Eco-evolutionary interactions with multiple evolving species reveal both antagonistic and additive effects

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Abstract

Most eco-evolutionary research focuses on ecological effects of single-species evolution. We therefore know little of eco-evolutionary dynamics when multiple species evolve simultaneously. We quantified evolution-mediated ecological effects in communities equivalent in genetic diversity and starting biomass, but different in selection background (heatwave exposure) of one or all four zooplankton species (three *Daphnia* and one *Scapholeberis* species). We observed transient eco-evolutionary effects that differed depending on which species in the community had evolved. Evolution did not always lead to higher abundances of the evolved species. Indirect effects on species abundances caused by evolution of another species could be as strong as direct effects mediated by its own evolution. The cumulative effect of evolution in multiple species was antagonistic for community composition and grazing pressure but additive for community-wide biomass. Our results imply that focusing on single species' evolutionary effects on ecology may lead to unreliable predictions when multiple species evolve simultaneously.

Introduction

The field of eco-evolutionary dynamics has expanded since evidence emerged for rapid evolution (Thompson 1998; Hendry & Kinnison 2001), occurring on similar time scales as ecological processes (Hairston *et al.* 2005; Fussmann *et al.* 2007; Hendry 2017). A surging number of studies documented effects of evolution on population dynamics (e.g., Johnson & Stinchcombe 2007; Becks *et al.* 2012), community composition (e.g., Farkas *et al.* 2013; Pantel *et al.* 2015) and ecosystem functioning (e.g., Bassar *et al.* 2010). However, most empirical studies so far quantified the effect of evolution on ecology focusing on the ecological consequences of evolutionary change in a single species (e.g., Palkovacs & Post 2009; Pantel *et al.* 2015). While these studies provided important insights into the role of evolution for ecological dynamics, focusing on evolutionary responses of a single species may underestimate complex eco-evolutionary interactions occurring in natural communities in which multiple species co-occur and simultaneously evolve (Faillace & Morin 2016; De Meester *et al.* 2019).

Adopting a multi-species eco-evolutionary approach is important to understand how multiple species within their community and ecosystem simultaneously evolve to environmental change, and how such multi-species evolutionary changes affect community and ecosystem features in changing environments (De Meester *et al.* 2019). One example involves the global increase in mean temperature and increased prevalence of heatwaves (IPCC 2012, 2021). Consequently, many species will endure higher temperatures and extreme temperature variability more often (Christidis & Stott 2014). Studies showed that species can respond to these warmer temperatures by means of rapid micro-evolutionary change in species' traits like body size, heat tolerance or behavior (e.g., Buckley & Kingsolver 2019; Salinas *et al.* 2019), which can have large consequences for the ecological functioning of communities and ecosystems (Fussmann *et al.* 2007; Hughes *et al.* 2008).

Traits play a central role in eco-evolutionary interactions, as they can evolve when (partly) heritable and govern ecological interactions and ecosystem properties. How traits are linked to these can differ among species with varying trait value ranges (Hughes *et al.* 2008; Wojtal-Frankiewicz 2012; Mensens

66 *et al.* 2017). For example, the grazing capacity of *Daphnia* species is known to be size-dependent with
67 larger-bodied species being more efficient grazers (Gliwicz & Kerfoot 1980). Species also vary in their
68 responses to environmental change (Parmesan 2006; Sheridan & Bickford 2011), including
69 evolutionary responses (Rapacciuolo *et al.* 2014), which may influence their effect on ecosystem
70 functions. As the evolutionary response of a focal species can be altered by species interactions
71 (Lawrence *et al.* 2012; Grainger *et al.* 2021), the ecological effect of the focal species' evolution might
72 also be mediated by other species and their evolution. Yet, so far few studies quantified whether and
73 to what extent evolutionary trait change of different species have similar or divergent ecological
74 effects within the same community and ecosystem.

75 Evolution-mediated ecological effects of multiple species can be independent leading to additive
76 cumulative effects, buffer one another leading to antagonistic interactions, or enhance each other
77 resulting in synergistic interactions. Hence, the total effect size of evolution on ecology in a system
78 where multiple species evolve simultaneously could diverge from a total effect size estimated from
79 species-specific evolutionary effects on ecology assessed in isolation (Piggott *et al.* 2015; Hendry 2019;
80 Fig. 1). To our knowledge, no study so far evaluated how evolution-mediated effects on ecology of
81 multiple co-occurring species in a guild combine to shape community and ecosystem features and
82 tested whether the cumulative effects of evolution of multiple species on ecology are additive,
83 synergistic or antagonistic.

84 [FIGURE 1]

85 Here, we adopt a multi-species eco-evolutionary approach to assess the cumulative effects of
86 evolution of multiple *Daphnia* species on ecology. We use *Daphnia* as a model organism as they are
87 important ecological interactors commonly found in ponds and shallow lakes. Micro-evolutionary trait
88 change in *Daphnia* can have substantial effects on freshwater communities and ecosystem functioning
89 (De Meester *et al.* 2011; Pantel *et al.* 2015). In our study, we quantify evolution-mediated ecological
90 effects in zooplankton communities that are equivalent in the number of clonal lineages and starting

biomass but differ in selection background (i.e., previous heatwave exposure, Vanvelk *et al.* 2021) of either one or of all four zooplankton species (three *Daphnia* and one *Scapholeberis* species). We test the overarching hypothesis that evolution-mediated ecological effects of different species may interact such that eco-evolutionary dynamics in a multi-species setting differ from expectations generated by adding up eco-evolutionary dynamics of single species. We also hypothesize that *Daphnia* species will differ in their evolution-mediated ecological effects on community structure and ecosystem functioning and that the way in which these effects of single species add up or interact will differ among the endpoint studied. Based on the fact that in *Daphnia* larger-bodied species tend to be competitively dominant, we furthermore expected that the eco-evolutionary effects among species might be transient. Finally, we expect the interaction between *Scapholeberis* and the *Daphnia* species to be weak due to the small-bodied *Scapholeberis* being a weak competitor and their diverging niches, with *Scapholeberis* being hyponeustic (i.e. clinging to the underside of the water surface) while *Daphnia* species are planktonic.

Material and methods

The overall study design consists of 1) establishing multiple clonal isolates of four Daphniidae species isolated from one single habitat, i.e. populations co-occurring in nature; 2) setting up species populations in mesocosms using these clonal isolates, which were then exposed to a heatwave; and 3) assembling four-species communities in a second mesocosm experiment, the focus of the current study, using either clonal isolates obtained from part 1 or part 2. We briefly outline all parts below, but details can be found in Supplement A.

Obtaining original and heatwave-evolved populations

We focused on four Daphniidae species (*Daphnia magna* Straus, *D. pulicaria* Forbes, *D. galeata* Sars and *Scapholeberis mucronata* Müller), frequently found to co-occur in natural communities (Altermatt *et al.* 2008; E. M. van den Berg pers. comm. in Langerodevijver), which cover a wide range of mean body size values i.e., 0.7mm (*S. mucronata*), 1.5mm (*D. galeata*), 1.95mm (*D. pulicaria*) to 2.4mm (*D. magna*). *Daphnia* clones were hatched from sexual dormant eggs extracted from sediment samples (Langerodevijver, Spring 2018). Each *Daphnia* clone represents a distinct genotype and the set of clones captures the natural genetic variation within species that could occur at the start of a growing season. *S. mucronata* individuals were sampled from the active population in Langerodevijver and kept as clonal lineages.

Heatwave-evolved populations for each of the four species were obtained from an outdoor mesocosm experiment in which populations experienced selection by a naturally occurring heatwave. Genetically equivalent populations of each species were kept under ambient temperatures for approximately six weeks (15th June 2018 – 27th July 2018), during which a naturally occurring heatwave increased temperatures in all mesocosms (daytime water temperature in the ambient mesocosms exceeded 25°C for 22 out of 37 days; Supplement A Figure S1). The occurrence of this natural heatwave led to the populations becoming heatwave-evolved.

Common gardening community assembly

We started a four-week community assembly experiment on August 30th 2018 by constructing six different four-species communities: 1) a community in which all populations consisted of clonal lineages directly isolated from the pond of origin, i.e. not exposed to the heatwave; the None-evolved community, 2) a community in which all populations were represented by clonal lineages isolated from the heatwave-evolved mesocosms; the All-evolved community, 3-6) four communities in which only one population consisted of clonal lineages isolated from the heatwave-evolved mesocosms, whereas the other populations were represented by clonal lineages directly isolated from the pond of origin. These communities are referred to as the *D. magna*-evolved community, the *D. pulicaria*-evolved community, the *D. galeata*-evolved community and the *S. mucronata*-evolved community. Prior to inoculation, all lineages were cultured under standardized conditions for four weeks (i.e. three to four generations depending on the species). This ensures we worked with genetically identical individuals per clonal lineage; as we did not perform genomic analyses on all clonal lineages, we cannot claim with certainty that all clonal lineages are genetically unique. Communities were inoculated with near-equal biomasses for all species and kept at constant temperature of approximately 30°C (more details in Supplement A).

Data collection

To assess the effect of evolution on ecology, we quantified several endpoints characterizing different ecological levels. We estimated species abundances from weekly zooplankton samples, from which we determined community composition and biomass. To quantify biomass, we measured the body length (mm; top of the eye until base of the spine) of 10 randomly selected adult females per species for each sample and estimated their weight using weight-length regressions (Bottrell *et al.* 1976; Rosen 1981). The weight of the juveniles was estimated with the same methodology, assuming that juvenile length is on average approximately half the length of a female adult. Biomass per life stage and species was calculated for each sample by multiplying absolute species abundance with its average estimated weight. We quantified the chlorophyll a concentration (a proxy for phytoplankton biomass) daily using a handheld fluorometer (Turner designs; San Jose, CA, USA). Given similar

nutrient and light conditions in the mesocosms, differences in chlorophyll a concentration reflect mesocosm-specific grazing pressures. We calculated the change in chlorophyll a concentration for each sampling time as the difference in chlorophyll a concentrations two days after and two days before the sampling moment. Grazing pressure was defined as the change in chlorophyll a multiplied by -1 to make this metric more intuitive, as grazing pressure entails a decrease in chlorophyll a concentration (i.e., decreases in algal concentrations due to grazing exceeding algal growth).

Statistical analyses

Statistical analyses were run using R Version 4.2.3 (R Core Team 2014) in RStudio and we used the 'ggplot2' R package (Wickham 2009) for all visualizations. We analyzed the experimental data in two steps. First, we analyzed the data on the community of the three *Daphnia* species only (i.e., not considering *Scapholeberis*), as these species strongly compete with each other, all being free-swimming in the medium and non-selective filter-feeders. Second we focused on reciprocal influences between the *Daphnia* species and *Scapholeberis* using the full dataset.

Evolution-mediated ecological effects

By contrasting the single species-evolved *Daphnia* communities to the None-evolved community, we quantified the species-specific evolution-mediated ecological effects and interspecific differences therein. Contrasting the None-evolved communities to the All-evolved communities allows quantifying the total effect size of evolution on ecology when multiple species in the community evolve. Specifically, we tested for differences in population abundances, population biomass, species' relative abundances, total community biomass and change in chlorophyll a concentration across the five different community types over time using a repeated-measures analysis of variance (ANOVA). Models were constructed using the 'lmer' function (lme4 package; Bates *et al.* 2015) with the ecological property as response variable, community type as fixed factor and time as repeated factor. Given that the observed eco-evolutionary patterns appeared transient (i.e. strong effect of time), we also carried out separate analyses per time point for the same ecological endpoints using type II

ANOVAs. Additional endpoints were grazing pressure and grazing efficiency, calculated as grazing pressure divided by total zooplankton biomass. When a significant effect of community type was found, we conducted pairwise post hoc tests using the 'glht' function (multcomp package; Hothorn *et al.* 2008) adjusted for multiple testing with the Holm correction. The community composition was analyzed with a permutational analysis of variance (PERMANOVA) with the 'adonis' function (vegan package; Oksanen *et al.* 2008). The model used community composition based on Hellinger-transformed abundances of all species as response variable and community type, time and their interaction as predictors. Differences in community composition were visualized using Hellinger-transformed abundances in a Non-metric Multi-Dimensional Scaling analysis (NMDS) with the 'metaMDS' function (vegan package; Oksanen *et al.* 2008).

Interaction between evolution-mediated ecological effect sizes

To test whether evolution-mediated ecological effects of distinct species are additive, synergistic or antagonistic, we calculated the predicted value of the evolution-mediated ecological effect size of the All-evolved community based on the effect sizes of the single species-evolved *Daphnia* communities assuming additivity of these effects. This was done for community composition and biomass as well as grazing pressure. Absolute effect sizes between the single species-evolved and the None-evolved communities and between the All-evolved and the None-evolved communities were calculated by creating 100 sets of random pairwise permutations between the six replicates of each respective community type. From each permutation, we calculated the mean difference across the six replicates, creating a distribution of 100 community differences. From this distribution, we quantified a mean effect size and a 95% confidence interval (CI) around the mean. By adding all the effect sizes of the difference between single species-evolved and None-evolved communities, we obtained the predicted effect sizes of total evolution and calculated the mean value and 95% CI. If the predicted effect is outside the 95% CI of the observed values, then the single species effects are considered non-additive. If the predicted effect is significantly larger than the 95% CI of the observed values, the

interaction is synergistic and if the observed effect is significantly smaller than the predicted effect, the interaction is considered antagonistic (Payne *et al.* 2001; Coors & De Meester 2008).

Scapholeberis *dynamics and interaction* Daphnia - Scapholeberis

To test if *Daphnia* evolution affects *Scapholeberis* abundances and vice versa, we constructed a regression model followed by type II ANOVA, assessing differences across communities over time and per time point. We used *Scapholeberis* abundance as response variable to assess possible evolution-mediated effects from heatwave exposure of any of the *Daphnia* species or of *Scapholeberis* itself. We used *Daphnia* species abundance as response variable to study effects of *Scapholeberis* evolution (i.e., *S. mucronata*-evolved communities). Lastly, we analyzed the other variables (i.e. relative abundance, community composition, change in chlorophyll a, grazing pressure and efficiency) to probe for parallels between our findings when using a three-species community versus the four-species community (i.e. including *S. mucronata*). We did not calculate *S. mucronata* biomass because we could not distinguish adults from juveniles due to its small size.

Results

Evolution-mediated ecological effects

Throughout the experiment, we found significant differences for *D. galeata* population biomass (p=0.002 Table 1; Supplement B Fig. S2D). The *D. galeata* biomass in the All-evolved communities was significantly higher compared to its biomass in the None-evolved (p=0.037; Table S2) and *D. galeata*-evolved communities (p=0.037). *D. galeata* biomass was also significantly higher in the *D. magna*-evolved communities compared to the None-evolved (p=0.028) and *D. galeata*-evolved communities (p=0.028; Table S2). We detected no significant differences for *D. pulicaria* and *D. magna* in their population biomass (Fig. S2E and S2F respectively) across community types (p=0.08 and p=0.79, respectively; Table 1). Statistical output for species' absolute and relative abundances are presented in Supplement B.

Total community biomass did not differ significantly over time among communities (p=0.72; Table 1; Fig. S2J). However, we did find significant differences in the community composition over time (p=0.001; Table 1) and visualization shows that communities changed from equal presence of all three *Daphnia* species (initial inoculum) to communities being mainly dominated by *Daphnia magna* (Fig. 2). Each pairwise time point comparison differed significantly in community composition (p<0.001; Table 1). Community type also differed significantly (p=0.001), but post-hoc testing did not reveal significant differences in the community composition between specific communities (Table S2).

[FIGURE 2]

We observed significant differences among community types for top-down control of phytoplankton, quantified by changes in chlorophyll a concentration through time (p<0.001; Fig. S2K). We found significant differences between the None-evolved communities and all other communities (all p<0.006; Table S2), between the *D. magna*-evolved communities and the *D. galeata*-evolved communities (p=0.003), and the *D. pulicaria*-evolved communities (p=0.013) and the All-evolved communities (p<0.001).

[TABLE 1]

When evaluating differences among community types for each time point separately, we observed no significant differences for population or total community biomass (Table 1; Fig. 3A-D). However, the community composition differed significantly at time point T2 ($p=0.04$; Table 1), specifically between the *D. magna*-evolved communities and the None-evolved communities ($p=0.01$; Table S3). This is visually confirmed in the NMDS plot (i.e., the red polygon of the *D. magna*-evolved community did not overlap with the green polygon of the None-evolved community; Fig. 3E). At time point T3, only tendencies of the pattern observed at T2 remained (Table 1; Fig. S4), and by time point T4 there was complete convergence of the communities (Table 1; Fig. S5).

The effects of evolution on top-down control of phytoplankton were also transient, with strong significant differences in grazing pressure at time point T2 ($p=0.001$; Table 1) followed by convergence toward the end of the experiment. At time point T2, the None-evolved communities showed significantly stronger grazing pressure than the *D. galeata*-, *D. pulicaria*-, *D. magna*- and the All-evolved communities ($p= 0.02$, 0.03, 0.01 and <0.001, respectively; Table S2; Fig. 3F). The statistical output for grazing efficiency can be found in Supplement B.

[FIGURE 3]

Interaction between evolution-mediated ecological effect sizes

Comparing the predicted and the observed total evolution-mediated ecological effect size in the All-evolved communities reveals that the single species effects at time point T2 are antagonistic for community composition and grazing pressure, since assuming additivity resulted in a predicted value that lies outside the 95% CI of the observed values (Fig. 4B-C). For community biomass, the predicted value fell within the 95% CI of observed values, therefore the hypothesis of additivity is not rejected (Fig. 4A).

[FIGURE 4]

Scapholeberis *dynamics and interaction* Daphnia - Scapholeberis

We did not observe an effect of *Scapholeberis* evolution on its own abundance nor on the abundances of the *Daphnia* species across time (p=0.399; Table S4; Fig. S6). At time point T1 we observed significantly higher densities of *Scapholeberis* (p=0.005; Fig. 5A) in the *S. mucronata*-evolved communities compared to the *D. galeata*- (p=0.024; Table S5) and *D. magna*-evolved communities (p=0.037; Table S5). We did not observe significant effects of *Scapholeberis* or *Daphnia* evolution on *Scapholeberis* abundances at subsequent time points (T2: p=0.396, T3: p=0.455, T4: p=0.283; Table S4). Evolution in *Scapholeberis* did not lead to any significant changes in abundance for *Scapholeberis* nor any of the *Daphnia* species compared to the None-evolved community (Fig. 5B). Focusing on the results for *Daphnia* while analyzing the four-species community including *Scapholeberis,* our overarching results on the *Daphnia* patterns are similar with the observations for the dataset focusing on the three *Daphnia* species only (see overview of the comparison between the three-species and four-species analysis in Table S6). Yet, several of the finer-scaled dynamics are lost (see Table S6), probably due to the fact that *Scapholeberis* is so indifferent to the different treatments.

[FIGURE 5]

Discussion

Global change induces eco-evolutionary responses of multiple species simultaneously (Norberg *et al.* 2012; Urban *et al.* 2016). To understand the impact of global change on natural communities, it is essential to quantify both the evolutionary responses of multiple species within these communities to the environmental change, and the ecological consequences of these evolutionary responses on the ecosystem and on the other species (De Meester *et al.* 2019). In this study, we go beyond a single-species approach and evaluate evolution-mediated ecological effects of four zooplankton species on a set of response variables connecting to population, community and ecosystem ecology. Our multi-species analysis of ecological implications of heatwave-induced evolution shows that there is a significant, but transient effect of evolution on ecology and that this effect varies among species. Our results also show that evolved populations not necessarily perform better in the community. When assessing the cumulative effect of evolution, we found that it was additive for community biomass, but antagonistic for community composition and grazing pressure, reflecting that the eco-evolutionary feedback of different species can counteract each other.

We observed significant evolutionary-mediated ecological effects for two of the three *Daphnia* species and only a weak effect for *Scapholeberis*. Heatwave-evolved *S. mucronata* did not affect the *Daphnia* abundances, and the effect of *Daphnia* evolution on *Scapholeberis* was moderate. This weak eco-evolutionary interaction between the *Daphnia* species and *Scapholeberis* may reflect the different niche use of these species in our experimental cultures as well as in nature (Błędzki & Rybak 2016). In our analysis of the data of the *Daphnia* species, significant but transient evolutionary-ecological effects were found. The effects of heatwave-induced evolution were strongest after two weeks, but diminished rapidly toward the end of the experiment. After four weeks, the largest species, *D. magna*, outcompeted *D. galeata* and *D. pulicaria*, irrespective of the selection background of populations. *D. magna* is known to be a strong competitor due to its large body size, which results in a lower metabolic demand per unit mass than for smaller species (Gliwicz 1990) and allows them to graze more efficiently (Cyr & Curtis 1999; Gianuca *et al.* 2016). In the absence of visual predators, it is indeed

observed that large-bodied zooplankton often dominate the community (Brooks & Dodson 1965; Hu & Tessier 1995). The eco-evolutionary effect observed in our experiment entails that this dominance is delayed in the evolved compared to the non-evolved communities.

We found that the ecological effects of evolution varied among the three *Daphnia* species we studied. For example, evolution of *D. magna* resulted in higher *D. galeata* abundances, and even more so than evolution of *D. galeata* itself. Given that competitive strength in daphniids is strongly related to body size (Hall *et al.* 1976; Gianuca *et al.* 2016), heatwave-induced evolution may have reduced the competitive dominance of *D. magna*. Warming is known to result in smaller body sizes of ectothermic organisms, through both phenotypic plasticity and evolution (Gardner *et al.* 2011; Vanvelk *et al.* 2021). Such trait evolution can occur in different species simultaneously (Daufresne *et al.* 2009; Sheridan & Bickford 2011; Franks & Hoffmann 2012), but to a different extent. In an earlier study, we showed that heatwave-induced evolution indeed reduced body size in the here studied *Daphnia* populations, which was strongest for *D. magna* (Vanvelk *et al.* 2021). In addition, heatwave-induced evolution often results in physiological responses such as increased heat shock protein and hemoglobin production (Zeis *et al.* 2013; Stillman 2019), leading to a reduced availability of energy for growth and reproduction (Chopelet *et al.* 2008; Walberg & Green 2021). This shift in energy allocation likely differs across zooplankton species, as the costs and ecological implications of evolution of such traits can show pronounced differences (Sheridan & Bickford 2011). *D. magna* ended up dominating all community types, suggesting that evolution upon heatwave exposure would not impact the equilibrium condition. Yet, within communities consisting of *D. magna*-evolved populations, *D. magna* experienced a transient competitive disadvantage, creating a window-of-opportunity for less competitive species, such as *D. galeata*, to reach higher abundances than in the communities with non-evolved *D. magna*. These transient changes need to be viewed in the context of rapidly changing dynamics typically characterizing spring and summer dynamics of phytoplankton and zooplankton in lakes and ponds (Lampert & Sommer 2007), possibly impacting resource-consumer dynamics in lakes (e.g., Schaffner *et al.* 2009). Transient competitive effects were also found in microalgae after salt

adaptation (Limberger & Fussmann 2021). We note that aspects of the transient nature of the eco-evolutionary dynamics observed in our study might to some extent be influenced by rapid micro-evolutionary processes occurring during the community assembly experiment. This is possible due to the short generation times of *Daphnia*, yet is unnecessary to explain the observed patterns, as the transient nature was predicted based on the observation that *D. magna* in such mesocosm experiments tends to strongly dominate community composition (e.g. Verreydt *et al* 2012; Almeida *et al* 2023). Even though the experiment was carried out at a high temperature (30°C; average summer temperatures in the study region are 20-22°C), it did not result in higher absolute or relative abundances of *D. magna* in the *D. magna*-evolved or All-evolved communities. This is not due to *D. magna* being more heat-sensitive than the other species, as our previous study showed that *D. magna* has the highest heat tolerance of these three species (Vanvelk *et al.* 2021). The response of *D. galeata* is more in line with adaptive evolution, as it reached a larger share in the *D. galeata*-evolved communities than in the None-evolved communities. However, the pattern is more complicated, as the abundance of *D. galeata* seems to be more enhanced when *D. magna* evolves than when *D. galeata* itself evolves. These findings illustrate that evolution-mediated ecological effects of competitors on the focal species might be stronger than the evolution-mediated ecological effect of the focal species itself. Such indirect effects of evolution were also found in a small modelled food web, where focal species persistence more often depended on the other species evolution than its own evolution (van Velzen 2023). It highlights the importance of studies considering the eco-evolutionary feedback in all (or at least the dominant) species of communities.

We found significant, but transient, differences in grazing pressure across communities. Grazing pressure was strongest in the None-evolved communities and weakened when one or all species consisted of heatwave-evolved populations. This decrease in grazing pressure could be driven by changes at the individual level (e.g., trade-off between metabolic activity and thermal adaptation; Wojtal-Frankiewicz 2012) or at the *Daphnia* community level (e.g., decrease in abundance of larger-bodied zooplankton; Gianuca *et al.* 2016). These mechanisms likely interacted in the present study,

with heatwave-evolved populations having overall smaller body sizes (Vanvelk *et al.* 2021) combined with a decreased abundance of larger *D. magna* in favor of higher abundances of the smaller-bodied *D. galeata* might result in a lower grazing in communities with evolved populations. This effect was most prevalent in communities consisting of *D. magna* heatwave-evolved populations. While this effect was transient, it can still have large implications for natural ponds and lakes. Heatwave exposure leading to a reduced grazing capacity of the zooplankton community could strongly increase the likelihood of harmful algal blooms and of shifts from clear to turbid water state occurring at high temperatures in shallow standing waters (Moss *et al.* 2011).

When multiple species within a community simultaneously evolve to environmental change, the cumulative effect of evolution can be either additive, synergistic or antagonistic depending on whether or not the single-species eco-evolutionary effects interact (De Meester *et al.* 2019). Using an additive model as null model, we found that the cumulative effect of evolution on community composition and grazing pressure was lower than expected based of the sum of single-species effects. This indicates to an antagonistic interaction for these ecological endpoints, where evolution of one species partially compensated for the effect of evolution of the other species. Conversely, the cumulative effect of evolution was additive for community biomass. While previous studies have found non-additive effects of interspecific interactions among species (terHorst *et al.* 2015; Thompson *et al.* 2018; Lürig *et al.* 2021), our study suggests that non-additivity can also occur between the ecological effects of evolution of multiple species. While Hendry (2019) suggested an asymptotic increase in the cumulative effect of evolution when increasing the number of species within a community that are genetically variable, the effect can likely take any shape. Our results, for instance, point to an interpretation that the cumulative effect of all species evolving is most similar to the effects of only *D. magna* evolving, suggesting that evolution of *D. magna* interferes with and reduces the impact of evolution of its smaller congeners. Interactive effects can greatly complicate the predictability of the total effect of evolution and bias predictions as it results in an under- or

386 overestimation of evolution's effect on ecology when cumulative effects are extrapolated from
387 separate single species effects (Billick & Case 1994; Simmons *et al.* 2021).

388 As outlined in De Meester *et al.* (2019), most studies fall short on studying eco-evolutionary
389 interactions in a multi-species framework because (i) evolution is only studied in one focal species; (ii)
390 the non-focal species are not being given the opportunity to evolve; and (iii) evolution itself is mostly
391 studied without embedding the species in realistic communities (De Meester *et al.* 2019). Our study
392 tackles the first two shortcomings, and indeed shows that the outcome of multi-species eco-
393 evolutionary interactions deviates from expectations based on single-species studies. Yet, our study
394 could still be further improved as the heatwave-evolved populations were exposed in a single-species
395 context and were not given the prior opportunity to evolve to interspecific competition. While
396 originally our study design included heatwave selection in a community context, this treatment failed
397 because all communities became rapidly dominated by *D. magna* during the selection experiment,
398 leaving no opportunity to obtain populations exposed to both a heatwave and interspecific
399 competition. Hence, as selection occurred in the absence of competitors, evolution of enhanced heat
400 tolerance was not modulated by selection for competitive strength, so that the degree to which heat
401 tolerance evolved or the pathway it followed may have resulted in a stronger cost in terms of
402 competitive ability (Lawrence *et al.* 2012; Barraclough 2015; Grainger *et al.* 2021). This can potentially
403 explain why heatwave-evolved *D. magna* population had a transient competitive disadvantage in the
404 first half of the experiment. If that interpretation holds, then eco-evolutionary dynamics in a system
405 in which all species are allowed to evolve in competition might give a different result from what we
406 observed here.

407 Our study provides a first step toward studying the effect of multiple simultaneously evolving species
408 on ecology. Moving beyond a focal-species approach toward a multi-species approach is required to
409 reveal interactive effects of evolution on ecology (De Meester *et al.* 2019) and obtain more realistic
410 estimates of cumulative effects of evolution (Hendry 2019). Effects of evolution on ecology in our

study were transient and varied among species, creating a window of competitive release and giving the inferior competitor species the opportunity to temporarily increase in abundance. This transient effect implies that solely assessing the effects of evolution at the end of the experiment would have overlooked the dynamical effects of evolution, as *D. magna* dominated all communities at the end. The nature of the interaction between effects of evolution differed across the studied ecological endpoints. While the study of multi-species eco-evolutionary interactions is challenging and the results may be more complex to interpret than single-species studies, it will be a crucial step to assess the real impact of eco-evolutionary interactions in complex, natural settings.

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582

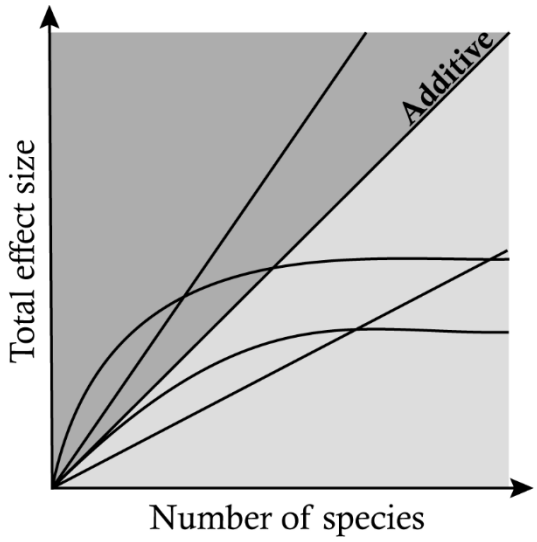
583 **Table 1**

	Over time	**Time point 1**	**Time point 2**	**Time point 3**	**Time point 4**
Population biomass					
D. magna	P= 0.793	P= 0.610	P= 0.495	P= 0.577	P= 0.781
D. pulicaria	P= 0.080	P= 0.540	P= 0.079	P= 0.589	P= /
D. galeata	**P= 0.002**	P= 0.112	P= 0.058	P= 0.173	P= 0.479
Total community biomass	P= 0.719	P= 0.911	P= 0.330	P= 0.560	P= 0.785
Community composition	Time: **P<0.001** Treatment: **P=0.001**	P= 0.482	**P= 0.036**	P= 0.100	P= 0.312
Change in Chla concentration	**P< 0.001**	P= /	P= /	P= /	P= /
Grazing pressure	P= /	P= /	**P= 0.001**	P= 0.858	P= 0.464

584

585 **Figure 1:**

586



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589 **Figure 2:**

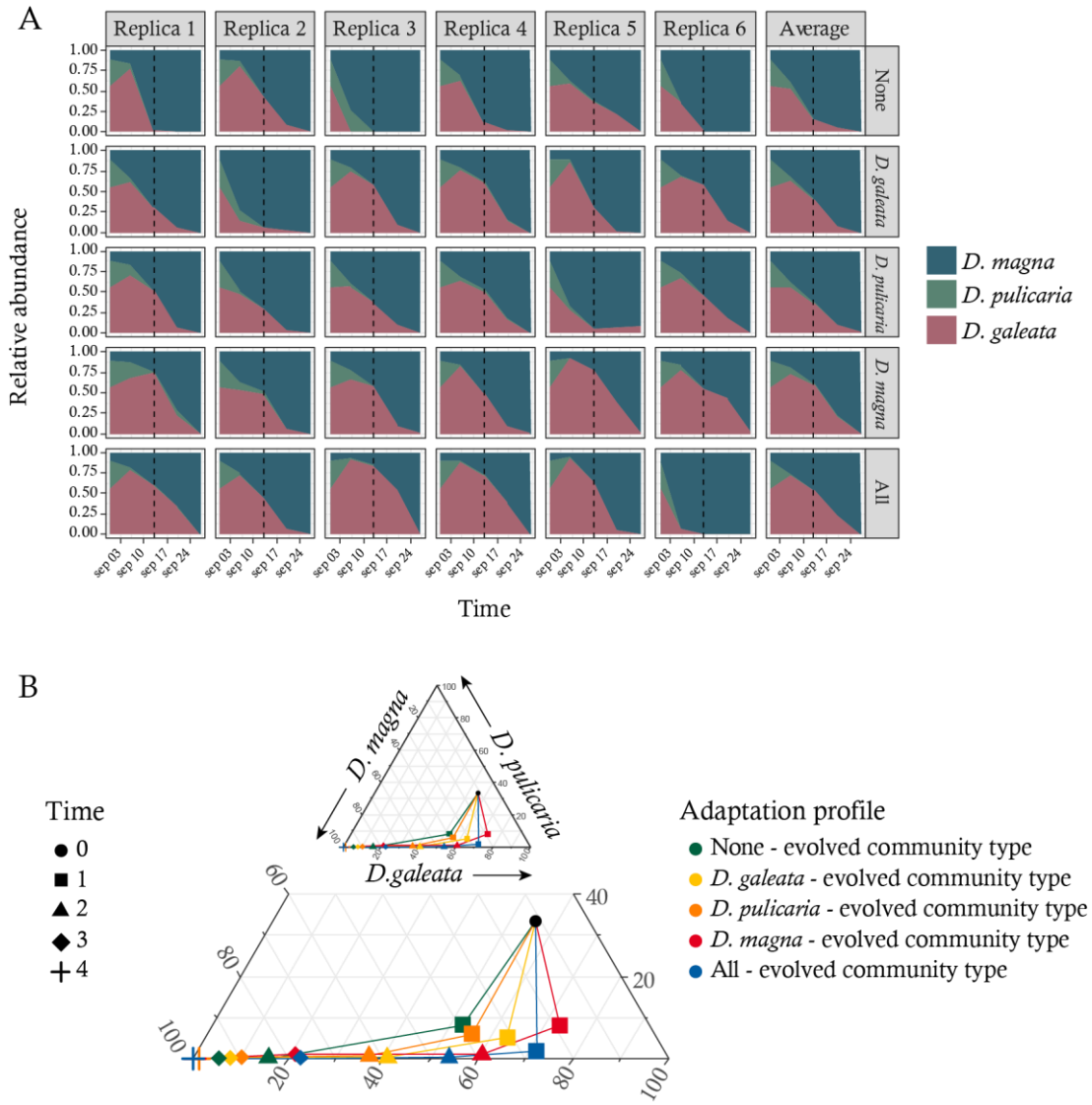

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Figure 3:

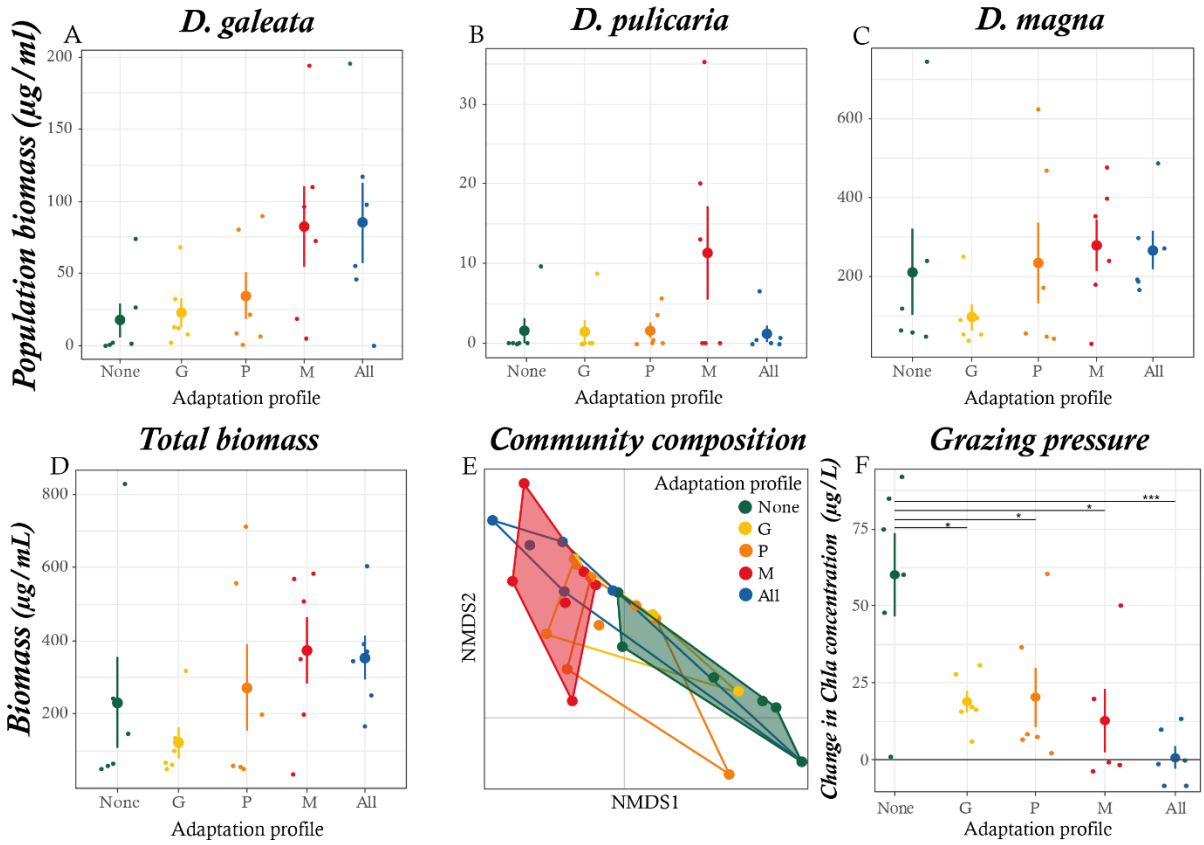

A
D. galeata
Population biomass (µg/ml)
Adaptation profile
None
G
P
M
All
B
D. pulicaria
C
D. magna
D
Total biomass
Biomass (µg/mL)
E
Community composition
NMDS1
NMDS2
Adaptation profile
F
Grazing pressure
Change in Chla concentration (µg/L)

Figure 4:

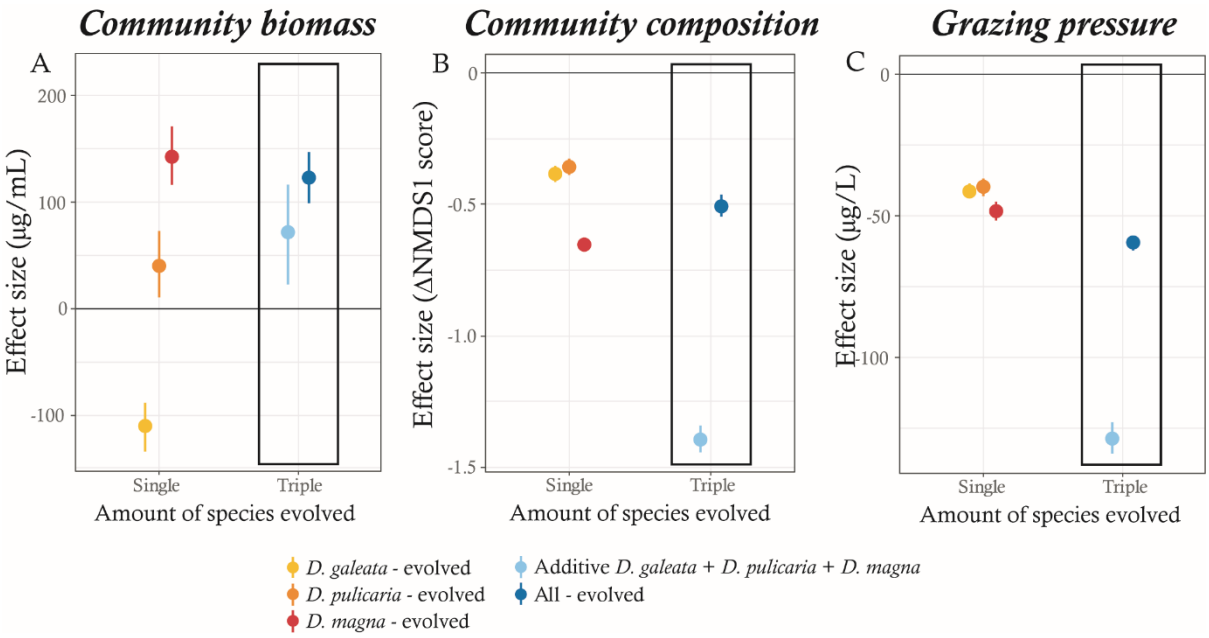
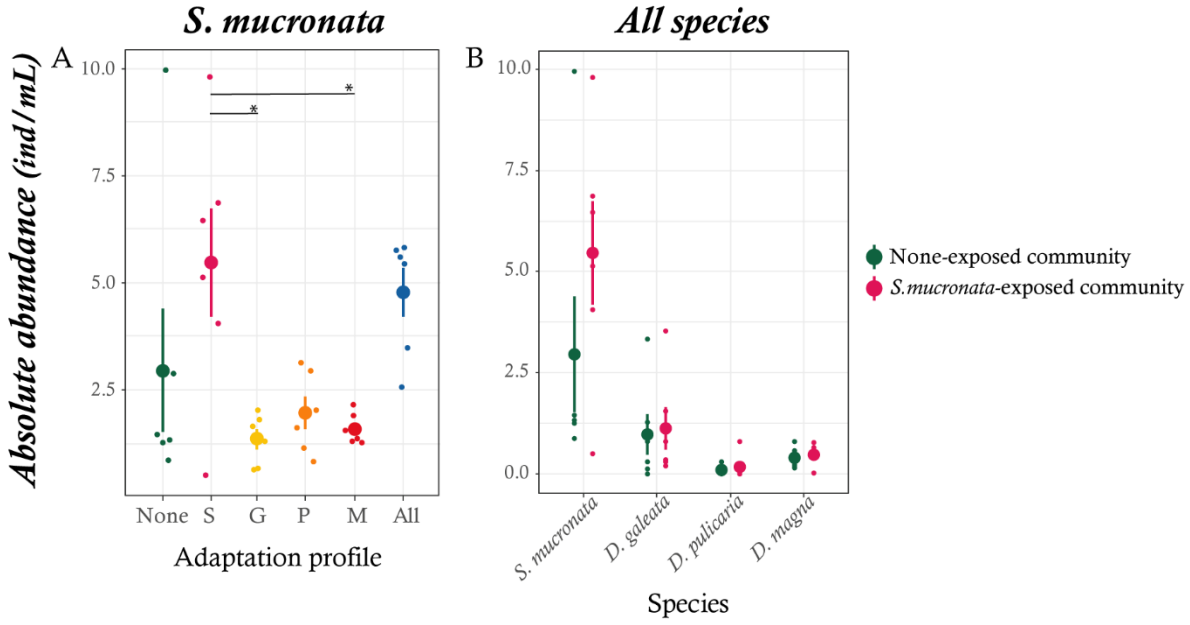


Figure 5:



Captions and Legends

Table 1: Summary output of the type II ANOVA on the ecological endpoints over time and per time point. Significant values (bold; $p < 0.05$) were further investigated with post hoc testing (Supplementary Information Table S2).

Figure 1: Conceptual figure showing possible scenarios for how the total effect of evolution on ecology of multiple evolving species might interact in a community of *n* species. The *x*-axis represents the number of species in the community of *n* species displaying an evolutionary response with a potential effect on an ecological property of interest. The *y*-axis represents the total effect size of evolution on ecology in the community of *n* species. The additive line assumes that evolution in all species, when studied in isolation, have an equal effect on ecology. Other potential relationships are depicted as lines. The darker area above the additive line reflects synergistic effects, i.e. where the total effect of evolution is accelerated compared to the scenario without interactions between the ecological effects of evolution in the different species. The lighter area below the line depicting additive effects reflects antagonistic effects, i.e. scenarios where the total effect of evolution is suppressed.

Figure 2: Schematic visualization of species' relative abundances over time. (A) shows the species composition in the different replicates (column 1-6) and their average value (column 7) per community type containing *D. magna* (blue), *D. pulicaria* (green) and *D. galeata* (red). The halfway point of the experiment (i.e. T2) is marked with the dashed vertical line. (B) shows averaged values across the different replicates. Each line reflects the average trajectory of a community type (None-evolved community in green, *D. galeata*-evolved community in yellow, *D. pulicaria*-evolved community in orange, *D. magna*-evolved community in red and All-evolved community type in blue). Every symbol represents a point in time.

Figure 3: Panels showing the effect of evolution on different ecological endpoints at time point T2 across community types (None: none-evolved community in green, G: *D. galeata*-evolved community in yellow, P: *D. pulicaria*-evolved community in orange, M: *D. magna*-evolved community in red and

All: all-evolved community type in blue). Line range plots have larger dots with error bars representing the mean value ±1 standard error. Significant differences between community type pairs are indicated with horizontal lines and symbols corresponding to the level of significance (*** $p<0.001$; * $p<0.05$). **Panel A-C**: Population biomass (µg/mL) of the three *Daphnia* species in each community type. **Panel D**: Total community biomass (µg/mL). **Panel E**: NMDS plot visualizing the community composition using Hellinger transformed abundances. **Panel F**: Grazing pressure (µg/L).

Figure 4: Effect size of evolution at time point T2 for (A) community biomass (µg/mL), (B) community composition (ΔNMDS1 score), and (C) grazing pressure (µg/L). Filled symbols with error bars represent the mean value of the effect size with the 95% CI. The single-species treatments include the *D. galeata*-evolved community (yellow), the *D. pulicaria*-evolved community (orange) and the *D. magna*-evolved community (red). The predicted additive cumulative effect of evolution quantified from the three species combined is given in light blue and is contrasted against the observed effect of evolution (dark blue). When 95% CIs do no overlap, there are non-additive interactions at play.

Figure 5: Absolute abundances (ind. /ml) for (A) *S. mucronata* across the different community types and (B) all Daphniidae species within the *S.mucronata*-evolved community type.