Title

Models and experiments of ecological and evolutionary dynamics

Author

Lyberger, Kelsey

Publication Date

2021

Peer reviewed|Thesis/dissertation

Models and experiments of ecological and evolutionary dynamics

By

KELSEY LYBERGER

DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Population Biology

in the

OFFICE OF GRADUATE STUDIES

of the

UNIVERSITY OF CALIFORNIA

DAVIS

Approved:

Thomas W. Schoener

Sebastian Schreiber

Steven Sadro

Committee in Charge

2021

Abstract

The building evidence that evolution can be extremely rapid leads us to consider the convergence of ecological and evolutionary timescales. The convergence of timescales implies that evolution has the potential to significantly affect ecological processes and vice versa. The models and experiments presented here fall under this shared framework of eco-evolutionary feedbacks. Chapter 1 uses a general model to consider one way in which evolution affects ecology. It asks the question how rapid evolution affects short-term population dynamics and extinction risk in a population facing extreme climate events. We found that evolution can increase extinction risk if the extreme event is short-lived. Chapters 2 and 3 consider the other direction: how ecological contexts drive evolutionary change. In a controlled laboratory experiment using the common freshwater zooplankton *Daphnia*, we explored two selective mechanisms through which predators can cause prey life-history evolution. We found that both density-dependent selection and size selection are important drivers of life-history evolution. Then, in an outdoor experiment, we explored to what extent rapid evolution in *Daphnia* is predictable in a natural environment. We found consistent changes across replicate populations that are potentially driven by the ability of different clones to utilize resources. Finally, Chapter 4 documents a purely ecological feedback. In a lab experiment, we exposed multiple naturally diverse communities of phytoplankton to zooplankton grazing and showed that the composition of primary producers was altered as a result of the changes they imposed on the composition and feeding behavior of consumers.

Acknowledgements

I owe so much to the wonderful community of ecologists and evolutionary biologists at UC Davis. In particular, I would like to thank my advisors, Sebastian Schreiber and Thomas W. Schoener, who have been my biggest advocates and provided unending support and mentorship. They have given me the freedom to pursue my ideas, the biggest being to build a *Daphnia* lab. Sebastian has been patient as I learned the ropes of theoretical ecology and I attribute my success and interest in mathematical modeling to his infectious love for it. Tom has taught me to think bigger picture about broad questions in ecology and evolution and instilled in me the importance of testing hypotheses in the field. My committee member Steve Sadro has also provided much guidance and feedback. I hope that over the years some of their intellectual acumen and encyclopedic knowledge of the literature has rubbed off on me.

I am thankful for members of my cohort who have become some of my closest friends. Victoria Watson-Zink, Yige Luo, Erin Calfee, and Anita To never hesitate to celebrate my ups and have been there to get me through the downs. I would also like to thank members of the Schreiber lab. Matt Osmond and Swati Patel have been inspiring collaborators. William Cuello, Michael Curshaw-Maurer, Sam Fleischer, and Dale Clement have often generously offered me their expertise and their time. Chapters 2 and 3 would not have been possible without support from Rick Grosberg/Rachel Bay's lab and advice from Brenda Cameron. Similarly, I felt very welcomed at the Seeon Limnological Station where I conducted the work presented in Chapter 4.

I could not have accomplished this without my fiancé Dayton Thorpe, who has done everything to help me through this journey from reading drafts to sampling in the field to offering words of wisdom. Finally, I would like to acknowledge my family, who have always encouraged me and given me lots of practice communicating my science to a wider audience.

Table of Contents

Introduction

Although it has long been recognized that ecological conditions set the stage for evolution, the traditional view has been that evolution did not have much impact on ecology because it occurred on a much longer timescale (Hutchinson 1965). More recently, the accumulating examples of rapid contemporary evolution in the wild (Hendry and Kinnison 1999) have renewed interest in the reciprocal interactions between ecology and evolution on short timescales (Ellner et al. 2011, Fussmann et al. 2007, Schoener 2011). This reciprocal interaction has been termed an eco-evolutionary feedback, in which ecological changes drive selection to produce rapid evolutionary change and the resulting evolutionary change impacts the environment. Eco-evolutionary feedbacks can operate within populations (e.g. density-dependent selection), among species (e.g. host-parasite coevolution), or at the community or ecosystem level (e.g. evolution impacting nutrient flux).

The need to better understand the interplay between ecological and evolutionary dynamics is especially important given the pace at which humans are modifying the environment. Anthropogenic activities can drive evolution directly, as in the case of the harvesting and exploitation of commercially important species (Olsen et al. 2004), or indirectly, as in the case of global atmospheric changes that lead to warming and increased frequency and intensity of extreme climate events like hurricanes and floods (Donihue et al. 2018). These changes provide opportunities to observe rapid evolution. In fact, evidence suggests that rates of phenotypic change in response to human-driven environmental change are faster than those in natural contexts (Hendry et al. 2008).

In my dissertation, I use theoretical and experimental approaches to study ecological and evolutionary dynamics in both natural and human-affected contexts. Multiple approaches are

necessary because of the inherent complexity of ecological and evolutionary processes; it is often intractable to manipulate or to simultaneously track phenotypes, genotypes, and population dynamics (Fussmann et al. 2007). Mathematical models provide a method of representing a system that can be used to explore how it responds to a suite of evolutionary and ecological conditions. For example, eco-evolutionary models using paired equations of population and trait dynamics have helped us understand when feedbacks between ecology and evolution stabilize or destabilize population and community dynamics (Patel et al. 2018, Sinervo et al. 2000). On the other hand, empirical studies allow us to observe natural phenomena and test predictions in nature. A growing number of experiments have demonstrated a significant effect of genetic variation and evolutionary change on ecological dynamics and vice versa (Hendry 2016). However, these have mostly been limited to microscopic organisms in carefully controlled laboratory environments, e.g., chemostats with rotifers and algae (Yoshida et al. 2003).

Study System

I have used the common freshwater zooplankton, *Daphnia*, as a model system to understand ecological and evolutionary dynamics. Not only are *Daphnia* particularly suited to studies of rapid evolution as they are cyclic parthenogens with short generation times but also studies of ecology as they have well-understood population dynamics and trophic interactions (Miner et al. 2012). The life cycle of a typical *Daphnia* species consists of reproducing clonally under favorable conditions, whereas during unfavorable conditions, typically wintertime, females parthenogenetically create males, who then mate with females to produce resting eggs that fall to the sediment and hatch out in spring (Decaestecker et al. 2009). Evolution experiments become especially tractable in clonally reproducing organisms because evolution can be measured as the change in genotype frequencies over time using genetic markers such as microsatellites

(Colbourne et al. 2004). For example, Duncan and Little (2007) show that, in response to a parasite epidemic, clonal diversity in a *Daphnia* population declined and the frequency of resistant clones increased. Beyond changes in genotype frequency, *Daphnia* are known to undergo rapid phenotypic changes. For example, some species show plastic changes in the formation of a helmet when in the presence of predator kairmones (Kruger and Dodson 1981, Hebert and Grewe 1985). Others undergo evolutionary changes in their life histories in response to different fish predation regimes (Walsh and Post 2011).

Additionally, *Daphnia* play a key ecological role in lake ecosystems. They form strong trophic interactions, acting as a primary consumer and as a food source for invertebrates and planktivorous fish. Populations of *Daphnia* exhibit both internally generated population cycles and seasonal cycles in response to algae availability (McCauley and Murdoch 1987, McCauley et al. 2008). They are efficient grazers, responsible for the clear water phase in lakes (Scheffer et al. 1997). Experiments manipulating planktivorous fish (Ives et al. 1999) and nutrients (Carpenter et al. 2001) have demonstrated *Daphnia*'s significant effect on food-web dynamics. More generally, zooplankton and phytoplankton share many qualities that make them ideal for studying ecological processes at different scales, from bottle experiments in the lab to large enclosures in lakes and ponds.

Effects of evolution on ecology

Rapid evolutionary change can have important ecological consequences. Previous studies have shown that evolution impacts population growth rates (Turcotte et al. 2011), within species genetic variation alters predator-prey cycles (Yoshida et al. 2003), and rapidly diverged phenotypes result in changes to ecosystem properties (Bassar et al. 2010). Within the eco-evolutionary dynamics literature, a subfield has emerged to study how and when evolutionary

responses can rescue populations experiencing environmental stress from going extinct (Carlson et al. 2014, Bell 2017). This concept has received attention from both the conservation perspective with the goal of preventing species extinction, as well as the medical and agricultural perspective with the goal of preventing drug and pesticide resistance (Alexander et al. 2014). Theoretical predictions have been made for a range of environments from a single drastic shift to cyclic and fluctuating environments (Gomulkiewicz and Holt 1995, Lande and Shannon 1996, Chevin 2013), but there are still many environmental scenarios left to explore. In Chapter 1, I model the effect of evolution in a population experiencing an extreme climatic event, defined as a short-term disturbance. Manipulating genetic variation, heritability, and the length and severity of the extreme event, I found that while variance can be useful in the generation of a severe event, if heritable it can induce maladaptation that increases extinction risk in the generations after the event. Hence, only when an extreme event is sufficiently long in duration does heritability help a population persist. In the future, I plan to extend this work to consider longer-term effects of repeated extreme events.

Effects of ecology on evolution

Organisms often face two major ecological challenges: avoiding predators and obtaining food. Each of these acts as a selective force and has the potential to drive rapid phenotypic changes in morphological, behavioral, or life history traits. In Chapter 2, I more deeply explore the mechanisms in which predators lead to life-history trait evolution. More specifically, when predators feed on prey they remove certain stages, imposing size-selection. However, they also reduce prey-density which increases per capita resources, imposing density-dependent selection. Despite density-dependent selection being one of the most basic interactions between ecology and evolution (Kokko and Lopez-Sepulcre 2007), it has largely been ignored as a potential

driving force of life-history evolution in response to predators. Using an artificial selection experiment in *Daphnia*, I showed that both size selection and density-dependent selection led to significant evolution.

My experimental results in Chapter 2 add to a longer list of studies documenting ecology affecting evolution, but we have limited knowledge about how predictable this evolution is (Losos 2017). Furthermore, much of what we do know comes from microorganisms like *E. coli* and viruses in controlled laboratory environments (Lenski et al. 1991, Bull et al. 1997). Therefore, the goal of Chapter 3 is to test to what extent rapid evolution in *Daphnia* is predictable in a natural environment. Using replicate field enclosures, I showed there was a parallel increase in the frequency of one genotype in all populations. The finding that evolution was relatively deterministic is encouraging for future field studies of eco-evolutionary dynamics.

Ecological feedbacks

Feedbacks are a unifying theme of my work. In the final chapter, I consider the purely ecological feedback between primary producers and consumers. The interplay between these trophic levels is essential for understanding the ecology of a system. So far, in freshwater aquatic systems, the effects of primary producers on consumers and the effects of consumers on primary producers have been studied independently and often in simplified communities. Studies have found that consumers change the composition of primary producers through selective feeding and nutrient regeneration (Sterner 1986, Peter and Sommer 2012). Another set of studies have found that primary producers change the growth, abundance, and diversity of consumers (Striebel et al. 2012, Marzetz et al. 2017). Such changes in consumers could lead to additional propagated direct and indirect effects on primary producers, however, they had not yet been investigated. Using natural communities of phytoplankton and zooplankton in a bottle

experiment, I showed there was a significant effect of a primary producer community on itself via the altered composition and feeding behavior of consumers (Chapter 4).

References

Alexander, H. K., Martin, G., Martin, O. Y., and S. Bonhoeffer. 2014. Evolutionary rescue: linking theory for conservation and medicine. Evolutionary Applications 7:1161–79.

Bassar, R. D., Marshall, M. C., López-Sepulcre, A., Zandonà, E., Auer, S. K., Travis, J., Pringle, C. M., Flecker, A. S., Thomas, S. A., Fraser, D. F. and D. N. Reznick. 2010. Local adaptation in Trinidadian guppies alters ecosystem processes. Proceedings of the National Academy of Sciences 107:3616-3621.

Bell, G. 2017. Evolutionary rescue. Annual Review of Ecology, Evolution, and Systematics 48:605–627.

Bull, J. J., Badgett, M. R., Wichman, H. A., Huelsenbeck, J. P., Hillis, D. M., Gulati, A., Ho, C. and I. J. Molineux. 1997. Exceptional convergent evolution in a virus. Genetics 147:1497-1507.

Carlson, S. M., Cunningham, C. J. and P. A. Westley. 2014. Evolutionary rescue in a changing world. Trends in Ecology & Evolution 29:521-530.

Carpenter, S. R., Cole, J. J., Hodgson, J. R., Kitchell, J. F., Pace, M. L., Bade, D., Cottingham, K. L., Essington, T. E., Houser, J. N. and D. E. Schindler. 2001. Trophic cascades, nutrients, and lake productivity: whole-lake experiments. Ecological Monographs 71:163-186.

Chevin, L. M. 2013. Genetic constraints on adaptation to a changing environment. Evolution 67:708-721.

Colbourne, J. K., Robison, B., Bogart, K. and M. Lynch. 2004. Five hundred and twenty-eight microsatellite markers for ecological genomic investigations using Daphnia. Molecular Ecology Notes 4:485-490.

Decaestecker, E., De Meester, L. and J. Mergeay. 2009. Cyclical parthenogenesis in Daphnia: sexual versus asexual reproduction. In Lost sex (pp. 295-316). Springer, Dordrecht.

Donihue, C. M., Herrel, A., Fabre, A. C., Kamath, A., Geneva, A. J., Schoener, T. W., Kolbe, J. J. and J. B. Losos. 2018. Hurricane-induced selection on the morphology of an island lizard. Nature 560:88-91.

Duncan, A. B. and T. J. Little. 2007. Parasite-driven genetic change in a natural population of Daphnia. Evolution 61:796-803.

Ellner, S. P., Geber, M. A. and N. G. Hairston Jr. 2011. Does rapid evolution matter? Measuring the rate of contemporary evolution and its impacts on ecological dynamics. Ecology Letters 14:603-614.

Fussmann, G. F., Loreau, M. and P. A. Abrams. 2007. Eco-evolutionary dynamics of communities and ecosystems. Functional Ecology 21:465-477.

Gomulkiewicz, R. and R. D. Holt. 1995. When does evolution by natural selection prevent extinction?. Evolution 49:201-207.

Hebert, P. D. N. and P. M. Grewe. 1985. Chaoborus-induced shifts in morphology of Daphnia ambigua. Limnology and Oceanography 30:1291-1297.

Hendry, A. P. and M. T. Kinnison. 1999. Perspective: the pace of modern life: measuring rates of contemporary microevolution. Evolution 53:1637-1653.

Hendry, A. P., Farrugia, T. J. and M. T. Kinnison. 2008. Human influences on rates of phenotypic change in wild animal populations. Molecular Ecology 17:20-29.

Hendry, A. P. 2016. Eco-evolutionary Dynamics, Princeton: Princeton University Press. https://doi.org/10.1515/9781400883080

Hutchinson, G. E. 1965. The ecological theater and the evolutionary play. Yale University Press.

Ives, A. R., Carpenter, S. R. and B. Dennis. 1999. Community interaction webs and zooplankton responses to planktivory manipulations. Ecology 80:1405-1421.

Kruger, D. A. and S. I. Dodson. 1981. Embryological induction and predation ecology in Daphnia pulex. Limnology and Oceanography 26:219-223.

Kokko, H. and A. López-Sepulcre. 2007. The ecogenetic link between demography and evolution: can we bridge the gap between theory and data?. Ecology Letters 10:773-782.

Lande, R. and S. Shannon. 1996. The role of genetic variation in adaptation and population persistence in a changing environment. Evolution 50:434-437.

Lenski, R. E., Rose, M. R., Simpson, S. C. and S. C. Tadler. 1991. Long-term experimental evolution in Escherichia coli. I. Adaptation and divergence during 2,000 generations. The American Naturalist 138:1315-1341.

Losos, J. B. 2017. Improbable destinies: Fate, chance, and the future of evolution. Penguin.

Marzetz, V., Koussoroplis, A.-M., Martin-Creuzburg, D., Striebel, M. and A. Wacker. 2017. Linking primary producer diversity and food quality effects on herbivores: a biochemical perspective. Scientific Reports 7:1–9.

McCauley, E. and W. W. Murdoch. 1987. Cyclic and stable populations: plankton as paradigm. The American Naturalist 129:97-121.

McCauley, E., Nelson, W. A. and R. M. Nisbet. 2008. Small-amplitude cycles emerge from stage-structured interactions in Daphnia–algal systems. Nature 455:1240-1243.

Miner, B. E., De Meester, L., Pfrender, M. E., Lampert, W. and N. G. Hairston Jr. 2012. Linking genes to communities and ecosystems: Daphnia as an ecogenomic model. Proceedings of the Royal Society B: Biological Sciences 279:1873-1882.

Olsen, E. M., Heino, M., Lilly, G. R., Morgan, M. J., Brattey, J., Ernande, B. and U. Dieckmann. 2004. Maturation trends indicative of rapid evolution preceded the collapse of northern cod. Nature 428:932-935.

Patel, S., Cortez, M. H. and S. J. Schreiber. 2018. Partitioning the effects of eco-evolutionary feedbacks on community stability. The American Naturalist 191:381-394.

Peter, K. H. and U. Sommer. 2012. Phytoplankton cell size: intra-and interspecific effects of warming and grazing. PloS one 7:e49632.

Scheffer, M., Rinaldi, S., Kuznetsov, Y. A. and E. H. van Nes. 1997. Seasonal dynamics of Daphnia and algae explained as a periodically forced predator-prey system. Oikos 80:519-532.

Schoener, T. W. 2011. The newest synthesis: understanding the interplay of evolutionary and ecological dynamics. Science 331:426-429.

Sinervo, B., Svensson, E. and T. Comendant. 2000. Density cycles and an offspring quantity and quality game driven by natural selection. Nature 406:985-988.

Sterner, R. W. 1986. Herbivores' direct and indirect effects on algal populations. Science 231:605–607.

Striebel, M., Singer, G., Stibor, H. and T. Andersen. 2012. "Trophic overyielding": phytoplankton diversity promotes zooplankton productivity. Ecology 93:2719–2727.

Turcotte, M. M., Reznick, D. N. and J. D. Hare. 2011. The impact of rapid evolution on population dynamics in the wild: Experimental test of eco-evolutionary dynamics. Ecology Letters 14:1084-1092.

Walsh, M. R. and D. M. Post. 2011. Interpopulation variation in a fish predator drives evolutionary divergence in prey in lakes. Proceedings of the Royal Society B: Biological Sciences 278:2628-2637.

Yoshida, T., Jones, L. E., Ellner, S. P., Fussmann, G. F. and N. G. Hairston. 2003. Rapid evolution drives ecological dynamics in a predator–prey system. Nature 424:303-306.

Chapter 1

Is evolution in response to extreme events good for population persistence?

Kelsey P. Lyberger[1], Matthew M. Osmond[1,2], Sebastian J. Schreiber[1]

[1]Department of Evolution and Ecology and Center for Population Biology, University of California Davis, Davis, California 95616

[2]Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, Ontario M5S 3G5, Canada

Abstract

Climate change is predicted to increase the severity of environmental perturbations, including storms and droughts, which act as strong selective agents. These extreme events are often of finite duration (pulse disturbances). Hence, while evolution during an extreme event may be adaptive, the resulting phenotypic changes may become maladaptive when the event ends. Using individual-based models and analytic approximations that fuse quantitative genetics and demography, we explore how heritability and phenotypic variance affect population size and extinction risk in finite populations under an extreme event of fixed duration. Since more evolution leads to greater maladaptation and slower population recovery following an extreme event, greater heritability can increase extinction risk when the extreme event is short. Alternatively, when an extreme event is sufficiently long, heritability often helps a population persist. We also find that when events are severe, the buffering effect of phenotypic variance can outweigh the increased load it causes.

Introduction

Globally, humans are causing substantial environmental perturbations, and these perturbations are likely to become more severe in the future. In particular, climate change is projected to lead to more extreme weather events, including droughts and major storms (Ummenhofer and Meehl 2017). With more severe events comes the potential for dramatic demographic and genetic consequences.

In the process of causing mass mortality, extreme events can act as catalysts of evolutionary change. In fact, there are many examples of rapid evolution in response to extreme events (reviewed in Grant et al. 2017), such as droughts causing finches to evolve larger beaks (Grant and Grant 2014) and hurricanes causing lizards to evolve larger toepads (Donihue et al. 2018). Extreme events are sudden transient changes in the environment, i.e., pulse disturbances (Bender et al. 1984). In the ecological literature, pulse disturbances lie at the crossroads of two other forms of environmental change: press perturbations, a sudden long-term change in the environment (Ives and Carpenter 2007, Kefi et al. 2019, Yodzis 1988), and continuously fluctuating environments (Lande 1993, Ozgul et al. 2012). Despite their transient nature, pulse disturbances can have strong and diverse impacts on ecological systems, ranging from transient ecological dynamics to permanent shifts in ecological states (Fox and Gurevitch 2000, Hastings et al. 2018, Holling 1973, 1996, Holt 2008, Ives and Carpenter 2007). Here we study the most extreme form of a permanent shift – species extinction – examining how extinction risk depends on the length of a pulse event (see also Figure 1 in Holt 2008). Understanding short-term extinction risk after a single disturbance is critical for conservation and management.

Previous work on evolution in changing environments can provide intuition for how evolution might affect extinction risk during or after a pulse disturbance. One focus of the

evolutionary rescue literature has been on understanding the consequences of phenotypic change in the context of a sudden, long-term or permanent environmental shift (a press perturbation). These studies, some of which account for demographic stochasticity, underline the importance of genetic variance for increasing the probability of rescue (Gomulkiewicz and Holt1995), reviewed in (Alexander et al. 2014, Bell 2017). Similarly, studies of adaptation in fluctuating environments suggest that if an environment is predictable, such as the case of positively autocorrelated fluctuations, genetic variation reduces lag load (Charlesworth 1993, Chevin 2013, Lande and Shannon 1996). This reduction in the lag load leads to higher population per-capita growth rates and, consequently, is expected to reduce extinction risk. Whereas, when the environment is unpredictable, genetic variance typically increases the lag load. These studies calculated lag load and per-capita growth rates in a number of environmental contexts including a press perturbation, randomly fluctuating environments, and cyclic environments. However, they did not account for demographic stochasticity, the ultimate cause of extinction. As higher long-term growth rates need not imply lower extinction risk (Ellner et al. 2010, Pande et al. 2020, Yahalom and Shnerb 2019), it is unclear whether intuition provided by these earlier studies extends to extinction risk following a pulse disturbance.

To understand extinction risk during and following a pulse disturbance, we introduce an individual-based model that fuses population demography with quantitative genetics. Using a mixture of computational and analytical methods, we examine how phenotypic variation and the heritability of this variation influences population growth, lag load, and extinction risk during and following a pulse perturbation. Moreover, we examine how the magnitude and direction of these effects depend on the duration and intensity of the pulse perturbation.

Model

We use an individual-based model that combines the infinitesimal-model of an evolving quantitative trait with density-dependent demography. To gain insights beyond simulating the model, we derive analytical approximations of the probability of extinction using a mixture of deterministic recursion equations and branching process theory (Harris 1964). We assume discrete, non-overlapping generations. The life cycle starts with viability selection. In each generation *t*, we impose stabilizing selection around some optimal trait value θ_t, which is set by the environment in that generation, by making the probability of survival

$$s_t(z) = \exp\left(\frac{-(\theta_t - z)^2}{2\omega^2}\right), \quad (1)$$

a Gaussian function of phenotype, *z*, with a strength of selection proportional to $1/\omega^2$.

Following viability selection, survivors randomly mate and produce a Poisson number of offspring with mean 2λ. The habitat supports at most *K* individuals. If more than *K* offspring are produced, only *K* are randomly chosen. An offspring's breeding value is a draw from a normal distribution centered on the mean of its parents' breeding values and with a fixed segregation variance V_0, i.e. the infinitesimal model (Fisher 1918, Turelli 2017). Its phenotype, z, is this breeding value, *g*, plus a random environmental component, *e*, which is a draw from a normal distribution with mean 0 and variance V_e. We ignore dominance and epistasis, thus the phenotypic variance in generation *t* is the additive genetic variance plus the environmental variance, $V_{p,t} = V_{g,t} + V_e$. At equilibrium, $\hat{V}_{p,t} = \hat{V}_{g,t} + V_e$.

Prior to experiencing an extreme event, the populations in the individual-based simulations start with a 100-generation burn-in from an initial state where all $N = K$ individuals have breeding value $\theta = 0$ corresponding to the optimal trait value θ_t during this period (Figure S1.1). To model the extreme event of length τ, the optimum trait value increases by $\Delta\theta$ and

reverts back to its original value after τ generations (Figure 1.1). Unless otherwise stated, we use the parameter values ω^2 =1, $\hat{V}_p$ =1, $\Delta\theta$ = 2.5, λ = 2, and K = 500. These ω^2 and $\hat{V}_p$ values represent strong selection and large phenotypic variance relative to those estimated in (Turelli 1984). Reducing the strength of selection or phenotypic variance reduces lag load but does not otherwise change our qualitative results. For this set of parameter values, the optimum shift ($\Delta\theta$ = 2.5) corresponds to two and a half standard deviations beyond the mean of the trait distribution, and consequently, we expect roughly 85% of the population to die in the first generation. We have chosen a high growth rate, λ, to reduce extinction from demographic stochasticity in the absence of disturbance. We chose a large enough starting population size and carrying capacity, K =500, to make approximations reasonable (e.g. normal distribution of traits).

Approximations

Approximating the evolutionary and population size dynamics

In Appendix A, we derive deterministic approximations for the dynamics of the mean breeding value $\bar{g}_t$, genetic variance $V_{g,t}$, and population size N_t. If we assume the distribution of breeding values remains normally distributed, then we show

$$\bar{g}_{t+1} = \bar{g}_t\left(1 - \frac{V_{g,t}}{V_t}\right) + \theta_t \frac{V_{g,t}}{V_t} \quad (2)$$

$$V_{g,t+1} = \frac{V_{g,t}V_s}{V_t}\frac{1}{2} + V_0, \quad (3)$$

where $V_t = V_{g,t} + V_s$, with $V_s = \omega^2 + V_e$ the inverse of the effective strength of selection. We also show that the population size in the next generation is

$$N_{t+1} = \min(N_t \bar{s}_t \lambda, K), \quad (4)$$

where the mean survival probability, $\bar{s}_t$, is $\sqrt{\omega^2/V_t}\exp\left[-(\theta_t - \bar{g}_t)^2/(2V_t)\right]$.

Regardless of the trait or environmental dynamics, the genetic variance approaches an equilibrium $\hat{V}_g = \left(2V_0 - V_s + \sqrt{4V_0^2 + 12V_0V_s + V_s^2}\right)/4$. In a constant environment, $\theta_t = \theta$for all *t*, the mean breeding value approaches the optimum, $\hat{\bar{g}} = \theta$, and, provided $\lambda > 1$, $N_0\bar{s}_0$is large enough, and $\hat{V}_p = \hat{V}_g + V_e$ is small enough, the population size reaches carrying capacity, $\overline{N} = K$. Starting from this equilibrium, we can then approximate the response of the population to a shift in the optimum using Equations (2)-(4).

Approximating Extinction Risk

We next approximate the probability of extinction using branching processes (Harris 1964). The probability generating function for an individual with the average survivorship in generation *t* is $f_t(x) = 1 - \bar{s}_t \exp\left[-(1-x)\lambda\right]$. Assuming that the effects of density-dependence are negligible, we can approximate the probability of extinction by the end of generation *T* since an extreme event of length τ began as

$$P_{extinct}(\tau, T) = (f_1 \circ f_2 \circ \ldots f_T(0))^K. \quad (5)$$

To calculate $\bar{s}_t$, we assume $V_{g,t} = \hat{V}_g$ and use Equation (2) to get $\bar{g}_t$, which together give $\bar{s}_t$ (Equation (1)).

Results

Demographic recovery

We first explore extreme events lasting a single generation. To characterize the impact of phenotypic variance and heritability on population size, we compare the demographic response of populations with low or high phenotypic variance, $\hat{V}_p$, across a range of heritabilities, $h^2 = \hat{V}_g / \hat{V}_p$. During the event, heritability has no effect on population size (Figure 1.1A) but

phenotypic variance does (Figure 1.2A). A population with higher phenotypic variance has a smaller population size immediately following a low severity extreme event, but a larger population size following a high severity event. This pattern stems from the dual role of phenotypic variance, in that it both increases variance load and contributes individuals with extreme traits who are able to survive an extreme event. High phenotypic variance therefore reduces both mean fitness within a generation and the variance in fitness across generations – a form of short-term bet-hedging which can increase the geometric mean of fitness in the generations during and after the disturbance event.

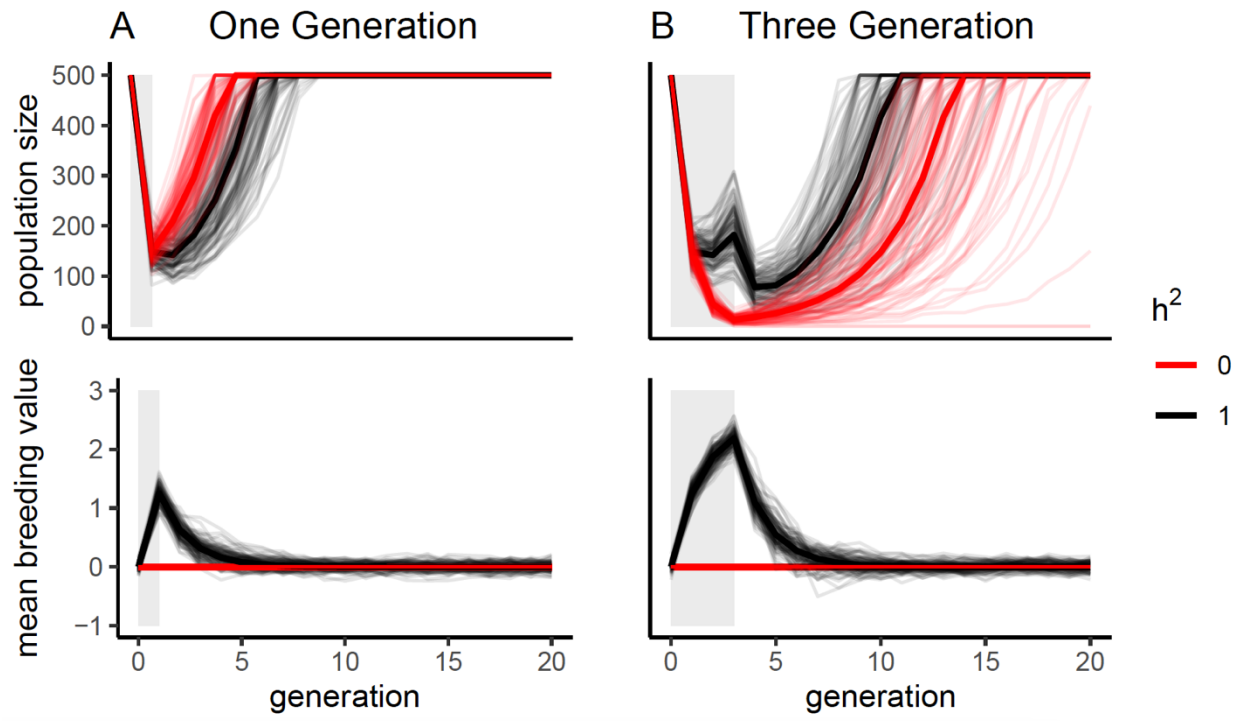


Figure 1.1. Population size over time for populations with ($h^2 = 1$, black) and without ($h^2 = 0$, red) evolution after (A) a single-generation extreme event or (B) a three-generation extreme event (event length shaded in gray). Phenotypic variance is the same for both populations ($\hat{V}_p = 1$). Faded lines are 100 simulations and solid lines are the model predictions using Equations (2) and (4). Parameters: $\omega = 1, \lambda = 2, \Delta\theta = 2.5$. Red: $V_0 = 0, V_e = 1$, Black: $V_0 = 3/4, V_e = 0$.

While heritability has no effect on survival during the event, it has a strong effect on population recovery in subsequent generations. In particular, heritability dampens the growth rate in subsequent generations (Figure 1.1A) as evolution in the generation of the event induces future maladaptation. The maladaptation induced by heritability continues past the generation after the event, generally slowing population recovery. For longer events, evolution can help population recovery (Figure 1.1B). While evolution still increases maladaptation after the event, the increase it causes in growth rates during the event can more than compensate.

Extinction Risk

When a single-generation extreme event is severe enough, increasing phenotypic variation lowers extinction risk both during and after the event (Figure 1.2B). The biological intuition behind this pattern is the same as in Figure 1.2A, where increased variance means more individuals survive the extreme event. However, at such large population sizes the extinction risk is essentially zero during a mild event. In other words, while having too much variance leads to considerable reduction in population size when events are mild, it is very unlikely to lead to extinction unless there is extremely high phenotypic variance or if carrying capacity is very low. In the former case load will cause extinction in the absence of extreme events (Supplementary Figure S1.2).

To isolate the effect of evolution, we next compare extinction risk for populations with the same phenotypic variance but different heritabilities. When the extreme event lasts only one generation (Figure 1.3A), heritability increases short and long-term extinction risk. However, for two generation events, long-term extinction risk is lowest at intermediate heritabilities (Figure 1.3B). For three generation events (or longer), long-term extinction risk decreases with heritability (Figure 1.3C). These patterns hold for milder $\Delta\theta = 2.5$) and more severe ($\Delta\theta = 4.5$)

extreme events (Figures S1.3-S1.4).

While Equation (5) gives a good approximation of extinction risk, the function itself is too complex to give us intuition. Next, by writing down the geometric mean fitness of a population, we reproduce the general trends in long-term extinction risk, but with added clarity for how maladaptation contributes to these outcomes.

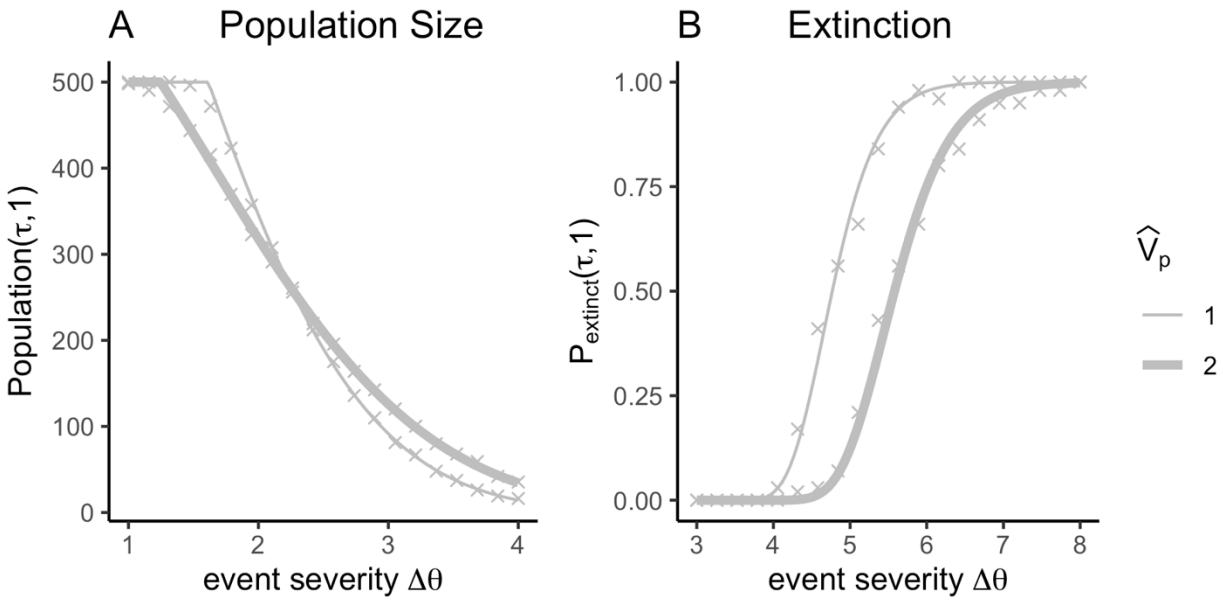


Figure 1.2. Population size (A) and extinction risk (B) during the generation of a single-generation extreme event across a range of event severities Δθ. Expectations using Equation (4) as curves and simulation results (mean of 100 replicates) as crosses. Parameters: $\omega = 1, \lambda = 2, h^2 = 0.5$. Thick gray: $V_0 = 2/3, V_e = 1$. Thin gray: $V_0 = 5/16, V_e = 1/2$.

Contribution of Lag Load

To better understand how evolution affects the probability of extinction, we can approximate the geometric mean fitness of a population as

$$\bar{W}(\tau, T) = \lambda\sqrt{\omega^2/\hat{V}}\exp\left[-\frac{1}{2\hat{V}T}\sum_{t=1}^{T}(\theta_t - \bar{g}_t)^2\right] \quad (6)$$

where $\sum_{t=1}^{T}(\theta_t - \bar{g}_t)^2/(2\hat{V})$ is the cumulative lag load. This cumulative lag load over $T > \tau$ generations is

$$L(\tau, T) = \frac{\Delta\theta^2}{2\hat{V}} \frac{[1-(1-v)^2][2-(1-v)^{2(T-\tau)}+(1-v)^{2T-\tau}]}{(2-v)v}, \quad (7)$$

where $v = \hat{V}_g/\hat{V}$ is a measure of evolvability (Charlesworth 1993, see Appendix C). In the long term, the cumulative lag load is

$$L_\infty(\tau) \equiv \lim_{T\to\infty} L(\tau, T) = \frac{\Delta\theta^2}{\hat{V}} \frac{1-(1-v)^\tau}{(2-v)v}. \quad (8)$$

Equation (8) generalizes a result of (Chevin 2013), who considered the special case of weak selection and a press perturbation $\tau \to \infty$).

Noting that $v = h^2\hat{V}_p/(\hat{V}_p + \omega^2)$, Equation (8) determines how heritability affects the long-term cumulative lag load (Figure 1.3D). When the extreme event only lasts one generation (τ =1), the cumulative lag load simplifies to $\frac{\Delta\theta^2}{2(\hat{V}_p+\omega^2)-h^2\hat{V}_p}$. Hence, increasing heritability increases the cumulative lag load (solid blue curve in Figure 1.3D), a trend consistent with the extinction probabilities for τ =1 (Figure 1.3A). Alternatively, when the extreme event lasts two generations (τ =2), the cumulative lag load becomes $\frac{\Delta\theta^2}{\hat{V}_p+\omega^2}$ and is independent of heritability (solid green curve in Figure 1.3D). Finally, when the extreme event lasts for more than two generations, the cumulative lag load decreases with heritability (solid red curve in Figure 1.3D), a trend consistent with extinction probabilities decreasing with heritability when $\tau \geq 3$ (Figure 1.3C).

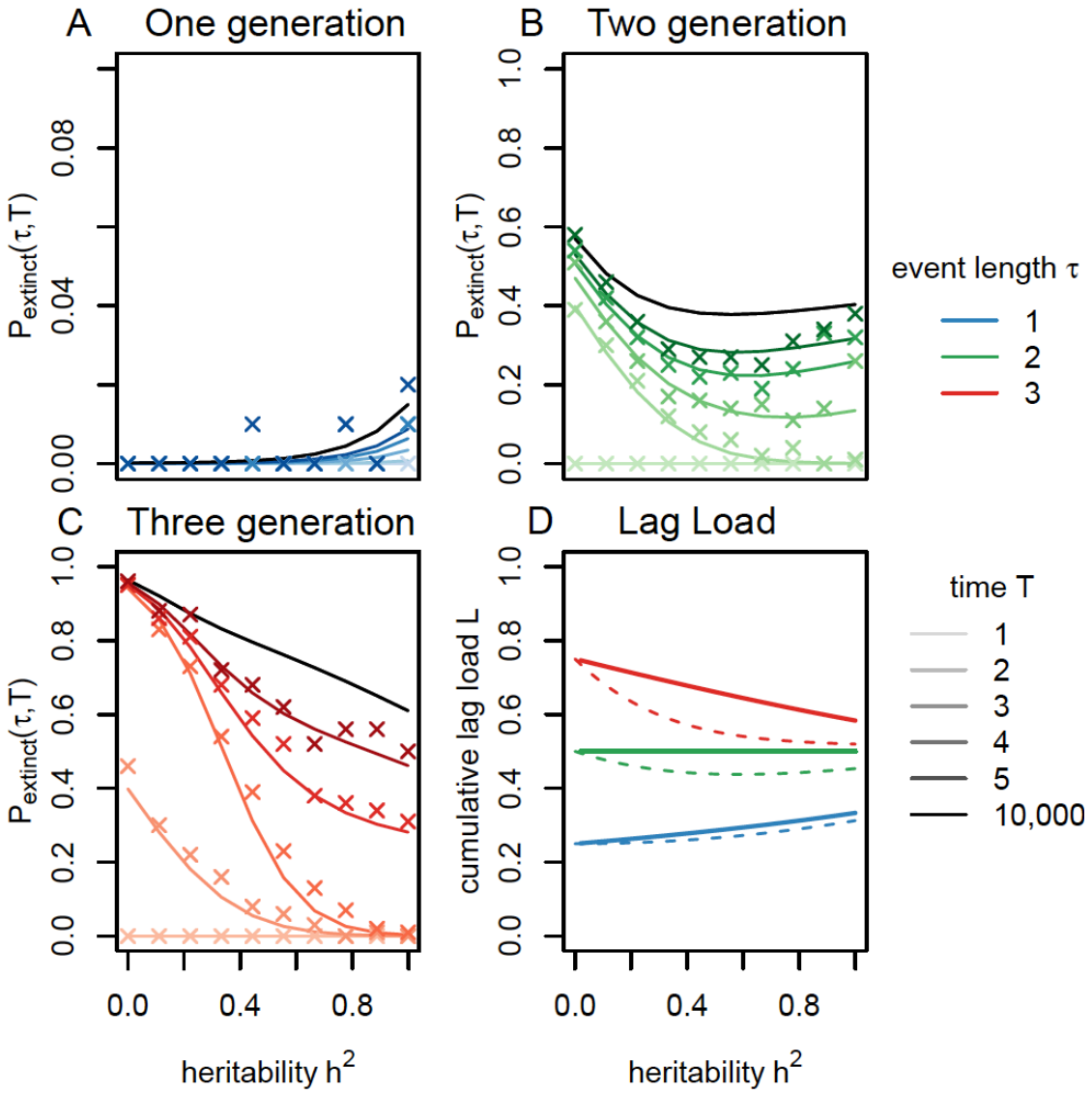


Figure 1.3.(A-C) Extinction risk through time T across a range of heritability for extreme events lasting 1, 2, or 3 generations. $T = 0$ is when the event began. Note, the y-axis in (A) has a maximum at 0.1. Expectations using Equation (5) as curves and simulation results (mean of 100 replicates) as crosses. (D) Cumulative lag load as a function of heritability. Dashed curves show the cumulative load immediately following the event (Equation (7) with $T = \tau + 1$ and solid curves show the cumulative load in the long term (Equation (8)). Parameters: For A-C, $\hat{V}_p = 1, \omega = 1, \lambda = 2, \Delta\theta = 3.5$. For D, $\hat{V}_p = 1, \omega = 1, \lambda = 2, \Delta\theta = 1$.

Discussion

Although it has long been recognized that evolution may affect a population's response to a changing environment, previous studies have primarily focused on understanding this effect over the long term following a non-reversing environmental shift (a press disturbance) or a continuously fluctuating environment. Here, we are concerned with the short-term effect of a pulse disturbance on population growth and extinction risk. By allowing pulses to be of any duration we connect our results with this existing literature while providing new insights into the transient dynamics following a single disturbance. Our results provide two general conclusions about the effect of trait variation and its heritability on population growth and extinction risk during and following a pulse disturbance. First, trait variance, whether it is heritable or not, is a double-edged sword: it adds a variance load due to stabilizing selection, yet also provides individuals with extreme traits who can survive large shifts in the environment. Second, while variance can be useful in the generation of a severe event, if heritable it can induce maladaptation that slows demographic recovery and therefore increases extinction risk in the generations after the event.

Phenotypic Variance

Phenotypic variance, whether heritable or not, can be beneficial or deleterious. A simultaneous reduction in the mean and variance in fitness before and during an extreme event can increase the short-term geometric mean of fitness (Figure 1.2). This increase only occurs when disturbances are sufficiently severe. Furthermore, variation in survival rates reduces variation in the total number of offspring produced by the population (Kendall and Fox 2002) and thereby lowers extinction risk (Lloyd-Smith et al. 2005). Prior studies of evolutionary rescue have emphasized the beneficial aspect of genetic variance (Alexander et al. 2014, Barfield and

Holt 2016, Bell and Collins 2008, Charlesworth 1993, Gomulkiewicz and Holt 1995), but not non-heritable phenotypic variance, in rescuing a population from an abrupt shift in environment. Here, by teasing out the effects of heritability and phenotypic variance, we emphasize the costs and benefits of each.

Heritability

Contrary to evolutionary rescue of populations experiencing a press-perturbation (Barfield and Holt 2016, Gomulkiewicz and Holt 1995), we find that heritability increases extinction risk for short pulse perturbations. We can gain some intuition for why this is by considering the limiting cases of traits not evolving versus tracking the optimal trait perfectly with a one generation lag. When the population is adapted to the original environment, but does not evolve in response to the extreme event, it experiences a reduction in fitness for the duration τ of the extreme event. In contrast, when selection tracks the optimal trait with a one generation lag, the population experiences a reduction in fitness only in the first and last generation of the extreme event. Hence, when the extreme event lasts one generation, extinction risk is higher for the evolving populations and when the extreme event lasts more than two generations, extinction risk is higher for the non-evolving populations. A similar understanding can be gained by adapting a classic population genetic model of allele frequency change with time-varying selection (Dempster 1955, Felsenstein 1976, see Appendix D).

In general, the trends in short-term extinction risk are parallel to the lag load predictions (Figure 1.3). However, they differ in two ways. First, when the extreme event lasts exactly two generations, the non-evolving population experiences the reduction in fitness in successive generations while the evolving population experiences this reduction in alternate generations. Hence, the evolving population is slightly less likely to go extinct (see Appendix B). Second,

when a population exhibits an intermediate amount of tracking of the optimum, the variance in survival from year to year is reduced and therefore can lower the overall extinction probability (Figure 1.3B).

While previous studies of temporally variable selection have focused on large populations in the long term, calculating lag load and growth rates when rare, they provide intuition for our results on short-term extinction risk after a one-time event. A single-generation extreme event functions like a negatively autocorrelated fluctuating environment where a strong genetic response to selection in one generation is likely maladaptive in the next generation (Benaim and Schreiber 2019, Charlesworth 1993, Chevin 2013, Lande and Shannon 1996). However, an extreme event lasting three or more generations acts like a positively autocorrelated environment where evolution tends to reduce maladaptation.

Future Challenges and Directions

Our models include a number of simplifications to both evolutionary and demographic processes. First, we do not model the erosion of genetic variance with decreasing population size (Barfield and Holt 2016, Lande and Barrowclough 1987). Furthermore, we have limited our analysis to truly continuous traits, but different genetic architectures, such as a few loci of large effect, likely will respond differently (Barghi et al. 2020). Second, our model ignores the potential for phenotypic plasticity, which has variable effects on evolution and extinction risk (Kopp and Matuszewski 2014, Lande 2015). Third, we used the simplest possible model for density-dependence, the ceiling model, as used in previous evolutionary rescue studies (Burger and Lynch 1995). For other models of compensating density-dependence, we expect similar results. However, over-compensatory density-dependence can result in oscillatory population-dynamics for which the timing of the extreme event may play a subtle role.

An important next step will be to understand evolution and extinction risk under repeated extreme events. Extreme events, or catastrophic events, can be characterized by causing abrupt, infrequent, and large reductions in biomass or population size. Hence, prior work on adaptation and persistence using autoregressive processes to model environmental fluctuations (Benaim and Schreiber 2019, Charlesworth 1993, Chevin 2013, Lande and Shannon 1996), do not accurately capture the nature of repeated, pulsed disturbances of the type considered here. We hope future studies exploring the impact of disturbance regime on evolution and extinction risk will benefit from the detailed understanding, like that provided here, of an evolving population's response to a single extreme event.

Acknowledgments

This work was supported by the U.S. National Science Grant DMS-1716803 to SJS, Center for Population Biology (University of California - Davis) and Banting (Canada) fellowships to MMO, and a National Institute of General Medical Sciences of the National Institutes of Health grant (NIH R01 GM108779) to Graham Coop.

References

Alexander, H. K., G. Martin, O. Y. Martin, and S. Bonhoeffer. 2014. Evolutionary rescue: linking theory for conservation and medicine. Evolutionary Applications 7:1161–79.

Barfield, M., and R. D. Holt. 2016. Evolutionary rescue in novel environments: towards improving predictability. Evolutionary Ecology Research 17:771–786.

Barghi, N., J. Hermisson, and C. Schlötterer. 2020. Polygenic adaptation: a unifying framework to understand positive selection. Nature Reviews Genetics 1–13.

Barton, N. H., A. M. Etheridge, and A. Véber. 2017. The infinitesimal model: Definition, derivation, and implications. Theoretical Population Biology 118:50–73.

Bell, G. 2017. Evolutionary rescue. Annual Review of Ecology, Evolution, and Systematics 48:605–627.

Bell, G., and S. Collins. 2008. Adaptation, extinction and global change. Evolutionary Applications 1:3–16.

Benaïm, M., and S. Schreiber. 2019. Persistence and extinction for stochastic ecological models with internal and external variables. Journal of Mathematical Biology 79:393–431.

Bender, E. A., T. J. Case, and M. E. Gilpin. 1984. Perturbation experiments in community ecology: theory and practice. Ecology 65:1–13.

Bürger, R., and M. Lynch. 1995. Evolution and extinction in a changing environment: a quantitative-genetic analysis. Evolution 49:151–163.

Charlesworth, B. 1993. Directional selection and the evolution of sex and recombination. Genetics Research 61:205–224.

Chevin, L. M. 2013. Genetic constraints on adaptation to a changing environment. Evolution 67:708–21.

Dempster, E. R. 1955. Maintenance of genetic heterogeneity. Cold Spring Harbor Symposium 20:25–32.

Donihue, C. M., A. Herrel, A. C. Fabre, A. Kamath, A. J. Geneva, T. W. Schoener, J. J. Kolbe, and J. B. Losos. 2018. Hurricane-induced selection on the morphology of an island lizard. Nature 560:88–91.

Ellner, S. P., R. E. Snyder, P. B. Adler, G. Hooker, and S. J. Schreiber. 2020. Technical comment on Pande et al.(2020): Why invasion analysis is important for understanding coexistence. Ecology Letters 23:1721–1724.

Felsenstein, J. 1976. The theoretical population genetics of variable selection and migration. Annual Review of Genetics 10:253–280.

Fisher, R. A. 1918. The correlation between relatives on the supposition of mendelian inheritance. Transactions of the Royal Society of Edinburgh 52:399–433.

Fox, G. A., and J. Gurevitch. 2000. Population numbers count: tools for near-term demographic analysis. The American Naturalist 156:242–256.

Gomulkiewicz, R., and R. D. Holt. 1995. When does evolution by natural selection prevent extinction? Evolution 49:201–207.

Grant, P. R., and B. R. Grant. 2014. 40 years of evolution: Darwin's finches on Daphne Major Island. Princeton University Press.

Grant, P. R., B. R. Grant, R. B. Huey, M. T. J. Johnson, A. H. Knoll, and J. Schmitt. 2017. Evolution caused by extreme events. Philosophical Transactions of the Royal Society B: Biological Sciences 372.

Harris, T. E. 1964. The theory of branching process. Rand Corporation.

Hastings, A., K. C. Abbott, K. Cuddington, T. Francis, G. Gellner, Y.-C. Lai, A. Morozov, S. Petrovskii, K. Scranton, and M. L. Zeeman. 2018. Transient phenomena in ecology. Science 361.

Holling, C. S. 1973. Resilience and stability of ecological systems. Annual review of ecology and systematics 4:1–23.

———. 1996. Engineering resilience versus ecological resilience. Pages 31–44 in P. Schulze, ed. Engineering Within Ecological Constraints. National Academy Press, Washington DC.

Holt, R. D. 2008. Theoretical perspectives on resource pulses. Ecology 89:671–681.

Ives, A. R., and S. R. Carpenter. 2007. Stability and diversity of ecosystems. Science 317:58–62.

Kéfi, S., V. Dominguez-García, I. Donohue, C. Fontaine, E. Thébault, and V. Dakos. 2019. Advancing our understanding of ecological stability. Ecology Letters 22:1349–1356.

Kendall, B. E., and G. A. Fox. 2002. Variation among individuals and reduced demographic stochasticity. Conservation Biology 16:109–116.

Kopp, M., and S. Matuszewski. 2014. Rapid evolution of quantitative traits: theoretical perspectives. Evolutionary Applications 7:169–91.

Lande, R. 1993. Risks of population extinction from demographic and environmental stochasticity and random catastrophes. The American Naturalist 142:911–927.

———. 2015. Evolution of phenotypic plasticity in colonizing species. Molecular Ecology 24:2038–2045.

Lande, R., and G. Barrowclough. 1987. Effective population size, genetic variation, and their use in population management. Pages 87–123 in S. ME, ed. Viable populations for conservation. Cambridge Univ Press, New York.

Lande, R., and S. Shannon. 1996. The role of genetic variation in adaptation and population persistence in a changing environment. Evolution 50:434–437.

Lloyd-Smith, J. O., S. J. Schreiber, P. E. Kopp, and W. M. Getz. 2005. Superspreading and the effect of individual variation on disease emergence. Nature 438:355–359.

Ozgul, A., T. Coulson, A. Reynolds, T. C. Cameron, and T. G. Benton. 2012. Population responses to perturbations: the importance of trait-based analysis illustrated through a microcosm experiment. The American Naturalist 179:582–594.

Pande, J., T. Fung, R. Chisholm, and N. M. Shnerb. 2020. Mean growth rate when rare is not a reliable metric for persistence of species. Ecology Letters 23:274–282.Turelli, M. 1984.

Heritable genetic variation via mutation-selection balance: Lerch's zeta meets the abdominal bristle. Theoretical population biology 25:138–193.

———. 2017. Commentary: Fisher's infinitesimal model: a story for the ages. Theoretical Population Biology 118:46–49.

Ummenhofer, C. C., and G. A. Meehl. 2017. Extreme weather and climate events with ecological relevance: a review. Philosophical Transactions of the Royal Society B: Biological Sciences 372:20160135.

Yahalom, Y., and N. M. Shnerb. 2019. Phase diagram for logistic systems under bounded stochasticity. Physical Review Letters 122:108102.

Yodzis, P. 1988. The indeterminacy of ecological interactions as perceived through perturbation experiments. Ecology 69:508–515.

Am. Nat. 2021. Vol. 198, pp. 44–52.

Appendix A Dynamics of the breeding value distribution and population size

$$V_{p,t} = V_{g,t} + V_e$$

Let the trait value of an individual be the sum of a genetic component (breeding value) and an environmental component, $z = g + e$. Assume we start with a population, in generation t, that has a normal distribution of breeding values, $p_g(g,t)$, with mean $\bar{g}_t$and variance $V_{g,t}$. And assume each environmental component is independently chosen from a normal distribution, $p_e(e)$, with mean 0 and variance V_e. The joint distribution of g and e, $p_{g,e}(g,e,t)$, , is then initially multivariate normal with mean $(\bar{g}_t, 0)$, variances $V_{g,t}$ and V_e, and no covariance.

Let the probability of survival for an individual with trait value z in generation t be

$$s(z,t) = exp\left[-\frac{(\theta_t - z)^2}{2\omega^2}\right], \quad \text{(A1)}$$

where θ_t is the optimum trait value in generation t and ω^2 is the strength of selection.

The joint distribution of g and e following viability selection is

$$p'_{g,e}(g,e,t) = \frac{s(z,t)p_{g,e}(g,e,t)}{\bar{s}_t}, \quad \text{(A2)}$$

where

$$\bar{s}_t = \int_{-\infty}^{\infty}\int_{-\infty}^{\infty} s(z,t)p_{g,e}(g,e,t)dgde \quad \text{(A3)}$$

$$= \sqrt{\frac{\omega^2}{\mathrm{V_t}}}\exp\left[-\frac{(\theta_t - \bar{g})^2}{2\mathrm{V_t}}\right]$$

is the expected fraction of the population that survives in generation t (i.e., the population mean survival probability), with $\mathrm{V_t} = \mathrm{V_{g,t}} + \mathrm{V_s}$ and $\mathrm{V_s} = \omega^2 + \mathrm{V_e}$ the inverse of the effective strength of selection. Integrating over environmental effects then gives the distribution of breeding values amongst the survivors

$$p'_g(g,t) = \int_{-\infty}^{\infty} p'_{g,e}(g,e,t)de, \quad \text{(A4)}$$

which is normal with mean $\bar{g}_t\left(1-\mathrm{V_{g,t}}/\mathrm{V_t}\right) + \theta_t\mathrm{V_{g,t}}/\mathrm{V_t}$ and variance $\mathrm{V_{g,t}}\left(1-\mathrm{V_{g,t}}/\mathrm{V_t}\right)$. The mean breeding value is thus shifted towards θ_t with a weight of $V_{g,t}/V_t$ and the genetic variance has been reduced by this fraction.

We next assume that the breeding value is determined by a large number of small effect loci, such that the distribution of breeding values amongst siblings, $\mathrm{p_{g,sibs}(g|g_{mid})}$, is normal with a mean equal to the midpoint of the parental breeding values, $\mathrm{g_{mid}}$, and a variance, V_0, that does not depend on the parental genotypes or trait values (i.e., the infinitesimal model; Barton et al. 2017, Fisher 1918). The distribution of breeding values among the offspring is then

$$p_g(g, t+1) = \int_{-\infty}^{\infty}\int_{-\infty}^{\infty} p'_g(g_m, t)p'_g(g_p, t)\mathrm{p}_{\mathrm{g,sibs}}\left(\mathrm{g}\middle|(g_m + g_p)/2\right)dg_m dg_p, \qquad \text{(A5)}$$

which is normal with mean

$$\bar{g}_{\mathrm{t}+1} = \bar{\mathrm{g}}_{\mathrm{t}}\left(1 - \frac{\mathrm{V_{g,t}}}{\mathrm{V_t}}\right) + \theta_{\mathrm{t}}\frac{\mathrm{V_{g,t}}}{\mathrm{V_t}} \qquad \text{(A6)}$$

and variance

$$V_{g,t+1} = \frac{\mathrm{V_{g,t}}}{\mathrm{V_t}}\frac{1}{2} + V_0. \qquad \text{(A7)}$$

That is, the mean breeding value remains constant through reproduction while the variance before reproduction is first halved (due to essentially "blending inheritance" between the parents) and then increased by segregation, V_0.

So we see that given the initial distribution of breeding values is normal, with Gaussian selection the breeding value distribution remains normal, allowing us to track the entire distribution of breeding values (and therefore phenotypes) across generations by keeping track of only its mean and variance. The variance dynamics are independent of the environment (θ_{t}) and the breeding values; solving Equation (A7) gives the genetic variance in generation any t. This expression is rather complicated (see Mathematica file), however it reaches an equilibrium

$$\hat{V}_g = \frac{2V_0 - V_s + \sqrt{4V_0^2 + 12V_0V_s + V_s^2}}{4} \qquad \text{(A8)}$$

Holding genetic variance constant at its equilibrium (which is reasonable given the variance is not expected to change with the environment or breeding values), in a constant environment, $\theta_{\mathrm{t}} = \theta$, the mean breeding value in any generation t is found by solving Equation (A6),

$$\bar{g}_t = \theta - (\theta - \bar{g}_0)\left(1 - \frac{\hat{V}_g}{V_s + \hat{V}_g}\right)^t, \qquad \text{(A9)}$$

implying a geometric approach to $\bar{g}_t = \theta$ that becomes faster with $\hat{V}_g/(V_s + \hat{V}_g)$.

We assume each individual that survives viability selection produces λ offspring, and that if more than K offspring are produced then K of these are randomly chosen to start the next generation. If the population size in generation t was N_t then the population size in generation $t+1$ is expected to be

$$N_{t+1} = \min\left(N_t \bar{s}_t \lambda, K\right) \tag{A10}$$

Appendix B Extinction Risk in Single and Two Generation Events

In this Appendix, we examine the effect of long-term extinction risk when populations are either not evolving or are perfectly tracking, with a one-generation lag behind the optimal trait value. Let s_o and s_m be the survivorship of individuals with the optimal trait or the maladaptive trait. The offspring probability generating functions for these individuals are $\mathrm{f_o(x)} = \mathrm{f(x, s_o)}$ and $\mathrm{f_m(x)} = \mathrm{f(x, s_m)}$, respectively, where $\mathrm{f(x, s)} = 1 - \mathrm{s} + \mathrm{s}\exp\big(\lambda(1-\mathrm{x})\big)$. Let $\mathrm{x_o^*}$ and $\mathrm{x_m^*}$ be the asymptotic extinction probability for the lineage of a single individual if it always exhibits the optimal trait and if it always is maladapted, respectively. Namely, $\mathrm{x_o^*}$ and $\mathrm{x_m^*}$ are the smallest fixed points of $\mathrm{f_o}$ and $\mathrm{f_m}$ respectively, on the interval $0 \le x \le 1$.

If a disturbance event lasts $\boldsymbol{\tau} \ge 1$ generations, then the eventual extinction probability of the lineage of a non-evolving individual equals

$$e_m := \lim_{T\to\infty} \mathrm{f_m^{\boldsymbol{\tau}}}(\mathrm{f_o^{T-\boldsymbol{\tau}}}(0)) = \mathrm{f_m^{\boldsymbol{\tau}}}(\lim_{T\to\infty} \mathrm{f_o^{T-\boldsymbol{\tau}}}(0)) = \mathrm{f_m^{\boldsymbol{\tau}}}(\mathrm{x_o^*}) \tag{A11}$$

While the eventual extinction probability of the lineage of an individual with a one-generation lagged tracking of the optimal trait equals

$$e_m := \lim_{T\to\infty} \mathrm{f_m}(\mathrm{f_o^{\tau-1}}(\mathrm{f_m}(\mathrm{f_o^{T-\tau-1}}(0)))) = \mathrm{f_m}(\mathrm{f_o^{\tau-1}}(\mathrm{f_m}(\lim_{T\to\infty} \mathrm{f_o^{T-\tau-1}}(0)) = \mathrm{f_m}(\mathrm{f_o^{\tau-1}}\big(\mathrm{f_m}(\mathrm{x_o^*})\big)). \tag{A12}$$

As $s_o > s_m$, we have $\mathrm{f_o(x)} < \mathrm{f_m}(x)$ for all $0 \le x \le 1$, and $\mathrm{x_o^*} < \mathrm{x_m^*}$. Furthermore, $\mathrm{f_i}(x)$ are strictly increasing functions of x, $\mathrm{f_i}(x) > x$ for $x < \mathrm{x_i^*}$, and $\mathrm{f_i}(x) < x$ for $x > \mathrm{x_i^*}$ for $i = o, m$. Now suppose $\boldsymbol{\tau} = 1$. Then $\mathrm{e_m} = \mathrm{f_m}(\mathrm{x_o^*})$ and $\mathrm{e_o} = \mathrm{f_m}\big(\mathrm{f_m}(\mathrm{x_o^*})\big)$. As $\mathrm{x_o^*} < \mathrm{x_m^*}$, $\mathrm{f_m}(\mathrm{x_o^*}) > \mathrm{x_o^*}$. As $\mathrm{f_m}$ is an increasing function, it follows that $\mathrm{e_o} = \mathrm{f_m}\big(\mathrm{f_m}(\mathrm{x_o^*})\big) > \mathrm{f_m}(\mathrm{x_o^*}) = \mathrm{e_m}$. Now suppose $\boldsymbol{\tau} = 2$. Then $\mathrm{e_m} = \mathrm{f_m^2}(\mathrm{x_o^*})$ and $\mathrm{e_o} = \mathrm{f_m}\left(\mathrm{f_o}\big(\mathrm{f_m}(\mathrm{x_o^*})\big)\right)$. As $\mathrm{f_m}(\mathrm{x_o}*) > \mathrm{x_o^*}$, $\mathrm{f_o}\big(\mathrm{f_m}(\mathrm{x_o^*})\big) < \mathrm{f_m}(\mathrm{x_0^*})$. As $\mathrm{f_m}$ is increasing, it follows that $\mathrm{e_o} = \mathrm{f_m}\left(\mathrm{f_o}\big(\mathrm{f_m}(\mathrm{x_o^*})\big)\right) < \mathrm{f_m}\big(\mathrm{f_m}(\mathrm{x_o^*})\big) = \mathrm{e_m}$.

Appendix C Cumulative lag load

Here we show how to derive Equation (7). Our goal is to develop a formula for the cumulative squared displacement, $C(\tau, T) = \sum_{t=1}^{T}(\theta_t - \bar{g}_t)^2$, given event length τ. First note that Equation (2) implies that, with constant genetic variance $\hat{V}_g$, the mean trait displacement in the next generation is $g_{t+1} - \theta_{t+1} = (g_t - \theta_t)(1 - v)$, where $v = \hat{V}_g/\hat{V}$ is a measure of evolvability. Thus, if the optimum is fixed at some arbitrary value for τ generations then the displacement in generation t, $d_t = g_t - \theta_t$, is $d_t = d_0(1 - v)^t$ and the cumulative squared displacement over those $\boldsymbol{\tau}$ generations is $\mathrm{d_0^2}\ \sum_{t=0}^{\tau-1}(1 - v)^{2t}$. If the optimum then reverts to its original value for a further $T - \tau > 0$ generations then the initial displacement is $d_0(1 - v)^{\boldsymbol{\tau}} - d_0$ and the cumulative squared displacement over this period is $\mathrm{d_0^2}\ [(1 - \mathrm{v})^{\tau} - 1]^2 \sum_{t=\tau}^{\mathrm{T}-1}(1 - v)^{2(t-\tau)}$. Combining these two sums we get

$$
\begin{aligned}
&C(\tau, T) = \sum_{t=1}^{T}(\theta_t - \bar{g}_t)^2 \\
&= \mathrm{d_0^2}\ \sum_{t=0}^{\tau-1}(1 - v)^{2t} + \mathrm{d_0^2}\ [(1 - \mathrm{v})^{\tau} - 1]^2 \sum_{t=\tau}^{\mathrm{T}-1}(1 - v)^{2(t-\tau)} \qquad \text{(A13)}
\end{aligned}
$$

Multiplying by $\frac{1-v}{2V_s}$, evaluating the sums, and setting the initial displacement as $d_0 = \Delta\theta$ gives Equation (7) in the main text.

Appendix D Adapting a Population Genetic Model

To gain a better understanding of why cumulative lag load depends on event length, we adapt previous population genetic models of temporally variable selection. Consider a haploid case with the ratio of the initial frequencies of two alleles being $\frac{q_0}{1-q_0}$. The ratio of the frequencies of the alleles at time $T+1$, $\frac{q_{T+1}}{1-q_{T+1}}$, equals the product of the selection coefficients s_t from $t=0$ to time $t=T$, multiplied by the ratio of the initial frequencies (Dempster 1955, Felsenstein 1976)

$$\frac{q_{T+1}}{1-q_{T+1}} = \prod_{t=0}^{T}(1+s_t)\frac{q_t}{1-q_t}. \quad \text{(A14)}$$

Here, rather than allele frequency change, we consider the product of fitnesses over one extreme event of length τ, where s is the selection coefficient of the new optimum trait relative to the initial trait during the event (or vice versa before or after the event). When a population starting at the original optimum perfectly tracks the extreme event, the product of fitnesses is

$$(1-s)(1)^{\tau-1}(1-s)(1)^{T-\tau} = (1-s)^2. \quad \text{(A15)}$$

Fitness is reduced by s initially when the environment shifts to a new optimum, and then again when the environment returns to the original optimum. On the other hand, when a population starting at the original optimum does not track the extreme event, the product of fitnesses is

$$(1-s)(1-s)^{\tau-1}(1)(1)^{T-\tau} = (1-s)^{\tau}. \quad \text{(A16)}$$

In the case of a two generation event, the product of fitnesses is $(1-s)^2$ regardless of whether it is a perfectly tracking population or a population that does not track the event at all. In events longer than two generations, perfectly tracking the environment is better.

Supplementary Information

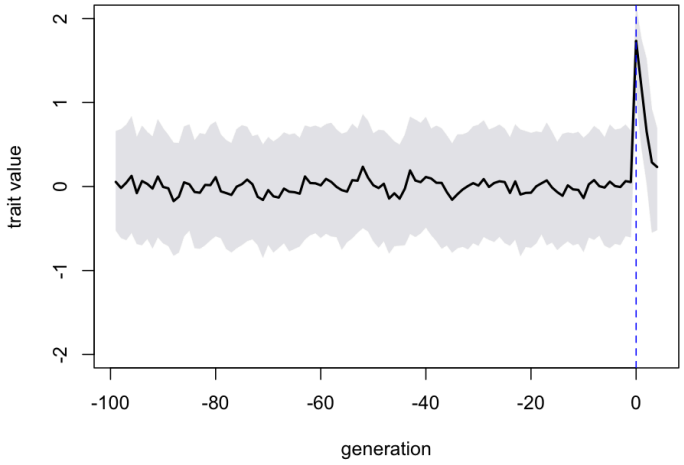


Figure S1.1. Rapid expansion and stabilization of phenotypic variance during the 100 generation burn-in with $V_e = 0$, $V_0 = 1$. Black line is mean trait value and gray shaded region extends from minimum to maximum trait values. The dashed blue curve indicates a one generation extreme event. Parameters: $\omega = 1$, $\lambda = 2$.

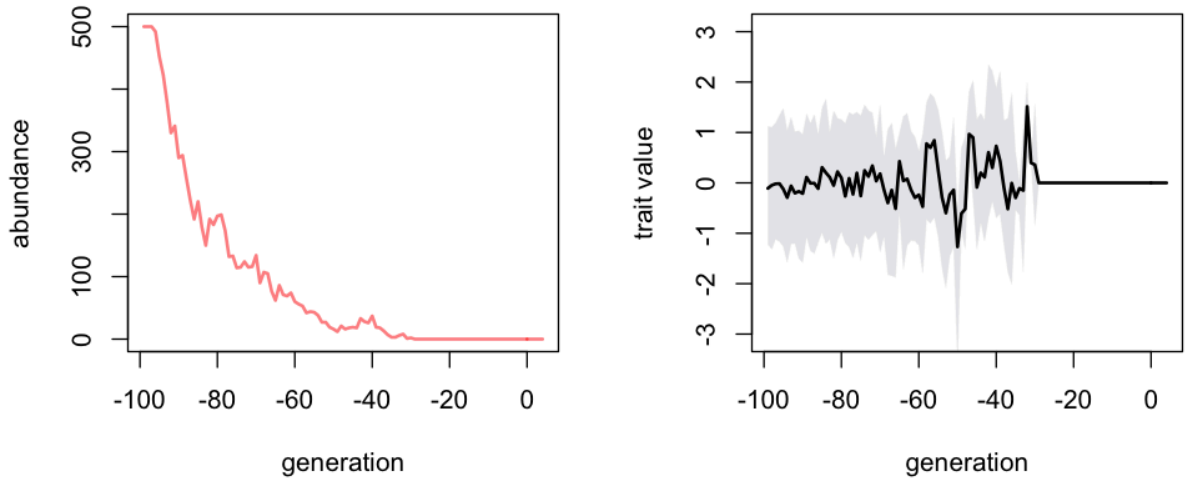


Figure S1.2. Extinction in a population with high variance load with V0 = 3, Ve = 0. Black line is mean trait value, grey shaded region extends from minimum to maximum trait values. Parameters: $\omega = 1$, $\lambda = 2$.

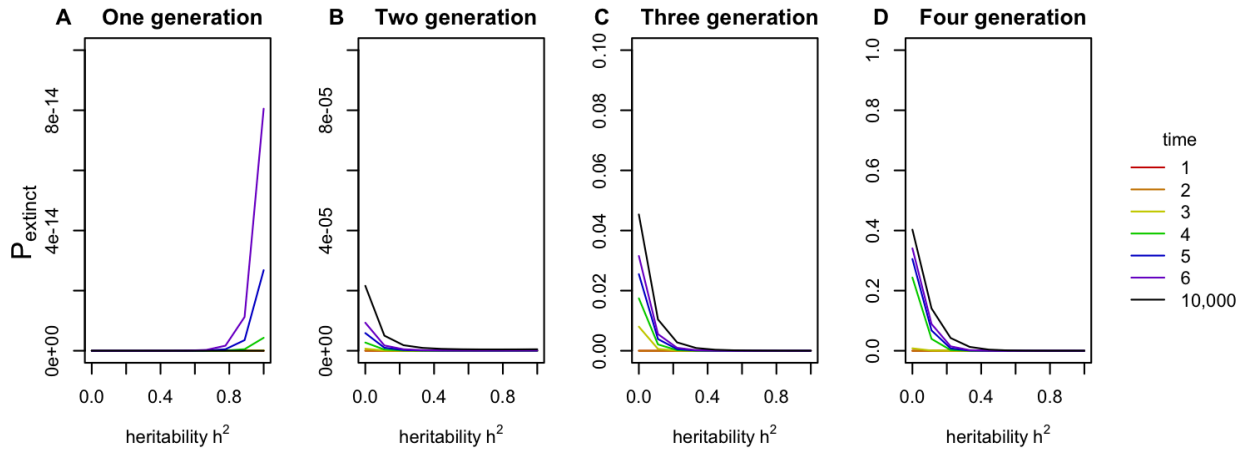


Figure S1.3. Extinction risk through time across a range of heritability for extreme events lasting 1, 2, 3, or 4 generations. Time starts the generation the event began. Parameters: $\hat{V}_p = 1, \omega = 1, \lambda = 2, \Delta\theta = 2.5$.

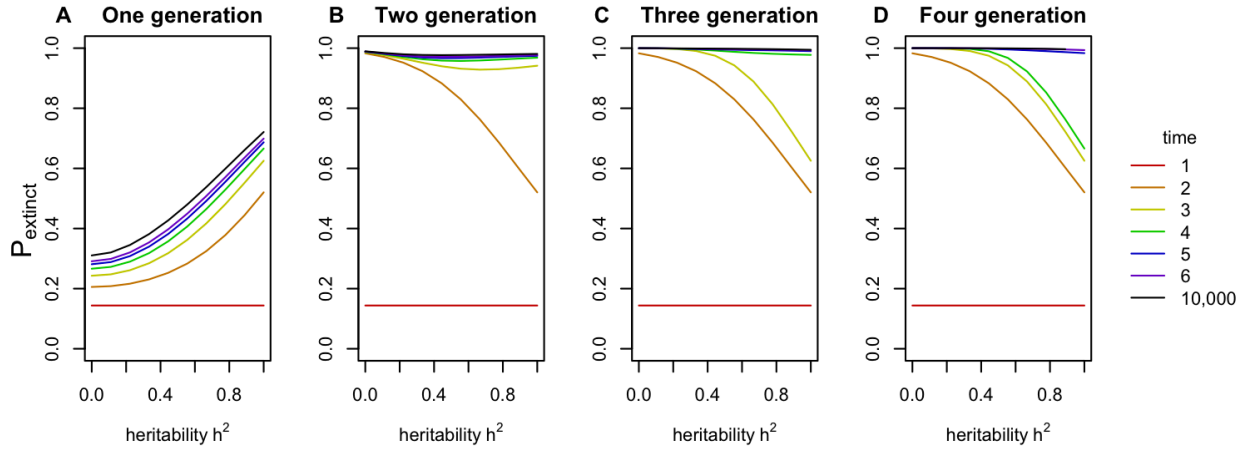


Figure S1.4. Extinction risk through time across a range of heritability for extreme events lasting 1, 2, 3, or 4 generations. Time starts the generation the event began. Parameters: : $\hat{V}_p = 1, \omega = 1, \lambda = 2, \Delta\theta = 4.5$.

Chapter 2

Effects of size selection versus density dependence on life histories: a first experimental probe

Kelsey Lyberger, Thomas W. Schoener, Sebastian J. Schreiber

Department of Evolution and Ecology and Center for Population Biology, University of California, Davis, Davis, CA 95616, U.S.A.

Abstract

When prey experience size-based harvesting by predators, they are not only subject to selection due to larger individuals being preferentially harvested but also selection due to reductions in population density. Density-dependent selection represents one of the most basic interactions between ecology and evolution. Yet, the reduction in density associated with exploitation has not been tested as a possible driving force of observed evolutionary changes in populations harvested size-dependently. Using an artificial selection experiment with a mixture of *Daphnia* clones, we partition the evolutionary effects of size-based harvesting into the effects of removing large individuals and the effects of lowering the population density. We show that both size selection and density-dependent selection are significant drivers of life-history evolution. Importantly, these drivers affected different life-history traits with size-selective harvesting selecting for slower juvenile growth rates and a larger size at maturity, and low density selecting for reduced reproductive output.

Introduction

Rapid evolution of key life-history traits in harvested populations has been documented repeatedly across a range of species (Haugen and Vøllestad 2001, Walsh et al. 2006). Although this phenomenon is particularly concerning in economic contexts such as fisheries, we lack a mechanistic understanding of what drives these rapid evolutionary changes. One such mechanism is size selection, which occurs, for example, when harvesting or predation leads to greater losses of larger individuals thereby shifting the distribution of prey to smaller sizes (Brooks and Dodson 1965). Another mechanism is density-dependent selection, defined as occurring when different genotypes are favored as population density changes (Travis et al. 2013). Here, we focus on density-dependent selection due to intraspecific competition, e.g., per-capita availability of resources increases due to a reduction in their population size. For example, this form of density-dependent selection is responsible for some of the phenotypic differences in Trinidadian guppies at low and high predation sites (Travis et al. 2014). Despite this evidence for both forms of selection, their relative importance for natural systems is not well understood.

Both observation and theory have focused almost exclusively on size selection by predators and other agents as being the major selective mechanism of life-history evolution in harvested populations (Haugen and Vøllestad 2001, Walsh et al. 2006, Kuparinen and Merilä 2007), even though the accompanying reduction in prey population density could be equally or more important. Although the latter has been mentioned in conceptual discussions of this issue (Abrams and Rowe 1996, Reznick et al. 2002, Walsh et al. 2013) and investigated empirically in one system (Walsh and Reznick 2008, Bassar et al. 2012), it has never been experimentally manipulated along with size selection. Determining the relative magnitude of these effects has

many practical implications, for example, whether to set size limits and/or catch limits for harvested populations.

Models of size selection, in which mortality of large individuals increases, predict some combination of a reduction in age or size at maturity, an increase in reproduction, or a reduced growth rate, but exactly which traits evolve depends on which traits are flexible and what trade-offs exist among them (Abrams and Rowe 1996, Williams 1966). Density-dependent selection may also promote these same traits, favoring current investment over future investment if it increased mortality of adults or of all age classes (Charlesworth 1980, Reznick et al. 2002). But density dependence can also act to increase mortality of juveniles, reduce growth rates, or reduce fecundity.

Here we examine the evolutionary impact of density-dependent selection and size selection on life-history traits using a controlled selection experiment in the zooplankter *Daphnia melanica*, collected from a fishless lake. *Daphnia* are particularly suited to studies documenting genetically-based trait changes because of their clonal nature and short generation time. Also, zooplankton are a classic example in which the presence of fish predators leads to smaller body size (Brooks and Dodson 1965), and *Daphnia* in particular are known to show life-history evolution in response to introduced fish predators (Fisk et al. 2007). We raised eight populations and subjected them to culling treatments over several generations. We initiated all populations with identical mixtures of eight clones, thereby controlling for any confounding effects of initial differences in genetic composition that would be present among natural populations, and we manipulated size selection by removing either large individuals or randomly-selected individuals and density-dependent selection by maintaining a high or low density. Subsequent to these manipulations, we raised the surviving clones individually for two generations before

phenotyping to reduce maternal and grand-maternal effects. This approach allowed us to separate the two selective forces predators impose on prey. Further, by comparing phenotypic differences between populations, we quantified the extent to which both density-dependent selection and size selection lead to the evolution of life-history traits.

Methods

Sample collection

To inoculate our microcosms, we collected *Daphnia melanica* from the fishless lake Gable 4 (Sierra Nevada, CA 37°19'41.4"N, 118°41'17.4"W). We chose to use a source population that lacked fish predators to eliminate the possibility that the population had already been subjected to predator-imposed selection. Each field-collected gravid female was placed into its own beaker and allowed to reproduce clonally. From these females, eight clonal lines were established and maintained for 5-6 generations before the start of the selection experiment. To propagate lines across generations, neonates from the second or third clutch were taken and reared individually in100ml containers. No males or resting eggs were observed in the clonal lines prior to or during the experiment, suggesting that individuals only reproduced clonally. By genotyping at five microsatellite loci, we confirmed that the clonal lines were genetically diverse, consisting of five unique multilocus genotypes (supplementary material, Table S2.1). Lines belonging to the same multilocus genotype are potentially unique but indistinguishable across our microsatellite loci. We verified that our lines were clonal by genotyping 3-6 individuals per line. There was no indication of contamination (N=39). All *Daphnia* were maintained in FLAMES media (Celis-Salgado et al. 2008) under constant temperature (16°C) and light (16L:8D) and fed with *Cryptomonas* every other day.

To understand the initial variation among clonal lines, we phenotyped the life-history traits of multiple individuals per clonal line (see *Phenotyping* below). We reared six individuals from each clonal line. As six individuals died before reaching reproductive maturity, sample sizes for the eight clonal lines were: 6, 6, 6, 4, 3, 5, 6, and 6 individuals. We observed variation among clonal lines in all traits (Figure S2.1), with reproduction having the highest coefficient of variation (CV = 0.32), followed by growth rate (CV = 0.19), age at maturity (CV = 0.11), maximum size (CV = 0.05), and size at maturity (CV = 0.03).

Experimental Conditions

We experimentally partitioned the effects of size selection, density-dependent selection, and their interaction using genetically identical populations raised in microcosms. We initiated eight one-liter microcosms, split into two blocks. After enough neonates had been birthed from each clonal line to create genetically identical starting populations, blocks were started two days apart. These starting populations consisted of an equal number of individuals from our eight clonal lines. We manipulated the effect of density-dependent selection by initiating populations with either 16 or 72 neonates per liter. While we do not have a density measure from this lake due to weather conditions, we found density ranges from 0.1 per liter to 100 per liter in lakes in the same species in the same region. More broadly, *Daphnia* in lakes can reach densities up to 4,000 per liter (Kvam and Kleiven 1995) but are not typically that high. Neonates were between 36 and 48 hours old when placed in the mesocosms. All mesocosms were fed the same amount, so that individuals in the high-density treatment were competing more strongly for food. Previous studies have shown that *Daphnia* are typically food-resource limited (McCauley et al. 1988), although other negative effects of crowding have also been documented (Burns 2000).

To manipulate the effect of size selection, we artificially selected out and discarded 20% of adults, either based on size or at random on days 7, 14 , and 21. For the size-selective culling, we measured all adults using an ocular micrometer to sort individuals based on size into 0.2 mm increments and disposed of the top 20%. When populations became larger than roughly 150 individuals, we split the mesocosm into two and the process was repeated for both halves to reduce the time spent out of the environmental chamber. Because mesocosms differed in *Daphnia* density, we removed a different number of individuals from each. Doing so was likely one reason the density treatment did not last very long, as we removed more individuals from the high-density treatments than the low-density treatments.

Although *Daphnia* populations in all microcosms increased over time, the high- and low-density treatments persisted for the first 11 days, but thereafter densities were volatile, with high-density populations generally, but not always, having higher densities than low-density populations (supplementary material, Figure S2.2). We stopped the experiment once the populations began declining. Given that *D. melanica* on average reproduce at 14 days old but can reproduce as young as 10 days old, the experiment lasted one to two generations but may have been as long as three generations. No males nor resting eggs were observed throughout the experiment, suggesting that individuals only reproduced clonally. At the end of the 21-day period, low-density populations ranged from 102 to 180 individuals (mean ± s.d. = 140 ± 37), and high-density populations ranged from130 to 253 individuals (mean ± s.d. = 217 ± 58). We then propagated an average of 10.5 individuals (s.d. = 0.93, min = 10, max = 13) from each experimental population individually for three additional generations after the end of the 21-day period, from each of which we measured traits.

Phenotyping

We measured the life-history traits of the third generation in a controlled environment using standard methods (Lynch 1989). Neonates aged 36-48 hours were taken from the mother's second clutch and reared individually. For 30 days, each individual's size and reproductive status (including number of eggs) was captured with photographs every other day (when media and algae were replaced) with a Cannon EOS Rebel T3i mounted to a microscope at 20x magnification. Size was measured manually using ImageJ by drawing a line segment from the base of the tail to top of the eye. Measurements were calibrated using photographs of a micrometer taken under the same conditions. We measured the same image ten times, repeated for 3 images to obtain measurement error (s.d. = 0.009mm). To find error due to our photographing method, we took 2 photographs of the same individual on the same day, repeated for 5 individuals, and found the average of the 5 ranges was 0.08mm. To determine age at maturity, we recorded the day of the first photo in which eggs appeared, then classified the stage of those eggs as early or late. Early-stage eggs are round, whereas late-stage eggs are oblong with an eyespot. Reproduction was measured as the sum of all eggs produced by Day 30. Ten individuals died before reaching maturity and were excluded from the analysis. In block 1, the samples sizes were 7 from the randomly culled low density treatment, 9 from the large-size culled low density treatment, 11 from the randomly culled high density treatment, and 6 from the large-size culled high density treatment. Sample sizes for block 2 were 9 the randomly culled low density treatment, 9 from the large-size culled low density treatment, 9 from the randomly-culled high density treatment, and 10 from the large-size culled high density treatment.

Statistical Analysis

To estimate the juvenile growth rate and maximum size, an asymptotic regression model was fit to the time series of an individual's growth over time using SSAsympOrig function in the

nlme package in R version 3.3.3 (Pinheiro and Bates 2000). The SSAsympOrig function is $size = Asym * \left(1 - e^{-e^{lrc} * day}\right)$, where *lrc* is the natural log of the growth rate constant and Asymp is the asymptotic (maximum) size. Because the parameter *lrc* is not an intuitive measure of growth, we transformed *lrc* to the time in days to reach half maximum size i.e., $\frac{log2}{e^{lrc}}$. Note that this measure of growth rate is, in part, dependent on the maximum size of an individual. We also assess an alternate measure of growth rate (i.e., size on the fourth measurement minus size on the first measurement divided by six) and alternate measure of maximum size (i.e., the maximum size observed on any day). These results are reported in Table S2.2.

To test for differentiation in traits between high-density and low-density treatments, and between randomly and size-based culling treatments, we performed a two-sided three-way MANOVA using the *manova* function in R version 3.3.3. There was no indication of a deviation from normality. We report the phenotypic correlation structure among traits in Table S2.3. This was followed by two-sided univariate three-way ANOVAs. For all models, we included density treatment, culling treatment, the interaction between density and culling treatment, and block as our predictor variables.

Results

The density treatment, culling treatment, and block were significant explanatory variables for the combined set of life-history traits (MANOVA in Table 2.1). The interaction between density and culling treatment was not significant. Individual tests (ANOVA in Table 2.1) showed density had a significant effect on reproduction, but not on juvenile growth rate, age at maturity, size at maturity, or maximum size. Culling treatment had a significant effect on juvenile growth rate and size at maturity, but not on age at maturity, reproduction, nor maximum size. Neither

block nor the interaction between culling and density treatments was significant in any of the univariate tests.

	Density			Culling			Density x Culling			Block		
	F	d.f.	P	F	d.f.	P	F	d.f.	P	F	d.f.	P
MANOVA (Wilk's)	3.28	5	**0.01**	3.90	5	**0.004**	0.55	5	0.73	2.40	5	**0.05**
ANOVAs												
Growth rate (lrc)	0.29	1	0.59	9.94	1	**0.002**	1.42	1	0.24	3.23	1	0.08
Age at maturity (days)	2.72	1	0.10	1.97	1	0.17	0.09	1	0.77	0.75	1	0.39
Reproduction (eggs in 30 days)	8.81	1	**0.004**	1.16	1	0.29	0.13	1	0.72	2.07	1	0.15
Size at maturity (mm)	1.31	1	0.26	6.66	1	**0.01**	1.18	1	0.28	1.46	1	0.23
Maximum size (mm)	0.56	1	0.46	3.45	1	0.07	1.10	1	0.30	1.18	1	0.28

Table 2.1. Statistical analysis of life-history traits. Results of the MANOVA and univariate linear models, testing for the effect of density treatment, culling treatment, the interaction between density and culling treatment, and block on life-history traits. P-values marked in bold indicate numbers that are significant at the 0.05 level or below. Effect sizes for the MANOVA, calculated as Wilks' Lambda, the multivariate partial η^2 are $\eta^2 = 0.23$ for density, $\eta^2 = 0.25$ for culling, $\eta^2 = 0.27$ for block, and $\eta^2 = 0.05$ for the interaction of density and culling.

Individuals in the size-based culling treatment evolved slower juvenile growth rates and a larger size at maturity (Figure 2.1A, D). The alternate measure of juvenile growth similarly showed size-based culling slowed juvenile growth rate (Table S2.2). The less expected outcome of our study is that individuals in the high-density treatment evolved increased reproductive output (Figure 2.1C).

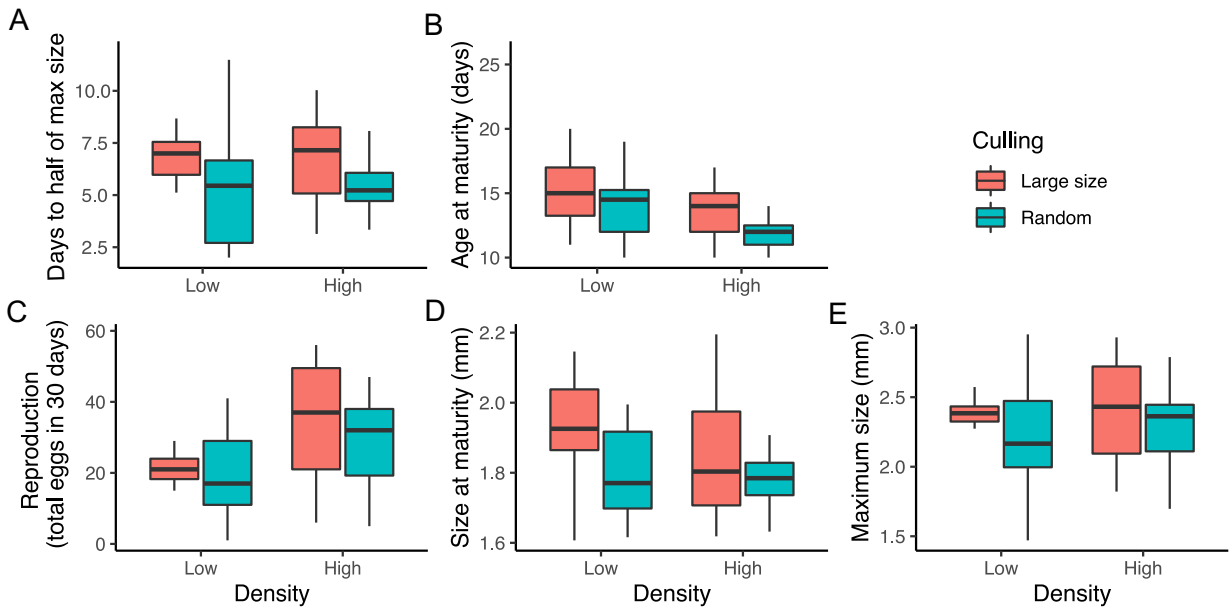


Figure 2.1. Comparison between low- and high-density treatments and large-size-culling and random-culling treatments for (a) days to half maximum size, (b) age at maturity, (c) reproduction, (d) size at maturity, and (e) maximum size. The horizontal line in each grey box is the median, the boxes extend to the first and third quartiles, and the whiskers extend 1.5 times the interquartile range from the boxes. Sample sizes for block 1 are low density/random culling (N = 7), low density/large size culling (N = 9), high density/random culling (N = 11), and high density/large size culling (N = 6). Sample sizes for block 2 are low density/random culling (N = 9), low density/large size culling (N = 9), high density/random culling (N = 9), high density/large size culling (N = 10).

Discussion

While density-dependent selection and size selection are likely operating concurrently in many systems, the evolutionary effects of each have not been partitioned. We found density-dependent selection and size selection have different effects on different life-history traits. Also, we found the two types of selection differ in their magnitude of effects. Density-dependent

selection led to moderately significant differences in reproduction ($P < 0.05$). Size-selection led to very significant differences in growth rate ($P < 0.001$), and, to a lesser extent, differences in size at maturity ($P < 0.05$). These results may reflect differences in the selection intensity on each trait and the amount of initial genetic variation in each trait. Because reproduction was so variable among clonal lines, it is perhaps not surprising that density selection resulted in significant differences. Similarly, growth rate had the second highest coefficient of variation and showed significant effects of size selection. Size at maturity did not vary much among lines but showed significant effects of size selection. It is unlikely growth rate and size at maturity jointly evolved because of linkage disequilibrium, as these traits have a correlation of -0.05.

The evolutionary predictions of density-dependent selection rely on knowing the age(s) at which competition causes increased mortality or reduced fecundity (Charlesworth 1980). We observed the evolution of a higher reproductive rate in the high-density selection treatment. Theory predicts that a greater investment in reproduction is favored when competition increases mortality across all age classes (Reznick et al 2002). The biological intuition is if a high-density environment increases mortality on all stages, the chance of surviving to an older age is lowered; therefore, those able to invest more in offspring will be favored. However, if there was increased mortality on all age classes, we would also predict that an earlier age at maturity would be favored, which is not observed in our data.

The direction of evolution in response to our treatments which mimic harvesting is sometimes but not always the same as the evolutionary effect of harvesting found in other taxa as follows. Previous research by Edley and Law (1988) simulating fish predation in *Daphnia* similarly found culling large individuals favored slower growing clones. While that study did not separate the effects of size selection and density selection, our results suggest that their finding

was the result of size selection. One explanation for the size-selection-driven slower juvenile growth rates is that organisms should linger in stages that have lower mortality rates (Williams 1966), which in our experiment are the younger stages. A similar response to artificial size selection was found in the males of Mozambique tilapia (*Tilapia mossambica)* that evolved reduced growth rates compared to those harvested randomly (Silliman 1975). In another empirical test of size-selective harvesting, Atlantic silversides (*Menidia menidia*) evolved slower juvenile growth rates and reduced fecundity (Conover and Munch 2002, Walsh et al. 2006), although these results differ from what has been observed in fisheries data (Hilborn 2006). In sum, across the various empirical studies of size selection a common outcome has been observed, i.e., slower growth rates.

In contrast, there have been few empirical studies of density-dependent selection. We found *Daphnia* evolved increased reproduction in response to density-dependent selection. Our result differed from that of an experiment evaluating density-dependent selection in Trinidadian guppies (*Poecilia reticulata*), which showed that individuals mature later and have fewer offspring in a high-density, low-predation environment (Bassar et al. 2012, Travis et al. 2014). High density environments can reduce fecundity or increase mortality in specific age classes, which will depend on how intraspecific competition acts within the species. Because of these variable impacts of density on different ages or stages, predictions of life-history evolution by density-dependent selection are particularly sensitive to the specifics of a system. In a review of studies testing density regulation in vertebrates, Bassar et al. (2010) found that reduced fecundity was more common than reduced juvenile survival, which was more common than reduced adult survival, and that most studies found significant effects on more than one demographic variable. Therefore, the effects of density-dependent selection and size selection in other natural systems

may strengthen one another, cancel one another out, or, as we observed, affect different traits. Thus, a major future challenge is understanding how life-history traits of different populations and species of *Daphnia* as well as of different taxa respond to both forms of selection.

Implications for eco-evolutionary feedbacks, food webs, and harvesting

Our findings have important implications for eco-evolutionary feedbacks, food web dynamics, and fisheries management.

First, density-dependent selection represents a direct link between evolution and population dynamics. This link has two components: population density influences the relative fitness of different individuals, and the absolute fitness of individuals influences population growth rate and population size. When population size and fitness influence each other, there is the potential for eco-evolutionary feedback loops (Kokko and López-Sepulcre 2007). Thus far, there are few complete empirical demonstrations of these feedback loops (Schoener 2011, Schoener 2013, Schoener et al. 2014). Here, we have shown the first condition, in which changes in an ecological variable, i.e., population density, drive evolutionary changes in life-history traits. The next step to complete the feedback loop is to show the second condition; that evolutionary changes in life-history traits (which translate into changes in absolute fitness) drives ecological changes at the population level. We believe this condition is likely to be satisfied. In particular, we hypothesize that the higher reproductive rates that evolved in the high-density treatment would increase population-level growth rates and increase density, triggering subsequent evolution in life-history traits.

We did not find any significant interactions between density and culling treatments. If this lack of interaction occurs for harvested fish species, fisheries would be able to manage for these two selective mechanisms separately. The absence of an interaction term, however, need

not exclude eco-evolutionary feedbacks involving both density-dependent selection and size-selection. For example, the life-history changes of reduced growth rate and larger size at maturity induced by size selection will likely impact ecological processes such as population growth rate and population density. More work is needed to understand how far the effects of size selection might also indirectly lead to density-dependent selection.

Second, the joint effects of density-dependent selection and size selection may propagate through the food web with unexpected consequences. For example, in a previous study (Pantel et al. 2015) *Daphnia* that were locally adapted to conditions with or without fish significantly altered zooplankton community assembly. In another study (Park et al. 2018) *Daphnia* that diverged in life history in response to fish predation not only increased their resistance to being eaten but also had distinct grazing preferences. Hence, density-dependent selection and size selection acting on a single species has the potential to change the composition of primary producers and reduce the abundance of their consumers. Both of these changes can have cascading effects to species in other trophic levels of the community (Wootton and Power 1993, Ripple et al. 2016).

Third, our results have implications for how we think about the evolutionary effects of harvesting. Previous work has advocated that the evolutionary effects of size-based harvesting should be considered in the management of exploited systems (Kuparinen and Merilä 2007). Changes in life-history traits have been repeatedly documented in harvested populations (Edley and Law 1988, Haugen and Vøllestad 2001, Walsh et al. 2006). This harvest-induced change is often a combination of a plastic and evolutionary response (Eikeset et al. 2016, Gislason et al. 2018, Wilson et al. 2019). There has been some debate over whether some of the best-known examples of harvesting-induced changes are evolutionary versus demographic and over the

speed at which evolution is taking place. For example, Traill et al. (2014) argue that smaller body mass and horn size in trophy-hunted bighorn sheep were mostly demographic responses rather than evolutionary (see however Pigeon et al. 2016) and that the speed of evolution was likely slower than empirically reported (Coulson et al. 2018). In light of this debate, our study offers one example that documents a rapid evolutionary effect of simulated harvesting, which occurs over a very short amount of time and has been separated from any potential demographic effects. Here, we show a reduction in population size can lead to evolutionary changes in a harvested population. Therefore, not only do we need to consider the evolutionary effects of which size classes are removed in an exploited population, but also the evolutionary effects of reducing density.

Acknowledgments

This work was supported by U.S. National Science Grant DMS-1716803 to SJS and TWS, a grant from the University of California Valentine Eastern Sierra Reserve to KL, and a grant from UC Davis Center for Population Biology to KL. Comments by the three reviewers and the editor were very helpful in improving the manuscript.

References

Abrams, P. A., and L. Rowe. 1996. The effects of predation on the age and size of maturity of prey. Evolution 50:1052-1061.

Bassar, R. D., López-Sepulcre, A., Walsh, M. R., Turcotte, M. M., Torres-Mejia, M., and D.N. Reznick (2010). Bridging the gap between ecology and evolution: integrating density regulation and life-history evolution. Annals of the New York Academy of Sciences 1206:17-34.

Bassar, R. D., Lopez-Sepulcre, A., Reznick, D. N., and J. Travis. 2012. Experimental evidence for density-dependent regulation and selection on Trinidadian guppy life histories. The American Naturalist 181:25-38.

Brooks, J. L. and S. I. Dodson. 1965. Predation, body size, and composition of plankton. Science 150:28-35.

Burns, C. W. 2000. Crowding-induced changes in growth, reproduction and morphology of Daphnia. Freshwater Biology 43:19-29.

Charlesworth, B. 1980. Evolution in age-structured populations. Cambridge University Press. Cambridge, U.K.

Celis-Salgado, M. P., Cairns, A., Kim, N. and N. D. Yan. 2008. The FLAMES medium: a new, soft-water culture and bioassay medium for Cladocera. Internationale Vereinigung für theoretische und angewandte Limnologie: Verhandlungen 30:265-271.

Conover, D. O., and S. B. Munch. 2002. Sustaining fisheries yields over evolutionary time scales. Science 297:94-96.

Coulson, T., Schindler, S., Traill, L., and B. E. Kendall. (2018). Predicting the evolutionary consequences of trophy hunting on a quantitative trait. The Journal of Wildlife Management, 82: 46-56.

Edley, M. T. and R. Law. 1988. Evolution of life histories and yields in experimental populations of Daphnia magna. Biological Journal of the Linnean Society 34:309-326.

Eikeset, A. M., Dunlop, E. S., Heino, M., Storvik, G., Stenseth, N. C. and U. Dieckmann. 2016. Roles of density-dependent growth and life history evolution in accounting for fisheries-induced trait changes. Proceedings of the National Academy of Sciences 113:15030-15035.

Fisk, D. L., Latta, L. C., Knapp, R. A., and Pfrender, M. E. 2007. Rapid evolution in response to introduced predators I: rates and patterns of morphological and life-history trait divergence. BMC Evolutionary Biology 7:1-11.

Gíslason, D., McLaughlin, R. L., Robinson, B. W., Cook, A. and E. S. Dunlop. 2018. Rapid changes in age and size at maturity in Lake Erie yellow perch (Perca flavescens) are not explained by harvest. Canadian Journal of Fisheries and Aquatic Sciences 75:211-223.

Haugen, T. O. and L. A. Vøllestad. 2001. A century of life-history evolution in grayling. Genetica 112:475-491.

Hilborn, R. 2006. Faith-based fisheries. Fisheries 31:554-555.

Kokko, H. and A. López-Sepulcre. 2007. The ecogenetic link between demography and evolution: can we bridge the gap between theory and data? Ecology Letters 10:773-782.

Kuparinen, A. and J. Merilä. 2007. Detecting and managing fisheries-induced evolution. Trends in Ecology & Evolution 22:652-659.

Kvam, O. V. and O. T. Kleiven. 1995. Diel horizontal migration and swarm formation in Daphnia in response to Chaoborus. In Cladocera as model organisms in biology (pp. 177-184). Springer, Dordrecht.

Lynch, M. 1989. The life history consequences of resource depression in Daphnia pulex. Ecology 70:246-256.

McCauley, E., Murdoch, W. W., and S. Watson. 1988. Simple models and variation in plankton densities among lakes. The American Naturalist 132:383-403.

Pantel, J. H., Duvivier, C., and L. De Meester. 2015. Rapid local adaptation mediates zooplankton community assembly in experimental mesocosms. Ecology Letters 18:992-1000.

Park, J. S., and Post, D. M., 2018. Evolutionary history of Daphnia drives divergence in grazing selectivity and alters temporal community dynamics of producers. Ecology and Evolution 8:859-865.

Pigeon, G., Festa-Bianchet, M., Coltman, D. W., and F. Pelletier. 2016. Intense selective hunting leads to artificial evolution in horn size. Evolutionary Applications 9:521-530.

Pinheiro, J. C., and D. M. Bates. 2000. Linear mixed-effects models: basic concepts and examples. Mixed-effects models in S and S-Plus 3-56.

Reznick, D., Bryant, M. J., and F. Bashey. 2002. r-and K-selection revisited: the role of population regulation in life-history evolution. Ecology 83:1509-1520.

Ripple, W. J., Estes, J. A., Schmitz, O. J., Constant, V., Kaylor, M. J., Lenz, A., Motley, J. L., Self, K. E., Taylor, D. S.,and C. Wolf. 2016. What is a trophic cascade? Trends in Ecology and Evolution 31:842-849.

Schoener, T. W. 2011.The newest synthesis: Understanding the interplay of evolutionary and ecological dynamics. Science 330: 426-429.

Schoener, T. W. 2013. Eco-Evolutionary dynamics. In Oxford Bibliographies in Ecology. Ed. David Gibson. Oxford University Press, online resource.

Schoener, T. W., Moya-Larano, J., and J. Rowntree. 2014. Eco-evolutionary dynamics. PREFACE. Advances in Ecological Research, 50, pp. XIII-XX.

Silliman, R. P. 1975. Experimental exploitation of competing fish populations. Fishery Bulletin 73:872-888.

Traill, L. W., Schindler, S., and T. Coulson 2014. Demography, not inheritance, drives phenotypic change in hunted bighorn sheep. Proceedings of the National Academy of Sciences 111:13223-13228.

Travis, J., Leips, J., and F. H. Rodd 2013. Evolution in population parameters: density-dependent selection or density-dependent fitness? The American Naturalist 181: S9-S20.

Travis, J., Reznick, D., Bassar, R. D., López-Sepulcre, A., Ferriere, R., and T. Coulson. 2014. Do eco-evo feedbacks help us understand nature? Answers from studies of the Trinidadian guppy. In Advances in Ecological Research (Vol. 50, pp. 1-40). Academic Press.

Walsh, M. R., Munch, S. B., Chiba, S., and D. O. Conover. 2006. Maladaptive changes in multiple traits caused by fishing: impediments to population recovery. Ecology Letters 9:142-148.

Walsh, M. R. and D. N. Reznick. 2008. Interactions between the direct and indirect effects of predators determine life history evolution in a killifish. Proceedings of the National Academy of Sciences 105:594-599.

Walsh, M. R., 2013. The evolutionary consequences of indirect effects. Trends in Ecology & Evolution 28:23-29.

Williams, G. C. 1966. Adaptation and natural selection: A critique of some current evolutionary thought (Vol. 61). Princeton university press.

Wilson, K. L., De Gisi, J., Cahill, C. L., Barker, O. E. and J. R. Post. 2019. Life-history variation along environmental and harvest clines of a northern freshwater fish: Plasticity and adaptation. Journal of Animal Ecology 88:717-733.

Wootton, J. T., and M. E. Power. 1993. Productivity, consumers, and the structure of a river food chain. Proceedings of the National Academy of Sciences 90: 1384-1387.

Ecology Letters 2021. Vol. 24, pp. 1467-1473.

Supplementary Information

Clonal Line	CAA8	GTT3	CAA27	CAA2	CAA14
1[+]	136/148	198	207	220/224	240
2	136/148	198/204	207	220/224	240
3	136/148	198/204	206/207	220/224	240/251
4*	145/148	198	206/207	214/224	240
5[+]	136/148	198	206/207	220/224	240
6*	145/148	198	207	214/224	240
7*	145/148	198	206/207	214/224	240
8	136/148	198	207	214/220	240

* denotes lines 4, 6, and 7 are the same multilocus genotype
[+] denotes lines 1 and 5 are the same multilocus genotype.

Table S2.1. Genetic composition of the 8 clonal lines that make up the starting populations, identified using 5 microsatellite markers (Latta et al. 2010). There are 5 unique multilocus genotypes.

	Density			**Culling**			**Density x Culling**			**Block**		
	F	d.f.	P	F	d.f.	P	F	d.f.	P	F	d.f.	P
Growth rate (mm day^{-1})	3.61	1	0.06	14.01	1	0.0004	9.59	1	0.003	0.001	1	0.98
Maximum size (mm)	0.40	1	0.53	1.52	1	0.22	1.68	1	0.23	1.50	1	0.20

Table S2.2. Results of univariate linear models of an alternate measure of growth rate (i.e. size on the fourth measurement minus size on the first measurement divided by six) and alternate measure of maximum size (i.e. the maximum size observed on any day), testing for the effect of density treatment, culling treatment, the interaction between density and culling treatment.

Growth rate (lrc)	-0.25	-0.43	-0.05	-0.87
	Age at maturity (days)	-0.32	0.53	-0.06
		Reproduction (eggs in 30 days)	-0.05	0.71
			Size at maturity (mm)	0.27
				Maximum size (mm)

Table S2.3. Correlations among the life-history traits.

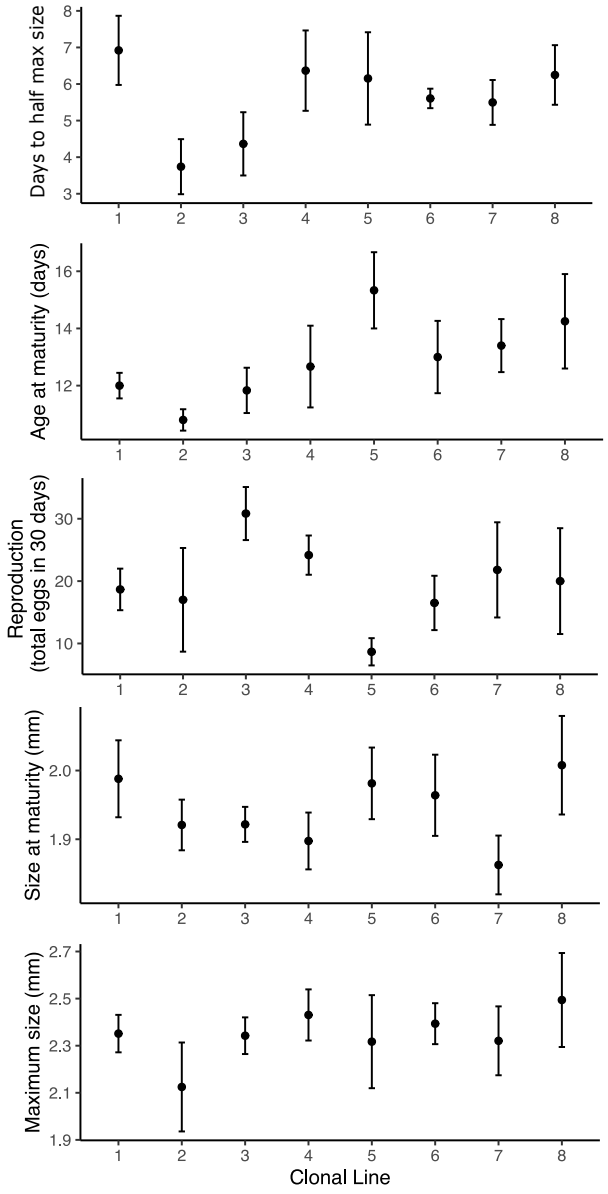


Figure S2.1. Mean ± s.e. for the life history traits of the eight clonal lines that make up the starting population.

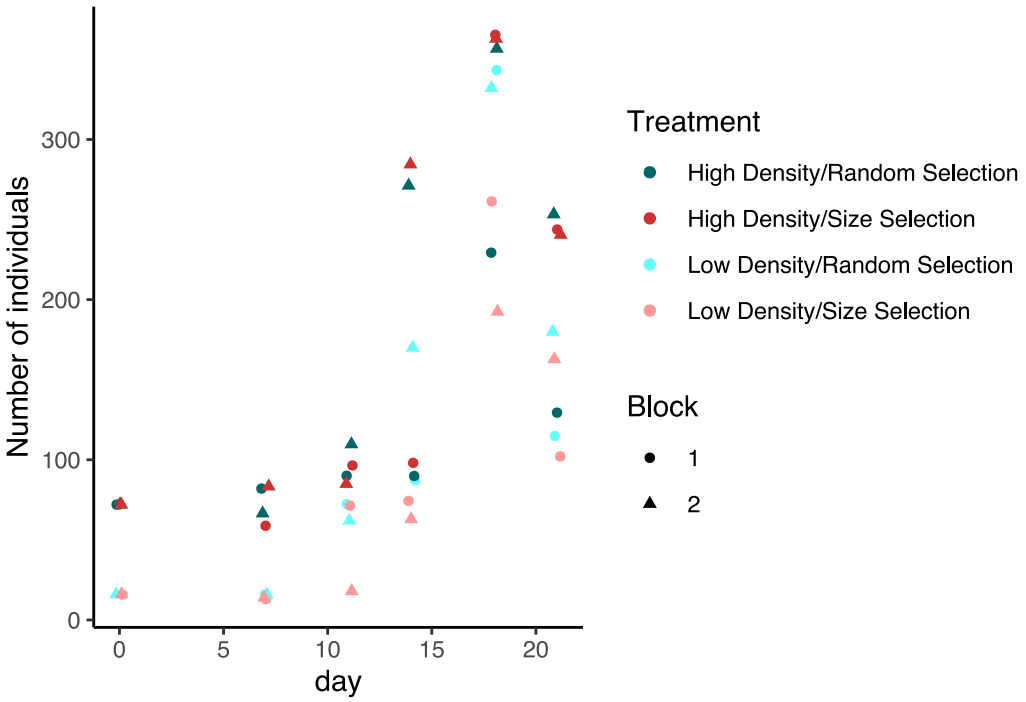


Figure S2.2. Densities of *Daphnia* populations throughout the experiment.

Supplementary Information Reference:

Latta, L.C., Fisk, D.L., Knapp, R.A. and M.E. Pfrender. 2010. Genetic resilience of Daphnia populations following experimental removal of introduced fish. Conservation genetics 11:1737-1745.

Chapter 3

A trade-off in resource exploitation helps explain parallel evolution in *Daphnia*

Kelsey Lyberger

Department of Evolution and Ecology and Center for Population Biology, University of California Davis, Davis, CA

Abstract

Under controlled laboratory conditions, previous studies have shown that selection can produce repeatable evolutionary trajectories, but the question remains if this is also true in the wild or if random chance comes to dominate. In this study, we investigated the extent to which rapid evolution in the wild is predictable by monitoring the genetic composition of replicate populations of Daphnia in field mesocosms containing two clonal genotypes. We found parallel changes across all mesocosms, in which the same genotype increased in frequency. To test whether a trade-off in resource exploitation could be a driver of this frequency change, we conducted a life history assay under high and low resource conditions. We found that resource exploitation differed by genotype, in that, while one genotype (the winner in the field mesocosms) was more fit than the other genotype at high resources, the opposite was true at low resources. These findings suggest that a resource-based trade-off may not only be driving the observed parallel evolution, but also may play a role in maintaining variation within the population.

Introduction

A growing body of work in experimental evolution, spurred by Stephen Jay Gould's idea of "replaying life's tape", has begun to evaluate whether evolution follows a deterministic route in response to some environmental pressure (reviewed in Lobkovsky and Koonin 2012, Losos 2017, Blount et al. 2018). The question remains: does the deterministic force of selection produce repeatable evolutionary trajectories or does random chance dominate? Previous experiments, typically limited to microbes in lab settings and highly controlled environments, have shown a relatively high degree of repeatability in evolutionary responses (e.g. Lenski et al. 1991, Bull et al. 1997). In this study, we are interested in understanding the extent to which rapid evolution in the wild is predictable and whether a trade-off in resource exploitation may be an important selective mechanism that shapes this evolutionary trajectory.

The freshwater crustacean, *Daphnia pulex*, provide an excellent model system in which to address these questions because of their short generation times and cyclically parthenogenetic mode of reproduction. Under favorable conditions females reproduce clonally, then switch to sexual reproduction typically during winter months. Additionally, *Daphnia* populations are easily maintained in field mesocosm experiments in which they are exposed to ambient temperature, light, and natural phytoplankton communities. These advantages make conducting a "parallel replay experiment" in the wild feasible. We are able to rear multiple genetically-identical populations within a pond. While previous studies have observed genotype turnover in the wild (e.g. Steiner and Nowicki 2019) and demonstrated similarity across multiple locations within a lake and across years (Carvalho and Crisp 1987), to our knowledge, there has yet to be a study documenting parallel genotype frequency changes across replicated populations in the wild.

The most likely driver of a deterministic outcome in *Daphnia* is resource availability. Resource availability is a constraining feature of many environments, under which differences in a genotype's ability to exploit resources will be directly reflected in its fitness. However, a high-exploitation strategy may not always be favored, in that performing especially well in a high resource environment may trade off with performing especially poorly in a low resource environment. In resource poor environments, an "efficient" strategy that performs better under low resource conditions would be favored. If this power-efficiency trade-off exists (Reznick et al. 2000), there is the potential for fluctuations in resources to maintain genetic variation.

Here, we present the results of two interrelated experiments. First, to understand to what extent genotype frequency change is predictable, we monitored the genetic composition of replicate populations in field mesocosms initiated with an equal proportion of 2 genotypes. With strong selection we expect frequencies to change in the same direction to favor one genotype over the other in all populations; whereas, without selection we expect frequencies to drift randomly. Second, to understand whether resource availability is a potential driver of this frequency change, we conducted a life history assay to assess whether the genotypes exhibit fitness differences under high and low resource conditions.

Methods

Sample collection and rearing

In May 2018, we sampled *Daphnia pulex* clones from a mesotrophic pond at UC McLaughlin Reserve, CA. Located in a mediterranean climate, this pond remains habitable for *Daphnia* year-round. The pond has a maximum depth of 6 meters and a surface area of 3600 m^2. During our initial sample, the chlorophyll a content was 3.0 ug L^{-1} and the mean across all 6 weeks of the experiment was 4.9 ug L^{-1} (sd = 1.4). We genotyped a random sample of 16 clones

at 5 microsatellite markers (see Latta et al. 2010 for a detailed description of these markers). We found the population was dominated by 2 multilocus genotypes (Table S3.1). These genotypes can be distinguished at a single locus using gel electrophoresis, as either a single band or two bands, from here on, genotype A and B. This method allowed for the genotyping of thousands of individuals (N = 2874). Starting from a single gravid female, we clonally propagated thousands of *Daphnia* of genotype A. We did the same for genotype B. These batch cultures were used to initiate our mesocosm experiment and to run life-history assays. There were no males nor resting eggs in the batch cultures, suggesting that all individuals stayed genetically identical.

Experimental design

In June 2018, we set out a total of 27 floating mesocosms, divided into three treatments: an equal mix of the two genotypes, and each genotype alone. The former allowed us to determine whether selection was acting to favor one genotype over the other or whether genotype frequency change was driven by random chance. The latter were used as controls which allowed us to determine whether populations of each genotype alone were able to grow under these semi-natural conditions.

To create the mesocosms, we floated clear LDPE plastic bags in the pond and filled each with 50 liters of pond water that had been filtered through a 63µm mesh to remove all zooplankton. The dimensions of a mesocosm were approximately 0.8 m deep by 0.5 m wide. We covered them with mesh lids to prevent falling leaves or other detritus from entering. We waited 5 days then added 39 individual *Daphnia* of genotype A and 39 of genotype B to each of 9 mesocosms. As a single genotype control, we added 78 *Daphnia* of genotype A to a second set of 9 mesocosms and 78 *Daphnia* of genotype B to a third and final set of 9 mesocosms. All starting densities were approximately 1.56 L^{-1}.

We sampled all mesocosms every week for 6 weeks. To measure density, we sampled 9 liter columns of water with a 13cm diameter 153µm mesh zooplankton net towed upward from the bottom of the mesocosm, repeated 2-6 times. To characterize relative changes in chlorophyll a, we used the total algae sensor on a Yellow Springs Instrument EXO2 water quality sonde. To measure genotype frequency change, we genotyped the maximum of all collected individuals or 24 individuals for mixed genotype mesocosms and 8 individuals for those with only one genotype. We ended the experiment after six weeks, by which point populations in six mesocosms had declined to extinction and one mesocosm had been punctured by a fishing hook.

Life-history assay

In a controlled laboratory setting we crossed two genotypes with two levels of resources. We measured individual life-histories of genotype A and genotype B under high (16.5 ug L^{-1} chlorophyll a) and low (0.91 ug/L chlorophyll a) resource conditions. The resource conditions were chosen based on a previous study in *Daphnia* showing growth rates saturate around 15 ug L^{-1} (Müller-Solger et al. 2002). The chlorophyll a content in the pond falls in between the two conditions tested in the lab, as measured by the EXO2 water quality sonde.

We conducted the life-history assay on 10 individuals per genotype and treatment (N = 40). However, 3 individuals died before their growth rate could be measured. For genotype A, sample sizes were 8 high and 10 low and for genotype B, sample sizes were 10 high and 9 low. Prior to the start of the assay, to reduce maternal and grandmaternal effects, one adult from each batch culture was selected and propagated for three generations using the second clutch. To begin the assay, neonates <12 hours old were then selected to be measured. All *Daphnia* were reared in individual containers filled with 200 ul filtered pond water and kept under controlled

laboratory conditions (16L:8D, at 20°C). We fed and changed the water in each container every other day and the location of the containers in the incubation chamber was randomized daily.

Data were collected on growth rate, age at maturity, and reproduction. To measure growth rate, we photographed individuals on day 1 and day 4 with a Cannon EOS Rebel T3i camera mounted to a microscope at 20x magnification to produce a 3400x5100 pixel jpeg image. Size was measured manually using ImageJ by drawing a line segment from the base of the tail to top of the eye. By measuring a single image repeatedly, we found this method produces a measurement error of s.d. = 0.009mm. We then used these two sizes to estimate growth rate in mm/day. One photograph from day 1 (genotype B/low) was missing, so a growth rate could not be determined. To obtain an estimate of the age at maturity, we monitored individuals for the presence of eggs every 12 hours. Finally, to measure reproductive output, we summed the number of neonates produced in an individual's first 3 clutches.

Data analysis

All statistics were performed using R version 3.6 (R Development Core Team 2021). To test for selection on the two genotypes in the mesocosms, we ran a one-sample Wilcox signed-rank test to see if the final frequency of genotype A was significantly different from the initial frequency of 0.5.

For life-history assays, we evaluated the effects of genotype, resource condition, and their interaction on the measured life-history traits using a MANOVA test. To check there was no strong correlation among traits, we calculated the correlation structure (Table S3.2). To determine the effects of genotype, resource condition, and their interaction on specific traits, we followed the MANOVA with three separate two-way ANOVAs, one for each of the primary response variables: growth, age at maturity, and reproduction. To meet the assumption of

normality, data for age at maturity and reproduction were natural log transformed. While we expected to see an effect of resource condition alone on our measures of fitness, an interaction between genotype and resources would indicate a resource-based tradeoff among the two genotypes.

Results

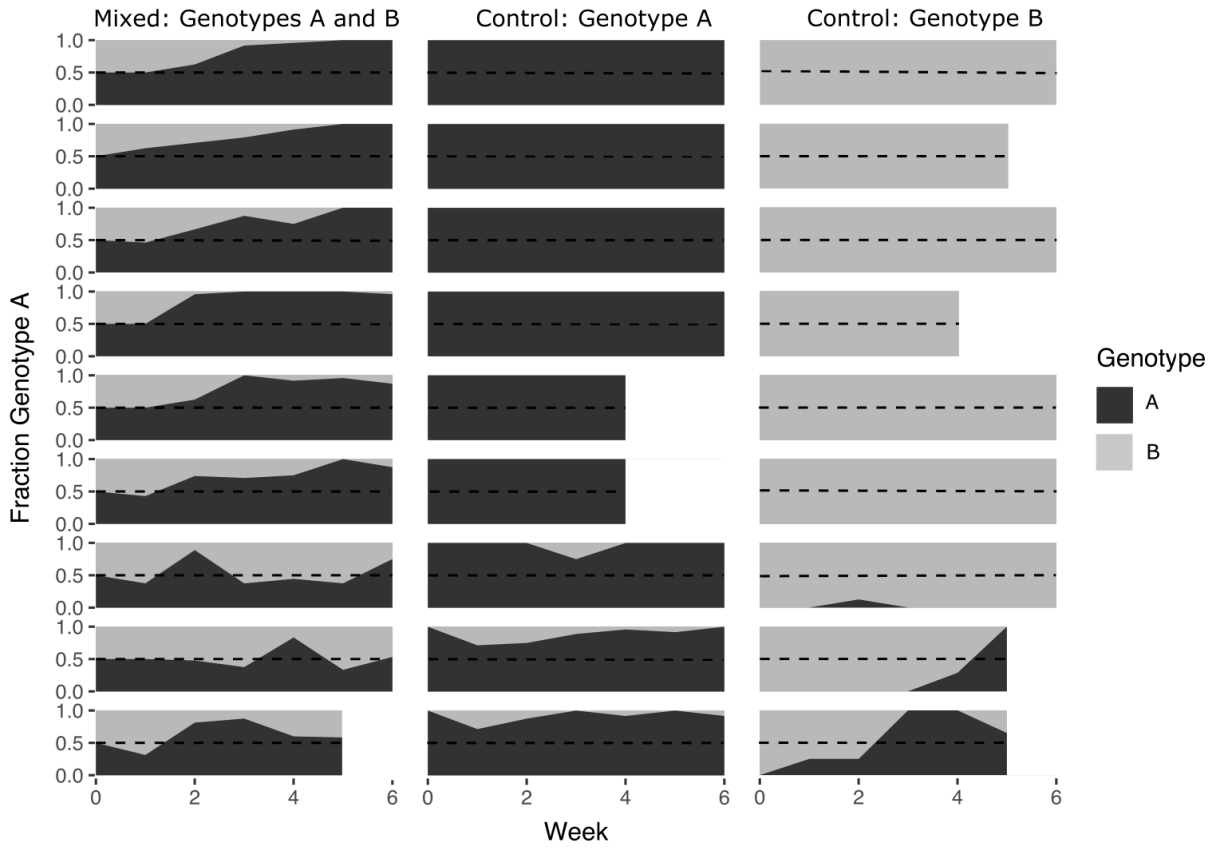


Figure 3.1. Changes in genotype frequency in mesocosms over six weeks, where genotype A is in black and genotype B is in gray. Dashed lines represent equal amounts of each genotype.

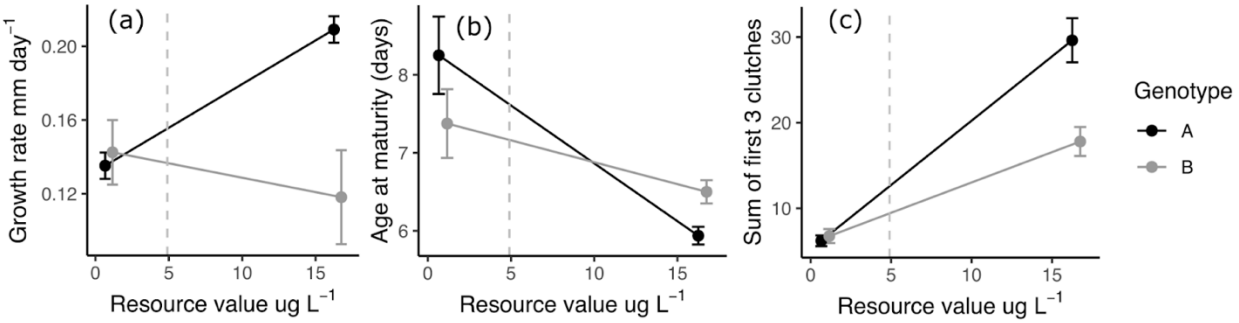


Figure 3.2. Plot of reaction norms across high and low resource values for (a) growth rate, (b) age at maturity, and (c) reproduction measured as the sum of neonates produced in the first three clutches. Points are means with error bars corresponding to ±1 SE. Genotype A is in black and genotype B is in gray. Vertical dashed lines correspond to the average resource value in field mesocosms.

Table 3.1. Results of the MANOVA followed by individual ANOVAs, testing the effects of genotype, resource condition, and their interaction on growth, ln-transformed age at maturity, and ln-transformed reproduction. P-values marked in bold indicate numbers that are significant at the 0.05 level or below.

		Genotype		Resource		Genotype x Resource	
	df	F	p-value	F	p-value	F	p-value
MANOVA	3	3.26	**0.035**	41.39	**<0.0001**	8.05	**0.0004**
Growth rate	1	6.01	**0.020**	2.10	0.16	8.27	**0.007**
Age at maturity	1	0.19	0.66	20.61	**<0.0001**	5.92	**0.02**
Reproduction	1	4.44	**0.043**	151.84	**<0.0001**	8.19	**0.007**

Genotype A increased in frequency in the mesocosms with a mixture of the two genotypes (Figure 3.1). In the final week of the experiment, the fraction of genotype A was significantly greater than the starting fraction of 0.5 according to the Wilcox signed-rank test (p

= 0.014). By that time, genotype A had reached fixation in 3 of the 8 remaining populations and was more abundant than genotype B in all populations.

Both genotypes were able to increase in density in the mesocosms when cultured individually (Figure S3.2), with the exception of two mesocosms containing genotype A alone. Compared to the initial density of 1.56 L^{-1}, populations with genotype A alone averaged a density of 2.7 L^{-1} (sd = 0.36), populations with genotype B alone averaged a density of 1.6 L^{-1} (sd = 0.28), and mixed populations averaged a density of 2.6 L^{-1} (sd = 0.30). Chlorophyll a in the mesocosms averaged 3.8 ug L^{-1} (sd = 1.73) and we saw a decline in chlorophyll a over time, especially in the mesocosms with genotype B alone (Figure S3.3).

There was evidence that some mesocosms that should have contained only a single genotype became contaminated (Figure 3.1). This occurred in 6 mesocosms in total. Although we rinsed our sampling gear with zooplankton-free water between each sample, the *Daphnia* may have been transferred between mesocosms in the process of sampling. Interestingly, in mesocosms in which a small number of individuals of genotype A were introduced, they were able to increase in frequency in 2 out of 3 of these populations. Whereas, in the mesocosms in which individuals of genotype B were introduced, they declined to make up only 8% of the population or went extinct entirely.

The life-history assays showed that resource exploitation differed by genotype. Genotype A was more fit in the higher resource treatment compared to genotype B, in that it had a higher growth rate, earlier age at maturity, and produced more offspring. In the low resource treatment, genotype B had an earlier age at maturity and similar growth rate and number of offspring. Figure 3.2 shows these crossing reaction norms. The MANOVA showed there was a highly significant interaction between genotype and resources ($F_{1,32} = 8.05$, $p < 0.001$), and a significant

interaction between genotype and resources for all three traits individually (Table 3.1). The MANOVA also showed the effect of genotype was significant ($F_{1,32} = 3.26$, $p < 0.05$) and the effect of treatment was highly significant ($F_{1,32} = 41.39$, $p < 0.0001$). More specifically, genotype A had a higher growth rate and more offspring overall compared to genotype B. There was not a significant difference in age at maturity between the two genotypes. Also, the high resource environment reduced the age at maturity and increased reproductive output, although it did not have a significant effect on growth rate.

Discussion

We find evidence of selection favoring one genotype over the other. The same genotype increased in frequency across all experimental mesocosms (Figure 3.1). Especially given that the modest population sizes we used could have been impacted by demographic stochasticity or genetic drift, the consistent trend across mesocosms suggests that changes in genotype frequencies are predictable, at least in the short-term. Our results shed light onto previous studies that have documented seasonal turnover in *Daphnia* genotypes in the wild (Carvalho and Crisp 1987, Pfrender and Lynch 2000, Steiner and Nowicki 2019), in that, this turnover is, in part, the result of selection favoring certain genotypes rather than being dominated by random chance. Establishing the deterministic aspect of turnover is a critical first step, as only after this has been established is investigating the underlying drivers worthwhile.

We also find evidence for one possible process driving this selection, a "power-efficiency" trade-off among the two dominant genotypes in the pond. We see crossing reaction norms across the two resource conditions (Figure 3.2). Specifically, genotype A had a higher growth rate, an earlier age at maturity, and more offspring compared to genotype B under high resource conditions; whereas genotype B responded slightly better to low resource conditions

compared to genotype A. Given that genotype B was only significantly better with respect to one trait, development time, this might not be enough of an advantage to allow it to persist. While chlorophyll a content in the pond during the experiment was intermediate compared to the low and high conditions in the life-history assays, we can hypothesize from the mesocosm results that June-August months reflected environmental conditions in which resources are high enough that genotype A has a higher fitness.

Crossing reaction norms have been previously observed across species of *Daphnia*, in which species differ in their sensitivity to a resource gradient (Tessier et al. 2000). This trade-off can be explained by differences in acquisition, in that certain species or genotypes, termed "superfleas", are better at acquiring nutrients but only when resources are abundant (Spitze et al. 1991, Reznick et al. 2000, Hall et al. 2012). Given the similar pattern seen in our results, we believe the same mechanisms operating at the species level are also operating at the genotype level. Genotype-specific differences in response to food availability have been previously documented in *Daphnia* (Steiner and Nowicki 2019) and other organisms (Turner et al. 1996, Osier and Lindroth 2006). However, our findings contrast with those of Crawford and colleagues (2020), which failed to show a power-efficiency trade-off between *Daphnia* clones in persisting versus spring-only populations, nor did they show a trade-off between spring and summer clones within the same population.

An important implication of a resource-based trade-off is its potential to maintain coexistence among clones and therefore, genetic variation within a population. Theory suggests coexistence is possible under one of two additional conditions. First, if the functional responses to a resource gradient are curved, both genotypes can persist on a single resource (Armstrong and McGehee 1980, see Fig. 2). In the case of nonlinear saturating functional response curves, each

genotype has an advantage when rare. If a small number of the low resource favored genotype enters a population of the high resource genotype, they can increase because the resource level is higher than what they require for positive growth, similar to the R* rule. If a small number of the high resource favored genotype enters a population of the low resource genotype which undergoes internally generated cycles, they can increase during periods of high resource levels. Second, genotype by environment interactions paired with temporally fluctuating resources, for example, driven by seasonality, or spatial variability in resources would also allow for coexistence through fluctuating selection (Haldane and Jayakar 1963, Lynch 1987, Gillespie and Turelli 1989, Schreiber 2020).

As resources in aquatic environments exhibit predictable seasonal patterns of algal succession (Sommer et al. 1986), there is the potential for seasonal niche partitioning. Carvalho and Crisp (1987) demonstrated that the dominant genotypes in their system were seasonal specialists, each favored in either summer, fall, or winter, and that this pattern was consistent across multiple locations within a lake and across multiple years. In ostracods, this seasonal niche partitioning is thought to allow for coexistence among lineages (Rossi et al. 2017). There are a vast number of seasonally-changing variables that could be responsible for the seasonal turnover of genotypes, with resource availability being just one possibility. For example, previous studies have documented genotype-specific responses to environmental variables such as temperature, phosphorus limitation, and salinity (Carvalho 1987, Sherman et al. 2017, Venâncio et al. 2018). One implication of this pattern is that we may expect climate change to induce changes in genetic diversity based on changes in seasonal or spatial niche partitioning associated with warming or changes in productivity or the phenology of productivity. Clones favored in winter may be permanently replaced by those favored in summer.

Biotic factors such as predation and parasitism, although not present in our mesocosms, may also play a role in maintaining genotypic variation in natural populations of *Daphnia* (Hall et al. 2007, Walsh and Post 2012) and other organisms (e.g., Jokela et al. 2003). Future studies should explore how these other variables drive selection, in addition to resource availability. Furthermore, we recognize our study is limited in scope to the two dominant genotypes in a single pond. Understanding the role of resource-based trade-offs in driving selection in additional populations and genotypes requires further investigation.

Acknowledgements

Sampling help was provided by J. Vang, A. Milkey, and X. Westworth. Funding was provided by the Mildred E. Matthias Grant to KL and UC Davis Center for Population Biology Grant to KL.

References

Armstrong, R. A. and R. McGehee. 1980. Competitive exclusion. The American Naturalist, 115:151-170.

Blount, Z. D., Lenski, R. E. and J. B. Losos. 2018. Contingency and determinism in evolution: Replaying life's tape. Science, 362: eaam5979.

Bull, J. J., Badgett, M. R., Wichman, H. A., Huelsenbeck, J. P., Hillis, D. M., Gulati, A., Ho, C. and I. J. Molineux. 1997. Exceptional convergent evolution in a virus. Genetics 147:1497–1507.

Carvalho, G. R. 1987. The clonal ecology of Daphnia magna (Crustacea: Cladocera): II. Thermal differentiation among seasonal clones. The Journal of Animal Ecology 56:469-478.

Carvalho, G. R. and D. J. Crisp. 1987. The clonal ecology of Daphnia magna (Crustacea: Cladocera): I. Temporal changes in the clonal structure of a natural population. The Journal of Animal Ecology 56:453-468.

Colbourne J. K., Robison B., Bogart K. and M. Lynch. 2004. Five hundred and twenty-eight microsatellite markers for ecological genomic investigations using Daphnia. Molecular Ecology Notes 4:485-490.

Crawford, J. W., Schrader, M., Hall, S. R. and C. E. Cáceres. 2020. Intraspecific variation in resource use is not explained by population persistence or seasonality. Oecologia 193:135-142.

Gillespie, J. H. and M. Turelli. 1989. Genotype-environment interactions and the maintenance of polygenic variation. Genetics 121:129–138.

Haldane, J. B. S. and S. D. Jayakar. 1963. Polymorphism due to selection of varying direction. Journal of Genetics 58:237–242.

Hall, S. R., Sivars-Becker, L., Becker, C., Duffy, M. A., Tessier, A. J. and C. E. Cáceres. 2007. Eating yourself sick: transmission of disease as a function of foraging ecology. Ecology Letters 10:207-218.

Hall, S.R., Becker, C.R., Duffy, M.A. and C.E. Cáceres. 2012. A power–efficiency trade-off in resource use alters epidemiological relationships. Ecology 93:645-656.

Jokela, J., Lively, C. M., Dybdahl, M. F. and J. A. Fox. 2003. Genetic variation in sexual and clonal lineages of a freshwater snail. Biological Journal of the Linnean Society 79:165-181.

Latta, L. C., Fisk, D. L., Knapp, R. A. and M. E. Pfrender. 2010. Genetic resilience of Daphnia populations following experimental removal of introduced fish. Conservation Genetics, 11:1737-1745.

Lenski R. E., Rose M. R., Simpson S. C. and S. C. Tadler. 1991. Long–term experimental evolution in Escherichia coli. I. adaptation and divergence during 2,000 generations. The American Naturalist 138:1315–1341

Lobkovsky, A. E. and E. V. Koonin. 2012. Replaying the tape of life: quantification of the predictability of evolution. Frontiers in Genetics 3:246.

Losos, J. B. 2017. Improbable destinies: Fate, chance, and the future of evolution. Penguin.

Lynch, M. 1987. The consequences of fluctuating selection for isozyme polymorphisms in Daphnia. Genetics 115:657-669.

Müller-Solger, A. B., Jassby, A. D. and D. C. Müller-Navarra. 2002. Nutritional quality of food resources for zooplankton (Daphnia) in a tidal freshwater system (Sacramento-San Joaquin River Delta). Limnology and Oceanography 47:1468-1476.

Osier, T. L. and R. L. Lindroth. 2006. Genotype and environment determine allocation to and costs of resistance in quaking aspen. Oecologia 148:293-303.

Pfrender, M. E. and M. Lynch. 2000. Quantitative genetic variation in Daphnia: temporal changes in genetic architecture. Evolution 54:1502-1509.

R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Reznick, D., Nunney, L. and A. Tessier. 2000. Big houses, big cars, superfleas and the costs of reproduction. Trends in Ecology & Evolution 15:421-425.

Rossi, V., Martorella, A., Scudieri, D. and P. Menozzi. 2017. Seasonal niche partitioning and coexistence of amphimictic and parthenogenetic lineages of Heterocypris barbara (Crustacea: Ostracoda). Canadian Journal of Zoology 95:7-14.

Schreiber, S. J. 2020. When do factors promoting genetic diversity also promote population persistence? A demographic perspective on Gillespie's SAS-CFF model. Theoretical Population Biology 133:141-149.

Steiner, C. F. and C. J. Nowicki. 2019. Eco-Evolutionary Dynamics in the Wild: Clonal Turnover and Stability in Daphnia Populations. The American Naturalist 194:117-123.

Sherman, R. E., Chowdhury, P. R., Baker, K. D., Weider, L. J. and P. D. Jeyasingh. 2017. Genotype-specific relationships among phosphorus use, growth and abundance in Daphnia pulicaria. Royal Society Open Science 4:170770.

Sommer, U., Gliwicz, Z. M., Lampert, W. and A. Duncan. 1986. The PEG-model of seasonal succession of planktonic events in fresh waters. Archiv für Hydrobiologie 106:433-471.

Spitze, K., Burnson, J. and M. Lynch. 1991. The covariance structure of life-history characters in Daphnia pulex. Evolution 45:1081-1090.

Tessier, A. J., Leibold, M. A. and J. Tsao. 2000. A fundamental trade-off in resource exploitation by Daphnia and consequences to plankton communities. Ecology 81:826-841.

Turner, P. E., Souza, V. and R. E. Lenski.1996. Tests of ecological mechanisms promoting the stable coexistence of two bacterial genotypes. Ecology 77:2119-2129.

Venâncio, C., Ribeiro, R., Soares, A. M. V. M. and I. Lopes. 2018. Multigenerational effects of salinity in six clonal lineages of Daphnia longispina. Science of the Total Environment 619:194-202.

Walsh, M. R. and D. M. Post. 2012. The impact of intraspecific variation in a fish predator on the evolution of phenotypic plasticity and investment in sex in Daphnia ambigua. Journal of Evolutionary Biology 25:80-89.

Supplementary Information

Table S3.1. Multilocus genotypes of the two dominant genotypes A and B. The two genotypes differ at all 5 microsatellite markers. Gel electrophoresis run on marker "CAA2" allowed us to distinguish between the two genotypes. Primers for these markers are from Colbourne et al. (2004).

	CAA8		GTT3		CAA27		CAA2		CAA14	
Genotype A	136	148	202		199	205	213	224	243	246
Genotype B	130	142	198	202	203	206	223	224	240	243

Table S3.2. Correlation structure among life-history traits.

Growth rate	-0.09	0.40
	Age at maturity	-0.58
		Reproduction

Figure S3.1. Mean chlorophyll a ±1 SE in mesocosms over time by treatment.

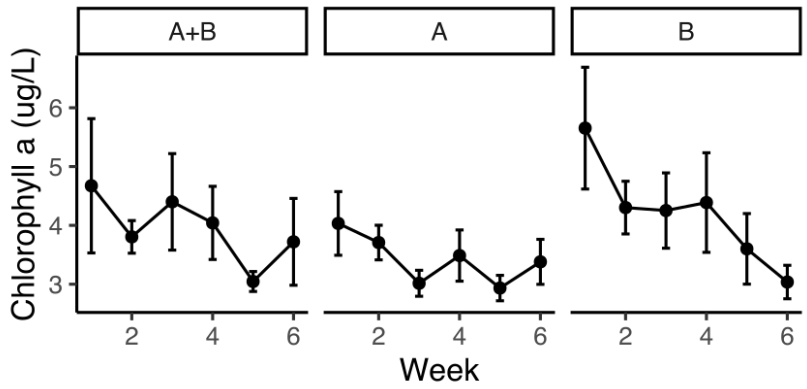
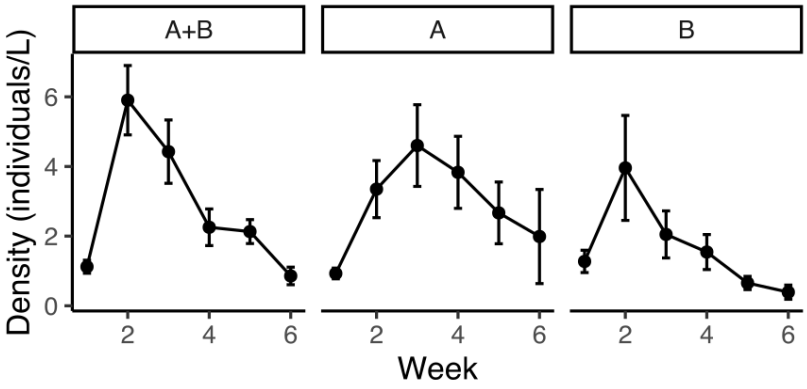


Figure S3.2. Mean *Daphnia* density ±1 SE in mesocosms over time by treatment.



Chapter 4

Feedback effects in natural phytoplankton communities: the role of mesozooplankton grazing

Kelsey Lyberger[1,§], Luna Benitez-Requena[2], Elisabeth Ramm[2,3], Herwig Stibor[2], Maria Stockenreiter[2]

[1]Department of Evolution and Ecology and Center for Population Biology, University of California, Davis, U.S.A.

[2]Aquatic Ecology, Department Biology II, Ludwig-Maximilians-Universität München, Germany

[3]Institute of Meteorology and Climate Research, Atmospheric Environmental Research (IMK-IFU), Karlsruhe Institute of Technology (KIT), Garmisch-Partenkirchen 82467, Germany

Abstract

The interactions between primary producers and consumers are well recognized as being essential to understand the ecology of a system. Importantly, there is the potential for a dynamic interplay between these two trophic levels. So far, the effects of phytoplankton on zooplankton and the effects of zooplankton on phytoplankton have been studied independently and often in simplified aquatic communities. Here, we use natural phytoplankton and mesozooplankton communities to investigate the feedback effect of a primary producer community on itself via the altered composition and feeding behavior of consumers. We found that diverse phytoplankton communities shaped zooplankton composition. Our results also show that grazing by zooplankton reduced biomass but increased diversity in phytoplankton. This effect was driven in part by elemental nutrient availability recycled by consumers, where recycled nutrients have feedback effects on phytoplankton. Altogether, our approach demonstrates how phytoplankton can be shaped by the changes they cause in zooplankton.

Introduction

Understanding the interaction between primary producers and consumers is one of the most pressing topics in ecological research. Previous studies have demonstrated that both, bottom-up and top-down control is responsible for shaping these communities (Chase et al. 2000, Sommer and Sommer 2006). At the same time, biodiversity has been found to serve as an important role in maintaining ecological services (Cardinale et al. 2012, Manning et al. 2019, van der Plas 2019). For example, increasing biodiversity in primary producer communities affect their biomass production and biochemical composition (Power and Cardinale 2009, Ptacnik et al. 2008, Marzetz et al. 2017), which in turn can significantly impact consumers (Jetz et al. 2009; Striebel et al. 2012; Marzetz et al. 2017). Whereas in terrestrial primary producer communities diversity manipulations are done by removal experiments, where certain species can be simply removed from natural communities, manipulating diversity in planktonic primary producers is challenging and experimentally rare (Flöder and Sommer 1999; Hammerstein et al. 2017).

Consequently, studies on the interactions between primary producers and consumers in freshwater aquatic communities are conducted using simplified and artificial communities, which often consist only of few species (e.g. Pan et al. 2014, Striebel et al. 2012; Marzetz et al. 2017). Yet, these studies show important evidence that diversity in phytoplankton and zooplankton influence the interaction between these two trophic levels. For example, zooplankton organisms like *Daphnia* sp. altered their growth, abundance, and diversity in response to changes in phytoplankton species diversity (Striebel et al. 2012, Marzetz et al. 2017). Such changes in zooplankton have the potential to lead to additional propagated effects back on phytoplankton, because consumers can have large direct and indirect effects on the quantity and

diversity of primary producers through consumption and nutrient recycling (Sterner et al. 1992, Thingstad and Lignell 1997).

Previous studies testing the top-down effects of consumers found *Daphnia* have strong direct and indirect effects on natural phytoplankton (Olsen et al. 1986, Sterner 1986) and bacteria (Jürgens 1994, Kamjunke and Zehrer 1999) in laboratory experiments. Sterner (1986) showed that phytoplankton mortality rates increased when exposed to *Daphnia*. This was due to higher grazing and reproduction rates, the latter of which was increased by nutrient regeneration. However, each of these effects varied among species of phytoplankton. Different size selectivity and nutrient recycling among zooplankton taxa favors certain phytoplankton groups over others (Peter and Sommer 2012). For example, while sloppy unselective filter feeding cladocerans have fast soluble excrements, selective copepods excrement via slowly dissolving pellet-like waste (Litchman et al. 2013, Sommer et al. 2001, Andersen and Hessen 1991). Traditionally, manipulations of phytoplankton diversity have been tested separately from the addition or removal of zooplankton grazers. Thus, the question arises how the impacts of different phytoplankton communities on zooplankton can lead to subsequent changes in phytoplankton. The feedback effect of a primary producer community on itself via the altered composition and feeding behavior of consumers has not been investigated so far in natural freshwater aquatic ecosystems. Most of our knowledge derives from a separated point of view where either the effect of primary producers on consumers or the effect of consumers on primary producers was investigated. Little is known about a full feedback loop in natural plankton communities, where phytoplankton have the potential to be shaped by the changes they cause in zooplankton. Therefore, there is a need of conducting experiments using natural communities to ensure a more natural biodiversity and community composition in both trophic levels.

Here we present the results of a small-scale laboratory experiment that we conducted using different diverse natural phytoplankton communities and a natural zooplankton community to investigate the following hypotheses.

H1: different diverse natural primary producers affect a natural consumer community composition.

H2: altered consumer communities result in feedback effects through altered nutrient recycling on primary producers.

Testing *H1*, we exposed a natural zooplankton community from a single lake to different diverse natural phytoplankton communities. We then measured the response of zooplankton to these different food sources. We also maintained a paired set of phytoplankton microcosms free of zooplankton. By comparing both treatments, with and without zooplankton, we measured the response of phytoplankton to predation pressure. In the second phase, we subsequently tested *H2* using our paired design to test whether the altered consumer community influenced the phytoplankton community through nutrient recycling. To estimate small changes in nutrient limitation we performed bioassays before and after zooplankton grazing (Anderson et al. 2007).

Methods

Sampling sites

We sampled the phytoplankton of five lakes (A-D, Figure 4.1; for coordinates see SI_Table 1), which vary in trophic status and are found close to the Seeon Limnological Station, Southern Bavaria, Germany. In each of the five lakes, we analyzed nutrients (total phosphorus (TP)) and phytoplankton composition. Zooplankton for the experiment were gathered from an additional lake (F, Figure 4.1) to avoid a bias of phytoplankton zooplankton interactions in one

of the treatments. Both sampling and the experiment took place in spring prior to any obvious stratification.

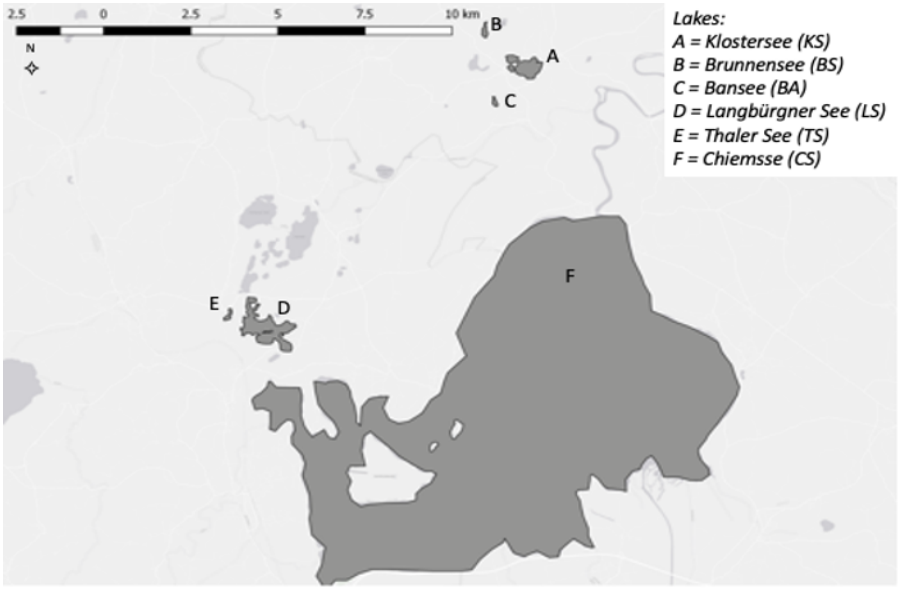


Figure 4.1. Lake sampling sites. Phytoplankton was sampled from smaller lakes A-E, zooplankton was sampled from lake F. The experiment was performed near lake A at Seeon Limnological Station (47°58'28"N, 12°27'36"E).

Experimental setup and measurements:

We designed the experiment using natural phytoplankton communities from the five lakes incubated in 650ml cell culture flasks (Cell Star, Greiner Biogene One, Germany). Collected phytoplankton samples were filtered through 250µm mesh size to exclude meso and macro zooplankton. In the first phase, phytoplankton from each lake was split in two treatments, one with and one without zooplankton added. Each combination of lake and treatment was replicated three times for a total of 30 flasks. For the grazing treatments (half of all flasks) we introduced zooplankton in naturally occurring densities from the additional lake Chiemsee. Zooplankton was sampled using a 100µm plankton net (Hydrobios, Kiel, Germany). Flasks were

gently mixed, rotated, and randomly positioned each day under 12h:12h light:dark cycle (60µmol photons $m^{-2}s^{-1}$) within a growth chamber at 20°C.

To assess the nutrient-mediated effects of zooplankton, we measured the growth response of ungrazed phytoplankton to water that was previously conditioned with zooplankton and phytoplankton or phytoplankton alone. In this second phase, we filtered out (Gf /F filters, VWR collection, Ismaning, Germany) all plankton from the experimental flasks and added just the nutrient-containing water to flasks that had received only phytoplankton. Each phase lasted one week.

Total phosphorus (TP)

12 mL of lake water was used to estimate TP by using the molybdenum blue reaction following digestion with 0.7 mL of sulfuric acid. 0.7 mL of ascorbic acid and 0.7 mL of a reagent mixture (sulfuric acid, antimony potassium tartrate and ammonium molybdate solution) were added. The blue color complex was measured after 30 mins with a spectrophotometer at 880 nm (Shimadzu UV-1700, Shimadzu Cooperation, Germany).

Chlorophyll a

Total chlorophyll a (Chla in µg L^{-1}) was estimated in vivo using fluorometric analyses with the AlgaeLabAnalyzer (bbe moldaenke, Schwentinental, Germany). This device measures total chlorophyll a of a phytoplankton community using different colored LED light sources to enhance light absorbance also by accessory pigments, which increases the sensitivity of estimating the total chlorophyll a amount. With this, in one measurement it is also possible to distinguish between major freshwater algal groups (Chlorophyta, Brown group (referring to Bacillariophyta and Dinoflagellates), Cyanobacteria and Cryptophyta) here named as functional groups in the following.

Plankton Identification

Lugol samples were taken from all treatments at the beginning and end of the first phase to identify phytoplankton species at genus level with the Utermöhl (1958) technique at 400 x microscopic magnification (Wild, Heerbrugg, Switzerland). A minimum of 100 individuals were counted by scanning a minimum of five perpendicular transects or 20 randomly distributed distinct fields to keep the counting error at less than 10% (Lund et al. 1958) to determine genus presence and abundance. We used a fairly comprehensive published dataset on the average biovolume of phytoplankton genera (Kremer et al 2014). For each of the five lakes, after the first phase, one sample per treatment was randomly chosen and counted.

Zooplankton were identified to genus level with a stereomicroscope at 20 x magnification (Wild, Heerbrugg, Switzerland) from preserved (4% sugar formol; Haney and Hall 1973) samples. Zooplankton were counted for two subsamples of the initial Lake Chiemsee community and the entire community in each flask at the end of the first week.

Bioassays

Additionally, to determine the strength and direction of the nutrient limitation caused by possible feedback effects on phytoplankton through zooplankton, we performed nutrient assays according to Andersen et al. (2007), Tamminen and Andersen (2007) and Ptacnik et al. (2010). Briefly, we ran time-series (4 day) experiments with a replicated factorial experimental design (adding 80 µg L^{-1} nitrogen (N) and 20 µg L^{-1} phosphorous (P) and a combination of N and P). Shortly, the procedure was as follows: 50ml of lake water was put in a cell culture vial and measured for chlorophyll a on day 0, then was given one of four nutrient treatments. Every 24 hours chlorophyll a was measured for four days. When measured, the position of flasks was randomized in a climate chamber (12:12h cycle, 90µmol m^{-2} s^{-1}, 20°C).

Data and statistical analyses

All statistical analyses were performed within the R computing environment, version 3.5.0 (R Core Team, 2019). From the zooplankton and phytoplankton data, we calculated species richness and diversity as well as evenness for phytoplankton. Shannon diversity index was calculated following Shannon's formulae (Shannon and Weaver 1948); for evenness we used Pielou´s evenness index (Pielou 1966). To analyze the compositional differences among communities, non-metric multidimensional scaling (NMDS) was performed using the "vegan" package (Oksanen et al. 2019) in R. NMDS is an ordination technique used to visualize patterns among samples in a reduced number of axes. Bray-Curtis was employed as the distance metric. To test for significant differences between groups, either lake, grazing treatment, or time, we performed an ANOSIM test.

We built linear mixed models with phytoplankton biomass, phytoplankton diversity, and phytoplankton evenness as response variables and grazing treatment and TP as predictor variables. For genus-level biovolume data, when only one observation per treatment per lake was collected, TP and the interaction between treatment and TP were excluded as predictors. Lake was treated as a random predictor. Phytoplankton biomass was log transformed to improve normality and homogeneity of variance. We also built a linear mixed model to better understand the nutrient feedback effect. The difference in phytoplankton biomass in pairs of samples receiving grazed and ungrazed water was the response variable and the number of zooplankton residing in the source of grazed water was a continuous predictor variable. Again, lake was a random predictor. We ran the linear mixed models with restricted maximum-likelihood estimation "lme4" package (Bates et al. 2015) in R and reported results for the best fitting model.

We also report the marginal and conditional R^2 values for the mixed models "MuMIn" package (Barton 2016) in R. All replicates were included in our analyses.

To interpret the bioassay data, we calculated effect sizes as *ln* ($Chla_N/Chla_C$), where $Chla_N$ represents the Chla (µg L^{-1}) for treatments with added P or N or NP and $Chla_C$ is the control treatment without added nutrients after 72h. We used this approach of natural log ratio, because of its clear biological meaning in a normal, least biased sampling distribution after Hedges et al. (1999); Stibor et al. (2004).

Results

Lake characteristics

Our experiment contained two oligotrophic lakes (Brunnensee BS, Langbürgnersee LS), two meso-oligotrophic lakes (Thalersee TS, Klostersee KS) and one eutrophic lake (Bansee, BA). Phytoplankton biomass has a strong positive correlation with TP (linear regression biomass = 0.82*TP - 4.18: $R^2 = 0.96$, P = 0.003, Figure 4.2a). Phytoplankton diversity also increased with increasing trophicity, with a minimum richness of 11 genera in BS and maximum richness of 26 genera in TS (Figure 4.2b,c). All lakes are phosphorus limited but differ on their extent of this nutrient limitation (Figure 4.3a). Further characteristics and differences between all six lakes included in this study are given in the supplementary information material (Table S4.1).

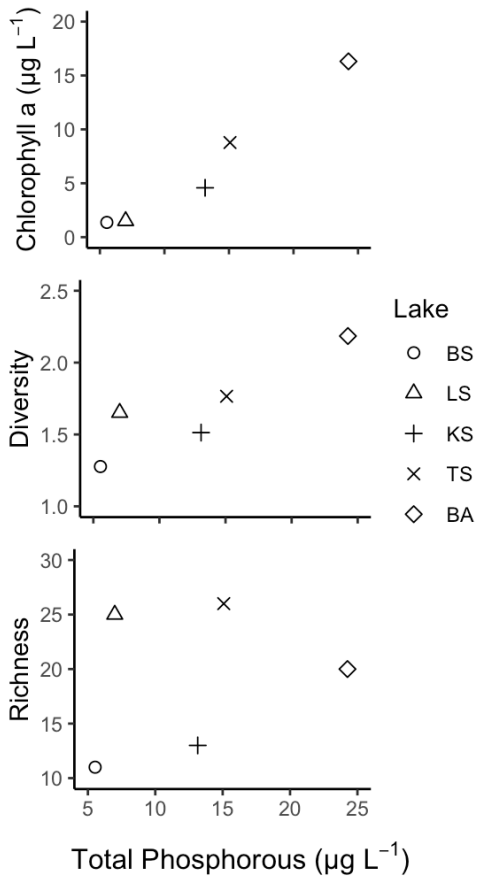
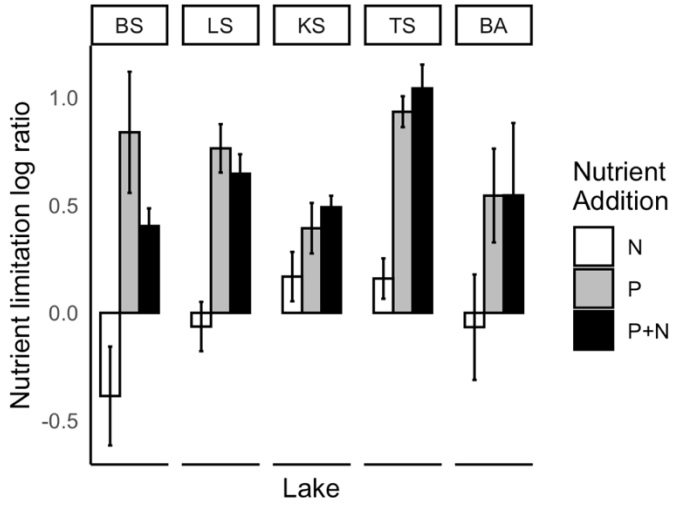


Figure 4.2. Chlorophyll a content (µg L^{-1}), phytoplankton diversity measured using the Shannon Index, and phytoplankton genus-level richness are related to total phosphorus (µg L^{-1}) in the five focal lakes.

a



b

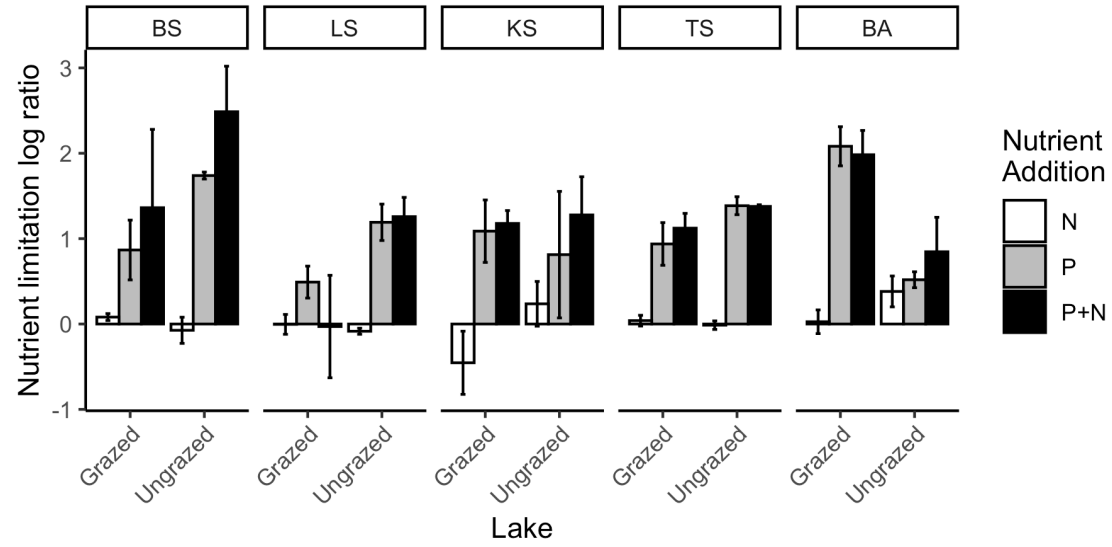


Figure 4.3. Mean log ratio of nutrient limitation (+/- SE) computed from (a) the first bioassay, at the start of the experiment, and (b) the second bioassay, after the grazing treatment. Samples from each lake were spiked with either no nutrients (i.e. controls), phosphorus (P), nitrogen (N), or both P and N.

Phase 1: Zooplankton changes

Zooplankton from Lake Chiemsee comprised a community with seven genera. The most abundant zooplankton initially were nauplii larvae, calanoid copepods, and *Daphnia* (Table S4.2). Grazing on different phytoplankton communities had a strong effect on the zooplankton community composition. Zooplankton replicates exposed to the same phytoplankton community cluster together in NMDS space (Figure 4.4). In an analysis of similarity we found that these clusters, each defined by one of the five phytoplankton communities, differed significantly from each other (ANOSIM, $R^2 = 0.73$, $P = 0.0001$). However, zooplankton communities grazing on different phytoplankton communities differed significantly from the starting community after zooplankton grazed on different phytoplankton communities and showed a range of dissimilarity from 0.27 to 0.89 (from lowest to highest: BS, LS, TS, BA, KS). In more detail, these altered zooplankton communities differed from the starting community in their cladoceran to copepod ratios, which ranged from 0.10 to 7.8 (from lowest to highest: CS, BS, BA, TS, KS, LS).

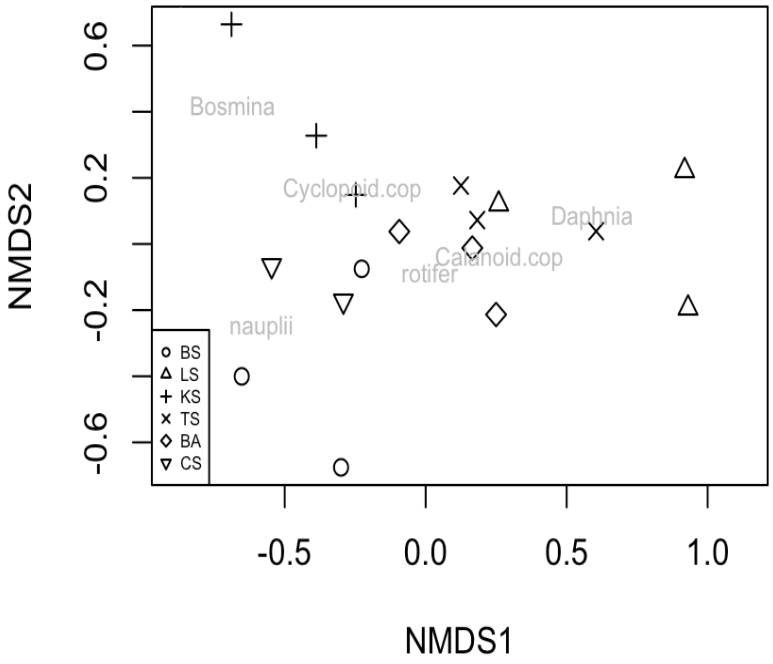


Figure 4.4. The ordination plot produced by nonmetric multidimensional scaling of zooplankton communities (stress = 0.084). Symbols refer to the lake (and associated phytoplankton) to which the zooplankton were exposed. Zooplankton taxa scores are shown in gray.

Phase 1: Phytoplankton changes

In the first phase of the experiment, phytoplankton were strongly modified by zooplankton grazers. In the linear mixed models testing for patterns in phytoplankton biomass and diversity, we found significant treatment, TP, and treatment x TP effects (Table 4.1), where treatment refers to the addition of zooplankton. Phytoplankton exposed to zooplankton grazing showed reduced biomass compared to those without (Figure 4.5a). Phytoplankton biomass was higher for samples from lakes with higher TP, but this effect was not significant. Including an interaction between TP and grazing treatment did not improve model fit for phytoplankton biomass (log-likelihood ratio = 1.54, P = 0.21), hence it was removed from the model. Phytoplankton exposed to zooplankton grazing showed increased functional group diversity (based on AlgaeLabAnalyser data), compared to those without (Figure 4.5b). We also found a positive effect of TP, and strong interaction between TP and grazing treatment on functional group diversity (based on AlgaeLabAnalyser data). However, the interaction between TP and grazing treatment was driven by the lake with the highest TP, BA. as it is the only lake in which grazed phytoplankton have lower diversity. When this lake is removed, the best fitting model only includes treatment, where grazing significantly increased diversity (Table S4.3). We also observed an increase in evenness as a result of grazing (linear mixed model, $R^2\_c = 0.56$, P = 0.002, Table S4.3). Genus-level biovolume data also suggested grazing increased diversity, but not significantly so (Table 4.1).

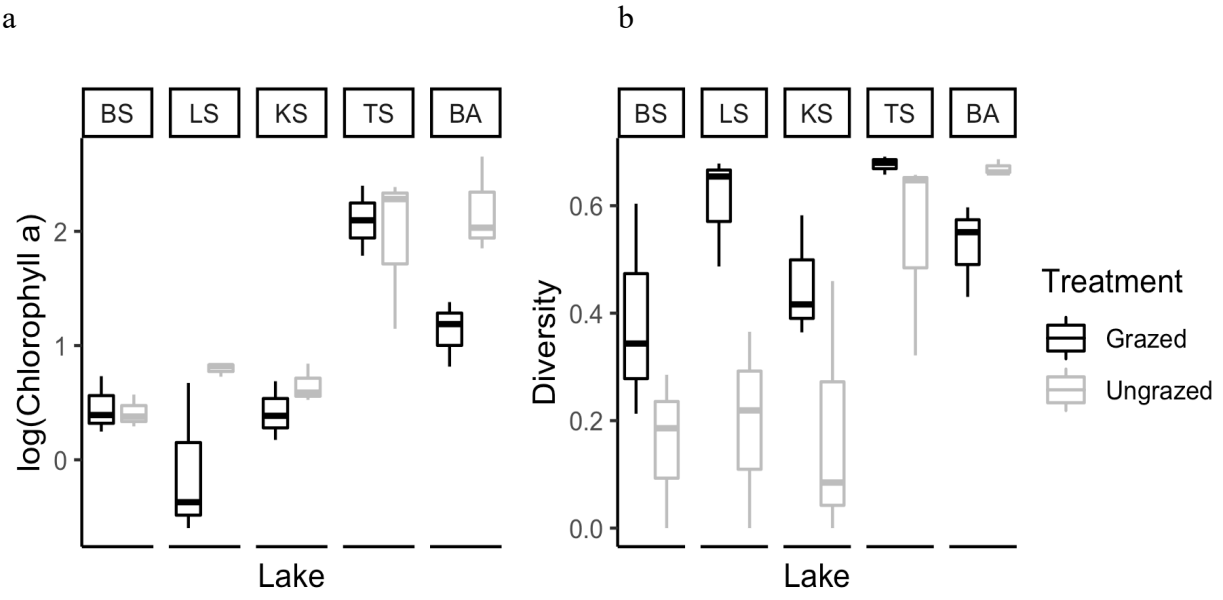


Figure 4.5. Boxplot showing grazing effects on (a) log-transformed phytoplankton biomass and (b) phytoplankton functional group diversity measured using the Shannon Index. Samples with zooplankton are in black and samples without zooplankton are in gray.

Table 4.1. Results of best fitting linear mixed effect models predicting log-transformed biomass, Shannon diversity for phytoplankton functional groups measured using an AlgaeLabAnalyzer, and Shannon diversity for biovolume-transformed phytoplankton genera. Grazing treatment, TP, and their interaction are treated as fixed effects and lake is treated as a random effect.

	Biomass				**Diversity-Lab Analyzer**				**Diversity-Biovolume**			
Effect	Estimate (s.e)	ndf, ddf	t-value	P-value	Estimate (s.e)	ndf, ddf	t-value	P-value	Estimate (s.e.)	ndf, ddf	t-value	P-value
Fixed effects	marginal R^2 = 0.39, conditional R^2 = 0.76				marginal R^2 = 0.47, conditional R^2 = 0.63				marginal R^2 = 0.15, conditional R^2= 0.64			
intercept	-0.22 (0.58)	1,24	-0.38	0.71	0.48 (0.13)	1,23	3.76	0.001	1.58 (0.24)	1,4	6.42	0.003
Treatment (UG)	0.40 (0.16)	1,24	2.44	0.022	-0.50 (0.12)	1,23	-4.25	0.0003	-0.44 (0.22)	1,4	0.12	0.12
TP	0.079 (0.039)	1,3	1.99	0.14	0.0038 (0.0087)	1,3	0.44	0.69	----------	--------	--------	--------
Treatment x TP	----------	--------	--------	--------	0.025 (0.0081)	1,23	3.05	0.0056	----------	--------	--------	--------
Random effects												
Lake variation	0.31				0.0097				0.18			
Residual variation	0.19				0.022				0.13			

Additionally, grouping phytoplankton genera as determined via microscopic biovolume-transformed counting data into their functional groups. This highlights differences between grazed communities and ungrazed communities (Figure S4.1) and permits a better understanding of which phytoplankton groups are changing to allow for a higher diversity with grazing pressure. In lake KS and in lake LS, grazed samples consisted of lower proportion of Cyanophyta and higher proportion of Chlorophyta compared to the ungrazed samples. In contrast, the grazed sample from lake TS consisted of a slightly higher proportion of Cyanophyta and lower proportion Chlorophyta than the ungrazed sample. In BA, there are small changes across many functional groups. In lake BS, the largest proportional change was an increase in *Pseudopedinella* sp. in the grazed sample. Overall, there was a shift towards a more even distribution across functional groups in the grazed samples (mean grazed = 0.49, mean ungrazed = 0.36, linear mixed model, R^2 =0.60, P =0.16, Table S4.3), which is consistent with the positive effect of grazing on diversity in phytoplankton groups.

Next, we determined whether there was a consistent direction of change among lakes in terms of the composition of phytoplankton genera that were present or abundant. As there were 107 different genera, we reduced the dimensionality of our dataset. The phytoplankton communities did not form visually distinct clusters based on grazing treatment in the ordination plot generated using non-metric multidimensional scaling (ANOSIM R = -0.19, P = 0.95; Figure S4.2). However, phytoplankton at the start of the experiment cluster together as do those after one week of treatment, regardless of treatment (Figure S4.2).

Phase 2: Phytoplankton alteration and nutrient feedback

The second phase of the experiment, in which nutrients from grazed and ungrazed samples were transferred into ungrazed phytoplankton, showed zooplankton grazing had an

effect on nutrient recycling. We found that a week after the transfer the Bray-Curtis dissimilarity of phytoplankton composition in the pairs of samples receiving grazed and ungrazed water was highest in KS, TS, and BA, lakes with higher nutrient availability (Figure 4.6). We also found that the difference in phytoplankton biomass in pairs of samples receiving grazed and ungrazed water was not significantly different from zero (linear mixed model, $R^2\_c = 0.48$, $P = 0.59$, Table S4.4). However, including the number of zooplankton previously residing in the grazed vials significantly improved the model fit (log-likelihood ratio = 21.81, $P < 0.0001$), where more zooplankton had a significant positive feedback effect on phytoplankton biomass (linear mixed model, $R^2\_m = 0.79$, $R^2\_c = 0.79$, $P < 0.0001$, Table S4.4).

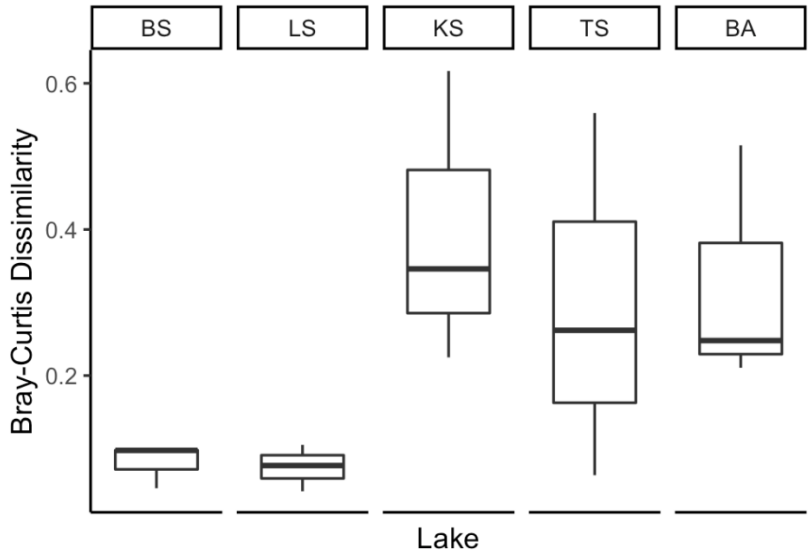


Figure 4.6. Boxplot showing the Bray-Curtis dissimilarity between phytoplankton communities one week after receiving grazed water versus ungrazed water.

Another line of evidence measuring the extent of nutrient feedbacks is the second bioassay, which was performed immediately after the week of grazing in the first phase. The effect size of nutrient limitation was smaller in grazed samples compared to ungrazed samples from all lakes for at least one type of nutrient addition, suggesting grazing reduced nutrient

limitation (Figure 4.3b). BS, LS, and TS phytoplankton that had been grazed were less P limited and less jointly limited, whereas KS and BA phytoplankton that had been grazed were less N limited. Although, for BA, grazed phytoplankton were also more P limited and more jointly limited.

Discussion

Our results add to the findings of previous studies investigating the ways in which phytoplankton affect zooplankton. We found consistent changes in the zooplankton community when exposed to phytoplankton from a single lake. On the other hand, zooplankton communities grazing on phytoplankton from different lakes were dissimilar to each other and to the starting Lake Chiemsee community (Figure 4.4). Phytoplankton can influence zooplankton because species differ in their biochemical composition (Klausmeier and Litchman 2004; Martin-Creuzburg and von Elert 2009, Schälicke et al. 2019) and in their resistance to being eaten (Sterner 1989, Sarnelle 2005). Here, we've sampled lakes that display a wide range of phytoplankton richness, diversity, and trophic status (Figure 4.2, Figure 4.3a). Additionally, phytoplankton diversity itself has been shown to influence consumer growth, abundance, and diversity (Striebel et al. 2012, Martzetz et al. 2017). By using multiple natural phytoplankton communities, we expand on previous results of simplified artificial phytoplankton communities, which are generally low in diversity.

Our results demonstrate that zooplankton changed both the quantity and composition of phytoplankton. While zooplankton grazing reduced phytoplankton biomass, it also increased phytoplankton diversity (Table 4.1). Both genus and functional diversity of phytoplankton increased, although the trend observed in functional diversity was reversed for the most eutrophic lake, BS. While models of predator-mediated coexistence have suggested that

consumers can increase the diversity of producers, most experimental studies in freshwater systems have failed to find evidence supporting this (McCauley and Briand 1979, reviewed in Hillebrand et al. 2007). Our study expands on the few exceptions in which zooplankton grazing increased phytoplankton diversity (Leibold et al. 2017, Sarnelle 2005), by demonstrating this pattern holds across several different natural communities of phytoplankton. Similar to Sarnelle (2005), we found an increase in diversity due to higher evenness and no clear shift towards dominance by resistant taxa. Our results suggest a multiple grazer theory, in which each group of zooplankton in our speciose community reduces a specific class of phytoplankton (Leibold et al. 2017, Sommer et al. 2001).

The second phase of the experiment focused on the importance of elemental nutrient availability for primary producers recycled by consumers. The results of our second bioassay provide evidence that consumers reduce nutrient limitation (Figure 4.3b). Brunnensee (BS) and Langburgnersee (LS), the lakes with the lowest trophic statuses, showed the largest reductions in phosphorous limitation and joint limitation in grazed samples. Whereas Bansee (BA), the lake with the highest trophic status, is the only lake to show an increase in nutrient limitation in grazed samples. This could suggest the reduction in nutrient limitation by consumers is more likely when nutrients are low. On the other hand, we found that transferring nutrients from grazed versus ungrazed samples into ungrazed phytoplankton led to dissimilar phytoplankton communities in the three lakes with the highest trophic statuses. This suggests the top-down pressure in these lakes is stronger compared to lakes where the nutrients are already limiting bottom up. Our result aligns with previous studies showing top-down control of primary producers is dependent on nutrient status (McQueen et al. 1989, Brett and Goldman 1997); e.g., consumers have the larger effects in nutrient rich lakes (Bakker and Nolet 2014). We also found

that the benefit from released nutrients, as measured by an increase in biomass, was most apparent in phytoplankton receiving nutrients from samples with higher zooplankton concentrations, suggesting a dependent relationship between zooplankton concentrations and nutrient release.

In addition to zooplankton concentration, changes in cladoceran to copepod ratios could have influenced which and how much nutrients were released. For example, P limitation was reduced in grazed samples from Lake Brunnensee (BS), which also had the lowest cladoceran to copepod ratio. In contrast, N limitation was reduced in grazed samples of Lake Klostersee, which had the second highest cladoceran to copepod ratio. Copepods, which have a high N:P body ratio, are expected to reduce the N:P ratio based on the stochiometric model (Sterner et al. 1992). In contrast, *Daphnia* have been found to reduce the advantage of N_2 fixing Cyanophyta by differentially recycling N to P, because of *Daphnia*'s low body N:P ratio (MacKay and Elser 2003). Nutrient recycling by an altered consumer community is just one mechanism through which the feedback loop is completed.

Another mechanism is through the altered consumer community directly consuming different phytoplankton groups. Cladocerans suppress small phytoplankton whereas copepods are thought to feed mainly on large phytoplankton (Peter and Sommer 2012, Sommer et al. 2001, Yoshida et al. 2001). The large differences in phytoplankton between grazed and ungrazed samples in phase 1 are likely driven by a combination of direct (selective consumption) and indirect effects (nutrient recycling) (see also Sterner 1986). Besides the differences in zooplankton composition and the differences in the strength of nutrient recycling across lakes, one reason we did not observe parallel changes in phytoplankton may be due to the potential trade-off between top-down and bottom-up effects. The most nutrient competitive species may

also be the most suppressed by grazing (Mandal et al. 2018), hence it is difficult to predict which groups will benefit.

Ecologists have long recognized the consumers' and primary producers' potential to reciprocally alter each other's composition. Our experiment disentangles the different stages of this feedback loop. We document the effect of different primary producer communities on consumers, the effect of the altered consumers on producers, and finally, the importance of nutrient recycling by consumers. An important limitation of our study is the small scale of microcosms, which were chosen in order to control the zooplankton community and in order to feasibly replicate treatments across five natural phytoplankton communities. Larger-scale and longer-term experiments will further assess the generality of our findings. A next step would be to investigate the feedback loop further, i.e., what effects altered phytoplankton after nutrient recycling have on zooplankton in return. In summary, our study points out the multiple stages of feedback between consumers and producers in a freshwater ecosystem and works towards further understanding the complex direct and indirect effects involved.

Acknowledgements

We would like to thank Achim Weigert for technical help in the laboratory. This work was supported by H2020 project AQUACOSM (project number 731065) and Deutsche Forschungsgemeinschaft (DFG) to Herwig Stibor (STI 180/10-1 and Maria Stockenreiter (STO 1075/1-1; STO 1075/2-1) within the SPP 1704 DynaTrait.

References

Andersen, T. and D. O. Hessen. 1991. Carbon, nitrogen, and phosphorus content of freshwater zooplankton. Limnology and Oceanography 36:807–814.

Andersen, T., Saloranta, T. M. and T. Tamminen. 2007. A statistical procedure for unsupervised classification of nutrient limitation bioassay experiments with natural phytoplankton communities. Limnology and Oceanography: Methods 5:111–118.

Bakker, E. S., and B. A. Nolet. 2014. Experimental evidence for enhanced top-down control of freshwater macrophytes with nutrient enrichment. Oecologia 176:825-836.

Barton, K. 2016. Multi-model inference. R package version 1.15. 6.

Bates, D. Maechler, M., Bolker, B., Walker, S., Christensen, R.H.B., Singmann, H., Dai, B., Scheipl, F. et al. 2015. Package 'Ime4'.

Brett, M. T., and C. R. Goldman. 1997. Consumer versus resource control in freshwater pelagic food webs. Science 275:384-386.

Cardinale, B. J. Duffy, J. E., Gonzalez, A., Hooper, D. U., Perrings, C., Venail, P., Narwani, A., Mace, G. M. Tilman, D., Wardle, D. A. and A. P. Kinzig. 2012. Biodiversity loss and its impact on humanity. Nature 486:59–67.

Chase, J. M., Leibold, M. A., Downing, A. L. and J. B. Shurin. 2000. The Effects of Productivity, Herbivory, and Plant Species Turnover in Grassland Food Webs. Ecology 81:2485–2497.

Haney, J. F. and D. J. Hall. 1973. Sugar-coated Daphnia: A preservation technique for Cladocera 1. Limnology and Oceanography 18:331–333.

Hedges, L. V., Gurevitch, J. and P. S. Curtis. 1999. The meta-analysis of response ratios in experimental ecology. Ecology 80:1150-1156.

Hillebrand, H., Dürselen, C. D., Kirschtel, D., Pollingher, U., and T. Zohary. 1999. Biovolume calculation for pelagic and benthic microalgae. Journal of Phycology 35:403–424.

Hillebrand, H., Gruner, D. S., Borer, E. T., Bracken, M. E., Cleland, E. E., Elser, J. J., Harpole, W. S., Ngai, J. T., Seabloom, E. W., Shurin, J. B. and J. E. Smith. 2007. Consumer versus resource control of producer diversity depends on ecosystem type and producer community structure. Proceedings of the National Academy of Sciences 104:10904–10909.

Jetz, W., Kreft, H., Ceballos, G., and J. Mutke. 2009. Global associations between terrestrial producer and vertebrate consumer diversity. Proceedings of the Royal Society B: Biological Sciences 276:269-278.

Jürgens, K. 1994. Impact of Daphnia on planktonic microbial food webs- a review. Marine Microbial Food Webs 8:295–324.

Kamjunke, N. and R. F. Zehrer. 1999. Direct and indirect effects of strong grazing by Daphnia galeata on bacterial production in an enclosure experiment. Journal of Plankton Research 21: 1175–1182.

Klausmeier, C. A., Litchman, E., and S. A. Levin. 2004. Phytoplankton growth and stoichiometry under multiple nutrient limitation. Limnology and Oceanography 49:1463-1470.

Kremer, C. T., Gillette, J. P., Rudstam, L. G., Brettum, P. and R. Ptacnik. 2014. A compendium of cell and natural unit biovolumes for> 1200 freshwater phytoplankton species: Ecological Archives. Ecology 95:2984–2984.

Leibold, M. A., Hall, S. R., Smith, V. H. and D. A. Lytle. 2017. Herbivory enhances the diversity of primary producers in pond ecosystems. Ecology 98:48–56.

Litchman, E. , Ohman, M. D., and T. Kiørboe. 2013. Trait-based approaches to zooplankton communities. Journal of Plankton Research 35:473–484.

Liu, J. 2016. Interspecific biodiversity enhances biomass and lipid productivity of microalgae as biofuel feedstock. Journal of Applied Phycology 28:25–33.

Lund, J. W. G., Kipling, C. and E. D. Le Cren. 1958. The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. Hydrobiologia 11:143–170.

MacKay, N. A. and J. J. Elser. 1998. Nutrient recycling by Daphnia reduces N2 fixation by cyanobacteria. Limnology and Oceanography 43:347–354.

Mandal, S., Abbott Wilkins, R. and J. B. Shurin. 2018. Compensatory grazing by Daphnia generates a trade-off between top-down and bottom-up effects across phytoplankton taxa. Ecosphere 9:e02537.

Martin-Creuzburg, D. and E. von Elert. 2009. Good food versus bad food: the role of sterols and polyunsaturated fatty acids in determining growth and reproduction of Daphnia magna. Aquatic Ecology 43:943-950.

Marzetz, V., Koussoroplis, A.-M., Martin-Creuzburg, D., Striebel, M. and A. Wacker. 2017. Linking primary producer diversity and food quality effects on herbivores: a biochemical perspective. Scientific Reports 7:1–9.

McCauley, E. and F. Briand. 1979. Zooplankton grazing and phytoplankton species richness: Field tests of the predation hypothesis 1. Limnology and Oceanography 24:243–252.

McQueen, D. J., Johannes, M. R., Post, J. R., Stewart, T. J., and D. R. Lean. 1989. Bottom-up and top-down impacts on freshwater pelagic community structure. Ecological Monographs 59:289-309.

Oksanen, J., Guillaume Blanchet, F., Friendly, M., Kindt, R. and P. Legendre. 2019. McGlinn 439 D et al. Vegan: Community Ecology Package. R package version, 2.5-5.

Olsen, Y., Jensen, A., Reinertsen, H., BØrsheim, K. Y., Heldal, M. and A. Langeland. 1986. Dependence of the rate of release of phosphorus by zooplankton on the P: C ratio in the food supply, as calculated by a recycling model 1. Limnology and Oceanography 31:34–44.

Pan, Y., Zhang, Y. and S. Sun. 2014. Phytoplankton–zooplankton dynamics vary with nutrients: a microcosm study with the cyanobacterium Coleofasciculus chthonoplastes and cladoceran Moina micrura. Journal of Plankton Research 36:1323–1332.

Peter, K. H. and U. Sommer. 2012. Phytoplankton cell size: intra-and interspecific effects of warming and grazing. PloS one 7:e49632.

Power, L. D. and B. J. Cardinale. 2009. Species richness enhances both algal biomass and rates of oxygen production in aquatic microcosms. Oikos 118:1703–1711.

Ptacnik, R., Andersen, T. and T. Tamminen. 2010. Diversity predicts stability and resource use efficiency in natural phytoplankton communities. Proceedings of the National Academy of Sciences 105:5134-5138.

Ptacnik, R., Solimini, A. G., Andersen, T. and T. Tamminen. 2010. Performance of the Redfield ratio and a family of nutrient limitation indicators as thresholds for phytoplankton N vs. P limitation. Ecosystems 13:1201–1214.

Sarnelle, O. 2005. Daphnia as keystone predators: effects on phytoplankton diversity and grazing resistance. Journal of Plankton Research 27:1229–1238.

Schälicke, S., Teubner, J., Martin-Creuzburg, D. and A. Wacker. 2019. Fitness response variation within and among consumer species can be co-mediated by food quantity and biochemical quality. Scientific Reports 9:1-9.

Shannon, C. E. and W. Weaver. 1948. Biodiversity measurements. The Mathematical Theory of Communication. Urbana University Press, Illinois, 117–27.

Sommer, U., Sommer, F., Santer, B., Jamieson, C., Boersma, M., Becker, C. and T. Hansen. 2001. Complementary impact of copepods and cladocerans on phytoplankton. Ecology Letters 4:545–550.

Sommer, U. and F. Sommer. 2006. Cladocerans versus copepods: the cause of contrasting top–down controls on freshwater and marine phytoplankton. Oecologia 147:183–194.

Sterner, R. W. 1986. Herbivores' direct and indirect effects on algal populations. Science 231:605–607.

Sterner, R. W. 1989. The role of grazers in phytoplankton succession. Plankton ecology. Springer, pp. 107–170.

Sterner, R. W., Elser, J. J. and D. O. Hessen. 1992. Stoichiometric relationships among producers, consumers and nutrient cycling in pelagic ecosystems. Biogeochemistry 17:49–67.

Stibor, H., Vadstein, O., Diehl, S., Gelzleichter, A., Hansen, T., Hantzsche, F., Katechakis, A., Lippert, B., Løseth, K., Peters, C. and W. Roederer. 2004. Copepods act as a switch between alternative trophic cascades in marine pelagic food webs. Ecology Letters 7:321-328.

Striebel, M., Singer, G., Stibor, H. and T. Andersen. 2012. "Trophic overyielding": phytoplankton diversity promotes zooplankton productivity. Ecology 93:2719–2727.

Tamminen, T. and T. Andersen. 2007. Seasonal phytoplankton nutrient limitation patterns as revealed by bioassays over Baltic Sea gradients of salinity and eutrophication. Marine Ecology Progress Series 340:121–138.

Thingstad, T. F. and R. Lignell. 1997. Theoretical models for the control of bacterial growth rate, abundance, diversity and carbon demand. Aquatic Microbial Ecology 13:19–27.

Utermöhl, H. 1958. Zur vervollkommnung der quantitativen phytoplankton-methodik: Mit 1 Tabelle und 15 abbildungen im Text und auf 1 Tafel. Internationale Vereinigung für theoretische und angewandte Limnologie: Mitteilungen 9:1–38.

Yoshida, T., Gurung, T., Kagami, M. and J. Urabe. 2001. Contrasting effects of a cladoceran (Daphnia galeata) and a calanoid copepod (Eodiaptomus japonicus) on algal and microbial plankton in a Japanese lake, Lake Biwa. Oecologia 129:602–610.

Supplementary Information

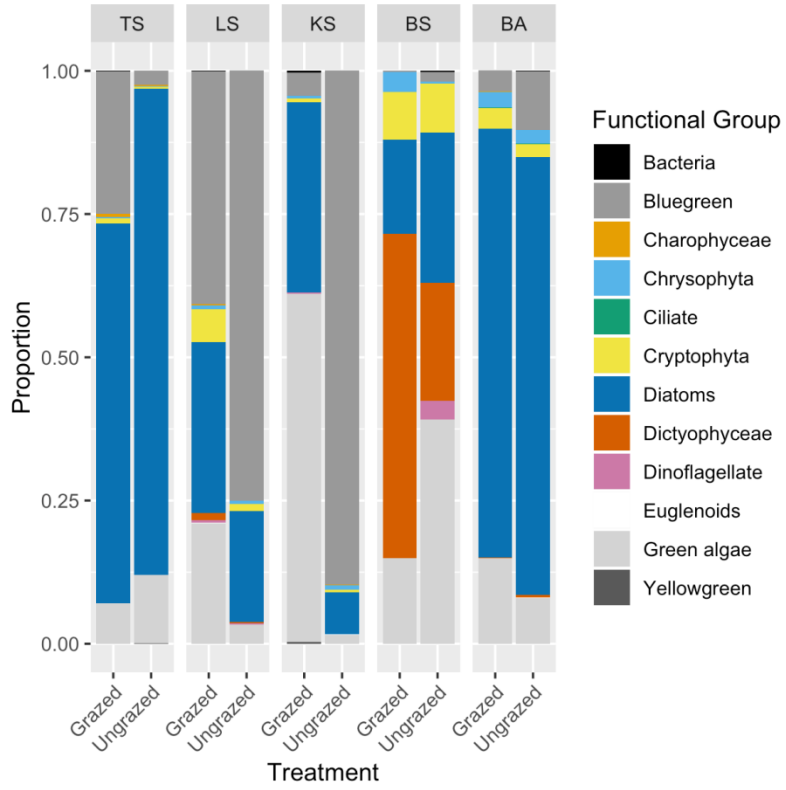


Figure S4.1. Phytoplankton composition in grazed and ungrazed samples for each lake. Functional groups have been aggregated from biovolume-transformed counts at the genus level. Each vertical bar represents a sample divided up by the proportion of each functional group.

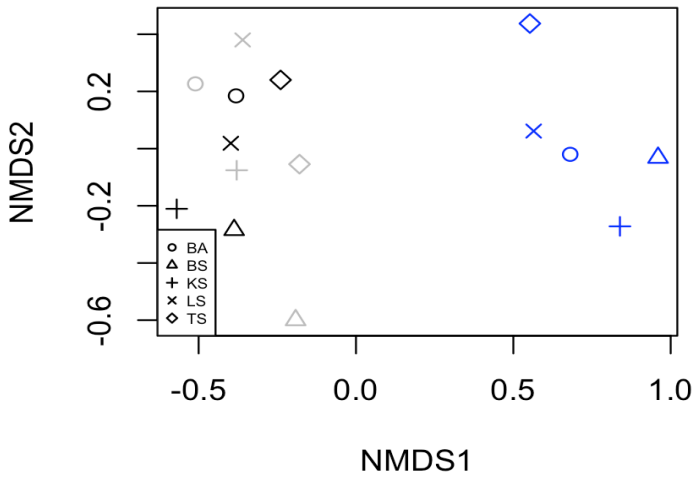


Figure S4.2. The ordination plot produced by nonmetric multidimensional scaling of phytoplankton communities (stress = 0.12). Symbols refer to the lake from which the phytoplankton were originally sampled. Colors refer to treatment and time, where starting samples are in blue, samples grazed by zooplankton are in black, and samples without zooplankton grazing are in gray.

Table S4.1. For each lake, GPS location, total phosphorus (TP), particulate phosphorus (PP), chlorophyll-a, and the depth (in meter) of our zooplankton haul at our sampling location (roughly at the center of the lake or lake arm) were recorded. Total phosphorus (TP) and particulate phosphorus (PP) were directly measured via standard acid-persulfate digestion procedures and photometrical light absorbance measurements (performed at Seeon Limnological Station).

Lake	Latitude	Longitude	TP	chl-a [µg L^{-1}]	Depth [m]
Bansee (BA)	47.964301	12.440403	24.259	16.31	3.5
Brunnensee (BS)	47.983587	12.436525	5.5367	1.37	10+
Klostersee (KS)	47.973733	12.455108	13.15	4.58	10+
Langbürgnersee (LS)	47.89639	12.360254	6.9937	1.51	10+
Thalersee (TS)	47.906215	12.338978	15.08	8.78	8
Chiemsee (CS)	47.881596	12.355663	21.783	4.28	6

Table S4.2. Zooplankton community composition for Lake Chiemsee counted. A vertical haul of zooplankton from 10m or just above the lake bottom was taken. At least 500 zooplankton individuals from two samples were counted.

Taxon	**ind. L^{-1}**
Daphnia	2.7
Diaphanosoma	0.032
Bosmina	0.037
Cydorus	0.005
calanoid copepod	8.3
cyclopoid copepod	0.50
copepod nauplii	21
rotifer	1.9

Table S4.3. Results of the best fitting linear mixed effect models predicting Shannon diversity for phytoplankton functional groups measured using an AlgaeLabAnalyzer with data from BA removed, evenness for phytoplankton functional groups measured using an AlgaeLab Analyzer, and evenness for biovolume-transformed phytoplankton genera.

	Diversity-Lab Analyzer (BA removed)				**Evenness-Lab Analyzer**				**Evenness-Biovolume**			
Effect	Estimate (s.e)	ndf, ddf	t-value	P-value	Estimate (s.e)	ndf, ddf	t-value	P-value	Estimate (s.e.)	ndf, ddf	t-value	P-value
Fixed effects	marginal R^2 = 0.29, conditional R^2 = 0.58				marginal R^2 = 0.42, conditional R^2 = 0.56				marginal R^2 = 0.13, conditional R^2= 0.60			
intercept	0.53 (0.082)	1,19	6.51	<0.0001	0.69 (0.14)	1,23	4.96	0.0001	0.49 (0.077)	1,4	6.41	0.003
Treatment (UG)	-0.26 (0.066)	1,19	-4.0	0.0008	-0.61 (0.17)	1,23	-3.56	0.002	-0.12 (0.072)	1,4	-1.72	0.16
TP	----------	-------	-------	-------	0.0056 (0.0095)	1,3	0.58	0.60	----------	--------	-------	-------
Treatment x TP	----------	-------	-------	-------	0.031 (0.011)	1,23	2.80	0.01	----------	--------	-------	-------
Random effects												
Lake variation	0.018				0.0066				0.016			
Residual variation	0.026				0.032				0.014			

Table S4.4. Results of linear mixed effect models predicting nutrient feedback effects, with and without the concentration of zooplankton residing in the source of grazed water as a continuous predictor variable.

Phytoplankton biomass (samples receiving grazed nutrients – samples receiving ungrazed nutrients)								
Effect	Estimate (s.e)	ndf, ddf	t-value	P-value	Estimate (s.e)	ndf, ddf	t-value	P-value
Fixed effects	marginal R^2 = NA, conditional R^2 = 0.48				marginal R^2 = 0.79, conditional R^2 = 0.79			
intercept	0.20 (0.37)	1,10	1.9	0.59	-0.81 (0.18)	1,9	-4.40	0.001
number zoop	----------	-------	-------	-------	0.014 (0.0019)	1,9	7.15	0.0001
Random effects								
Lake variation	0.49				$1.4\ e^{-10}$			
Residual variation	0.54				0.21			