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1 **Feeding in the frequency domain: Coarser-grained environments increase consumer**
2 **sensitivity to resource variability, covariance and phase**

3 Apostolos-Manuel Koussoroplis*^{1,2}, Svenja Schällicke*¹, Michael Raatz*^{1,3}, Moritz Bach¹,
4 and Alexander Wacker^{1,4}
5

6 ¹Theoretical Aquatic Ecology and Ecophysiology group, Institute of Biochemistry and
7 Biology, University of Potsdam, Potsdam, Germany.

8 ²Aquatic Food Web Interactions group (I.R.T.A), Microorganisms Genome and Environment
9 Lab (L.M.G.E.) UMR CNRS 6023, Université Clermont Auvergne, Aubière, France.

10 ³Department of Evolutionary Theory, Max Planck Institute for Evolutionary Biology, Plön,
11 Germany.

12 ⁴Animal Ecology, Zoological Institute and Museum, University of Greifswald, Greifswald,
13 Germany.
14

15 *These authors contributed equally.

16

17 †Corresponding author:

18 Université Clermont Auvergne, L.M.G.E. UMR CNRS 6023

19 Campus Universitaire des Cézeaux

20 1 Impasse Amélie Murat

21 TSA 60026 CS 60026

22 63178 AUBIERE Cedex

23 FRANCE

24 a-manuel.koussoroplis@uca.fr

25 Phone: +33 4 73 40 77 51

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28

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31 AK, MR and MB analyzed the model. AK, SS and MR wrote the first draft of the manuscript.
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41 **Abstract**

42 Theory predicts that resource variability hinders consumer performance. How this effect
43 depends on the temporal structure of resource fluctuations encountered by individuals remains
44 poorly understood. Combining modelling and growth experiments with *Daphnia magna* we
45 decompose the complexity of resource fluctuations and test the effect of resource variance,
46 supply peak timing (i.e. phase) and co-limiting resource covariance along a gradient from
47 high to low frequencies reflecting fine to coarse-grained environments. Our results show that
48 resource storage can buffer growth at high frequencies, but yields a sensitivity of growth to
49 resource peak timing at lower ones. When two resources covary, negative covariance causes
50 stronger growth depression at low frequencies. However, negative covariance might be
51 beneficial at intermediate frequencies, an effect that can be explained by digestive
52 acclimation. Our study provides a mechanistic basis for understanding how alterations of the
53 environmental grain size affect consumers experiencing variable nutritional quality in nature.
54

55 **Introduction**

56 The nutritional traits of prey such as the composition of limiting resources or
57 secondary metabolites strongly influence consumer performance and population dynamics
58 (Sternler & Elser 2002; Simpson & Raubenheimer 2012; Hunter 2016; Sperfeld *et al.* 2016;
59 Raatz *et al.* 2017). Spatiotemporal variability in the nutritional quality of food resources is
60 inherent to natural consumer-resource systems (Orians & Jones 2001; Park *et al.* 2004; Junker
61 & Cross 2014; Grosbois *et al.* 2016), and most consumers face intense and frequent
62 fluctuations in food quality during their lifetime. Accumulating evidence shows that such
63 resource variability strongly influences consumers at the individual (Stockhoff 1993; Hood &
64 Sternler 2010; Pearse *et al.* 2018), population, and community level (Underwood 2004; Riolo
65 *et al.* 2015) and may have far-reaching implications such as the control of herbivore pest
66 populations in agroecosystems (McArt & Thaler 2013; Wetzel *et al.* 2016). Resource
67 variability effects manifest as a deviation between performance achieved under fluctuating
68 resource supply and performance that would be achieved under the same average, yet constant
69 resource supply (*variance effect*, **Fig. 1A**). If performance is a non-linear saturating function
70 of resource concentration, non-linear averaging predicts that performance should decrease
71 with resource variability, proportionally to resource variance and function curvature (Jensen's
72 inequality (Ruel & Ayres 1999; Wetzel *et al.* 2016)).

73 To date, studies on resource variability focused on single nutritional traits (Carlotti *et*
74 *al.* 2010; Hood & Sternler 2010; Wagner *et al.* 2017). Yet, consumer performance can be
75 simultaneously driven by several nutritional traits of their food (*e.g.* concentrations of various
76 mineral and biochemical resources or secondary metabolites), a situation known as co-
77 limitation (Sperfeld *et al.* 2012, 2016). Hence, when feeding in nutritionally variable
78 environments, consumers need to acquire, store and use multiple co-limiting resources which
79 might, or might not, co-occur temporally and spatially. Non-linear averaging predicts that,

80 when resources act non-additively, consumers should perform differently in positive and
81 negative covariance scenarios despite temporal means and variances of the co-limiting
82 resources being equal (*covariance effect*, Koussoroplis & Wacker 2016; **Fig. 1B**). Given the
83 propensity of co-limiting resources acting non-additively on consumer performance (Simpson
84 & Raubenheimer 2012; Sperfeld *et al.* 2016), the importance of resource variability for
85 consumers cannot be addressed without considering the interplay of co-limitation and the
86 spatiotemporal covariance of the co-limiting resources in the landscape.

87 Independent of whether the consumer is sessile in a dynamic environment (e.g.
88 bivalves in tidal estuarine systems) or mobile in a heterogeneous landscape (e.g. daily
89 vertically migrating zooplankton), spatial and temporal nutritional variance and covariance
90 are always experienced by the consumer as temporal fluctuations in resource supply. Hence,
91 changes in spatial or temporal contrasts of resource availability influence the variance and
92 covariance of resource supply, whereas the grain size of the environment (i.e. size or duration
93 of food patches), the motility and life span, as well as the foraging behavior of the consumer
94 influence the frequency and phase of the perceived fluctuations. While the fluctuation
95 frequency and phase of the nutritional environment can be highly different across consumer
96 taxa and ecological contexts, non-linear averaging approaches cannot, by definition,
97 accommodate their potential effects on consumer performance (Kingsolver & Woods 2016;
98 Wetzal & Thaler 2016; Kremer *et al.* 2018; Pearse *et al.* 2018). Thus, for the same resource
99 variance or covariance, differences in resource supply frequency or phase could yield
100 performances that differ from non-linear averaging predictions (Koussoroplis *et al.* 2017a). In
101 order to construct a general theory on the importance of resource variability for consumers,
102 we thus need to understand the responses to resource supply fluctuation frequency and phase
103 as well as the physiological mechanism mediating them.

104 In most living organisms, assimilated resources are first stored into a metabolically
105 inactive pool (hereafter *reserves*) and subsequently mobilized and used for maintenance,
106 growth, or reproduction (Kooijman 2010). Such reserves may buffer the effects of high
107 frequency resource fluctuations on performance (hereafter *reserve effect*) (Muller & Nisbet
108 2000; Fujiwara *et al.* 2003; Hood & Sterner 2010). Furthermore, many organisms
109 dynamically acclimate to fluctuating resources by adjusting nutrient extraction and transport
110 through gut enzyme modifications (Karasov *et al.* 2011; Koussoroplis *et al.* 2017b), thereby
111 improving the assimilation efficiency of the most limiting resource (Clissold *et al.* 2010;
112 Urabe *et al.* 2018). However, acclimation processes may also have negative consequences if
113 they involve energetic costs (Wetzel & Thaler 2016) or if acclimation lags behind the changes
114 in environmental conditions (Niehaus *et al.* 2012; Kingsolver & Woods 2016; Koussoroplis *et*
115 *al.* 2017a; Kremer *et al.* 2018). To date, the roles of reserves and acclimation in mediating
116 organismal responses to fluctuating environments are studied in isolation. Yet, the combined
117 effects of the two processes on resource-determined growth, where they are likely to co-occur,
118 remains unstudied.

119 Here, we test the hypothesis that for the same mean and variance of resource
120 fluctuations, environments with different temporal patterns (i.e. resource covariance,
121 frequency, and phase, **Fig. 1C**) lead to altered consumer growth responses that do not
122 necessarily comply with non-linear averaging predictions. We used a two-resource Dynamic
123 Energy Budget (DEB) model (**Fig. 2**) to predict the somatic growth of a consumer facing
124 constant versus fluctuating resource supply and investigate how performance differences are
125 mediated by the temporal structure of resource availability. The effects of the temporal
126 patterns in limiting and co-limiting resource supply on somatic growth were tested
127 experimentally using juvenile *Daphnia magna* reared on variable phosphorus (P) and
128 cholesterol supply. Our study offers theoretical and empirical evidence that the temporal

129 structure of resource availability can strongly decrease consumer growth in fluctuating
130 environments and provides a mechanistic understanding of such negative effects.

131 **Materials and Methods**

132 *Design of the study*

133 For the same total duration, modelled or experimental organisms are exposed either to
134 different constant resource supply conditions (**Fig. 1A,B**; open and black filled circles), or to
135 sequences of resource regimes alternating high and low supply of a single or two co-limiting
136 resources, thereby experiencing the same resource supply mean and variance over time.

137 Constant treatments are used to obtain non-linear averaging predictions of consumer growth
138 (**Fig. 1A,B**; filled grey, red, and blue circles). Fluctuating resource treatments are used to
139 study variance in a single resource alternating in low or high supply, while the other resource
140 is kept at constant high supply (**Fig. 1A**), or covariance in two co-limiting resources
141 alternating in their supply, resulting in negative or positive covariance (**Fig. 1B**). Fluctuations
142 are applied at different fluctuation frequencies (i.e. time spans of high or low supply) and
143 different phases (i.e. starting with high or low supply) (**Fig. 1C**).

144 *Model structure*

145 Based on DEB Theory (Kooijman 2010), we constructed an ordinary differential
146 equation (ODE) model that predicts the juvenile somatic growth of an organism (**Tab. 1**). The
147 three important aspects of the model are (i) co-limitation by two interactively essential
148 resources, (ii) independent storage of these resources in two reserves and (iii) regulation of
149 assimilation efforts depending on the relative filling of the reserves with a trade-off between
150 the two resources.

151 The state variables are the structural volume of the organism V , the resource density in
152 the reserve E_i ($i = \text{resource 1 or 2}$) and the assimilation effort ϕ , which determines the

153 assimilation efficiencies for the two resources $\varepsilon_i(\phi)$ (**Fig. 2**). Resources are assimilated from
154 the food proportionally to the available resource concentrations, the organism's surface and
155 the assimilation efficiency. Assimilates are then added to the reserves at rates p_{Ai} . Reserves
156 are consumed at rates p_{Ci} , proportionally to their density. The synthesizing unit (SU)
157 (Kooijman 1998) combines the two consumption fluxes at a fixed ratio with rate p_{SU} , which,
158 after subtracting maintenance costs p_M , results in structural growth. If influxes to the SU are
159 imbalanced, excessive resources are rejected with rate p_{Ri} , of which a fraction returns to the
160 reserves while the rest is excreted. Fluxes in and out of the reserves are expressed as resource
161 density fluxes in units of resources per structural volume per time.

162 We assume that the production of nutrient-specific digestive enzymes is energetically costly
163 and that it involves trade-offs hindering the capacity of the consumer to maximize
164 assimilation efficiency for all resources simultaneously (Zera & Harshman 2001). We
165 therefore implemented a trade-off in the assimilation efficiencies of the two co-limiting
166 resources (*see Appendix 1, Fig. A1, A2* for further details). Along this trade-off the organism
167 allocates the assimilation effort (i.e. production of nutrient-specific enzymes) towards the
168 most limiting resource as a compensatory mechanism maintaining nutrient uptake
169 homeostasis (Clissold *et al.* 2010). Evidence suggests that in invertebrates such digestive
170 processes are controlled by the nutrient status of the hemolymph (Bede *et al.* 2007).

171 Accordingly, in the model, the allocation of assimilation effort is controlled by the balance of
172 resources in the reserves. The adjustments of the assimilation effort are not immediate. We
173 therefore implemented a characteristic switching time that determines the speed at which an
174 organism acclimates to changed resource conditions (*see Appendix 1, Fig. A1, A2* for further
175 details). The model equations and parameter estimates are provided in **Table 1**.

176 *Obtaining modelled growth rates*

177 The resulting system of ODEs is numerically integrated using the *odeint* package from
178 the *Scipy* library (Jones *et al.* 2001) in Python (version 3.7) for the different constant or
179 fluctuation treatments. Starting from low initial volume and low, balanced reserve densities,
180 the unsaturated increase in structural volume within the first four days yields an estimate for
181 the somatic growth rate of juveniles that may be compared to experimental observations.
182 Different parameter combinations, which represent limiting cases of (i) no reserves, no
183 variable assimilation (null model), (ii) no variable assimilation, (iii) no reserves, and (iv) the
184 full model with reserves and variable assimilation, give rise to submodels that combine the
185 three important model components (i.e. Reserve, Acclimation, SU). Reserves are excluded by
186 increasing the reserve conductance 1000-fold. At such high values, the resources are stored
187 for a negligibly short time-interval before being passed on to the SU, thereby preventing any
188 significant reserve build-up. Acclimation is excluded by fixing the assimilation efficiencies at
189 the intermediate assimilation effort $\phi = 0.5$ along the trade-off curve. A sensitivity analysis
190 for the three most influential parameters is presented in **Appendix 2 (Fig. A3-5)**.

191 *Experiments*

192 Stock cultures of *D. magna* were kept as in (Koussoroplis & Wacker 2016). For the
193 experiments, we used P-replete (SynP+) and P-deficient (SynP-) *Synechococcus elongatus*
194 (SAG 89.79), a non-toxic cyanobacterium lacking essential sterols, using the culture protocol
195 described in (Lukas *et al.* 2011). By mixing SynP+ and SynP- cultures the appropriate P:C
196 ratios of food suspensions were obtained. Cholesterol containing liposomes were produced
197 following the protocol described in (Wacker & Martin-Creuzburg 2012) and supplemented to
198 the food suspensions to obtain desired cholesterol concentrations.

199 Third-clutch juveniles of *D. magna* (< 24 h old) were born on *S. elongatus* with low
200 resource supply (1.25 mmol P molC⁻¹, 0.25 µg cholesterol mgC⁻¹). In the constant treatments
201 daphnids experienced either constant high or low P (6.66; 1.25 mmol molC⁻¹) each in

202 combination with constant high or low cholesterol (8; 0.25 $\mu\text{g mgC}^{-1}$), or average conditions
203 (3.95 mmol P molC⁻¹; 4.125 $\mu\text{g cholesterol mg C}^{-1}$). In the variance treatments high and low
204 supply of either P or cholesterol fluctuated, while the other resource was at constant high
205 supply. In the covariance treatments high concentrations coincided or alternated, respectively
206 for positive and negative covariance. For a total duration of 4 days, three different fluctuation
207 frequencies of the resource supply (0.25 d⁻¹, 0.5 d⁻¹, 1 d⁻¹, corresponding to 1.25, 2.5, and 5
208 cycles per generation of approximately 5 days, respectively (Giebelhausen & Lampert 2001))
209 were applied for variance and covariance treatments, where animals experienced different
210 fluctuation phases, i.e. either high or low supply in the respective varying resource(s) first.
211 Each treatment consisted of four replicates with six juvenile *D. magna* per replicate. The
212 cholesterol variance experiment was performed twice and the data were pooled resulting in
213 eight replicates. The experiments were conducted at 20°C in the dark using glass beakers
214 filled with 200 ml of food suspensions prepared with ADaM medium (Klüttgen *et al.* 1994).
215 Food suspensions of *S. elongatus* with targeted resource concentrations were prepared daily.
216 Food quantity was kept constant at *ad-libitum* levels (2 mgC L⁻¹). Twice (for 1 d⁻¹ fluctuation
217 treatments) or at least once per day (for constant, 0.25 d⁻¹ and 0.5 d⁻¹ fluctuation treatments)
218 daphnids were transferred into renewed food suspensions to avoid food quantity limitation
219 and to simulate (co)variance in P and cholesterol. Initial dry mass (M_0) of animals was
220 determined using three subsamples of 10 juveniles ($\pm 1\mu\text{g}$, CP2P; Sartorius, Göttingen,
221 Germany). Somatic growth rate g (d⁻¹) was calculated as increase in dry mass using the
222 equation

$$223 \quad g = \frac{\ln M_T - \ln M_0}{T},$$

224 where T is the duration of the experiment (4 days) and M_T the final dry mass of animals.

225 *Data analysis*

226 To test for effects of fluctuation frequency and phase (variance experiments) or
227 fluctuation frequency and covariance direction (covariance experiment) on growth, two-way
228 ANOVAs were applied followed by Tukey HSD post-hoc tests. A nested-design two-way
229 ANOVA was used to additionally test for effects of fluctuation phase nested within positive
230 and negative covariance in the covariance experiment. Here, only the lowest fluctuation
231 frequency of 0.25 d^{-1} was considered. Means and associated 95% confidence intervals (CI)
232 were estimated using a bootstrapping approach with 1000 repetitions (Efron & Tibshirani
233 1993). We tested whether our observations in the various fluctuation treatments are
234 significantly different from the growth rate under constant average resource supply (i.e. $g(\bar{R})$,
235 $g(\bar{R}_1, \bar{R}_2)$) and from that predicted by non-linear averaging (i.e. $\overline{g(R)}$, $\overline{g(R_1, R_2)}_+$,
236 $\overline{g(R_1, R_2)}_-$), by comparing their respective CI. A significant difference was concluded in the
237 absence of overlap.

238 **Results**

239 **Reserve dynamics predict fluctuation frequency-dependent effects of resource supply** 240 **variability and phase on consumer growth**

241 With the SU alone, the model behaves as predicted by non-linear averaging, that is, frequency
242 independent (**Figs. 3A** and **4A**). However, including reserves increases growth across all
243 fluctuation frequencies compared to the null model, most dominantly at high frequencies (**Fig.**
244 **3B** and **Fig. 4B**). As fluctuation frequency increases growth increases from the non-linear
245 average prediction $\overline{g(R)}$ to the growth predicted from constant average resource supply $g(\bar{R})$
246 in the variance treatments. In the covariance treatments, growth moves from the non-linear
247 average prediction for positive, $\overline{g(R_1, R_2)}_+$, and negative covariance, $\overline{g(R_1, R_2)}_-$, to the
248 growth predicted for average co-limiting resource supply, $g(\bar{R}_1, \bar{R}_2)$. This model version also
249 predicts a resource phase effect since growth is higher when the organism experiences a high

250 resource supply first in the nutritional sequence. This phase effect is strong at low fluctuation
251 frequencies and decreases with increasing fluctuation frequency.

252 **Acclimation dynamics yield maladaptive consumer responses at high resource** 253 **fluctuation frequencies**

254 In our model, acclimation acts when only one resource fluctuates or the two resources are
255 fluctuating asynchronously, i.e. in the variance (**Fig. 3C**) and negative covariance treatments
256 (**Fig. 4C**), respectively. The positive effect of acclimation on growth becomes apparent at low
257 frequencies and on the non-linear average predictions $\overline{g(R)}$ and $\overline{g(R_1, R_2)}$, where growth
258 rates increase compared to the null model (e.g. **Fig. 3A** vs. **Fig. 3C**). For higher fluctuation
259 frequencies the lag of acclimation dynamics behind nutritional fluctuations becomes too
260 strong for organisms to fully acclimate. This leads to a decrease in growth below $\overline{g(R)}$ and
261 $\overline{g(R_1, R_2)}$. As resource fluctuations become very fast, assimilation efficiencies remain in an
262 intermediate, non-specialized range and growth collapses towards the null model predictions.

263 **Combining reserves and acclimation yields inversions in the direction of the covariance** 264 **effect and frequency gradient**

265 Including storage and acclimation combines the traits of the two previous model
266 versions, thereby enhancing growth relative to the null model both at the highest and lowest
267 fluctuation frequencies (**Fig. 3D** and **Fig. 4D**). At the intermediate frequency range, however,
268 the full model predicts a novel pattern where growth under single resource variance or
269 negative covariance can surpass that expected under constant conditions ($g(\bar{R})$ or $g(\bar{R}_1, \bar{R}_2)$,
270 respectively) (**Fig. 3D** and **Fig. 4D**). For fluctuations in both resources, this yields an
271 inversion of the covariance effect where the growth rates for negatively covarying resources
272 exceed those for positively covarying ones, as opposed to the non-linear average predictions
273 (**Fig. 4D**). As for the reserves model version, the full model also predicts changes in phase
274 effect size along the frequency gradient. However, as we parametrized both resources

275 identically, phase affects growth only in the positive covariance scenario, where an initially
276 low resource supply decreases growth at low fluctuation frequencies compared to initially
277 high resources (**Fig. 4D**). These model outcomes are robust for broad parameter ranges
278 (**Appendix 2, Fig. A3-5**).

279 **Experiments confirm the effect of fluctuation frequency, phase effects and maladaptive** 280 **responses**

281 We found a strong agreement between experimental results and most predictions of
282 the full model. In the experiment with fluctuating cholesterol supply, growth rates were
283 affected both by fluctuation frequency (two-way ANOVA, $F_{2,41} = 20.21$, $p < 0.001$) and phase
284 ($F_{1,41} = 41.17$, $p < 0.001$). As predicted, there were no growth differences between different
285 fluctuation phases at high frequencies (**Fig. 3E**), where growth rates were comparable to
286 $g(\bar{R})$. At low frequencies, however, the growth rate was more reduced (by 30.0%) when
287 exposed to low cholesterol concentrations first, than when experiencing high concentrations
288 first (11.8% reduction) (interaction: $F_{2,41} = 9.56$, $p < 0.001$).

289 In the experiment with fluctuating P supply, growth rates were only marginally
290 affected by fluctuation frequency (two-way ANOVA, $F_{2,18} = 2.63$, $p = 0.10$; **Fig. 3F**). A
291 marginal interaction between frequency and phase ($F_{2,18} = 3.01$, $p = 0.07$) suggests the
292 predicted increase in growth differences between the two phases from no difference at the
293 high frequency to 17.4% difference at the low frequency. In agreement with the model,
294 growth rates were generally lower when animals experienced low P supply first (factor
295 fluctuation phase, $F_{1,18} = 33.86$, $p < 0.001$): When exposed to high P supply first, growth rates
296 were high across all fluctuation frequencies tested and did not differ from $g(\bar{R})$ (**Fig. 3F**).
297 When experiencing low P supply first, growth rates decreased by 8.4%, 12.8% and 18.4%
298 with decreasing fluctuation frequency compared to $g(\bar{R})$, a pattern also predicted by the
299 model. At the 0.25 d^{-1} frequency growth rates were even 11.5% lower than $\overline{g(\bar{R})}$ (**Fig. 3F**).

300 Lower growth rates than $\overline{g(R)}$ are also predicted by the full model for certain parameter
301 ranges (**Appendix 2, Fig. A6**).

302 **Experiments partially confirm covariance effect inversion along the frequency gradient**

303 The experiment confirmed the majority of model predictions for the two resource
304 covariance scenarios (**Fig. 4E**). Similar to the model predictions, the frequency effect was less
305 pronounced for positive (from 7.3% to 15.0% lower growth rates) than for negative
306 covariance (from 1.3% to 23.7% lower growth rates), which is supported by a significant
307 interaction of fluctuation frequency and covariance direction ($F_{2,18} = 15.14$, $p < 0.001$). As
308 predicted, we observed an inversion of covariance effects; at the 0.25 d^{-1} frequency, negative
309 covariance yielded a stronger growth rate decrease (23.7%) relative to $g(\overline{R}_1, \overline{R}_2)$ than positive
310 covariance (10.5%) (Tukey HSD, $p < 0.05$), whereas at the 1 d^{-1} frequency the decrease was
311 stronger for positive covariance (14.9%) than for negative covariance (1.7%) (Tukey HSD, p
312 < 0.05). The overshoot of growth rates above $g(\overline{R}_1, \overline{R}_2)$ was not observed at the frequencies
313 applied in the experiment. Consistent with the model prediction, growth was affected by
314 fluctuation phase under positive covariance (18.7 % difference) (nested ANOVA, $F_{2,12} =$
315 38.07 , $p < 0.001$; Tukey HSD, $p < 0.001$), but was similar under negative covariance (Tukey
316 HSD, $p = 0.09$), which was tested experimentally for the 0.25 d^{-1} frequency (**Fig. 4E**).

317 **Discussion**

318 Our results highlight the importance of fluctuation frequency for the effect of
319 nutritional variability on consumer's growth. The nutritional variability constraint becomes
320 stronger in coarser-grained environments where perceived nutritional quality fluctuates
321 slowly. As such, our study confirms the hypothesis that non-linear averaging cannot predict
322 nutritional variability effects across all fluctuation frequencies. The simulations suggest that
323 non-linear averages are accurate only when fluctuation frequency is slow enough to empty the

324 reserves and allow for a full dietary acclimation of the consumer. We further show that the
325 effect of resource phase and covariance on growth increases for lower frequencies. This
326 finding stresses the need to consider the timing of resource peaks and the spatiotemporal
327 correlation of co-limiting resources in order to understand how nutritionally complex
328 environments constrain consumers. Below we discuss the causes of the observed patterns and
329 their ecological implications.

330 **Non-linear averages and consumer performance in nutritionally variable environments**

331 In a seminal study, (Hood & Sterner 2010) showed that the growth responses of
332 different *Daphnia* species exposed to a given P fluctuation pattern fall within a gradient
333 ranging from “growth integration” to “resource integration”. These two terms correspond to
334 the non-linear average, $\overline{g(R)}$ and the growth under average constant conditions $g(\overline{R})$,
335 respectively (Koussoroplis *et al.* 2017a). While these terms are very intuitive, using them to
336 define a strategy *vis-à-vis* dietary variability can be misleading (Hood & Sterner 2010;
337 Wagner *et al.* 2017). As shown in our study, whether an animal exhibits growth or resource
338 integration depends on the frequency at which resources fluctuate.

339 Animals typically experience variability at a multitude of temporal scales. For
340 growing herbivorous zooplankton, these range from diurnal variability induced by vertical or
341 horizontal migration between regions differing in seston quality (Park *et al.* 2004) to daily or
342 few days variability driven by changes in the physiology and species composition of
343 phytoplankton communities (Jonasdottir *et al.* 1995; Martin-Platero *et al.* 2018). Here, we
344 show that within this range the same species can exhibit either growth or resource integration.
345 Hence, the question is not whether an organism *is* a growth integrator (i.e. whether non-linear
346 averaging can predict growth) or not. Instead we have to ask under which conditions
347 organisms are *acting* as such and if these conditions actually occur in their natural habitats. As

348 we will discuss below, answering this question requires to understand how physiology shapes
349 growth responses to the various scales at which resources fluctuate.

350 **Nutritional variability effects: Reserves and acclimation shape growth responses across**
351 **frequencies**

352 Reserves explain the decrease in somatic growth with decreasing fluctuation frequency
353 as we observed in our experiments. Hence, reserve capacity likely determines whether an
354 organism will respond as a *resource* or a *growth integrator* when exposed to a given resource
355 fluctuation frequency. Furthermore, a higher reserve capacity (i.e. lower reserve conductance
356 (Kooijman 2010)) translates into higher flexibility in somatic stoichiometric or biochemical
357 composition and encompasses the capability to grow as fast as $g(\bar{R})$ over a wider range of
358 decreasing fluctuation frequencies. Hood & Sterner (2010) found that, when exposed to the
359 same P fluctuation pattern, *Daphnia* species with more flexible C:P stoichiometry tend
360 towards resource integration (or $g(\bar{R})$). These stoichiometric flexibility differences suggest
361 that *Daphnia* species differ in their P reserve capacity. Here, our model predicts that an
362 increase in reserve capacity should come at the cost of a decrease in growth rate under
363 constant or nearly constant resource supply (very high frequency fluctuations). More data is
364 needed to quantify interspecific variability in reserve capacity and to clarify whether it
365 involves trade-offs.

366 As for reserves, the capacity to acclimate to environmental changes might not be
367 beneficial under all circumstances. The existence of trade-offs, such as the one we
368 implemented in our model, offers a general explanation for frequently observed maladaptive
369 responses across taxa. The model predicts that maladaptive responses in digestive acclimation
370 decrease growth when the resource fluctuations are too slow for reserves to buffer, yet too fast
371 for acclimation to track. Under such circumstances, maladaptive acclimation can yield lower
372 growth rates than a growth integrator would achieve (i.e. below the non-linear average,

373 $\overline{g(R)}$), a situation we also observed in our P experiment. On the other hand, the consumer
374 benefits from acclimation when fluctuations are significantly slower than the time required for
375 full acclimation. In the model, this benefit manifests as a higher non-linear average than what
376 would be achieved without acclimation. Acclimation capacities are unlikely to evolve if they
377 regularly yield maladaptive responses (Botero *et al.* 2015). An intriguing hypothesis is that in
378 the historical environment of a consumer with acclimation capacity, the frequencies leading to
379 non-linear average growth rates are more common than those yielding a growth rate below the
380 non-linear average.

381 **Phase effect: When obtaining resources earlier in life is advantageous**

382 Size differences during juvenile stages can drive intraspecific interactions (dominance
383 or cannibalism) and may determine survival in the case of gape-size-limited predation. We
384 show that for the same temporal mean and variance in cholesterol and P supply *Daphnia*
385 neonates released within a high quality food patch achieve larger body sizes during the course
386 of our experiments than those experiencing high quality later. This phase-dependent growth
387 rate difference becomes significant when the nutritional conditions persist for 1 day (0.5 d^{-1}
388 frequency), a duration that roughly corresponds to 20% of a generation time (ca. 5 days at
389 20°C and saturating food, Giebelhausen & Lampert 2001). Hence, relatively modest
390 differences in dietary history can generate substantial intra-individual differences in size-at-
391 age of juveniles experiencing the same dietary temporal mean and variance. In accordance
392 with our findings, insect larvae experiencing high quality food early in ontogeny can better
393 cope with low quality resources later (Stockhoff 1992; Stoyenoff *et al.* 1994). Our results
394 suggest that the importance of fluctuation phase should increase in coarser-grained
395 environments. Furthermore, our model predicts that at least during the juvenile period this
396 phase effect becomes stronger with higher reserve capacity (**Appendix Fig. A4, but see**
397 Fujiwara *et al.* 2003 for longer periods). This context specificity of phase effects could

398 partially explain why selection of oviposition sites that insure early access to high quality
399 food to offspring is not systematically observed across species (Wetzel & Strong 2015).

400 **Covariance effect: Linking prey diversity and consumer performance across**
401 **environmental grain sizes**

402 In terrestrial systems, negative relationships between plant species diversity and
403 herbivore density are frequently observed (Crutsinger 2006; McArt & Thaler 2013). Recent
404 hypotheses explain this pattern by the fact that plant diversity translates into nutritional
405 variance. By moving from one leaf or host plant to another, insect herbivores experience the
406 plants' inherent intra- and inter-individual variance in nutrient contents (Orians & Jones 2001;
407 Herrera 2017). Because of non-linear effects (Wetzel *et al.* 2016) and the need to
408 continuously acclimate to the variable plant biochemistry (Wetzel & Thaler 2016; Pearse *et*
409 *al.* 2018) herbivore growth is constrained (*variance hypothesis*). On the other hand,
410 experimental studies, typically at the phytoplankton-zooplankton interface, show that plant
411 diversity improves consumer growth (Striebel *et al.* 2012; Marzetz *et al.* 2017; Urabe *et al.*
412 2018). The hypothesized explanation is that diverse prey assemblages translate into increased
413 biochemical diversity which enables herbivores to obtain the optimal blend of co-limiting
414 resources (Marzetz *et al.* 2017) while diluting potential secondary metabolites (Simpson &
415 Raubenheimer 2012) (*co-limitation hypothesis*). Both of the above hypotheses are potentially
416 correct. Nevertheless, the two are boundary cases that either assume the absence of nutritional
417 co-limitation (*variance hypothesis*) or a very-fine grained and well-mixed nutritional
418 environment thus excluding temporal nutritional variance (*co-limitation hypothesis*).
419 Covariance effects can accommodate both the nutritional variance that is inherent to coarser-
420 grained environments and nutritional co-limitation. This allows merging negative and positive
421 prey diversity effects on consumers within a common framework. Furthermore, covariance
422 effects account for the way prey species in a community differ in terms of nutritional quality.

423 Negative covariance describes a situation when prey species in a community differ mostly in
424 terms of co-limiting resource *ratios* whereas in a positive covariance situation species have
425 similar ratios but vary mostly in terms of co-limiting resource *concentrations*.

426 Our study provides the first proof of principle for dietary covariance effects (but see
427 (Chambers *et al.* 1996)). While we find the expected change of covariance effects with
428 fluctuation frequency, this change is more complex than anticipated (Koussoroplis *et al.*
429 2017a). An important and entirely novel result in our study is the possibility of inversions in
430 the direction of the covariance effect along a frequency gradient. Our model suggest that this
431 inversion could be explained by the interaction between reserve and imperfect acclimation
432 dynamics. Yet, the inversion pattern in the model presents some differences compared to the
433 experimental results suggesting that certain aspects of the acclimation dynamics might be
434 missing. Future work should explore how potential acclimation to absolute resource supply,
435 rather than ratios, or the inclusion of energetic acclimation costs can improve the match of the
436 predictions with our results.

437 **Conclusions**

438 In this study, we measured the qualitative differences in the growth response along a
439 gradient of dietary fluctuation frequencies. We demonstrated that these frequencies modulate
440 the effect sizes of resource supply variance, phase and covariance. Our model shows how
441 physiological mechanisms (i.e. reserves, acclimation), which may have evolved in response to
442 different temporal scales of variation, can be coupled. This is a necessary step to understand
443 organismal response to fluctuating environments (Dillon *et al.* 2016). The good agreement
444 with our experimental results suggests that the model indeed captures the most important
445 aspects. In order to achieve this mechanistic understanding, however, we had to trade control
446 over complexity. Thus, extrapolation of our findings to a natural context requires caution.
447 Unlike our experiments and simulations, in which resource supply fluctuates periodically,

448 natural nutritional noise is more likely to be stochastic. Consumers should therefore
449 simultaneously experience nutritional variance over a wide range of superimposed
450 frequencies. Overall, our results suggest that lower frequencies need less amplitude to yield an
451 effect on growth than high frequencies. Yet, whether and how the superimposed frequencies
452 that compose a stochastic signal act on growth remains unanswered (Dillon *et al.* 2016), and
453 presents fertile grounds for future investigations.

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- 583

584 **Data accessibility**

585 The experimental data as well as a Python script allowing to reproduce Fig. 3 and Fig. 4 are
586 available at [figshare link here].

587 **Acknowledgements**

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State variables				
Symbol	Description	Differential equation	Initial condition	Unit
ϕ	Assimilation effort	$\frac{d\phi}{dt} = \frac{1}{\tau}(\phi_T(x) - \phi)$	0.5	1
E_i	Reserve density	$\frac{dE_i}{dt} = p_{Ai} - p_{Ci} + \kappa p_{Ri} - (p_{SU} - p_M) E_i$	10^{-11}	mol mm ⁻³
V	Structural volume	$\frac{dV}{dt} = (p_{SU} - p_M) V$	0.008	mm ³
Functions				
Symbol	Description	Equation		
$\phi_T(x)$	Target assimilation effort at reserve balance $x = \frac{y_1 E_1}{y_2 E_2}$	$\phi_T(x) = \begin{cases} -\frac{1}{2}x^\gamma + 1, & x \leq 1 \\ \frac{1}{2}x^{-\gamma}, & x > 1 \end{cases}$		
$\varepsilon_i(\phi)$	Assimilation efficiency	$\begin{aligned} \varepsilon_1 &= \phi^\alpha (\varepsilon_{max} - \varepsilon_{min}) + \varepsilon_{min} \\ \varepsilon_2 &= (1 - \phi)^\alpha (\varepsilon_{max} - \varepsilon_{min}) + \varepsilon_{min} \end{aligned}$		
Fluxes				
Name	Equation	Unit		
Assimilation flux	$p_{Ai} = p_{Am} \varepsilon_i n_i V^{-1/3}$	mol mm ⁻³ d ⁻¹		
Catabolic flux	$p_{Ci} = u E_i V^{-1/3}$	mol mm ⁻³ d ⁻¹		
Outflow of SU	$p_{SU} = \frac{1}{\frac{1}{g_{max}} + \frac{1}{y_1 p_{C1}} + \frac{1}{y_2 p_{C2}} - \frac{1}{y_1 p_{C1} + y_2 p_{C2}}}$	d ⁻¹		
Rejection flux	$p_{Ri} = \begin{cases} p_{Ci} - p_{SU}/y_i, & p_{Ci} - p_{SU}/y_i > 0 \\ 0, & p_{Ci} - p_{SU}/y_i \leq 0 \end{cases}$	mol mm ⁻³ d ⁻¹		
Maintenance flux ^a	$p_M = 0.3 \text{ d}^{-1}$	d ⁻¹		
Parameters - fixed				
Symbol	Description	Value		
τ	characteristic switching time of assimilation ^b	0.1 d		
α	curvature of assimilation trade-off	3		
γ	sensitivity for reserve balance	5		
n_i	resource concentrations in the food	$7 \times 10^{-3} \text{ mol molC}^{-1}$ (high cond.) $3 \times 10^{-4} \text{ mol molC}^{-1}$ (low cond.)		
p_{Am}	maximum ingestion rate ^c	$4 \times 10^{-6} \text{ molC mm}^{-2} \text{ d}^{-1}$		
y_i	yield of structural volume per mole resource ^d	$1.5 \times 10^8 \text{ mm}^3 \text{ mol}^{-1}$		
g_{max}	maximum volume-specific growth rate ^e	0.6 d^{-1}		
κ	fraction of resources rejected by the SU and returned to the reserves	0.3		
Parameters - submodel-specific (null model, with storage, with acclimation, full model)				
ε_{max}	maximum assimilation efficiency	0.2, 0.2, 0.9, 0.9		
ε_{min}	minimum assimilation efficiency	0.2, 0.2, 0.1, 0.1		
u	Reserve conductance ^f	1000, 1, 1000, 1 mm d ⁻¹		

592

593 **Table 1: Model equations and parameter estimates.** The model is loosely parametrized for *Daphnia*, when
594 available, published values are given. All other values are set within reasonable biological ranges. ^a from
595 (Vanoverbeke 2008); ^b inferred from (Koussoroplis *et al.* 2017b); ^c calculated from (Koussoroplis & Wacker
596 2016); ^d inferred from (Sperfeld & Wacker 2009) for cholesterol; ^e Authors' personal observation on the *D.*
597 *magna* clone used; ^f slightly modified from AmP *Daphnia magna* version 2016/02/04
598 bio.vu.nl/thb/deb/deblab/add_my_pet/.

599 **Figure legends**

600 **Figure 1: Hypotheses.** (A) For single resource limitation, non-linear averaging predicts that
601 the average growth rate of a consumer experiencing fluctuating supply in a limiting resource
602 R , $\overline{g(R)}$, should be lower than the growth rate of a consumer experiencing a constant resource
603 environment with same average resource conditions, $g(\bar{R})$. Consumer growth rate should
604 decrease with increasing resource fluctuation amplitude. (B) When expanding to two co-
605 limiting resources (R_1, R_2), the average growth rate of a consumer experiencing positively
606 covarying co-limiting resources, $\overline{g(R_1, R_2)_+}$, should be higher than that of a consumer
607 experiencing negative covariance, $\overline{g(R_1, R_2)_-}$. (C) Because of reserve and acclimation effects
608 (see text), the realized growth and therefore the exactitude of non-linear averaging predictions
609 (panels A and B) should be modulated by the resource fluctuation phase and frequency (for
610 simplicity, only single resource cases are depicted).

611 **Figure 2: Dynamic Energy Budget (DEB) model.** Arrows indicate fluxes and rectangles
612 indicate the state variables. Numbered circles indicate implemented physiological
613 mechanisms: (i) The organism has the capacity to acclimate by adjusting its assimilation
614 effort towards the resource that has the lower reserves (is most limiting). The acclimation
615 process involves a time lag and implies a trade-off shown in the left panel below. (ii) The
616 organism has a reserve compartment for each resource that buffers the amplitude of higher
617 frequency fluctuations in ingested resources (middle panel below). (iii) The synthesizing unit
618 (SU) merges the resource fluxes that are liberated from the reserves into new biomass with a
619 fixed resource ratio. The rate at which the SU produces biomass from the reserves depends on
620 the magnitude and the balance of the mobilized resources fluxes. The SU was implemented
621 such that under constant resource conditions, the two resources act as interactively essential
622 resources thereby enabling resource covariance effects.

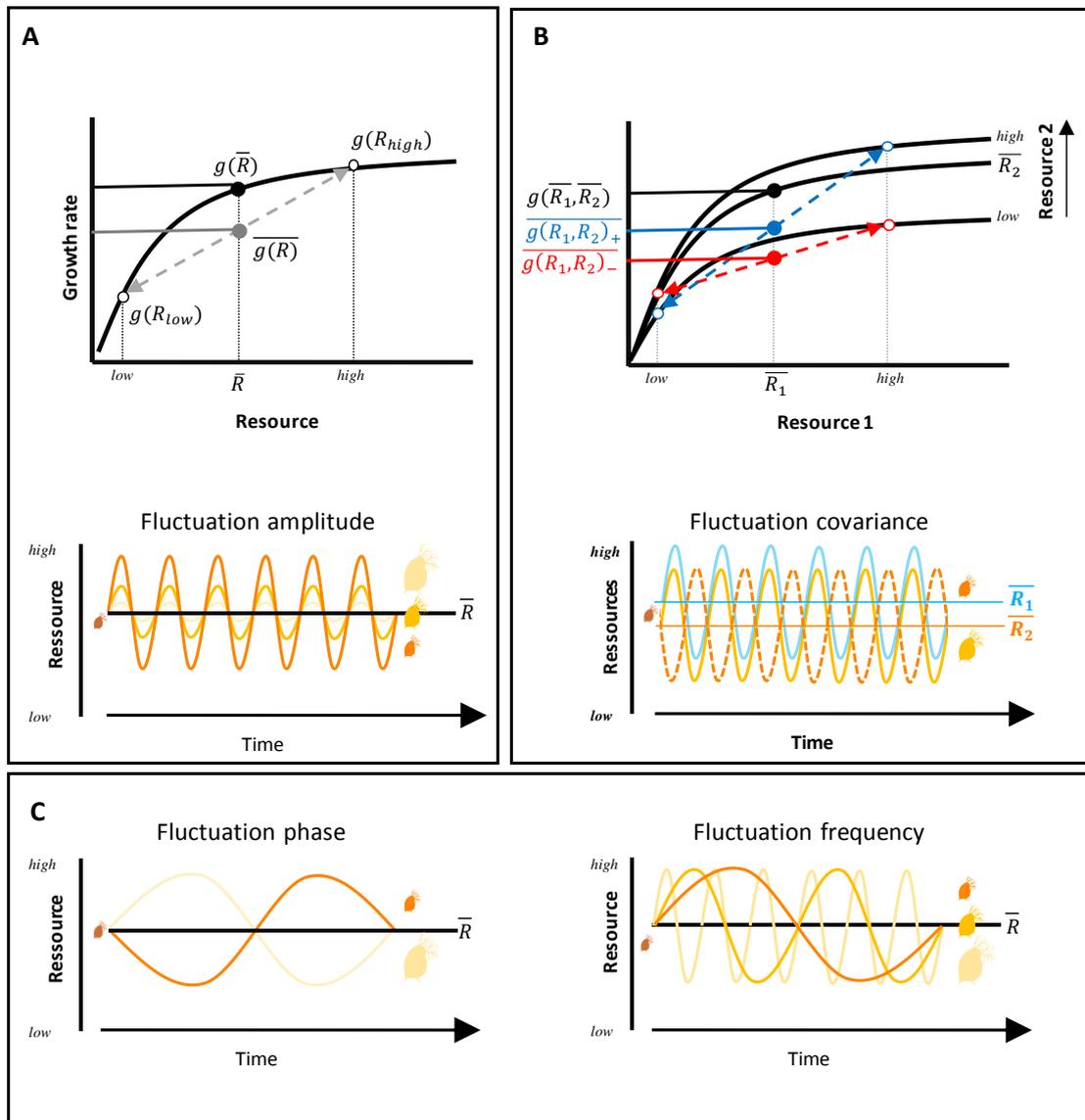
623 **Figure 3: Effects of frequency and phase for fluctuations of one limiting resource. (A-D)**
624 Model results for the somatic growth rate of a consumer for the four different submodels. The
625 dark grey horizontal lines are the predictions for growth under the same average constant
626 resource supply, $g(\bar{R})$, whereas the lighter horizontal line is the non-linear averaging
627 prediction of growth under variable resource supply, $\overline{g(R)}$ (see Fig. 1A). **(E-F)** Experimental
628 observations of juvenile growth rate of *Daphnia magna* (mean \pm 95% confidence interval,
629 C.I.) under **(E)** fluctuating cholesterol or **(F)** phosphorus supply, with different starting
630 conditions representing different fluctuation phases experienced after birth. The darker shaded
631 area is the 95% confidence interval (C.I.) of $g(\bar{R})$. The lighter shaded area is the 95% C.I. of
632 $\overline{g(R)}$. The other (non-fluctuating) resource (phosphorus or cholesterol in the experiments) is
633 kept at saturating supply.

634 **Figure 4: Effects of frequency, phase, and covariance for fluctuations of two co-limiting**
635 **resources. (A-D)** Modelled somatic growth rate of a consumer for the different submodels
636 with positively (blue) or negatively (red) covarying supply in the two co-limiting resources.
637 The black horizontal line shows the predicted growth under the same average constant
638 resource supply, $g(\bar{R}_1, \bar{R}_2)$, whereas the red and blue lines are the non-linear averaging
639 predictions of growth under variable resource supply, $\overline{g(R_1, R_2)}_{+/-}$ (see Fig. 1B). **(E)**
640 Experimentally observed juvenile growth rate of *Daphnia magna* (mean \pm 95% C.I.) under
641 positively or negatively covarying phosphorus and cholesterol supply. The combined effect of
642 fluctuation phase and covariance was tested experimentally for 0.25 d^{-1} fluctuations. The
643 grey shaded area is the 95% C.I. of $g(\bar{R}_1, \bar{R}_2)$. Colored areas are the 95% C.I. of $\overline{g(R_1, R_2)}_+$
644 (blue) and $\overline{g(R_1, R_2)}_-$ (red). Filled circles denote high starting resource supply, whereas open
645 circles indicate low resource supply experienced after birth.

646

647 **Figures**

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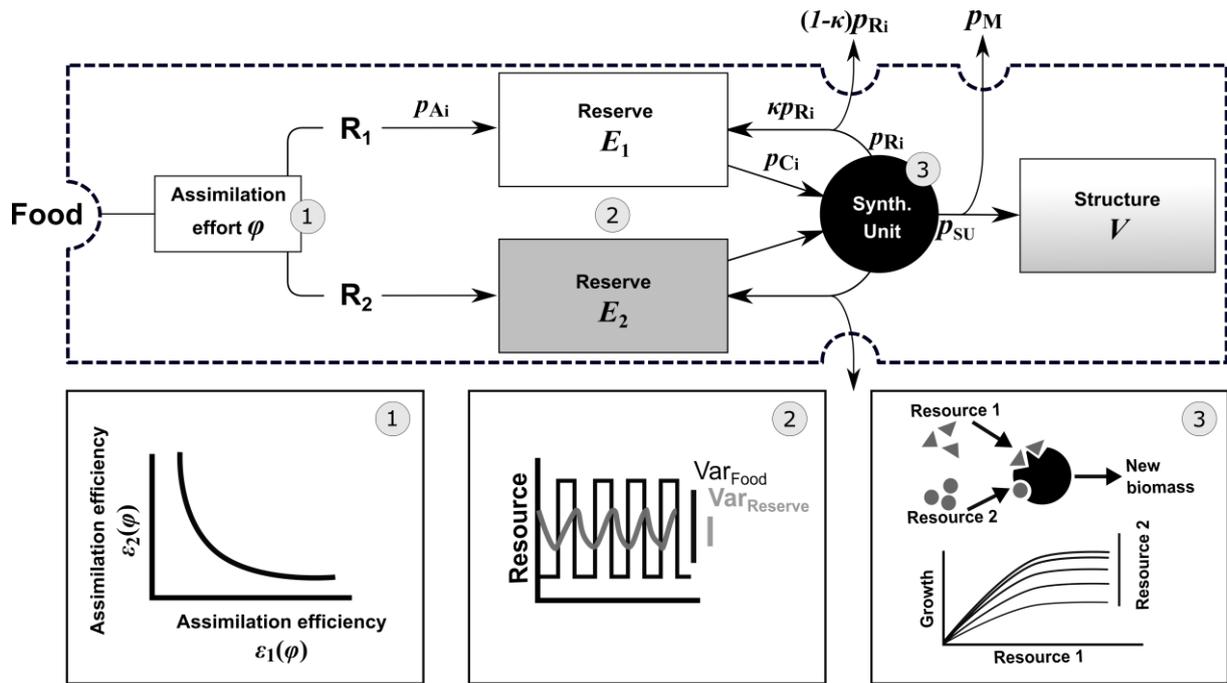


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650 **Figure 1**

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655 **Figure 2**

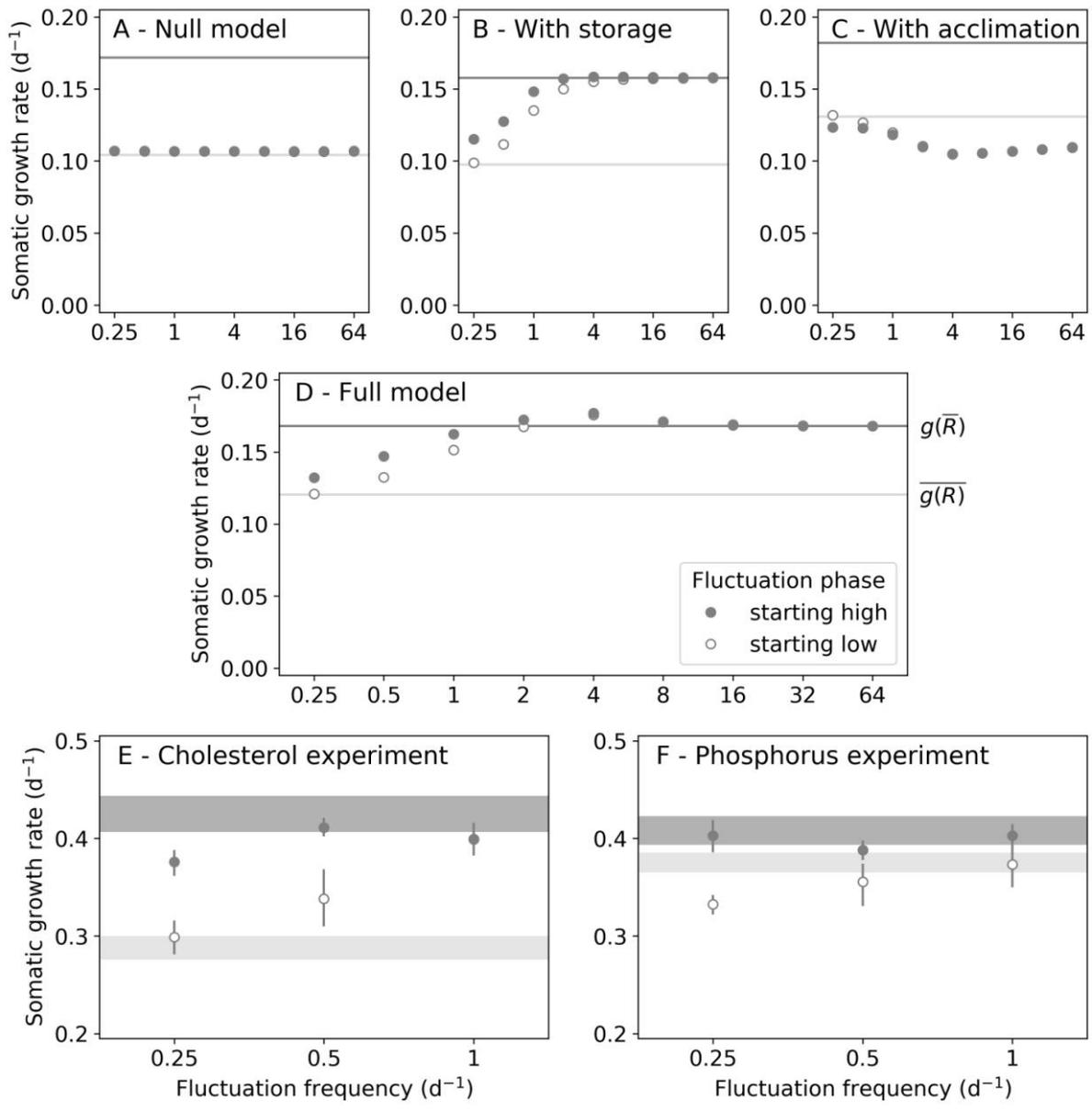
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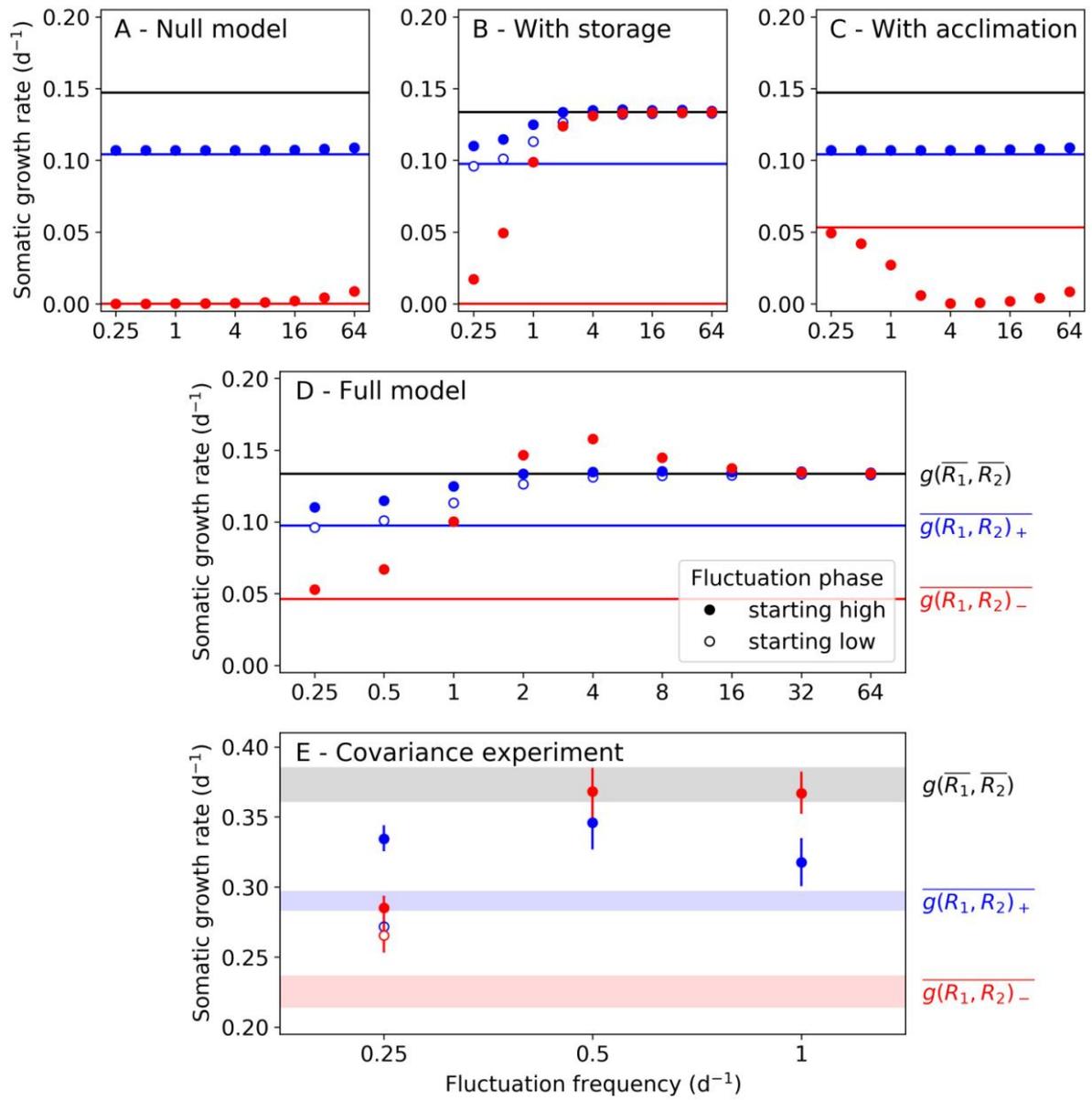
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662 **Figure 3**

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665 **Figure 4**

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