Adaptation and evolutionary rescue in a community context

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Abstract

Evolution might mitigate negative effects of environmental change on diversity by rescuing populations from extinction. However, biotic interactions could affect adaptation to abiotic change, and conversely, adaptation to an abiotic stressor could influence biotic interactions. We used experimental evolution of microalgae to investigate reciprocal effects of competition and adaptation to salt stress. We found that evolutionary history influenced competitive interactions and community properties. In high-salt environments, communities assembled from lines with a history of salt stress declined more slowly in diversity than communities assembled from lines without prior exposure to salt. We also found that a history of salt stress can lead to reduced competitive ability in the ancestral environment. While competition had no effect on adaptation to salt, we found that competition can hinder evolutionary rescue by preventing that adaptation translates into population persistence. Collectively, our results highlight the importance of considering evolutionary rescue in a community context.

Introduction

Abiotic environmental change can render populations maladapted, leading to their decline, and ultimately, extinction (Ceballos & Ehrlich 2002; Stuart *et al.* 2004; Both *et al.* 2006). Adaptive evolution may rescue populations from extinction if genotypes rise in frequency that have positive growth rates under the new environmental conditions (evolutionary rescue; Gomulkiewicz & Holt 1995; Bell & Gonzalez 2009). Laboratory experiments have demonstrated that microorganisms can adapt rapidly to a variety of abiotic stressors, including acidification (Lohbeck et al. 2012), salt stress (Lachapelle & Bell 2012), resource limitation (Bell 2013), and warming (Schaum *et al.* 2018). Experimental evolution has also helped to reveal the demographic, genetic, and extrinsic factors that influence the probability of evolutionary rescue (Carlson *et al.* 2014). For example, evolutionary rescue is facilitated by larger population size (Bell & Gonzalez 2009), higher genetic variation (Lachapelle & Bell 2012), and a slower rate of environmental change (Lindsey *et al.* 2013). However, most empirical studies on evolutionary rescue have considered single species in isolation. Yet, interactions with other species might influence (i) whether populations adapt to abiotic stressors, and (ii) whether adaptation translates into population persistence (Fig. 1a). Moreover, within communities, the ecological consequences of evolutionary rescue could go beyond population persistence and include effects on species interactions and community dynamics (Fig. 1b). This potentially complex interplay of biotic interactions and adaptation to abiotic stressors limits our understanding of whether evolution can contribute to the maintenance of diversity in changing environments.

Theory suggests several mechanisms whereby interspecific competition can influence adaptation to abiotic change. First, competition can constrain adaptation to environmental change by reducing population size, and thus the amount of standing genetic variation (Bocedi *et al.* 2013) and the supply of beneficial mutations (Johansson 2008). Second,

competition can reduce the time available for adaptation as species are outcompeted by better adapted competitors (de Mazancourt *et al.* 2008). Third, competition can alter the strength and direction of selection (Osmond & de Mazancourt 2013); depending on whether competition and environmental change impose selection on a trait in the same or in the opposite direction, competition can speed up or hinder adaptation to environmental change (Osmond & de Mazancourt 2013). Finally, negative genetic correlations between traits that mediate competition and stress tolerance could constrain adaptation in environments with both biotic and abiotic selective agents (Lau & Terhorst 2015). Empirical tests of these theoretical predictions are rare and focused on biotic interactions other than interspecific competition (Collins 2011; Hiltunen *et al.* 2018) or on environmental amelioration rather than deterioration (Kleynhans *et al.* 2016).

Competition could not only alter how species adapt to abiotic change, but could also modulate the effect of adaptation on population dynamics. When competition alters the selection environment generated by abiotic change, adaptation of a focal species in isolation may not translate into increased population size in a community context. Accordingly, an experiment that manipulated CO_2 and diversity of a plant community showed that interspecific competition altered the selection environment created by CO_2 (Kleynhans *et al.* 2016): adaptation to elevated CO_2 was only observed when the plants were assayed in the same community context in which selection had occurred. Similarly, an experiment with a marine alga revealed that adaptation to acidification in isolation was not associated with increased population size within communities (Bach *et al.* 2018). In the context of evolutionary rescue, competition could prevent that adaptation translates into population persistence and thus preclude that evolution mitigates negative effects of environmental change on diversity.

The field of evolutionary rescue primarily considers the effect of evolution on population dynamics, but within communities, adaptation of a focal species to the local environment may affect interactions with other species (terHorst *et al.* 2014; Pantel *et al.* 2015; Gomez *et al.* 2016). Evolution can influence community dynamics directly, by altering traits that underlie species interactions, and indirectly, by influencing population dynamics (Matthews *et al.* 2011; Hendry 2019). In a scenario with an abiotic stressor as the selective agent (Fig. 1b), adaptation could influence species interactions if traits that confer adaptation to the stressor are correlated with traits that affect biotic interactions. Some traits may even simultaneously mediate stress tolerance and species interactions. For example, in *E. coli* reduced membrane permeability increases resistance to antibiotics but decreases the ability to take up nutrients, such that adaptation to antibiotics results in lower competitive ability (Phan & Ferenci 2013). In addition to such trait-mediated effects, adaptation of a focal species to an abiotic stressor might influence biotic interactions by increasing its population size. Despite this range of possibilities of how adaptation to abiotic change could affect species interactions, the ecological consequences of evolutionary rescue have been rarely considered in a community context.

A further complexity of adaptation in a multi-species context is that species might vary in their ability to adapt to environmental change, which could hamper maintenance of diversity. The rate of evolution depends on selection pressure, genetic variance, and covariance (Lande 1979; Agrawal & Stinchcombe 2009). Variation among species in ecological tolerance, life-history traits, and genomic architecture could thus result in variation in evolvability (Barraclough 2015). Species with narrow tolerance of the abiotic stressor will have a higher risk of extinction, but will also experience stronger selection pressure and might thus evolve more than species with broader tolerance (Osmond & de Mazancourt 2013). Species also vary widely in population density (Damuth 1987), mutation rate (Drake *et al.* 1998; Baer *et al.*

2007), recombination rate (Stapley *et al.* 2017), and genome complexity (Lynch *et al.* 2011), all of which influence the rate of evolution. Such variation among species in evolutionary potential could lead to changes in the composition and interaction structure of communities in deteriorating environments.

Here we investigated if adaptation to abiotic change influences competitive interactions and community properties, and, vice versa, if competition influences adaptation to deteriorating environmental conditions. In a laboratory experiment, we propagated monocultures of six species of microalgae for ~ 180 generations with and without increasing salt stress. We then quantified monoculture growth rates of all lines in a reciprocal transplant assay to test the hypothesis that species vary in their evolutionary response to salt stress (Hypothesis 1). We then assembled communities, either from lines with a history of salt stress or from lines without prior exposure to salt, and investigated if evolutionary history affected community dynamics in environments with and without salt, respectively. We predicted that adaptation to salt in isolation would translate into longer persistence of species within salt-stressed communities and consequently into maintenance of diversity (Hypothesis 2). Alternatively, competitive interactions could prevent that adaptation of monocultures translates into persistence within communities. In a second set of experiments, we propagated two algae species for ~ 200 generations as monocultures and as pairs, respectively, in environments with and without increasing salt. We then re-isolated the two species, and measured monoculture growth rates and competitive abilities in reciprocal transplant assays. We predicted that interspecific competition during the selection experiment would constrain adaptation to salt stress (Hypothesis 3). As in the first set of experiments, we expected that a history of salt stress would lead to increased competitive ability at high salt (Hypothesis 4). Our results highlight the complexities that arise from adaptation to abiotic change in a community context. We found that adaptation to abiotic stress influenced competitive

interactions and community-level properties, but we observed no effect of competition on adaptation to salt stress.

Material and methods

Model organisms

We tested our hypotheses using six species of microalgae from three different taxonomic groups: the cyanobacteria *Synechococcus leopoliensis* (Canadian Phycological Culture Centre (CPCC) 102) and *Anabaena variabilis* (CPCC 105), the diatoms *Navicula pelliculosa* (CPCC 552) and *Nitzschia palea* (CPCC 160), and the chlorophytes *Pseudokirchneriella subcapitata* (CPCC 37) and *Scenedesmus acutus* (CPCC 10). The algae were cultivated in Bold's basal medium (Bold 1949), which was modified by adding silicate (0.58 g L^{-1} of Na_2SiO_3) and vitamins (Low-Décarie *et al.* 2011) to allow growth of diatoms. In all experiments, the algae were cultivated at 25 °C with light continuously provided at 100 µE m^{-2} s^{-1}. Cultures were propagated under sterile conditions. We did not make cultures isogenic at the start of the experiment; the observed evolutionary responses could thus have resulted either from *de novo* mutations or from sorting of standing genetic variation.

Salt tolerance of ancestral lines

We determined the limit of salt tolerance of the six species by measuring their growth along a salt gradient implemented on 48-well plates. The length of the gradient was based on results of a preliminary experiment, and was 0 to 10 g NaCl L^{-1} in 1 g increments for *Nitzschia* and *Pseudokirchneriella*, and 0 to 20 g NaCl L^{-1} in 2 g increments for the remaining species. After six days of acclimation at the respective salt concentration, we inoculated fresh plates with acclimated cultures (20 µl culture in 1 ml medium), sealed the plates with sterile air-permeable membranes (VWR), and cultivated them on shakers (350 rpm). Fifteen times over

the course of ten days, we measured absorbance at 660 nm on an optical plate reader (Synergy-HT, BioTek, Winooski, VT). We estimated the limit of salt tolerance for each species as the salt concentration where we observed no increase in absorbance. Each salt × species combination was replicated twice.

Adaptation to salt in monoculture

In a serial transfer experiment, we propagated monocultures of the six species with and without increasing concentrations of NaCl (Fig. S1, S2). The algae were cultured in 125-ml glass flasks filled with 50 ml of Bold's medium and continuously shaken at 250 rpm. Every 3.5 days, we transferred 1 ml of culture to 50 ml of fresh medium for a total of 37 transfers (~ 180 generations). For each of the six species, three replicate control lines were cultivated without addition of salt and three replicate selection lines were cultivated with increasing salt. We increased the salt concentration in the selection lines by 0.25 g L^{-1} every transfer until reaching 4 g NaCl L^{-1} at transfer 16 (Fig. S2). We maintained this salt concentration for 10 transfers and then continued the increase in salt until reaching 6 g NaCl L^{-1} at transfer 34. We chose a final salt concentration of 6 g NaCl L^{-1} because this was the limit of salt tolerance for the species with the lowest salt tolerance among the six species.

To test if the six species of algae differed in their evolutionary response to salt stress (Hypothesis 1), we measured the growth rate of each line in the two final salt environments (0 and 6 g NaCl L^{-1}). Before starting the growth assay, we removed non-evolutionary effects by cultivating all lines at identical environmental conditions for three transfer cycles, that is two cycles in the ancestral environment (0 g NaCl L^{-1}) and one cycle in the assay environment (0 and 6 g NaCl L^{-1}). We then inoculated flasks containing 50 ml medium of either 0 or 6 g NaCl L^{-1} with 1 ml of acclimated culture and measured absorbance through time. Depending on the time of individual cultures to reach carrying capacity, we measured absorbance over 2.5 to 7.5

weeks with 23 to 34 measurements. Absorbance was measured twice a day during the first 5 days, once a day during the intermediate phase of the experiment, and once every other day towards the end of the experiment. At each measurement, we removed 200 µl of culture from each flask and measured absorbance in 96-well plates on an optical plate reader.

Community assay

To test if evolutionary history can influence community dynamics (Hypothesis 2), we assembled communities either of control or of selection lines, transplanted the communities into the two final salt environments of the selection experiment, and quantified species abundances and diversity of the community over four transfers. Prior to the community assay, the lines had been cultivated for 37 transfers with or without increasing salt, followed by two transfers in the ancestral environment (see above). We assembled communities with equal biovolume of the six species. To this end, we determined species abundances in the monocultures by counting Lugol-fixed samples with an inverted microscope, and then converted abundances to biovolume using cellular biovolume estimates based on measurements of 20 individuals per species (Hillebrand *et al.* 1999). We assembled three replicate communities of control lines and three replicate communities of selection lines. From each of the six communities, we inoculated two flasks, one with a salt concentration of 0 and one of 6 g NaCl L^{-1}. Every four days, we transferred 1 ml of the community into 50 ml of fresh medium, with the flasks continuously shaken at 250 rpm. Over the course of four transfers, we sampled the communities at each transfer, fixed the samples with Lugol's solution, and counted species abundances with an inverted microscope.

Adaptation in monoculture vs. adaptation in pairs

To test if competition can influence adaptation to salt stress (Hypothesis 3), we propagated monocultures and pairs of *Anabaena* and *Scenedesmus* with and without increasing salt for 46 transfers, then re-isolated the two species and quantified their fitness with and without salt (Fig. S1b). The monocultures and pairs of the two species were part of the selection experiment described above and experienced the same changes in salt concentration (Fig. S2). To quantify how strongly competition affected the abundances of the two species during the selection experiment, we sampled the pairs and monocultures of *Anabaena* and *Scenedesmus* ten times over the course of the selection experiment and microscopically determined the abundances of the two species in Lugol-fixed samples. We chose the combination of *Anabaena* and *Scenedesmus* because in other species combinations the inferior competitor was outcompeted quickly both with and without increasing salt.

After 46 transfers in the selection experiment, we re-isolated *Anabaena* and *Scenedesmus* by plating the cultures on agar and isolating individual colonies. Monocultures were treated the same way as the pairs. Individual colonies were resuspended in liquid Bold's medium on well plates, and microscopically checked for contamination after a week of growth. We then merged eight clean isolates per sample to one culture. The two isolation steps were conducted in medium without addition of NaCl. We then acclimated the cultures to the two assay environments (0 and 6 g NaCl L^{-1}) for two transfer cycles. Hence, before starting the growth and competition assays, the cultures were cultivated under identical environmental conditions for four cycles (two isolation cycles without salt, two transfer cycles in the assay environment) to remove any non-evolutionary effects.

The acclimated cultures were used to start growth and competition assays. Growth rates of all lines were determined in monoculture assays in the two final salt environments by measuring absorbance through time as described above. To test if salt history and community

history influenced competitive interactions (Hypothesis 4), we assembled pairs of *Anabaena* and *Scenedesmus* in the two final salt environments and determined changes in biomass of the two species through time. We conducted two competition assays that differed in how we assembled the pairs. First, we assembled pairs of co-evolved *Anabaena* and *Scenedesmus*, i.e. lines that had the same community and salt history. However, because both competitors differed between flasks in this assay, differences among treatments could not unequivocally be attributed to one of the two species. We thus conducted a second competition assay where we competed each line of *Anabaena* with the same reference line of *Scenedesmus*, and each line of *Scenedesmus* with the same reference line of *Anabaena*. As reference line, we randomly selected one of the three replicate control lines (i.e. community history: monoculture, salt history: no salt). The second competition assay was started three weeks after the first competition assay; cultures were stored meanwhile as monocultures at their respective salt selection environment and then acclimated for one cycle to the salt assay environment.

In the competition assays, we measured chlorophyll a concentrations of the two competitors as proxy for biomass. To quantify chlorophyll a concentrations of the two species, we used a FluoroProbe (bbe Moldaenke, Kiel-Kronshagen, Germany), a fluorometer which can discriminate among algal groups (Catherine *et al.* 2012). Before the start of the assay, we calibrated the FluoroProbe using cultures of *Anabaena* and *Scenedesmus* with known chlorophyll a concentrations. We started the competition assay with equal chlorophyll a concentrations (2 µg) of both species in flasks filled with 50 mL of Bold's medium. We transferred the cultures every 3.5 days for ten transfers in the first competition assay and for four transfers in the second competition assay and measured chlorophyll a concentrations of both species at each transfer.

Data analysis

We estimated growth rates in monoculture assays by fitting linear models to the exponential part of the absorbance vs. time curves, using ln-transformed absorbance data as response variable. We computed two-way ANOVAs to investigate effects of salt history and assay environment on growth rates of the six species. We analyzed the time series data of the community assay with linear mixed models, with ln-transformed abundance as response variable, transfer, salt history and assay environment as fixed effects, and flask as random effect to account for repeated sampling of communities. We used the same approach to investigate treatment effects on species richness. To test whether competition affected adaptation of *Anabaena* and *Scenedesmus* to increasing salt, we computed three-way ANOVAs and investigated the effects of community history, salt history and assay environment on monoculture growth rates (growth assay) and relative chlorophyll a (competition assay). All analyses were computed with R version 3.4.1 (R Core Team 2015); the package *lme4* was used to calculate linear mixed effect models.

Results

Salt tolerance of ancestral lines

Among the six species, *Nitzschia* and *Pseudokirchneriella* had the lowest limit of salt tolerance (5 and 6 g NaCl L^{-1}, respectively), *Anabaena*, *Scenedesmus* and *Navicula* had intermediate limits of salt tolerance (12-16 g NaCl L^{-1}), and *Synechococcus* had the highest limit of salt tolerance (18-20 g NaCl L^{-1}).

Adaptation of monocultures

After 37 transfers with and without increasing salt, respectively, we detected adaptation to salt in two of six species, *Nitzschia* and *Pseudokirchneriella* (Fig. 2). In both species, the growth response to the assay environment depended on salt history: selection lines had higher growth

rates at 6 g NaCl L^{-1} than control lines, but did not differ from control lines in the no-salt assay environment (Fig. 2 c, d). The effect of salt history was particularly strong in *Nitzschia*; the control lines were unable to grow at the final salt concentration, while all three replicate selection lines had positive growth rates. Among the remaining four species, *Scenedesmus* and *Synechococcus* were affected neither by salt history nor assay environment, while *Navicula* and *Anabaena* had reduced growth rates at high salt, irrespective of salt history (Fig. 2).

Community assay

When assaying communities of either control or selection lines, we found that the effect of salt history varied among species and assay environment (Table 1, Fig. 3). The abundances of most species declined over the four transfers except for *Synechococcus,* which increased. Assay environment had no effect on the abundances of *Navicula* and *Synechococcus*, while the other four species declined faster when the community was assayed at high salt than without salt. Salt history affected three of the six species (*Nitzschia*, *Pseudokirchneriella*, and *Scenedesmus*), but the effect depended on the assay environment (Table 1). The strongest effect of salt history was observed for *Pseudokirchneriella* (Fig. 3): in the high-salt assay environment, selection lines declined more slowly in abundance than control lines, resulting in extinction being delayed by one transfer. Similarly, selection lines of *Scenedesmus* declined more slowly at high salt than control lines, but the effect was of small magnitude (Fig. 3). In contrast, selection lines of *Nitzschia* declined slightly faster than control lines when assayed in the no-salt environment.

The effect of evolutionary history on species abundances translated into an effect on the diversity of the community (Table S1, Fig. 4). Species richness declined with time (transfer: $P < 0.001$), and this decline was faster when communities were assayed with than without salt

(assay: $P < 0.001$). However, communities assembled from selection lines declined more slowly in species richness than communities assembled from control lines (salt history × assay; $P = 0.047$), because of slower extinction of *Pseudokirchneriella*.

Adaptation in monoculture vs. adaptation in pairs

Interspecific competition strongly influenced species abundances during the selection experiment, but did not lead to any evolutionary responses. In the selection experiment, *Anabaena* reduced the abundance of *Scenedesmus* as long as salt concentration was low (Fig. S3). However, at the final salt concentration of 6 g NaCl L^{-1} the competitive hierarchy reversed and *Anabaena* was negatively affected by *Scenedesmus* (Fig. S3). When assayed as monocultures after the selection experiment, neither of the two species showed evolutionary responses (Table S2, Fig. 5a, b). Salt had a negative effect on the growth rate of both species ($P < 0.001$), irrespective of salt history and community history.

The two competition assays revealed that lines of *Anabaena* with a history of salt stress had reduced competitive ability in the ancestral environment. When assayed with co-evolved competitors, high-salt lines of *Anabaena* had lower relative chlorophyll a than no-salt lines in the no-salt assay environment, and consequently high-salt lines of *Scenedesmus* had higher relative chlorophyll a than no-salt lines (salt history × assay: $P = 0.002$; Table S3, Fig. S4). Because both competitors differed among treatment combinations, this experiment did not allow detecting if the observed pattern was due to altered competitive ability of *Anabaena* or of *Scenedesmus*. However, when assayed in competition with a reference line, only *Anabaena* was affected by salt history, again with lower relative chlorophyll a of high-salt than no-salt lines in the no-salt assay environment (salt history × assay: $P = 0.029$, Table S4, Fig. 5c, d).

Discussion

We found that adaptation to an abiotic stressor can influence competitive interactions and community-level properties, but we observed no effect of competition on adaptation to salt. Communities assembled from lines with a selection history of salt stress maintained diversity longer under salt stress than communities assembled from control lines. The positive effect of salt history on diversity resulted from longer persistence of selection lines of *Pseudokirchneriella* in salt-stressed communities compared with control lines. However, we also found that adaptation of monocultures to salt stress did not necessarily translate into longer persistence within communities: *Nitzschia* adapted to salt in isolation, but selection and control lines went extinct equally fast within salt-stressed communities. This result indicates that competition can override the effect of evolution on population dynamics. Finally, the second set of experiments revealed that a history of salt stress can lead to reduced competitive ability in benign conditions. When assayed in competition, selection lines of *Anabaena* performed worse than control lines in the no-salt assay environment. Taken together, we found different scenarios of how adaptation to an abiotic stressor can play out in a community context, ranging from a positive effect of adaptation on performance within stressed communities, over no effect, to a negative effect on performance under benign conditions.

Species varied in their evolutionary response to salt stress. The two species with the lowest limit of salt tolerance adapted to salt, while species that were less negatively affected by salt showed no sign of adaptation (Fig. 2). This finding is in line with the prediction that species with narrow tolerance of a stressor should evolve more than species with broader tolerance (Osmond & de Mazancourt 2013) because they experience stronger selection pressure. Species-specific differences in genetic variance and covariance could be additional reasons for variation among species in the rate of evolution (Lande 1979; Barraclough 2015). However, given that only two species adapted, our sample size is not large enough to evaluate

if species-specific differences other than ecological tolerance influenced the evolutionary response to salt. To elucidate such effects, future experiments could expose a set of species to their respective limit of tolerance and then investigate if differences in life-history traits (e.g. population size) influence the rate of adaptation. In our experiment, however, we simulated a scenario where all species experienced the same amount of stress.

Evolution transiently mitigated the negative effect of salt stress on diversity, but did not maintain diversity at unstressed levels. In high-salt environments, diversity declined more slowly when communities were assembled from lines with a history of salt stress (Fig. 4), because selection lines of *Pseudokirchneriella* persisted for one transfer longer than control lines (Fig. 3). In *Nitzschia*, however, adaptation to salt in isolation was not associated with increased fitness in salt-stressed communities. Consequently, even in communities of selection lines, diversity declined faster in the high-salt than the no-salt assay environment (Fig. 4). Our results thus suggest that competitive interactions can hinder evolutionary rescue by preventing that adaptation translates into persistence of the population. The scenario of adaptation in isolation followed by competition with pre-adapted species might seem artificial, but it is a plausible scenario in a spatial context. Species may adapt to changes in their local environment before immigration of better-adapted species from the regional species pool (Urban *et al.* 2012). Depending on how well the resident species manage to adapt to the new environmental conditions, they may coexist with or even dominate over pre-adapted, immigrating species (De Meester *et al.* 2016). In our experiment, however, the positive effect of salt history on persistence of *Pseudokirchneriella*, and thus on the diversity of the community, was only transient. By the end of the experiment, this species had gone extinct at high salt irrespective of salt history. However, *Pseudokirchneriella* also declined to low or zero abundance in the no-salt assay environment, indicating that this species was generally a weak competitor, irrespective of salt concentration. The effect of adaptation on

diversity and community dynamics might be more pronounced and persistent for species with high interaction strengths and strong ecosystem effects, such as superior competitors or efficient predators (Pantel *et al.* 2015; Matthews *et al.* 2016).

Competition had no effect on adaptation to salt stress, neither in terms of growth rate nor in terms of competitive ability (Fig. 5). We had predicted that competition during the selection experiment would constrain adaptation to salt because of reduced population size and/or increased genetic constraints (Johansson 2008; Lau & Terhorst 2015). Alternatively, competition could facilitate adaptation to abiotic stress by increasing the strength of selection (Osmond & de Mazancourt 2013). The two species that we used to test this question, *Anabaena* and *Scenedesmus*, adapted to salt neither in monoculture nor in pairs. Given that these species had not reached their limit of salt tolerance in the selection experiment, low selection pressure could explain why the monocultures did not adapt to salt. However, the combination of salt and competition in the selection experiment drove the inferior competitor to the brink of extinction (Fig. S3), suggesting that the inferior competitor experienced strong selection pressure to evolve faster growth rates. Yet, we did not find any effect of community history on adaptation. One possible explanation is that the competitive hierarchy in flasks with increasing salt changed shortly before the end of the selection experiment when the final salt concentration had been reached, from dominance of *Anabaena* to dominance of *Scenedesmus*. Maybe relaxed selection pressure on *Scenedesmus* towards the end of the experiment and only a short phase of strong selection on *Anabaena* explain why competition had no effect on the evolutionary response of these two species.

Evolutionary change in response to salt influenced competitive interactions (Fig. 5c). We had predicted that a history of salt stress would lead to increased competitive ability at high salt. In contrast, however, strains of *Anabaena* with a history of salt stress had reduced competitive ability in the no-salt assay environment. Such a pattern is an indication of

conditionally deleterious mutations (Mee & Yeaman 2019), i.e. mutations that are neutral in the local environment and deleterious in an alternative environment. Because we have neither molecular nor trait data, we cannot elucidate potential mechanisms behind the observed mal-adaptation in the ancestral environment. The evolutionary response of *Anabaena* was independent of community history and of whether fitness was assayed in competition with a co-evolved or a naïve competitor (Fig. 5, Fig. S4). Importantly, however, we observed reduced fitness of selection lines of *Anabaena* only when assayed in competition, not in the monoculture growth assay. It thus seems that the evolutionary response of *Anabaena* to salt was associated with changes in traits that influenced competitive interactions. Similar evidence comes from a functional genomics study on *Daphnia*, which suggested that evolution of salt tolerance could entail fitness costs in environments with predators (Latta *et al.* 2012). Overall, our results suggest that evolutionary responses to abiotic change can influence biotic interactions, such that populations with different histories of abiotic stress might vary in their effects on communities and ecosystems.

The emerging field of eco-evolutionary dynamics investigates the effects of evolution on populations, communities, and ecosystems (Hendry 2016). Evolutionary rescue, as one component of this field, has its focus on the effect of evolution on population dynamics, specifically on population persistence. Here we showed that the ecological consequences of evolutionary rescue can include effects on species interactions and the diversity of communities. Moreover, we found that competition can override the effect of evolution on population dynamics, illustrating that community context can modulate the ecological consequences of evolutionary change. We have observed a range of complexities arising from a multi-species context despite using a fairly simple model system. Higher diversity and trophic structure of natural systems will pose an even greater challenge to understanding eco-evolutionary dynamics (De Meester *et al.* 2019; Hendry 2019). Field studies have

demonstrated evolutionary rescue of populations embedded in diverse communities (Reid *et al.* 2016; Oziolor *et al.* 2019), but it remains to be determined if stress tolerant genotypes have the same ecosystem effects as ancestral genotypes. If adaptation to an abiotic stressor is associated with changes in traits that mediate biotic interactions, evolution may maintain populations but fail to maintain community and ecosystem structure. Collectively, our results suggest that considering community context is of fundamental importance if we want to evaluate the capacity of evolution to maintain diversity and species interactions in changing environments.

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Table 1: Community assay. Results (i.e. P-values) of linear mixed models testing for effects of transfer (T), assay environment (A), and salt history (H) on the abundances of the six species within communities assembled either from control or selection lines. Transfer was treated as a continuous variable. Abundances of species were ln-transformed prior to analysis, P < 0.05 in bold.

Species	Transfer	Assay	History	A × H	T × A	T × H	T × A × H
Anabaena	**< 0.001**	**< 0.001**	0.807	0.538	**0.048**	0.770	0.822
Navicula	**< 0.001**	0.836	0.947	0.749	**0.010**	0.128	0.664
Nitzschia	**< 0.001**	**< 0.001**	0.285	**0.031**	**0.004**	0.151	0.648
Pseudokirchneriella	**< 0.001**	**< 0.001**	0.100	**0.005**	0.502	0.450	0.843
Scenedesmus	**< 0.001**	**< 0.001**	0.682	**0.017**	**0.005**	0.901	0.189
Synechococcus	**< 0.001**	0.289	0.781	0.545	0.328	0.611	0.633

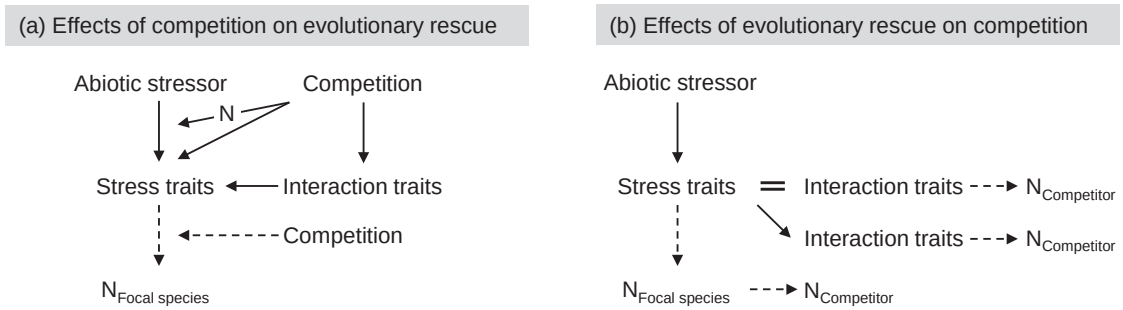

Figure 1: Conceptual figure of how evolutionary rescue from an abiotic stressor (a) is affected by competition, and (b) affects competition. Evolutionary effects are denoted with solid lines, ecological effects with dashed lines. Stress traits indicate traits that mediate the response to a stressor, interaction traits indicate traits that mediate competitive interactions. Evolutionary rescue from a stressor occurs when the focal species responds with the evolution of stress traits such that population size (N) increases and the population persists. (a) Competition can influence adaptation to an abiotic stressor by reducing population size and thus genetic variation of the focal species, by imposing selection on stress traits, or by imposing selection on interaction traits that are genetically correlated with stress traits. Competition could also influence evolutionary rescue by altering the effect of adaptation on population size. (b) Evolutionary rescue can affect competition when stress traits simultaneously act as interaction traits, when stress traits are correlated with interaction traits, or when increased population size of the focal species influences the competitor.

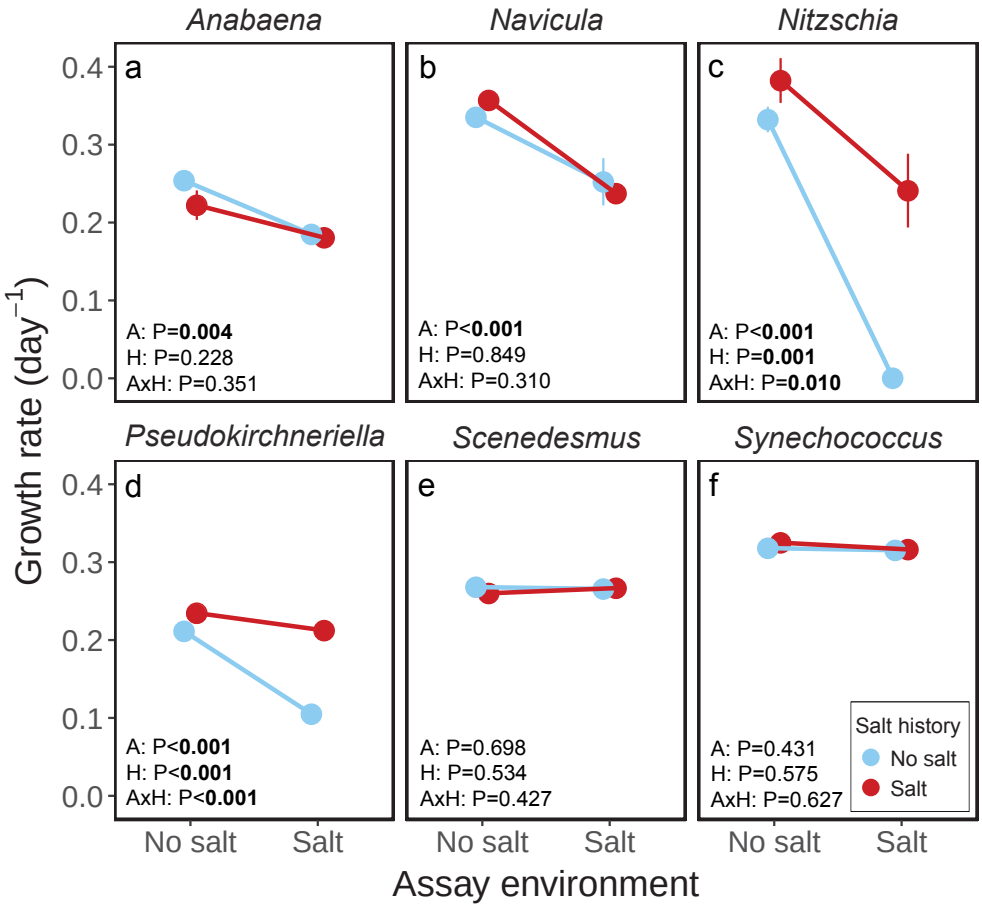

Figure 2: Effects of salt history and assay environment on the monoculture growth rates of six species of microalgae. Control lines (blue) have a salt history of 37 transfers at 0 g NaCl L^{-1}, selection lines (red) have a salt history of 37 transfers at increasing salt stress with a final concentration of 6 g NaCl L^{-1}. Salt concentrations in the two assay environments were the same as in the final selection environments, i.e. 0 and 6 g NaCl L^{-1}, respectively. Inserts give the P-values for assay environment (A), salt history (H), and their interaction (AxH). Bold font denotes P-values < 0.05. Values are means ± SE, n = 3.

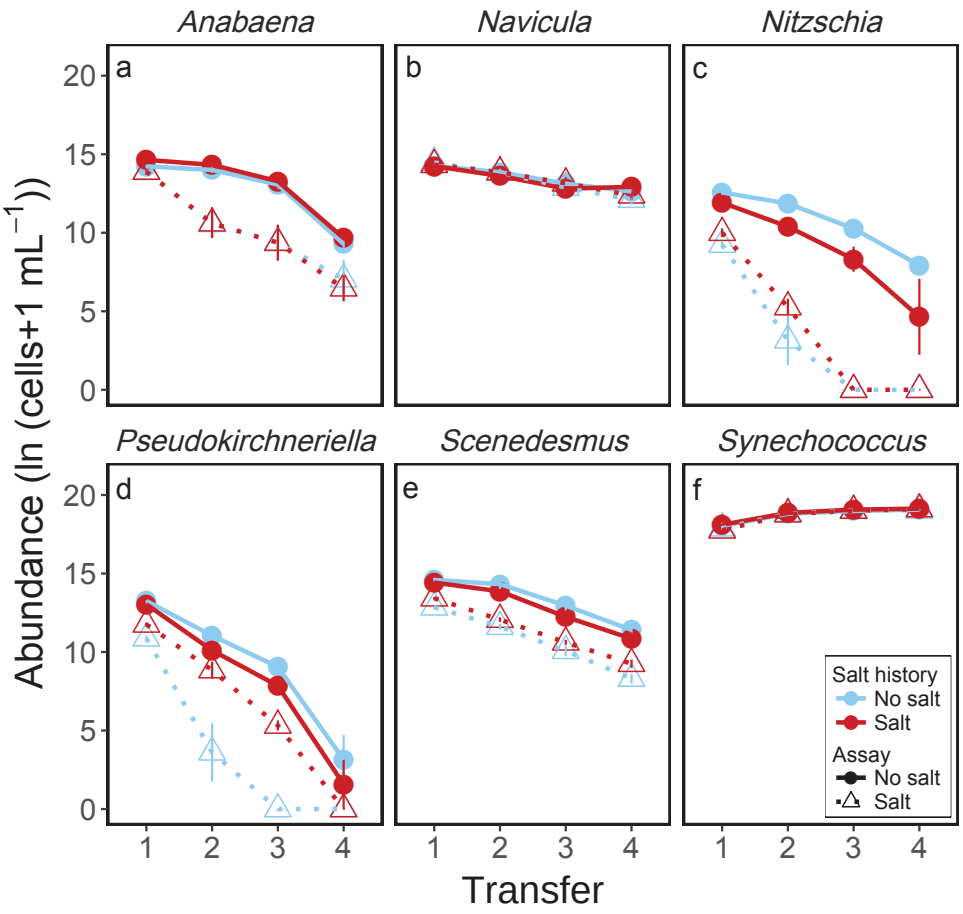

Figure 3: Effects of salt history and assay environment on species abundances in the community assay. Communities of six species were assembled either from control lines (blue) or from selection lines (red). Species abundances were quantified over four transfers in assay environments without salt (solid circles, solid lines) and with salt (open triangles, dotted lines), respectively. Values are means ± SE, n = 3.

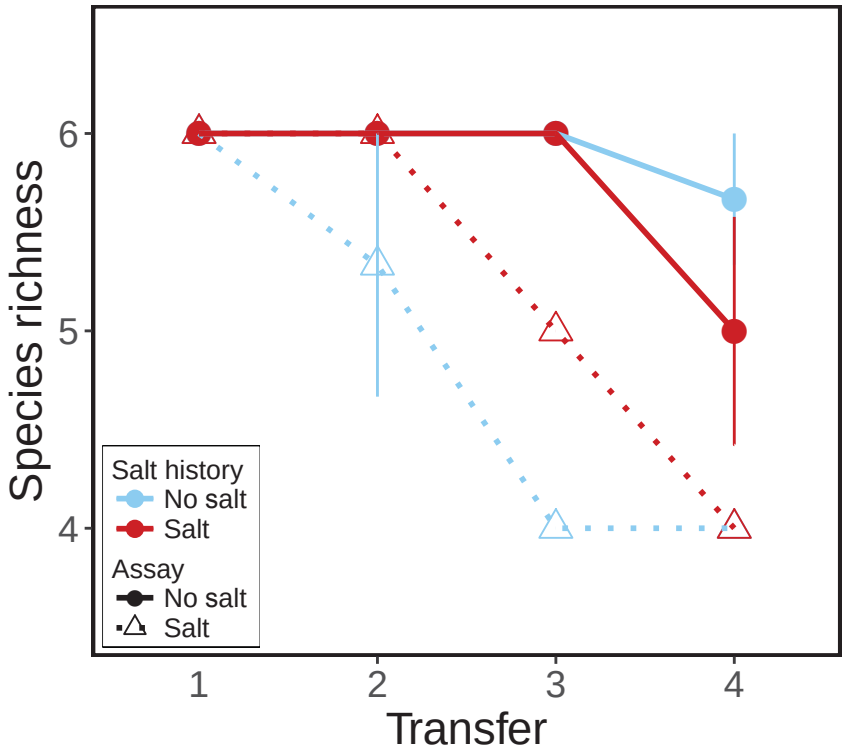

Figure 4: Effects of salt history and assay environment on species richness in the community assay. Communities of six species were assembled either from control lines (blue) or from selection lines (red). Species richness was quantified over four transfers in assay environments without salt (solid circles, solid lines) and with salt (open triangles, dotted lines), respectively. Values are means ± SE, n = 3.

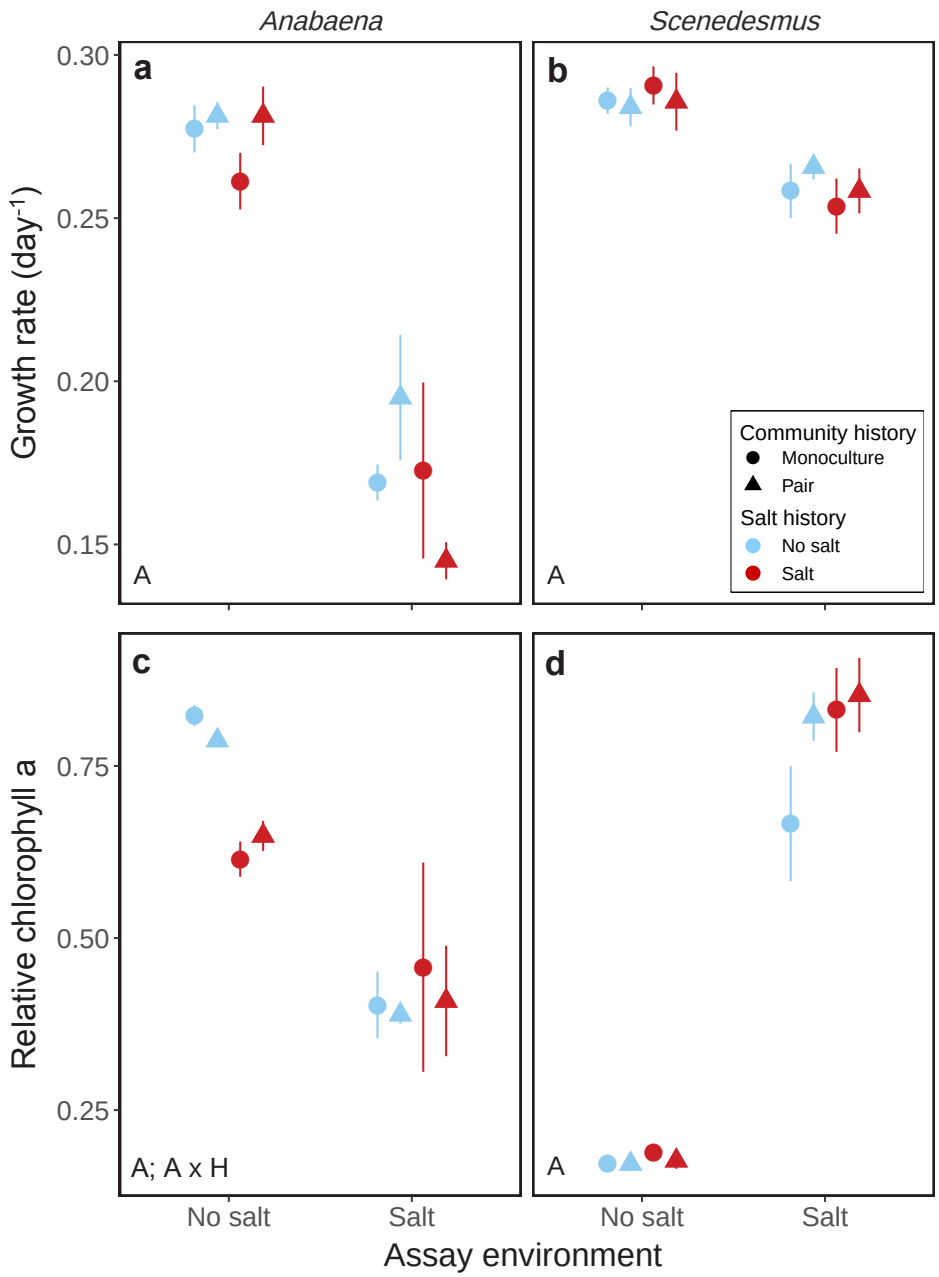

Figure 5: Effects of community history, salt history, and assay environment on the growth rates and competitive abilities of Anabaena and Scenedesmus. Monoculture growth rate of (a) Anabaena and (b) Scenedesmus, relative chlorophyll a of (c) Anabaena in competition with a reference line of Scenedesmus and (d) of Scenedesmus in competition with a reference line of Anabaena. Inserts indicate significant effects ($P < 0.05$) of community history (C), salt history (H), assay environment (A), or their interactions. Values are means ± SE, n = 3.