Zoological Research 35 (4): 332−337 DOI:10.13918/j.issn.2095-8137.2014.4.332

Effects of light intensity on activity in four sympatric anuran tadpoles

Guo-Hua DING, Zhi-Hua LIN*, Li-Hua ZHAO, Xiao-Li FAN, Li WEI

College of Ecology, Lishui University, Lishui Zhejiang 323000*, China*

Abstract: Though light conditions are known to affect the development and anti-predation strategies of several aquatic species, relatively little is known about how different species react to light, or how light can affect these species during different points in their life-cycle. In this study, we used four sympatric anuran tadpoles (*Bufo gargarizans*, *B. melanostictus*, *Pelophylax nigromaculatus* and *Microhyla fissipes*) as animal system to examine species-specific activities of the underdoing different light intensity treatments, so as to better understand how they respond to light. We exposed four different species of tadpoles to 1660 and 14 lux light intensity treatments and then measured several parameters including development stage, body length and tail length, and as well as their basic activities. The results of this observation and analysis showed that the activities of tadpoles were significantly greater in *B. gargarizans* and *B. melanostictus* than in *P. nigromaculatus* and *M. fissipes*; and were also significantly greater during times of high light intensity as compared to during low light intensity. Moreover, the observed relationship between species and light intensity was significant. The activities of *B. gargarizans* and *B. melanostictus* tadpoles were greater in high light, while the activity of *P. nigromaculatus* tadpoles was greater in low light intensity, while *M. fissipes* tadpoles showed no differences in either low or high intensity light. Furthermore, the activities of *B. gargarizans*, *B. melanostictus* and *M. fissipes* tadpoles in terms of developmental stage, body size or tail length did not seem to differ with light intensity, but during early larval developmental period of *P. nigromaculatus*, the activity of tadpoles was negatively correlated with development stage, but irrelevant to either body size or tail length in different light intensities. These results lead us to conclude the observed activities of the four sympatric anuran tadpoles are closely correlated with their specific anti-predation strategies.

Keywords: Anura; Activity; Developmental stage; Light intensity; Tadpole

Activities of anuran tadpoles are known to be finely sculpted by aquatic predators (Relyea, 2005; Smith et al, 2008; Smith & Awan, 2009; Nelson et al, 2011a, b), as well as several additional factors such as group size (Spieler, 2005) or developmental stage (Golden et al, 2000), but one of the most intriguing is light conditions (Beiswenger, 1977; Griffiths et al, 1988; Warkentin, 1992; McClure et al, 2009). Light conditions during the day or night can induce both the spatial and temporal differences in the distribution and feeding behaviors of tadpoles (Branch, 1983). Tadpoles could likewise initiate orientation behaviors to match the photoperiodic changes they sense in surrounding environments (Justis & Taylor, 1976). Such phototactic behavior is common not only in adult anurans (Jaeger & Hailman, 1976), but also in their larvae, e.g., in tadpoles of *Rana temporaria* (Ashby, 1969), *Ascaphus truei* (de Vlaming & Bury, 1970) and *Bufo americanus* (Beiswenger, 1972).

Among the various parameters of light that can anuran affect tadpoles, light intensity is one of the most important ones in shaping behavior, growth and development. Indeed, light intensity is one of the key factors in differing anti-predation to counter both diurnal and nocturnal predators (Taylor, 1983; Holomuzki, 1986). High light intensity can, for example, increase the range of vision in tadpoles and enhance their ability in

Received: 08 October 2013; Accepted: 27 November 2013

Foundation items: This work was supported by the National Natural Science Foundation of China (30970435, 31270443), the Zhejiang Provincial National Science Foundation of China (C030403), the Open Research Fund program of Laboratory of Lishui University (2014-26-10) and the Scientific Research Foundation of Ph.D. in Lishui University (QD1301).

*Corresponding author, E-mail: zhlin1015@126.com

identifying predators, accordingly aiding in better anti-predation reaction and higher levels of activity. For example, in wood frog tadpoles (*R. sylvatica*), light conditions do not influence the activity level in undisturbed tadpoles, but do affect the response to the shadow stimulus, with the greatest responses being during conditions of bright light (McClure et al, 2009). Better light conditions also cause environmental effects that can be beneficial, such as an increase water temperature which benefits the growth and development of thermophilic tadpoles (Wright et al, 1988), and also induces tadpoles' aggregation (Beiswenger, 1977).

Despite the previous observations, it is not entirely clear how species-specific some of these effects are, nor is it clear how different species respond to different light conditions. In this study we used tadpoles from four sympatric anuran amphibians—*B. gargarizans, B. melanostictus, Pelophylax nigromaculatus* and *Microhyla fissipes*—all of which are endemic to China in Zhejiang, Jiangxi and Fujian province. All four species have breeding seasons from April to May, and their larvae are also sympatric (Fei et al, 2009). Using tadpoles from these four sympatric anurans as an animal system, we focused on investigating two key unanswered questions: (1) Are there species-specific behavior to light intensities in sympatric anuran tadpoles under different light intensities; and (2) Are there correlations between light intensity, activity and developmental stage, tail length and body length among these four tadpoles.

MATERIALS AND METHODS

Animal collection and rearing

From March to April in 2011, tadpoles of *B. gargarizans, B. melanostictus, P. nigromaculatus* and *M. fissipes* were collected by net from the water bodies of suburban farmlands in Lishui, Zhejiang (N28°27', E119°54') and brought back to our laboratory at Lishui University. Tadpoles of each species were housed separately in four dedicated breeding tanks (60 cm×40 cm×40 cm, length×width×height) containing 20 cm of 24-hour-decholorinated tap-water. Aquatic plants, such as confervoid and algae were added to the tanks to imitate the natural environment of the tadpoles. Tadpoles were fed with abundant egg yolk and fish powder. Prior to experimentation, one week was allowed for tadpoles to become acquainted with the new tank environment.

Experimental design

Totally, 20 tadpoles with intact tails of each species were randomly selected from the four tanks and housed in 80 small plastic containers (30 cm×30 cm×10 cm, length×width×height) containing 3 cm of water. These containers were placed in a psychrometric room with air temperature adjusted to 30±0.5 °C, and the light cycle (light period: 0700h−1900h) mimicked by fluorescence lights (28 W, 169 lux). During the trials, lighting conditions were created by turning off the fluorescence light and to create different intensities, light from an artificial camera (1 300 W, 1 660 lux) was used to mimic high lighting and a calico covered desk lamp (15 W, 14 lux) to mimic low lighting. Trials were conducted from 1100h. After a one-hour acclimation period, six one-minute observations were conducted every 20 minutes to determine the swimming activities of tadpoles. Only swimming activities lasting 10 seconds and longer were considered as valid, otherwise they were discarded as being irrelevant.

Following the light intensity trials, high and low lighting were turned off and the fluorescence light was switched back on. Tadpoles were *ad libitum* for 3 hours, and then water was changed. Before next day's trials, tadpoles were under fasting conditions for 18 hours. Tadpoles movement was evaluated based on its valid swimming activities during the 6 observations (Individual's activity=valid swimming activities/times of observation×100%). Each lighting treatment combination was duplicated twice, yielded a total timeframe of four days. At the end of the experiment, tadpoles were examined under a Nikon XTS30 lens and staged according to the staging tables developed by Gosner (1960). Body length (from snout to vent) and tail length (from base to tip of the caudal fin) were determined using ImageJ 1.44 (±0.01 mm).

Data analyses

All statistical analyses were conducted using Statistica 6.0 (Tulsa, OK, USA). All variables were tested for normality and homogeneity before further analysis. As the lighting conditions had no effects on the activity of duplicate measurement of the tadpoles from each species (paired-sample *t*-test; all $P>0.58$), the data from each duplicated experiment were pooled for analysis. Multiple regression, paired-sample *t*-test, ANOVA and repeated-measures ANOVA were used to evaluate relevant data. Variables with significant differences were analyzed by Tukey's *post hoc* comparisons.

Throughout this study, all values are expressed as mean± *SE*, with α=0.05 being significant.

RESULTS

The descriptive statistics of the developmental stage, body length and tail length of the four sympatric anuran tadpoles are shown in Table 1. Taking species as factor, ANOVA analysis was used to evaluate the linear regression residuals for the developmental stage, body length and tail length of the four sympatric anuran tadpoles, which showed significant interspecies differences in both the developmental stage and morphological traits (all $P<0.0001$). Under the differing light treatments, the developmental stage was more advanced in *B. gargarizans* tadpoles than in the other three tadpoles (Tukey's test; all $P<0.001$; Figure 1). In general, there was also different observed mean values in terms of body length, ranging from the smallest, *P. nigromaculatus*, to *B. gargarizans*, *B. melanostictus* and finally *M. fissipes* tadpoles being the largest (Tukey's test; all $P<0.001$; Figure 1). Similarly, *P. nigromaculatus* and *M. fissipes* tadpoles had much longer tails than those of *B. gargarizans* and *B. melanostictus* tadpoles (Tukey's test; all $P<0.001$; Figure 1).

Table 1 Descriptive statistics of developmental stage, body length and tail length in the four sympatric anuran tadpoles

	B. gargarizans	*B. melanostictus*	*P. nigromaculatus*	*M. fissipes*
Sample size (*n*)	20	20	20	20
Developmental stage	35.4±0.2 (34–37)	30.6±0.2 (29–32)	30.2±0.2 (29–32)	30.1±0.2 (29–32)
Body length (mm)	8.76±0.13 (7.74–9.63)	7.52±0.14 (6.36–8.90)	10.61±0.16 (9.25–12.25)	4.08±0.11 (2.97–4.70)
Tail length (mm)	11.27±0.23 (9.18–12.92)	9.36±0.25 (6.53–11.74)	18.19±0.45 (14.59–22.30)	5.98±0.33 (3.33–8.91)

Note: data presented as means±*SE*

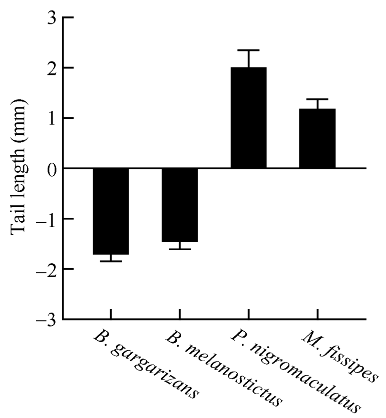

Figure 1 Mean regression residuals of tail length against body length in four sympatric anuran tadpoles

Taking species as between-subject factor and light intensity as within-subject factor, the activities of the four sympatric anuran tadpoles were evaluated by repeated-measures ANOVA, which indicated significant interspecies differences ($F_{3,76}$=34.25, $P<0.0001$). The activities of *B. gargarizans* and *B. melanostictus* tadpoles were significantly greater than those of *P. nigromaculatus* and *M. fissipes* tadpoles (Tukey's test; both $P<0.001$). The activities of tadpoles under different light intensity treatments were also significantly different ($F_{3,76}$=52.23, $P<0.0001$), being greater during high light intensity than in low light intensity (Tukey's test; $P<0.001$). Moreover, the activity was also affected by the interaction between species and light intensity (Tukey's test; $P<0.001$) (Figure 2). Intraspecific analyses showed the activities of *B. gargarizans*, *P. nigromaculatus* and *B. melanostictus* tadpoles were significantly affected by light intensity (pair-sample *t* test, all $P<0.04$), though not for *M. fissipes* tadpoles (pair-sample *t* test, t=0.95, df=19, P=0.356). *B. gargarizans* and *P. nigromaculatus* tadpoles were likewise more active under high light intensity, while *B. melanostictus* tadpoles seemed more active low light intensity (Figure 2) and *M. fissipes* tadpoles acted no differently under either.

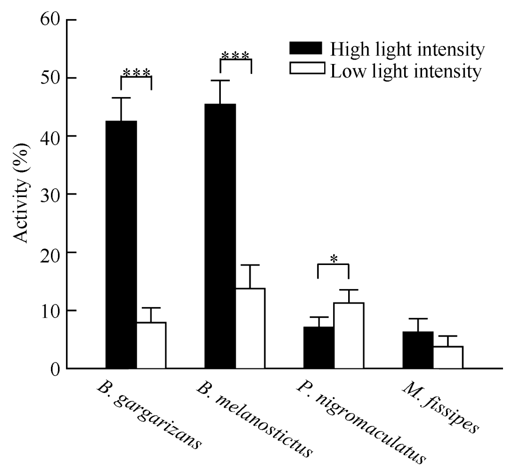

Figure 2 Mean activity of four sympatric anuran tadpoles under different light intensity treatments
***: $P<0.0001$; *: $P<0.05$.

Taking the developmental stage, body length and tail length as independent variables, and taking activity as dependent variable, multiple regression showed that under different light intensity treatments, the activities of *B. gargarizans*, *P. nigromaculatus* and *M. fissipes* tadpoles were not associated with their developmental stage, body length nor tail length (all P>0.216), but the activity of *B. melanostictus* tadpoles was negatively correlated with its developmental stage, and not connected with its body length and tail length (all P>0.137).

DISCUSSION

The results of our study showed that *B. gargarizans*, *B. melanostictus* tadpoles are more active under high light intensity treatments, while their activities were reduced 35% and 32% under low light intensity treatments, respectively (Figure 2). *B. rufus* and *B. bufo* tadpoles were previously shown to be inactive at night, but when a light is shined on them they become active (Eterovick & Sazima, 1999; Griffiths et al, 1988). Similarly, the activity of *B. americanus* tadpoles increase as light increases, and decreases on overcast days (Beiswenger, 1977), so much so that typical daytime behaviors could be induced in inactive tadpoles by creating artificial lighting conditions during nighttime (Branch, 1983). These studies not only suggest the specific activity rhymes of Bufonidae tadpoles, but also indicate that their activity patterns are likely influenced by light conditions. Conversely, in *P. nigromaculatus* tadpoles activity under high light intensity treatment was found to be some 4% lower than under low light intensity (Figure 2), which was in accordance with the results previously reported for *R. palmipes* and *R. catesbeiana* tadpoles in caliginous environment, such as during night or evening (McIntyre et al, 2004; Smith et al, 2007). Compared with Bufonidae and Ranidae tadpoles, different light intensity treatments showed no significant effects on the activities of *M. fissipes* tadpoles (Figure 2), but with no other species to test against, it is not possible to determine if this phenomenon is universal in *Microhyla* tadpoles still needs further study and more data.

Under different light intensity treatments, the activity of *P. nigromaculatus* tadpoles in their early developmental period was all negatively correlated with developmental stage (Figure 3), which manifested in impacts of developmental stage on individual's activity (Golden et al, 2000). Huey (1980) reported that the swimming ability of tadpoles before Gosner stage 40 were significantly positively correlated with their developmental stage and Anholt et al (2000) found that tadpoles with low swimming ability moved more frequently to increase preying rate. As such, the high observed activity level of *P. nigromaculatus* tadpoles in their early developmental period could function as compensation to their low swimming abilities. A similar phenomenon was found in *Xenopus laevis* tadpoles (Golden et al, 2000). But in our study, this specific correlation between activity and developmental stage was not found in the other three anuran tadpoles (all P>0.05), though this may be due to the small sample size.

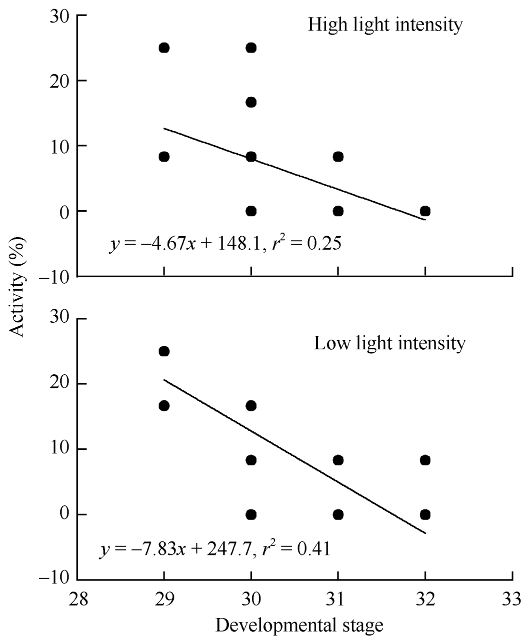

Figure 3 Relationships between activity and developmental stage in *P. nigromaculatus* tadpoles under different light intensity treatments

In this study, the mean values for activities of *B. gargarizans* and *B. melanostictus* tadpoles were significantly greater than those of *P. nigromaculatus* and *M. fissipes* tadpoles (Figure 2), which were correlated with their specific anti-predation strategies. There may be some factors that help explain this difference. Bufonidae tadpoles can release toxic chemicals to decrease their palatability as prey, so some aquatic predators would accordingly choose to avoid Bufonidae tadpoles after they have tasted them once (Laurila, 1998; Álvarez & Nicieza, 2009; Nelson et al, 2011a, b; Wei et al, 2013). Likewise, by moving more frequently, Bufonidae tadpoles can accelerate the release of those toxic chemicals and alert other individuals, thus decreasing their chances of being preyed upon (Hews, 1988; Van Buskirk, 2001). In our study, a high active level was found in *B. gargarizans* and *B. melanostictus* tadpoles (Figure 2). Paired alongside their ability to release toxic chemicals such activity could effectively increase both their individual and species defense against predators. Compared to the Bufonidae tadpoles, Ranidae tadpoles are usually pleasant food for aquatic predators (Van Buskirk & McCollum, 2000; Laurila et al, 2002; Wilson et al, 2005), and accordingly they decrease their active levels to lower risks of encountering predators and increase their survival rates (Lefcort, 1996; Anholt et al, 2005). Similarly, in this study, tail morphological traits of *P. nigromaculatus* and *M. fissipes* tadpoles were much more apparent than those of the other two Bufonidae tadpoles (Figure 1). This makes sense—longer tails endow individuals with greater burst speed (Lardner, 2000; Van Buskirk & McCollum, 2000; Dayton et al, 2005; Arendt, 2010). Presumably then, the decrease in activity and increase in burst speed may offer effective anti-predation strategies of the two Ranidae tadpoles.

In conclusion, our study suggests three things. First, that *B. gargarizans* and *B. melanostictus* tadpoles are more active under high lighting conditions, and the activity of *P. nigromaculatus* tadpoles decrease as light intensity increase, whereas, light intensity had no effects on the activity of *M. fissipes* tadpoles. Second, that during the early developmental period, the activity of *P. nigromaculatus* tadpoles is negatively correlated with their developmental stage. And finally, that the specific activity patterns of these four sympatric anuran tadpoles under different light intensities are closely correlated with their anti-predation strategies.

References

Álvarez D, Nicieza AG. 2009. Differential success of prey escaping predators: tadpole vulnerability or predator selection? *Copeia,* **2009**(3): 453-457.

Anholt BR, Negovetic S, Rauter C, Som C. 2005. Predator complement determines the relative success of tadpoles of the *Rana esculenta* complex. *Evolutionary Ecology Research,* **7**(5): 733-741.

Anholt BR, Werner E, Skelly DK. 2000. Effect of food and predators on the activity of four larval ranid frogs. *Ecology,* **81**(12): 3509-3521.

Arendt J. 2010. Morphological correlates of sprint swimming speed in five species of spadefoot toad tadpoles: Comparison of morphometric methods. *Journal of Morphology,* **271**(9): 1044-1052.

Ashby KR. 1969. The population ecology of a self-maintaining colony of the Common frog (*Rana temporaria*). *Journal of Zoology,* **158**(4): 453-474.

Beiswenger RE. 1972. Aggregative Behavior of Tadpoles of the American Toad, *Bufo americanus* in Michigan. Ph.D. thesis, University of Michigan, Ann Arbor MI.

Beiswenger RE. 1977. Diel patterns of aggregative behavior in tadpoles of *Bufo americanus*, in relation to light and temperature. *Ecology,* **58**(1): 98-108.

Branch LC. 1983. Social behavior of the tadpoles of *Phyllomedusa vaillanti*. *Copeia,* **1983**(2): 420-428.

Dayton GH, Saenz D, Baum KA, Langerhans RB, DeWitt TJ. 2005. Body shape, burst speed and escape behavior of larval anurans. *Oikos,* **111**(3): 582-591.

de Vlaming VL, Bury RB. 1970. Thermal selection in tadpoles of the tailed frog, *Ascaphus truei*. *Journal of Herpetology,* **4**(3/4): 179-189.

Eterovick PC, Sazima I. 1999. Description of the tadpole of *Bufo rufus* with notes on aggregative behavior. *Journal of Herpetology,* **33**(4): 711-713.

Fei L, Hu SQ, Ye C, Huang YZ. 2009. Fauna Sinica: Amphibia. Beijing: Science Press (in Chinese).

Golden DR, Smith GR, Rettig JE. 2000. Effects of age and group size on habitat selection and activity Level in *Xenopus laevis* tadpoles. *Nebraska Academy of Sciences,* **26**: 23-27.

Gosner KL. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica,* **16**(3): 183-190.

Griffiths RA, Getliff JM, Mylotte VJ. 1988. Diel patterns of activity and vertical migration in tadpoles of the common toad, *Bufo bufo*. *Herpetological Journal,* **1**(1): 223-226.

Huey RB. 1980. Sprint velocity of tadpoles (*Bufoboreas*) through metamorphosis. *Copeia,* **1980**(3): 537–540.

Hews DK. 1988. Alarm response in larval western toads, *Bufo boreas:* release of larval chemicals by a natural predator and its effect on predator capture efficiency. *Animal Behaviour,* **36**(1): 125-133.

Holomuzki JR. 1986. Predation avoidance and diel patterns of microhabitat use by larval tiger salamanders. *Ecology,* **67**(3): 737-748.

Jaeger RG, Hailman JP. 1976. Phototaxis in anurans: Relation between intensity and spectral preferences. *Copeia,* **1976**(1): 92-98.

Justis CS, Taylor DH. 1976. Extraocular photoreception and compass orientation in larval bullfrogs, *Rana catesbeiana*. *Copeia,* **1976**(1): 98-105.

Lardner B. 2000. Morphological and life history responses to predators in larvae of seven anurans. *Oikos*, **88**(1): 169-179.

Laurila A. 1998. Breeding habitat selection and larval performance of two anurans in freshwater rock-pools. *Ecography*, **21**(5): 484-494.

Laurila A, Pakkasmaa S, Crochet PA, Merila J. 2002. Predator-induced plasticity in early life history and morphology in two anuran amphibians. *Oecologia,* **132**(4): 524-530.

Lefcort H. 1996. Adaptive, chemically mediated fright response in tadpoles of the southern leopard frog, *Rana utricularia*. *Copeia,* **1996**(2): 455-459.

McClure KV, Mora JW, Smith GR. 2009. Effects of light and group size on the activity of wood frog tadpoles (*Rana sylvatica*) and their response to a shadow stimulus. *Acta Herpetologica,* **4**(1): 103-107.

McIntyre PB, Baldwin S, Flecker AS. 2004. Effects of behavioral and morphological plasticity on risk of predation in a neotropical tadpole. *Oecologia,* **141**(1): 130-138.

Nelson DWM, Crossland MR, Shine R. 2011a. Behavioural responses of native predators to an invasive toxic prey species. *Austral Ecology,* **36**(6): 605-611.

Nelson DWM, Crossland MR, Shine R. 2011b. Foraging responses of predators to novel toxic prey: effects of predator learning and relative prey abundance. *Oikos,* **120**(1): 152-158.

Relyea RA. 2005. The heritability of inducible defenses in tadpoles. *Journal of Evolutionary Biology,* **18**(4): 856-866.

Smith GR, Awan AR. 2009. The roles of predator identity and group size in the antipredator responses of American toad (*Bufo americanus*) and Bullfrog (*Rana catesbeiana*) tadpoles to different predators. *Behaviour,* **146**(2): 225-243.

Smith GR, Burgett AA, Sparks KA, Temple KG, Winter KE. 2007. Temporal patterns in bullfrog (*Rana catesbeiana*) tadpole activity: a mesocosm experiment on the effects of density and bluegill sunfish (*Lepomis macrochirus*) presence. *Herpetological Journal,* **17**(3): 199-203.

Smith GR, Burgett AA, Temple KG, Sparks KA, Winter KE. 2008. The ability of three species of tadpoles to differentiate among potential fish predators. *Ethology,* **114**(7): 701-710.

Spieler M. 2005. Can aggregative behaviour of *Phrynomantis microps* tadpoles reduce predation risk. *Herpetological Journal,* **15**(3): 153-157.

Taylor J. 1983. Orientation and flight behavior of a neotenic salamander (*Ambystoma gracile*) in Oregon. *American Midland Naturalist,* **109**(1): 40-49.

Van Buskirk J. 2001. Specific induced responses to different predator species in anuran larvae. *Journal of Evolutionary Biology,* **14**(3): 482-489.

Van Buskirk J, Mccollum SA. 2000. Functional mechanisms of an inducible defence in tadpoles: morphology and behaviour influence mortality risk from predation. *Journal of Evolutionary Biology,* **13**(2): 336-347.

Warkentin KM. 1992. Effects of temperature and illumination on feeding rates of green frog tadpoles (*Rana clamitans*). *Copeia,* **1992**(3): 725-730.

Wei L, Lin ZH, Zhao RY, Chen ST. 2013. Prey selection by tiger frog larvae (*Hoplobatrachus chinensis*) of two sympatric anuran species' tadpoles. *Zoological Research,* **34**(3): 209-213. (in Chinese)

Wilson RS, Kraft PG, Van Damme R. 2005. Predator-specific changes in the morphology and swimming performance of larval *Rana lessonae*. *Functional Ecology,* **19**(2): 238-244.

Wright ML, Jorey ST, Myers YM, Fieldstad ML, Paquette CM, Clark MB. 1988. Influence of photoperiod, day length, and feeding schedule on tadpole growth and development. *Development, Growth & Differentiation*, **30**(3): 315–323.