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Effects of temperature on embryonic and early larval growth and development in the rough-skinned newt (*Taricha granulosa*)

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ABSTRACT

We investigated the effects of temperature on the growth and development of embryonic and early larval stages of a western North American amphibian, the rough-skinned newt (*Taricha granulosa*). We assigned newt eggs to different temperatures (7, 14, or 21 °C); after hatching, we re-assigned the newt larvae into the three different temperatures. Over the course of three to four weeks, we measured total length and developmental stage of the larvae. Our results indicated a strong positive relationship over time between temperature and both length and developmental stage. Importantly, individuals assigned to cooler embryonic temperatures did not achieve the larval sizes of individuals from the warmer embryonic treatments, regardless of larval temperature. Our investigation of growth and development at different temperatures demonstrates carry-over effects and provides a more comprehensive understanding of how organisms respond to temperature changes during early development.

1. Introduction

Temperature is among the most indelible and long-studied factors influencing growth and development in animals (Angilletta et al., 2004; Gillooly et al., 2002; Huey and Stevenson, 1979; Lillie and Knowlton, 1897; Zuo et al., 2012). Temperature can play especially important roles at early life-history stages, where it is known to profoundly influence embryonic and larval growth and development (Brown et al., 1992; Howe, 1967; Pepin, 1991), and have important fitness consequences for later life (Blanckenhorn, 2000; Chamaille-Jammes et al., 2006; Huey and Berrigan, 2001).

One of the ways that temperature can influence organisms throughout their lives is through carry-over effects, where the effects of temperature experienced at one life-history stage pass to the next discrete life stage. Carry-over effects of embryonic temperature have been observed in a wide variety of taxa (reviewed by Hopkins et al. (2014)), ranging from arthropods (Ernsting and Isaaks, 1997; Geister et al., 2009; Giménez, 2006) and tunicates (Thiyagarajan and Qian, 2003) to fish (Johnston et al., 1998; Martell et al., 2005, 2006) and reptiles (Brooks et al., 1991; Elphick and Shine, 1998; O'Steen, 1998).

Amphibians are excellent models for studying the effects of temperature at, and across, early development. Temperature has long been known to affect rates of embryonic and larval growth in these animals (Moore, 1939; Wilbur and Collins, 1973; Harkey and Semlitsch, 1988; Newman, 1989; Smith-Gill and Berven, 1979; Álvarez and Nicieza, 2002), and the discrete life stages of amphibians make them attractive models for the study of carry-over effects. Carry-over effects have been found in amphibians exposed to ultraviolet radiation (Belden and Blaustein, 2002; Pahkala et al., 2001), salinity (Wu et al., 2012; Hopkins et al., 2014), and acidic conditions (Räsänen et al., 2002), but the carry-over effects of temperature across early life-history stages are still largely unknown. Given that the world is undergoing unprecedented anthropogenic change (Steffen et al., 2007), including global climate change (IPCC, 2014), and amphibians are known to be particularly sensitive to changes in their environments (Hopkins, 2007), investigating the implications of temperature shifts on the development and growth of amphibians at and across early life-history stages is crucial for conservation efforts (Walther et al., 2002).

We investigated the effects of temperature on embryonic and early larval growth and development in the rough-skinned newt (*Taricha granulosa* Skilton; Caudata: Salamandridae), a common

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amphibian inhabiting ponds and streams along the west coast of North America. We predicted that increasing temperatures would result in more rapid growth and development, and that the temperature at which embryos developed would affect growth and development after hatching, providing evidence for carry-over effects.

2. Materials and methods

2.1. Animal collection, housing, and egg deposition

Gravid *T. granulosa* females (16) were collected from Hunter Creek, Curry County, Oregon (42°22′07.30″N, 124°24′16.64″W) by dip net, minnow trap, or by hand in May 2013. Animals were housed individually at Utah State University in plastic containers filled with 200 ml filtered water. The containers were kept at 14 °C and newts were fed blackworms (*Lumbriculus* spp.) ad libitum.

Females were injected with 10 μl luteinizing hormone releasing hormone ([des-Gly10, D-His(Bzl)6]-LHRH ethylamide; Sigma #L2761, Sigma-Aldrich, St. Louis, MO, USA) to induce oviposition onto pieces of polyester fiber. Eggs were collected within 12 h of deposition and placed in different cups with 200 ml of filtered water that were designated to one of three environmental control chambers (7 °C, 14 °C, or 21 °C) using an equal-probability method to ensure that each female's offspring were represented equally in all treatments. The temperature treatments chosen for this study reflect the natural variation the animals experience at the site from which they were collected (Hopkins, unpublished data). Generally, water temperatures are cooler toward the beginning of the breeding season in spring and warmer throughout the summer as the offspring grow and develop. However, there is considerable variation even within a small reach of the sample stream driven by water depth, microhabitat complexity, and weather patterns. Eggs and larvae of *T. granulosa* can be subject to the temperatures chosen for this study in the wild, depending on local conditions.

2.2. Temperature treatments and measuring growth and development

For each individual egg, time (in days) from oviposition to hatching was recorded, and any egg that failed to hatch was removed from the experiment. Once hatched and free-swimming, the larvae were placed in individual cups with 200 ml of filtered water. At least 60 larvae from each embryonic temperature treatment were re-designated to larval treatments (7 °C, 14 °C, or 21 °C), again using an equal-probability method so that any effect of the female would be balanced across treatments. The combination of embryonic and larval temperatures created a total of nine treatments with at least 20 individuals (and a maximum of 26) in each treatment and a total of 200 larvae. Specifically, $N_{7,7\ (\text{embryonic temperature, larval temperature})}=24$, $N_{7,14}=20$, $N_{7,21}=22$, $N_{14,7}=23$, $N_{14,14}=22$, $N_{14,21}=22$, $N_{21,7}=26$, $N_{21,14}=20$, and $N_{21,21}=22$.

Each larva was measured and staged immediately after hatching and then weekly for four weeks, using a stereo-microscope (Olympus Corporation, Shinjuku, Tokyo, Japan). Total length was recorded using an ocular micrometer. Larvae were staged following the standard salamander early life-history developmental staging protocol of Harrison (1969). Once the larvae grew beyond the Harrison staging protocol, the Watson and Russell (2000) larval staging scheme was utilized. However, the numbering system for Harrison (1969) was continued instead of using the Watson and Russell (2000) numbering system. The equivalent of stage 7 in Watson and Russell's (2000) system was stage 46 in Harrison's protocol (1969), so stage 8 according to Watson and Russell's (2000) system was considered to be stage 47 in this study.

Although larvae open their mouths at stage 44, they retain residual yolk until stage 46 (Harrison, 1969). Supplemental feeding (which could confound the effects of temperature alone on growth and development) was thus unnecessary for the time frame of this study, which continued for four weeks if the group mean did not reach stage 46. Animals were exempt from further analysis if the mean developmental stage for the treatment group reached stage 46. Larvae were euthanized in 5% MS-222 at the conclusion of the experiment.

2.3. Statistical analyses

The effect of temperature on time to hatching was assessed using Friedman's Test (blocking on individual female) due to a lack of normality in these data. Where an overall significant difference was found, we compared the effects of different treatments using Dunn's Multiple Comparisons. The effect of embryonic temperature on length and developmental stage at hatching was assessed using two-way ANOVAs with female incorporated as a random effect, and post-hoc Tukey-adjusted multiple comparisons. Because we examined length and development of all larvae for three weeks post-hatching, and larvae at 7 and 14 °C for four weeks post-hatching, we ran two separate analyses: the first analyzing the effects of embryonic temperature, larval temperature, time, and their interactions on larval growth (total length) and development (developmental stage) for all three larval temperatures for three weeks post-hatching, and the second analyzing these effects for 7 and 14 °C for four weeks post-hatching. Both models were mixed model two-way factorial incomplete random block designs with repeated measures modeled using a first-order autoregressive structure. Individual female was treated as a random block effect, and within each female values for multiple larvae in each treatment combination were averaged and the means were subsequently used as response data. Depending on fecundity, each female contributed 0–5 offspring to each treatment, with a mean of 1.62. Where an overall significant effect of embryonic temperature was found, we conducted post-hoc comparisons using the "simulate" adjustment to determine differences in embryonic treatment at each week at each larval treatment (Edwards and Berry, 1987). Statistical analyses were completed in SAS v9.3 (Cary, North Carolina, USA), and significance was set at $\alpha=0.05$.

3. Results

3.1. Effect of embryonic temperature on hatching timing, body length and developmental stage at hatching

There was a significant effect of embryonic temperature on time to hatching (Friedman's $\chi^2=161.67$, $p<0.001$) (Fig. 1A). Eggs reared at 21 °C took the shortest time to hatch (mean ± SE = 15.41 ± 0.18 days), followed by eggs reared at 14 °C (29.82 ± 0.29 days) and 7 °C (109.3 ± 1.18 days) (Fig. 1A). There was a significant effect of embryonic temperature on body length ($F_{2,182}=4.74$, $p<0.01$), and developmental stage ($F_{2,182}=24.37$, $p<0.001$) at hatching, with larvae hatching significantly larger at 7 °C than at 14 °C (Tukey-adjusted multiple comparison, $p<0.01$) (Fig. 1B), and more developed at 21 °C than at 7 °C or 14 °C (Tukey-adjusted multiple comparisons, $p<0.001$) (Fig. 1C).

3.2. Effects of temperature on larval growth and development

There was a significant effect of embryonic temperature, larval temperature, time, and their interactions on larval newt growth