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Staffan Jacob[1], Léonard Dupont[1], Bart Haegeman[2], Mélanie Thierry[1], Julie L. M. Campana[1], Delphine Legrand[1], Julien Cote[3], Allan Raffard[4*]

[1] Station d'Écologie Théorique et Expérimentale, UAR2029, CNRS, 09200, Moulis, France.

[2] CNRS/Sorbonne Université, UMR7621 Laboratoire d'Océanographie Microbienne, Banyuls-sur-Mer, France

[3] CNRS, UPS, IRD, Laboratoire Évolution et Diversité Biologique, UAR 5174, CNRS, UPS, IRD, 31062, Cedex 9 Toulouse, France

[4] Université catholique de Louvain, Earth and Life Institute, Biodiversity Research Centre, Louvain-la-Neuve, Belgium

[*] present address: Univ. Savoie Mont Blanc, INRAE, CARRTEL, 74200 Thonon-les-Bains, France

Corresponding author: *Staffan Jacob: staffan.jacob@sete.cnrs.fr*

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Abstract

Classical theories predict that relatively constant environments should generally favor specialists, while fluctuating environments should select for generalists. However, theoretical and empirical results have pointed out that generalist organisms might on the contrary perform poorly under fluctuations. In particular, if generalism is underlaid by phenotypic plasticity, performance of generalists should be modulated by the temporal characteristics of environmental fluctuations. Here, we used experiments in microcosms of *Tetrahymena thermophila* ciliates and a mathematical model to test whether the period or autocorrelation of thermal fluctuations mediate links between the level of generalism and the performance of organisms under fluctuations. In the experiment thermal fluctuations consistently impeded performance compared to constant conditions. However, the intensity of this effect depended on the level of generalism: while the more specialists strains performed better under fast or negatively autocorrelated fluctuations, plastic generalists performed better under slow or positively autocorrelated fluctuations. Our model suggests that these effects of fluctuations on organisms' performance may result from a time delay in the expression of plasticity, restricting its benefits to slow-enough fluctuations. This study points out the need to further investigate the temporal dynamics of phenotypic plasticity to better predict its fitness consequences under environmental fluctuations.

Introduction

Organisms inhabit environments that are constantly changing, leading to variations of selective pressures affecting their performance, and consequently their ecology and evolution [1]. Given the ubiquity of environmental fluctuations, understanding how organisms deal with such changing conditions has attracted much attention [2,3]. A common first step in this investigation process consists in quantifying tolerance curves, *i.e.,* variations in how organisms perform across a gradient of environmental conditions [4–6]. Tolerance curves allow to place organisms on a continuum ranging from specialists to generalists depending on the breadth of environmental conditions they manage to live in (*i.e.,* their niche width).

Theory classically predicts that constant environments should favor specialists, while organisms able to tolerate a broader range of environmental conditions should be selected in fluctuating environments [7–11]. However, empirical and theoretical studies have revealed that generalism is not always favored under environmental fluctuations [1,6,12–14]. This discrepancy suggests that evolutionary strategies providing fitness benefits under constant conditions may be neutral or even disadvantageous in fluctuating environments, and conversely [5,6,11,15]. For instance, Botero and colleagues [11] showed that the evolution of adaptive mechanisms underlying tolerance curves (*i.e.,* phenotypic plasticity, bet-hedging, adaptive tracking) should depend on the characteristics of environmental fluctuations, *i.e.,* the predictability and timescale of fluctuations relative to generation time. Especially, phenotypic plasticity, the ability of a given genotype to produce multiple phenotypes depending on the environment, is classically considered to underlie the degree of generalism or organisms, and requires environmental changes to evolve [11,16–19].

However, whether generalist organisms perform well in fluctuating environments should depend on the interaction between the characteristics of fluctuations and the mechanisms underlying generalism. In the case of phenotypic plasticity, implementing changes in trait

87 expression most often takes time and notably depends on the speed at which underlying
88 mechanisms occurs (*i.e.,* the rate of plasticity; [20–26]). Consequently, if generalism results
89 from adaptive plastic changes occurring at a rate below that of environmental fluctuations,
90 generalist organisms might attain only low performance under temporally fluctuating
91 conditions [6,13,20,25–27]. Although considered in some theoretical works (*e.g.,*
92 [19,20,28,29]), whether the rate of plastic changes could determine the effects of environmental
93 fluctuations on how generalists and specialists perform remains experimentally unexplored
94 [26].

95 In this study, we used experiments in microcosms and a mathematical model to explore
96 how phenotypic plasticity affects the relationship between the degree of generalism and
97 performance under thermal fluctuations. We performed experiments using 15 strains of the
98 ciliate *Tetrahymena thermophila* that differ in their degree of thermal generalism and capacity
99 of phenotypic plasticity for morphological and movement traits [30,31] (Figure 1). Cell
100 morphology (cell size) was previously related to resource acquisition and metabolic rate in
101 protists [32], while cell movement (velocity) tends to be associated with dispersal [33,34]. We
102 first tested whether the strains' level of generalism correlated with morphological and
103 movement plasticity. Then, each isolated strain was independently exposed to two fluctuation
104 gradients: the first varied in the timescale of fluctuations (*i.e.,* period) and the second in the
105 temporal autocorrelation of fluctuations (*i.e.,* as a proxy of predictability [2,18]; Figure 1A),
106 with a timing of fluctuation centered on average generation time. Phenotypically-plastic
107 generalists are expected to perform better under rapidly changing thermal conditions (*i.e.,* low
108 period), and to be favored by positively autocorrelated fluctuations (*i.e.,* predictable) compared
109 to less plastic genotypes. However, if phenotypic plasticity incurs time delays larger than the
110 rate of environmental changes, fluctuations might become detrimental for plastic generalists
111 [26]. Finally, we used a model to test whether effects of fluctuations on the performance of

specialists and generalists can result from a rate of plasticity. We incorporated the temporal dynamics of the plastic response into a simple model of tolerance to fluctuations and compared the predictions to the effects of fluctuations on performance found in the experiment.

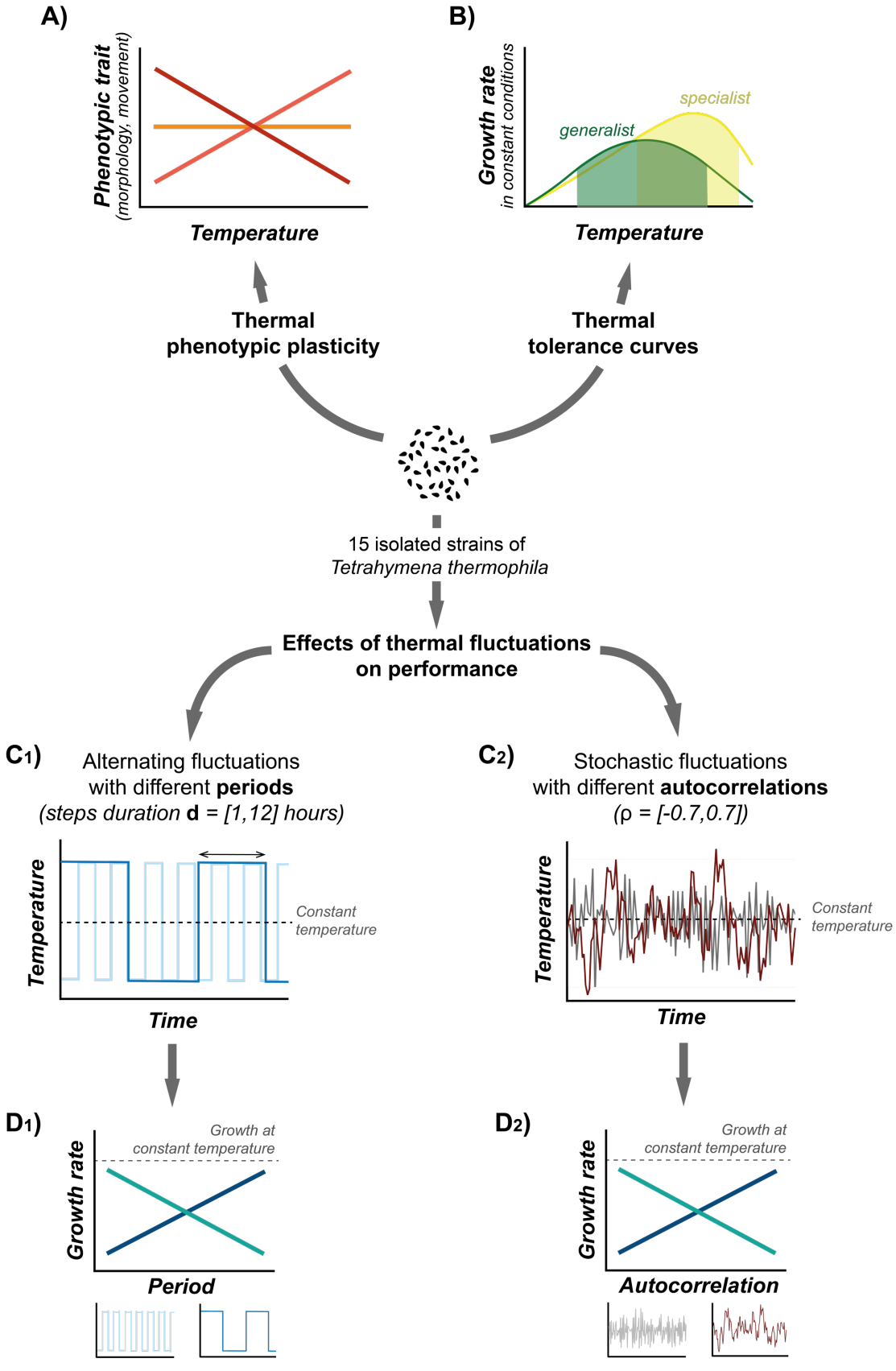
Methods

Study system

Tetrahymena thermophila is a 20 to 50µm ciliate naturally living in freshwater ponds and streams [35,36]. Previous studies provided evidence for differences between genotypes in thermal tolerance curves [30,31] and phenotypic plasticity of morphological and movements traits [30,31,37–39]. Moreover, thermal fluctuations are known in this species to affect population dynamics and the evolution of heat shock protein Hsp90 expression [40]. Here we used 15 strains originally sampled in the early 2000' from different locations in North America [41]. Isogenic strains reproduce clonally in laboratory conditions, meaning that for a given clonal strain, differences in trait values after two hours between replicated environmental conditions result from the expression of phenotypic plasticity [31,39,42]. Cells were maintained in axenic liquid growth media (0.6% Difco proteose peptone, 0.06% yeast extract) at 23°C, a classic laboratory maintenance condition for this species [43,44]. All manipulations were performed in sterile conditions under a laminar flow hood.

Figure 1: **Illustration of the key steps of the experimental design**. Using 15 isolated strains of *T. thermophila*, we quantified (A) morphological and movement plasticity following two hours of exposure to a gradient of thermal conditions to reconstruct thermal reaction norms for each trait and (B) tolerance curves across constant temperatures. Colors in A) and B) illustrate a diversity of possible forms of plasticity and tolerance curves expected based on previous studies [30,31] (see Figure S1). The same 15 isolated strains were separately exposed for two weeks to gradients of either period (C_1) or autocorrelation (C_2) of thermal fluctuations (period: from 1 to 12 hours; autocorrelation: from -0.7 to 0.7 with changes every 3 hours; average generation time across genotypes and

temperatures: 3 to 8 hours). During the two weeks of thermal fluctuations, population growth was measured to quantify the effects of fluctuation period (D_1) and autocorrelation (D_2) on population growth (*i.e.,* growth rate and maximal density).



142

143 ***Growth along gradients of fluctuation period and autocorrelation***

144 We quantified the influence of thermal fluctuations on the 15 isolated genotypes of *T.*
145 *thermophila* by inoculating a small number of cells (~100) from each isolated strain into 250
146 µL of growth media in 96-well plates, and exposing them for two weeks to treatments of
147 different fluctuation period and autocorrelation (Figure 1).

148 First, we quantified the role of fluctuation period by setting up a regime of alternating
149 temperatures: 19 and 31°C, corresponding to the margins of 80% of the area under a Gaussian
150 distribution representative of averaged thermal tolerance curve in this species [45]. We
151 generated a gradient of fluctuation period from 1 to 12 hours (1, 2, 3, 4, 6, 8, 10, 12h; the
152 average generation time of *T. thermophila* is ~3-8h in our experimental conditions [45] and
153 depends on temperature; Fig. S1C). We performed three replicates per strain and fluctuating
154 period. Second, we defined a gradient of fluctuation autocorrelation by generating time
155 sequences where temperature changed every 3h, was distributed following a Gaussian
156 distribution of mean 25°C, and was comprised between 11 and 39°C (considered as maximal
157 viable margins). We defined two negatively autocorrelated fluctuation regimes (-0.7 and -0.4),
158 one without temporal autocorrelation (autocorrelation = 0), and two positively autocorrelated
159 regimes (0.4 and 0.7) by generating 10,000 time-series for each autocorrelation value, and
160 selecting the series that best matched the requirements (mean, autocorrelation and variance of
161 temperature through time). To avoid time series where parameters may change through the
162 growth phase (*e.g.,* with lower mean value during earlier growth than at stationary phase [46]),
163 we performed the selection of best matches by computing desired parameters for each 24h time
164 window. As for the fluctuation period, we performed three replicates per strain and level of
165 autocorrelation.

166 We quantified population growth rate, a classic proxy of fitness, through absorbance
167 measurements at 450nm using a microplate reader (TECAN Infinite 200) twice a day until the
168 stationary phase was reached. Growth curves were smoothed using General Additive Modelling
169 (GAM; *gam* R-package [47]) to avoid any bias due to slight technical variability in absorbance
170 measurements. For each strain and each fluctuation treatment, we computed performance using
171 the *growth rate* measured as the maximum slope of population growth using the *gcfit* function
172 (*grofit* R-package [48]) with spline fit. We additionally quantified the *maximal population*
173 *density* reached at the plateau, which was highly correlated to growth rate (Pearson correlation:
174 0.893; df=583; t=47.91; p<0.001) and was therefore not included in the following analyses,
175 thus focusing on the exponential phase.
176
177 ***Thermal tolerance curves***
178 We additionally reconstructed thermal tolerance curves (Figure 1) by quantifying the growth
179 rate of each isolated strain across a gradient of eight constant temperatures (11, 15, 19, 23, 27,
180 31, 35, 39°C; Figure S1), as done previously (*e.g.,* [30,31]). We quantified population growth
181 through absorbance measurements as explained for growth under fluctuations: ~100 cells from
182 each genotype into 250 µL of 96-well plates. For this part, we performed four replicates in
183 different plates for each temperature, each being technically duplicated on each plate that were
184 latter on averaged for analyses. We fitted the relationship between temperature and growth rate
185 using GAMs, and computed the width of thermal tolerance curves as 90% of the area under the
186 curve (other cutoffs leading to similar results [30]). We furthermore quantified the thermal
187 optimum as the temperature corresponding to maximal growth rate, which did not significantly
188 correlate with thermal niche width (Pearson correlation coefficient = -0.241; t = -0.894, p =
189 0.388).
190

191 ***Thermal plasticity***

192 We quantified reaction norms of cell morphology and movement following [31], by exposing
193 five replicates of one-week old cultures (close to asymptotic density) from each strain for two
194 hours (less than the generation time) to five different temperatures: 11, 19, 25, 31 and 39°C
195 (Figure 1). Immediately after the two-hour exposure, we recorded 20s videos of two samples
196 of 10µl of cells placed in counting slides under dark-field microscopy to measure cell
197 morphology and movement characteristics using the BEMOVI R-package [49]. We described
198 cell morphology as cell *size* (measured as mean cell area in videos), a commonly measured trait
199 known to be linked to resource acquisition and metabolic rate in protists [32]. In standard
200 conditions, variability of cell size among strains in this species is not significantly correlated to
201 population growth rate [50,51]. Cell movement was measured as *velocity*, defined as the total
202 distance travelled by cells divided by the duration of the trajectory, a trait classically used to
203 describe movement in microorganisms [33,34]. Averaged cell size and velocity across all cells
204 of each experimental replicate (*i.e.,* two videos, see above) were then used to compute the
205 morphological and movement thermal plasticity of each strain as the slope of the reaction norm
206 of the scaled trait along temperature [31] (Figure S1). We summarized plasticity through linear
207 slopes since quadratic relationships were not significant (morphology: temperature^2 x strain:
208 $F_{14,171} = 1.192$; $p = 0.286$; temperature^2: $F_{1,171} = 0.018$; $p = 0.894$; movement: temperature^2 x
209 strain: $F_{14,171} = 1.380$; $p = 0.168$; temperature^2: $F_{1,171} = 0.003$; $p = 0.987$). Slopes close to zero
210 indicate flat reaction norms (and hence, no plasticity), while positive or negative values
211 respectively denote increase and decrease in traits along temperature (Figure S1).

212

213 ***Statistical analyses***

214 We first tested whether tolerance curve width correlated with the plasticity of morphological
215 and movement traits using linear regressions (*lm* function, with 1/standard error of the reaction

norm slope as weights). Then, we tested for the role of thermal tolerance width in response to thermal fluctuations using linear simple and mixed models, separately for periodic and autocorrelated fluctuations. First, we used the growth rate under fluctuations relative to constant temperature as dependent variable in models, allowing to quantify the effects of thermal fluctuations independently from differences of mean growth rate among genotypes using linear models. Second, we tested for effects of thermal tolerance width, thermal fluctuations (either period or autocorrelation) and their interaction on growth rate under fluctuations using linear mixed models with strain as a random factor. All analyses were performed using R (version 4.1.0; R Core Team 2021).

Model

We investigated how the rate of phenotypic plasticity affects growth in fluctuating environments by modifying the model of [19]. This model describes a population of N individuals experiencing a time-varying environment E, here temperature. Their phenotype P, the same for all individuals, varies as a deterministic function of the variation of the environment (*i.e.,* reversible plasticity).

The fitness landscape specifies the instantaneous growth rate $r(P,E)$ of the population with phenotype P at temperature E. Population growth is assumed density-independent:

$$\frac{dN}{dt} = r(P,E)N \qquad (1)$$

For a fixed temperature E, the growth rate is maximal at a specific phenotype $P = \varphi(E)$. Away from this optimal phenotype the growth rate decreases quadratically (see supplementary material).

We incorporated into this model a rate of plasticity, specifying the dynamical response of the phenotype to the thermal fluctuations:

$$\frac{dP}{dt} = -\frac{1}{\tau_P}(P - \psi(E)) \qquad (2)$$

where $\psi(E)$ is the target phenotype at a constant temperature E, and τ_P is the time-lag of the plasticity. The function $\psi(E)$ determines the reaction norm, and together with the fitness landscape $r(P,E)$ the thermal performance curve $r(\psi(E),E)$. In particular, for generalists the reaction norm $\psi(E)$ is close to the optimal phenotype $\varphi(E)$, leading to a wide thermal niche (see supplementary material).

For simplicity, we considered periodic thermal fluctuations with a sine wave:

$$E(t) = c_E + a_E \sin(2\pi\frac{t}{\tau_E}) \qquad (3)$$

where c_E is the mean temperature, a_E the amplitude and τ_E the period of the fluctuations. By combining equations (1-3) and averaging the instantaneous growth rate r over time, we obtained the long-term population growth rate R (see supplementary material):

$$R = c_R - a_R \frac{(1-a_\psi)^2+(2\pi\frac{\tau_P}{\tau_E})^2}{1+(2\pi\frac{\tau_P}{\tau_E})^2} a_E^2 \qquad (4)$$

where c_R is the growth rate in the constant environment c_E, and a_R is a positive constant independent of the degree of generalism a_ψ.

We used equation (4) to construct tolerance curves for generalists and specialists, characterized by a high and low degree of plasticity $a\psi$, respectively. This allowed us to investigate how the rate of plasticity affects the relationship between fluctuation period τ_E and growth rate R in generalists and specialists.

The model predicts an increase of R along τ_E both for generalists and specialists. Since the experimental results showed that the relationship between growth rate and fluctuation period can also decrease in specialists (Figure 2), we added to the model a mechanism that can generate such a pattern: we assumed that the transmission of the temperature to the individuals is buffered by a thermal inertia. The simplest model for thermal transmission with inertia is:

$$\frac{dE'}{dt} = -\frac{1}{\tau_I}(E' - E) \qquad (5)$$

where E is the requested temperature (e.g., the temperature set on the incubator), E' is the body

265 temperature of the individuals, and τ_I is the time-lag of the thermal transmission. The
266 transmitted fluctuations E' can differ substantially from the intended fluctuations E if τ_I is
267 comparable or larger than τ_E. For the sine-wave fluctuations considered above, the reduction in
268 amplitude from the requested E to the transmitted E' is given by:

269 $$\frac{a_{E'}}{a_E} = \frac{1}{\sqrt{1 + \left(2\pi\frac{\tau_I}{\tau_E}\right)^2}}$$

270 The long-term population growth rate becomes:

271 $$R = c_R - a_R \frac{(1-a_\psi)^2 + (2\pi\frac{\tau_P}{\tau_E})^2}{1 + (2\pi\frac{\tau_P}{\tau_E})^2} \frac{a_E^2}{1 + \left(2\pi\frac{\tau_I}{\tau_E}\right)^2} \quad (6)$$

272

273

274 # Results

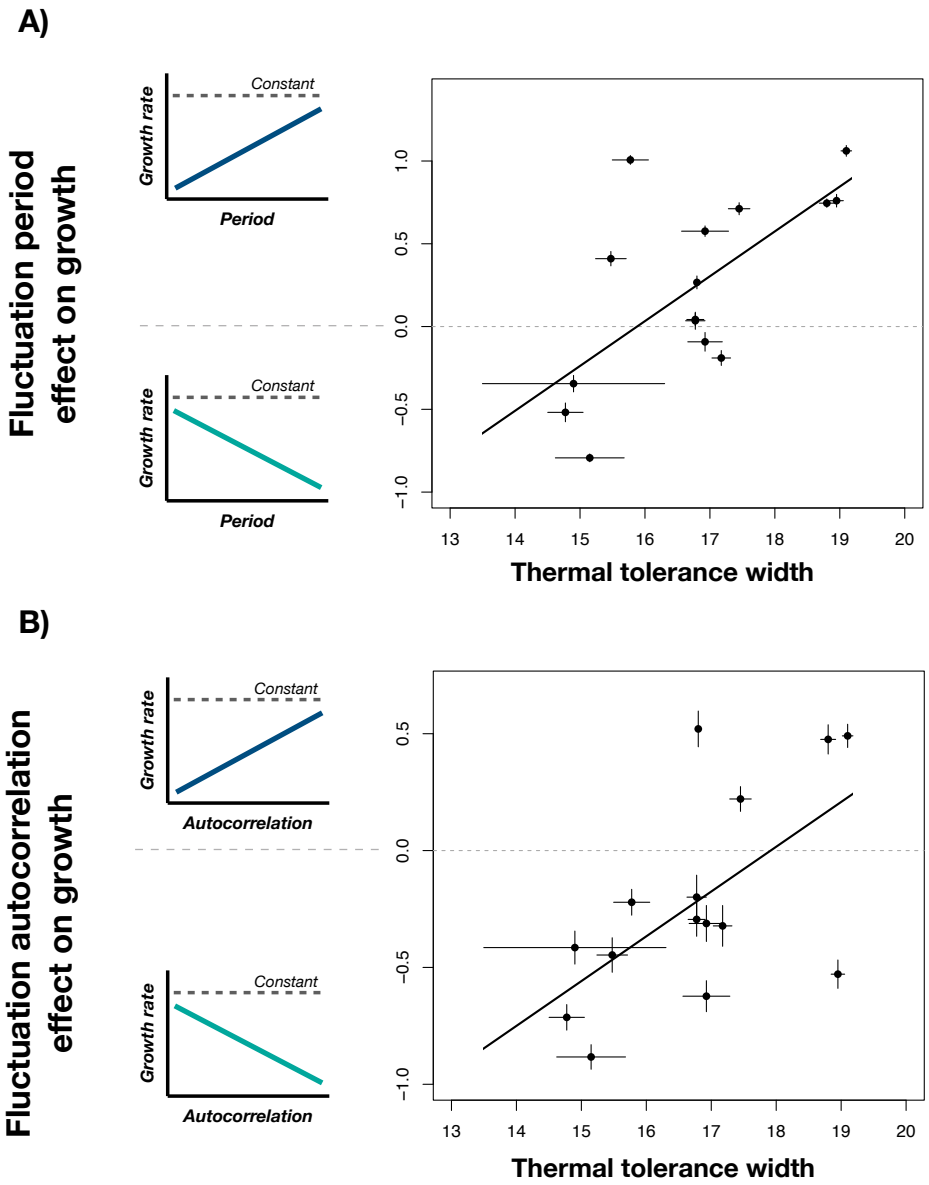
275 We first quantified the width of thermal tolerance curves and the plastic capacity of 15 strains
276 of the ciliate *T. thermophila* (Figure S1). We refer to strains with broader tolerance curves as
277 the most generalists, and to those with narrower thermal tolerance as the most specialists. The
278 level of thermal generalism of strains was positively correlated with cell size plasticity (estimate
279 ± SE = 0.170 ± 0.053; df = 1,13; t = 3.222; p = 0.007), but not with the plasticity of cell velocity
280 (-0.044 ± 0.063; df = 1,13; t = -0.688; p = 0.503). The most generalist strains showed higher
281 size plasticity with positive reaction norm slopes (*i.e.,* cells became larger with increasing
282 temperature), while most specialists strains appeared less plastic or even showed negative
283 slopes (Figure S2). Note that strains' plasticity did not significantly correlate with thermal
284 optimum (-0.090; t = -0.327, p = 0.749).

285 We then separately exposed the 15 strains to two independent gradients of period and
286 temporal autocorrelation of thermal fluctuations (Figure 1). Fluctuations impeded the growth
287 of all strains compared to constant conditions: when averaged across all period and

autocorrelation levels, growth rates were respectively reduced by 66.5 ± 1.6% (mean ± SE; period), and by 59.1 ± 2.5% (autocorrelation; Figure S3). This averaged sensitivity of strains to thermal fluctuations did not significantly correlate with their degree of generalism (periodic fluctuations: -0.004 ± 0.009; df = 1,358; t = -0.484; p = 0.629; autocorrelated fluctuations: -0.013 ± 0.014; df = 1,223; t = -0.952; p = 0.342).

However, the extent to which the period and autocorrelation of fluctuations impeded performance significantly depended on the degree of generalism (tolerance curve width * period: $F_{1,343} = 38.234$; $p < 0.001$; tolerance curve width * autocorrelation: $F_{1,208} = 9.912$; $p = 0.002$; Figure 2). Specifically, the effect of the fluctuation period on performance reversed along the degree of thermal specialization (Figure 2A). The most specialist strains (*i.e.,* with narrowest thermal tolerance) performed better under fast fluctuations compared to slower ones (*i.e.,* negative effect of fluctuation period on growth rate; Figure 2A). Conversely, the most generalist strains performed better under slow fluctuations (*i.e.,* positive effect of period on growth rate; Figure 2A). Similarly, the effect of autocorrelation depended on thermal tolerance width: the most specialist strains showed higher growth rates under negatively autocorrelated fluctuations compared to positively autocorrelated ones, while the most generalist ones performed better under positively autocorrelated fluctuations (Figure 2B). As expected from the correlation between thermal generalism and morphological plasticity, equivalent analyses using phenotypic plasticity of cell size instead of thermal tolerance width as explanatory variable gave similar results (fluctuation period * morphological plasticity: $F_{1,356} = 24.048$; $p < 0.001$; fluctuation autocorrelation * morphological plasticity: $F_{1,221} = 6.114$; $p = 0.014$). Finally, the interactions between fluctuation period or autocorrelation and thermal optimum had non-significant effects on growth rate (thermal optimum * period: $F_{1,343} = 0.074$; $p = 0.787$; thermal optimum * autocorrelation: $F_{1,208} = 2.070$; $p = 0.152$).

Figure 2: **Relationships between tolerance width and effects of fluctuation period (A) and autocorrelation (B) on performance (*i.e.*, growth rate)**. Each point in the main plots corresponds to a strain, with bars showing standard error. Values on the y-axis are the effect of fluctuation period or autocorrelation on growth, measured as the slope of the relationship between fluctuations and growth (either positive or negative, as illustrated by the schematic relationships on the left of the axes; see also Figure S3). They are computed as a *Z*-score effect size derived from the linear relationship between growth rate and the fluctuation gradients [52]: positive values indicate that strains are more affected by fast fluctuations compared to slow ones, or by negatively autocorrelated fluctuations compared to positively autocorrelated, while negative values show that strains are more affected by slow or positively autocorrelated fluctuations.



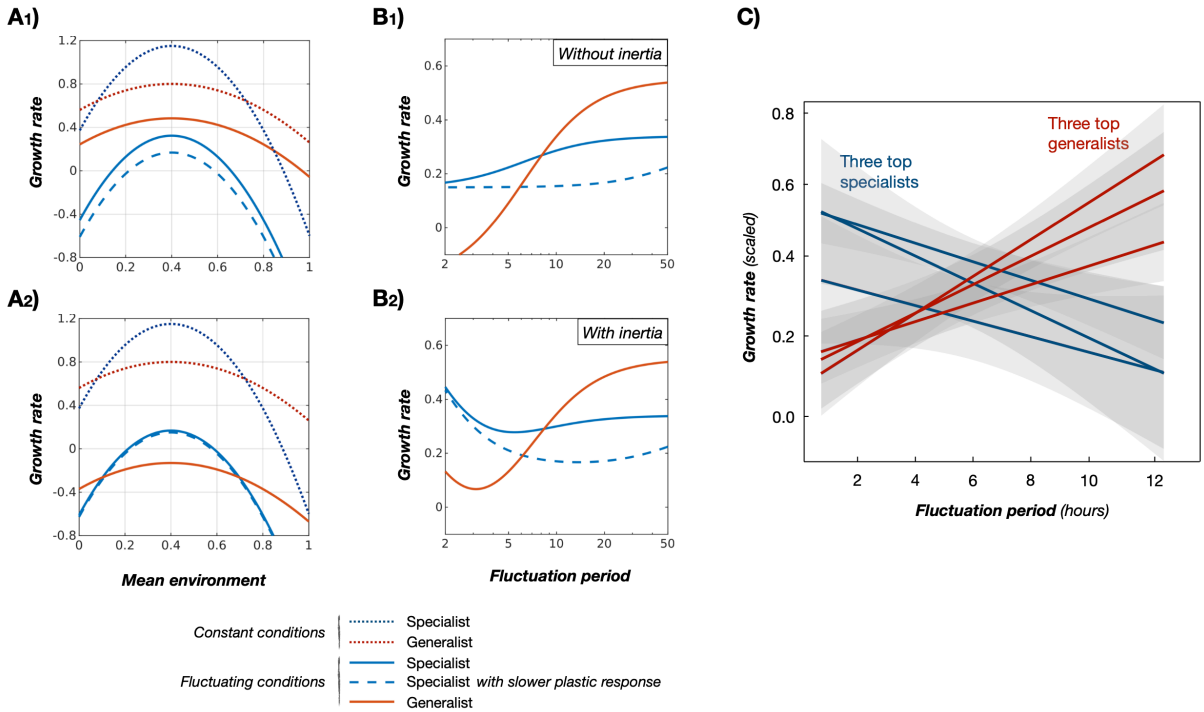
To explicitly test the potential role of the speed of plasticity in how organisms responded to environmental fluctuations, we used a simple model that included a rate in the adaptive plastic response underlying tolerance curves. We restricted the model to a simple gradient of fluctuation period, since period and autocorrelation of fluctuations led to similar effects in the experiment and gave similar results in the model (Figure 2). In the model, generalists have steeper reaction norms (*i.e.,* higher plasticity capacity) and therefore reached wider tolerance curves but with a reduced maximal performance compared to specialists (here due to a cost of plasticity; Figure $3A_1$). This leads to a classical specialist-generalist trade-off [53], already known in the experimental system we used in this study [30].

In slowly fluctuating environments, specialists were more affected by fluctuations compared to constant environments than generalists: because their tolerance curve is narrower, excursions from their optimal environment led to stronger fitness reductions (Figure $3A_1$). Without a rate of plasticity (*i.e.,* in a case of immediate plasticity), the fluctuation period had no effect at all on generalist growth rates (Figure 3). When the rapidity of environmental fluctuations increased, the rate of plasticity constrained the expression of adaptive plasticity to lesser degrees, leading generalists to suffer stronger fitness reduction compared to specialists (Figure $3A_2$). This trend reproduced what we observed in the experiment, where generalists suffered more from fast fluctuations than slower ones (Figure 3C).

It however did not reproduce the pattern of decreasing growth rates along increasing fluctuation period, as observed for specialists in the experiment. One simple mechanism that might generate such a pattern is environmental inertia, which would buffer to some degree the effects of rapid fluctuations on organisms. Implementing this simple mechanism in the model (see Methods) resulted in a negative relationship between growth rate and fluctuation period in specialists for fast fluctuations, while the positive relationship in generalists remained unchanged (Figure $3B_2$). Interestingly, this decrease in specialists was even steeper if we

considered that, in addition to their smaller degree of plasticity, specialists also had a slower plastic response than generalists (*i.e.,* slow rate of plasticity, as expected if plasticity rate and capacity correlate [26]; Figure 3B_2).

Figure 3: **The differential effects of environmental fluctuations on the performance of specialists and generalists depend on the underlying rate of phenotypic plasticity**. In the mathematical model, tolerance curves of specialists and generalists along the gradient of mean environments (*i.e.,* either constant temperatures or means of temporally fluctuating conditions) are modified by environmental fluctuations. **A_1)** while the maximal performance of specialists was higher than for generalists in a constant environment (respectively dotted blue and red lines), generalists reached higher maximal performance in environments fluctuating relatively slowly (high period: $\tau_E = 20$, solid red and blue curves); **A_2)** in rapidly fluctuating environments ($\tau_E = 2$), generalists suffered stronger fitness reduction due to not fast enough plastic response compared to specialists; **B_1)** In slowly fluctuating environments (i.e., long fluctuation period), specialists were more affected by fluctuations than generalists because deviations from their optimal environment lead to stronger fitness reductions. When fluctuations became faster (i.e., low period), phenotypic plasticity was not fast enough to follow environmental fluctuations, which is particularly detrimental for generalists. In a case of immediate plasticity (*i.e.,* infinite rate), there was no effect of the fluctuation period on growth rate. **B_2)** When environmental fluctuations are to some extent buffered as in the case of a thermal inertia, very fast fluctuations had small effects on fitness compared to constant conditions. When fluctuation period increased, generalists again showed increasing fitness as without inertia. However, specialists now showed decreasing fitness with increasing fluctuation period, as observed in the experiment (see panel C). This negative relationship was steeper if we assumed that specialists also showed slower plastic response than generalists (dashed blue line). **C)** Illustration of growth rate variations along fluctuation period in the three most specialist (red) and generalist (blue) genotypes (see Figure S3 for all strains). As expected with a lag time of phenotypic plasticity, generalists performed better under slow fluctuations compared to fast ones.



Discussion

Although fluctuating environmental conditions are a necessary condition for plastic generalists to evolve [7–11], some theoretical and empirical studies showed that generalists able to live in a wide range of relatively stable conditions might in contrast perform badly under fluctuations [1,6,54]. In this study, we experimentally showed that while thermal fluctuations always decreased performance relative to constant conditions, the magnitude and direction of their effects depended on the interaction between the width of tolerance curves and the characteristics of fluctuations. In particular, the most specialist strains performed better under fast or negatively autocorrelated fluctuations, while the most generalist strains performed better under slower or positively autocorrelated fluctuations. Using a mathematical model, we showed that a time delay in the expression of phenotypic plasticity can generate such effects of fluctuations on organisms' performance.

Strains with broader thermal tolerance curves showed higher morphological plasticity with positive reaction norm slopes (cells became larger with increasing temperature). On the contrary, the most specialist strains appeared less morphologically plastic, or became smaller with increasing temperatures. Our results thus provide correlative support for the hypothesis that the cell-size plasticity in response to temperature may underlie part of the ability to tolerate broad thermal conditions. Incidentally, the link between temperature sensitivity and body size is at the core of the metabolic theory in ecology [55]. Body size is also commonly related to demography and species interactions [55–57], including in protists [32,57,58]. However, whether cell size plasticity is adaptive, neutral or maladaptive in *T. thermophila* and other ciliates, and whether and how it might affect species interactions are still unsolved questions [31]. Answering them would especially require establishing causal relationships between cell size, thermal tolerance and how organisms perform under a diversity of environmental fluctuation scenarios.

If generalism is achieved through phenotypic plasticity as suggested in this study, the performance of generalists should depend on the characteristics of fluctuations, and especially their rapidity [1,6,54]. In the experiment, the most generalist strains suffered more from fast fluctuations than from slow ones, the reverse being true for specialists. This pattern might have resulted from the existence of a rate of plasticity [25,26]. Changes of performance metrics across a given environmental gradient (*i.e.,* tolerance curves) indeed often depend on acute plastic responses and acclimation mechanisms, either adaptive or not [1,59]. These plastic responses to changes in environmental conditions likely takes some amount of time [1,21–23,25–27]. This rate of phenotypic change may for instance depend on the rapidity of underlying mechanisms, such as transcriptional or hormonal changes, that precede variations in the phenotypic traits of interest [25,26]. Our model accordingly suggested that a time delay in the expression of phenotypic plasticity may restrict its benefits to slow-enough fluctuations.

An organism with a broad tolerance curve under a range of constant conditions might thus perform badly under too-rapid fluctuations if the underlying mechanisms involve significant time delays relative to the speed of environmental changes [6,13,25–27]. These results point out that considering the rate at which phenotypic plasticity takes place, together with the rate of environmental changes, is key to understand the conditions under which phenotypic plasticity is expected to be favored [25,26].

Interestingly, generalists performed better under positively autocorrelated fluctuations compared to negatively autocorrelated ones, and the reverse for specialists. These results therefore match with the general expectation that plasticity should be beneficial in predictable environments (*sensus* positively autocorrelated), as recently demonstrated experimentally [18]. Yet, positively autocorrelated fluctuations do not only translate into environmental predictability: they are also associated with a reduced degree of environmental change through time, which somehow leads to the perception of slower fluctuations than non-autocorrelated or negatively autocorrelated fluctuations. In our experiment, generalists performed better in both slow fluctuations and positively autocorrelated ones. Our results thus confirm that is that the predictability of environmental fluctuations is probably not an intrinsic property of the environmental fluctuations alone, but should rather be understood relative to the considered organisms, and especially to their rate of phenotypic plasticity [25,26].

To conclude, our study revealed that the effect of fluctuations on performance depended on the width of thermal tolerance curves: plastic generalists performing better under slow or positively autocorrelated fluctuations became poor performers under fast and negatively autocorrelated fluctuations. As reminded by our model, such dependence to fluctuations may result from the temporal dynamics of phenotypic plasticity. The speed of plasticity might thus play a major role in organisms' response to environmental fluctuations. A better understanding of the relationship between classical measures of generalism and the response of organisms to

environmental fluctuations would thus require investigating the temporal dynamics of plasticity [25,26]. Whether phenotypic plasticity and the associated width of tolerance curves are adaptive strategies to face environmental fluctuations [5,6,15] is likely to depend on the interplay between the characteristics of fluctuations and the speed of phenotypic plasticity [25,26]. Exploring further into the mechanisms that underlie tolerance curves and the timing of phenotypic plasticity is therefore of key importance to understand the response of organisms to the different types of environmental fluctuations they face.

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