

Negative frequency-dependent growth underlies the stable coexistence of two cosmopolitan aquatic plants

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Abstract. Identifying and quantifying the mechanisms influencing species coexistence remains a major challenge for the study of community ecology. These mechanisms, which stem from species' differential responses to competition and their environments, promote coexistence if they give each species a growth advantage when rare. Yet despite the widespread assumption that co-occurring species stably coexist, there have been few empirical demonstrations in support of this claim. Likewise, coexistence is often assumed to result from interspecific differences in life-history traits, but the relative contributions of these trait differences to coexistence are rarely quantified, particularly across environmental gradients. Using two widely co-occurring and ecologically similar species of freshwater duckweed plants (*Spirodela polyrrhiza* and *Lemna minor*), we tested hypotheses that interspecific differences in facultative dormancy behaviors, thermal reaction norms, and density-dependent growth promote coexistence between these species, and that their relative influences on coexistence change as average temperatures and fluctuations around them vary. In competition experiments, we found strong evidence for negative frequency-dependent growth across a range of both static and fluctuating temperatures, suggesting a critical role of fluctuation-independent stabilization in coexistence. This negative frequency dependence could be explained by our observation that for both species, intraspecific competition was over 1.5 times stronger than interspecific competition, granting each species a low-density growth advantage. Using an empirically parameterized competition model, we found that while coexistence was facilitated by environmental fluctuations, fluctuation-independent stabilization via negative frequency dependence was crucial for coexistence. Conversely, the temporal storage effect, an important fluctuation-dependent mechanism, was relatively weak in comparison. Contrary to expectations, differences in the species' thermal reaction norms and dormancy behaviors did not significantly promote coexistence in fluctuating environments. Our results highlight how coexistence in two ubiquitous and ostensibly similar aquatic plants is not necessarily a product of their most obvious interspecific differences, and instead results from subtle niche differences causing negative frequency-dependent growth, which acts consistently on both species across environmental gradients.

Key words: aquatic plants; coexistence; competition; dormancy; invasibility; Lemnaceae; negative frequency dependence; storage effect; temperature.

INTRODUCTION

Widespread associations of ecologically similar taxa in so-called *functional guilds* (Simberloff and Dayan 1991) present a paradox: why do ostensibly similar species regularly co-occur in a particular habitat when they also compete for the same finite resources? A rich body of theory has developed to address this problem, identifying the central roles of fitness equivalence, niche differences, environmental fluctuations, and neutral drift in the long-term coexistence of competing species (MacArthur and Levins 1967, Vandermeer 1975, Levins

1979, Turelli 1981, Chesson 1990, 2003, Hubbell 2001). Nonetheless, explicit empirical demonstrations of species coexistence remain quite rare, and only a subset of these studies proceed to identify, or better still, quantify, various coexistence-promoting mechanisms (Wilson et al. 1999, Stomp et al. 2004, Dybzinski and Tilman 2007, Siepielski and McPeck 2010). Of these, a smaller minority measure the effects of spatiotemporal environmental variation on coexistence, despite its central importance in modern theory (Cáceres 1997, Sears and Chesson 2007, Gremer et al. 2014, Usinowicz et al. 2017). Given these deficiencies, further development of coexistence theory would benefit from empirical approaches for mapping interspecific trait variation to coexistence outcomes across environmental gradients, environmental fluctuations, and, ultimately, real-world geography.

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Strictly speaking, for two species to coexist, they must satisfy the mutual invasibility criterion (Turelli 1978, Chesson and Ellner 1989). That is, each species must have a long-term low-density growth rate advantage over the resident species. Satisfaction of this criterion requires species' growth exhibit negative frequency dependence, which arises naturally when growth is more strongly regulated by conspecifics than by heterospecifics. For empiricists, proof of this criterion commonly involves reciprocally "invading" a real-world or model community with taxa of interest and tracking the invaders' low-density growth rates (Huisman et al. 1999, Wilson et al. 1999, Adler et al. 2006, Narwani et al. 2013, Cothran et al. 2015). Such approaches have demonstrated the beneficial effects of environmental fluctuations on species coexistence (Descamps-Julien and Gonzalez 2005, Jiang and Morin 2007, Letten et al. 2018).

In cases where multiple putative coexistence-promoting mechanisms may operate, their relative contributions to low-density growth rates (and therefore coexistence) might vary across environments or even become redundant. This is especially relevant in cases where the fitness benefits of a trait depend on its environmental context. Dormancy behavior is an example of one such trait, and many organisms must constantly navigate the uncertainty of provisioning current vs. future reproductive potential in the forms of active and dormant individuals, respectively (Bradshaw et al. 1998, Cáceres and Tessier 2003, Lennon and Jones 2011, Gremer et al. 2012). Failure to appropriately partition this growth may result in a species' inability to coexist as its environment fluctuates. However, by pairing mortality-minimizing dormancy with interspecific differences in environmental responses, environmental fluctuations can facilitate or even "rescue" a competitive subordinate, provided certain conditions are met (Chesson and Warner 1981, Chesson and Huntly 1989).

Communities of duckweed plants (Lemnaceae) are a promising model system for quantifying and manipulating putative coexistence-promoting traits such as dormancy and differential environmental responses. These floating, freshwater angiosperms occur throughout much of the world and are commonly encountered in mixed communities (Landolt 1986). In the United States and Europe, two of the most common species of duckweeds are *Lemna minor* L., and *Spirodela polyrhiza* (L.) Schleiden (Fig. 1). Where the species' ranges overlap, they show remarkably consistent co-occurrence patterns; *S. polyrhiza* is found much more frequently in the presence of *L. minor* than in monoculture, whereas *L. minor* is commonly encountered in the absence of *S. polyrhiza*, suggesting that *Spirodela* may be competitively subordinate to *L. minor* (Jacobs 1947, Docauer 1983; Appendix S1; Fig. S1; Metadata S1). Despite having indistinguishable nutrient and habitat requirements (Clatworthy and Harper 1962, Docauer 1983, Landolt 1986), *L. minor* and *S. polyrhiza* differ in two key ways. First, while *L. minor* exhibits higher growth rates at

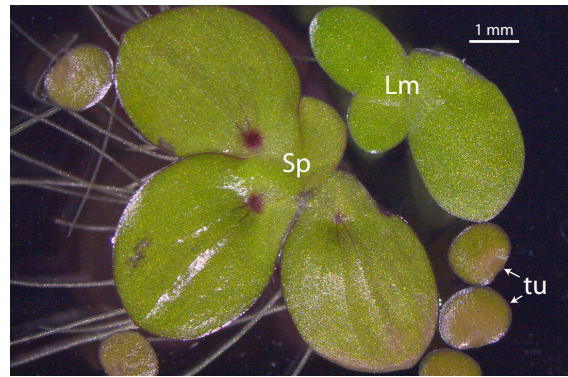


FIG. 1. Photograph of the duckweeds *Spirodela polyrhiza* (Sp) and *Lemna minor* (Lm). Each oval-shaped lobe is a distinct reproductive individual. Also present are dormant turions (tu) of *S. polyrhiza*.

most temperatures, *S. polyrhiza* is capable of sustaining growth at higher temperatures than *L. minor* (Wolek 1974, Docauer 1983, Lemon et al. 2001). Second, *S. polyrhiza* has the ability to invest in dormant, mortality-resistant fronds (called turions) that overwinter in the pond surface sediments and germinate in the spring (Jacobs 1947; Fig. 1). In contrast, *L. minor* has no such dormancy mechanism besides rarely produced seeds (Jacobs 1947).

We hypothesized that these differences in environmental responses and dormancy behaviors will facilitate coexistence when temperatures fluctuate. We further postulated that, in static temperatures, coexistence cannot occur without negative frequency-dependent growth counteracting a species' fitness disadvantage. This effect, called stabilization, is the hallmark of coexistence and, in static environments, is predicted to arise primarily from interspecific niche differences (Chesson 2000, Adler et al. 2007). In fluctuating environments, stabilization can be bolstered by a combination of buffered population growth and differential responses to the environment (Chesson and Warner 1981, Chesson and Huntly 1989, Chesson 1994). Called the *storage effect*, this fluctuation-dependent coexistence mechanism is capable of overriding the competitive disadvantage an invading species may otherwise experience in static environments (Chesson and Warner 1981). For our duckweed species, we predicted that interspecific differences in dormancy behaviors and thermal tolerances would increase the importance of the storage effect on species coexistence.

Here, we followed the pluralistic approach advocated by Siepielski and McPeck (2010) for assessing coexistence between *L. minor* and *S. polyrhiza*. To do so, we combined experimental competition assays and mathematical modeling to pursue three related goals. First, we quantified the extent of fitness (here, per capita growth rate) equivalence and stabilization in competition assays in static and fluctuating environments. Next, we tested the hypotheses that particular interspecific differences in dormancy, thermal growth responses, and density-dependent

regulation facilitate coexistence between these species. Finally, we investigated whether the relative effects of putative coexistence-promoting traits change with environmental context. In doing so, we identified environmental state spaces where traits' effects on coexistence can become redundant or disappear completely, and quantified how these interspecific differences influence the magnitude of the temporal storage effect.

MATERIALS AND METHODS

Duckweed collection and maintenance

Specimens of the duckweeds *Spirodela polyrhiza* and *Lemna minor* were collected from 15 temporary and permanent woodland ponds in St. Joseph County, Indiana, USA. In all cases, *S. polyrhiza* was found co-occurring with *L. minor* and occasionally with two other duckweed species, the minute *Wolffia borealis* and *W. columbiana*, whereas *L. minor* were found in monocultures as often as in mixed assemblages. Although two other duckweed species, *Lemna turionifera* and *Lemna trisulca*, also occur in this region, neither were detected at our sampling locations. Living fronds of *S. polyrhiza* and *L. minor* from each pond were vigorously washed with deionized (DI) water and surface sterilized via 2–5 min immersion in a dilute (10% v:v) bleach solution. Bleached fronds were rinsed twice in sterile Milli-Q water (Millipore, Bedford, Massachusetts, USA) and placed in sterile 12-well plates containing UTCC Hoagland's E-medium supplemented with 10 g/L sucrose and 0.6 g/L tryptone (medium recipe *available online*).² Strains from wells showing no microbial growth were considered axenic and moved into sterile Erlenmeyer flasks containing 100 mL of the aforementioned medium. Axenic duckweed lines were maintained in a growth chamber experiencing constant 23°C temperatures under full-spectrum fluorescent lights (300 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ photon flux) on a 16:8 hour light:dark cycle.

Prior to the start of the experiments, strains of each species were moved into new flasks containing 100 mL of a second sterile growth medium designed to closely mimic the chemical properties of ponds in the region (Docauer 1983; formulation in Appendix S1: Section 1.1). Henceforth called Docauer medium, it contained low concentrations of inorganic nutrients, a bicarbonate buffer, and no added carbohydrates nor proteins. We used this medium for all subsequent experiments. Focal duckweed strains were allowed to acclimate to the medium under aforementioned environmental conditions for at least 28 d prior to the start of the experiments. To avoid complete depletion of nutrients, the medium was completely refreshed every 14 d using sterile technique.

Measuring thermal growth responses

We constructed thermal reaction norms for each species by measuring the growth rates of six randomly

selected axenic strains of each species originating from different ponds. Six sterile flasks containing 100 mL Docauer medium were each inoculated with five fronds of a strain. Flasks were distributed across growth chambers set to six different temperatures (3°C, 15°C, 20°C, 25°C, 30°C, and 37°C) and identical, 16:8 light regimes. In total, we measured the growth rates of 72 cultures. After 8 d, we counted the total number of living and dead vegetative fronds and turions in each culture flask to estimate the maximum low-density per capita growth rates, $\mu_{js}(T)$, of each strain s and species j at temperature T with the equation

$$\mu_{js}(T) = \frac{\log N_{jsT}(t_2) - \log N_{jsT}(t_1)}{t_2 - t_1} \quad (1)$$

where $N_{jsT}(t)$ are the number of living vegetative fronds at days t_1 and t_2 . Following convention (Landolt 1957), all visible individuals in a population were counted, regardless of whether they remained attached to their parental frond. We fit cubic models describing each species' temperature-dependent reproductive rates following van der Heide et al. (2006):

$$\mu_j(T) = c_j T (T - T_{\min,j})(T_{\max,j} - T), \quad 0 \leq T_{\min,j} < T_{\max,j} \quad (2)$$

where T is the temperature (in degrees Celsius), $T_{\min,j}$ and $T_{\max,j}$ are the minimum and maximum temperature thresholds of species j , respectively, and c_j is a scaling constant. We fit our experimental data to Eq. 2 using nonlinear least squares in R v.3.3 (R Development Core Team 2017) and assessed the models' fits using observed vs. predicted coefficients of determination (R^2).

The proportion of growth devoted to turion production by *S. polyrhiza* was estimated using the ratio of vegetative to total production and modeled as a decreasing logistic function of temperature (T):

$$d_j(T) = 1 - \frac{e^{T_{d_j}+T}}{1 + e^{T_{d_j}+T}} \quad (3)$$

where T_{d_j} is the temperature at which 50% of growth is devoted to turions. We used previously published data (Jacobs 1947, Docauer 1983) to model temperature-dependent turion germination rates ($g_j(T)$) according to the function

$$g_j(T) = \frac{1}{10} \frac{e^{T_{g_j}+T}}{1 + e^{T_{g_j}+T}} \quad (4)$$

where T_{g_j} is the temperature at which 50% of the turions germinated after 10 d.

Competition in static environments

We assembled experimental communities with the goal of quantifying the effects of species identity, relative

² www.mobot.org/jwcross/duckweed/media.htm

frequency, and temperature on duckweed growth rates (following Levine et al. 2008, Hart et al. 2018). We assembled flasks containing 100 mL Docauer medium with 100 total duckweed fronds where each species had one of three different relative starting frequencies: 5%, 50%, or 95%. These flasks were distributed equally across six temperature treatments: 10°C, 15°C, 20°C, 25°C, 30°C, and 35°C. Each frequency \times temperature treatment was replicated four times. Lighting conditions were as previously described. The growth medium was refreshed on day 17, and total growth was estimated on day 28 by counting each species' vegetative fronds and turions. We calculated vegetative and total (vegetative + turion) growth rates for both species (note that both rates are equal for *L. minor*) in each frequency \times temperature treatment.

Competition in fluctuating environments

We conducted a second competition experiment to assess how temperature fluctuations influence species coexistence at longer timescales. Species were seeded into sterile flasks containing 100 mL Docauer medium at either low (5%) or high (95%) relative frequencies, with the total number of individual fronds standardized to 100. We created a total of 40 communities this way, one-half of which were dominated by *L. minor* and half by *S. polyrhiza*. These communities were placed in a growth chamber programmed to fluctuate around a mean of 20°C, with maximum and minimum temperatures of 37°C and 3°C, respectively. Each day, the temperature increased or decreased 1°C. In order to assess whether turion germination influences per capita growth rates, one half of each of these communities was assigned to the turion replacement treatment, wherein at each sampling period, dormant *Spirodela* turions were replaced with an equal number of fresh vegetative fronds. This was done in order to simulate turion germination without the need for cold stratification. Light conditions were as previously described, and the experiment was run for 103 d (equal to 1.5 temperature cycles and approximately 13–15 generations of each species). Cultures were sampled every 17 d, wherein the medium was replenished, dead fronds removed, and living vegetative fronds and turions counted. Per capita growth rates (including both vegetative fronds and turions) during each census period were estimated using Eq. 1. The population dynamics from this experiment are shown in Appendix S1: Fig. S2.

Testing for negative frequency-dependent growth

We tested for negative frequency-dependent population growth, a signal of interspecific niche differentiation, in both static and fluctuating temperature treatments. For both static and fluctuating temperature experiments, we modeled species' total growth rates using multiple regression. We initially included temporal autocorrelation error and random effects of

community replicate nested within census period to account for repeated measures in the latter experiment, but found that these effects did not improve model fit compared to a standard, fixed-effects model, and do not consider them further here. Our candidate models, detailed in the Appendix S1: Table S1, also included interaction terms to account for potential interspecific differences in frequency effects. Models were pluralistically assessed using the coefficient of determination (R^2) and the Bayesian Information Criterion, a metric that ranks models based on fit and parsimony (Schwarz 1978). Additionally, we used Tukey post-hoc analyses to assess statistical differences among species' growth responses at different frequencies. This was done to identify whether negative frequency-dependent growth was strong enough to overcome a species' overall growth rate disadvantage.

An alternative explanation for growth rate differences in polycultures compared to monocultures could stem from some sort of environmental modification (e.g., pH) or exudate (e.g., carbohydrates, auxins) by heterospecifics. To test for these effects, we grew monocultures containing approximately 500 individuals of *L. minor* or *S. polyrhiza* for 14 d at 25°C. The spent media from these cultures was removed, amended with sterile nutrients, and added in 15 mL aliquots to multiwell culture plates. An equal number of wells were filled with media originating from *L. minor* cultures, from *S. polyrhiza* cultures, or with a 50:50 mixture of the two. Using individual plants from each species' low-frequency treatments, we inoculated these plates with three to four vegetative fronds of *S. polyrhiza* or *L. minor* plants for a total of 24 replicates per medium per species. Plates were incubated as described above and their growth rates measured after 15 d (Eq. 1). The effect of medium origin was evaluated using a linear regression containing a species \times medium interaction term of the form $\hat{r} = \beta_0 + \beta_1 \text{Species} + \beta_2 \text{Medium} + \beta_3 (\text{Species} \times \text{Medium}) + \epsilon$, where \hat{r} is the growth rate estimate. The sign and magnitude of these terms indicate how a species' growth rate is impacted by habitat-conditioning effects of conspecifics or heterospecifics.

Dynamic population model and invasion analyses

We used our results from these competition experiments to develop an empirically parameterized competition model between our focal species. This model took the form of a system of differential equations:

$$\begin{aligned} \frac{dN_j}{dt} &= N_j \mu_j(T) \left[1 - \sum_{k=1}^2 \alpha_{jk}(T) \log(N_k + 1) \right] (1 - d_j(T)) \\ &\quad - N_j m_j + S_j g_j(T) \\ \frac{dS_j}{dt} &= N_j \mu_j(T) \left[1 - \sum_{k=1}^2 \alpha_{jk}(T) \log(N_k + 1) \right] d_j(T) \\ &\quad - S_j g_j(T), \end{aligned} \quad (5)$$

where N_j and S_j are, respectively, the populations of vegetative fronds and dormant turions of species j . The production of new individuals (which become either vegetative fronds or turions) is governed by a temperature-dependent maximum per capita reproductive rate, $\mu_j(T)$ (Eq. 2). The parameters $\alpha_{jk}(T)$ are temperature-dependent terms describing intraspecific ($k = j$) and interspecific ($k \neq j$) competition (Appendix S1: Eq. S5). Mortality, m_j , was represented by a species' average per capita mortality rate. The dynamics of each species' dormant turions, S_j , are governed by additional parameters: $d_j(T)$, the temperature-dependent proportion of total growth dedicated to turion production (Eq. 3), and $g_j(T)$, the temperature-dependent rate of turion germination (Eq. 4). Because *L. minor* does not produce turions but can still overwinter when submerged, we set this species' d_j to a small constant representing the tiny fraction of the population capable of surviving the winter (Jacobs 1947). We further assumed that, unlike vegetative fronds, dormant vegetation did not experience mortality. This is not an unrealistic assumption, given our own and others' observations of long-term (>1 yr) turion viability.

Our process for fitting this and alternative models is detailed in Appendix S1: Section 1.2. Briefly, parameter estimates for $\mu_j(T)$, $d_j(T)$, and $g_j(T)$ were obtained from nonlinear regression fits of Eqs. 2, 3, and 4 to data from our experiments or the literature. Competition parameters were estimated using regressions on data from our fluctuating temperature experiment. We considered alternative competition terms to Eq. 5 that omitted logarithms and temperature-dependent competition parameters. Fits of all candidate models were compared using R^2 values and BIC rankings.

We used our model to test the potential for coexistence based upon the reciprocal invasibility criterion. To do this, we followed the approach of Chu and Adler (2015). First, we generated 10-yr temperature time series spanning a range of averages (-10°C to 34°C) and amplitudes (0 – 24°C) that approximate climatic conditions across the Earth. Next, for each temperature series, we simulated the population dynamics of each species in monoculture for the entire ten-year period. In all cases, this led to each species j reaching a dynamic population equilibrium where its long-term per capita growth rate, $\bar{r}_{j\setminus r}$, was approximately zero. Here, we use the subscripts $\setminus r$ and $\setminus i$ to distinguish a species in its resident or invader state. For instance, the notation $\bar{r}_{j\setminus r}$ indicates the average growth rate of species j in its resident state (i.e., in the absence of any invading species). We used the resident species' equilibria $N_{k\setminus r}^*$ and $S_{k\setminus r}^*$, saved from the final 365 d of the simulation, to calculate the instantaneous, low-density ($N_j = 1$) growth rates of an invading focal species j (where $k \neq j$) at each time step, and then used these values to calculate the focal invader's long-term average invasion growth rate, $\bar{r}_{j\setminus i}$. This value is the average growth rate of the species in its invasion state (i.e., its low-density growth rate in the presence of a resident

at its environmentally determined equilibrium), and must be greater than zero for invasion to be successful. We used this approach to identify environmental spaces in which both species had positive growth rates as invaders, implying they are able to stably coexist. Following Warner and Chesson (1985), we use the geometric average for \bar{r}_j , but note that our results are quantitatively consistent using arithmetic averages.

To assess the relative contributions of species differences to coexistence, we simulated invasions using versions of our population dynamic model modified to remove particular interspecific differences. First, we assessed the effect of replacing each species' interspecific competition coefficient and temperature modifier with its intraspecific value, keeping all other parameters the same. Doing so removes the effects of fluctuation-independent stabilization (i.e., niche differences), and therefore nullifies the growth advantages a species gains when rare. Second, we assessed how removing differences in thermal growth differences changed species' invasion abilities. To do so, we shifted each species' thermal reaction norm such that they shared a common intermediate peak. This resulted in the growth curve of *L. minor* completely overlapping that of *S. polyrhiza*. Finally, we removed the ability of *Spirodela* to produce dormant turions at low temperatures, and instead replaced its dormancy rate with a constant equal to that of *L. minor*. Furthermore, in order to identify the relative importance of single interspecific factors, we removed combinations of these species differences (e.g., turions + temperature) and then performed the invasion analysis to determine how a single factor acts on coexistence in the absence of other interspecific differences.

Finally, we used a recently developed Monte Carlo simulation-based approach (Ellner et al. 2016) to identify environmental states where the temporal storage effect contributed positively to species' invader growth rates. We also used this approach to identify how the removal of interspecific differences in thermal reaction norms, dormancy behaviors, and negative frequency-dependent growth affected the strength of the temporal storage effect. The method underlying this estimation technique is detailed in Appendix S1: Section 1.3.

RESULTS

Empirical findings

In monoculture, duckweed species had different average growth rates across temperature treatments, with *Lemna minor* having a significantly higher average low-density growth rates under the majority of census periods and temperatures (Table 1, Fig. 2a). These differences were reflected in our modeled thermal reaction norms, which provided good fits to both species' data ($R^2 = 0.88$ and 0.97 for *Spirodela* and *Lemna*, respectively), and identified interspecific differences in average growth rates and thermal maxima (Fig. 2a). Generally

TABLE 1. Terms and parameter estimates for best-fitting linear models describing duckweed species' per capita growth rates in monoculture and in competition in both static and fluctuating temperatures.

Environment and term	Estimate	95% CI	Significance	R^2
Static temperatures, monoculture ($n = 72$)				0.70
Intercept	1.80×10^{-1}	1.10×10^{-2}	***	
Temperature	-8.50×10^{-4}	4.60×10^{-4}	***	
Temperature ²	-4.28×10^{-4}	3.70×10^{-5}	***	
Species (<i>Spirodela</i> = 1)	-2.21×10^{-2}	9.07×10^{-3}	***	
Census period	various	various	***	
Static temperatures, competition ($n = 40$)				0.67
Intercept	6.59×10^{-2}	3.61×10^{-3}	***	
Temperature	9.75×10^{-4}	2.29×10^{-4}	***	
Temperature ²	-2.28×10^{-4}	3.16×10^{-5}	***	
Frequency	-1.04×10^{-2}	5.35×10^{-3}	***	
Species (<i>Spirodela</i> = 1)	4.43×10^{-3}	3.94×10^{-3}	*	
Fluctuating temperatures, competition ($n = 55$)				0.83
Intercept	6.68×10^{-2}	2.12×10^{-3}	***	
Frequency	-7.13×10^{-3}	1.79×10^{-3}	***	
Species (<i>Spirodela</i> = 1)	-4.57×10^{-3}	1.61×10^{-3}	***	
Census period	various	various	***	

Notes: Temperatures and frequencies were centered at their median values prior to fitting. Frequency reflects species' relative frequencies at the start of each census period. Values of n refer to numbers of independent communities used in each experiment.

* $P < 0.05$; *** $P < 0.001$.

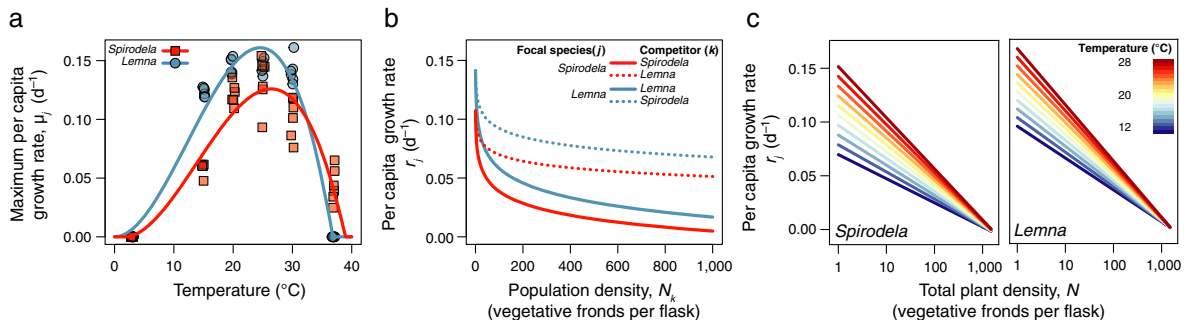


FIG. 2. (a) Thermal reaction norm curves for each species of duckweed. Curves are fit to strains' low-density growth rates (d^{-1}) from static monocultures following Eq. 2. (b) Estimated effects for intraspecific (solid lines) and interspecific (dotted lines) density dependence for each species in the fluctuating temperature experiment. For clarity, these responses are shown at average temperatures (20°C) and with the other competitor species' populations fixed at zero. (c) The impacts of vegetative duckweed density on each species' per capita growth rate covaries with temperature. For clarity, responses are shown as pooled over total population densities, but are allowed to vary depending on the competitor species (Appendix S1: Table S1). All growth rate estimates include both vegetative and turion fronds.

speaking, *S. polyrhiza* grew more slowly than *L. minor* below temperatures of 30°C , an observation in line with other studies (Wołek 1974, Docauer 1983, Lemon et al. 2001, Ziegler et al. 2014).

In competition, we identified growth trends similar to those of the species in monoculture, with *L. minor* dominating at intermediate temperatures and *S. polyrhiza* growing fastest at very high and low temperatures (though this brief advantage at low temperatures will not persist, as senescing vegetative fronds cease producing new turions). Best-fitting models of growth rates in competition included terms for species and frequency effects, but not turion replacement treatments or

interactions between any covariates (Appendix S1: Table S1). Significantly negative impacts of relative frequencies on both species' growth rates were detected most static temperatures, implying that negative frequency-dependent growth occurs in the absence of temperature fluctuations (Table 1, Fig. 3a). In our fluctuating temperature competition experiment, we found significantly negative parameter estimates for the impacts of frequency on species' per capita growth rates (Table 1, Fig. 3b). Furthermore, the frequency parameters in static and fluctuating temperatures were comparable in magnitude, indicating similar effects of negative frequency dependence across our experiments (Table 1).

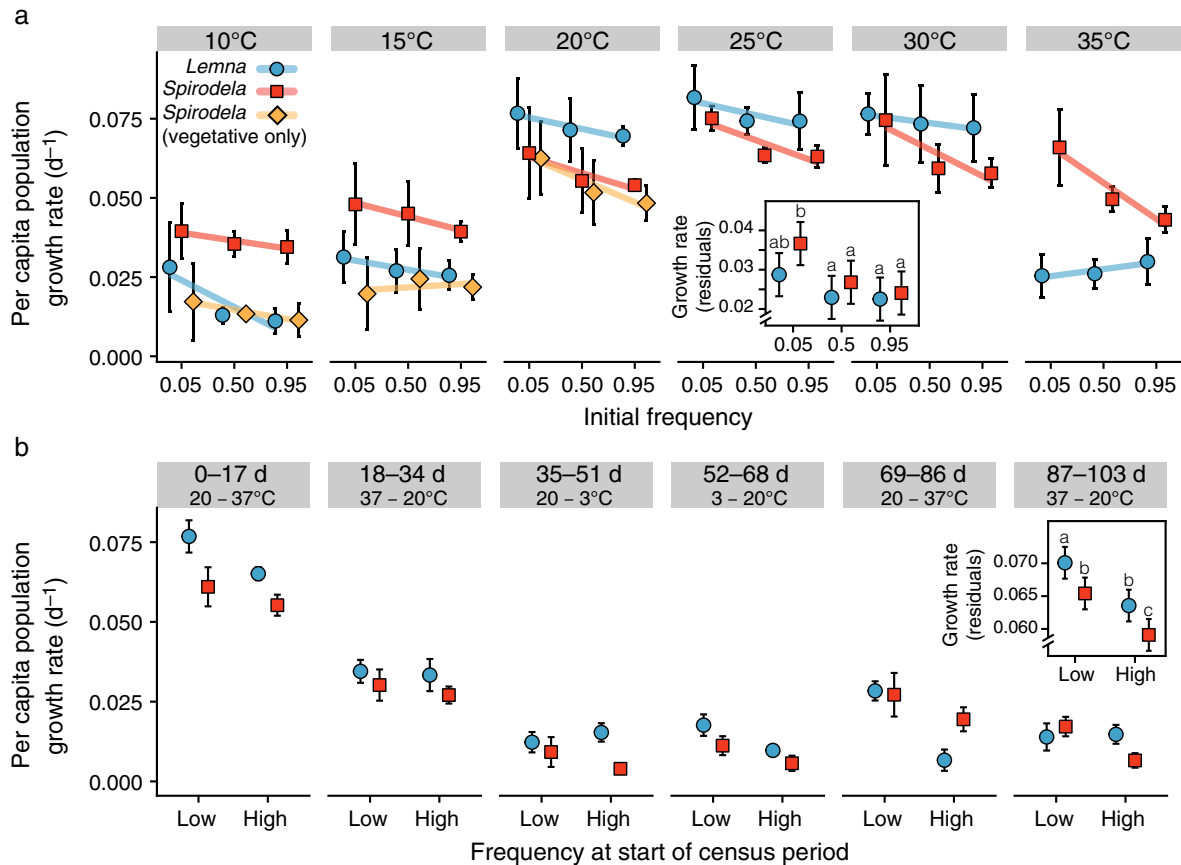


Fig. 3. (a) Average per capita growth rates (d^{-1}) of duckweed populations in static temperature environments and at different relative starting frequencies. Lines denote regression fits for frequency effects on growth rates of individual cultures. (b) Growth rates for each species in competition under fluctuating temperatures. Facets represent measurements at each census period. Rates are grouped based on initial relative frequency at the beginning of each census period. For the low-frequency category, this value was never greater than 0.25. Inset panels compare partial residual growth rates for each species \times frequency, controlling for temperature or census period. Letters above points represent groups differing significantly at $P < 0.001$. In both static and fluctuating temperatures, both species exhibit statistically significant negative frequency-dependent population growth. Error bars represent 95% confidence intervals.

Post-hoc analyses of frequency effects revealed that the growth rates of *S. polyrhiza* and *L. minor* at low relative frequencies were greater than or, at minimum, approximately equal to the mid- and high-frequency growth rates of heterospecifics (Fig. 3, insets). Thus, negative frequency-dependent growth appears to stabilize these communities across a wide range of temperatures by providing a low-frequency advantage to *S. polyrhiza*.

In a follow-up experiment comparing species' growth rates in media previously conditioned by either conspecifics or heterospecifics, we found little evidence for feedbacks between a species' growth response and the medium's previous occupant (Appendix S1: Fig. S3). However, growth rates of *Lemna* in media conditioned by *Spirodela* were significantly greater than average ($t = 2.17, P < 0.05$), but the partial effect size of medium origin was small ($\eta^2 = 0.03$), relative to the effects of species' frequencies ($\eta^2 = 0.10$ and 0.12 in static and fluctuating temperatures, respectively). Therefore, we conclude that the effects of habitat conditioning by the

dominant species cannot fully explain both species' low-frequency growth advantages.

Using data from the fluctuating temperature experiment, our best-fitting model for density-dependent growth included separate terms for intraspecific and interspecific competition ($R^2 = 0.77$ and 0.80 for *S. polyrhiza* and *L. minor*, respectively; Appendix S1: Table S2, Fig. S4). In this model, per capita growth rates were decreasing functions of log-transformed conspecific and heterospecific population densities (Fig. 2b). For both *L. minor* and *S. polyrhiza*, intraspecific competition coefficients were larger than the interspecific parameters and did not overlap in their 95% confidence bounds (Appendix S1: Table S3), implying that conspecific densities had stronger regulatory effects on growth than did heterospecific densities. Furthermore, our best-fit model also included temperature-dependent competition coefficients (Fig. 2c), signifying a positive linear relationship between the strength of density-dependent regulation and ambient temperature.

Because our model includes only two competing species, and is of the Lotka-Volterra type, we can use our competition coefficients to calculate an index of niche overlap, $1 - \rho(T)$, at a particular static temperature T with the equation

$$1 - \rho(T) = \sqrt{\frac{\alpha_{12}(T)\alpha_{21}(T)}{\alpha_{11}(T)\alpha_{22}(T)}}. \quad (6)$$

Values of $1 - \rho(T) < 1$ indicate stabilization via niche differences (Chesson 2013). Plugging our duckweed species' competition terms into Eq. 6 returns values of $1 - \rho(T)$ less than one under static temperatures greater than 8°C. Above this temperature, these values averaged 0.612 ± 0.09 (mean \pm SD), implying stabilization via niche differences contributes to coexistence across a wide range of static temperatures (Appendix S1: Fig. S5).

Simulation outcomes

In static temperature simulations with all interspecific differences in place, duckweed species only coexisted at temperatures above 10°C (Fig. 4) in general agreement with our findings from the previous section. Increasing temperature fluctuations permitted coexistence across progressively lower average temperatures to a minimum of approximately 0°C. Overall, *L. minor* experienced higher invader growth rates across more of the temperature state spaces than did *S. polyrhiza*, consistent with its overall growth advantages across most temperatures. Long-term invader growth rates of both species were maximized between 20°C and 30°C, though *Lemma* had a second invasion optimum at 13°C.

Contrary to our expectations, aligning the species' temperature differences did not significantly impact coexistence compared to the baseline, implying the species' thermal responses were already similar enough to minimize the coexistence-promoting effects of differential environmental responses (Fig. 4). Likewise, removal of turion production by *S. polyrhiza* did not appreciably change its low-density growth rates, but shifted the region of highest growth by *L. minor* from 13°C to 25°C and suppressed its maximum invasion growth rate (Fig. 4). On the other hand, making inter- and intraspecific competition equal eliminated the ability of *S. polyrhiza* to successfully invade a population of resident *L. minor*, and at the same time markedly reduced the invasion growth rates of *L. minor* over most thermal regimes (Fig. 4). Upon further removal of thermal differences and turion production, invasion growth rates of *L. minor* declined to near-zero levels across the entire range of thermal regimes. Therefore, *L. minor* was able to maintain low albeit positive invader growth even in the absence of fluctuation-independent negative frequency-dependent growth and interspecific differences in thermal performance and dormancy. Invasion success of *Spirodela*, on the other hand, was entirely dependent

on stabilization resulting from negative frequency dependence.

We found little evidence that the temporal storage effect strongly contributed to coexistence dynamics in this system. Across all environments, we found no cases where the temporal storage effect was responsible for maintaining species' positive invasion growth rates when fluctuation-independent mechanisms (such as negative frequency dependence) were removed. However, the positive storage effects in *L. minor* were not inconsequential at temperatures between 5°C and 18°C, and under certain environments contributed to up to 50% of its invader growth rate (Appendix S1: Figs. S6, S7). In contrast, all storage effects for *S. polyrhiza* remained near zero, indicating that fluctuation-independent stabilization was primarily responsible for its positive invader growth rates.

DISCUSSION

In this study, we used two similar and widely co-occurring aquatic plant species (*Lemna minor* and *Spirodela polyrhiza*) to confront the contradictory and commonly encountered assumptions that (1) ecologically similar species should not stably coexist and (2) species that regularly co-occur must be stably coexisting. To address whether, how, and under what conditions coexistence is possible between *L. minor* and *S. polyrhiza*, we followed the recommendation of Siepielski and McPeck (2010) to provide three different lines of evidence supporting coexistence. These lines of evidence include (1) mutual invasibility, (2) negative frequency-dependent population regulation, and (3) phenotypic differences underlying negative frequency dependence. Furthermore, we asked how these lines of evidence varied across environments, and determined whether putative phenotypic differences influenced coexistence similarly across environmental gradients.

Satisfaction of the mutual invasibility criterion is a critical test of species coexistence, but one that most empirical studies fail to conduct (Siepielski and McPeck 2010). Based on our experimental and modeling results, duckweed species *S. polyrhiza* and *L. minor* satisfy the invasibility criterion across a wide range of static and fluctuating temperature regimes. While field validation of these results was not conducted, we note that duckweed species are commonly observed invading or increasing in relative frequency within a water body (Docauer 1983, Dickinson and Miller 1998, Paolacci et al. 2016), implying a potential for duckweed to increase from (or persist at) low abundances. Of course, field validation of mutual invasibility is preferable and, while challenging, could be accomplished for duckweed species by introducing an invader to established (and preferably contained) resident communities where the invader is known to be absent. To date, very few studies have experimentally assessed invasibility in situ (but see Huisman et al. 1999, Narwani et al. 2013), and instead

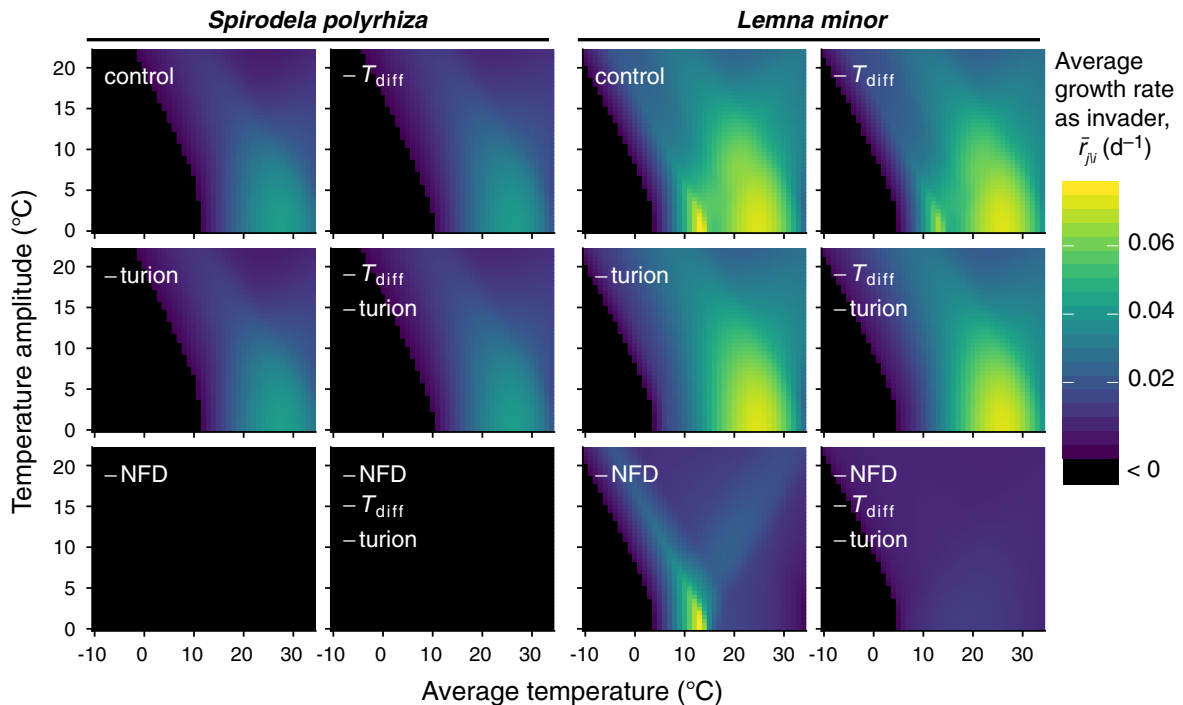


FIG. 4. Average invasion growth rates, \bar{r}_{ji} , for each species of duckweed under various average temperatures and fluctuation amplitudes. Coexistence occurs in regions where both species have positive low-density growth rates. Each panel shows invader growth rates simulated in the presence or absence of particular interspecific differences. Control, standard conditions with all species-level differences (Eq. 5); $-T_{diff}$, species' thermal growth differences removed; $-turion$, turion production by *Spirodela* removed; $-NFD$, differences between inter- and intraspecific competition removed and replaced with average values.

take our approach of combining the outcomes of controlled experiments with model simulations (Wilson et al. 1999, Descamps-Julien and Gonzalez 2005, Jiang and Morin 2007, Letten et al. 2018).

Negative frequency-dependent growth can occur when intraspecific competition more strongly regulates a species' growth rate than does interspecific competition (Adler et al. 2007). Though inter- and intraspecific competition coefficients are infrequently measured in competition experiments, meta-analyses have found interspecific competition to be consistently weaker than intraspecific competition across a wide variety of plant and animal communities (Connell 1983, Adler et al. 2018). Likewise, the few studies (including our own) to address this question in submerged and floating macrophytes agree with this finding (Keddy 1976, McCreary et al. 1983, Kautsky 1991, Barrat-Segretain and Elger 2009, Gérard and Triest 2018). That we found no increase in the strength of negative frequency-dependent growth between static and fluctuating temperatures, and that contributions of the temporal storage effect were minimal, implies that stabilization in this system acts primarily in a fluctuation-independent manner.

In our experiments, the mechanism of competition was most likely exploitation of dissolved nutrients in the liquid growth medium. Preliminary results of an ongoing experiment suggest that both species exhibit

approximately equal rates of dissolved N and P draw-down, with nutrient concentrations approaching lower detection limits (0.1 $\mu\text{g/mL}$ N, 0.005 $\mu\text{g/mL}$ P) after one week. Mutual crowding or shading is a second potential mechanism of competition in this system, but appears minimal in our cultures, since thick, multilayered surface mats of plants did not develop. We note, however, that light limitation, pathogens, and predators are also capable of mediating competitive outcomes, and their prevalence in natural systems merits further study.

For a positive storage effect to occur and promote coexistence, three key conditions must be satisfied (Chesson and Huntly 1989, Chesson 1994). First, there must be positive covariation between a species' environmental response and the competition it experiences (EC covariance). In our system, both species exhibit positive (albeit low) EC covariance at low temperatures with increasing fluctuations (Appendix S1: Fig. S8). Second, species' growth responses to joint changes in the environment and competition must be *subadditive*. That is, species' growth rates in suboptimal environments are buffered from the suppressive impacts of competition that they experience in more favorable environments. In our system, subadditivity occurs and acts similarly on both species by decreasing the impacts of competition as temperatures move away from their optima (Appendix S1: Fig. S9). Lastly, the strength of the storage effect is

proportional to the correlation between species' responses to their environment. In our system, despite species' differences in dormancy and thermal reaction norms, their environmental responses were still highly correlated (Appendix S1: Fig. S10), thus minimizing the storage effect contribution for both species.

Outside of the storage effect, a second fluctuation-dependent coexistence mechanism called *relative nonlinearity* can occur when species' differential consumption of shared resources generates variation in the strength of competition (Chesson 1994). The strength of this mechanism critically depends on species possessing differential, nonlinear responses to shared resources. While differential, nonlinear uptake kinetics of growth-limiting nutrients would seem like the most obvious source of relative nonlinearity in our system, our species exhibit indistinguishable nutrient uptake curves for both nitrogen and phosphorus (Lüönd 1980, Docauer 1983). Likewise, *L. minor* and *S. polyrhiza* have nearly identical tissue N:P ratios (3.37 ± 1.19 vs. 4.42 ± 0.67 , respectively; Reddy and De Busk 1985), which suggests that they will respond similarly to nutrient fluctuations. Finally, we found the curvature of species' responses to conspecific and heterospecific densities (the competitive factors in our models) to be nearly equal, and so are not expected to exhibit strong differential responses to fluctuations in competition.

Phenotypic dissimilarity arising from niche differences is critical for enabling species' differential responses to their environments. In static environments with no predators or pathogens, resource partitioning is a potential source of coexistence-promoting niche differences (Tilman 1982). As previously mentioned, there do not appear to be obvious differences in our species' responses to variable N and P concentrations. However, there is some evidence for temperature-dependent differential responses to macro- and micronutrient concentrations, light limitation, and NP stoichiometry (Landolt 1957, 1986, Docauer 1983, McCann 2016). Given the limited utility of single traits for predicting coexistence (Kraft et al. 2015), it is not surprising that no single niche axis can easily explain coexistence in our system. Regardless, more research is clearly needed to identify other, more cryptic axes of interspecific trait divergence among the Lemnaceae.

Fluctuation-dependent coexistence can also be caused by interspecific differences in species' environmental responses. Although we posited that differences in species' dormancy behaviors and thermal reaction norms would strengthen the storage effect, we found little evidence that either was particularly important in this system. Therefore, despite the ability of *S. polyrhiza* to avoid mass mortality and competition by producing dormant turions, it cannot be considered an important coexistence-promoting trait at the scale of the local community. Again, this is likely due to the high correlation between turions' and the competitor's environmental responses. We also note that turion production by

Spirodela facilitated the invader growth rates of *L. minor*, which stems from the fact that *Lemna* plants do not directly compete with *Spirodela* turions, and so experience a net growth benefit when they are being produced. However, given the propensity for duckweeds to experience periodic mass-mortality events from herbivory and freezing, turion production is still likely critical for the species' continued persistence, as we discuss below.

In our competition experiments and models, invading *S. polyrhiza* never achieved relative frequencies greater than 15%. These results are consistent with our own and others' observations that *S. polyrhiza* is typically encountered at low frequencies relative to *Lemna* spp. and *Wolffia* spp. (Jacobs 1947, Docauer 1983). In our survey ponds, at the height of the growing season, relative frequencies of *S. polyrhiza* averaged only $5.83\% \pm 4.16\%$ (mean \pm SD). Even when occurring in monocultures, *S. polyrhiza* does not commonly achieve the numerical abundances typical of *Lemna* (Jacobs 1947, Ziegler et al. 2014). The cosmopolitan distribution of *S. polyrhiza* is noteworthy, however, given the species' relative rarity at finer spatial scales. Keddy (1976) made a similar observation of *Lemna trisulca*, which was explained, in part, by strong limitation, but not exclusion, by the superior competitor *L. minor*. With sufficient stabilization, species with low relative growth rates can persist when rare (Yenni et al. 2012). However, these small populations may become more prone to extinctions resulting from ecological drift (Gravel et al. 2011). We posit that production of resistant turions by *S. polyrhiza* buffers the species from stochastic extinctions and allows for continued coexistence at low average relative frequencies. Though challenging in its design, such a prediction could feasibly be experimentally tested in natural duckweed communities.

CONCLUSIONS

The duckweeds *Lemna minor* and *Spirodela polyrhiza* are ubiquitous residents of temperate fresh waters in North America, Europe, and parts of Asia, Africa, and Oceania where they commonly co-occur in their local communities. Owing to differences in the species' thermal growth and dormancy responses, we investigated whether these species stably coexist across a range of realistic thermal regimes. We determined that stable coexistence among these species is indeed possible across a wide range of environments, and is primarily driven by fluctuation-independent stabilization resulting from strong self limitation. In contrast, there was remarkably little evidence that interspecific differences in thermal reaction norms and dormancy behaviors meaningfully promoted coexistence in fluctuating environments. We hope future work will build on these results by using our approach to predict where species coexistence (or invasion) is possible across real-world geographic gradients. Such predictions could then be used, for example, to test range limit hypotheses or to identify areas where

reintroduction efforts or invasions may have higher probabilities of success.

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