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# Climate warming mediates negative impacts of rapid pond drying for three amphibian species

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**Abstract.** Anthropogenic climate change will present both opportunities and challenges for pool-breeding amphibians. Increased water temperature and accelerated drying may directly affect larval growth, development, and survival, yet the combined effects of these processes on larvae with future climate change remain poorly understood. Increased surface temperatures are projected to warm water and decrease water inputs, leading to earlier and faster wetland drying. So it is often assumed that larvae will experience negative synergistic impacts with combined warming and drying. However, an alternative hypothesis is that warming-induced increases in metabolic rate and aquatic resource availability might compensate for faster drying rates, generating antagonistic larval responses. We conducted a mesocosm experiment to test the individual and interactive effects of pool permanency (permanent vs. temporary) and water temperature (ambient vs. +~3°C) on three anurans with fast-to-slow larval development rates (Great Basin spadefoot [*Spea intermontana*], Pacific chorus frog [*Pseudacris regilla*], and northern red-legged frog [*Rana aurora*]). We found that although tadpoles in warmed pools reached metamorphosis 15–17 days earlier, they did so with little cost (<2 mm) to size, likely due to greater periphyton growth in warmed pools easing drying-induced resource competition. Warming and drying combined to act antagonistically on early growth ( $P = 0.06$ ) and survival ( $P = 0.06$ ), meaning the combined impact was less than the sum of the individual impacts. Warming and drying acted additively on time to and size at metamorphosis. These nonsynergistic impacts may result from cotolerance of larvae to warming and drying, as well as warming helping to offset negative impacts of drying. Our results indicate that combined pool warming and drying may not always be harmful for larval amphibians. However, they also demonstrate that antagonistic responses are difficult to predict, which poses a challenge to proactive conservation and management. Our study highlights the importance of considering the nature of multiple stressor interactions as amphibians are exposed to an increasing number of anthropogenic threats.

**Key words:** global warming; life history; metamorphosis; phenotypic plasticity; *Pseudacris regilla*; *Rana aurora*; *Spea intermontana*; wetland drying.

## INTRODUCTION

Over the past two decades, amphibian declines have been documented across diverse taxa and geographical regions (Houlahan et al. 2000, Stuart et al. 2004). Researchers have proposed many drivers of decline, including infectious disease, chemical pollutants, invasive species, overexploitation, and habitat loss (reviewed in Collins 2010). However, climate change may pose the greatest threat to amphibians because all ectotherms show varying degrees of sensitivity to changes in climate (Walther et al. 2002, Carey and Alexander 2003, Hof et al. 2011). In the last 50 years, global average surface temperature increased by 0.10–0.16°C per decade and is projected to rise by 1.8–4.0°C by 2099 (relative to 1999;

IPCC 2007). Increased surface temperatures will drive changes in humidity, precipitation, and water temperature, variables that directly affect amphibian development (e.g., Browne and Edwards 2003), reproduction (Waldman 1982), behavior and morphology (Jameson et al. 1973), movement patterns (Reading 1998), and species interactions (Walther et al. 2002).

Warmer water temperatures at amphibian breeding sites can elicit a wide range of responses among larvae of pool-breeding species (Newman 1992). Temperature increases that shift species closer to but not beyond their thermal optima may increase larval metabolic and development rates (Dillon et al. 2010). If resources are limited, higher metabolic demand and intraspecific competition during growth can result in smaller size at metamorphosis (Newman 1998, Enriquez-Urzelai et al. 2013), which can subsequently decrease juvenile survivorship (e.g., Morey and Reznick 2001). However, warmer water temperatures may also stimulate aquatic

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primary and secondary productivity. Such increased resource availability with higher metabolic demand could allow larvae greater opportunities for growth (Cushing 1990, Winder and Schindler 2004) and mediate negative effects of intraspecific competition (Newman 1998). By contrast, water temperatures that exceed species' thermal optima compromise enzymatic and physiological functions, causing acute or chronic negative effects on growth, development, and mobility (e.g., Somero 2010, Duarte et al. 2012).

Climate change effects on larval amphibians will be generated not only by higher pool temperatures but also by associated hydrologic changes due to more frequent drought (McMenamin et al. 2008), reduction in snowpack (Mote et al. 2005), and altered precipitation and humidity patterns (Winter 2000). In the lower mainland of British Columbia (B.C.), Canada, averaged models predict a 10% decline in summer precipitation by 2099 (relative to 1999; IPCC 2007). The resulting decreased diversity and availability of wetlands (Mote et al. 2003, Elsner et al. 2010) is predicted to become a source of stress for pool-breeding amphibians (e.g., Walther et al. 2002). In particular, temporary pools are expected to dry more rapidly or disappear, and species that inhabited these may have to disperse (if possible) to more permanent breeding ponds (Araujo et al. 2006). Further, permanent pools may become temporary (Ryan et al., *in press*). Pool drying can be lethal if the drying rate exceeds individuals' ability to accelerate development (Semlitsch and Wilbur 1988, Leips et al. 2000). A wealth of research has also documented nonlethal effects of drying on metamorphs. For example, during pool drawdown, threat of desiccation often leads larvae to metamorphose earlier but at a smaller size (Wilbur and Collins 1973, McMenamin and Hadly 2010, Richter-Boix et al. 2011). Alternatively, smaller size but without earlier emergence may occur in response to increased density-dependent, intraspecific competition in shrinking habitats (Wilbur and Collins 1973).

Pool warming and drying are individually well studied, but few studies have evaluated the physiological and ecological challenges they simultaneously present for larval amphibians. When multiple stressors interact, they may generate additive effects, synergies, or antagonisms. Additive interactions occur when the combined impact of stressors is simply the sum of their individual impacts (Folt et al. 1999). Synergies are nonadditive interactions that occur when the combined impact of stressors is greater than the sum of their individual effects (Folt et al. 1999). In the context of climate change impacts on larvae, it is assumed that synergies will result from warmer pool temperatures exacerbating the challenges imposed on larvae by rapid drying alone (McMenamin et al. 2008; Ryan et al., *in press*). This assumption has been based on the fact that pool warming and drying are nonindependent physical processes and that climate projections indicate that warmer water temperatures combined with decreased

water inputs will lead to earlier and faster wetland drying in the summer (McMenamin et al. 2008). If true, we would expect to see a reduction in size at metamorphosis and higher larval mortality exceeding what would occur if the negative effects of higher temperatures and faster drying rate were additive. Alternatively, antagonisms (when the combined impact of stressors is less than the sum of their two effects; Breitbart et al. 1998, Didham et al. 2007) may occur if warming-induced increases in metabolic rate and resource availability benefit species in permanent environments or compensate for faster drying rates. Such antagonistic effects could result in earlier emergence with a lower cost on size at metamorphosis than if individual effects of warming and drying on size at metamorphosis were additive.

Predicting the type of interaction between these two stressors is also challenging because, across species, larvae have varying tolerances to warming and sensitivities to desiccation (Walther et al. 2002). For pool-breeding amphibians, larvae must develop at a rate that allows them to metamorphose before their pool becomes dry (Wellborn et al. 1996). Larvae with sufficient phenotypic plasticity (as defined by Newman 1992) may metamorphose early in response to pool drying but delay emergence when in long-duration pools (e.g., Loman and Claesson 2003, Richter-Boix et al. 2011). Therefore, how pool warming and drying will impact larval development also depends on whether species exhibit phenotypic plasticity in response to pool drying.

Here, we conducted a mesocosm experiment to evaluate the effects of water temperature (ambient vs.  $\pm 3^{\circ}\text{C}$ ) and habitat permanency (permanent vs. temporary) on three anuran species along a continuum of fast-to-slow larval development rates: Great Basin spadefoot (*Spea intermontana*), Pacific chorus frog (*Pseudacris regilla*), and northern red-legged frog (*Rana aurora*). Our four treatments simulated alternative climate-change scenarios, allowing us to assess the independent and combined impacts of warming and permanency on early larval growth, size and mass at metamorphosis, time to metamorphosis, and survival to metamorphosis. We tested whether warming and faster drying would interact synergistically or antagonistically, rather than additively. We also tested the hypothesis that species with longer larval development (at the slow end of the life-history continuum) will be more constrained in their ability to respond to shorter hydroperiods expected with warming climates.

## METHODS

### *Species*

In natural environments, the larvae of our study species develop within 1–3 months. Great Basin spadefoot (*S. intermontana*) have a relatively short larval developmental period (on average 6–8 weeks in B.C.) and breed in temporary pools in variable, arid environments (COSEWIC 2007). Pacific chorus frogs (*P. regilla*)

are habitat generalists, breed in temporary and permanent pools, and have an intermediate larval period (8–10 weeks; Nussbaum et al. 1983). Northern red-legged frogs (*R. aurora*) are cold water specialists, with a long larval developmental period (10–12 weeks) and low thermal tolerance limits (COSEWIC 2002). We collected egg masses of *S. intermontana* from White Lakes Grasslands Protected Area (49°16'04.43" N, 119°35'48.18" W), *P. regilla* from Lower Seymour Conservation Reserve (49°15'02.97" N, 123°00'49.28" W), and *R. aurora* from Pinecrest wetlands (50°01'56.96" N, 123°07'17.76" W) in late spring. We housed egg masses in shallow artificial pools under common outdoor conditions in natal pond water until hatching.

#### Experimental design

We established experimental ponds in 48 1000-L plastic cattle tanks at the University of British Columbia's Experimental Pond Facility (Vancouver, British Columbia, Canada) in spring 2012. We filled the tanks with city water to a depth of 28 cm (~500 L) on 12 April and added 2 kg of dried deciduous leaf litter to each tank. We added 2 L of pond water and 1.5 L of concentrated plankton collected by a 64- $\mu$ m conical tow net from a local natural pond to each tank. To initiate primary production, we added 10  $\mu$ g/L phosphorous as  $\text{KH}_2\text{PO}_4$  and 160  $\mu$ g/L nitrogen as  $\text{NaNO}_3$  per tank (Thompson and Shurin 2012). On 8 May, we randomly assigned 30 tadpoles at stage 26 (Gosner 1960) to each tank (one species per tank). Mesh tops on all tanks prevented macroinvertebrate colonization.

We randomly assigned water temperature (no warming or warming), pool permanency/drying (permanent or temporary), and species in a  $2 \times 2 \times 3$  factorial design and replicated each treatment four times. We added 300 W submersible heaters (Hagen, Baie d'Urfé, Quebec, Canada) to warmed tanks prior to introducing plankton. The heaters ran constantly so that warmed tanks were ~3°C warmer than ambient tanks at all times and followed the same diurnal temperature fluctuations (Appendix A). We simulated temporary pools by removing water with buckets once a week (Appendix B). We did not remove water from permanent tanks but similarly disturbed them to control for water removal activity. The warmed tanks had some associated natural increase in drying rate and the temporary tanks had some associated natural increase in temperature (Appendices A and B). Therefore, our warming and drying treatments refer to treatments in which the primary manipulation is warming or drying.

#### Temperature, depth, chlorophyll *a* measurements

We recorded temperature hourly with temperature loggers (Maxim/Dallas Semiconductor, Dallas, Texas, USA;  $n = 19$  temperature loggers) until the tanks dried or the experiment ended. We took weekly water depth measurements. To sample periphyton as a metric of food availability, we hung plastic flagging tape from the

inside of each tank on 17 May and sampled it after 25 and 55 days. We froze the periphyton samples until analysis, at which time we scraped, filtered them with distilled water onto Whatman GF/C filters (GE Healthcare Life Sciences, Baie d'Urfé, Quebec, Canada) and estimated chlorophyll *a* content ( $\mu\text{g}/\text{cm}^2$ ) by methanol extraction fluorometry (Steinman and Lamberti 1996).

#### Tadpole growth and metamorphosis

We estimated tadpole early growth rates at day 31 from a subsample of 15 randomly sampled tadpoles (or the maximum number remaining). From day 37, we monitored tanks every other day for emerging frogs. We removed *P. regilla* and *R. aurora* metamorphs at stage 45–46 (<1 mm tail; Gosner 1960). Because *S. intermontana* tadpoles frequently cannibalize metamorphs (S. M. O'Regan, *personal observation*), we removed them at stage 43 and housed them in separate containers outdoors without food until stage 45. We weighed metamorphs (g) and measured snout-to-vent length (mm, SVL) before they were euthanized with MS222 (Western Chemical, Ferndale, Washington, USA). Just prior to temporary pools completely drying, we removed and counted any remaining individuals.

#### Statistical analysis

We compared the effects of warming and drying on four response variables by fitting linear mixed-effects models (LMEs) to individual tadpole responses with a random intercept for the individual tank to control for pseudoreplication (Pinheiro and Bates 2000; Appendix C). The four response variables were tadpole growth to day 31 (mm/d), SVL at metamorphosis (mm), mass at metamorphosis (g), and mean time to metamorphosis (d). For simplicity, we present the analysis of mass at metamorphosis in Appendix D, as responses were nearly identical to SVL at metamorphosis. To examine treatment effects on the proportion of tadpoles that survived to metamorphosis and periphyton availability, we fit a generalized linear model (GLM) with a binomial error distribution and a logit link function, and a GLM with a gamma error distribution and a log link function, respectively (McCullagh and Nelder 1989). We corrected for overdispersion in the survival data by calculating the variance-inflation factor ( $\hat{c}$ ), which we used to adjust the coefficient standard errors (Burnham and Anderson 2002).

Our models expressed response variables as a function of temperature, permanency, species identity, and all two-way interactions. We considered whether our data supported incorporating a three-way interaction between these factors by comparing support for the models using Akaike's information criterion adjusted for small sample size ( $\text{AIC}_c$ ; Akaike 1974). However, models with the three-way interaction never received stronger support than models with only two-way interactions (except in the case of growth to day 31,  $\Delta\text{AIC}_c = 0.7$  and SVL,  $\Delta\text{AIC}_c = 2.1$ , where  $\Delta\text{AIC}_c \lesssim 2$  indicates equivalent



PLATE 1. Great Basin spadefoot (*Spea intermontana*) metamorphs from a (left) permanent versus (right) temporary pool. *S. intermontana* displayed greater phenotypic plasticity in size and mass than Pacific chorus frog (*Pseudacris regilla*) and northern red-legged frog (*Rana aurora*). Photo credit: S. C. Anderson.

support [Burnham and Anderson 2002; Appendix E]). Therefore, in our main analysis we only included two-way interactions as a consistent and parsimonious approach across all response variables. We fit all LMEs using the nlme package for R (R Development Core Team 2012, Pinheiro et al. 2013) with restricted maximum likelihood (REML). For AIC<sub>c</sub> model comparison we fit the models with maximum likelihood.

To improve the interpretability of the species-level interaction coefficients (species  $\times$  warming and species  $\times$  drying) we extracted species-level treatment effects of warming and drying by adding the contrast coefficients to the main effect reference-level coefficients (Schielzeth 2010). For example, we calculated the effect of warming on *S. intermontana* by adding the coefficient of the *S. intermontana*  $\times$  warming interaction term to the coefficient of the warming term for the reference species (*R. aurora*). We calculated the variance for these species-level treatment effects as in Schielzeth (2010) by subtracting the variance of the reference-level coefficients from that of the contrast coefficients.

We used the warming  $\times$  drying interaction to test whether the combined effects of warmer pool temperature and faster pool drying were antagonistic, additive, or synergistic across species. We considered an additive interaction the null hypothesis (warming  $\times$  drying coefficient = 0; Darling and Côté 2008). If the warming

$\times$  drying interaction was different from zero and the opposite sign of the warming and drying effects, we considered the effect antagonistic. If the interaction was different from zero and of the same sign then we considered it synergistic.

## RESULTS

### *Temperature and pool depth*

The temperature and permanency treatments resulted in four climate scenarios with unique temperature and drying profiles (Appendices A and B). Over the course of the experiment, warmed pools were  $2.8^\circ \pm 0.8^\circ\text{C}$  (mean  $\pm$  SD) higher in temperature than ambient conditions in the permanent pools and  $3.1^\circ \pm 0.7^\circ\text{C}$  higher in the temporary pools. Experimental temperatures fell within the range that we observed in natural breeding sites (Appendix A). Permanent pools increased in depth until early July due to precipitation, before decreasing through evaporation in late summer. Warmed temporary pools dried completely by mid-July (day 60) and ambient-temperature temporary pools by August (day 76).

### *Effects of pool warming*

By day 31 of the experiment, tadpoles had grown faster in warmed pools (Figs. 1a, 2a, b; see Appendix F for table of coefficients). *S. intermontana*, *P. regilla*, and

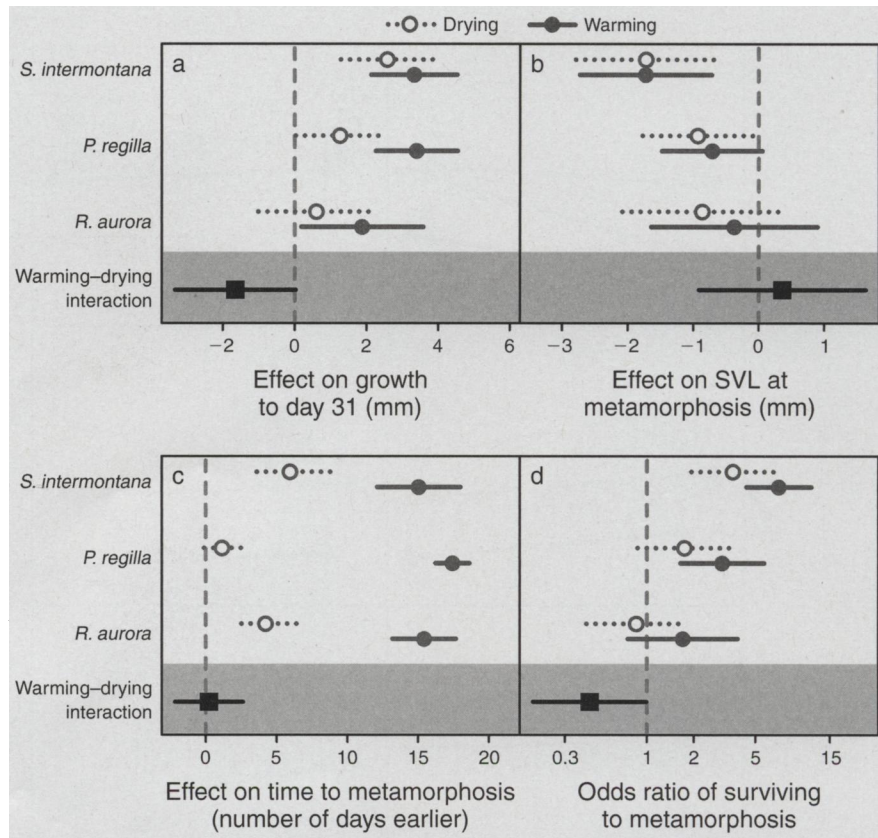


FIG. 1. Species-level and interactive effects of warming and drying (coefficient values and 95% confidence intervals) on larval fitness-related traits. We show the main effects of warming (solid circles) and drying (open circles) for each species and the species-independent interaction coefficient for the warming and drying effects (solid squares on shaded background). The observed effect of warming and drying together is the sum of the warming effect, drying effect, and interaction coefficient. Therefore, a warming-drying interaction of 0 represents the null hypothesis of an additive effect of warming and drying, an antagonistic effect has the opposite sign of the main effects, and a synergistic effect has the same sign as the main effects. In panel d, odds ratios >1 mean that the treatment increased the odds of surviving to metamorphosis. Species are Great Basin spadefoot (*Spea intermontana*), Pacific chorus frog (*Pseudacris regilla*), and northern red-legged frog (*Rana aurora*). SVL stands for snout-vent length.

*R. aurora* grew  $3.3 \pm 0.6$ ,  $3.4 \pm 0.6$ , and  $1.9 \pm 0.9$  mm longer (mean  $\pm$  SE), respectively, in warmed compared to ambient-temperature pools. By metamorphosis, such differences disappeared and *S. intermontana* showed slightly reduced SVL (Fig. 1b) and mass at metamorphosis (Appendix D) when pools were warmed ( $1.7 \pm 0.5$  mm smaller and  $0.2 \pm 0.06$  g less). Warming had a strong negative effect on time to metamorphosis for all species (Figs. 1c, 2e, f). Larval period was reduced by  $15.0 \pm 1.5$ ,  $17.4 \pm 0.6$ ,  $15.4 \pm 1.1$  d with warming for *S. intermontana*, *P. regilla*, and *R. aurora*, respectively, compared to the ambient treatment. The odds of surviving to metamorphosis were significantly greater in warmed pools for *S. intermontana* and *P. regilla*, but not for *R. aurora* (Fig. 1d; odds ratios: *S. intermontana* =  $7.0$  [95% confidence interval (CI) =  $4.36$ – $11.40$ ], *P. regilla* =  $3.0$  [95% CI =  $1.64$ – $5.67$ ], *R. aurora* =  $1.7$  [95% CI =  $0.76$ – $3.83$ ]). By 11 June, periphyton availability ( $\mu\text{g}/\text{cm}^2$ ) was almost two times greater ( $1.8$  [95% CI =  $0.9$ – $3.3$ ]) in warmed compared to ambient-temperature pools (Fig. 3a, b, c).

Effects of pool drying

Of the three species, only *S. intermontana* tadpoles grew more by day 31 when in temporary rather than permanent pools ( $2.6 \pm 0.7$  mm more; Fig. 1a). Time to metamorphosis was significantly reduced for *S. intermontana* ( $-6.0 \pm 1.4$  d) and *R. aurora* ( $-4.2 \pm 1.1$  d) when reared in temporary vs. permanent pools, but not for *P. regilla* (Fig. 1c). *S. intermontana* and *P. regilla* emerged from temporary pools with  $1.7 \pm 0.6$  and  $0.9 \pm 0.4$  mm smaller SVLs, respectively, compared to tadpoles in permanent pools (Figs. 1b, 3c, d; see Plate 1). *S. intermontana* also emerged  $0.2 \pm 0.06$  g lighter in temporary pools compared to permanent pools (Appendix D). In temporary pools, *S. intermontana* had significantly higher survival and *P. regilla* marginally higher survival (odds ratios: *S. intermontana* =  $3.37$  [95% CI =  $1.93$ – $6.60$ ], *P. regilla* =  $1.74$  [95% CI =  $0.87$ – $3.47$ ]; Fig. 1d). There was little evidence for an effect of drying on *R. aurora* survival (Fig. 1d). All *P. regilla* and most *R. aurora* and *S. intermontana* metamorphosed prior to the pools completely drying. In temporary pools, the

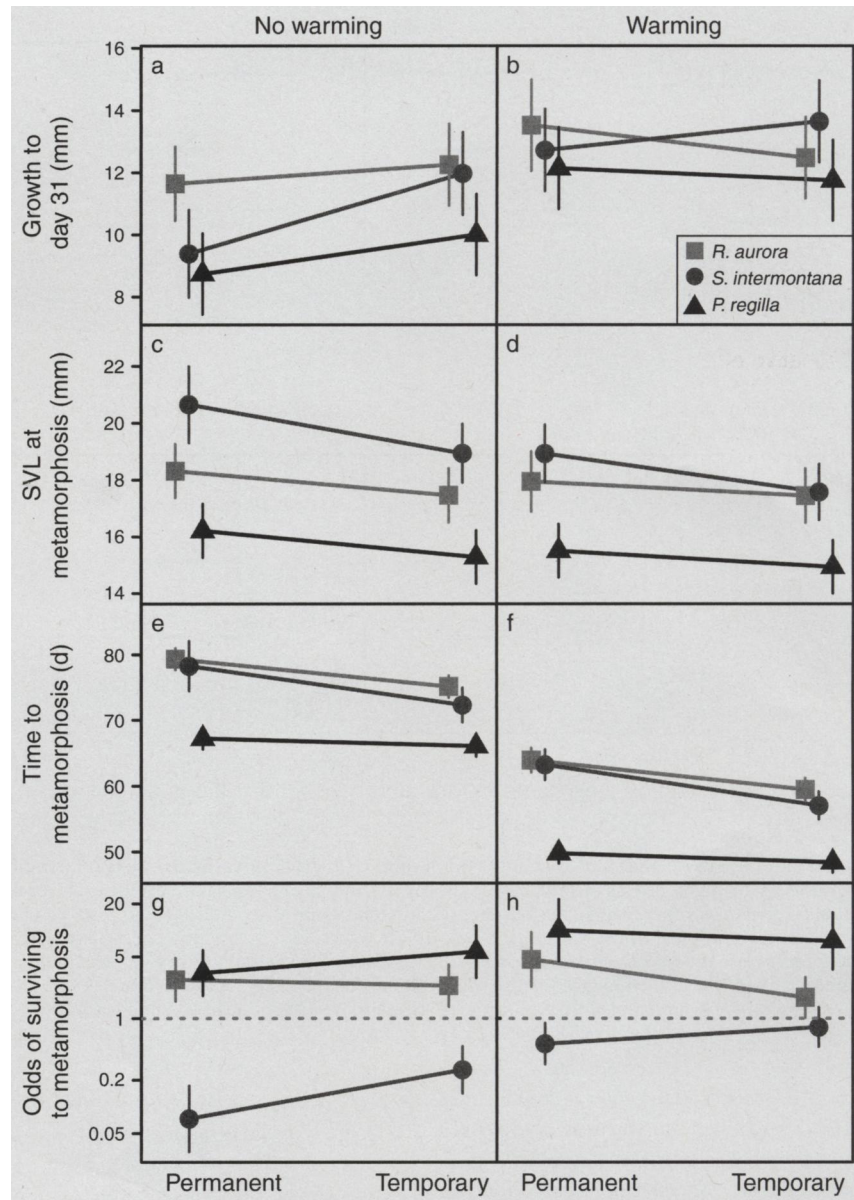


FIG. 2. Model-predicted means and 95% confidence intervals of larval fitness-related traits across three amphibian species in permanent and temporary pools, with and without warming. The dashed horizontal line in panels (g) and (h) represents equal odds; odds >1 mean that the tadpoles were more likely to survive to metamorphosis than to not survive.

median proportion of tadpoles caught by drying were 0.03 and 0.13 for *R. aurora* in warmed and not warmed pools, respectively; and 0.02 and 0.05 for *S. intermontana* in warmed and not warmed pools, respectively (Appendix G).

#### Combined effects of warming and rapid drying

The combined effects of warming and drying on larvae were antagonistic or additive, not synergistic. Warming and drying alone both increased early growth across species, but together had antagonistic effects on early growth. Tadpoles grew  $1.7 \pm 0.8$  mm less by day 31 than that expected if the individual warming and

drying effects were additive (Fig. 1a). This means, for example, that *S. intermontana* in warmed temporary pools grew 4.3 mm more on average than those in ambient-temperature permanent pools, but we would have expected an increase in 5.9 mm ( $3.3 + 2.6$  mm) if the drying-warming interaction had been additive. In contrast, the combined effects of warming and drying on SVL at metamorphosis and time to metamorphosis were close to additive: SVL at metamorphosis was only 0.4 mm (SE) greater (Fig. 1b) and time to metamorphosis was only 0.2 d (SE) less (Fig. 1c) than if the effects were additive. Warming and drying together had antagonistic effects on survival (Fig. 1d). The odds of surviving to

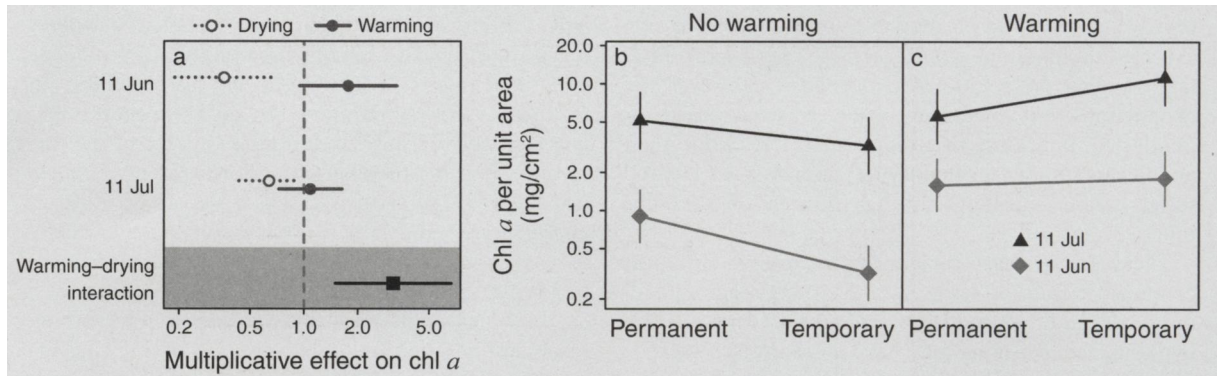


FIG. 3. The effect of warming and drying treatments on chlorophyll *a* content per unit substrate ( $\mu\text{g}/\text{cm}^2$ ) at two sampling times. (a) We show the main effects (coefficient values and 95% confidence intervals) of warming (solid circles) and drying (open circles) as well as the interaction coefficient for warming and drying combined (solid square on shaded background). Because we fit the model with a log link, a warming–drying interaction of 1 represents the null-hypothesis of an additive effect of warming and drying on a log scale. (b, c) Model-predicted means for chlorophyll *a* content across treatments and 95% confidence intervals. Samples are from 11 June (diamonds) and 11 July (triangles) 2012.

metamorphosis generally increased under the warming or drying treatments on their own, but when combined, the odds of surviving were less than the additive expectation (odds ratio = 0.43 [95% CI = 0.21–0.99]). Our alternative analysis with three-way interactions did not change our main conclusions: all interactions remained additive or antagonistic, although with increased uncertainty and some heterogeneity across species (Appendix H). Periphyton decreased with drying and increased with warming. When warming and drying were combined, periphyton availability was 3.2 (95% CI: 1.5–6.6) times greater than if their individual effects were additive on a log scale (Fig. 3a, c). We cannot define this interaction as strictly antagonistic or synergistic given that periphyton strongly decreased with drying but increased with warming.

#### DISCUSSION

An increasingly important challenge in conservation biology is predicting the cumulative impact of multiple stressors on ecological systems (Sutherland et al. 2009). When nonadditive interactions between stressors occur, the magnitude and direction of their combined impacts are difficult to anticipate. Mitigating the consequences of these ecological surprises (Paine et al. 1998, Lindenmayer et al. 2010) requires empirical evaluations of stressor interactions. Here, we quantified the individual and interactive effects of increased water temperature and pool drying rate on three larval amphibians (*S. intermontana*, *P. regilla*, and *R. aurora*) that exhibit fast-to-slow larval development. Future climate change is projected to increase surface temperatures, result in more droughts and variable precipitation, and cause coincident increases in water temperatures and decreases in wetland availability and permanency (Carey and Alexander 2003). We tested whether the naturally linked processes of pool warming and drying generate synergistic larval responses. If so, we expected larvae exposed

to both stressors would metamorphose far earlier and at much smaller size or experience greater mortality than what would occur if the individual effects of warming and drying were simply summed. This hypothesis was not empirically supported. Under our experimental conditions, we found additive or antagonistic effects of warming and drying on all larval response variables, which suggest that at worst, warming did not multiply the negative effects of drying (additive), and at best, may have alleviated effects of faster drying (antagonistic). Individually, warming and drying resulted in rapid growth within the first 31 days of development, earlier metamorphosis with little to no cost on size and mass at metamorphosis, and increased survival to metamorphosis. Relative differences in the magnitude of these effects are consistent with the species' contrasting fast-to-slow life histories.

We found the independent effects of warming and drying to be largely consistent with our a priori expectations. The effects of warming on time to metamorphosis and early growth support the hypothesis that higher water temperatures accelerate larval growth and development by increasing metabolic rate (Newman 1998) as well as resource availability. Likewise, larvae in temporary pools accelerated growth and development, likely in response to desiccation cues (Leips et al. 2000, Tejedo et al. 2010). The effect of warming in reducing time to metamorphosis was about three times stronger than the effect of drying, suggesting that drying rate may not have imposed as strong a constraint on development. Alternatively, increasing density-dependent competition as temporary pools lost water may have had an opposing effect of lengthening the time for larvae to reach the minimum size needed for metamorphosis (Wilbur and Collins 1973). We saw little difference in size or mass between permanent and temporary pools except in *S. intermontana*, perhaps because density-dependent effects also tempered beneficial effects of



drying and warming on growth by metamorphosis. We expected drying alone would decrease the proportion of tadpoles that survived to metamorphosis. However, the proportion that metamorphosed was increased or unaffected, indicating that the species were within their abilities to respond to the drying, though *R. aurora* did suffer greater mortality through desiccation than the other species.

There are at least three possible reasons why the combined effects of warming and drying were antagonistic or additive but not synergistic. First, as we hypothesized, warming may have helped offset negative effects of drying. Warming strongly increased periphyton availability, which may have promoted earlier emergence with little reduced size and mass at metamorphosis. Indeed, larvae in warmed temporary pools emerged ~18–21 d earlier but only ~0.9–1.2 mm smaller (~3 mm for *S. intermontana*) than those in permanent, ambient-temperature pools (Fig. 1b, c). Moreover, acceleration of development with warming may have allowed larvae to keep pace with the faster rate of drying in temporary pools and avoid drying-induced mortality. For example, the slowest developing *R. aurora* in temporary pools were unable to metamorphose before the pools dried. However, this mortality was over four times greater when the temporary pools were not warmed (Appendix G). Second, antagonistic interactions between stressors are likely when a species' tolerance to the first stressor is positively correlated with tolerance to the second stressor, such that the cumulative impact is less than the sum of both effects (Vinebrook et al. 2004). This could be the case with larval amphibians, where thermal and desiccation tolerance are often linked (Denver 1997; Gerick et al., *in press*). Additionally, exposure of larvae to higher temperatures should increase responsiveness to drying because larvae respond similarly to both cues in temporary environments (Wilbur 1990). These redundant responses may generate antagonistic interactions. Third, there may be a physiological limit to a species' response to co-occurring stressors that prevent synergistic interactions (Vinebrook et al. 2004), such as minimum size thresholds larvae must reach to undergo the energetically demanding process of metamorphosis (Wilbur and Collins 1973).

Relative differences in the effects of warming and drying across our three study species are consistent with what we understand about their life-history strategies. *S. intermontana*, which experienced the greatest increase in survival to metamorphosis with warming or drying, is strongly associated with warm, temporary habitats; whereas *R. aurora*, which showed no difference in (or perhaps lower) survival with warming or drying, has low thermal limits and is thought to be best adapted to permanent, colder pools (Licht 1971). Additionally, differences in early growth between the species are in keeping with the expectation that amphibians associated with uncertain environments, such as *S. intermontana*,

will exhibit greater phenotypic plasticity; whereas species that tend to inhabit more stable environments, such as *R. aurora*, will respond the least (Wilbur and Collins 1973). In contrast to our expectation, *S. intermontana* did not develop most quickly of the three species, but began emerging shortly after *P. regilla* (range of 37–80 d for *P. regilla* vs. 45–85 d for *S. intermontana*) and largely overlapped in time with *R. aurora* (range of 49–109 d). *S. intermontana* grow to a larger size and mass than the other two species (Fig. 2c, d); therefore, it is possible they experienced stronger resource limitation. Alternatively, the drying rate may not have been rapid enough to initiate a more accelerated development response. In amphibians, life-cycle transitions often depend on degree days, a metric of thermal time, as an environmental cue. Unlike the other two species, *S. intermontana* tadpoles accumulated far more degree days than we found was necessary for the species before metamorphosing when in permanent pools (Appendix A), which suggests that they slowed development in the absence of a rapid-drying cue.

Our results have the potential to inform the relative vulnerability of our study species to future climate change. *R. aurora*, with its stronger affiliation for cool, permanent habitats, may be more constrained in its ability to respond to rapid drying in having to accumulate a greater minimum number of degree days to metamorphose (Appendix A). As we observed, warming may have beneficial effects on tadpoles by increasing periphyton availability, which may help offset the challenge of accelerated drying rates. However, if *R. aurora* are subject to more variable and faster drying wetlands, they may suffer greater lethal effects than recorded in this experiment. In contrast, *P. regilla* is a habitat generalist that may be more resilient to habitat loss and fragmentation, and our results suggest they may not be especially vulnerable to more rapid drying rates expected in the future. It is more difficult to draw conclusions about the vulnerability of *S. intermontana* to climate change. In our experiment, they appeared able to display a much larger range of responses than the other species (see Plate 1). However, they are also restricted to arid, temporary wetlands that are already highly threatened by urbanization and agriculture and are likely to be at greater risk of disappearing with climate change (COSEWIC 2007).

The interactive effects of stressors are often context- and magnitude-specific (Darling and Côté 2008). If the magnitude of warming or drying had been greater, we might have drawn different conclusions. Future work is needed to assess whether the type of interaction between these two stressors changes along a gradient of drying rates and temperatures from antagonistic to additive to synergistic interactions and whether this shift is linear or nonlinear. Similarly, we found that periphyton availability was sensitive to warming, but it also varies across natural pools as a function of abiotic conditions, food web structure, and

resource competition (e.g., Shurin et al. 2012). We also acknowledge that our experiment explored a limited range of contrasts. First, we selected our species based on average rates of larval development; however, plasticity in larval development traits can reflect interannual variability in breeding habitat permanency and vary across populations (Loman and Claesson 2003, Richter-Boix et al. 2006, but see Richter-Boix et al. 2011). Though we lacked detailed information on the variability of developmental traits for our focal populations, incorporating this into future work would help identify regional differences in plasticity and sensitivity to warming. Second, though all three species demonstrated some plasticity in time to metamorphosis in response to warming and drying with little cost to size or mass, we can draw limited inference about survival in subsequent life stages or population-level consequences. Larger spadefoot metamorphs (Newman and Dunham 1994, Morey and Reznick 2001) and *P. regilla* (Schaub and Larsen 1978) lose less water and experience greater survival than smaller individuals. Further, earlier metamorphosis can allow individuals more time to grow and build energy stores before hibernation (Reading and Clarke 1999). However, altered larval phenology can also lead to temporal shifts in niche overlap, with consequences for trophic and competitive interactions. For example, in Britain, earlier breeding by newts (*Triturus* sp.) has exposed early breeding frog embryos and larvae to increased predation by newts (Beebee 1995). Despite this context dependency, our study sets a precedent for evaluating the interactive effects of warming and drying on larvae that others can adapt to study the responses of species in diverse systems.

Our study emphasizes the importance of considering the nature of interactions between pool warming and drying in predicting consequences of climate change for pool-breeding amphibian larvae. We identified that despite the linked nature of these two physical processes, they had nonsynergistic impacts on larvae in our experiment and may not always be harmful for larval amphibians. However, antagonisms, like synergies, are ecological surprises that can be just as difficult for conservation biologists to anticipate and manage (Doak et al. 2008). Indeed, we observed that the interactive effects of warming and drying on early larval growth and survival to metamorphosis were not predictable based on the magnitude and direction of their independent effects. These results reveal that climate change will present both opportunities and challenges for amphibian larvae. Our data suggest that whether larvae adequately respond to faster and earlier wetland drying is likely to hinge on the degree of warming and whether primary production can keep pace with metabolic demand. We expect that the relative sensitivity of species to warming and drying will vary with their habitat specificity, and this reinforces the conclusion that protecting a diversity of productive wetlands that are less vulnerable to future

thermal stress should be a priority for climate adaptation planning (Lawler 2009).

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#### SUPPLEMENTAL MATERIAL

##### Appendix A

Temperature data and degree days across treatments (*Ecological Archives* E095-071-A1).

##### Appendix B

Mean pool depths over the duration of the experiment (*Ecological Archives* E095-071-A2).

##### Appendix C

Model-fit residuals for each of the response variables (*Ecological Archives* E095-071-A3).

##### Appendix D

Species-level and interactive effects of warming and drying on mass at metamorphosis (*Ecological Archives* E095-071-A4).

##### Appendix E

Comparison of support for candidate models with up to two- or three-way predictor interactions (*Ecological Archives* E095-071-A5).

##### Appendix F

Table of model coefficients for each of the response variables (*Ecological Archives* E095-071-A6).

##### Appendix G

Boxplot of mortality due to drying in temporary pools (*Ecological Archives* E095-071-A7).

##### Appendix H

Three-way interaction coefficients (*Ecological Archives* E095-071-A8).