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► To cite this version:

Thomas Ruiz, Apostolos-manuel Koussoroplis, Michael Danger, Jean-pierre Aguer, Nicole Morel-desrosiers, et al.. Quantifying the energetic cost of food quality constraints on resting metabolism to integrate nutritional and metabolic ecology. *Ecology Letters*, 2021, 24 (11), pp.2339-2349. 10.1111/ele.13855 . hal-03408463v1

HAL Id: hal-03408463

<https://hal.science/hal-03408463v1>

Submitted on 29 Oct 2021 (v1), last revised 7 Feb 2024 (v2)

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1 **Title:**

2 Quantifying the energetic cost of food quality constraints on resting metabolism to
3 integrate nutritional and metabolic ecology

4
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15 **Short title:** Linking nutritional and metabolic ecology

16
17 **Keywords:** Metabolic ecology, Nutrition, Secondary production, Daphnia, stoichiometry, fatty acids,
18 sterols

19 **Article Type:** Letters

20 **Statement of authorship:**

21 TR, AMK, MD and AB conceived the idea and developed the experimental protocol. TR ran the
22 experiment and acquired the data. TR, AMK and AB led the writing of the manuscript. All authors
23 contributed critically to the draft and gave the final approval for publication.

24
25 **Data accessibility statement:**

26 The data supporting the paper conclusions and R scripts associated are available on Figshare
27 (doi:10.6084/m9.figshare.13234277).

28 **Words in abstract:** 150

29 **Words in main text:** 4863

30 **Number of references:** 50

31 **Number of figures:** 3 + **Supplementary figures:** 1

32
33 **Number of Tables:** 1 + **Supplementary tables:** 1

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38

39 **Abstract**

40 Consumer metabolism controls the energy uptake from the environment and its
41 allocation to biomass production. In natural ecosystems, available energy in food often
42 fails to predict biomass production which is also (co)limited by the relative availability of
43 various dietary compounds. To date, the link between energy metabolism and the effects
44 of food chemical composition on biomass production remains elusive. Here, we measured
45 the resting metabolic rate (RMR) of *Daphnia magna* along ontogeny when undergoing
46 various (non-energetic) nutritional constraints. All types of dietary (co)limitations (Fatty
47 acids, Sterols, Phosphorus) induced an increase in mass-specific RMR up to 128% between
48 highest and lowest quality diets. We highlight a strong negative correlation between RMR
49 and growth rate indicating RMR as a promising predictor of consumer growth rate. We
50 argue that quantifying the energetic cost imposed by food quality on individual RMR may
51 constitute a common currency enabling the integration of nutritional and metabolic
52 ecology.

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60 Introduction

61 Resting metabolic rate (RMR), sometimes reported as standard metabolic rate, is a
62 central component of individuals' energetics since it reflects the necessary cost of self-
63 maintenance subsequently constraining the energy allocation to other functions such as
64 growth or reproduction (McNab 2002). This key role made RMR a central component of
65 metabolic ecology from the Kleiber's power law (Kleiber 1932) through to the more recent
66 metabolic theory of ecology (MTE – (Brown *et al.* 2004)). MTE uses the allometric scaling
67 of RMR with body size and temperature to assess the rates at which organisms expend
68 (metabolism) and store energy as biomass (growth and reproduction). Thus, MTE
69 constitutes a quantitative approach that aims to predict the fluxes and stores of energy
70 between and within trophic levels (Brown *et al.* 2004). Yet, focusing on energy as sole
71 currency fails to explain the high spatial and temporal variability in energy transfer
72 efficiencies observed between trophic levels, particularly at the plant-animal interface
73 (Brett & Goldman 1997).

74 To explain this variability, one needs to consider that energy transfer is coupled to
75 and driven by material transfer (Cebrian *et al.* 2009). Material can obviously be converted
76 into energy to fuel metabolism but is also required to build structure and ensure vital
77 functions (Clarke 2019). The non-substitutability of some essential compounds in
78 organism's physiology coupled with their inability to synthesize them *de-novo* require the
79 availability, at appropriate proportions, of such compounds in food resources. The disparity
80 between the consumer's nutritional demand for and the resources supply in essential
81 compounds -often referred to as *food quality*- drives the energy transfers between trophic

82 levels via its effect on consumer growth and reproductive rates. Over the last decades,
83 identifying quantitative markers of food quality became an important task in ecological
84 research (Elser *et al.* 2000; Müller-Navarra *et al.* 2000; Ravet *et al.* 2012). For example, a
85 highly unsaturated fatty acid (HUFA) has been suggested to predict secondary production
86 in lakes (Müller-Navarra *et al.* 2000). At the same time, Elser *et al.* (2000) showed that the
87 carbon (C) to phosphorus (P) ratio of food can also constrain herbivore growth efficiency.
88 Following these early works, the biochemical view of trophic interactions unveiled a great
89 variety of dietary compounds such as n-3 and n-6 polyunsaturated fatty acids (PUFA)
90 (Wacker & von Elert 2001) and phytosterols (Martin-Creuzburg *et al.* 2006) as important
91 determinants of food quality limiting aquatic consumer growth either alone or in
92 combination (Sperfeld *et al.* 2012; Marzetz *et al.* 2017). The great variety of determinants
93 of food quality has split nutritional ecology into a patchwork of sub-disciplines which
94 focused on material transfer, often forsaking energy transfers of early works of trophic
95 ecology (Lindeman 1942). Several authors identify this segregation as an obstacle to our
96 understanding of ecosystem structure and functioning and call for the integration of these
97 two currencies into a common framework (Allen & Gillooly 2009; Hillebrand *et al.* 2009;
98 Sperfeld *et al.* 2017; Barnes *et al.* 2018). We argue that the first step towards this goal is to
99 understand how limitation by material transfer (food quality) affects the way consumers
100 dissipate (RMR) and store energy (growth / reproduction).

101 In that aim, we used *Daphnia magna* to test whether and how individual RMR can
102 be affected by food quality. Besides allowing the exclusion of genetic differences of RMR,
103 the use of *Daphnia magna* is advantageous since the nutrition of this key aquatic organism
104 has been extensively studied over the last decades. Thanks to this rich literature we know

105 that daphnids commonly face both stoichiometric (high food C:P) and biochemical (low
106 PUFA and/or sterols contents) dietary limitations in the field (Elser *et al.* 2000; Müller-
107 Navarra *et al.* 2004; Sperfeld *et al.* 2012). Here, we measured individual resting metabolic
108 rate (RMR) and juvenile growth rate (i.e., from birth to sexual maturity) along a gradient
109 of realistic food qualities incorporating various degrees of biochemical and stoichiometric
110 limitation, as well as their combination. We used a direct microcalorimetric method to
111 precisely measure how energy is dissipated by RMR (Ruiz *et al.* 2018). We hypothesized
112 that independently of its proximal cause (*i.e.* PUFA, Sterol or stoichiometry), sub-optimal
113 food quality may impose additional energetic costs to maintain organismal homeostasis,
114 ultimately inducing an increase of individual RMR. We argue that quantifying the energetic
115 cost imposed by food quality on individual RMR may constitute a common currency
116 further enabling the integration of food quality and metabolic ecology.

117

118 **Materials and Methods**

119

120 *Selection of Daphnia diets*

121 The quality of food for *Daphnia* is typically evaluated by measuring its effect on juvenile
122 growth rate (Wacker & von Elert 2001; Martin-Creuzburg *et al.* 2006; Ravet *et al.* 2012)
123 in feeding experiments where the food quantity is unrestricted (eg above the incipient
124 limiting level, 0.7 mgCL⁻¹). Chlorophyte algae grown on P-limited medium are often used
125 to study stoichiometric limitations whereas different phytoplanktonic species are used in
126 light of their biochemical composition to assess limitations in sterol, polyunsaturated fatty

127 acids (PUFA: 2 or 3 double bonds) and highly unsaturated fatty acids (HUFA: more than
128 3 double bonds).

129 Here, our aim was to study the relationship between RMR and food quality as
130 reflected in *Daphnia* growth rate. To do so we created a food quality gradient based on 7
131 different diets for which nutritional quality was previously assessed in growth rate
132 experiments. These 7 treatments were used as training dataset to define the relation linking
133 RMR and growth rate. Besides the fact that these 7 diets could be ranked in terms of
134 expected growth rate they also differed in the proximate cause of growth limitation: i.e.
135 unbalanced stoichiometry, sterol, PUFA and HUFA limitation. To induce differences in
136 stoichiometric (P) balance we used the chlorophyte *Chlamydomonas reinhardtii* SAG 7781
137 grown either on P-replete (*Chlamydomonas* P+) or P-limited medium (*Chlamydomonas* P-
138) (Ruiz *et al.* 2018). To create contrasts in biochemical nutritional constraints, we used
139 different phytoplankton strains: *Cryptomonas sp.* SAG 2680 (*Cryptomonas* P+), a
140 cryptophyte rich in sterols, PUFA and HUFA constituting an excellent quality food for
141 daphnids (Bec *et al.* 2006) and *Chlamydomonas reinhardtii* SAG 7781 (*Chlamydomonas*
142 P+) considered as a good quality food due to its high PUFA content (especially 18-3 ω 3)
143 (Denoux *et al.* 2017). The lower food quality of chlorophytes when compared to
144 cryptophytes is mainly explained by the absence of the HUFA 20:5 ω 3 (Martin-Creuzburg
145 & von Elert 2009). *Synechococcus sp.* strain BO8809, a cyanobacteria of poor dietary
146 quality was used to induce HUFA, PUFA and sterol limitations of *Daphnia* growth (Bec
147 *et al.* 2006). We gradually alleviated sterol limitation by supplementing *Synechococcus sp.*
148 with liposomes containing cholesterol (see below liposomes preparation) to create a
149 gradient of cholesterol availability with 2, 4, 8 and 12 μ g cholesterol per mg C in the final

150 food suspension. In addition to the diets mentioned above, we further included 3 other diets
151 for which the food quality rank within our gradient (see above) is unknown: P-limited
152 *Cryptomonas sp.* SAG 2680 (Cryptomonas P-), a mixed diet composed of *Chlamydomonas*
153 *reinhardtii* SAG 7781 (representing 20% of food C) and *Synechococcus sp.* strain BO8809
154 (representing 80% of food C) as well as a lyophilized sample of natural lake seston (i.e.
155 biotic and/or abiotic matter floating in the water column, here the fraction < 50µm) sampled
156 during spring in a lake from the floodplain of the Allier river (France). These 3 dietary
157 treatments were considered as more complex diets since we have no *a priori* regarding
158 their consequences on consumer growth. They were thus used as test dataset to assess the
159 robustness of our regression with diets of unknown quality. All these dietary treatments are
160 summarized in **Table 1**.

161 All phytoplankton species were grown on a modified WC medium (Von Elert &
162 Wolffrom 2001) with 7.1 mg.L⁻¹ or 0.35 mg.L⁻¹ phosphates at 20°C and under a permanent
163 artificial light flux of 120 µmol quanta.m⁻².s⁻¹. They were cultured semi-continuously at a
164 dilution rate of 0.2 d⁻¹ in aerated (compressed sterile-filtrated air) 5 L vessels. Food
165 suspensions were prepared by centrifugation and resuspension of the cultured cells in
166 Volvic® mineral water. The carbon concentrations of the food suspensions were estimated
167 from photometric light extinction (800 nm) and from carbon-extinction regressions
168 determined prior to the experiment.

169 Experimental setup:

170 We used the same clonal line of *Daphnia magna* (clone IRTA 2) in our experiment
171 to exclude any genotypic variability. The stock cultures of daphnids were kept in Volvic

172 water© at 20°C on a 14:10h day:night cycle and fed with *Chlamydomonas reinhardtii* (at
173 3mgC.L⁻¹) for more than 3 generations before the beginning of the experiments. Due to the
174 fact we could only run 12 RMR measurements simultaneously (see below), the experiments
175 were run sequentially in 3 groups of 3 or 4 dietary treatments (Chlamydomonas P+,
176 Chlamydomonas P-, Cryptomonas P+ / Cryptomonas P-, mixed diet, natural seston /
177 *Synechococcus sp* + 2-4-8-12 µg cholesterol). The same temperature – light conditions
178 were kept for the experiments. To reduce inter-individual differences and permit
179 experiment reproducibility, all the daphnids used in the experiment were third-clutch
180 neonates (born within 8 hours) of the same clonal line (see details in appendix Figure S1).
181 During the first 24h, neonates were fed on a suspension of *Chlamydomonas reinhardtii* (3
182 mgC.L⁻¹) to ensure that all individuals reached a sufficient size to, later, perform
183 measurements of metabolic rate. After this first day, daphnids were randomly distributed
184 into 3 replicate jars for each dietary treatment with 12 daphnids per jar. Each of these jars
185 was filled with 500mL Volvic© water and with ad-libitum food suspensions (3mgC.L⁻¹).
186 This breeding medium was renewed daily to avoid any food quantity limitation and
187 crowding effects during all the experiment duration. Each day of measurement, one
188 individual was randomly selected in each replicate of each dietary treatment to determine
189 its RMR by microcalorimetry (see below). After measuring its RMR, the individual was
190 placed into a pre-weighed aluminum container, dried 48h at 60°C and weighed (Sartorius;
191 ME36S; Goettingen-Germany). The dry weight reported here was used to define the
192 growth rate of daphnids as:

193
$$GR = \frac{\ln(Wt) - \ln(W0)}{t} \quad Eq.1$$

194 Where **GR** is the growth rate, **Wt** is the weight (in μg) at age **t** (in days) and **W0** the weight
195 (in μg) of neonates reflecting the mean dry weight of 3 pools of 15 individuals at birth
196 (n=45).

197 In each treatment, the experiment was stopped when daphnids reached maturity (i.e. after
198 eggs deposition in the brood pouch) to avoid misleading measurement of metabolic rate
199 due to the development from eggs to juvenile in daphnids brood pouch. Mature organisms
200 were weighed as described above and the juvenile growth rates were calculated using
201 equation 1.

202 *Resting metabolic rate measurement by microcalorimetry:*

203 Resting metabolic rate (RMR) of daphnids was determined through the
204 measurement of the heat produced by each individual using a TAM III microcalorimetric
205 system of the heat-conduction type (Waters, TA Instruments; Newcastle, United States)
206 enabling the continuous and simultaneous monitoring of 12 individuals. Before RMR
207 measurement, daphnids were rinsed three times with Volvic® water to remove all food
208 particles and they were kept 1h in clean water to exclude excretory products. They were
209 then placed into specific disposable 4mL ampoules containing 2mL of Volvic® water and
210 2mL of air. The ampoules were then sealed and introduced in the 12 minicalorimeters. The
211 data collection was performed with the TAM Assistant Software (Water, TA Instruments;
212 Newcastle, United States): for each individual, a heat-flow value was collected every
213 second during 8h. The last two hours of measurement (i.e. 6-8h after insertion) were used
214 to define individual RMR, this method ensuring the stability of the heat-flow measurement
215 and confirms that the RMR value excludes residual digestive activity (Ruiz *et al.* 2018)

216 Liposome preparation:

217 The 1-palmitoyl-2-oleoyl-phosphatidylglycerol (POPG, Sigma-Aldrich) and 1-
218 palmitoyl-2-oleoyl-phosphatidylcholin (POPC; Sigma-Aldrich) were dissolved in ethanol
219 at respective concentrations of 3 and 7mg.mL⁻¹. In parallel, cholesterol was dissolved in
220 ethanol at a concentration of 20mg.mL⁻¹. A mix containing 1mL of POPG and POPC
221 solution plus 400μL of cholesterol solution was dried using a rotary evaporator and re-
222 suspended in 10 mL buffer (20 mmol.L⁻¹ NaPi , 150 mmol.L⁻¹ NaCl). This suspension was
223 then sonicated and centrifuged (150 000 g, 90 min, 4°C). The pellet was collected and
224 resuspended in buffer to remove free cholesterol from the liposome suspension (Martin-
225 Creuzburg *et al.* 2008; Denoux *et al.* 2017). Concentration of cholesterol was measured on
226 the final liposomes suspension used to supplement dietary treatment.

227 Mineral and Biochemical analyses of food treatment:

228 The mineral analyses were performed on suspensions of our phytoplankton cultures
229 adjusted to an absorbance of 0.8 at 800 nm. These food suspensions were diluted 10 times
230 and their C content measured with a CHN analyser (NA-2100-Protein, ThermoQuest-CE
231 Instruments, Italy). Phosphorus content was estimated based on molybdenum blue reaction
232 after potassium persulfate oxidation (Ruiz *et al.* 2018) directly on the sample and on the
233 sample diluted 10 times. In parallel food suspensions with the similar concentration (i.e.
234 absorbance) were dried and weighted to report the C and P content per unit of dry mass.
235 Total lipids were extracted from food suspensions containing 1mgC using
236 chloroform/methanol (see Denoux *et al.* 2017 for further details). Lipids extract used to
237 fatty acid analysis were then submitted to an acid-catalysed transesterification producing
238 fatty acid methyl esters (Denoux *et al.* 2017). For sterol analysis, lipids extract undergone

239 an alkaline hydrolysis (saponification) to specifically extract sterols. The fatty acid methyl
240 esters and sterols extracts were analysed by gas chromatography (6850-Network-GC,
241 Agilent-technologies, USA) (Martin-Creuzburg *et al.* 2006). Mineral and biochemical
242 composition of diets are given in **Table 1**.

243 Statistical analysis:

244 The daphnids' juvenile growth rates were log transformed to meet ANOVA
245 assumptions. The differences between dietary treatments were tested using ANOVA
246 analysis followed by a post hoc Tukey test.

247 In order to estimate the ontogenetic mass-scaling exponent (*b*) for each dietary
248 treatment, the RMR of daphnids was plotted against body mass and fitted to the following
249 linear model (Brown *et al.* 2004) for each dietary treatment separately using least squares
250 regression:

251
$$\log(RMR) = \log(a) + b * \log(M) \quad Eq.2$$

252 where *a* is the proportionality constant and *b* the scaling exponent linking **RMR** to body
253 mass (*M*). To check for the effect of replicate jar on our results we used the R package
254 “rstanarm” to fit a model including jar as random effect as:

255
$$\log(RMR) \sim \log(Mass) * Treatment + (1 | Jar) \quad Eq.3$$

256 And a model excluding the effect of jars as:

257
$$\log(RMR) \sim \log(Mass) * Treatment \quad Eq. 4$$

258 Equation 3 and 4 were run on the data including all dietary treatments. We used the leave-
259 one-out-cross validation (LOO) procedure (R package “loo”) to test which model offered
260 a better fit to our data. LOO information Criterion (LOOIC) is appropriate to handle model
261 uncertainty and reduces the potential overfitting of models with more parameters. Here the

262 model excluding “jar” (equation 4) appears as the top-ranked model since it exhibits an
263 expected log predictive density (ELPD) of 0 and ΔLOOIC of 1.2 with the model including
264 jars (equation 3) (Vehtari *et al.* 2017). We thus used the model excluding “jar” parameter
265 to test the effect of mass and dietary treatments on consumers RMR. In addition, we used
266 non-parametric bootstrapping with replacement (n=10000) to estimate the uncertainty
267 around the **b** estimates for the different dietary treatments (Efron & Tibshirani 1986). For
268 each bootstrap iteration, the **a** and **b** estimates for each dietary treatments were also used
269 to predict the mass-specific RMR for individuals of 100 μg (MS-RMR₁₀₀). Significant
270 differences in bootstrapped **b** and MS-RMR₁₀₀ estimates between dietary treatments were
271 concluded in the lack of overlap between their 95%CI.

272 In order to evaluate the ability of RMR metrics to predict food quality, we constructed
273 linear models relating the mass-specific RMR measurements at a given age (5 days old;
274 MS-RMR_{5d}), MS-RMR₁₀₀ estimates and **b** estimates to log-transformed growth rate using
275 the training dataset (N= 21, see above). We then used these models on the above-mentioned
276 RMR metrics measured in the test dataset (N=9) to predict the growth rates. The goodness
277 of the predictions was evaluated by regressing the observed (in the test dataset) to predicted
278 values (Piñeiro *et al.* 2008).

279

280 **Results**

281 Effects of dietary treatments on growth:

282 The differences in availability of essential biochemical and mineral dietary compounds in
283 our treatments significantly affected individuals’ growth rate (ANOVA, $F_{(9,110)}=9.94$;
284 $p<0.001$, **Figure 1.B**). Individuals exhibited the lowest growth rate of 0.32d^{-1} when fed

285 with *Synechococcus sp.* supplemented with $2\mu\text{g}\cdot\text{mgC}^{-1}$ cholesterol. The supplementation
286 of *Synechococcus sp.* with cholesterol concentrations up to $12\mu\text{g}\cdot\text{mgC}^{-1}$ alone significantly
287 increased individual's growth rate up to 0.41d^{-1} (Tukey post-hoc test versus *Synechococcus*
288 *sp.* + $2\mu\text{g}\cdot\text{mgC}$ cholesterol: $p<0.001$). Individual's growth rate further increased to 0.5d^{-1}
289 when fed with *Chlamydomonas P+*, which contains PUFAs, (Tukey post-hoc test versus
290 *Synechococcus sp.* + $12\mu\text{g}\cdot\text{mgC}$ cholesterol: $p<0.001$) and reached a maximum value of
291 0.64d^{-1} when both PUFAs and HUFAs were available on a diet composed of *Cryptomonas*
292 *P+* (Tukey post-hoc test versus *Chlamydomonas P+*: $p<0.001$). From a stoichiometric
293 viewpoint, the deficiency of nutrient (phosphorus) significantly reduced individuals growth
294 rate in *Cryptomonas sp.* treatments (growth declines from 0.64d^{-1} (P+) to 0.49d^{-1} (P-),
295 Tukey post-hoc test, $p<0.001$) but not in *Chlamydomonas reinhardtii* treatments (growth
296 declines from 0.5d^{-1} (P+) to 0.45d^{-1} (P-), Tukey post-hoc test, $p=0.06$). Individuals fed with
297 mixed diet and natural seston exhibited growth rates of 0.38d^{-1} and 0.39d^{-1} respectively,
298 which correspond to the lower range of growth rates reported in this study (**Figure 1.A-B**).

299 Effects of dietary treatments on resting metabolic rate:

300 The progressive increase in availability of essential biochemical compounds
301 induced significant decrease of individuals resting metabolic rate (**Figure 1.C-E**). The
302 worst dietary treatment (*Synechococcus sp.* with $2\mu\text{g}\cdot\text{mgC}^{-1}$ cholesterol) resulted in the
303 highest MS-RMR₁₀₀ of $0.048\mu\text{W}\cdot\mu\text{g}^{-1}$ (95%CI [0.047; 0.05]) (**Figure 1.E**). The successive
304 supplementations of *Synechococcus sp.* with cholesterol (from $2\mu\text{g}\cdot\text{mgC}^{-1}$ to $12\mu\text{g}\cdot\text{mgC}^{-1}$)
305 gradually alleviated the sterol deficiency with the consequence of decreasing the mass-
306 specific MS-RMR₁₀₀ down to $0.035\mu\text{W}\cdot\mu\text{g}^{-1}$ (95%CI [0.034; 0.036]). The MS-RMR₁₀₀
307 reached values of $0.029\mu\text{W}\cdot\mu\text{g}^{-1}$ (95%CI [0.028; 0.03]) when PUFAs were available and

308 0.021 $\mu\text{W}\cdot\mu\text{g}^{-1}$ (95%CI [0.021; 0.022]) when both PUFA and HUFA were available.
309 Deficiency of phosphorus also significantly increased individuals MS-RMR₁₀₀ which
310 shifted from 0.029 $\mu\text{W}\cdot\mu\text{g}^{-1}$ (95%CI [0.028; 0.03]) to 0.034 $\mu\text{W}\cdot\mu\text{g}^{-1}$ (95%CI [0.031; 0.036])
311 between Chlamydomonas P+ and Chlamydomonas P- treatments respectively. In a
312 comparable fashion to what was reported for *Chlamydomonas*, the P limitation in
313 *Cryptomonas* shifted the MS-RMR₁₀₀ from 0.021 $\mu\text{W}\cdot\mu\text{g}^{-1}$ (95%CI [0.021; 0.022]) (P+) to
314 0.029 $\mu\text{W}\cdot\mu\text{g}^{-1}$ (95%CI [0.028; 0.03]) (P-). The two other diets from the test dataset
315 exhibited intermediate values with 0.042 $\mu\text{W}\cdot\mu\text{g}^{-1}$ (95%CI [0.04; 0.045]) for the mixed diet
316 and 0.036 $\mu\text{W}\cdot\mu\text{g}^{-1}$ (95%CI [0.035; 0.037]) for the natural seston.

317 Effects of dietary treatments on metabolic scaling during ontogeny:

318 We also reported a significant variation of the RMR-mass scaling during ontogeny
319 in response to dietary quality. Here, the worst diet composed of *Synechococcus sp.* with
320 2 $\mu\text{g}\cdot\text{mgC}^{-1}$ cholesterol exhibited a scaling exponent (coefficient **b**) of 1 (95% CI [0.98;
321 1.02]) which is the highest value reported. The supplementations of *Synechococcus sp.*
322 with cholesterol significantly reduced the value of **b** which, however, remained close to 1
323 when 4 $\mu\text{g}\cdot\text{mgC}^{-1}$ (0.9; 95% CI [0.88; 0.92]) and 8 $\mu\text{g}\cdot\text{mgC}^{-1}$ (0.9; 95% CI [0.89; 0.92])
324 cholesterol were added. In *Synechococcus sp.* supplemented with 12 $\mu\text{g}\cdot\text{mgC}^{-1}$ cholesterol
325 the value of **b** reached 0.79 (95%CI [0.76; 0.81]). The treatments composed of
326 Chlamydomonas P+ which contains PUFA exhibited **b** value of 0.67 (95%CI [0.65; 0.69]).
327 The lowest value for **b** was 0.46 (95% CI [0.44; 0.48]) and was noted for daphnids fed on
328 Cryptomonas P+ (**Figure 1.D**).

329 Individual RMR and growth rate:

330 Our linear models linking growth rate to MS-RMR fit well with the data both for
331 MS-RMR_{5d} ($F_{(1:19)}=167.9$, $p\text{-value}<0.001$, $R^2=0.92$) and MS-RMR₁₀₀ ($F_{(1:19)}=456.6$, $p\text{-}$
332 $\text{value}<0.001$, $R^2=0.96$) (**Figure 2**). Similarly, our linear model linking growth rate and
333 RMR scaling exponent (*b*) also fits well with our data ($F_{(1:19)}=506.9$, $p\text{-value}<0.001$,
334 $R^2:0.96$) (**Figure 2**). The high correlation between RMR and growth reported here when
335 all dietary treatments of our training dataset are included highlights a strong correlation
336 between RMR and growth rate regardless of the nature of the nutritional constraint.

337 Growth rate predictions from individuals RMR:

338 Using the linear model drawn above we predicted individual growth rate on the test
339 dataset from MS-RMR_{5d}, MS-RMR₁₀₀ and *b*. The scaling exponent (*b*) appeared as a good
340 predictor of individual growth since the “observed versus predicted” linear regression
341 exhibits a slope of 0.97 (CI95% [0.61; 1.32]), an intercept of 0.01 (CI95%[-0.14, 0.16])
342 and a high R^2 (0.80). The MS-RMR₁₀₀ also provide useful prediction of individual growth
343 (slope 0.81 (CI95%[0.45;1.16]); intercept: 0.06 (CI95%[-0.09; 0.23]) and $R^2:0.74$). The
344 MS-RMR_{5d} was the less useful predictor of growth rate as reported by the low R^2 (0.43)
345 and the uncertainty in slope (1.25 (CI95%[0.08; 2.42])) and intercept (-0.14 (CI95%[-0.71;
346 0.41])). Note that these regressions were based on a small number of datapoints (3
347 treatments×3 replicates = 9) which explains the large confidence intervals in the regression
348 parameter estimates.

349

350 **Discussion**

351

352 Here we found that regardless of their nature (biochemical and/or elemental
353 constraint) food quality constraints systematically increase RMR. Between some dietary
354 treatments, the difference in RMR at a given mass (100 μ g) can be as large as 128% which
355 is comparable to the effect on RMR caused by a mass difference of one order of magnitude
356 (assuming $b=0.75$ (Brown *et al.* 2004)). We found a tight negative log-linear relationship
357 between mass-specific resting metabolic rate (MS-RMR₁₀₀) and growth rate of *Daphnia*
358 *magna* submitted to various diets of contrasting quality. This relationship predicts
359 relatively well the growth rate from MS-RMR₁₀₀ in the complex diets of unknown food
360 quality used as test dataset (Natural seston, Mixed diet, Cryptomonas P-).

361 Due to its deficiency in sterols, polyunsaturated fatty acids (PUFA) and highly
362 unsaturated fatty acids (HUFA) the cyanobacterium *Synechococcus sp.* is known to be of
363 very low dietary quality for *Daphnia* (Martin-Creuzburg & von Elert 2009). The diet
364 composed of *Synechococcus sp.* with the lowest supplementation of cholesterol induced
365 the lowest growth rate and the highest MS-RMR₁₀₀ in this study. From a biochemical
366 viewpoint, the stepwise supply of sterols, PUFA (*Chlamydomonas reinhardtii*) and HUFA
367 (*Cryptomonas sp.*) gradually improved food quality (as reflected by the increase of juvenile
368 growth rate) inducing a decrease of individual MS-RMR₁₀₀. As predicted by ecological
369 stoichiometry (Elser *et al.* 2000), the increasing availability of P in Chlamydomonas P+
370 (i.e. the reduction of dietary C:P) enhanced food quality and slightly decreased MS-
371 RMR₁₀₀ in agreement with Ruiz *et al.* (2018). Phosphorus limitation had a much stronger
372 effect on *Daphnia* growth and MS-RMR₁₀₀ when fed on *Cryptomonas sp.* which contains
373 HUFA. Despite its high HUFA content, P-limited *Cryptomonas sp.* (Cryptomonas P-) did
374 not improve growth relative to both Chlamydomonas P+ and Chlamydomonas P-. This

375 suggests that *Daphnia* fed on P-limited chlorophytes (Boersma 2000) are actually co-
376 limited by P and HUFA supply. Interestingly, this also indicates that the relationship
377 between MS-RMR₁₀₀ and food quality likely holds for situations where multiple nutrients
378 co-limit growth.

379 Taking a stoichiometric perspective, dietary limitations can be viewed as an
380 imbalance leading to excess in the supply of a nutrient relative to another (limiting)
381 nutrient. To maintain internal homeostasis, consumers facing P limitation should actively
382 release excess C through respiration and/or excretion (Darchambeau *et al.* 2003; Jeyasingh
383 2007) resulting in a higher RMR (Ruiz *et al.* 2018). In this case, the excess carbon is lost
384 to growth and the increased RMR would reflect the cost of strict stoichiometric
385 homeostasis. In the same way, a biochemical limitation could be seen as an imbalance in
386 essential lipids supply (PUFA, HUFA, sterols) relative to other lipids. In this case too,
387 consumers might need to actively dispose lipids in excess and/or synthesize the required
388 compounds from their precursors (e.g. HUFA from PUFA) in order to maintain membrane
389 homeostasis. Yet, the lipid composition of cell membranes is not strictly homeostatic and
390 depends to a certain extent on dietary supply (Clamp *et al.* 1997). Such diet-induced
391 modifications in membrane composition can affect the efficiency of membrane transport
392 proteins (NaKATPase, Ca²⁺ATPase,...) (Pepe *et al.* 1999) and overall mitochondrial
393 energetic efficiency (Stanley *et al.* 2012). Given that these transporters consume nearly
394 half of the energy (ATP) used by resting organisms (Rolfe & Brown 1997) any decrease in
395 their efficiency could imply an energy expense that contributes to the increase of RMR we
396 observe in the case of dietary lipid limitation.

397 Our results also highlight important dietary-induced differences in the ontogenetic
398 RMR-mass scaling coefficient (b). This scaling coefficient is a central component of the
399 metabolic theory of ecology (Brown *et al.* 2004) or ontogenetic growth models (West *et*
400 *al.* 2001) and has long been assumed constant across the entire animal reign (Savage *et al.*
401 2004). Yet, over the last decade, the universality of this scaling coefficient has largely been
402 questioned (Glazier 2006; Burton *et al.* 2011). Here we show that food quality alone can
403 induce variations of this coefficient ranging from 1 (95% CI [0.98; 1.02]) (Poor diet -
404 *Synechococcus sp.* +2 $\mu\text{g}\cdot\text{mgC}^{-1}$ cholesterol) to 0.46 (95% CI [0.44; 0.48]) (Rich diet -
405 *Cryptomonas P+*) which is as much as the entire range of interspecific variation in mass
406 scaling exponents reported in literature (White & Seymour 2003; Glazier 2006). Our data
407 shows strong linear relationship between b and growth rate. In *Daphnia*, juvenile growth
408 rate is usually tightly correlated to fecundity (Martin-Creuzburg *et al.* 2009). Hence, the
409 observed differences in b likely reflect shifts in the temporal dynamic of the ratio between
410 structural and reproductive tissues along ontogeny. At the onset of sexual maturity,
411 *Daphnia* incorporates reproductive tissues (ovaries/eggs) in addition to the structural tissue
412 that constitutes its body mass. Compared to structural tissues, the production and
413 maintenance of reproductive tissues require considerably less energy *per* unit mass
414 (Kooijman 2009). Hence, the increasing contribution of reproductive tissues to body mass
415 during ontogeny gradually decreases the whole-body mass-specific energy requirements
416 ultimately reducing the scaling of RMR to body mass (coefficient b). While fecundity was
417 not measured here, several lines of published evidence support this hypothesis. In the
418 dietary treatments composed of *Synechococcus sp.* solely supplemented with cholesterol,
419 reproduction should not be possible or very reduced in *Daphnia* (Martin-Creuzburg *et al.*

420 2006; Martin-Creuzburg & von Elert 2009; Ravet *et al.* 2012). In our experiments, *Daphnia*
421 fed *Synechococcus sp.* + 2 μ g.mgC⁻¹ cholesterol exhibits a nearly isometric RMR-mass
422 scaling ($b=1$) which is in agreement with what is expected if the observed ponderal growth
423 would only be due to an increase of structural tissue (Glazier 2006). To be able to reproduce
424 *Daphnia* ideally requires HUFA, or at least their PUFA precursors (Martin-Creuzburg &
425 von Elert 2009) at the cost of reduced reproductive output however. Hence,
426 *Chlamydomonas*-based diets which contain PUFA should enable reproduction yet to a
427 lesser degree compared to the HUFA-rich *Cryptomonas sp.* which usually maximizes
428 fecundity in *Daphnia* (Müller-Navarra *et al.* 2004; Bec *et al.* 2006; Denoux *et al.* 2017).
429 The difference between the b estimates for the two treatments found here (*Chlamydomonas*
430 P+: 0.65, *Cryptomonas* P+: 0.46) could therefore reflect their different reproductive
431 outputs.

432 Overall, our results show that nutritional constraints cause a rise of RMR that is
433 proportional to the intensity of the constraint regardless of its nature (stoichiometry, PUFA,
434 sterols, co-limitation). Resting metabolic rate has been proposed as a transversal currency
435 underpinning ecological processes from individuals to the biosphere (Brown *et al.* 2004),
436 suggesting that our conclusions could be transposable to other taxa. By measuring the
437 dissipation of energy associated with food quality, regardless of whether one considers
438 MS-RMR_{5d}, MS-RMR₁₀₀ or RMR mass-scaling (b), our study shows that RMR can
439 quantify the energetic cost imposed by food quality constraints. The fact that complex diets
440 such as natural seston fall within the regression obtained from our food quality gradient
441 suggests that it might be possible to develop an instantaneous marker of food quality in
442 natural systems based on energy dissipation by RMR. For example, if food quality

443 fluctuates at longer temporal scales than consumers developmental time, estimating
444 ontogenetic b from coexisting cohorts of differently-sized individuals could provide a
445 proxy for food quality constraints on growth rate. Furthermore, quantifying diverse food
446 quality constraints in a unique energetic currency may also facilitate the integration of food
447 quality into metabolic theories. For example, Dynamic Energy Budget (DEB) theory offers
448 the possibility to explicitly model the flows of each limiting dietary compounds into the
449 organism (Kooijman 2009; Koussoroplis *et al.* 2019). Yet, this level of detail comes at the
450 cost of a high complexity and specificity as well as a large number of independent
451 parameters. In this regard, Sperfeld *et al.* (2017) stress the need to maintain DEB models
452 as simple as possible. Our results suggest that integrating food-quality dependence in the
453 DEB model parameters that account for energy dissipation (*e.g.* structural maintenance)
454 could be a simplified alternative to multi-reserve models.

455 Under pressure from global changes such as warming and eutrophication, food
456 quality in aquatic ecosystems is expected to decline as phytoplanktonic communities
457 become increasingly dominated by chlorophytes and cyanobacteria (Freeman *et al.* 2020).
458 Higher water temperature (Schulte 2015) and lower food quality -as we show here- lead to
459 increases of individual RMR that reflect a rising oxygen demand. Yet, warming and
460 eutrophication also lead to a decrease in water oxygen availability (Thomas *et al.* 2019;
461 Sokolova 2021). Hence, the combination of high temperature, low food quality and low
462 oxygen might exacerbate the risk of hypoxia for aquatic animals. In the same manner, poor
463 food quality might also interact with other stressors known to affect RMR such as food
464 quantity (Auer *et al.* 2015), pollutants (Rowe 1998) or predation stress (Glazier *et al.* 2020).

465 Focus on the bioenergetic consequences of poor food quality stress enables its integration
466 into a multiple stressors framework based on a common energetic currency.

467

468 **Acknowledgments**

469

470 Research project “PASSCAL” funded by I-site Clermont under the “Emergence” initiative
471 for the CAP 20-25 (awarded to AB). We thank Neil Metcalfe and two anonymous
472 reviewers for their insightful comments that helped improve this manuscript. We also thank
473 Franck Jabot for his thoughtful reading and comments on a very first version of this
474 manuscript.

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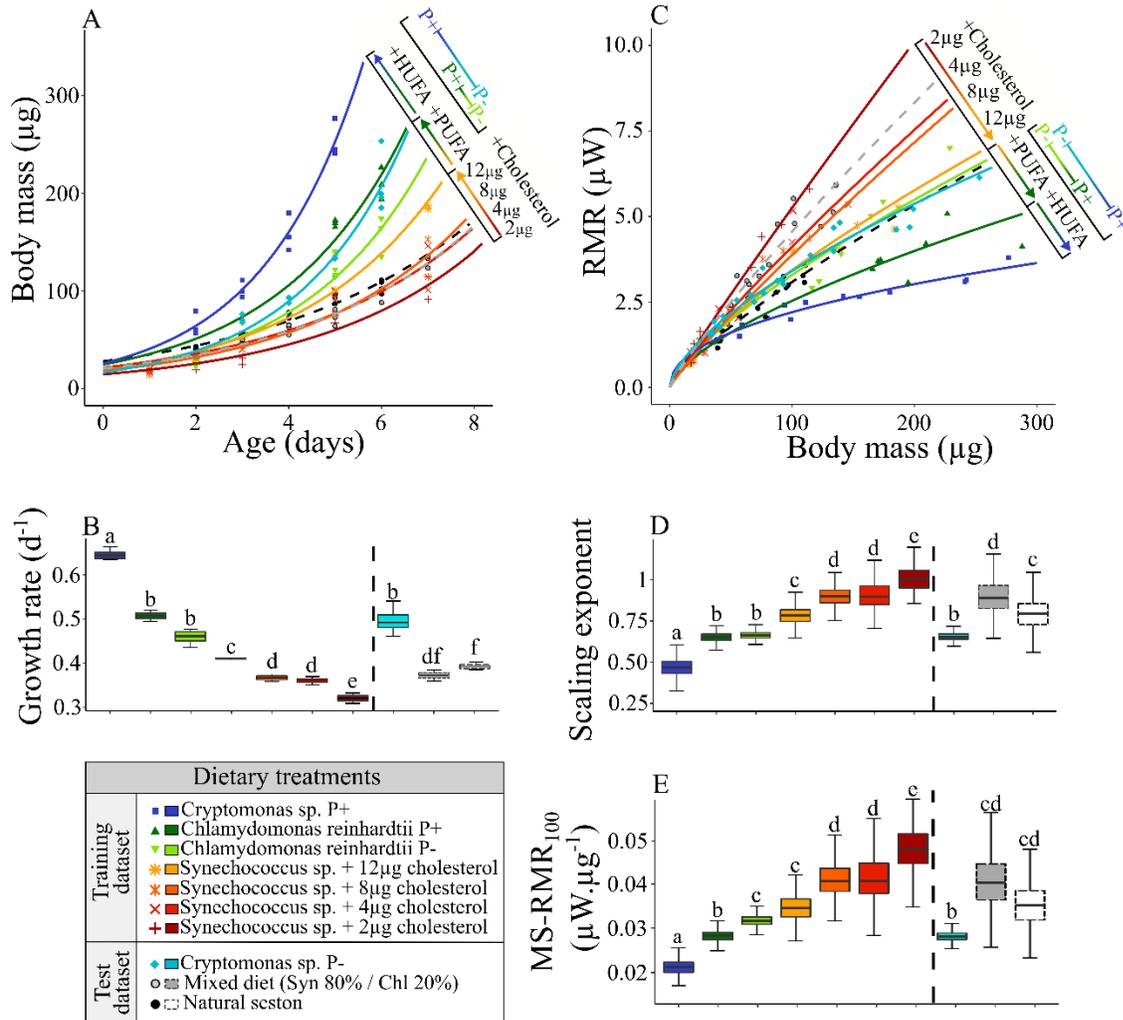
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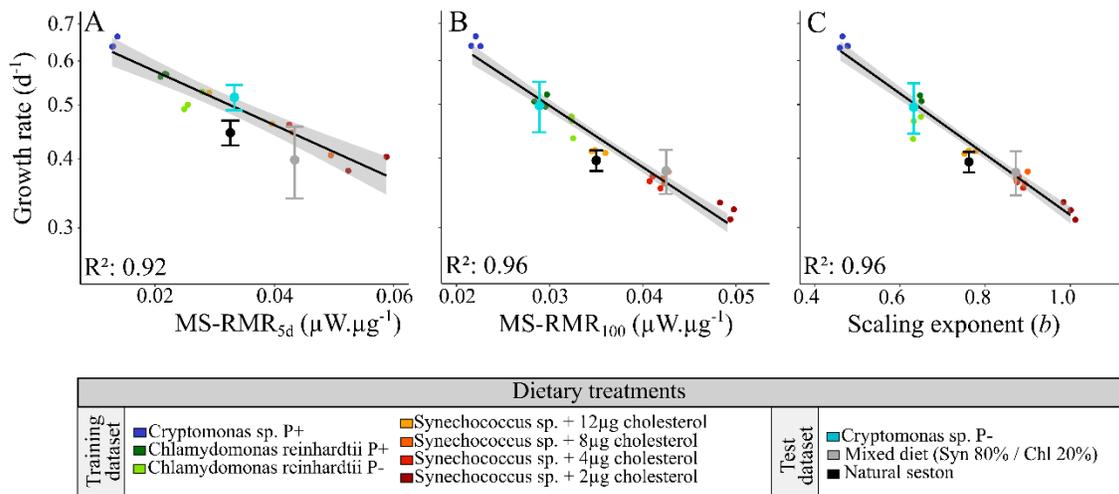
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601



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 605
 606 **Figure 1.** Individual growth and resting metabolic rate measured along dietary gradient. Left side
 607 panels show the evolution of individuals body mass versus age depending on dietary treatments (A)
 608 and the corresponding juvenile growth rate (B). Right side panels show the resting metabolic rate
 609 (RMR) of individuals depending on their body mass and diet (C), the coefficients defining the RMR
 610 scaling (D) and the mass-specific RMR (MS-RMR) of an individual of 100µg (E). Values for panel
 611 D and E were predicted from log-linear regression between RMR and body mass. In each panel,
 612 colors represent dietary treatments. In panel A and C the dietary gradient exhibits the main limiting
 613 dietary compounds. Boxes represent interquartile ranges and letters are significant differences
 614 based on post-hoc Tukey test (panel B) or based on 95% confidence intervals (panel D & E, see
 615 Table S1 for details).
 616

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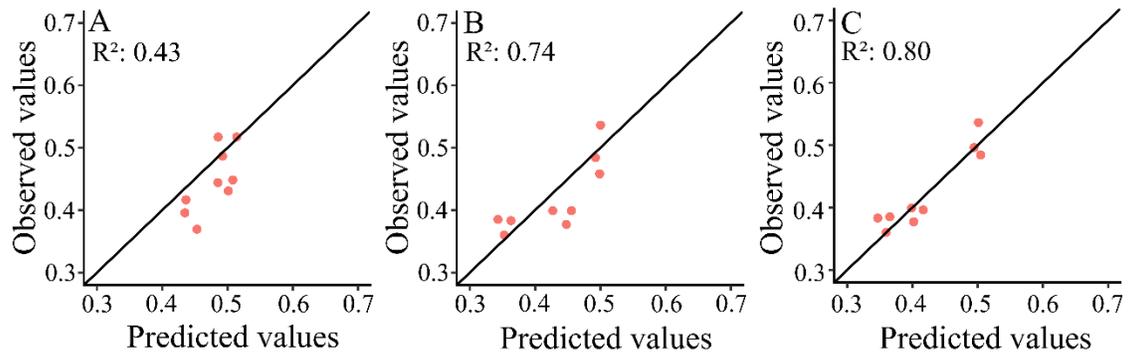


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619 **Figure 2:** *Daphnids* juvenile growth rate (from birth to maturity) versus the mass specific
 620 RMR of 5 days old individuals ($MS-RMR_{5d}$ - panel A), versus MS-RMR of 100µg
 621 individuals ($MS-RMR_{100}$ - panel B) or versus the scaling exponent b (panel C). In each
 622 panel, the black line is the linear model fitted from the training dataset with its 95%CI
 623 (shaded area). For the 7 treatments of the training dataset each point reflects the
 624 measurements of one replicate jar. The 3 diets from the test dataset are reported with their
 625 mean values and 95% CI.

626

627



628

629 **Figure 3:** Observed versus predicted growth rates of the test dataset. Growth rate were
630 predicted using the linear models of figure 2 linking growth with $MS-RMR_{5d}$ (panel A),
631 $MS-RMR_{100}$ (panel B) or scaling exponent b (panel C). The correlation between predicted
632 and observed values is given as R^2 . Black line reflects the 1:1 line.

633

634

635

	Dietary Treatment	HUFA		PUFA		Sterol	C:P ratio
		20:5n-3	22:6n-3	18:2n-6	18:3n-3		
Training data-set	<i>Cryptomonas sp. P+</i>	8.8 ± 1.2	2.7 ± 0.5	7.9 ± 0.1	8.4 ± 0.4	10-12 *	111.9 ± 6.7
	<i>Chlamydomonas sp. P+</i>	Und.	Und.	14.5 ± 0.3	10.9 ± 0.2	3-5 *	166.3 ± 4.1
	<i>Chlamydomonas sp. P-</i>	Und.	Und.	27.4 ± 5.7	11.2 ± 1.9	3-5*	1439.6 ± 174.8
	<i>Synechococcus + 12µg cholesterol</i>	Und.	Und.	Und.	Und.	12	195 ± 6.9
	<i>Synechococcus + 8µg cholesterol</i>	Und.	Und.	Und.	Und.	8	195 ± 6.9
	<i>Synechococcus + 4µg cholesterol</i>	Und.	Und.	Und.	Und.	4	195 ± 6.9
	<i>Synechococcus + 2µg cholesterol</i>	Und.	Und.	Und.	Und.	2	195 ± 6.9
Test data-set	<i>Cryptomonas sp. P-</i>	7.6 ± 0.7	2.1 ± 0.4	8.3 ± 0.4	8 ± 0.2	10-12 *	453.5 ± 59.4
	Mixed diet	Und.	Und.	2.9	2.2	0.6-1*	171.8
	Natural seston	Und.	Und.	4.7 ± 1.1	3.2 ± 0.8	N.A.	76.4 ± 3.8

636

637 **Table 1.** Dietary treatments used in the experiment and their main characteristics in term of
638 biochemical and stoichiometric composition. Fatty acid and sterol contents are given in $\mu\text{g}\cdot\text{mgC}^{-1}$.
639 Und. stands for undetected compounds and N.A. for not measured parameters. Values with
640 asterisks were taken from Martin-Creuzburg & Merkel (2016).

641

642