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Effects of Temperature on Growth, Development and Survival in Larvae of *Pelophylax ridibundus* (Pallas, 1771) (Amphibia: Anura): Linking Global Warming to Amphibian Development

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Abstract: Amphibian life histories are exceedingly sensitive to temperature and there are good evidences that recent climate change may influence their growth, development and survival. The effects of water temperature on growth, age and size at metamorphosis and survival were studied in larvae of marsh frog *Pelophylax ridibundus*. Exposure of larval *P. ridibundus* to two different water temperatures (18 and 21°C) during an experiment of 22 weeks of showed that larvae grown at high temperature, exhibited an earlier metamorphosis and the greatest snout to vent length (SVL). Also, our data showed that in larvae grown at relatively low temperature, the metamorphosis time was longer and the body size (SVL) was larger compared with individuals metamorphosed at relatively high temperature. Although the results of the present study showed that the temperature had significant effects on SVL ($p<0.0001$), size ($p<0.001$) and time ($p<0.001$) to metamorphosis, no effect of temperature was detected on survival rate ($p<0.88$).

Key words: *Pelophylax ridibundus,* global warming, development, metamorphosis, survival

Introduction

Declining of global amphibian populations is well documented (e.g. Wake 1991, Stuart et al. 2008). Various factors are known to cause such declines. These include habitat loss and modification, introductions of non-indigenous species, over-harvesting, UV-B radiation, chemical contaminants, emerging infectious diseases, local and international trade and climate change (Hayes et al. 2010). In the 1970s, environmental biologists and atmospheric scientists predicted that two significant human-induced environmental changes, global warming and ozone depletion, could potentially affect the biology of a wide array of plants, animals and microorganisms (Blaustein et al. 2010). Scientists projected that species might respond to these global changes by altering their behaviour and by shifting their ranges. However, if they are unable to adapt to these environmental changes, they may experience increased mortality and significant sublethal effects. Additionally, a number of scientists suggested that global warming and ozone depletion will affect entire ecological communities (Cockell & Blaustein 2001). It is now undoubtedly clear that the Earth's climate is changing in response to anthropogenic greenhouse-gas emissions. The recent report of the Intergovernmental Panel on Climate Change confirmed a global mean warming of 0.6 °C during the 20th century and predicted a mean global temperature increase between 1.4 and 4.8 °C between 1990 and 2100 (IPCC 2007).

Temperature affects development and growth of all living organisms. Populations of ectotherm animals have a stronger dependence on temperature because they do not have an efficient mechanism for physiological thermoregulation (Brattstrom 1963). Many researchers have shown a strong relationship between growth rate and temperature (Castañeda et al. 2006, Bancroft et al. 2008, Gonçalves et al. 2012). Higher temperatures are well known to accelerate larval growth and development (Hayes et al. 1993, Álvarez & Nicieza 2002). Laboratory studies have shown a pattern in which tadpoles develop and grow more slowly but undergo metamorphosis with a larger body size at low temperatures (Smith–Gill & Berven 1979, Hayes et al. 1993; Álvarez & Nicieza 2002, Sanuy et al. 2008). Moreover, the effects of temperature on growth and development may interact with other factors, such as food availability (Álvarez & Nicieza 2002, Vaissi & Sharifi 2016) and hydroperiod (Loman 2002). Survival of larvae is also influenced by temperature in a complex way. For example, with increasing temperature survival rates of larvae of the Pacific tree frog (*Pseudacris regilla* *Baird & Girard*, 1852) at low density is high, but at high density, is low (Govindarajulu & Anholt 2006).

Due to high aridity, relatively few species of amphibians occur in Iran: there are reports of the presence of at least 21 species (Kami and Baluch 1994). *Pelophylax ridibundus* is reported from all over Iran except from the south-east and central deserts (Mohammadi et al. 2015). The main purposes of this study are to examine the influence of temperature on the (1) growth, (2) development and (3) survival of the larval stages of *P. ridibundus*.

Materials and Methods

Eggs from a single cohort of *P. ridibundus* were collected in Parvan (35º 58′ N, 49º 17′ E; 2320 masl), Ghazvin Province, Iran. In the laboratory they were placed in a chamber with controlled temperature and immediately after hatching larvae (Gosner Stage 22, N= 200) were exposed to two different water temperatures (18 and 21ºC) under a constant 12-hour light/12 hours dark photoperiod. Each treatment was replicated four times for a total of eight containers (26 cm height and 13 cm wide), each filled with 3L of dechlorinated tap water. Each container had 25 larvae. Larvae were fed with raw spinach: 3 g twice per week during the first month, 4 g three times per week during the second month and after the third month 4 g raw spinach and 4 g chicken gizzard four times per week until the end of the experiment. We monitored experimental containers daily and removed the bodies of larvae that had died. Containers were cleaned once a week during which time all tadpoles were removed. Photos were taken with a digital camera (SONY, DSC-HX9V, 3.6V) on a tripod at a fixed height (30 cm). The larvae were put in a petri dish which was located over latticed paper. Immediately after photos were taken the larvae were released into their containers. All pictures were analysed using AUTOCAD software 2014. We measured the snout to vent length (SVL, in mm). SVLs were calculated by drawing a line from the tip of the snout to the tip of the vent. Measurements were performed at days 7, 11, 14, 18, 21, 25, 28, 32, 35, 39, 42, 46, 49, 53, 56, 60, 68, 74, 83, 92, 101, 110, 118, 136, 157. After the experiment was completed (experimental time: 157 days), the surviving tadpoles were returned to the pond where they were collected.

Growth data for the SVL of *P. ridibundus* were fitted to the logistic equation as follows: Logistic equation = a / (1 + b × exp[-k×t] (, where a is the asymptotic value, b is the growth rate, exp is the exponent for natural logarithms, k is the growth coefficient (shape of the growth curve) and t is the time (age) in weeks. In order to define changes in growth patterns and trajectories, we adopted an early differential equation (Krebs, 1972; Sharifi and Vaissi, 2012) used as a description of logistic growth as follows: $dX/dt = rX (K - X) / K$, where X is the variable size at birth, t is the time, r is the early exponential growth rate for the variable larval period and K is the upper asymptote or maximal value of the variable X (SVL). The early exponential growth rate (r) was determined as the slope of a line fitted to the variable data in a graph in which the y-coordinate was $\ln ((K - X)/K)$ and the x-coordinate was time (Krebs 1972). Data were tested for homogeneity of variance and normal distribution with the Kolmogorov-Smirnov and the Levene tests, respectively. One-way ANOVA was used to examine the effects of temperature on growth, metamorphosis and survival of the test animals. Post-hoc comparisons (Tukey tests) were used to check for differences between means for the two treatments. The significance level was set at $\mu = 0.05$. All data are expressed as mean ± SD. The statistical program package SPSS (v. 16) was used for all analyses.

Results

The SVL at day 7 were approximately 3.25 ± 0.23 mm and 3.45±0.16 and had reached 23.21 ± 1.80 mm and 28.03±0.10 by the end of the larval period

Table 1. Summary statistics of analysis on the effects of temperature on snout-vent length, size at metamorphosis, time to metamorphosis and survival obtained for larvae of *Pelophylax ridibundus* at low and high temperature. SD: Standard Deviation.

Source of variation	Low temperature (Mean±SD)	High temperature (Mean±SD)	P-value
Snout to vent length (mm)	23.21±1.80	28.03±0.10	0.001
Size at metamorphosis (mm)	23.92±1.85	20.31±2.52	0.001
Time to metamorphosis (day)	162.32±11.82	113.54±18.54	0.001
Survival (%)	33±19.14	35±18	0.88

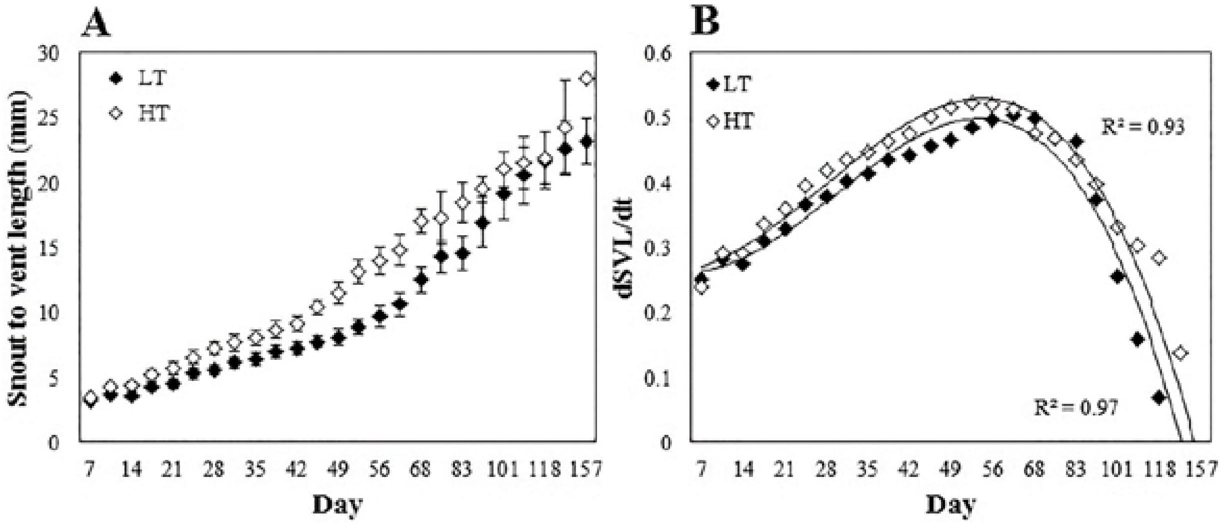

Fig. 1. Average and one standard deviation of the snout to vent length during growth from day 7 to day 157 (A) and the pattern of growth rate (B) (dSVL/dt) fitted to the growth values in *Pelophylax ridibundus* for the same period

in low temperature and high temperature, respectively (Fig. 1A). The growth rate pattern (Fig. 1B) for SVL was rapid during the first seven weeks but decreased rapidly at the end of the larval period. Larvae at high temperature exhibited greater mean growth rate for SVL as compared with those at low temperature. Different rearing temperatures caused a significant difference in size of larvae between temperature treatments ($p < 0.001$; Table 1). At the end of the experiment larvae reared at 21ºC were significantly larger than larvae reared at 18ºC, based on SVL (28.03±0.10 and 23.21±1.80, respectively; Fig. 1, Table 1).

Rearing temperature affected size of larvae of similar developmental age, with larvae reared at a lower temperature attaining larger size for developmental age than similarly developed siblings at higher temperatures (23.92±1.85 and 20.31±2.52, respectively). A lower rearing temperature also significantly increased the larval duration of *P. ridibundus*, slowing down developmental rate such that larvae reared at 18ºC required 25% more time to reach metamorphosis. Larvae reared at 21ºC metamorphosed at day 113.54±18.54, and those at 18ºC at day 162.33±11.82 ($p < 0.001$). Survival rate at both low and high temperature was low (33%±19.14 and 35 %±18, respectively). The results of our ANOVA also showed that temperature had not significant effect on survival rate ($p<0.88$; Table 1). A summary of growth parameters derived from the logistic model fitted to various growth parameters is given in Table 2. The coefficient of determination for logistic model

Table 2. Parameters (P) obtained from fitted logistic growth curve to the snout to vent length (SVL) of larval *Pelophylax ridibundus* in low and high temperature treatments. A: asymptotic SVL (mm); K: growth rate constant (d); R^2: correlation coefficient; SD: standard deviation.

Equation	P	Low temperature (SVL±SD)	High temperature (SVL±SD)
	K	0.03±0.001	0.03±0.002
Logistic	A	25.35±0.26	26.31±0.64
	R^2	0.99	0.98

fitted to SVL values in low and high treatments were 0.99 and 0.98 respectively (Table 2).

Discussion

As expected, results obtained from our experiment suggest that growth and development rates of *P. ridibundus* are influenced by temperature. Tadpoles metamorphosed at an older age and larger body size when reared at low temperature. In anuran populations, larger metamorphs may exhibit higher terrestrial survival since they can cope with different stressors in their terrestrial habitats, such as predators and desiccation (Reques & Tejedo 1997, Altwegg & Reyer 2003). This experiment also confirmed a general rule for ectotherms (Atkinson 1996): differentiation rates are more responsive to temperature than growth rates (Smith-Gill & Berven 1979). This impact of water temperature on metamorphic timing and body size at metamorphosis is similar to the temperature effects seen in other amphibian larvae. For example, larval anurans grown at cold temperatures take longer to develop but the metamorphs are also larger than conspecifics grown at warmer temperatures (Smith-Gill & Berven 1979, Voss 1993, Walsh et al. 2008).

This pattern of accelerated development has been observed in both anurans (Voss 1993, Álvarez & Nicieza 2002) and urodels (Beachy 1995, Hickerson 2005). Shorter larval periods can increase chances of survival in environments such as ephemeral ponds and streams by increasing the chance of successful emergence from a pond that is drying. For many species, however, a reduction in larval period also results in metamorphosis at a smaller size (Morand et al. 1977, Wilbur & Collins 1973, Werner 1986). This pattern suggests a likely trade-off between rate of development and growth, which might be exacerbated by an increase in temperature .

Although the present study shows that survival of larvae is not affected by high water temperature, significantly shortened metamorphosis period and larger body size at high temperature coupled with other threatening factors, such as habitat loss and degradation (Blaustein 2010), changes in the length and date of seasonal ice cover (Loman 2009), changes in water balance (Merilä et al. 2000, Maciel & Juncá 2009), altered nutrient cycling and productivity (IPPC 2007) and changes in stratification and eutrophication (IPPC 2007), are likely to result in significant loss of biodiversity in amphibians.

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Reference

Altwegg R. & Reyer H. U. 2003. Patterns of natural selection on size at metamorphosis in water frogs. Evolution 57: 872–882.

Álvarez, D. & Nicieza A. G. 2002. Effects of temperature and food quality on anuran larval growth and metamorphosis. Functional Ecology 16: 640-648.

Atkinson D. 1996. Ectotherm life-history responses to developmental temperature. In animals and temperature phenotypic and evolutionary adaptation. In: Johnston I. A. & Bennett A. F. (Eds.). Cambridge: Cambridge University Press, pp. 183–205.

Bancroft B. A., Baker N. J., Searle C. L., Garcia T. S. & Blaustein A. R. 2008. Larval amphibians seek warm temperatures and do not avoid harmful UVB radiation. Behaviour Ecology 19: 879-886.

Beachy C. K. 1995. Effects of larval growth history on metamorphosis in a stream-dwelling salamander (*Desmognathus ochrophaeus*). Journal of Herpetology 29: 375-382.

Berven K. A., Gill D. E. & Smith-Gill S. J. 1979. Countergradient selection in the Green Frog, *Rana clamitans*. Evolution 33: 609-623.

Blaustein A. R., Walls S. C., Bancroft B. A., Lawler J. J., Searle C. L. & Gervasi S. S. 2010. Direct and indirect effects of climate change on amphibian populations. *Diversity* 2: 281-313.

Brattstrom B. H. 1963. A preliminary review of thermal requirements of amphibians. Ecology 44: 238–255.

Castañeda L. E., Sabat P., Gonzalez S. P. & Nespolo R. F. 2006. Digestive plasticity in tadpoles of the Chilean giant frog (*Caudiverbera caudiverbera*): factorial effects of diet and temperature. Physiological and Biochemical Zoology 79: 919-926.

Cockell C. S. & Blaustein A. R. 2001. Ecosystems, Evolution and Ultraviolet Radiation; Springer: New York, NY, USA

Gonçalves R. V., Migowski P., Wender H., Eberhardt D., Weibel D. E., Sonaglio F. C., Zapata M. J. M., Dupont J., Feil A. F. & Teixeira S. R. 2012. Ta_2O_5 Nanotubes Obtained by Anodization: Effect of Thermal Treatment on the Photocatalytic Activity for Hydrogen Production. The Journal of Physical Chemistry C 116 (26): 14022–14030.

Govindarajulu P. P. & Anholt B. R. 2006. Interaction between biotic and abiotic factors determines tadpole survival rate under natural conditions. Ecoscience 13: 413-421.

Hayes T., Chan R. & Licht P. 1993. Interactions of temperature and steroid on larval growth, development, and metamorphosis in a toad (*Bufo boreas*). Journal of Experimental Zoology 299: 206–215.

Hayes T. B., Falso P., Gallipeau S. & Stice M. 2010. The cause of global amphibian declines: a developmental endocrinologist's perspective. Journal of Experimental Biology

213: 921-933.

Hickerson C. A. M., Barker E. L. & Beachy C. K. 2005. Determinants of metamorphic timing in the Black-bellied Salamander, *Desmognathus quadramaculatus*. Southeastern Naturalist 4: 33-50.

IPCC. 2007. Climate change 2007: the physical science basis. Geneva, Switzerland: Intergovernmental Panel on Climate Change.

Kami H. G. & Baluch M. 1994. Amphibians of Iran. 2nd Edn., Tehran University, Iran, ISBN: 964-03-353.

Loman J. 2002. Temperature genetic and hydroperiod effects on metamorphosis of brown frogs *Rana arvalis* and *R. temporaria*in the field. Journal of Zoology 258: 115-129.

Loman J. 2009. Primary and secondary phenology. Does it pay a frog to spawn early? Journal of Zoology 279: 64–70.

Maciel T. A. & Juncá F. A. 2009. Effects of temperature and volume of water on the growth and development of tadpoles of *Pleurodema diplolister* and *Rhinella granulosa* (Amphibia: Anura). Zoologia 26 (3): 413–418

Merilä J., Pahkala M. & Johanson U. 2000. Increased ultraviolet-B radiation, climate change and latitudinal adaptation-a frog perspective. *Annales Zoologici Fennici* 37: 129-134.

Mohammadi Z., Khajeh A., Ghorbani F. & Gholi Kami H. 2015. A biosystematic study of new records of the marsh frog *Pelophylax ridibundus* (Pallas, 1771) (Amphibia: Ranidae) from the southeast of Iran. Journal of Asia-Pacific Biodiversity 8: 178e182.

Morand A., Joly P. & Grolet O. 1997. Phenotypic variation in metamorphosis in five anuran species along a gradient of stream influence. Comptes Rendus de l'Académie des Sciences 320: 645-652.

Reques R. & Tejedo M. 1997. Reaction norms for metamorphic traits in Natterjack toads to larval density and pond duration. Journal of Evolutionary Biology 10: 829–851.

Sanuy D., Oromí N. & Galofré A. 2008. Effects of temperature on embryonic and larval development and growth in the Natterjack toad (*Bufo calamita*) in a semi–arid zone. Animal Biodiversity and Conservation, 31: 1.

Smith-Gill S. & Berven K. 1979. Predicting amphibian metamorphosis. The American Naturalist 113 (4): 563-585.

Stuart S., Hoffmann M., Chanson J., Cox N., Berridge R., Ramani P. & Young B. 2008. Threatened Amphibians of the World. 1st Edition.Lynx Edicions, IUCN, Barcelona, Spain; Gland, Switzerland; and Arlington, Virginia, USA.

Vaissi S. & Sharifi M. 2016. Changes in food availability mediate the effects of temperature on growth, metamorphosis and survival in endangered yellow spotted mountain newts: implications for captive breeding programs. *Biologia* 71 (4): 444-451.

Voss S. R. 1993. Relationship between stream order and length of larval period in the salamander Eurycea wilderae. *Copeia,* 1993: 736-742.

Wake D. 1991. Declining amphibian populations: A global phenomenon. Science 253: 860.

Walsh P. T, Downie J. R. & Monaghan P. 2008. Plasticity of the duration of metamorphosis in the African clawed toad. Journal of Zoology 274: 143-149.

Werner E. E. 1986. Amphibian metamorphosis: growth rate, predation risk, and the optimal size at transformation. The American Naturalist 128: 319–341.

Wilbur H. M. & Collin J. P. 1973. Ecological aspects of amphibian metamorphosis. Science 182: 1305–1314.

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