Rapid eco-phenotypic feedbacks and the temperature response of biomass dynamics

Jean P. Gibert[1,*], Daniel J. Wieczynski[1], Ze-Yi Han[1], Andrea Yammine[1]

[1]Department of Biology, Duke University, Durham, NC, USA

*corresponding author: jean.gibert@duke.edu

CONFLICTS OF INTERESTS

None

AUTHOR CONTRIBUTIONS

JPG conceived the study. JPG and AY designed the experimental work with support from ZYH.

AY collected the experimental data with support from JPG. JPG and DJW analyzed the data.

JPG wrote the first version of the manuscript and all authors contributed to subsequent versions.

All authors approved the final version for submission and agree to be held accountable for all

information within.

OPEN RESEARCH

All data and code can be accessed for peer-review (and will be permanently stored) at:

https://github.com/JPGibert/Temperature_-_Biomass_Dynamics

27   ABSTRACT

28   As biomass dynamics capture information on population dynamics and ecosystem-level

29   processes (e.g., changes in production over time), understanding how rising temperatures

30   associated with global climate change influence biomass dynamics is a pressing issue in ecology.

31   The total biomass of a species depends on its density and its average mass. Disentangling how

32   biomass dynamics may respond to increasingly warm and variable temperatures may thus

33   ultimately depend on understanding how temperature influences both density and mass

34   dynamics. Here, we address this issue by keeping track of experimental microbial populations

35   growing to carrying capacity for 15 days at two different temperatures in the presence and

36   absence of temperature variability. We show that temperature influences biomass through its

37   effects on density and mass dynamics, which have opposite effects on biomass and can offset

38   each other. We also show that temperature variability influences biomass, but that effect is

39   independent of any effects on density or mass dynamics. Last, we show that reciprocal effects of

40   density and mass shift significantly across temperature regimes, suggesting that rapid and

41   environment-dependent eco-phenotypic dynamics underlie biomass responses. Overall, our

42   results connect temperature effects on population and phenotypic dynamics to explain how

43   biomass responds to temperature regimes, thus shedding light on processes at play in

44   cosmopolitan and massively abundant microbes as the world experiences increasingly hot and

45   variable temperatures.

46

47   KEYWORDS: Biomass, Temperature, Temperature size rule, Temperature effects, Warming

48

49

50   INTRODUCTION

51   Understanding the biotic and abiotic factors that influence ecosystem function is a central goal of

52   ecology (Giller and O'Donovan 2002, Srivastava and Vellend 2005, Begon et al. 2006). While

53   censusing species presence/absence and abundances (or densities) provides a window into the

54   overall structure of a community (e.g., composition, richness, evenness, diversity), species

55   abundances alone do not contain information on the ecosystem-level functions performed by that

56   community. However, tracking biomass (or biomass density) over time –i.e., the total mass of all

57   individuals of a species or community (per unit area, if biomass density)– provides information

58   on production within trophic levels, and comparing biomass across trophic levels can yield

59   information on energy transfers within a food web (McKie and Malmqvist 2009, Trebilco et al.

60   2013, D'Alelio et al. 2016, Barneche et al. 2021). Because of that, biomass is a central concept

61   that both describes the state of an ecosystem and provides information on ecosystem-level

62   processes that influence overall function like production or energy transfers (Hatton et al. 2015).

63   As the planet warms, the structural and dynamical responses of ecosystems are reflected

64   in changes in biomass (Kortsch et al. 2015, Ullah et al. 2018, Bartley et al. 2019, Gibert 2019,

65   Barbour and Gibert 2021). For example, the biomasses of multiple taxa have been shown to

66   decline with temperature across systems (O'Connor et al. 2009, Carr et al. 2018, Larjavaara et al.

67   2021). However, biomass declines are not universal (Lin et al. 2010) and the mechanisms

68   through which warming influences species biomass are not well understood. Intuitively, because

69   biomass is the sum of the mass of all individuals in a species, it is possible to decompose

70   biomass into two main components: species' average masses and species' abundances

71   (densities). Indeed, biomass is often estimated in the field as the product of the average mass of

72   the individuals of a population and their abundance (or density). Consequently, any effects of

73   temperature on biomass should, at their core, result from temperature effects on the

74   abundance/density of a species or its average body size/mass.

75   Body size is an important functional trait that determines metabolic rates (Gillooly et al.

76   2001, Brown et al. 2004), demographic parameters (Savage et al. 2004, DeLong and Hanson

77   2009, Wieczynski et al. 2021), species interactions (DeLong 2014, DeLong et al. 2014, DeLong

78   et al. 2015), and even community and ecosystem-level structure and processes (Allen et al. 2005,

79   Gibert and DeLong 2014, Schramski et al. 2015, Wieczynski et al. 2021). Increasing temperature

80   generally reduces individual body sizes, an effect called the 'temperature-size rule' (TSR) that is

81   pervasive across systems and taxa (Atkinson 1994, Atkinson 1995, Atkinson et al. 2003, Forster

82   et al. 2012). For these reasons, body size and the temperature-size rule have clear consequences

83   for changes in biomass across all levels of ecological organization in a warming world (Brose et

84   al. 2012).

85   How temperature influences the other component of biomass –density– is less clear. The

86   Metabolic Theory of Ecology predicts that warming should decrease species' carrying capacities

87   –the maximum density attainable in a given environment– but proof of that decline remained

88   elusive until recently. Data-tested theoretical work has now shown that carrying capacity indeed

89   declines with temperature, but this effect can only be understood by integrating associated effects

90   on body size via the TSR (Bernhardt et al. 2018). Moreover, while carrying capacities may

91   indeed decline with temperature, it is unlikely that all species within a community will be at

92   carrying capacity at any given moment—rather transient, non-equilibrium dynamics are expected

93   (Hastings et al. 2018). Thus, addressing whether and how non-equilibrium densities are impacted

94   by temperature is important for understanding how temperature influences biomass.

4

95   Last, body size can influence population growth, and hence densities, through

96   relationships with demographic parameters like carrying capacity (K) and the intrinsic growth

97   rate (r) (Damuth 1981, Savage et al. 2004, DeLong et al. 2015). On the flip side, population

98   dynamics could, in theory, also influence body size, through associated effects on resource

99   levels, but these effects are less well understood. Recent work has shown that, as populations

100   grow to carrying capacity, rapid changes in body size can have a stronger effect on changes in

101   density than the other way around, suggesting important –albeit asymmetric– feedbacks between

102   population density and body size (Gibert et al. 2022). But how these reciprocal effects change

103   with temperature, or how they may influence biomass responses to warming, is not known.

104   Here, we tackle these unknowns by addressing the following questions: 1) How is

105   biomass affected by temperature and temperature variability as a species grows to carrying

106   capacity? 2) To what extent are the effects of temperature on biomass dependent on how density

107   and body size dynamics respond to temperature? 3) Does density or body size have a stronger

108   effect on biomass responses to temperature? And, 4) do the reciprocal impacts of density and

109   body size vary across temperature regimes? To address these questions, we recorded time series

110   of population dynamics in a microbial species and tracked changes in total biomass, density, and

111   body size in four different temperature regimes: constant 22ºC, constant 25ºC, and both

112   temperatures with fluctuations. We derive a simple mathematical expression to partition the

113   contribution of changes in density and body size to changes in biomass and assess how

114   temperature responses in either one influence biomass shifts. Last, we use time series analyses to

115   assess whether and how reciprocal effects of density and body size on biomass vary across

116   temperature regimes.

117

118 METHODS

119 *Microcosm growth assays*

120 We grew populations of the protist *Tetrahymena pyriformis* for 15 days at various temperature

121 treatments. To do so, we set up 24 experimental microcosms in 250 mL autoclaved borosilicate

122 jars containing 200 mL of Carolina protist pellet media (1L of autoclaved DI water per pellet)

123 previously inoculated with pond bacteria from Duke Forest (Gate 9/Wilbur pond, Lat=36.02°,

124 Long=-78.99°, Durham, NC) and a wheat seed as a carbon source for the bacteria (Altermatt et

125 al. 2015). All microcosms were started at 10 ind/mL protist densities and incubated in humidity-

126 controlled (65% humidity) growth chambers (Percival AL-22L2, Percival Scientific, Perry,

127 Iowa) on a 12hr night/day cycle. The entire replicated timeseries is therefore composed of 360

128 data points.

129 The 24 microcosms were subdivided into 4 experimental treatments: constant 22ºC,

130 constant 25ºC, variable 22ºC or variable 25ºC. Temperature variability was programmed into our

131 growth chambers to keep an average temperature of either 22ºC or 25ºC, but to fluctuate between

132 two temperatures that were ±1.5ºC of the average every 12 hours, therefore imposing variability

133 with a thermal amplitude of 3 ºC. A microcosm in the variable 22ºC treatment thus spent half of

134 the day at 19.5ºC and half of the day at 23.5ºC while one in the variable 25ºC spent half of the

135 day at 23.5ºC and half of the day at 26.5ºC. At each temperature change, temperature ramped

136 up/down for roughly 15 minutes. Neither water nor nutrients were replaced throughout the

137 course of this experiment. From now on we call these temperature treatments constant' (C) and

138 'variable' (V).

139

140 *Density, mass, and biomass estimates*

141   Densities (ind/mL) and trait dynamics were tracked daily for 15 days through fluid imaging of 1

142   mL subsamples of each microcosm (Fig 1a, FlowCam, Fluid Imaging Technologies,

143   Scarborough, ME, USA). The FlowCam captures images of particles ranging from 5-10 μm to

144   2mm in length. The procedure produced ~ 450k cell images, thus providing us a unique window

145   into how biomass, density, and body size, changed together over the course of this experiment.

146   Density was quantified as cell counts per volume sampled. Cell mass was quantified as the

147   product of cell volume (as the volume of a spheroid, in μm^3) and water density (1 g/cm^3, or 10-

148   12 g/μm^3). Sample biomass was measured as the sum of the masses of all individuals per sample

149   (in grams, g). However, the FlowCam can only census a fraction of each water sample. This

150   determines the efficiency of the machine (in our case ~ 0.33). Because of that, total biomass

151   needs to be corrected by the efficiency, as the observed number of individuals is a fraction of the

152   total that actually occur in our water samples. To do so, we linearly transform sample biomass

153   according to the observed relationship between the number of cell images and the actual

154   densities as detailed in Appendix 1. True biomass is therefore the observed biomass divided by

155   the sampling efficiency.

156

157   *Statistical analyses*

158   To assess how temperature regimes influenced biomass, density and mass dynamics, we used

159   Generalized Additive Mixed Models (GAMMs) with biomass, density, or mass as the response

160   variables, day is a smooth term, both temperature and the presence and absence of variation as

161   discrete predictors, and jar replicate as a random intercept. Additionally, because time series are

162   necessarily sampled in a repeated fashion within each replicate, temporal autocorrelation may

163   exist. To account for this temporal autocorrelation, we included an Autoregressive Moving

164   Average (ARMA) correlation structure of order one in our GAMMs using the R package mgcv

165   v.1.8 (Wood 2011, Wood et al. 2016).

166   While GAMM yields a good understanding of how time and treatments influence

167   dynamics, a finer understanding is possible by assessing what specific aspects of the dynamics

168   may have been influenced by the treatments. First, we assessed whether the imposed treatments

169   in any way influenced the peak observed biomass by running a multiple linear regression ('lm'

170   function in base R (R Core Team 2013)) with peak biomass (i.e., from days 3 to 5) as the

171   response variable and both additive and interactive effects of temperature and the

172   presence/absence of fluctuations as predictors. To quantify which differences between treatments

173   were significant, we also ran a separate ANOVA with a post-hoc Tukey test ('aov' and

174   'TukeyHSD' functions in base R (R Core Team 2013)) with peak biomass (i.e., from days 3 to 5)

175   as the response variable and all four temperature treatments as separate predictors. We used the

176   same statistical methods to assess whether demographic parameters controlling density –i.e.,

177   intrinsic growth rates, r, and carrying capacities, K– changed with treatment. Intrinsic growth

178   rates r where calculated as the natural log of the ratio of the density at day 1 and the density at

179   day 0 (Wieczynski et al. 2021, Gibert et al. 2022), and K was estimated as the densities measured

180   over the last 2 days of the dynamics in each jar.

181

182   *Decomposing change in biomass into change in density and mass*

183   To decompose the contribution of changes in density and mass to the observed changes in

184   biomass, we assume that the biomass, B, could be written as a function of density, N, and

185   average mass, M, as

186                              $$B = NM.$$ Eq. 1

187   The rate of change of B over time, $\frac{dB}{dt}$, can be found by taking time derivatives in both sides of

188   Eq. 1., which yields:

189    $$\frac{dB}{dt} = M\frac{dN}{dt} + N\frac{dM}{dt}.$$ Eq. 2

190   We then noticed that Eq 1 could be used to solve for either N or M, as $N = \frac{B}{M}$ and $M = \frac{B}{N}$, and

191   replaced both into Eq. 2 to get:

192    $$\frac{dB}{dt} = \frac{B}{N}\frac{dN}{dt} + \frac{B}{M}\frac{dM}{dt}.$$ Eq 3.

193   Eq. 3 coul be further simplified by factoring B, dividing both sides of the expression by B, then

194   using the relation $\frac{1}{x}\frac{dx}{dt} = \frac{d\,Ln(x)}{dt}$ to get:

195    $$\frac{d\,Ln(B)}{dt} = \frac{d\,Ln(N)}{dt} + \frac{d\,Ln(M)}{dt}\,.$$ Eq. 4

196   Eq. 4 links the rate of change in Ln(B), to that of Ln(N) and Ln(M). This equation can thus be

197   used to decompose the contributions of N (i.e., $\frac{d\,Ln(N)}{dt}$) and M (i.e., $\frac{d\,Ln(M)}{dt}$) to the rate of change

198   in B over time and across temperature treatments. We used our experimental time series to

199   calculate these contributions of N and M to changes in B for each individual jar on each day of

200   the experiment.

201

202   *Time series analysis*

203   Previous studies have shown that Convergent Cross Mapping (CCM) can be used to infer

204   causation between variables with available time series across ecological systems and

205   environmental conditions (Sugihara et al. 2012, Clark et al. 2015, Karakoç et al. 2020, Kondoh et

206   al. 2020, Rogers et al. 2020). A recent study used CCM to show that rapid plastic change in body

207   size influences population dynamics more than the other way around, which was then confirmed

208   through a manipulative experiment (Gibert et al. 2022). Following this literature, we therefore

209   used CCM to assess whether change in body size more strongly influenced changes in density, or

210   vice versa, across the temperature treatments.

211   CCM quantifies whether one time series (A) causally influences another (B) through the

212   estimation of how much information of A is contained in B (Takens 1981, Sugihara et al. 2012).

213   Conceptually, if a variable A causally influences variable B, but B does not influence A, then B

214   should contain information about A, but not the other way around. CCM assesses how much

215   information of the one variable is contained in the other by quantifying whether variable A can

216   be predicted from the time series of B (and vice-versa) for subsets of the time series of increasing

217   length (the length of these re-sampled time series is called the library size). If A more strongly

218   influences changes in B than the other way around, then B responds to A more strongly than A

219   responds to B. If the effect of A on B is causal, then the ability to predict A from B increases

220   with library size, while the error associated with the prediction decreases. If this 'predictability'

221   (or cross-mapping skill, ρ) is constant across library sizes, there is correlation, but not causation

222   (Sugihara et al. 2012). More details can be found in the now extensive literature on this

223   algorithm (Brookshire and Weaver 2015, Ye et al. 2015a, Ye et al. 2015b, Kaminski et al. 2016,

224   Hannisdal et al. 2017, Luo et al. 2017, Mønster et al. 2017, Tsonis et al. 2018, Vannitsem and

225   Ekelmans 2018, Liu et al. 2019, Barraquand et al. 2020). We used modified version of the CCM

226   algorithm (R package multispatialCCM v1.0 (Clark et al. 2015)) to analyze the time series for

227   each of the four temperature treatments because it allows for replicated times series.

228

229   RESULTS

230   *General dynamics*

231   Biomass increased steeply in the early days of the dynamics, then declined over time (Fig 2)

232   across temperatures. Density showed a typical logistic growth pattern of fast growth in the early

233   days followed by a plateau at around 6,000 ind/mL (Fig 2b). Average mass increased from Day 0

234   to Day 1, then decreased roughly monotonically over time (Fig 2c).

235

236   *Effects of temperature and variability on biomass, density, and average mass*

237   Biomass did not respond to either temperature (estimate=0.02±0.02, p=0.48, Fig 2d) or

238   temperature variability (estimate=-0.009±0.02, p=0.73, Fig 2d). Temperature had a positive

239   additive effect on density at 25ºC relative to 22ºC (estimate=0.05±0.02, p=0.018) while

240   temperature variability had no effect (estimate=0.003±0.02, p=0.89, Fig 2e). Temperature also

241   had a negative effect on mass (estimate=-0.006±0.003, p=0.002), but there was no effect of

242   variability (estimate=-0.03±0.01, p=0.06, Fig 2f). These results suggest that the effects of

243   temperature on density and mass likely cancel each other out, thus leading to an apparent lack of

244   biomass temperature response.

245   Once the time series were detrended (by subtracting a GAMM model only containing

246   time as a smooth term), additional effects of the treatments could be observed (Fig 2 g-i). In

247   particular, biomass and density showed similar strong effects of temperature (but not

248   fluctuations) in the first few days of the dynamics (Fig 2g & 2h). Mass temperature responses,

249   however, were most prevalent in the later dynamics, when the temperature size rule appears to

250   set in (Fig 2i).

251   Despite showing no effects of temperature or variability on overall biomass dynamics

252   (Fig 1d-f), peak biomass in the variable environment was higher than in the non-variable

253   environment across temperatures, and this difference was only slightly smaller in the high

254   temperature treatment, thus showing an effect of temperature variability but not temperature

255   alone on peak biomass (temp. effect = $4 \times 10^{-7} \pm 3 \times 10^{-6}$, p=0.906, var. effect = $9.492 \times 10^{-}$

256    $^{6} \pm 3.410 \times 10^{-6}$, p=0.007, interaction= $-5 \times 10^{-6} \pm 5 \times 10^{-6}$, p=0.314; ANOVA p = 0.02, Fig 3a).

257   Temperature and temperature variability also influenced simple descriptors of what are otherwise

258   complex density dynamics (Fig 3b, c). Indeed, temperature increased intrinsic growth rate

259   despite fluctuations having no effect (temp. effect = 1.03 ± 0.02, p<10^{-4}, var. effect = -0.08 ± 0.02,

260   p=0.7, interaction= -0.17 ± 0.28, p=0.6; ANOVA p < 10^{-4}, Fig 3b; calculated using the first two

261   days). Carrying capacity, on the other hand, decreased with variability but only at the low

262   temperature and showed no significant differences between temperatures (temp. effect = -

263   304 ± 166.1, p=0.074, var. effect = -696 ± 166, p<10^{-3}, interaction= 667.2 ± 234.9, p=0.007;

264   ANOVA p = 0.002, Fig 3c).

265

266   *Decomposing the effects of density and mass on biomass across treatments*

267   Density and mass dynamics contributed distinctly to biomass dynamics, especially in the first

268   three days (Fig 4). For day $\leq$ 2, rapid density increases strongly and positively influenced

269   biomass, while mass only positively influenced biomass dynamics on day 1, then made mostly

270   negative contributions (GAMM smooth term = p<10^{-16}, Fig 4a; ANOVA p<10^{-16}, Fig 4g), likely

271   due to the monotonous decline in mass from day 1 on (Fig 2c, f).

272   Despite temperature and temperature variability influencing both density and mass

273   dynamics, their effects on the contributions of either one to biomass dynamics –i.e., $\frac{d\,Ln(N)}{dt}$ and

274    $\frac{d\,Ln(M)}{dt}$ – was surprisingly low, especially in the long-term. Initially (day $\leq$ 2), density had a

275   large, positive affect on biomass that remained high until day 2 at 22ºC (Fig 4a) but declined

276   sharply after day 1 at 25ºC (Fig 4b, thin lines). Beyond day 2, the contributions of either density

277   or mass to biomass dynamics were small but different in sign (positive for density, negative for

278   mass, Fig 4e–g). These results suggest that, while temperature treatment effects on biomass are

279   most notable in the early dynamics, small, opposing effects of density and mass dynamics on

280   biomass dynamics persist in the long term but are mostly unaffected by temperature and

281   temperature fluctuations. Moreover, small temperature effects in the contributions of mass and

282   density in the early dynamics are enough to produce larger effects later on.

283

284   *The temperature response of the coupling between density and mass*

285   We observed that changes in mass more strongly influenced change in density than the other way

286   around (consistent with a recent study (Gibert et al. 2022)) across all temperature treatments (Fig

287   5 and Fig S2 Appendix2). However, the strength of these reciprocal effects varied among

288   treatments in specific ways. Temperature variability weakened the effect of mass on density

289   across temperatures, and this effect was slightly stronger at 25 ºC compared to 22ºC (Fig 5, temp.

290   effect = 0.013 ± 0.001, p=0.15, var. effect = -0.05 ± 0.009, $p<10^{-6}$, interaction= -0.05 ± 0.01, $p<10^{-4}$).

291   In contrast, the effect of density on mass weakened from 22ºC to 25ºC but got stronger with

292   temperature fluctuations (Fig 5, temp. effect = -0.05 ± 0.01, $p<10^{-6}$, var. effect = 0.20 ± 0.007,

293    $p<10^{-16}$, interaction= -0.02 ± 0.01, p=0.29). These results suggest that rapid feedbacks between

294   density and mass (or "eco-phenotypic feedbacks") may themselves depend on environmental

295   conditions—especially the effect of density on mass, which seems to respond more strongly to

296   environmental variability than the effect of mass on density (Fig 5).

297

298

299

300   DISCUSSION

301   Understanding how changes in environmental conditions influence biomass dynamics is

302   paramount in ecology. Here, we argue that doing so requires understanding how temperature and

303   temperature variability influence density and mass dynamics, then determining how those, in

304   turn, influence biomass dynamics. Our results show that, while density and mass dynamics are

305   independently susceptible to changes in temperature regimes (Fig 2 and 3), these effects may

306   cancel each other out and not always translate to changes in biomass in response to temperature

307   (Fig 2). We also show that different aspects of density-mass-biomass dynamics respond

308   differentially to variation in environmental conditions (Fig 3), even when environmental effects

309   on overall dynamics are less obvious (Fig 2). We show that density and mass have mostly

310   opposite effects on biomass and their contributions are nuanced and likely stronger in earlier

311   dynamics (Fig 4). Last, we show that temperature and temperature variability can alter the

312   strength of feedbacks between mass and density (Fig 5), suggesting that rapid eco-phenotypic

313   feedbacks may play and important but poorly understood role in biomass change in novel

314   environments.

315   Previous research has shown that temperature often reduces body size, a phenomenon

316   widely known as the Temperature-Size Rule (or TSR, e.g., (Atkinson 1994, Atkinson et al.

317   2003)). This phenomenon is widespread in myriad organisms, including mammals (Ozgul et al.

318   2009), birds (Weeks et al. 2020, Jirinec et al. 2021), invertebrates (Ghosh et al. 2013) and

319   unicellular organisms (Atkinson et al. 2003, DeLong 2012, Tabi et al. 2020). The TSR has long

320   been suggested to play an important role in the responses of populations (Ozgul et al. 2009),

321   communities (Brose et al. 2012, Forster et al. 2012, Gibert and DeLong 2017) and ecosystems

322   (Brose et al. 2012) to warming, as body size can directly impact reproductive and mortality rates

14

323   and species-interaction parameters through its effect on metabolic rates (Gillooly et al. 2002,

324   Brown et al. 2004, Savage et al. 2004). Our results show that the onset of the TSR occurs very

325   early in population dynamics as species grow towards carrying capacity (Fig 1). Our results also

326   suggest that, despite the numerous hypothesized effects of the TSR on ecological processes and

327   dynamics, the TSR represents at most 5% of the observed variation in mass over time, with

328   transient changes in mass being much larger in magnitude than the observed TSR (Fig 1).

329   However, recent work has shown that, without accounting for the TSR, predictions about

330   how temperature influences long-term species densities (i.e., at carrying capacity) may be

331   inaccurate (Bernhardt et al. 2018). Our results further imply that, without accounting for the

332   TSR, inferring changes in biomass from changes in density alone may lead to equivocal

333   estimates, as the effects of temperature on density and mass can cancel each other out (Fig 2).

334   These results are important because they imply that environmental perturbations may –

335   sometimes rapidly– change populations not just numerically (e.g., changes in densities), but also

336   phenotypically. Although the ecological consequences of these rapid, plastic, phenotypic

337   responses are still very poorly understood, our results emphasize the need to improve this

338   understanding.

339   A recent study showed that rapid, plastic changes in body size more strongly influence

340   changes in density than the other way around (Gibert et al. 2022), thus establishing the existence

341   of important, but poorly understood, rapid feedbacks between body size and density. We

342   observed the same pattern across in our study. Additionally, our results show that the strength of

343   these feedbacks vary across temperature regimes (Fig 4) and that both mean temperature and

344   temperature variability may be important. This result further emphasizes the need to study rapid

15

345   phenotypic change –evolutionary or not– as a fundamental ecological response mediating how

346   species cope with novel environmental conditions.

347   While our results provide novel insights about how rapid eco-phenotypic dynamics may

348   mediate changes in biomass, density, and mass in response to warming and temperature

349   variability, we caution against extrapolating these results beyond the range of temperatures

350   studied here. Indeed, temperature effects are well known to have canonically unimodal effects on

351   many demographic rates (Amarasekare and Savage 2012, Amarasekare and Coutinho 2013,

352   Luhring and DeLong 2017, DeLong et al. 2018, Wieczynski et al. 2021). Thus, eco-phenotypic

353   responses to a wider range of temperatures may be more complex than the results reported here.

354   Moreover, the regimes of temperature fluctuations imposed here were less variable than the

355   random fluctuations expected in an increasingly warmer world (Vasseur et al. 2014). Because of

356   this, we also caution against interpreting our results to say that average temperatures cause

357   stronger species-level responses than temperature variability and, in fact, some of our results

358   even suggest that variability does have important effects (Fig 3a, Fig 5). Last, while CCM has

359   long been used to infer effects of one time series on another (e.g. (Sugihara et al. 2012, Clark et

360   al. 2015, Ye et al. 2015a, Ye et al. 2015b, Tsonis et al. 2018)), other unobserved variables like

361   reductions in available nutrients, effects of regular sampling, or even physiological and

362   metabolic changes as the ecological dynamics unfold, may affect and even weaken the CCM

363   inference. A silver lining is that our results are consistent with those obtained by Gibert et al.

364   (2022) which were validated with additional body size and density manipulations and showed

365   that CCM correctly inferred reciprocal effects between size and density based only on their time

366   series, as was done here (Fig 5).

16

367   Overall, our results shed light on how rapid eco-phenotypic dynamics in density and mass

368 may influence how biomass responds to changes in temperature regimes. Our study emphasizes

369 the need to consider rapid phenotypic change as an important –but poorly understood–

370 mechanism through which organisms cope with changes in environmental conditions, with

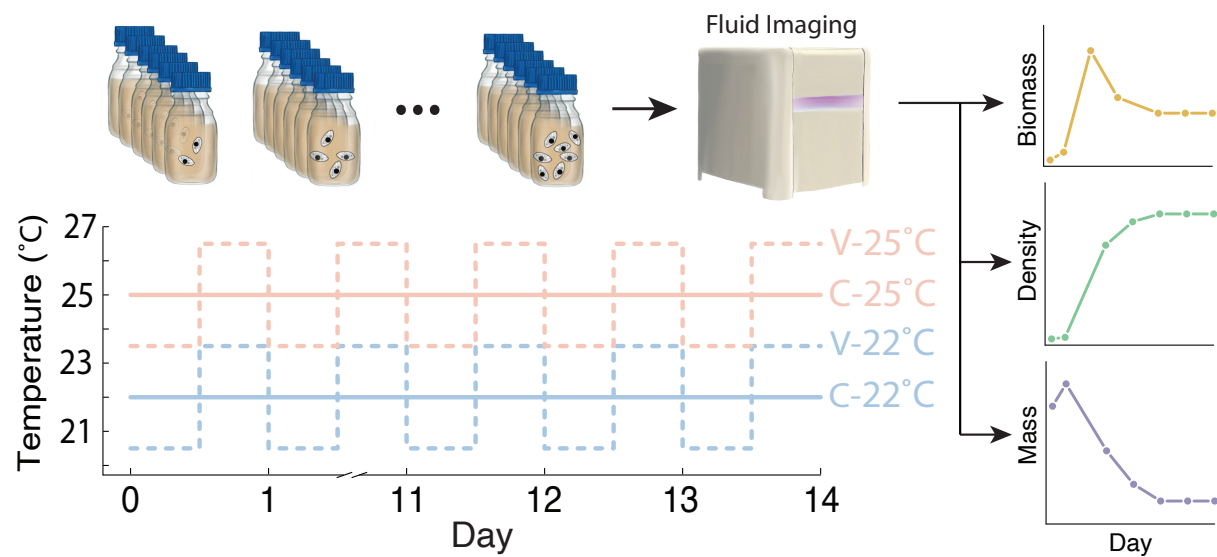371 important implications for species responses to a rapidly changing and increasingly warm world.

372

373

374

375

376

377

378

379

380

381

382

383

384

385

386

387

388

389

390   FIGURES

391

392   Fig 1: Microcosms where initialized at Day 0 and kept in four possible temperature treatments

393   (Constant-22ºC, Variable-22ºC, Constant-25ºC or Variable-25ºC) for 15 days. Each day, a 1mL

394   sample of media was taken for fluid imaging (FlowCam) to estimate total biomass, density, and

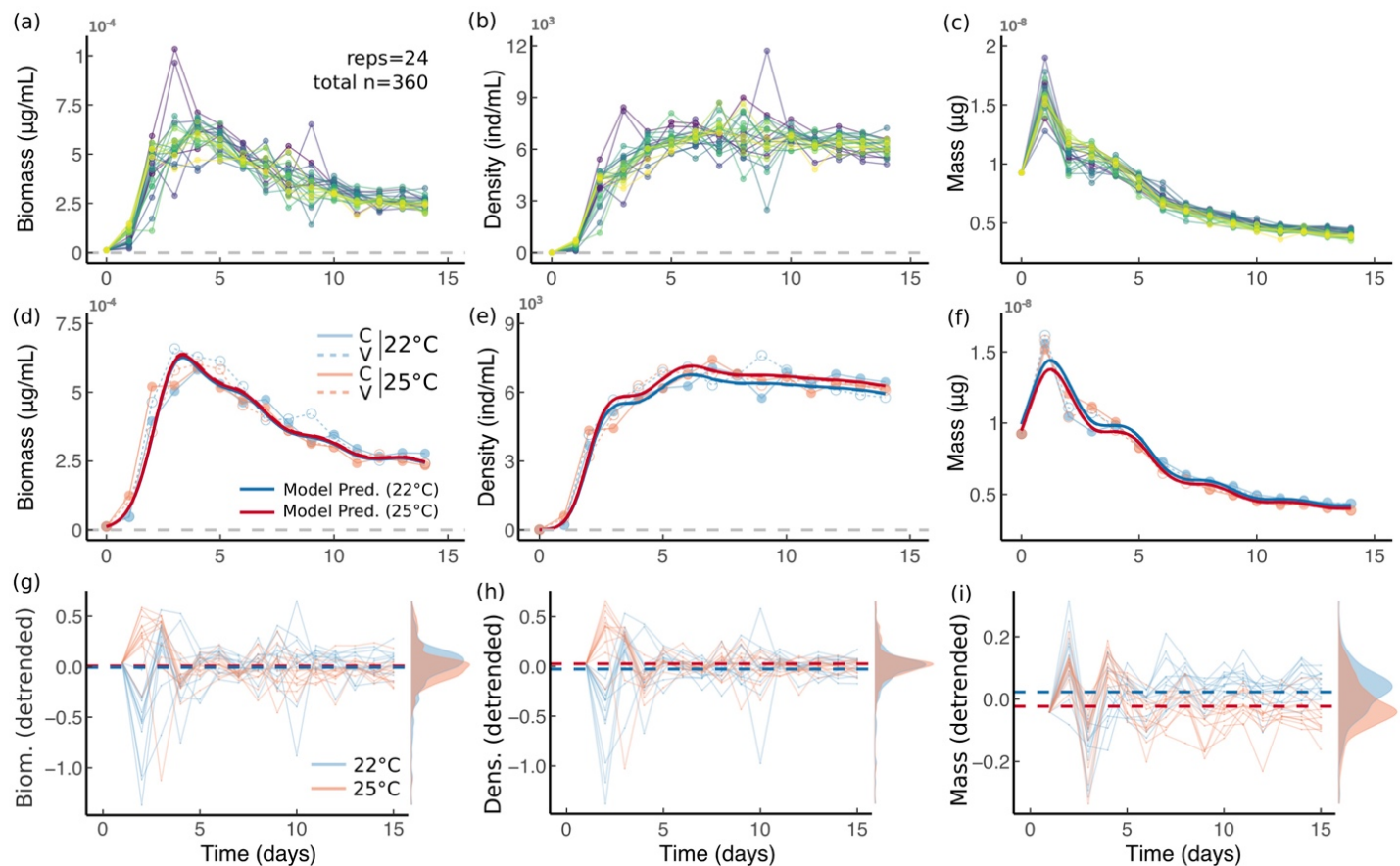395   average mass as the species grew to carrying capacity.

396

397

398

399

400

401

402

Fig 2: (a) Biomass over time for all 24 experimental jars. (b)-(c) as in (a) but for density and average mass respectively. (d) Biomass change over time (dots represent average biomass across all 6 replicates within each experiment, blue represents 22°C treatments, red represent 25°C treatments, while solid lines represent constant temperature treatments, C, dashed lines represent variable temperature treatments, V). Solid bold lines represent GAMM model predictions. (e-f) as in (d) but for density and mass. (g) Detrended biomass dynamics across all temperature treatments (but only color coded for mean temperature as temperature variability had no effect in d-f) with color coding as in (d). Bold dashed lines represent mean biomass for both temperature treatments. The distribution that biomass values take over time are shown on the right. (h-i) as in (g) but for density and mass.
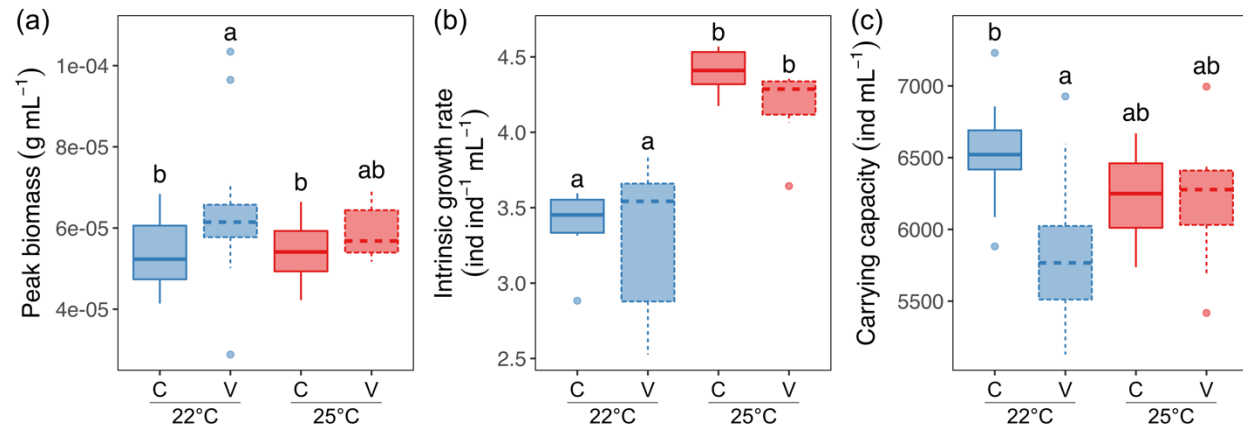
Fig 3: (a) Peak biomass, measured at days 3-5 with significant differences indicated as letters above the boxes. Variable temperatures lead to higher peak biomass, and that peak is higher at 22ºC than at 25ºC. (b) Intrinsic growth rate increases at 25ºC. (c) Carrying capacity is higher at constant 22ºC than in variable temperature but that difference disappears at 25ºC. Color coding as in Fig 1.
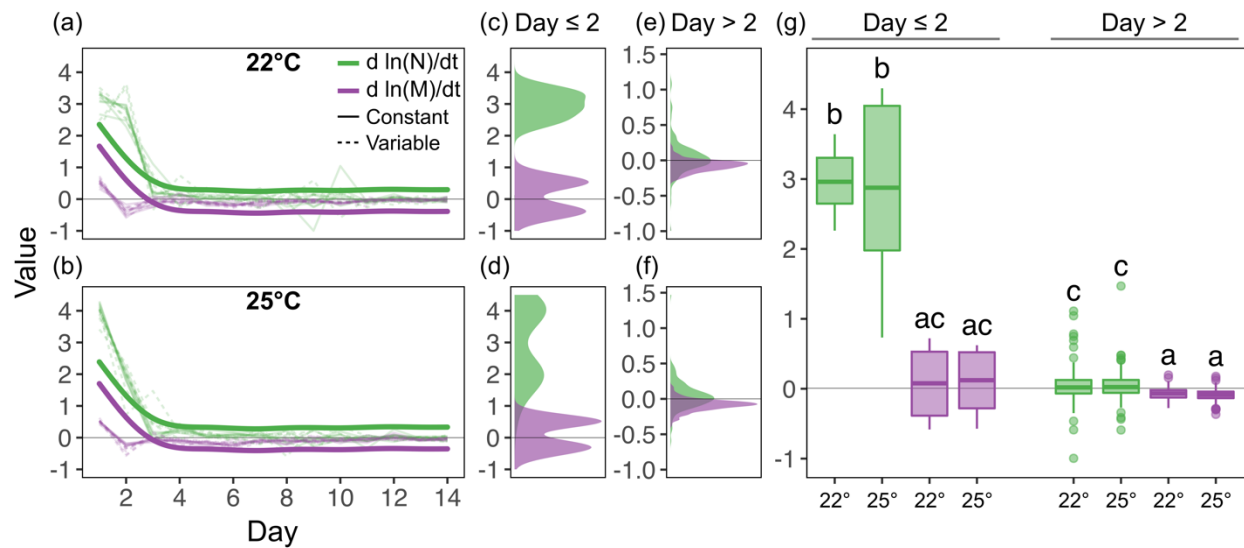
20

Fig 4: Contributions of density (d ln(N)/dt) and mass (d ln(M)/dt) to biomass dynamics at (a,c,e) 22ºC and (b,d,f) 25ºC. (a) and (b) show the contributions of density and mass over time at 22ºC and 25ºC, respectively. Thin lines represent replicate populations (jars) and thick lines are GAMM fits to these data. (c,d) and (e,f) show distributions of density and mass contributions over time for Days 0–2 and Days > 2, respectively. (g) shows differences in the contributions of density and mass across both temperatures for Day ≤ 2 and Day >2, evaluated using an ANOVA with Tukey's HSD post-hoc test (p < 10^{-5}).
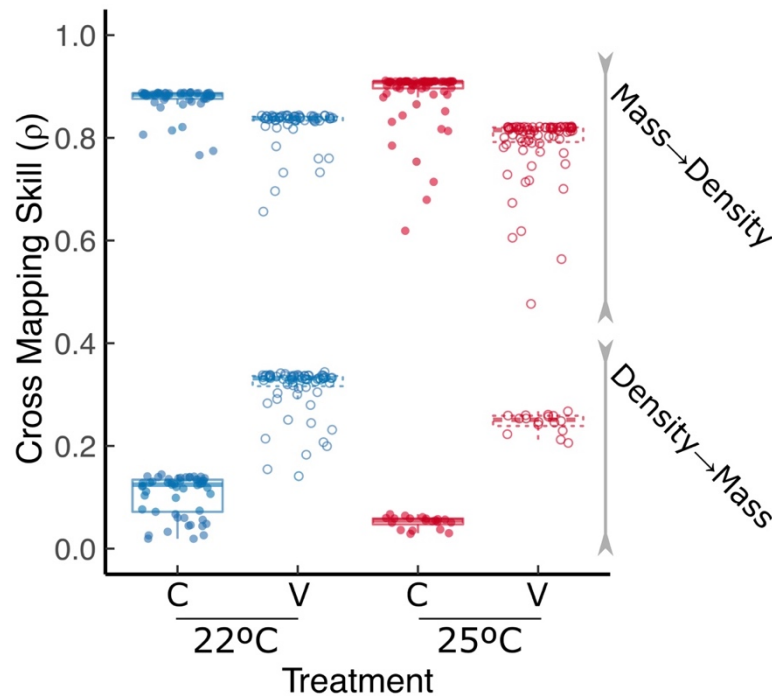
449

Fig 5: We show the cross-mapping skill (ρ) for all possible library sizes across temperature treatments (here represented as individual dots). Package multispatialCCM performs CCM on 800 total bootstrap replicates of the time series for each library size and yields an average value for the cross mapping skill. Effects of mass on density are indicated as 'Mass→Density' and effects of density on mass are indicated as 'Density→Mass'. Mass more strongly influences density than the other way around, but the reciprocal effects of mass and density respond to both average temperature and temperature fluctuations. Colors as in Figs 1-3.

457

458

459

460

461

462

463

464

REFERENCES

Allen, A. P., J. F. Gillooly, and J. H. Brown. 2005. Linking the global carbon cycle to individual metabolism. Functional Ecology **19**:202-213.

Altermatt, F., E. A. Fronhofer, A. Garnier, A. Giometto, F. Hammes, J. Klecka, D. Legrand, E. Mächler, T. M. Massie, F. Pennekamp, M. Plebani, M. Pontarp, N. Schtickzelle, V. Thuillier, and O. L. Petchey. 2015. Big answers from small worlds: A user's guide for protist microcosms as a model system in ecology and evolution. Methods in Ecology and Evolution **6**:218-231.

Amarasekare, P., and R. M. Coutinho. 2013. The intrinsic growth rate as a predictor of population viability under climate warming. Journal of Animal Ecology **82**:1240-1253.

Amarasekare, P., and V. M. Savage. 2012. A framework for elucidating the temperature dependence of fitness. The American Naturalist **179**:178-191.

Atkinson, D. 1994. Temperature and Organism Size–A Biological Law for Ectotherms? Advances in Ecological Research **25**:1-58.

Atkinson, D. 1995. Effects of temperature on the size of aquatic ectotherms: exceptions to the general rule. Journal of Thermal Biology **20**:61-74.

Atkinson, D., B. J. Ciotti, and D. J. S. Montagnes. 2003. Protists decrease in size linearly with temperature: ca. 2.5% degrees ºC-1. Proceedings of the Royal Society B: Biological Sciences **270**:2605-2611.

Barbour, M. A., and J. P. Gibert. 2021. Genetic and plastic rewiring of food webs under climate change. Journal of Animal Ecology.

Barneche, D. R., C. J. Hulatt, M. Dossena, D. Padfield, G. Woodward, M. Trimmer, and G. Yvon-Durocher. 2021. Warming impairs trophic transfer efficiency in a long-term field experiment. Nature **592**:76-79.

Barraquand, F., C. Picoche, M. Detto, and F. Hartig. 2020. Inferring species interactions using Granger causality and convergent cross mapping. Theoretical Ecology **14**:87-105.

Bartley, T. J., K. S. McCann, C. Bieg, K. Cazelles, M. Granados, M. M. Guzzo, A. S. MacDougall, T. D. Tunney, and B. C. McMeans. 2019. Food web rewiring in a changing world. Nature Ecology & Evolution **3**:345-354.

Begon, M., C. R. Townsend, and J. L. Harper. 2006. Ecology: from individuals to ecosystems. Fourth Edi edition. BLACKWELL PUBLISHING.

Bernhardt, J. R., J. M. Sunday, and M. I. O'Connor. 2018. Metabolic theory and the temperature-size rule explain the temperature dependence of population carrying capacity. American Naturalist **192**:687-697.

Brookshire, E. N., and T. Weaver. 2015. Long-term decline in grassland productivity driven by increasing dryness. Nature Communications **6**:7148.

Brose, U., J. A. Dunne, J. M. Montoya, O. L. Petchey, F. D. Schneider, and U. Jacob. 2012. Climate change in size-structured ecosystems. Philosophical transactions of the Royal Society of London. Series B, Biological sciences **367**:2903-2912.

Brown, J. H., J. F. Gillooly, A. P. Allen, V. M. Savage, and G. B. West. 2004. Toward a Metabolic Theory of Ecology. Ecology **85**:1771-1789.

Carr, L. A., R. K. Gittman, and J. F. Bruno. 2018. Temperature Influences Herbivory and Algal Biomass in the Galápagos Islands. Frontiers in Marine Science **5**:1-10.

508   Clark, A. T., H. Ye, F. Isbell, E. R. Deyle, J. Cowles, G. D. Tilman, and G. Sugihara. 2015. Spatial
509   convergent cross mapping to detect causal relationships from short time series. Ecology
510   **96**:1174-1181.
511   D'Alelio, D., S. Libralato, T. Wyatt, and M. Ribera D'Alcalà. 2016. Ecological-network models link
512   diversity, structure and function in the plankton food-web. Scientific Reports **6**:1-13.
513   Damuth, J. 1981. Population density and body size in mammals. Nature **290**:699-700.
514   DeLong, J. P. 2012. Experimental demonstration of a ' rate – size ' trade-off governing body size
515   optimization. Evolutionary Ecology Research **14**:343-352.
516   DeLong, J. P. 2014. The body-size dependence of mutual interference. Biology Letters
517   **10**:20140261-20140261.
518   DeLong, J. P., G. Bachman, J. P. Gibert, T. M. Luhring, K. L. Montooth, A. Neyer, and B. Reed.
519   2018. Habitat, latitude, and body mass influence the temperature dependence of
520   metabolic rate. Biology Letters.
521   DeLong, J. P., B. Gilbert, J. B. Shurin, V. M. Savage, B. T. Barton, C. F. Clements, A. I. Dell, H. S.
522   Greig, C. D. G. Harley, P. Kratina, K. S. McCann, T. D. Tunney, D. A. Vasseur, and M. I.
523   O'Connor. 2015. The Body Size Dependence of Trophic Cascades. The American
524   Naturalist **185**:354-366.
525   DeLong, J. P., T. C. Hanley, and D. A. Vasseur. 2014. Competition and the density dependence of
526   metabolic rates. The Journal of Animal Ecology **83**:51-58.
527   DeLong, J. P., and D. T. Hanson. 2009. Metabolic rate links density to demography in
528   Tetrahymena pyriformis. The ISME journal **3**:1396-1401.
529   Forster, J., A. G. Hirst, and D. Atkinson. 2012. Warming-induced reductions in body size are
530   greater in aquatic than terrestrial species. Proceedings of the National Academy of
531   Sciences of the United States of America **109**:19310-19314.
532   Ghosh, S. M., N. D. Testa, and A. W. Shingleton. 2013. Temperature-size rule is mediated by
533   thermal plasticity of critical size in Drosophila melanogaster. ZooKeys **298**.
534   Gibert, J. P. 2019. Temperature directly and indirectly influences food web structure. Scientific
535   Reports **9**:5312-5312.
536   Gibert, J. P., and J. P. DeLong. 2014. Temperature alters food web body-size structure. Biology
537   Letters **10**:20140473-20140473.
538   Gibert, J. P., and J. P. DeLong. 2017. Phenotypic variation explains food web structural patterns.
539   Proceedings of the National Academy of Sciences of the United States of America
540   **114**:11187-11192.
541   Gibert, J. P., Z.-Y. Han, D. J. Wieczynski, S. Votzke, and A. Yammine. 2022. Feedbacks between
542   size and density determine rapid eco-phenotypic dynamics. Functional Ecology.
543   Giller, P. S., and G. O'Donovan. 2002. Biodiversity and Ecosystem Function: Do Species Matter?
544   Biology and Environment: Proceedings of the Royal Irish Academy **102B**:129-139.
545   Gillooly, J. F., J. H. Brown, G. B. West, V. M. Savage, and E. L. Charnov. 2001. Effects of size and
546   temperature on metabolic rate. Science (New York, N.Y.) **293**:2248-2251.
547   Gillooly, J. F., E. L. Charnov, G. B. West, V. M. Savage, and J. H. Brown. 2002. Effects of size and
548   temperature on developmental time. Science **417**:70-73.
549   Hannisdal, B., K. A. Haaga, T. Reitan, D. Diego, and L. H. Liow. 2017. Common species link global
550   ecosystems to climate change: dynamical evidence in the planktonic fossil record.
551   Proceedings of the Royal Society B **284**.

552 Hastings, A., K. C. Abbott, K. Cuddington, T. Francis, G. Gellner, Y.-C. Lai, A. Morozov, S.
553   Petrovskii, K. Scranton, and M. L. Zeeman. 2018. Transient phenomena in ecology.
554   Science **361**:1-9.
555 Hatton, I. A., K. S. McCann, J. M. Fryxell, T. J. Davies, M. Smerlak, a. R. E. Sinclair, and M. Loreau.
556   2015. The predator-prey power law: Biomass scaling across terrestrial and aquatic
557   biomes. Science **349**:aac6284-aac6284.
558 Jirinec, V., R. C. Burner, B. Amaral, R. O. Bierregard Jr., G. Fernández-Arellano, A. Hernández-
559   Palma, E. I. Johnson, T. E. Lovejoy, L. L. Powell, C. Rutt, J. D. Wolfe, and P. Stouffer. 2021.
560   Morphological consequences of climate change for resident birds in intact Amazonian
561   rainforest. Science Advances **7**: eabk1743.
562 Kaminski, M., A. Brzezicka, J. Kaminski, and K. J. Blinowska. 2016. Measures of Coupling
563   between Neural Populations Based on Granger Causality Principle. Frontiers in
564   Computer Sciences **10**:114.
565 Karakoç, C., A. T. Clark, and A. Chatzinotas. 2020. Diversity and coexistence are influenced by
566   time-dependent species interactions in a predator-prey system. Ecology Letters **23**:983-
567   993.
568 Kondoh, M., K. Kawatsu, Y. Osada, and M. Ushio. 2020. Theoretical Ecology, concepts, and
569   applications: A data-driven approach to complex ecological systems. Pages 117-133 *in* K.
570   S. McCann and G. Gellner, editors. Theoretical Ecology, Concepts and Applications.
571   Oxford University Press, Oxford.
572 Kortsch, S., R. Primicerio, M. Fossheim, A. V. Dolgov, and M. Aschan. 2015. Climate change
573   alters the structure of arctic marine food webs due to poleward shifts of boreal
574   generalists. Proceedings. Biological sciences / The Royal Society **282**:20151546--
575   20151546-.
576 Larjavaara, M., X. Lu, X. Chen, and M. Vastaranta. 2021. Impact of rising temperatures on the
577   biomass of humid old-growth forests of the world. Carbon Balance Management **16**:31.
578 Lin, D., J. Xia, and S. Wan. 2010. Climate warming and biomass accumulation of terrestrial
579   plants: a meta-analysis. New Phytologist **188**:187-198.
580 Liu, H., M. Lei, N. Zhang, and G. Du. 2019. The causal nexus between energy consumption,
581   carbon emissions and economic growth: New evidence from China, India and G7
582   countries using convergent cross mapping. PLOS ONE **14**:e0217319.
583 Luhring, T. M., and J. P. DeLong. 2017. Scaling from Metabolism to Population Growth Rate to
584   Understand How Acclimation Temperature Alters Thermal Performance. Integrative and
585   Comparative Biology **57**:103-111.
586 Luo, L., F. Cheng, T. Qiu, and J. Zhao. 2017. Refined convergent cross-mapping for disturbance
587   propagation analysis of chemical processes. Computers & Chemical Engineering **106**:1-
588   16.
589 McKie, B. G., and B. Malmqvist. 2009. Assessing ecosystem functioning in streams affected by
590   forest management: increased leaf decomposition occurs without changes to the
591   composition of benthic assemblages. Freshwater Biology **54**:2086-2100.
592 Mønster, D., R. Fusaroli, K. Tylén, A. Roepstorff, and J. F. Sherson. 2017. Causal inference from
593   noisy time-series data — Testing the Convergent Cross-Mapping algorithm in the
594   presence of noise and external influence. Future Generation Computer Systems **73**:52-
595   62.

O'Connor, M. I., M. F. Piehler, D. M. Leech, A. Anton, and J. F. Bruno. 2009. Warming and resource availability shift food web structure and metabolism. PLoS Biology **7**:3-8.

Ozgul, A., S. Tuljapurkar, T. G. Benton, J. M. Pemberton, T. H. Clutton-Brock, and T. Coulson. 2009. The dynamics of phenotypic change and the shrinking sheep of St. Kilda. Science (New York, N.Y.) **325**:464-467.

Rogers, T. L., S. B. Munch, S. D. Stewart, E. P. Palkovacs, A. Giron-Nava, S. S. Matsuzaki, and C. C. Symons. 2020. Trophic control changes with season and nutrient loading in lakes. Ecology Letters **23**:1287-1297.

Savage, V. M., J. F. Gillooly, J. H. Brown, and E. L. Charnov. 2004. Effects of body size and temperature on population growth. The American Naturalist **163**:429-441.

Schramski, J. R., A. I. Dell, J. M. Grady, R. M. Sibly, and J. H. Brown. 2015. Metabolic theory predicts whole-ecosystem properties. Proceedings of the National Academy of Sciences USA **112**:2617-2622.

Srivastava, D. S., and M. Vellend. 2005. Biodiversity-Ecosystem Function Research: Is It Relevant to Conservation? Annual Review of Ecology, Evolution, and Systematics **36**:267-294.

Sugihara, G., R. May, H. Ye, C. H. Hsieh, E. Deyle, M. Fogarty, and S. Munch. 2012. Detecting causality in complex ecosystems. Science **338**:496-500.

Tabi, A., A. Garnier, F. Pennekamp, and C. White. 2020. Testing multiple drivers of the temperature-size rule with nonlinear temperature increase. Functional Ecology **34**:2503-2512.

Takens, F. 1981. Detecting strange attractors in turbulence. Dynamical Systems and Turbulence. . Springer, Berlin, Heilderberg.

Team, R. C. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. R Foundation for Statistical Computing, Vienna, Austria.

Trebilco, R., J. K. Baum, A. K. Salomon, and N. K. Dulvy. 2013. Ecosystem ecology: size-based constraints on the pyramids of life. Trends in Ecology & Evolution **28**:423-431.

Tsonis, A. A., E. R. Deyle, H. Ye, and G. Sugihara. 2018. Convergent Cross Mapping: Theory and an Example. Pages 587-600 Advances in Nonlinear Geosciences.

Ullah, H., I. Nagelkerken, S. U. Goldenberg, and D. A. Fordham. 2018. Climate change could drive marine food web collapse through altered trophic flows and cyanobacterial proliferation. PLoS Biology **16**:1-21.

Vannitsem, S., and P. Ekelmans. 2018. Causal dependences between the coupled ocean–atmosphere dynamics over the tropical Pacific, the North Pacific and the North Atlantic. Earth System Dynamics **9**:1063-1083.

Vasseur, D. A., J. P. DeLong, B. Gilbert, H. S. Greig, C. D. G. Harley, K. S. McCann, V. Savage, T. D. Tunney, and M. I. O'Connor. 2014. Increased temperature variation poses a greater risk to species than climate warming Proceedings of the Royal Society B: Biological Sciences **281**:20132612-20132612.

Weeks, B. C., D. E. Willard, M. Zimova, A. A. Ellis, M. L. Witynski, M. Hennen, and B. M. Winger. 2020. Shared morphological consequences of global warming in North American migratory birds. Ecology Letters **23**:316-325.

Wieczynski, D. J., P. Singla, A. Doan, A. Singleton, Z. Y. Han, S. Votzke, A. Yammine, and J. P. Gibert. 2021. Linking species traits and demography to explain complex temperature

640   responses across levels of organization. Proceedings of the National Academy of
641   Sciences **118**:e2104863118.
642 Wood, J. L., M. C. Yates, and D. J. Fraser. 2016. Are heritability and selection related to
643   population size in nature? Meta-analysis and conservation implications. Evolutionary
644   Applications **9**:640-657.
645 Wood, S. N. 2011. Fast stable restricted maximum likelihood and marginal likelihood estimation
646   of semiparametric generalized linear models. Journal of the Royal Statistical Society B
647   **73**:3-36.
648 Ye, H., R. J. Beamish, S. M. Glaser, S. C. Grant, C. H. Hsieh, L. J. Richards, J. T. Schnute, and G.
649   Sugihara. 2015a. Equation-free mechanistic ecosystem forecasting using empirical
650   dynamic modeling. Proceedings of the National Academy of Sciences **112**:E1569-1576.
651 Ye, H., E. R. Deyle, L. J. Gilarranz, and G. Sugihara. 2015b. Distinguishing time-delayed causal
652   interactions using convergent cross mapping. Scientific Reports **5**:14750.
653