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Variation and Diversity of the Fatty Acid Composition in Anuran Larvae in the Different Aquatic Environments

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ABSTRACT

The study on trophic composition is important to understand the environmental adaptability in amphibians in ecology. The compositional differences of fatty acids in tadpoles that live in different altitudes of aquatic environment can reflect the variation of the dietary sources, which provide an important physiological understanding and prediction of ecological adaptation. The tadpoles were obtained in the stream at 500 m for *Bufo gargarizans,* at 1000 m for *Rana chensinensis,* at 1500 m for *Feirana quadranus*, and 2000 m for *Oreolalax popei.* We compared the types and composition of fatty acids in these four anuran species by using gas chromatography-mass spectrometry (GC-MS) analysis. For these four anuran species, the types of fatty acids were seven, seven, six and ten, respectively. The dominant components of fatty acids were $C_{17}H_{34}O_2$(16:0), $C_{19}H_{34}O_2$(18:2), $C_{19}H_{36}O_2$(18:1) and $C_{19}H_{38}O_2$(18:0), and they had different rates among them. The results showed that the composition of anuran larvae in different altitudes of aquatic environment exhibited varied and diverse values.

INTRODUCTION

Vertebrates obtain the lipid nutrition from the living environment. Lipids provide energy source and nutrients for tissue development and maintain functions in the body (Noble & Cocchi 1990). Additionally, essential lipids are the key biochemical compounds and have important nutritional value for aquatic animals living in different aquatic environment (Gladyshev et al. 2014, Doherty et al. 2015).

Among aquatic animals, tadpoles play a variety of ecological roles in showing morphological diversity, and inhabiting a wide variety of microhabitats. The water environments of tadpoles are different and complex, and it is difficult to observe them how to diet, though we know that their diets include various foods, such as animal tissue, plant, dissolved organic matter, protozoans and algae (Kupferberg 1997, Huang et al. 2003).

The variation of fatty acid composition in tadpoles may reflect its physiological and ecological significance (Huang et al. 2003). Tadpoles have a limited ability to synthesize and modify fatty acids. Numerous dietary fatty acids are directly or indirectly incorporated into their adipose tissue. The fatty acid compositions can effectively reflect dietary source, lipid utilization, living environment and physiology of tadpole. The analysis of fatty acids has become a useful tool to study the environment and physiology of tadpoles in different aquatic environments (Gladyshev et al. 2014, Doherty et al. 2015).

The research on fatty acids has mainly focused on the role of lipids during the metamorphosis (Kupferberg 1997), the lipid mobilization (Das 1996), and the accumulation of lipid in the organs (Scott et al. 2007). These investigations, however, mainly focused only on pre-metamorphic, metamorphic larvae or adult amphibians in the same living environment. In addition, there is still no information regarding the fatty acid composition of larvae in amphibians from different altitude aquatic environments. Whether the changes of fatty acid composition are associated with their living environment is unknown in tadpoles. Because the taxa and living environments of amphibians are different, the diets are diverse and vary widely, moreover, the nutritional quality of diets vary greatly. Based on these, we hypothesise that tadpoles living in different altitude aquatic environments may have different types and contents of fatty acid. Accordingly, the larvae of *Bufo gargarizans, Rana chensinensis, Feirana quadranus* and *Oreolalax popei* were

used in this study to evaluate whether there exists changes in the diet of amphibian larvae, from different altitude aquatic environments, by examining the compositions of fatty acid.

MATERIALS AND METHODS

Tadpoles (*Feirana quadranus*, *Oreolalax popei*, *Rana chensinensis* and *Bufo gargarizans*) were taken from the different altitudes of Qinlin Mountains, Shannxi province in China. The tadpoles were obtained in the stream at 2000 m for *O. popei,* at 1500 m for *F. quadranus*, at 1000 m for *R. chensinensis* and at 500 m for *B. argarizans.* All tadpoles were identified according to the development stage. Ten individuals of each species in the GS 36 were chosen for the experiment (Gosner 1960).

At the end of the experiment, tadpoles were anaesthetized with buffered MS222 and tissues (muscle, kidney, liver and intestine) were removed, frozen in liquid N_2 quickly, and then stored at -80°C for later lipid extraction. Total lipids of the tissues were extracted with chloroform/methanol (2:1, v/v) according to the method of Folch et al. (1957). Isolated lipids were methylated with 1.0% H_2SO_4 in methanol. The resulting fatty acid methyl esters (FAME) were then extracted with hexane and subjected to gas chromatography-mass spectrometry (GC-MS) analysis. GC-MS analysis was conducted by using a Shimadzu GC 2010 system (Shimadzu, Japan) equipped with a CBP capillary column (50 mm × 250 μm × 0.25 μm). The oven temperature program was as follows: the initial temperature was 130°C, then raised to 230°C at 8°C/min, then to 235°C at 1°C/min, and with a holding time of 1 min. The injector and detector temperatures were both set at 250°C. The fatty acid content was then expressed as percentages of the total fatty acid peak areas recognized in the GC analysis.

For identification of branched-chain saturated fatty acids, FAME were further prepared into pyrrolidide derivatives by the method of Andersson & Holman (1974) and identified by GC-MS. The amounts of each fatty acid composition were represented by the mole percentage. Polyunsaturated fatty acids (PUFA) represented the dienoic, trienoic, tetraenoic, pentaenoic, and hexaenoic fatty acids. Unsaturation index (USI) and mean chain length (MCL) were calculated as previously described by LeBlanc et al. (1995).

All statistics used the Statistica 6.0 (StatSoft, Tulsa, USA) to compare the significance. Data were expressed as means ± error (SE). One-way ANOVA and Turkey's multiple tests were used to examine the differences among the four species of tadpoles, and the statistical significance used the level of 0.05 to express ($P < 0.05$).

RESULTS

Here, we mainly identified 11 common fat acids by GC-MS including $C_{15}H_{30}O_2$(14:0), $C_{17}H_{32}O_2$(16:1), $C_{17}H_{34}O_2$(16:0), $C_{18}H_{36}O_2$(17:0), $C_{19}H_{34}O_2$(18:2), $C_{19}H_{36}O_2$(18:1), $C_{19}H_{38}O_2$(18:0), $C_{20}H_{40}O_2$(19:0), $C_{21}H_{34}O_2$(20:3), $C_{21}H_{34}O_2$(20:4) and $C_{23}H_{34}O_2$(22:5) respectively (Fig. 1). In the different aquatic environments, the composition of fatty acid had significant difference ($P < 0.05$). The fatty acids were mainly detected of seven types ($C_{15}H_{30}O_2$(14:0), $C_{17}H_{32}O_2$(16:1), $C_{17}H_{34}O_2$(16:0), $C_{19}H_{34}O_2$(18:2), $C_{19}H_{36}O_2$(18:1), $C_{19}H_{38}O_2$(18:0) and $C_{20}H_{40}O_2$(19:0)) for *B. Argarizans* at 500 m, seven types ($C_{15}H_{30}O_2$(14:0), $C_{17}H_{32}O_2$(16:1), $C_{17}H_{34}O_2$(16:0), $C_{19}H_{34}O_2$(18:2), $C_{19}H_{36}O_2$(18:1), $C_{19}H_{38}O_2$(18:0), and $C_{20}H_{40}O_2$(19:0)) for *R. Chensinensis* at 1000 m, six types ($C_{17}H_{32}O_2$(16:1), $C_{17}H_{34}O_2$(16:0), $C_{19}H_{34}O_2$(18:2), $C_{19}H_{36}O_2$(18:1), $C_{19}H_{38}O_2$(18:0) and $C_{21}H_{34}O_2$(20:4)) for *F. Quadranus* at 1500 m, and ten types ($C_{15}H_{30}O_2$(14:0), $C_{17}H_{32}O_2$(16:1), $C_{17}H_{34}O_2$(16:0), $C_{18}H_{36}O_2$(17:0), $C_{19}H_{34}O_2$(18:2), $C_{19}H_{36}O_2$(18:1), $C_{19}H_{38}O_2$(18:0), $C_{20}H_{40}O_2$(19:0), $C_{21}H_{34}O_2$(20:3) and $C_{23}H_{34}O_2$(22:5)) for *O. popei* at 2000 m (Fig. 1). For these four anuran species, the $C_{17}H_{34}O_2$(16:0), $C_{19}H_{34}O_2$(18:2), $C_{19}H_{36}O_2$(18:1) and $C_{19}H_{38}O_2$(18:0) were all the predominant fatty acids, and they have different rate of fatty acids among the four species ($P < 0.05$; Fig. 1), moreover, the fatty acids with greater than 22 carbon atoms (very long chain fatty acids, VLCFA) are present only in *O. Popei*.

The fatty acids include saturated fatty acid (SFA) and unsaturated fatty acid (UFA), and the UFA is made of two types: monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA). Among the four tadpoles, the content of PUFA in *B. gargarizans* at 500 m is the most than others ($P < 0.05$), however, the content of SFA in *B. gargarizans* is the least ($P < 0.05$) (Fig. 2).

DISCUSSION

Tadpoles have diverse diets, including animal larvae, aquatic plant, plant pollen and dissolved organic matter (Kupferberg 1997). These diverse diets have a wide variation in the nutritional quality across taxa and environments (Gladyshev et al. 2014, Doherty et al. 2015).

Many earlier studies have repeatedly documented that food types in water have key roles in the growth, development, and metamorphosis of anuran larvae (Kupferberg 1997), especially the compositions of fatty acids in the anuran larvae (Huang et al. 2003, Hixson et al. 2014, Gladyshev et al. 2014, Doherty et al. 2015, Egeler et al. 2016). For example, Huang et al. (2003) document that the compositions are different in the fatty acids between *Chirixalus eiffingeri* tadpoles feeding chicken egg yolk or

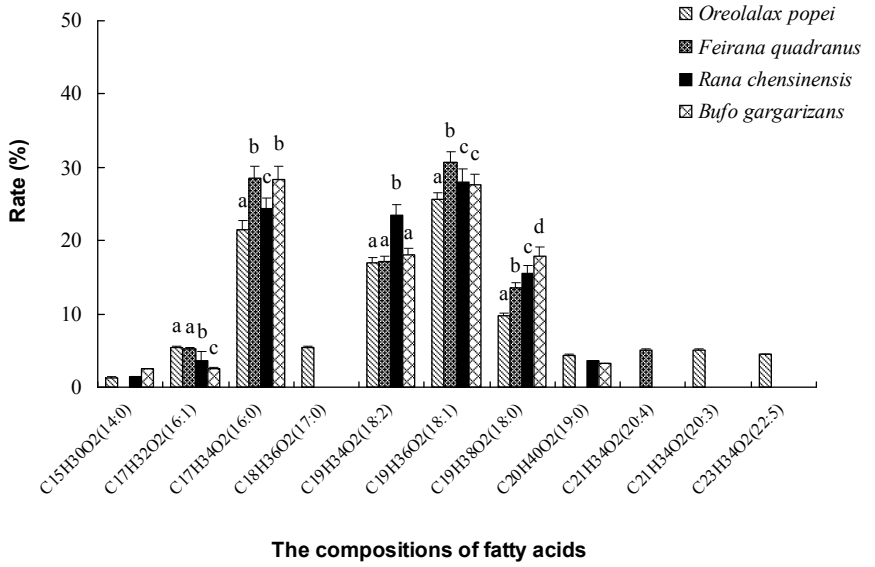

Fig. 1: The composition of fatty acids in four anuran amphibians (*B. Gargarizans* at 500 m, *R. chensinensis* at 1000 m, *F. quadranus* at 1500 m and *O. Popei* at 2000 m) in the different aquatic environments. Data are mean ± SE. Treatments labeled with different letters.

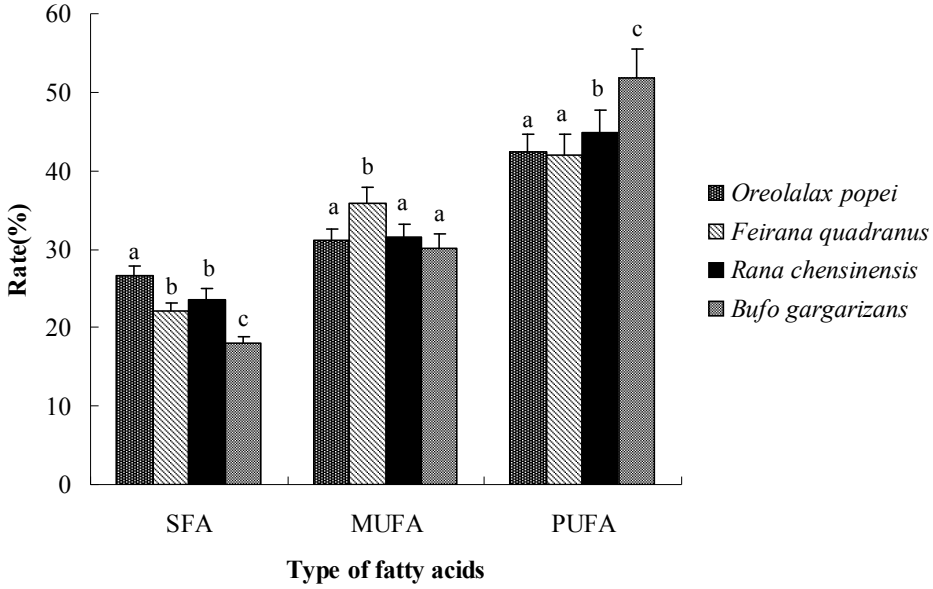

Fig. 2: The different contents of fatty acids in four anuran amphibians (*B. Gargarizans* at 500 m, *R. chensinensis* at 1000 m, *F. quadranus* at 1500 m and *O. Popei* at 2000 m) in the different aquatic environments. Data are mean ± SE. Treatments labeled with different letters were significantly different *($P < 0.05$).*

egg yolk of three anuran species (*Chirixalus eiffingeri*, *Rhacophorus moltrechti* and *Buergeria robustus*), which can reflect the variations in the diets. This result indicates that tadpoles can incorporate directly the fatty acids into their body without minimal modification. Our study also documented the similar results. The compositions of fatty acids are different among the tadpoles from different altitudes, which reflects the variation of the dietary sources. For the four anuran species, they live respectively in four different altitudes from 500 m to 1500 m for *B. gargarizans, R. chensinensis, F. quadranus* and *O. popei.* For example, the streams at low altitude have rich foods, while the streams

at high altitude have poor foods. Moreover, different foods have different types of fatty acids (Hixson et al. 2014, Gladyshev et al. 2014, Doherty et al. 2015, Egeler et al. 2016).

Fatty acids include SFA and UFA. SFA do not contain double bonds, and UFA includes MUFA and PUFA. Among them, MUFA contains one double bond, PUFA contains two or more double bonds in the acyl chain and the position of the first double bond counted from the methyl terminus (Calder 2009). SFA and UFA are known to be essential for the growth, development and metamorphosis of anuran larvae, in which the PUFA is the essential fatty acid in the living organism coming from the food. The function of PUFA can ensure the cell's normal physiological functions and improve brain cell activity, memory and thinking ability. Our study also documented that the content of SFA and UFA had a significant difference among the four anuran species. Among the four tadpoles, the content of PUFA in *B. gargarizans* at the relative low altitude (500 m) is the most compared to others. The reasons may be that in the low altitude, the resources of food are rich, and *B. gargarizans* can obtain rich food in the rivers.

It is known that the fatty acids with very long chain fatty acids greater than 22 carbon atoms (VLCFA) are present in small amounts in most animal tissues. Saturated and monoenoic VLCFA are major components of brain, while the polyenoic VLCFA occur in significant amounts in certain specialized animal tissues such as retina and spermatozoa (Poulos 1995). In our results, the VLCFA existed only in the content of *O. popei* at the relative high altitude (2000 m). The possible reasons include: 1) Different foods have different types of fatty acids, especially the UFA (Hixson et al. 2014, Gladyshev et al. 2014, Doherty et al. 2015, Egeler et al. 2016), and 2) temperature in waters has an important effects on the composition of fatty acids. For example, Gladyshev et al. (2014) observed that the two taxa (Cladocera and Copepoda) have higher percentages of 18:0 and lower percentages of 14:0 and 18:4 in warm waters than those in cold waters.

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