The importance of intraspecific diversity on duckweed growth with and without salt stress

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Abstract

The pollution of freshwater ecosystems is threatening freshwater plant species diversity worldwide. Freshwater plants, such as duckweed (*Lemna minor*), are potentially sensitive to novel stressful environments. To test if intraspecific diversity could increase resistance to stressful environments, I used seven *L. minor* populations and assessed their growth rates in the absence and presence of moderate salt stress across an intraspecific diversity gradient. I grew the populations (ecotypes) of *L. minor* over five months in 92 experimental mesocosms in a glasshouse either in ecotype monocultures or in polyculture with either one or three conspecific ecotypes (23 unique compositions). The experiment was conducted in semi-natural conditions, including a natural community of algae and microbes. After assessing the duckweed growth rate in unperturbed conditions, the cultures were subjected to moderate salt stress (50mM NaCl) for several weeks. Population abundances were assessed weekly, both on the ecotype level and the whole-population level.

Throughout the experiment, the ecotypes differed in their growth rates, the fastest growing at twice the rate of others. Whether the ecotypes grew in monoculture or in polyculture with other conspecifics further shaped the ecotype growth rates. Ecotype polycultures showed higher abundances towards the end of the experiment, indicating that over time, as the environment deteriorated, intraspecific diversity gained in importance. These findings show that intraspecific variation in growth rates can translate to a positive effect of intraspecific diversity on whole-population abundance. Exposure of *L. minor* to moderate salt levels did not significantly impact growth rates.

Keywords

aquatic plant, diversity experiment, glasshouse experiment, growth rate, Lemna minor

INTRODUCTION

Freshwater environments are increasingly under pressure from human-mediated climate change and activities. Freshwater biodiversity is consequently in decline as aquatic communities scramble to adapt rapidly to their deteriorating environments (Dudgeon *et al.* 2006; Tickner *et al.* 2020). Not only species diversity is in decline but even within species, the genetic variability is increasingly being lost (Leigh *et al.* 2019). However, intraspecific (genetic) diversity is key for the persistence of communities in changing environments (Des Roches *et al.* 2018; Stange *et al.* 2021). It is an essential component of a population's fitness and extinction risk and thus key for evolutionary adaptation (Booy *et al.* 2000). Intraspecific diversity can increase a species' performance in varying environments because different genotypes have different traits that allow them to perform well in varying environments (Bell 1991), thus creating intraspecific response diversity.

Stressful environmental conditions lead to a reduction in fitness in populations, thus stress is defined as a loss of absolute fitness resulting in declining populations (Hoffmann and Hercus 2000; Agrawal and Whitlock 2010). Intraspecific diversity can increase resistance to biotic stressors, such as for example pathogens, and abiotic stressors (Jump *et al.* 2009). As an example, Zeller et al. found increased disease resistance in populations of wheat consisting of different genotypes (Zeller *et al.* 2012). Others observed that intraspecific diversity can maintain ecosystem functioning in the presence of stressors but did not have an effect in the absence of disturbance (Hughes and Stachowicz 2004). However, there is still limited evidence as to how intraspecific diversity can increase the response diversity (Elmqvist *et al.* 2003) within a single species. Here, I tested whether intraspecific diversity can increase a single species' resistance to environmental stress.

To this aim, I used one of the world's smallest angiosperms, *Lemna minor* L. (common or lesser duckweed) as a model system. *L. minor* grows very fast and is easy to culture, and is a convenient and cheap model system to test ecological and evolutionary theory. This species has recently gained substantial interest to be used as a model organism in community ecology and eco-evolutionary dynamics (Laird and Barks 2018). To create intraspecific diversity, I collected seven populations of *L. minor* from different waterbodies under the assumption that they represent different ecotypes (or potentially genotypes).

The aim was to test two hypotheses: First, whether the ecotype identity influences ecotype-level growth rates in the absence and presence of salt stress. This response diversity would be a prerequisite to be able to test the second hypothesis, if intraspecific diversity could increase the resistance of *L. minor* to moderate salt stress. Finally, I tested whether the growth rates of ecotypes were influenced by the diversity context in which the ecotype was growing.

I grew the seven ecotypes alone or in the presence of either one or three other ecotypes, thus creating a diversity gradient ranging from monoculture to a 4-ecotype-polyculture. This allowed studying the effects of intraspecific diversity on total population abundance. I carried out the experiment in two phases. Initially I allowed *L. minor* to vegetatively grow with ample nutrients, light, and space for several weeks (phase 1). Subsequently I subjected them to stress using sodium chloride (NaCl or salt) as a stressor commonly found in *L. minor*'s urban freshwater habitats (phase 2). In many northern areas where duckweeds are common, large amounts of salt are applied to roads and other surfaces in winter. The application of road salt can significantly increase the salinity of waterbodies in urban areas (Schuler *et al.*

2017), with negative consequences for not only the aquatic ecosystems but also terrestrial ones. In contrast to previous experiments that investigated the influence of NaCl on duckweed growth rate (Sree *et al.* 2015), I used wild populations (ecotypes) and not strains that had been grown under laboratory conditions for a prolonged period of time. I conducted the experiment in a semi-natural environment with a community of algae and microbes in the water.

METHODS AND MATERIALS

Study species and collection

Lemna minor mostly reproduces clonally, producing new plants by budding, although in the wild it also occasionally flowers. It has a near-global distribution and occurs at high densities in slow-moving freshwater bodies in a wide range of environmental conditions (Landolt 1975).

The seven ecotypes were collected at different locations in and around Zurich, Switzerland. I sampled in Thalwil (ecotype 1), Neeracher Ried (ecotype 2), Fällanden (ecotype 3), Zurich, Rehalp (ecotype 4), Zurich, Irchel park (ecotype 5), Zurich, Seebach (ecotype 6), and Dübendorf (ecotype 7) in late summer/early fall 2020 (Fig. S1 for a map with the sampling locations, Table S1 for additional information including the coordinates). The conductivity was measured in a water sample using a handheld probe (Hanna instruments). Conductivity ranged from 183 µS/cm (Seebach, Zurich) to 552 µS/cm (Rehalp, Zurich) between collection areas but was < 1000 µs/cm in all locations, which corresponds to freshwater conditions (Table S2). Duckweed ecotypes were collected with approximately 5 L of water from the water body they were collected from. From each location, several hundred to several thousand individuals (fronds) were collected, capturing any intraspecific diversity present. The duckweed populations were then moved to the glasshouse facility at University of Zurich and kept in plastic tubs in their own water for ~6 weeks and then transferred into tap water to reduce algal growth.

Glasshouse experiment

The experiment was carried out at the glasshouse facilities of the University of Zurich, Switzerland in opaque plastic tubs (Universalwanne 9L, PP, Semadeni, Switzerland) containing 6 L of tap water and nutrients (see below). In total, there were 92 tubs which were divided into four compartments using black plastic containers (10 x 11 cm, GVZ rossat, Switzerland) to track growth of each ecotype individually (Fig. S2). A large hole was cut out from the bottom of the containers to maximize the underwater connection. The ecotypes were thus separated on the surface to prevent them from floating into each other's areas but shared the same water and freshwater microbial community. At the beginning of the experiment, the fronds were placed inside the containers (Fig. S2) using an inoculation loop. *L. minor* individuals were not sterilized prior to the experiment to maintain the natural epimicrobial community. Artificial light was programmed to be turned on from 10 am to 4 pm if the natural light was below 30 klux. The temperature was set at minimum 20° C during the day, 15°C during the night. The experimental design included seven monocultures (single-ecotype communities), nine 2-ecotype mixtures, and seven 4-ecotype mixtures for a total of 23 unique culture compositions. Like this, I avoided using all available ecotypes for the highest

diversity treatment and instead could replicate the highest diversity treatment by using different compositions. The design aimed to be as balanced as possible: All ecotypes were grown in monoculture, in multiple 2-ecotype and multiple 4-ecotype settings. For ecotypes 4 and 5, there were not enough fronds (individuals) available, therefore, they appeared less frequently in the design. Each unique composition was replicated four times at the beginning of the experiment (23 * 4 = 92 containers). For the full experimental design, see Table S4.

The experiment was started on 11 Nov 2020. On this day, each of the four containers per tub received on average 13 fronds (+/- 3 fronds) for a total of an average of 51 fronds (+/- 5 fronds) per tub as initial population size. In the monocultures, all four containers received the same ecotype. The tubs were then covered with transparent plastic boards (4 mm Hobbyglas, Coop, Switzerland**)** to reduce evaporation. One board covered a group of 4 tubs. 9 mL (0.7ml / L) of fertilizer (100% Hoagland's E Media (Cowgill and Milazzo 1989) was added nine days after the start on 20 Nov for a final concentration of 0.125% fertilizer, which corresponded to approximately 0.124 mg/L of nitrogen (N) and 0.019 mg/L of phosphorus (P). To mitigate algal growth, the communities were transferred into fresh tap water with Hoagland's E medium every two weeks. After the transfer, fresh Hoagland solution was added to the tap water and thoroughly mixed. Initially, 9 mL / 6 L of nutrients (0.7 ml/ L) were added but then the concentration was increased to 18 mL / 6L (1.4 mL/ L) from January 2021. Consequently, nutrients were never limiting for the duration of the experiment.

The experiment was then carried out in two phases. During phase 1 (11 November 2020 – 14 February 2021), the populations were grown without any experimental treatment. During this time, I recorded population abundances for all ecotypes and cultures under unperturbed conditions. On 14, 15 and 16 February 2021, phase 2 of the experiment was initiated by establishing moderate salt stress using sodium chloride (NaCl). To ensure that all four independent replicates started with relatively equal abundances, I standardized *L. minor* abundance among the four replicates of the same composition. To do so, I pooled all the duckweed individuals per ecotype and collected them in a global pool from which I redistributed the fronds to the replicates at roughly equal abundances. After this standardization process, I subjected half of the communities to salt stress. I added NaCl (Sigma-Aldrich, 99.5% purity) to half of the tubs (17.53 +/– 0.01 g / 6L) for a final concentration of 50 mM. This salt concentration has been shown to be harmful but not lethal to *L. minor* (Sree *et al.* 2015; O'Brien *et al.* 2020). The salt was added to pre-labelled replicates 3 and 4 for all experimental cultures. The NaCl was resuspended in the water by mixing. Phase 2 ran from February 2021 to March 2021. The duckweed populations continued to be transferred into fresh tap water with the same salt concentrations and nutrient concentrations. However, despite these frequent transfers, the experiment had to be terminated after six weeks of phase 2 to due algal growth causing high duckweed mortality in both treatment and control tubs. On 3 March, 2021, the tubs had to be moved to an adjacent compartment due to construction work being carried out at the glasshouse facilities. The new compartment had adiabatic cooling but had otherwise similar conditions. Groups of four tubs who were in close proximity in the first compartment (termed a "group") stayed together after the move. Duckweed abundance was estimated using photographs (iPhone SE camera) and

by counting all individual fronds with the counter function in Image J (Rasband, W.S. 1997). The tubs were photographed in total 16 times from November 2020 to March 2021, resulting in 16 time points.

Data analysis

During phase 1, ecotype population growth rates were calculated as $\ln(N_2/N_1)/(t_1-t_2)$. For population growth rates in phase 1, I used the initial population abundance at the start of the experiment (t_1) and the final time point of phase 1 (t_2, 8.2.2021). For ecotype-level analyses, the average growth rate was calculated across all individual black plastic containers with a specific ecotype growing in it (n = 56 for ecotypes 1, 2, 3, 5 and 6, n=40 for ecotype 4, and n=48 for ecotype 5). In phase 2, the duckweed populations stopped growing and even slightly declined. Therefore, calculating the growth rate would not be insightful. Instead, I used the mean abundance during phase 2 as response variable.

To test the outcome of the intraspecific diversity manipulation for the full duration of the experiment, I used abundance as response variable. Total abundance per tub was summed for the four black containers to get an abundance estimate per tub (n=92).

To account for the effect of spatial position in the glasshouse, a factor "group" was created that corresponded to a group of 4 tubs that were covered by the same plastic board throughout the experiment and thus had similar light conditions. "Group" was used as random-effect in statistical models when appropriate.

For the ecotype growth rates in phase 1, the effect of the treatment variables on growth rates (n=368) was analyzed with linear mixed models. Fixed-effect factors were intraspecific diversity (1, 2 or 4 ecotypes), ecotype identity (ecotype 1, 2, 3, 4, 5, 6 or 7) and their interaction. Group was included as random-effect factor to account for spatial variation. In addition, I nested the container (unique composition) within group. Mixed models using restricted maximum likelihood (REML) were fitted using the function lmer in the R-package lme4 (Bates *et al.* 2015). To further investigate the significant effect of ecotype identity a post-hoc test (Tukey, lsmeans (Lenth 2016)) was used. Mean abundance per population in phase 2 (n=368) was log-transformed and consequently analyzed using the same mixed models as explained above.

For whole-population abundances (n=92) the time series was also split into the two phases. Whole-population abundance was log-transformed for all statistical analyses. Here, the fixed-effects were intraspecific diversity either as factor (1, 2 or 4 ecotypes), a linear term or a contrast between monocultures and polycultures, and time (linear, n = 16 time points). Group (to account for spatial variation) and community composition were included as random effects. Mixed models using restricted maximum likelihood (REML) were fitted using the function lmer in the R-package lme4 (Bates *et al.* 2015). Due to the imbalanced design (Table S3), test statistics were obtained with a type 3 ANOVA using the R package lmerTest (Kuznetsova *et al.* 2017). All analyses were conducted in R v 4.1.0 (R Development Core Team 2021).

RESULTS

Ecotype responses in the absence and presence of moderate salt stress

I found strong and significant effects of ecotype identity on growth rates in both mono- and polyculture in phase 1 (Fig. 1, 3A, Table S5). However, the diversity context in which the ecotypes were growing and the interaction between the diversity context and ecotype identity were not significant (Table S5). Thus, ecotype growth rates were not influenced by either diversity or the presence or absence of a specific different ecotype.

During phase 1, most ecotypes showed continued exponential growth (Fig. 1). An exception was ecotype 7, which stopped growing after only a few weeks and then maintained its population size. A Tukey post-hoc test confirmed that ecotype number 2 significantly outperformed all the others. In contrast, ecotype number 7 showed a significantly lower growth rate than the others (but its growth rate was comparable to the one of ecotype 4). Ecotype 6 grew significantly better than ecotypes 3, 4, 5 and 7 but its growth rate was similar to the one of ecotype 1 and still significantly lower than the one of ecotype 6.

In phase 2 (Fig. 2, 3B), when salt exposure was combined with (unintentional) stress from algal contamination (Fig. S2), I also found strong and significant effects of ecotype identity on growth rates in both mono- and polyculture (Table S5). In addition, there was a significant effect of the diversity context for a subset of the ecotypes (significant interaction ecotype x diversity, table S5). When diversity is added as a contrast between monocultures and polycultures in the model, the contrast term was also significant (data not shown). In other words, ecotype growth varied significantly, and some ecotypes were influenced by the diversity context, especially the difference between growing alone vs growing in polyculture (Fig. 3B). However, salinity did not impact population growth rates and on average, diversity did also not influence growth rates.

Whole-population responses in the absence and presence of moderate salt stress

For whole-population analyses the population abundances for the four ecotypes growing in the black containers were summed to get a total population abundance per culture. For phase 1, I could not find significant effects of diversity on total abundance (Table 1). In phase 1, over time ecotype monocultures started to have on average lower total abundance and polycultures (both 2-ecotype and 4-ecotype polycultures) were on average more productive. (Fig. 4, Table 1). However, throughout phase 1 the best performing community composition was the monoculture of ecotype 2. The interaction between the comparison between mono- and polycultures and time was significant for all three diversity terms but the effect was strongest for the comparison between monocultures and polycultures ($P = 0.009$, Time x Diversity interaction, Table 1). In phase 2, the diversity effect (only the contrast between mono- and polycultures) was significant, with 2- and 4-ecotype polycultures being significantly more productive than ecotype monocultures ($P = 0.021$, Table 2). This positive effect of diversity was consistent during phase 2 (Fig. 4). There was no significant effect of salt addition on total abundance in phase 2 (Table 2, Fig. 4).

DISCUSSION

Effects of intraspecific diversity on whole-population abundance

Intraspecific diversity can have strong effects on community and ecosystem functioning (Des Roches *et al.* 2018). Intraspecific diversity is the basis for response diversity of a single population in the face of novel environmental conditions (Hughes and Stachowicz 2004) similar to species response diversity with consequences for whole-community resilience (Baskett *et al.* 2014). Therefore, I hypothesized that intraspecific diversity would lead to greater population abundances in a stressful environment.

Indeed, I found that as the experiment progressed, the positive effect of diversity started to emerge (see significant interaction term Date x Diversity in Table 1 and significant diversity term in Table 2). The strengthening of the positive effect of diversity came as the growing conditions worsened, both due to the exposure to salt in phase 2 but also the appearance of increasingly more algae in the containers. The algae formed a dense biofilm covering the fronds and roots of the *L. minor* individuals. Therefore, the moderate salt stress was accompanied by a secondary stressor, the algal biofilm (Fig. S3) which has led to significant reductions in growth rates and mortality in all ecotypes, even those which were not subjected to salt stress. At the end of the experiment, polycultures were consistently more abundant, though not more abundant than the most productive monocultures (no evidence for transgressive overyielding). The positive effect of intraspecific diversity could be due to facilitative mechanisms driven by both the dilution of algae and dilution of hosts. Mixing four ecotypes together resulted in the dilution of growth-inhibiting algae which were associated more with some ecotypes and less with others. The host-dilution effect is based on the assumption that there were co-evolved harmful algae present that were specialized on a specific host (Sallinen *et al.* 2020). In polycultures, ecotypes under attack by such algae could have experienced a reduction in pathogenic load because of a host-dilution effect, similarly to the positive effect of plant species richness on soil pathogens (Schnitzer *et al.* 2011; Maron *et al.* 2011). Interestingly, it has previously been found that *L. minor* associates with a diverse and mostly beneficial microbial community (Ishizawa *et al.* 2017; O'Brien *et al.* 2020). However, the placing of the *L. minor* populations in tap water under greenhouse conditions may have selected for a subset of algae, which were on average growth-inhibiting. Host genetic diversity can increase parasite resistance (Altermatt and Ebert 2008), it is thus conceivable that similar mechanisms were at play in this study.

In contrast to the stress imposed by the algae in the experiment, salt addition had no significant effects on population abundances. Previous studies conducted in laboratory conditions found that >50 mM of NaCl significantly reduced growth in *L. minor* (Sree *et al.* 2015). However, there is also evidence that *L. minor* can grow well under sustained salt stress in the laboratory (Ullah *et al.* 2021). Here, I showed that *L. minor* can grow in near-brackish water, which adds to previous evidence that *L. minor* can be grown under a wide range of environmental conditions, including in saline environments.

Ecotype identity effects on growth rates

I showed that ecotypes from the same species collected from different waterbodies of a maximum distance of 40 km showed differential growth rates in a new environment, i.e., a

glasshouse compartment. In particular ecotype 2 outperformed all the other ecotypes. In contrast, ecotype 7 grew significantly slower than all the other ecotypes. The differential response of the ecotypes could be because different ecotypes are locally adapted to their environments, which resulted in varying degrees of maladaptation to the novel conditions in the greenhouse. Despite the dominance of asexual reproduction, populations of duckweed maintain relatively high levels of genetic diversity. It is known that for *L. minor* there is commonly high genetic variation among populations, but low genetic diversity within populations (Cole and Voskuil 1996; Xu *et al.* 2015). Thus populations of duckweed usually represent different genotypes/clones, even when they occur in close distance to each other (Vasseur *et al.* 1993; Ho 2018; Hart *et al.* 2019; Tan *et al.* 2021) and are potentially adapted to different environmental conditions. This was further evidenced in a study demonstrating that three genotypes of *L. minor* collected in France showed varying growth rates in a common garden experiment as well as differential responses to copper pollution (Roubeau Dumont *et al.* 2019). Thus, it is conceivable that the different populations collected in the field are different genotypes that could also show differential growth rates in the experimental setting.

Alternatively, it could be that the associated epimicrobial community that hitchhiked on the surface of the duckweed leaves into the experiment had a very strong effect on duckweed growth and survival. Recent studies have shown that there are strong interactions between the duckweed microbiome and the plants fitness as well as response to stressors (O'Brien *et al.* 2020; Tan *et al.* 2021). Together, these findings suggest that experiments conducted with *L. minor* in axenic conditions may overestimate growth rates and other fitness components but underestimate the strength of population dynamics over time. Future experiments should consider more natural conditions, in particular, those studying stress tolerance of *L. minor* to evaluate their potential for phytoremediation.

Not only did the ecotypes vary in their growth rates but the ecotype growth rate also varied over time. For example, the best-performing ecotype in phase 1 (ecotype 2) could not maintain its growth rate in phase 2 and its higher population abundance was not buffering the impact of moderate salt stress, or the secondary stressor induced by the algal biofilm. Contrastingly, the best-performing ecotype at the end of phase 2 was only average during phase 1 (ecotype no. 6). In phase 2, the significant interaction term (P= 0.012, Table S5) between ecotype identity and diversity showed that in some instances, ecotypes grew better when they were in the presence of other ecotypes, whereas for some ecotypes it was beneficial to be growing in a monoculture. Those that profited from growing alone tended to be ecotypes that performed well in general. The two low-performers, ecotypes 5 and 7, however, grew better when they were in the presence of a second ecotype, indicating some facilitative mechanisms (Le Bagousse-Pinguet *et al.* 2014). It is possible that the low-performers (e.g., ecotypes 5 and 7) have been associated more with growth-inhibiting algae than others. Consequently, the facilitation was most likely driven by a dilution effect (see above). The "dilution" of pathogenic algae and other microbes in polycultures may have had a positive effect on all constituent ecotypes, but particularly on those low-performers which experienced a reduction in the load of their growth-inhibiting associated algae and microbes. Regardless of the mechanism, these results show that associated algae and other microbes can

strongly influence population dynamics of *L. minor*. Further research needs to be conducted that carefully disentangles the effect of the microbiome from the effect of ecotype identity.

Conclusions

L. minor is a promising candidate for many applications such e.g. as biofuel (Van Hoeck *et al.* 2015), bioremediators (Alvarado *et al.* 2008), crop (Chakrabarti *et al.* 2018), or protein source (Ullah *et al.* 2021). More studies need to be conducted with greater intraspecific diversity gradients and a separation of the effects of genotype vs. the effects of epimicrobial flora on duckweed population fitness. Knowing more about the effects of its intraspecific diversity on abundance and growth rate will help to maximize yields for food production and to choose ecotypes (genotypes) best suited for local cost-effective growing conditions.

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SUPPLEMENTARY MATERIAL

Supplementary Tables S1–S5 and Figures S1–S3 are available online.

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DATA ACCESSIBILITY STATEMENT

Data and code will be made publicly available upon final acceptance.

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Table 1. Type III ANOVA results for linear-mixed model with log-transformed whole population abundance as response variable in phase 1. Fixed-effect terms were time point and diversity (factorial, linear and contrast between monocultures and polycultures). Group was included as random effect to account for spatial variation in the glasshouse and community composition was added as random effect to account for the variation induced by specific combinations of ecotypes. p-values < 0.05 are shown in bold.

Source of variation	**Sum Sq**	**Mean Sq**	**NumDF**	**DenDF**	**F value**	**Pr(>F)**
Diversity as factor						
Time	179.967	179.967	1	956.97	3078.0939	**<0.001**
Diversity	0.054	0.027	2	27.67	0.4623	0.635
Time x Diversity	0.406	0.203	2	956.97	3.469	**0.032**
Random terms	**Variance**	**St.Dev**				
Group (n=23)	0.01249	0.1118				
Composition (n=23)	0.02602	0.1613				
Residual (n = 1008 observations)	0.05847	0.2418				
Diversity as linear term						
Time	19.7685	19.7685	1	957.82	337.5893	**<0.001**
Linear diversity	0.0479	0.0479	1	29.56	0.818	0.37307
Time x linear diversity	0.2651	0.2651	1	957.82	4.5267	**0.034**
Random terms	**Variance**	**St.Dev**				
Group (n=23)	0.01245	0.1116				
Composition (n=23)	0.02476	0.1574				
Residual (n = 1008 observations)	0.05856	0.242				
Diversity contrast monocultures vs. polycultures						
Time	149.442	149.442	1	957.82	2558.3577	**<0.001**
Diversity contrast	0.055	0.055	1	29.33	0.9345	0.341597
Time x Diversity contrast	0.404	0.404	1	957.82	6.91	**0.009**
Random terms	**Variance**	**St.Dev**				
Group (n=23)	0.01245	0.1116				
Composition (n=23)	0.02476	0.1574				
Residual (n = 1008 observations)	0.05841	0.2417				

Table 2. Type III ANOVA results for linear-mixed model with log-transformed whole-population abundance as response variable in phase 2. The fixed effects was diversity (either factorial, linear or as a contrast between monocultures and polycultures) and salinity (control vs. 50mM). Time point and nested within each time point community composition were added as random effects. Group was excluded from the model because spatial variation did not influence the results in phase 2. p-values < 0.05 are shown in bold.

Source of variation	**Sum Sq**	**Mean Sq**	**NumDF**	**DenDF**	**F value**	**Pr(>F)**
Diversity as factor						
Diversity	0.232742	0.116371	2	108.030	2.743	0.069
Salinity	0.036214	0.036214	1	341.05	0.8536	0.356
Diversity x Salinity	0.04379	0.021895	2	341.05	0.5161	0.597
Random terms	**Variance**	**St.Dev**				
Community x time point	0.25873	0.5087				
Time point	0.04256	0.2063				
Residual (n= 459 observations)	0.04242	0.206				
Diversity as linear term						
Linear Diversity	0.152737	0.152737	1	109.04	3.6001	0.060
Salinity	0.002686	0.002686	1	342.04	0.0633	0.801
Linear diversity x Salinity	0.000834	0.000834	1	342.06	0.0197	0.889
Random terms	**Variance**	**St.Dev**				
Community x time point	0.26084	0.5107				
Time point	0.04246	0.2061				
Residual (n= 459 observations)	0.04243	0.206				
Diversity contrast monocultures vs. polycultures						
Diversity contrast	0.233605	0.233605	1	109.01	5.5127	**0.021**
Salinity	0.02165	0.02165	1	342.04	0.5109	0.475
Diversity contrast x Salinity	0.018084	0.018084	1	342.04	0.4268	0.514
Random terms	**Variance**	**St.Dev**				
Community x Time point	0.26084	0.5107				
Time point	0.04246	0.2061				
Residual (n= 459 observations)	0.04243	0.206				

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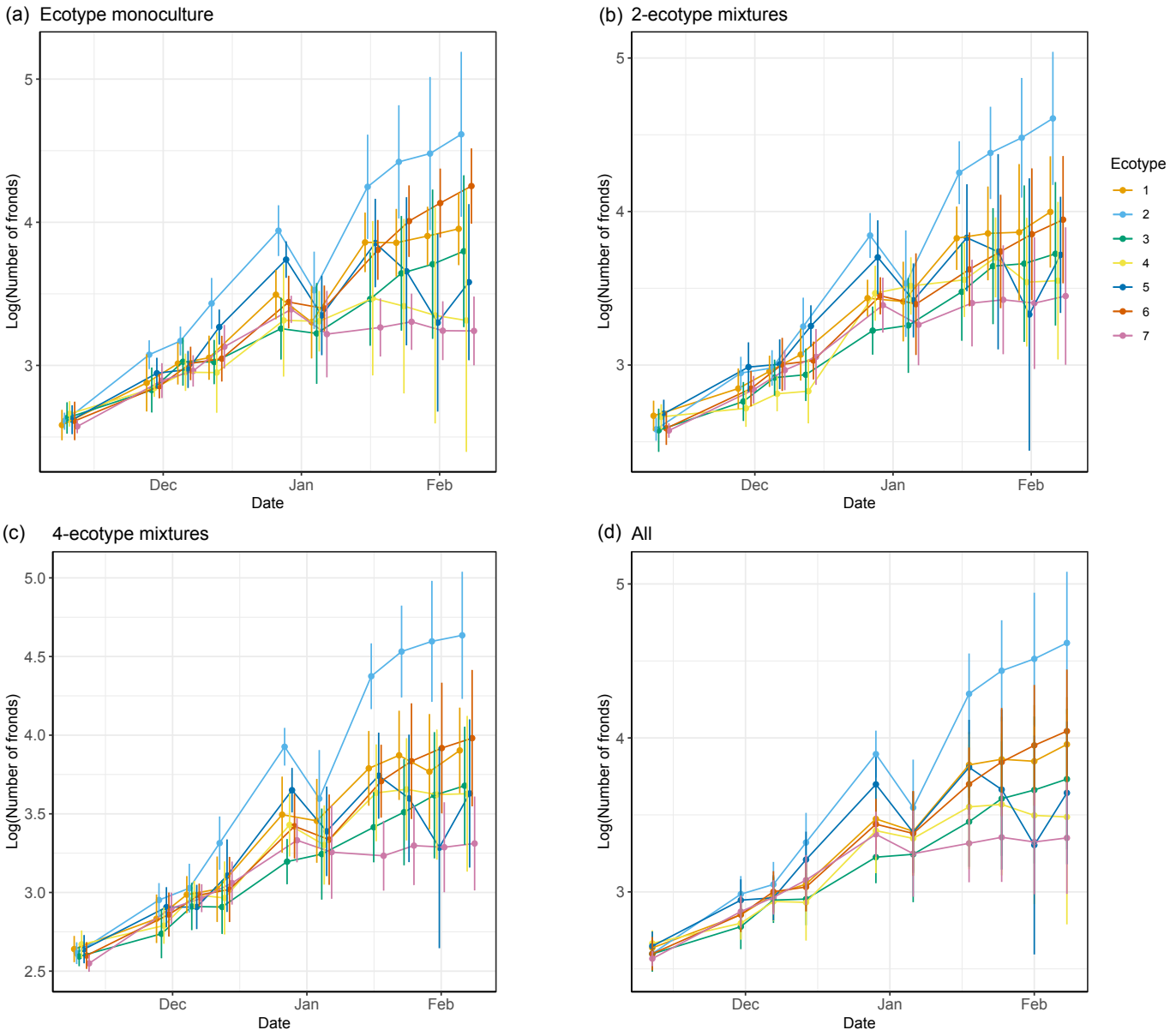


481 **Figure 1:** Population growth (log-transformed number of fronds) during phase 1. **a** Ecotype
482 monoculture, **b** 2-ecotype polyculture, **c** 4-ecotype polyculture and **d** across all three diversity
483 levels. Shown are means and standard errors. Fronds were counted based on image analysis
484 using the counter function in ImageJ.
485
486

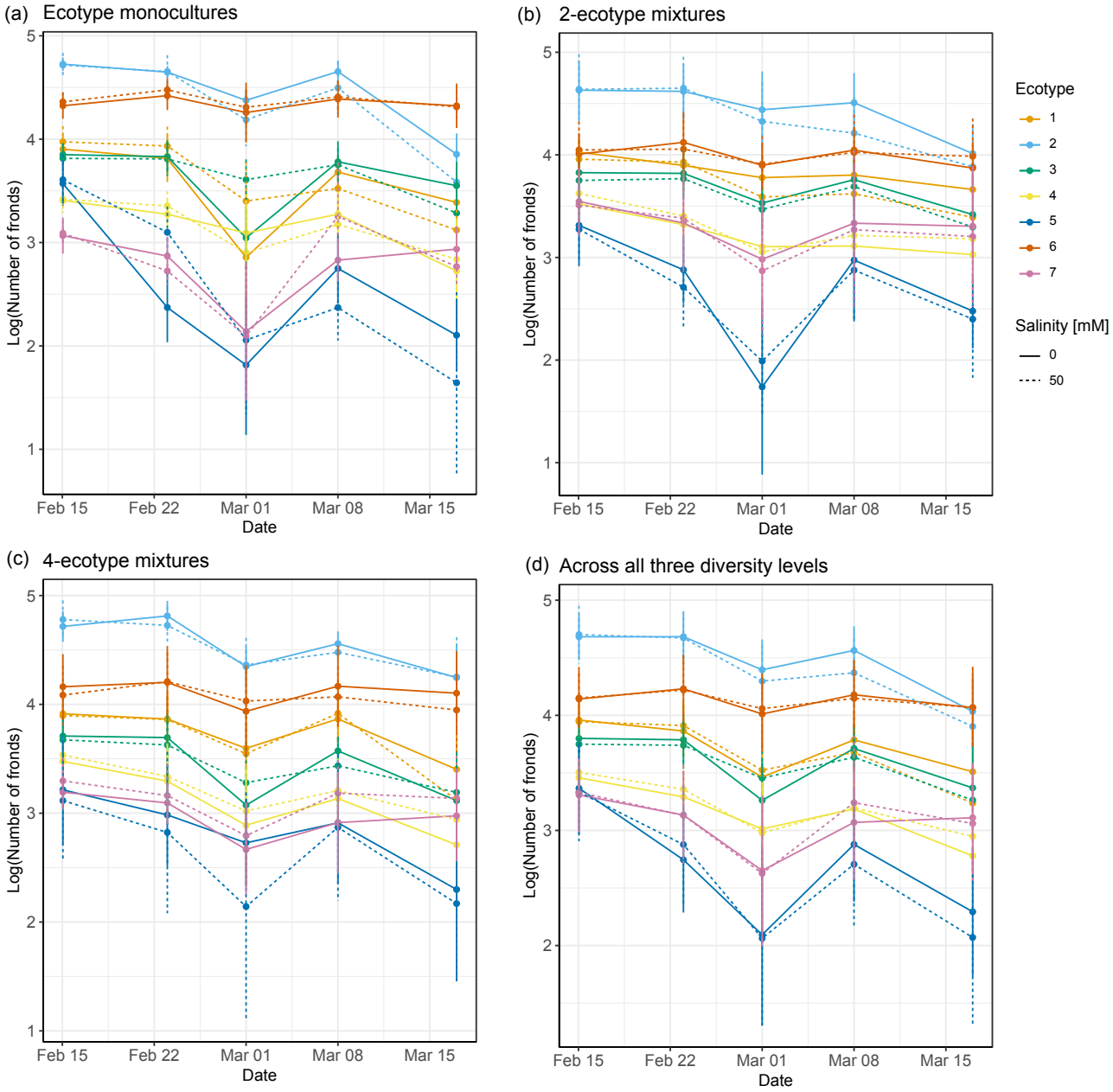


Figure 2: Population growth (log-transformed number of fronds) during phase 2. **a** Ecotype monoculture, **b** 2-ecotype mixtures, **c** 4-ecotype mixtures and **d** across all three diversity levels. Shown are means and standard errors. Note that abundances declined due to a secondary stressor induced by algal biofilms in all experimental cultures. Salt addition did not significantly decrease population growth (Table S5).

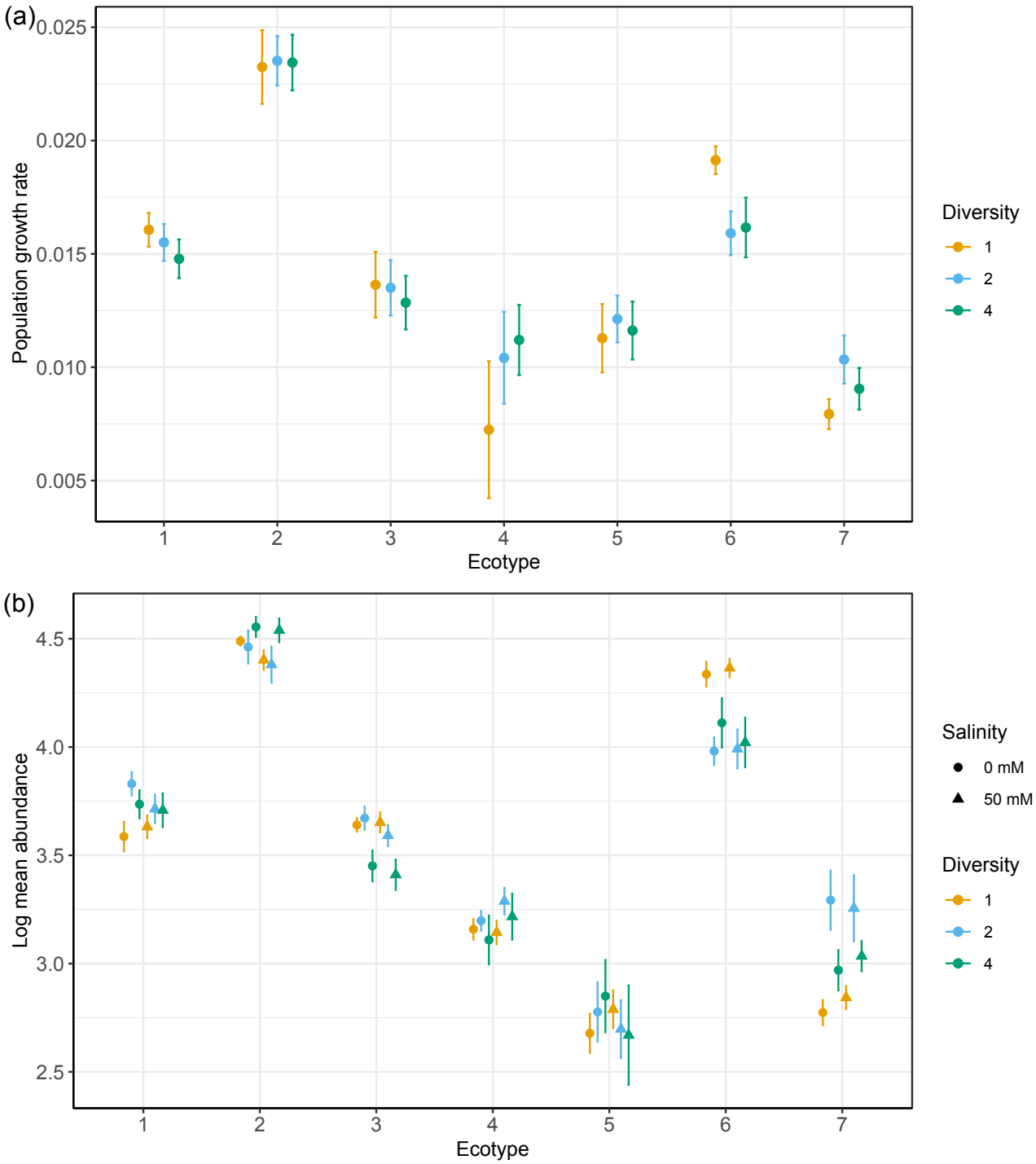
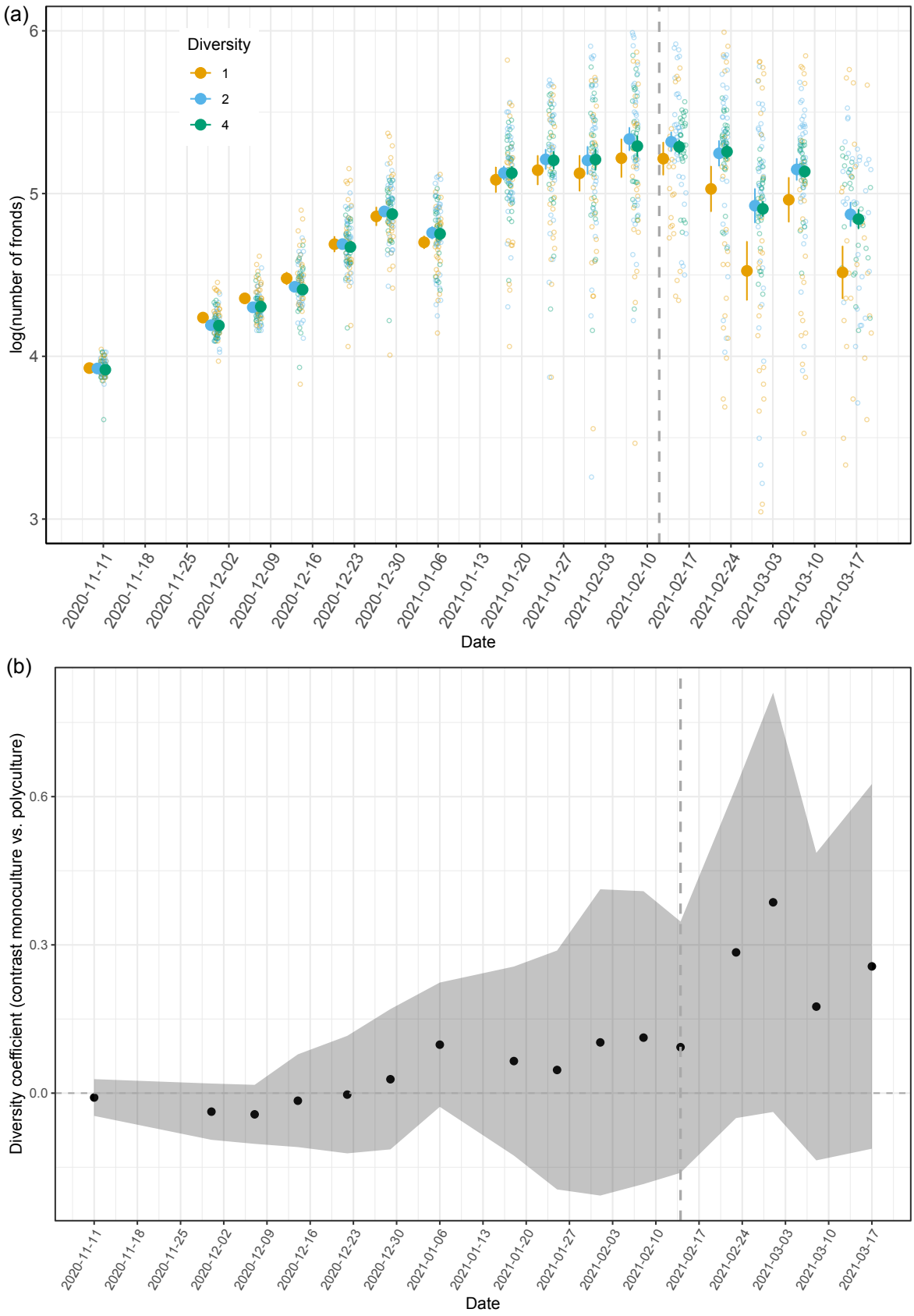


Figure 3: a Growth rates across phase 1. Shown are means and associated standard errors across cultures per diversity level. **b** Log-transformed mean abundance across phase 2 in the presence of salt (triangles) and in the absence of salt (round points). Shown are means and associated standard errors across cultures per diversity level and salinity treatment. For associated ANOVA test statistics see table S5.



500 **Figure 4: a** Community abundance (log-transformed number of individual duckweed fronds)
501 over time for each diversity level. The start of phase 2 (i.e., the addition of 50mM of NaCl to
502 half of the cultures) is indicated with a vertical dashed line. Shown are means and associated

standard errors per sampling date (14 time points) and diversity (n= 28 for monocultures, n = 36 for 2-ecotype polycultures, n= 28 for 4-ecotype monocultures, total n= 92) . Ecotype monocultures: orange; 2-ecotype polycultures: blue; 4-ecotype polycultures: green. For corresponding test statistics see Tables 1 and 2. **b** Model coefficients for the contrast between monocultures and polycultures from a linear-mixed model including salinity as fixed-effect factor and group as random-effect factor for each date. The horizontal dashed line at 0 indicates that there is no diversity effect. Below 0, diversity had a negative impact on community abundance (in the beginning of the experiment), above 0, diversity positively influenced community abundance (from the middle to the end of the experiment). Shown are the model estimates and the 95% confidence intervals as shaded areas. The start of phase 2 (i.e., the addition of 50mM of NaCl to half of the cultures) is indicated with a vertical dashed line.