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Skeletochronological Age Determination and Comparative Demographic Analysis of Two Populations of the Gold-spotted Pond Frog (*Rana chosenica*)

Cheong, Seokwan*, Daesik Park[1], Ha-Cheol Sung, Jung Hyun Lee[1] and Shi-Ryong Park

Department of Biology Education, Korea National University of Education, Cheongwon, Chungbuk 363-791, Korea

[1]Department of Science Education, Kangwon National University, Chuncheon, Gangwon 200-701, Korea

ABSTRACT: To obtain demographic information on threatened gold-spotted pond frog (*Rana chosenica* Okada, 1931) populations, we determined the ages of 45 male and 13 female frogs (20 males and 9 females from Cheongwon and 25 males and 4 females from Tae-an) and compared the age structures and growth patterns of the two populations in 2006. The snout-vent length (SVL) and body weight of female frogs were greater than those of male frogs in both populations. Male frogs' ages ranged 2 to 7 years old and females' ages ranged 3 to 6 years old. In both populations, 4 years old male frogs were the most abundant age-sex class. The age structures of the two populations were significantly different and the growth coefficients of male frogs from the Cheongwon population were greater than those from the Tae-an population. The mean age of males from the Tae-an population was higher than that from the Cheongwon population. However, the SVL and body weights of male frogs were not different between two populations and there was no difference between the two populations in the mean male SVL at any age. The results could increase our understanding of the life-history of this threatened frog and may be useful in conservation planning.

Key words: Age determination, Growth rate, *Rana chosenica*, Skeletochronology

INTRODUCTION

Researchers require basic biological information about wildlife populations including population density, age structure, dispersion, and adult sex ratio to be able to understand and monitor population changes in a species over time. The ability to accurately estimate the ages of individual animals, in turn, is essential for the acquisition of information about the demographic characteristics of a population including mortality, longevity and other demographic variables (Halliday and Verrell 1988). Individual growth rates and population growth patterns of most amphibians are influenced by environmental factors such as resource availability as well as genetic factors (Miaud et al. 1999). Therefore, the age structure of an amphibian population may also provide information on the effects of past and current environmental conditions such as climatic variables, pollution gradients, and human activities on the population and how the population will change in the future.

Estimation of the age of amphibians from measurements of snout-vent length (SVL) or using mark-recapture records (extrapolating from size frequency data) is laborious and unreliable because of inter- and intra-population size variation (Halliday and Verrell 1988). On the contrary, skeletochronology is a reliable technique to determine ages of amphibians and reptiles (Castanet and Smirina 1990), and has been used for numerous species (Misawa and Matsui 1999, Kumbar and Pancharatna 2001, Homan et al. 2003, Snover and Hohn 2004). To determine age of individuals, one counts the number of lines of arrested growth (LAGs) in the cross sections of phalanges formed by differences in bone growth rates between time periods. In temperate zones, the period of winter hibernation leads to the formation of clear LAGs as a result of relatively slow, but compact bone growth during hibernation as compared with faster rates during the active period (Driscoll 1999). Therefore, it is possible to determine how many winters individuals have survived (i. e. their age in years) by counting the LAGs without killing the animals (Lai et al. 2005).

The gold-spotted pond frog (*Rana chosenica* Okada, 1931) is a medium-sized frog that was once abundant in paddy fields and marshes throughout western and southern Korean peninsula (Kang and Yoon 1975). However recent surveys showed that the species is rapidly declining in many habitats (Lee 2003). The Ministry of Environment in Korea has listed the species as an endangered species since 1975 and the IUCN (the International Union for the Conservation of Nature and Natural Resources) listed it as vulner-

* Corresponding author; Phone: +82-43-230-3848, Fax: +82-43-233-6263, e-mail: skcheong@empal.com

able to extinction in the 2006 Red Book of Threatened Species.

In this paper, we determined the ages of male and female gold-spotted pond frogs using skeletochronology and compared the age structures and growth patterns of two isolated field populations to obtain basic population information which could be useful in the development of management and conservation plans.

MATERIALS AND METHODS

We collected field data from two populations of gold-spotted pond frogs in Cheongwon, Chungbuk (N 36°36'03", E 127°21'41") and Tae-an, Chungnam (N 36°48'05", E 126°9'19") in South Korea. The population in Cheongwon was located in a deserted paddy field that was not used to grow a rice crop for at least six years. The area included several small ponds and some dry land, measured about 3966.3 m^2 and was surrounded by steep banks (0.3 to 1.2 m height) on three sides, with a low hillside on the remaining side. The population in Tae-an was found in a pond and nearby paddy fields, in an area measuring about 330.3 m^2. The main study area, a pond which was covered by water chestnuts (*Trapa japonica* Flerow) was surrounded by paddy fields except on one side, which was adjacent to a pine forest. The maximum depth of the pond was about 1.5 m. The pond was surrounded by banks of rice cut grass (*Leersia oryzoides* SW) and threelobe beggarticks (*Bidens tripartita*).

We captured gold-spotted pond frogs between May and September 2006 using a hand net for the Tae-an population and pitfall traps placed around the ponds, as well as a drift fence for the Cheongwon population. Following capture we determined the frogs' sexes using presence/absence of nuptial excrescence, measured their SVLs to the nearest 0.1 mm with a vernia caliper and determined their body weight to the nearest 0.1 g using an electric field balance and then clipped their toes. Following the collection of biological data and toe samples, we released the frogs at the site of capture. The clipped toes were fixed in 70% ethanol at the site for storage until laboratory processing (which occurred at least one month later for all samples).

In the laboratory, the clipped toes were washed in running water for 24 hrs, and then decalcified by submersion in 5% nitric acid for 20~30 min. After another 24 hrs, the toes were washed again using tap water. Following this initial preparation, the toes were paraffin-embedded after a serial dehydration process, sectioned to 10 μm sections using a rotary microtome, stained following the Harris Eosin-Haematoxylin method, and examined under a microscope. Growth zones and LAGs were visible in the cross sections of phalanges. The number of the LAGs was counted, and the ages of individuals were determined independently by two of the authors. When the determinations of the two authors differed, we recounted the LAGs and drew a consensus conclusion.

It is known that amphibians display an S-shaped growth curve like most ectotherms (Atkinson and Sibly 1997). We estimated the growth curve for gold-spotted pond frogs using the model of von Bertalanffy (1938):

$$S_t = S_m - (S_m - S_0)e^{-K(t-t_0)}$$

where S_t=average body length at age t; S_m=asymptotic body length; S_0=body length at metamorphosis; t=number of growing seasons experienced; t_0=age at metamorphosis; and K=growth coefficient (i. e. shape of the growth curve). We used 18.1 mm (n=10) as the body length at metamorphosis and 0.3 as the age at metamorphosis (unpublished data).

The von Bertalanffy growth model was fitted to the average growth curves using the dynamic fitting method with SigmaPlot 10.0 (Systat software Inc.). To compare physical parameters such as frog SVL, body weight and age between the sexes and the two populations, we first determined whether the data met the assumptions for parametric analysis using Shapiro-Wilk test. Data meeting the assumption of normality were analyzed using the independent sample t-test, while data not meeting this assumption were analyzed using the non-parametric Mann-Whitney U test. Numerical data in the text are presented as mean ± SD.

RESULTS

Twenty males and 9 females from the Cheongwon population and 25 males and 4 females from the Tae-an population were captured during the research period and their ages were determined. LAGs were found in the phalanges of various fingers, but were most clearly discriminated in the third finger. The line surrounded endosteal bone (EB) was interpreted to be the line of endosteal resorption (ER). LAGs were found in concentric hematoxylinophilic lines outward of the ER (Fig. 1). The presence of LAGs in the phalanges of both sexes was confirmed.

Within each study population, females had larger mean SVLs (Cheongwon, t=10.32, df=27, p<0.001; Tae-an, t=5.99, df=27, p<0.001) and had higher mean body mass (Cheongwon, t=12.59, df=27, p<0.001; Tae-an, t=9.99, df=27, p<0.001) than did males, while the differences in SVL (male, t=1.91, df=43, p=0.06; female, Mann-Whitney U test, Z=0.31, p=0.825) and body mass (male, t=1.20, df=43, p=0.24; female, Mann-Whitney U test, Z=0.46, p=0.71) between two populations were not significant (Table 1).

We estimated ages for all frogs captured from both populations. The ages of captured males ranged from 2 to 7 years and female's ages ranged from 3 to 6 years. Four-year-old frogs were the most abundant age-sex class in both populations. In the Cheongwon population, male frogs were on average younger than females (males: mean age=3.7 ± 0.73, n=20; females: mean age=5.0 ± 0.87, n=9; Mann-Whitney U test, Z=3.25, p<0.001), while in the Tae-an population, ages of males (mean age=4.24 ± 0.88, n=25) and females (mean age=4.00 ± 0.82, n=4) were not significantly different (Mann-Whitney U test, Z=0.45, p=0.69). Male frogs captured from the Tae-an population were on average significantly older than those from the Cheongwon population (Mann-Whitney U test, Z=2.04, p<0.05) while females' mean ages did not differ in the two populations (Mann-Whitney U test, Z=1.702, p=0.09, Table 1). The age structures for both sexes differed significantly between the two populations (males: χ^2=21.87, df=4, p<0.001; females: χ^2=62.85, df=3, p<0.001, Fig. 2).

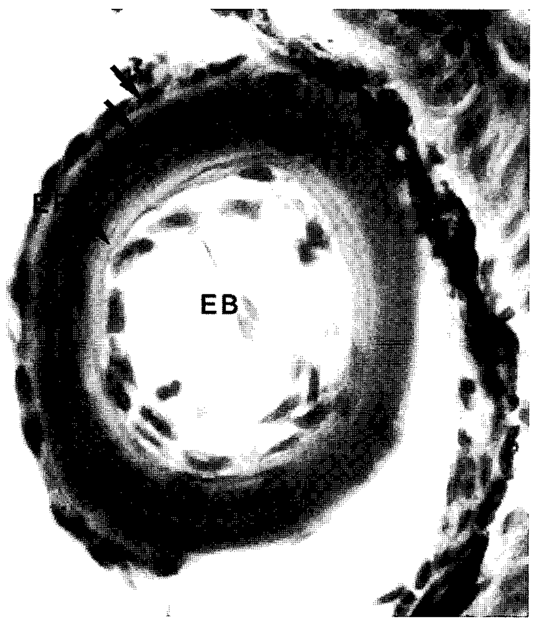

Fig. 1. Phalangeal cross section of the gold-spotted pond frog (*Rana chosenica*): a male with 31.0 mm SVL, 4 LAGs, i. e. 4 years old. Arrows indicate the LAGs; RL represents the resorption line. × 400.

Table 1. The snout-vent length (SVL), body weight, and age of the gold-spotted pond frogs captured in the Cheongwon and Tae-an populations. The data are given in mean ± SD.

Population	Sex	N	SVL (mm)	Body weight (g)	Age (year)
Cheongwon	M	20	34.15 ± 2.75	3.90 ± 1.02	3.70 ± 0.73
	F	9	51.80 ± 6.57	18.21 ± 4.96	5.00 ± 0.87
Tae-an	M	25	36.72 ± 5.45	4.47 ± 1.94	4.24 ± 0.88
	F	4	53.81 ± 3.91	17.13 ± 4.45	4.00 ± 0.41

The SVLs of male frogs of the same age were not different in the two populations for any age class. The SVL at age 3 was 35.08 ± 3.41 mm (n=6) in the Cheongwon population and 31.73 ± 3.03 mm (n=4) in the Tae-an population (Mann-Whitney U test, Z=1.49, p=0.17). For males of age 4, the SVL at Cheongwon was 34.00 ± 2.34 mm (n=11) and that at Tae-an was 37.10 ± 4.79 mm (n=13, Mann-Whitney U test, Z=1.80, p=0.07). The SVL of 5-year-old males was 34.65 ± 1.20 mm (n=2) in Cheongwon and 39.56 ± 6.19 mm (n=7) in Tae-an (Table 2).

In further analyses of population age structures and population growth patterns, we excluded data from females from the Tae-an population due to inadequate sampling (n=4).

The growth of male frogs from the Cheongwon population was more rapid during early life time than that of the Tae-an population (Fig. 3A). Males in the Cheongwon population reached their asymptotic size at about 3～4 years old age, while males in the Tae-an population reached asymptotic size at around 5～6 years old

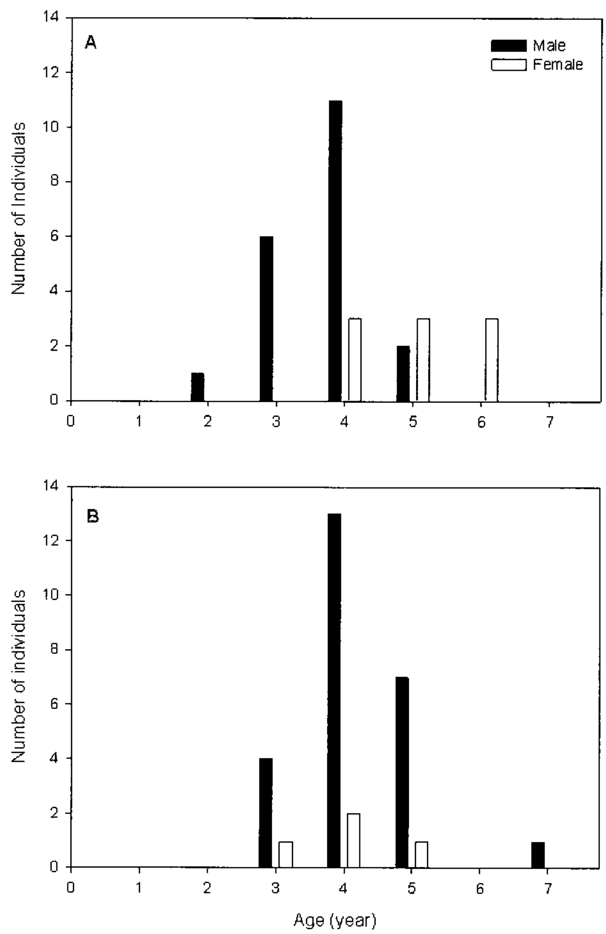

Fig. 2. Age distribution in the Cheongwon (A) and Tae-an (B) populations.

Table 2. The snout-vent length (SVL) and body weight of the frogs at a specific age captured from the Cheongwon and Tae-an populations. The data are given in mean ± SD.

Population	Sex	Age (year)	N	SVL (mm)	Body weight (g)
Cheongwon	M	2	1	29.3	1.8
		3	6	35.08 ± 3.41	3.95 ± 0.81
		4	11	34.00 ± 2.34	4.00 ± 1.02
		5	2	34.65 ± 1.20	4.20 ± 1.13
	F	4	3	47.72 ± 6.50	15.03 ± 5.66
		5	3	51.83 ± 7.91	18.37 ± 4.48
		6	3	55.83 ± 4.27	21.22 ± 4.19
Tae-an	M	3	4	31.73 ± 3.03	3.28 ± 0.64
		4	13	37.10 ± 4.79	4.36 ± 1.64
		5	7	39.56 ± 6.19	5.76 ± 2.19
		7	1	32.0	3.1
	F	3	1	54.2	16.6
		4	2	56.43 ± 0.67	20.35 ± 1.77
		5	1	48.2	11.2

age (Fig. 3A). The asymptotic size of males from the Cheongwon population was smaller than that of the Tae-an population (t=5.51, df=43, p<0.001) but the growth coefficient of males from the Cheongwon population was greater than that of the Tae-an population (t=4.29, df=43, p<0.001, Table 3). In the Cheongwon population, the asymptotic sizes and growth coefficients of male and female frogs were significantly different (asymptotic size, t=4.00, df=27, p<0.001; growth coefficient, t=36.28, df=27, p<0.001, Table 3).

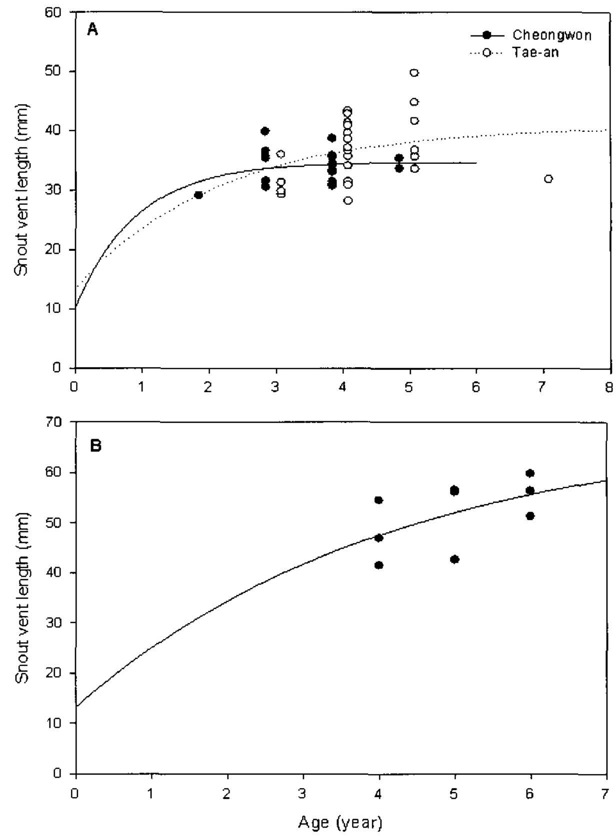

Fig. 3. Growth patterns of male Gold-spotted pond frogs from the Cheongwon and Tae-an populations (A) and female frogs from the Cheongwon population (B) fitted to the von Bertalanffy's equation (1938).

Table 3. Asymptotic size and growth coefficient of male frogs in the Cheongwon and Tae-an populations.

Population	Sex	N	Asymptotic size (mm)	Growth coefficient
Cheongwon	M	20	34.72 ± 1.15	1.11 ± 0.61
	F	9	68.86 ± 25.59	0.24 ± 0.22
Tae-an	M	25	40.75 ± 5.32	0.47 ± 0.30

DISCUSSION

We successfully determined the ages of wild-caught male and female gold-spotted pond frogs by counting the LAGs in their phalanges. This is the first study on anuran ages conducted in Korea although skeletochronology has been widely used to determine amphibian ages in other areas (Castanet and Smirina 1990). LAGs are produced as a result of differences in bone growth rates between the period of winter hibernation and period of the breeding or feeding (Kleinenberg and Smirina 1969, Hemelaar 1985, Halliday and Verrell 1988). The age estimates produced by two authors using the number of LAGs from each phalangeal section were identical for 49 out of 58 individuals. In the remaining 9 cases, the initial differences in the estimated ages of two authors were within 2 years and the authors were able to reach a consensus decision by recounting the LAGs and exchanging opinions. In some phalangeal sections, the EB was lost during haematoxylin-eosin staining process, but this did not affect our ability to determine ages.

Our results indicate that male gold-spotted pond frogs reach sexual maturity at 2~3 years of age and female frogs reach maturity at age 3~4 years. These results are consistent with the results of previous studies, which show that male frogs of many Ranidae species, such as *R. temporaria* (Miaud et al. 1999), *R. nigromaculata* (Khonsue et al. 2001a), *R. rugosa* (Khonsue et al. 2001b) and *R. latastei* (Guarino et al. 2003), start to reproduce at age 2~3 years and that females reach sexual maturity 1 year later than males (Hemelaar 1983, Reading 1991). Our data show a rapid growth pattern of males in the Cheongwon population during the first 1~2 years of age, which supports the argument that males become sexually mature at 2~3 years of age. Usually, females require more time to reach sexual maturity, and as a consequence of this delayed maturity and a higher juvenile growth rate, the body size of females is larger than that of males (Reading 2001). This pattern appears to be applicable for female gold-spotted pond frogs as well, and to explain differences in the SVLs and body weights of females and males.

In our study, growth coefficients of male frogs from the Cheongwon population were greater than those of Tae-an male frogs, while asymptotic size of male frogs in the Cheongwon population was smaller than that from the Tae-an population. Our data reveal several dimensions of variation in growth patterns of gold-spotted frogs in different populations on the Korean peninsula. Males in the Cheongwon population grew more rapidly in their early lives (ages 0~2 years; Fig. 3A), and then breed earlier in their lives than did males in the Tae-an population. In the Cheongwon population, a 2-year-old male participated in breeding (Table 2) and males reached their asymptotic size at around 3~4 years of age, resulting in a relatively smaller asymptotic size of about 35 mm SVL. Conversely, in the Tae-an population male frogs showed relatively slow growth from age 0 to 2 years old, but then kept growing until about 6~7 years of age (Fig. 3A) resulting in much larger asymptotic size of about 41 mm SVL. Individual growth rates are influenced by numerous factors including food availability, individual variation in efficiency of prey capture, parasitic infections, and abiotic factors such as temperature and light conditions (Richards and Lehman 1980, Kluge 1981, Minchella and Scott 1991, Duellman and Trueb 1994, Rehage et al. 2002). In this study, although the two study populations show quite different growth patterns and overall asymptotic sizes of male frogs, we could not determine which ecological factors may have played a role in producing the observed differences. Further study of the relationship between habitat conditions and other factors which may affect individual growth in gold-spotted pond frogs will be required to clarify this issue.

Males captured in the Tae-an population were on average older than those in the Cheongwon population. How can we explain this difference? Several factors such as age at sexual maturity, growth rate, and habitat-related factors such as nutritional conditions and levels of competition can affect longevity. Several previous studies have shown that in anurans, lower growth rates are associated with reduced mortality rates (Arendt 1997, Mangel and Stamps 2001, Schiesari et al. 2006). Our result is consistent with these previous results. The growth coefficient of males in the Tae-an population was lower than that of males in the Cheongwon population, which may result in lower mortality and longer lifespan. Males in the Tae-an population were also on average larger than those in the Cheongwon population, suggesting that males in the Tae-an population may be exposed to milder environmental conditions such as relatively warmer temperatures, high quality of foods, and reduced competition. For many frog populations, food availability affects overall growth and mortality of individual frogs (Duellman and Trueb 1994). Here, we propose and discuss several factors that might explain differences in the mean ages of the two populations. Those factors are not mutually exclusive, and further study will be necessary to clarify the ecological and environmental factors affecting patterns of age-specific growth and mortality.

In conclusion, our results about age structure and growth rate of gold-spotted pond frog populations may be useful for constructing conservation strategies for this threatened frog species. Further study of the biotic and abiotic factors affecting habitat conditions of gold-spotted pond frog populations will be necessary to develop a more detailed understanding of their ecology.

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