Agriculture, Ecosystems and Environment 279 (2019) 74–79

Contents lists available at ScienceDirect

Agriculture, Ecosystems and Environment

journal homepage: www.elsevier.com/locate/agee

Breeding habitat influences abundance and body condition of rice frog (*Fejervarya multistriata*) in agricultural landscape of Shanghai, China

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ARTICLE INFO

Keywords:
Rice frog
Farmland
Nutrient condition
Population density
Irrigation ditches

ABSTRACT

Amphibians are threatened by the intensification of agriculture throughout the world. Several studies have considered the morphology of animals to be an indicator of the health of a population, but differences in anuran morphology (especially body condition) in different breeding habitats in agricultural landscapes remain largely unknown. In this study, we investigated differences in the abundance and morphology of the rice frog (*Fejervarya multistriata*) in three waterbody types (ten farmland irrigation ditches, ten fruit forest drainage ditches, and ten lotus ponds) in agricultural landscapes in Shanghai, China. We sampled the snout–vent length, body mass, and body condition index for 206 individual rice frogs from the three types of waterbody. Our results showed that the abundance of rice frogs was higher in farmland irrigation ditches than in the other two habitats, whereas the snout–vent length, body mass, and body condition index of rice frogs were lower in farmland irrigation ditches than in the other two habitats. Our results suggest that breeding habitats in agricultural landscapes have different effects on rice frog abundance and morphology, and that constructing diverse waterbodies (other than farmland irrigation ditches) in the same agricultural landscape might benefit the condition of rice frogs.

1. Introduction

Amphibians are the most threatened vertebrates in the world. The 2018 IUCN Red List showed that 40% of amphibian species are threatened, far higher than mammals (25%) and birds (14%) (IUCN, 2018). Global urbanization is regarded as the main threat to amphibian populations (Hamer and McDonnell, 2008). Shanghai is the city with the highest levels of urbanization in China and there has been a rapid decline in the species richness, abundance, and body condition of amphibians due to urbanization in this area (Li et al., 2016; Zhang et al., 2016). As an important habitat for amphibians in a highly urbanized city such as Shanghai (Li et al., 2017), it is vital to conserve amphibian biodiversity in agricultural landscapes because it has the greater potential to support amphibian biodiversity than urban environments and is considered a substitute for natural habitats (Donald, 2004).

However, agricultural intensification has led to a rapid decline of anuran biodiversity worldwide (Benton et al., 2003; Hamer et al., 2004; Kato et al., 2010). In China, amphibians, especially rice frogs and toads, in agricultural landscape face a rapid decline because of agrochemical contaminants (Nanjing Institute of Environmental Sciences, 2018). In addition, previous studies also concluded that landscape structure could affect amphibian biodiversity in agricultural landscapes (Suárez et al., 2016; Collins and Fahrig, 2017). Amphibians in farmlands depend on waterbodies during the early stage of their life cycle, although adults also need waterbodies in farmlands to breed and migrate (Watabe et al., 2012; Kidera et al., 2018). Therefore, waterbodies have an important role in maintaining amphibian populations in agricultural landscapes.

Environmental differences are considered to generate stresses that can lead to changes in the morphology and nutritional status of organisms (Sumner et al., 1999; Matías-Ferrer and Escalante, 2015), whereas environmental change can also favor phenotypic variation and differences in the nutritional status among populations even at a landscape scale (Guillot et al., 2016). In agricultural landscapes, amphibians are readily harmed by agrochemicals (Hamer et al., 2004) and intense physical disturbances (Cayuela et al., 2017). For instance, Guillot et al. (2016) found that the hind legs and forelegs of male toads were less symmetrical in an agricultural landscape compared with those in a forest landscape, indicating that the amphibians were exposed to more environmental stress in the agricultural landscape. However, whether breeding habitat, which is on a smaller spatial scale than the

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https://doi.org/10.1016/j.agee.2019.04.003
Received 17 December 2018; Received in revised form 1 April 2019; Accepted 2 April 2019
Available online 15 April 2019

overall agricultural landscape, affects amphibian morphology and nutritional status is unclear.

The rice frog (*Fejervarya multistriata*) is one of the most widely distributed anuran species in China and South Asia (van Dijk et al., 2004). According to terrestrial wildlife studies conducted in Shanghai during 2013–2015, the rice frog is the dominant amphibian in Shanghai, where it is widely distributed in rural areas (Li et al., 2017). In particular, farm fields are an important habitat for the rice frog in rural Shanghai (Li et al., 2017). However, the agricultural landscape of Shanghai has decreased in size over the past 30 years, whereas the use of pesticides and fertilizers has increased (Shanghai Municipal Statistics Bureau, 2016). This frog species has been negatively affected by urbanization and it is rarely seen in urban areas of Shanghai (Zhang et al., 2016; Li et al., 2018). Previous studies examined the variations in the body condition of the rice frog in different seasons and with age (Li et al., 2016), but it is not known how farm patterns and breeding habitat might influence its morphology (including body condition), where appropriate changes could be designed to improve amphibian biodiversity and further assess agricultural habitat quality in Shanghai. Hence, the objectives of this study were to understand the differences in the abundance and morphology (snout–vent length, body mass, and body condition) of the rice frog in three common breeding habitats (farmland irrigation ditches, fruit forest drainage ditches, and lotus ponds) in the agricultural landscape of Shanghai.

2. Materials and methods

2.1. Study species

The rice frog is an ideal candidate for testing the effects of breeding habitat quality on the health of amphibian populations in agricultural landscapes. It is one of the most abundant amphibians in Shanghai farmlands, which enabled us to obtain a suitable sample size for statistical tests. In addition, rice frogs are distributed widely in farmland ditches, lotus ponds, and fruit forest drainage ditches in agricultural landscapes (Li et al., 2017); thus, we could compare differences in morphology in diverse breeding habitats in agricultural landscapes. Although few studies have focused on the ability of the rice frog to move across habitats, its small body size ($<$ 50 mm in this study) might limit its ability to move any significant distance across agricultural landscapes.

2.2. Study site

Shanghai is located in eastern China in the southeastern Yangtze River Delta. The total and rural areas of Shanghai are 6340.5 and 3700 km^2, respectively. The common crops grown in the agricultural landscapes of Shanghai are rice and wheat. In addition, various vegetables, watermelon, peach, citrus, and lotus root are cultivated. Three common water bodies are constructed by different crop patterns in agricultural landscapes of Shanghai: farmland irrigation ditches are grass ditches along farmlands cultivated with rice, wheat, watermelon, and/or diverse vegetables; fruit forest drainage ditches are grass ditches along fruit woodlands cultivated with peach and citrus; and lotus ponds are mainly cultivated with lotus (Fig. 1).

We selected 30 1-km radius agricultural landscapes in Shanghai that contained $>$ 50% farmland (mainly paddy fields). Rice frogs were sampled along a single transect measuring 500 m (length) $\times$5 m (width) along permanent or semi-permanent water bodies in each landscape. Each waterbody type was sampled in ten of the 30 landscapes: farmland irrigation ditches, fruit forest drainage ditches, and lotus ponds. These three waterbody types are considered the main suitable breeding habitats for rice frogs in rural Shanghai (Li et al., 2017). To avoid the effect of crop type on rice frog abundance and morphology (Suárez et al., 2016; Collins and Fahrig, 2017), we chose farmland irrigation ditches mainly cropped by rice and fruit forest drainage ditches mainly cropped by citrus. The distance between the edges of each landscape was $>$ 3 km to ensure spatial independence.

2.3. Rice frog surveys

Rice frogs emerge from hibernation at the end of March and early April. Breeding occurs between the end of April and the end of August (Huang, 1990). Therefore, we surveyed rice frogs and collected individual samples from May to September. We conducted four visual surveys (Crump and Scott, 1994) along each transect during May to June (breeding season: two surveys) and September (nonbreeding season: two surveys) in 2016. Surveys were conducted at least 0.5 h after sunset (19:00–24:00 h) when there was no rain and the wind speed was $<$ 30 km/h. The surveys involved three people walking at a steady walking speed of 1.5 km/h along each transect in a group with flashlights to search for rice frogs. The rice frog abundance in each landscape was the average rice frog population density detected in the four surveys.

During the surveys, a total of 206 rice frogs (120 adult females and 86 adult males) were captured by hand in the 30 landscapes and placed in individual bags. Their body condition was assessed based on measurements of body mass and snout–vent length, where body mass was measured to the nearest 0.01 g with a portable electronic balance and snout–vent length was recorded to the nearest 0.1 mm with an electronic digital caliper. The body condition index (BCI) of each frog was taken as its residual value based on a regression of log body mass on log snout–vent length across all individuals (Welsh et al., 2008; Băncilă et al., 2010; Li et al., 2016). Each individual was sexed based on the presence or absence of nuptial pads (Fei et al., 2009). The rice frog BCI was used to represent the body condition in this study.

2.4. Statistical analyses

One-way ANOVA was used to test the differences in the abundance of rice frogs in the three common breeding habitats (farmland irrigation ditches, fruit forest drainage ditches, and lotus ponds) in the agricultural landscape. Tukey's honestly significant difference (HSD) test was used to conduct post hoc multiple comparisons. Given the high naive occupancy of rice frog in this study and fewer changes in occupancy during the survey period, we did not run an occupancy model with species presence–absence in this study (Mackenzie et al., 2006).

To test the differences in the snout–vent length, body mass, and BCI of the rice frog in the three common breeding habitats in the agricultural landscape, the values of each of these measurements for each rice frog were used as the response variables. Given the potential differences in anuran snout–vent length, body mass, and BCI according to the season and sex (Băncilă et al., 2010), general linear mixed models (GLMMs) were used to test potential differences in these variables with respect to season, sex, and breeding habitat, including all three factors and the interactions among them; the capture site was recorded as a random factor. Tukey's HSD test was used to conduct post hoc multiple comparisons between the three different breeding habitats.

All statistical analyses were performed using R 3.5.1 (R Core Team, 2018), GLMMs were performed in R with the nlme package (Pinheiro et al., 2018). Shapiro–Wilk test was used to test the normality of rice frog population density in each landscape and the snout–vent length, body mass, and BCI for each of the 206 rice frogs.

3. Results

In total, we observed 1957 rice frogs and the species was recorded in all study sites during each survey. The rice frog population density in farmland irrigation ditches (mean $\pm$ SE = 0.140 $\pm$ 0.018 ind. m^{-1}) was significantly higher than that in fruit forest drainage ditches (mean $\pm$ SE = 0.027 $\pm$ 0.003 ind. m^{-1}) and lotus ponds (mean $\pm$ SE = 0.029 $\pm$ 0.006 ind. m^{-1}) ($P < 0.01$) (Fig. 2).

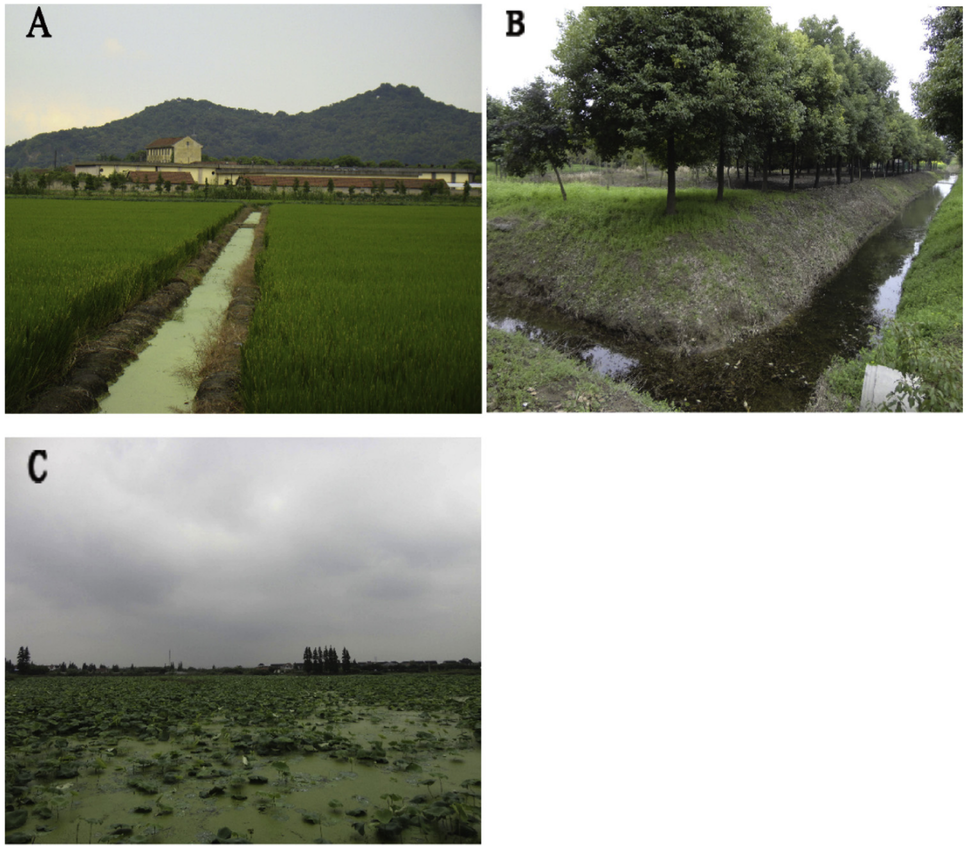

Fig. 1. Three common water bodies result from different crop patterns in the agricultural landscapes of Shanghai: (A) farmland irrigation ditches, (B) fruit forest drainage ditches, and (C) lotus ponds.

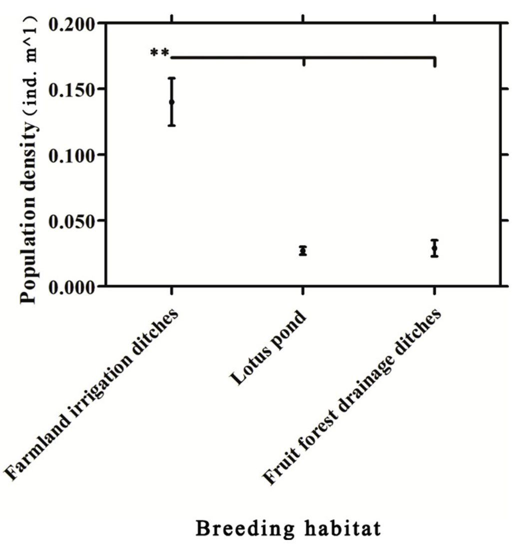

Fig. 2. Differences in the population density of rice frogs among breeding habitats: farmland irrigation ditches, fruit forest drainage ditches, and lotus ponds. Results represent the mean ± 1 standard error (SE) of population density (ind. m^{-1}). Horizontal lines indicate significant pairwise differences in the body condition index of rice frogs between breeding habitats. **Significantly different at $P < 0.01$.

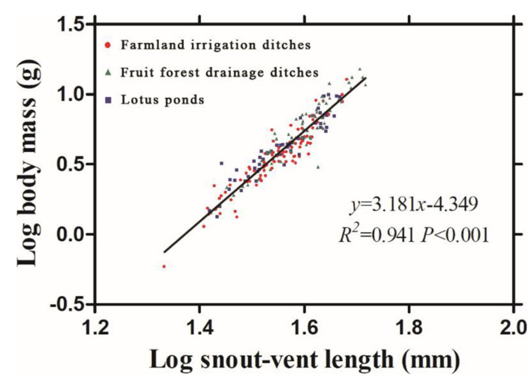

Fig. 3. Relationship between log10-transformed body mass and snout–vent length in rice frogs ($n = 206$).

The regression used to estimate body condition was: log body mass = –4.349 + 3.181 log snout–vent length ($R^2 = 0.941$, $P < 0.001$, Fig. 3). All response variables were normally distributed ($n = 206$, all $P > 0.05$). Snout–vent length, body mass, and BCI of rice frog were significantly different between breeding habitats according to GLMMs (all $P < 0.05$, Table 1, Appendix A). The rice frog body mass and BCI also differed significantly between season ($P < 0.001$, $P < 0.05$, Table 1). The rice frogs in farmland irrigation ditches exhibited smaller snout-vent length and lower body mass than those in fruit forest drainage ditches (Tukey's HSD test, $P < 0.01$, Fig. 4A and B). Tukey's HSD test also showed that BCI values for rice frogs in lotus ponds and fruit forest drainage ditches were significantly higher than those in farmland irrigation ditches ($P < 0.01$, $P < 0.05$, Fig. 4C).

4. Discussion

In this study, we found that rice frog abundance in farmland irrigation ditches was higher than that in fruit forest drainage ditches and lotus ponds (Fig. 2). However, snout–vent length and body mass of rice frogs in farmland irrigation ditches were lower than those in fruit forest

Table 1
General linear mixed model results with respect to variations in rice frog body mass, snout–vent length, and body condition index measured as the residual index[a].

Factor	*df*	*F*	*P*
Snout–vent length			
Breeding habitat	2, 196	3.801	**0.024**
Sex	1, 196	0.188	0.665
Season	1, 196	1.524	0.219
Breeding habitat * Sex	2, 196	0.320	0.727
Breeding habitat * Season	2, 196	5.286	0.135
Sex * Season	1, 196	2.400	0.096
Body mass			
Breeding habitat	2, 196	5.473	**0.020**
Sex	1, 196	7.510	0.070
Season	1, 196	13.220	**< 0.001**
Breeding habitat * Sex	2, 196	0.360	0.698
Breeding habitat * Season	2, 196	3.462	0.333
Sex * Season	1, 196	22.086	0.115
Body condition index			
Breeding habitat	2, 196	3.968	**0.020**
Sex	1, 196	3.574	0.060
Season	1, 196	3.989	**0.047**
Breeding habitat * Sex	2, 196	0.690	0.503
Breeding habitat * Season	2, 196	3.977	0.220
Sex * Season	1, 196	0.530	0.468

[a] The factors included season (breeding season:104 individuals, nonbreeding season: 102 individuals), sex (female: 120 individuals, male: 86 individuals), breeding habitat (farmland irrigation ditches: 91 individuals, fruit forest drainage ditches: 63 individuals, lotus ponds: 52 individuals) and the interactions among them. Capture site ID was used as a random factor.

drainage ditches, and BCI of rice frogs in farmland irrigation ditches was lower than that of those in fruit forest drainage ditches and lotus ponds (Fig. 4).

Farmland is the most important habitat for rice frogs in Shanghai (Li et al., 2017) and rice frogs tend to choose farmland irrigation ditches as their breeding habitat (Fei et al., 2009), because of the low water depth and mud bank (Huang et al., 2018). In addition, as semi-permanent water bodies, lotus ponds are often drained in the winter to gather louts, which could have a negative effect on rice frog abundance in lotus ponds. Therefore, more rice frogs were found in farmland irrigation ditches than in two other waterbodies due to the microhabitats and farming mode. Although rice frogs were detected in all study sites during each survey in this study, the detectability and error in the surveys of rice frogs in the three waterbody types might vary due to differences in vegetation cover, water depth, and other environmental factors (Mazerolle et al., 2007). For instance, lotus ponds have the deepest water depth and highest aquatic vegetation cover of these three waterbody types, making it much more different to survey and catch anurans than in the other two waterbody types.

Rice frogs are an aquatic anuran species with little or no resistance to water loss compared with terrestrial and arboreal anuran species (Young et al., 2005). Fruit forest drainage ditches and lotus ponds are characterized by higher soil and air moisture levels, and are similar to the natural habitat of this species. Thus, anurans living in these habitats might have a lower rate of water loss (i.e. higher body mass and body condition) under microclimate conditions with more cover and less disturbance (Mazerolle and Desrochers, 2005). Besides, we also need to explore whether the differences in environmental conditions could delay the development of this species in the farmland irrigation ditches compared to the other habitats, such information could help us to understand the effect of microclimate factors on anuran body condition.

In addition, the lower snout–vent length, body mass, and BCI of rice frogs showed that these frogs experienced greater environmental stress during their larval and post-metamorphic growth stages in the farmland irrigation ditches (Söderman et al., 2007). This environmental stress could result from agricultural intensification, which is characterized by the greater use of fertilizers, herbicides, and insecticides, and mechanized cultivation methods (Hamer et al., 2004). Anuran species in farmland irrigation ditches are affected by many agrochemical substances (Berger et al., 2012). However, farm managers seldom use agrochemical substances in lotus ponds and some highly toxic pesticides are also banned in Chinese orchards (Yao et al., 2010; Fang et al., 2013). Previous studies showed that agrochemical substances have negative effects on the juvenile stages of amphibians in water bodies (Boyer and Grue, 1995; Ortiz et al., 2004; Mann et al., 2009) and can also harm amphibians on the land (Oldham et al., 1997; Ortiz et al., 2005). Moreover, because of the human-dominated landscape, the high levels of human disturbance in farmlands might have changed the land-use structure to directly and/or indirectly affect anuran morphology, especially mechanized cultivation (Fujioka and Lane, 1997), which should be assessed in future studies.

Finally, lower food availability and higher intraspecific competition might be the most important explanations for the low snout–vent length, body mass, and BCI of rice frogs in farmland irrigation ditches. Tews et al. (2004) showed that resources are scarcer and poorer in farmland environments than in natural habitats. In addition, Ousterhout et al. (2015) demonstrated the negative relationship between intraspecific anuran density and body size. Moreover, the intraspecific competition among rice frogs in farmland irrigation ditches is more intense than in fruit forest drainage ditches and lotus ponds because of the higher abundance of rice frogs in farmland irrigation ditches, according to the results of this study. Therefore, our study in addition highlights a shortcoming of using abundance and morphology separately to assess habitat quality and anuran population health.

In Chinese traditional agricultural landscapes, orchards and lotus pond are set as mosaics in an irrigated farmland-dominant landscape. However, intensive agriculture, which is a simple farming mode with fewer waterbody types and more homogeneous agricultural landscape, tends to be more prevalent in rapidly urbanized cities in Asia, such as Shanghai. Therefore, we suggest that waterbody type diversity should be improved in agricultural landscapes to support the growth of anuran populations. However, the effects of agricultural landscape heterogeneity on anuran populations still require further study.

5. Conclusion

In this study, we found that the abundance of rice frogs was higher in farmland irrigation ditches than in fruit forests drainage ditches and lotus ponds, whereas the snout–vent length, body mass, and BCI of rice frogs were lower in farmland irrigation ditches than in the other two habitats. We conclude that breeding habitats in agricultural landscape can influence rice frog abundance and morphology. Constructing diverse waterbodies in the same agricultural landscape could improve rice frog nutrient condition in Asian agricultural landscapes.

Acknowledgments

This work was supported by the Shanghai Landscaping and City Appearance Administrative Bureau (No. F131508), and the Science and Technology Commission of Shanghai Municipality (No.18DZ1205001). We thank Dr. Xiaoxiao Shu for her assistance with fieldwork, and the two anonymous reviewers for their insightful comments on earlier versions of this manuscript.

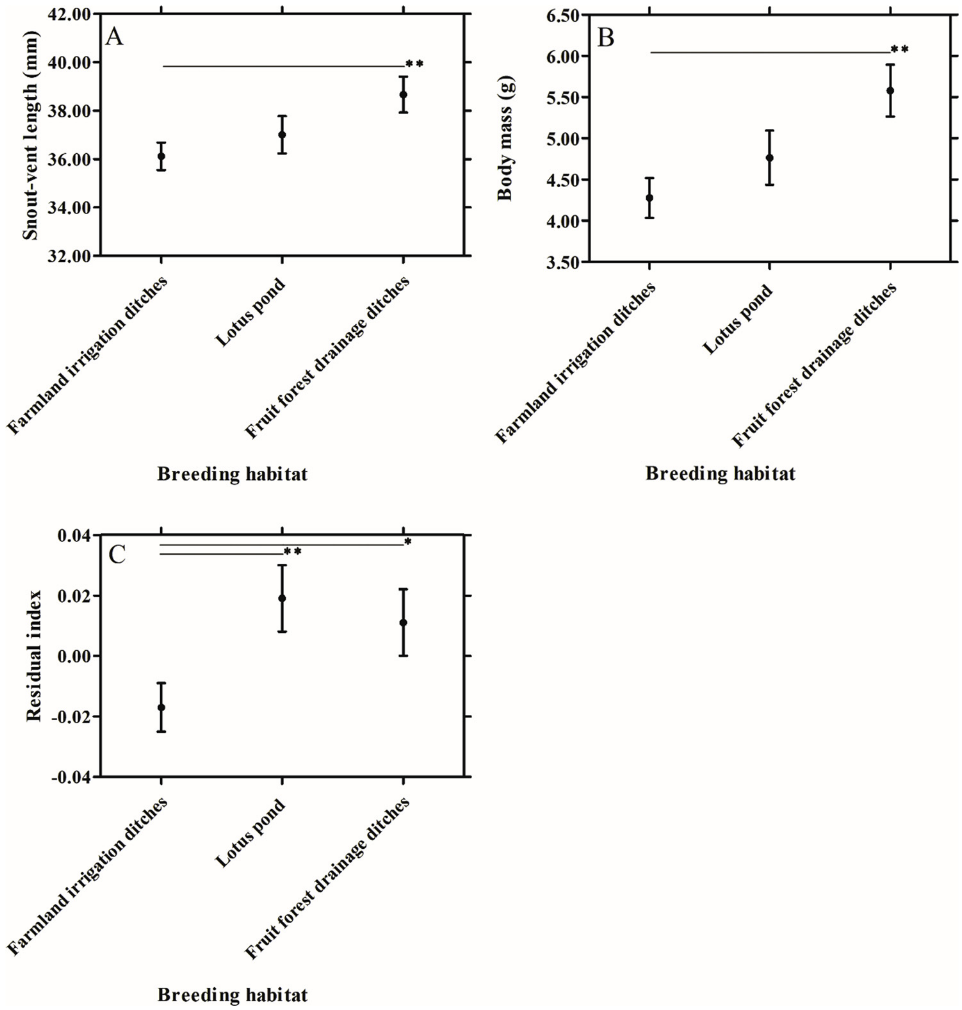

Fig. 4. Differences in the snout–vent length (A), body mass (B), and body condition index (C) of rice frogs among breeding habitats: farmland irrigation ditches, fruit forest drainage ditches, and lotus ponds. Results represent the mean ± 1 standard error (SE) snout–vent length, body mass, body condition index (residual index) based on general linear mixed models. Horizontal lines indicate significant pairwise differences in the snout–vent length, body mass, and body condition index of rice frogs between breeding habitats. **Significantly different at $P < 0.01$.*Significantly different at $P < 0.05$.

Appendix A. Summarized morphology data indicating mean ± 1 SE of snout–vent length, body mass, and body condition index of rice frogs in farmland irrigation ditches, fruit forest drainage ditches, and lotus ponds

Morphological character	Farmland irrigation ditches	Fruit forest drainage ditches	Lotus pond
Snout–vent length (mm)	36.122 ± 0.569	38.672 ± 0.741	37.011 ± 0.771
Body mass (g)	4.276 ± 0.242	5.579 ± 0.315	4.765 ± 0.328
Body condition index	−0.017 ± 0.008	0.011 ± 0.011	0.019 ± 0.011

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