Combined influence of hydroperiod and parasitism on larval amphibian development

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Abstract: Hydroperiod strongly influences the breeding period and development time of many amphibians, and larvae of various species display developmental plasticity in response to habitat drying. Hydrologic alterations associated with anthropogenic activities potentially can influence host–parasite interactions in humans and wildlife, but few investigators have examined this possibility. Pathogens can delay amphibian development, so infections could constrain the ability of parasitized larvae to respond to a shortened hydroperiod under drying conditions. We examined the individual and joint effects of hydroperiod and infection with a pathogenic trematode parasite (*Ribeiroia ondatrae*) on larval development of Pacific chorus frogs (*Pseudacris regilla*) in mesocosms. Tadpoles subjected to accelerated drying were twice as likely to emerge early. However, this tendency was most pronounced among uninfected individuals (96% of early-emerging individuals) and may have arisen from differences in competitive ability between infected and uninfected conspecifics in mesocosms. Our findings indicate the importance of investigating hydrologic variables associated with environmental perturbations, such as climate change, particularly as they relate to the ability of organisms to respond to multiple stressors that include diseases.
Key words: evaporation, tadpole, metamorphosis, development, plasticity, global change, amphibian decline, trade-off, infectious disease

Hydrologic regimes are important for many freshwater communities, and can have substantial impacts on organisms, e.g., survival and abundance of juvenile fish (Freeman et al. 2001). One aspect of the hydrologic regime, hydroperiod, is critical for amphibians because lack of moisture reduces breeding habitat and decreases the time available for successful larval development and metamorphosis (Pounds et al. 1999, Daszak et al. 2005, Kupferberg et al. 2012). Many species use a range of breeding habitats and show phenotypic plasticity in their development as an adaptation to potentially unpredictable conditions. The remarkable flexibility of many amphibian species in reducing their developmental periods or size at metamorphosis when exposed to accelerated drying regimes is documented in an extensive body of experimental research (e.g., Márquez-García et al. 2009, Richter-Boix et al. 2011). Among habitat generalists, the ability to alter development time in response to current hydrologic conditions is an added advantage that enables larvae to survive by escaping poor habitat conditions associated with low water levels, including depleted O_2 or increased salinity (Leips et al. 2000). Therefore, factors that negatively affect the developmental flexibility of larval amphibians under altered hydroperiods are cause for concern, particularly in light of increasing hydrologic alterations associated with human activities (see Barnett et al. 2008).

Many climate-change models indicate greater variability in precipitation, including an increase in extreme events, such as flooding and drought (e.g., Christensen and Christensen 2003, Huntington 2006) that are likely to affect amphibian development via altered wetland hydroperiod. In some cases, hydrologic alterations resulting from climate change already have been linked to amphibian population declines. For example, wetland desiccation in Yellowstone National Park has been cited as a contributing factor in the decline of formerly common native species that has decreased local populations and amphibian diversity (McMenamin et al. 2008). The hydroperiods of many ponds in same region are now shorter than the developmental period required for salamander larvae to reach minimum size required for metamorphosis (McMenamin and Hadly 2010). Thus, depending on the range of developmental flexibility and the rates of local adaptation among species, ongoing shifts in climate and hydroperiod could have important effects on amphibian community composition.

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Additional stressors, including infectious diseases, could influence amphibians' capacity for developmental plasticity and their ability to respond flexibly to current and forecasted changes in hydroperiod. Pathogens can interact with other stressors, such as contaminants, competition, and predation, with detrimental effects on individual condition and survival that may result in population-level effects (e.g., Parris and Cornelius 2004, Johnson et al. 2006, Koprivnikar 2010). Precipitation and hydrologic regimes can interact with some types of pathogens to affect disease dynamics. For instance, Kiesecker et al. (2001) suggested that a decreased winter snow pack resulted in shallower waters and higher exposure of embryonic amphibians to ultraviolet-B radiation, which ultimately increased infection and mortality by a pathogenic water mold. Changes in means of climate variables and increased variation in either (or both) temperature or precipitation can alter hydrologic regimes, with consequences for amphibian diseases. Outbreaks of a thermophilic pathogenic copepod in a northern California river were more severe during unusually warm summers that followed winters with low precipitation. This pattern led investigators to suggest that transmission among amphibian larvae was enhanced by their forced aggregation by lower water levels (Kupferberg et al. 2009).

Infection by trematode parasites (flatworms) is common among wetland-breeding amphibian populations and can have negative effects depending on infection intensity and host developmental stage at infection (reviewed by Koprivnikar et al. 2012). The trematode *Ribeiroia ondatrae* is of particular concern in North America because it can cause high larval amphibian mortality and severe malformations (Johnson et al. 1999, Schotthoefer et al. 2003). *Ribeiroia ondatrae* often causes developmental delays (e.g., Johnson et al. 2001, 2012), probably because hosts spend resources to repair damaged tissues. This parasite has a wide geographic range, and prevalence of infection can be high (reviewed by Johnson and McKenzie 2008). Thus, *R. ondatrae* might affect the ability of larval amphibians to alter their developmental regimes in response to shortened hydroperiods, especially if a trade-off exists between increasing developmental rate and tolerating infection.

We used a factorial manipulation of *R. ondatrae* infection (present or absent) and drying regime (accelerated or ambient) in experimental mesocosms to investigate their separate and combined effects on larval amphibian development. We chose the Pacific chorus frog (*Pseudacris regilla*) for our experiment because this species is affected by *R. ondatrae* and uses a wide variety of habitats, including those prone to drying (Nussbaum et al. 1983, Johnson et al. 1999). We were particularly interested in possible interactive effects between parasitism and shortened hydroperiod that might indicate limited ability of larval amphibians to respond to multiple threats. We examined mean developmental stage in each treatment combination, and considered variation among individuals because of its possible population-level implications. We exposed only half the larvae in each experimental mesocosm to *R. ondatrae* to allow the possibility of differential competitive abilities between infected and uninfected tadpoles so that we could better simulate field conditions and the strong competitive stress associated with drying environments (Semlitsch 1987, Rogers and Chalcraft 2008). We hypothesized that *R. ondatrae* would reduce developmental plasticity of infected tadpoles subjected to accelerated drying and increase developmental variability in infected vs uninfected individuals.

METHODS

Experimental design and mesocosms

We used a 2 × 2 factorial design of *R. ondatrae* exposure (all hosts unexposed or half exposed) and water drawdown (ambient drying or 15 L removed every other day [ambient or accelerated, respectively]), resulting in 4 treatment combinations, each with 5 mesocosm replicates. We randomly assigned treatments to twenty 1000-L plastic cattle water tanks that we used as experimental mesocosms. We initially filled the mesocosms with 336 L of water and covered them with weighted mesh to prevent entry by other organisms and potential escape by *P. regilla* metamorphs. We added 35 g of rabbit chow and ~240 mL (1 cup) of mud collected from a local pond to each mesocosm to promote algal growth based on standard methods of mesocosm establishment (e.g., Semlitsch and Boone 2010). We assigned 16 tadpoles to each mesocosm (initial density = 0.05 tadpoles/L) to ensure that *P. regilla* larval densities did not become artificially high by the end of the experiment. We exposed only half of the tadpoles in each mesocosm assigned the parasite treatment to *R. ondatrae* infectious stages (see below).

We based the accelerated evaporation regime on drying curves used in previous studies of amphibian developmental plasticity (e.g., Wilbur 1987). Some larval amphibians have a limited window for developmental flexibility in response to environmental stressors, such as food deprivation and predators, and are incapable of developmental plasticity after reaching the midpoint of metamorphosis (Hensley 1993, Leips and Travis 1994). We chose a drawdown regime that resulted in almost total dry-down in ~30 d because *P. regilla* typically take 50 to 80 d to metamorphose (Jameson 1956). We removed 15 L of water every 2 d from mesocosms in the accelerated drying treatment and marked the resulting water level on the inside of the tanks. We corrected for additions via precipitation in the accelerated drying treatment mesocosms by removing excess water until the level corresponded to the line. Mesocosms assigned to the ambient drying treatment were allowed to evaporate naturally. We used automatic

Hobo data loggers (Onset Computer Corporation, Bourne, Massachusetts) to record the water temperature at the bottom of the mesocosms every 15 min in 6 tanks (3 in each drying treatment). We measured the final water level in each mesocosm at the end of the experiment. A tree that fell on a mesocosm caused the loss of 1 replicate from the ambient drying/no-parasitism treatment.

Amphibian infection

The life cycle of *R. ondatrae* involves multiple hosts (see Johnson et al. 2004 for a review). Adult worms reside in the digestive tracts of bird or mammal final hosts and release eggs that enter the environment when hosts defecate, hatch into miracidia that seek out an appropriate gastropod 1st intermediate host, and then undergo asexual development. Free-swimming cercariae are produced, which emerge and encyst in 2nd intermediate hosts, such as larval amphibians and fish. The life cycle is completed upon consumption of the 2nd intermediate host by a suitable final host. We used field-infected snails (*Helisoma trivolvis*) collected from sites in California.

We obtained *P. regilla* eggs from Oregon in early June 2012 and raised them in the laboratory until they reached the desired stage for exposure to parasites. Young tadpoles experience high *R. ondatrae*-associated mortality, so we waited until they had reached a mean ± SD Gosner developmental stage of 28.8 ± 1.3 (Gosner 1960) in late June before exposing them to parasites. We assigned tadpoles to 2.5-L plastic bins of water (9/bin; 11 exposure and 11 no-exposure bins). We added 180 cercariae to each exposure bin, corresponding to an average dose of 20 parasites/tadpole but allowing for natural variation in infection intensity, and left parasites to infect tadpoles overnight. Tadpoles in no-exposure bins were treated in the same manner only without cercariae addition. The following day, we randomly added 8 exposed and 8 unexposed tadpoles to mesocosms in the parasitism treatments, and 16 unexposed tadpoles to mesocosms in the no-parasitism treatments. Exposed tadpoles that were not assigned to a treatment and remained alive 5 d post-exposure were preserved and examined for infection (n = 15). We first removed water from mesocosms assigned to the accelerated drying treatment the next day on 30 June 2012.

Data collection and statistical analysis

We monitored tadpoles throughout the experiment. We removed metamorphs and noted their date of emergence and developmental stage. At the end of the experiment 28 d later (27 July), we removed all remaining larvae with dipnets and transported them to the laboratory. We found and collected 3 tadpoles remaining in the tanks on July 31 and August 3. We euthanized larvae in a solution of buffered MS-222, weighed them, and preserved them in 10% neutral buffered formalin. At a later date, we assessed the developmental stage (Gosner 1960) of each individual. We also examined every tadpole from the parasitism-treatment mesocosms via necropsy for parasite infection (present or absent) and malformations. In addition, we randomly chose 5 individuals from each of the no-parasitism mesocosms and performed necropsies to confirm that no infections were present.

We categorized individuals that emerged before the experiment end date, or had achieved the final stage in larval development (Gosner stage 46) on that day, as early emergers. We log(x)-transformed tadpole developmental stage prior to analysis. We examined the fixed effects of our treatments on normally distributed response data, such as tadpole developmental stage, with linear mixed-effects models (LMM) with mesocosm as a random effect (i.e., individual tadpoles were nested within their mesocosm number), sampling day as a covariate, and an identity link function. We excluded the 3 late tadpoles found after July 27 from all stage analyses. We analyzed treatment effects on binomially distributed response data, such as individual categorization as an early-emerger (yes/no) with general LMMs (GLMMs) with mesocosm as a random effect and a logit link function. All tadpoles were included in analyses for early emergence. We also calculated the coefficient of variance (CV) in tadpole developmental stage for each mesocosm and used a general linear model (GLM) to explore within-mesocosm variability as a function of parasitism or drying treatment.

We were interested in competitive ability as a potential driving force, so we used parasitism as a fixed effect in 2 ways in all analyses: 1) whether an individual was assigned to a mesocosm with *R. ondatrae* exposure (i.e., parasitism) and 2) whether an individual amphibian in a mesocosm was actually infected (i.e., infection status), which we assessed by necropsies. Thus, we conducted 2 sets of analyses (individual- vs mesocosm-level treatments) for each dependent variable, which allowed us to examine factorial combinations of the parasitism and drying-speed treatments and of infection status and drying-speed treatments. These analyses contrasted the effects of parasite presence overall (at the mesocosm level) vs the effects of infection on individuals. Infection success was similar in the experimental tadpoles exposed to *R. ondatrae* and the 15 tadpoles exposed but not introduced into mesocosms (see Results), but we cannot completely eliminate the possibility that individuals categorized as uninfected had lost their infections over the period of our experiment. However, larval *P. regilla* have a very low rate of *R. ondatrae* cyst clearance (Lafonte and Johnson 2013), and it is unlikely that individuals categorized as uninfected had lost their infections. We explored the importance of competitive ability by comparing developmental stage and likelihood of early emergence of uninfected tadpoles compet-

ing against uninfected conspecifics in the no-parasitism mesocosms vs uninfected tadpoles competing against a mixture of infected and uninfected conspecifics in the parasitism mesocosms.

We used a *t*-test to quantify the difference in mean water depth between the ambient and accelerated drying mesocosms to check the effectiveness of our manipulation. We evaluated how the water-level manipulation influenced water temperature by testing the effects of treatment on the daily average, minimum, and maximum temperatures with general additive mixed models (GAMMs), which allow for nonlinear relationships between a response and ≥1 predictors with a smoothing function. We applied the smoothing function to day and treatment (ambient or accelerated drying) as fixed effects. Mesocosm identity was included as a random effect. We expected variation in temperature responses to be temporally autocorrelated, so we used Autoregressive Moving Average Models (ARMA) to incorporate autoregressive or moving-average terms at the appropriate lag (identified iteratively considering all values of p and q between 0 and 2). We used Akaike's Information Criterion (AIC) to select among candidate models, including nonARMA models (i.e., $p = q = 0$). Last, we calculated the overall CV for water temperature across the 6 monitored mesocosms and used a *t*-test to examine whether the drying treatment significantly influenced thermal variation. For all model-based analyses, we dropped additive models containing nonsignificant main effects and interactive models containing nonsignificant interaction terms. We ran analyses in SPSS (version 21.0; IBM, Armonk, New York) or R (version 2.15.3; R Project for Statistical Computing, Vienna, Austria).

RESULTS

Mesocosm conditions

The mean water level at the end of the experiment (12.4 ± 0.5 cm [SE]) was 40% lower in the accelerated-drying mesocosms than in the ambient-drying treatment (20.8 ± 0.3 cm) ($t = 15.134$, df = 18, $p < 0.005$). After accounting for the nonlinear relationship between temperature and time in the GAMM analyses (s[day] effective df all > 8, $p < 0.005$), accelerated drying reduced mean daily and minimum temperatures, but not maximum daily temperatures. Mesocosms tended to warm as the experiment progressed, but mean and minimum water temperatures were slightly lower in accelerated-drying than in ambient-drying mesocosms (22.9 ± 0.1 vs 23.4 ± 0.2°C), primarily because accelerated-drying mesocosms experienced greater nighttime cooling (Fig. S1). ARMA models outperformed nonARMA models, but the specific values of p and q varied among response variables (Fig. S1). Variability in mesocosm water temperature did not differ between drying regimes over the experimental period ($t = -1.755$, df = 4, $p = 0.154$).

Overall tadpole mortality was low. We recovered a mean of 85.5 ± 3.5% of tadpoles from each mesocosm (n = 19 mesocosms), with no significant effects of drying regime or parasitism treatment on tadpole recovery. In parasitism mesocosms, ~25% of all individuals collected at the end of the study showed signs of *R. ondatrae* infection (50% were exposed to parasites). The mean prevalence of infection was 22.5 ± 7.3% for tadpoles in the parasitism mesocosms (3.6 ± 1.2 infected tadpoles/mesocosm), well within the range of 0–100% reported in field-collected *P. regilla* (Johnson et al. 2002). Of the infected tadpoles, 63.9% had malformations typically associated with *R. ondatrae* infection (Johnson et al. 2004). We found no infected tadpoles in the no-parasitism mesocosms (n = 45), nor did any uninfected tadpoles show signs of malformations. Infection rate 5 d after exposure for parasite-exposed tadpoles that were not used in mesocosms (n = 15) was ~60%, relative to 50% for exposed tadpoles collected from mesocosms at the end of the experiment.

Responses to infection and drying

The accelerated-drying treatment increased the rate of host development (LMM, $p = 0.009$; Fig. 1). Tadpoles were more developed (mean stage = 42.1 ± 2.9 [SD]) in the accelerated-drying mesocosms than in the ambient-drying mesocosms (mean stage = 40.6 ± 3.8). Development was negatively affected by individual *R. ondatrae* infection (LMM, $p = 0.002$). In the parasitism mesocosms, uninfected individuals were more developed than infected individuals (LMM, $p < 0.005$). These effects were additive,

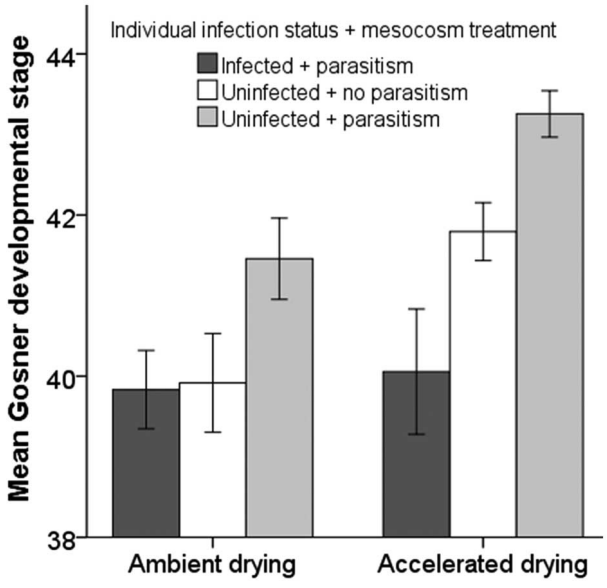

Figure 1. Mean (±1 SE) mesocosm-level Gosner developmental stage of larval Pacific chorus frogs in mesocosms that differed in drying regime, individual infection status, and mesocosm parasitism treatments.

such that uninfected individuals in the accelerated-drying mesocosms were the most mature (additive LMM, accelerated drying, $p = 0.02$; infection, $p = 0.004$). Mean developmental stage of uninfected tadpoles was 42.3 ± 3.3 vs 39.9 ± 2.7 for infected individuals. By the end of the experiment, twice as many individuals emerged early from mesocosms in the accelerated- than in the ambient-drying treatment (GLMM, $F_{1,256} = 4.739$, $p = 0.030$; Fig. 2A). This positive effect of accelerated drying was additive with infection status (full additive GLMM, $F_{2,255} = 5.075$, $p = 0.007$). Thus, 94% of early-emergers were uninfected because *R. ondatrae* infection decreased the likelihood of early emergence (GLMM, $F_{1,256} = 6.333$, $p = 0.012$; Figs 2B, S2).

Uninfected tadpoles competing against a mixture of infected and uninfected conspecifics (parasitism mesocosms) were no more likely to emerge early than those competing only against other uninfected individuals in the no-parasitism mesocosms (GLMM, $F_{1,220} = 1.692$, $p = 0.195$). However, uninfected tadpoles in the parasitism mesocosms had some advantage because their developmental stage was marginally more advanced than that of uninfected individuals in mesocosms with no parasite exposure (and no infected individuals) (LMM, $p = 0.058$; Fig. 3).

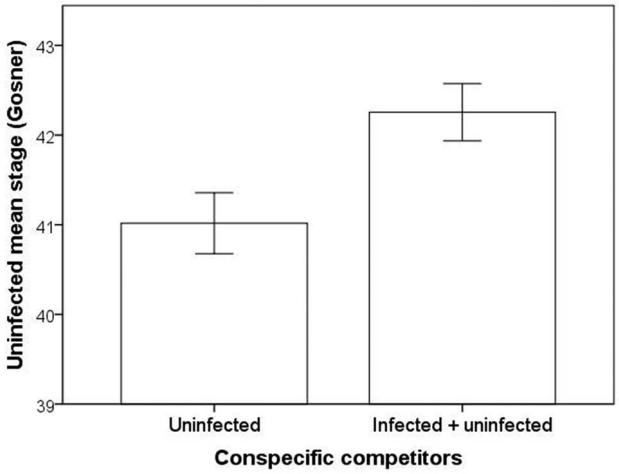

Figure 3. Mean (±1 SE) Gosner developmental stage of uninfected larval Pacific chorus frogs facing different competitive environments: only uninfected conspecifics or a mixture of infected and uninfected competitors.

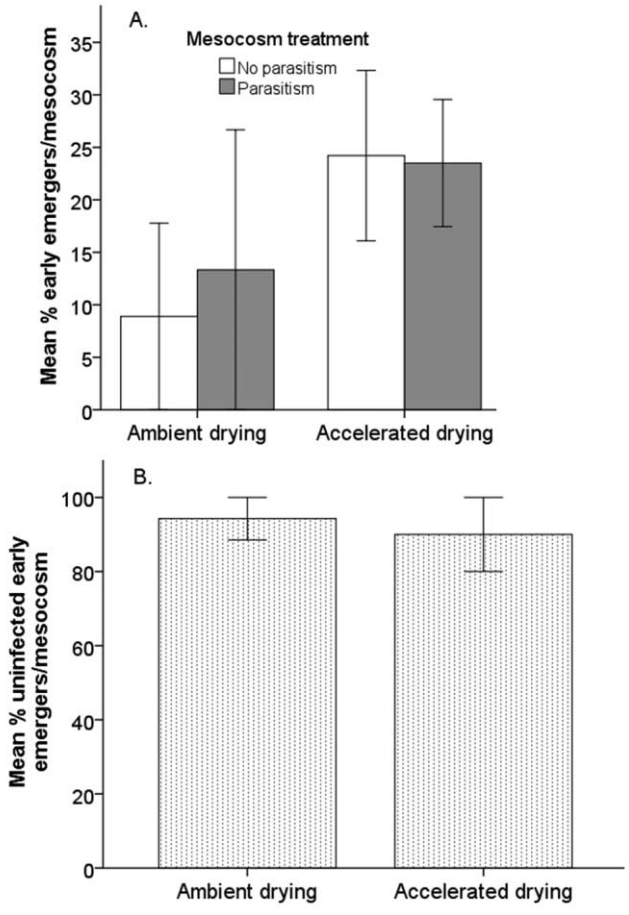

Figure 2. Mean (±1 SE) % larval Pacific chorus frogs emerging early from each experimental mesocosm (A) and % uninfected larval Pacific chorus frogs emerging early (B) from mesocosms that differed in drying regime and mesocosm parasitism treatment.

Responses to parasitism and drying

Parasitism and drying treatment had an interactive effect on early emergence (full interactive GLMM, $F_{3,254} = 3.091$, $p = 0.028$). In the ambient-drying treatment, tadpoles were more likely to emerge early from the parasitism mesocosms than from the no-parasitism mesocosms. However, in the accelerated-drying treatment, tadpoles were more likely to emerge early from the no-parasitism mesocosms than from the parasitism mesocosms. Based on the mesocosm-level CVs, tadpoles showed marginally more intraspecific (within-mesocosm) variation in developmental stage in the accelerated- than in the ambient-drying mesocosms (GLM: $F_{1,17} = 3.964$, $p = 0.063$). However, mesocosm-level variation in development was not affected by the parasitism treatment (GLM: $F_{1,17} = 0.056$, $p = 0.815$), nor were additive or interactive effects of drying and parasitism significant.

DISCUSSION

Our findings support the expectation that hydroperiod reductions affect amphibian development time and also indicate that the ability of larvae to respond flexibly to drying conditions is influenced by parasitic infection. Larval Pacific chorus frogs accelerated their development in response to a shortened hydroperiod, consistent with findings for other amphibian species (e.g., Wilbur 1987, Márquez-García et al. 2009). Individuals in the accelerated-drying treatment were almost 2 Gosner developmental

stages ahead of and twice as likely to emerge early as those in the ambient-drying treatment. This effect probably can be attributed mostly to changes in hydroperiod, rather than temperature, because the monitored ambient-drying mesocosms had a slightly higher mean temperature than the accelerated-drying mesocosms. However, daily maxima did not differ between ambient and accelerated mesocosms. Rather, nighttime cooling was greater in the accelerated-drying mesocosms because of their smaller volumes (see Leips et al. 2000) and the high specific heat of water. Therefore, we think that tadpoles were responding to cues associated with shortened hydroperiod, such as reduced swimming volume, altered water chemistry, and increased larval density (Newman 1989, 1992, Denver et al. 1998, Morey and Reznick 2004).

The ability of tadpoles to respond to accelerated drying was adversely affected by infection at the individual-host level. Tadpoles with *R. ondatrae* infection at the end of the experiment developed more slowly and had a lower likelihood of emerging early, regardless of the drying treatment (i.e., treatment effects were additive but in opposite directions). This finding is consistent with previous laboratory experiments in which investigators found delayed larval development in several amphibian species after exposure to *R. ondatrae* (Johnson et al. 2001, 2012). Uninfected larvae were twice as likely to emerge early under accelerated drying and made up most of the early-emerging individuals. This observation raises the intriguing possibility that uninfected tadpoles in the parasite treatment were better able to respond to accelerated drying than were co-occurring infected individuals, possibly because of a competitive disadvantage associated with infection. This result may be driven by a trade-off in the ability to speed up development vs tolerance of *R. ondatrae* infection.

Our results suggest that tadpoles infected with *R. ondatrae* may have difficulty responding rapidly to high rates of evaporation via accelerated development and that this combination of stressors could be detrimental under altered hydrologic conditions. However, the effects of parasite treatment at the mesocosm level differed from actual infection status at the individual level, highlighting interesting dissimilarities in population vs individual responses to parasite occurrence. At the mesocosm level, drying and parasitism had an interactive effect in contrast to the single or additive effects of infection status at the individual level. This interaction was evident because larvae in the parasitism treatment were more likely to emerge early than those without *R. ondatrae* exposure under ambient drying, but this pattern was reversed under accelerated drying.

Competitive pressure intensifies greatly for larval amphibians under drying conditions because contacts among individuals increase at the same time that algal food supplies diminish (e.g., Wilbur 1987, Leips et al. 2000, Rogers and Chalcraft 2008). *Pseudacris regilla* tadpoles consume both periphyton and phytoplankton (Kupferberg et al. 1994), so tadpoles subjected to the accelerated drying regime probably experienced stronger competition via 2 mechanisms. Exploitative competition would occur as food availability decreased because of reduced surface area and volume available for algal growth, as seen in other studies (e.g., Rogers and Chalcraft 2008). This reduced water volume would also increase contact among tadpoles, driving interference competition (Steinwascher 1978). By examining the development of uninfected tadpoles in different competitive environments (uninfected conspecifics only vs a mixture of infected and uninfected competitors), we found that uninfected individuals had some competitive advantage over infected tadpoles via slightly accelerated development. Koprivnikar et al. (2008) found that the competitive ability of tadpoles was not affected by infection with the trematode *Echinostoma trivolvis* in a study of larval density and parasitism, but we used a more pathogenic parasite and accelerated drying, which may have increased the likelihood of observing competitive interactions.

The nature of the mesocosm-level interaction between the drying and parasitism treatments on early emergence was unexpected. If uninfected individuals enjoyed a competitive advantage, it should have been even greater under accelerated drying, but we did not see such an effect. Parasite-exposed tadpoles found to be uninfected via necropsy may have still experienced stress from the presence of cercariae, their attempts to penetrate (Rohr et al. 2010), or the possible cost of clearing light infections. In the minimally stressful ambient-drying regime, parasite-exposed but uninfected individuals may have fared well enough to emerge early, resulting in a greater number of early emergers from the parasitism than from the no-parasitism mesocosms. The additional stress of accelerated drying could have reduced the ability of parasite-exposed but uninfected tadpoles to speed up development. Food in the accelerated drying mesocosms may have also become so limited that all tadpoles fared poorly and the competitive advantage of uninfected individuals was limited.

The interactive effects of the mesocosm-level drying and parasitism treatments on early emergence also could have arisen from the independent processes of competition and mesocosm-level capacity for phenotypic plasticity. Infected individuals were less developed than their uninfected counterparts in both drying regimes, but the mesocosm-level mean developmental stage of tadpoles did not differ between parasitism treatment in either of the 2 drying conditions. This result suggests that development of uninfected individuals in parasite-exposed mesocosms must have been especially rapid to make up for a deficit caused by infected conspecifics, further support for the notion that they were at a competitive advantage. The far higher likelihood that uninfected individuals would emerge

early could account for the greatest occurrence of early emergence from no-parasite mesocosms under accelerated drying (i.e., fewer uninfected tadpoles were present in parasitism mesocosms to respond to drying). Thus, the effect of the parasitism × drying interaction on early emergence may be the result of 2 simultaneous but independent processes: intraspecific competition and mesocosm-level ability to respond to drying.

We did not find that *R. ondatrae* treatment or infection increased variability in development as initially predicted, but accelerated drying had a marginally negative effect on this variation. This type of variation is important because similar means among treatments could have rather different biological implications if a measure of condition has a bimodal distribution in one group (i.e., very good or very poor) but a normal distribution in another (most individuals in moderate condition). The more rapid drying in accelerated- than in ambient-drying mesocosms may have been a harsher environmental filter that reduced within-mesocosm variation in developmental stage. This reduced variation could have population-level effects if consecutive years of shorter hydroperiod selected against certain phenotypes, and probably genotypes, to reduce genetic variation.

The ability of the parasite *R. ondatrae* to affect flexibility of larval amphibian development under variable hydroperiod is a matter of concern because altered hydrologic regimes also are likely to affect tadpole exposure and susceptibility to this and other parasites. For example, reductions in water levels can increase host exposure to parasite infectious stages by making contacts more likely in smaller water volumes (Kiesecker and Skelly 2001). The higher rates of infection and warmer temperatures experienced by amphibians in temporary ponds probably led to reduced performance of gray tree frog larvae compared to larvae in permanent ponds (Kiesecker and Skelly 2001). However, evaporation could potentially negatively influence amphibian trematode infections by reducing infected snail densities in the field through parasite egg mortality (O'Connor et al. 2008, Martinaud et al. 2009, van Dijk et al. 2010), or by inducing estivation or mortality of snail hosts (Thomas and McClintock 1996, Sandland and Minchella 2004). Evaporation also could negatively affect amphibian infection by reducing parasite output in snails exposed to desiccation (Badger and Oyerinde 1996, Zekhnini et al. 2002) or by hastening amphibian development and, thus, reducing the time for exposure (e.g., Newman 1992, Denver et al. 1998, Doughty and Roberts 2003).

Hydrologic conditions can have important implications for human and wildlife diseases (e.g., Curriero et al. 2001, Epstein 2001), including those of already-imperiled amphibians (Kiesecker et al. 2001). Our study illustrates the need for further study in this regard. Precipitation is a critical consideration for many pathogens, as shown by the links between hantavirus outbreaks and El Niño episodes (Yates et al. 2002), and tick-borne diseases and drought (Jones and Kitron 2000), but alterations to hydroperiod also must be considered. For example, mosquito breeding is dependent on the availability of suitable aquatic habitat, but droughts can lead to mosquito population booms by removing competitors and predators (Chase and Knight 2003), with implications for mosquito-vectored diseases. We showed that hydroperiod and parasitism can combine and interact in unexpected ways, with consequences for larval amphibian development and likelihood of surviving pond drying. Thus, changes to hydroperiod could pose an additional problem for amphibians trying to metamorphose or escape poor conditions if they face the added burden of pathogens. Given the critical nature of hydroperiod for amphibian breeding and survival, hydrologic aspects are now being integrated into amphibian conservation and management solutions (Shoo et al. 2011). Future research in which larval densities are manipulated in field enclosures across a natural gradient of hydroperiod variability will be a valuable next step to elucidate how pathogens interact with this important habitat factor.

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