



The influence of visual cues of conspecifics based on density and habitat features on the growth of *Bufo gargarizans minshanicus* larvae: an experimental approach

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Anuran larvae may use chemical, visual and tactile cues to assess habitat features, and subsequently mediate their growth and development. Of the three cues, chemical ones have been analysed the most, but little is known about the role of visual cues and the extent to which tadpoles rely on their vision for intraspecific social assessment. In this study, we investigated whether conspecific visual cues affect development and growth of *Bufo gargarizans minshanicus* tadpoles, and analysed whether they use visual cues as indicators of density. The tadpoles did not significantly alter their growth and development in response to low visual stimulation. However, tadpoles under high visual stimulation were significantly smaller than single tadpoles without visual cues. Therefore, we suggest that *B. g. minshanicus* tadpoles are susceptible to high visual stimulation when the environment changes (little vegetation and clear water), allowing for decreased growth in the presence of high-density conspecifics.

Key words: body mass, visual cues, density, intraspecific competition, habitat features, *Bufo gargarizans minshanicus*

INTRODUCTION

Visual signals play important roles in foraging, mate choice, anti-predator defence and social interactions in many vertebrates (Liao & Lu, 2009; Møller and Erritzøe, 2010; Liao & Lu, 2011; Yu et al., 2009). For instance, eye size in birds has evolved as a means of predator avoidance since larger eyes allow early detection of an approaching predator (Møller & Erritzøe, 2010). In toads, males prefer larger females as mates using visual signals (Liao & Lu, 2009; Liao & Lu, 2011; Yu et al., 2009). In fish, while experiencing visual social stressors, the smaller male uses an opportunistic strategy, acting like a subordinate male while maintaining the physiology of a dominant male (Chen & Fernald, 2011). In amphibian species, larvae may use chemical, visual, and tactile cues to assess features of their habitats (reviewed by Rot-Nikcevic et al., 2006). Many previous studies demonstrated that chemical cues are associated with predator detection (Petranka et al., 1987; Kats et al., 1988; Semlitsch & Reyer 1992; Kiesecker, Chivers & Blaustein, 1996; Laurila, 2000; Benard, 2006), kin recognition (Waldman, 1985, 1986; Blaustein & Walls, 1995; Gramapurohit et al., 2006; Eluvathingal et al., 2009), growth and survival (Crossland & Shine, 2012). Anuran larvae use visual cues to find their prey, competitors, and predators when water is clear (Hettyey et al., 2012). Moreover, Rot-Nikcevic et al. (2006) suggested that visual stimuli were perceived as stressful ones, therefore

inducing tadpoles to become more active in response to enhanced visual stimuli, resulting in less energy available for growth.

Tadpoles forming aggregations with kin or non-kin may gain many of the advantages of group living (reviewed by Blaustein & Waldman, 1992). For example, group-living animals forage more efficiently and detect or avoid predators more effectively (reviewed by Blaustein & Waldman, 1992). Although visual cues are not sufficient for discriminating between kin and non-kin (Wassersug, 1973; Wassersug & Hessler, 1971; Wassersug et al., 1981; O'Hara, 1981), they are important in schooling with conspecifics (Blaustein & O'Hara, 1982). Conversely, crowding may reduce growth, development rate, and survival of metamorphosis in most amphibians (Smith-Gill & Berven, 1979; Semlitsch & Caldwell, 1982; Smith, 1990; Hokit & Blaustein, 1994, 1997; Girish & Saidapur, 1999; Relyea & Hoverman, 2003).

We tested whether visual cues affect fitness-related metamorphic traits (e.g., age and size at metamorphosis, growth rates, and body condition) in a laboratory experiment with *Bufo gargarizans minshanicus*. This animal is a typical explosive breeder (Wells, 2007), and is distributed at high elevations on the eastern Tibetan plateau (Fei & Ye, 2001). The breeding season is extremely short (7-15 days), and once the eggs hatch, the larvae typically group together as they grow and mature in permanent wetlands (although it is not known whether

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these groups are composed of kin or non-kin). Our aim was to test whether visual cues would lead to reduced larval growth and development in response to habitat change.

MATERIALS AND METHODS

Study area

Our field study was carried out at two altitudes in Gahai-Zecha National Nature Reserve of Luqu County, Gansu, China in April of 2014 and 2015. The length of *B. g. minshanicus* breeding season at individual sites is as follows: (1) Site A, Gaihai Lake (34°12'N, 102°19'E, altitude 3477 m), from mid- to late April; and (2) Site B, Guomao Lake (34°18'N, 102°18'E, altitude 3449 m), from early to mid-April. At site A, water quality is usually clear because fencing generally excludes yaks from drinking at this water source. On the contrary, at site B, water quality is often muddy because yaks often frequent this water source for drinking and/or feeding.

Data collection

During peak reproductive periods, we captured a total of 60 amplexant pairs of *B. g. minshanicus* by hand at site A and site B. We then placed amplexant pairs into individual 5-liter plastic containers filled with 12 cm of water until females oviposited. We randomly selected 20 fertilised eggs from each clutch for our experiment, and placed each egg mass into separate opaque plastic vessels containing 3000 ml of water. Adult toads and the remaining eggs were released back into the wetlands. We carried out this experiment in the laboratory, which allowed us to avoid confounding environmental effects and predation pressure.

Experiment design

Once the tadpoles reached Gosner stage 25 (absorption of external gills and fully formed spiracle; Gosner, 1960), we created two treatments designed to provide tadpoles with different visual cues. In the first set of treatments (in 2014), each tadpole was kept in an individual transparent glass beaker (250 ml). Then, two tadpoles, still housed individually, were placed adjacent to one another and wrapped together with an opaque paper. In this case, the tadpoles from the two adjacent containers could see one another. In 2015, all containers with single tadpole were arranged into a 7 row × 8 column grid. In this arrangement, 4 containers (i.e., the containers in the corners of the grid) were placed adjacent to 2 other containers, 22 containers (i.e., the outside rows excluding the corners) were immediately adjacent to 3 other containers, and 30 containers (i.e., the inner rows) were placed adjacent to 4 other containers. Therefore, visual signals varied between 2 and 4 other tadpoles being visible.

In the second set of treatments (2014 and 2015), tadpoles were housed individually in opaque glass beakers (250ml, n = 30). Each tadpole was on a single shelf by itself. The tadpoles in each treatment were placed on the same food regimen (50 mg of commercial fish food per tadpole per day at the beginning, and two pieces of water weed, *Potamogeton crispus*). As the larval period progressed,

ration levels were increased based on tadpole mass to keep up with the normal demands of growth and development. Tadpoles were exposed to an 11L:13D photoperiod throughout the study period, and room temperature was kept at $25.80 \pm 1.39^\circ\text{C}$ (2014) and 27.72 ± 1.12 (2015). The water in the containers was changed weekly.

After the first metamorphosis (defined as the emergence of the first forelimb, stage 42) was discovered, the 60 glass beakers were checked daily in both treatments. We excluded 4 or 5 cases in visual or non-visual treatments (all from site B) because they indicated a developmental abnormality, and therefore were not a suitable candidate for the experiment. Several variables were measured: (1) development time (number of days from the beginning of the experiment until metamorphosis, Gosner stage 42); (2) body mass (weighed with an electric balance to the nearest 0.001g); (3) snout-vent length (SVL, using digital callipers to 0.01 mm); (4) growth rate (measured as the mass at metamorphosis divided by the age at metamorphosis); (5) body condition at the start or the end metamorphosis (defined as body condition = $(\text{mass}/\text{SVL}^3) \times 1000$), and (6) the overall metamorphosis survival rate (the percentage of surviving tadpoles that metamorphosed).

Data analysis

All data were log-transformed to meet the assumptions of parametric analysis of variance. Difference between treatments in development time, body mass, body length, body condition at the start of metamorphosis and growth rate were analysed using Student's t-tests for each site. The Pearson Chi-square test was employed to test the metamorphosis survival rate. We did not analyse differences in metamorphic traits between years because of different rearing temperatures. All data were analysed with SPSS 19.0, SPSS Inc., 2004, Chicago, IL, USA. All p-values given are two-tailed, with values presented as means \pm standard error.

RESULTS

The development time was not significantly different between the two treatments for both sites (Student's t-test: site A, $t_{41} = 1.17$, $p = 0.25$; site B, $t_{43} = -0.12$, $p = 0.90$). Although tadpoles in both treatments had similar body mass at site A ($t_{41} = 0.104$, $p = 0.92$), tadpoles from site B raised in non-visual treatments were significantly larger than those raised with visual cues ($t_{43} = 2.06$, $p = 0.045$). Compared with tadpoles raised in visual treatments, the tadpoles raised in non-visual conditions showed two opposing tendencies for body length, but the differences were not significant in either site (site A: $t_{41} = 0.72$, $p = 0.48$; site B: $t_{43} = -1.50$, $p = 0.14$).

The tadpoles raised in non-visual treatments did not have significantly larger mean growth rate or body condition at metamorphosis than that of tadpoles raised in individual containers with visual cues (growth rate, site A, $t_{41} = 0.09$, $p = 0.93$; site B, $t_{43} = 1.44$, $p = 0.16$; body condition, site A, $t_{41} = 0.80$, $p = 0.43$; site B, $t_{43} = 0.14$, $p = 0.89$). Finally, the tadpoles raised in both treatments had similar survival rate at site A (Pearson Chi-Square test: $\chi_1^2 = 0.12$, $p = 0.73$) and site B ($\chi_1^2 = 0.00$, $p = 1.00$; Table 1).

Table 1. Summary of laboratory experiment data for *Bufo gargarizans minshanicus* following treatments

Variables	Year	Single, visual	Single, non-visual
		Mean(SE)	Mean(SE)
Development time	2014	26.30(0.21)	25.91(0.26)
	2015	10.78(0.31)	10.77(0.20)
Body mass	2014	0.147(0.006)	0.148(0.008)
	2015	0.128(0.005)	0.144(0.005)
Body length	2014	9.47(0.15)	9.32(0.116)
	2015	9.64(0.16)	9.99(0.17)
Growth rate	2014	5.60(0.25)	5.73(0.32)
	2015	12.24(0.65)	13.48(0.63)
Body condition	2014	0.14(0.005)	0.18(0.008)
	2015	0.14(0.006)	0.15(0.007)
Survival rate	2014	66.67	76.67
	2015	90	90

Significant effects have P-values in bold

DISCUSSION

Rot-Nikcevic et al. (2006) found that *Bufo americanus* tadpoles did not respond to visually stimulated increases in conspecific density by altering either their development or growth. Our results showed that visual cues did not affect any fitness-related metamorphic traits of *B. g. minshanicus* tadpoles under low visual stimulation. Interestingly, we found tadpoles with high visual stimulation were significantly smaller than tadpoles without visual cues. Thus, our results support the findings of other authors (e.g., *Rana sylvatica*, Rot-Nikcevic et al., 2005, 2006; *Rana kukunoris*, Yu & Lambert, 2015) that high visual stimulation, better perceived by compound eyes, results in detrimental effects on tadpole growth and development (Land, 1997).

Previous studies showed that increased competition promotes decreased growth rate in anuran larvae (e.g. Wilbur & Collins, 1973; Smith-Gill & Berven, 1979; Berven & Chandra, 1988; Scott, 1990). We found that growth rate was not statistically different between the tadpoles raised in non-visual treatments and that of tadpoles raised with visual cues. Moreover, our results also showed that the tadpoles with visual stimuli had similar development times with those of tadpoles raised without visual cues. Importantly, body mass of the tadpoles with high visual stimuli were smaller than tadpoles in non-visual treatments. Thus, this may suggest that *B. g. minshanicus* tadpoles may use their vision for environment assessment, and that they are able to modify their growth and development in response to sensory enrichment. Generally, *Bufo* larvae may be composed of tens of thousands of individuals from numerous sibships (Wassersug, 1973; Waldman, 1982). Rot-Nikcevic et al. (2005) found that visual cues do not play a role in affecting growth and development in *Bufo americanus* tadpoles because these cues were redundant in their natural habitats. This result was not consistent with our study. In this study, we speculated that the effect of visual cues may be related to features from the original habitat that impact differences in visual

stimulation. If their habitat consisted of turbid water and dense vegetation, vision is often dismissed as unimportant (reviewed by Hettyey et al., 2012). On the contrary, if *Bufo* larvae aggregated in habitats with little vegetation and clear water, visual information may be perceived as stressful. In site B, water quality is often muddy because yaks go there to drink water or feed on aquatic plants. In this case, the tadpoles usually form denser aggregates and rely on olfactory cues; visual cues are redundant in their natural habitats. However, *B. g. minshanicus* tadpoles were raised in two treatments with clean water, which was different from their original habitat. Therefore, we suggested that high visual stimulation with altered habitat features (e.g., water clarity) may induce negative effects on tadpole growth and development.

In conclusion, *B. g. minshanicus* tadpoles that typically form high-density schools are susceptible to visual stimulation, when the environment changes, especially if water quality becomes clear. In this case, vision of conspecifics is a source of stress for tadpoles and may have detrimental effects on tadpole growth and development.

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