

The Effects of Pond Drying and Predation on Blanchard's Cricket Frogs (*Acris blanchardi*)

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Pond hydroperiod and predators play a central role in structuring aquatic communities. Because of predicted changes in precipitation and temperature patterns associated with climate change, pond hydroperiods will likely be altered. Reduced hydroperiods can impact amphibian populations by restricting the amount of time available for larval growth and by altering predatory interactions via increased predator densities. We investigated how pond drying and predation singularly and interactively affected growth and survival of *Acris blanchardi* (Blanchard's Cricket Frogs). We reared recently hatched tadpoles through metamorphosis in outdoor mesocosms using a factorial design incorporating three hydroperiods (fast-drying, slow-drying, or constant) and three larval odonate predator treatments (caged, uncaged, or absent). Caged and uncaged predator treatments were implemented to evaluate both consumptive and non-consumptive effects. There were no differences in survival, time to metamorphosis, or size at metamorphosis in response to drying or predation treatments. Although pond drying rates in this study did not induce measurable responses, it is possible that more extreme conditions could impact metamorphosis. The lack of response to odonate predator presence and cues suggests tadpoles of *A. blanchardi* may rely on other behavioral and phenotypic defenses, such as the tail spot, to escape predation. Developing a more comprehensive understanding of how *A. blanchardi* responds to altered hydroperiod, predators, and their potential interactions is important to predict how this species and other amphibians that breed in diverse aquatic habitats may respond to the influence of climate change on aquatic ecosystems.

WETLAND hydroperiod, the length of time a wetland retains water, plays a critical role in structuring communities by influencing the local distribution of aquatic species and mediating biotic interactions (Wellborn et al., 1996). For example, the composition of amphibian predators changes along a gradient from ephemeral to permanent ponds, which affects the distribution and diversity of amphibians in these wetlands (Smith, 1983; Werner and McPeck, 1994). Along this gradient, larval amphibians face a trade-off between predation pressure and the threat of desiccation (Söderström and Nilsson, 1987). As a response to these challenges, many species have evolved plasticity in behavior, morphology, and development (e.g., Newman, 1992; Relyea, 2004; Touchon et al., 2013). Hydrological regimes may be altered in the future because of changing precipitation patterns associated with climate change (Matthews, 2010); thus, the suitability of current habitats and the ability of species to balance predator and desiccation risks may be affected (Lawler et al., 2009; Matthews, 2010). Reduced hydroperiod could increase densities and intensify predator–prey interactions. More specifically, larval amphibians often reduce activities and therefore have slower growth and developmental rates in the presence of predators (e.g., Skelly, 1995; Anholt et al., 2000). However, longer developmental times could impact the risk of desiccation in a drying environment, where individuals must reach metamorphosis quickly. Conversely, increasing the hydroperiod could increase the abundance or diversity of predators, while reducing the risk of desiccation. Understanding how species balance and respond to these risks will enable us to evaluate potential threats to population persistence that are associated with a changing climate.

Species that have large geographic distributions and breed in both permanent and ephemeral ponds, such as *Acris blanchardi* (Blanchard's Cricket Frogs; Gray et al., 2005), could experience a variety of hydrological scenarios and be

negatively affected by climate change across their range. *Acris blanchardi* is of particular interest because of recent declines for ambiguous reasons, progressing from the northern boundaries of its range (Gray et al., 2005). Furthermore, as an annual species (Lehtinen and MacDonald, 2011), populations of *A. blanchardi* may be more susceptible to changes in juvenile recruitment, which depends on the quality of the larval environment. Understanding how individuals respond to multiple stressors is vital for providing insight on current declines and predicting future population dynamics. Furthermore, because *A. blanchardi* have a large distribution and generalized habitat requirements, they could act as an indicator species for environmental change. Changes in population dynamics of *A. blanchardi* might serve as a warning for changes in other pond-breeding anurans—especially for species with smaller geographic ranges or specialized habitat requirements.

The objective of our study was to investigate how pond drying and predation singularly and interactively affect the development of larval *A. blanchardi* and therefore evaluate potential threats to population persistence posed by altered pond hydroperiod associated with global climate change. To address our central question, we reared recently hatched tadpoles through metamorphosis in mesocosms using a factorial design of three hydroperiods (fast-drying, slow-drying, or constant) and three odonate naiaid predator treatments (caged, uncaged, or absent). We implemented both caged and uncaged predator treatments so that consumptive and non-consumptive effects could be incorporated into the understanding of predatory influences. Non-consumptive effects have been reported to strongly influence population dynamics in multiple systems (e.g., McPeck and Peckarsky, 1998; Peckarsky et al., 2008); for larval anurans, risk of predation can induce reduced algal grazing and consequently slow growth and development (Peacor and Werner, 1997; Relyea, 2004). We predicted that

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caged and uncaged predator treatments under constant pond hydroperiod would result in increased time to metamorphosis, while uncaged treatments would also experience decreased survival. For pond drying treatments, we predicted that shortened hydroperiods, in the absence of predators, would result in decreased time to metamorphosis and reduced mass at metamorphosis. By combining predator and pond drying treatments, we aimed to reveal which variable is most influential for larval development of *A. blanchardi*.

MATERIALS AND METHODS

Animal collection and care.—We collected ten amplexed pairs of *A. blanchardi* on 21 May 2013 from a permanent pond at Miami University's Ecology Research Center (ERC; Butler County, Ohio) that laid egg masses in the laboratory. Eggs were mixed in a rearing chamber at the ERC where they remained for approximately two weeks before addition to experimental mesocosms. The rearing chamber was created by filling a 1300 L mesocosm with 220 L (10 cm depth) aged tap water, leaf litter, and algal inoculates from a fishless pond.

For predator treatments, we collected dragonfly naiads in late instars (body length between 10 and 14 mm; Currie, 1961) of the species *Erythemis simplicicollis* from the same pond at the ERC where the breeding pairs were captured. *Erythemis simplicicollis* is commonly found in Ohio (Glotzhober and McShaffrey, 2002) and has a larval diet consisting of a wide variety of small aquatic animals including tadpoles (Currie, 1961). Prior to implementing experimental treatments, we ensured that naiads of *E. simplicicollis* consumed tadpoles of *A. blanchardi* by enclosing tadpoles with naiads and observed the number remaining after 24 hours; we also observed direct consumption by caged predators over the course of the experiment.

Experimental design.—To test the effects of predation and pond drying on development of larval *A. blanchardi*, we used a randomized block design with three dragonfly naiad predator treatments (caged, uncaged, or absent) and three pond hydroperiods (42 days, 63 days, or constant). We replicated each of the nine treatment combinations 3–4 times to total 34 experimental units (two mesocosms were eliminated because of no or low survival). We filled experimental mesocosms with water to a depth of 45 cm (1000 L) on 20 May 2013 at the ERC. The following day, we added 1 kg of leaf litter. We established planktonic communities by inoculating the mesocosms with pond water from a fishless pond at the ERC every other day until the start of the experiment. To prevent the invasion of undesired predators and anuran colonists, we covered each mesocosm with 2 mm screen-mesh lids. We added tadpoles on 6 June 2013 (Experimental Day 0) at a density of 30 tadpoles per mesocosm. After allowing one week for tadpole acclimation, we added dragonfly naiads on Day 7 and began pond drying treatments on Day 11.

To observe both non-consumptive and consumptive effects, we used caged and uncaged predator treatments. We released three dragonfly naiads into each mesocosm receiving uncaged predator treatments, establishing a low density of predators relative to natural densities (McPeck, 1990) that is comparable to densities used in other studies (1–4 naiads per 1000 L; e.g., Peacor and Werner, 1997; Relyea, 2004). Within caged predator mesocosms, we floated three enclosures, each containing one naiad. We

also placed three empty enclosures in predator-absent and uncaged predator mesocosms to account for any enclosure effects on tadpole development. Each enclosure was made out of a 12 cm long piece of drainage pipe (10 cm diameter) covered on each end with screen-mesh secured by a rubber band. Throughout the experiment, caged dragonfly naiads were fed tadpoles of *A. blanchardi* (~60 mg) three times a week. During the first week following predator addition, we replaced dragonfly naiads found dead or fully emerged. Dragonflies emerged an average of 24 days after addition to mesocosms (range: 8–41 days). None of the predator treatment mesocosms lost all dragonfly naiads until Day 32, and seven predator mesocosms had zero naiads remaining by the end of the experiment (Day 63).

We created a mesocosm drying schedule by following the curve $D_j = 1 - (j/t)a^P$ (Wilbur, 1987), where D_j is the desired depth for day j , t is the target date for a depth of zero ($t = 42$ for fast-drying and $t = 63$ for slow-drying), a is the shape parameter for the curves ($a = 2$), and P is the starting depth ($P = 45$ cm). Average rates of depth reduction were 1.1 cm/day for the 42 day hydroperiod and 0.7 cm/day for the 63 day hydroperiod. We selected these particular hydroperiods so that the fast-drying treatment terminated near the average initiation of metamorphosis in *A. blanchardi* (Gray et al., 2005; Youngquist et al., 2015). Every three to four days, we bailed water with buckets to achieve the desired water depth (as measured from the center of the mesocosm) until the water level reached 15 cm, after which ponds were drained three days later and remaining tadpoles collected. To equalize the effects of water agitation, we also disturbed the water in non-drying ponds by scooping and replacing two buckets of water. We collected metamorphs at the emergence of at least one forelimb (Gosner stage 42; Gosner, 1960) and held them in the laboratory until tail resorption (Gosner stage 46). Upon tail resorption, we recorded the time to metamorphosis and mass of each metamorph. At the conclusion of the fast-drying treatments on Day 42 and the slow-drying and non-drying treatments on Day 63, we drained the appropriate ponds and collected any remaining tadpoles or metamorphs.

Statistical analysis.—Our responses were proportion surviving to metamorphosis, time to metamorphosis, and mass at metamorphosis. We tested responses for normality using the Shapiro-Wilk test; survival and time to metamorphosis were not normally distributed. Therefore, we aligned-rank transformed our data and used analysis of variance (ANOVA) to test for main and interactive effects of pond drying and predation on survival, time to metamorphosis, and mass at metamorphosis (Wobbrock et al., 2011; Oliver-Rodriguez and Wang, 2015). As opposed to using rank transformed data in a Friedman test, which is a non-parametric version of ANOVA that only tests for main effects, the aligned-rank transformation procedure allows researchers to test for interactive effects. Mesocosm means were used in analyses. We conducted all analyses in R (version 3.1.0; R Core Team, 2014); we used the package ARTool (Kay and Wobbrock, 2014). Our power to detect significant differences if they existed was moderate (replicates 3–4; effect size 1.25, $\alpha = 0.05$, power = 0.58–74).

RESULTS

Predator and hydroperiod treatments did not affect survival to metamorphosis ($F_{4,25} < 1.39$, $P > 0.2668$), which was

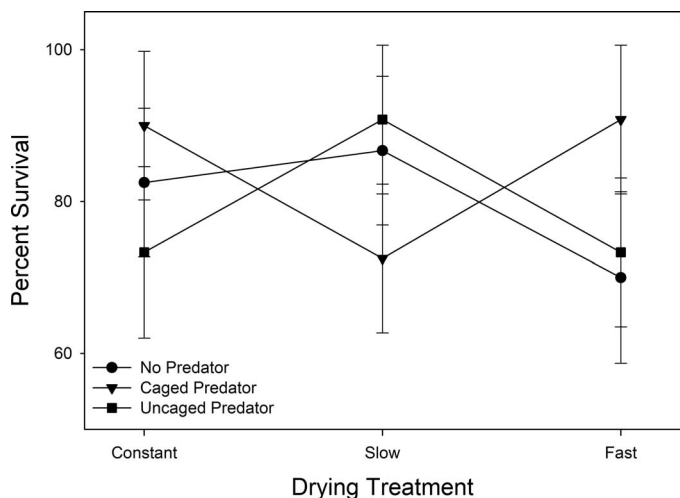


Fig. 1. Survival to metamorphosis of *A. blanchardi* across all nine experimental treatments.

relatively high across all treatments ($81.7 \pm 0.03\%$; Fig. 1). Neither predator nor hydroperiod treatments significantly affected time to metamorphosis ($F_{2,25} < 1.39$, $P > 0.2675$; 42.5 ± 0.8 days) or mass at metamorphosis ($F_{4,25} < 1.27$, $P > 0.3091$; 0.216 ± 0.009 g; Fig. 2).

DISCUSSION

Our results indicate that *A. blanchardi* are robust to odonate predation up to 3 naiads/1000 L and pond hydroperiod of 42 days or greater. Because many amphibians species are sensitive to hydroperiods and show developmental plasticity (e.g., Newman, 1992; Denver, 1997; Koprivnikar et al., 2014), we predicted that *A. blanchardi* would also be sensitive to changing hydroperiods. However, we did not observe any differences in response to the hydroperiods used in this study. This was surprising given the rapid rate of water loss: only 25% volume remained by Day 36 and Day 54 in the fast-drying and slow-drying treatments, respectively (this corresponds to a depth of ~ 11 cm). One possible reason is that *A. blanchardi* generally metamorphose in 35–90 days (Gray et al., 2005) and may have a short enough larval period that they could tolerate the experimental hydroperiods; the fast-drying treatment may not have been short enough to elicit a response. The wide range of larval period reported in the literature represents variation across their range as well as within a population. For example, this study observed a range of 33–63 days from mesocosm addition to metamorphosis. High individual variation in rates of development, within and among treatments and populations, might indicate that *A. blanchardi* have evolutionary potential to respond to future climate and environmental changes. Alternatively, *A. blanchardi* prefer permanent and semi-permanent ponds (Shulse et al., 2010) and may not have evolved an adaptive response to desiccating environments; similar results have been observed in other species that breed in permanent wetlands (e.g., Leips et al., 2000; Cook et al., 2013). Furthermore, populations of species that are distributed across a gradient of pond permanence can develop local adaptations to a single hydroperiod. For example, Amburgey et al. (2012) observed that hydroperiod of origin determined developmental rate for *Pseudacris maculata* (Boreal Chorus Frog), as opposed to experimental hydroperiods, which did not induce a plastic response. The breeding pairs used in our

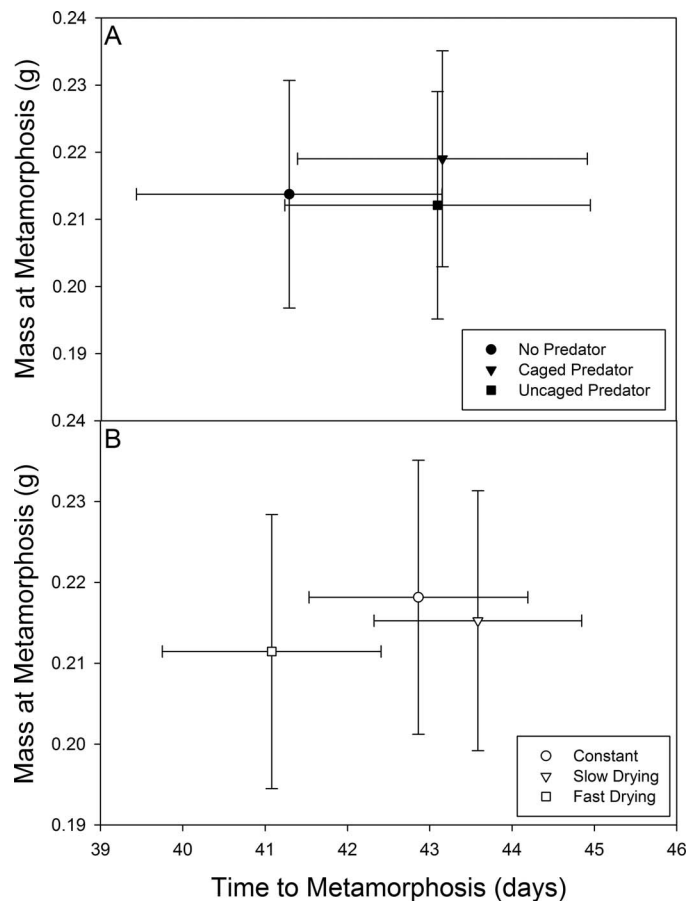


Fig. 2. Mass and time to metamorphosis of *A. blanchardi* in response to (A) predation and (B) hydroperiod.

study were collected from a permanent pond, and thus pond of origin could be a contributing factor to the lack of response.

Non-consumptive and consumptive predatory treatments used in this study did not result in any significant effects on the timing of metamorphosis and size at metamorphosis, despite other studies reporting significant reductions in tadpole developmental rate and larval activity in response to similar predator densities (e.g., Peacor and Werner, 1997; Relyea, 2004). However, these studies investigated other species and responses to predators are unlikely to be universal. The lack of response observed in our study may be attributable to an inducible phenotypic defense mechanism that makes *A. blanchardi* less vulnerable to odonate predators. Larval *A. blanchardi* possess notable dark coloration on the latter portion of their tail, referred to as a tail spot, unless they are exposed to fish cues (Carfagno et al., 2011). The tail spot is thought to help direct odonate naiad attacks towards the tail and thus away from the more critical parts of the body (Caldwell, 1982). When the naiads of *E. simplicicollis* were added to the mesocosms following the tadpole acclimation period, the tadpoles were approximately three weeks old and some appeared to be larger than the dragonfly naiads. Thus, if attacks were directed to the tail spot, individuals may have been able to escape from the predator because of more advanced swimming ability; this could explain why mortality was not significantly decreased and why predation stress may not have been severe enough to alter the timing or size at metamorphosis in predator treatments. Predation by *E. simplicicollis* on *A. blanchardi* may

be most effective only when tadpoles of *A. blanchardi* are very small. Furthermore, while *A. blanchardi* will reduce activity in response to predator cues (Carfagno et al., 2011), there may have been ample resources in the mesocosms to offset reduced activity, resulting in no overall predator effects on growth or development (e.g., Peacor, 2002).

Populations of *A. blanchardi* across the species' range may experience increased or decreased seasonal precipitation as well as changes in the timing of precipitation over the next few decades (Pryor et al., 2014; Shafer et al., 2014); thus, understanding the adaptive capabilities of this species to changing pond hydroperiods is of ecological importance. Although the pond drying regimes and dragonfly naiad predation levels used in our study did not significantly alter survival, growth, and developmental rate in *A. blanchardi*, other amphibian species have been shown to respond to odonate predation and pond hydroperiod at the cost of compromising one developmental response over the other (Laurila and Kujasalo, 1999; Altwegg, 2002). These data indicate that developmental responses of larval anurans are species and context specific. Studies like this one are needed to better predict the response of populations to changing environments and are especially important for species, like *A. blanchardi*, that are experiencing declines. With the reported dwindling of amphibian populations across their ranges, studies exploring the biological responses of species to natural and anthropogenic factors are critical to understanding and mitigating declines.

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