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SYMPOSIUM

Developmental Stage Affects the Consequences of Transient Salinity Exposure in Toad Tadpoles

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Synopsis Development can play a critical role in how organisms respond to changes in the environment. Tolerance to environmental challenges can vary during ontogeny, with individual- and population-level impacts that are associated with the timing of exposure relative to the timing of vulnerability. In addition, the life history consequences of different stressors can vary with the timing of exposure to stress. Salinization of freshwater ecosystems is an emerging environmental concern, and habitat salinity can change rapidly due, for example, to storm surge, runoff of road deicing salts, and rainfall. Elevated salinity can increase the demands of osmoregulation in freshwater organisms, and amphibians are particularly at risk due to their permeable skin and, in many species, semi-aquatic life cycle. In three experiments, we manipulated timing and duration of exposure to elevated salinity during larval development of southern toad (*Anaxyrus terrestris*) tadpoles and examined effects on survival, larval growth, and timing of and size at metamorphosis. Survival was reduced only for tadpoles exposed to elevated salinity early in development, suggesting an increase in tolerance as development proceeds; however, we found no evidence of acclimation to elevated salinity. Two forms of developmental plasticity may help to ameliorate costs of transient salinity exposure. With early salinity exposure, the return to freshwater was accompanied by a period of rapid compensatory growth, and metamorphosis ultimately occurred at a similar age and size as freshwater controls. By contrast, salinity exposure later in development led to earlier metamorphosis at reduced size, indicating an acceleration of metamorphosis as a mechanism to escape salinity stress. Thus, the consequences of transient salinity exposure were complex and were mediated by developmental state. Salinity stress experienced early in development resulted in acute costs but little long-lasting effect on survivors, while exposures later in development resulted in sublethal effects that could influence success in subsequent life stages. Overall, our results suggest that elevated salinity is more likely to affect southern toad larvae when experienced early during larval development, but even brief sublethal exposure later in development can alter life history in ways that may impact fitness.

Introduction

Organisms exist in environments that are constantly changing, often in unpredictable ways or at unpredictable times. The dynamic nature of the environment poses challenges to fitness, and development can play a critical role in mediating how organisms are affected by environmental change. Plasticity—in physiology, behavior, and development—is an important mechanism by which many organisms cope with unpredictable environmental variation. In particular, developmental plasticity can adjust the phenotype in response to the environment by inducing different developmental pathways or trajectories (West-Eberhard 2003; Sultan 2017). However, development can also impose constraints on an organism's responses to environmental change. For example, the ability to tolerate an environmental perturbation or stress can vary with developmental stage (e.g., Boege and Marquis 2005; Bowler and Terblanche 2008) or plasticity can be limited to a particular window of development (Hoverman and Relyea 2007; Fischer et al. 2014). Thus,

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understanding how changes in the environment impact organisms requires understanding not only the nature of environmental variation but also how development shapes potential responses.

Increased and variable salinity in freshwater ecosystems is an emerging environmental concern (Kaushal et al. 2018; Cañedo-Argüelles et al. 2019). Influx of salts can result from a variety of sources including runoff of road deicing salts, coastal storm surge, agricultural practices, and land use change (Gornitz 1991; Williams 2001; Morton and Barras 2011). Salinity levels within affected environments can be dynamic, with runoff, flooding, rainfall, and evaporation rapidly changing the concentrations of salts in the water (Gornitz 1991; Morton and Barras 2011). For example, coastal freshwater wetlands can be inundated with water of higher salinity during storm surge events, leading to a rapid increase in salinity (Gunzburger et al. 2010); salinity in these habitats can also change due to rainfall, evaporation, and water management techniques (Gordon et al. 1989; Moreira et al. 2015). Consequently, coastal freshwater habitats can be at risk of rapid and potentially dramatic fluctuations in salinity. This issue is particularly timely, as climate change can contribute to and intensify fluctuations in habitat salinity, due to sea level rise and an increase in extreme weather events. For freshwater organisms, a rise in salinity can increase the demands of osmoregulation and ultimately pose a threat to survival, as many species cannot maintain homeostasis beyond a narrow range of salinities (Burggren and Just 1992).

Amphibians with aquatic life stages are at particular risk of freshwater salinization given their permeable skin and eggs, and relatively poor osmoregulatory abilities (Burggren and Just 1992; Gomez-Mestre et al. 2004), though species vary in their salinity tolerance (reviewed in Alexander et al. 2012; Hopkins and Brodie 2015). Exposure to elevated salinity can result in reduced survival (reviewed in Alexander et al. 2012), suppressed larval growth (e.g., Christy and Dickman 2002; Chinathamby et al. 2006), increased risk of predation (Squires et al. 2008), and smaller size at and/or delayed metamorphosis (e.g., Gomez-Mestre and Tejedo 2003; Sanzo and Hecnar 2006; Wu and Kam 2009). However, few studies have investigated the consequences of transient salinity changes during amphibian development.

For amphibians, the effects of salinity fluctuation are likely to depend on developmental context in multiple ways. First, salinity tolerance may change during an organism's development, such that the consequences of salinity exposure may depend on timing. Second, various forms of developmental plasticity may be induced by exposure to elevated salinity, with consequences for performance later in life. For example, acclimation may equip an individual to cope with greater salt concentrations later in development. Another form of developmental plasticity that may result from salinity exposure is compensatory growth (Box 1), a period of accelerated growth in response to improved conditions, which can offset a growth deficit incurred earlier in development (Metcalfe and Monaghan 2001; Hector and Nakagawa 2012). Though limited, existing studies in amphibians suggest that salinity tolerance increases during larval development (Wu et al. 2012; Kearney et al. 2014) and that compensatory growth may occur following release from salinity stress experienced early in development (Squires et al. 2010; Kearney et al. 2014; Hsu et al. 2018).

Developmental plasticity in response to changing environmental conditions can also result in life history shifts. For organisms with complex life cycles, age and size at metamorphosis are predicted to show adaptive plasticity in response to conditions in the larval environment (Wilbur and Collins 1973; Newman 1992). In high quality environments, individuals are predicted to maximize size at metamorphosis via an extended period of larval growth, while in poor environments individuals are predicted to accelerate development resulting in smaller size at metamorphosis (Wilbur and Collins 1973). When the quality of the environment changes during development, amphibian larvae are predicted to respond with altered developmental trajectories, although the capacity for or form of plasticity may be modulated by developmental stage (Box 1). These model predictions have been applied to various axes of environmental quality, including food availability, competition, pond drying, and predation (reviewed in Newman 1992; Rose 2005). Although metamorphic plasticity in response to changes in habitat salinity has been observed in a few recent studies (Wu et al. 2012; Kearney et al. 2014; Hsu et al. 2018), how these effects are influenced by developmental stage is not yet clear.

To better understand the consequences of salinity fluctuation for amphibians, we examined the effects of short-term salinity increases occurring at different points during larval development. In three complementary experiments, we manipulated the timing and duration of exposure to elevated salinity during larval development of the southern toad, *Anaxyrus terrestris*, to investigate the following questions. Does salinity tolerance vary during larval development? Does developmental plasticity occur that can help

Box 1. Predictions arising from models of adaptive developmental plasticity

Growth rates during and after salinity stress: Tadpoles exposed to elevated salinity are expected to experience decreased growth rates, from increased energetic costs of osmoregulation (Gomez-Mestre et al. 2004) as well as depressed feeding rates (Sanzo and Hecnar 2006; Wood and Welch 2015). Because these sources of growth reduction are reversible, if rainfall or another influx of freshwater decreases salinity, growth rates are expected to recover. Variation in the timing and extent of this expected growth recovery generates alternative predictions for the life history consequences of exposure to a transient stress during larval development.

Transient salinity stress early in larval development: Consequences of a transient exposure to salinity early in larval development may depend on the capacity for compensatory growth (Fig. 1A). Salinity stress may be followed by a period of compensatory growth (Metcalfe and Monaghan 2001; Hector and Nakagawa 2012), allowing body size to "catch up" to unexposed controls such that metamorphosis is reached at an equivalent size and age. Alternatively, growth rate may recover to meet but not exceed that of controls, allowing the larva to continue growing in this now-favorable environment until metamorphosis occurs at the maximum size, but at some delay relative to controls (Wilbur and Collins 1973; Day and Rowe 2002).

Transient salinity stress late in larval development: The outcomes of transient salinity stress late in development may depend on the timing of the stress relative to the window for metamorphic plasticity (Fig. 1B). If the period of salinity stress ends before the minimum threshold for initiating metamorphosis has been reached, growth rate may recover leading to delayed metamorphosis at the maximum size, as predicted for stress experienced earlier in the larval period. If the minimum threshold is reached before or during the period of salinity stress, development may be accelerated (Wilbur and Collins 1973; Day and Rowe 2002) such that metamorphosis is reached at a smaller size and possibly—but not necessarily—earlier in time relative to controls, depending on the severity and duration of growth reduction experienced. If the period of salinity stress begins after the maximum threshold for initiating metamorphosis has been reached (Hensley 1993; Hentschel 1999), some amount of growth reduction may be incurred during the remainder of larval development, leading to smaller metamorphic size but no difference in time to metamorphosis relative to controls. Thresholds may be based on size, developmental stage, and/or other elements of body condition (Morey and Reznick 2000).

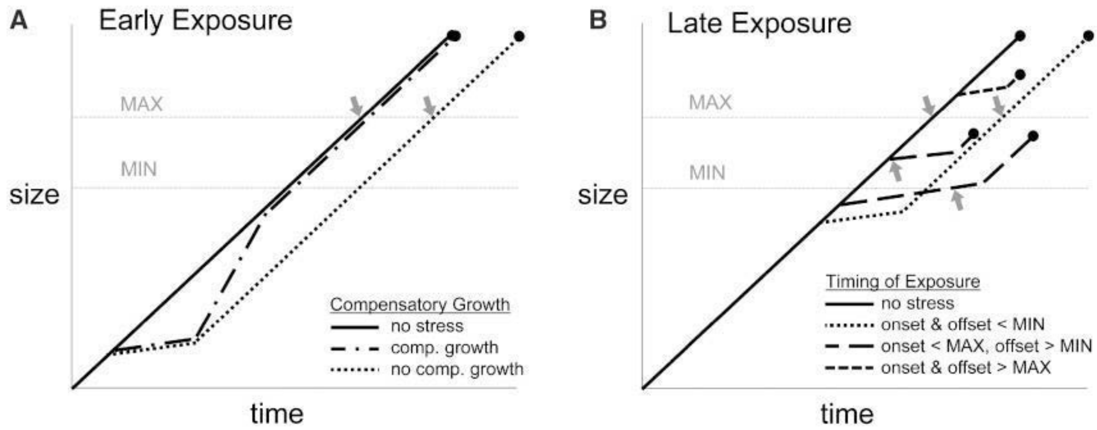

Fig. 1 Simplified growth trajectories representing alternative predictions for the consequences of transient salinity stress experienced at different points during larval development. The endpoint of each trajectory represents age and size at the completion of metamorphosis, while the arrow represents the point at which metamorphosis is initiated, resulting in fixation of developmental period. Although growth typically slows during the period preceding metamorphosis, we represent growth as linear throughout development to highlight the effects of salinity on growth and for consistency with previous authors. Thus, periods of salinity stress correspond with reduced growth rate (shallower slope). For the sake of visualization, the minimum and maximum thresholds for initiating metamorphosis (MIN and MAX, respectively) are represented in terms of body size, and the period between MIN and MAX defines the window of plasticity. In both panels, the solid line represents the growth trajectory under constant freshwater (i.e., control) conditions. (**A**) Early in larval development, the consequences of transient salinity stress depend on whether compensatory growth does (alternating dot-dash line) or does not (dotted line) occur. (**B**) Late in larval development, metamorphic outcomes depend on whether the period of stress begins and ends before MIN (dotted line, analogous to A), ends after MIN but begins before MAX (long-dashed lines, representing two different scenarios), or begins and ends after MAX (short-dashed line).

Source: Adapted from Alford and Harris (1988); Hensley (1993); Rose (2005).

to ameliorate the potential costs of transient salinity exposure, through compensatory growth, acclimation, or adaptive metamorphic plasticity? Does the degree or form of plasticity in response to salinity exposure vary with developmental stage? Box 1 describes specific predictions for developmental plasticity in response to salinity stress experienced at different stages of development. Ultimately, we seek to understand how developmental context mediates the consequences of salinity fluctuation for amphibian populations in dynamic environments.

Methods

Animals

The southern toad (*A.* [*Bufo*] *terrestris*) is an anuran species common to the coastal plain of the southeastern United States, where it may come into close proximity with seawater. This species has been observed on barrier islands, on beaches, and in coastal wetlands with elevated salinity (up to 9.3 parts per thousand [ppt], mean 4.2 ppt) following overwash from hurricane storm surge (Neill 1958; Gibbons and Coker 1978; Gunzburger et al. 2010; Hopkins and Brodie 2015). Southern toads breed sporadically from late winter through early autumn, often triggered by rainfall and often in dense breeding aggregations (Jensen 2005). Large clutches (2500–4000 ova) are oviposited in various shallow wetlands including ditches, canals, impoundments, ponds, and ephemeral pools (Jensen 2005), and resulting larval densities can be very high particularly in small ephemeral pools. Hatching occurs in ~4 days and larval development can take from 1 to 2 months or more (Jensen 2005). In a study of six coastal plain anurans, *A. terrestris* was reported to have moderate salinity tolerance (Brown and Walls 2013).

Tadpoles were obtained by collecting newly fertilized *A. terrestris* embryos from a permanent urban water feature in Charleston, SC (32.782356, −79.937546) on April 29, 2017 (Experiment 1), May 24, 2017 (Experiment 2), and May 29, 2018 (Experiment 3). Because eggs are released in strands rather than distinct masses and because dense breeding aggregations occur at this site, it was not possible to identify individual clutches; however, we estimate at least six clutches were included in each of the first two Experiments and one to two clutches in third experiment. We reared embryos and tadpoles in 10% modified Holtfreter's solution (3.46 g NaCl, 0.05 g KCl, 0.2 g $NaHCO_3$, 0.2 g $MgSO_4$, 0.1325 g $CaCl_2$ per L; Armstrong et al. 1989), an amphibian culture medium constituted in charcoal-filtered tap water with a final salinity of 0.4 ppt. Embryos were housed in groups of several hundred in 10–20 L of solution in polypropylene tubs (80.2 cm × 49.7 cm × 42.9 cm); after hatching, tadpoles were housed in groups of up to 200 in 12 L of solution in polypropylene tubs (40.2 cm × 27.8 cm × 23.0 cm) until experiments began. Salinity experiments commenced on May 16, 2017 (Experiment 1), June 12, 2017 (Experiment 2), and June 6, 2018 (Experiment 3), with tadpoles at Gosner stages (GS; Gosner 1960) 25–26 (i.e., early, post-hatchling tadpoles). All procedures were conducted in a temperature-controlled laboratory (20°C ± 1.5°C) under protocols approved by the College of Charleston's Institutional Animal Care and Use Committee (IACUC 2015-013 and 2018-011).

Experimental procedures

Tadpoles were exposed to elevated salinity during different portions of the larval period across three separate experiments, detailed below and in Supplementary Fig. S1, to examine the ontogeny of salinity tolerance and the potential for developmental plasticity in response to salinity exposure. During periods of salinity exposure, tadpoles were held in charcoal-filtered tap water mixed with Instant Ocean Sea Salt (Spectrum Brands, Blacksburg, VA) adjusted to the selected salinity—6.0 ppt (10.60 mS/cm) for Experiments 1 and 2 and 4.0 ppt (7.30 mS/cm) for Experiment 3—using a YSI EC300 conductivity meter. We selected these salinities based on preliminary experiments with this species. When tadpoles were not being exposed to elevated salinity, they were held in a 0.4 ppt control solution (10% modified Holtfreter's solution for Experiment 1; 0.4 ppt Instant Ocean solution for Experiments 2 and 3).

To begin each experiment, we selected tadpoles of similar size and stage (GS 25–26). Tadpoles were randomly assigned to treatments and housed in 1 L of the appropriate solution in containers (18 cm × 13 cm × 8.5 cm, polypropylene) arranged on laboratory shelves in a randomized block design. Every 4 days throughout each experiment, we replaced the solution in each container and fed tadpoles a pre-measured ration of a mixture of three parts pulverized Kaytee Fortified Daily rabbit food (16% protein, 2% fat, and 13% fiber) with one part pulverized TetraMin® Tropical Flakes (46% protein, 11% fat, and 3% fiber). Food rations increased as tadpoles grew, to simulate ad libitum feeding, but were held constant across treatments. We checked tadpoles daily and recorded any mortality. Upon metamorphosis (GS 42; emergence of at least one forelimb), each tadpole was moved to a container propped at a ~10° angle, with ~2 cm of the 0.4 ppt solution at the lower end, until the tail was resorbed (GS 46).

Tadpole mass and developmental stages were determined periodically (see details below). We determined GS using a Zeiss Stemi 2000-C stereomicroscope. Mass of tadpoles and metamorphs (at GS 46; resorption of tail) was measured to the nearest 0.1 mg on a Mettler AT261 DeltaRange balance, after gently blotting on a paper towel. Tadpoles were blotted through a small piece of fiberglass screening to protect their delicate skin.

Experiment 1—timing and duration of salinity exposure

Experiment 1 investigated effects of timing and duration of salinity exposure. The experiment consisted of nine treatments, each with exposure to elevated salinity (6.0 ppt) for a different portion of the first 32 days of the experiment (Supplementary Fig. S1), to include the rapid growth portion of the larval period. Four of the nine treatments consisted of 8 days exposure periods, one during each quarter of the 32 day period, hereafter referred to as 8d-1, 8d-2, 8d-3, and 8d-4. Three treatments featured 16 day exposures at the beginning, middle, and end of the 32 day period (16d-12, 16d-23, and 16d-34), and one treatment included salinity exposure for the entire 32 day period (32d-1234). Tadpoles in the control treatment were never exposed to elevated salinity. Each treatment was replicated across 10 separate containers, with the exceptions of 32d-1234 and the control, each of which was replicated across 20 containers, for increased statistical power in comparisons with the control and in anticipation of higher mortality in the 32 day exposure. Each container housed three tadpoles in 1 L solution, for a total of 330 tadpoles across 110 containers. In order to maintain tadpole density within containers, whenever possible, we consolidated tadpoles among containers within the same treatment from which an individual had died. This approach prioritized maintaining tadpole density over keeping tadpoles in their original groups. We weighed tadpoles from each container at the beginning of the experiment and every 7–8 days thereafter for the first 38 days.

Experiments 2 and 3—developmental stage during salinity exposure

Amphibian larval development can proceed at different rates among individuals such that age and developmental stage become decoupled, particularly later in larval development (e.g., Newman 1992; Morey and Reznick 2000). Thus, to examine effects of salinity exposure beginning at different developmental stages, in Experiments 2 and 3 we initiated salinity exposures when each tadpole reached a given developmental stage rather than at a particular age (Supplementary Fig. S1). Tadpoles assigned to begin salinity exposure at GS 25 were exposed from the outset of the experiment, while those assigned to later salinity exposures were monitored for developmental stage and switched to elevated salinity accordingly. To control for duration of exposure, salinity exposures ended after a given number of days rather than at a particular developmental stage.

In Experiment 2, tadpoles were exposed to elevated salinity (6.0 ppt) for 8 days beginning at GS 25 (“early”), for 8 days beginning at GS 38 (“late”), or for the entire larval period (i.e., GS 25–42; “continuous”). Tadpoles in the control treatment were never exposed to elevated salinity. Tadpoles were housed individually in 1 L solution, and each treatment was replicated across 30 containers, for a total of 120 individually-housed tadpoles. We weighed tadpoles at the beginning of the experiment in groups of 12 (to minimize damage due to blotting individually), and then individually every 8 days for 72 days.

Experiment 3 also investigated effects of salinity exposure beginning at different developmental stages but used a lower salinity, to focus on sublethal effects, and included exposure periods initiated early, midway, or late in the larval period (Supplementary Fig. S1). Tadpoles were exposed to elevated salinity (4.0 ppt) for 8 days beginning at GS 25 (“early”), or for 12 days beginning at GS 34 (“mid”) or GS 38 (“late”); tadpoles in the control treatment were never exposed to elevated salinity. We used a longer exposure period for mid and late treatments to compensate for the milder salinity stress, compared to Experiments 1 and 2, anticipating weaker effects on growth and thus allowing longer for treatments to diverge. Tadpoles were housed individually in 1 L solution. The mid, late, and control treatments were replicated across 30 containers, while the early treatment was replicated across 40 containers in case of elevated mortality, for a total of 130 individually-housed tadpoles. We weighed four representative groups of 5 tadpoles at the beginning of the experiment. Beginning on Day 20 of the experiment, we weighed tadpoles individually every 4 days until Day 48.

Data analysis

Within each experiment, we analyzed effects of salinity treatments on survival, tadpole mass at several points, mass at metamorphosis, duration of larval period, and larval growth rate. Because tadpole growth is non-linear, with body size decreasing immediately prior to metamorphosis (Wilbur and Collins 1973), we limited our analyses of tadpole mass to time points before any decrease in growth. For survival to metamorphosis (or to the end of the experiment, for the few that did not reach metamorphosis), we used generalized linear models with a binomial distribution and complementary log-log link. For larval period, we used generalized linear models with a gamma distribution and log link.

Table 1 Survival of tadpoles exposed to elevated salinity for different portions of larval development across three experiments

Salinity onset	**-**	**Day 1 (GS 25)**				**Day 9**		**Day 17**		**Day 25**	**GS 34**[a]	**GS 38**[a]
Duration (days)	-	8	16	32	Until metamorphosis	8	16	8	16	8	12	8[c], 12[d]
Treatment name(s)	Control	8d-1[b], early[c,d]	16d-12	32d-1234	Continuous	8d-2	16d-23	8d-3	16d-34	8d-4	Mid	Late
Experiment (salinity)						**Survival (proportion)**						
1 (6 ppt)	51/60 (0.85)	**13/30 (0.43)**	**10/30 (0.33)**	**22/60 (0.37)**		23/30 (0.77)	21/30 (0.70)	21/30 (0.70)	18/30 (0.60)	25/30 (0.83)		
2 (6 ppt)	29/30 (0.97)	**3/30 (0.10)**			**0/30 (0)**							25/29 (0.86)
3 (4 ppt)	27/30 (0.90)	32/39 (0.82)									25/28 (0.89)	26/29 (0.90)

Number that survived to metamorphosis (or to the end of the experiment, for the few that did not reach metamorphosis) out of the total number per treatment is shown, with proportion surviving in parentheses. Treatments significantly different (adjusted $P<0.001$) from the control treatment in each experiment are indicated in bold. These results are presented visually in Supplementary Fig. S3.

[a] See Supplementary Fig. S1 for the range of days corresponding to GS 34 and GS 38 in each experiment.

[b] Experiment 1.

[c] Experiment 2.

[d] Experiment 3.

One-way ANOVA was used for mass variables and larval growth rate to metamorphosis (i.e., mass at metamorphosis/larval period); to meet assumptions of normality and homoscedasticity, we log-transformed tadpole mass in Experiment 2 and at the first time point in Experiment 3 as well as larval growth in Experiment 3. Sequential Bonferroni adjustment was used for post-hoc analyses comparing individual treatments with the control treatment. For Experiment 1, in which tadpoles were reared and weighed in groups, analyses of tadpole mass were conducted on group means, but analyses of metamorphic data were conducted on individual masses and larval periods, using mixed models with a random effect to account for the potential lack of independence among individuals emerging from the same container. All analyses were conducted using SPSS version 24.0 (IBM 2016).

Results

Experiment 1—timing and duration of salinity exposure during early larval development

Tolerance to elevated salinity (6 ppt) was lower during the first 8 days of the experiment, as evidenced by reduced survival relative to later exposure periods. Survival differed significantly among salinity treatments (likelihood ratio $\chi^2 = 56.7$, $P < 0.001$): those treatments experiencing elevated salinity during the first 8 day period (i.e., 8d-1, 16d-12, and 32d-1234) showed a 47.5% reduction in survival relative to the controls (pairwise comparisons, adjusted $P < 0.001$, Table 1, Supplementary Fig. S2). In a two-way analysis, timing of the onset of salinity exposure significantly affected survival ($\chi^2 = 21.3$, $P < 0.001$), but neither the duration of exposure ($\chi^2 = 1.6$, $P = 0.44$) nor the interaction of timing with duration ($\chi^2 = 0.1$, $P = 0.96$) had a significant effect.

Tadpole growth was impaired by salinity exposure during the first 24 days of the experiment, but upon return to the freshwater solution, tadpoles rapidly reached a size equivalent to the control tadpoles, consistent with compensatory growth (Fig. 2A). Immediately after the first 8 day exposure period, tadpoles that had been exposed to salinity (treatments 8d-1, 16d-12, and 32d-1234) were 23% smaller in mass relative to the control tadpoles ($F = 4.8$, $P < 0.001$, $\eta^2 = 0.28$; pairwise comparisons, adjusted $P < 0.05$; Fig. 2A, Supplementary Fig. S3). Similarly on Day 16, tadpoles that had been exposed to salinity during the previous 8 days (8d-2, 16d-12, 16d-23, and 32d-1234) were 30% smaller than the control tadpoles ($F = 5.8$, $P < 0.001$, $\eta^2 = 0.34$; pairwise comparisons, adjusted $P < 0.001$; Fig. 2A,

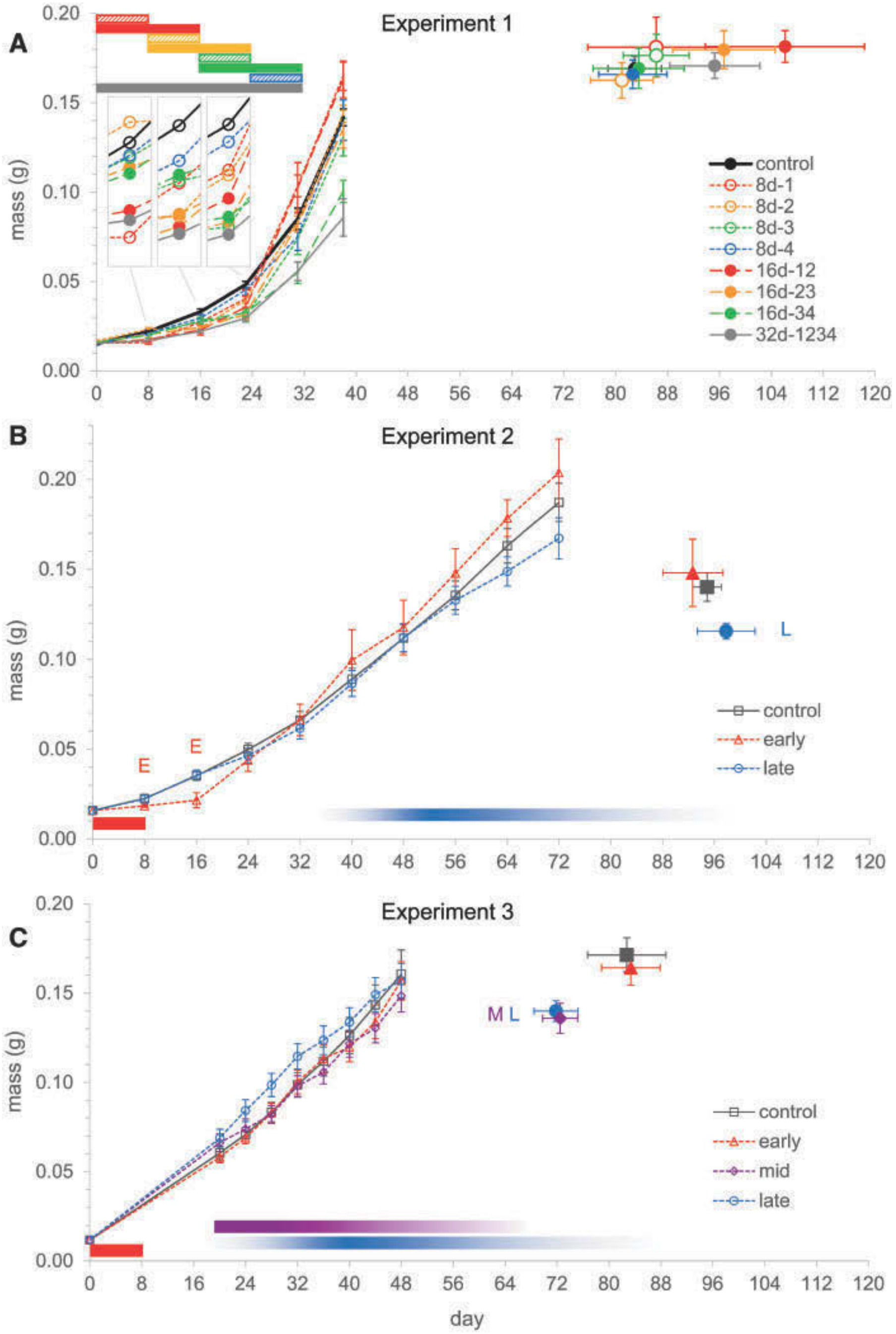

Fig. 2 Developmental trajectories of tadpoles exposed to elevated salinity for different periods during three experiments: (**A**) Experiment 1, exposure to 6 ppt salinity for different 8, 16, and 32 day periods during early larval development. (**B**) Experiment 2, exposure to 6 ppt for 8 day early or late in larval development. (**C**) Experiment 3, exposure to 4 ppt for 8 day early or for 12 day beginning at GS 34 (mid) or GS 38 (late). Lines show tadpole mass during development (mean ± SE), with insets to magnify the relative treatment means for three time points in Experiment 1, to better visualize effects. Isolated points represent mass and time at metamorphosis (mean ± SE). For Experiments 2 and 3, letters identify treatments significantly different from the control treatment (see text for details). For Experiment 1, significant treatment effects are described in the text and illustrated in more detail in Supplementary Fig. S3. Horizontal bars indicate periods of salinity exposure for each treatment, with shading indicating the approximate proportion of tadpoles receiving the mid and late exposures at a given time. Treatments are further described in the text and in Supplementary Fig. S1.

Supplementary Fig. S3) while those that were exposed only for the first 8 days (8d-1) were only 17% smaller (adjusted $P = 0.07$). On Day 23, tadpoles exposed during the third 8 day period (8d-3, 16d-23, 16d-34, and 32d-1234) were 36% smaller while those exposed for the second 8 days (8d-2, 16d-12) were 21% smaller than the control tadpoles ($F = 8.3$, $P < 0.001$, $\eta^2 = 0.45$; pairwise comparisons, adjusted $P < 0.05$ and $P < 0.001$ for second and third 8 day periods, respectively; Fig. 2A, Supplementary Fig. S3). On Days 31 and 38, tadpoles exposed during the final 16 days of the exposure period (16d-34 and 32d-1234) were 35% smaller than the control tadpoles (Day 31: $F = 4.4$, $P < 0.001$, $\eta^2 = 0.33$; pairwise comparisons, adjusted $P = 0.01$; Day 38: $F = 7.5$, $P < 0.001$, $\eta^2 = 0.47$; pairwise comparisons, adjusted $P < 0.01$; Fig. 2A, Supplementary Fig. S3).

Salinity exposure did not significantly affect mass at metamorphosis ($F = 0.41$, $P = 0.92$, $\eta^2 = 0.018$, Fig. 2A), duration of larval period ($F = 1.23$, $P = 0.29$, $\eta^2 = 0.051$, Fig. 2A), or growth rate to metamorphosis ($F = 0.35$, $P = 0.95$, $\eta^2 = 0.015$).

Experiment 2—salinity exposure at early versus late developmental stages

Tadpoles exposed to elevated salinity (6 ppt) at early stages of development were less tolerant than those exposed during late stages. Salinity exposure beginning at GS 25 resulted in a 90% reduction in survival relative to the control treatment, while exposure beginning at GS 38 did not affect survival (Table 1, Supplementary Fig. S2). Only 3 of 30 tadpoles exposed to elevated salinity for the first 8 days of the experiment ultimately survived, and none that were exposed continuously survived, with most perishing within 16 days. Survival differed significantly among treatments (likelihood ratio $\chi^2 = 113.2$, $P < 0.001$), with the late exposure and control treatments showing significantly greater survival than the early exposure and continuous salinity treatments (adjusted $P < 0.001$, Table 1, Supplementary Fig. S2).

Exposure to salinity during the first 8 days initially reduced growth, with some evidence of accelerated growth after release from salinity stress (Fig. 2B). Considering all tadpoles, those in both the early exposure and the continuous exposure treatments were 30% smaller than tadpoles in the control treatment on Day 8 ($F = 18.4$, $P < 0.001$, $\eta^2 = 0.33$; pairwise comparisons, adjusted $P < 0.001$) and 51% smaller on Day 16 ($F = 19.2$, $P < 0.001$, $\eta^2 = 0.46$; pairwise comparisons, adjusted $P < 0.001$). By Day 24, tadpoles in the early exposure treatment were no longer significantly smaller than controls, but the two remaining tadpoles in the continuous exposure were 42% smaller ($F = 2.4$, $P = 0.08$, $\eta^2 = 0.11$; pairwise comparisons, early: adjusted $P = 0.54$, continuous: adjusted $P = 0.04$). By Day 32 and beyond, no tadpoles remained in the continuous exposure treatment and there was no significant difference in tadpole mass among the remaining treatments (all $P > 0.2$), a pattern consistent with compensatory growth. Because this pattern could also occur if small individuals are more likely to succumb to salinity stress, we re-analyzed tadpole growth considering only individuals that survived through Day 40 (i.e., the end of salinity-related mortality). In the latter analysis, there were no significant differences in tadpole mass for Days 8, 24, or 32 (all $P > 0.4$), while the effect was marginally non-significant for Day 16 ($F = 3.1$, $P = 0.054$, $\eta^2 = 0.10$) at which point tadpoles from the early exposure treatment were 39% smaller than tadpoles from the control treatment (adjusted $P = 0.036$; Fig. 2B).

Metamorphic traits were affected by salinity exposure late but not early in development. At metamorphosis, tadpoles exposed to salinity during late development were 17% smaller than the control tadpoles ($F = 3.8$, $P = 0.028$, $\eta^2 = 0.13$; pairwise comparison, adjusted $P = 0.025$; Fig. 2B), with a 17% reduced growth rate over the larval period ($F = 3.3$, $P = 0.046$, $\eta^2 = 0.12$; pairwise comparison, adjusted $P = 0.042$), while those exposed during early development were similar in size to the control tadpoles (adjusted $P = 0.7$) with no difference in growth rate (adjusted $P = 0.7$). Larval period did not vary significantly among treatments ($F = 0.3$, $P = 0.73$, $\eta^2 = 0.012$; Fig. 2B).

Experiment 3—mild salinity exposure at early, mid- and late developmental stages

Tadpoles exposed to a milder salinity elevation (4 ppt) for a portion of development showed high survival regardless of developmental stage (Table 1, Supplementary Fig. S2), with no significant differences among salinity treatments (likelihood ratio $\chi^2 = 1.3$, $P = 0.72$). Tadpole mass did not vary among treatments at any point from Day 20 through Day 48 (all $P > 0.1$; Fig. 2C). We were unable to collect tadpole mass data before Day 20, so while there is no evidence of a growth reduction due to early salinity exposure or subsequent compensatory growth, neither can these possibilities be ruled out.

At metamorphosis, tadpoles exposed to the mid and late salinity treatments were 20% smaller than tadpoles in the control treatment ($F = 4.3$, $P = 0.007$, $\eta^2 = 0.12$; pairwise comparisons, adjusted $P < 0.02$;

Fig. 2C). Larval period was 13% shorter for the mid and late exposure treatments ($F = 2.7$, $P = 0.047$, $\eta^2 = 0.07$; Fig. 2C), although neither was significantly different from the control treatment in pairwise comparisons (adjusted $P > 0.1$). Although mid and late salinity exposures affected metamorphic size and timing, salinity exposure did not significantly affect growth rate over the larval period ($F = 1.4$, $P = 0.26$, $\eta^2 = 0.04$).

Discussion

Our results indicate that the timing of salinity exposure relative to development can have important, potentially complex consequences for amphibian larvae. Early in the tadpole stage, a temporary increase in habitat salinity may be lethal due to low tolerance. However tadpoles that survive an early, transient salinity stress may have few lasting consequences, as a result of compensatory growth, reaching metamorphosis at similar age and size as unaffected individuals. Later in development, on the other hand, tadpoles are more likely to survive a salinity influx but, unable to recover from growth reduction, may be more subject to subtle costs associated with small metamorphic size and its potential consequences for success in future life history stages. Our findings offer insights into the ontogeny of salinity tolerance, the capacity for different forms of developmental plasticity in response to salinity exposure, and the potential consequences of salinity fluctuations for amphibian populations.

Ontogeny of salinity tolerance

In our study, tolerance to salinity increased during early stages of larval development. In Experiments 1 and 2, tadpoles exposed to elevated salinity (6 ppt) during the first 8 days of the experiment showed dramatically reduced survival, but later salinity exposures did not affect survival comparable to control tadpoles. Interestingly, in Experiment 1, the reduction in survival was similar among tadpoles exposed for the first 8 days, the first 16 days, or the first 32 days of the experiment, while tadpoles exposed only for the second 8 day period showed no decrement in survival, providing strong evidence that vulnerability decreased rapidly after the earliest portion of the larval period. A similar increase in salinity tolerance during tadpole development has been observed in two previous studies. Tadpoles of *Litoria ewingii* exposed to moderate salinity from GS 25 through approximately GS 30 showed dramatically reduced survival relative to those exposed later in development (Kearney et al. 2014). In *Fejervarya limnocharis*, survival was reduced when salinity was increased early in tadpole development (beginning at GS 26) but not later in development (beginning at GS 30 or GS 38; Wu et al. 2012), although tadpoles exposed to elevated salinity early in development only experienced notable mortality when the salinity stress continued through much of the larval period (i.e., GS 25–38 but not GS 25–30; Wu et al. 2012). Considering our results alongside these previous findings suggests that salinity tolerance increases during larval development and that the timing of exposure relative to development may be a more important predictor of vulnerability than the duration of exposure.

The observed increase in tolerance to salinity stress during tadpole development may result from maturation in larval osmoregulatory ability. The primary location for ion regulation in anuran larvae appears to be the internal gills (Burggren and Just 1992; Uchiyama and Yoshizawa 1992). Mitochondria-rich cells, which are thought to be involved in osmoregulatory ion exchange, are sparse in the transient external gills present during the hatchling stage (GS 21–24), but common in the persistent internal gills present during the tadpole stage (Uchiyama and Yoshizawa 1992; Brunelli et al. 2004). Within the internal gills, Na^+/K^+-ATPase, a transmembrane pump responsible for sodium export, appears to play an important role in salinity tolerance. Gill Na^+/K^+-ATPase expression has been shown to increase following acclimation to elevated salinity in *F. limnocharis* and *F. cancrivora* (Wu et al. 2014; Lai et al. 2019), and has been shown to be higher in *Bufo balearicus* than in its less salt-tolerant congener *B. bufo* (Bernabò et al. 2013). The rapid increase in salinity tolerance observed in our study appears to correspond roughly with the timing of external gill regression and internal gill development (Viertel 1991; Uchiyama and Yoshizawa 1992), consistent with a critical role of internal gills in osmoregulation.

Acclimation

Our results do not provide evidence of acclimation to salinity, in contrast to previous results in certain salinity-tolerant species. In Experiment 1, acclimation would predict that the impacts of salinity exposure during a particular period would be ameliorated by previous exposure. However, similar growth reductions were observed for tadpoles exposed during a given 8 day period regardless of whether they had been exposed in the previous period, indicating that prior salinity exposure was not protective.

Contrary to our results, salinity acclimation is well-documented in the euryhaline *F. cancrivora* (Uchiyama and Yoshizawa 1992, Hsu et al. 2012; Lai et al. 2019) and has also been demonstrated in its moderately salinity-tolerant congener, *F. limnocharis* (Wu et al. 2014). However, tadpoles of the Gulf Coast toad, *Incilius nebulifer*, exposed to sublethal salinity were subsequently less, rather than more, tolerant to a further increase in salinity (Hua and Pierce 2013), suggesting a cumulative impact of salinity exposure rather than acclimation. This discrepancy could reflect a difference in when the *I. nebulifer* and *Fejervarya* tadpoles were exposed (GS 24 vs. GS 27 or later, respectively) or could reflect a difference among species. Based on the divergent results among species, we hypothesize that the capacity for acclimation is stronger in species that have evolved with salinity as a routine stressor than in those with less frequent salinity stress. Thus, future research should aim to elucidate the effects of evolutionary history as well as developmental stage on the ability to acclimate to elevated salinity.

Compensatory growth

Our results provide evidence of compensatory growth in *A. terrestris* following release from sublethal salinity stress during early stages of larval development, but not during later stages. Tadpoles exposed to elevated salinity early in development in Experiments 1 and 2 showed an initial reduction in growth, but upon return to freshwater quickly reached a size at least equivalent to control tadpoles, with no difference in time to or size at metamorphosis. On the other hand, tadpoles exposed to, and subsequently released from, salinity stress later in development did not compensate for reduced growth, ultimately metamorphosing at a smaller size than control tadpoles. Previous studies in *L. ewingii* (Kearney et al. 2014) and *F. cancrivora* (Hsu et al. 2018) have also documented compensatory growth following a period of salinity exposure during early larval development but not later in development. During later developmental stages, compensatory growth may be precluded as development shifts from a phase of rapid growth to a phase of morphological change in preparation for metamorphosis (Wilbur and Collins 1973; Harris 1999). However, even during early development, compensatory growth may not always occur following a release from salinity stress, as in *F. limnocharis* tadpoles, which did not exhibit accelerated growth following either early or late release from salinity stress and ultimately experienced delayed metamorphosis in both treatments (Wu et al. 2012).

Compensatory growth may benefit tadpoles by reducing the risk of predation by gape-limited predators (Richards and Bull 1990; Jara and Perotti 2010), by increasing the likelihood of reaching a minimum size for metamorphosis before the growing season ends or an ephemeral pond dries (Newman 1992; Dmitriew 2011), or by capitalizing on brief periods of favorable conditions before another influx of salinity. Accelerated growth could be facilitated by increased food intake, improved conversion efficiency, or changes in body composition (Metcalfe and Monaghan 2001; Dmitriew 2011). However, these mechanisms may also underpin costs of compensatory growth, for example increased predation risk from elevated foraging activity or later fitness costs due to decreased allocation to energy stores or cellular damage accumulated during rapid growth (Metcalfe and Monaghan 2001; Mangel and Munch 2005; Dmitriew 2011). A cost of compensatory growth was indicated in a study with *L. ewingii*, which quickly caught up in size following salinity exposure early in larval development, but then experienced delayed metamorphosis (Squires et al. 2010). Future research should seek to clarify the potential costs and limits of compensatory growth in amphibian larvae following release from salinity stress.

Metamorphic plasticity

Whereas tadpoles in our study were able to compensate via accelerated growth following salinity exposure early in development, those subjected to transient salinity stress during later development did not recover from the growth reduction and ultimately reached metamorphosis at a smaller size. This result is consistent with the prediction that a shift to poorer conditions can hasten the transition to the terrestrial juvenile stage by initiating metamorphosis before the maximum threshold is reached (Box 1). Because elevated salinity can increase energetic costs and decrease food intake (Gomez-Mestre et al. 2004; Sanzo and Hecnar 2006; Wood and Welch 2015), reduced growth, rather than direct effects of osmotic stress, could be the proximate signal of this change in environmental quality.

Our results provide evidence of both a minimum and a maximum threshold for initiating metamorphosis in response to elevated salinity. Because tadpoles exposed to salinity stress beginning at either GS 34 or GS 38 showed reduced size at metamorphosis and, in Experiment 3, accelerated metamorphosis, our results suggest that the maximum threshold for metamorphosis had not been reached by GS 38. Although these salinity exposures were transient, we infer that by the time the tadpoles were returned to freshwater, they had already initiated the metamorphic process and were unable to decelerate development to exploit the newly improved conditions and maximize metamorphic size. Consequently, our results suggest that the minimum threshold for initiating metamorphosis had been surpassed, and thus the developmental trajectory had become fixed, by 12 days after GS 34. Unfortunately, we did not record developmental stages at the end of the salinity exposure period for each tadpole, so we are unable to identify the minimum threshold more precisely. Although a minimum threshold for initiating metamorphosis in response to water level reduction has been reported (Denver et al. 1998), and both minimum (Morey and Reznick 2000) and maximum (reviewed in Harris 1999; Rose 2005) thresholds have been investigated with changes in food availability, to our knowledge, our study is the first to report evidence of an upper threshold for amphibian metamorphic plasticity in response to an abiotic stressor.

Adaptive metamorphic plasticity in response to changes in salinity imposed relatively late in development has also been observed in other species. Similar to our findings and consistent with adaptive plasticity persisting through at least GS 38, *F. limnocharis* tadpoles exposed to increased salinity starting at either GS 30 or GS 38 reached metamorphosis earlier and at smaller size (Wu et al. 2012). Meanwhile, tadpoles that were released from salinity stress at GS 30 or GS 38 experienced delayed metamorphosis with no reduction in size (Wu et al. 2012), suggesting an adaptive response to improved conditions, with prolonged development enabling growth to maximize size. In *L. ewingii*, tadpoles subjected to a transient salinity exposure in mid- or late-development showed a delay in metamorphosis with no size decrement (Kearney et al. 2014). Although the authors did not report developmental stages, the timing of salinity exposure relative to the length of the larval period suggests that the exposures occurred through at least GS 38 and likely later, indicating that the minimum threshold for initiating metamorphosis may not have been reached by that point. The thresholds for metamorphic plasticity in response to salinity changes could vary among species, depending, for example, on their history of salinity exposure or—as has been shown with respect to food limitation (Morey and Reznick 2000)—their degree of adaptation to ephemeral larval habitats. Future studies are warranted, across a variety of species and stressors, to better understand the capacity for and constraints on metamorphic plasticity in response to shifting environmental conditions.

Implications

As our results highlight, the effects of transient salinity stress depend not only on the level of salinity and duration of exposure but also on the timing of exposure relative to development and the capacity for different forms of plasticity. An influx of salinity is more likely to lead to mortality when experienced during early larval stages, and embryos may be even more vulnerable (Albecker and McCoy 2017) and less able to acclimate (Uchiyama and Yoshizawa 1992). Consequently, increased habitat salinity may be more likely to affect population dynamics when experienced early in development, although strong density dependence may help to mitigate population effects by releasing survivors from the effects of intraspecific competition (Karraker et al. 2008). Following a sublethal early salinity exposure, compensatory growth may enable individuals to recover rapidly and complete larval development with few apparent long-term costs. However in some cases, the deleterious effects of early salinity exposure may be irreversible (Wu et al. 2012), particularly at higher salinities (Karraker and Gibbs 2011). Thus, population-level impacts are likely to be complex, depending on a variety of factors that may vary among species and habitats.

While a mild increase in salinity experienced early in development may have no long-term effect due to compensatory growth, a similar increase in salinity could be detrimental if experienced later in development. During later developmental stages, salinity stress can lead to accelerated metamorphosis thereby facilitating a more rapid transition out of the aquatic habitat. Among amphibians, earlier metamorphosis can be imperative for survival when faced with a deteriorating larval environment (Newman 1992) and has been linked to improved post-metamorphic survival (Smith 1987; Berven 1990; Altwegg and Reyer 2003). However, accelerated metamorphosis comes at the cost of smaller size at metamorphosis, which may ultimately diminish fitness by reducing survival in subsequent life stages, delaying reproductive maturity, and restricting body size at maturity (Smith 1987; Berven 1990; Altwegg and Reyer 2003). Importantly, this metamorphic plasticity was observed even at levels of salinity that had no impact on survival earlier in development. Thus, the risks posed by transient salinity stress may depend on a complex interaction between salinity level, timing of exposure, and developmental stage, such that the relative vulnerability of different stages depends on the salinity level experienced.

Our study demonstrates that developmental plasticity can be an important way for larval amphibians, and potentially other organisms with complex life cycles, to cope with fluctuating or unpredictable environments. Like salinity, other environmental stressors may have complex consequences as a result of various forms of developmental plasticity including acclimation, compensatory growth, and adaptive plasticity in the timing of life history transitions. Furthermore, species are likely to vary in phenology, tolerance, and the capacity for different forms of plasticity, suggesting the potential for complex population- and community-level responses to environmental fluctuations. Thus, development can play a crucial role in shaping responses to dynamic environmental stress, which may be increasingly important in the context of global change.

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Supplementary data

Supplementary data available at *ICB* online.

References

Albecker MA, McCoy MW. 2017. Adaptive responses to salinity stress across multiple life stages in anuran amphibians. Front Zool 14:40.

Alexander LG, Lailvaux SP, Pechmann JHK, DeVries PJ. 2012. Effects of salinity on early life stages of the Gulf Coast toad, *Incilius nebulifer* (Anura: Bufonidae). Copeia 2012:106–14.

Alford RA, Harris RN. 1988. Effects of larval growth history on anuran metamorphosis. Am Nat 131:91–106.

Altwegg R, Reyer HU. 2003. Patterns of natural selection on size at metamorphosis in water frogs. Evolution 57:872–82.

Armstrong JB, Duhon ST, Malacinski GM. 1989. Raising the axolotl in captivity. In: Armstrong JB, Malacinski GM, editors. Developmental biology of the axolotl. New York (NY): Oxford University. p. 220–7.

Bernabò I, Bonacci A, Coscarelli F, Tripepi M, Brunelli E. 2013. Effects of salinity stress on *Bufo balearicus* and *Bufo bufo* tadpoles: tolerance, morphological gill alterations and Na+/K+-ATPase localization. Aquat Toxicol 132–133:119–33.

Berven KA. 1990. Factors affecting population fluctuations in larval and adult stages of the wood frog (*Rana sylvatica)*. Ecology 71:1599–608.

Boege K, Marquis RJ. 2005. Facing herbivory as you grow up: the ontogeny of resistance in plants. Trends Ecol Evol 20:441–8.

Bowler K, Terblanche JS. 2008. Insect thermal tolerance: what is the role of ontogeny, ageing and senescence? Biol Rev Camb Philos Soc 83:339–55.

Brown ME, Walls SC. 2013. Variation in salinity tolerance among larval anurans: implications for community composition and the spread of an invasive, non-native species. Copeia 2013:543–51.

Brunelli E, Perrotta E, Tripepi S. 2004. Ultrastructure and development of the gills in *Rana dalmatina* (Amphibia: Anura). Zoomorphology 123:203–11.

Burggren WW, Just JJ. 1992. Developmental changes in amphibian physiological systems. In: Feder ME, Burggren WW, editors. Environmental physiology of the Amphibia. Chicago (IL): University of Chicago. p. 467–530.

Cañedo-Argüelles M, Kefford B, Schäfer R. 2019. Salt in freshwaters: causes, effects and prospects – introduction to the theme issue. Philos Trans R Soc Lond B Biol Sci 374:20180002.

Chinathamby K, Reina RD, Bailey PCE, Lees BK. 2006. Effects of salinity on the survival, growth and development of tadpoles of the brown tree frog, *Litoria ewingii.* Aust J Zool 54:97–105.

Christy MT, Dickman CR. 2002. Effects of salinity on tadpoles of the green and golden bell frog (*Litoria aurea*). Amphib Reptil 23:1–11.

Day T, Rowe L. 2002. Developmental thresholds and the evolution of reaction norms for age and size at life-history transitions. Am Nat 159:338–50.

Denver RJ, Mirhadi N, Phillips M. 1998. Adaptive plasticity in amphibian metamorphosis: response of *Scaphiopus hammondii* tadpoles to habitat desiccation. Ecology 79:1859–72.

Dmitriew CM. 2011. The evolution of growth trajectories: what limits growth rate? Biol Rev Camb Philos Soc 86:97–116.

Fischer B, van Doorn GS, Dieckmann U, Taborsky B. 2014. The evolution of age-dependent plasticity. Am Nat 183:108–25.

Gibbons JW, Coker JW. 1978. Herpetofaunal colonization patterns of Atlantic coast barrier islands. Am Midl Nat 99:219–33.

Gomez-Mestre I, Tejedo M. 2003. Local adaptation of an anuran amphibian to osmotically stressful environments. Evolution 57:1889–99.

Gomez-Mestre I, Tejedo M, Ramayo R, Estepa J. 2004. Developmental alterations and osmoregulatory physiology of a larval anuran under osmotic stress. Physiol Biochem Zool 77:267–74.

Gordon DH, Gray BT, Perry RD, Prevost MB, Strange TH, Williams RK. 1989. South Atlantic coastal wetlands. In: Smith LM, Pederson RL, Kaminski RM, editors. Habitat management for migrating and wintering waterfowl in North America. Lubbock (TX): Texas Tech University. p. 57–92

Gornitz V. 1991. Global coastal hazards from future sea level rise. Glob Planet Change 3:379–98.

Gosner KL. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16:183–90.

Gunzburger MS, Hughes WB, Barichivich WJ, Staiger JS. 2010. Hurricane storm surge and amphibian communities in coastal wetlands of northwestern Florida. Wetl Ecol Manag 18:651–63.

Harris RN. 1999. The anuran tadpole: evolution and maintenance. In: McDiarmid RW, Altig R, editors. Tadpoles: the biology of anuran larvae. Chicago (IL): University of Chicago. p. 279–94.

Hector KL, Nakagawa S. 2012. Quantitative analysis of compensatory and catch-up growth in diverse taxa. J Anim Ecol 81:583–93.

Hensley FR. 1993. Ontogenetic loss of phenotypic plasticity of age of metamorphosis in tadpoles. Ecology 74:2405–12.

Hentschel BT. 1999. Complex life cycles in a variable environment: predicting when the timing of metamorphosis shifts from resource dependent to developmentally fixed. Am Nat 154:549–58.

Hopkins GR, Brodie ED Jr. 2015. Occurrence of amphibians in saline habitats: a review and evolutionary perspective. Herpetol Monogr 29:1–27.

Hoverman JT, Relyea RA. 2007. How flexible is phenotypic plasticity? Developmental windows for trait induction and reversal. Ecology 88:693–705.

Hsu W-T, Wu C-S, Lai JC, Chiao YK, Hsu CH, Kam Y-C. 2012. Salinity acclimation affects survival and metamorphosis of crab-eating frog tadpoles. Herpetologica 68:14–21.

Hsu W-T, Wu C-S, Hatch KA, Chang Y-M, Kam Y-C. 2018. Full compensation of growth in salt-tolerant tadpoles after release from salinity stress. J Zool 304:141–9.

Hua J, Pierce BA. 2013. Lethal and sublethal effects of salinity on three common Texas amphibians. Copeia 2013:562–6.

Jara FG, Perotti MG. 2010. Risk of predation and behavioural response in three anuran species: influence of tadpole size and predator type. Hydrobiologia 644:313–24.

Jensen JB. 2005. *Bufo terrestris* (Bonnaterre, 1789): southern toad. In: Lannoo M, editors. Amphibian declines: the conservation status of United States species. Berkeley (CA): University of California. p. 436–8.

Karraker NE, Gibbs JP. 2011. Road deicing salt irreversibly disrupts osmoregulation of salamander egg clutches. Environ Pollut 159:833–5.

Karraker NE, Gibbs JP, Vonesh JR. 2008. Impacts of road deicing salt on the demography of vernal pool-breeding amphibians. Ecol Appl 18:724–34.

Kaushal SS, Likens GE, Pace ML, Utz RM, Haq S, Gorman J, Grese M. 2018. Freshwater salinization syndrome on a continental scale. Proc Natl Acad Sci U S A 115:E574–83.

Kearney BD, Pell RJ, Byrne PG, Reina RD. 2014. Anuran larval developmental plasticity and survival in response to variable salinity of ecologically relevant timing and magnitude. J Exp Zool A Ecol Genet Physiol 321:541–9.

Lai JC, Kam YC, Lin HC, Wu CS. 2019. Enhanced salt tolerance of euryhaline tadpoles depends on increased Na+, K+-ATPase expression after salinity acclimation. Comp Biochem Physiol A Mol Integr Physiol 227:84–91.

Mangel M, Munch SB. 2005. A life-history perspective on short- and long-term consequences of compensatory growth. Am Nat 166:155–76.

Metcalfe NB, Monaghan P. 2001. Compensation for a bad start: grow now, pay later? Trends Ecol Evol 16:254–60.

Moreira LFB, Knauth DS, Maltchik L. 2015. Intermittently closed estuaries and tadpole communities: influence of artificial breaching. Estuaries Coast 38:979–87.

Morey S, Reznick D. 2000. A comparative analysis of plasticity in larval development in three species of spadefoot toads. Ecology 81:1736–49.

Morton RA, Barras JA. 2011. Hurricane impacts on coastal wetlands: a half-century record of storm-generated features from southern Louisiana. J Coast Res 27:27–43.

Neill WT. 1958. The occurrence of amphibians and reptiles in saltwater areas, and a bibliography. Bull Mar Sci 8:1–97.

Newman RA. 1992. Adaptive plasticity in amphibian metamorphosis. Bioscience 42:671–8.

Richards SJ, Bull CM. 1990. Size-limited predation on tadpoles of three Australian frogs. Copeia 1990:1041–6.

Rose CS. 2005. Integrating ecology and developmental biology to explain the timing of frog metamorphosis. Trends Ecol Evol 20:129–35.

Sanzo D, Hecnar SJ. 2006. Effects of road de-icing salt (NaCl) on larval wood frogs (*Rana sylvatica*). Environ Pollut 140:247–56.

Smith DC. 1987. Adult recruitment in chorus frogs: effects of size and date at metamorphosis. Ecology 68:344–50.

Squires ZE, Bailey PCE, Reina RD, Wong B. 2008. Environmental deterioration increases tadpole vulnerability to predation. Biol Lett 4:392–4.

Squires ZE, Bailey PCE, Reina RD, Wong B. 2010. Compensatory growth in tadpoles after transient salinity stress. Mar Freshw Res 61:219–22.

Sultan SE. 2017. Developmental plasticity: re-conceiving the genotype. Interface Focus 7:20170009.

Uchiyama M, Yoshizawa H. 1992. Salinity tolerance and structure of external and internal gills in tadpoles of the crab-eating frog, *Rana cancrivora*. Cell Tissue Res 267:35–44.

Viertel B. 1991. The ontogeny of the filter apparatus of anuran larvae (Amphibia: Anura). Zoomorphology 110:239–66.

West-Eberhard MJ. 2003. Developmental plasticity and evolution. New York (NY): Oxford University.

Wilbur HM, Collins JP. 1973. Ecological aspects of amphibian metamorphosis. Science 182:1305–14.

Williams WD. 2001. Anthropogenic salinisation of inland waters. Hydrobiologia 466:329–37.

Wood L, Welch AM. 2015. Assessment of interactive effects of elevated salinity and three pesticides on life history and behavior of southern toad (*Anaxyrus terrestris*) tadpoles. Environ Toxicol Chem 34:667–76.

Wu C-S, Kam Y-C. 2009. Effects of salinity on survival, growth, development, and metamorphic traits of *Fejervarya limnocharis* tadpoles living in brackish water. Zool Sci Contrib N Y Zool Soc 26:476–82.

Wu C-S, Gomez-Mestre I, Kam Y-C. 2012. Irreversibility of a bad start: early exposure to osmotic stress limits growth and adaptive developmental plasticity. Oecologia 169:15–22.

Wu C-S, Yang W-K, Lee T-H, Gomez-Mestre I, Kam Y-C. 2014. Salinity acclimation enhances salinity tolerance in tadpoles living in brackish water through increased Na+/K+-ATPase expression. J Exp Zool A Ecol Genet Physiol 321:57–64.