Demography of Two Small Breeding Populations of Taipei Grass Frog, *Rana taipehensis* van Denburgh (Amphibia, Anura)

Chi-Tai Lien[1], Hwa-Ching Lin[2], Kuang-Yang Lue[1*]
[1]Depatment of Life Sciences, National Taiwan Normal University
Taipei, Taiwan
[2]Taipei Zoo, Taipei, Taiwan

(Received: 29 December 2006, accepted: 2 March 2007)

ABSTRACT

In 2003 and 2004, we surveyed different populations of Taipei grass frog (*Rana taipehensis*) living at Sanchih and Yangmei, Taiwan, using waist-band and toe-clipping methods. Sex ratios of breeding populations of this frog were skewed to males. The ratio of male to female frogs from Sanchih population (16.13:1) was almost twice that from Yangmei (6.97:1). Estimated population sizes, using the Schnabel method, were declining in 2004 both at Sanchih and Yangmei. We defined one line of arrested growth (LAG) as one year old. The oldest frog was 4 years old according to skeletochronology of both males and females. The age of females (2.56±0.09 LAGs) was significantly older than that of males (2.30± 0.03 LAGs). Based on age structure, some males reached sexual maturity and joined the breeding population in 1 year. For females it was in 2 years. From age 2 to 3, females living at Yangmei had the highest survival rate (0.68), but at Sanchih had the lowest survival rate (0.22). From the survival rates of females, we supposed that disturbed habitats (at Sanchih) may have a negative effect on females. The sex ratio may be affected by human disturbance indirectly.

Key words: demography, breeding population, LAG

Introduction

The Taipei grass frog (*Rana taipehensis* van Denburgh, 1909) is a small to medium size frog. Adult males are usually smaller than 3 cm in snout-vent length (SVL). Females are seldom bigger than 4 cm. Its most unique character is on both lateral sides of the body: a golden dorsolateral fold with black stripes. In Taiwan, the frog's breeding season is from April to September. The metamorphosis of tadpoles occurred near the end of August, after which all adults and froglets disappeared from the breeding sites (Yang, 2002).

Although the Taipei grass frog is widely distributed throughout South China, Vietnam, India, its distribution is fragmented in Taiwan (Chou *et al.*, 1993). It is also on the Taiwan's endangered species list (Wildlife Conservation Law, Taiwan, R. O. C., 1989). However these are what we know about this frog, we do not know anything about its longevity, demography, and survival rate. Before the Taipei grass frog extinct in Taiwan, we should get these informations in the hope that these data may help us to conserve this species.

Since 1970s, skeletochronology has been used to determinate the age of amphibians and reptiles (Tsai, 2002b). In temperate regions, annual temperature fluctuations have strong influence on the growth-mark formation of long bones of frogs and lizards (Esteban *et al*., 1996). In subtropical and tropical regions, food availability caused by seasonal climatic change will also affect the formation of lines of arrested growth (LAGs) (Guarino *et al.*, 1998; Kumbar and Pancharatna, 2001a, 2001b, 2004; Lin and Hou, 2002). Because of its precision in age determination, skeletochronology techniques were applied to several subjects, including classifying fossil species (Esteban *et al.*, 1998); investigating sexual size dimorphism (Marquez *et al.*, 1997; Tsai, 2002a;

*Corresponding author: Kuang-Yang Lue; FAX:886-2-29312904; E-mail: luky66@ntnu.edu.tw

Monnet and Cherry, 2002; Schäuble, 2004); studying life history (Eggert and Guyétant, 1999; Esteban *et al.*, 1999, 2004; Guarino *et al.*, 2003; Lima *et al.*, 2000; Marunouchi *et al.*, 2002; Tsiora and Kyriakopoulou-Sklavounou, 2002); and evaluating age structure of populations at different altitudes (Esteban and Sanchiz, 2002; Morrison *et al.*, 2004). In this paper, we used the skeletochronology to study the demography, longevity, and survival rate of *R. taipehensis*.

Material and methods

We chose two populations for investigation: Sanchih (N 25^{o} 15' 41.4", E 121 ^{o} 31' 21.8") and Yangmei (N 24 ^{o} 56' 10.4", E 121 ^{o} 10' 4.2"), Taiwan (Fig. 1). The site at Sanchih is a terraced rice paddy with water lilies. The farmer did some agricultural activities like regular weeding and harvest of water lilies. At Yangmei, the study site includes two abandoned ponds and one farm. No one else except us would be there. We surveyed both sites twice every month from April to September in 2003 and 2004. Each survey, we began to search and capture Taipei grass frogs at 7 o'clock pm. After almost frogs we could find were captured, we marked every captured frog a unique number. In 2003, we used the waist-band method (Chen, 1992) to mark individual frogs. In 2004, we used toe-clipping method (Martof, 1959). In 2003, we clipped the fourth toe of the left hind leg from all captured frogs for skeletochronology. In 2004, we clipped the fourth toe of the right hind leg. After marking, we released them. Mark-recaptured data were used to estimate the size of the Sanchih and Yangmei populations with the Schnabel method (Seber, 1982).

We fixed the second phalange of the fourth toe in 95% alcohol in the field. Following the methods of Tsai (2002a) and Marunouchi *et al.* (2002), we first decalcified the frog phalanges in 3% nitric acid for 3 hours, and then soaked them in tap water for another 3 hours. We cross-sectioned the phalanges (20 μm thick) with a freezing microtome and stained the sections with Erich's haematoxylin for 30 minutes. After rinsing sections with tap water for

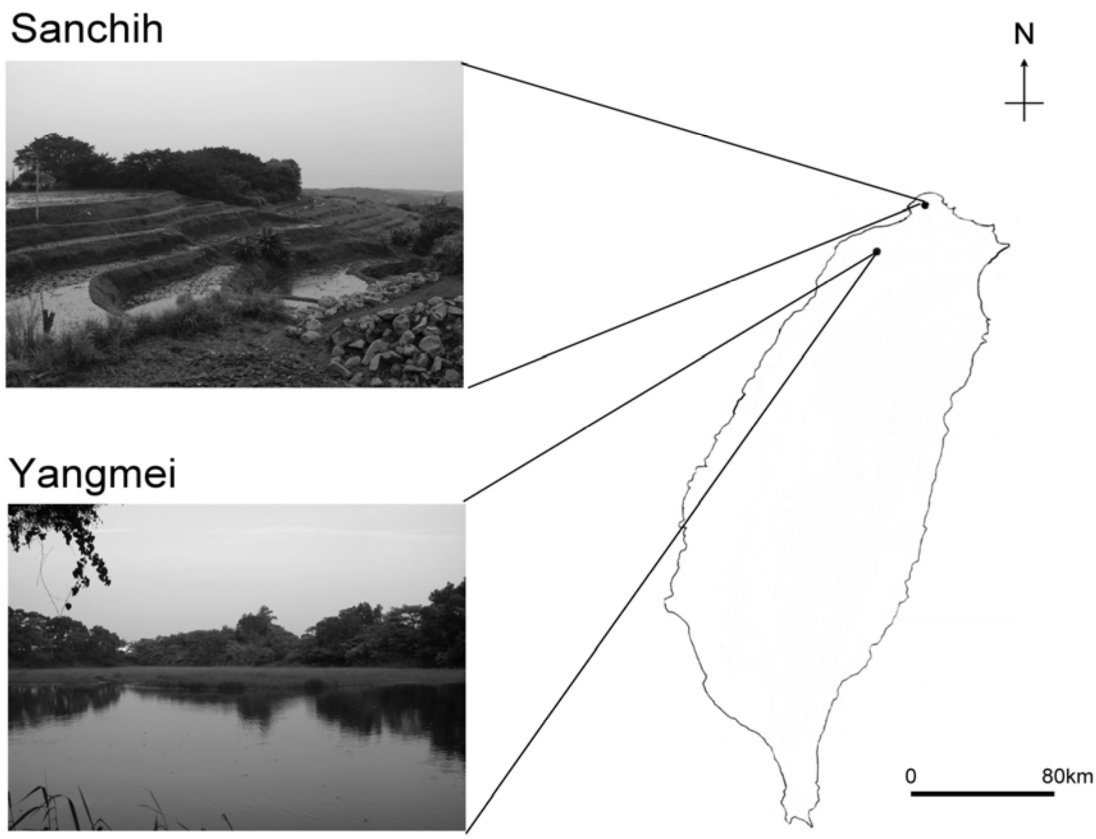

Figure 1. The study sites using for the studies of demographic, longevity, and survivorships of the Taipei grass frog (*Rana taipehensis*) were investigated in 2003 and 2004: Sanchih, and Yangmei.

30 minutes, we examined the entire section under an optical microscope to determine the age of frogs. All data were analyzed with the statistical program JMP (Lehman *et al*., 2002).

After constructing the demographies from known-aged frogs based on the phalange cross-sections, we extrapolated by the Schnabel method to estimate the demographies of the Sanchih and Yangmei populations. We estimated the relative frequency of each age class. Then, we calculated the cross year survival rate for each age class.

Results

The dorsum of Taipei grass frog is usually green, but sometimes we found brown individuals. Based on air field observations, the green or brown individuals did not change the color with environment.

In the breeding seasons of 2003 and 2004, we totally marked 371 adult males and 23 females in Sanchih and 258 males and 37 females in Yangmei. Sex ratios from both study sites were significantly skewed towards males (Table 1).

Because some waist-band marks were lost in the later part of the 2003 breeding season, we used the Schnabel method to estimate the size of the Sanchih and Yangmei populations. In Sanchih, the population sizes were 347 in 2003 and 203 in 2004. In Yangmei, they were 544 in 2003 and 226 in 2004.

Under the microscope, we examined phalange sections from 689 individuals. Of these, the age of 620 frogs could be determined easily; they showed clear LAGs (Fig. 2). The males were 1 to 4 years old. Females were 2 to 4 years old (Fig. 3). With analyzing the effect of different years, places, and

Table 1. Number and sex ratio of Taipei grass frog (*Rana taipehensis*) marked at Sanchih and Yangmei, Taiwan, from April to September 2003 and 2004.

Year	Sanchih				Yangmei			
	Male (N)	Female (N)	Sex Ratio	χ^2	Male (N)	Female (N)	Sex Ratio	χ^2
2003	207	17	12.18:1	161.16*	159	20	7.95:1	107.94*
2004	164	6	27.33:1	146.85*	99	17	5.82:1	57.97*
Total	371	23	16.13:1	307.37*	258	37	6.97:1	165.56*

*$P < 0.0001$

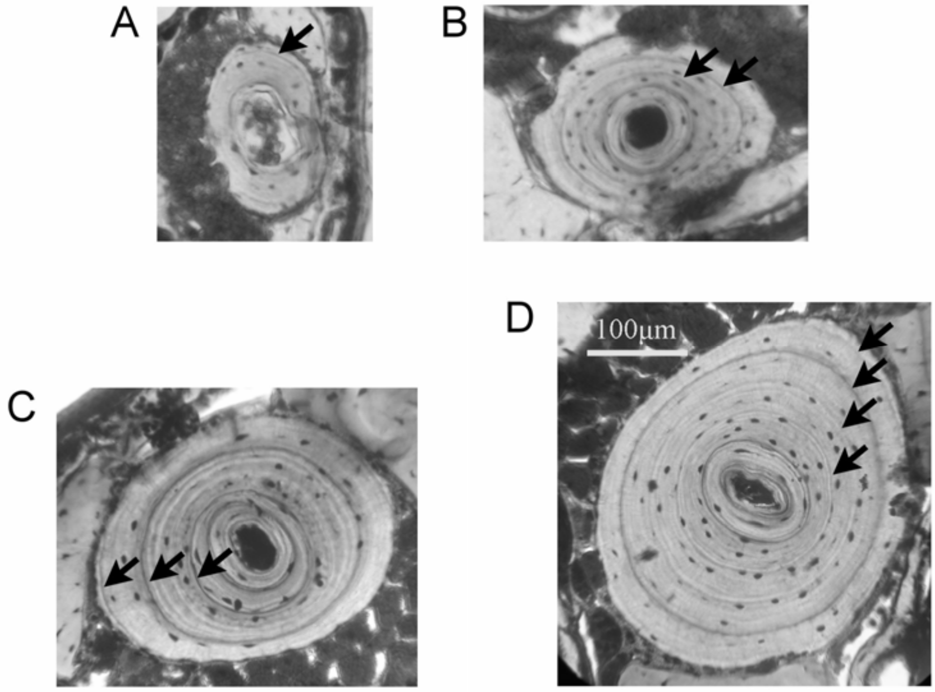

Figure 2. Phalange sections from Taipei grass frog (*Rana taipehensis*) trapped at Sanchih and Yangmei, Taiwan, from April to September 2003 and 2004: A) 1-year-old male, B) 2-year-old male, C) 3-year-old male, and D) 4-year-old female. Arrows indicate the lines of arrested growth (LAGs) formed during the winter.

sexes on age, there were only significant age differences between the sexes (ANOVA: F=8.19, *P*=0.0044). The mean age of females (2.56±0.09 LAGs) was significantly older than that of males (2.30±0.03 LAGs). Contingency analysis revealed is no significant difference between female age structure in the two study sites (χ^2=2.38, *P*=0.8820). For males, the difference was significant (χ^2=31.37, *P*=0.0003).

We calculated the cross year survival rate for each age class (Table 2). The survival rate from age 2 to 3 was highest of females living in Yangmei, but lowest of females in Sanchih. In Sanchih, males had higher survival rate than females. But in Yangmei, females had higher survival rate than males.

Discussion

The sex ratios of Taipei grass frog during breeding seasons at Sanchih and Yangmei were skewed towards males. At Sanchih, ratio of males to females was almost twice that in Yangmei. The difference may be caused by different environmental conditions. In Sanchih, the study site is of terraced fields planted with water lilies. Regular weeding and harvest of water lilies eliminated the vegetation cover. Surrounding vegetation is sparse compared to that at Yangmei where the abandon ponds were surrounded by shrubs and weeds. The vegetation cover may provide shelters for females, giving the female frogs at Yangmei a higher survival rate. This higher female survival rate at Yangmei increased the sex ratio during the breeding seasons (Table 1).

Worldwide amphibian declines are mainly due to habitat loss and over harvesting (Stuart *et al*., 2004). According to our study, the sizes of Sanchih and Yangmei populations in 2004 estimated using the Schnabel method were almost half of those in 2003.

Table 2. Survival rates of each age class to next class of Taipei grass frog (*Rana taipehensis*) trapped in Sanchih and Yangmei, Taiwan, from April to September of 2003 and 2004.

Age*	Sanchih		Yangmei	
	Male	Female	Male	Female
2 → 3	0.33	0.22	0.23	0.68
3 → 4	0.02	0	0.06	0.12
>4	0	0	0	0

*data not collected for ages 0→1 and 1→2.

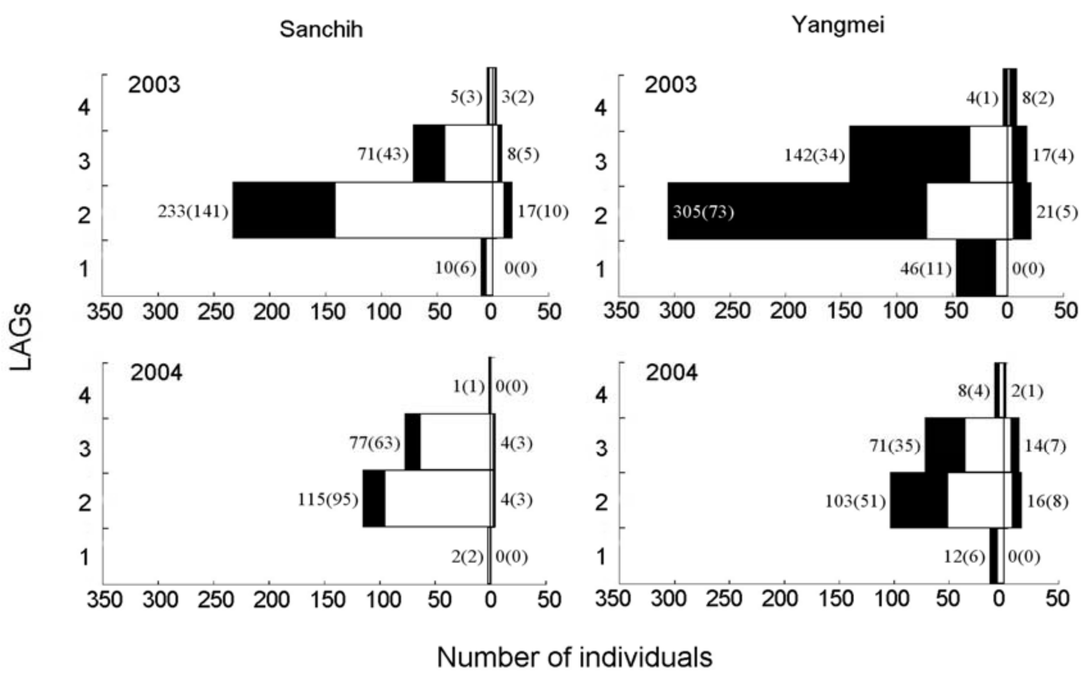

Figure 3. Demography of Taipei grass frog (*Rana taipehensis*) according to skeletochronology of those trapped in Sanchih and Yangmei, Taiwan, from April to September 2003 and 2004. Bars on left indicate males. Bars on right indicate females. Numbers in parenthesis are from skeletochronology. Numbers outside of parenthesis indicate population estimated using Schnabel method.

The Taipei grass frog populations are also declining. Since this small-sized frog is not harvested for food, the declining can not be caused by over harvesting. At Sanchih, the main culprit is human disturbance. In July and August in 2004, there was a constructing work in the upstream that affected the main water source of the water lily fields. As water quality declined and the number of frog was also declined. At Yangmei, the reason for the frog decline may be habitat loss. June 2004, low month rainfall (1.5mm) caused one pond to dry out. Frogs disappeared from the dried pond.

In determining the LAGs, we used 1-year-old individuals as baseline since that the first line outer of the cavity is the boundary between endosteal and periosteal (Esteban *et al.*, 1996; Guarino *et al.*, 1998). The oldest male and female frogs were 4 years old, indicating that this species had a short life span. Analysis of their demography revealed that 1-year-old males were scarce. We did not find 1-year-old females. It may be that only sexually mature individuals appeared at the breeding sites, with immature frogs staying away from the breeding ponds. Female Taipei grass frogs may become sexually mature at 2 years of age. Some males may reach maturity at 1 year of age. For some anuran species, delayed sexual maturity not only results in female frogs having a larger body size than males (Esteban *et al.*, 1996; Guarino *et al.*, 1998), but it also means the female breeding population is older than that of the males (Eggert and Guyétant, 1999; Ento and Matsui, 2002). We found that the age structure of male frogs varied with location and year. If a frog is sensitive to environmental change and human disturbance, the age structure may fluctuate.

If we calculate the cross year survival rate of a population from known-aged frogs, the result might be incorrect if the ratios of the marked population to the total population are different in different years. We do not know exactly how many individuals are in our study sites. We estimated population by first using the Schnabel method to estimate the breeding population. Then we extrapolated the relative frequency of each age class to estimate the total population (Marunouchi *et al.*, 2002). Although this method might create some error within an age class which no individuals were found, it should be more accurate for those classes for which individuals were found. Therefore, our estimates of 2 and 3-year-old frogs should be fairly accurate.

Because there were fewer 1-year-old male or female frogs from each study site than there were 2-year-old male or female frogs. We could not calculate the survival rate from age 1 to 2. Since we had no information about the cross winter survival rate of froglets, we could not calculate survival rates of age 0 to 1.

Marunouchi *et al.* (2002) studied Japanese brown frogs (*Rana japonica*) in a valley from 1993 to 1997. They found that females have a significantly higher annual survival rate (0.475) than males (0.305). But in our study, only females living at Yangmei had higher survival rate than males. When comparing the male survival rate to that of females (age 2 to 3), we found that the females had highest survival rate at Yangmei. At Sanchih, males had a higher survival rate than females. But at Yangmei, females had a higher survival rate than males. The reason may be due to human disturbance. The vegetation was less disturbed by human at Yangmei than at Sanchih. A good vegetation cover may provide suitable hiding places that will decrease the opportunities for female frogs to be found by predators such as birds and snakes. Male frogs will also benefit from habitats with good coverage. In the male's habitats, however, there are many small predators like spiders (Pisauridae). Spiders could easily catch male frogs (Lien, C.-T., personal observation), but might not catch the larger females. Therefore, females had a higher survival rate than males at Yangmei. In a disturbed habitat like Sanchih, females will lose their hiding places and their larger bodies will be easily found by predators. Thus females had a lower survival rate than males there.

Acknowledgments

We especially thank colleagues from the Taipei City Zoo for help with field work. Part of this work was supported by the National Science Council of Taiwan (grant number: NSC92-2311-V-003-007).

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二個小繁殖族群台北赤蛙的族群統計研究

連志台 [1] 林華慶 [2] 呂光洋 [1*]
[1]國立臺灣師範大學生命科學系
[2]台北市立動物園

(收稿日期：2006.12.29，接受日期：2007.3.2)

摘　要

從 2003 至 2004 年，我們調查了二個台北赤蛙(*Rana taipehensis*)的繁殖族群，分別位於三芝及楊梅。繁殖族群的性別比明顯的偏向雄性，三芝族群的雄雌性別比(16.13:1)約是楊梅族群的二倍(6.97:1)。以 Schnabel 法估算的二個族群數量，在 2004 年皆呈下降的現象。骨骼鑑齡上定義每一生長停滯線(LAG)代表一歲，族群 中年紀最大的個體在雌性及雄性皆為 4 歲，雌蛙的年齡(2.56±0.09 LAGs)顯著的大於雄蛙(2.30± 0.03 LAGs)。依據年齡結構的分析結果，一些雄性個體在 1 歲時就能達到性成熟且進入到繁殖族群內，雌蛙則要到 2 歲。楊梅族群中雌蛙在 2 歲至 3 歲的存活率最高(0.68)，但是此階段在三芝族群有最低的存活率(0.22)。從雌蛙的存活率，我們認為棲地干擾(三芝)對雌蛙有負面效應。性別比可能會受到人為擾動的間接影響。

關鍵詞：族群統計、繁殖族群、生長停滯線

*通信作者：呂光洋（Kuang-Yang Lue）；FAX:886-2-29312904；E-mail：luky66@ntnu.edu.tw