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Long-term heavy metal pollution varied female reproduction investment in free-living anura, *Bufo raddei*

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ABSTRACT

Environment contamination is known to affect the growth, reproduction, and even mortality of anuran species, and hence modulate their life history traits. Although knowledge of the ability of amphibians to cope with harsh environments has gained ongoing research, the reproductive strategy of free-living amphibians subjected to long-term heavy metal pollution is largely unknown. This study aimed to explore the variation in the life history traits, including age structure, maturation age, reproductive investment, and reproduction trade-off, in female *Bufo raddei*, a widespread anuran in Baiyin (BY) in northwest of China, subjected to sublethal heavy metal pollution. *B. raddei* collected from Liujiaxia (LJX), a relatively unpolluted area, were used as control. Skeletochronological analysis revealed variation in the average breeding age of females: more than 70% of females from BY began to breed 1 year before the toads collected from LJX. Females from BY tended to prioritize reproduction over survival and invested more in their first reproductive event. Further, females in BY with a high fluctuating asymmetry index showed a relatively lower reproductive investment. For trade-off in offspring number and size, BY population optimize larger clutch sizes with smaller egg size compared with population in LJX. Changes in female reproductive investment caused by heavy metal pollution might ultimately alter the structural stability of amphibian population.

1. Introduction

Amphibians populations are declining at a faster rate than any other vertebrate group (Stuart et al., 2004), and environmental contaminants are known to contribute remarkably to this rapid decline (Collins, 2010; Araujo et al., 2014). Surface water is a primary sink for most of the environmental pollutants; hence, acqutic animals, especially amphibians with high skin permeability, are at a particular risk to be exposed to contaminants directly via the aquatic medium or indirectly via the food chain (Bentley and Yorio, 1976; Hamlin and Guillette, 2010).

Environmental conditions can be a strong factor driving the evolution of life history traits (Hoffmann and Parsons, 1997). For species that do not provide parental care, maternal investment in the trade-off between egg size and egg number would directly affect the fitness of the offspring under adverse environmental conditions (Hendry et al., 2001; Liao et al., 2014), since the initial development of the young depends mainly on egg provisioning. Hence, females tend to optimize the allocation between quality and quantity of offspring such that the offspring survival is maximized (Smith and Fretwell, 1974; Fleming and Gross, 1990).

Iteroparous organisms need to trade-off between the time and energy allotted to the present reproduction and those directly related to future reproduction, growth, and survival. Severe environmental pollution caused by natural or anthropogenic sources has threatened or already imposed strong adverse effects on the growth, development, as well as survival of numerous species, including amphibians (Bruhl et al., 2013), this has become a focus of current researches on life history traits. Under such pressures, reproductive output is predicted to be optimized according to classical life history theory. Studies across vertebrates and macro-invertebrates such as birds, fishes, and spiders have provided some understanding on life history evolution under stressful conditions (Hendrickx et al., 2003a; Provencher et al., 2016). Organic or inorganic pollutants have been shown to reshape life history traits, such as leading to the decline in egg size or reduction of female reproductive output, due to the variation in body size, body condition (Eraly et al., 2011; Provencher et al., 2016), or changes in prey species composition and decrease in food availability, thereby resulting in reduced resource availability (Waelti and Reyer, 2007; Dmowski et al., 2015).

Common species with wide distribution are valuable for

Abbreviations: SVL, Snout-vent length; FA, fluctuating asymmetry; BY, Baiyin; LJX, Liujiaxia

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ecotoxicology researches, because of the convenient to obtain sufficiently large number of samples and to conduct research at a fine spatial scale. *Bufo raddei*, the most widespread species in northwest China, has long been used for assessing environmental pollution in the region. It is also an ideal species to learn the reproductive strategies and trade-offs in the region, since it excluded the impact of several complicated factors in life history research, such as multiple clutches in a single breeding season and parental care of offspring. Studies in the upper Yellow River region showed that this species has long been exposed to toxicity stress and genetic damage because of anthropogenic environmental contamination (Huang et al., 2007; Zhang et al., 2016). However, to the best of our knowledge, no study has investigated how growth and reproduction trade-off is influenced, or how reproductive strategies differ under environmental stress caused by contaminants, especially heavy metals that are easily enriched, but difficult to degrade in organisms, in free-living amphibians.

For organisms with indeterminate growth, body size reflects age as well as the past growth trajectory (Hasumi, 2010). In addition, in most amphibians and reptiles, females with larger body size are expected to have better fitness in reproduction than those with a small body size (Tejedo, 1992; Lardner and Loman, 2003). However, the limited reproductive resources and maternal abdominal space restrict the simultaneous increase of clutch size and egg size (Shine and Schwarzkopf, 1992; Sakai and Harada, 2004). For most amphibians, larger initial body size would improve larval survival, stress tolerance, and metamorphic performance (Altwegg and Reyer, 2003; Rasanen et al., 2005). However, under fluctuating environmental conditions, the allocation rule is flexible (Philippi and Seger, 1989).

This study aimed to elucidate the effect of long-term heavy metal pollution on the life history traits of free-living anuran, *B. raddei*. Furthermore, to provide insights into the fitness consequences of variation in female reproduction investment, as well as the trade-off between growth and reproduction in *B. raddei* under long-term heavy metal stress.

2. Materials and methods

2.1. Ethics statement

Animals were cared for in accordance with the guidelines of the ethics committee of the School of Life Sciences, Lanzhou University, which approved this study.

2.2. Study species and study site

The breeding season of *B. raddei* in Gansu Province lasts from the end of April up to the beginning of July, and the peak reproduction occurs in mid-May to early June. Females lay one clutch of eggs per year (approximately 800–4000 eggs). The aquatic larvae develop and metamorphose in 2 months and then become largely terrestrial.

Liujiaxia (LJX) and Baiyin (BY) are two cities in Gansu Province in the northwest of China; they are about 110 km apart. BY is a resource-exhausted city and was once a major non-ferrous metal mining and smelting base of Gansu Province. Large amounts of industrial pollutions from BY are discharged into the Yellow River through Dongdagou and Xidagou ditches. Among which heavy metals are the main environmental pollutions, mainly Cu, Zn, Pb, and Cd (Dai et al., 2012; Zhang et al., 2016). LJX, an upstream site with similar fauna and natural characteristics as those of BY, is a water source protection area and is relatively unpolluted. All the toads were captured from the Dongdadou ditch in Silong town, BY (E104°23′, N36°25′) and from the wetland in Weijiachuan village in LJX (E103°15′, N35°56′) (Fig. 1). Long-term monitoring by our group showed that the temperature and pH of the water bodies at the two study areas were very similar every month.

2.3. Experimental procedures

From 2014 to 2017, during the breeding peak (mid- to the end of May), amplectant male and female *B. raddei* were caught by hands at night from BY and LJX. The gravid body mass of female was measured using an electronic balance to the nearest 0.01 g. Subsequently, pairs were placed in separate tanks (60 × 40 cm, 30 cm tall) with shallow water and left in the wetland of the habitat for overnight. Spawning usually initiated several hours later, and egg deposition would finish by the following morning. Every morning, the entire clutch was collected. Egg strings were carefully coiled in a glass dish to minimize impact on the string and photographed using a digital camera (Canon EOS M; Canon Inc., Tokoy, Japan). The body mass of the spend female was measured using an electronic balance with a precision of 0.01 g. The snout-vent length (SVL) was measured using a digital caliper with a precision of 0.01 mm. In 2016 and 2017, 8 morphological features (listed in Section 2.3.3) of all samples were recorded for fluctuating asymmetry (FA) analyses, which is a measurement of the developmental stress experienced by individuals in different environments. Besides, skeletochronological analysis was performed to explore the age distribution of the two populations. The longest phalanges from the right hind lib of the females were removed for marking and preserved in 10% neutral buffered formalin solution. Finally, all the individuals were released near the capture site, and all the eggs were released together.

2.3.1. Body condition and maternal reproductive investment

Body condition, taken as an indicator of the health and fitness of an individual, was estimated as the residual of a regression line of the post-spawn body mass against SVL (Schulte-Hostedde et al., 2005). Clutch size was measured using the cell-counter function of ImageJ (1.47 q; National Institutes of Health, Bethesda, MD) to count the total eggs in the digital pictures. Clutch mass was indirectly estimated as the difference of female body mass before and after spawning. The mean egg mass was calculated as clutch mass divided by clutch size. In this study, the clutch mass/post-spawn body mass was used as an indicator of maternal reproductive investment.

2.3.2. Age determination

The skeletochronological analysis was performed as previously described (Bo Liao and Lu, 2010). Cross sections (12–15 μm thick) of the phalanx with the narrowest medullar cavity and the thickest cortical bone were selected and mounted on glass slides. Two observers independently counted the number of lines of arrested growth (LAG) in the sections by using a light microscope; the results were then compared.

2.3.3. Fluctuating asymmetry analyses

The FA characteristics of the toads collected in 2016 and 2017 were analyzed using CFA 2 (the second composite index of FA) described by Leung et al. (2000). The following morphological features of all samples were recorded and analyzed: (1) number of spots on the dorsal side of the shank; (2) number of spots on the dorsal side of the thigh; (3) number of spots on the dorsal side of the forearm; (4) number of spots on the plantar side of the third finger of the hind leg; (5) length of the forearm; (6) width of the forearm; (7) length of the hind leg; (8) length of the third finger of the hind leg.

2.4. Statistical analyses

Statistical analyses were performed using SPSS 19.0 (SPSS Inc., Chicago, IL, USA). The normal distribution of the data was checked using Kolmogorov-Smirnov test. Body mass, SVL, post-spawning body mass, clutch size, body condition, clutch mass, and egg mass were normally distributed. The FA index was log-transformed to achieve normality. A general linear model (GLM) was used to investigate the factors affecting female fecundity and reproductive output of the toads,