showed an increase or decrease during ontogeny (table
168 1, figure 1a). Inverse relationships between b_R and b_L , also seen among all of the species
169 averaged together (figures 1b, c), follow SA theory (figure 1b), but contradict all existing RTN
170 models (figure 1c). Although ontogenetic stages with a mean $b_L \sim 3$ (2.97 ± 0.21 95% CI)
171 exhibited a mean b_R (0.72 ± 0.08) not significantly different from $2/3$ or $3/4$, as predicted by both
172 SA and RTN theory, stages with a mean $b_L \sim 2.3$ (2.30 ± 0.12) exhibited a mean b_R (0.98 ± 0.07)
173 not significantly different from 1 and significantly greater than $2/3$ and $3/4$, as predicted by SA
174 theory, but in contradiction to all RTN models, which predict b_R values $< 2/3$ or $3/4$ (figures 1b,
175 c).

176 Since the scaling exponent for body surface area (b_A), as inferred from Euclidean geometry,
177 is inversely related to b_L (figure 1a; and equations 3.1 and 3.2), it follows that ontogenetic shifts
178 in b_R and b_A should be positively correlated, as observed in seven of the eight species sampled
179 (table 1, figure 2a). As predicted by SA theory, b_R and b_A are also positively correlated for all
180 species averaged together (figure 2b), and when these exponents were compared pairwise among
181 each of the ontogenetic phases of each species (figure 2c). The slope for the latter correlation
182 (1.14) is not significantly different from 1, as expected if b_R varied in direct proportion to b_A
183 (figure 2c).

184 Similar ontogenetic shifts are seen when b_R , b_L and b_A values are compared between nauplii
185 and copepodites/adults averaged among several species of copepods (table 2, figure 1a). The
186 heterospecific, inversely related shifts in b_R and b_L almost exactly parallel those observed for the
187 single copepod species *Mesocyclops brasiliensis* (figure 1a).

188 The spiny lobster *Sagmariasus verreauxi* nicely exemplifies how ontogenetic changes in
189 metabolic rate and body shape correlate. The phyllosoma larvae are very thin and flat and show
190 nearly 2D growth, until they metamorphose into adult-looking benthic juveniles that are much
191 thicker and show 3D growth. This marked shift in growth pattern and body form is represented
192 by an abrupt ontogenetic shift in b_L values: from 2.142 ± 0.260 (95% CI) in the phyllosoma
193 larvae to 2.991 ± 0.037 in the juveniles (figure 3a, table 1). The phyllosoma b_L value is not
194 significantly different from 2, whereas the juvenile b_L value is not significantly different from 3.
195 As a result, the scaling exponents for surface area (b_A) can be inferred (see Supplementary
196 Information) to be 0.91 for the phyllosomas and 0.67 for the juveniles (table 1). Like b_A , the b_R
197 values for RMR are also higher in the phyllosomas (1.002 ± 0.081) than in the juveniles ($0.829 \pm$
198 0.157) (figure 3b, table 1). Comparisons of the 95% CI [19, 28] reveal that the b_L and b_R values
199 are both significantly different between the two life-history stages. The phyllosoma b_R value is
200 not significantly different from 1, but is significantly greater than $1/2$, $5/8$, $2/3$ and $3/4$. By
201 contrast, the juvenile b_R value is significantly less than 1, not significantly different from $3/4$, and
202 significantly greater than $1/2$, $5/8$ and $2/3$ (just barely).

203

204 5. Discussion

205 The parallel changes in the scaling exponents for surface area (SA) and routine metabolic rate
206 (RMR) observed in seven of the eight species sampled supports the importance of SA changes in
207 the observed ontogenetic shifts in metabolic scaling, but contradicts predictions of all current
208 models emphasizing internal resource-transport networks. SA theory predicts positive
209 correlations between the scaling exponents for SA (b_A) and RMR (b_R), as observed, whereas
210 current RTN theory incorrectly predicts negative correlations between b_A and b_R . Although RTN

211 theory has been claimed to be universally applicable to all of life [1, 15] or at least to
212 macroscopic multicellular eukaryotes [29], RTN theory may not apply to animals without
213 circulatory systems or with open or incompletely closed circulatory systems [2, 4, 18], which are
214 far more common taxonomically than those with completely closed circulatory systems
215 (including only vertebrates, cephalopod mollusks, and some annelid and nemertean worms [30,
216 31]. In fact, two of the animal species included in our study have no or very rudimentary
217 circulatory systems (a ctenophore and scyphozoan), whereas the other six have open or
218 incompletely closed circulatory systems (the bivalve, crustacean and thaliacean species) [31].
219 Nevertheless, Hirst et al. [8] have shown that the similarly steep ontogenetic scaling of metabolic
220 rate and surface area in squids (cephalopods), which have closed circulatory systems, is also
221 consistent with SA theory, but not with RTN theory. This additional deviation from RTN theory
222 may be because squids use both their skin and gills for gas exchange and also distribute resource-
223 laden blood by means of multiple hearts found throughout the body [31], rather than from a
224 single centralized heart or distribution center, as assumed by the theory [1, 2, 15, 16]. If current
225 RTN theory does not apply to squids for these reasons, then annelid worms that use multiple
226 hearts to pump blood [31] and nemertean worms that have no heart at all [31] may also be
227 exceptions. Therefore, current RTN theory appears to apply to only a small subset of animals:
228 perhaps only vertebrates with single central hearts in a completely closed vascular system. Even
229 the application of RTN theory to vertebrates may be of limited use, because currently it cannot
230 explain large variation in the metabolic scaling exponent observed in various vertebrate classes
231 that appears to be related to physiological state, ecological lifestyle, or environmental conditions
232 [11, 32-35]. Perhaps next generation resource exchange and supply theory may resolve these
233 problems (also see section 6).

234 In any case, the positive associations between b_A and b_R observed in this study are
235 remarkable because they occur in animals with very different body designs from five different
236 phyla, and furthermore, they occur regardless of whether b_A increases, or decreases, during
237 ontogeny. Therefore, it is unlikely that these correlations are merely side-effects of b_A and b_R
238 being independently related to other unmeasured factors associated with developmental
239 maturation or ontogenetic age. Rather, it is more likely that shape shifting directly affects
240 metabolic scaling via changes in surface area available for resource uptake and waste excretion.

241 This hypothetical mechanism for how shape-shifting may affect metabolic scaling seems
242 especially applicable to the thin-skinned pelagic animals (larval or adult) that we have studied
243 here and in a related paper [8]. Phyllosoma, veliger and other pelagic larvae of many marine
244 animals that show biphasic or triphasic metabolic scaling appear to be ‘skin-breathers’, i.e., they
245 can absorb oxygen and expel dissolved waste products such as CO_2 through their thin, permeable
246 integuments [36, 37]. Some can even absorb nutrients through their body surfaces and
247 metabolize them in their tissues [36, 38]. Remarkably, the body-mass scaling slope for
248 metabolism in larvae of the Pacific oyster, *Crassostrea gigas*, is not significantly different from
249 that for uptake of the amino acid alanine [36]. However, it is not known how much cutaneous
250 absorption of nutrients contributes to the overall energy budget of these larvae and other pelagic
251 animals under natural conditions [39]. Our findings point toward the importance of further
252 research on integumentary energy and material exchange by aquatic animals, as a way to better
253 understand variation in their metabolic scaling.

254 Although parallel shifts in b_A and b_R values have been typically seen in this study, possible
255 exceptions invite further scrutiny. For example, although b_A and b_R decrease in tandem in the
256 common mussel *Mytilus edulis*, as juveniles mature into adults, more research is needed to

257 determine whether shifts in SA and metabolic scaling also match as veliger larvae develop into
258 juveniles. This is because, although the inferred b_A shift from 0.57 to 0.71-0.83 parallels the shift
259 of b_R from 0.77 (based on an average of four values) to 0.89, the individual b_R estimates for
260 veligers are highly variable, ranging from 0.59 to 0.90 (table 1). Some of this variation appears
261 to be related to temperature, because as temperature increases from 6 to 18° C, b_R decreases from
262 0.90 to 0.59 (see Supplementary Information), as predicted by the metabolic-level boundaries
263 hypothesis [11]. Sampling error may also be important because the body-size range of growing
264 veligers is small (< 2 orders of magnitude), thus potentially increasing variation in b_R estimates
265 [32, 40, 41] relative to those of juveniles and adults with larger body-size ranges (> 3 and 4
266 orders of magnitude, respectively; see table 1). A similar explanation may apply to the variable
267 b_R estimates (0.35 and 1.01) for two ephyra samples of the scyphozoan *Aurelia aurita* that have
268 body-size ranges < 1.5 orders of magnitude, and that deviate markedly from the inferred b_A value
269 of 0.64 (table 1). By contrast, the b_R estimate (0.63) for a third sample of ephyra larvae, with a
270 body-size range > 2 orders of magnitude, is very close to the estimated b_A value. The b_R value
271 (0.65) for ephyra larvae of *A. aurita*, estimated from the metabolic data of several studies taken
272 together, is also not significantly different from 0.64 [42]. It is also possible that our simple
273 Euclidean estimates of SA scaling (b_A) do not adequately represent the ontogenetic SA changes
274 occurring in a mussel veliger or an ephyra larva. A veliger's velum (foot), which has an
275 extensive, highly permeable surface [36], can extend far beyond the measured shell length used
276 in calculating b_L and b_A [43]. In addition, the ephyra larva shows complex changes in body
277 shape as it grows, thus making it difficult to accurately estimate the scaling of its surface area
278 (see footnote 6 in Supplementary Information for table 1).

279 Another major pattern evident in our results is that, although b_R is significantly correlated
280 with b_A , it is usually greater than that predicted by b_A alone (see tables 1 and 2, and figures 1a, b
281 and 2). Two major factors may help account for these upward deviations of b_R . First,
282 metabolically costly growth may elevate b_R values, as has been observed in other animals and
283 plants [3-7, 19]. These growth effects prompt the question: what are the relative influences of
284 resource supply versus metabolic demand by growth and other biological processes on
285 ontogenetic metabolic scaling [4, 9-12]? For example, does the steep scaling of SA (and
286 presumably resource uptake) of many kinds of pelagic animals (including larvae) documented
287 here and by Hirst et al. [8] permit or even drive the steep scaling of metabolism, or is steep SA
288 scaling a secondary adjustment to steep metabolic scaling that is driven by high resource demand
289 (e.g., high mass-specific growth rates that often occur in pelagic animals [4, 44, 45]).
290 Attempting to answer this critical question brings into focus the importance of understanding the
291 various factors influencing all of the steps of energy flow through an organism, and their
292 integration, as a way to improve our knowledge of how and why metabolic rate varies with body
293 size (also see [4]).

294 A second major factor that may help to explain apparent upward deviations in b_R from that
295 predicted by our SA model (figure 1a) is that we may have underestimated SA changes during
296 ontogeny in our study species. Our geometric estimates of b_A based on b_L do not allow the
297 detection of increases in SA related to increased convolutions (or fractal dimensionality) of the
298 body surface, or to the development of special respiratory and nutrient-absorption structures
299 (e.g., the velum in veligers, and the gills in other larval or juvenile forms). In addition, half of
300 our inferred b_A values (see Table 1) may have been underestimated because they were based on
301 the midpoint of a potential range of values, whereas in most cases the upper limit of this range,

302 which involves shape flattening, is probably closer to the actual b_A value than the lower limit,
303 which involves shape elongation (also see section 3 and [8]). This claim is supported by two
304 observations. First, when data on both b_L and the scaling of body width versus length were
305 available (Supplementary Information), the inferred b_A value was almost always closer (in 9 of
306 10 cases) to the predicted upper limit involving mainly 2D growth (flattening) than the lower
307 limit involving mainly 1D growth (elongation) (see table 1). Second, the mean empirical
308 relationship between b_L and b_R (and by association b_A : see figures 2a, b, c) for all of our study
309 species more closely parallels the upper predicted boundary involving flattening than the lower
310 boundary involving elongation (figure 1b).

311 Other data consistent with effects of ontogenetic shifts in body shape and SA scaling on
312 metabolic scaling include significant correlations between b_L and b_R values among diverse ‘skin-
313 breathing’ pelagic animals, for which species-specific values also show a closer match to the
314 upper boundary curve for predicted b_A than to the lower boundary curve [8], and parallel steep
315 scaling of SA and metabolic rate in echinoid larvae [46] and epipelagic squid [47]. Steep (often
316 near isometric) scaling of metabolic rate in larval fishes may not only be related to their rapid
317 growth rates [4], but also to their ability to absorb oxygen and possibly nutrients through their
318 entire body surface [48, 49]. In addition, shifts from isometric to negatively allometric
319 metabolic scaling seen in plants may be related, at least in part, to decreases in their relative
320 ‘leafiness’ (i.e., reductions in the relative contribution of high SA leaves to total biomass) as they
321 grow [6].

322

323 6. Conclusions

324 Ontogenetic shifts in metabolic scaling may often be linked to developmental changes in body
325 shape and SA scaling. Our SA model predicts that this should happen in any organism that relies
326 heavily on body surfaces for resource uptake, such as many kinds of thin-skinned pelagic
327 animals and leafy plants. Ontogenetic shifts in metabolic scaling in other organisms with
328 impermeable integuments may be related to shifts in the SA scaling of specialized respiratory
329 structures (as occur for gills in developing fish [48] and for tracheae during molting in insect
330 larvae [12]) or to other metabolically demanding developmental changes unrelated to body shape
331 (e.g., shifts from ectothermy to endothermy in developing mammals [4]). Nevertheless,
332 evidence reported here and elsewhere suggests that the effect of external surface area on
333 metabolic scaling may often outweigh or modify the influence of resource-transport networks [4,
334 8, 11, 46, 47], contrary to currently influential general models [1, 2]. New resource transport
335 theory is needed that can accommodate organisms that distribute resources from external
336 surfaces rather than from (or in addition to) internal central “pumps” (also see [8]). Therefore,
337 reports of the demise of surface-area theory in the field of metabolic scaling are incorrect [50,
338 51]. We argue that surface-area theory is alive and strong, and is essential to a comprehensive
339 explanation of metabolic scaling.

340

341 References

- 342 1. West GB, Brown JH, Enquist BJ. 1997. A general model for the origin of allometric scaling laws in
343 biology. *Science* **276**, 122-126. (doi:10.1126/science.276.5309.122)
- 344 2. Banavar JR, Moses ME, Brown JH, Damuth J, Rinaldo A, Sibly RM, Maritan A. 2010. A general basis
345 for quarter-power scaling in animals. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 15816-15820.
346 (doi:10.1073/pnas.1009974107)
- 347 3. Riisgård HU. 1998 No foundation of a ‘3/4 power scaling law’ for respiration in biology. *Ecol. Lett.* **1**,
348 71-73. (doi:10.1046/j.1461-0248.1998.00020.x)
- 349 4. Glazier DS. 2005 Beyond the ‘3/4-power law’: variation in the intra- and interspecific scaling of
350 metabolic rate in animals. *Biol. Rev.* **80**, 611-662. (doi:10.1017/S1464793105006834)

- 351 5. Czarnoleski M, Kozłowski J, Dumiot G, Bonnet J-C, Mallard J, DuPont-Nivet M. 2008 Scaling of
352 metabolism in *Helix aspersa* snails: changes through ontogeny and response to selection for
353 increased size. *J. Exp. Biol.* **211**, 391-399. (doi:10.1242/jeb.013169)
- 354 6. Peng Y, Niklas KJ, Reich PB, Sun S. 2010 Ontogenetic shift in the scaling of dark respiration with
355 whole-plant mass in seven shrub species. *Funct. Ecol.* **24**. 502-512. (doi:10.1111/j.1365-
356 2435.2009.01667.x)
- 357 7. Kutschera U, Niklas KJ. 2011 Ontogenetic changes in the scaling of cellular respiration with respect to
358 size among sunflower seedlings. *Plant Signal. Behav.* **6**, 72-76. (doi:10.4161/psb.6.1.14001)
- 359 8. Hirst AG, Glazier DS, Atkinson D. 2014 Body shape shifting during growth permits tests that
360 distinguish between competing geometric theories of metabolic scaling. *Ecol. Lett.*
361 (doi:10.1111/ele.12334).
- 362 9. Darveau C-A, Suarez RK, Andrews RD, Hochachka PW. 2002 Allometric cascade as a unifying
363 principle of body mass effects on metabolism. *Nature* **417**, 166-170. (doi:10.1038/417166a)
- 364 10. Hofmeyr JHS, Rohwer JM. 2011. Supply-demand analysis: a framework for exploring the regulatory
365 design of metabolism. *Methods Enzymol.* **500**, 533-554. (doi: 10.1016/B978-0-12-385118-5.00025-6)
- 366 11. Glazier DS. 2010 A unifying explanation for diverse metabolic scaling in animals and plants. *Biol. Rev.*
367 **85**, 111-138. (doi:10.1111/j.1469-185X.2009.00095.x)
- 368 12. Callier V, Nijhout HF. 2012 Supply-side constraints are insufficient to explain the ontogenetic scaling
369 of metabolic rate in the Tobacco Hornworm, *Manduca sexta*. *PLoS ONE* **7**, e45455.
370 (doi:10.1371/journal.pone.0045455)
- 371 13. Rubner M. 1883 Über den Einfluss der Körpergrösse auf Stoff- und Kraftwechsel. *Zeit. Biol.* **19**, 535-
372 562.
- 373 14. Okie JG. 2013 General models for the spectra of surface area scaling strategies of cells and
374 organisms: fractality, geometric dissimilitude, and internalization. *Am. Nat.* **181**, 421-439.
375 (doi:10.1086/669150)
- 376 15. West GB, Brown JH, Enquist BJ. 1999 The fourth dimension of life: fractal geometry and allometric
377 scaling of organisms. *Science* **284**, 1677-1679. (doi:10.1126/science.284.5420.1677)
- 378 16. Dodds PS. 2010 Optimal form of branching supply and collection networks. *Phys. Rev. Lett.* **104**,
379 048702. (doi:10.1103/PhysRevLett.104.048702)
- 380 17. Savage VM, Deeds EJ, Fontana W. 2008. Sizing up allometric scaling theory. *PLoS Comp. Biol.* **4**,
381 e1000171 (doi:10.1371/journal.pcbi.1000171)
- 382 18. Price CA, Weitz JS, Savage VM, Stegen J, Clarke A, Coomes DA, Dodds PS, Etienne RS, Kerkhoff
383 AJ, McCulloh K, Niklas KJ, Olf H, Swenson NG. 2012 Testing the metabolic theory of ecology. *Ecol.*
384 *Lett.* **15**, 1465-1474. (doi:10.1111/j.1461-0248.2012.01860.x)
- 385 19. Glazier DS, Butler EM, Lombardi SA, Deptola TJ, Reese AJ, Satterthwaite EV. 2011 Ecological
386 effects on metabolic scaling: amphipod responses to fish predators in freshwater springs. *Ecol.*
387 *Monogr.* **81**, 599-618. (doi.org/10.1890/11-0264.1)

- 388 20. Kearney MR, White CR 2012 Testing metabolic theories. *Am. Nat.* **180**, 546-565.
389 (doi:10.1086/667860)
- 390 21. Zeuthen E. 1953 Oxygen uptake as related to body size in organisms. *Q. Rev. Biol.* **28**, 1-12.
391 (<http://www.jstor.org/stable/2810299>)
- 392 22. Kinoshita J, Hiromi J, Kadota S. 1997 Do respiratory metabolic rates of the scyphomedusa *Aurelia*
393 *aurita* scale isometrically throughout ontogeny in a sexual generation? *Hydrobiologia* **347**, 51-55.
394 (doi:10.1023/A:1002942806113)
- 395 23. Svetlichny LS, Abolmasova GI, Hubareva ES, Finenko GA, Bat, L, Kideys AE. 2004 Respiration rates
396 of *Beroe ovata* in the Black Sea. *Mar. Biol.* **145**, 585-593. (doi:10.1007/s00227-004-1336-4)
- 397 24. Hirst AG. 2012 Intra-specific scaling of mass to length in pelagic animals: Ontogenetic shape change
398 and its implications. *Limnol. Oceanogr.* **57**, 1579-1590. (doi:10.4319/lo.2012.57.5.1579)
- 399 25. Glazier DS. 2013 Log-transformation is useful for examining proportional relationships in allometric
400 scaling. *J. Theor. Biol.* **334**, 200-203. (doi.org/10.1016/j.jtbi.2013.06.017)
- 401 26. Jensen MA, Fitzgibbon QP, Carter CG, Adams LR. 2013a Effect of body mass and activity on the
402 metabolic rate and ammonia-N excretion of the spiny lobster *Sagmariasus verreauxi* during ontogeny.
403 *Comp. Biochem. Physiol., Part A* **166**, 191-198. (doi.org/10.1016/j.cbpa.2013.06.003)
- 404 27. Jensen MA, Fitzgibbon QP, Carter CG, Adams LR. 2013b The effect of stocking density on growth,
405 metabolism and ammonia-N excretion during larval ontogeny of the spiny lobster *Sagmariasus*
406 *verreauxi*. *Aquaculture* **376-379**, 45-53. (doi.org/10.1016/j.aquaculture.2012.10.033)
- 407 28. Cumming G. 2008 Inference by eye: reading the overlap of independent confidence intervals. *Stat.*
408 *Med.* **28**, 205-220. (doi:10.1002/sim.3471)
- 409 29. DeLong JP, Okie JG, Moses ME, Sibly RM, Brown JH. 2010 Shifts in metabolic scaling, production
410 and efficiency across major evolutionary transitions of life. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 12941-
411 12945. (doi:10.1073/pnas.1007783107)
- 412 30. Reiber CL, McGaw IJ. 2009 A review of the “open” and “closed” circulatory systems: new terminology
413 for complex invertebrate circulatory systems in light of current findings. *Int. J. Zool.* **2009** (Article ID
414 301284), 1-8. (doi:10.1155/2009/301284)
- 415 31. McMahon BR, Wilkens JL, Smith PJS. 2011 Invertebrate circulatory systems. *Compr. Physiol.*, 931-
416 1008. (doi:10.1002/cphy.cp130213)
- 417 32. Bokma F. 2004 Evidence against universal metabolic allometry. *Funct. Ecol.* **18**, 184-187.
418 (doi:10.1111/j.0269-8463.2004.00817.x)
- 419 33. White CR, Phillips NF, Seymour RS. 2006 The scaling and temperature dependence of vertebrate
420 metabolism. *Biol. Lett.* **2**, 125-127. (doi:10.1098/rsbl.2005.0378)
- 421 34. Glazier DS. 2008 Effects of metabolic level on the body-size scaling of metabolic rate in birds and
422 mammals. *Proc. Roy. Soc. Lond B* **275**, 1405-1410. (doi:10.1098/rspb.2008.0118)

- 423 35. Killen SS, Atkinson D, Glazier DS. 2010 The intraspecific scaling of metabolic rate with body mass in
424 fishes depends on lifestyle and temperature. *Ecol. Lett.* **13**, 184-193. (doi:10.1111/j.1461-
425 0248.2009.01415.x)
- 426 36. Manahan DT. 1990 Adaptations by invertebrate larvae for nutrient acquisition from seawater. *Am.*
427 *Zool.* **30**, 147-160. (doi:10.1093/icb/30.1.147)
- 428 37. Rodriguez Souza JC, Strüssmann CA, Takashima F, Satoh H, Sekine S, Shima Y, Matsuda H. 2010
429 Oral and integumental uptake of free exogenous glycine by the Japanese spiny lobster *Panulirus*
430 *japonicas* phyllosoma larvae. *J. Exp. Biol.* **213**, 1859-1867. (doi: 10.1242/jeb.040030)
- 431 38. Haond C, Charmantier G, Flik G, Bonga SE. 2001 Identification of respiratory and ion-transporting
432 epithelia in the phyllosoma larvae of the slipper lobster *Scyllarus arctus*. *Cell Tissue Res.* **305**, 445-
433 455. (doi:10.1007/s004410100405)
- 434 39. Gomme, J. 2001 Transport of exogenous organic substances by invertebrate integuments: the field
435 revisited." *J. Exp. Zool.* **289**, 254-265. (doi:10.1002/1097-010X(20010401/30)289:4<254::AID-
436 JEZ6>3.0.CO;2-F)
- 437 40. White CR, Seymour RS. 2005 Sample size and mass range effects on the allometric exponent of
438 basal metabolic rate. *Comp. Biochem. Physiol. A* **142**, 74-78. (doi.org/10.1016/j.cbpa.2005.07.013)
- 439 41. Moses ME, Hou C, Woodruff WH, West GB, Nekola JC, Zuo W, Brown JH. 2008 Revisiting a model of
440 ontogenetic growth: estimating model parameters from theory and data. *Am. Nat.* **171**, 632-645.
441 (<http://www.jstor.org/stable/10.1086/587073>)
- 442 42. Gambill M, Peck MA. 2014 Respiration rates of the polyps of four jellyfish species: Potential thermal
443 triggers and limits. *J. Exp. Mar. Biol. Ecol.* **459**, 17-22. (doi:10.1016/j.jembe.2014.05.005)
- 444 43. Hamburger K, Møhlenberg F, Randløv A, Riisgård HU. 1983 Size, oxygen consumption and growth in
445 the mussel *Mytilus edulis*. *Mar. Biol.* **75**, 303-306. (doi:10.1007/BF00406016)
- 446 44. Glazier DS. 2006 The 3/4-power law is not universal: evolution of isometric, ontogenetic metabolic
447 scaling in pelagic animals. *Bioscience* **56**, 325-332. (doi:10.1641/0006-
448 3568(2006)56[325:TPLINU]2.0.CO;2)
- 449 45. Hirst AG, Forster J. 2013 When growth models are not universal: evidence from marine invertebrates.
450 *Proc. R. Soc. B* 280: 20131546. (doi.org/10.1098/rspb.2013.1546)
- 451 46. McEdward LR. 1984 Morphometric and metabolic analysis of the growth and form of an
452 echinopluteus. *J. Exp. Mar. Biol. Ecol.* **82**, 259-287. (doi.org/10.1016/0022-0981(84)90109-6)
- 453 47. Seibel BA. 2007 On the depth and scale of metabolic rate variation: scaling of oxygen consumption
454 rates and enzymatic activity in the Class Cephalopoda (Mollusca). *J. Exp. Biol.* **210**, 1-11.
455 (doi:10.1242/jeb.02588)
- 456 48. Post JR, Lee JA. 1996 Metabolic ontogeny of teleost fishes. *Can. J. Fish. Aquat. Sci.* **53**, 910-923.
457 (doi:10.1139/f95-278)
- 458 49. Glover CN, Bucking C, Wood, CM. 2013 The skin of fish as a transport epithelium: a review. *J. Comp.*
459 *Physiol. B* 183, 877-891. (doi: 10.1007/s00360-013-0761-4)

- 460 50. Peters RH. 1983 *The ecological implications of body size*. Cambridge, UK: Cambridge University
461 Press.
- 462 51. Savage, VM, Gillooly JF, Woodruff WH, West GB, Allen AP, Enquist BJ, Brown JH. 2004 The
463 predominance of quarter-power scaling in biology. *Funct. Ecol.* **18**, 257-282. (doi:10.1111/j.0269-
464 8463.2004.00856.x)
- 465
- 466
- 467
- 468
- 469
- 470
- 471
- 472
- 473
- 474
- 475
- 476
- 477
- 478
- 479
- 480
- 481
- 482
- 483
- 484
- 485
- 486
- 487
- 488
- 489
- 490
- 491
- 492
- 493
- 494
- 495
- 496

497 Table 1. Ontogenetic scaling exponents from least squares regressions of \log_{10} body mass in relation to
 498 \log_{10} body length (b_L) and \log_{10} routine metabolic rate in relation to \log_{10} body mass (b_R) for larvae,
 499 juveniles, and (or) adults of the Atlantic ctenophore *Beroe ovata*, moon jellyfish *Aurelia aurita*, Pacific
 500 oyster *Crassostrea gigas*, common mussel *Mytilus edulis* and spiny lobster *Sagmariasus verreauxi*; for
 501 nauplii, copepodites and adults of the copepod *Mesocyclops brasiliensis*; and for solitary and aggregate
 502 life-cycle stages of the salps (pelagic tunicates) *Salpa fusiformis* and *S. thompsoni*. Values of b_L and b_R
 503 were taken or calculated from data in sources listed in the Supplementary Information, where additional
 504 data and methodological information can be found. Values of b_A for \log_{10} body surface area in relation to
 505 \log_{10} body mass were estimated from b_L values and scaling exponents of body width versus length
 506 (Supplementary Information). When data for scaling of width versus length were not available, ranges of
 507 potential b_A values are given (based on equations 3.1 and 3.2). Note the parallel changes in b_A and b_R
 508 values (shown in bold), regardless of whether decreases or increases in b_R were observed during
 509 ontogeny (except possibly for the transition from veliger larvae to juveniles in *M. edulis*; also see text).
 510 Statistically significant ontogenetic shifts in b_L and b_R values occur in all species, except for between
 511 solitary and aggregate life stages of *S. thompsoni*.

Species	Stage	b_L	b_A	b_R
<i>Beroe ovata</i>	juveniles	2.92	0.67 (0.67-0.68)	0.62
	adults	2.47	0.80 (0.70-0.81)	0.99
		2.23	0.87 (0.72-0.90)	1.04
		1.78	1.09 (0.78-1.00)	0.86
<i>Aurelia aurita</i>	ephyra larvae	3.14	0.64 (0.64-0.66)	0.63
				0.35
				1.01
	medusae	2.50	0.80 (0.70-0.80)	0.93
		2.72	0.74 (0.68-0.74)	1.11
				1.01
<i>Crassostrea gigas</i>	veliger larvae	2.12	0.95 (0.74-0.94)	0.96
	adults	2.79	0.68-0.72	0.77
<i>Mytilus edulis</i>	veliger larvae	3.49	0.57 (0.57-0.71)	0.90
				0.90
				0.70
	juveniles	2.42	0.71-0.83	0.89

541		adults	3.17	0.63-0.68	0.66
542					0.68
543				
544					
545					
546	<i>Sagmariasus</i>	phyllosoma larvae	2.14	0.91 (0.73-0.93)	1.00
547	<i>verreauxi</i>				
548		juveniles	2.99	0.67	0.83
549				
550	<i>Mesocyclops</i>	nauplii	2.15	0.73-0.93	1.08
551	<i>brasilianus</i>				
552		copepodites & adults	3.12	0.64-0.68	0.56
553				
554	<i>Salpa</i>	solitary zooids	2.40	0.71-0.83	1.15
555	<i>fusiformis</i>				
556		aggregate zooids	2.78	0.68-0.72	0.68
557				
558	<i>Salpa</i>	solitary zooids	2.28	0.72-0.88	0.84
559	<i>thompsoni</i>				
560		aggregate zooids	2.41	0.71-0.83	0.92
561				
562					
563					
564					
565					
566					
567					
568					
569					
570					
571					
572					
573					
574					
575					
576					
577					
578					
579					
580					
581					
582					
583					
584					

585 Table 2. Mean ontogenetic scaling exponents from least squares regressions of \log_{10} body mass in
 586 relation to \log_{10} body length (b_L) and \log_{10} routine metabolic rate in relation to \log_{10} body mass (b_R) for
 587 nauplii, copepodites and adults of several copepod species (b_L and b_R values were taken from the
 588 indicated sources, whereas the ranges of potential b_A values for \log_{10} body surface area in relation to
 589 \log_{10} body mass were estimated from b_L values using equations 3.1 and 3.2 in the text). Mean scaling
 590 exponents were calculated by averaging mean conspecific values among species. The 95% confidence
 591 intervals (CI) and number of species sampled (n) are given for each scaling exponent. Note the parallel
 592 changes in b_A and b_R values (shown in bold). Data sources are given in the Supplemental Information.

593
 594

595 Stage	$b_L \pm 95\% \text{ CI (n)}$	b_A	$b_R \pm 95\% \text{ CI (n)}$
597 Nauplii	$2.28 \pm 0.27^1 (17)$	0.72-0.88	1.00 \pm 0.16² (4)
599 Copepodites 600 and adults	$2.84 \pm 0.20^1 (20)$	0.68-0.70	0.72 \pm 0.22² (7)

601

602 ¹ Based on dry mass, carbon mass or nitrogen mass.

603 ² Based on dry body mass

604
 605
 606
 607
 608
 609
 610
 611
 612
 613
 614
 615
 616
 617
 618
 619
 620
 621

622 Figure legends

623 **Figure 1.** Ontogenetic shifts of b_R in relation to b_L of several species of aquatic invertebrates in five
 624 different phyla (a) and for all species averaged together (b, c), where b_R is the scaling exponent for \log_{10}
 625 routine metabolic rate in relation to \log_{10} body mass, and b_L is the scaling exponent for \log_{10} body mass in
 626 relation to \log_{10} total body length (data from tables 1 and 2). Multiple b values for the life-history stage of
 627 a species or group of species (copepods) were averaged. Arrows indicate the direction of ontogenetic
 628 change. In (b, c) the upper left and lower right points were calculated by averaging all of the paired b_R
 629 and b_L values that occurred in ontogenetic stages with the higher versus lower b_R , respectively. The 95%
 630 confidence limits are shown for each mean value of b_R and b_L . Also shown in (b) are the bounded range
 631 of values of b_A (\log_{10} body surface area in relation to \log_{10} body mass) in relation to b_L (depicted as light
 632 purple lines) calculated using equations 3.1 and 3.2 based on Euclidean geometry (also see [8]); and in
 633 (c) the predicted effects of body-shape changes (b_L) on b_R (depicted as dashed colored lines) according
 634 to the resource-transport network models of West et al. (1999)[15], Banavar et al. (2010)[2] and Dodds
 635 (2010)[16] (also see [8]). Note that in 7 of 8 species sampled, ontogenetic shifts in b_L are accompanied by
 636 inverse shifts in b_R , as predicted by surface-area scaling theory (b), but in contradiction to resource-
 637 transport network scaling theory (c).

638

639 **Figure 2.** Ontogenetic shifts of b_R in relation to b_A of several species of aquatic invertebrates (a) and for
 640 all species averaged together (b), where b_R is the scaling exponent for \log_{10} routine metabolic rate in
 641 relation to \log_{10} body mass, and b_A is the scaling exponent for \log_{10} body mass in relation to \log_{10} total
 642 body length (data from table 1). Values of b_A were calculated using b_L values and additional data in the
 643 Supplementary Information. Multiple b_R values for the life-history stage of a species were averaged,
 644 whereas the midpoint was used when only a range of b_A values was available. In (b) the lower left and
 645 upper right points were calculated by averaging all of the paired b_R and b_A values that occurred in
 646 ontogenetic stages with the lower versus higher b_R , respectively. The 95% confidence limits are shown
 647 for each mean value of b_R and b_A . As predicted by surface-area scaling theory (also see [8]), in 7 of 8
 648 species sampled, ontogenetic shifts in b_A are accompanied by positively correlated shifts in b_R . Also
 649 shown in (c) is a significant positive correlation between b_R and b_A for each ontogenetic stage of each
 650 species. The equation for the least squares regression line is: $b_R = 1.14 \pm 0.64$ (95% CI) (b_A) - 0.0092 (r^2
 651 = 0.454; $P = 0.0018$, $n = 17$). In (a, b, c), the dotted diagonal lines represent $b_R = b_A$.

652

653 **Figure 3.** Scaling of \log_{10} wet body mass in relation to \log_{10} total body length (a) and \log_{10} routine
 654 metabolic rate (RMR) in relation to \log_{10} wet body mass (b) in phyllosoma larvae (open points) and
 655 juveniles (solid points) of the spiny lobster *Sagmariasus verreauxi* maintained at 21-23° C. Each point in
 656 (a) and (b) is based on 4-11 replicate measurements, respectively [26, 27]. The phyllosoma points

657 represent instars 1, 3, 6, 9, 12, 15 and 17. The standard errors for each RMR value are all less than 20%
658 of the mean. The least squares regression equations for the scaling lines and their coefficients of
659 determination (r^2) and significance levels (p) for phyllosoma larvae and juveniles are: (a) $Y = -3.931 +$
660 $2.142 (X)$, $r^2 = 0.988$, $p = 0.00001$; $Y = -4.574 + 2.991 (X)$, $r^2 = 1.000$, $p < 0.00001$; and (b) $Y = -0.773 +$
661 $1.002 (X)$, $r^2 = 0.995$, $p < 0.00001$; $Y = -0.673 + 0.829 (X)$, $r^2 = 0.993$, $p = 0.00353$, respectively. The
662 number by each line is the scaling slope (exponent). Linear extrapolations of the empirical scaling lines
663 for the phyllosoma larvae are shown as dotted lines to highlight the ontogenetic scaling shifts for both
664 body shape (a) and metabolic rate (b).





