Temperature and nutrients drive complex eco-phenotypic dynamics in a microbial food web

Ze-Yi Han¹, Daniel J. Wieczynski¹, Andrea Yammine¹, Jean P. Gibert¹

¹Department of Biology, Duke University, Durham, North Carolina, USA

Statement of authorship: ZH and JPG conceived the study; ZH and JPG designed the experimental work. ZH collected the data with support from AY. ZH analyzed the data with support from DJW and JPG; ZH wrote the first version of the manuscript; all authors contributed substantially to subsequent versions.

Data accessibility statement: All annotated code and data is available at our dedicated repository (https://github.com/ZeYiHan/Nut_Temp_Pheno_Eco).

Running title: Consequences of thermal asymmetries

Keywords: Global warming, Food-web temperature responses, Eutrophication, Anthropogenic impacts, Communities, Trait-based Ecology

Type: Letter

Number of words: abstract (150 words), main text (3983 words), boxes (0 words)

Number of figures: 5

Number of references: 48

Number of tables: 0

Number of boxes: 0

To whom correspondence should be addressed: Ze-Yi Han, Department of Biology, Duke University, Durham, NC, USA, 402-405-3888, zeyihanw@gmail.com

ABSTRACT

Anthropogenic increases in temperature and nutrient loads will likely impact food web structure and stability. Though their independent effects have been well studied, their joint effects—particularly on coupled ecological and phenotypic dynamics—remain poorly understood. Here we experimentally manipulated temperature and nutrient levels in microbial food webs and used time-series analysis to quantify the strength and causality of reciprocal effects between ecological and phenotypic dynamics across species. We found that i) temperature and nutrients have more complex effects on ecological dynamics at higher trophic levels, ii) temperature and nutrients interact antagonistically to shift the relative strength of top-down vs. bottom-up control, iii) phenotypic dynamics have stronger impacts on ecological dynamics than vice versa (especially at higher temperature), and iv) rapid phenotypic change mediates observed ecological responses to changes in temperature and nutrients. Our results expose how feedbacks between ecological and phenotypic dynamics mediate food web responses to environmental change.

INTRODUCTION

Understanding how rapid global climate change (GCC) will affect the structure and dynamics of communities is a pressing goal of ecology (Karl 2003). Increasing temperatures associated with GCC influence the metabolism of individuals (Clarke 2006), which strengthens species interactions (Barton *et al.* 2009; O'Connor 2009; Gounand *et al.* 2016), alters community structure (Bartley *et al.* 2019; Gibert 2019; Gauzens *et al.* 2020), and ecosystem function (Dossena *et al.* 2012). Additionally, increasing nutrient loads from agricultural runoffs can result in eutrophication and destabilize natural communities (Rosenzweig 1971; Fussmann 2000; Hautier *et al.* 2014), often leading to species loss (Hautier *et al.* 2009). Warming and eutrophication may therefore independently and jointly impact food web structure and stability (Malcolm *et al.* 2006; Tabi *et al.* 2019). Counterintuitively, however, simultaneous increases in temperature and nutrient load can produce outcomes that are qualitatively different from the combined negative effects of each variable on its own (Binzer *et al.* 2012, 2016). These non-additive (interactive) outcomes are still poorly understood, but central to honing our predictions about GCC impacts on food web structure and dynamics in a highly anthropogenized world.

The mechanisms through which warming and increasing nutrient loads independently influence food webs are relatively well understood (McClelland & Valiela 1998; Carlier *et al.* 2008; Gibert 2019), specifically regarding their impacts on the relative strength of bottom-up and top-down effects (Binzer *et al.* 2012, 2016; Shurin *et al.* 2012). For example, warming can increase predation pressure (Kratina *et al.* 2012), thus decreasing resource biomass, while increasing the proportion of top predators (Vasseur & McCann 2005; O'Connor 2009; Shurin *et al.* 2012). Alternatively, warming can increase metabolic demands while reducing conversion efficiency

(Barneche *et al.* 2021), leading to predator starvation at high temperatures, loss of top predators, and reduced food chain length (Petchey *et al.* 1999) Unlike warming, increasing nutrient loads tend to increase bottom-up effects, often resulting in unstable dynamics and species loss (i.e., paradox of enrichment, Rip and McCann, 2011; Rosenzweig, 1971). Increasing nutrient loads can alter consumer-resources interactions, sometimes resulting in top-heavy, unstable food webs (Rip & McCann 2011). Eutrophication resulting from increasing nutrient loads can also change consumer trophic position, leading to changes in species interactions and food web structure (Lee *et al.* 2021).

While warming and nutrients can independently influence food webs, they can also have non-additive (interactive) effects when acting together (Binzer *et al.* 2012, 2016; Tabi *et al.* 2019). For example, at low temperatures, small temperature increases can stabilize nutrient-induced instabilities (Binzer *et al.* 2012, 2016), although temperature increases are themselves considered destabilizing because they strengthen consumer-resource interactions (Barton *et al.* 2009). At high temperatures, increasing nutrient loads can counteract warming-induced consumer starvation by increasing carrying capacity and predator attack rates (Binzer *et al.* 2012). Warming also weakens nutrient-induced increases in community biomass (negative interactive effects, Tabi *et al.*, 2019), in turn influencing food web structure (Sentis *et al.* 2014), species richness, and community composition (Wang *et al.* 2016).

Temperature and nutrients can both determine the physiological and morphological traits of organisms (Rosenblatt & Schmitz 2016; Tabi *et al.* 2019). For example, higher temperatures often result in smaller sizes (Atkinson 1995; Atkinson *et al.* 2003), while nutrient enrichment

leads to larger organisms (Irwin *et al.* 2006; Mara $\acute$ on *et al.* 2013). Additionally, temperature and nutrients can interactively affect body size, where body size may increase with nutrients at low temperature but decrease at high temperature (Tabi *et al.* 2019). Although body size is often considered a response variable, its strong influences on population growth (Fenchel 1974; Savage *et al.* 2004) and species interactions (Ferenc & Sheppard 2020) mean that rapid phenotypic responses to temperature, nutrients, or both, may have consequences for food web structure and dynamics in warmer climates (Brose *et al.* 2012; Bernhardt *et al.* 2018; Wieczynski *et al.* 2021). However, whether body size only responds to environmental conditions, whether rapid changes in body size influences how environmental conditions affect food webs, or whether shifts in body size in a species can influence body size responses to environmental conditions in the species they interact with, are not well understood.

Here, we study how temperature and nutrients influence the feedbacks between ecological and body size dynamics in a tractable microbial food web by first describing observed food web and body size responses to temperature and nutrients across trophic levels, then studying the mechanisms of response. Specifically, we ask: 1) do temperature and nutrients independently or interactively influence ecological dynamics within microbial food webs?, 2) do these effects alter the relative importance of top-down vs. bottom-up control?, 3) do these effects vary across trophic levels?, and 4) is body size only responding to temperature and nutrients, as suggested elsewhere (e.g., Binzer *et al.* 2016, Tabi *et al.* 2019), or does that response play a role in determining how the food web itself responds to environmental change. To address these questions, we manipulate nutrient inputs and temperature in a microbial food web composed of bacteria, a bacterivorous protist (*Tetrahymena pyriformis*), and an omnivorous top predator

(*Euplotes sp.*), then track the changes in population densities and body sizes of over time. We use state-of-the-art time-series analysis to evaluate the relative strength of top-down vs. bottom-up processes across trophic levels, as well as whether and how the observed ecological and phenotypic dynamics influence one another. Our results reveal complex temperature and nutrient effects on food web dynamics that vary predictably across trophic levels by altering the relative strength of trait-mediated bottom-up and top-down effects.

METHODS

Culture care

Euplotes sp. and *Tetrahymena pyriformis* stock cultures were acquired from Carolina Biological Supply (Burlington, NC, USA). Both species were kept in autoclaved liquid protist medium (Carolina Biological Supply), supplemented with one autoclaved wheat seed as a carbon source (Altermatt et al 2014). Protists were fed a mixture of pond bacteria collected from an ephemeral pond at Duke Forest (Wilbur/Gate 9 pond, Lat 36.013914, -Long 78.979720). We maintained all cultures on a 16:8 light:dark cycle at 22°C and 65% humidity in AL-22L2 Percival growth chambers (Perry, IA, USA).

Experimental design

Microcosms were set up in autoclaved 250mL borosilicate jars filled with 200mL of protist media. We manipulated temperature and nutrient loads by imposing two temperature levels (22°C/25°C) and two nutrient levels (normal protist media concentration plus one wheat seed – i.e., high nutrients–, or half concentration plus half a wheat seed, –i.e., low nutrients) in a

factorial design with 4 treatments and 6 replicates per treatment. Day/night cycle and humidity levels mimicked rearing conditions and were kept constant. We inoculated the bacterial communities from 2 ml of stock culture, the same used to rear the protists. The intermediate consumer species, *T. pyriformis*, was introduced at a starting density of 37 ind/ml, and the larger protists species, *Euplotes sp.*, at a starting density of 0.24 ind/ml in all microcosms. We recorded the density of both protist species through fluid imaging (FlowCam; Yokogawa Fluid Imaging Technologies, Portland, ME, USA), Monday through Friday, for 16 days. Fluid imaging generates individual cell raster images that were used to quantify changes in protist size (measured as cell area, in μm^2) over time. Bacteria density was quantified as optical density at a wavelength of 600nm (OD600), using a BioTEK Epoch-2 microplate spectrophotometer (Winooski, VT, USA).

Data analysis

To test for possible effects of temperature and nutrients on ecological dynamics, we fitted Generalized Additive Mixed Models (GAMM) to time series of species density (OD600 for bacteria) and protist body size, across all treatments, using the ‘mgcv’ package (version 1.8-31,(Wood 2011)) in R (version 4.0.2, R Core Team 2020). To control for temporal autocorrelation, we used an Autoregressive Moving Average (ARMA) correlation structure in the GAMM models using the ‘nlme’ R package (version 3.1-148). We compared models with additive and/or interactive effects temperature and nutrients effects, as well as different ARMA correlation structures, using AICc (Appendix I Table S1-2). We discarded *T. pyriformis* phenotypic data from days on which less than 10 individuals were measured after populations collapsed.

Characterization of ecological dynamics

To assess environmental effects on microcosm food web dynamics, we characterized multiple aspects of the population dynamics of bacteria and protists and their responses to environmental conditions. Specifically, we quantified: 1) initial growth rate (day^{-1}) as $[\ln(N_f) - \ln(N_i)]/\text{time}$ for early-dynamics data, (up to day 1 for *T. pyriformis* & bacteria and day 8 for *Euplotes sp.*, due to much slower growth), 2) maximum density (individual/ml for protists and OD₆₀₀ for bacteria), using the highest average daily population density across replicates but within treatments for protists, and the top three observed OD600 values for each replicate from each treatment for bacteria because their population dynamics remained relatively stable, 3) the coefficient of variation ($\text{CV} = \text{standard deviation}/\text{mean}$) of the temporal population dynamics within treatments (typically used as a measure of stability (McCann 2012)), 4) the time to population collapse in days (only *T. pyriformis*), and 5) the time to population peak in days (only *Euplotes sp.*).

Quantifying top-down/bottom-up effects and feedbacks between ecological and body size dynamics

To understand the mechanisms through which temperature and nutrients affected the observed food web dynamics, we quantified the reciprocal effects of ecological and body size dynamics using Convergent Cross Mapping (CCM) from the R package ‘rEDM’ (version 1.5.0; Park et al 2020). CCM quantifies the strength of causation of one dynamical variable onto another by measuring the extent to which the time series of one variable X can reliably estimate the state of another variable Y in a future time step (Sugihara *et al.* 2012). The higher the ability of X to

predict Y, the higher the causal effect of Y on X. Meanwhile, a variable Y that does not influence a variable X cannot be predicted from the dynamics of X (Sugihara *et al.* 2012). This procedure yields a ‘predictability’ value that can be interpreted as a correlation coefficient between past and future states of the two dynamical variables (Sugihara *et al.* 2012). Whether there is a cause-effect relationship between two dynamical variables, as opposed to simple correlation, further depends on whether the average predictability value increases with library size (i.e., number of data points taken into account to run the analysis, Sugihara *et al.*, 2012). We took this into account to infer causation in our data (see Appendix II Fig. S1-14).

CCM is often applied to relatively long time series (Sugihara *et al.* 2012). However, the dynamics in our system occurred much too fast (Fig. 1), precluding us from collecting longer time series. To resolve this issue, we used CCM on the GAMM model predictions of changes in density (for all species) and body size (for protists) with time, for each treatment. These GAMM model predictions considered the effects of each treatment as a whole—i.e., each combination of nutrient and temperature levels—because they more closely reproduced the observed dynamics (Appendix I Table S1). We refer to these GAMM predictions as ‘time-series’ in the context of CCM analyses. Using CCM on the density time series, we calculated the predictability of changes in prey density from changes predator density (i.e., bottom-up control, as predator density predicting prey density means changes in prey density causes changes in predator densities, Sugihara *et al.*, 2012) and the predictability of changes in predator density from changes in prey (i.e., top-down control). We dubbed these “eco-eco” effects, for simplicity. To assess feedbacks between body size and ecological dynamics, we calculated the predictability of changes in protist body size from changes in species density (i.e., causal effects of changes in

body size on densities, or ‘pheno-eco’ effects), and vice versa (‘eco-pheno’ effects). Last, we calculated the predictability of changes in predator and prey body sizes on each other (‘pheno-pheno’ effects).

RESULTS

General Ecological and Phenotypic dynamics

Overall, bacterial density increased to carrying capacity early (Fig. 1a, light grey), *T. pyriformis* increased rapidly, then decreased (Fig. 1a, dark grey), while *Euplotes sp.* increased almost monotonically to carrying capacity (Fig. 1a, black), ultimately resulting in a two-species state. Temperature and nutrients affected all three species and led to significantly different dynamics across treatments (Fig. 1b-d, Appendix I Table S2). The body sizes of both protists changed rapidly over time (Fig. 1e, f) and responded to both temperature and nutrients (Fig. 1e, f, Appendix I Table S2).

Temperature and nutrient effects change across trophic levels in systematic ways

Temperature significantly affected a larger number of dynamical descriptors than nutrients (Fig. 2, 3). Individual temperature effects were more frequent in the upper trophic levels than in bacteria (Fig. 3), as were interactions between temperature and nutrients (Fig. 3). In particular, higher temperature alone i) strongly increased initial growth rate in *Tetrahymena* ($T = 0.50025$, $p = 1.16 \times 10^{-6}$, Fig. 2b), ii) decreased the maximum density of bacteria and *T. pyriformis* ($T_B = -0.004917$, $p = 0.00419$; $T_T = -975.3$, $p < 0.0155$; Fig. 2d-e) but increased that of *Euplotes sp.* ($T_E = 39.83$, $p = 0.02668$, Fig. 2f), iii) increased the variability in *T. pyriformis* density (i.e. CV, $T =$

0.20033, $p = 0.00554$, Fig. 3h) but decreased that of *Euplotes sp.* ($T = -0.07689$, $p = 0.01692$, Fig. 3i), iv) accelerated the time to collapse in *T. pyriformis* ($T = -2.7576$, $p = 1.59 \times 10^{-5}$; Fig. 2j), and v) accelerated the time to peak density in *Euplotes sp.* ($T = -2.1667$, $p = 0.0196$; Fig. 2k). Higher nutrients alone i) decreased the initial growth rate of *Euplotes sp.* ($N = -0.087$, $p = 5.02 \times 10^{-4}$ Fig. 2c), ii) increased maximum density of all species ($N_B = 0.012717$, $p = 2.03 \times 10^{-10}$; $N_T = 1002.2$, $p = 0.0132$; $N_E = 60.67$, $p = 0.00157$, Fig. 2d-f), and iii) increased the CV of bacteria and *Euplotes sp.* ($N_B = 0.17521$, $p = 8.54 \times 10^{-5}$, Fig. 2g; $N_E = 0.15853$, $p = 2.94 \times 10^{-5}$, Fig. 2i) while decreasing that of *T. pyriformis* ($N_T = -0.18727$, $p = 0.00875$, Fig. 2h). Interestingly, increased temperature and nutrients had opposite effects on the CV of *T. pyriformis* and *Euplotes sp.*, indicating antagonistic additive effects of temperature and nutrients on food web stability.

Temperature and nutrients had complex interactive effects on ecological dynamics of both protist consumers. Nutrients had a negative effect on the initial growth rate of *Euplotes sp.* at low temperature, but a positive effect at high temperature ($N \times T = 0.12621$, $p = 0.000391$; Fig. 2c). The maximum density of *T. pyriformis* increased with nutrients, but this effect was stronger at high temperature ($N \times T = 1526.7$, $p = 0.0083$, Fig. 2e). Nutrients also had i) a negative effect on *T. pyriformis* population variability (CV) at low temperature but no effect at high temperature ($N \times T = 0.19492$, $p = 0.04500$, Fig. 2h), and ii) a positive effect on *Euplotes sp.* CV that was stronger at low temperature ($N \times T = -0.12045$, $p = 0.00913$, Fig. 2i). Additional model stats can be found in Appendix I Table S3. Taken together, these results imply that temperature and nutrients individually and interactively influence population dynamics at all trophic levels, with more of both types of effects occurring at higher trophic levels (Fig. 3).

Temperature and nutrients influence top-down and bottom-up effects

Using CCM analysis, we estimated changes in the relative strength of bottom-up and top-down ecological effects across treatments (Fig. 4a). In general, top-down and bottom-up effects between the top predators were the strongest (Fig. 4a, yellow). Top-down effects involving bacteria were the most strongly influenced by temperature and nutrients (Fig. 4a, green & purple dashed lines). Importantly, temperature and nutrients showed interactive effects on top-down and bottom-up control. Most notably, top-down control by both protists on bacteria decreased with nutrients at low temperature but increased with nutrients at high temperatures (Fig. 4a, open dots and dashed lines).

The most dominant species interactions in the overall food web also changed according to shifts in the strength of top-down and bottom-up control between species pairs (Figure 5a). To be conservative and increase the chance of picking up biologically relevant effects, we only present effects whose CCM predictability value was above 0.75 in Figure 5 (Appendix III Table S1). Specifically, increasing nutrients weakened top-down control from *T. pyriformis* to bacteria at low temperatures (Fig. 5a left panel, dashed purple line), but strengthened top-down control from both protists to bacteria at high temperatures (Fig. 5a right panel, dashed lines). In other words, top-down effects were strongest when temperature and nutrients were both low, or both high, suggesting that shifts in the relative importance of top-down and bottom-up effects across trophic levels explain some effects of temperature and nutrients on the overall food web.

Temperature and nutrients alter the reciprocal effects of ecological and phenotypic dynamics

Protist size not only responded to temperature and nutrients, but also played an important role in determining the effects of temperature and nutrients on ecological dynamics (Fig. 4b-c). On average, higher temperature generally led to stronger eco-pheno effects (mean CCM at 22°C = 0.55, mean CCM at 25°C = 0.66, Fig. 4b, Appendix II Table S1) and pheno-eco interactions (size driving ecological dynamics, mean CCM at 22°C = 0.71, mean CCM at 25°C = 0.79, Fig. 4c, Appendix II Table S1), indicating stronger coupling between ecological and phenotypic dynamics at higher temperatures. Pheno-eco effects were generally stronger (14 strong effects, i.e., CCM predictability > 0.75, Fig. 4b) than eco-pheno effects (11 strong effects, Fig. 4c), suggesting stronger effects of body size on ecological dynamics than the other way around. For example, *Euplotes sp.* size had stronger effects on its own population dynamics than the opposite (Fig. 4b-c, black dotted lines).

Both eco-pheno and pheno-eco effects generally responded interactively to changes in temperature and nutrients. For instance, *T. pyriformis* eco-pheno effects on *Euplotes. sp* (yellow solid lines in Fig.4b) decreased with nutrients at low temperature but remained relatively unchanged at high temperature. Also, nutrients increased the effect of *T. pyriformis* size on bacterial dynamics and this pattern was more exaggerated at higher temperature (Fig. 4c, dashed purple lines). Meanwhile, nutrients decreased the effects of *Euplotes sp.* size on bacteria dynamics at low temperature and this pattern was reversed at high temperature (Fig. 4c, dashed green lines). In 22 out of the 25 eco-pheno or pheno-eco interactions, we found strong support for causation (Appendix II Fig. S1-14).

Pheno-pheno effects between protists (Fig. 4d) were relatively weaker than pheno-eco or eco-pheno effects. In general *T. pyriformis* size more strongly affected *Euplotes sp.* size than the other way around (Fig. 4d). Additionally, temperature generally increased body size effects between protists and we observed the strongest phenotypic coupling between protists when temperature and nutrients were both high (Fig. 4d), indicating that higher temperatures may increase the role of phenotypic dynamics in consumer-resource interactions.

Overall, these results indicate that the effect of temperature and nutrients on ecological dynamics may be mediated by changes in body size (Fig. 5). Temperature and nutrients showed negative interactions on strong eco-eco effects (CCM predictability > 0.75), leading to stronger eco-eco effects when temperature and nutrients were both low or both high (Fig. 5a). However, the number of strong causal effects between ecological and phenotypic dynamics increased with temperature but were weakened by nutrients (Fig. 5b).

DISCUSSION

Our results reveal complex but systematic effects of temperature and nutrients on the ecological and phenotypic dynamics of a microbial food web. We show that temperature and nutrients can each independently influence different aspects of food web dynamics (Fig. 1, 2), while their joint effects get increasingly complex at higher trophic levels (Fig. 2, 3). We also found that changes in the relative strength of top-down and bottom-up effects likely drive observed responses to temperature and nutrients (Fig. 4, 5), and that rapid changes in body size mediate these effects (Fig. 4, 5). These results suggest that body size not only responds to shifts in environmental

conditions, but also plays a role in determining ecological responses to such shifts. Evaluating feedbacks between ecological and phenotypic dynamics is therefore integral to understanding food web responses to environmental change.

Increasingly complex effects of temperature and nutrients at higher trophic levels

Our results reveal the pervasive effects of temperature and nutrients across all species, but these effects are more numerous and increasingly complex (temperature-nutrient interactions) at higher trophic levels (Fig. 2, 3). The effects of temperature and nutrients on dietary preference, species interactions, and foraging behavior may help explain why these interactive effects may be more likely among consumer species (Fig. 1d; Fig. 2c).

Because energy enters at the bottom of a food web, basal species are more likely to be influenced by direct effects of nutrients while species at higher trophic levels may be more strongly affected by temperature (Petchey *et al.* 1999; Voigt *et al.* 2003). As energy flows from the basal species to the consumers, the temperature and nutrient effects that act on basal species could be integrated by consumers, leading to additional indirect temperature and nutrient effects on consumers. Additionally, these effects may be exaggerated in omnivores due to the complex nature of their trophic interactions. As temperature and nutrients change species interactions and foraging behaviors (Sentis *et al.* 2014), omnivorous consumers may shift diets between basal and intermediate resources (Sentis *et al.* 2014), altering the strength/prevalence of competitive vs. predatory interactions (Vandermeer 2006), thus rewiring food webs (Bartley *et al.* 2019). Taken together, this may indicate why the joint effects of temperature and nutrients may be more complex at higher trophic levels in food webs with higher prevalence of omnivory.

Top-down control, bottom-up control, and food web stability

Consistent with our results (Fig. 2d), theory predicts that higher nutrient loads should increase energy flux in food webs, increasing basal species density (Rip & McCann 2011; Shurin *et al.* 2012). Theory also predicts that increasing energy flux can destabilize population oscillations (Rosenzweig 1971; Rip & McCann 2011), but increasing temperatures should stabilize oscillations by weakening top-down effects (Vasseur & McCann 2005; Binzer *et al.* 2016; Tabi *et al.* 2019). We found that temperature and nutrients interact to have divergent impacts on population stability—with temperature stabilizing, and nutrients destabilizing *Euplotes sp.* densities, but the opposite being true for *T. pyriformis* (Fig. 2h-i). We also observed that top-down control of bacteria by both consumers was strongest when temperature and nutrients were both high (and both low), while bottom-up effects were relatively invariant across treatments (Fig. 4a, Fig. 5). Interestingly, these shifts in top-down control across environments (Fig. 4a) coincided with significant changes in population stability that varied across trophic levels (Fig. 2i). These results thus indicate that changes in the strength of top-down effects between basal resources and consumers—instead of bottom-up effects—could be the dominant mechanism through which higher temperature stabilize unstable fluctuations caused by nutrients.

Phenotypes mediate temperature and nutrients effects on food web dynamics

Our results indicate that phenotypic dynamics play a larger role in mediating environmental impacts on food web dynamics than previously thought (Fig. 5, Appendix III Fig. S1). Previous studies showed that predator-prey body size ratios significantly influence temperature and nutrients effects (Binzer *et al.* 2012) and that body size responds to changes in nutrients,

temperature, and ecological dynamics in specific ways (Tabi *et al.* 2019). Our results support—
but also extend—those results by showing that 1) phenotypic effects on ecological dynamics
may be stronger than the other way around, as also recently reported elsewhere (Gibert *et al.*
2021), 2) changes in prey size may more strongly drive changes in predator size than the other
way around, and 3) interactions involving phenotypic dynamics (eco-pheno, pheno-eco, or
pheno-pheno) vary in complex ways across environmental conditions, but seem to be generally
stronger at higher temperature (Fig. 4b-d, Fig. 5). Rapid phenotypic change has been suggested
as a main driver of food web rewiring in future climates (Barbour & Gibert 2021), but little
experimental evidence exists. Here, we found that strong feedbacks between ecological and
phenotypic dynamics vary with temperature and nutrients, suggesting that rapid phenotypic
change influences food web responses to changing environmental conditions. Together, our
results emphasize the need to incorporate phenotypic dynamics in future studies of food web
responses to warming and eutrophication in a changing world.

ACKNOWLEDGMENTS

This work was supported by a U.S. Department of Energy, Office of Science, Office of
Biological and Environmental Research, Genomic Science Program Grant under Award Number
DE-SC0020362 to JPG.

Figures

Figure 1. Population and phenotypic dynamics within experimental microcosms. (a) The average population density across treatments shows the general population dynamics of each species. (b-d) show GAMM smoothed population dynamics of bacteria, *T. pyriformis* and *Euplotes* sp.. (e-f) show the changes in the area (body size) of both protists body over time. *T. pyriformis* were effectively extinct by day 9. Greyed area indicates days for which data are insufficient to estimate trait distributions.

Figure 2. Interactive plot of effects of temperature and nutrients on descriptors of population dynamics. Open circles and triangles represent data points from each replicate. Solid shapes are the mean across 6 replicates. Grey letters T, N, and (T×N) represent statistically significant effects from temperature, nutrients, and interactive effects between temperature and nutrients, respectively.

Fig. 3. Significant temperature and nutrients effects on ecological dynamics. T, N, and T×N denote effects of temperature, nutrients, and interactive effects of temperature and nutrients, respectively.

Figure 4. CCM predictabilities ecological and phenotypic dynamics across treatments for each species pair. Circles represents CCM predictability of one species' effect on another species, while the triangles representing intraspecific. Solid circles and solid lines represent CCM predictability of bottom-up effects and open circles and dashed lines represent top-down effects. Interactions with CCM predictability higher than 0.75 are considered to be strong interactions.

Figure 5. Strong effects (i.e., CCM predictability higher than 0.75) within food webs across treatments. Panel (a) shows strong eco-eco effects (Fig. 4a). Panel (b) shows all strong interactions between phenotypic (Ph) and ecological (E) dynamics (pheno-eco, eco-pheno and pheno-pheno effects; Fig. 4b-d). Dashed and solid lines represent top-down and bottom-up effects respectively. Dotted lines represent intraspecific effects. Color code is the same as in Fig. 4a.

References:

- Atkinson, D. (1995). Effects of temperature on the size of aquatic ectotherms: Exceptions to the general rule. *J. Therm. Biol.*, 20, 61–74.
- Atkinson, D., Ciotti, B.J. & Montagnes, D.J.S. (2003). Protists decrease in size linearly with temperature: $ca. 2.5\% \text{ }^{\circ}\text{C}^{-1}$. *Proc. R. Soc. Lond. B Biol. Sci.*, 270, 2605–2611.
- Barbour, M.A. & Gibert, J.P. (2021). Genetic and plastic rewiring of food webs under climate change. *J. Anim. Ecol.*, 1365–2656.13541.
- Barneche, D.R., Hulatt, C.J., Dossena, M., Padfield, D., Woodward, G., Trimmer, M., *et al.* (2021). Warming impairs trophic transfer efficiency in a long-term field experiment. *Nature*.
- Bartley, T.J., McCann, K.S., Bieg, C., Cazelles, K., Granados, M., Guzzo, M.M., *et al.* (2019). Food web rewiring in a changing world. *Nat. Ecol. Evol.*, 3, 345–354.
- Barton, B.T., Beckerman, A.P. & Schmitz, O.J. (2009). Climate warming strengthens indirect interactions in an old-field food web. *Ecology*, 90, 2346–2351.
- Bernhardt, J.R., Sunday, J.M. & O'Connor, M.I. (2018). Metabolic Theory and the Temperature-Size Rule Explain the Temperature Dependence of Population Carrying Capacity. *Am. Nat.*, 192, 687–697.
- Binzer, A., Guill, C., Brose, U. & Rall, B.C. (2012). The dynamics of food chains under climate change and nutrient enrichment. *Philos. Trans. R. Soc. B Biol. Sci.*, 367, 2935–2944.
- Binzer, A., Guill, C., Rall, B.C. & Brose, U. (2016). Interactive effects of warming, eutrophication and size structure: impacts on biodiversity and food-web structure. *Glob. Change Biol.*, 22, 220–227.
- Brose, U., Dunne, J.A., Montoya, J.M., Petchey, O.L., Schneider, F.D. & Jacob, U. (2012). Climate change in size-structured ecosystems. *Philos. Trans. R. Soc. B Biol. Sci.*, 367, 2903–2912.
- Carlier, A., Riera, P., Amouroux, J.-M., Bodiou, J.-Y., Desmalades, M. & Grémare, A. (2008). Food web structure of two Mediterranean lagoons under varying degree of eutrophication. *J. Sea Res.*, 60, 264–275.
- Clarke, A. (2006). Temperature and the metabolic theory of ecology. *Funct. Ecol.*, 20, 405–412.
- Dossena, M., Yvon-Durocher, G., Grey, J., Montoya, J.M., Perkins, D.M., Trimmer, M., *et al.* (2012). Warming alters community size structure and ecosystem functioning. *Proc. R. Soc. B Biol. Sci.*, 279, 3011–3019.
- Fenchel, T. (1974). Intrinsic rate of natural increase: The relationship with body size. *Oecologia*, 14, 317–326.
- Ferenc, V. & Sheppard, C.S. (2020). The stronger, the better – trait hierarchy is driving alien species interaction. *Oikos*, 129, 1455–1467.
- Fussmann, G.F. (2000). Crossing the Hopf Bifurcation in a Live Predator-Prey System. *Science*, 290, 1358–1360.
- Gauzens, B., Rall, B.C., Mendonça, V., Vinagre, C. & Brose, U. (2020). Biodiversity of intertidal food webs in response to warming across latitudes. *Nat. Clim. Change*, 10, 264–269.
- Gibert, J.P. (2019). Temperature directly and indirectly influences food web structure. *Sci. Rep.*, 9, 5312.
- Gibert, J.P., Han, Z.-Y., Wieczynski, D.J. & Yammine, A. (2021). Rapid plastic shifts in body size precede and determine population growth. *BioRxiv*. doi: 10.1101/2021.07.15.452551

- Gounand, I., Kéfi, S., Mouquet, N. & Gravel, D. (2016). Trait selection during food web assembly: the roles of interactions and temperature. *Theor. Ecol.*, 9, 417–429.
- Hautier, Y., Niklaus, P.A. & Hector, A. (2009). Competition for Light Causes Plant Biodiversity Loss After Eutrophication. *Science*, 324, 636–638.
- Hautier, Y., Seabloom, E.W., Borer, E.T., Adler, P.B., Harpole, W.S., Hillebrand, H., *et al.* (2014). Eutrophication weakens stabilizing effects of diversity in natural grasslands. *Nature*, 508, 521–525.
- Irwin, A.J., Finkel, Z.V., Schofield, O.M.E. & Falkowski, P.G. (2006). Scaling-up from nutrient physiology to the size-structure of phytoplankton communities. *J. Plankton Res.*, 28, 459–471.
- Joseph Park, Cameron Smith, George Sugihara and Ethan Deyle (2020). rEDM: Empirical Dynamic Modeling ('EDM'). R package version 1.5.0. <https://CRAN.R-project.org/package=rEDM>
- Karl, T.R. (2003). Modern Global Climate Change. *Science*, 302, 1719–1723.
- Kratina, P., Greig, H.S., Thompson, P.L., Carvalho-Pereira, T.S.A. & Shurin, J.B. (2012). Warming modifies trophic cascades and eutrophication in experimental freshwater communities. *Ecology*, 93, 1421–1430.
- Lee, G.H., Vonk, J.A., Verdonchot, R.C.M., Kraak, M.H.S., Verdonchot, P.F.M. & Huisman, J. (2021). Eutrophication induces shifts in the trophic position of invertebrates in aquatic food webs. *Ecology*, 102.
- Malcolm, J.R., Liu, C., Neilson, R.P., Hansen, L. & Hannah, L. (2006). Global Warming and Extinctions of Endemic Species from Biodiversity Hotspots. *Conserv. Biol.*, 20, 538–548.
- Marañón, E., Cermeño, P., López-Sandoval, D.C., Rodríguez-Ramos, T., Sobrino, C., Huete-Ortega, M., *et al.* (2013). Unimodal size scaling of phytoplankton growth and the size dependence of nutrient uptake and use. *Ecol. Lett.*, 16, 371–379.
- McCann, K.S. (2012). *Food webs*. Monographs in population biology. Princeton University Press, Princeton, N.J.
- McClelland, J. & Valiela, I. (1998). Changes in food web structure under the influence of increased anthropogenic nitrogen inputs to estuaries. *Mar. Ecol. Prog. Ser.*, 168, 259–271.
- O'Connor, M.I. (2009). Warming strengthens an herbivore–plant interaction. *Ecology*, 90, 388–398.
- Petchey, O.L., McPhearson, P.T., Casey, T.M. & Morin, P.J. (1999). Environmental warming alters food-web structure and ecosystem function. *Nature*, 402, 69–72.
- R Core Team (2020). R: A language and for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- Rip, J.M.K. & McCann, K.S. (2011). Cross-ecosystem differences in stability and the principle of energy flux: Cross-ecosystem differences in stability. *Ecol. Lett.*, 14, 733–740.
- Rosenblatt, A.E. & Schmitz, O.J. (2016). Climate Change, Nutrition, and Bottom-Up and Top-Down Food Web Processes. *Trends Ecol. Evol.*, 31, 965–975.
- Rosenzweig, M.L. (1971). Paradox of Enrichment: Destabilization of Exploitation Ecosystems in Ecological Time. *Science*, 171, 385.
- Savage, V.M., Gillooly, J.F., Brown, J.H., West, G.B. & Charnov, E.L. (2004). Effects of Body Size and Temperature on Population Growth. *Am. Nat.*, 163, 429–441.

- Sentis, A., Hemptinne, J.-L. & Brodeur, J. (2014). Towards a mechanistic understanding of temperature and enrichment effects on species interaction strength, omnivory and food-web structure. *Ecol. Lett.*, 17, 785–793.
- Shurin, J.B., Clasen, J.L., Greig, H.S., Kratina, P. & Thompson, P.L. (2012). Warming shifts top-down and bottom-up control of pond food web structure and function. *Philos. Trans. R. Soc. B Biol. Sci.*, 367, 3008–3017.
- Sugihara, G., May, R., Ye, H., Hsieh, C. -h., Deyle, E., Fogarty, M., *et al.* (2012). Detecting Causality in Complex Ecosystems. *Science*, 338, 496–500.
- Tabi, A., Petchey, O.L. & Pennekamp, F. (2019). Warming reduces the effects of enrichment on stability and functioning across levels of organisation in an aquatic microbial ecosystem. *Ecol. Lett.*, 22, 1061–1071.
- Vandermeer, J. (2006). Omnivory and the stability of food webs. *J. Theor. Biol.*, 238, 497–504.
- Vasseur, D.A. & McCann, K.S. (2005). A Mechanistic Approach for Modeling Temperature-Dependent Consumer-Resource Dynamics. *Am. Nat.*, 166, 184–198.
- Voigt, W., Perner, J., Davis, A.J., Eggers, T., Schumacher, J., Bährmann, R., *et al.* (2003). TROPHIC LEVELS ARE DIFFERENTIALLY SENSITIVE TO CLIMATE. *Ecology*, 84, 2444–2453.
- Wang, J., Pan, F., Soininen, J., Heino, J. & Shen, J. (2016). Nutrient enrichment modifies temperature-biodiversity relationships in large-scale field experiments. *Nat. Commun.*, 7, 13960.
- Wieczynski, D., Singla, P., Doan, A., Singleton, A., Han, Z., Yammine, A., Gibert, J.P. (2021). Simple traits predict complex temperature responses across scales. Research Square. doi:10.21203/rs.3.rs-116110/v1
- Wood, S.N. (2011). Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models: Estimation of Semiparametric Generalized Linear Models. *J. R. Stat. Soc. Ser. B Stat. Methodol.*, 73, 3–36.