Amphibians under stress: life history, density dependence, and differences in vulnerability

by

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Abstract

Numerous anthropogenic stressors are known drivers of amphibian declines. Nonetheless, research has revealed few lessons for preventing declines in advance of their occurrence. This thesis presents a conceptual framework for identifying when spatial and temporal overlap of density-dependent bottlenecks, life-history traits, and stressors increase decline risk. I evaluated this framework using published empirical amphibian density-dependence data, and found that population dynamics and life-history theory could be useful in prioritizing vulnerability to stressors, though current data deficiencies limit evidence of correlations between these factors. In an experimental test with three frog species, I found that not all species share the same sensitivities to combined climate warming and habitat permanency scenarios. These results suggest larval life-history requirements can influence species' responses to climate change. Integrating theoretical and empirical tests provides useful tools for estimating species vulnerability and helps identify gaps in our knowledge of the dynamics that govern amphibian responses to stressors.

Keywords: climate warming; density dependence; intrinsic sensitivity; life history; phenotypic plasticity; risk correlates

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General Introduction

One of the greatest challenges conservationists face is quantifying the cumulative impact of multiple stressors on ecological systems (Sutherland *et al.* 2009). Stressors interacting together may either mitigate or exacerbate negative impacts in comparison to single-stressor impacts (Folt *et al.* 1999). The need to resolve the conditions under which multiple stressors cause population declines is perhaps greatest with the amphibian decline problem, which has arguably become the crisis discipline within conservation biology (Lawler *et al.* 2006)

Over the past 50 years, we have witnessed the decline of amphibian populations across the globe (Wyman 1990; Houlahan *et al.* 2000). The IUCN Red List now lists 41% of amphibian species as threatened, over 420 of which are Critically Endangered (Stuart *et al.* 2004; Hoffmann *et al.* 2010). Numerous anthropogenic stressors have been suggested as drivers of decline or even correlated with extinction events, including habitat loss (Gallant *et al.* 2007), overexploitation (Baillie *et al.* 2004), infectious disease (Berger *et al.* 1998; Lips *et al.* 2003), climate warming (Duarte *et al.* 2012), invasive species (Kats and Ferrer 2003), chemical pollutants (Davidson *et al.* 2002), UV-B radiation (Blaustein *et al.* 1994), and acidification of freshwater habitats (Leuven *et al.* 1986). In spite of the progress that has been made in understanding the mechanistic processes that lead these stressors to cause lethal and non-lethal effects on amphibians of various life stages, identification of patterns of intrinsic sensitivity among species as well as their overlap with extrinsic drivers of decline has been lacking. There is therefore a critical need to develop tools for prioritizing vulnerability of amphibians to anthropogenic stressors.

In this thesis, I aim to advance our thinking on amphibian population dynamics and life-history theory and how it can be linked to the sensitivity of amphibians to multiple stressors. I accomplish this by applying cross-disciplinary ecological theory and integrating modeling and empirical methods. As Ricklefs (2000) argues, combining modeling and empirical methods is often lacking in life-history studies but allows us to show what data need to be collected to verify assumptions and quantify constraints. I perform a detailed review of amphibian density-dependence data and apply two modeling approaches to examine whether relationships exist between the occurrence and magnitude of density-dependence within amphibian life cycles and life-history traits; and conduct an experimental test of larval responses to two interacting stressors with species under different life-history constraints.

In Chapter 1, I present a conceptual framework for identifying when spatial and temporal overlap of density-dependent bottlenecks, life-history traits, and stressors increase decline risk. I demonstrate that insights gained from marine fish, where density-dependence and life-history information are regularly incorporated into stock assessments to predict responses of populations to exploitation, might likewise be applied to amphibians. I adapt and expand this fisheries management model to reflect the complexity of amphibian life cycles and the diversity of stressors impacting amphibians. I conduct the first comprehensive assessment of the amphibian density-dependence literature and a first evaluation of potential correlates of risk to illustrate the potential of this framework. Finally, I conclude by identifying next steps amphibian ecologists need to take to advance this approach, including creating a database for amphibian population and life-history data, which has lagged behind what is available for other taxa.

In Chapter 2, I examine the impacts of climate change on the larval stage of three anurans in greater detail. Increased surface temperatures are projected to warm water temperatures and decrease water inputs, leading to earlier and faster wetland drying (Carey and Alexander 2003), 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. Further, amphibian larvae have varying tolerance to warming and responsiveness to desiccation (e.g. Duarte *et al.* 2012); therefore, how these two stressors will impact larval development also depends on whether species may exhibit phenotypic plasticity in response to pool drying.

I conduct a mesocosm experiment to assess the individual and interactive effects of pool permanency and water temperature 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*)). I find that although tadpoles in warmed pools reached metamorphosis 15–17 days earlier, they did so with little cost (<2mm) to size, possibly because greater periphyton growth in warmed pools mediated drying-induced resource competition. Warming and drying combined to act antagonistically on early growth and survival, meaning the combined impact was less than the sum of the individual impacts, and additively on time to and size at metamorphosis. These non-synergistic impacts may result from cotolerance of larvae to warming and drying as well as warming helping to offset negative impacts of drying, and indicate that combined pool warming and drying may not always be harmful for larval amphibians.

I also find that relative differences in the magnitude of individual warming and drying effects are consistent with the species' contrasting fast-to-slow life-history strategies. Chorus frogs may not be especially vulnerable to rapid drying exacerbated by climate warming. The slow developing red-legged frog, however, is more constrained in its ability to respond to rapid drying and demonstrates a higher physiological thermal time requirement than the other two species. These findings highlight the importance of considering the nature of multiple-stressor interactions as amphibians are exposed to an increasing number of anthropogenic threats.

Both chapters were written as manuscripts to be submitted for publication in scientific journals and were therefore written in the first person plural. "We" refers to myself and my co-authors. I executed the field and experimental work, and participated in a primary role in the conceptualization, analysis and writing of this thesis. W.J. Palen supervised both chapters. S.C. Anderson assisted in analysis and data visualization for Chapter 2.

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1. A framework for prioritizing amphibian decline risk: linking density-dependent bottlenecks to stage-specific stressors

1.1. Abstract

Enigmatic amphibian declines have stimulated tremendous research into drivers of decline. Over a decade of research, however, has revealed few lessons for preventing declines in advance of their occurrence. We propose a conceptual framework for assessing amphibian decline risk that is based on identifying when spatial and temporal overlap of stage-specific density-dependent bottlenecks, life-history traits, and stressors lead to increased vulnerability. We illustrate the potential utility of this framework by summarizing empirical amphibian density-dependence data from the literature (n=105 studies). While data deficiencies currently limit a full evaluation of this framework, we identify measures to advance this prioritization approach, including changes to study designs and creation of a relational database for amphibian population and life-history data. Such information could accelerate our ability to evaluate threats, even to poorly-studied amphibians, extending the value of individual studies and broadening the scope of conservation efforts beyond species-by-species triage.

1.2. Amphibian Declines

Amphibians are declining globally (Houlahan et al. 2000) and have been identified as one of the world's most imperiled taxonomic groups. According to the IUCN Red List, 41% of amphibians are threatened (sum of the categories Critically Endangered, Endangered, and Vulnerable plus a proportion of the Data Deficient species), a figure that exceeds that for birds or mammals (Hoffmann et al. 2010). Overexploitation and habitat loss are among the primary causes of amphibian population decline; however, of the 427 amphibian species listed as Critically Endangered, 92.4% are undergoing enigmatic declines (Stuart et al. 2004). Extensive research has sought to determine the stressors driving enigmatic declines. For example, studies have attributed population extinctions in Australia and the Neotropics to emerging infectious disease, climate change, and synergistic interactions between these and other stressors (reviewed in Collins 2010). Other efforts have linked declines to elevated solar UV-B (Blaustein et al. 1994; but see Palen and Schindler 2010), chemical pollutants (Davidson et al. 2002), or acidification (Leuven et al. 1986). The case-specific, and often species-specific, nature of these and many other studies suggest that we have a limited ability to anticipate which amphibian populations are most likely to decline.

1.3. The Need for a Predictive Framework

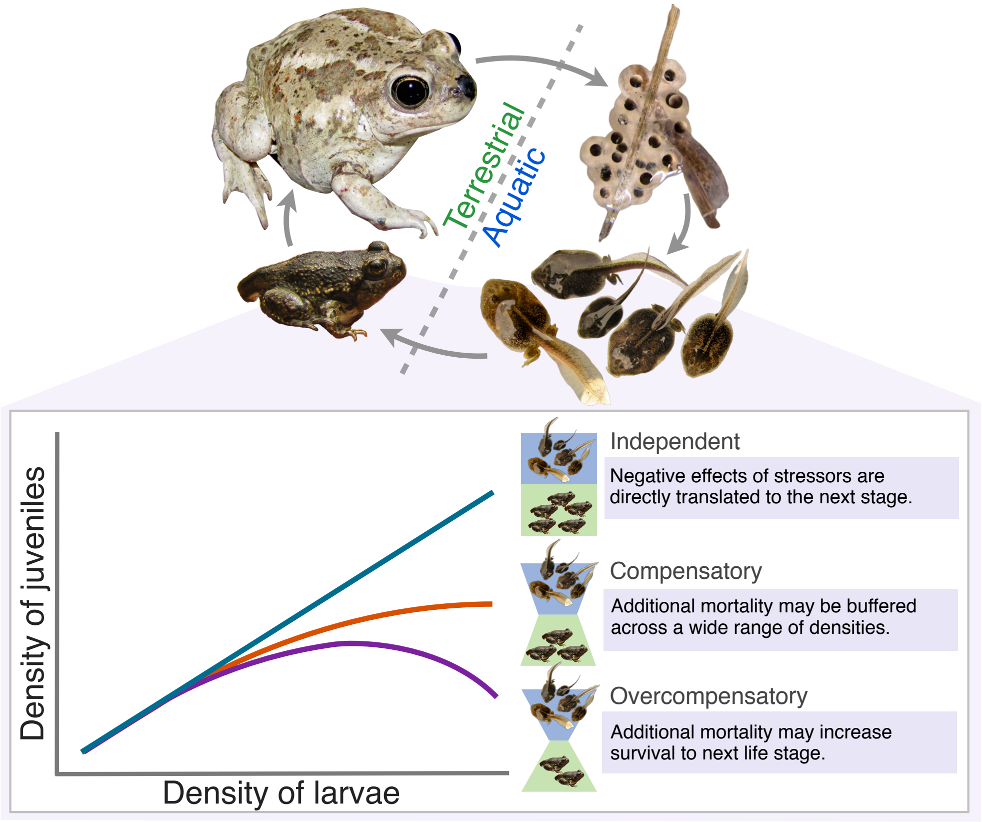
Moving beyond retrospective amphibian decline studies and reactionary management requires tools to categorize patterns of intrinsic sensitivity among species as well as their overlap with potential extrinsic drivers of decline. We propose a conceptual framework that links the assessment of amphibian risk to anthropogenic stressors with identification of important density-dependent bottlenecks in species' population dynamics. We begin building this framework by outlining how amphibian life-history diversity and tradeoffs in life-history traits may be correlated with decline risk, similar to that reported for fish, mammals, and birds (Anderson et al. 2011). We highlight that we have given less thought to how the complex life cycles of many amphibians may institute stage- or transition-based intrinsic sensitivities unique to this vertebrate group. Complex life cycles lead to stage-based density-dependent processes, which may be strong enough to act as bottlenecks that limit survival to subsequent life stages. Drawing on theory from other taxa, we argue that the spatial and temporal interplay between these density-dependent bottlenecks and stressors ultimately determines the risk of a stressor causing negative population-level impacts.

Life-history theory posits that every organism's population dynamics are fundamentally constrained by trade-offs in life-history traits (Law 1979). Organisms allocate energy to survival, growth, and reproduction in ways that reflect diverse solutions to producing on average one offspring that successfully reproduces per individual. Because metabolism obeys the laws of mass, energy balance, and thermodynamics, not all combinations of traits are energetically possible (Brown et al. 2004). For example, species with rapid growth and early maturation typically achieve smaller maximum body sizes. Those with higher rates of mortality and shorter lifespans must produce more offspring per reproductive event. In total, these traits generate birth and death schedules that form the basis of population demography and set the stage for different responses of populations to stressors (Dulvy and Forrest 2010).

Amphibians exhibit exceptional diversity in life-history traits, and consequently species are not expected to share the same intrinsic sensitivities to stressors. Recent analyses have correlated several amphibian life-history traits and environmental variables with increased IUCN threat status or population decline, including small geographic range (Cooper et al. 2008, Sodhi et al. 2008), large body size (Sodhi et al. 2008), and—specific to *Batrachochytrium dendrobatidis* fungus-related decline—possession of aquatic life stages (Bielby et al. 2008). These studies make coarse-scale recommendations for prioritizing conservation effort (e.g., protection of areas with abundant range-restricted species) but do not predict when a stressor will cause negative population-level effects. The coarse scale of such analyses necessitates that fundamental controls on population dynamics, such as the presence of complex life cycles, are not explicitly considered. A majority of amphibians exhibit complex life cycles, where population-level stability is dependent on individuals successfully transitioning through many life stages. These transitions often occur between different habitat types, creating physiological and ecological challenges. This complexity also leads to sequential stage-specific density-dependent processes, which mediate the connection between life history and population-level dynamics to produce the realized population growth rate.

Survival, growth, and reproduction are said to be density-dependent processes if these rates change as a function of the density of individuals. In amphibians, negative responses to high densities, including decreased survivorship and growth, are attributed to increased intraspecific competition for food (e.g., Brockelman 1969), cannibalism (e.g., Wildy et al. 2001), and growth inhibition cues released by larval conspecifics (Licht 1967). Amphibian ecologists have long studied density-dependent processes, yet the role of density dependence as a fundamental regulator of population growth in an applied context is comparatively poorly assessed.

In stage-structured life cycles, density-independent survival is characterized by a linear relationship between survival to the next life stage and density. However, bottlenecks of strong intraspecific competition can occur along a continuum of density-dependent survival, which is often categorized as one of two forms: *Compensatory*, characterized by an asymptotic curve where survival rises less steeply with increasing density; and *Overcompensatory*, characterized by a dome-shaped relationship between survival and density, whereby survival eventually declines as density increases (Hilborn and Walters 1992). Thus far, most studies have examined mechanistic stressor effects on specific stages with less consideration for how stage-specific density-dependent bottlenecks might confer population-level resilience to perturbation (Fig 1.1). With density-independent survival, the effects of stressors on one stage are directly translated to the next, and potentially to emergent population dynamics. In contrast, a life stage that exhibits compensatory density-dependence may buffer additional mortality across a wide range of densities. For instance, simulations of empirical data by Vonesh and De la Cruz (2002) showed that when egg-stage survival was reduced to simulate the impact of a stressor, the stressor did not necessarily result in fewer individuals metamorphosing to the terrestrial stage (metamorphs) because it sufficiently reduced larval competition to yield the same number of surviving individuals as at higher larval densities. A life stage that exhibits overcompensatory density dependence at high densities acts as an even stronger bottleneck; stressor-induced mortality at these high densities may actually increase survival to the next life stage. For example, Govindarajulu et al. (2005) demonstrated that overcompensatory density dependence may occur in larval invasive American bullfrogs (*Lithobates catesbeianus*) in British Columbia. Therefore, control efforts that remove tadpoles can lead to higher numbers of tadpoles reaching metamorphosis due to decreased density-dependent competition. Overall, the potential for stressors to negatively impact population growth rates is a function of the form and strength of density dependence, the magnitude of stressor impacts, and whether stressors occur before or after density-dependent bottlenecks.



Negative effects of stressors are directly translated to the next stage.

Additional mortality may be buffered across a wide range of densities.

Additional mortality may increase survival to next life stage.

Figure 1.1. Both density-independent and density-dependent processes may operate within any stage of complex life cycles. For instance, a density-independent response of larval survival to increasing larval density is linear; more larvae present results in more metamorphs produced. Density-dependent survival, either of the compensatory or overcompensatory form, is evidence of strong intraspecific competition that may act as a population bottleneck within a complex life cycle, dampening the population-level effects of stressors in that life stage.

1.4. Lessons From Other Taxa

In other taxa, we have gained insight into population responses to perturbations by combining population dynamics theory with knowledge of life-history trade-offs. In fisheries management, incorporating density dependence into stock assessments has been standard practice for over half a century (Beverton and Holt 1957). Stock assessments make quantitative predictions about the response of fish populations to harvest and inform authorities about how much fishing to permit. Stock-recruitment models are fit to empirical data to quantify the relationship between the number of individuals reproducing in a given year (i.e., spawning stock size) and recruitment of juveniles (recruits, typically one to three year olds) produced in a single year class. Fisheries stock-recruitment models include density-dependent parameters because it is recognized that many biological processes (resource competition, disease transfer, etc.) have density-dependent effects on survival of eggs, juveniles, or adults (Hilborn and Walters 1992). They also increasingly incorporate considerations of habitat availability (Bello et al. 2005) and potential trophic feedbacks between exploited species (Pauly et al. 2000). Typically, stocks with the strongest density dependence withstand the greatest mortality levels and provide the largest yields. However, the specific form and strength of density dependence is difficult to estimate in wild populations, so life-history theory has provided a bridge between demography and species' biology (Beverton and Holt 1957). For example, body size and age at maturity have been correlated with the strength of density-dependent recruitment and are used to predict how species' will respond to exploitation (Goodwin et al. 2006). This approach has also been extended to chondrichthyan management (Dulvy and Forrest 2010). Chondrichthyans are typically slow growing, late maturing, and dependent on high survival of few young. When producing fewer young, survival of early life stages must be greater to maintain a stable population size, and consequently we expect the strength of density-dependent survival to be weak. This limited compensatory density-dependent response of most chondricthyans to early mortality makes them especially vulnerable to fishing impacts, which is why they are second only to amphibians in threat status (Hoffman et al. 2010).

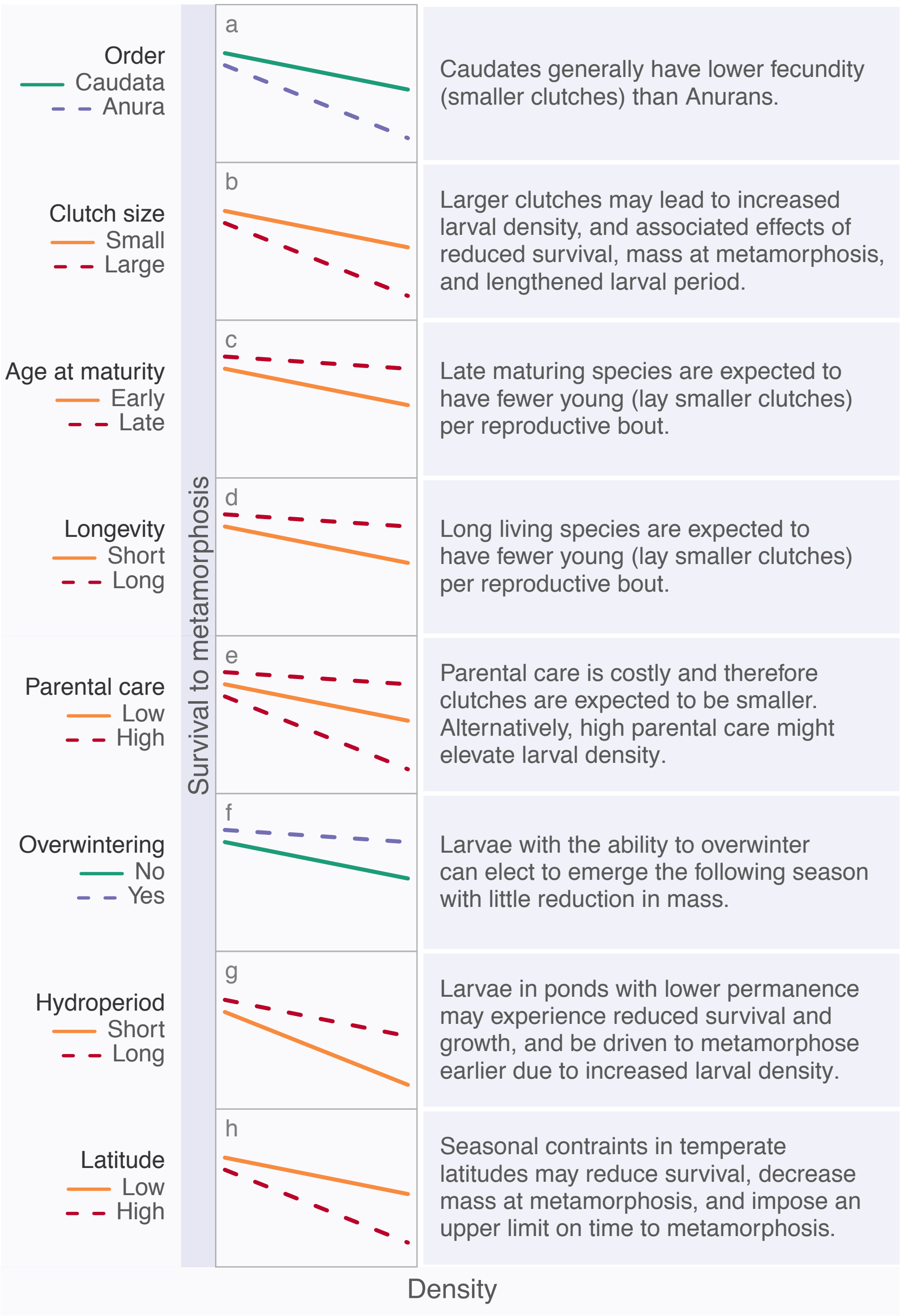
Here, we focus on the fisheries model because it is the most studied and well developed. However, the principles underlying fisheries models are common biological phenomena. The relationship between density dependence and life-history strategies has been acknowledged to have implications for understanding fundamental controls on population growth rates and for making predictions about how populations or species will respond to environmental change for mammals (Fowler 1981), insects (Stubbs 1977), and birds (Sibly et al. 2005). Recently, Vonesh and De la Cruz (2002) have shown that amphibian species with larger clutch sizes experience stronger larval density dependence. This suggests that the relationships that exist for fish between life-history traits (besides fecundity) and density-dependent population dynamics may similarly exist for amphibians. Yet, to date, such analyses have not been expanded upon to investigate if these relationships might also exist for amphibians.

1.5. Tailoring a Priority-setting Framework for Amphibian Conservation

The development of life history and density dependence correlates of species' sensitivity for use in fish management highlights the potential to develop a similar framework for amphibians. However, such a framework would require substantial expansion to reflect the complexity of amphibian life histories and the diversity of stressors impacting amphibians.

Fisheries management typically focuses on a single stressor (fishing) and aims to maximize sustainable removal of adults from populations over time. Reproducing adults, not other life stages, are thus generally controlled through management. Consequently, fisheries stock-recruitment modeling is usually generational rather than stage-specific, aggregating across many life-history transitions (Hilborn and Walters 1992). In contrast, in addition to exploitation, many classes of often stage-specific and geographically non-random stressors are known to cause amphibian mortality (Table 1.1). As a result, to understand patterns in species' sensitivities to stressors and predict population-level effects, we must explicitly consider the spatial and temporal overlap of stressors with individual life stages. For example, if mortality or reproductive output in a given life stage due to chemical pollutants or habitat loss (Table 1.1) can be estimated, then it is important to have some knowledge of the strength of density dependence operating in the impacted life stage to accurately project population-level consequences of that mortality.

Risk frameworks have proposed that vulnerability to stressors is a function of exposure to the stressor and intrinsic sensitivity (Williams et al. 2008). Likewise, we propose that we could develop correlates of vulnerability to stressors by (1) identifying extrinsic patterns of stressor occurrence, and (2) using the strength and stage-specificity of density dependence as proxies for intrinsic population-level sensitivity. We can then evaluate how life-history traits and phylogeny strengthen or weaken this density dependence. Though life-history traits may be plastic, we posit they may interact predictably with density dependence and that the two, together, are determinants of amphibian survival to subsequent life stages (Fig. 1.2).



Caudates generally have lower fecundity (smaller clutches) than Anurans.

Larger clutches may lead to increased larval density, and associated effects of reduced survival, mass at metamorphosis, and lengthened larval period.

Late maturing species are expected to have fewer young (lay smaller clutches) per reproductive bout.

Long living species are expected to have fewer young (lay smaller clutches) per reproductive bout.

Parental care is costly and therefore clutches are expected to be smaller. Alternatively, high parental care might elevate larval density.

Larvae with the ability to overwinter can elect to emerge the following season with little reduction in mass.

Larvae in ponds with lower permanence may experience reduced survival and growth, and be driven to metamorphose earlier due to increased larval density.

Seasonal contraints in temperate latitudes may reduce survival, decrease mass at metamorphosis, and impose an upper limit on time to metamorphosis.

Figure 1.2. Hypothetical predictions (a-h) and their mechanistic explanations (text) about how we expect life-history traits (a-f) and environmental factors (f-h) to affect the strength of density-dependent response in larval survival. Dashed lines and colors within life-history-trait panels represent the anticipated effect of different continuous (orange and red) and categorical (green and blue) life-history-trait values.

1.6. A First Evaluation

We conducted the first comprehensive assessment of the utility of this approach for amphibians by (1) summarizing currently published patterns in amphibian density dependence across taxa and life stages, (2) fitting stage-specific density-dependent models to the subset of studies with enough data points ($n \geq 4$), and (3) identifying necessary next steps to advancing a density-dependence- and life-history-based approach to prioritize population vulnerability to stressors.

1.6.1. Literature search

To characterize the state of understating of amphibian density dependence, we conducted an exhaustive search of peer-reviewed literature (published 1930-2010) in ISI Web of Science and Google Scholar for studies where stage-specific densities of amphibian populations had been manipulated in experimental or natural settings and that estimated stage-specific responses (e.g., survival, time to metamorphosis) to density, or that quantitatively followed cohorts of individuals through time (time-series).

We identified 71 papers, comprising 105 studies. The majority of studies were experimental (82%, either field enclosure, cattle tank, or laboratory), yet there were also time-series datasets (18%) that tracked cohorts entering and leaving life stages. Strong patterns in the focus of these studies are evident (Fig. 1.3), with studied species almost exclusively from families Ranidae (32%), Ambystomatidae (22%), and Bufonidae (18%). Over 90% of studies were single-stage studies, and did not examine the carryover effects of density dependence in subsequent stages. Almost 80% pertained to larval density dependence, primarily pond-breeding Anurans from temperate latitudes. We also found that studies have measured density effects for 29 different response variables, from locomotor capacity to the occurrence of cannibal morphs (Table 1.2). But, the most commonly studied of these is density impacts on larval survival. To fit basic density-dependent models, we determined that a minimum of four density levels is required, and less than 30% of all 105 studies met this criteria (Fig 1.3). Given this, only 10 studies were suitable for this purpose; seven of which were larval-stage studies, and four of which were North American Ambystomatids. Because of this current data limitation, we used these studies to illustrate the potential of this framework and the methods required to estimate the strength of stage-specific density dependence and evaluate correlations between density dependence and life-history traits.

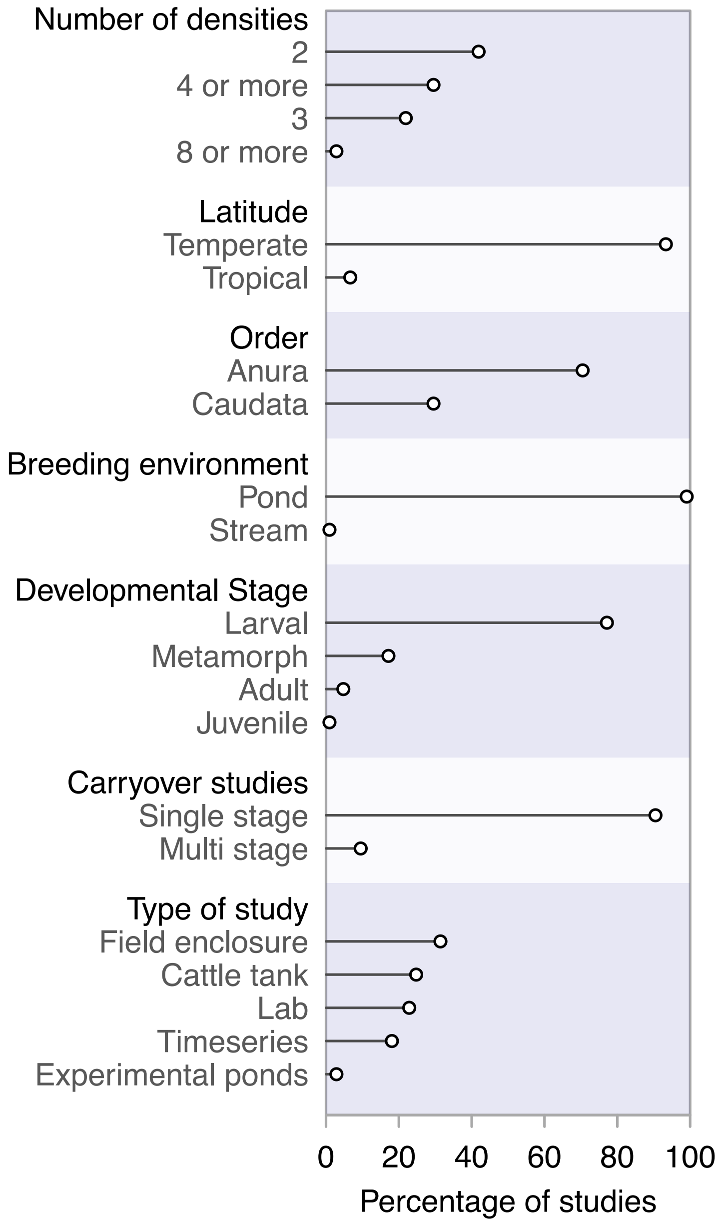


Figure 1.3. Summary of 105 peer-reviewed amphibian density-dependence studies. Points represent the percentage of the studies that had a specific study characteristic, within seven broader study attributes.

1.6.2. *Evaluating density dependence and life history correlations*

To illustrate the potential for correlations between life-history traits and the strength of density dependence, we focused on the seven larval-stage studies that met the above criteria (n ≥ 4). The sample size (n=3) for terrestrial-stage studies was too small to include. We quantified the functional relationship between density and survival to metamorphosis for these seven studies by calculating Compensation Ratios (CRs), a simpler method to estimate the form of density dependence than fitting stock-recruitment models that also requires less empirical data. CRs are calculated as the ratio of the slope at maximum (density-independent) survival, to the slope at the point of minimum survival (Goodyear 1977). A larger CR indicates stronger density dependence (stronger population bottleneck), and greater capacity to buffer stressor-induced mortality in that or earlier life stages. For example, a CR of 200 means that survival in that life stage would be 200 times greater in the absence of density dependence. The CR was then used as a comparative metric to evaluate correlations between density-dependent survival and taxonomic groupings (Anura, Caudata), life-history traits (fecundity, age at maturity, larval period), and environmental variables (breeding habitat type).

Many studies evaluated the effect of density on mass at metamorphosis and time to metamorphosis (Table 1.2), two response variables used to make inferences about survival and fitness. Again, the majority of these had too few density levels to calculate CRs. However, we collated available life-history information (clutch size, parental care, mean female size) for the species in these studies and evaluated correlations between life-history traits and the strength of density-dependent responses in these two response variables using an alternative method. We estimated the relative slope of the change in the response variable across the study densities to examine whether combinations of life-history traits predicted the magnitude of the relative slope (n = 34 for mass at metamorphosis, n = 34 for time to metamorphosis). We did all analyses with R (version 2.13.2, R Development Core Team 2011).

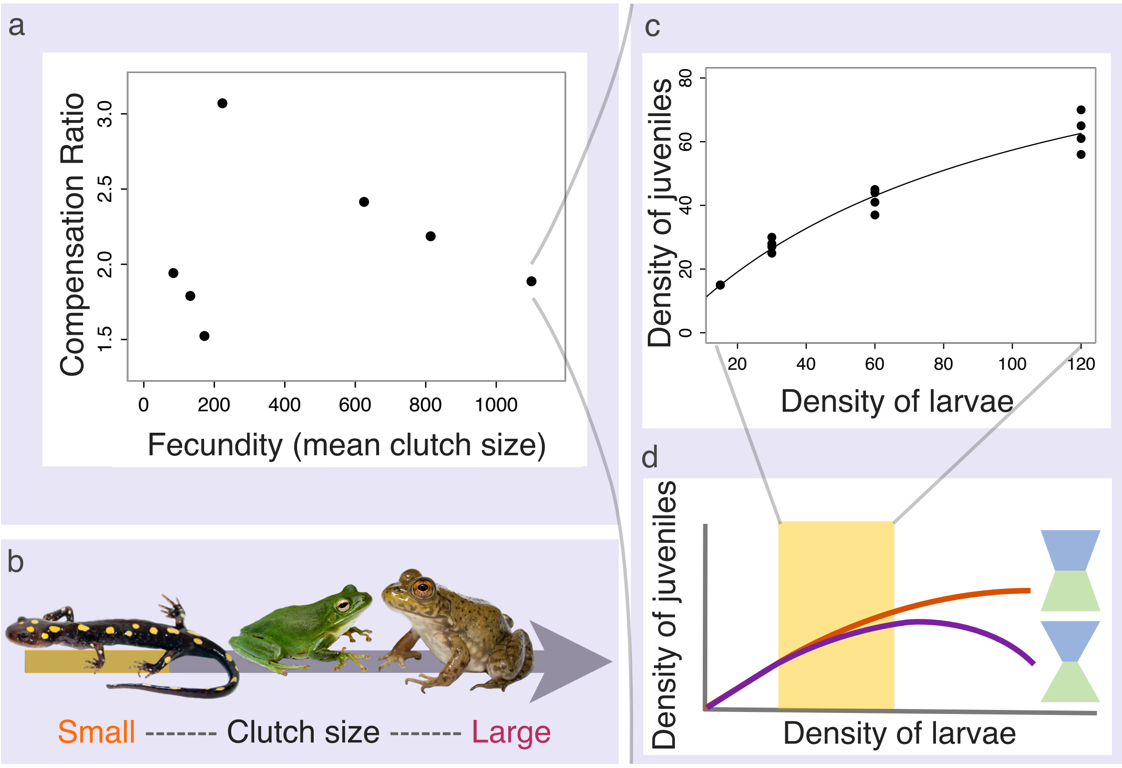


Figure 1.4. (a) Compensation ratios (CRs, n=7) that we could estimate from 105 published amphibian density-dependence studies. (b) Studies from which CRs were calculated span only a short portion of the possible life-history continuum (highlighted in yellow), and possess few data points for CR estimation. When data used to calculate a CR only reflect change in survival over a small range of densities (c), it can be difficult to make inferences about whether compensatory or overcompensatory density dependence is at work (as highlighted in yellow) (d). Photos © Brian Gratwicke.

Our analyses did not reveal clear patterns between the strength of density dependence and the life-history traits we examined. For example, we did not see the positive relationship between larval-stage CR and mean clutch size that life-history theory and Vonesh and De la Cruz (2002) predict (Fig. 1.4). Likewise, we could not support our predictions about how life-history traits might strengthen or weaken density-dependent effects on time to metamorphosis and mass at metamorphosis (Fig. 1.5). However, given the limited number of suitable studies we had available, these relationships were not assessed with any power and should be reevaluated with more data (e.g., from unpublished work). We did find that amphibian CRs for the 10 usuable studies ranged from 1.5 to 4.7, which is much lower than the range observed for other taxa (5-20 for elasmobranchs (Forrest and Walters 2009) and typically 3-100 for teleosts (Goodwin et al. 2006)). This suggests that amphibian compensatory response may be more constrained than in fish, though with few species included in our analyses this should be considered a preliminary finding.

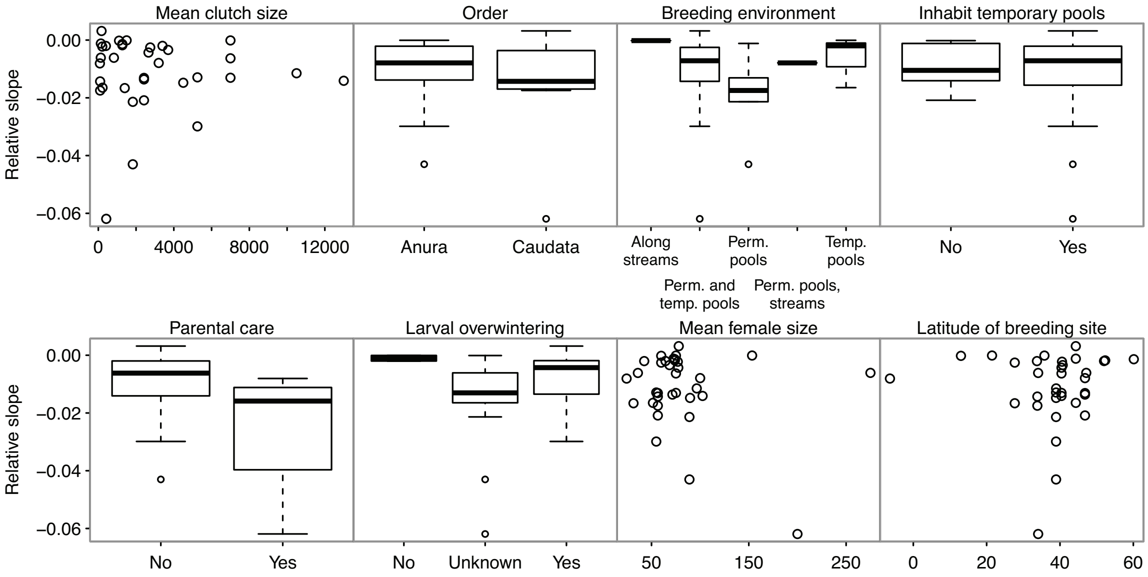


Figure 1.5. Relative slopes calculated from studies that measured the response of mass at metamorphosis to increasing density (n=34) plotted against life-history traits, phylogenetic, and environmental factors. A more negative relative slope indicates a stronger effect of increased density on mass at metamorphosis (stronger density dependence).

1.7. Research priorities in life histories, demography, and density dependence

An increasing weight of theoretical and empirical evidence suggests density-dependent bottlenecks and life-history strategies strongly structure amphibian lifecycles (e.g., Vonesh and de la Cruz 2002; Harper and Semlitsch 2007; Berven 2009). Though we found limited quantitative evidence for the link between life-history traits and stage-specific density dependent bottlenecks, our results should be seen as an illustration of the methods given an extremely limited sample size. A full evaluation of whether this framework will serve as a useful tool for prioritizing vulnerability requires overcoming three main challenges: (1) expanding traditional study designs, (2) adapting methods of density-dependence estimation for amphibian datasets, and (3) facilitating access to amphibian population and life-history data.

First, most existing empirical studies are species-specific and conducted using diverse methodologies, which makes comparative analyses difficult. Such comparisons help extend the utility of single-species studies to conserve poorly-studied species. Experiments often manipulate densities and control other variables that might otherwise confound density-specific responses, but rarely include four or more densities, which are needed to quantitatively estimate density dependence (stock-recruitment models or CRs). Though experiments with fewer densities can yield mechanistic insights into responses of amphibians to changes in density, they have limited value in the current context because they cannot be used to quantitatively estimate developmental stages' sensitivity to stressors. Similarly, time-series datasets can also be excellent sources for obtaining data suitable for estimating density dependence, but must span four or more years, include a range of densities, and record the number of individuals both entering and leaving a life stage (see Berven 2009 for example). Promoting long-term population monitoring of this nature may be more useful than density-dependence experiments because data from such studies can be used for a large variety of purposes beyond the current context (e.g., to monitor general population trends or to parameterize population models). Indeed, we foresee our proposed framework to be most useful for population-level management. Amphibian populations within a species may have different density-dependent dynamics, and density-dependence may vary from year to year depending on site-specific environmental variables (e.g., Saether et al. 1996). Regardless, populations within a species will share common life-history constraints that can serve as important baselines for making management decisions when detailed population-specific demographic data is lacking.

Second, amphibian ecologists may be better able to estimate density dependence with methods that are more forgiving given existing data limitations. Fisheries population time-series are typically longer and more common than most amphibian population data. Therefore, not all stock-recruitment functions developed for fisheries will be suitable for modeling more data-limited amphibian density-dependent processes. For example, the Shepherd model—which has been applied in a few amphibian studies (e.g., Vonesh and De la Cruz 2002, Harper and Semlitsch 2007)—has three parameters and therefore would require far greater than four densities or years to estimate. We recommend the theta-logistic model as alternative to the Shepherd model because it has only two parameters to estimate (Appendix A.1). Likewise, CRs require less data to quantitatively estimate density-dependence than stock-recruitment functions.

Third, only limited amphibian population data exists in published literature. We expect that amphibian biologists have collected datasets that remain unpublished. Such studies may potentially transform the utility of this framework given present data availability and we propose ecologists would benefit from a centralized online database to collect unpublished and published population and life-history data. Relational databases such as Fishbase (www.fishbase.org) and the Ram Legacy Stock Assessment Database (Ricard et al. 2012) have been foundational in advancing the same types of analyses for fish as we attempted here for amphibians. The Species Information Service (SIS), which informs the Amphibian IUCN Red List, contains some population and life-history data, however, the public cannot upload or retrieve this data. We hypothesize that concerns regarding attribution, rather than technological constraints, have largely impeded progress on data transparency and sharing, despite the responsibility to return products of publicly-funded research to the public domain. The amphibian decline problem has arguably become *the* crisis discipline within conservation biology (Lawler et al. 2006), and establishing such an outlet for information will no doubt accelerate research and conservation action.

1.8. Conclusions

The current gaps in our knowledge of the dynamics that govern amphibian life stages detract from our ability to prioritize which anthropogenic impacts are more or less likely to matter for populations and species. However, amphibian conservation may be able to advance from being a crisis discipline. We have identified key steps to advancing a more pro-active risk assessment framework based on knowledge of life-history theory, density dependence, and stressor occurrence. Central to this is the development of a public online database, which has thus far lagged behind that for other taxa. Moving forward in these areas will leave us better equipped to integrate empirical and theoretical knowledge and improve the efficiency of conservation efforts. Working alone to tackle any one of the many challenges amphibians face is a humbling experience, but by joining approaches and data from multiple researchers, taxa, and disciplines we can apply more innovative theoretical approaches to evaluate threats and anticipate population trends in advance of declines.

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1.11. Tables

Table 1.1. Stressors that cause amphibian mortality and their known patterns of occurrence across spatial, temporal, and ecological scales.

Threat	Life stage	Life-history traits	Habitat	Geographic pattern
Exploitation				
Food	Adults[1]	Large body size[1]	Asia[1]	
Pet trade	Adults Juveniles[1]		South America, Madagascar[1]	
Habitat loss	All		Tropical and temperate rainforests[2]	Small geographic ranges[3]
Climate warming	All		Aquatic habitats Drier habitats[4]	High latitudes, Low elevations[4] Low latitudes[5]
Acidification	Aquatic stages[6]		Temporary ponds[6]	
UV radiation	Aquatic embryos, larvae			High latitudes and elevations[4]
Chemical pollutants	All		Aquatic habitats	Proximity to agriculture[4]
Invasive species	Aquatic stages[7]		Permanent water bodies[7]	
Chytridiomycosis	Species with aquatic stage(s)[8,9]			Small geographic range[10] High elevation[9]

Sources: [1]Baillie et al. 2004, [2]Gallant et al. 2007, [3]Cooper et al. 2008, [4]Davidson et al. 2002, [5]Duarte et al. 2012, [6]Leuven et al. 1986, [7]Kats and Ferrer 2003, [8]Piotrowski et al. 2004, [9]Lips et al. 2003,[10]Bielby et al. 2008

Table 1.2. Density effects have been measured for 29 different response variables in the published amphibian density-dependence literature.

Response variable	Number of studies
Larval survival	67
Time to metamorphosis	37
Mass at metamorphosis	36
Size at metamorphosis	15
Growth rate	10
Body length by end of experiment	9
Mass at spring emergence	6
Body mass by end of experiment	5
Maximum mass reached before metamorphosis	3
Cannibalism (% larvae eaten)	2
Occurrence of cannibal morph	2
Mean Gosner stage by end of experiment	2
Offspring per tank	2
Reproductive development	2
Proportion of eggs that hatched	1
Final density by end of experiment	1
Number of adult females	1
Cumulative adult survival	1
Survival to first reproduction	1
Adult growth	1
Maximum size reached before metamorphosis	1
Time to maximum size	1
Locomotor capacity	1
Clutch size	1
Number of females returned to breed	1
Number of ova in paedomorphic females	1
Survival until end of September	1
Average size by spring	1
Survival until spring	1

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

2.1. 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 temperatures 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 (<2mm) to size, possibly because greater periphyton growth in warmed pools mediated drying-induced resource competition. Warming and drying combined to act antagonistically on early growth ($p = 0.06$) and survival ($p = 0.05$), meaning the combined impact was less than the sum of the individual impacts, and additively on time to and size at metamorphosis. These non-synergistic 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 *a priori,* which poses a challenge to

proactive conservation and management strategies. This study highlights the importance of considering the nature of multiple-stressor interactions as amphibians are exposed to an increasing number of anthropogenic threats.

2.2. Introduction

Over the past two decades, amphibian declines have been documented across diverse taxa and geographical regions (Wyman 1990, 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 ecotherms 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 0.1–0.16°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—climatic variables that directly affect amphibian development (Moore 1939, Smith-Gill and Berven 1979, 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 and rearing sites can elicit a wide range of responses among larvae of pool-breeding species (e.g., Berven 1990, Semlitsch et al. 1996). Temperature increases that shift species closer to but not beyond their thermal optima (Duarte et al. 2012) may increase larval metabolic and development rates. If resources are limited, higher metabolic demand and intraspecific competition during growth generally comes at the cost of 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 primary productivity. Such increases in resource availability with higher metabolic demand could allow larvae greater opportunities for growth (Cushing 1990, Winder and Schindler 2004), as well as mediate negative effects of intraspecific competition on size at metamorphosis (Newman 1998). By contrast, water temperatures that exceed species' thermal optima compromise enzymatic and physiological function, causing acute or chronic negative effects on growth, development, and swimming performance (Huey et al. 2009, Somero 2010).

The effects of climate change 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 patterns of precipitation and humidity (Winter 2000). 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 (Stewart 1995, Donnelly and Crump 1998, IPCC 2007). In particular, temporary pools are expected to dry more rapidly or disappear, and species that inhabited these environments (with sufficient dispersal ability), may have to move to find more permanent breeding ponds (Araujo et al. 2006). Further, permanent pools may become temporary (Lee et al. *unpublished manuscript*). Pool drying can be lethal for species if the rate of drying exceeds individuals' ability to accelerate development (Semlitsch and Wilbur 1988, Leips et al. 2000). A wealth of research documenting the effects of drying rate on larval amphibian physiology has shown that drying can also have non-lethal effects on post-metamorphic fitness. For example, with shrinking habitat during pool drawdown, the threat of desiccation often leads larvae to metamorphose earlier but at a smaller size (Wilbur and Collins 1973; Crump 1989). Alternatively, smaller size at metamorphosis 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 well studied individually, but few studies have evaluated the physiological and ecological challenges they present simultaneously 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 non-additive 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. *unpublished manuscript*). This assumption has been based on the fact that pool warming and drying are physical processes that naturally aggravate each other, 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 than under current climatic conditions (McMenamin et al. 2008, Lee et al. *unpublished manuscript*). 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 (Breitburg 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. We would therefore expect 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 in advance is also challenging because, across species, amphibian larvae have varying tolerance to warming and sensitivity to desiccation (Walther et al. 2002). For larval pool-breeding amphibians, successful metamorphosis depends on developing at a rate that reflects the permanency of their environment (Wellborn et al. 1996). Larvae with sufficient phenotypic plasticity (as defined by Newman 1992) may metamorphose earlier in response to a rapidly drying pool but delay metamorphosis when in long-duration pools (e.g., Semlitsch et al. 1990, Loman and Claesson 2003). Therefore, how pool warming and drying will impact larval development also depends on whether species may exhibit phenotypic plasticity in response to pool drying.

Here, we conducted a factorial mesocosm experiment to evaluate the effects of water temperature (ambient vs. +~3°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 examine 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 the combined effects of 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.

2.3. Methods

2.3.1. Species

In natural environments, the larvae of our three 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 BC) and prefer to breed in temporary pools in variable, arid environments (COSEWIC 2007). Pacific chorus frog (*P. regilla*) are habitat generalists, breed in both temporary and permanent pools, and have an intermediate larval period (8–10 weeks) (Nussbaum et al. 1983). Northern red-legged frog (*R. aurora*) are cold-water specialists, with a long larval developmental period (10–12 weeks) and low maximum and minimum 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 (April 26–May 6, 2012). We housed egg masses in shallow artificial pools under common outdoor conditions in natal pond water until they hatched.

2.3.2. Experimental design

We established experimental pond communities in 48 1000 L plastic cattle tanks at the University of British Columbia's Experimental Pond Facility (Vancouver, Canada) during spring 2012. We filled the tanks with city water to a depth of 28 cm on April 12 (~500 L) 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 64 μm conical net tow from a local natural pond to each tank on April 18. To initiate primary production, we added 10 $\mu g\ L^{-1}$ phosphorous as KH_2PO_4 and 160 $\mu g\ L^{-1}$ nitrogen as $NaNO_3$ per tank (Thompson and Shurin 2012). On May 8, we randomly assigned 30 tadpoles (1 species per tank) at Gosner stage 26 (Gosner 1960) to each tank. We installed mesh tops on all tanks to prevent macroinvertebrate colonization.

We randomly assigned the treatments of water temperature (no warming or warming), pool permanency (temporary or permanent), and species in a 2×2 factorial design and replicated each treatment four times for each species. We added 300 W submersible heaters (Hagen, Montreal, Canada) at the maximum temperature setting to warmed treatments one day prior to the introduction of plankton. Temporary pools were simulated by manually removing water with buckets once a week. We did not remove water from permanent tanks but they were disturbed with the same buckets to control for the effect of water removal activity.

2.3.3. Temperature, depth, chlorophyll-a measurements

Temperature loggers (Maxim/Dallas Semiconductor, Dallas, TX) (n=19) recorded hourly temperature until the tanks dried or the experiment ended. We also recorded temperatures in three egg mass collection sites to compare experimental temperatures with natural pond temperatures. We took weekly water depth measurements in all tanks. To sample periphyton growth as a metric of food availability, we installed plastic flagging tape along the side of each tank on May 17. We removed the flagging tapes on June 11 and July 11 (25 and 55 days into the experiment), wrapped them in tinfoil and froze them. We later scraped and filtered the periphyton from the tapes with distilled water onto Whatman GF/C filters, and measured chlorophyll-a concentration using methanol extraction fluorometry. We calculated periphyton availability as the chl-a content per unit substrate (µg cm^{-2}).

2.3.4. Tadpole growth and metamorphosis

On June 8 (day 31), we took photos of the first 15 tadpoles seen (or the maximum number remaining) in each tank to estimate early growth rates. We checked tanks every other day for metamorphosing frogs from day 37 until the experiment ended on August 29. We removed *P. regilla* and *R. aurora* metamorphs at stage 45–46 (Gosner 1960). Because *S. intermontana* tadpoles frequently cannibalize metamorphs (*personal observation*), we removed them at stage 43 and housed them in separate containers outdoors without food until stage 45. We weighed each metamorph to 0.001 g and measured snout-to-vent length (SVL) before they were euthanized with MS222. Just prior to temporary pools completely drying, we weighed, measured the SVL, and recorded the developmental stage of any remaining individuals.

2.3.5. Statistical analysis

We compared the effects of warming and drying on four response variables among species 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 B, Fig. B.1). The four response variables were tadpole growth to day 31, SVL at metamorphosis, mass at metamorphosis, and mean time to metamorphosis. For simplicity, we present the analysis of mass at metamorphosis in Appendix B, Fig. B.4, 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 (chl-a on June 11 and July 11), 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. We corrected for over-dispersion in the survival data by calculating the variance-inflation factor ($\hat{c}$), which was used to adjust the coefficient standard errors (McCullagh and Nelder 1989).

Our models expressed the 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 (AICc) (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 where ΔAICc = 0.7 and SVL where ΔAICc = 2.1, where ΔAICc $\lessapprox$ 2 indicates equivalent support (Burnham and Anderson 2002; Table B.1). Therefore, we only included two-way interactions as a consistent and parsimonious approach across all response variables. We fit all LMEs using the nlme package (Pinheiro et al. 2013) for R

(R Core Team 2012) with restricted maximum likelihood (REML). For AICc model comparison we fit the models with maximum likelihood.

To improve the interpretability of the species-level interaction coefficients (species × warming and species × 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* × 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 × 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 × drying coefficient = 0) (Darling and Côté 2008). If the warming × 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.

2.4. Results

2.4.1. Temperature and pool depth

The temperature and permanency treatments resulted in four climate scenarios with unique temperature and drying profiles (Fig. B.2; Fig. B.3). Over the course of the experiment, warmed pools experienced 2.8±0.8°C (mean ± SD) higher temperatures compared to ambient conditions in the permanent tanks, and a 3.1±0.7°C increase in the temporary tanks. Our experimental temperatures fell within the range that we observed in natural breeding sites (Fig. B.2). Permanent pools increased depth until early July due to precipitation, before decreasing through evaporation in late summer. Warmed, temporary pools dried completely by July 16 (day 60) and ambient temperature, temporary pools by August 1 (day 76).

2.4.2. Effects of pool warming

By day 31 of the experiment, tadpoles had grown faster in warmed pools (Fig. 2.1a, Fig. 2.2a,b, see Table B.2 for coefficients). *S. intermontana*, *P. regilla*, and *R. aurora* grew 3.3±0.6 (mean ± SE), 3.4±0.6, and 1.9±0.9 mm longer, respectively, in warmed compared to ambient temperature pools. In contrast, by metamorphosis such differences had disappeared and *S. intermontana* showed slightly reduced SVL (Fig. 2.1b) or mass at metamorphosis (Fig. B.4) when pools were warmed (1.7±0.5 mm smaller and 0.2±0.06 g less). Warming had a strong negative effect on time (days) to metamorphosis for all species (Fig. 2.1c, 2.2f). Larval period was reduced by 15.0±1.5, 17.4±0.6, 15.4±1.1 days 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 the warmed pools for *S. intermontana* and *P. regilla*, but not for *R. aurora* (Fig. 2.1d, 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 June 11 (day 34), periphyton availability (µg cm^{-2}) was almost two times greater (1.8 [95% CI = 0.9–3.3]) in warmed compared to ambient temperature pools (Fig. 2.3a,b).

2.4.3. 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±0.7 mm more; Fig. 2.1a). The time required to metamorphose was significantly reduced for *S. intermontana* (-6.0±1.4 days) and *R. aurora* (-4.2±1.1 days) when reared in temporary versus permanent pools, but not for *P. regilla* (Fig. 2.1c). *S. intermontana* and *P. regilla* emerged from temporary pools at significantly smaller sizes (SVL) compared to tadpoles in permanent pools (*S. intermontana* = -1.7±0.6 mm, *P. regilla* = -0.9±0.4 mm; Fig. 2.1b, 2.2c,d). *S. intermontana* also emerged 0.2±0.06 g lighter in temporary pools compared to permanent pools (Fig. B.4). 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. 2.1d). There was little evidence for an effect of drying on *R. aurora* survival (Fig 2.1d). All *P. regilla* and the majority of *R. aurora* and *S. intermontana* metamorphosed prior to the pools completely drying. In temporary pools, the 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 (Fig. B.5).

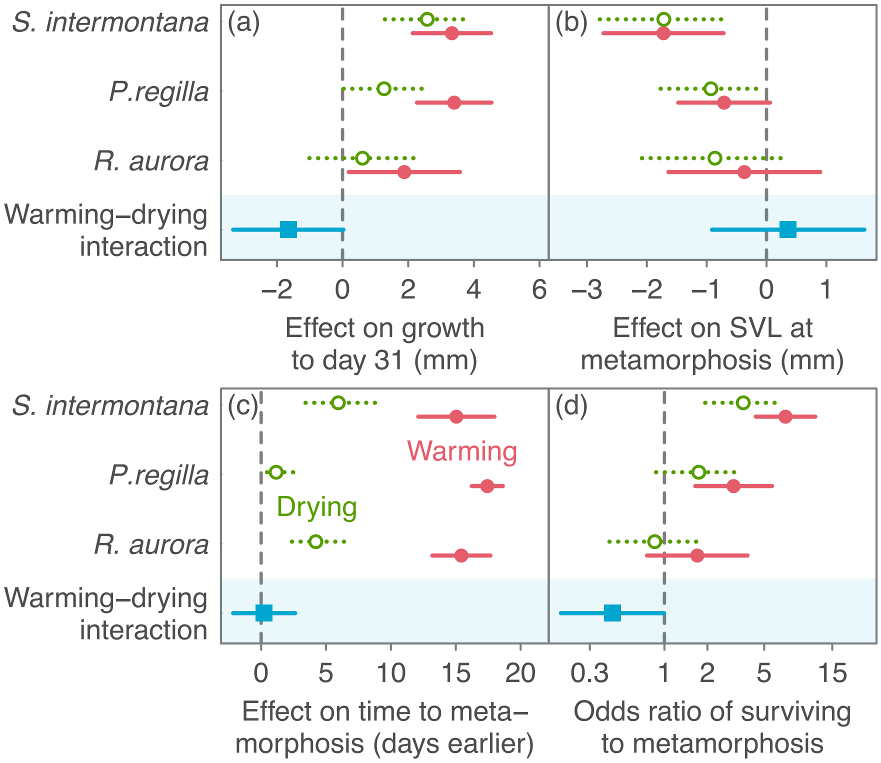

Figure 2.1. Species-level and interactive effects of warming and drying (coefficient values and 95% confidence intervals) on larval fitness-related traits. We show the main effects of warming (solid circles) and drying (open circles) for each species individually relative to no warming and permanent treatments (dashed vertical lines). In contrast, we show the species-independent interaction coefficient (shaded box) for warming x drying (solid squares) as a relative rather than absolute value. The interaction coefficient represents the deviation from an additive assumption of the two single factor effects (warming, drying), where a warming-drying interaction of 0 represents the null-hypothesis (additive).

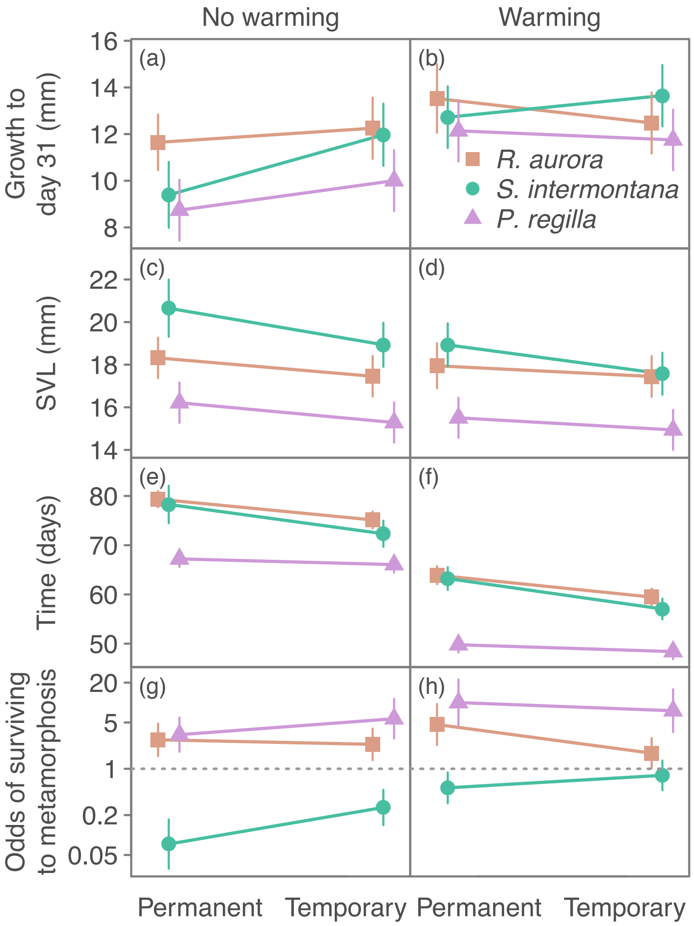

Figure 2.2. Model predicted means and 95% confidence intervals of larval fitness-related traits across three amphibian species. The dashed horizontal line in panels (g) and (h) represents equal odds; odds greater than one mean that the tadpoles were more likely to survive to metamorphosis than not survive.

2.4.4. *Combined effects of warming and drying*

Models including the three-way interaction of species, warming, and drying were not supported by AICc model selection (Table B.1). The combined effects of warming and drying on larvae were either antagonistic or additive. Warming and drying alone both increased early growth across species, but together had antagonistic effects on early growth. Tadpoles grew 1.7±0.8 mm less by day 31 than what we would expect if the individual warming and drying effects were additive (Fig. 2.1a). 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 mm + 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. 2.1b) and time to metamorphosis was only 0.2 days (SE) less (Fig. 2.1c) than if the effects were additive. Therefore, larvae in warmed temporary pools metamorphosed ~18–21 days earlier but only ~0.9–1.2 mm smaller (~3 mm for *S. intermontana*) than those in permanent, ambient temperature pools. Warming and drying together had antagonistic effects on survival (Fig. 2.1d). The odds of surviving to metamorphosis generally increased under the warming or drying treatments on their own, but when combined, the odds of surviving were less (odds ratio = 0.43 [95% CI = 0.21–0.99]) than the additive expectation. 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. 2.3a,c). We cannot define this interaction as antagonistic or synergistic given that periphyton strongly decreased with drying but increased with warming.

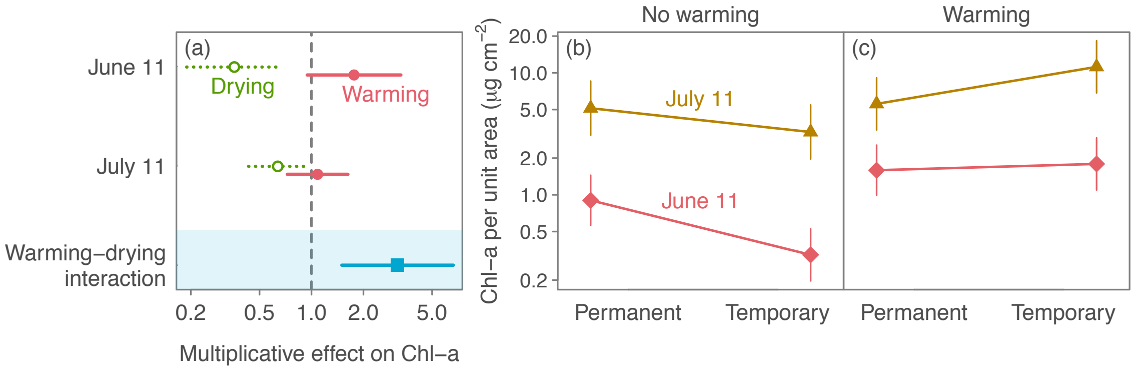

Figure 2.3. The effect of warming and drying treatments on chlorophyll-a content per unit substrate (µg cm^{-2}) at two time intervals. (a) We show the main effects (coefficient values and 95% confidence intervals) of warming (solid circles) and drying (open circles). The interaction coefficient for warming and drying combined (shaded box, square) represents the deviation from an additive assumption of the two single factor effects, where a warming-drying interaction of 0 represents the null-hypothesis (additive). Since this GLM was fit with a log link, the effects are multiplicative. (b, c) Model predicted means for chorophyll-a content across treatments and 95% confidence intervals. Samples are from June 11 (diamonds) and July 11 (triangles), 2012.

2.5. Discussion

An increasingly important challenge in conservation biology is predicting the cumulative impact of multiple stressors on ecological systems (Sutherland et al. 2009). When non-additive 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 sought to quantify the individual and interactive effects of increased water temperature and pool drying rate on three larval amphibians (*Spea intermontana*, *Pseudacris regilla*, and *Rana aurora*) that occur along a continuum of 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 to metamorphose far earlier and at much smaller size than what would occur if the individual effects of warming and drying were simply summed, or suffer greater mortality due to desiccation. This hypothesis was not empirically supported. We found additive or antagonistic effects of warming and drying on all larval response variables, which suggest that at worst, warming does not multiply the negative effects of drying (additive), and at best, may alleviate 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-history strategies.

We found the independent effects of warming and drying to be largely consistent with our *a priori* expectations. The effect 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). The effect of warming in reducing time to metamorphosis was about three times stronger than the effect of drying, suggesting that the drying rate may not have imposed as strong a constraint on development. Alternatively, increasing density-dependent competition as water was lost in the temporary pools may have had an opposing effect of lengthening the time required for larvae to grow to the minimum size needed for metamorphosis (Brockelman 1969, 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 the 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, survival was increased or unaffected, indicating that the species were within their abilities to respond to the drying environment.

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 larval emergence with little impact on size and mass at metamorphosis. Indeed, larvae in warmed temporary pools metamorphosed ~18–21 days earlier but only ~0.9–1.2 mm smaller (~3 mm for *S. intermontana*) than those in permanent, ambient temperature pools (Fig. 2.1b,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 (Fig. B.5). Second, antagonistic interactions between stressors are likely when the tolerance of a species 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 would be the case with larval amphibians, where thermal and desiccation tolerance are often linked (Newman 1992, Denver 1997). 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 would 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). Although our models did not assess species-specific three-way interactions because our data did not support the added model complexity, we propose that interpreting species-independent interaction coefficients is still informative because the response of each species to the main-effects were consistently in the same direction (Fig. 2.1). If any of our results should be interpreted with caution it would be whether we can label the interaction effects on *R.aurora* growth to day 31 and survival to metamorphosis as being antagonistic given that the main effects weren't both strongly in the same direction for this species.

The relative differences in the effects of warming and drying across our three study species are consistent with what is understood about their life-history strategies. *S. intermontana*, which experienced the greatest increase in survival to metamorphosis with warming or drying, is most 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. Additionally, differences in early growth response between the species are in keeping with the expectation that amphibians which exploit uncertain environments, such as *S. intermontana*, will respond with greater phenotypic plasticity; whereas species that tend to inhabit relatively certain 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: 37 to 80 days for *P. regilla* versus 45 days 85 for *S. intermontana*), and largely overlapped in time with *R. aurora* (range: 49 to 109 days). *S. intermontana* grow to a larger size and mass than the other two species (Fig. 2.2e,f); therefore, it is possible that they became resource limited under our experimental conditions. Alternatively, the drying rate may not have been rapid enough to initiate a more accelerated development response. In amphibians, life-cycle transitions are often dependent 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 before metamorphosing than was shown to be necessary for the species when in permanent pools (Fig. B.2), which suggests that they slow development in the absence of a rapid-drying cue.

Our results have the potential to provide insight into 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 and must accumulate a greater minimum number of degree-days to metamorphose (Fig. B.2). As we observed, warming may have beneficial effects on tadpoles by increasing periphyton availability, which may act to offset the challenge of accelerated drying rates. However, if *R. aurora* are subject to more variable and faster drying we project that they could suffer greater lethal effects than measured in this experiment. As a habitat generalist, *P. regilla* is expected to 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 to either of the two treatments than the other species. However, they are also restricted to arid, temporary wetlands that are already highly threatened by urbanization and agriculture, and that 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 (Fig. B.6). Future work could 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 non-linear. Additionally, we found that periphyton availability was highly 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 only a limited range of contrasts. First, we selected our three species based on average rates of larval development. However, plasticity in larval development traits often reflects the interannual variability in breeding habitat permanency and can vary across populations, temperatures, and pool permanence (Loman 2002, Loman and Claesson 2003, Richter-Boix et al. 2006, Maciel and Junca 2009). 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 consequences for population growth rates. Larger spadefoot metamorphs (Newman and Dunham 1994, Morey and Reznick 2001) and *P. regilla* (Schaub and Larsen 1978) have been found to 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 can be adapted to study the responses of other 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 have non-synergistic impacts on larvae 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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Appendices

Appendix A.

Supporting material for Chapter 1

Appendix A.1. Stock-recruitment model performance with amphibian density-dependence data

The traditional method of estimating the form and strength of density dependence is to fit stock-recruitment models to time-series data. We tested the performance of five commonly-used stock-recruitment models with the subset of 10 suitable amphibian studies we collated from the literature to determine whether any models stood out as being more appropriate for amphibian datasets. We followed a model selection approach to fit the models using nonlinear least squares regression and ranked support for the models using Akaike’s information criterion adjusted for small sample size (AICc). The five models were (1) linear $Y = aX + b$; (2) Beverton-Holt $Y = (aX)/(1+bX)$ (Beverton and Holt 1957); (3) Ricker $Y = aXe^{-bX}$ (Ricker 1954); (4) Theta-logistic $Y = X-(X^2/a)^b$ (Nelder 1961); and (5) Shepherd $Y = (aX)/(1+bX^c)$ (Shepherd 1982).

We found that the Theta-logistic model consistently emerged as a top-ranked model, whereas the Shepherd function, which has been applied in a few studies (e.g., Vonesh and De la Cruz 2002), rarely converged due to the small number of data points and the need to estimate three rather than two parameters, as with all other candidate models. When the Shepherd model did converge, there was high uncertainty in the parameter estimates and it was never the best-supported model because of the penalty imposed by the additional parameter.

Appendix B.

Supporting material for Chapter 2

Figure B.1. *Model fit residuals for each of the response variables across individual pools. Growth to day 31, SVL at metamorphosis, mass at metamorphosis, and time to metamorphosis are modeled with linear mixed effects models. Survival to metamorphosis is modeled with a GLM (generalized linear model) with a quasibinomial error structure and a log link. Colours indicate the warming and drying treatments.*

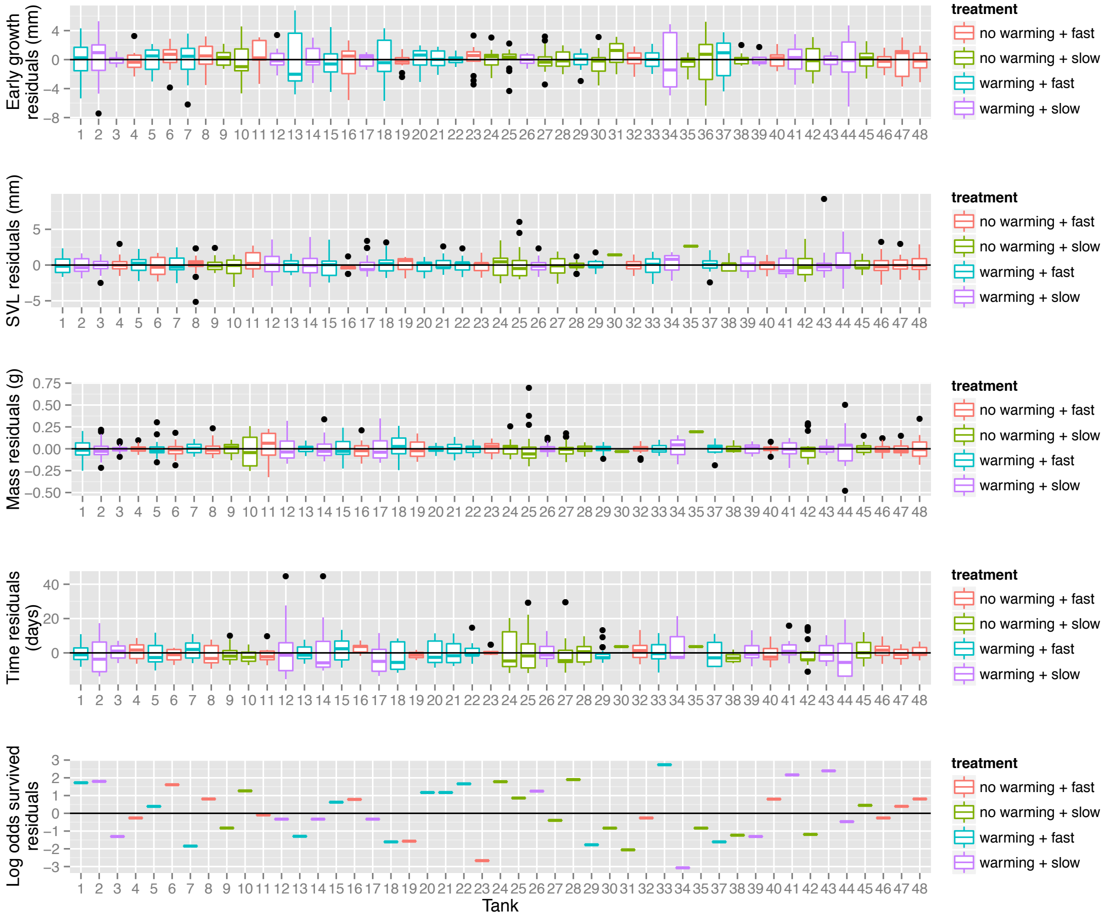

Table B.1. *AICc and QAICc rankings of candidate models with up to two or three-way predictor interactions. Models for growth to day 31, SVL at metamorphosis, mass at metamorphosis, and time to metamorphosis were compared with AICc. Models for survival were fit with a quasibinomial error structure and so are compared with QAICc. DF refers to degrees of freedom. Models with a maximum of two-way interactions model a common warming x drying interaction coefficient. Models with a maximum of three-way interaction model a separate warming x drying interaction coefficient for each species.*

Response	Log likelihood	DF	AICc or QAICc	ΔAICc or ΔQAICc	Maximum interactions
Early growth	-1457	14	2942.6	0	Three-way
Early growth	-1459.4	12	2943.3	0.7	Two-way
SVL	-1438.6	14	2905.8	0	Three-way
SVL	-1441.7	12	2907.8	2.1	Two-way
Mass	722.2	12	-1420.1	0	Two-way
Mass	723.8	14	-1419.2	0.9	Three-way
Time	-2842.7	12	5709.7	0	Two-way
Time	-2841	14	5710.6	0.9	Three-way
Survival	-120.2	10	131.7	0	Two-way
Survival	-119.1	12	138.3	6.6	Three-way

Appendix B.2. Temperature data and degree-days across treatments

Using the temperature data we logged for our pool treatments, we calculated degree-days as the number of degrees Celsius that the daily mean temperature exceeded a pre-specified developmental threshold of 10°C (Charnov and Gillooly 2003).

R. aurora and *P. regilla* degree-days corresponding with the median proportion metamorphosed was nearly the same (range of 567.17 to 599.36 and 440.26 to 491.26 degree-days for *R. aurora* and *P. regilla*, respectively), regardless of drying treatment (Fig. B.2). It appears that *P. regilla* has the lowest thermal time requirement for metamorphosis (440.26 to 491.26 degree-days). Warming hastened development time by allowing more rapid accumulation of degree-days. *S. intermontana* tended to remain in the pools longer when they were permanent, accumulating more than the minimum degree-days before metamorphosing, which suggests that these tadpoles slow development in the absence of a rapid-drying cue (range of 533 to 650 degree-days).

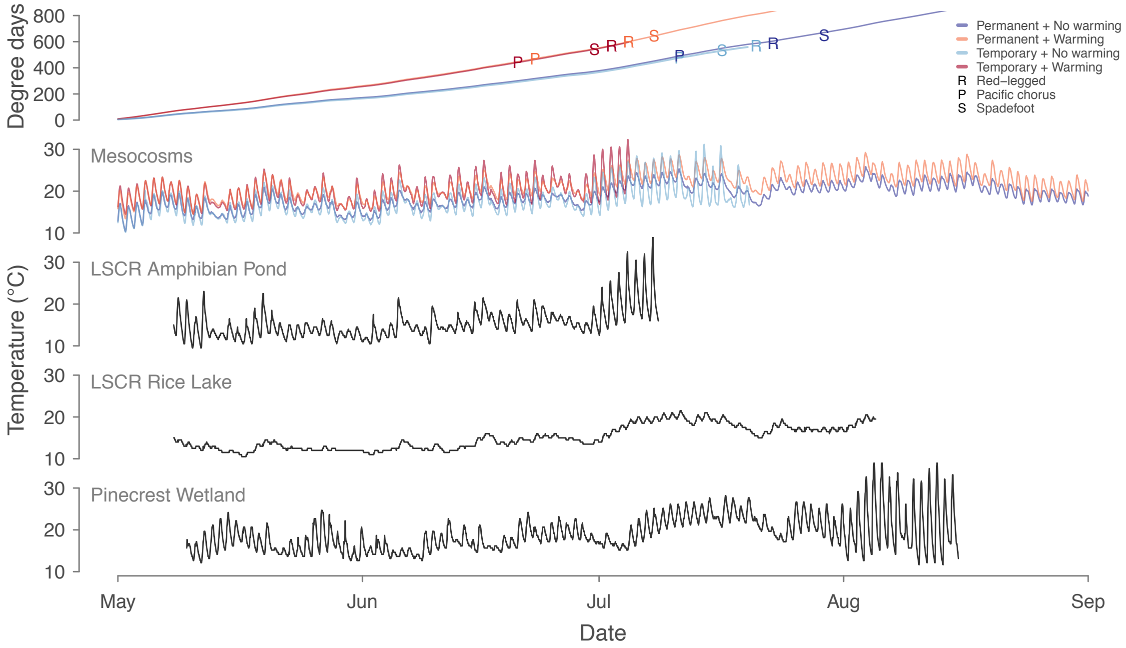

Figure B.2. *Temperature data logged hourly over the course of the experiment in the mesocosms and at three breeding sites where egg masses used in the experiment were collected. Mean temperatures of the treatments were also translated into cumulative degree-days. The cumulative degree-day on the date corresponding with the median proportion of tadpoles metamorphosed in each treatment is overlaid for each species (S, P, R).*

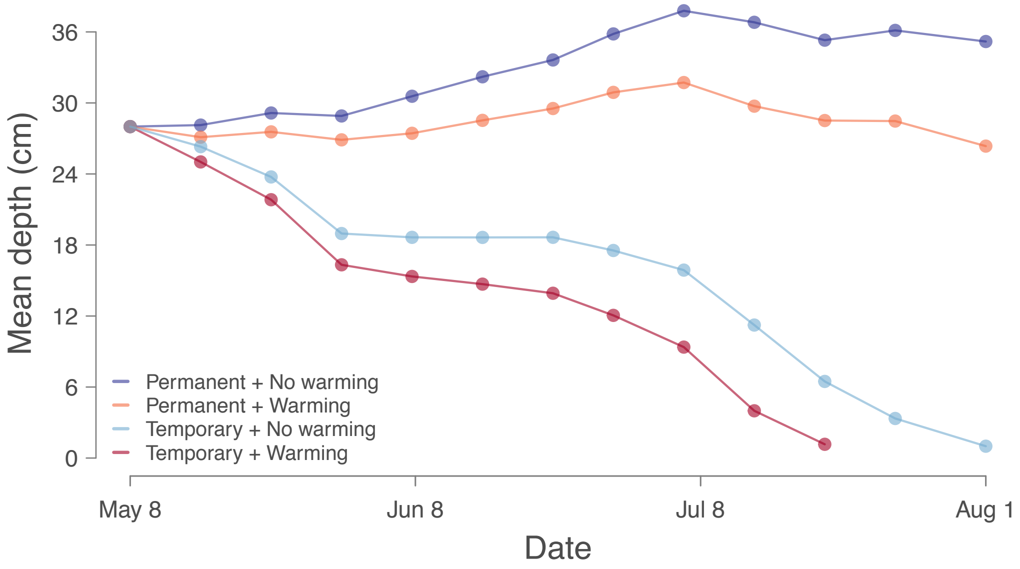

Figure B.3. *Mean depth (cm) of the pools in each treatment over time.*

Table B.2. *Coefficient estimates and 95% confidence intervals for each of the five response variables. Confidence intervals were calculated as the estimate ±1.96 times the standard error. Intercept, warming, and drying coefficients have been adjusted to reflect species-level means. For example, the intercepts for SVL at metamorphosis reflect the mean SVL without warming or drying for each species; the warming coefficients for SVL at metamorphosis reflect the mean change in SVL compared to the intercept for each species.*

Response	Coefficient	Species	Estimate	Lower	Upper
Growth to day 31 (mm)	Intercept	R. aurora	11.64	10.45	12.84
	Intercept	S. intermontana	9.39	8.09	10.69
	Intercept	P. regilla	8.74	7.54	9.94
	Warming	R. aurora	1.88	0.18	3.57
	Warming	S. intermontana	3.33	2.13	4.53
	Warming	P. regilla	3.40	2.26	4.54
	Drying	R. aurora	0.61	-1.01	2.23
	Drying	S. intermontana	2.58	1.29	3.87
	Drying	P. regilla	1.26	0.03	2.50
	Interaction		-1.65	-3.33	0.03
SVL at metamorphosis (mm)	Intercept	R. aurora	18.32	17.37	19.27
	Intercept	S. intermontana	20.65	19.43	21.87
	Intercept	P. regilla	16.21	15.37	17.05
	Warming	R. aurora	-0.37	-1.64	0.90
	Warming	S. intermontana	-1.72	-2.73	-0.72
	Warming	P. regilla	-0.71	-1.48	0.06
	Drying	R. aurora	-0.86	-2.08	0.36
	Drying	S. intermontana	-1.72	-2.79	-0.64
	Drying	P. regilla	-0.93	-1.77	-0.09
	Interaction		0.36	-0.91	1.64
Mass at metamorphosis (g)	Intercept	R. aurora	0.50	0.39	0.62
	Intercept	S. intermontana	1.05	0.91	1.18
	Intercept	P. regilla	0.38	0.28	0.48
	Warming	R. aurora	-0.05	-0.21	0.10
	Warming	S. intermontana	-0.23	-0.34	-0.12
	Warming	P. regilla	-0.06	-0.16	0.03
	Drying	R. aurora	-0.04	-0.19	0.10
	Drying	S. intermontana	-0.24	-0.36	-0.12
	Drying	P. regilla	-0.07	-0.17	0.04
	Interaction		0.05	-0.10	0.21
Time at metamorphosis (days)	Intercept	R. aurora	79.35	77.70	81.00
	Intercept	S. intermontana	78.27	74.69	81.86
	Intercept	P. regilla	67.22	65.79	68.64
	Warming	R. aurora	-15.43	-17.67	-13.19
	Warming	S. intermontana	-15.05	-17.99	-12.11

	Warming	P. regilla	-17.43	-18.63	-16.23
	Drying	R. aurora	-4.22	-6.39	-2.04
	Drying	S. intermontana	-5.95	-8.78	-3.12
	Drying	P. regilla	-1.16	-2.47	0.15
	Interaction		-0.23	-2.62	2.16
Survival to metamorphosis (odds)	Intercept	R. aurora	2.74	1.55	4.83
	Intercept	S. intermontana	0.07	0.03	0.16
	Intercept	P. regilla	3.27	1.88	5.7
	Warming	R. aurora	1.7	0.76	3.83
	Warming	S. intermontana	7.05	4.36	11.4
	Warming	P. regilla	3.05	1.64	5.67
	Drying	R. aurora	0.85	0.41	1.78
	Drying	S. intermontana	3.57	1.93	6.6
	Drying	P. regilla	1.74	0.87	3.47
	Interaction		0.43	0.19	0.99

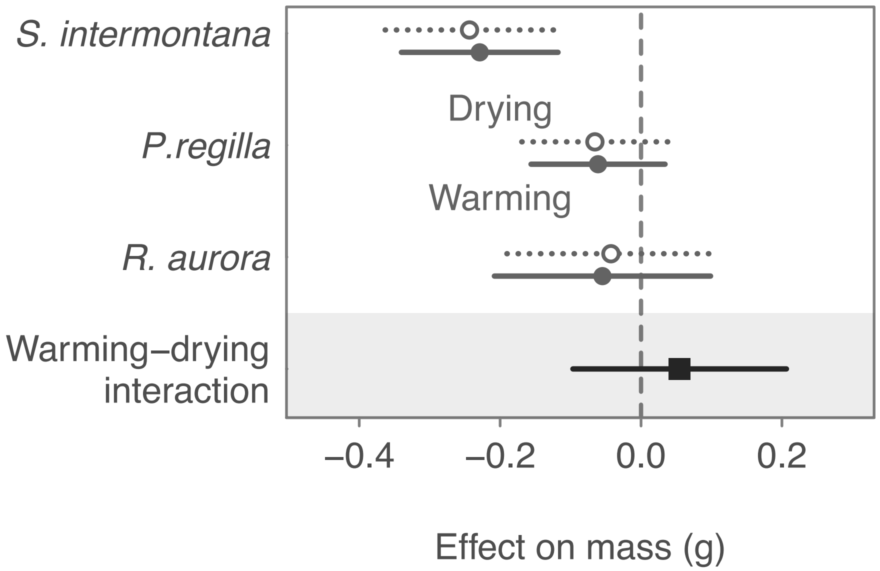

Figure B.4. *Species-level and interactive effects of warming and drying (model coefficient values and standard errors) on mass at metamorphosis.*

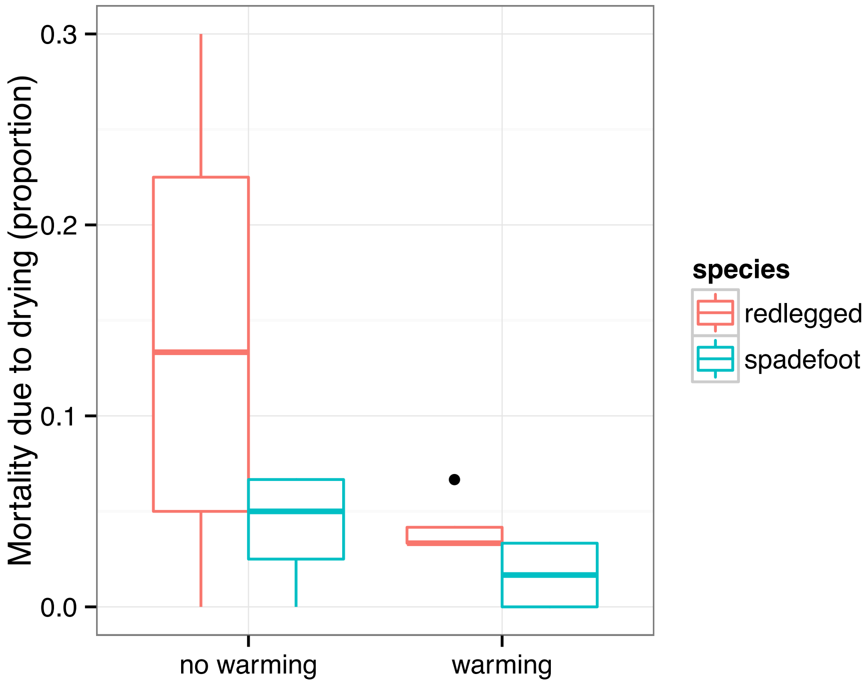

Figure B.5. *Boxplot of the proportion of Northern red-legged frog (R. aurora) and Great Basin spadefoot (S. intermontana) tadpoles that died due to drying in temporary pools (by tank). Midline is the median and upper and lower hinges refer to the 1*st *and 3*rd *quartiles. Upper and lower whiskers extend to 1.5 times the interquartile range.*

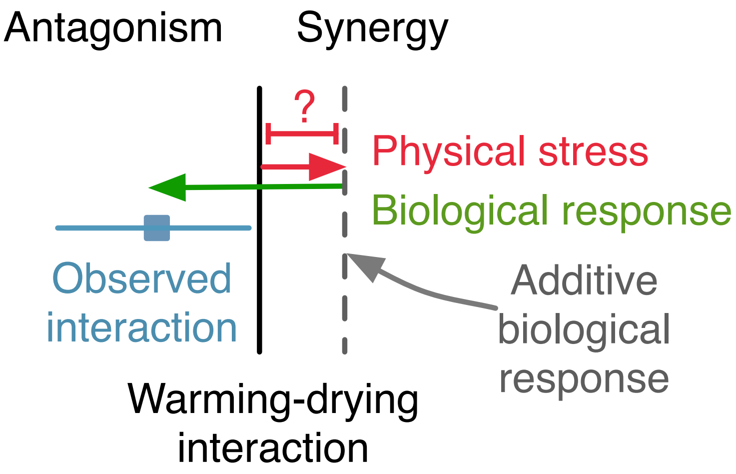

Figure B.6. *The warming-drying interactions we estimated likely underestimate the level of antagonism in the underlying biological response. Pool warming and drying are physical processes that exacerbate each other—warmer water evaporates faster and shallower water can warm to greater temperatures. The null-hypothesis for an additive biological interaction of warming and drying (grey dashed line) is therefore not the same as the null-hypothesis we tested (solid vertical line) which assumed that the warming and drying treatments did not exacerbate each other. Our estimates of antagonism are therefore likely underestimates of the biological antagonism (green arrow pointing to the left) since the physical synergy of warming and drying acts in the opposite direction (red arrow pointing to the right).*