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## EFFECTS OF HYDROPERIOD DURATION ON SURVIVAL, DEVELOPMENTAL RATE, AND SIZE AT METAMORPHOSIS IN BOREAL CHORUS FROG TADPOLES (*PSEUDACRIS MACULATA*)

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**ABSTRACT:** Understanding the relationship between climate-driven habitat conditions and survival is key to preserving biodiversity in the face of rapid climate change. Hydroperiod—the length of time water is in a wetland—is a critical limiting habitat variable for amphibians as larvae must metamorphose before ponds dry. Changes in precipitation and temperature patterns are affecting hydroperiod globally, but the impact of these changes on amphibian persistence is poorly understood. We studied the responses of Boreal Chorus Frog (*Pseudacris maculata*) tadpoles to simulated hydroperiods (i.e., water level reductions) in the laboratory using individuals collected from ponds spanning a range of natural hydroperiods (Colorado Front Range, USA). To assess the effects of experimental hydroperiod reduction, we measured mortality, time to metamorphosis, and size at metamorphosis. We found that tadpoles grew at rates reflecting the hydroperiods of their native ponds, regardless of experimental treatment. Tadpoles from permanent ponds metamorphosed faster than those from ephemeral ponds across all experimental treatments, a pattern which may represent a predation selection gradient or countergradient variation in developmental rates. Size at metamorphosis did not vary across experimental treatments. Mortality was low overall but varied with pond of origin. Our results suggest that adaptation to local hydroperiod and/or predation and temperature conditions is important in *P. maculata*. Moreover, the lack of a plastic response to reduced hydroperiods suggests that *P. maculata* may not be able to metamorphose quickly enough to escape drying ponds. These results have important implications for amphibian persistence in ponds predicted to dry more quickly due to rapid climate change.

*Key words:* Amphibian decline; Climate change; Hydroperiod; Larval growth rate; Metamorphosis; *Pseudacris maculata*

AMPHIBIAN populations are experiencing declines worldwide at rates significantly higher than most other vertebrate taxa (Stuart et al., 2004; IUCN, 2011), with multiple factors associated with this decline, including habitat loss (Cushman, 2006; Becker et al., 2009), invasive species (Kats and Ferrer, 2003; Knapp, 2005), exploitation for food and the pet trade (Frías-Alvarez et al., 2010), and disease (Collins, 2010; Kilpatrick et al., 2010). Climate change has been proposed as an additional explanation for amphibian declines (Pounds, 2001; Pounds and Crump, 2006). Because of the predicted severity of its impacts on ecosystems and biodiversity, assessing impacts of global climate change on taxa is a pressing issue (McCarty, 2001; Thomas et al., 2004). In addition to more direct effects, changes in climate may alter

two components important to amphibian persistence: disease dynamics (Pounds and Crump, 2006) and habitat suitability (Matthews et al., in press). Adult and larval amphibians have been shown to play integral roles in community structure and food web dynamics, highlighting their importance in the overall conservation of wetland ecosystems and maintaining healthy habitats (Ranvestel et al., 2004).

Climate change presents a particular challenge for amphibians because of their vulnerability to environmental conditions, especially water availability (Thorson, 1955; Bucklet and Jetz, 2007). Hydroperiod, the duration of water in a wetland (Ryan and Winne, 2001), is a critical pond characteristic that directly affects survivorship of pond-breeding amphibians (i.e., desiccation risk; Pechmann et al., 1989). Hydroperiods and pond conditions will likely be altered by climate change as hydrological and seasonal weather patterns shift (MacCracken et al., 2003; Thomas et al., 2004; McMenamin et al., 2008; Brooks, 2009),

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altering habitat suitability. Temperature is expected to increase (Michener et al., 1997; MacCracken et al., 2003), which would increase evaporation rates, thereby decreasing hydroperiod (Matthews, 2010). Less overall snowfall and snowmelt earlier in the spring will result in less water available for summer and autumn use (Barnett et al., 2005), a time period critical for larval development (Corn, 2005). In regions dependent on snowfall for pond filling, this shift in timing of precipitation could have severe consequences on amphibian breeding (Corn, 2005). Researchers have already observed declines in amphibian populations associated with altered precipitation as a result of changing climate (Pounds et al., 1999; Pounds, 2001; McMenamin et al., 2008, but see Patla et al., 2009).

To escape suboptimal pond conditions, tadpoles must rapidly metamorphose. If the rate of pond desiccation exceeds maximum developmental rates, tadpoles can suffer significant mortality (Denver et al., 1998). Some amphibian species display developmental plasticity and speed up metamorphosis in response to pond drying (Denver et al., 1998; Loman and Claesson, 2003; Gervasi and Foufopoulos, 2008), which could limit the impact of truncated hydroperiod on pond-breeding amphibian persistence. However, there are potential tradeoffs between time to metamorphosis (developmental rate) and size at metamorphosis (Merilä et al., 2000), where tadpoles that develop more quickly may metamorphose at a reduced size (Denver et al., 1998; Merilä et al., 2000). Accelerated development may lead to compromised tadpole immune system function (Gervasi and Foufopoulos, 2008), and reduced size at metamorphosis is linked to both reduced juvenile and adult survival (Terentyev, 1960; Rudolf and Rödel, 2007; Márquez-García et al., 2009). Despite these potential costs, developmental plasticity is one mechanism that could facilitate amphibian survival under abbreviated hydroperiod conditions due to climate change.

Understanding hydroperiod influence on larval survival and developmental rate is key for predicting the potential effects of climate change on amphibians. Because of the importance of water in the life history of pond-

breeding amphibians, hydroperiod has been postulated to be a major cue in driving metamorphosis (Merilä et al., 2000). If hydroperiod plays an important role in regulating developmental rate and metamorphosis, the ability of amphibians to respond to altered water availability may determine the persistence of populations. However, little is known regarding the proximate cues (reduction in water volume, change in temperatures, altered water quality, or other factors) that trigger metamorphosis relative to hydroperiod (Denver, 1997). Some of these cues, such as increased water temperature, may affect tadpole development physiologically by speeding development in a certain temperature range (Harkey and Semlitsch, 1988). In addition, developmental plasticity may vary by both species and populations within species. If developmental plasticity is locally adapted (Loman, 2001), one would expect ponds with highly variable hydroperiod conditions to have tadpoles with a higher degree of developmental plasticity and be more capable of responding plastically to climate change shifts in hydroperiod (Lind and Johansson, 2007). In contrast, ponds that exhibit more consistent hydroperiods may have tadpoles with a limited ability to respond to highly variable conditions and, therefore, a limited ability to respond plastically to climate change altered hydroperiods.

Our goal was to test the effects of simulated hydroperiod reductions on mortality, developmental rate, and size at metamorphosis using wild caught Boreal Chorus Frog tadpoles (*Pseudacris maculata*). *P. maculata* are ideal for this investigation because they occupy a wide range of breeding habitats, including ephemeral wetlands, ponds, lakes, and small, permanent agricultural reservoirs (Hammerson, 1999). Their wide use of habitats with different hydrological conditions and limited dispersal distance (Spencer, 1964) suggests that they may be adapted to local hydroperiods and/or be phenotypically plastic with respect to hydroperiod. Using this versatile species, we tested three hypotheses: (1) if hydroperiod reduction is sufficiently rapid, tadpoles will be unable to develop fast enough to metamorphose before the pond dries, resulting in mortality; (2) a threshold

level of water reduction is needed to elicit a plastic response in developmental rate, resulting in a tradeoff with size at metamorphosis; and (3) tadpoles from ephemeral ponds will develop faster than tadpoles from permanent ponds, regardless of treatment, reflecting a response to the natural hydroperiod of the tadpoles' pond of origin.

## MATERIALS AND METHODS

### *Field Sampling*

We collected 36 early stage tadpoles (Gosner stages 24–31; Gosner, 1960) using random net sweeps from each of 11 ponds across mid to high elevations (1923–3014 m) in Larimer County, Colorado, USA, during late May through mid-June 2009. *P. maculata* do not lay eggs in singular, large egg masses like many other amphibians, increasing the difficulty of visual detection and collection of eggs (Corn and Muths, 2002), and making collections of tadpoles more plausible. Tadpoles were brought into the laboratory over approximately 1 mo (26 June–17 July 2009) due to asynchronous dates of egg deposition across elevations. During collection, each pond's natural hydroperiod was designated as ephemeral or permanent based upon observations of wetland drying from early to mid-season across 2 yr (2009–2010), wetland photographs, depth of pond on the date of collection, and the source of water (ground water, stream, precipitation/snowmelt) as determined by observations during visits. Ephemeral ponds were defined as those that dried completely by the end of the breeding season, and permanent ponds were those that retained water for the entire duration of the summer breeding season. Six middle-elevation sites (two permanent, four ephemeral) and five high elevation sites (three permanent, two ephemeral) were sampled (Appendix).

### *Experimental Design and Animal Care*

To assess the degree of water reduction needed to elicit any potential plastic response and to assess if a potential threshold exists, the tadpoles from each pond were assigned randomly to three experimental hydroperiod treatments with three replicates each (3 treatments  $\times$  3 replicates  $\times$  11 ponds  $\times$  4 animals/container = 396 tadpoles total) within

1 to 2 d of collection. "Experimental hydroperiod" refers to water reduction treatments, whereas "natural hydroperiod" refers to the hydroperiod in the ponds where tadpoles were collected. In treatment 1 (control), water levels were maintained at 1.5 L for the duration of the experiment. In treatment 2 (intermediate water level reduction), water levels were reduced by 150 mL every week until a minimum volume of 150 mL was reached. In treatment 3 (fast reduction), water levels were dropped by 250 mL each week until 150 mL remained. These amounts were deemed an appropriate volume in order to signify a substantial, natural water reduction while not solely eliciting a mortality response. All containers were maintained at their final water levels until all animals metamorphosed or died. Mortalities and metamorphosis were recorded daily for each container, yielding an average size and date of metamorphosis per container.

Tadpoles were housed in plastic containers measuring 22 cm  $\times$  22 cm  $\times$  9.5 cm, with lids ventilated using multiple small holes cut into the plastic in order to minimize evaporation (Lemmon and Lemmon, 2010). Containers were filled with pH-neutral, dechlorinated, and ammonia-eliminated tap water (using AmQuel® plus) that sat for at least 24 h prior to use (Lemmon and Lemmon, 2010).

Containers were randomized on 21 shelves, and tadpoles were maintained at room temperature (21–24°C). Light cycles were adjusted monthly to mimic local seasonal conditions (with an average cycle of 14 h light, 10 h dark). Tadpoles ( $n = 4$  per container) were fed raw organic spinach (0.1 g) and rabbit pellets (0.2 g) twice per week (Lemmon and Lemmon, 2010).

All treatments were maintained at constant water levels (1.5 L) for the first week of the experiment for acclimation before treatment. After acclimation, containers were cleaned, water was changed completely, and water volume was reduced once every 7 d. When half of the tadpoles in a given container reached Gosner stage 36, a partial water change was added each week to avoid confounding the effects of water reduction and water cleanliness. During this second

water change, only half of the total container volume was replaced with fresh, treated water.

#### *Data Analysis and Collection*

*Mortality.*—Containers were checked once per day for mortalities. A mortality event was designated as the death of any individual within a container. These individuals were removed from the container upon discovery and were recorded daily. Mortality data were skewed due to a high overall survival. Therefore, the data were analyzed by random effects logistic regression to correct for pond effects. Survival was indicated binomially for every individual in the experiment, and analysis focused on the response of survival by pond, experimental treatment, and natural hydroperiod. Significance was measured at the 0.05 level for all analyses.

*Correcting for Starting Stage.*—All tadpoles were photographed to determine approximate initial Gosner stage (Gosner, 1960) prior to assigning to treatments. Because starting stage may impact both time to metamorphosis and size at metamorphosis, we used linear regression to establish the amount of variation in time to metamorphosis and size at metamorphosis that was explained by Gosner stage at the start of the experiment.

Time to metamorphosis was significantly negatively correlated with initial Gosner stage (adjusted  $r^2 = 0.71$ ,  $df = 97$ ,  $P < 0.0001$ ). Size at metamorphosis had a weak but significantly positive correlation with initial stage (adjusted  $r^2 = 0.03$ ,  $df = 97$ ,  $P < 0.04$ ). Since the regression was significant in both cases, we used the residuals between Gosner stage/time to metamorphosis and Gosner stage/size at metamorphosis in all subsequent analyses of variance (ANOVAs).

*Time to Metamorphosis.*—All containers were checked daily for metamorphosed individuals, defined as both hind limbs and at least one forelimb emerged (Lemmon and Lemmon, 2010). To assess the effect of experimental hydroperiod on time to metamorphosis, we conducted a randomized block design ANOVA using pond as the block effect to control for pond-specific variation (Zar, 1998; R Development Core Team, 2009). To assess the effects of natural hydroperiod, experimental hydroperiod, and any potential interaction, we conducted a

two-way ANOVA including both natural and experimental hydroperiod effects (pond is not a block effect in this case as each pond is not subjected to each natural hydroperiod “treatment”; Zar, 1998; R Development Core Team, 2009). Potential interactions between elevation and natural hydroperiod are possible; however, the spread of natural hydroperiod type by elevation reduced the likelihood of this interaction. In order to test for any potential confounding effect of changing tadpole density due to removal of dead tadpoles throughout the experiment, the above analyses were conducted again using only the containers that experienced no tadpole mortality.

*Size at Metamorphosis.*—Animals were photographed at metamorphosis with a size standard (ruler). Snout–vent length, a commonly used metric of size for frogs, was measured using ImageJ (Rasband, 2009). Size at metamorphosis was averaged for each container ( $n \leq 4$ ).

To assess effect of experimental hydroperiod on size at metamorphosis, we conducted a randomized block design ANOVA analogous to the one used above for time to metamorphosis (Zar, 1998; R Development Core Team, 2009). To assess the effects of natural hydroperiod, experimental hydroperiod, and potential interaction on size at metamorphosis, we conducted a two-way ANOVA analogous to the analysis used for time to metamorphosis above (Zar, 1998; R Development Core Team, 2009). Again, all of the above analyses were done a second time with containers that had no mortality in order to remove any potential effects of reduced tadpole density.

## RESULTS

### *Mortality*

Mortality was minimal throughout the experiment (6.4%), generally occurring during the first 7 d of the experiment (69% of all mortality). Mean mortality varied with pond of origin (11–19.4%), with the majority of the mortality occurring in ponds categorized as having ephemeral hydroperiods. However, in the random effects logistic regression, when survival was corrected for pond effects, individual mortality by natural hydroperiod

TABLE 1.—Analysis of variance of experimental effects on residuals of time to metamorphosis. Pond effect includes natural hydroperiod effects among other variables. ExpHydro is the experimental hydroperiod treatment effect.

Response: time	Degrees of freedom	Sum of squares	Mean square	F value	P value
ExpHydro	2	0.49	0.25	0.07	0.94
Pond	10	364	36.4	10.0	$6.51 \times 10^{-10a}$
Replicate	1	8.55	8.55	2.35	0.13
ExpHydro $\times$ pond	20	71.3	3.56	0.98	0.50
Residuals	65	237	3.64		

<sup>a</sup>  $P < 0.001$ .

was nonsignificant. Mortality was also not impacted significantly by experimental hydroperiod treatment or pond of origin.

#### Time to Metamorphosis

In our first analysis, there was a significant effect of pond on time to metamorphosis when correcting for initial Gosner stage ( $F = 10.0$ ,  $df = 10$ ,  $P = 6.51 \times 10^{-10}$ ; Table 1). There was no effect of experimental hydroperiod ( $F = 0.07$ ,  $df = 2$ ,  $P = 0.94$ ; Table 1). For the second analysis including natural hydroperiod, time to metamorphosis was protracted, on average, for individuals from permanent ponds compared to individuals from ephemeral ponds ( $F = 10.4$ ,  $df = 1$ ,  $P = 1.72 \times 10^{-3}$ ; Table 2; Fig. 1), with no significant effect of experimental hydroperiod on time to metamorphosis ( $F = 0.04$ ,  $df = 2$ ,  $P = 0.96$ ; Table 2).

Our results did not change qualitatively when the analyses were conducted only using containers with no tadpole mortality. As above, all values were corrected for initial Gosner stage. The first analysis showed a significant effect of pond on time to metamorphosis ( $F = 8.17$ ,  $df = 10$ ,  $P = 2.35 \times 10^{-7}$ ) and no effect of experimental hydroperiod ( $F = 0.21$ ,  $df = 2$ ,  $P = 0.81$ ). In the second analysis that included natural hydroperiod, time to metamorphosis was still shorter for individuals from permanent natural hydroperiods ( $F = 6.86$ ,  $df = 1$ ,  $P = 0.01$ ), and there was no significant effect of experimental hydroperiod ( $F = 0.11$ ,  $df = 2$ ,  $P = 0.89$ ).

#### Size at Metamorphosis

We found a significant relationship between size at metamorphosis and pond ( $F = 4.38$ ,  $df = 10$ ,  $P = 1.07 \times 10^{-4}$ ; Table 3), even after accounting for the effect of initial Gosner stage. However, there was no significant relationship between size at metamorphosis

and experimental treatment ( $F = 0.42$ ,  $df = 2$ ,  $P = 0.66$ ; Table 3; Fig. 2) or natural hydroperiod ( $F = 0.02$ ,  $df = 1$ ,  $P = 0.89$ ; Table 4).

Our analysis of size at metamorphosis also did not change qualitatively when analyses were conducted using only containers with no mortality. After correction of initial Gosner stage, the first analysis showed a significant effect of pond on time to metamorphosis ( $F = 4.17$ ,  $df = 10$ ,  $P = 4.11 \times 10^{-4}$ ) but no effect of experimental hydroperiod ( $F = 0.16$ ,  $df = 2$ ,  $P = 0.85$ ). The second analysis including natural hydroperiod showed no significant effect of experimental hydroperiod ( $F = 0.11$ ,  $df = 2$ ,  $P = 0.90$ ) or natural hydroperiod ( $F = 0.21$ ,  $df = 1$ ,  $P = 0.65$ ).

#### DISCUSSION

Altered hydroperiods resulting from changes in climate norms will affect amphibians globally (Matthews et al., in press). Our results show that time to metamorphosis and survival of *P. maculata* tadpoles are explained more by source pond and its natural hydroperiod rather than experimental hydroperiod treatments. This indicates that the ability to respond to climate change may be determined by adaptation to local pond conditions rather than developmental plasticity, stressing the importance of site-specific conditions for conservation strategies.

#### Mortality

Mortality was not a major factor in our experiment, with high survival rates shown by all experimental treatments over the course of the study. Experimental treatment did not affect mortality significantly, but a more severe water reduction regime in addition to initiation of treatments at an earlier stage might have increased mortality. Ryan and

TABLE 2.—Analysis of variance of experimental and natural hydroperiod effects on residuals of time to metamorphosis. Pond block effect has been removed. ExpHydro is the experimental hydroperiod treatment effect. ExpHydro is the experimental hydroperiod treatment effect; NatHydro is the natural hydroperiod effect.

Response: time	Degrees of freedom	Sum of squares	Mean square	F value	P value
ExpHydro	2	0.49	0.25	0.04	0.96
NatHydro	1	66.8	66.8	10.4	$1.72 \times 10^{-3a}$
ExpHydro $\times$ NatHydro	2	17.7	8.85	1.38	0.26
Residuals	93	596.2	6.41		

<sup>a</sup>  $P < 0.01$ .

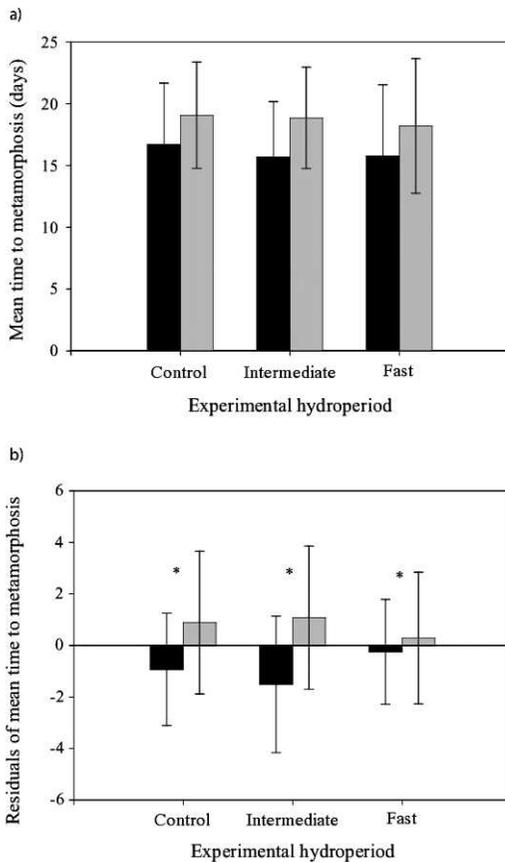


FIG. 1.—(a) Mean time to metamorphosis (raw data) of *Pseudacris maculata* tadpoles from permanent (black) and ephemeral (grey) natural hydroperiods by experimental hydroperiod. (b) Residuals of mean time to metamorphosis of *P. maculata* tadpoles from permanent and ephemeral natural hydroperiods by experimental hydroperiod. Experimental hydroperiod was designated as control (1.5 L constant), intermediate (150 mL reduction/wk), and fast (250 mL reduction/wk). Error bars represent standard errors. Asterisks indicate significance at the 0.05 level.

Winne (2001) did not see a significant difference in mortality between different experimental hydroperiod reductions, but observations in nature associate shortened hydroperiods with increased mortality due to desiccation (Newman, 1987; Loman and Claesson, 2003). The limited observed mortality in this study was higher in tadpoles from ponds with ephemeral natural hydroperiods compared to those from permanent natural hydroperiods, but this was nonsignificant when accounting for random pond effects. This could be due to stress from disease and parasites imposed by suboptimal conditions associated with specific ponds used (Kiesecker and Skelly, 2001) in addition to other sources of variation not factored into the experiment.

#### *Time to Metamorphosis*

We found no effect of experimental hydroperiod on time to metamorphosis; these results were contrary to our predictions and to previous work that reported an increased rate of development in response to reduced water levels (Denver et al., 1998; Loman and Claesson, 2003; Gervasi and Foufopoulos, 2008). Plasticity may be energetically expensive (Terentyev, 1960; Gervasi and Foufopoulos, 2008), and plastic responses may occur only if conditions reach a specific threshold (Morey and Reznick, 2000; Márquez-García et al., 2009). Our experimental manipulations of water level may not have been drastic enough to achieve an accelerated growth response. Denver et al. (1998) used more severe water reductions (35% weekly volume reduction compared to our 25%), which elicited a plastic response from *Scaphiopus hammondi* tadpoles. Leips et al. (2000) manipulated hydroperiod reductions in a manner similar to our own and saw no direct effect of reduced hydroperiod on time to metamorphosis of two sister species of

TABLE 3.—Analysis of variance of experimental effects on residuals of size at metamorphosis. Pond effect includes natural hydroperiod effects among other variables. ExpHydro is the experimental hydroperiod treatment effect.

Response: size	Degrees of freedom	Sum of squares	Mean square	F value	P value
ExpHydro	2	0.003	0.002	0.42	0.66
Pond	10	0.16	0.026	4.38	$1.07 \times 10^{-4a}$
Replicate	1	0.0002	0.0002	0.06	0.81
ExpHydro $\times$ Pond	20	0.06	0.003	0.77	0.74
Residuals	65	0.24	0.004		

<sup>a</sup>  $P < 0.001$ .

Hylidae. However, the natural hydroperiod each species utilizes (permanent versus ephemeral) may affect development as seen in our experiment. Doughty and Roberts (2003) saw

no effect of experimentally reduced hydroperiod on time to metamorphosis in *Crinia georgiana* tadpoles unless in combination with restricted access to food, suggesting that other cues in addition to reduction of volume may be needed to elicit a plastic response.

Natural hydroperiod of the pond of origin was a predictor of time to metamorphosis whereas experimental hydroperiod reduction was not. Tadpoles from ponds with permanent natural hydroperiods metamorphosed significantly faster than those in ephemeral natural hydroperiods (Fig.1), regardless of the experimental treatment. In addition, pond of origin was also consistently a significant predictor variable, indicating that hydroperiod designation (natural or experimental) did not explain all the variation in time to metamorphosis.

The effect of natural hydroperiod on time to metamorphosis could be due to several factors. Many deeper sites with more permanent hydroperiods contain dangerous predators, such as fish (Skelly, 1996; Hecnar and M'Closkey, 1997), which may result in stronger selection on those individuals to metamorphose even more rapidly. Because tadpoles were collected rather than eggs, tadpoles may have also had time to experience predation stress before initiation of the experiment, plastically affecting developmental rate postremoval (Relyea, 2007). The faster metamorphosis observed in tadpoles from permanent ponds may also reflect counter-gradient variation (Berven, 1982; Loman, 2002; Skelly, 2004), where individuals from colder, deeper ponds (i.e., those with permanent hydroperiods) have metabolic and growth rates already accelerated to compensate for adverse pond conditions (i.e., thermal deficits). Newman (1989) found that deeper ponds were colder than ephemeral, shallow ponds, and these ephemeral ponds increased

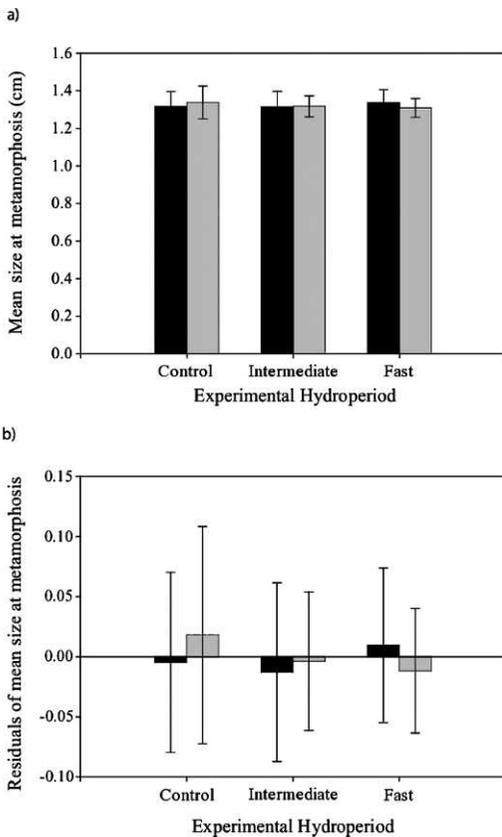


FIG. 2.—(a) Mean size at metamorphosis (raw data) of *Pseudacris maculata* tadpoles from permanent (black) and ephemeral (grey) natural hydroperiods by experimental hydroperiod. (b) Residuals of mean size at metamorphosis of *P. maculata* tadpoles from permanent and ephemeral natural hydroperiods by experimental hydroperiod. Experimental hydroperiod was designated as control (1.5 L constant), intermediate (150 mL reduction/wk), and fast (250 mL reduction/wk). Error bars represent standard errors.

TABLE 4.—Analysis of variance of experimental and natural hydroperiod effects on residuals of size at metamorphosis. Pond block effect has been removed. ExpHydro is the experimental hydroperiod treatment effect. NatHydro is the natural hydroperiod effect.

Response: size	Degrees of freedom	Sum of squares	Mean square	F value	P value
ExpHydro	2	0.003	0.002	0.32	0.73
NatHydro	1	$9.00 \times 10^{-5}$	$9.00 \times 10^{-5}$	0.02	0.89
ExpHydro $\times$ NatHydro	2	0.008	0.004	0.81	0.45
Residuals	93	0.46	0.005		

in temperature as they became shallower due to drying. In addition, ponds with a higher percentage of overstory coverage have been found to be colder and may retard amphibian larval growth, arguing that thermal characteristics play an important role in amphibian development (Skelly et al., 2002). General site fidelity and presumably low gene flow exhibited by Boreal Chorus Frogs (*P. maculata*) and other anurans (Tordoff and Pettus, 1977; Sinsch, 1990) would facilitate local adaptation, which could result in countergradient variation. For example, *Rana temporaria* tadpoles have been found to have a genetically based developmental time based upon the hydroperiod of the breeding site (Lind et al., 2008). Due to the limitations of our experiment, these two hypotheses cannot be teased apart with the available data.

Maternal effects could potentially explain the differences observed in time to metamorphosis (Bernardo, 1996), especially the significant pond effect observed in all tests. However, maternal effects may not be independent of hydroperiod and local pond conditions. Clutch size and allocation (egg size) have been observed to vary with hydroperiod, potentially another type of local adaptation to pond drying (Loman, 2001). Finally, there are other sources of variation that we did not address, including stress from tadpole crowding, temperature, and the presence and abundance of predators (Newman, 1989; Petranka, 1989; Higginson and Ruxton, 2010).

#### *Size at Metamorphosis*

In contrast to time to metamorphosis, size at metamorphosis was unaffected by both the experimental treatment and the natural hydroperiod. This is contrary to previous studies showing that a rapid drying regime resulted in an accelerated developmental rate and, therefore, a smaller size at metamorphosis (Rudolf

and Rödel, 2007; Márquez-García et al., 2009). Doughty and Roberts (2003) saw no effect of hydroperiod reduction by itself on size at metamorphosis but did when varying food availability and quality in combination with water reductions. Altered larval densities may better explain smaller size at metamorphosis rather than water reduction alone (Leips et al., 2000), though our analyses did not see a response even when including containers with mortalities (altered tadpole densities). There was again a significant pond effect, suggesting that other sources of variation not taken into account by our hydroperiod categorization still exist. Many variables could have differed between sites and affected tadpoles before collection. For example, predators within a community are capable of altering the developmental rate and size at metamorphosis of their tadpole prey (Higginson and Ruxton, 2010), potentially altering tadpoles prior to their removal.

#### *Future Work*

We saw significant variation in size and time to metamorphosis that could not be explained by natural or experimental hydroperiod, suggesting further consideration is needed. Our results suggest that Boreal Chorus Frogs may not have a plastic response to hydroperiod reductions. More research is required to test if potential plasticity is species-specific. In addition, if *P. maculata* are capable of responding plastically to hydroperiod, it may mean that more severe cues (such as harsher water reductions) or additional cues (other than water level) may be required to stimulate a plastic response. For example, odonate larvae common within these sites prey upon tadpoles and have been shown to release a predator cue that may alter developmental rates (Eklöv, 2000). If predator-prey interactions affecting timing of metamorphosis are

also altered by a change in hydroperiod, then this could significantly alter the predicted effects on amphibian populations (Walther et al., 2002; Gilman et al., 2010). Ecological responses to changes in hydroperiod are likely complex, and understanding the effects will require a synthesis of knowledge concerning the effects of climate change on the habitat and species overall. Nonetheless, understanding the effects of single factors, such as hydroperiod reduction, is a necessary step for unraveling interactions between tadpoles and their pond environments.

#### *Conservation Implications*

Our results suggest that some species of amphibians may have highly variable rates of metamorphosis depending upon hydroperiod conditions in their native ponds. If the ability to respond plastically to hydroperiod reduction is limited in some species such as *P. maculata*, then the inability to respond plastically could cause high levels of tadpole mortality as hydroperiods decrease as climate changes. This could result in severe impacts upon populations and species across the landscape. In addition, if response to hydroperiod is as variable as is suggested by our results, then reactions to climate change may vary from population to population. Based on our findings on *P. maculata*, tadpoles developing in ephemeral wetlands will be even more strongly impacted by climate change than those species that can plastically respond. Climate change is expected to impact ephemeral ponds more severely (Matthews et al., in press), thus increasing the risk of desiccation for individuals who already appear to develop more slowly than those from permanent ponds. If these plastic responses are limited or locally adapted, then a species' or population's survival will depend on the extent or direction of the climate change effects. By understanding the role of hydroperiod in tadpole development and survival, better predictions can be made as to the effects such shifts will have on populations. In addition, more realistic and biologically significant conservation and management plans can be developed and implemented. If a hydroperiod response varies between populations or species, then policies specific to each circum-

stance would be required rather than overall similar management plans.

Several wetland surveys have generated information regarding wetland health (USEPA, 2011), especially at the state level within the United States, but additional long-term wetland monitoring initiatives would enhance the ability to track trends in population persistence and hydroperiod consistency to better understand future effects and conditions (Brooks, 2009). In addition, identifying natural interactions and their importance and understanding how these will change in response to a changing climate is critical to conservation (Gilman et al., 2010).

Climate change and its potential impact on biodiversity is a pressing issue in ecological research (Parmesan, 2006; Ackerly et al., 2010; Blaustein et al., 2010). Amphibian life cycles are strongly tied to water availability because of the dependency of the tadpole stages of most amphibian species on an aquatic environment (Pechmann et al., 1989). With predicted alterations in water availability and shifts in precipitation patterns globally (MacCracken et al., 2003; Thomas et al., 2004; Barnett et al., 2005), understanding how altered hydroperiods may affect amphibian growth and survival is likely to be a cornerstone in amphibian conservation.

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## APPENDIX

Ponds by elevational grouping where tadpoles were collected. Geographic coordinates were based on the WGS84 datum.

Site name	Elevation (m)	Latitude and longitude	Natural hydroperiod
High-elevation sites			
Phantom Lake	2513	40°46'39.40"N 105°34'17.80"W	Permanent
Upper Pingree 2	2854	40°34'5.70"N 105°36'2.03"W	Ephemeral
Upper Pingree 1	2858	40°34'6.28"N 105°36'4.49"W	Ephemeral
Surprise Pond	2975	40°33'39.06"N 105°36'41.71"W	Permanent
Sylvatica	3014	40°34'5.00"N 105°50'57.50"W	Permanent
Middle-elevation sites			
Cherokee B	1923	40°50'28.77"N 105°20'8.71"W	Permanent
Maloo's Pond	2116	40°37'53.05"N 105°19'38.36"W	Ephemeral
Colard 1	2188	40°36'44.36"N 105°21'10.67"W	Permanent
Blue House 1	2196	40°38'27.74"N 105°18'35.52"W	Ephemeral
Lost Lake	2429	40°50'51.40"N 105°31'33.84"W	Ephemeral
Nairdad	2432	40°50'34.24"N 105°31'35.00"W	Ephemeral