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

Thomas Ruiz, Apostolos-manuel Koussoroplis, Michael Danger, Jean-pierre Aguer, Nicole Morel-desrosiers, Alexandre Bec

► 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-03408463

HAL Id: hal-03408463

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

Submitted on 29 Oct 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Title:

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

Authors:

Thomas RUIZ¹ (thomas.ruiz@uca.fr),
Apostolos-Manuel KOUSSOROPLIS¹ (A-Manuel.KOUSSOROPLIS@uca.fr),
Michael DANGER² (michael.danger@univ-lorraine.fr),
Jean-Pierre AGUER¹ (J-Pierre.AGUER@uca.fr),
Nicole MOREL-DESROSIERS¹ (Nicole.MOREL@uca.fr),
Alexandre BEC¹ (alexandre.BEC@uca.fr)

Affiliations : ¹ Université Clermont Auvergne, CNRS, LMGE, F-63000 Clermont-Ferrand, France.
² Université de Lorraine, CNRS, LIEC, F-57000 Metz, France

Short title: Linking nutritional and metabolic ecology

Keywords: Metabolic ecology, Nutrition, Secondary production, Daphnia, stoichiometry, fatty acids, sterols

Article Type: Letters

Statement of authorship:

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

Data accessibility statement:

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

Words in abstract: 150

Words in main text: 4863

Number of references: 50

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

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

Corresponding author: Thomas RUIZ; LMGE 1 Impasse Amélie Murat 63170 Aubière FRANCE;
thomas.ruiz@uca.fr ; 0634194327

Abstract

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

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

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

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

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

Materials and Methods

Selection of *Daphnia* diets

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

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

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

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

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

Experimental setup:

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

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

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

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

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

Resting metabolic rate measurement by microcalorimetry:

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

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

Statistical analysis:

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

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

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

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

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

And a model excluding the effect of jars as:

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

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

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

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

Results

Effects of dietary treatments on growth:

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

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

Effects of dietary treatments on resting metabolic rate:

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

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

Effects of dietary treatments on metabolic scaling during ontogeny:

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

Individual RMR and growth rate:

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

Growth rate predictions from individuals RMR:

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

Discussion

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

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

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

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

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

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

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

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

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

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

Acknowledgments

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

References

- Allen, A.P. & Gillooly, J.F. (2009). Towards an integration of ecological stoichiometry and the metabolic theory of ecology to better understand nutrient cycling. *Ecol. Lett.*, 12, 369–384.
- Auer, S.K., Salin, K., Rudolf, A.M., Anderson, G.J. & Metcalfe, N.B. (2015). Flexibility in metabolic rate confers a growth advantage under changing food availability. *J. Anim. Ecol.*, 84, 1405–1411.
- Barnes, A.D., Jochum, M., Lefcheck, J.S., Eisenhauer, N., Scherber, C., O’Connor, M.I., *et al.* (2018). Energy Flux: The Link between Multitrophic Biodiversity and Ecosystem Functioning. *Trends Ecol. Evol.*, 33, 186–197.
- Bec, A., Martin-Creuzburg, D. & von Elert, E. (2006). Trophic upgrading of autotrophic picoplankton by the heterotrophic nanoflagellate *Paraphysomonas* sp. *Limnol. Oceanogr.*, 51, 1699–1707.
- Boersma, M. (2000). The nutritional quality of P-limited algae for *Daphnia*. *Limnol. Oceanogr.*, 45, 1157–1161.
- Brett, M.T. & Goldman, C.R. (1997). Consumer Versus Resource Control in Freshwater Pelagic Food Webs. *Science*, 275, 384–386.
- Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M. & West, G.B. (2004). TOWARD A METABOLIC THEORY OF ECOLOGY. *Ecology*, 85, 1771–1789.
- Burton, T., Killen, S.S., Armstrong, J.D. & Metcalfe, N.B. (2011). What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? *Proc. R. Soc. B Biol. Sci.*, 278, 3465–3473.
- Cebrian, J., Shurin, J.B., Borer, E.T., Cardinale, B.J., Ngai, J.T., Smith, M.D., *et al.* (2009). Producer Nutritional Quality Controls Ecosystem Trophic Structure. *PLoS ONE*, 4, e4929.

501 Clamp, A.G., Ladha, S., Clark, D.C., Grimble, R.F. & Lund, E.K. (1997). The influence of dietary
 502 lipids on the composition and membrane fluidity of rat hepatocyte plasma membrane.
 503 *Lipids*, 32, 179–184.
 504 Clarke, A. (2019). Energy Flow in Growth and Production. *Trends Ecol. Evol.*, 34, 502–509.
 505 Darchambeau, F., Faervig, P.J. & Hessen, D.O. (2003). How *Daphnia* copes with excess carbon in
 506 its food. *Oecologia*, 136, 336–346.
 507 Denoux, C., Martin-Creuzburg, D., Koussoroplis, A.-M., Perriere, F., Desvilletes, C., Bourdier, G.,
 508 *et al.* (2017). Phospholipid-bound eicosapentaenoic acid (EPA) supports higher fecundity
 509 than free EPA in *Daphnia magna*. *J. Plankton Res.*, 39, 843–848.
 510 Efron, B. & Tibshirani, R. (1986). Bootstrap Methods for Standard Errors, Confidence Intervals,
 511 and Other Measures of Statistical Accuracy. *Stat. Sci.*, 1, 54–75.
 512 Elser, J.J., Fagan, W.F., Denno, R.F., Dobberfuhl, D.R., Folarin, A., Huberty, A., *et al.* (2000).
 513 Nutritional constraints in terrestrial and freshwater food webs. *Nature*, 408, 578–580.
 514 Freeman, E.C., Creed, I.F., Jones, B. & Bergström, A. (2020). Global changes may be promoting a
 515 rise in select cyanobacteria in nutrient-poor northern lakes. *Glob. Change Biol.*, 26,
 516 4966–4987.
 517 Glazier, D.S. (2006). The 3/4-Power Law Is Not Universal: Evolution of Isometric, Ontogenetic
 518 Metabolic Scaling in Pelagic Animals. *BioScience*, 56, 325.
 519 Glazier, D.S., Borrelli, J.J. & Hoffman, C.L. (2020). Effects of Fish Predators on the Mass-Related
 520 Energetics of a Keystone Freshwater Crustacean. *Biology*, 9, 40.
 521 Hillebrand, H., Borer, E.T., Bracken, M.E.S., Cardinale, B.J., Cebrian, J., Cleland, E.E., *et al.* (2009).
 522 Herbivore metabolism and stoichiometry each constrain herbivory at different
 523 organizational scales across ecosystems. *Ecol. Lett.*, 12, 516–527.
 524 Jeyasingh, P.D. (2007). Plasticity in metabolic allometry: the role of dietary stoichiometry. *Ecol.*
 525 *Lett.*, 10, 282–289.
 526 Kleiber, M. (1932). Body size and metabolism. *Hilgardia*, 6, 315–353.
 527 Kooijman, B. (2009). *Dynamic Energy Budget Theory for Metabolic Organisation*. 3rd edn.
 528 Cambridge University Press, Cambridge.
 529 Koussoroplis, A., Schälicke, S., Raatz, M., Bach, M. & Wacker, A. (2019). Feeding in the frequency
 530 domain: coarser-grained environments increase consumer sensitivity to resource
 531 variability, covariance and phase. *Ecol. Lett.*, 22, 1104–1114.
 532 Lindeman, R.L. (1942). The Trophic-Dynamic Aspect of Ecology. *Ecology*, 23, 399–417.
 533 Martin-Creuzburg, D., Bec, A. & von Elert, E. (2006). Supplementation with Sterols Improves
 534 Food Quality of a Ciliate for *Daphnia magna*. *Protist*, 157, 477–486.
 535 Martin-Creuzburg, D. & von Elert, E. (2009). Good food versus bad food: the role of sterols and
 536 polyunsaturated fatty acids in determining growth and reproduction of *Daphnia magna*.
 537 *Aquat. Ecol.*, 43, 943–950.
 538 Martin-Creuzburg, D., von Elert, E. & Hoffmann, K.H. (2008). Nutritional constraints at the
 539 cyanobacteria- *Daphnia magna* interface: The role of sterols. *Limnol. Oceanogr.*, 53,
 540 456–468.
 541 Martin-Creuzburg, D., Sperfeld, E. & Wacker, A. (2009). Colimitation of a freshwater herbivore
 542 by sterols and polyunsaturated fatty acids. *Proc. R. Soc. B Biol. Sci.*, 276, 1805–1814.
 543 Marzetz, V., Koussoroplis, A.-M., Martin-Creuzburg, D., Striebel, M. & Wacker, A. (2017). Linking
 544 primary producer diversity and food quality effects on herbivores: A biochemical
 545 perspective. *Sci. Rep.*, 7, 1–9.
 546 McNab, B.K. (2002). *The physiological ecology of vertebrates: a view from energetics*. Cornell
 547 University Press. New York.

548 Müller-Navarra, D.C., Brett, M.T., Liston, A.M. & Goldman, C.R. (2000). A highly unsaturated
 549 fatty acid predicts carbon transfer between primary producers and consumers. *Nature*,
 550 403, 74–77.
 551 Müller-Navarra, D.C., Brett, M.T., Park, S., Chandra, S., Ballantyne, A.P., Zorita, E., *et al.* (2004).
 552 Unsaturated fatty acid content in seston and tropho-dynamic coupling in lakes. *Nature*,
 553 427, 69–72.
 554 Pepe, S., Tsuchiya, N., Lakatta, E.G. & Hansford, R.G. (1999). PUFA and aging modulate cardiac
 555 mitochondrial membrane lipid composition and Ca²⁺ activation of PDH. *Am. J. Physiol.-*
 556 *Heart Circ. Physiol.*, 276, H149–H158.
 557 Piñeiro, G., Perelman, S., Guerschman, J.P. & Paruelo, J.M. (2008). How to evaluate models:
 558 Observed vs. predicted or predicted vs. observed? *Ecol. Model.*, 216, 316–322.
 559 Ravet, J., Persson, J. & Brett, M.T. (2012). Threshold dietary polyunsaturated fatty acid
 560 concentrations for *Daphnia pulex* growth and reproduction. *Inland Waters*, 2, 199–209.
 561 Rolfe, D.F. & Brown, G.C. (1997). Cellular energy utilization and molecular origin of standard
 562 metabolic rate in mammals. *Physiol. Rev.*, 77, 731–758.
 563 Rowe, C.L. (1998). Elevated standard metabolic rate in a freshwater shrimp (*Palaemonetes*
 564 *paludosus*) exposed to trace element-rich coal combustion waste. *Comp. Biochem.*
 565 *Physiol. A. Mol. Integr. Physiol.*, 121, 299–304.
 566 Ruiz, T., Bec, A., Danger, M., Koussoroplis, A.-M., Aguer, J.-P., Morel, J.-P., *et al.* (2018). A
 567 microcalorimetric approach for investigating stoichiometric constraints on the standard
 568 metabolic rate of a small invertebrate. *Ecol. Lett.*, 21, 1714–1722.
 569 Savage, V.M., Gillooly, J.F., Woodruff, W.H., West, G.B., Allen, A.P., Enquist, B.J., *et al.* (2004).
 570 The predominance of quarter-power scaling in biology. *Funct. Ecol.*, 18, 257–282.
 571 Schulte, P.M. (2015). The effects of temperature on aerobic metabolism: towards a mechanistic
 572 understanding of the responses of ectotherms to a changing environment. *J. Exp. Biol.*,
 573 218, 1856–1866.
 574 Sokolova, I. (2021). Bioenergetics in environmental adaptation and stress tolerance of aquatic
 575 ectotherms: linking physiology and ecology in a multi-stressor landscape. *J. Exp. Biol.*,
 576 224, jeb236802.
 577 Sperfeld, E., Martin-Creuzburg, D. & Wacker, A. (2012). Multiple resource limitation theory
 578 applied to herbivorous consumers: Liebig’s minimum rule vs. interactive co-limitation.
 579 *Ecol. Lett.*, 15, 142–150.
 580 Sperfeld, E., Wagner, N.D., Halvorson, H.M., Malishev, M. & Raubenheimer, D. (2017). Bridging
 581 Ecological Stoichiometry and Nutritional Geometry with homeostasis concepts and
 582 integrative models of organism nutrition. *Funct. Ecol.*, 31, 286–296.
 583 Stanley, W.C., Khairallah, R.J. & Dabkowski, E.R. (2012). Update on lipids and mitochondrial
 584 function: impact of dietary n-3 polyunsaturated fatty acids. *Curr. Opin. Clin. Nutr.*
 585 *Metab. Care*, 15, 122–126.
 586 Thomas, Y., Flye-Sainte-Marie, J., Chabot, D., Aguirre-Velarde, A., Marques, G.M. & Pecquerie, L.
 587 (2019). Effects of hypoxia on metabolic functions in marine organisms: Observed
 588 patterns and modelling assumptions within the context of Dynamic Energy Budget (DEB)
 589 theory. *J. Sea Res.*, 143, 231–242.
 590 Vehtari, A., Gelman, A. & Gabry, J. (2017). Practical Bayesian model evaluation using leave-one-
 591 out cross-validation and WAIC. *Stat. Comput.*, 27, 1413–1432.
 592 Von Elert, E. & Wolfffrom, T. (2001). Supplementation of cyanobacterial food with
 593 polyunsaturated fatty acids does not improve growth of *Daphnia*. *Limnol. Oceanogr.*, 46,
 594 1552–1558.

595 Wacker, A. & von Elert, E. (2001). Polyunsaturated fatty acids: Evidence for non-substitutable
596 biochemical resources in *Daphnia galeata*. *Ecology*, 82, 2507–2520.
597 West, G.B., Brown, J.H. & Enquist, B.J. (2001). A general model for ontogenetic growth. *Nature*,
598 413, 628–631.
599 White, C.R. & Seymour, R.S. (2003). Mammalian basal metabolic rate is proportional to body
600 mass^{2/3}. *Proc. Natl. Acad. Sci.*, 100, 4046–4049.

601

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

617

618

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-RMR₁₀₀ - 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</i> + 12µg cholesterol	Und.	Und.	Und.	Und.	12	195 ± 6.9
	<i>Synechococcus</i> + 8µg cholesterol	Und.	Und.	Und.	Und.	8	195 ± 6.9
	<i>Synechococcus</i> + 4µg cholesterol	Und.	Und.	Und.	Und.	4	195 ± 6.9
	<i>Synechococcus</i> + 2µg cholesterol	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 µg.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