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Environmental fluctuations restrict eco-evolutionary dynamics in predator–prey system

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Environmental fluctuations, species interactions and rapid evolution are all predicted to affect community structure and their temporal dynamics. Although the effects of the abiotic environment and prey evolution on ecological community dynamics have been studied separately, these factors can also have interactive effects. Here we used bacteria–ciliate microcosm experiments to test for eco-evolutionary dynamics in fluctuating environments. Specifically, we followed population dynamics and a prey defence trait over time when populations were exposed to regular changes of bottom-up or top-down stressors, or combinations of these. We found that the rate of evolution of a defence trait was significantly lower in fluctuating compared with stable environments, and that the defence trait evolved to lower levels when two environmental stressors changed recurrently. The latter suggests that top-down and bottom-up changes can have additive effects constraining evolutionary response within populations. The differences in evolutionary trajectories are explained by fluctuations in population sizes of the prey and the predator, which continuously alter the supply of mutations in the prey and strength of selection through predation. Thus, it may be necessary to adopt an eco-evolutionary perspective on studies concerning the evolution of traits mediating species interactions.

Subject Areas:
ecology, evolution

Keywords:
community dynamics, coevolution, eco-evolutionary dynamics, temporal fluctuations, *Tetrahymena thermophila*, *Pseudomonas fluorescens* SBW25

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1. Introduction

Understanding the rate and the direction of evolutionary change is a central aim in evolutionary biology. Changes in abiotic stressors have been shown to alter adaptive dynamics, and the strength of the stressor, the duration of the stress and its frequency determine the direction and rate of evolutionary change [1,2]. As populations are embedded into communities and food webs with different numbers and types of interactions, evolutionary change within one population as a response to an abiotic stressor might directly or indirectly alter the strength of species interaction [3,4]. Typically, harsh environmental conditions reduce population sizes and consequently encounter rates of interacting species, and ultimately weaken selection that results from species interaction [3]. Thus, the ecological and evolutionary dynamics of interacting species are intertwined, and predictions on whether and how an abiotic change might affect evolutionary trajectories require an eco-evolutionary perspective.

Most experiments studying evolutionary dynamics in consumer–resource systems (i.e. predator–prey or host–parasite) were conducted in a constant environment (e.g. [5–12]). However, natural environments are frequently unstable, and conditions might fluctuate over time [1,3,13]. As a consequence, organisms within a population experience conditions that are favourable and unfavourable at times, while the exact same conditions might have no direct effect, an opposite effect or only a weak effect on an interacting species. In general, fluctuating environments are suggested to lower population sizes and thus constrain evolutionary dynamics when only mutations are considered. On the other hand, with small population sizes, the role of drift might be more important.

Table 1. Environmental fluctuations and manipulations used on our experiment.

fluctuation type	resources $t_1 \rightarrow t_2 \rightarrow t_3 \cdots$	salinity $t_1 \rightarrow t_2 \rightarrow t_3 \cdots$
stable	5% → 5% → 5%	2.5 g l^{-1} → 2.5 g l^{-1} → 2.5 g l^{-1}
bottom-up	10% → 0.5% → 10%	0 g l^{-1} → 0 g l^{-1} → 0 g l^{-1}
top-down	10% → 10% → 10%	0 g l^{-1} → 5 g l^{-1} → 0 g l^{-1}
synchronous	0.5% → 10% → 0.5%	5 g l^{-1} → 0 g l^{-1} → 5 g l^{-1}
asynchronous	10% → 0.5% → 10%	5 g l^{-1} → 0 g l^{-1} → 5 g l^{-1}

Indeed, fluctuations in nutrient levels in consumer–resource systems showed constrained coevolutionary dynamics depending on the frequency of changes in the nutrient level [14–17]. What is important in these observations is that the fluctuating environment had a direct effect on species only at the lower trophic level (the host or prey). The consumers (phage or protozoan predator) were only indirectly affected through the supply of hosts or prey, but they played a significant role in the coevolutionary dynamics through changes in interaction strengths and the potential for selective sweeps [14]. Similarly, abiotic environmental changes might only affect species at the higher trophic level while indirectly affecting evolutionary dynamics at lower trophic levels. Thus far, the ecological roles of top-down (affecting the higher trophic level, typically the consumer) or bottom-up (affecting the lower trophic level, typically the resource) factors have been studied extensively (reviewed in Osenberg & Mittelbach [18]). However, different roles of fluctuating top-down and bottom factors in evolutionary dynamics have not been explored yet. In particular, we currently lack an understanding on whether and how top-down and bottom-up fluctuations occurring at the same time alter evolutionary dynamics.

Here we investigate the evolution of a prey defence trait within a microbial predator–prey system and the potential of the predator to counter-adapt in constant as well as fluctuating environments. Previous work with this experimental system demonstrated rapid evolution of a defence against predation in constant, favourable environments [9,19,20]. In these studies bacteria typically evolved to grow in aggregates, which increase handling time and/or decrease ingestion rates of ciliates. In fluctuating nutrient environments (bottom-up), we predict evolutionary constraints for the rate of adaptation as prey and predator population sizes will be small at times. As the evolutionary change in prey populations is determined by population sizes—either through supply of mutations or selection strength arising from predator–prey interactions, prey defence is predicted to evolve at a slower rate. Thus, when the environment of predators changes recurrently between favourable and unfavourable conditions, the rate of adaptation of the prey is predicted to be slower compared with environments where populations experience an intermediate but constant environment. When top-down and bottom-up factors change at the same time and in the same direction (i.e. favourable and unfavourable conditions for prey and predator coincide in time), population sizes of both might even become further reduced compared with environments with only one environmental parameter fluctuating, resulting in weaker selection (hereafter, synchronous changes). When the two environmental parameters alternating asynchronously (i.e. favourable condition for prey and unfavourable condition for predators) coincide in time and vice versa, prey can be abundant, but due to adverse physical conditions predators are unable consumer the prey efficiently. This can again lead to weaker selection on prey defence traits as a result of lowered predator pressure. We therefore hypothesize (i) that environments with both top-down and bottom-up fluctuations constrain the evolution of a defence in the prey compared with environments with only one of the two stressors fluctuating, and (ii) that this effect is greater when top-down and bottom-up fluctuations are asynchronized.

To test for the effect of environmental fluctuations, we conducted a series of microcosm experiments with a well-established microbial system [9,19,20], consisting of the bacterial prey *Pseudomonas fluorescens* and the ciliated predator *Tetrahymena thermophila*. Environments were manipulated by changing the concentrations of resources available to the prey (bottom-up; one change), salinity affecting the predator (top-down; one change) or top-down and bottom-up in a synchronous or asynchronous way (two changes; see also table 1). We transferred bacteria and ciliate every third day (2.5%) to a new microcosm with the respective medium, and followed up predator and prey densities (ecological dynamics) and the defence trait D of the prey over time (evolutionary dynamics). To detect and contrast evolutionary constraints across the different treatments, we studied both the rate of evolutionary changes in the prey's defence trait D and the maximum D reached.

2. Material and methods

(a) Model system

The bacterium *P. fluorescens* SBW25 [21] and the ciliated protozoan *T. thermophila* 1630/1U (CCAP) were used in the experiments. Prior to the experiments, ciliate stocks were cultured axenically in PPY medium containing 20 g of proteose peptone and 2.5 g of yeast extract in 1 l of deionized water. All treatments began with a single smooth colony of *Pseudomonas* from a frozen stock (to achieve minimal initial genetic variability in the prey population).

(b) Manipulating temporal changes in the environment

Experiments were conducted in standard 25 ml glass vials (a microcosm type previously used in [8,9,22–25]) with medium containing M9 salts and King's B nutrients, depending on the environment. For environments with bottom-up fluctuations, we alternated between two concentrations of King's B nutrients, 0.5% and 10%, between transfers (0.5% King's B: 0.1 g l^{-1} Peptone number 3 and 0.05 ml l^{-1} glycerol; 10% King's B: 2 g l^{-1} Peptone number 3 and 1 ml l^{-1} glycerol). For environments with top-down changes, we added NaCl in addition to King's B and alternated between 0 g l^{-1} and 5 g l^{-1} of NaCl between each transfer with a resource concentration of 10% King's B in each transfer. Prior to the experiment, we experimentally verified that 5 g NaCl l^{-1}

salinity had no adverse effects on *Pseudomonas* growth (prey daily growth rate in 0 g l^{-1} salinity (mean ± s.e.): 2.20 ± 0.04 and in 5 g l^{-1} salinity: 2.21 ± 0.03; ANOVA: $F = 0.04$, d.f. = 1, $p = 0.849$) while reducing *Tetrahymena* growth (predator daily growth rate in 0 g l^{-1} salinity: 4.04 ± 0.07 and in 5 g l^{-1} salinity: 2.85 ± 0.255; ANOVA: $F = 20.2$, d.f. = 1, $p = 0.002$). Finally, we performed a control treatment in a constant environment with an average resource concentration and salinity (5% King's B supplemented with 2.5 g l^{-1} of NaCl) over time (see also table 1). All treatments were replicated three times in 25 ml glass vials containing 6 ml of the corresponding King's B medium. Every 72 h, 2.5% of each culture was transferred to a new vial containing fresh culture medium. Microcosms were kept at 28°C (±0.1°C) and shaken constantly (50 r.p.m.). The duration of the experiment was 42 days, representing approximately 240 prey and 130 predator generations.

(c) Predator–prey dynamics

During each transfer, a 0.5 ml subsample from each vial was frozen with 0.5 ml of 80% glycerol and kept at −80°C for later analysis (ciliates do not survive freezing under these conditions). *Pseudomonas* numbers were estimated using optical density (OD) at 600 nm (UV-1800 spectrophotometer, Shimadzu, Japan; for details about converting OD values to *Pseudomonas* cell densities see [9]). *Tetrahymena* cell densities were enumerated directly from live 2.5 µl subsamples using a compound microscope (Zeiss Axioskop 2 plus, Oberkochen, Germany).

(d) Evolutionary changes in prey populations

Evolution of the prey defence trait against predator grazing was quantified with a simple, ecologically appropriate bioassay as described in detail by Hiltunen & Becks [9]. Briefly, after thawing cryopreserved *Pseudomonas* samples, we grew bacteria in liquid cultures (5% King's B without salt) for 24 h, corresponding to approximately 10 bacterial generations, so that phenotypic differences result from evolutionary change rather than an induced defence mechanism. We then added 2100 ciliates from the stock to each overnight bacterial culture; that is, we used naive predators as a standard for consumer feeding on genetically differentiated prey. Predator numbers were counted after 48 h, and differences in predator densities compared with predators grown on naive prey were taken as an estimate for the prey defence level *D*. Prey defence trait values were calculated as relative fitness by $D = 1 - \text{prey}_{evo}/\text{prey}_{anc}$, where prey_{evo} is predator density after feeding on evolved prey, and prey_{anc} is predator density after feeding on ancestral prey.

(e) Adaptation to salt and coevolutionary change in predator population

When testing for potential evolutionary adaptation to elevated salt concentrations and coevolutionary response in predator populations, we used the same method as described above, with the exception that the evolutionary history of the predators was not uniform; that is, we used naive ciliates from the stock and coevolved predators isolated on day 42 of the experiment. Coevolved predator lines were isolated from the control, the synchronous and the asynchronous treatments. Evolutionary change in predators was quantified as maximum daily growth rate when grown on naive and coevolved prey types (isolated on day 42 of the experiment) for 48 h in 0 g l^{-1} and 5 g l^{-1} salinities (full factorial design for salinity, prey evolution and predator evolution). Before these measurements, predators were cultured without the prey in axenic, non-saline PPY culture medium for one week, corresponding to several predator generations.

(f) Data analysis

Statistical analyses were performed with R, using the lme4 package [26], and PASW statistics (SPSS Inc., Chicago, IL, v. 20.0) software. Differences across environments—zero (control), one (bottom-up and top-down) or two fluctuating environments (synchronous and asynchronous) over time—were determined by ANOVA, and differences in one and two fluctuating environments were determined by Tukey *post hoc* tests. For correlations between environmental changes and prey or predator densities, we used liner mixed models (LMM) with the environmental condition (high or low resources, high or low salinity) as the fixed effect and transfer within replicate as the random effect. To evaluate the significance of the correlations, we compared models with and without the fixed effect.

3. Results

(a) Population dynamics

We found that environmental fluctuations had distinct effects on both predator and prey, independent of whether the fluctuation was top-down or bottom-up (figure 1*a–e*). With only resource fluctuations (bottom-up), we found significant correlations between environmental fluctuations and prey and predator population sizes, with predator and prey having higher densities at high nutrient levels (figure 1*b*; LMM: predator $\chi^2 = 23.02$, d.f. = 1, $p = 1.6 \times 10^{-6}$; prey $\chi^2 = 30.05$, d.f. = 1, $p = 4.28 \times 10^{-8}$). Predator and prey population sizes also changed at different salinity fluctuations (top-down), but the changes were less clear (figure 1*c*; LMM: predator $\chi^2 = 6.64$, d.f. = 1, $p = 0.01$; prey $\chi^2 = 3.5$, d.f. = 1, $p = 0.061$) and occurred in the opposite direction for prey and predator. The different effects of top-down and bottom-up manipulation indicate that the communities here were mainly driven by bottom-up fluctuations. For changes in both nutrient levels and salinity (two environmental changes), we again found correlations between the level of environmental stress and population sizes. For both treatments, synchronous and asynchronous, the predator density was negatively correlated with salt concentrations (synchronous: LMM $\chi^2 = 15.17$, d.f. = 1, $p = 9.85 \times 10^{-5}$; asynchronous: LMM $\chi^2 = 7.59$, d.f. = 1, $p = 0.006$) and positively correlated with nutrients (synchronous: LMM $\chi^2 = 15.12$, d.f. = 1, $p = 9.85 \times 10^{-5}$; asynchronous: LMM $\chi^2 = 7.59$, d.f. = 1, $p = 0.0059$). Prey population densities were significantly higher in low-salt environments with synchronous changes (LMM $\chi^2 = 21.52$, d.f. = 1, $p = 3.49 \times 10^{-6}$), and an opposite effect was found for asynchronous changes (LMM $\chi^2 = 14.78$, d.f. = 1, $p = 0.0001$). Prey densities were positively correlated with high nutrients in both environments (synchronous: LMM $\chi^2 = 21.52$, d.f. = 1, $p = 3.49 \times 10^{-6}$; asynchronous: LMM $\chi^2 = 14.78$, d.f. = 1, $p = 0.0001$). Predator and prey population sizes were more unstable in environments with two changes compared with the environments with no or one change (figure 2; ANOVA of coefficient of variation for the comparison no, one and two changes: ciliate: $F = 11.76$, d.f. = 2, $p = 0.0015$, bacteria: $F = 24.84$, d.f. = 2, $p = 5.4 \times 10^{-5}$; Tukey *post hoc* test: comparison between zero and two changes for ciliates, $p = 0.01$; one and two changes, $p = 0.002$; for bacteria: zero and two changes $p = 0.00012$; one and two changes, $p = 0.0003$). Furthermore, within the environments with one fluctuating stressor, we found that predator and prey were more stable

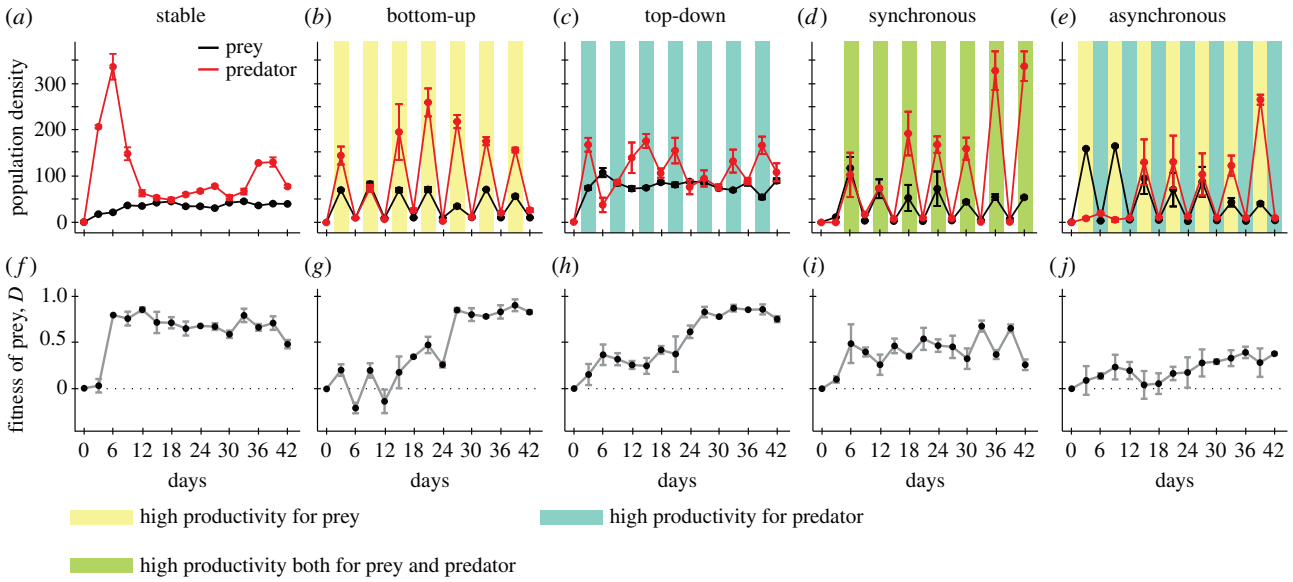

Figure 1. Eco-evolutionary community dynamics and prey fitness (defence trait *D*) among different fluctuation scenarios (table 1). Here, high productivity for prey refers to the increase in resources, whereas high productivity for predator refers to the decrease in salinity. (*a–e*) Population densities (black lines: prey *P. fluorescens* as 10^6 cells ml^{-1}; red lines: predator *T. thermophila* as 10^4 cells ml^{-1}). (*f–j*) Evolution of the prey defence trait *D* (mean ± s.e., $n = 3$).

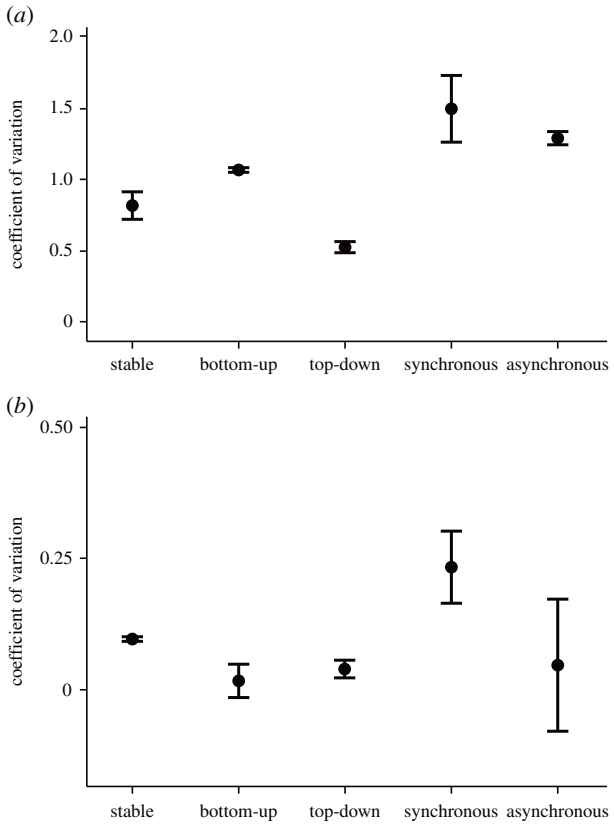

Figure 2. Stability of predator and prey population. (*a*) Coefficient of variation (mean ± s.d.) of the ciliate populations. (*b*) Coefficient of variation (mean ± s.d.) of the bacteria populations. For statistical analysis, see main text.

in the top-down treatment (compared with the bottom-up environments: ANOVA ciliate $F = 492.3$, d.f. $= 1$, $p = 2.44 \times 10^{-5}$; bacteria: $F = 703.4$, d.f. $= 1$, $p = 1.2 \times 10^{-5}$), whereas there was no difference between environments with synchronous and asynchronous changes (two changes).

(b) Prey evolution

When testing for the effects of one or two environmental changes on the evolution of the prey defence trait, we first compared the maximum trait and the time until the maximum was reached among the control (no change), one environmental change and two environmental changes. The maximum defence trait differed significantly between environments (ANOVA: $F = 11.7$, d.f. $= 2$, $p = 0.002$), having significantly lowest levels in the environments with two changes (Tukey *post hoc* test: comparison between zero and two changes, $p = 0.012$; one and two changes, $p = 0.002$) and similar levels in environments with zero and one change. Similarly, the number of environmental changes affected the speed, that is, the number of days before the maximum was observed (ANOVA: $F = 8.605$, d.f. $= 2$, $p = 0.005$). The maximum was reached at the significantly fastest rate in the environment with zero change (comparison between zero and one changes, $p = 0.004$; comparison between zero and two changes, $p = 0.03$), and there was no difference between environments with two changes and one change. In the environments with two changes, we found that the defence level with asynchronous changes evolved to lower levels compared with synchronous changes (ANOVA: $F = 11.44$, d.f. $= 1$, $p = 0.03$), whereas there was no difference in the level of defence between top-down and bottom-up in treatments with one environmental change.

(c) Eco-evolutionary link

To further disentangle the effects of different environmental changes, we looked at differences in population sizes and dynamics before the maximum trait (highest *D* value) was reached, as there was no or very little change in the prey's defence trait. Average predator density (ANOVA: $F = 25.94$, d.f. $= 2$, $p = 4.39 \times 10^{-5}$) and predator–prey ratios (ANOVA: $F = 28.05$, d.f. $= 2$, $p = 2.99 \times 10^{-5}$) differed significantly between the treatments (zero, one or two changes in the environment) before the maximum trait was reached,

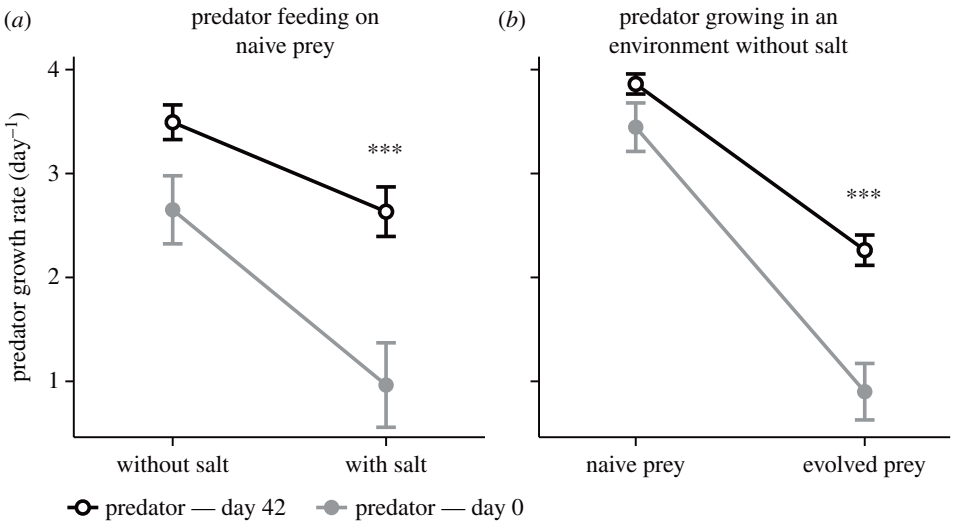

Figure 3. Evolved salinity adaptation and coevolutionary change in predator population after 42 days. (*a*) Evolution in salt tolerance: effects of the evolutionary history of predator (ciliates from day 0 and day 42) on predator growth rate when feeding on ancestral naive prey in without- and with-salt environments. (*b*) Coevolutionary change: effects of the evolutionary history of predator (ciliates from day 0 and day 42) on predator growth rate when feeding on ancestral naive prey and evolved prey isolated at the end of the experiment, both measured in non-saline environments.

which was also reflected in the predator–prey dynamics (figures 1 and 2). When comparing top-down and bottom-up fluctuations, prey density and predator–prey ratios were significantly lower in the bottom-up environment (ANOVA for prey: $F = 863.8$, d.f. $= 1$, $p = 7.98 \times 10^{-6}$; ANOVA for ratio: $F = 15.81$, d.f. $= 1$, $p = 0.0165$). Nevertheless, we did not find differences between synchronous and asynchronous environments when comparing mean population sizes (ANOVA: ciliate: $F = 0.145$, d.f. $= 1$, $p = 0.722$; bacteria: $F = 3.23$, d.f. $= 1$, $p = 0.147$) and predator–prey ratios (ANOVA: ciliate: $F = 0.223$, d.f. $= 1$, $p = 0.661$), as well as amplitudes before the prey defence trait reached its maximum (ANOVA: ciliate: $F = 4.618$, d.f. $= 1$, $p = 0.0981$; bacteria: $F = 2.131$, d.f. $= 1$, $p = 0.218$). Predator–prey ratios were significantly highest at the beginning of the experiment in the control treatment (Tukey *post hoc* test: comparison between zero and two changes, $p = 0.00007$; zero and one change, $p = 0.00004$), applying high selection on the prey population. In fluctuating environments, independent of one or two changes, predator–prey ratios and hence selection was significantly lower at the beginning (Tukey *post hoc* test: comparison between one and two changes, $p = 0.84$). Another important observation is that the predator populations remained at very low densities until transfer 5 (day 12) in the asynchronous environment, leading to the highest overall prey densities at high nutrient conditions; that is, the prey was only controlled by nutrients.

(d) Evolutionary and coevolutionary changes in the predator

The fluctuation treatments had no effect on the predator evolution (daily growth rate (mean ± s.e.) in stable conditions: 3.04 ± 0.31; asynchronous: 3.06 ± 0.26; synchronous: 3.10 ± 0.28; ANOVA for environmental treatments: $F = 0.007$, d.f. $= 2$, $p = 0.993$). After pooling data across fluctuation treatments, we found that predators isolated at the end of the experiment had evolved to tolerate high salinity better compared with naive stock predators (evolutionary change in salt tolerance; figure 3*a*; ANOVA: $F = 9.61$, d.f. $= 1$, $p = 0.005$). In addition, predators had coevolved to partly overcome prey defences (coevolution; figure 3*b*; ANOVA: $F = 11.4$, d.f. $= 1$, $p = 0.002$). We did not find any costs associated with these adaptations since the growth rates of naive stock and evolved predators did not differ when cultured in a non-saline environment with naive prey (figure 3; ANOVA: $F = 0.65$, d.f. $= 1$, $p = 0.44$).

4. Discussion

In this study, we tested for differences in adaptive evolutionary change of prey and predator populations with fluctuations of one or two stressors over time. We found that in environments with two fluctuating stressors, the evolution of an anti-predatory defence trait was slower compared with environments without fluctuations, and that the trait also reached lowest defence levels in comparison with environments with one stressor or no stressors fluctuating over time. Thus, our experiments confirmed the hypothesis that fluctuating environments constrain evolution and that this was even more so in environments with two stressors. Interestingly, evolutionary constraints were expressed here as lower rates of adaptation as well as the maximum defence level reached. Overall, our results suggest that the relative role of evolution on eco-evolutionary community dynamics might be smaller in more complex environments.

There are at least two important parameters that explain the observed rates and levels of evolution in the populations. First, the population sizes of prey differed significantly over time. In bottom-up, synchronous and asynchronous environments, prey densities altered recurrently between very small and large densities. Previous studies suggest that evolutionary adaptation is constrained in fluctuating environments because of a limited supply of novel mutations and the possibility of novel mutations to sweep to higher frequencies when populations are at low densities [14–17]. In our experiment, low nutrients reduced prey population sizes, restraining mutations (see also [27]). In addition, time periods of high densities were relatively short, constraining the possibility of sweeps (i.e. the time period was too short for a genotype

to sweep to fixation) and slowing down the evolution of the prey defence trait. Second, selection by the predator differed over time as a result of either top-down manipulation or lack of sufficient prey. Average predator densities were similar in all treatments, but in bottom-up, synchronous and asynchronous environments, predator densities alternated between very low and very high densities, creating temporal differences in strength of selection across treatments. In the stable environment, very high predator densities and predator–prey ratios at the beginning resulted in strong selection and very rapid evolution of a defence. In the asynchronous environments, ciliates did not grow to high densities during the initial four transfers, resulting in weak or no selection. Thus, the combination and timing of low prey densities (supply of adaptive mutations) and high predator densities (selection) seem to have been decisive for the evolution of the prey defence trait. We recognize that our initial experimental setting (i.e. starting with low/high salinity or resource levels) may have played a role in the evolution of the defence trait. However, we observed prey adaptation only after approximately 6 days, during which time there had been few environmental cycles, suggesting that the initial setting did not play a significant role on overall quantitative results that were the focus of our analyses.

Prey populations in our experiments were exposed to two different stressors: fluctuations in nutrient levels and predation pressure. Generally, adaptive evolution is slowed down when populations evolve in complex environments because genes for environment-specific effects (here, low or high resources, or high grazing rates) might have a smaller chance to become fixed [1]. Indeed, we observed slower adaptation in prey populations subjected to fluctuating environments compared with a constant environment. The observation that this occurred in all fluctuating environments, even though predator and prey population sizes were relatively constant in the top-down environment, suggests a role for complex environments in addition to the aforementioned changes in predator and prey densities. Similarly, predators were exposed to two environmental changes: evolving prey and salinity stress. We tested for salt adaptation of the ciliates at the end of the experiments, and found that they had evolutionarily adapted to high salt concentrations. The temporal dynamics of predator populations suggest that adaptation played an important role in eco-evolutionary dynamics. Especially in the asynchronous environment (figure 1*e*), the predator remained at a low density until day 12 even though the prey was very abundant during that time. As the initial adaptation to the salinity was relative rapid (figure 1*e*; very low predator densities for the first 12 days followed by a rapid increase similar to all other treatments) compared with other observations of *Tetrahymena* evolution with the same system [9,19], it is likely that an initial increase in salt tolerance (phenotypic plasticity) was later followed by an evolutionary adaptation. Generally, adaptation in the ciliate is predicted to be slower than in the bacteria as the ciliates have smaller population sizes and a longer generation time, and thus a lower supply rate of mutations.

Similar adaptation to a thermally fluctuating environment with the same protozoan species was found by Ketola *et al*. [28]. In comparison with other studies with similar predator–prey systems (e.g. [15–17]), we found comparable results in that a fluctuating environment constrains the evolution of anti-predator defences. However, an important difference between previous studies and the study presented here is that we manipulated both top-down and bottom-up stressors. Surprisingly, we found that changes in the bottom-up stressor were directly reflected in the population dynamics, whereas the top-down effects had little effect on the overall dynamics, suggesting that the stabilizing effect of evolutionary adaptation to the stressor seems to be only possible with fluctuations affecting the higher trophic level. Our results suggest that the co-occurrence of bottom-up and top-down fluctuations has important additive effects on the ecological and evolutionary dynamics of communities.

Our study underlines the important role of ecology in terms of the rate and the direction of adaptive evolution. Here environmental fluctuations altered prey and predator population densities in various ways, and depending on whether and how prey and predator densities changed, the evolution of the prey defence was limited by the supply of mutations and selection. The timing of strong selection and emergence of genetic variation might therefore be crucial factors, and are ultimately driven by eco-evolutionary dynamics. Finally, the slower (or even completely lacking) evolutionary adaptation owing to environmental stressors can alter the relative importance of ecological and evolutionary factors on community dynamics, and thus eco-evolutionary dynamics.

Data accessibility. The data used for this study are available in Dryad: doi:10.5061/dryad.vm6dh

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Authors' contributions. Conceived and designed experiment: T.H. and L.B. Preformed the experiment: T.H. and G.B.A. Analysed the data: L.B., G.B.A. and T.H. Wrote the paper: L.B., T.H. and G.B.A.

Competing interests. The authors declare no competing financial interest.

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