COMMUNITY ECOLOGY - ORIGINAL RESEARCH

Disentangling climate change effects on species interactions: effects of temperature, phenological shifts, and body size

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Abstract Climate-mediated shifts in species' phenologies are expected to alter species interactions, but predicting the consequences of this is difficult because phenological shifts may be driven by different climate factors that may or may not be correlated. Temperature could be an important factor determining effects of phenological shifts by altering species' growth rates and thereby the relative size ratios of interacting species. We tested this hypothesis by independently manipulating temperature and the relative hatching phenologies of two competing amphibian species. Relative shifts in hatching time generally altered the strength of competition, but the presence and magnitude of this effect was temperature dependent and joint effects of temperature and hatching phenology were non-additive. Species that hatched relatively early or late performed significantly better or worse, respectively, but only at higher temperatures and not at lower temperatures. As a consequence, climate-mediated shifts in hatching phenology or temperature resulted in stronger or weaker effects than expected when both factors acted in concert. Furthermore, consequences of phenological shifts were asymmetric; arriving relatively early had disproportional stronger (or weaker) effects than arriving relatively late, and this varied with species identity. However, consistent with recent theory, these seemingly idiosyncratic effects of phenological shifts could be explained by species-specific differences in growth rates across temperatures and concordant shifts in relative body size of interacting species. Our results emphasize the need to account for environmental conditions when predicting the effects of phenological shifts, and suggest that shifts in size-structured interactions can mediate the impact of climate change on natural communities.

Introduction

Predicting the impact of climate change on species and communities is a central challenge in ecology and conservation. Early studies have largely been focused on the direct effect of climate change on the life history and physiology of individual species. However, individuals do not exist in isolation and ecologists increasingly recognize the importance of species interactions for mediating the effects of climate change (reviewed in Tylianakis et al. 2008; Gilman et al. 2010; Walther 2010; Yang and Rudolf 2010).

Shifts in the phenology of species are expected to have important impacts on the interactions among species in seasonal environments (Yang and Rudolf 2010). Many species around the world show changes in their development and seasonal life history events (i.e., their phenology), such as the onset of breeding, timing of insect emergence, and flowering times (Beebee 1995; Parmesan and Yohe 2003; Root et al. 2003; Parmesan 2006). However, species within the same community often show different responses to climate change (Root et al. 2003; Visser and Both 2005; Parmesan 2006; Memmott et al. 2007; Guo et al. 2009). Some species show advances in their

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phenologies to varying degrees, some show no change, and others have demonstrated delayed seasonal phenologies (Beebee 1995; Gordo and Sanz 2005; Both et al. 2006; Martin 2007; Miller-Rushing and Primack 2008). As a consequence, climate change has altered the "phenology of species interactions" (the chronological progression of stage or size-structured interactions within a season) in many systems (Yang and Rudolf 2010). Although these studies clearly indicate that climate-mediated phenological shifts can disrupt species interactions, we still lack a detailed mechanistic understanding that links the phenology of species to the strength and temporal dynamics of species interactions (Yang and Rudolf 2010).

Climate change is associated with changes in a variety of abiotic factors, such as precipitation patterns and temperature, that can influence the phenologies and interactions of species. Because changes in these factors may or may not be correlated, it is unclear how these different aspects of climate change jointly affect species interactions. At a mechanistic level, climate change can alter the phenology of species interactions by: (1) altering the relative timing of emergence of species in a community (e.g., hatching of larvae or emergence of seedlings); and (2) changing relative growth/developmental rates. The strength of species interactions typically changes during the development of individuals due to changes in relative body size (reviewed in Yang and Rudolf 2010; Miller and Rudolf 2011). Thus, because both relative changes in emergence phenology and changes in developmental rates alter which size classes (or developmental stages) interact, they also alter the interaction strength between species (Yang and Rudolf 2010). For example, a species that hatches earlier will be relatively larger when interacting with species that hatch later. This size difference often provides a competitive advantage for the earlier species (e.g., Alford and Wilbur 1985; Lawler and Morin 1993; Werner 1994; Wilbur 1997; Schwinning and Weiner 1998; Asquith and Vonesh 2012). However, relative changes in species' time of appearance or development can be driven by different climatic changes (e.g., rainfall vs. temperature) that may or may not be correlated and it is largely unknown how both emergence phenology and temperature interact with each other to determine species interactions.

Here we suggest that the effects of phenological shifts on species interactions should strongly depend on the environmental conditions (i.e., temperature) species experience. Given a size-mediated competitive advantage, we predict that if an individual hatches early relative to its competitors it will generally perform better. However, the magnitude of this arrival effect should depend on growth rates and thus on the specific temperature: lower temperatures will reduce growth rates of early arrivers and should therefore reduce size differences and the associated

competitive advantage of relatively early arrival vs. late arrival. Here we test this hypothesis by using an experimental approach with amphibians as a model system. In particular we experimentally manipulated temperature regimes (including projected climate warming scenarios), the presence and absence of interspecific competition, and relative hatching time of two competing tadpole species to test:

1. Whether species differ in their responses to changes in temperature.
2. How phenological shifts mediated by either changes in the hatching phenology or developmental rates alter species interactions.
3. The interactive effect of temperature and hatching phenology on species.
4. Whether the observed effects of phenological shifts are correlated with shifts in relative body sizes of interacting species.

Materials and methods

Study species

Amphibians are an excellent focal group for studying effects of climate change. The responses of amphibians to climate change are among the strongest across all animal taxa (Root et al. 2003) and amphibian species within a community often differ in their responses to climate change, suggesting that mis-timing of species interactions is common (Beebee 1995; Saenz et al. 2006). In addition, there is increasing evidence for priority effects in anuran communities, in that the relative hatching time of tadpole species has strong impacts on the dynamics of species interactions and the structure of communities (e.g., Alford and Wilbur 1985; Wilbur and Alford 1985; Morin et al. 1990; Sredl and Collins 1991; Lawler and Morin 1993; Knight et al. 2009). Here we focus on two species, the southern leopard frog *Rana sphenocephala* and the western chorus frog, *Pseudacris triseriata*. These species commonly co-occur in small semi-permanent ponds in southeast Texas during early spring (Wiest 1982) but differ in their breeding behavior. *P. triseriata* breeds in winter and early spring (December–April) and experiences air temperatures ranging from ~ 4 to $20\,°C$ depending on the season and year (Wiest 1982; Saenz et al. 2006). *R. sphenocephala* also breeds mainly during winter-early spring and it also commonly reproduces throughout the year (October–September). Therefore, its tadpoles experience a larger temperature range (~ 4–$33\,°C$) (Wiest 1982; Saenz et al. 2006; Rudolf, personal observation). Furthermore, the breeding of *P. triseriata* is associated with temperature but not rainfall, while breeding of *R. sphenocephala* is mostly

associated with rainfall and typically not with temperature in our study area (Saenz et al. 2006). Because both species respond to different environmental cues, changes in either temperature or rainfall can alter the relative timing of breeding events and thereby also change the relative emergence phenology (i.e., hatching) of tadpoles in ponds. Thus, climate change is likely to impact interactions in the aquatic stages of both species through: (1) changes in the relative timing of the appearance of species, and (2) changes in the relative development and growth rates of tadpoles. Many tadpoles consume similar resources and food competition is known to be a major factor influencing interactions between tadpole species (Wilbur 1997). Because our species overlap in their resource use and natural populations in these temporary ponds are typically resource limited (Smith 1983; Wilbur 1997), both species are expected to compete in natural ponds. Because *P. triseriata* is much smaller it is expected to be an inferior competitor.

Experimental design

This experiment was designed to test the individual and combined effects of climate-mediated changes in emergence phenology (simulated by different hatching times) and growth rates on the dynamics and consequences of interspecific competition. The experiment consisted of 18 treatments representing two sets of experiments running concurrently. The first set raised each species in isolation at three different temperatures (10, 16, 20 °C) to determine how each species grows and responds to different temperature regimes in the absence of interspecific competition. The second set manipulated: (1) the relative hatching time of each species (early or late), and (2) the water temperature (10, 16, 20 °C) in a full factorial design. For logistical reasons, the single-species treatments were only performed with "early" hatching tadpoles. Each of the 18 treatments was replicated four times (=72 experimental units). The three temperatures reflected the average water temperatures tadpoles experience during the early breeding season (January–February, mean 10 °C, average range 4–15 °C), and the late breeding season (March–April, average mean 16 °C, average range 12–21 °C) in natural breeding ponds (Wiest 1982; Rudolf et al., unpublished data; Ruthig, unpublished data) and a 4 °C increase in average temperature (20 °C) projected for this region (south-eastern North America) in the next 100 years (Intergovernmental Panel on Climate Change 2008). All eggs were collected during a 7-day period from several local ponds where the species co-occur naturally. To avoid confounding genetic effects with hatching dates, we evenly divided eggs from all clutches [∼30 clutches (with ∼50 eggs per clutch) for *P. triseriata* and ∼six clutches

(with >500 eggs per clutch) for *R. sphenocephala*] and haphazardly assigned one half of the eggs to early treatments and the other half to late treatments. Within the early or late treatments, all eggs from different clutches were mixed and tadpoles were assigned randomly to a specific tank. Following previous studies (Alford and Wilbur 1985), eggs were incubated at different temperatures: early hatching treatment were incubated at 19 °C to accelerate hatching while eggs from the late hatching treatment were kept at 10 °C to delay hatching by 10 days. All tadpoles within a treatment (early or late) hatched within 1–2 days. Tadpoles were introduced to the respective replicates within 2 days of hatching. All tadpoles were allowed to slowly acclimate to new temperature conditions before the start of the experiment by submerging holding containers with tadpoles into the tanks until water temperatures reached target temperatures (∼4 h). There were no significant differences in average size [*P. triseriata*, average ∼6.5 mg (range 4.0–8.2 mg), $n = 15$ tadpoles; *R. sphenocephala,* average ∼10.52 mg (9.0–12.2 mg), $n = 15$ tadpoles] or developmental stage (Gosner stage 24–25) between early and late introductions of each species. This method has the advantage of accounting for any differences in genetic or maternal effects. However, differences in incubation temperatures could also affect tadpole performance (e.g., growth rate or mortality risk). To address this potential incubation effect, we compared the performance of a species when it was introduced early, simultaneously with the other species, to its performance when it was introduced late, simultaneously with the other species (see details below). In addition, all analyses accounted for potential confounding effects of delayed hatching by using the corresponding controls (see details below). We used 15 *P. triseriata* tadpoles and five *R. sphenocephala* tadpoles per tank (either alone in the single-species treatments or together in the competition treatment) to account for the natural abundance and body mass differences across both species. This allowed us to standardize and easily manipulate food levels across treatments and it reflects the natural differences in the abundance of both species observed in natural ponds. All densities are within the higher range (but well below the maximum) of densities observed in natural populations (Smith 1983; Rudolf, unpublished data).

All experiments were carried out in 5-L aquaria randomly arranged on shelving units housed in a climate-controlled environmental chamber at Rice University, Texas, set to a natural light:dark cycle (10 h:14 h) and 10 °C. Aquaria were filled with 3 L of reverse osmosis water reconditioned to mimic natural conductivity levels (specific conductivity mS/cm = 0.037, salinity = 0.02 p.p.t.). Each aquarium was individually heated with a submersible heater (±0.5 °C temperature precision; 75 W;

Eheim Jaeger). Aquaria were set up 1 week prior to the experiment to allow the temperature to adjust and were visually separated by opaque partitions.

We standardized initial food levels (ground up pellets of Purina Rabbit Chow) across treatments to represent the natural scenarios where two competing species colonize a pond but at different times. When both species arrive at a pond at the same time they both compete for a limited food resource. However, when one species arrives earlier than the other species it naturally experiences a short period without interspecific competition for the same food resource and therefore enjoys initially higher per unit biomass food levels. In the single-species controls (i.e., either five *R. sphenocephala*, or 15 *P. triseriata*), tadpoles received the equivalent of 5 % of their total wet body mass in food daily. In competition treatments (both species present) in which one species was added before the other (early treatments) food levels were equivalent to single-species controls until the second species was added. When both species were present, competition treatments received 2.5 % of the total combined biomass (of both species, five *R. sphenocephala* plus 15 *P. triseriata*) in food. This additive design (in which the density increases in two-species treatments while resources remain unchanged) is generally used in studies on priority effects (e.g., Alford and Wilbur 1985; Wilbur and Alford 1985; Warner et al. 1991; Lawler and Morin 1993; Blaustein and Margalit 1996; Hernandez and Chalcraft 2012). We kept food per unit biomass constant in this manner until the end of the experiment. This allowed us to isolate the consequences of climate-mediated changes in body size on species interactions (our main goal) without confounding differences in relative food levels across treatments. For example, if food levels were fixed and not adjusted to account for biomass gain due to growth, faster growing tadpoles at high temperatures would experience a relative decrease in food per unit biomass over time, thereby confounding size-specific differences in competitive ability with available food levels.

Food levels are comparable to other competition studies (e.g., Laurila 2000) and were based on pilot studies to ensure that all food was consumed within 3 days in the single-species treatments and to encourage competition when both species were present. Tadpoles were fed every 3 days, and food levels were adjusted every 3 days for survival and every 6 days for mass. To reduce tadpole stress, we weighed all tadpoles of each species from a replicate within a treatment combination that was haphazardly selected each time. Aquaria were cleaned every 14 days by vacuuming all feces and replacing 50 % of the water. To account for initial transplant mortality, we checked for tadpole survival daily for the first 4 days of each introduction (day 1–4 for early and day 10–14 for late

introduction) and replaced all dead individuals from the initial stock. After this period we checked tanks daily and removed dead tadpoles without replacement. The experiment was started on 21 February 2010 and ended on 17 April 2010 before any tadpole reached metamorphosis. Mortality rates of *P. triseriata* were within the range of mortality rates observed in other experiments and in natural populations (Smith 1983, 1990).

Response variables

Survival and mortality rates

We checked for survival every 3 days. To reduce disturbance, we visually counted all tadpoles of both species within a tank without removing individuals. Mortality rates were calculated as the number of tadpoles that died per day. Note that this response variable accounts for differences in the duration of the early and late hatching treatment. Mortality rates were calculated in two ways. First the number of dead tadpoles was either divided by the duration of the respective treatments if tadpoles survived to the end of the experiment, or by the number of days until no survivor was left in a replicate. Because this approach assumes a linear decline in survival, we also used a second approach in which we fitted a linear and exponential regression equation, both with fixed intercepts at $n = 15$, to the number of survivors in a tank across all time steps. The non-linear regression did not improve the fit of the model and we therefore present only results of the linear model here. Because the regression approach tended to be a little more robust to missing the exact date of extinction within a replicate or misidentification, we present only its estimates for analyses of *P. triseriata* which frequently went extinct during the experiment.

Size and growth rates

At the end of the experiment, we weighed each tadpole from all replicates individually to calculate the average final mass and growth rate [=(final mass − average of initial mass)/day] within a replicate for each species. While these growth rates and mass metrics are linearly related, we include statistics on final mass of tadpoles to emphasize that relative shifts in hatching time will alter relative size differences even if growth rates do not differ among arrival times. Due to the substantial mortality of *P. triseriata* at the end of the experiment we could only perform a meaningful analysis of final mass and growth rates for the no-competition control at 16 and 20 °C. All experiments were approved by the Institutional Animal Care and Use Committee (protocol no. A09022602).

Statistical analysis

We analyzed subsets of the data in two separate ways, each aimed to answer a different question. First, we analyzed single-species treatments using a 2 × 3 factorial design with species identity (*R. sphenocephala* or *P. triseriata*) and temperature as fixed effects to test how differences in temperature influenced mortality and growth rates and mass in the absence of interspecific competition and whether species differed in their responses. Second, we used all two-species treatments to examine how the relative hatching time of each species altered the strength and effects of interspecific competition on each species and whether this changed across temperatures by using a 3 (10, 16, 20 °C) × 2 (early vs. late hatching *R. sphenocephala*) × 2 (early vs. late hatching *P. triseriata*) factorial design, with temperature and each of the hatching times of both species as independent factors. Survival of *P. triseriata* was significantly lower when both species were introduced late than when both were introduced early ($P < 0.05$), indicating that delayed hatching had somewhat negative effects on tadpole survival in this species. Thus, the analysis was followed by orthogonal planned contrasts that compared the effect of relatively early or late hatching of a species to the respective control when both species arrived at the same time. Note that these are relative comparisons and thus account for any potential effects of experimentally delayed hatching.

Each factorial design was analyzed using separate general linear models (GLM) in SAS 9.3 (Littell et al. 2006). Significant GLMs were followed by planned orthogonal comparisons. To test whether observed differences in growth rates or size were due to changes in tadpole densities, analyses were carried out with and without mortality rates of *P. triseriata* as a covariate. Mortality of *R. sphenocephala* was never a significant covariate in initial analyses and thus is omitted from the results presented here. Due to differences in size among species and temperature treatments variances sometimes differed among certain treatments. To account for this heterogeneity in variances among treatments we used a GLM approach that modeled different variances for the respective treatments using a likelihood estimation in the proc mixed procedure in SAS and corrected the *df* using the Kenward-Roger correction (Littell et al. 2006). This approach has the advantage that it does not assume identical variances among treatments, but instead uses the observed data to estimate the real variances and corresponding SE for individual treatments and then uses these estimates to calculate corresponding *F*-statistics. We inspected all variances for significant differences across all treatment combinations to determine which variances were allowed to vary in the final analysis. Maximum likelihood tests were used to identify the most parsimonious model with the best fit to the data (Littell et al. 2006). All analyses met all underlying assumptions.

Results

Do species differ in their responses to temperature changes?

In the absence of interspecific competition, *R. sphenocephala* had three times higher growth rates (16 °C, 3.65 mg/day; 20 °C, 3.168 ± 0.371 SE) than *P. triseriata* (16 °C, 1.208 mg/day; 20 °C, 1.498 ± 0.371 SE) ($F_{1,12} = 30.75$, $P = 0.0001$) and was 2.4 times larger at the end of the experiment ($F_{1,12} = 32.95$, $P < 0.0001$) irrespective of temperature (interaction: $F_{1,12} = 1.09$, $P = 0.3165$) (16 °C, *R. sphenocephala* = 185.4 mg, *P. triseriata* = 64.5 mg; 20 °C, *R. sphenocephala* = 162.1 mg, *P. triseriata* = 78.4; SE = ± 17.8). A separate analysis for only *R. sphenocephala* across all temperatures (including 10 °C) revealed that the growth rate and final body mass of this species were significantly lower at 10 °C (47.5 mg) than at 16 and 20 °C (all $P < 0.0001$) but there was no significant difference between 16 °C or 20 °C ($P = 0.42$).

Mortality rate also differed significantly across species ($F_{1,18} = 466.94$, $P < 0.0001$) and temperature ($F_{2,18} = 28.71$, $P < 0.0001$), but species differed in their responses to temperatures (interaction: $F_{2,18} = 20.12$, $P < 0.0001$; Fig. 1a). Mortality rates of *P. triseriata* were significantly different among all temperature treatments (all $P \leq 0.006$); mortality was highest at 10 °C [0.312 individual (ind.)/day], lowest at 16 °C (0.141 ind./day), but significantly increased again at 20 °C (0.240 ind./day). Mortality of *R. sphenocephala* was always low (0.005–0.021 ind./day) and did not differ among temperature treatments (all $P > 0.38$).

How do changes in temperature and shifts in species' phenology interact to affect interspecific competition?

R. sphenocephala

The effect of interspecific competition on final body mass of *R. sphenocephala* depended on its own hatching time, the relative hatching time of the interspecific competitor and their individual interactions with temperature, even when accounting for the variation in survival of *P. triseriata* among treatments (Table 1). This three-way interaction clearly indicates that effects of changes in both factors (temperature and hatching phenology) were not independent. This interaction was driven by the significantly larger size of *R. sphenocephala* tadpoles when *P. triseriata* arrived late compared to treatments when both
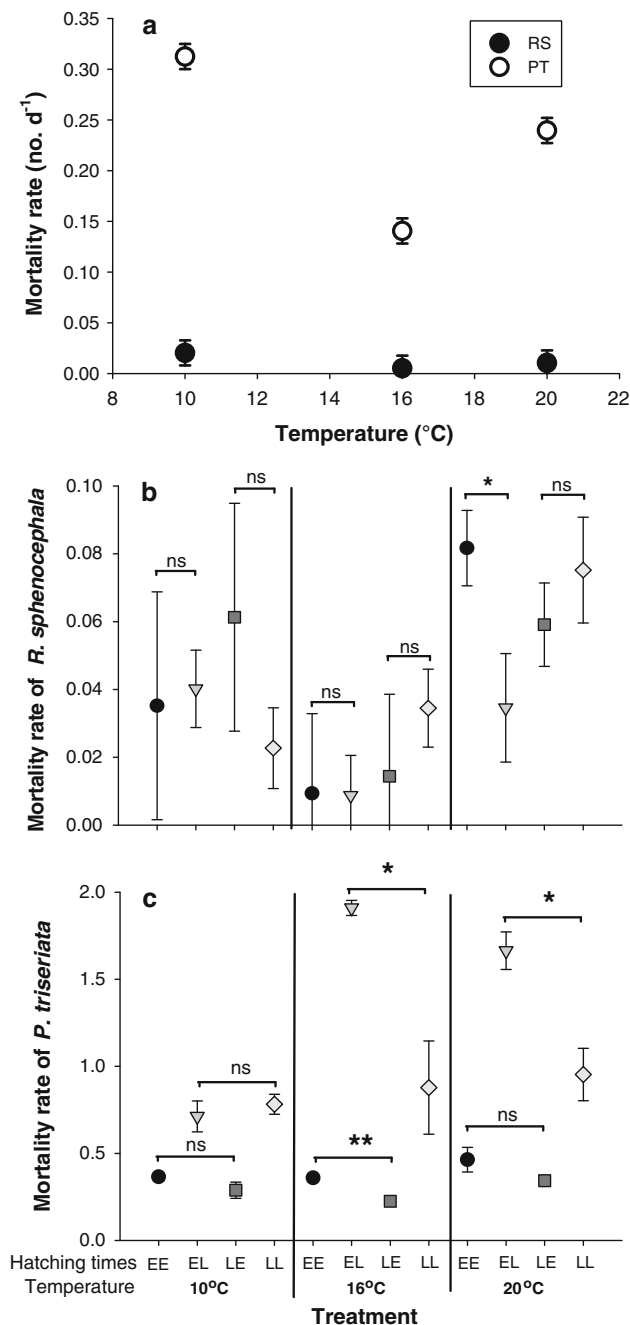
Fig. 1 **a** Effect of temperature on mean (±1 SE) daily mortality rates (number of dead individuals per day) of *Rana sphenocephala* (*RS*; *solid circles*) and *Pseudacris triseriata* (*PT*; *open circles*) in the absence of inter-specific competition (single-species treatments). Mortality rates differed significantly among species but only mortality rates of PT differed among temperatures. **b, c** Effect of temperature and relative hatching time on the mean (±1 SE) daily mortality rate (number of individuals that died each day) of two competing tadpole species when both species co-occur. Tadpoles either hatched early (*E*) at the start of experiment or late (*L*) by delaying hatching by 10 days. The *first letter* of the hatching-time treatment indicates hatching for RS and the *second letter* hatching of PT. Values are model estimates of the respective analysis indicated in Table 1 which also indicates significance of each treatment effect. *ns P* > 0.05, **P* < 0.05, ***P* < 0.01 (based on post hoc planned contrasts)

arrived early, but only at intermediate temperatures (post hoc planned contrast: $P = 0.0014$; Fig. 2). On the other hand, early hatching of the competitor had a negative effect on final body mass (post hoc planned contrast: $P < 0.05$) at 10 °C but no significant effect at higher temperatures (all post hoc planned contrasts: $P \geq 0.8$; Fig. 2).

Growth rates of *R. sphenocephala* were not significantly affected by their own hatching times, except at the lowest temperature (Table 1; Fig. 2). However, the relative hatching time of the interspecific competitor and its interaction with temperature significantly influenced growth rates even after correcting for mortality in the *P. triseriata* (Table 1). This was largely driven by the significant increase in growth rates at 16 °C when the interspecific competitor hatched later than *R. sphenocephala* compared to when both hatched at the same time, while relative early or late hatching of the competitor had no significant effect at 10 and 20 °C (Fig. 2). Note that these are relative comparisons that account for any potential differences in performance of *P. triseriata* between early and late introductions. Furthermore, there was no difference among treatments where both species hatched early or both hatched late in *R. sphenocephala* growth rates at 16 and 20 °C indicating that the observed differences in *R. sphenocephala* growth rates between relative early or relative late arrival were not simply driven by lower performance of late-arriving *P. triseriata* (Fig. 2). In general, this indicates that the projected increase in temperature due to climate change could alter the strength of interspecific competition for this species.

Mortality rates of *R. sphenocephala* differed significantly among temperature treatments (Table 1). Interestingly, the relative hatching time of the interspecific competitor only mattered for mortality rates of *R. sphenocephala* at the highest temperature (20 °C) when *R. sphenocephala* hatched early—here relative late hatching of the interspecific competitor reduced mortality rates (post hoc planned contrast: $P = 0.0395$) compared to equal hatching times. Relative differences in hatching time did not alter mortality rates in any other treatment comparisons (all post hoc planned contrasts: $P > 0.171$, Fig. 1b), again suggesting that a future increase in temperature due to climate change could alter the strength of interspecific competition for this species.

P. triseriata

The individual impact of interspecific competition on survival of *P. triseriata* was significantly affected by the interaction of relative hatching time of both species and temperature (three-way interaction: $P = 0.0126$, Table 1; Fig. 1c). On average, hatching early relative to the competitor significantly decreased mortality rates, while

Table 1 Impact of relative hatching time of two competing tadpole species *Rana sphenocephala* (*RS*) and *Pseudacris triseriata* (*PT*) and temperature (*Temp.*: 10 °C, 16 °C, 20 °C) on consequences of interspecific competition

| | PT | | RS | | | | | |
| | Mortality rate | | Mortality rate | | Growth rate | | Final mass | |
	F-value[a]	P-value	F-value[a]	P-value	F-value[a]	P-value	F-value[a]	P-value
Effect								
Temp.	$F_{2,7.15} = 18.18$	$P = 0.0016$	$F_{2,28.3} = 3.54$	$P = 0.0425$	$F_{2,15.8} = 67.81$	$P < 0.0001$	$F_{2,14.2} = 71.38$	$P < 0.0001$
RS	$F_{1,8.35} = 31.40$	$P = 0.0004$	$F_{1,31} = 2.89$	$P = 0.0994$	$F_{1,22.9} = 4.35$	$P = 0.0484$	$F_{1,16.5} = 82.95$	$P < 0.0001$
RS × Temp.	$F_{2,7.15} = 10.65$	$P = 0.072$	$F_{2,27.4} = 0.85$	$P = 0.4389$	$F_{2,15.3} = 3.16$	$P = 0.0710$	$F_{2,13.6} = 15.07$	$P = 0.0004$
PT	$F_{1,8.35} = 183.93$	$P < 0.0001$	$F_{1,33.9} = 2.60$	$P = 0.1158$	$F_{1,20.7} = 8.06$	$P = 0.0099$	$F_{1,20.1} = 8.99$	$P = 0.0071$
PT × Temp.	$F_{2,7.15} = 14.70$	$P = 0.0029$	$F_{2,27.9} = 0.30$	$P = 0.7429$	$F_{2,15.7} = 2.15$	$P = 0.1497$	$F_{2,13.9} = 3.99$	$P = 0.0426$
PT × RS	$F_{1,8.35} = 14.04$	$P = 0.0052$	$F_{1,28.2} = 0.06$	$P = 0.8144$	$F_{1,21.3} = 5.30$	$P = 0.0315$	$F_{1,14.9} = 6.85$	$P = 0.0195$
RS × PT × Temp.	$F_{2,7.15} = 8.58$	$P = 0.0126$	$F_{2,27.2} = 1.68$	$P = 0.2056$	$F_{2,15.3} = 4.48$	$P = 0.0294$	$F_{2,13.7} = 6.09$	$P = 0.0128$
PTmort.[b]			$F_{1,23.8} = 0.36$	$P = 0.5546$	$F_{1,7.42} = 7.42$	$P = 0.0297$	$F_{1,10.1} = 6.18$	$P = 0.0320$

Treatments RS and PT consist of two factors where the respective species arrived either early or late

[a] Variances were allowed to vary among certain treatments and *df* were adjusted accordingly (see section "Materials and methods" for details)

[b] To account for differences in the number of surviving PT present during the experiment across treatments (Fig. 3), mortality rate of PT (*Pmort.*) was used as covariate in all analyses for RS

hatching later than the competitor significantly increased mortality rates relative to the respective controls when both species arrived at the same time at 16 °C (post hoc planned contrast: $P \leq 0.02$) and 20 °C (post hoc planned contrast: $P \leq 0.04$), while it had no significant effect at 10 °C (post hoc planned contrasts: $P \geq 0.18$). However, the magnitude of the negative effect of hatching relatively late was significantly (post hoc planned contrasts: $P < 0.0001$) bigger (average both species hatching early vs. *R. sphenocephala* hatching early and *P. triseriata* hatching late $= -45.4$ %) than the positive effect of hatching relatively early (average both species hatching late vs. *R. sphenocephala* hatching late and *P. triseriata* hatching early $= 11.2$ %). In general, this indicates that shifts in the phenology of a species can have very different consequences which depend on the phenology of other species and the ambient temperature, and these effects were not additive.

Discussion

While ample evidence indicates that climate change has altered the phenologies of species worldwide, the consequences for species interactions are not well understood (Forrest and Miller-Rushing 2010). Here we show that relative shifts in the emergence phenology (e.g., hatching of tadpoles) of interacting species can alter the strength of species interactions, and that the presence and strength of these effects differed with temperature because of changes in growth rates. Importantly, the effects of shifts in relative hatching phenology and temperature were non-linear (non-additive), resulting in stronger or weaker effects than expected when both factors acted in concert. Thus, our results indicate that we need to account for the interactive effects of different aspects of climate change and species interactions when predicting the consequences of phenological shifts for species and dynamics of natural communities.

Species-specific responses to temperature change in the absence of interspecific competition

Increasing evidence suggests that species within a community often differ in their responses to climate change (Beebee 1995; Gordo and Sanz 2005; Both et al. 2006; Martin 2007; Miller-Rushing and Primack 2008), but which species will be affected more or less is typically unclear. In our study, the winter/early spring breeding species *P. triseriata* performed best at the average temperature expected during its natural breeding time, but survival significantly declined at both low temperature and at a simulated 4 °C increase in temperature predicted for future climate change scenarios (Intergovernmental Panel on Climate Change 2008). While mortality rates of *P. triseriata* may seem high, the observed mortality is completely consistent with other experiments (Smith 1990) and within the range of mortality rates in natural populations (Smith 1983), indicating that the observed mortality was not an experimental artifact. Indeed, natural populations are often strongly food limited and mortality due to intra-specific competition is known to be important in regulating natural tadpole populations in this species (Smith 1983). In contrast,
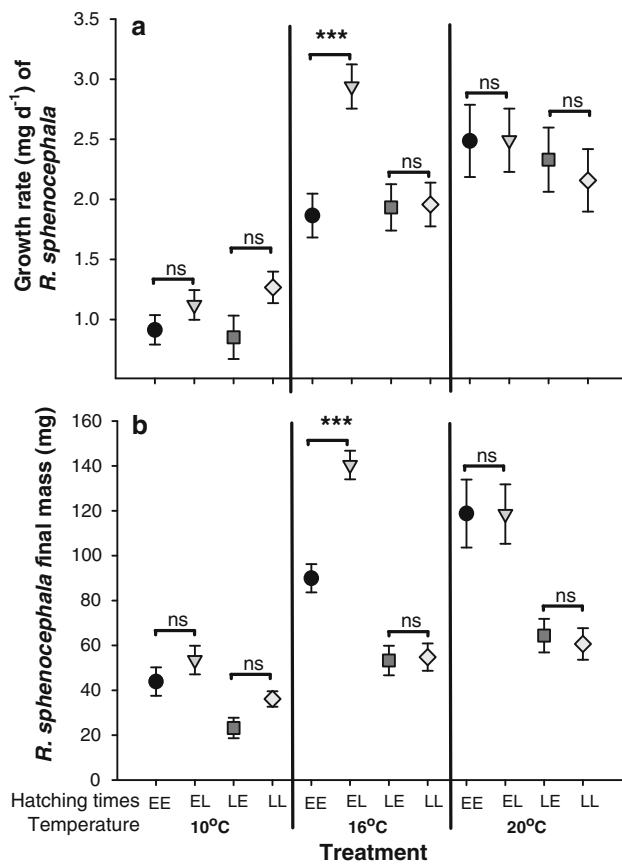
Fig. 2 Effect of temperature and relative hatching time of two competing species of tadpoles on the mean (±1 SE) **a** growth rate and **b** final body mass of RS. The *first letter* of the hatching time treatment indicates hatching for RS and the *second letter* hatching of PT. Values are model estimates of the respective analysis indicated in Table 1. For abbreviations, see Fig. 1

growth rates but not survival of *R. sphenocephala* differed among temperature treatments in the absence of interspecific competition. It is possible that this difference in temperature sensitivity between species stems from differences in the breeding phenology of both species. *P. triseriata* breeds only in winter and early spring while *R. sphenocephala* breeds throughout the year. Thus, *P. triseriata* tadpoles experience a narrower temperature range then *R. sphenocephala*. This could explain why tadpoles of the latter species are less negatively affected by temperature change, suggesting that "seasonal generalists" (i.e., species with life history events not restricted to specific seasons) may be less affected by climate change than "seasonal specialists" (i.e., species with life histories restricted to a short specific season). This notion is consistent with other studies. For example, in a community of Mongolian grasshoppers, changes in temperature had a stronger effect on the development of mid- and late-season species than on the early-season species (Guo et al. 2009). However, more studies are needed to confirm this hypothesis.

Phenological shifts, species interactions, and the role of body size

Phenological shifts in response to climate change often differ among species (including our study species) within a community (Root et al. 2003; Visser and Both 2005; Saenz et al. 2006; Memmott et al. 2007; Guo et al. 2009). Our results clearly demonstrate that such differential shifts in species' phenologies (i.e., relative timing of hatching) can alter the outcome of competitive interactions. This effect persisted even after accounting for changes in densities, indicating that phenological shifts altered the per capita interaction strength of interspecific competition. Consistent with previous studies on tadpole communities (Alford and Wilbur 1985; Wilbur and Alford 1985; Morin et al. 1990; Sredl and Collins 1991; Lawler and Morin 1993; Knight et al. 2009), we found that species typically (but not always) performed significantly better when they arrived relatively earlier than their competitors. However, the effects of phenological shifts were contingent on the specific temperature and the combined effect of temperature and phenological shifts was non-linear for both species. Moreover, the effects were species specific and asymmetrical; *R. sphenocephala* was much less affected by changes in relative arrival time than *P. triseriata*, and the costs of arriving late were much higher than benefits of arriving early for *P. triseriata*, while the opposite was generally true for *R. sphenocephala*. This suggests that the consequences of climate-mediated phenological shifts depend on the specific environmental conditions and species identity.

The observed effects of phenological shifts are consistent with recent theory which suggests that the effects of phenological shifts on species will depend on how these shifts alter the relative body size of interacting species (Yang and Rudolf 2010). We found that the growth rates of both species increased with temperature. Since growth rates determine the size difference of individuals between early and late hatching events, the effects of phenological shifts should scale with differences in growth rates. This was indeed the case; in a regression analyses we found that the mortality rate of *P. triseriata* (which showed the strongest response to phenological shifts) significantly increased with the growth rate of *R. sphenocephala* when it arrived late, while it tended to decrease (relative to the control) with its own growth rate when it arrived early (Fig. 3). Although the latter relationship was not significant, we did observe a significant increase in mortality across temperature treatments in the GLM analysis (Fig. 2; Table 1), suggesting that lack of significance was due to a lack of statistical power in this regression, likely because of its smaller sample size. This effect is consistent with a size-specific competitive advantage that has been observed in many other organisms including tadpoles (e.g., Wilbur 1977;
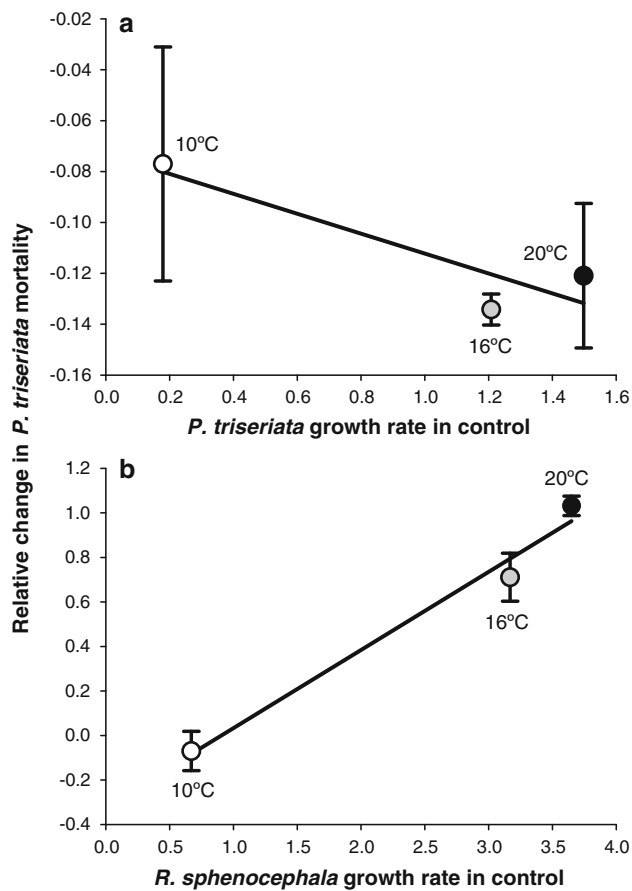
Fig. 3 Relationship between relative change in PT mortality and **a** growth rates of PT for treatments where PT arrived earlier than RS, and **b** growth rates of RS when PT arrived later. Note the differences in x-axis scales. *Symbols* indicate treatment means ± 1 SE. Growth rates were estimated from three different temperature treatments in single-species controls in the absence of inter-specific competition (see section "Materials and methods"). Because mortality rates at 10 °C prevented us from using the final mass to calculate growth rates for PT, growth rates were estimated using the body size measurements from the last available three subsamples periods for a given treatment. Relative change in mortality is the absolute difference between the mortality rate in a given treatment and the average of its respective control for that temperature when both species arrived at the same time (i.e., EE for **a** and LL for **b**); *positive values* indicate an increase in mortality rates relative to the control. PT decreased with PT growth rate in the control [$R^2 = 0.123$, regression coefficient (b) = 0.037, $P > 0.05$] and increased with RS growth rates in the control ($R^2 = 0.889$, $b = 0.346$, $P > 0.001$). All statistics are based on individual replicates. For other abbreviations, see Fig. 1

Steinwascher 1978; Alford and Wilbur 1985). Moreover, because *R. sphenocephala* has, overall, an up to four times higher growth rate than *P. triseriata*, the increase in mortality rates with *R. sphenocephala* growth rates was ~tenfold higher in magnitude than the decrease in mortality rate with *P. triseriata* growth rate (slope increase 0.346 vs. decrease 0.037; Fig. 3), which explains why the negative effects of arriving late were much worse than arriving earlier for this species. Note that the respective

growth rates of each species were estimated in the control and were thus independent predictors for the patterns observed in the competition experiment. Thus, simple differences in growth rates and concordant shifts in relative body size among temperature regimes and species could explain much of the complex and seemingly idiosyncratic effects of phenological shifts across different treatments.

While the duration of the interaction also differed between early and late hatching dates, this is unlikely to explain the pattern. First, tadpoles that hatched relatively late experienced competition for a shorter amount of time, yet hatching late always had a negative effect. In addition, the duration of the interaction did not vary among different temperature treatments, but body size did. Together, this clearly indicates that body size was the key factor driving the pattern and not duration of the interaction. To the best of our knowledge, this provides the first experimental evidence supporting recent theory that shifts in relative body size can determine the consequences of phenological shifts on interacting species (Yang and Rudolf 2010).

Climate change is associated with a range of biotic and abiotic factors that could influence the strength of species interactions directly (e.g., through changes in relative body sizes) or indirectly (e.g., change in resource density). As a consequence it is often unclear what the underlying mechanisms are that link climate change to the observed patterns. To avoid this problem we kept the per unit biomass food level constant in our experiment. This allowed us to isolate the consequences of climate-mediated changes in body size on species interactions without confounding changes in food resources with different temperatures or body sizes. It is unclear whether resources (i.e., food densities) of consumers in natural systems would increase in direct proportion to body size of consumers with temperature and over time. Recent studies suggest that this will likely depend on consumer and resources identity and respective metabolic rates (e.g., Petchey et al. 1999; O'Connor et al. 2009; Hoekman 2010). Such species-specific differences and complex feedbacks between consumers and resource dynamics may explain why the relative arrival time of tadpole species can sometimes have a positive, a negative, or no effect on late-arriving species and does not always seem consistent with simple size-dependent competitive advantages (e.g., Alford and Wilbur 1985; Wilbur and Alford 1985; Sredl and Collins 1991; Lawler and Morin 1993). Thus, future studies that examine the consequences of dynamic resources will be a logical and important extension of our experiment to multi-trophic systems.

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