High tolerance to zinc but limited evidence for local adaptation in the aquatic plant species *Lemna gibba/minor*

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Abstract

Duckweed, a widely distributed aquatic plant family, are economically important and have high potential for phytoremediation of polluted water bodies. We collected four *Lemna gibba/minor* populations from across Switzerland and assessed how their original vs. foreign environments influenced their growth. Additionally, we investigated their response to a metal pollutant (Zn) in both the original and foreign environment. Zn is found in freshwater systems and can become harmful at elevated concentrations. We hypothesized that growing in their original environment would help the plants buffer the negative effect of the metal pollutant.

To test this, we measured *Lemna* growth in a reciprocal transplant experiment in a glasshouse where the four plant populations were grown in each of the environments, as well as in three different concentrations of Zn. We sampled chlorophyll-a as a proxy for algal biomass, and also measured total nitrogen and total organic carbon.

The four *Lemna gibba/minor* populations exhibited significantly different growth rates across environments. However, the effect of the environment on duckweed growth was the same for all populations. We did thus not find evidence for local adaptation, and instead observed strong plastic responses in the populations. Zn increased duckweed growth rate but inhibited algal growth. Consequently, the positive effect of Zn on duckweed growth could be in part via reducing the competition with algae. We conclude that *L.gibba/minor* ecotypes may exhibit large differences in growth rate but that the species overall has a high Zn tolerance and strong plastic adaptive potential in novel environments.

Keywords: aquatic plant ecology, duckweed, heavy metal pollutant, home vs. away, Nitrogen, plant-algae interactions, reciprocal transplant experiment, TOC

INTRODUCTION

Understanding how species evolve to adapt to specific environmental conditions allows to better predict how environmental change may affect populations and communities, and thus find ways to prevent or mitigate its consequences more effectively. Within species, different populations experience different selective pressures and will thus adapt to become better suited to their own local environmental conditions (Joshi et al. 2001). When such local phenotypes demonstrate higher fitness in their local environment compared to members of populations at foreign locations and vice-versa, the population is locally adapted (Kawecki and Ebert 2004). Such a reciprocal home site advantage results in a fitness trade-off, given that adaptation to one environment can sacrifice performance in a different one (Kawecki and Ebert 2004). A spatially heterogeneous environment generating a heterogeneous selective pressure is essential for the development of local adaptation (Kawecki and Ebert 2004, Gibson et al. 2016). Accordingly, an environmental component can create divergent selection among different sites consistently and populations thus evolve in response.

Measuring local adaptation allows for the mechanisms of natural selection to be assessed (Ruiz Daniels et al., 2019), furthers our understandings of the interactions of natural selection and gene flow and is also vital for decision-making for land managers attempting ecosystem restoration (Gibson et al., 2016). Many environmental components can select for local adaptation (Leimu and Fischer 2008), including heavy metals (Eränen 2006), and interactions among species (Hoeksema and Forde 2008) such as plant-herbivore (Hargreaves et al. 2019) and host-parasite interactions (Kaltz and Shykoff 1998). To test for local adaptation, the fitness of a population in both its originaland foreign environments must be measured. Two criteria are used to predict whether populations are locally adapted to an environment. Within-genotype comparisons ('home vs away') require that members of a population will express higher relative fitness in their original habitat compared to members of the same population transplanted in other habitats (Blanquart et al., 2013). Between genotype comparisons ('local vs foreign') require that members of a native population will express higher fitness in their original habitat relative to individuals of foreign populations of the same species in that same environment (Kawecki & Ebert, 2004).

The fast-growing aquatic plant species complex *Lemna gibba/minor* belongs to the family Lemnaceae (duckweeds). Duckweeds have a simple structure composed of a frond (its vegetative body) and a very thin thallus-like structure (Landolt 1986, Ziegler et al. 2016). They are known as one of the smallest flowering plants in the world, but mainly reproduce asexually by budding (Landolt 1975, O'Brien et al. 2020b). Globally distributed, *Lemna gibba/minor* grow in ponds or bodies of very slow-moving water, where they coat the surface and reach high population densities. They can take up and accumulate trace metals in their roots and fronds (Newman 1991, Fritioff and Greger 2006, Subramanian and Turcotte 2020). The *Lemna* genus can tolerate a wide range of conditions, which makes it an important agent for the bioremediation of different aquatic bodies (Landolt 1996, Dirilgen 2011). They can remove excess macronutrients and many different substances from organic chemicals to heavy metals (Khellaf and Zerdaoui 2009), including zinc (Lahive et al. 2011b).

Zn pollution is common in urbanized areas, including in Switzerland (AWEL 2006), and elevated Zn concentrations inhibit plant growth (Lahive et al. 2011a). Heavy metal pollution is of particular relevance in freshwater ecosystems due to their long-term effects on the ecosystem integrity (Duruibe et al. 2007, Sasmaz et al. 2015). Phenotypic plasticity can arise as a fast response to lower levels of pollution, but in consistently highly toxic environments it may lead to maladaptation (Gienapp et al. 2008, Loria et al. 2019). However, organisms with short generation times, such as duckweed, have a higher probability to adapt to a fast-changing polluted environment (Vander Wal et al. 2013).

Isolation-by-distance can drive local adaptation (Wright 1943), especially in plants, which do not move post-dispersal. Therefore, we collected four populations of *L. gibba/minor* from four distant locations separated by several hundred kilometers across Switzerland to test for local adaptation. In addition, we asked if and how the presence of different levels of Zn affects the growth rates of the four, potentially locally adapted, *L. gibba/minor* populations. Finally, we tested how the addition of the metal pollutant influenced the algal biomass, and whether there exists a relationship between algal biomass and duckweed growth rate. Due to competition for light and nutrients, we expected a negative relationship between algal biomass and duckweed growth, but facilitative (Brooker et al. 2008) or mutualistic (O'Brien et al. 2020a) processes could also play a role. According to the theory of local adaptation, we expected growth rates of duckweed populations to be highest in their original environments and to be lower in all the other environments. We further anticipated populations to be negatively affected by an increase in Zn and that, due to being locally adapted to the biotic environment, populations will be more resistant to Zn when grown in their original environment.

MATERIALS AND METHODS

Study species

We consider all populations used in this study as belonging to the Lemna *gibba/minor* species complex. *L. minor* and *L. gibba* have inconsistent vegetative morphologies and, aside from the occasional gibbosity in *L. gibba*, their identification cannot be determined with certainty without genotyping (De Lange and Pieterse 1973, Kandeler 1975, de Lange and Westinga 1979, De Lange et al. 1981). In addition, despite molecular genotyping being the standard for duckweed classification, even these markers are sometimes not enough to differentiate between *Lemna* species (Braglia et al. 2021). Thus, such complex genetic analyses of our collected populations were not within the scope of this project.

Study sites and sample collections

We collected *Lemna gibba/minor* populations from four geographic region of Switzerland (Appendix S1: Figure S1). The distances between the populations maximized the likelihood that there was no recent mixing of genotypes. The four water bodies represented also different altitudes, pond sizes, and shading conditions (Appendix S1: Table S1). In

August 2021, at each site (Koblenz: 47°36’03.3” N, 8°13’32.0” E, Yverdon: 46°47'50.8"N, 6°37'59.6"E, Motto: 46°25’43.6” N, 8°58’03.4” E, Ramosch: 46°50'01.4"N, 10°24'04.0"E, Figure S1), we measured conductivity (WTW LF 325 conductivity meter), pH (WTW Multi 340i), water temperature, and dissolved oxygen (HQ40D Portable Multi Meter from Hach). At the same time, we collected thousands of individuals of the respective local *Lemna gibba/minor* population and 10 L of pond water. Subsequently, the water and plants were transported to the glasshouse at the University of Zurich in Zurich, Switzerland where the source water was first sieved to remove larger pieces of leaves, bark, and other aquatic organisms. A 50 mL sample of water from each location was frozen at -20° C for later analyses “pre-experiment” (natural source water) of their inorganic carbon, organic carbon, and N concentrations (TOC/TN Analyzer, details below). A second 50 mL sample was collected and analyzed using a Fluoroprobe (see below).

Experimental design and set up of the glasshouse experiment

To test for local adaptation, the fitness of a population in both its original and foreign environments must be measured (Kawecki and Ebert 2004). Therefore, we conducted a fully reciprocal transplant experiment: each population was matched to their original/home environment and received the environment from three other populations (foreign/away). Additionally, we crossed this design with the application of Zn (in the form of $ZnSO_4$). We used three Zn treatment levels: no Zn (control), low Zn and high Zn. For all populations each treatment combination was replicated four times, resulting in a total of 192 experimental units (four populations x four environments x three Zn treatments x four replicates; Appendix S1: Figure S2).

To create the Zn treatments, we mixed $ZnSO_4 \bullet 7H_2O$ (Alfa Aesar) with the filtered source water at a concentration of 3.4 mg [Zn]/L for the low treatment and 11.36 mg [Zn]/L for the high treatment. The high concentration level of Zn exceeded that found in a waterbody near a mining area in Turkey (7.23 mg/L of elemental Zn, Sasmaz et al., 2015). Thus, the high concentration used would represent a heavily polluted waterbody.

Each experimental unit was contained within a 150-mL plastic cups (Semadeni, Switzerland). All cups were located within a single glasshouse compartment, which was cooled to prevent excess algal growth, but the natural daily temperature change was maintained. 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 a minimum of 20° C during the day, and 15°C during the night.

To start the experiment, each cup received 100 mL of filter source water and 30 *L. gibba/minor* individuals. All individuals were rinsed in tap water to ensure that there would be no source water transferred into the cup, while maintaining the frond microbiomes. No nutrients were added to the cups. All 192 cups were spread onto four different tables, one table per replicate, and within each table all cups were randomly placed to account for potential variation in artificial lighting. The experiment ran for 22 days. Using a smartphone camera

(iPhone 11, Apple), we took pictures of each cup on days 1, 8, 15, and 22. Using ImageJ (https://imagej.nih.gov/ij/index.html), we manually counted the number of mature green fronds. For individuals to be considered as alive, they must have contained green pigmentation. Individuals that were entirely either yellow or white were considered dead.

Laboratory analyses

At the end of the experiment (day 22), an unfiltered 50-mL water samples from each cup was analyzed for chlorophyll-a concentration (μg/L) fluorometrically through a Fluoroprobe (bbe Moldaenke, Germany). This chlorophyll-a concentration we used as a proxy for total algal biomass (see e.g., van Moorsel et al. 2021). In addition, a 30-mL water sample was analyzed for its inorganic carbon, total organic carbon (TOC) and total nitrogen (TN) concentrations (Skalar Formacs HT – I TOC/TN Analyzer). Since the samples contained more inorganic carbon than organic carbon, we were not able to analyze TOC via subtraction (i.e., TOC = TC – IC). Thus, we measured non-purgeable organic carbon (NPOC), often reported as TOC since most samples contain a negligible amount of NPOC (NPOC = TOC – POC). Part of each sample (7 mL) was acidified with 100 μL of 10% HCl and purged for two minutes with N_2 gas prior to the analyzer measurement. TN was measured simultaneously in a parallel compartment of the analyzer. We then compared the pre-experiment and post-experiment elemental concentrations.

Statistical analyses

We use two metrics to evaluate population fitness. The first metric was initial population growth rate calculated as $\ln(N_2/N_1)/(t_1 - t_2)$ where N is the number of fronds, and $t_1=1$ and $t_2= 8$ represent the first and eighth day of the experiment. By focusing on population growth during the first week of the experiment (i.e., prior to reaching carrying capacity) we were able to reduce a possible effect of nutrient limitation on growth rates. The second metric was total population growth rate calculate as $\ln(N_2/N_1)/(t_1 - t_2)$, where $t_1=1$ and $t_2=22$, which represented the first and last days of the experiment.

Using additive three-way ANOVAs, we tested whether the environment and the Zn treatments significantly influenced either fitness metric of the experimental populations. Treatment variables were population, environment, Zn treatment, and their interactions. The population x environment interactions was further decomposed into a 'home vs. away' contrast (Joshi et al. 2001), i.e. we matched each population to its own environment a created a variable (home) for this. Zn treatment was also further decomposed into a contrast of control vs. Zn treatment followed by the comparison between the low and high Zn treatments. To tease apart which populations were driving interactions between treatments and the population factor, we also used contrasts.

Using both initial and total growth rates, we calculated selection coefficents for each population *i* relative to the best population in a particular environment as $S_i = 1 - (\lambda_i/\lambda_{max})$ (sensu Joshi et al. 2001). We then averaged the four selection coefficients per population and

calculated the standard error as measure of uncertainty. A selection coefficient of zero indicates that a population is the most successful one of that species in that environment, while a coefficient of 1 indicates complete maladaptation to that environment (see also McGraw and Antonovics 1983).

To investigate if algal biomass influenced total population growth rate of the duckweed, we used an ANOVA to assess the effect of total chlorophyll-a concentration and its interactions with population, environment, and Zn treatment on total duckweed population total growth rate. We used the same model to also test how the final TOC and TN concentrations influenced total growth rates of *Lemna.* Because both algal biomass and TOC and TN were only assessed at the end of the experiment, we limited these analyses to total growth rates.

Finally, using three-way ANOVAs we assessed the effect of population, environment, and Zn treatments on total chlorophyll-a concentration (log-transformed) as a proxy for algal biomass and on the final TOC and TN concentrations (proxy for nutrient levels). In the ANOVA testing the influence of the treatment variables on total chl-a concentrations, we included "block" as a random factor and used lme() from the package nlme (Pinheiro et al. 2019) to run the mixed linear models. For all other linear models, we used the 'lm ()' function in R. All analyses were conducted in R v 4.1.0 (R Development Core Team 2021).

RESULTS

Differences between the populations were strong and consistent across environments but there was limited evidence for local adaptation

The effects of population identity and the four environments were strong (Figure 1, Table 1, significant main terms for environment and population). But, overall, we found no evidence for local adaptation using the home vs. away approach (Table 1, non-significant main term for the contrast home vs. away). However, the significant interaction term with population ($P = 0.005$ for total growth rate in Table 1 and $P = 0.016$ for initial growth rate, see Appendix S1: Table S2) shows that for a subset of the populations, there was an effect of home vs. away. Decomposing the population factor into the individual populations revealed that this was driven by population 4 (Table 1, $P = 0.019$ for the interaction term home vs. away x population 4) and by population 3 (Table 1, $P = 0.008$ for the interaction term home vs. away x population 3). Populations 1 and 2 did not show a home vs. away effect (Table 1, $P = 0.15$ and $P = 0.084$, for population 1 and 2, respectively). Thus, population 3 showed evidence for local adaptation through the home vs. away approach (Figure 1). Conversely, population 4 had significantly lower growth in its own environment compared to the away environments.

Through the local vs foreign approach, we found limited evidence that the local population would outperform all others (Appendix S1: Table S3). Population 1 had significantly higher initial and total growth rates in its original environment (environment 1)

compared to the other three populations in that same environment for both Zn treatments (Figure 1, $P < 0.001$ for the contrast term "population 1" fitted in front of population in a linear model using only data from environment 1). However, this was driven by the control and the low Zn treatments and the effect was gone in the high Zn treatment (Figure 1).

Population 1 had on average the highest growth rates and population 4 had the lowest growth rates across all treatments and environments (Figure 1). Environment 4 generally produced the weakest growth rates across all populations and treatments (Figure 1), thus being the least suitable habitat for *Lemna* growth.

Zn increased duckweed growth rates but reduced algal growth

The Zn treatments only marginally affected growth rates in the early stages of the experiment (Figure 1A, Appendix S1: Table S2, marginally significant effect of the control vs. Zn contrast, $P = 0.081$) and low vs. high Zn treatment had no effect (Appendix S1: Table S2, $P = 0.142$ for the contrast 'low vs. high Zn treatment'). Addition of Zn significantly increased total growth rates (Figure 1B, Table 1B, significant term for the Zn contrast 'control vs. Zn treatment', $P < 0001$). However, there was no significant difference between the low and high Zn treatments (Figure 1B, Table 1B, $P = 0.116$).

Zn significantly reduced algae growth (Figure 2, Appendix S1: Table S4, $P < 0.001$) and also modified community composition as resolved to the major algal group levels (Appendix S1: Figure S3b). In the high Zn treatment, the subgroups diatoms and cryptophyta went extinct in nearly all environments (environment 2 had a very small concentration of diatoms remaining).

Algal biomass was influenced by population and environment and decreased over time

Population identity had significant and strong effects on mean total chl-a concentration (Figure 2, Appendix S1: Table S4, $P < 0.001$ for the term 'population'). Algal biomass was highest in the cultures in which they were competing with population 2 and lowest in those in which they were competing with population 3 (Figure 2). In addition, the interaction with Zn treatment was significant (Appendix S1: Table S4, $P < 0.001$ for the interaction term 'Zn treatment x population'), indicating that the severity of the negative response to Zn addition further depended on the identity of the competitor in the culture.

Finally, mean chlorophyll concentrations strongly differed between the environments at the end of the experiment (Appendix S1: Table S4, $P < 0.001$ for the term 'environment') but also already in the field samples (Appendix S1: Figure S3a) and. Notably, environment 2 had the highest chlorophyll-a concentration at both the beginning and end of the experiment, whereas environment 3 had very low concentrations of chlorophyll-a and also exclusively algae belonging to the group "green algae". Chlorophyll-a concentrations decreased across all environments (Appendix S1: Figure S3a vs. b). In other words, the field samples had on

average more than double the chlorophyll-a concentrations than the samples taken after the three weeks of experiment, with most belonging to the green algae group.

Algae negatively affected Lemna growth

Across all levels of Zn, total growth rate generally decreased as chlorophyll-a concentration increased (Figure 3, Appendix S1: Table S5, $F_{1,94} = 5.7206$, $P = 0.012$). In the high Zn treatment, the different populations responded very differently to increasing algal growth (Appendix 1: Figure S4c). Specifically, population 4 had a sharp decrease in growth rate with increasing algal biomass, whereas populations 1 and 2 had a flatter response. However, the interaction between mean chlorophyll-a concentration and population was not significant (Appendix S1: Table S5, $F_{3,94} = 1.3462$ and $P = 0.265$) and neither was the interaction between mean chlorophyll-a concentration and Zn treatment (Appendix S1: Table S5, $F_{2,94} = 0.7059$ and $P = 0.496$).

Treatment variables influenced TOC and TN concentrations

Lemna total growth rates increased with increasing TN (Appendix S1: Figure S5a, linear model, $F_{1, 184} = 4.172$, $P = 0.043$), whereas TOC had no effect on *Lemna* growth (Appendix S1: Figure S5b, $F_{1, 184} = 2.835$, $P = 0.094$). TOC and TN varied significantly between environments (Appendix S1: Table S6, $P < 0.001$ for both TN and TOC, Figure S6). Generally, environment 2 had the highest concentrations of both TN and TOC while environment 3 had the lowest (Appendix S1: Figure S6). The Zn treatments significantly reduced TOC but did not have a significant effect on TN (Appendix S1: Table S6, $F_{2,144} = 6.8561$, $P = 0.001$ for TOC and $F_{2,144} = 2.3663$, $P = 0.097$ for TN, see also Figure S6). For TOC, there was a significant effect of population ($P = 0.00015$, Appendix S1: Table S6). TN concentrations were reduced more than TOC concentrations over the course of the experiment. This was evident because the source water samples contained much higher TN and only slightly higher TOC than post-experiment samples (Appendix S1: Figure S7).

DISCUSSION

We investigated local adaptation and the response to the metal pollutant Zn in the *Lemna gibba/minor* species complex using a fully reciprocal transplant approach. We asked 1) whether there is evidence for local adaptation; 2) if and how the presence of different levels of Zn affects the growth rates of the *Lemna gibba/minor* populations; 3) how the level of local adaptation influences the response to the metal pollutant; and 4) how the treatments influenced the algal biomass and the algal biomass influenced *Lemna* growth rates in turn. There were significant differences between duckweed growth rates in the different treatment groups. However, we did not find supportive significant evidence of local adaptation for neither of the four populations. The populations were positively affected by an increase in Zn in their environment, and thus had higher growth rates in the low and high Zn treatments.

No evidence for local adaptation but strong main effect of environments and populations

We expected to see higher growth rates when populations were raised in their original environments. Instead, we observed that a population's original environment rarely was significantly better than others. Assessing the results through the 'local vs foreign' lens, population 1 had higher growth rates in its own environment than all other populations had in that same environment. However, this was only the case in the presence of Zn, thus in the modifieds environments. Consequently, strictly speaking this would constitute a false signal of adaptation potentially due to the amelioration of negative biotic interactions, such as competition with algae (Hargreaves et al. 2020). Population 4 had the lowest growth rates across all environments and treatments, and its lowest growth rates were in its own original environment, suggestion maladaptation. However, lower growth rates of the other populations in environment 4 than in other environments could also indicates that it generally a less hospitable environment for *Lemna*. Environment 4 had a relatively high amount of TOC and a low amount of TN, which could have contributed to the negative performance trend of the duckweed populations. Additionally, the drastically lower growth rates in all environments could be a sign that population 4 was maladapted not specifically or only to its own environment but more generally to the other conditions in the glasshouse environment, e.g., light intensity or temperature. To our knowledge, our study is the first to test for local adaptation in multiple geographically distanced populations of *L. gibba/minor*. However, a study examining the closely related and morphologically similar *L. turionifera* came to similar conclusions, i.e. they could also not demonstrate local adaptation to environmental conditions (Barks et al. 2018). Contrastingly, Muranaka *et* al. observed local adaptation to the photo period in *L. aequinoctialis* growing in rice paddies (Muranaka et al. 2022), suggesting that some traits may exhibit local adaptation, which may not be picked up when merely using growth rate as fitness metric.

We explain the lack of local adaptation in these geographically very distant populations of *Lemna gibba/minor* with the well-known phenotypic plasticity of the *Lemna* family (Vasseur and Aarssen 1992a, Roubeau Dumont et al. 2019, Hitsman and Simons 2020). Phenotypic plasticity can arise faster than local adaptation as because a single genotype can express different phenotypes (de Villemereuil et al. 2018) and can be an important adaptive strategy for clonal plants (Riis et al. 2010). Indeed, the clonally reproducing duckweed are known to grow under many different environmental conditions (Laird and Barks 2018) and to persist and acclimate to environmental stress from salinity (e.g., van Moorsel 2022), to water pollutants (e.g. copper, Roubeau Dumont et al. 2019). Thus, the lack of local adaptation furthers adds evidence that populations and genotypes of the *Lemna minor/gibba* can be grown in many different environments likely due to their high levels of phenotypic plasticity (Vasseur and Aarssen 1992b). In extension this means that many *L. minor/gibba* genotypes could be used for heavy metal removal of polluted waterbodies. However, at the same time, we did find strong within-species variation in population growth rates, thus if high growth rates are desirable, evaluating several ecotypes may be advisable.

A more methodological explanation for the lack of strong population x environment interactions could be because we only reciprocally manipulated the water conditions. Light and other environmental parameters such as air temperature may be equally important. Our experiment possibly underestimated the degree of local adaptation because we did not test for local adaptation to light conditions, e.g., the level of sun exposure, which may be higher in higher altitudes or the south of Switzerland, or the percent of the water body being shaded by vegetation. In future experiments, it would be worthwhile to reciprocally transplant the populations in the field sites to include all environmental conditions paramount for plant fitness.

Despite the lack of local adaptation, we did find population-level differences across the environments. We noted that populations 1 and had generally higher growth rates and populations 2 and 4 generally lower growth rates. A possible explanation for the overall different growth rates between populations 1/ 3 and populations 2/ 4 could be in the species level. Due to the morphological similarity of many duckweed species, the taxonomy of the four populations was assessed based on microscopic analyses. According to this taxonomical analysis, populations 1 and 3 were *Lemna minor*, while populations 2 and 4 were *Lemna gibba* in its flat form (Walter Lämmler, personal communication). However, *L. gibba* is rarely observed in Switzerland (IUCN status: critically endangered; (InfoFlora 2022), and thus finding new *L. gibba* populations is unexpected. It is, however, possible that there is a cryptic presence of *L. gibba* in Switzerland, which could be revealed using molecular barcoding (Senevirathna et al. 2021). Future studies using wild populations of *L.* minor or other morphologically similar duckweed species such as *Lemna japonica, Lemna turionifera* and *Lemna minuta* should be aware that cryptic species may be potentially present.

Zn increased duckweed growth but reduced algal growth

We expected Zinc to be a stressful pollutant to *L. minor*, based on previous research with this species reporting that Zn impacted plant growth (O'Brien et al. 2020a). Here, Zn treatments significantly boosted *Lemna gibba/minor* total growth rates. Therefore, we could not test one of our main hypotheses, which was that growing in the original environment would aid in the stress response. This outstanding hypothesis could be addressed in future studies using 1) a different chemical that elicits an actual stress response in the duckweed species complex *L. gibba/minor* or 2) Zn levels at higher concentrations. Here, we wanted to keep Zn levels in somewhat realistic concentrations that may be relevant for phytoremediation, which is why we did not use extremely high concentrations.

The positive effect of Zn on duckweed growth rates could be due to its negative affect on most algae, which compete with *Lemna* for resources. In the presence of abundant nutrients and similar glasshouse conditions, algae took over *Lemna* populations and significantly reduced their growth rates (van Moorsel 2022). Another explanation stems from the fact that plants require Zn for their chlorophyll and protein production. Zn is an essential trace element for most organisms and plays important roles in metabolic processes in plants (Lahive et al.

2011a). This may explain the increased growth rates we observed in both low and high Zn treatments. In a different study, at the same concentrations as in this experiment, Zn increased growth rates of three *Lemna* species also under sterile conditions, i.e. in the absence of algae (Lanthemann and van Moorsel 2022). Other studies also report positive correlations between the presence of Zn and duckweed growth (Khellaf and Zerdaoui 2009, Jayasri and Suthindhiran 2017), suggesting efficacy in the uptake of this metal by these macrophytes. Jayasri & Suthindhiran (2017) found high tolerance of *L. minor* to Zn^{2+} concentrations of up to 10 mg/L. A second study found *L. minor* to tolerate Zn concentrations above 100 mg [Zn]/L, whereas the gibbous duckweed *Lemna gibba* only tolerated concentrations up to 10 mg [Zn]/L (Lahive et al. 2011a). Taken together, these previous and our findings indicate that *Lemna* may be a candidate species for the removal of excess Zn metal and derivatives from water bodies, as long as metal concentrations in the water are not toxic to the duckweeds themselves (Ziegler et al. 2016). We did, however, not measure Zn concentrations in the water at the end of the experiment to assess the amount of it that had been taken up by the plants.

In contrast to the positive effect of Zn on the plants, algal biomass and biodiversity was significantly reduced in the presence of Zn. Zn is known to negatively affect various algal groups even at levels lower than 30 µg/L (Kayser 1977, Wong and Chau 1990). Zn can alter the permeability of the algal cell membrane, leading to a steep decrease in potassium and sodium cell contents, inhibition of cell multiplication, photosynthesis, and N fixation (Kostyaev 1981). Furthermore, it has been demonstrated that algae become more sensitive to pollutants such as Zn when in competition with other plant species (Kayser 1977).

By the end of the experiment, TN had been significantly reduced to levels below the minimum needed for continuous *Lemna* growth (about 0.2 mg/L, Roijackers et al., 2004) in all environments except for environment 2. Interestingly, environment 2 had the highest algal biomass both prior and after the experiment, compared to the other environments. This suggests that *Lemna* may have played a larger role in TN uptake than the algae present. Although TOC concentrations decreased, there was still a significant amount left by the end of the experiment. However, there is not enough research on the effects of dissolved organic carbon on macrophytes, thus we do not know how it may have affected the *Lemna* populations. Initial *Lemna* growth rates were high, which together with the higher algal biomass, explains the strong TN decrease and shows that lower levels of N can have limited *Lemna* growth rates after the first week. After day 8, populations could have reached carrying capacity, given that their growths afterwards were slower, reduced, or decreased.

In conclusion, despite large effects of population identity and the tested environments, we did not find significant evidence for local adaptation. Instead, *Lemna* populations grew very well in Zn-contaminated waters, which prevented us from testing an actual stress response. Our findings suggests that for phytoremediation of heavy-metal polluted waters, many *Lemna gibba/minor* ecotypes may be suitable even though large within-species differences in growth rates should be expected.

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Data accessibility statement. We intend to make the publicly available on Data Dryad upon final acceptance of the manuscript.

Author contributions. SV and SJVM designed research. SV ran the experiment and collected data. SV and SVJM analyzed data. SV wrote the initial draft of the manuscript. SJVM wrote the final draft of the paper.

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585 **TABLE 1.** Results for ANOVA testing the effect of population, environment and Zn
586 treatments and their interactions on *Lemna* total growth rates (22 days of experiment). *P* -
587 values < 0.05 are shown in bold. For initial growth rates, see Appendix S1: Table S2.

Source of variation	**Df**	**Sum Sq**	**Mean Sq**	***F value***	***P***
Environment	3	0.00435	0.00145	41.55	**< 0.001**
Population	3	0.01306	0.00435	124.75	**< 0.001**
Home vs. away	1	0.00002	0.00002	0.49	0.485
Control vs. Zn treatment (Zn contrast)	1	0.00402	0.00402	115.11	**< 0.001**
Low vs. high Zn (Zn treatment)	1	0.00009	0.00009	2.50	0.116
Population x Home vs. away	3	0.00047	0.00016	4.48	**0.005**
Environment x Population	5	0.00022	0.00004	1.28	0.278
Population x Zn contrast	3	0.00063	0.00021	6.00	**0.001**
Population x Zn treatment	3	0.00006	0.00002	0.58	0.631
Environment x Zn contrast	3	0.00063	0.00021	6.03	**0.001**
Environment x Zn treatment	3	0.00013	0.00004	1.21	0.310
Home vs. away x Zn contrast	1	0.00009	0.00009	2.64	0.107
Environment x Population x Zn contrast	8	0.00045	0.00006	1.62	0.125
environment:population:zinc.treatment	9	0.00025	0.00003	0.81	0.612
Residuals	144	0.00503	0.00003		

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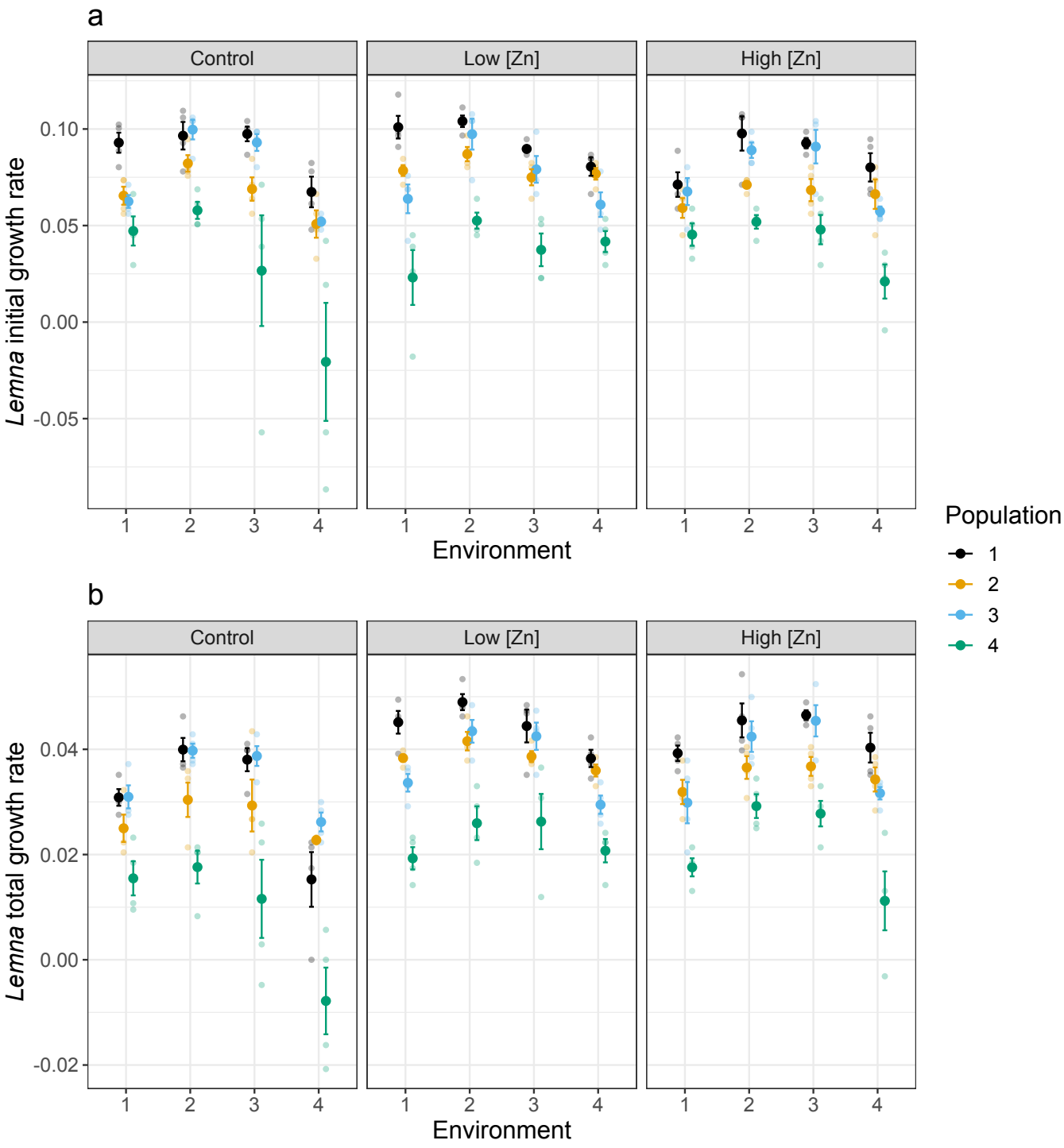

590 **FIGURE 1**. ***Lemna*** **growth rates per population in response to environment and Zn**
591 **treatments.** Mean initial (a) and total (b) growth rates of all four populations across all four
592 environments and the three Zn treatments with the associated standard errors. For test statistics
593 see Table 1 and Appendix S1: Table S2.

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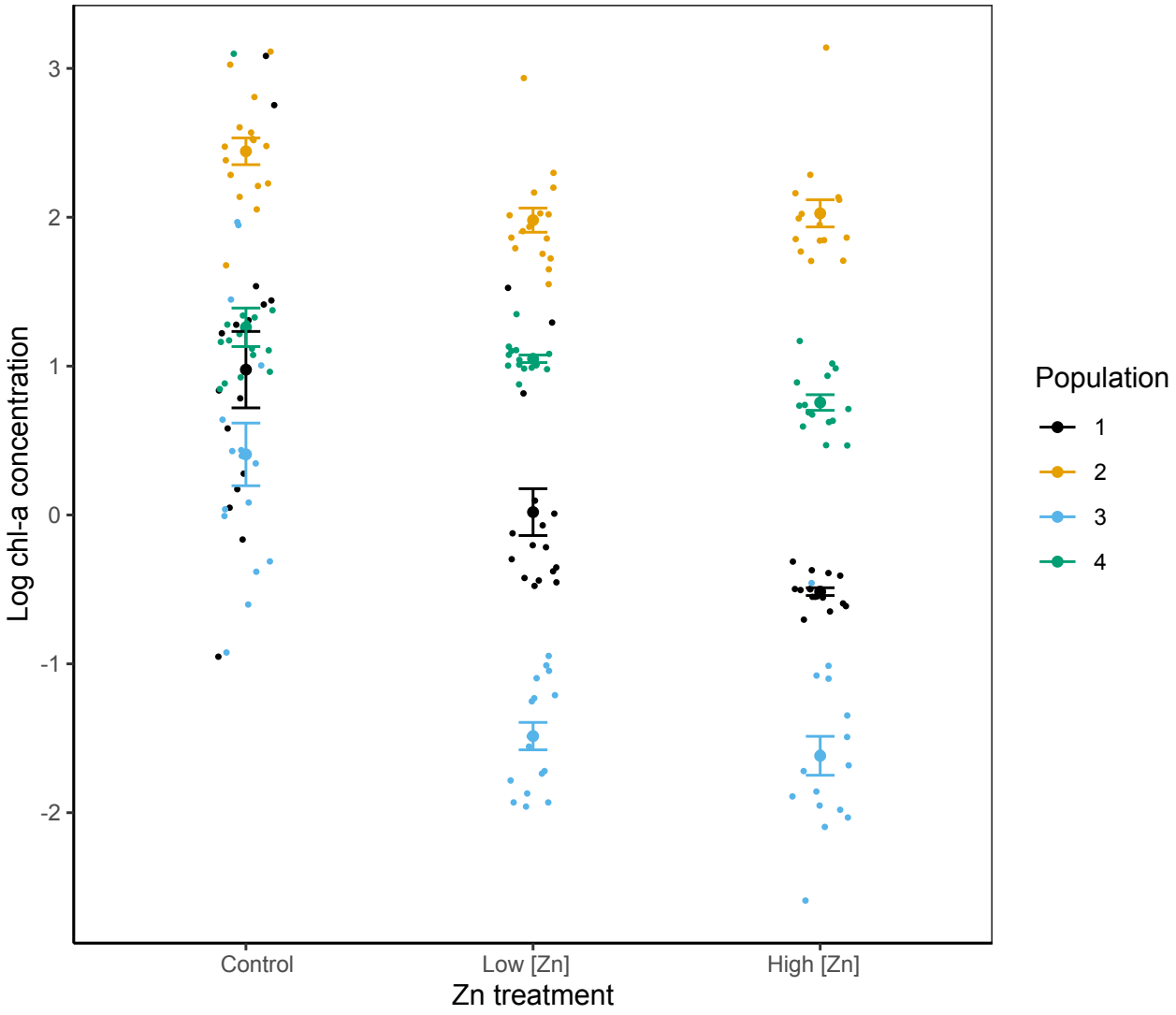
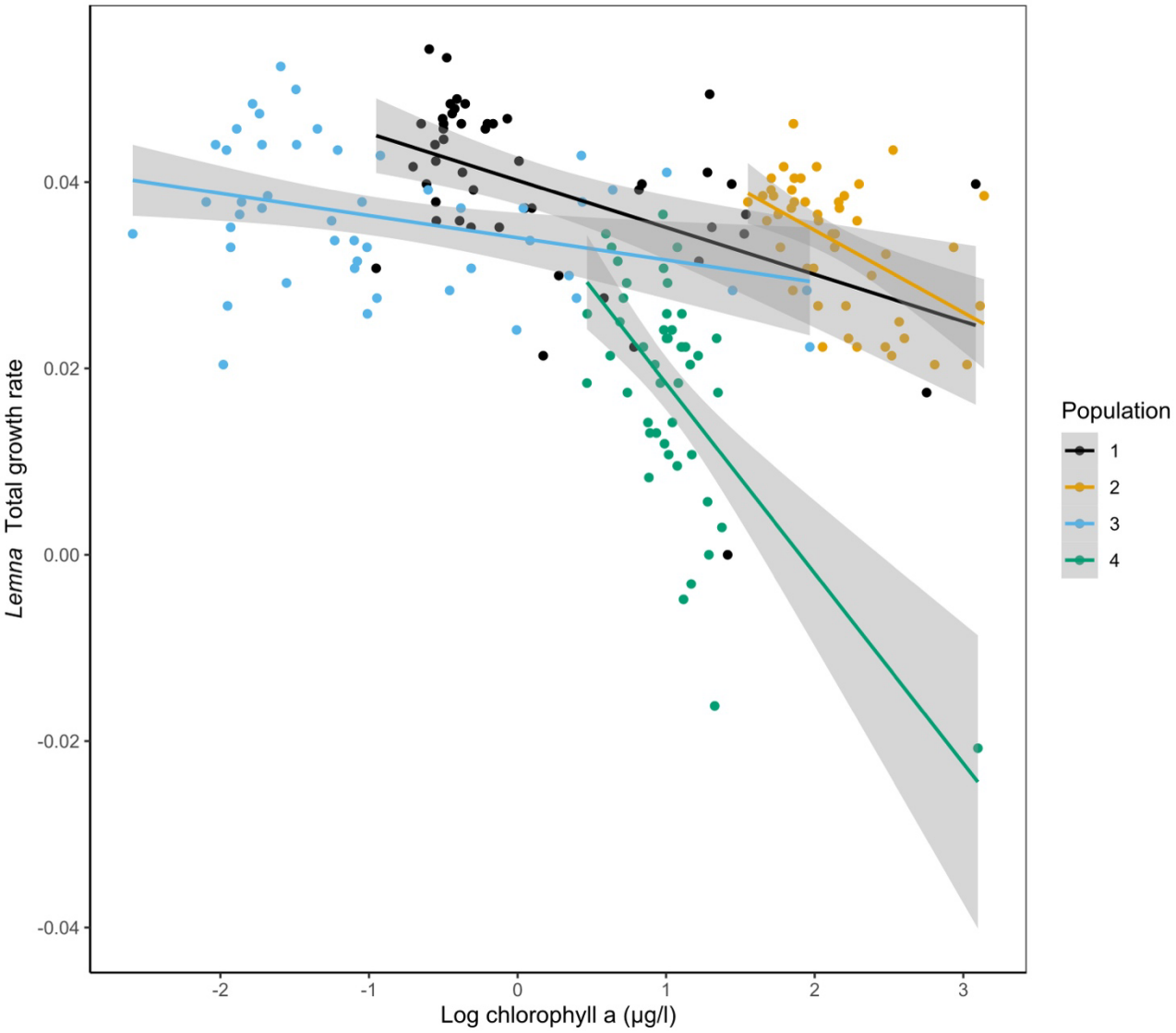


595 **FIGURE 2**. Response of algal growth (log-transformed mean chl-a concentration) to Zn
596 treatments and population identity (i.e., competitor identity). Shown are means and associated
597 standard errors. For test statistics see Appendix S1: Table S4.

598



599 **FIGURE 3.** *Lemna* growth rates per population in response to chlorophyll-a. Total growth
600 rates of all populations vs total algal biomass (log-transformed mean chl a-concentration)
601 across all Zn treatments. See Appendix for the regressions for each Zn separately (Figure S2).
602 Shaded areas correspond to 95% confidence intervals.
603

Supporting Information to:

High tolerance to zinc but limited evidence for local adaptation in the aquatic plant species *Lemna gibba/minor*

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611
612 **Table S1. Limnological properties of sampled ponds.** Date: Sampling and measuring date,
613 Cond.: Conductivity, DO: Dissolved oxygen, TC: Total carbon, TOC: total organic carbon,
614 IC: inorganic carbon, TN: total N.

615

Date	Site	Lat, Long	Air temp. (in °C)	Water temp. (in °C)	pH	Cond. (µS/cm)	DO (mg/L)	TC (mg/L)	TOC (mg/L)	IC (mg/L)	TN (mg/L)
7/8/21	Koblenz, AG	47.6011354, 8.2252776	25	16.8	7.61	89.4	1.85	5.69	5.69	0	0.33
7/8/21	Yverdon, VD	46.797448, 6.6332092	16	15.9	7.4	105.8	0.78	20.53	20.04	0.49	1.68
7/8/21	Motto, TI	46.25436, 8.58034	14	15.8	7.77	117.9	8.33	6.02	5.77	0.25	0.61
8/8/21	Ramosch, GR	46.833729, 10.401105	18	13.2	7.41	244	0.5	14.47	14.31	0.16	0.34

616

Table S2. Results for ANOVA testing the effect of population, environment and Zn treatments and their interactions on *Lemna* initial growth rates (8 days of experiment). *P* - values < 0.05 are shown in bold.

Source of variation	Df	Sum Sq	Mean Sq	*F value*	*P*
Environment	3	0.02210	0.00737	25.65	**< 0.001**
Population	3	0.07437	0.02479	86.32	**< 0.001**
Home vs. away	1	0.00002	0.00002	0.05	0.815
Control vs. Zn treatment (Zn contrast)	1	0.00089	0.00089	3.09	**0.081**
Low vs. high Zn (Zn treatment)	1	0.00063	0.00063	2.18	0.142
Population x Home vs. away	3	0.00308	0.00103	3.58	**0.016**
Environment x Population	5	0.00190	0.00038	1.32	0.257
Population x Zn contrast	3	0.00113	0.00038	1.32	0.271
Population x Zn treatment	3	0.00138	0.00046	1.60	0.191
Environment x Zn contrast	3	0.00505	0.00168	5.86	**0.001**
Environment x Zn treatment	3	0.00092	0.00030	1.06	0.367
Home vs. away x Zn contrast	1	0.00022	0.00022	0.76	0.383
Environment x Population x Zn contrast	8	0.00352	0.00044	1.53	0.151
Environment x Population x Zn treatment	9	0.00304	0.00034	1.18	0.314
Residuals	144	0.04135	0.00029		

Table S3. Selection coefficients for initial and total growth rates. A selection coefficient of zero indicates that a population is the most successful in a particular environment site with a selection advantage over all other populations in that environment. Near-zero selection coefficients that indicate potential local adaptation (i.e., a value near 0 in a home pairing of environment and population) are highlighted in grey. Bold type indicates "home". Shown are means and the associated standard errors across the four replicates per treatment combination. P = population.

Initial growth rates

Control	Environment 1		Environment 2		Environment 3		Environment 4	
P1	**0.092**	(+/– 0.051)	0.118	(+/– 0.065)	0.064	(+/– 0.036)	0.182	(+/– 0.096)
P2	0.360	(+/– 0.046)	**0.249**	(+/– 0.039)	0.338	(+/– 0.058)	0.384	(+/– 0.086)
P3	0.389	(+/– 0.032)	0.089	(+/– 0.046)	**0.107**	(+/– 0.042)	0.369	(+/– 0.022)
P4	0.539	(+/– 0.078)	0.471	(+/– 0.040)	0.744	(+/– 0.275)	**1.250**	(+/– 0.371)
Low Zn								
P1	**0.143**	(+/– 0.050)	0.064	(+/– 0.027)	0.0899	(+/– 0.018)	0.071	(+/– 0.055)
P2	0.334	(+/– 0.024)	**0.217**	(+/– 0.033)	0.239	(+/– 0.042)	0.114	(+/– 0.035)
P3	0.458	(+/– 0.063)	0.124	(+/– 0.072)	**0.198**	(+/– 0.070)	0.298	(+/– 0.073)
P4	0.804	(+/– 0.121)	0.527	(+/– 0.037)	0.620	(+/– 0.086)	**0.519**	(+/– 0.063)
High Zn								
P1	**0.197**	(+/– 0.072)	0.093	(+/– 0.082)	0.110	(+/– 0.025)	0.154	(+/– 0.077)
P2	0.334	(+/– 0.058)	**0.340**	(+/– 0.016)	0.343	(+/– 0.055)	0.300	(+/– 0.081)
P3	0.238	(+/– 0.078)	0.173	(+/– 0.037)	**0.127**	(+/– 0.084)	0.394	(+/– 0.026)
P4	0.489	(+/– 0.066)	0.518	(+/– 0.033)	0.540	(+/– 0.073)	**0.778**	(+/– 0.093)

Total growth rates

Control	Environment 1		Environment 2		Environment 3		Environment 4	
P1	**0.171**	(+/– 0.043)	0.137	(+/– 0.048)	0.124	(+/– 0.051)	0.490	(+/– 0.173)
P2	0.328	(+/– 0.070)	**0.343**	(+/– 0.071)	0.325	(+/– 0.114)	0.240	(+/– 0.009)
P3	0.168	(+/– 0.059)	0.141	(+/– 0.029)	**0.108**	(+/– 0.043)	0.126	(+/– 0.060)
P4	0.584	(+/– 0.087)	0.619	(+/– 0.067)	0.733	(+/– 0.171)	**1.261**	(+/– 0.211)
Low Zn								
P1	**0.087**	(+/– 0.044)	0.082	(+/– 0.029)	0.082	(+/– 0.064)	0.094	(+/– 0.039)
P2	0.224	(+/– 0.014)	**0.221**	(+/– 0.033)	0.202	(+/– 0.021)	0.149	(+/– 0.027)
P3	0.320	(+/– 0.034)	0.186	(+/– 0.041)	**0.122**	(+/– 0.054)	0.302	(+/– 0.041)
P4	0.610	(+/– 0.043)	0.513	(+/– 0.060)	0.457	(+/– 0.109)	**0.509**	(+/– 0.053)
High Zn								
P1	**0.071**	(+/– 0.035)	0.162	(+/– 0.059)	0.112	(+/– 0.018)	0.128	(+/– 0.061)
P2	0.245	(+/– 0.055)	**0.327**	(+/– 0.040)	0.299	(+/– 0.034)	0.259	(+/– 0.050)
P3	0.293	(+/– 0.093)	0.218	(+/– 0.054)	**0.133**	(+/– 0.057)	0.316	(+/– 0.026)
P4	0.584	(+/– 0.041)	0.462	(+/– 0.042)	0.470	(+/– 0.046)	**0.758**	(+/– 0.121)

Table S4. Results for a three-way ANOVA testing the effect of population, environment and Zn treatments on log-transformed mean total chlorophyll-a concentration (proxy for algal biomass). *P*-values < 0.05 are shown in bold. 'Block' was included as a random factor to account for the fact that the blocks (corresponding to the four replicates) were sampled and measured sequentially.

Source of variation	**Df**	**denDF**	***F***	***P***
Zn treatment	2	139	112.1934	**<.0001**
Environment	3	139	11.6966	**<.0001**
Population	3	139	386.3488	**<.0001**
Zn treatment x Environment	6	139	0.4813	0.8214
Zn treatment x Population	6	139	15.7432	**<.0001**
Environment x Population	9	139	1.9266	0.0529
Zn treatment x Environment x Population	18	139	1.7619	**0.0359**

Table S5. Results for four-way ANOVA testing the effect of mean total algae concentration, population, environment, Zn treatments and all interactions thereof on *Lemna* total growth rates.

Source of variation	Df	Sum Sq	Mean Sq	*F*	*P*
Zn treatment	2	0.0041675	0.0020837	57.2259	**< 0.001**
Environment	3	0.0044639	0.001488	40.8643	**< 0.001**
Population	3	0.0127925	0.0042642	117.1076	**< 0.001**
Mean total chla concentration	1	0.0002083	0.0002083	5.7206	**0.012**
Zn treatment x environment	6	0.0007184	0.0001197	3.2881	**0.006**
Zn treatment x population	6	0.0006723	0.0001121	3.0773	**0.009**
Environment x population	9	0.0007055	0.0000784	2.1528	**0.032**
Zn treatment x mean total chla concentration	2	0.0000514	0.0000257	0.7059	0.496
Environment x mean total chla concentration	3	0.0002108	0.0000703	1.9293	0.130
Population x mean total chla concentration	3	0.0001467	0.0000489	1.3426	0.265
Zn treatment x environment x population	18	0.0007089	0.0000394	1.0815	0.383
Zn treatment x environment x mean total chla concentration	6	0.0000858	0.0000143	0.3925	0.882
Zn treatment x population x mean total chla concentration	6	0.0002316	0.0000386	1.0599	0.392
Environment x population x mean total chla concentration	9	0.0003879	0.0000431	1.1836	0.315
Zn treatment x environment x population x mean total chla concentration	18	0.0004179	0.0000232	0.6376	0.861
Residuals	94	0.0034228	0.0000364		

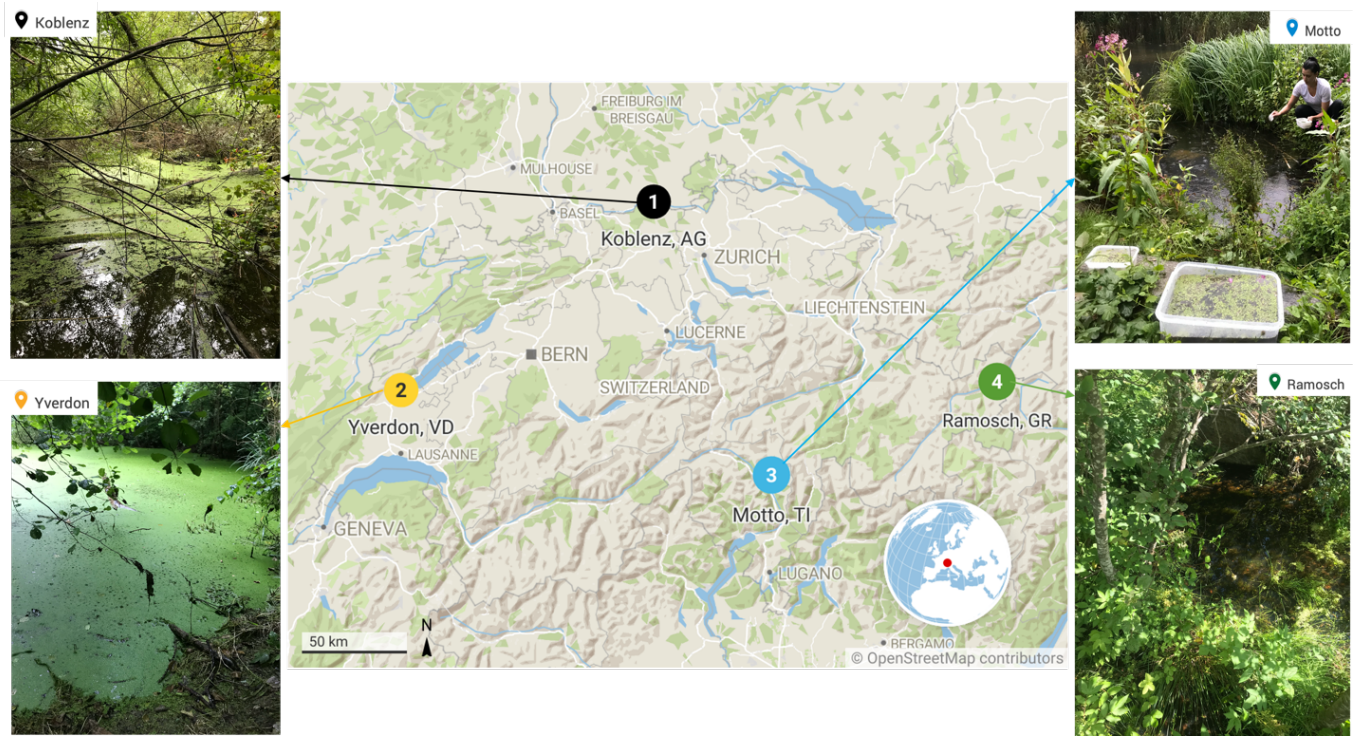
Table S6. ANOVAs with total organic carbon as response variable (a), and with total N as response variable (b). Significant p-values are shown in bold.

(a) Total Organic Carbon (TOC)

Source of variation	Df	Sum Sq	Sq	*F*	*P*
Zn treatment	2	72.6	36.31	6.8561	**0.001**
Environment	3	3416.7	1138.89	215.0299	**< 0.001**
Population	3	114.5	38.15	7.2032	**0.00015**
Zn treatment x environment	6	30.4	5.07	0.9581	0.456
Zn treatment x population	6	23.8	3.97	0.7496	0.611
Environment x population	9	10.7	1.18	0.2236	0.991
Zn treatment x environment x population	18	22	1.22	0.231	1.000
Residuals	144	762.7	5.3		

(b) Total N

Source of variation	Df	Sum Sq	Sq	*F*	*P*
Zn treatment	2	0.0149	0.00745	2.3663	0.097
Environment	3	2.25615	0.75205	238.7987	**< 0.001**
Population	3	0.02125	0.00708	2.2496	0.085
Zn treatment x environment	6	0.01039	0.00173	0.5497	0.770
Zn treatment x population	6	0.00258	0.00043	0.1367	0.991
Environment x population	9	0.01858	0.00206	0.6555	0.748
Zn treatment x environment x population	18	0.01221	0.00068	0.2154	1.000
Residuals	144	0.4535	0.00315		



647 **Figure S1.** Field collection locations across Switzerland. Map created with Datawrapper.
648

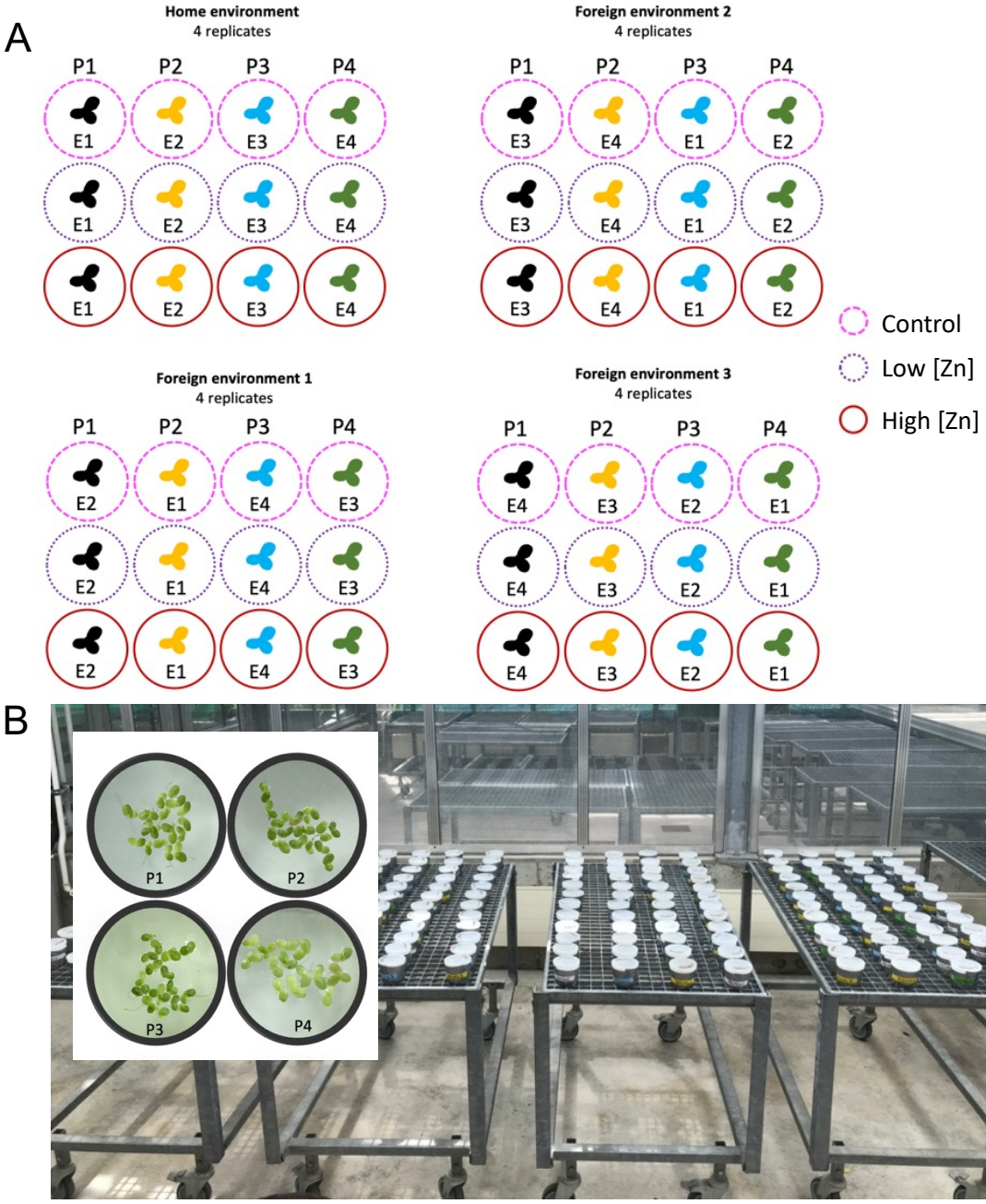
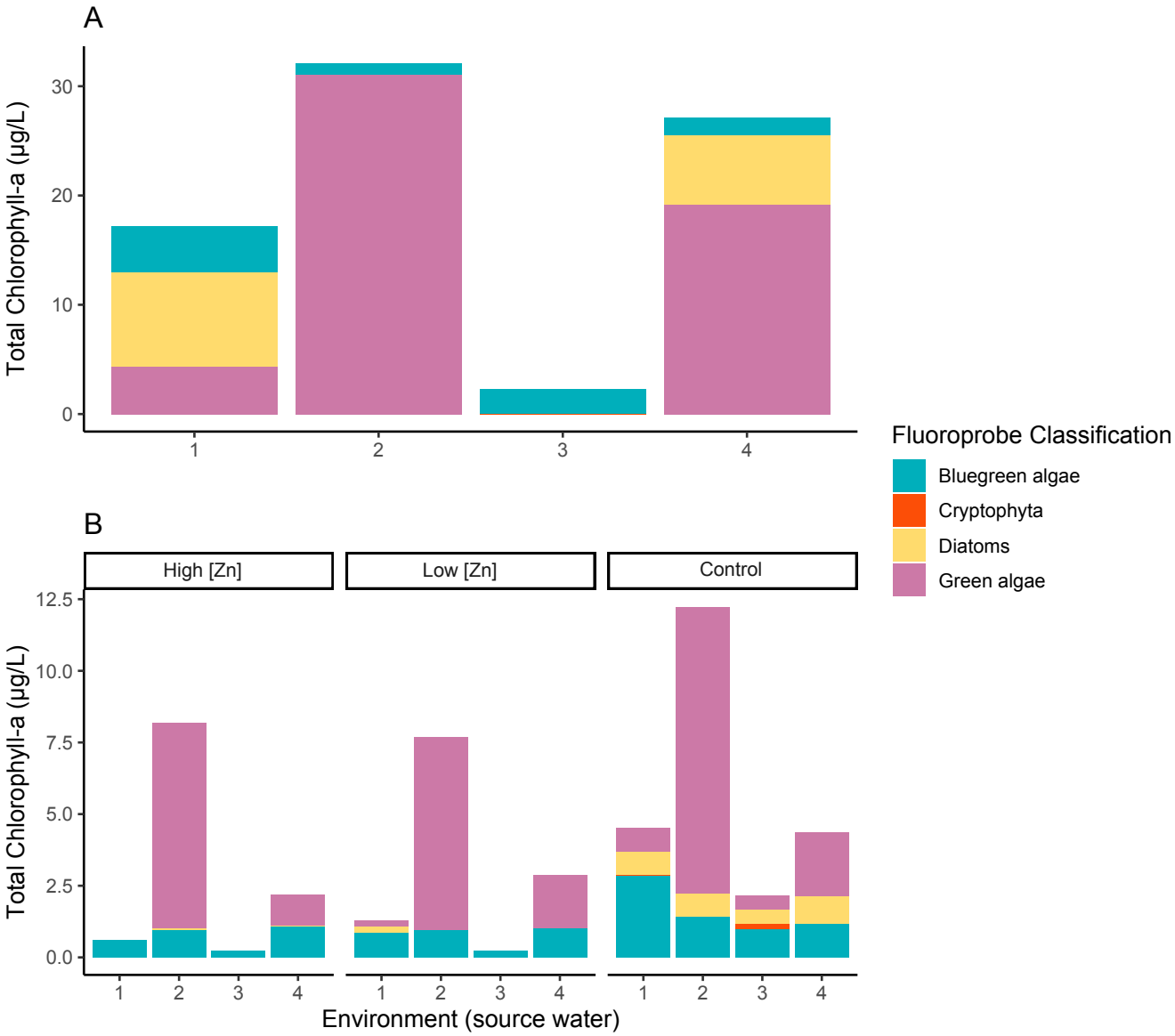


Figure S2. Schematic of the fully reciprocal transplant study design. (**A**) *Lemna* fronds in different colors to represent each population (P1-P4); Circles represent the cups, and the different outlines represent the Zn treatment. E1-E4 represent each environment. (**B**) Photo of the experimental set-up in the glasshouse with one replicate per table. Inset in (B): Photo taken of each population from above. P1-P4 represent each collected population: 1. Koblenz, 2. Yverdon, 3. Motto, 4. Ramosch.



657 **Figure S3. (a)** Field fluoroprobe measurements. **(b)** Post-experiment fluoroprobe
658 measurements in all three Zn treatments and for each sourced environment water. Note the
659 different scale on the y-axes.
660

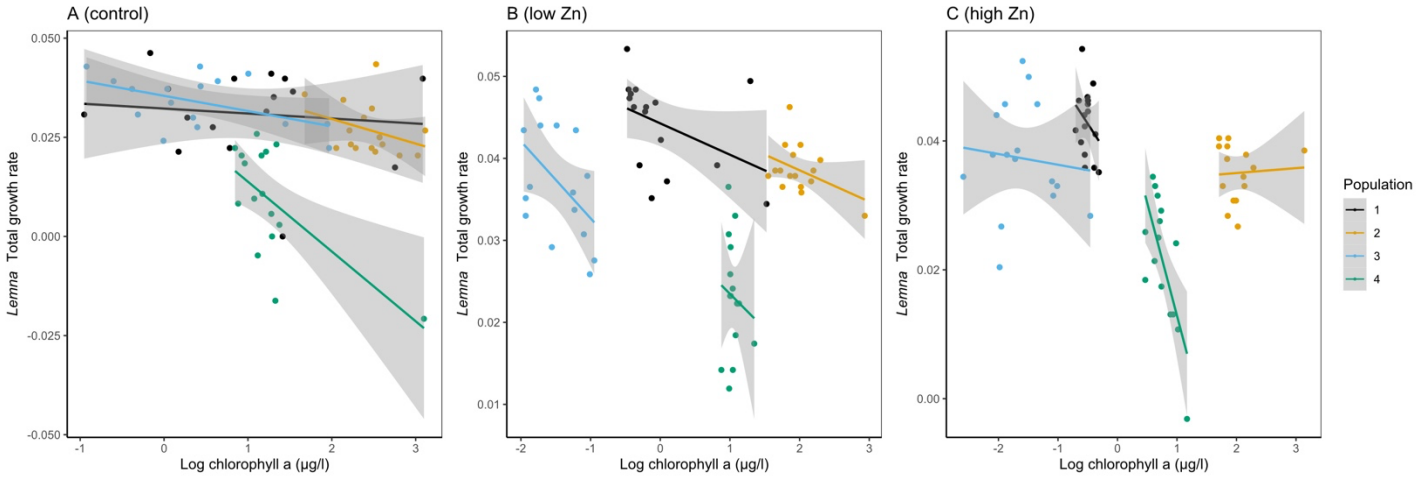


Figure S4. *Lemna* growth in response to mean Chlorophyll-a-concentration for the three Zn treatments separately. (**a**) Total growth rates of all populations vs total algal biomass in the control. (**b**) Total growth rates of all populations vs total algal biomass in the low Zn treatment. (**c**) Total growth rates of all populations vs total algal biomass in the high Zn treatment. A linear regression is fitted per population (colored lines). Note the change in scale for the y-axes of panels (a) and (b). The relationship was significant for the mean concentration of chlorophyll-a and all Zn treatments in all environments and for all populations ($P < 0.05$ for mean total concentration of chlorophyll-a and $P < 0.001$ for the rest) and Zn treatment interaction with mean total chlorophyll-a concentration did not have a significant effect on the total growth rate of the duckweed ($P = 0.4963$).

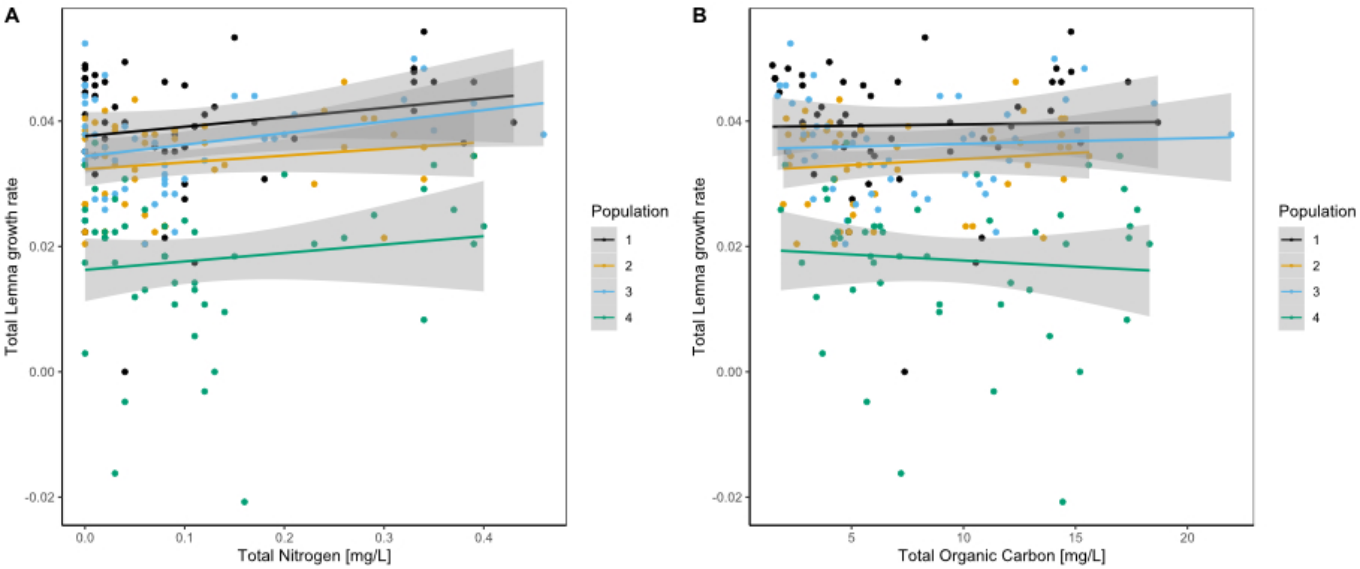
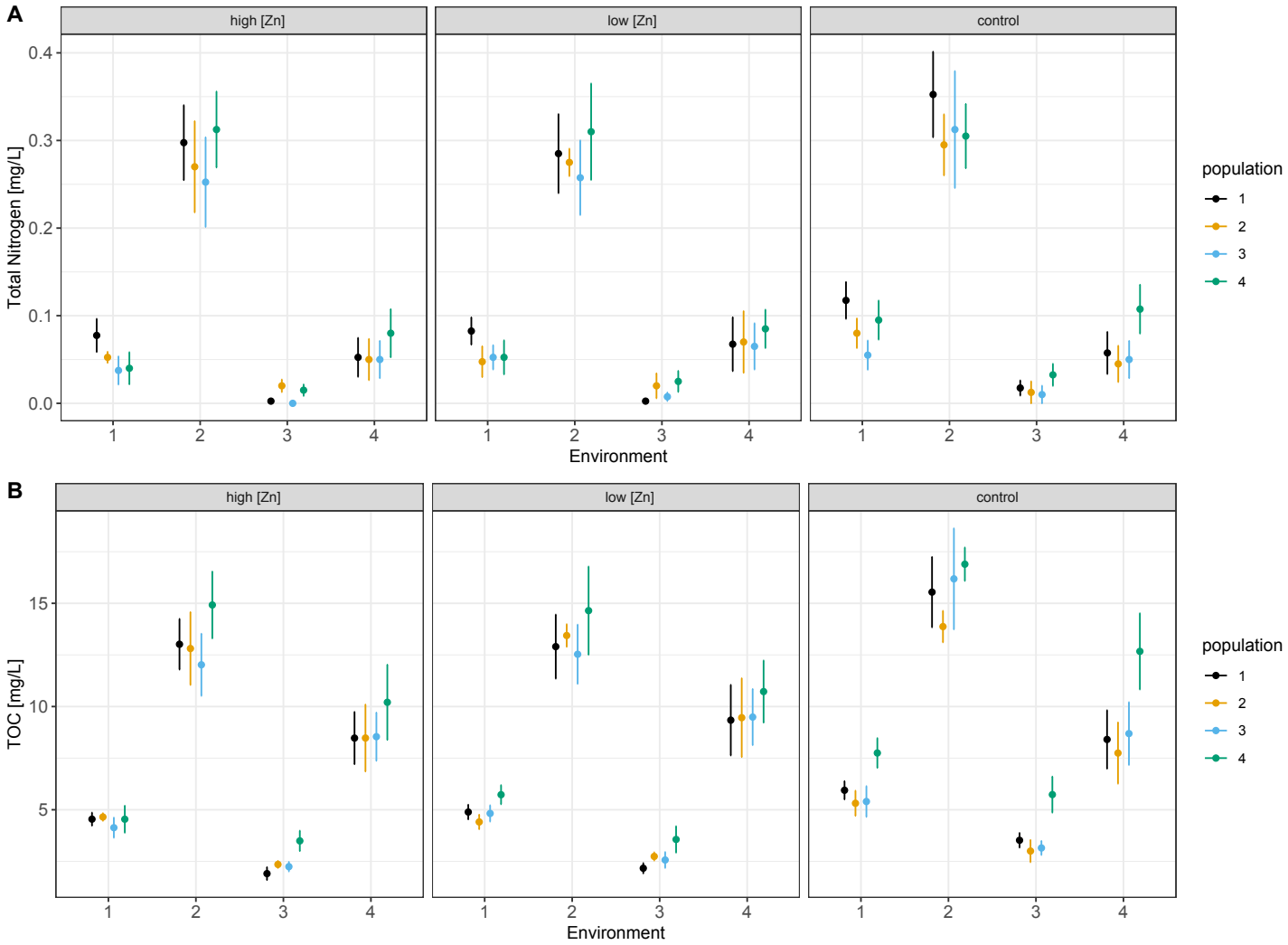
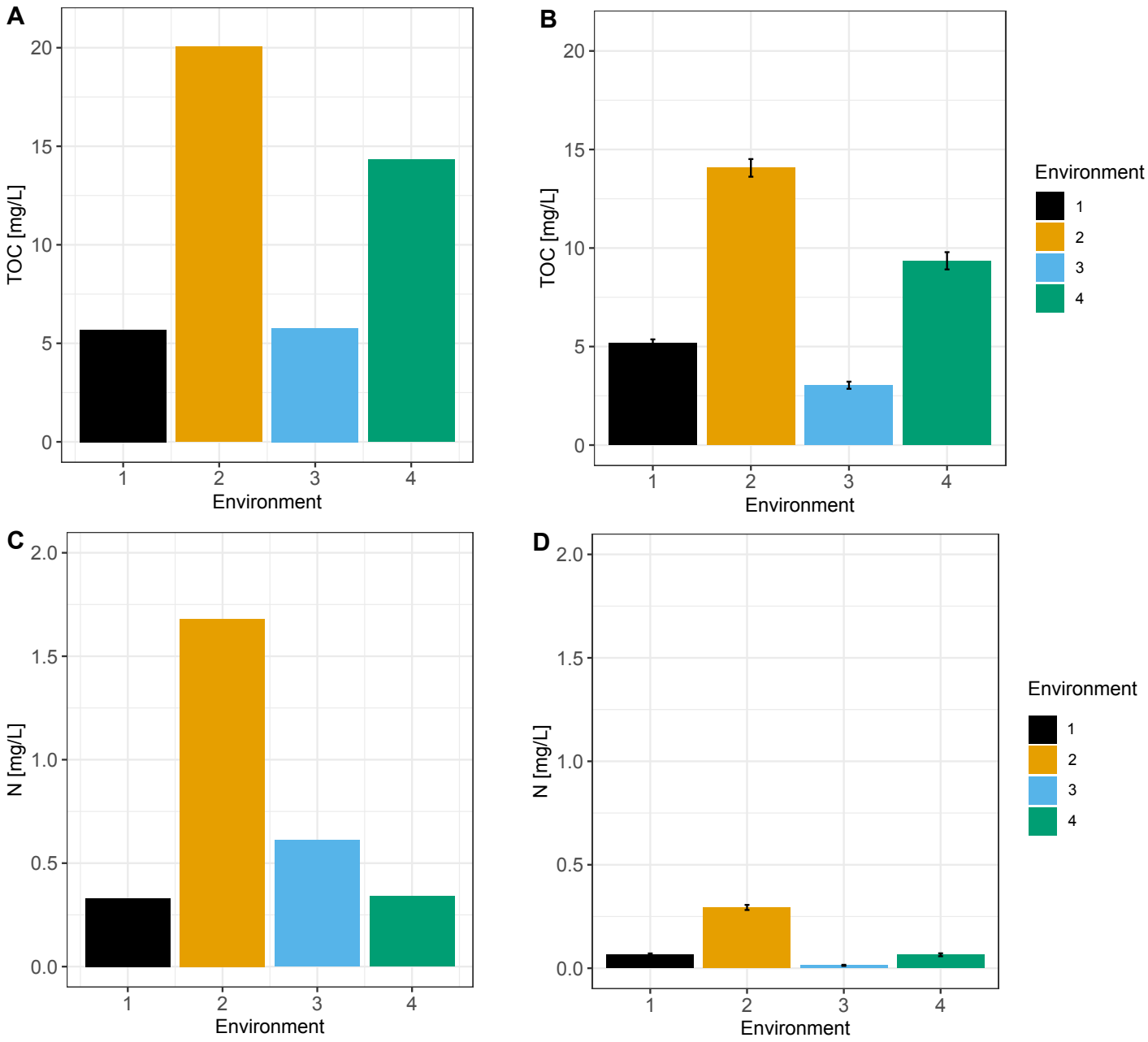


Figure S5. Total growth rate in response to final (a) TN and (b) TOC concentrations. A linear regression is fitted per population (colored lines). Growth rates tended to be greater in higher N concentrations (linear model, $F_{1, 184} = 4.172$, $P = 0.043$) but TOC had no effect on growth rates ($F_{1, 184} = 2.835$, $P = 0.094$). Shaded areas correspond to 95% confidence intervals.



685 **Figure S6. TOC and TN at the end of the experiment in all environments and Zn**
686 **treatments.** (**a**) Mean total N (TN) for each environment and population in each Zn treatment
687 (high, low and control). (**b**) TOC for each environment and population in each Zn treatment
688 (high, low and control). Shown are means and associated standard errors.



689 **Figure S7. Mean TOC and mean TN concentrations (mg/L).** (**a**) TOC before the
690 experiment, measured in field source samples. (**b**) TOC at the end of the experiment. (**c**) TN
691 before the experiment. (**d**) TN at the end of the experiment. Error bars are shown for panels (b)
692 and (d) to account for variation among the Zn treatments, replicates, and populations from the
693 experiment.

694

695