

## Interactive effects of temperature and food availability on the Marsh Frog (*Pelophylax ridibundus*) tadpoles in Western Iran

Alireza, Pesarakloo<sup>1\*</sup>; Nasrullah, Rastegar-Pouyani<sup>2</sup>; Eskandar, Rastegar-Pouyani<sup>3</sup>; Masoumeh, Najibzadeh<sup>4</sup>; Ahmad, Gharzi<sup>5</sup>; Alireza, Shayestehfar<sup>6</sup>

<sup>1,2,4,5</sup>Iranian Plateau Herpetology Research Group (IPHRG), Department of Biology, Faculty of Science, Razi University, 6714967346 Kermanshah, Iran

<sup>3</sup>Department of Biology, Faculty of Science, Hakim Sabzevari University, Sabzevar, Iran

<sup>6</sup>Department of Biology, Faculty of Science, Arak University, Arak, Iran

(Accepted November 25, 2015)

### ABSTRACT

Knowledge of how interactions between food availability and temperature influence larval growth, development and survivorship may also be of value to amphibian conservation. In the present study, to examine the effect of temperature and food availability on larval survival, growth and development, a 2 × 2 factorial design was used. The experiment involved two rearing temperatures (20 and 27°C) and two feeding regimens (constant and stochastic food availabilities). The effects of food availability and water temperature on tadpole size were determined by measuring individual body length and photographed by Stereo microscope equipped with a digital camera. Based on our results, larval growth rate was highest at the constant food and warmer temperature (27°C) treatment, whilst larval survivorship rate was highest at the constant food and cooler temperature (20°C). Furthermore, the longest metamorphosis time was observed on constant food and temperature (20°C) whilst the shortest metamorphosis time occurred on stochastic food and temperature (27°C). These results study strongly suggest that, environmental differences in food availability and water temperature and their interactions, cause differences in growth, development and survivorship of tadpoles in marsh frog.

**Key words:** Constant food; Development; Growth; Metamorphosis; Stochastic food; Survivorship.

### INTRODUCTION

Amphibians are affected by numerous environmental stresses that often act in complex ways (Blaustein & Kiesecker, 2002). Globally, amphibians are declining faster than any other vertebrate group (Stuart *et al.*, 2004). Populations of ectothermic animals have a strong dependence on ambient temperature because they do not have an efficient mechanism for physiological thermoregulation (Brattstrom, 1963). Changes in global temperature and precipitation may contribute to amphibian population declines; Alterations to temperature may result in mortality events. Changes in ambient temperature may influence amphibian behaviors, including those related to reproduction. Potentially, changes in this factor on a global scale could disrupt the timing of breeding, periods of hibernation, and the ability to find food (Donnelly & Crump, 1998; Blaustein *et al.*, 2001). Temperature affects amphibian larval developmental rates directly (Hayes *et al.*, 1993). Exposure to higher temperatures shortens the larval period in many species (Morand *et al.*, 1997). Survival and developmental rates of larvae are also influenced by temperature (Govindarajulu & Anholt 2006). Empirical studies suggest that changes in food availability have long-term consequences for various life-history traits due to a reduction in the amount of energy that can be allocated to

somatic growth (Yoneda & Wright, 2005; Inatsuchi *et al.*, 2010; Enriquez-Urzelai *et al.*, 2013). Knowledge of how interactions between food availability and temperature influence larval growth, development and survivorship may be of value to amphibian conservation. The Eurasian water frog (genus: *Pelophylax*) occurs widely from Central Europe, northwards to the Baltic Sea and southwards to the Mediterranean regions, eastwards to Asiatic Russia, and southwards to the Middle East (Frost, 2011), successive droughts threaten the Iranian water frog population. The Iranian basin is a large triangular depression flanked by the Elbourz Mountains in the north and the Zagros Range in the west. The Zagros Range extends diagonally from eastern Turkey to north of the Persian Gulf and Pakistan border. This range is part of a greater geographic unit arising from the east of the Anatolian Plateau of Turkey and extending southward to include Iran, Afghanistan, Pakistan, and further east to the western edge of the Tibetan Plateau. The Zagros Range acts as a barrier to incoming air masses from the west, and receives precipitation according to the elevation and longitude. In general, the northern and western portions of the range receive considerably more rainfall than areas in the south and east. Elbourz is a mountain range in northern Iran that stretches from the border of Azarbaijan along the western and entire southern coast of the Caspian Sea and finally runs northeast

\*Corresponding Author's E-mail: a.pesarakloo@gmail.com

and merges into the Aladagh mountain in the northern parts of Khorasan province. This mountain range is divided into Western, Central, and Eastern Elbourz Mountains; Mount Damavand, the highest mountain in Iran and the Middle east, is located in the Central Elbourz Mountains. The Elbourz Mountains acts as a barrier to incoming air masses from the north, while the southern slopes of the Elbourz Mountains are usually semiarid or arid with irregular and low precipitation, the northern slopes of the range are usually humid especially in the western parts of the Central Elbourz. In the southern slopes or the Elburz range forest steppe ecoregion, the higher elevations are arid with few trees. Furthermore, Central portion of Iran is dry (minimum precipitation in year), and *Pelophylax ridibundus* cannot observe in these parts. Two-thirds of Iran is located in the arid and semi-arid Iranian Plateau while suitable breeding habitats for this frog are temporary in Iran. Average annual global temperatures have risen 0.7 °C over the last century (Blaustein *et al.*, 2010). With this condition, understanding how environmental factors interact may assist in amphibian conservation by improving the number of tadpoles generated in captive breeding programs. The aim of the present study was to investigate the independent and interactive effects of long-term exposure to stochastic food availability and water temperature on larval survivorship, growth and development of *Pelophylax ridibundus*.

## MATERIALS AND METHODS

Four egg clutches of *Pelophylax ridibundus* were collected from 22 to 23 March 2015 from a breeding site in the Aligudarz region of south-western Iran (33°24'34"N 49°45'42"E, Elevation: 2196m). Clutches were collected by hand and stored in separate polyethylene tubs (600 mm × 350 mm × 250 mm) filled with water from the natural habitat and transported to the Iranian Plateau Herpetology Research Group (IPHRG), Razi University, Kermanshah, Iran (33°37'N 45°20'E, Elevation: 1330m). Clutches were maintained in these tubs in natural light conditions at  $\sim 25 \pm 2^\circ\text{C}$  for a 10 day acclimation period. This period was imposed to ensure that tadpoles were viable before being entered into the experiment. To ensure no build-up of nitrogenous waste in tubs during the acclimation period, one-third of the water was replaced every fifth day, resulting in two water changes during the acclimation period (Stephanie *et al.*, 2015). Tadpoles hatched from eggs 1–2 days after collection, and once tadpoles had hatched the egg jelly was removed from the tubs. Tadpoles were fasted during this acclimation period and were provided with food only at the time when they were entered into experimental treatments; Tadpoles were fed with boiled spinach, lettuce and potato (Pesarakloo *et al.*, 2008). Upon entry into the experimental treatments, ( $n = 300$ ; split between four rearing tanks) the tadpoles at various times were measured (body length) by a digital caliper and photographed by a Stereo microscope equipped with a digital camera. To examine the effect of temperature and food availability on larval survival, growth and development, a  $2 \times 2$  factorial design was used. The experiment involved two rearing temperatures (20 and 27°C) and two feeding regimens

(constant and stochastic food availabilities), resulting in four experimental treatments referred to as follows: (i) constant 20°C; (ii) constant 27°C; (iii) stochastic 20°C; and (iv) stochastic 27°C. A split-clutch design was used with tadpoles from each clutch being randomly allocated to an experimental treatment (i.e. 300 tadpoles per each treatment, total: 1200 tadpoles). The experimental period lasted 15 weeks, because the larval period in populations of *P. ridibundus* in Lorestan province typically lasts a maximum 105 days (Najibzadeh, 2010), and during this time the tadpoles were monitored daily. The experiment was commenced on 3 April 2015 and terminated on 16 July 2015. During the experimental period, tadpoles were housed in plastic rearing tanks (300 mm × 200 mm × 110 mm), and an aquarium water heater (50W) was placed in the plastic rearing tanks to set the experimental treatment temperature; Each plastic rearing tank had 50L water. These water volume adjustments were carried out on a weekly basis, and partial water changes ( $\sim 30\%$ ) were made once per week. Experimental samples were kept in a temperature and light-controlled room maintained at  $18 \pm 2^\circ\text{C}$  ambient temperature and a 12 h–12 h light–dark period. The two water temperature treatments (20 and 27°C) in which tadpoles were reared were selected because our observation had shown that, they reflected the average lower and upper estimates of temperatures that *P. ridibundus* tadpoles experience in Aligudarz systems during the period between March and July. To ensure that temperatures were maintained at treatment temperatures throughout the entire experimental period, water temperatures were monitored on a weekly basis using a calibrated digital thermometer probe. Animals were exposed to one of two feeding regimens: constant food availability or stochastic food availability. Constant food availability treatments supplied food (i.e. no food restrictions applied) throughout the entire experimental period. The stochastic food availability treatment had randomly allocated fasting periods of up to 3 days during which no fresh food was provided. Food consisted of a mixture of boiled spinach, lettuce and potato.

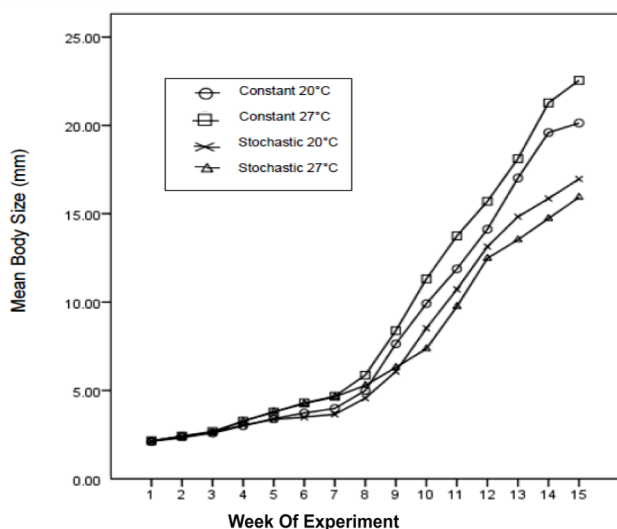
## RESULTS

The effects of food availability and water temperature on tadpole size were determined by measuring individual body length (in millimeters) and photographed by Stereo microscope with digital camera. Our observations showed that there are significant differences in body size over the 15 week experimental period between experimental groups. Comparisons were made on a weekly basis (weeks 0–3, 4–7, 8–11 and 12–15) to examine the additive and interactive effects of food availability and water temperature on tadpole size. Tadpole size was measured as body length and concludes mean size in each period (Table 1, Figure 1) During 0–3 weeks, there were almost no significant differences in baseline body size in all treatment groups. In weeks 4–7, body size was largest in tadpoles from treatments with warmer water temperatures, regardless of whether food availability was constant or stochastic. During the weeks of 8–11, Body size was largest in tadpoles from treatments with water temperatures (20 and 27°C) and constant food availability than water temperatures (20 and 27°C) and stochastic

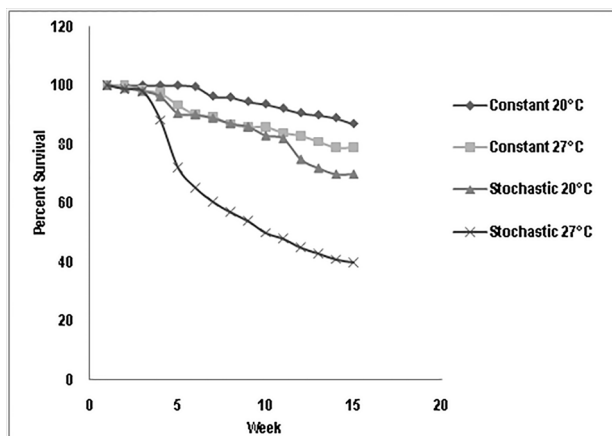
**Table 1.** Effects of food availability and water temperature on tadpole size MSW: Mean size in weeks.

Food avail-ability	Temperature°C	MSW(0–3) (mm±SD)	MSW(4–7) (mm±SD)	MSW(8–11) (mm±SD)	MSW(12–15) (mm±SD)
Constant	20	2.36±0.21	3.52±0.38	8.60±2.67	17.71±2.51
	27	2.41±0.23	4.00±0.56	9.82±3.12	19.4±2.82
Stochastic	20	2.35±0.22	3.38±0.25	7.46±2.48	15.19±1.50
	27	2.39±0.21	3.98±0.55	7.17±1.78	14.16±1.37

food availability. In weeks 12–15, body size was largest in tadpoles from treatments with food availability constant 20°C (Table1, Figure 1). Over the experimental period, tadpoles from constant food availability treatments had the highest number of metamorphoses. There were significant differences in survival rate between water temperature treatments, with higher survivorship in the 20°C treatment than in the 27°C treatments. On the other hand, survivorship was lowest in the warmer stochastic food treatments (27°C) and highest in the cooler constant food treatments (20°C). Survivorship was intermediate in the constant 27°C and stochastic 20°C treatments (Tables 2& 3, Figures 2& 3).



**Figure 1.** Effects of food availability and water temperature on tadpole size: Mean size in weeks .



**Figure 2.** Effects of food availability and water temperature on tadpole survivorship, over a period of 15 weeks (through in time) in the four experimental treatments.

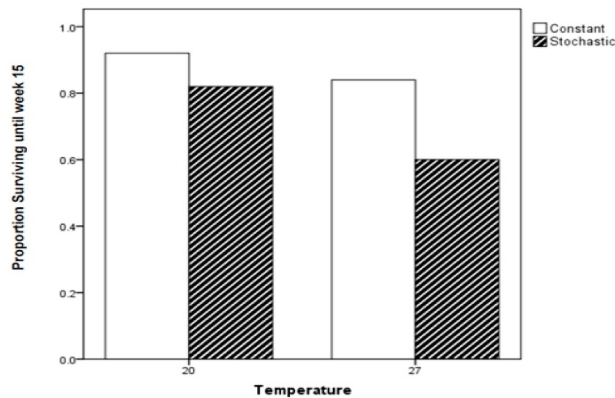
Generally, 7.5% of tadpoles (90 of 1200) of all treatment groups reached metamorphosis and completed it. Over the 15 week experimental period only 45 individuals of constant food availability and temperature 20°C, 30 of constant food availability and temperature 27°C, 12 individuals of stochastic food availability and temperature 20°C and 3 of stochastic food availability and temperature 27°C successfully metamorphosed (Table 4).

**Table 2.** Effects of food availability and water temperature on tadpole survivorship (Percentage of tadpoles surviving to week 15).

Treatment	Food avail-ability	Tempera- ture (°C)	Sample size	Survival at week 15(%)
Constant	Constant	20	300	87
		27	300	79
Stochastic	Stochastic	20	300	70
		27	300	40

**Table 3.** Effect of food availability and water temperature on percentage of tadpoles surviving mean at experimental period in the marsh frog (*P. ridibundus*). Values is means ± SD.

Food availability	Tempera- ture (°C)	Sample size	Survival mean at experimental period (%)
Constant	20	300	95.26±4.63
	27	300	88.94±7.42
Stochastic	20	300	85.84±10.39
	27	300	64.06±22.09



**Figure 3.** Effect of food availability and water temperature on proportion marsh frog (*P. ridibundus*) tadpoles surviving to week 15. Stochastic food availability treatments are represented by dark grey bars and constant food availability treatments by light grey bars, Values are shown as Means.

**Table 4.** Effects of food availability and water temperature on tadpoles' development

Treatment			
Food availability	Temperature (°C)	Percent of metamorphosis	Metamorphosis time (days)
Constant	20	15	103
	27	10	90
Stochastic	20	4	89
	27	1	70

## DISCUSSION

Changes in climate may push some individuals or populations past these thresholds, resulting in mortality or sublethal effects such as reduced growth. In amphibians, temperature acts as a controlling factor for many physiological processes, including rates of oxygen uptake, heart rate (beat), locomotion, water balance, digestion, developmental rate, sex determination, and immune function (Rome *et al.*, 1992). Changes in ambient temperature may influence amphibian behaviors, including those related to reproduction. Potentially, changes in ambient temperature on a global scale could disrupt the timing of breeding, periods of hibernation, and the ability to find food (Donnelly & Crump, 1998; Blaustein *et al.*, 2001). The aim of this study was to investigate the independent and interactive effects of food availability and water temperature on larval growth, development and survival in the Marsh frog, *Pelophylax ridibundus*. Variation in food availability was found to impact larval size and development, with smaller larval size and slower developmental rates in stochastic food availability treatments. Furthermore, survival and developmental rates of larvae are also influenced by temperature. The effects of temperature on survival of larvae are likely to be complex. For example, survival of Pacific tree frog (*Pseudacris regilla*) larvae under high temperatures is dependent on tadpole density. When tadpole density is low, higher temperatures increase survival rates (Govindarajulu & Anholt, 2006). However, when tadpole density is high, higher temperatures result in reduced survival (Govindarajulu & Anholt, 2006). Survival of larvae can influence population dynamics to a larger degree than embryonic survival (Vonesh & De la Cruz, 2002). The effects of increasing temperature on larval amphibians may include a reduction in time to metamorphosis, a decrease in size at metamorphosis, or both. Moreover, the effects of temperature on development may interact with other factors such as food availability (Álvarez & Nicieza, 2002). According to the temperature-size rule (Kozłowski *et al.*, 2004), growth rate is expected to increase with increasing water temperature because temperature regulates metabolism, growth and differentiation in ectothermic species (Álvarez & Nicieza, 2002; McLeod *et al.*, 2013). According to our observations *Pelophylax ridibundus* display increased growth with increased water temperature in constant food availability conditions. A previous study examining the effects of long-term changes to food availability and water temperature on coral fish species also reported low survivorship in warmer waters and

suggested that survival may have been compromised due to starvation. Consequently, when exposed to high food availability conditions, coral fish survivorship increased (McLeod *et al.*, 2013). In present study, there were significant differences in body size over the 15 weeks experimental period between treatment groups. Larval growth rate was highest at the constant food and warmer temperature (27°C) treatment, while survivorship was highest, food constant, and cooler temperature (20°C). In this experiment, it was determined that food availability was slightly more important than water temperature for survivorship, growth and development. Stochastic food availability would decrease larval survivorship, growth and development. The reported effects of food availability on larval growth support the predictions of the 'general optimization model' (Day & Rowe, 2002), which predicts slower growth in stochastic food availability conditions. In a study investigating the effects of water temperature and food quality on growth and development in Iberian painted frogs (*Discoglossus galganoi*), it was found that water temperature had persistent effects on development and metamorphic traits, with larvae metamorphosing later and at larger body size when reared at lower temperatures (Álvarez & Nicieza, 2002). In the present study, survivorship decreased in the warmer temperature (27°C) treatments and stochastic food availability conditions. Warmer waters may have compromised survival because of decreased oxygen availability (O'Connor *et al.*, 2007; Blaustein *et al.*, 2010). Temperature affects amphibian larval developmental rates directly (Hayes *et al.*, 1993). Exposure to higher temperatures shortens the larval period in many species (Morand *et al.*, 1997). For example, *Scaphiopus couchii* and *Spea multiplicata* are desert toads that show accelerated time to metamorphosis with increasing temperature (Buchholz & Hayes, 2000). This pattern of accelerated development has been observed in both anurans (Álvarez & Nicieza, 2002; Voss, 1993; Berven, 1979) and urodeles (Beachy, 1995; (Hickerson *et al.*, 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 (Duellman & Trueb, 1986; Morand *et al.*, 1997; Wilbur & Collins, 1973; Werner, 1986). Furthermore, Atkinson (1994) reported, larvae growing at cold temperatures have prolonged developmental periods but they may increase their size at metamorphosis. This phenomenon is considered a general rule for ectotherms. Negative relationship between growth and metamorphosis has been reported in three spade foot toad species (genus: *Scaphiopus*) (Morey & Reznick, 2000). According to our observations, the longest metamorphosis time was constant food and temperature (20°C) treatment whilst the shortest metamorphosis time was stochastic food and temperature (27°C). Furthermore, Morey & Reznick (2000) stated that, in low food availability conditions, larvae underwent earlier development to evade the resource-poor environment. However, the length of larval period can be regulated by other environmental factors (Álvarez & Nicieza, 2002). Moreover, over the experimental period, tadpoles from constant food



availability treatments had the highest number of metamorphosis. Generally, 7.5% of tadpoles of all treatment groups reached metamorphosis and completed it. Although mortality in the juvenile life stages of amphibians is typically high (Canessa *et al.*, 2014), there should be future studies about reasons for low rate of the metamorphosis in this species. Most authors agree that the principal causes of amphibian declines are habitat loss that the event is happening in the Iranian marsh frog population; At least in south-south east and central portion of Iran. Our observations suggest that, marsh frog population may have the ability to adapt to environmental stressors. For researchers, future efforts need to determine the mechanisms for captive breeding program and reintroduction in suitable sites. These programs for this species require detecting: survival and recruitment rates in the captive population, Reasons for low rate of the metamorphosis, survival of released animals, Growth rate of the reintroduced or augmented population, Who Establishment of a viable wild population.

In conclusion, the aim of this study was to use a manipulative laboratory experiment to examine the independent and interactive effects of long-term stochastic food availability and water temperature on larval *P. ridibundus* survivorship, growth and development. Based on results provided here, larval growth rate was highest at the constant food and warmer temperature (27°C) treatment, while survivorship rate was highest in conditions with food constant and cooler temperature (20°C). Furthermore, the longest metamorphosis time was observed on constant food and temperature (20°C) while the shortest metamorphosis time occurred in stochastic food and temperature (27°C). The results of this study strongly suggest that, environmental differences in food availability and water temperature, and their interaction, cause differences in growth, development and survivorship. Such advances have the potential to improve the output of amphibian captive breeding programs and may be of value to amphibian conservation. Conservation efforts such as captive breeding that no concentrate environmental factors likely will fail.

## ACKNOWLEDGMENTS

We are very grateful to Steven C. Anderson (Professor Emeritus Department of Biological Sciences University of the Pacific Stockton, California) for technical comments and improving our manuscript.

## REFERENCES

- Álvarez, D. and Nicieza, A.G. 2002. Effects of temperature and food quality on anuran larval growth and metamorphosis. *Functional Ecology* 16: 640-648.
- Atkinson, D. 1994. Temperature and organism size – a biological law for ectotherms? *Advances in Ecological Research* 25: 1–58.
- 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. and Smith-Gill, S.J. 1979. Countergradient selection in the Green Frog, *Rana clamitans*. *Evolution* 33: 609-623.
- Blaustein, A.R., Belden, L.K., Olson, D.H., Green, D.L., Root, T.L., and Kiesecker, K.M. 2001. Amphibian breeding and climate change. *conservation biology* 15: 1804-1809.
- Blaustein, A.R. and Kiesecker, J.M. 2002. Complexity in conservation: Lessons from the global decline of amphibian populations. *Ecology Letters* 5: 597-608.
- Blaustein, A.R., Walls, S.C., Bancroft, B.A., Lawler, J.J., Searle, C.L. and 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.
- Buchholz, D.R. and Hayes, T.B. 2000. Larval period comparison for the spadefoot toads *Scaphiopus couchii* and *Spea multiplicata* (Pelobatidae: Anura). *Herpetologica* 56: 455-468.
- Canessa, S., Hunter, D., McFadden, M., Marantelli, G. and McCarthy, M.A. 2014. Optimal release strategies for cost-effective reintroductions. *Journal of Applied Ecology* 51: 1107–1115.
- Day, T. and Rowe, L. 2002. Developmental thresholds and the evolution of reaction norms for age and size at life-history transitions. *The American Naturalist* 159: 338–350.
- Donnelly, M.A. and Crump, M.L. 1998. Potential effects of climate change on two Neotropical amphibian assemblages. *Climatic Change* 39: 541-561.
- Duellman, W.E. and Trueb, L. 1986. *Biology of Amphibians*, Johns Hopkins University Press: Baltimore, MD, USA.
- Enriquez-Urzelai, U., San Sebastián, O., Garriga, N. and Llorente, G. 2013. Food availability determines the response to pond desiccation in anuran tadpoles. *Oecologia* 173: 117–127.
- Govindarajulu, P.P. and 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. and Licht, P. 1993. Interactions of temperature and steroids in growth, development, and metamorphosis in a toad (*Bufo boreas*). *Journal of Experimental Zoology* 266: 206-215.
- Hickerson, C.M., Barker, E.L. and Beachy, C.K. 2005. Determinants of metamorphic timing in the black-bellied salamander, *Desmognathus quadramaculatus*. *Southeast. Nature* 4: 33-50.
- Inatsuchi, A., Yamato, S. and Yusa, Y. 2010. Effects of temperature and food availability on growth and reproduction in the neustonic pedunculate barnacle *Lepas anserifera*. *Marine Biology* 157: 899–905.
- Kozłowski, J., Czarnołęski, M. and Dańko, M. 2004. Can optimal resource allocation models explain why ectotherms grow larger in cold? *Integrative and Comparative Biology* 44: 480–493.
- McLeod, I.M., Rummer, J.L., Clark, T.D., Jones, G.P., McCormick, M.I., Wenger, A.S. and Munday, P.L. 2013. Climate change and the performance of larval coral reef fishes: the interaction between

- temperature and food availability. *Conservation Physiology*. 1, doi:10.1093/conphys/cot024.
- Morand, A., Joly, P. and Grolet, O. 1997. Phenotypic variation in metamorphosis in five anuran species along a gradient of stream influence. *C.R. Acad. Sci. Paris Life Sciences* 320: 645-652.
- Morey, S.R. and Reznick, D.N. 2000. A comparative analysis of plasticity in larval development in three species of spadefoot toads. *Ecology* 81: 1736-1749.
- Najibzadeh, M. 2010. A biosystematic study of Anuran amphibian in Lorestan province, MS thesis, Ferdowsi University, Iran [in Persian].
- O'Connor, M.I., Bruno, J.F., Gaines, S.D., Halpern, B.S., Lester, S.E., Kinlan, B.P. and Weiss, J.M. 2007. Temperature control of larval dispersal and the implications for marine ecology, evolution, and conservation. *Proceedings of the National Academy of Sciences of the United States of America*. 104: 1266-1271.
- Pesarakloo, A., Gharezi, A. and Kami, H.G. 2008. A study of embryonic stages and metamorphosis in the jungle frog (*Rana pseudodalmatina*) in Golestan province (Minudasht city), *Research Journal of University of Isfahan (Science)* 35(6): 209-222 [in Persian].
- Rome, L.C., Stevens, E.D. and John-Alder, H.B. 1992. Temperature and thermal acclimation and physiological function. In *Environmental Physiology of the Amphibia*, Feder, M.E., Burggren, W.W., Eds.; University of Chicago Press: London, UK., pp. 183-205
- Stephanie, K.C.J., Adam, J.M., Trent, D.P. and Phillip, G.B. 2015. Long-term changes in food availability mediate the effects of temperature on growth, development and survival in striped marsh frog larvae: implications for captive breeding programmes, *Conservation Physiology* 3: 1-12.
- Stuart, S.N., Chanson, J.S., Cox, N.A., Young, B.E., Rodrigues, A.S., Fischman, D.L. and Waller, R.W. 2004. Status and trends of amphibian declines and extinctions worldwide. *Science* 306: 1783-1786.
- Vonesh, J.R. and De la Cruz, O. 2002. Complex life cycles and density dependence: assessing the contribution of egg mortality to amphibian declines. *Oecologia* 133: 325-333.
- Voss, S.R. 1993. Relationship between stream order and length of larval period in the salamander *Eurycea wilderae*. *Copeia* 1993: 736-742.
- 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. and Collins J.P. 1973. Ecological aspects of amphibian metamorphosis. *Science* 182: 1305-1314.
- Yoneda, M. and Wright, P. 2005. Effect of temperature and food availability on reproductive investment of first-time spawning male Atlantic Cod, *Gadus morhua*. *ICES J Mar Sci* 62: 1387-1393.