Phenotypic Plasticity of Geographically Central and Peripheral Populations of Jefferson Salamanders (Ambystoma jeffersonianum) in Response to Simulated Climate Change

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PHENOTYPIC PLASTICITY OF GEOGRAPHICALLY CENTRAL AND PERIPHERAL POPULATIONS OF JEFFERSON SALAMANDERS (AMBYSTOMA JEFFERSONIANUM) IN RESPONSE TO SIMULATED CLIMATE CHANGE

BY

AARON L. DEVINE

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BY

AARON L. DEVINE

Submitted to the Faculty of the Graduate School of
Eastern Kentucky University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

2022
DEDICATION

For Dale and Delores
ACKNOWLEDGEMENTS

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ABSTRACT

Climate change will elicit various species responses as it causes habitats to change. Species with limited dispersal must respond to these changes “in place”, yet some biogeographical hypotheses indicate isolated and less abundant populations experience reduced gene flow and genetic diversity that may limit phenotypic plasticity, contrasting with evidence suggesting more variable habitats select for increased plasticity in peripheral populations. To gain insight into associations between range position and intensity of plasticity, mesocosm experiments were done to assess plasticity in larval growth, size at, and time to, metamorphosis, and survivorship in larval *Ambystoma jeffersonianum* salamanders from geographically core and peripheral populations. These populations were exposed to longer and shorter hydroperiods reflecting current and predicted future air temperatures, respectively, consistent with the A2 Climate Scenario for 2050. Growth rates and times to metamorphosis of edge populations were faster, yet larvae metamorphosed at smaller sizes. Populations experiencing future climate treatments were smaller and exhibited faster times to metamorphosis, and had higher survivorship than populations exposed to current climate treatments. In 2020, metamorphs were smaller on average than in 2021. Though these differences indicate plasticity in some larval traits, no differences in the magnitude of plasticity were observed between populations or years, tentatively suggesting populations may exhibit similar levels of genetic diversity, or that habitats may not be far enough away or differ enough in stochasticity between collection sites to detect variation in plasticity. These results improve our understanding of population-level differences in responses to environmental change, and the potential needs to incorporate population-level variation in future conservation and management efforts in the face of global climate change.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>SECTION</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Study Site</td>
<td>12</td>
</tr>
<tr>
<td>Experimental Design</td>
<td>12</td>
</tr>
<tr>
<td>Sampling</td>
<td>18</td>
</tr>
<tr>
<td>Analysis</td>
<td>20</td>
</tr>
<tr>
<td>Results</td>
<td>23</td>
</tr>
<tr>
<td>Biotic Factors</td>
<td>24</td>
</tr>
<tr>
<td>Abiotic Factors</td>
<td>33</td>
</tr>
<tr>
<td>Discussion</td>
<td>38</td>
</tr>
<tr>
<td>References</td>
<td>51</td>
</tr>
</tbody>
</table>
LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1. US EPA Evaporation Equation Estimated Rates of Evaporation During Study Period</td>
<td>18</td>
</tr>
<tr>
<td>Table 2. MANOVAs and Subsequent ANOVAs of Population Location, Climate Treatment, Year, and Their Interactions on Larval Growth, Size at Metamorphosis, Time to Metamorphosis, and Survivorship</td>
<td>27</td>
</tr>
<tr>
<td>Table 3. Means and Standard Errors of Larval Growth Rates, Size at Metamorphosis, Time to Metamorphosis, and Survivorship Between Climate Treatments, Population Locations, and Years in <em>A. jeffersonianum</em></td>
<td>30</td>
</tr>
<tr>
<td>Table 4. MANOVAs and Subsequent ANOVAs of Population Location, Year, and Their Interactions on Plasticity Intensity in Larval Growth, Size at Metamorphosis, Time to Metamorphosis and Survivorship in <em>A. jeffersonianum</em></td>
<td>31</td>
</tr>
<tr>
<td>Table 5. MANOVAs and Subsequent ANOVAS of Population Location, Climate Treatment, Year, and Their Interactions on Temperature, Dissolved Oxygen Levels, and pH</td>
<td>34</td>
</tr>
<tr>
<td>Table 6. Means and Standard Errors of Temperature, Dissolved Oxygen Levels, and pH Between Climate Treatments, Population Locations, and Year for Abiotic Factors</td>
<td>36</td>
</tr>
<tr>
<td>Table 7. MANOVAs and Subsequent ANOVAS of Population Location, Year, and their interactions on Differential Values of Temperature, Dissolved Oxygen Levels, and pH for Abiotic Factor Differentials</td>
<td>37</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>FIGURE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1. Collection Localities of Core and Peripheral Populations of <em>A. jeffersonianum</em> in 2020 and 2021 Study Periods</td>
<td>14</td>
</tr>
<tr>
<td>Figure 2. 2020 Randomized Block Design Mesocosm Array</td>
<td>17</td>
</tr>
<tr>
<td>Figure 3. 2021 Randomized Block Design Mesocosm Array</td>
<td>17</td>
</tr>
<tr>
<td>Figure 4. Graphical Representation of Comparison of Climate Treatments Within and Between Populations using a MANOVA</td>
<td>22</td>
</tr>
<tr>
<td>Figure 5. 2020 Larval Temporal Pattern of Metamorphosis</td>
<td>23</td>
</tr>
<tr>
<td>Figure 6. 2021 Larval Temporal Pattern of Metamorphosis</td>
<td>24</td>
</tr>
<tr>
<td>Figure 7. Mean Larval Body Girth and Length Growth Rates (mm/day) of Core and Edge Populations</td>
<td>28</td>
</tr>
<tr>
<td>Figure 8. Mean Size at Metamorphosis of Body Girth and Length (mm) of Core and Edge Populations</td>
<td>28</td>
</tr>
<tr>
<td>Figure 9. Magnitudes of Plasticity in Larval Growth Rates, Size at Metamorphosis, Time to Metamorphosis, and Survivorship Between Population Locations and Years</td>
<td>32</td>
</tr>
</tbody>
</table>
Introduction

Climate change is predicted to negatively impact species worldwide due to increased extremes and variability in temperature and precipitation (Corn 2005; Cahill et al. 2013). Recent rates of temperature increase (1985 - 2005) are almost twice those of historic rates (1905 - 2005; Blaustein et al. 2010), with temperatures warming as much as ~3°C for mid-latitudes (A2 Climate Scenario, IPCC 2018). Climate change has severe implications for species because of its diverse ecological effects on factors such as genetic diversity (Hampe & Petit 2005; Sexton et al. 2009), phenology (Parmesan 2006), species interactions (Pecl et al. 2017), adaptive potential (Evans et al. 2018; Nadeau & Urban 2019), and geographic range position (Lawler et al. 2009; Pecl et al. 2017). Consequently, it is difficult to predict species’ net responses to current, and predicted future, climate change (Evans et al. 2018). Although many other causes for species declines exist, such as land use changes, habitat loss, and disease, climate change may exacerbate the impacts of most, if not all, of these factors. Species are expected to elicit a variety of responses to climate change, such as redistribution to form entirely new shapes, sizes, and positions of geographic ranges (Araújo et al. 2006; Visser 2008; Lawler et al. 2009), or alterations in phenology that may impact species’ ecosystem functions (Pecl et al. 2017).

Climate-induced phenological shifts are some of the best understood responses to climate change, partly because such changes are easily observed (Parmesan 2006). Phenological shifts can disrupt population ecological processes and resultant community structure (Visser 2008) by creating phenological mismatches, or desynchronization of life cycle events between species and their abiotic environment, as
well as among species (Parmesan 2006). For example, marmots are emerging earlier than their usual food plants (Parmesan 2006), which could require them to use alternative food resources used by other species, resulting in increased niche overlap and resultant competition. Birds are advancing egg laying dates in response to warmer spring temperatures (Charmantier et al. 2008), and warming alters timing of migration in both birds and insects (Visser 2008). Warming temperatures also alter adult amphibian breeding phenology (Corn 2005; Kohli et al. 2019) and subsequent larval development rates (Blaustein et al. 2010), such that adults breed earlier and larvae develop faster due to reduced hydroperiods in their aquatic habitats. Such rapid development may directly impact community structure and body size-mediated ecosystem structure and function (Carlson & Langkilde 2017). In habitats such as ephemeral wetlands, warming temperatures may cause extended drought, ultimately eliminating aquatic ecosystem structure and function altogether (Araújo et al. 2006; Blaustein et al. 2010). In such cases, species may disperse to more suitable habitats (Parmesan 2006; Collins 2010), and theoretical models of climate-induced movement predict that ~ 25-38% of species in the Western Hemisphere will alter their geographic distributions as climates warm (Lawler et al. 2009).

Despite predictions of widespread movement in response to climate change, species with limited dispersal capabilities may not move because climate change-induced habitat loss may disconnect habitat patches, decreasing dispersal opportunities (Parmesan 2006; Evans et al. 2018). However, separate populations within species are likely to be affected unequally across their geographic range (Sexton et al. 2009), as many species are distributed unevenly, with abundances being highest at the geographic
range center and steadily decreasing towards the periphery (i.e., the "abundant center hypothesis"; Sagarin & Gaines 2002). Population densities are higher in range cores because of increased habitat suitability (Sagarin et al. 2006), with isolation and limiting factors increasing with closer proximity to range edges (Sexton et al. 2009). Limiting factors can be unique depending on geographic location, with abiotic stressors impacting southern ranges edge more than northern, cooler range edges (Paquette & Hargreaves 2021), where competition (Lyu & Alexander 2022) and predation (Baer & Maron 2018) may confine or promote range edges. Such limitations may also be genetic, because gene flow is often reduced among more isolated populations at geographic range margins (Sexton et al. 2009; Willi & Van Buskirk 2019), resulting in increased inbreeding (Hargreaves & Eckert 2019). General reductions of genetic diversity in peripheral populations can also result from peripheral populations consisting of new inhabitants of range edges exhibiting reduced genetic diversity due to recent dispersal from core populations (Hampe & Petit 2005). Closely related species may also hybridize in areas where range edges overlap, causing niche invasion (Willi & Van Buskirk 2019) and potentially decreasing already-low genetic diversity. In fact, Hargreaves and Eckert (2019) found that although edge populations may exhibit some adaptive potential outside of their usual range positions, core populations generally exhibit advantages over edge populations under climate warming scenarios.

Since gene flow is reduced in many isolated populations, climate change effects could be more severe for peripheral populations (Sexton et al. 2009). Isolation can increase local adaptation in individual populations, but localized adaptation does not promote adaptation to variability outside of local conditions, thus decreasing the ability
to respond to increasingly variable conditions produced by climate change (Hampe & Petit 2005). Low-latitude peripheral populations (i.e., “trailing edges”) in particular will be the first populations impacted by climate change (Nadeau & Urban 2019), and such change may affect them more severely because they may already be exhibiting their maximum physiological potential to mitigate effects of climatic extremes (Riddell et al. 2019). If peripheral populations are currently existing at their climatic limits, their responses to warming temperatures may be constrained due to low genetic diversity (Sexton et al. 2009; Hoffmann & Sgró 2011; Willi & Van Buskirk 2019).

It is unclear how climate change may shape the genetic diversity of peripheral populations as temperatures warm, causing the centers of geographic ranges to either shift or contract, which could increase or decrease genetic diversity at the periphery (Hampe & Petit 2005). Genetic diversity in low-latitude peripheral populations (i.e., “trailing edges”) will decrease if range cores “shrink away” from the periphery because peripheral populations will lose sources of genetic variation, causing higher incidence of genetic drift or population bottlenecks (Hampe & Petit 2005; Nadeau & Urban 2019). Conversely, high-latitude peripheral populations (i.e., “leading edges”) may not expand poleward as temperatures increase, generating issues such as gene swamping from migrating populations or genetic drift (Nadeau & Urban 2019), and/or creating increasingly limited distributions as trailing edge populations move poleward as current habitats warm beyond conditions suitable for survival (Parmesan 2006). If geographic range cores shift or expand, gene flow may increase in peripheral populations as cores expand to the periphery, benefiting leading populations at higher latitudes (Hampe & Petit 2005). Under such conditions, species must exhibit high tolerance to habitat
heterogeneity, especially in trailing populations at the range periphery, or move to more suitable habitats to compensate for increasing variability (Hoffmann & Sgró 2011; Pecl et al. 2017).

Habitat heterogeneity occurring prior to climate change at the range periphery may be currently advantageous to edge populations, because historical variability facilitates local adaptation to buffer against future climate change-induced heterogeneity (Hernández-Pacheco et al. 2019). Because of the rapid nature of climate change, however, phenotypic plasticity, wherein single genotypes can express any one of multiple possible phenotypes in response to local selective pressures, may need to occur to buffer against changing habitats (Merilä & Hendry 2014). As habitats become more heterogenous, such as those near range peripheries, phenotypic plasticity may be more important to population persistence than long-term natural selection (Agrawal 2001). Because phenotypic plasticity occurs within single generations, it may represent a more adaptive response to environmental stimuli than other responses, such as local adaptation (Duputié et al. 2015). For example, birds have exhibited adaptive phenological plasticity in egg-laying dates in response to warming temperatures (Charmantier et al. 2008). Phenotypic plasticity in response to climatic factors has also been observed in amphibians; plastic physiological responses assist with behavioral thermoregulation under the stress of warming temperatures in arid regions (Ruiz-Aravena et al. 2014), and some amphibians have advanced dates of breeding and egg deposition by several weeks in relatively short time spans (Corn 2005; Todd et al. 2011). In fact, Levis et al. (2018) and Hoffman & Sgró (2011) suggest that adaptive
phenotypic plasticity may occur prior to natural selection, acting as a buffer to assist with long-term microevolution.

In some cases, phenotypic plasticity represents a viable response to climate change for peripheral populations if plasticity maintains current levels of reproductive fitness (Gienapp et al. 2008). Plasticity does not always increase fitness, however, as it is sometimes predicted to be maladaptive for some species under future climate change conditions (Duputié et al. 2015). So that plasticity maintains adaptivity, it must balance tradeoffs to maintain or increase individual fitness (Visser 2008; Mägi et al. 2011; Merilä & Hendry 2014). For example, plasticity may facilitate earlier egg laying dates, but food availability may be lower at such times (Visser 2008). Requirements for maintaining adaptivity are likely different for populations throughout the geographic range, as leading edge populations experience indirect pressure from climate change via increases in competition (Hampe & Petit 2005; Sexton et al. 2009) and hybridization (Willi & Van Buskirk 2019) as they move poleward, while trailing edge populations experience warming temperatures that exceed their tolerance capabilities (Nadeau & Urban 2019) or change their phenology (Visser 2008).

Geographic variation in plasticity across species’ ranges is not well understood (Valladares et al. 2014). Decreases in population abundance and/or density towards range peripheries should reduce gene flow (Sexton et al. 2009; Willi & Van Buskirk 2019) and therefore the likelihood of locally adaptive plastic responses (Mägi et al. 2011). However, the incidence and/or intensity of plasticity in some cases may actually be higher at geographic range margins because peripheral populations live in more heterogeneous habitats, which may select for higher rates of plasticity (Agrawal 2001,
Snell-Rood et al. 2010, Hernández-Pacheco et al. 2019). As temperatures warm, species will experience new ecological constraints, thus increasing the likelihood of phenotypic plasticity as an adaptive response to habitat changes (Agrawal 2001).

In addition to core-periphery dynamics that may impact population densities and associated plasticity at broad scales across geographic ranges, fine-scale processes may be at play as well, such as local landscape features (Funk et al. 2005) and their associated impacts on genetic drift (Vucetich & Waite 2003). Local adaptation may impede species’ responses to new constraints, such as climate change, because populations may be more responsive to local landscape features and other selective pressures within their habitat, relative to new or changing constraints outside their current niche (Hargreaves & Eckert 2019). Local landscape features such as predator-prey interactions (Urban 2007), proximity to other ponds (Rubbo & Kiesecker 2005), and elevation (Funk et al. 2005) contribute to such local adaptation, thus altering the fitness of populations when new constraints arise or current constraints change.

Increased relative fitness could be inferred if plasticity occurs within populations (Valladares et al. 2014), and because genetic diversity is impacted by dispersal, tests of plastic capabilities would benefit from the use of species with limited dispersion. Movement between populations, or to previously uncolonized habitats, is a less viable response to climate change for many species (Evans et al. 2018), especially when considering local landscape features (Rubbo & Kiesecker 2005) and elevation (Funk et al. 2005). Phenotypic plasticity could therefore represent an appropriate response to changing habitats in such species (Hoffmann & Sgró 2011; Valladares et al. 2014).
Amphibians exhibit limited dispersal (Evans et al. 2018) but relatively high incidence of developmental plasticity (Urban et al. 2014), and are also susceptible to warming temperatures during larval development (Corn 2005; Riddell et al. 2018). Amphibians express plasticity in response to reduced hydroperiod from warming by decreasing time to metamorphosis but experience tradeoffs for developmental rate plasticity and reduced larval periods in the form of decreased size at metamorphosis (Brannelly et al. 2019), which is compensated for by rapid post-metamorphic growth (Bredeweg et al. 2019). Warming temperatures can exert long-term indirect effects on amphibian life history via body size, because body size scales with dispersal potential (Bredeweg et al. 2019), causing limited gene flow and higher likelihood of local adaptation in some parts of the geographic range (Amburgey et al. 2012; Valladares et al. 2014). Plastic responses that reduce body size at metamorphosis may also be maladaptive, because smaller body sizes may be selected against by increased susceptibility to predators (Carlson & Langkilde 2017) and altered dietary constraints due to gape-limitations (Urban 2008). Desiccation potential scales positively and negatively with temperature and body size, respectively, in amphibians (Riddell et al. 2019), which may further limit habitat connectivity for amphibian populations when coupled with effects of decreased body size on dispersal. However, Riddell et al. (2019) suggests that in terrestrial salamanders, some physiological plasticity occurs to reduce water loss potential, though increasing temperatures may ultimately push beyond the limits of plasticity. Also, Kohli et al. (2019) suggests that decreased size at metamorphosis from reduced hydroperiod could impact disease susceptibility later in adult life.
Experimental studies of plasticity are pertinent to understanding how species may realistically respond to climate change, as theoretical models predicting species responses may be unable to accurately predict future variability species may experience under pressures from warming temperatures and/or their adaptive responses (Lawler et al. 2009). Amphibians exhibit high levels of plasticity (Urban et al. 2014), and therefore represent an appropriate system to study plastic responses to climate change via reductions in hydroperiod to simulate increased temperatures. This study used Jefferson salamanders (*Ambystoma jeffersonianum*) to determine if plasticity in rates of growth, size at metamorphosis, and rates of successful metamorphosis differ between geographically central and peripheral populations in Southern Indiana and Kentucky, respectively, in response to simulated climate change causing reduced hydroperiods.

Jefferson salamanders inhabit ephemeral ponds throughout the upper Midwest, upper South, and Northeast United States and Ontario, Canada (Petranka 1998), where they tend to occur in forested wetlands with other ambystomatid salamanders (Rubbo & Kiesecker 2005), such as the spotted salamander, *Ambystoma maculatum* (Brodman 1996). Interspecific competition can impact larval survivorship for *A. jeffersonianum*, but they also hatch earlier and develop faster than *A. maculatum* (Brodman 1996). Other variables, such as egg mass size (Brodman 1995), hydroperiod (Rowe & Dunson 1995), and hybridization (Willi & van Buskirk 2019) may contribute to overall survivorship, plasticity, and metamorphosis in populations of *A. jeffersonianum* in my study. With respect to hybridization, *A. jeffersonianum* hybridizes with unisexual ambystomatid salamanders found in Northern Kentucky and Southern Indiana (Petranka 1988). Unisexual salamanders breed with *A. jeffersonianum* through kleptogenesis (Bogart et
al. 2007), wherein the former “steal” sperm of *A. jeffersonianum* to initiate reproduction, which could subsequently reduce the fitness of *A. jeffersonianum* and other ambystomatid salamanders by gene swamping of sexually reproducing ambystomatid populations (Todesco et al. 2016). This likelihood is strengthened by unisexual salamanders generally being more abundant than the sexually reproducing ambystomatids with which they interact (Bogart & Klemens 2008). In addition, historical landscape features such as latitudes of the last glacial maxima (Hampe & Petit 2005) impact genetic diversity of populations, with southernmost populations often acting as glacial refugia, and therefore exhibiting higher genetic diversity due to their relative temporal longevity (Duncan et al. 2015). However, it is unlikely that the last glacial maxima impacted populations in this study, as *A. jeffersonianum* historically resided south of the Wisconsin Glacial Border (Uzzell 1967) and therefore would not have been displaced and/or extirpated.

This study determined if plasticity is occurring at equivalent intensities in multiple core and peripheral populations of *A. jeffersonianum*, and whether such patterns in plasticity lead to fitness differences among populations. My results contribute to our understanding of how climate change may differentially affect populations across species’ geographic ranges, especially concerning phenotypic responses, and will also assist with improving recent “phenotype conservation” and management efforts (Lesica & Allendorf 1995; Watters et al. 2003) by highlighting possible differences in fitness between core and peripheral populations, as current conservation methods may not be suitable for every population across a species’ geographic range (Hampe & Petit 2005). Data obtained by this study may be
representative for how congeners *A. maculatum* or *A. opacum*, may respond to reductions in hydroperiod via climate change. These species tend to occur syntopically with *A. jeffersonianum*, are exposed to similar stressors (Rubbo et al. 2006b) interact with each other (Cortwright 1998; Mott & Maret 2011), and share similar resources (Cortwright 1988). Recent phenological shifts under climate change may also blur ecological interactions between species (Todd et al. 2011) by increasing niche overlap, and my results may provide insight into how traits affected by hydroperiod may impact these interactions. It was expected that differences in plasticity depended upon population location within the *A. jeffersonianum* geographic range (Figure 1), and I predicted that peripheral populations would exhibit reduced plasticity because such populations are typically considered to be less dense and more isolated than core populations (Hampe & Petit 2005), which can reduce genetic diversity and gene flow, thereby hindering plastic potential (Nadeau & Urban 2019; Willi & Van Buskirk 2019).
Materials and Methods

Study Site

Experiments occurred at Taylor Fork Ecological Area (TFEA) at Eastern Kentucky University, in Richmond, Kentucky (37.7166 °N, 84.2958 °W). Egg masses were collected at peripheral and central portions of the Jefferson Salamander geographic range, with five sites in Kentucky and three sites in Indiana respectively, between 2020 and 2021.

Experimental Design

The experiment utilized 20-gallon (~ 75 liters) mesocosms (MacCourt Products, Denver, CO, USA), because mesocosms provide the capability to control for confounding variables such as light, temperature, and predator abundance relative to field-based approaches (Merilä & Hendry 2014). To mimic conditions in natural ponds, mesocosms were stocked with rainwater and 850 mL of pond sediment, 1 cm³ of green filamentous algae, and 50 mL of concentrated zooplankton collected from a nearby pond in TFEA. ~1,800 cubic centimeters of thatch (grass clippings) were deposited into each container, as well as 20 oak leaves (Quercus palustris) to act as refugia for larvae. The pond sediment, algae, and concentrated zooplankton ensured larval salamanders had viable food resources at the beginning of the experimental periods (Relyea 2002; Urban 2008; Anderson & Whiteman 2015a). It was expected that invertebrates such as Chironomus and Chaoborus would naturally colonize tanks, further contributing to realistic representations of natural fishless pond ecosystems.
Collection of ~50-100 embryos per pond (~3-5 egg masses) of *A. jeffersonianum* occurred between January and March 2020 from six ponds at the core and edge of the species’ range (Figure 1). In 2020 egg masses were collected from peripheral populations in the Daniel Boone National Forest (37.805418°N, -83.669137°W; 37.819132°N, -83.681594°W), and Miller-Welch Central Kentucky Wildlife Management Area, Madison County, Kentucky (37.6212992°N, -84.2010809°W), with core populations being sampled from populations in Big Oaks National Wildlife Refuge, Madison, Indiana (38.977422 N, -85.369435 W), Clark State Forest, Henryville, Indiana (38.566025 N, -85.843159 W), and Splinter Ridge Fish & Wildlife Area, Jefferson County, Indiana (38.744467 N, -85.206094 W; 38.746900 N, -85.212625 W). In 2021 egg masses were collected from core populations in Clark State Forest, Henryville, Indiana (38.576220 N, -85.847406 W) and peripheral populations in Clay Hill Memorial Forest, Campbellsville, Kentucky (37.441190 N, -85.359531 W) and Mammoth Cave National Park, Hart County, Kentucky (37.141 N, -86.081 W).

Given that Clark State Forest was sampled in both years, ponds sampled in the first and second years were separated by distances exceeding the known maximum dispersal distance for *A. jeffersonianum* (~250 m; Williams 1973; Douglas and Monroe 1981) to minimize the possibility of sampling eggs from the same females across years.
Figure 1. Collection sites for egg masses of *Ambystoma jeffersonianum*. The western portion of the range of *A. jeffersonianum* is demarcated with orange, with both geographically core and peripheral populations occurring within Kentucky and Indiana. Core populations are found along the southern border of Indiana, with peripheral populations occurring in central Kentucky. Circles and triangles represent collection sites in 2020 and 2021, respectively. Range map from *IUCN Redlist*. ([https://www.iucnredlist.org/species/59059/56458965](https://www.iucnredlist.org/species/59059/56458965))

Egg masses were temporarily housed in environmental chambers under a 12L:12D photoperiod at ~7-10°C in the vivarium at Eastern Kentucky University, and kept in separate containers (15cm x 15cm, 728 cm³) based on specific pond collection locality, where they were kept separated in this manner throughout the experiments. Upon hatching, larvae were separated into groups of five and assigned to one of four
treatments. Larvae were transported to TFEA in clear plastic containers, which were placed into assigned mesocosms for 10 - 15 minutes to acclimate prior to release. Treatments were designed to compare larval salamander responses to future climate change scenarios and current climate patterns, wherein future climate change scenarios experienced reductions in hydroperiod to simulate increased temperatures and rates of evaporation. These treatments therefore simply addressed changes in predicted evaporation rates based on temperature increases, and not rainfall patterns that may become increasingly stochastic as climate changes. The four treatments included: 1) salamanders from core populations, with drying (i.e., Core Future Treatment); 2) salamanders from core populations without drying (control; i.e., Core Current Treatment); 3) salamanders from edge populations, with drying (i.e., Edge Future Treatment), or; 4) salamanders from edge populations, without drying (control; i.e., Edge Current Treatment). Treatments were arranged in a randomized block design, with four tanks comprising future and current climate treatments from both core and edge populations within each block (Figure 2; Figure 3). The future climate treatment was simulated using the anticipated ~3°C increase in temperature for mid-latitudes (A2 Climate Scenario, IPCC 2018). To simulate faster evaporation rates, water was removed from mesocosms under treatments 1 and 3 (O’Regan et al. 2014; Brannelly et al. 2019) using 1000 ml containers (BPA free plastic, Nalgene). The volume of water removed from mesocosms under climate warming treatments was calculated using the U.S. EPA Evaporation Equation \( E = \frac{7.4PA(0.477W^{0.78})}{T+459.67} \) \( E \) = evaporation rate (gallons/day); \( A \) = surface area (ft\(^2\)); \( P \) = water vapor pressure (mmHg; constant based on recorded temperature) at ambient temperature; \( W \) = wind speed above water (mph; based on
historical monthly averages for Lexington, KY; NOAA); and T= temperature (°F; based on monthly historical averages for Richmond, KY; NOAA); all calculations converted to cm³/2 weeks; Table 1).

Water level reduction in future climate treatments (i.e., core future treatment and edge future treatment; hereafter generally referred to as future climate treatment unless discussing one population group) began on April 30, 2020 and April 11, 2021 after all larvae were placed into tanks, and reduced biweekly. Withdrawal concluded on June 3, 2020 and May 5 in 2021 at a water depth of ~127 mm to prevent desiccation of larvae that had not yet metamorphosed. After this point, water levels were retained at the height recorded on June 3, 2020 and May 5, 2021 for future climate treatments and retained full for current climate treatments (i.e., core current treatment and edge current treatment; hereafter generally referred to as current climate treatment unless discussing one population group).
Figure 2. 2020 Mesocosm Array. Each block contained four tanks, each tank contained five *Ambystoma jeffersonianum* larvae from edge current, edge future, core current, or core future climate treatments.

Figure 3. 2021 Mesocosm Array. Each block contained four tanks, each tank contained five *Ambystoma jeffersonianum* salamander larvae from edge current, edge future, core current, or core future climate treatments.
Table 1. Evaporation rates calculated with the U.S. EPA Evaporation Equation. Listed rates indicate the volume of water that was drawn from each tank/mesocosm every two weeks.

<table>
<thead>
<tr>
<th>Month</th>
<th>Estimated Evaporation Rate of Future Climate Scenarios (cm³/ 2 weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>April</td>
<td>10,447</td>
</tr>
<tr>
<td>May</td>
<td>13,929</td>
</tr>
<tr>
<td>June</td>
<td>12,199</td>
</tr>
</tbody>
</table>

**Sampling**

Larval densities were recorded at the middle of the larval period (between April and May) and once prior to the onset of metamorphosis (between May and July) in tandem with determination of larval growth rates by measuring larval snout-vent length (SVL) and girth (i.e., maximum width at midsection of body). Larval densities were determined to estimate survival rates under future and current climate scenarios by placing collapsible minnow traps (1.6 mm mesh; Cabela’s Inc., Sidney, NE) into mesocosms for 24 hours, and body girth and SVL were measured using ImageJ (Schneider et al. 2012) for all captured larvae (1-5 individuals per mesocosm) to provide an estimate of body condition and growth rates with which to measure plastic responses to altered hydroperiod. In cases where no individuals were captured in minnow traps, hand nets were used for ~1 minute in an attempt to collect larvae from tanks, and after measurement, larvae were placed back into tanks to complete metamorphosis. Differences in metamorphic body girth and SVL between both
treatments and populations were measured because these traits can be affected by reductions in hydroperiod (Brannelly et al. 2019) and as such, should indicate differences in plasticity and differences in fitness among amphibian populations (Anderson & Whiteman 2015b). Dates of metamorphosis for each salamander were recorded, and metamorphosed salamanders were removed from mesocosms (Anderson & Whiteman 2015a), anesthetized and euthanized in 250 mg L\(^{-1}\) of benzocaine, and accessioned into Eastern Kentucky University’s Branley A. Branson Museum of Zoology.

Water temperatures, dissolved oxygen, and pH were recorded five times during the experiment with a YSI Pro20 Dissolved Oxygen Meter (YSI Incorporated, Yellow Springs, OH) for temperature and dissolved oxygen, and a pH 5+ pH 6+ Ion 6+ Meter for pH (Oakton Instruments, Vernon Hills, IL). Abiotic variable measurements occurred at roughly one-week intervals, and water depths in mesocosms were monitored every two weeks to maintain constant rates of water loss in future climate change treatments. While current climate change treatments likely experienced low evaporation rates from sun and wind exposure, they were maintained at constant water levels over the course of the experiment. During the five recording intervals, temperatures were recorded between 14:00 – 16:00 to account for anticipated maximum differences between current and future climate treatments. These measurements also accounted for effects of temperature on plasticity resulting from interactions between temperature and hydroperiod or evaporation (O’Regan et al. 2014) and effects of dissolved oxygen concentrations on larval development (Blaustein et al. 2010). Measurements of pH in
each tank were taken because increases in pond acidity diminishes the success of larval *A. jeffersonianum* (Horne and Dunson 1994).

**Analysis**

Statistical analyses were performed using RStudio version 3.6.1 (R Core Team 2019) using an alpha level of 0.05 for all analyses. General differences in larval growth, size at metamorphosis, time to metamorphosis, and survivorship were compared using MANOVA and, upon identification of a significant overall treatment effect, subsequent univariate ANOVA between populations, treatments, and years (Figure 4). These analyses were performed prior to calculating differences in responses between current and future climate treatments within core and peripheral populations and between years, and as such, were not testing for plasticity. Instead, general differences in traits indicated if individual populations or treatment groups grew larger or faster, exhibited reduced times to metamorphosis, or greater survival rates. Larval growth and survivorship were tested with separate MANOVAs and ANOVAs, respectively, due to frequent cases wherein lack of survivors or captures in tanks produced data on survivorship (as 0%) but not larval growth rates or sizes at metamorphosis.

While the previous analysis tested for “general” differences between variables, differences in larval growth rates (mm/day), time to metamorphosis (days), size at metamorphosis (mm), and survivorship (% of five larvae in a tank) between current and future climate treatments (i.e., subtracting current climate treatment measurements from those of future climate treatments) within populations and years were compared using MANOVA to test for plasticity (Figure 4). Although the original intent was to compare future and current climate “difference values” (there is currently no established term for
this in literature, though “differentials” were also considered to describe values where
current climate treatment measurements were subtracted from future climate treatment
measurements) within each block, some replicates from separate blocks were compared
if no data were present for opposing treatments within a single block. Due to natural
variation in larval success between replicates that caused data inconsistencies (i.e., no
larval survival caused an inability to record time to metamorphosis), datapoints were
assigned an arbitrary number and were randomly removed from the dataset using a
random number generator so each variable had an equivalent number of datapoints.

Temperature, dissolved oxygen, and pH differences between populations,
treatments, and years, as well as the difference values of the abiotic variables (i.e.,
subtracting current climate treatment measurements from those of future climate
treatments) were also analyzed to determine if abiotic variables influenced larval
responses, or if synergistic, non-synergistic, or antagonistic effects among hydroperiod
and abiotic variables affected larval responses (O'Regan et al. 2014). While five
measurements of dissolved oxygen and temperature were recorded, only three
measurements of pH were taken, because pH was not a variable initially expected to be
recorded. Due to the unequal numbers of observations between the three variables,
dissolved oxygen and temperature were analyzed with MANOVA, whereas pH was
analyzed separately with ANOVA.
Comparisons of climate scenarios within and between core and edge populations were made using MANOVA. The “General MANOVA” analyzed response variables from each of the four treatment groups, whereas the “Plasticity MANOVA” analyzed the differences in responses between current and future climate treatments.
Results

Larval *A. jeffersonianum* were placed into mesocosms between March 26 – April 24, 2020 and March 18 – March 30, 2021. Metamorphosis occurred between May 31 – July 22 in 2020 (Figure 5) and May 19 – July 12 in 2021 (Figure 6). Upon metamorphosis in 2020, it was observed that eggs collected from one pond in Daniel Boone National Forest were those of spotted salamanders (*Ambystoma maculatum*); therefore, tanks containing this species were removed from all datasets. Across all treatments, ~32% of *A. jeffersonianum* larvae (111 of 340) in 2020 and ~40% of larvae (64 of 160) in 2021 survived to metamorphosis.

![Figure 5. Temporal pattern of metamorphosis among *A. jeffersonianum* across all treatments in 2020. Line graphs depicting temporal patterns of metamorphosis for each treatment were also considered, but decided against.](image)
Figure 6. Temporal pattern of metamorphosis among *A. jeffersonianum* across all treatments in 2021. Line graphs depicting temporal patterns of metamorphosis for each treatment were also considered, but decided against.

**Biotic Factors**

Larval growth (i.e., SVL growth and body girth growth) was recorded on May 31, 2020 and May 7, 2021 when larvae were nearing metamorphosis (2020: N = 150 total larvae, $\bar{x} = 2.5$ / mesocosm; 2021: N= 54 total larvae, $\bar{x} = 2.25$ / mesocosm). Larval growth rates were influenced by source population (MANOVA $F_{2, 76} = 3.97$, $P = 0.02$) and the interaction between climate treatment and year (MANOVA $F_{2, 72} = 3.02$, $P = 0.05$) but not by climate treatment, year, or other interactions (Table 2, Column a). Growth rates of body girth among edge populations were significantly higher than those of core populations, (ANOVA $F_{1, 79} = 6.86$, $P = 0.01$) while rates of growth in body length did not differ between core and edge populations (Table 2, Column a; Figure 7).
Size at metamorphosis was generally influenced by population (MANOVA F₃,₆₅ = 9.08, P < 0.001), climate treatment (MANOVA F₃,₆₅ = 6.35, P < 0.001), year (MANOVA F₃,₆₅ = 14.45, P < 0.001), and an interaction between population and year (F₃,₆₅ = 10.93, P < 0.001), but not other interactions between population, treatment, and year (Table 2, Column b). Specifically, body lengths at metamorphosis were greater in core versus edge populations (ANOVA F₁,₇₃ = 4.39, P = 0.03); however, no differences in body girth at metamorphosis between populations were observed (Table 2, Column b; Figure 8). Body girths at metamorphosis were greater in current climate treatments (ANOVA F₁,₇₃ = 5.90, P = 0.01), whereas body lengths at metamorphosis were not significantly different between climate treatments, although a general trend was observed wherein body girth and length at metamorphosis were greater and longer, respectively, in populations exposed to current climate treatments (Table 2, Column b; Table 3). Metamorphs in 2021 had larger girths (ANOVA F₁,₇₃ = 3.75, P = 0.05) and longer lengths (ANOVA F₁,₇₃ = 25.52, P < 0.001) than in 2020 (Table 1, Column b; Table 2).

Time to metamorphosis was influenced by population (MANOVA F₃,₆₅ = 9.08, P < 0.001), climate treatment (MANOVA F₃,₆₅ = 6.35, P < 0.001), and year (MANOVA F₃,₆₅ = 14.45, P < 0.001) in a similar manner to size at metamorphosis (Table 2, Column b). Edge populations generally achieved metamorphosis sooner than core populations (ANOVA F₁,₇₃ = 11.77, P < 0.001). Populations exposed to future climate treatments metamorphosed more quickly than populations exposed to current climate treatments (ANOVA F₁,₇₃ = 4.26, P = 0.04), indicating larvae exhibited a plastic response to
hydroperiod reductions, and populations in 2020 metamorphosed more quickly than those in 2021 (ANOVA $F_{1,73} = 5.71, P = 0.01$; Table 2, Column $b$; Table 3).

Survivorship was influenced by treatment (ANOVA $F_{1,96} = 21.07, P < 0.001$), but not population, year, or any interactions between the three independent variables (Table 2, Column $c$). Individuals survived to metamorphosis more frequently in future climate treatments than current climate treatments, and though there was no significant difference between populations, core populations tended to exhibit higher survival than edge populations (Table 2, Column $c$; Table 3). A trend was also observed wherein survival tended to be higher in 2021 than in 2020, though no significant difference between years was observed (Table 2, Column $c$; Table 3).
Table 2. \(a\) MANOVA of population location, climate treatment, and year on larval growth. \(b\) MANOVA of population location, climate treatment, and year on size at metamorphosis and time to metamorphosis. \(c\) ANOVA of population location, climate treatment, and year on survivorship. Main effects and interactions analyzed using MANOVA are in lefthand column, indented variables indicating subsequent univariate ANOVAs for individual response variables.

<table>
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<th>(a)</th>
<th></th>
<th>(b)</th>
<th></th>
<th>(c)</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>(F)</td>
<td>(n, d)</td>
<td>(P)</td>
<td>(F)</td>
<td>(n, d)</td>
</tr>
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<td>0.02</td>
<td>9.08</td>
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<td>1, 79</td>
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<td>Larval Girth Growth</td>
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<td>1, 79</td>
<td>(0.01)</td>
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</tr>
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<td>Metamorph Length</td>
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<td>1, 73</td>
<td>(0.03)</td>
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<td>0.38</td>
<td></td>
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</tr>
<tr>
<td>Time to Metamorphosis</td>
<td>11.77</td>
<td>1, 73</td>
<td>(&lt;0.001)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Survivorship</td>
<td></td>
<td></td>
<td></td>
<td>0.00</td>
<td>1, 96</td>
</tr>
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<td>Climate Treatment</td>
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<td>0.70</td>
<td>6.35</td>
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<tr>
<td>Larval Length Growth</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Larval Girth Growth</td>
<td></td>
<td></td>
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<td></td>
</tr>
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<td>4.26</td>
<td>1, 73</td>
<td>(0.04)</td>
<td></td>
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</tr>
<tr>
<td>Survivorship</td>
<td></td>
<td></td>
<td></td>
<td>21.07</td>
<td>1, 96</td>
</tr>
<tr>
<td>Year</td>
<td>2.27</td>
<td>2, 72</td>
<td>0.10</td>
<td>14.45</td>
<td>3, 65</td>
</tr>
<tr>
<td>Larval Length Growth</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Larval Girth Growth</td>
<td></td>
<td></td>
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<tr>
<td>Metamorph Length</td>
<td>25.52</td>
<td>1, 73</td>
<td>(&lt;0.001)</td>
<td></td>
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<tr>
<td>Metamorph Girth</td>
<td>3.75</td>
<td>1, 73</td>
<td>(0.05)</td>
<td></td>
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<td>Time to Metamorphosis</td>
<td>5.71</td>
<td>1, 73</td>
<td>(0.01)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Survivorship</td>
<td></td>
<td></td>
<td></td>
<td>1.62</td>
<td>1, 92</td>
</tr>
<tr>
<td>Population x Treatment</td>
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<td>2, 76</td>
<td>0.67</td>
<td>0.68</td>
<td>3, 65</td>
</tr>
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<td>Population x Year</td>
<td>1.10</td>
<td>2, 72</td>
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<td>3, 65</td>
</tr>
<tr>
<td>Treatment x Year</td>
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<td>2, 72</td>
<td>(0.05)</td>
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<td>3, 65</td>
</tr>
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<td>Population x Treatment x Year</td>
<td>0.48</td>
<td>2, 72</td>
<td>0.59</td>
<td>0.83</td>
<td>3, 65</td>
</tr>
</tbody>
</table>
Figure 7. Mean growth rates of body girth and length (both in mm/day) of geographically core and edge populations of larval *A. jeffersonianum*. Error bars represent ± 1 S.E.

Figure 8. Mean size at metamorphosis of body girth and length (both in mm) of geographically core and edge populations of *A. jeffersonianum* metamorphs. Error bars represent ± 1 S.E.
The aforementioned analyses tested for general differences in larval growth and body size at metamorphosis, time to metamorphosis, and survivorship, but not differences in the magnitude of plasticity of these traits between current and future climate scenarios (Figure 4: “General MANOVA”). Differences in the magnitude of responses between current and future climate treatments were tested using a MANOVA to determine if plasticity occurred (Figure 4; “Plasticity MANOVA”). Plasticity was observed in some traits (Figure 9); however, no differences in the magnitude of plasticity were found in survivorship, larval growth, time to metamorphosis, or size at metamorphosis in response to climate treatment between core and edge populations of *A. jeffersonianum*, or the interaction between population and year. Magnitude of plasticity varied by year in some traits (MANOVA F\(6, 26 = 2.56, P = 0.05\)), where plasticity of length at metamorphosis was greater in 2021 than 2020 (ANOVA F\(1, 28 = 6.98, P = 0.01\)), and plasticity in larval girth growth was greater in 2020 than 2021 (ANOVA F\(1, 28 = 4.60, P = 0.04\)), but no differences were observed among the other response variables between 2020 and 2021 (Table 3; Table 4).
Table 3. Larval growth rates (mm/day), size at and time to metamorphosis (days), and survivorship (% of the five larvae in a tank), between treatments of core and edge populations in *Ambystoma jeffersonianum*. ± 1 S.E.

<table>
<thead>
<tr>
<th></th>
<th>2020</th>
<th>2021</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Core</td>
<td>Edge</td>
</tr>
<tr>
<td></td>
<td>Current</td>
<td>Future</td>
</tr>
<tr>
<td>Larval Girth Growth</td>
<td>0.06 ± 0.01</td>
<td>0.06 ± 0.00</td>
</tr>
<tr>
<td>Larval Length</td>
<td>0.22 ± 0.02</td>
<td>0.20 ± 0.01</td>
</tr>
<tr>
<td>Metamorph Girth</td>
<td>5.76 ± 0.22</td>
<td>5.21 ± 0.16</td>
</tr>
<tr>
<td>Metamorph Length</td>
<td>33.38 ± 0.90</td>
<td>31.47 ± 0.71</td>
</tr>
<tr>
<td>Time to Metamorphosis</td>
<td>86.62 ± 2.60</td>
<td>77.01 ± 1.60</td>
</tr>
<tr>
<td>Survivorship</td>
<td>0.19 ± 0.04</td>
<td>0.48 ± 0.06</td>
</tr>
</tbody>
</table>
Table 4. MANOVA on the effect of population location and year on “current-future differences” between survivorship, larval growth, time to metamorphosis, or size at metamorphosis. Significant results are italicized, and indented variables indicate subsequent ANOVAS for individual response variables.

<table>
<thead>
<tr>
<th></th>
<th>F</th>
<th>n, d</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
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<td>6, 26</td>
<td>0.13</td>
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<tr>
<td>Year</td>
<td>2.56</td>
<td>6, 26</td>
<td><strong>0.05</strong></td>
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<tr>
<td>Length at metamorphosis</td>
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<td>1, 28</td>
<td><strong>0.01</strong></td>
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<td>Girth at metamorphosis</td>
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<td>1, 28</td>
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<td>Larval Length</td>
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<td><strong>0.04</strong></td>
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<td>0.70</td>
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<td>0.95</td>
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<tr>
<td>Population x Year</td>
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<td>6, 26</td>
<td>0.36</td>
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Figure 9. Mean differences in larval length (a), larval girth (b), metamorph length (c), metamorph girth (d) survivorship (e), and time to metamorphosis (f), between core and edge populations in 2020 and 2021. Differences were calculated by subtracting response variable values in current climate treatments from those in future climate treatments. Plasticity is observed when mean values and error bars do not overlap zero on the y-axis.
**Abiotic Factors**

Temperature and dissolved oxygen were both influenced by year (MANOVA $F_{2, 571} = 56.02$, $P < 0.001$) and the interaction between treatment and year (MANOVA $F_{2, 571} = 3.37$, $P = 0.03$; Table 5, Column $a$), with increased temperatures in 2020 (ANOVA $F_{1, 578} = 79.05$, $P < 0.001$) and increased dissolved oxygen in 2021 (ANOVA $F_{1, 578} = 44.48$, $P < 0.001$; Table 6), though dissolved oxygen and temperature were not influenced by population, treatment, or interactions between population and treatment, population and year, or population, treatment, and year (Table 5, Column $a$). pH was influenced by year (ANOVA $F_{1, 340} = 65.73$, $P < 0.001$) and treatment (ANOVA $F_{1, 340} = 9.28$, $P = <0.001$), wherein pH was higher in 2021 and in current climate treatments (Table 5, Column $b$; Table 6). However, pH was not influenced by population location, the interaction between population and treatment, population and year, treatment and year, or population, treatment, and year (Table 5, Column $b$).
Table 5. *a.* MANOVA of population location, climate treatment, and year on dissolved oxygen and temperature. *b.* ANOVA of population location, climate treatment, and year on survivorship. Main effects are interactions that are presented in the lefthand column, with indented variables indicating subsequent univariate ANOVAs for individual response variables.

<table>
<thead>
<tr>
<th></th>
<th>a</th>
<th>b</th>
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<td>pH</td>
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<td>Population x Year</td>
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<td>2, 571</td>
</tr>
<tr>
<td>Treatment x Year</td>
<td>3.37</td>
<td>2, 571</td>
</tr>
<tr>
<td>Population x Treatment x Year</td>
<td>0.06</td>
<td>2, 571</td>
</tr>
</tbody>
</table>

A MANOVA indicated significant differences in the differentials in temperature, dissolved oxygen, and pH between years (MANOVA $F_{13, 42} = 12.87$, $P < 0.001$; Table 6; Table 7), with higher temperature differentials between current and future climate treatments observed in 2020 (ANOVA $F_{1, 288} = 43.63$, $P < 0.001$), but higher dissolved oxygen (ANOVA $F_{1, 288} = 6.9$, $P = 0.009$) and pH (ANOVA $F_{1, 172} = 65.73$, $P < 0.001$).
4.34, P = 0.03) differentials between current and future climate treatments in 2021. No significant differences were observed in temperature, dissolved oxygen, or pH differentials of core and edge populations or the interaction between population and year (Table 6; Table 7).
Table 6. pH, Dissolved oxygen, and temperature between populations and treatments for five sampling periods. Sampling periods are separated by gaps between the measured variables, and are in chronological order from top to bottom, with the first sampling period listed at the top of the table, and the fifth sampling period at the bottom of the table. D.O. = Dissolved Oxygen.

<table>
<thead>
<tr>
<th></th>
<th>2020</th>
<th>2021</th>
<th></th>
<th>2020</th>
<th>2021</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Core</td>
<td>Edge</td>
<td>Core</td>
<td>Edge</td>
<td>Core</td>
</tr>
<tr>
<td>pH</td>
<td>9.70 ± 0.17</td>
<td>8.55 ± 0.40</td>
<td>9.67 ± 0.15</td>
<td>8.62 ± 0.17</td>
<td>9.66 ± 0.20</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>30.50 ± 0.12</td>
<td>30.80 ± 0.16</td>
<td>30.50 ± 0.16</td>
<td>30.70 ± 0.18</td>
<td>30.70 ± 0.16</td>
</tr>
<tr>
<td>pH</td>
<td>8.80 ± 0.19</td>
<td>8.97 ± 0.30</td>
<td>9.05 ± 0.14</td>
<td>9.05 ± 0.20</td>
<td>9.10 ± 0.26</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>30.50 ± 0.16</td>
<td>30.80 ± 0.18</td>
<td>30.50 ± 0.12</td>
<td>30.70 ± 0.14</td>
<td>30.70 ± 0.16</td>
</tr>
<tr>
<td>pH</td>
<td>9.66 ± 0.20</td>
<td>9.66 ± 0.23</td>
<td>9.73 ± 0.34</td>
<td>9.73 ± 0.34</td>
<td>9.73 ± 0.34</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>30.50 ± 0.16</td>
<td>30.80 ± 0.18</td>
<td>30.50 ± 0.12</td>
<td>30.70 ± 0.14</td>
<td>30.70 ± 0.16</td>
</tr>
<tr>
<td>pH</td>
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<td>9.66 ± 0.23</td>
<td>9.73 ± 0.34</td>
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<tr>
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<td>30.70 ± 0.16</td>
</tr>
<tr>
<td>pH</td>
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<td>9.66 ± 0.23</td>
<td>9.73 ± 0.34</td>
<td>9.73 ± 0.34</td>
<td>9.73 ± 0.34</td>
</tr>
</tbody>
</table>
Table 7. MANOVA of population and year on the “current – future differences” between dissolved oxygen, temperature, and pH within the mesocosms. Indented variables indicate subsequent ANOVAS for the dependent variables.

<table>
<thead>
<tr>
<th></th>
<th>F</th>
<th>n, d</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population</td>
<td>0.50</td>
<td>13, 42</td>
<td>0.91</td>
</tr>
<tr>
<td>Year</td>
<td>12.87</td>
<td>13, 42</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Dissolved Oxygen</td>
<td>6.9</td>
<td>1, 288</td>
<td>0.009</td>
</tr>
<tr>
<td>Temperature</td>
<td>43.63</td>
<td>1, 288</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>pH</td>
<td>4.34</td>
<td>1, 172</td>
<td>0.03</td>
</tr>
<tr>
<td>Population x Year</td>
<td>0.78</td>
<td>13, 42</td>
<td>0.66</td>
</tr>
</tbody>
</table>
Discussion

Larval *A. jeffersoniaum* salamanders exhibited differences in growth, body size, time to metamorphosis, and survival reflective of plasticity in response to altered hydroperiod, but no differences in the magnitude of plasticity were observed between larvae from core and peripheral populations. These findings suggest that geographically central and peripheral populations of larval *A. jeffersonianum* will likely respond equivalently to predicted climate change-induced reductions in hydroperiods. Larvae from peripheral populations exhibited faster rates of body girth growth and earlier metamorphosis than larvae from geographically core populations, though metamorphs from core populations were typically longer than those of peripheral populations, and body size at metamorphosis differed between years. Salamanders from current climate treatments had larger body girths than those in future climate treatments, but salamanders exposed to future climate treatments more rapidly attained metamorphosis, a finding consistent with previous observations of tradeoffs in plasticity of the amphibian larval period (Brannelly et al. 2019; Bredeweg et al. 2019; Amburgey et al. 2012; Wilbur and Collins 1973). Though salamanders were smaller and metamorphosed faster under future climate treatments, they exhibited greater rates of survival than those in current climate treatments. While these findings tentatively suggest larval salamanders will exhibit increased survivorship under future climate scenarios, tanks were not completely dried to prevent desiccation of larvae, which may be overestimating total survivorship. Smaller larval body sizes of peripheral populations specifically, and smaller body sizes between both population groups under future climate conditions, is a concerning factor as climatic variables increase in intensity and
variability as climate change progresses (Pounds 2001; Brooks 2004; Blaustein et al. 2010).

Reduced hydroperiods are predicted to increase pond temperatures (Noland & Ultsch 1981; Brooks 2004), increase pH via increased primary production (Boeckman & Bidwell 2007), and reduce dissolved oxygen concentration via increased decomposition, which all elicit similar negative effects on larval fitness (Horne & Dunson 1994, 1995; Blaustein et al. 2010). However, similarities in temperature and dissolved oxygen between population groups and climate scenarios, as well as generally alkaline conditions, suggest larval fitness was unaffected by these variables. Hydroperiod reductions did not produce the hypothesized outcomes for abiotic variables, such as temperature increases under reduced hydroperiods leading to reduced dissolved oxygen and increased pH. Such results may be due to extreme stratification that can occur in even the shallowest ponds because abiotic data were not collected far below the water surface in tanks (Boeckman and Bidwell 2007), or results may be due to rapid cooling and heating of the relatively small tanks that might limit detectability of differences in abiotic variables (Song et al. 2013).

Temperature, pH, and dissolved oxygen fluctuate frequently in aquatic systems across seasons and years, due to isolated weather events and general seasonal variation (Black 1976; Boeckman & Bidwell 2007; Earl & Semlitsch 2013). Although the two study periods began towards the onset of summer, differences in temperature, dissolved oxygen, and pH observed between years may be the result of a ~ 1 month difference in introduction of larvae into tanks, drawdown schedules, and subsequent collection of abiotic data between years (i.e., one month earlier in 2021). The delay in 2020 resulted
from a die-off of larval *A. jeffersonianum* tanks just prior to the study period, likely caused by unintentional use of leaf litter from invasive Amur honeysuckle (*Lonicera maackii*), which can dramatically increase larval salamander mortality (Watling et al. 2011; Berta 2019; Robison et al. 2021). Though temperature differences may have resulted from the delay, average air temperatures in Richmond, KY during the study periods were also warmer in 2020 (18.79°C ± 0.08) than in 2021 (17.72°C ± 0.08; NOAA NCEI 2022), which mirrors my water temperature data. It is likely overall warmer temperatures, in tandem with the delay in data collection, may have partly caused greater temperature differentials between current and future climate treatments in 2020 because tanks with lower water levels in current climate treatments may have warmed more rapidly in warmer periods. Warmer temperatures in 2020 and increased dissolved oxygen concentrations in 2021 support observations of inverse relationships between temperature and dissolved oxygen (Blaustein et al. 2010). Additions of leaf litter, and use of grass substrate and its subsequent rapid decomposition, can decrease dissolved oxygen concentrations and pH, respectively, contributing to lower concentrations of dissolved oxygen and lower pH in 2020 because new leaf litter was not added in 2021 (Rubbo et al. 2006a; Earl & Semlitsch 2013). Surface pH may also have been higher in current climate treatments because water bodies can be greatly stratified even at minimal depths (Boeckman and Bidwell 2007), which would allow variation in pH to be more extreme in current climate treatments due to greater water volume than future climate treatments.

Climatic variables mediate larval growth, size at and time to metamorphosis, and survival in many amphibian species (Semlitsch et al. 1988; Laurila et al. 2002;
Denver & Middlemis-Maher 2010; Amburgey et al. 2012; Brannelly et al. 2019; Kohli et al. 2019), which in turn impact intra- and interspecific interactions (Parmesan 2006; Visser 2008; Todd et al. 2010; Jara et al. 2019). If natural populations of *A. jeffersonianum* exhibit smaller body sizes found in populations exposed to future climate treatments observed in my results, the aggressive nature *A. jeffersonianum* (Mott and Maret 2011) may diminish their predatory potential under climate change if syntopic species do not also decrease in body size, especially in peripheral populations where my data suggests that *A. jeffersonianum* larvae are already small. Such changes in body size can influence both competitive and predator-prey interactions; rapid growth may shift predation towards competition if prey outgrow predation risk by rapidly achieving size refugia (Travis 1983; Lannoo et al. 1989; Urban 2007). Faster growth rates among peripheral populations of *A. jeffersonianum* observed in my results may facilitate increased predation on smaller, later-hatching *A. maculatum*, although competition may instead increase among species both at the edge and across the geographic range due to ultimately smaller body sizes in *A. jeffersonianum* as climate change progresses, but faster metamorphosis in peripheral populations may decrease the likelihood of this happening. Faster growth can increase survivorship, especially in young larvae (Werner 1986), though the duration of larval periods are mediated by resource availability and/or habitat viability (Wilbur & Collins 1973; Semlitsch et al. 1988).

Variation in habitat and food availability can shape larval population size structure and survival, and food availability indirectly influences time to metamorphosis through its effects on size structure (Wilbur & Collins 1973; Shoop 1974; Urban 2007;
Levis et al. 2018). Smaller metamorph sizes in 2020 may reflect limited opportunities for invertebrate colonization due to recent construction of mesocosms prior to introduction of larvae, though I am unable to verify relationships between invertebrate prey densities and larval salamander body sizes. Limited food resource availability may partially explain the interaction between population and year on time to, and size at, metamorphosis due to potentially less invertebrate prey in 2020, which could subsequently reduce larval growth rates and increase time required to attain minimum body size thresholds for metamorphosis. However, longer larval periods and greater metamorph size in 2021 may have also been facilitated by earlier dates of larval introduction into mesocosms (Wilbur and Collins 1973; Shoop 1974). Typically, minimum body size thresholds must be met before undergoing metamorphosis (Wilbur and Collins 1973), but there were delays in time to metamorphosis in 2021, though metamorphs were larger than those in the previous year. Mesocosms in 2021 therefore may have contained relatively increased food resources, promoting larger body sizes that allowed larvae to remain in mesocosms until they reached maximum body sizes at which metamorphosis must be initiated (Wilbur and Collins 1973). Larger body sizes in 2021 may have facilitated increased survival due to increased size refugia from invertebrate predation or the ability to outcompete smaller individuals (Shoop 1974; Travis 1983), though predator and conspecific interactions were not recorded over the course of the study periods.

Climate change-induced reductions in metamorph body size may limit potential for dispersal among ponds (Sexton et al. 2009; Bredeweg et al. 2019), causing localized extirpation or increased isolation of peripheral populations as habitat suitability
decreases (Semlitsch et al. 1998). Trailing edge (i.e., low latitude) populations are predicted to face the greatest pressures from limited dispersal, as warmer temperatures drive populations poleward while simultaneously reducing body size, therefore limiting dispersal (Hampe and Petit 2005; Blackburn et al. 1999). Biotic constraints may also exist at trailing edges that are absent elsewhere in the range (Paquette & Hargreaves 2021), as trailing edge populations are more prone to experience habitat invasions as geographic ranges shift. Isolation will increase across the geographic range if future populations of *A. jeffersonianum* mirror smaller body sizes of current peripheral populations, although impacts of isolation from reduced body sizes may be less concerning for leading edge populations if high latitude core populations can buffer leading edge populations as geographic ranges shift poleward (Hampe & Petit 2005; Sexton et al. 2009; Nadeau & Urban 2019; Kottler et al. 2021), especially if high latitude populations exhibit larger body sizes than lower latitude populations. Latitudinal gradients in body size (e.g., Bergmann’s Rule) are not typical in amphibian taxa (Adams & Church 2007), yet my results indicate trailing edge populations of *A. jeffersonianum* are smaller at metamorphosis than core populations even under current climatic conditions, though individuals may compensate for reduced sizes at metamorphosis with increased growth as juveniles (Bredeweg et al. 2019). Further studies using high latitude populations may determine if such body size gradients exist, though average metamorphic snout-vent lengths I observed in trailing edge populations are smaller than either late-stage larvae or metamorphic snout-vent lengths in New York (Bishop 1941, 1943), and Pennsylvania (Mott 2004). Warmer low-latitude temperatures may indirectly explain size differences, as reduced hydroperiods driven by increased
temperatures may select for reduced body sizes in southern peripheral habitats of *A. jeffersonianum*.

The abundant center hypothesis identifies isolation as a key characteristic of peripheral populations, yet peripheral habitats also tend towards increased temporal and spatial variability (Hampe & Petit 2005; Lazaro-Nogal et al. 2015; Hernández-Pacheco et al. 2019). Due to their isolated nature, I hypothesized that peripheral populations would exhibit decreased plasticity relative to core populations, yet my results do not support my hypothesis or assumptions of the abundant center hypothesis, despite previous observations from peripheral populations indicating lower larval densities than core populations (C.L. Mott, unpublished data from same ponds used in this study). Biogeographical hypotheses assume that isolation and low abundances that characterize peripheral populations result in low genetic diversity and high genetic drift (Vucetich & Waite 2003), but isolated populations can harbor unique genetic assemblages (Lesica & Allendorf 1995; Hampe & Petit 2005; Provan & Maggs 2011) and may exhibit relatively high heterozygosity (Lesica & Allendorf 1992). If unique genetic variation occurs among isolated peripheral populations, it may result from relatively increased environmental stochasticity selecting for greater genotypic variation to buffer against change through local adaptation (Lesica & Allendorf 1992; Eckert et al. 2008; Savolainen et al. 2013; Hernández-Pacheco et al. 2019), or plasticity shaped by selection maintaining trait variation that perform best in temporally and/or spatially diverse habitats (Henn et al. 2018). However, my results indicate magnitude of plasticity in response to reduced hydroperiod is similar between core and peripheral populations of *A. jeffersonianum*, which suggests minimal genetic differentiation.
between populations, because genetic variability scales positively with plastic potential (Eckert et al. 2008). Assessments of genetic diversity across the geographic range would provide valuable insight to how genes mediate plastic responses of amphibians to climate change and may also lead to unexpected conclusions regarding their biogeographical and population genetic patterns. Similarities in the magnitude of plasticity between populations may also suggest: a) larval habitats of *A. jeffersonianum* are not variable enough to produce strong shifts in plasticity in either peripheral or core regions (Reed et al. 2011); b) peripheral and core populations used in this study may not be distant enough from one another to reflect differences in plastic potential across greater spatial scales (Broitman et al. 2021); and/or c) traits used to identify plastic responses in this study may be less plastic than other traits associated with larval fitness (Villellas et al. 2021), such as tail fin depth (Van Buskirk 2002) and tail color (Caldwell 1982). However, the traits measured in my study are most commonly used in studying plasticity as it relates to hydroperiod. Although salamander, zooplankton, and macroinvertebrate densities scaled latitudinally within natural ponds study populations were collected from (C.L. Mott, unpublished), hydroperiod may be influenced more at local landscape levels such as soil permeability than latitudinal position (Grillas et al. 2021), and such local landscape-level variation can influence population-level processes even at small scales (Arietta & Skelly 2021). While core populations used in this study are centrally located within a large subsection of the range (~24% of the total range; AmphibiaWeb 2022; Figure 1), they lie further west than the centroid of the *A. jeffersonianum* geographic range, and based on their relative position, my “core populations” may behave more like, and are likely exposed to more similar habitats as
peripheral populations than their counterparts in the range centroid in the northeastern United States (Gibson et al. 2009; Paquette & Hargreaves 2021). Simultaneous exposure of trailing edge populations to extreme stochastic weather events and residing in consistently warmer climates may reduce their plasticity, whereas poleward populations may exhibit increased plastic responses because more temperate climates may promote greater plastic capabilities (Sexton et al. 2009; Burggren 2018; Barley et al. 2021; Broitman et al. 2021).

Some evolutionary processes may occur too slowly to track anthropogenic climate change; thus, selection will favor traits with the greatest propensity to adapt across environments, and concern will lie in how climatic variables may shape plasticity (Henn et al. 2018). Greater temperature variability during climate warming may facilitate stronger plastic responses (Barley et al. 2021), but only if populations have previously experienced such stochasticity (Reed et al. 2011; Burggren 2018). The trade-off hypothesis predicts that as populations approach physiological limits of tolerance to environmental stressors, plasticity can decrease due to energetic costs, thus enforcing trade-offs between plasticity and physiological limits (Barley et al. 2021). However, the critical thermal maxima of *A. jeffersonianum* (~34 °C) is well above recorded average temperatures of the 2020 (28.45°C ± 0.42) and 2021 (25.11 °C ± 0.16) study periods (Gatz 1971), suggesting the trade-off hypothesis may not explain increased plasticity in metamorph length in the cooler 2021 study period. Interestingly, an inverse relationship between plasticity of metamorph length and plasticity of larval girth growth was observed across years. The causes of this relationship are ambiguous, though it may indicate trade-offs between plasticity of separate traits; Van Buskirk (2002) observed
that body girth and length are negatively correlated, but it is unclear if plasticity of these traits are similarly related, and the potential for trade-offs in plasticity between or among separate traits has not been explored to my knowledge. Directional differences in trait plasticity in my study may be due to use of different peripheral populations between experimental periods, and differences in trait plasticity may therefore simply represent natural variation among populations. Future studies should explore the potential for shifting selection of trait plasticity over time with increasing environmental stochasticity (Burggren 2018).

Amphibians exhibit plasticity across ontogeny, with embryonic plasticity permitting embryos to evade predation and reduced hydroperiods (Ferrari & Chivers 2009; Warkentin 2011; Delia et al. 2019; Ferrari et al. 2019), and adult plasticity largely concerning reproductive success (Touchon & Warkentin 2008; Becker et al. 2018). Plastic responses exhibited early in ontogeny may result in trade-offs affecting later life history stages (i.e., carry-over effects), such as decreased larval body size, feeding capabilities, and swimming capacity resulting from embryonic plasticity leading to earlier hatching (Delia et al. 2019), or reduced dispersal capabilities and smaller body sizes in adults resulting from larval plasticity leading to earlier metamorphosis (Bredeweg et al. 2019). My results indicate such trade-offs of adaptive plasticity may occur as carry-over effects in *A. jeffersonianum* populations experiencing the pressures of climate change. Responses may become fixed if individuals are exposed to stressors first as embryos, preventing larvae from adaptive responses to potential predation or other stressors later in life (i.e., behavioral mismatches; Ferrari et al. 2019). Mismatches will likely be exacerbated by climate change as species shift geographic ranges to track
suitable habitat, which may expose embryos to novel predators, thereby increasing the
difficulty of distinguishing predator cues (Ferrari et al. 2019). Adult plasticity may
reduce effects of some stressors for offspring, as some adults shift egg oviposition
location or cease oviposition altogether based on desiccation risk to egg masses
(Touchon & Warkentin 2008; Takahashi & McPhee 2016), or increase reproduction in
high rainfall events with the cost of increased adult mortality, with reduced
reproduction and decreased mortality during dry periods (Becker et al. 2018). While my
results indicate metamorphs exhibit decreases in body size in response to reduced
hydroperiods, and individuals exposed to reduced hydroperiods may compensate for
these smaller sizes at metamorphosis with faster post-metamorphic growth (Bredeweg
et al. 2019), it is uncertain how carry-over effects or trade-offs may affect larvae or
embryos simultaneously exposed to future climate scenarios and predation, or if cues
for separate stressors interfere with one another.

Both plasticity and local adaptation can mediate adaptive potential to climate
change (Mägi et al. 2011; Lazaro-Nogal et al. 2015; Hargreaves & Eckert 2019), yet
plasticity typically permits more flexible responses. For example, plasticity can create
niche shifts within populations to reduce competition for food resources (Levis et al.
2017), and maintain fitness when experiencing limited resource availability in variable
habitats (Lazaro-Nogal et al. 2015). Local adaptation typically facilitates adaptation to
current environments, though it may hinder populations experiencing conditions
straying from habitat norms (Hargreaves and Eckert 2019). In my study, plasticity
would promote faster metamorphosis to evade decreasing habitat suitability, whereas
responses shaped by local adaptation would be fixed in traits between populations,
treatments, and years. Faster metamorphosis occurred in my experiments under conditions of reduced hydroperiods, yet fixed (i.e., not plastic) differences in size at, and time to, metamorphosis between core and peripheral populations regardless of climatic scenario suggest these population groups are locally adapted. However, substantial habitat variability may permit locally adapted populations to respond with greater plasticity to environmental pressures such as climate change (Lozaro-Nogal et al. 2015). While habitat variation can promote increased plasticity, climate change may drive environmental stochasticity beyond the limits of adaptive plastic responses, thus requiring conservation and management plans to maintain phenotypic variation (Watters et al. 2003).

It is unknown whether phenotypic plasticity will sufficiently mitigate climate change effects, though my results do provide limited evidence for how populations associated with specific range positions may respond. While the current magnitude of plasticity may be adaptive under future climatic conditions, smaller size at metamorphosis could negatively impact later life stages by limiting dispersal potential and contribute to population isolation, even though smaller individuals can exhibit greater post-metamorphic growth to compensate for plasticity-induced reductions in body size (Bredeweg et al. 2019). Constrained migratory potential may be exacerbated by decreases in habitat availability as wetland hydroperiods are truncated with increasing rainfall stochasticity (Brooks 2004). Therefore, conserving plasticity in populations will require management for increased aquatic habitat diversity (Watters et al. 2003), while also ensuring that such variation does not become unpredictable (Chevin & Lande 2011). Conservation efforts must consider management of multiple
variable habitats with high connectivity to promote genetic diversity and concomitantly wider phenotypic breadth (Hoffmann & Sgro 2011; Reed et al. 2011; Nadeau & Urban 2019). For larval amphibians, managing multiple aquatic habitats in this manner may promote selection for greater phenotype diversity (Semlitsch 2000). Future studies should continue to assess relationships between climate change and ecological interactions, and determine if interactions result in phenotypic mismatches or shifts in the intensity and/or direction of phenotypic plasticity (Parmesan 2006; Charmantier et al. 2008; O’Regan et al. 2014; Henn et al. 2018; Riddell et al. 2019; Arietta & Skelly 2021). Such approaches may provide more realistic perspectives of how plastic responses may be shaped within natural systems, as many biotic variables can alter body size, time to metamorphosis, survival, growth, and their plasticity in similar manners to reduced hydroperiods (Scott 1994; Van Buskirk 2002; Bond et al. 2021).
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