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**LIFE HISTORY SWITCH POINT PLASTICITY IN RESPONSE TO POND
DRYING ALYERS METAMORPH ALLOMETRY AND JUMPING
PERFORMANCE**

A thesis submitted in partial fulfillment of the requirements for the degree of Master of
Science at Virginia Commonwealth University.

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ABSTRACT

LIFE HISTORY SWITCH POINT PLASTICITY IN RESPONSE TO POND DRYING ALTERS METAMORPH ALLOMETRY AND JUMPING PERFORMANCE

By Julie Charbonnier, B.A.

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biology at Virginia Commonwealth University.

Virginia Commonwealth University, 2012

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Animals with complex life cycles can cope with environmental uncertainty by altering life history switch points through developmental plasticity. Pond drying is an important factor which may alter life history switch points in aquatic organisms. Many amphibians can plastically respond to changes in pond drying by emerging earlier, but few studies have examined the post-metamorphic consequences for performance. To investigate the potential carry-over effects of plasticity to pond drying, we studied the túngara frog, *Physalaemus pustulosus*, a tropical anuran that breeds in highly ephemeral habitats. We conducted a field study with three different water depth treatments in 60 L mesocosms and measured time and size at metamorphosis, hind limb length and jumping

performance. We also conducted a complimentary laboratory study that manipulated resources and water depth. In the field experiment, metamorphs from dry-down treatments emerged earlier, but at a similar size to constant volume treatments. In the laboratory experiment, metamorphs from the low depth and dry-down treatments emerged both earlier and smaller. In both studies, frogs from dry-down treatments had relatively shorter hind limbs, which negatively impacted their jumping performance. Reductions in resources delayed and reduced size at metamorphosis, but had no effect on jumping performance. We demonstrate that conditions experienced early in ontogeny can transcend the metamorphic boundary by erasing the relationship between hind limb length and jumping performance.

INTRODUCTION

Many organisms have complex life cycles and pass through ecologically distinct phases during ontogeny (Werner 1988). During ontogenetic switch points, such as hatching and metamorphosis, organisms undergo dramatic changes in morphology and behavior, and often relocate to different habitats (Werner 1988). When environmental conditions are uncertain, organisms can change the timing of switch points through phenotypic plasticity, and escape mortality in the current life stage (Newman 1992). For instance, organisms can accelerate or delay hatching and metamorphosis in response to changes in predation risk across life stages (Benard 2004, Vonesh and Warkentin 2006, Warkentin 2011). However, a response to environmental conditions in an early life stage may negatively impact survival and performance in subsequent life stages (Pechenik 2006) that persist later in life (Madsen and Shine 2000). The majority of studies that quantify carry-over effects focus on post-metamorphic size, because size is a fitness proxy (Stearns 1992, Honeck 1993) and do not focus on size-independent effects on organismal phenotype. However, the allometry of an animal may also be an important factor driving fitness differences between individuals and populations (Losos 1990). Understanding the consequences of both size and size-independent effects of plasticity can elucidate the linkages between life stages.

The challenge in understanding the role of size-independent effects is that size and shape are intimately linked phenotypic effects: both are governed by changes in growth rates (increases in mass) and developmental rates (differentiation of tissues) and

their interaction during ontogeny. Amphibian larvae are an ideal system for understanding size and size independent carry-over effects. First, amphibian growth and developmental rates are largely decoupled through ontogeny (Leips and Travis 1994): e.g., tadpoles can increase their mass but remain at a similar developmental stage. Additionally, amphibian larvae are highly sensitive to heterogeneous conditions during larval development (Leips and Travis 1994), and may plastically alter their growth or developmental in response to different risks during ontogeny (Wells 2007). Such plasticity enables amphibian larvae to lengthen or shorten their larval period in response to environmental heterogeneity. However, environmental factors may have similar impacts on larval duration, but result in different carry-over effects (Gomez-Mestre et al. 2010), depending on whether they modulate growth and/or developmental rates. For example, both low food levels and low temperatures lengthen larval duration. However, because food limitation restricts growth, while low temperatures restrict development, they ultimately have different effects on size and shape at metamorphosis (Gomez-Mestre et al. 2010, Tejedo et al. 2010). Conditions in the larval environment which alter growth and/or developmental rates differently may result in multiple, non-additive and opposite carry-over effects on allometry (Tejedo et al. 2010).

Carry-over effects on allometry may also have lasting effects through locomotor performance. Locomotor performance is one of the key links between morphology and fitness (Arnold 1983) and a functionally relevant trait (Irschick and Garland 2001). In anurans, jumping performance is an important fitness proxy since it can determine predator avoidance (Wassersug and Sperry 1977, Henein and Hammond 1997). This is especially true for juvenile frogs, who suffer high mortality risk because of their small

size (Carrier 1996, Toledo et al. 2007). Size at metamorphosis is an important determinant of jumping performance, but allometry differences, such as differences in relative hindlimb limb, are an important driver of locomotor performance across taxa (Harris and Steudel 2002), and a functionally relevant trait in amphibian lineages. However recent work suggests that carry-over effects on size-independent morphology may have little impact on performance (Gomez-Mestre et al. 2010).

In this study, we examined size and size independent carry-over effects of pond drying on metamorphic phenotype and locomotor performance in an anuran. In response to pond drying, many aquatic organisms can plastically accelerate development to escape drying ponds (Wellborn 1996), which has been shown to result in smaller size at metamorphosis (Newman 1992). Pond drying primarily impacts developmental rates (Leips and Travis 1994, Wilbur and Collins 1973, Denver et al. 1998), although it may depend on tadpoles having sufficiently high growth rates, and thus may be influenced by resource levels in the larval environment. We focused our investigation on the túngara frog *Physalaemus pustulosus*, a species that utilizes highly ephemeral breeding habitats, as our model system to study how conditions experienced during early ontogeny carry over to impact performance in later life stages. We conducted complimentary field and laboratory experiments to assess how water depth and resource levels impacted size, hindlimb allometry, and jumping performance.

MATERIALS AND METHODS

This study was conducted at the Smithsonian Tropical Research Institute in Gamboa, Panama (9°17'17" N, 79°42'11" W) between 17 - June and 4 - Aug- 2011. Túngara frogs (*Engystomops = Physalaemus pustulosus*, Family: Leptodactylidae) are found throughout Central America and are locally abundant at this study site. Adults are 23-33 mm in length, nocturnal and typically breed between April and December and breed in puddles, partially dried stream beds, small swamps, tree holes and frequently use artificial water sources (Rand 1983). Adults frequently utilize extremely ephemeral habitats that may dry completely during the rainy season (Marsh 1999) between 0-10 cm (personal observation) although they may also lay clutches on the banks of the larger water bodies. Eggs are laid in foam nests (clutch size: 234 eggs \pm 97.6, $\bar{x} \pm \sigma$, Ryan 1985) and hatch within 2 - 4 days. Five egg nests were collected immediately after laying on 17 - June - 2011 in Gamboa for both field and laboratory experiments. Egg nests were kept in separate containers until hatching in an ambient-temperature laboratory. Three days after hatching, tadpoles were haphazardly sampled and randomly assigned to field treatments. Four days after hatching, tadpoles were sampled and randomly assigned to lab treatments. All tadpoles were photographed and digitally measured using ImageJ software (<http://rsbweb.nih.gov.proxy.library.vcu.edu/ij/>) at the start of experiment and one week into the experiment to assess larval growth.

Field

We conducted a field experiment to determine how pond drying may impact development and post-metamorphic performance, given natural variation in daily temperature and water levels. To distinguish between the effects of water depth versus pond drying (reduction in water depth), we included two control treatments, a constant high depth treatment (10 cm of water, 15.2 L), a constant low depth treatment (2 cm, 3 L) and a decreasing (dry-down) water depth treatment (10 - 1 cm, 15.2 - 1.5 L). In the dry-down treatment, water levels were reduced by one centimeter every other day for 19 days and subsequently maintained at 1 cm until metamorphs had emerged (20 additional days). Water was disturbed in control treatments every two days to mimic the intervention we used when reducing water depth in the dry-down treatment. Treatments were replicated ten times. Treatments were randomly assigned to 60- L plastic tub containers (40 cm deep x 44 cm diameter) arranged in three rows of ten tubs in a partially forested canopy field. Tubers were filled with a mix of filtered aged rain and tap water and stocked with 10 *Inga sp.* tree leaves for cover and with 50 ml of concentrated marsh inoculate. Marsh inoculate was collected by repeatedly sweeping a plankton net through the water column of Kent's Marsh (9°07'13" N, 79°41'46" W) and then filtering it through a 1 mm mesh filter to exclude invertebrates. Tanks were covered with fine nylon mesh and secured with elastic bands to prevent colonization by other organisms. Each treatment received fifteen tadpoles. Tadpoles were fed 10 rabbit chow pellets (primarily alfalfa, mass: 0.27g \pm 0.01, $\bar{x} \pm \sigma$, ~17% protein) weekly for the first two weeks. Tadpoles were fed twice a week after the second week. Tanks were checked and maintained at experimental water levels, although could vary freely within a 24 hour period. Metamorphs were removed

upon forelimb emergence (stage 42, Gosner 1960). We randomly assigned three HOBO data loggers per treatment to monitor temperature at half hour intervals across treatments.

Lab

To determine how both resources levels and pond drying may impact post-metamorphic performance, we conducted a 3 x 2 factorial experiment where we manipulated resource levels (high and low food) and water depth. Lab treatments were conducted in 1.5 L (15.7 cm x 10.9 cm) plastic containers filled with aged tap water. Treatments were randomly assigned and one tadpole was randomly assigned to each container. Depth treatments were consistent with field treatments and included one constant high depth (10 cm of water, 1.5 L), constant low depth (2 cm, 0.35 L) and a dry-down treatment (10-1 cm, 1.5 - 0.17 L). In the dry-down treatment, water levels were reduced by one centimeter every other day for 19 days and subsequently maintained at 1 cm until metamorphs had emerged (3 additional days). Water was disturbed in control treatments every two days to mimic the intervention we used when reducing water depth in the dry-down treatment. Tadpoles in high food treatments were fed 2 *Nutrafin Max*® fish flakes every day for the first week of the experiment and four fish flakes daily during the second week (mass of fish flake: $0.021\text{g} \pm 0.003$, $\bar{x} \pm \sigma$, ~44% protein). Tadpoles in low food treatments were fed the same amount but every two days. Although food types differed in quality, fish flakes enabled us to allot smaller quantities of food per tadpole, and per capita rates in the low food treatments were similar to per capita rates in the field treatment (Field: $0.36\text{ g wk}^{-1}\text{;indiv}^{-1}$, High food lab: $0.60\text{ g wk}^{-1}\text{;indiv}^{-1}$, Low food $0.30\text{ g wk}^{-1}\text{;indiv}^{-1}$). Feces and unconsumed food were removed every other day and water was

changed weekly. Laboratory conditions were 12:12 photoperiod cycle in an ambient temperature laboratory.

Juvenile Performance

Metamorphs were collected daily at forelimb emergence (stage 42, Gosner 1960) and weighed on a digital scale to the nearest milligram. Metamorphs were maintained individually in 240 ml plastic cups in the laboratory and misted with water and checked daily for tail reabsorption (stage 46, Gosner 1960). We measured snout-vent length (SVL) and tibiofibula length using digital calipers and weighed frogs upon tail reabsorption. To assess jumping performance, each frog was stimulated to jump in a circular artificial array with 0.5 cm markings. Jumping was initiated by lightly prodding the frog's urostyle (Goater 1993, Niehaus et al. 2006). Frogs were jumped three times per trial and three trials were conducted per frog (for a total of nine jumps), with a 5 minute rest period in between trial. Maximum and average jump lengths were calculated. To ensure we appropriately assessed maximum jumping performance, a subset of 24 frogs were jumped 21 times to examine whether nine jumps gave a viable estimate of maximum jumping performance. Maximum jump length of nine jumps was a significant predictor of maximum jump length of twenty one jumps ($F_{1, 22} = 337.6$, $P < 0.001$, $R^2 = 0.94$, $n = 24$). Average jump length of nine jumps was a significant predictor of average jump length of twenty one jumps ($F_{1, 22} = 249.2$, $P < 0.001$, $R^2 = 0.92$, $n = 24$).

Statistical Analyses

All statistical analyses were conducted on tank means using R version 2.14.0 (R Development Core Team 2011). We analyzed larval growth and survival using ANOVAs. To examine effects of pond drying on metamorph phenotype, we examined

how time to metamorphosis, SVL, tibiofibula length and final mass at metamorphosis were affected using a MANOVA. To examine effects of water depth on metamorph performance, we conducted stepwise linear models on maximum and average jump length with tibiofibula length, SVL, water depth and food (in the laboratory experiment) as predictor variables. When a significant multivariate effect was detected ($P < 0.05$), we examined the univariate effects. Our dependent variables were normally distributed as assessed by the Shapiro-Wilk test with one exception. Time to metamorphosis in the laboratory was not normally distributed, but for simplicity of analysis and because MANOVA is fairly robust to violation of non-normality (Fox 1997), we have included time to metamorphosis within the MANOVA analysis. There was homogeneity of variance between groups as assessed by Levene's test for equality of error variances with the exception of maximum and average jump length in the field experiment, which was log transformed. To allow comparisons across experiments, we log transformed maximum jump length in the laboratory experiment. Post-hoc comparisons were conducted using Tukey's HSD.

RESULTS

Field Experiment

The mean daily temperature from 23 June - 16 July was 26.73 ± 0.12 ($\bar{x} \pm \sigma$) and similar across treatments ($F_{2,6} = 1.5$, $P = 0.29$). Maximum daily temperature differed between treatments ($F_{2,6} = 5.2$, $P = 0.049$) but only between dry-down ($33.4 \text{ }^\circ\text{C} \pm 1.5$) and high depth ($30.9 \text{ }^\circ\text{C} \pm 0.62$) treatments ($P = 0.04$). Tadpole total length did not differ among treatments at the start of the experiment ($F_{2,25} = 0.24$, $P = 0.79$). Tadpoles in low depth treatments had on average 12% lower growth during the first week of the experiment than high depth and dry-down treatments ($F_{2,23} = 4.59$, $P = 0.02$). Mean survivorship through metamorphosis in treatments was high ($90 \pm 10\%$, $\bar{x} \pm \sigma$), although low depth treatments had 5% higher survival than high depth and dry-down treatments ($\chi^2 = 6.3$, $P = 0.04$). Two replicates of each control treatments were lost during the experiment ($n = 10$ for dry-down treatments, $n = 9$ for high and low depth treatments). Water depth affected metamorphic phenotype (Pillai = 0.80, $F_{8,46} = 3.85$, $P = 0.02$). Water depth affected time to metamorphosis ($F_{2,25} = 7.90$, $P = 0.002$), with frogs from dry-down treatments emerging 16% earlier (~ 4 days) than high depth frogs ($P = 0.002$) and 13% earlier (~ 3 days) than low depth frogs ($P = 0.03$; Fig. 1a). Water depth did not affect SVL ($F_{2,25} = 1.25$, $P = 0.30$) or mass ($F_{2,25} = 1.53$, $P = 0.24$) of metamorphs (Fig. 1b). Although there were no differences in size, treatment did affect absolute tibiofibula length ($F_{2,25} = 5.30$, $P = 0.01$). Specifically, the tibiofibula of frogs from dry-down

treatments were 6% shorter than those from high depth treatments ($P = 0.013$) and 4.8% shorter than frogs from low depth treatments, although this effect was marginal ($P = 0.057$). Water depth also altered relative tibiofibula length ($F_{2,25} = 8.20$, $P = 0.002$; Fig. 1c), with frogs from dry-down having on average 3% shorter relative tibiofibula length than low depth frogs ($P = 0.01$) and high depth frogs ($P = 0.003$).

Effects of pond drying on metamorph performance

Treatment did not affect maximum jump ($F_{2,25} = 2.542$, $P = 0.098$; Figure 1d) or average jump ($F_{2,25} = 2.483$, $P = 0.104$) beyond its effect on tibiofibula. The stepwise linear model examined the effects of tibiofibula length, SVL and water depth on the log of maximum jump length. Only tibiofibula and a tibiofibula by water depth entered the model, and this model explained 84% of the variation ($F_{6,21} = 24.19$, $P < 0.001$, $R^2 = 0.84$, Fig. 3a). There was an effect of tibiofibula length ($F_{1,21} = 14.99$, $P < 0.001$) and a tibiofibula by water depth interaction ($F_{2,21} = 3.39$, $P = 0.05$). In both the constant water depth treatments, increases in tibiofibula length were correlated in higher maximum jumps, while in dry-down treatments there was no relationship between tibiofibula length and maximum jump length. Results for average jump parallel those of maximum jump and are not presented for sake of brevity.

Laboratory Experiment

We identified three extreme outliers out of 48 as defined by (Hoaglin and Welsh 1978, Fox 1997) across three different treatments (low food & high depth, high food & low depth, high food & dry-down) that had high leverage and influence. These outliers were excluded from analysis. Analyses gave quantitatively similar patterns when outliers were not excluded. Mean daily temperature in the laboratory was $26.4\text{ }^{\circ}\text{C} \pm 0.26$ ($\bar{x} \pm$

σ). The initial length of tadpoles did not differ between treatments ($F_{5,42} = 1.68$, $P = 0.16$). Only resources affected growth during the first week of the experiment ($F_{1,43} = 36.4$, $P < 0.001$), with frogs in high food treatments growing 36 % faster than frogs from low food treatments. All frogs survived to forelimb emergence (Gosner stage 42). Eighty-percent of frogs survived until the completion of metamorphosis and there were no differences in survival among treatments ($\chi^2 = 4.2$, $P = 0.52$). Both resources and water depth affected metamorphic phenotype (Table 1). Larval duration was affected by resources and water depth. A 50% reduction in food resulted in 17% (~3 days) longer larval duration ($P < 0.0001$; Fig. 2a). Frogs from dry-down and low depth treatments emerged on average 10% earlier (~2 days) than frogs from high depth treatments ($P = 0.005$, $P = 0.018$; Fig. 2a). Mass and SVL at metamorphosis were affected by both resources and water depth. Frogs from high food treatments had 14% longer SVL and 40% higher mass than frogs from low food treatments ($P < 0.0001$, $P < 0.0001$; Fig. 2b). Frogs from dry-down ($P = 0.025$) and low depth treatments ($P = 0.02$) had on average 10% smaller SVL than high volume frogs. Frogs from dry-down treatments were 20% smaller than frogs from high depth treatments, although this effect was marginal ($P = 0.07$; Fig. 2b). Frogs from low depth treatments were 27% smaller than frogs from high depth treatments ($P = 0.03$, Fig. 2b). Absolute tibiofibula length was shaped by the interaction of resources and water depth ($P = 0.005$, Fig. 2c; Table 1). Frogs from dry-down treatments had 36% shorter tibiofibulas than frogs from high depth treatments, and 14% smaller tibiofibulas from low depth treatments ($P < 0.0001$, $P = 0.04$) but there was no such pattern in high food treatments. In high food treatments, there was no difference between dry-down frogs and either control treatment, but low water depth frogs had 16% smaller tibiofibulas than

frogs from high depth treatments ($P = 0.023$). Both resources and water depth affected relative tibiofibula length. A 50 % reduction in food resulted in 17% longer relative tibiofibula length ($P < 0.0001$). Frogs from dry-down treatments had 10 % ($P = 0.021$) smaller relative tibiofibula than high depth frogs (Fig. 2c). There was no difference in relative tibiofibula length between frogs from dry-down and low depth treatments, or between high and low depth treatments.

Effects of pond drying on metamorph performance

Reduction in water depth had a negative effect on maximum jumping performance ($F_{2,31} = 8.53$, $P = 0.001$; Fig. 2d). Frogs from dry-down treatments had 33% lower maximum jump length ($P = 0.004$) and frogs from low depth treatments had 23% lower maximum jump length than frogs from high volume treatment ($P = 0.002$, Fig. 2d). We found similar results for average jumping performance, ($F_{2,31} = 7.90$, $P < 0.001$) with low depth ($P < 0.001$) and dry-down frogs having on average 34% smaller average jump length than high volume frogs ($P < 0.001$).

The stepwise linear model examined the effects of tibiofibula length, SVL, water depth and food on the log of maximum jump length ($F_{15, 21} = 2.9$, $P = 0.01$, $R^2 = 0.45$). Only the three way interaction between water depth, tibiofibula and SVL entered the model ($F_{2,21} = 5.07$, $P = 0.016$). To facilitate comparison with the field experiment, we log-transformed maximum jump length and found a similar pattern ($F_{13, 23} = 2.104$, $P = 0.058$, $R^2 = 0.25$), with a water depth by tibiofibula by SVL interaction ($F_{2,23} = 3.76$, $P = 0.04$ Fig. 3b).

DISCUSSION

Phenotypic plasticity enables organisms to persist in ephemeral environments by adjusting their growth and developmental rates, altering the timing of ontogenetic switch points (Werner 1980, Newman 1992). Our results provide evidence that developmental plasticity experienced early in ontogeny can impact performance in later stages by altering size and/or the allometry of metamorphs at metamorphosis. In both the field and laboratory experiments, changes in water depth in the larval environment negatively impacted metamorphic jumping performance. A fundamental relationship across taxa is that increases in tibiofibula are correlated with increases in locomotor performance (Harris and Steudel 2002). While frogs from high and low depth environments followed this pattern, tibiofibula and maximum jump were not correlated in frogs from dry-down treatments (Fig. 3a). We demonstrate that conditions experienced early in ontogeny can transcend the metamorphic boundary by erasing the relationship between tibiofibula and jumping performance. The change in this relationship was not driven by differences in sizes, as we did not observe differences in SVL and mass across treatments in the field (Fig. 1b).

In the laboratory experiment, our resource manipulation generated differences in length and mass across treatments (Fig. 2b). Maximum jump was dependent on the relationships between tibiofibula, body length and water depth. We found that the effect of body length on jumping performance is mediated by tibiofibula length (i.e., small frogs

with long legs performed better than larger frogs with short legs). Previous studies have emphasized the role of body length alone on jumping performance (Taigen and Pough 1981, John-Adler and Morin 1990, Goater et al. 1993). However, we found that water depth in the larval environment negatively impacts jumping performance, by changing the allometric relationship between body length and tibiofibula. As a result, frogs from dry-down and constant low water treatments had smaller relative tibiofibulas (Fig. 2c) and shorter jumps (Fig. 2d). The permanency of the larval environment likely determines maximum jump length through its effects on the relationship between body length and tibiofibula, rather than through the effects on body length alone.

Both experiments strongly suggest that size-independent effects may directly impact locomotor performance. Previous work suggests that change in relative morphology under 10% should have little impact on locomotor performance (Emerson 1978, Gomez Mestre et al. 2010). We found that relatively small changes in allometry (~ 3%) in the field experiment will impact the morphology-performance relationship. In the laboratory experiment, we observed larger shifts in allometry (~ 10 %) that resulted in a 33 % reduction in maximum jumping performance. Recent work suggests that size at metamorphosis is incomplete metric of fitness in juvenile anurans. Later compensatory growth may erase initial differences in size at metamorphosis (Boone 2005) and mark-recapture work suggests that survival during the first year of life may not be related to size at metamorphosis (Schmidt et al. 2012). Although size and time to metamorphosis remain important proxy of overall fitness, our results suggest that understanding size-independent effects can improve our understanding of the linkages between life stages.

This study provides potential insight into the mechanisms driving the developmental acceleration to pond drying because we utilized a low water depth treatment that controlled for the effects of constant low water depth. Previous laboratory studies have found that animals in constant low treatments have shorter (Denver et al. 1998, Spieler 2000) or similar larval duration (Szekely et al. 2010) than animals in dry-down treatments. The only exception to our knowledge is that of Crump (1988) who used a “low depth” control that had an approximately intermediate depth between the high depth and dry-down treatment. In this experiment, low depth animals had longer larval duration than animals from dry-down treatments. In our laboratory experiment, dry-down and low depth frogs had similar larval duration, and emerged earlier than high depth frogs (Fig. 2a). These results, combined with the previously aforementioned studies, suggest that constant low water depth will also trigger the hormonal cascade signaling developmental acceleration. Such developmental acceleration is thought to be mediated by reductions in vertical swimming volume and by proximity to the water surface (Denver et al. 1998). We propose that in laboratory studies conducted in low depth (0.27 cm to 2 cm) and low volume containers (0.35 L to 1.5 L), may similarly cause a developmental acceleration and shorten larval duration. Selection may have favored developmental acceleration in low volumes; as such habitats tend to desiccate more quickly in nature. However, in our field experiment, dry-down frogs had shorter larval duration than both the high and low depth controls. This pattern may be explained by the periodic refilling of the low depth treatments. When pools are frequently refilled by rain, the sequential reductions in swimming volume may be necessary to induce the acceleration of development. To our knowledge, we do not know of other studies who

utilize a low depth control treatment in the field. Most pond drying studies do not include a low depth control treatments, making it difficult to determine how dry-down and constant low depth impact the response to pond drying in field conditions.

The traditional model of metamorphosis predicts that animals that metamorphose early should emerge smaller (Wilbur and Collins 1973, Newman 1992). However, many studies do not observe this predicted trade-off, with frogs from dry-down treatments emerging at similar sizes than frogs in control treatments (Wells 2007). We also did not observe this pattern in our field experiment and in the high food treatments in our laboratory experiment (Figures 2a, 2b). We did observe the expected pattern in the low food treatments in the laboratory experiment, with animals from dry-down treatments having smaller mass and SVL (Fig 2b). We hypothesize that the lower and upper limits of size at metamorphosis modulated by growth rates may prevent the expected time and size trade-off. Thus, if tadpoles have high growth rates (e.g. lab experiment, high food), dry-down frogs may reach the maximum size at metamorphosis quickly, and metamorphose at large and equal sizes than frogs from control treatments. If tadpoles have low growth rates (e.g. field experiment), dry-down frogs reach the minimum size at metamorphosis, and thus metamorphose at small and equal size than frogs from control treatments. If animals are raised at intermediate growth rates, trade-offs between size and time at metamorphosis are more likely to be evident. Ideally, a factorial experiment manipulating a range of food availability and pond drying could confirm this prediction. However, past studies typically do not report specific amounts of food, making it difficult to directly infer growth rates and thus relate them to size at metamorphosis. As we have demonstrated, even if there are no size differences among treatments, size-independent

effects (caused by changes in relative tibiofibula) can carry-over and have consequences on locomotion.

Our study contributes to previous literature on the effects of pond drying on amphibian larvae. Past studies have largely focused on the response of desert species, as they are thought to be more likely to demonstrate plasticity to pond drying (Newman 1992, Szekely et al. 2011, Wells 2007). Certain families of amphibians (most notably spadefoot toads) have been the traditional model system for exploring plasticity to pond drying and its proximal and ultimate drivers (Denver et al. 1998, Buchholz and Hayes 2002). However, utilizing closely related species can confound our understanding of the relative contributions of phylogeny and breeding habitat on the evolution of plasticity (Buchholz and Hayes 2002, Wells 2007). Plasticity to pond drying is likely selectively advantageous if the mean hydroperiod is low (e.g., temporary habitats) and if there is variations in the mean hydroperiod (e.g, through differences in habitat selection, or frequent rainfall). Desert species experience low mean hydroperiod, but little variation in rainfall. Kulkarni et al. (2012) suggests that within the spadefoot species, desert-adapted species have gradually evolved less plasticity to pond drying than non-desert species, because selection favors a fixed fast developmental time. Thus, species which breed in temporary habitats, but experience variation in hydroperiod due to frequent rainfall, can provide novel insight into the common mechanisms and carry-over effects of plasticity in larval duration.

Pond drying is an ideal factor for examining the potential consequences of carry-over effects. Pond drying is a key factor shaping aquatic communities (Wellborn 1996). Local hydroperiod regimes will likely be altered as climate change is expected to change

precipitation and temperatures regimes worldwide (Brooks 2002, Bauder 2005, Brooks 2009, Christensen et al. 2007). In Central America, climate models predict that total annual precipitation will decrease, specifically during the wet season (June - August) during amphibian breeding and tadpole development (Hulme 1998, Neelin et al. 2006, Lau and Wu 2007). At our study site in Gamboa, Panama, long term meteorological data indicates that the gaps between rainfall events are increasing. Larger gaps in rainfall (4 days without rain) are occurring more frequently (Touchon, in prep). For our study species, rainfall gaps of a few days could greatly increase the chance of desiccation. While the túngara frog possesses other adaptations to survive in ephemeral habitats (e.g., foam nests), response to pond drying may become more important for determining juvenile phenotype and recruitment.

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Table 1. MANOVA results for the effects of resource levels and pond drying on metamorphic phenotype. Multivariate tests are in boldface type, and univariate tests are in lightface type.

Source	Df	F	P
Food	4,28	13.3207	<0.001
Larval Duration	1,31	22.8796	< 0.001
SVL	1,31	31.9287	< 0.001
Mass	1,31	34.6222	< 0.001
Tibiofibula	1,31	0.1350	0.7157
Tib/SVL	1,31	29.4707	< 0.001
Water Depth	8,58	3.0399	0.006
Time to metamorphosis	2,31	39.006	0.0066
SVL	2,31	2.8546	0.01161
Mass (stage 46)	2,31	4.0225	0.02798
Tibiofibula	2,31	17.5791	<0.001
Tib/SVL	2,31	4.0515	0.0273
Interaction	8,58	1.2917	0.2659
Time to metamorphosis	2,31	0.0145	0.9856
SVL	2,31	1.8528	0.17374
Mass (stage 46)	2,31	1.6541	0.20771
Tibiofibula	2,31	6.2096	0.005395
Tib/SVL	2,31	1.8349	0.17654

Figure 1. Effect of water depth on (a) larval duration (b) mass at metamorphosis (c) relative tibiofibula length and (d) maximum jump length ($\bar{x} \pm \sigma$) in *Physalaemus pustulosus* in the field experiment. High depth were maintained in 10 cm of water (n = 9), low depth were maintained in 2 cm of water (n = 9), and dry-down in 10 cm - 1 cm of water (n = 10).

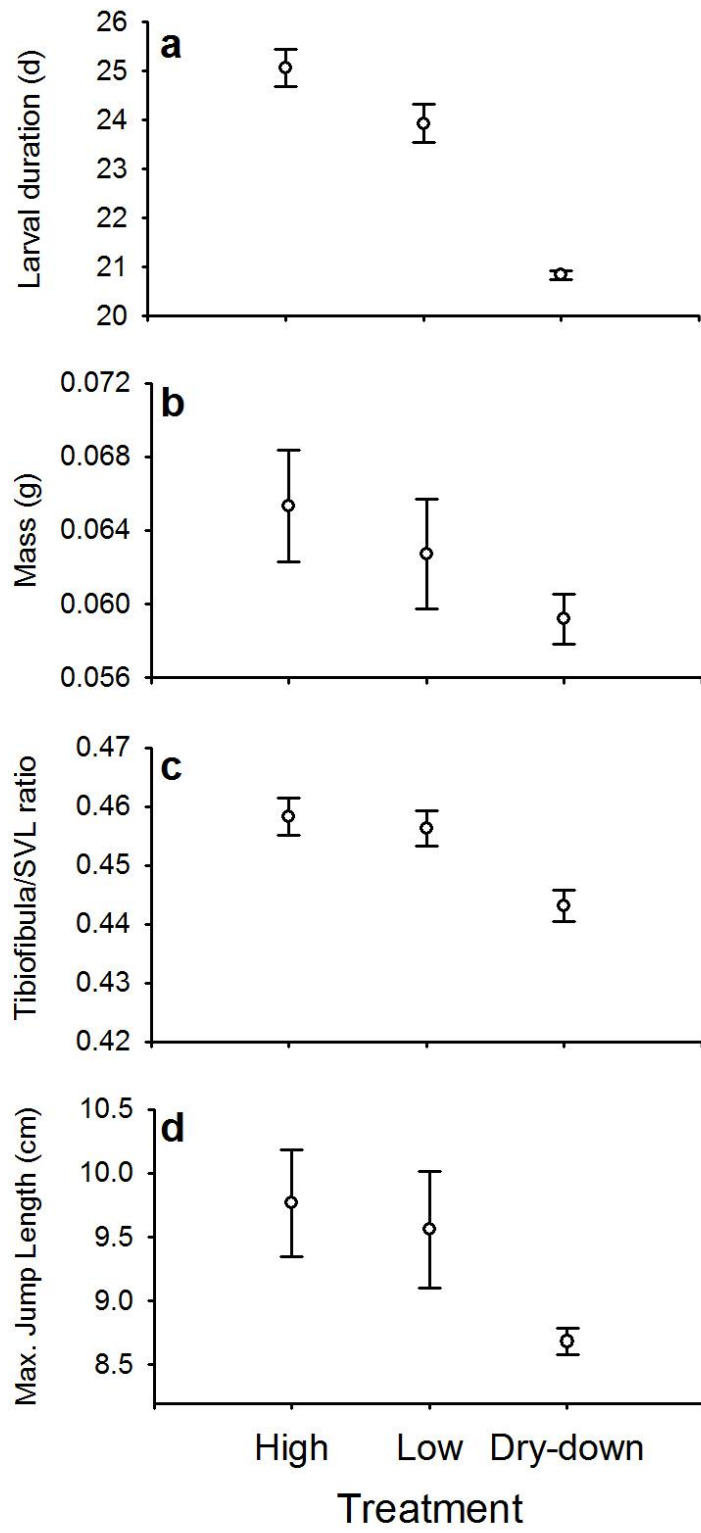


Figure 2. Effect of water depth on (a) larval duration (b) mass at metamorphosis (c) relative tibiofibula length and (d) maximum jump length ($\bar{x} \pm \sigma$) in the túngara frog in the laboratory experiment. Animals in high food treatments (closed symbols) received twice as much food (fish flakes) compared to animals in low food treatments (open symbols). High depth were maintained in 10 cm of water , low depth were maintained in 2 cm of water, and dry-down in 10 cm - 1 cm of water (n = 8 per treatment).

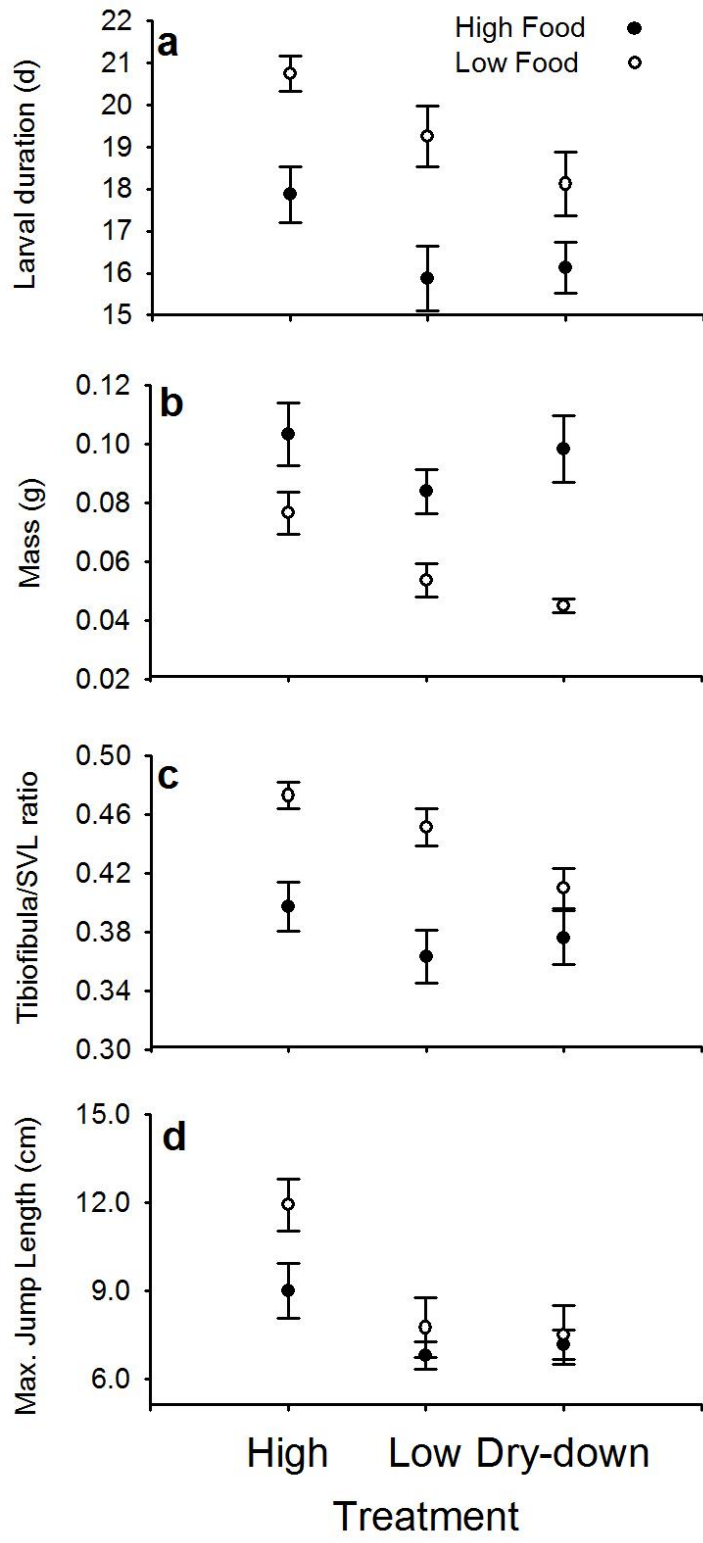
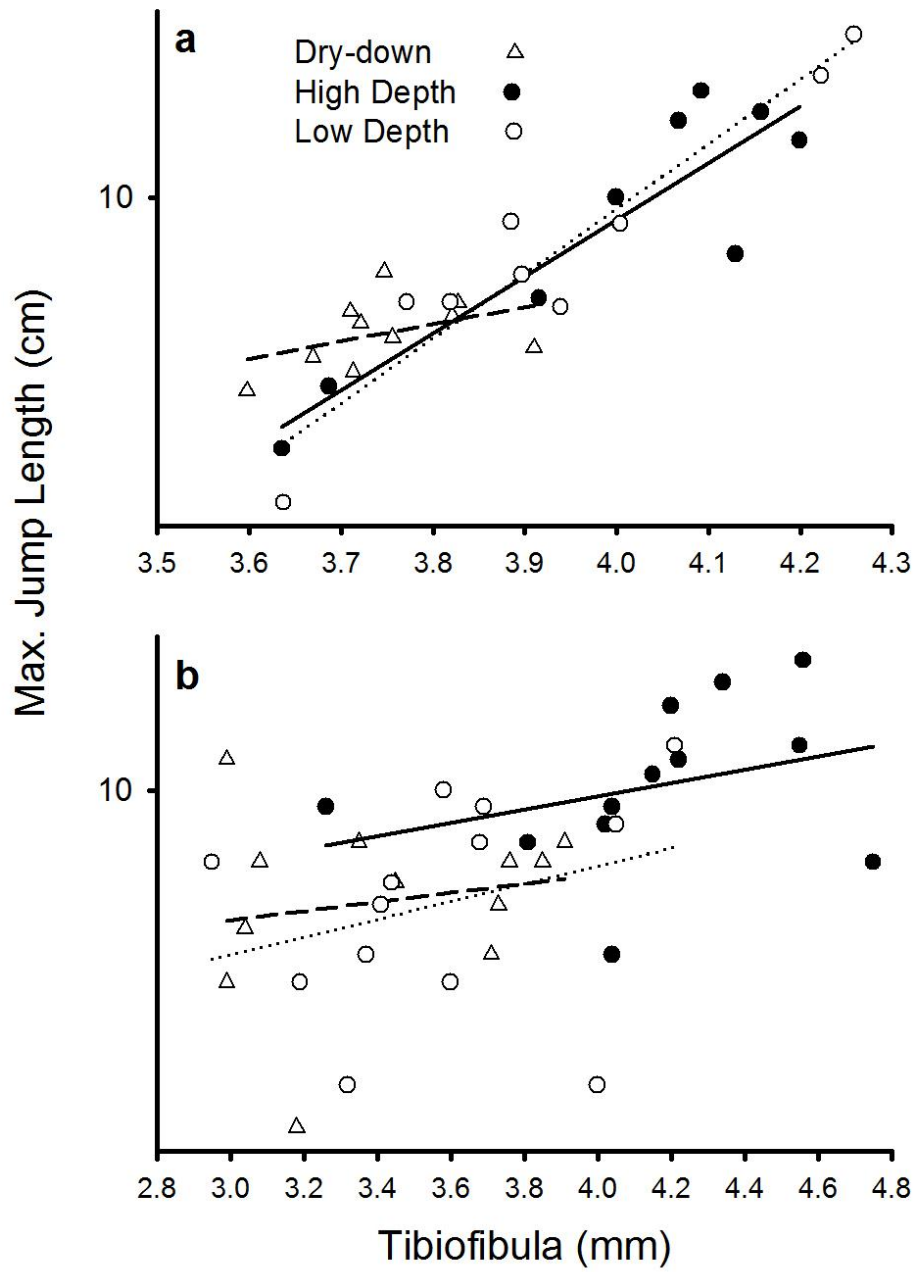


Figure 3. Relationship between tibiofibula and maximum jump length in the túngara in high depth (●, solid line), low depth (○, dotted line), and dry-down (Δ, medium dashed line) as a function water depth in (a) the field experiment and the (b) laboratory experiment. Field experiment (a): **High depth:** Overall model: $F_{1,8} = 30.5$, $P < 0.001$, $R^2 = 0.79$; slope = 0.60, $t_7 = 5.52$, $P < 0.001$; intercept, -0.10, $t_8 = -0.23$, $P = 0.82$. **Low Depth:** Overall model: $F_{1,7} = 64.9$, $P < 0.001$, $R^2 = 0.89$; slope = 0.68, $t_7 = 8.057$, $P < 0.001$; intercept, -0.42, $t_8 = -1.27$, $P = 0.25$. **Dry-down:** Overall model: $F_{1,8} = 1.80$, $P = 0.22$, $R^2 = 0.08$; slope = 0.17, $t_8 = 1.34$, $P = 0.22$; intercept, 1.49, $t_8 = 2.98$, $P = 0.02$. Laboratory experiment (b): **High depth:** Overall model: $F_{1,8} = 1.088$, $P = 0.32$, $R^2 = 0.0079$; slope = 0.21, $t_{10} = 1.04$, $P = 0.32$; intercept, 1.46, $t_{10} = 1.77$, $P = 0.11$. **Low depth:** Overall model: $F_{1,11} = 1.0$, $P = 0.33$, $R^2 = 0.003$; slope = 0.27, $t_{11} = 0.983$; $P = 0.33$; intercept, -0.96, $t_{11} = 0.98$, $P = 0.35$. **Dry-down:** Overall model: $F_{1,10} = 0.324$, $P = 0.58$, $R^2 = -0.07$, slope = 0.14, $t_{10} = 0.57$, $P = 0.58$; intercept, 1.47, $t_{10} = 1.72$, $P = 0.116$



Vita

Julie F. Charbonnier was born on July 25, 1988 in Paris, France. She moved to Philadelphia when she was 10 years old. She graduated from Central High School in 2006 and attended the University of Pennsylvania University from which she graduated in 2010 cum laude with a B.A. in Biology. She matriculated to Virginia Commonwealth University in September 2010 to pursue a M.S. degree in Biology and conducted her research in the Summer of 2010 in Panama. After graduation, she will pursue a Fulbright Fellowship in Seville, Spain.