



A Study on Age Determination and Life Cycle of *Pelophylax ridibundus* (Pallas, 1771) by Skeletochronology Method

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ABSTRACT

In this study, it was aimed to determine the ages of adult male and female *Pelophylax ridibundus* individuals captured from the around of Gölbaşı Lake. The age of tailless frogs was determined according to the skeletal chronology method. In this method, the fourth toes of the frogs hind legs are used. Age was determined counted on the Resting Lines (RL) of the phalanges. The mean ages of the frogs were found to be 3.72 ± 0.97 years for females (range 2-7) and 3.77 ± 0.87 years for males (range 3-6), respectively. There was no significant difference between the ages of female and male frogs ($P>0.05$). The mean weights of female and male frogs were found to be 62.9 ± 18.57 g and 42.77 ± 10.74 g, respectively. This difference among the weights was significant ($P<0.05$). The mean snout-vent length (SVL) value was measured as 74.71 ± 11.91 mm for females and 71.7 ± 7.25 mm for males. There was no significant difference in SVL among both sexes ($P>0.05$). In addition, it was defined a significant correlation among the SVL of female ($r=0.889$, $P<0.01$, $N=36$) and male frogs ($r=0.833$, $P<0.01$, $N=18$) age. As a result of the study, it was seen that the frogs have a young population structure.

Pelophylax ridibundus'un (Pallas, 1771) İskeletkronoloji Yöntemiyle Yaş Tespiti ve Yaşam Döngüsü Üzerine Bir Araştırma

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ÖZ

Bu çalışmada, Gölbaşı Gölü çevresinden yakalanan yetişkin erkek ve dişi *Pelophylax ridibundus* bireylerinin yaşlarının belirlenmesi amaçlanmıştır. Kuyruksuz kurbağaların yaşı iskelet kronolojisi yöntemine göre belirlenmiştir. Bu yöntemde, kurbağaların arka bacaklarının dördüncü ayak parmakları kullanılmaktadır. Yaş, falanjlardaki Dinlenme Halkaları (RL) üzerinden sayılarak belirlenmiştir. Kurbağaların ortalama yaşları sırasıyla dişi 3.72 ± 0.97 yıl (2-7 aralığı) ve erkek 3.77 ± 0.87 yıl (3-6 aralığı) bulunmuştur. Dişi ve erkek kurbağaların yaşları arasında anlamlı bir fark görülmemiştir ($P>0.05$). Dişi ve erkek kurbağaların ortalama ağırlıkları sırasıyla 62.9 ± 18.57 g ve 42.77 ± 10.74 g olarak bulunmuştur. Ağırlıklar arasındaki bu fark anlamlıdır ($P<0,05$). Burun deliği-Kloak arası uzunluğu (SVL) ortalama değeri dişilerde 74.71 ± 11.91 mm, erkeklerde ise 71.7 ± 7.25 mm olarak ölçülmüştür. Her iki cinsiyet arasında SVL açısından anlamlı bir fark yoktur ($P>0.05$). Ayrıca, dişi ($r=0.889$, $P<0.01$, $N=36$) ve erkek kurbağaların ($r=0.833$, $P<0.01$, $N=18$) yaşları ile SVL arasında anlamlı bir korelasyon olduğu belirlenmiştir. Çalışma sonucunda, kurbağaların genç bir nüfus yapısına sahip olduğu görülmüştür.

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1. Introduction

The reason for the decrease in the number of amphibians in the recent years shown as global warming, increase in harmful chemicals, accumulation of toxic substances and destruction of the habitats of these creatures (Houlahan et al., 2000; Reaser, 2000; Cohen, 2001; Kiesecker et al., 2001). Thus, the fact that frog production continues only with hunting will cause the extinction of frog species and the loss of sustainability on the ecosystem. This is unfavorable for amphibians, which are known to be important components of terrestrial and aquatic ecosystems (Toledo et al., 2007; Mohneke and Rodel, 2009). These negative effects also threaten the morphological character of frogs closely (Alford et al., 2007; Fagotti et al., 2007).

The impact of climate conditions on metabolic activities of amphibians observed with changes in bone tissue, which allows for individual age estimation in free living populations in nature. Some methods have been developed to estimate the age of amphibians. According to Halliday and Verrell (1988), extrapolation from size with frequency data, testicular lobation, marking and capture, skeletochronology methods were recommended, while Castanet and Smirina (1990) stated that only the marking and capture or skeletochronology method were reliable and indicated that the age in frogs. Both techniques have been used frequently (Leclair and Castanet, 1987; Ryser, 1988; Tsiora and Kyriakopoulou-Sklavounou, 2002).

In age estimation studies, the method called skeletochronology is used and it known to be a reliable method for amphibians as well as for many cold-blooded vertebrates (Castanet, 2002). Some differentiations that occur in the soft and hard tissues of living things throughout their lives show an effective persistence in the histomorphological structure of the bone. It gives information about the animals life span, sexual maturity age and physiology (Erişmiş, 2004). *Pelophylax ridibundus* is distributed throughout the world from Central and Southern Europe to North Africa, Anatolia and West Asia (Tok et al., 2000; Berezovikov et al., 2001; Kuzmin et al., 2009; IUCN, 2010). However, in Turkey just a few research has been carried out on the age composition of *Pelophylax ridibundus* (Yılmaz et al., 2005; Erişmiş, 2011; Gül et al., 2011). The purpose of study was to defined the ages of lifespans of male and female frog, and to know the relationship among body size and age.

2. Material and Methods

In this study, 54 adult specimens (36 ♂♂, 18♀♀) of *Pelophylax ridibundus* was collected from Gölbaşı Lake in March 2015 in Hatay (36°30'16.4"N 36°29'39.0"E). For each captured frog, measured length to with a digital caliper. Then, estimated sexes by observing the secondary sexual character male and female frog (Nuptial pads or black swelling on thumbs during breeding period adult males and ova in adult females). For age determination during this study; skeletochronology method was applied and the fourth toes of the hind legs were used to determine the ages of tailless frogs by the

skeletochronology method (Leclair and Castenet, 1987; Plytyez and Bigaj, 1993; Marunouchi et al., 2000). Age composition was defined by counting on the RL of the phalanges. After the bone tissue samples were taken from the frogs, fixation was performed by using 10% formalin solution. Then, the formalin-free samples were taken into 6% nitric acid and decalcification was performed. The tissues were then dehydrated through a series of graded alcohols, cleared in xylene, infiltrated, and embedded into the paraffin. Paraffin wax blocks were cut at 4 μm thickness and stained with hematoxylin and eosin, and they were examined under Olympus CX 41 microscope. The data was not normally distributed, the Man-Whitney (U) test used $P=0,05$ significance level, and the correlation between SVL values and ages determined by Spearman Rank analysis were used in SPSS the 17.0 program.

3. Results and Discussion

The mean ages of adult female and male frogs collected from Gölbaşı Lake were found to be 3.72 ± 0.97 years (age range 2-7), 3.77 ± 0.87 years (age range 3-6), respectively (Table 1). The difference among ages of female and male is not significant ($P>0.05$). In this study, it was determined that the maximum age of male frogs was 6 years, while female frogs was 7 years old. In this study, RL sections were visualized by staining the hindfoot fourth toe phalanges of frogs of different ages with hematoxylin-eosin (Figures 1, 2, 3, 4). RL was easily detected and counted in all samples examined, with the exception of some samples. The average weights of female and male frogs were 62.9 ± 18.57 g and 42.77 ± 10.74 g, respectively. This difference was significant ($P<0.05$). The mean SVL value was measured as 74.71 ± 11.91 mm for females and 71.7 ± 7.25 mm for males. The SVL both sexes were no significant differences ($P>0.05$).

Table 1. The number of sample (N), SVL (mean \pm SD) and age (mean \pm SD) distribution of *P. ridibundus* individuals collected from Gölbaşı Lake.

	Weight (g)		SVL (mm)		Age	
	Female	Male	Female	Male	Female	Male
N	36	18	36	18	36	18
Min-Max	35-125	35-65	50.43-115	65.18-87.9	2-7	3-6
Mean	62.9 ^a	42.77 ^b	74.91 ^a	71.7 ^a	3.72 ^a	3.77 ^a
Standart Deviation (SD)	18.57	10.74	11.91	7.25	0.97	0.87

*Different letters on the same line show significant differences for each group ($P<0.05$).

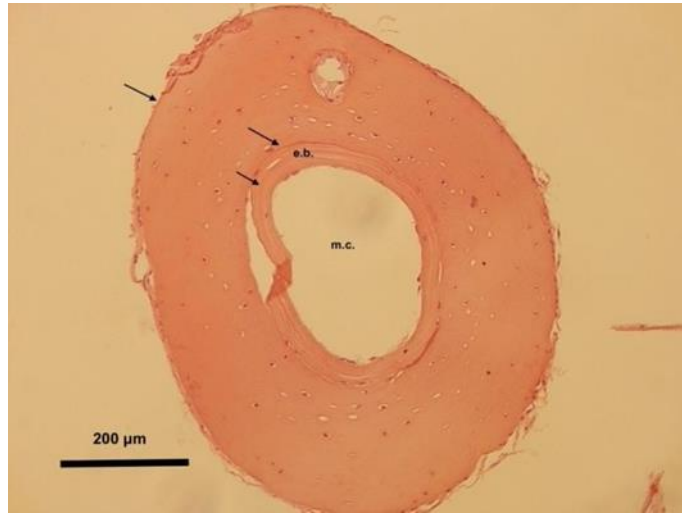


Figure 1. Cross section of hematoxylin-eosin-stained phalanges in a 3-year-old male with SVL 65.18 mm (arrows: RL, e.b: endosteal bone, m.c: marrow cavity. Magnification: 20X, original).

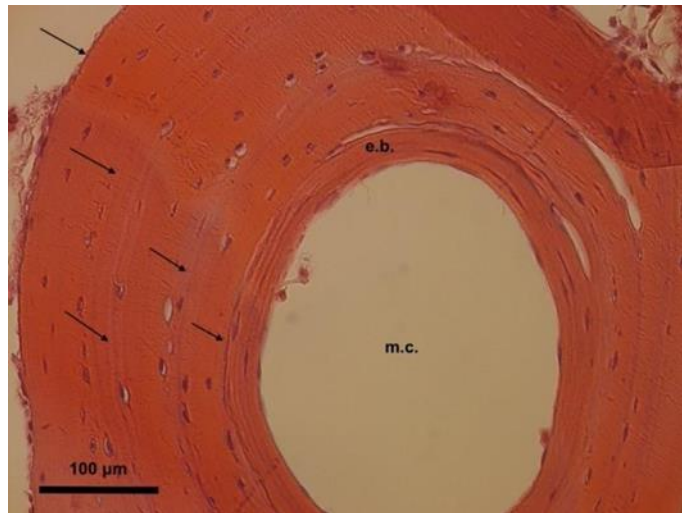


Figure 2. Cross section of hematoxylin-eosin-stained phalanges in a 5-year-old male with SVL 83.24 mm (arrows: RL, e.b: endosteal bone, m.c: marrow cavity. Magnification: 20X, original).

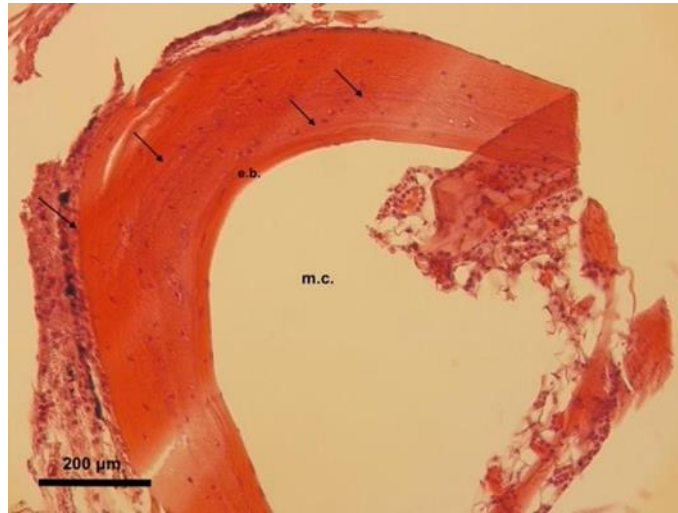


Figure 3. Cross section of hematoxylin-eosin-stained phalanges of a 4-year-old female with SVL 80.11 mm (arrows: RL, e.b: endosteal bone, m.c: marrow cavity. Magnification: 20X, original).

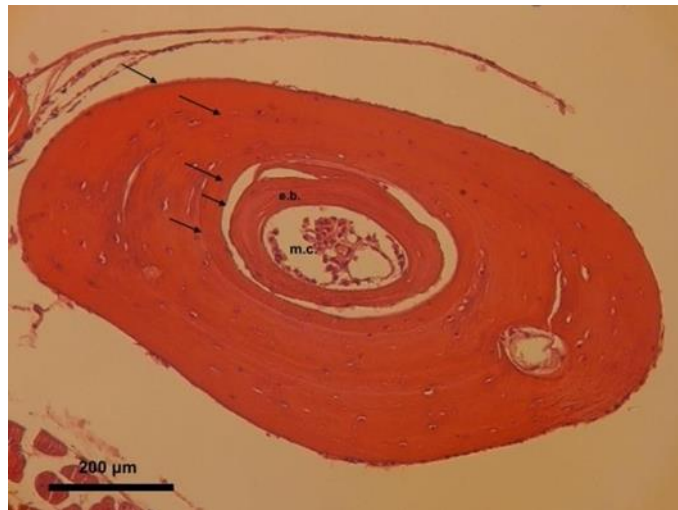


Figure 4. Cross section of hematoxylin-eosin-stained phalanges of a 5-year-old female with SVL 90.56 mm (arrows: RL, e.b: endosteal bone, m.c: marrow cavity. Magnification: 20X, original).

In this study, a positive correlation was found among age and SVL values of female ($r=0.889$, $P<0.01$, $N=36$) and male frogs ($r=0.833$, $P<0.01$, $N=18$) (Fig. 5). It was determined that the frog with the highest SVL value (87.9 mm) among male frogs was at the oldest age (6 years) (Fig. 5). Likewise, it was determined that female frogs with the highest SVL value (115.13 mm) among female frogs was found to be at the oldest age (7 years) (Fig. 5).

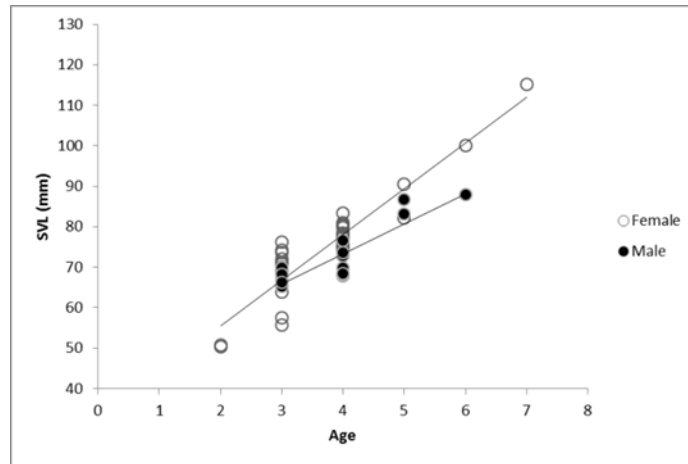


Figure 5. The relationship between the age of *P. ridibundus* and the SVL

Skeletal chronology is used in age estimation studies and it has been reported to be a reliable method for amphibians as well as for many cold-blooded vertebrates (Castanet, 2002). In this study, age determination of male and female frogs was made according to this method. Generally, the long bones of the extremities were determined as the most suitable skeletal parts for skeletal chronology studies. Moreover, the fourth toes of the hind legs are mostly used to determine the age of tailless frogs using the skeletal chronology method (Leclair and Castanet, 1987; Plytyez and Bigaj, 1993; Marunouchi et al., 2000). Similarly, the fourth toe of the right arm leg of the adult frogs was used for age determination in the study.

As a result of the study, the mean ages of female and male frogs collected from Gölbaşı Lake were found to be 3.72 ± 0.97 years (range 2-7 years), 3.77 ± 0.87 years (range 3-6 years), respectively. It was determined that the difference among the frogs in terms of average age was not significant ($P > 0.05$). The mean SVL value of female frogs was 74.71 ± 11.91 mm, and male frogs were 71.7 ± 7.25 mm, and the difference was not significant ($P > 0.05$). The mean age was determined as 3.90 ± 1.37 years (1-7 years) for male frogs and 3.72 ± 1.00 years (2-6 years) for female frogs in the *P. ridibundus* by Yılmaz et al. (2005). In another study with *P. ridibundus*, the average age was calculated as 3.5 years (1-7) for males and 4.3 years for females (İsmail and Çiçek; 2017).

Socha and Ogielska (2010) reported that the age distribution of male individuals as 2-6 years and the age distribution of females as 3-7 years in the population of *Pelophylax kl. esculentus* in Europe, a native crossbreed among *P. ridibundus* and *P. lessonae*. According to Gül et al. (2011) investigated the age composition of *P. ridibundus* in two different populations (Karagöl-Dörtüol) located at different altitudes in Turkey. In the Karagöl population, the age of the male individuals varies between 2-8, the age of the female individuals varies between 2-7. In Dörtüol population the age of male individuals varied between 4-11 years, and the age of female individuals between 3-7 years. It was determined that the average age we obtained as a result of the study was similar to the result of the

study, except for the male frogs in the Dörtüyl population.

In another study, Erişmiş (2011) determined the mean age of *P. ridibundus* as 5.73 ± 1.06 years in females and 4.82 ± 1.08 years in males. Çiçek et al. (2011) mean age in the *P. ridibundus* population was determined as 2.5 ± 0.65 years (age range; 2-4) and 2.95 ± 0.99 years (age range; 2-5) in male and female frogs. Moreover, their study reported mean SVL of male frogs was 56.1 ± 7.7 mm and the mean SVL of female frogs was 64.5 ± 14.8 mm. Thus, in this study, we determined that the differences between the mean ages, age ranges and mean SVL values and the values found in the other study are due to the samplings (Çiçek et al., 2011; Erişmiş, 2011).

In this study, it was determined that the minimum age is 3 years and the maximum age is 6 years in male frogs, while the minimum age is 2 years and the maximum age is 7 years in female frogs. Khonsue et al. (2000) found in their study with *Sylvirana nigrovittata* found the maximum age to be 9 years in males and 6 years in females. Yılmaz (2001) reported that the maximum age of *P. ridibundus* in Yıldız Stream (Trabzon) population was 7 years in males and 6 years in females. On the other hand, Olgun (2012) determined that the minimum age as 3 years and the maximum age as 10 years for both males and females, and reported that the mean age for females 6.69 ± 0.44 and 4.31 ± 0.21 for males.

As with most anuran species, the age of adult frogs has been reported to be positively correlated with size in both sexes (Ryser, 1996; Lu et al., 2006; Liao and Lu, 2010a; Liao et al., 2010; Liao and Lu, 2014). Hemelaar (1986) reported that there was a positive correlation between SVL value and age in both genders. Ryser (1988) found a positive correlation between SVL and age in both female and male frogs for *R. temporaria*. Guarino and Erişmiş (2008) reported that age determination and development in *R. holtzi* an endemic species that there was a positive correlation between SVL and age in both female and male individuals. Kyriakopoulou- Sklavounou et al. (2008) stated in their study with *P. ridibundus* that there was a significant relationship between SVL and age for both genders. Similar to the studies, a positive correlation was found between SVL values and ages of female ($r=0.889$, $P<0.01$, $N=36$) and male frogs ($r=0.833$, $P<0.01$, $N=18$) collected around Gölbaşı Lake.

Female individuals of sexually mature adult *Lithobates catesbeianus* are larger than males in terms of both weight and SVL (Kaefer et al., 2007). Bura et al. (2007) measured that the average SVL value of female frogs to be 80.08 mm and the average SVL value of male individuals to be 60.17 mm. Also, the average weight of female individuals (43.89 g) is higher than that of male individuals (18.45 g). Spigonardi et al. (2011) determined the sexes of individuals from six different populations belonging to *P. perezii* and revealed the relationship between body weights and SVL values; reported that female individuals were heavier and longer than male individuals. In this study, similarly it was determined that both the average SVL value and the average weight of female were higher than that of male.

Nayak et al. (2008) reported that male individuals were shorter than females in all age groups in their age determination and SVL study on the *Euphlyctis hexadactylus* population. In this study, it was determined that male frogs in the same age group are not always shorter than female frogs. Wei Chen et al. (2013) stated that there is a significant correlation among age and size (weight and length) in *R.*

kukunoris, but size is not a good indicator for age and similarities in body size of frogs in different age classes. In this study, SVL values of some frogs in different age groups collected from nature was found to be close to each other.

4. Conclusion

When the average age and age range of the adult are examined, it has been determined that the population has a young population structure. It is thought that this species is exposed to constant hunting pressure due to its economic importance and as a result its average longevity is shortened. It is thought that the average longevity of individuals living in nature will increase as a result of breeding *P. ridibundus* and reducing the hunting pressure on the species.

Conflict of interests

Authors declare that they have no conflict of interest.

Ethics committee approval

This study was carried out accordance with animal welfare and the ethics of trial.

Author Contribution Declaration

The authors declare that they have contributed equally to the article.

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