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Abundance, demography and population structure of *Pelophylax ridibundus* (Anura: Ranidae) in 26-August National Park (Turkey)

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Abstract. Demography and population structure aspects of the eurasian marsh frog *Pelophylax ridibundus* were studied in 26-August National Park (Turkey) for a duration of four years (2005-2008). The mark-recapture study showed that population size varied from year to year, and declined more than threefold in 2005 in comparison with the preceding years. Sexual maturity was reached at 2 years of age in males and at 3 years in females. The mean age of male and female frogs were 4.82 ± 1.08 and 5.73 ± 1.06 years. The growth was rapid during the early years in both sexes, and tended to slow down substantially when they reached 1-2 years for males, and 2-3 years for females. There was a significant positive corelation between SVL of female and males in amplexus (r=0.71, df=21, p<0.001). The minimum and maximum recorded values of egg number were 1255 eggs for a three-year old frog with a SVL of 62.48 mm and 2610 eggs for an eight-year old frog with a 98.46 mm of SVL. The mean ± SD of the eggs diameter calculated in 1.62 ± 0.49 mm, varied from 1.30 mm to 2.55 mm.

Key words: *Pelophylax ridibundus*, population size, skeletochronology, reproduction.

Introduction

There are apparent declines and extinction of the herpetofaunal communities throughout the world (Blaustein & Wake 1990, Alford & Richards 1999, Gibbons et al., 2000, Antal & Puttonen 2006). The causes may include habitat loss and degradation, unsustainable use, invasive species, environmental pollution, disease and global climate change. Habitat loss appears to be the most serious threat to herpetofauna as they are more affected than other vertebrates by serious human encroachment on their habitats (Capula 1989, Gibbons et al. 2000). Habitat destruction, wetland draining and/or pollution represent actual threats to amphibian populations and their reproduction. Furthermore, it is also apparent that some species are declining in number in certain areas, but thriving in other locations (Wyman 1990, Blaustein & Wake 1990, Blaustein et al. 1994, Blaustein et al. 2001, Stuart et al. 2004). However, it is now generally accepted that the decline in amphibian biodiversity is real and poses a serious threat to the global diversity of herpetofauna.

Pelophylax ridibundus, also known by its synonym of *Rana ridibunda,* was described for the first time from Guryev in Kazakhistan (terra typica restricta) and has a wide distribution in Central and Southern Europe and Western Asia (Bodenheimer 1944, Başoğlu & Özeti 1973, Arıkan 1988, Baran & Atatür 1998). It is listed in Appendix III of the Bern Convention [Convention on the Conservation of European Wildlife and Natural Habitats- 2006], and categorized as Least Concern (LC) in the IUCN List of Threatened Animals (http://www.iucnredlist.org).

Studies on the distribution, morphometry, serology, population size of *P. ridibundus* in Anatolia (Turkey) have been conducted by various researchers (Arıkan 1988, Baran et. al 1992, Atatur et. al 1999, Kumlutaş et al. 1999, Sinsch & Schneider 1999, Kaya & Erismis 2001, Tosunoğlu et. al 2005, Ayaz et al. 2007). Moreover, age determination studies have been completed successfully applying the skeletochronological method on the different populations of the same species that inhabit different environments (for example, *Rana ridibunda*: Shaldybin 1976, Aleksandrovskaya & Kotova 1986, Yılmaz et al. 2005, Kyriakopoulou et al. 2007). But there is an important lack of information on the age determination of *P. ridibundus* situated within the distribution area in the Anatolia region of Turkey.

Although it is well recognised that the estimation of declines and the detection of threats for small population of amphibians may be difficult to assess without long-term monitoring (Collins & Halliday 2005), the research on the trends in population size and structure is still scarce for amphibians of Turkey. In 1999, an initial study to determine the population size of the Eurasian marsh frog (has a commercial importance), was conducted in 26-August National Park by Kaya & Erişmiş (2001). Using this study as baseline, further studies on the population structure were conducted in four consecutive years (2005, 2006, 2007 and 2008) to assess age, sexual maturity, growth and the effect of human induced changes in the environment on the population density of the Eurasian marsh frog. The aims of this study were: (1) to elucidate whether its population size varies by the years studied; (2) to determinate age structure, sexual maturity, and (3) to give information regarding the reproductive biology of *P. ridibundus* in mid Anatolia. This research will also give some information for prospective researches on the major factors influencing biodiversity and its protection in the research area..

Materials and methods

Field Work

Akören Lake, located in 26-August National Park in the internal-West Anatolia Region of Turkey (Fıg. 1), is at cca. 1060 m altitude and is approximately 150 m long, 110 m wide and 2.5 m deep.

The present study of population size of *Pelophylax ridibundus* was conducted during August and September for each of the study years (2005 - 2006 - 2007 - 2008) and took place between the hours of 8:00 P.M. and 5:00 A.M. To estimate the population size of marsh frogs, all individuals caught were marked, released and recaptured on consecutive days by using Begon's triple catch method which helps in calculating the survival rate (Begon 1979). During the study, air and water temperature, pH of water were measured and recorded separately for fieldwork (Table 1).

Calls were recorded using Olympus digital voice recorder (W–10, Olympus) within 10–20 cm from calling males. The air temperature was 21.70 ± 1.45°C (range: 20–23.6°C). On four days, calls recorded from six individuals were analyzed using Cool Edit Pro.

Pelophylax ridibundus individuals were caught by hand. Captured individuals were labeled above their knees and elbows with different color elastic bands for each day (Elmberg 1989). The snout-vent length (SVL) to the nearest mm using a ruler (0.01 mm) was measured for each specimen captured. This study was approved by the Animal Ethics Committee of Afyon Kocatepe University (permit number for research: 0034).

Change in Population Size

In this study, the capture-recapture method has been studied to estimate the population size in closed populations. In this populations, it is assumed that the number of individuals do not change with respect to factors such as births, deaths and migrations during the sampling process. Population size was estimated using the double, and triple catch method (Begon 1979: BE). The chi-square goodness-of-fit test was used to assess whether the number of males versus females deviated from a 1 : 1 ratio. The change in population size is measured by using ΔN (Houlahan et al. 2000). $\Delta N = \log (N_t + 1) - \log (N_{t-1} + 1)$. Where N_{t-1} and N_t are population size for marsh frogs species at time t-1 and t, respectively. ΔN is positive when populations increase and negative when they decline. Software SPSS 10.0 for Windows was used for the calculations. The level of significance used was $p<0.05$.

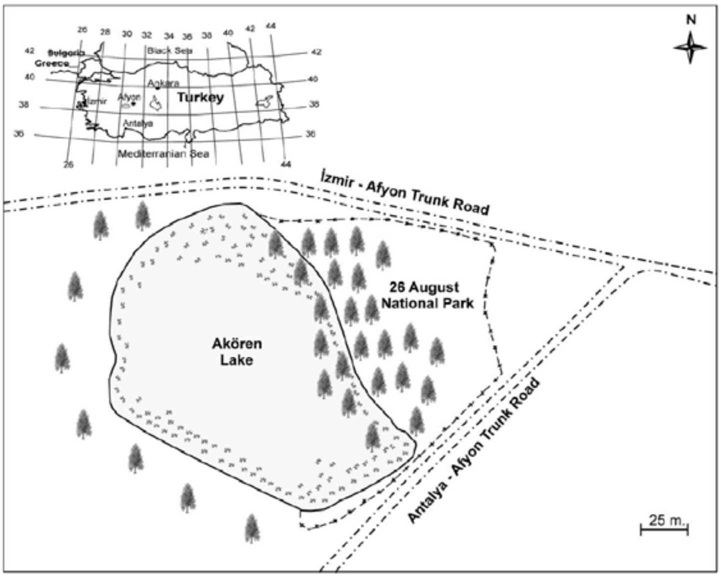


Figure 1. Illustration of the Akören Lake sampling site and the 26 August National Park's border.

Age – Growth Estimation - Eggs Diameters – Fecundity - Reproductive effort

Non-amplectant males (N=10)/females (N=14), and the amplectant pairs (N=22) of the eurasian marsh frog, collected in the breeding seasons throughout four years, were studied in terms of their age and reproductive characters. Stages of larval development were determined following Gosner (1960), and froglets of Stage 46 or older were used to compare SVL. The frogs were sexed using secondary sexual characteristics such as vocal sacs, the formation of nuptial pad on the forearms of males. The distal third phalanx of fourth toe of the left forelimb was amputated after local anaesthesia by Cloraethyl-sprey. The cut toe was transferred to 10% neutral buffered formol and the animal was released on site.

The skeletochronological technique used for age determination is based on counting the lines of arrested growth (LAGs) formed in bone tissue (Castanet et al. 1993). For preparing bone section, third phalanx stored in 10 % neutral buffered formol was washed in running water for 12 hours and then was decalcified in 3 % nitric acid (3h) before it was soaked overnight in running water. Successively, they were processed for paraffin embedding. The central region of diaphysis of each phalangeal bone was cross-sectioned at a thickness of 10 - 12 µm by a microtome (Surgipath RS4800 Microtome). Histological sections of bones were stained for 20 min in Mayer's acid hemalum and mounted in aqueous resin. For each phalanx, at least four mid-diaphyseal sections having the smallest medullary cavity at mid-diaphyseal level and thickest cortical bone were selected for observation under a light microscope and photographed with a digital photocamera. It is assumed that one zone and one LAG correspond to growth during a single year, with the LAGs representing periods of hibernation (Paton et al. 1991, Esteban et al. 2002, Guarino et al. 2003). Furthermore, the methodology developed by Sagor et al. (1998) was utilized to show conclusively that there were no instances of resorption of earlier LAGs. Thus, applying skeletochronology, the ages of all the individuals were assessed directly from the thin sections of the bones without the need for back-calculations.

Growth models were estimated using Von Bertalanffy's equation (1934) as performed in other studies on amphibians (Hemelaar 1998, Lima et al. 2000, Olgun et al. 2001, Cogalniceanu & Miaud 2003). The following equation was used: $SVL_t = SVL_{max} - (SVL_{max} - SVL_0)\ e\text{-}k(t\text{-}t_0)$, where SVL_t = average body size at age_t, SVL_{max}= Asymptotic maximum body size, SVL_0 = Body size at metamorphosis (t_0) fixed to mean 21.58 ± 4.49 mm, t = age, t_0 = age at metamorphosis (0.3 year) and k = growth coefficient that defines the shape of the curve. In order to estimate the mean SVL at metamorphosis, field data taken during metamorphosis at the end of summer (end of August) were used. Distributions of age and SVL were compared with the Manny - Whitney U-test and were used the Student's t-test to compare mean SVL among age classes.

As egg clutches from females were obtained under laboratory conditions (each amplectant pair laid a clutch in a separate aquarium), the SVL of both sexes were determined. For these purposes, a total of 11 amplexus (11 females, and 11 males) were caught between 21.00-01.00 hours of the day and transferred to the lab, and after completing our experiments, they were left to the same locations collected from. The egg diameter was measured to an accuracy of 0.01 mm using a binocular microscope with an ocular micrometer. Fecundity was estimated from the total volume (or mass) of an egg clutch. Reproductive effort (RE) was calculated as RE= F x ED^3 / SVL3, where F is fecundity, ED is egg diameter, and SVL is snout-vent length (Cherdantsev et all. 1997).

Results

The capture-recapture data from the field work according to years (2005 - 2008) are summarized in Table 1.

In the study carried out in 2005, 2 - 4 September, a total of 327 (134 male, 116 females, and 77 subadult) the eurasian marsh frogs were marked and 22 individuals were recaptured (Table 1). On two days, the population size with regard to Begon's estimator (BE) was estimated at 1773 (95%CI: 1856-1689). The triple catch of BE was used to calculate population size. Accordingly, the results were found with population sizes being 2007 (95% CI: 1918-2097) for BE on 3 days, and assumed that the survival and the gain rates were constant and that the interval between the samples was the same (Begon 1979). Mean ± SD of the estimated population size over the study period according to day 2 and day 3 was 1890 ± 165 (95%CI: 1803-1976). Accordingly, the survival rate and the population gain were estimated to be 1.25 and -0.15 respectively

Population density of the all captured frogs during the three-day study period was 1.98 individuals/100 m^2 and the mean rate was 7.78 per capita/hr. Distribution of size classes for this year revealed that samples consisted mainly of 76.45 % adults.

The sex-ratio of all captured adult frogs was biased in favor of males but there are significant differences between males and females on account of sex ratio (1.15 males : 1 female, χ^2= 23.68, df = 1, P<0.001). Females were bigger than males: o mean ± SD of adult SVL, were 68.16 ± 5.04 mm in males (n=134, range from 42.48 to 97.86 mm) and 77.05 ± 6.09 mm. in females (n=116 range from 46.36 to 102.36 mm). SVL mean differed significantly between the sexes (t-test, t_{249}= 3.64, P< 0.05).

In the study carried out in 2006, 7 - 9 September 2006, a total of 290 frogs (126 males, 99 females and 65 subadults) were marked and 15 individuals were recaptured (Table 1). According to the calculated data on day 2 of BE, population size was 1087 (95%CI: 1152 - 1021). On day 3, the estimated population size was 1508 (95%CI: 1584 - 1430) for BE. Accordingly, mean ± SD of the estimated population on day 2 and day 3 was 1297 ± 210 (95%CI: 1225 - 1369). The survival rate (Fig. 2) and the population gain were estimated to be 0.96 and 0.32, respectively. Population density of the all captured frogs was 1.75 individuals/100 m² and the mean rate was 6.9 per capita/hr. Distribution of size classes revealed that samples consisted mainly of 77.5 % adults (Fig. 3). The sex-ratio of all captured adult frogs was slightly biased in favor of males (1.27 males : 1 female, χ^2= 6.18 df= 1, P<0.001). Mean ± SD of adult SVL was 66.84 ± 6.74 mm in males (n= 126, range from 43.72 to 97.92 mm) and 74.62 ± 5.31 mm in females (n= 99 range from 44.66 to 101.58 mm). SVL means differed significantly between the sexes (t-test, t_{224} = 2.14, P<0.05).

Table 1. Habitat variables and captured - recaptured data of *Pelophylax ridibundus* population of Akören Lake native site (NC: Number caught, NR: Number recaptures, NNM: Number newly marked, PS: Population size, EP: Ecologicial parameters).

DATE	NC	NR		NNM	SVL (mm)	EP	Reference
		Day-1 recaptures	Day-2 recaptures				
1999							
Day 1	122	-	-	122			Kaya & Erismis (2001)
Day 2	115	6	-	109			Present study
Day 3	114	6	3	105			Present study
PS (on 3 days)	**3532**						
2005							
Day 1	121	-	-	122	Subadult (N: 77): 38.42 ± 3.73	Ph (water): 6.83	Present study
Day 2	111	9	-	102	Male (N=134) : 68.16 ± 5.04	°C (water): 16	Present study
Day 3	95	8	5	82	Female (116): 77.05 ± 6.09	°C (air): 13	Present study
PS (on 3 days)	**2007**						
2006							
Day 1	110	-	-	110	Subadult (N=65) 35.80 ± 3.63	Ph (water): 7.0	Present study
Day 2	94	8	-	86	Male (N=126): 66.84 ± 6.74	°C (water): 14	Present study
Day 3	86	4	3	79	Female (N=99): 74.62 ± 5.31	°C (air): 9	Present study
PS (on 3 days)	**1138**						
2007							
Day 1	119	-	-	119	Subadult (N=78): 37. 73 ±4.72	Ph (water): 7.3	Present study
Day 2	75	10	-	65	Male (N=115): 63.34 ± 4.29	°C (water): 21	Present study
Day 3	79	5	3	71	Female (N=80): 71.02 ±5.72	°C (air): 15.5	Present study
PS (on 3 days)	**1508**						
2008							
Day 1	112	-	-	112	Subadult (N=68): 34.73 ± 5.51	Ph (water): 6.9	Present study
Day 2	94	12	-	82	Male (N=110): 62.73 ± 3.09	°C (water): 16	Present study
Day 3	46	7	7	32	Female (N=72): 70.21 ± 4. 69	°C (air): 11	Present study
PS (on 3 days)	**426**						

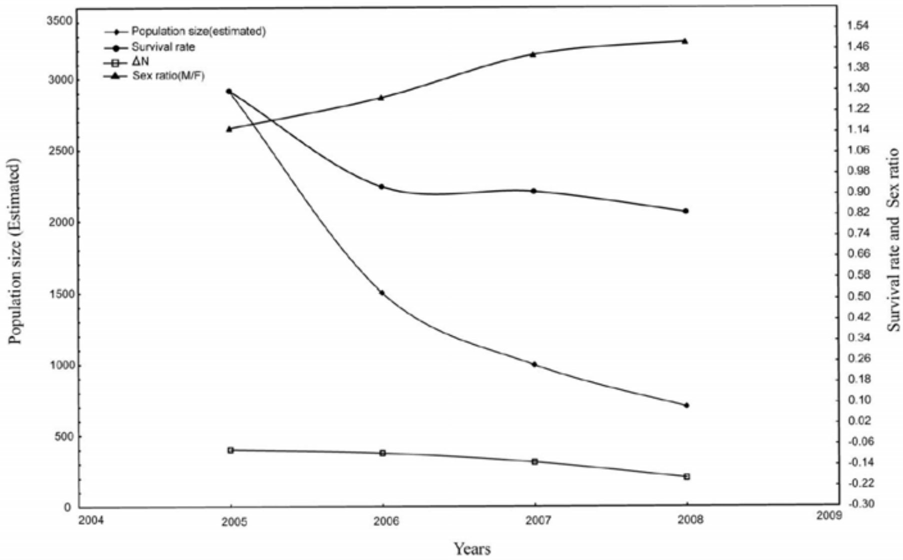


Figure 2. Population size (estimated), survival rate, ΔN and sex ratio of Eurasian marsh frogs (*P. ridibundus*) living in 26 August National Park's, taken from the capture mark-recapture, ΔN was calculated as log (Nt + 1) – log(Nt-1 + 1).

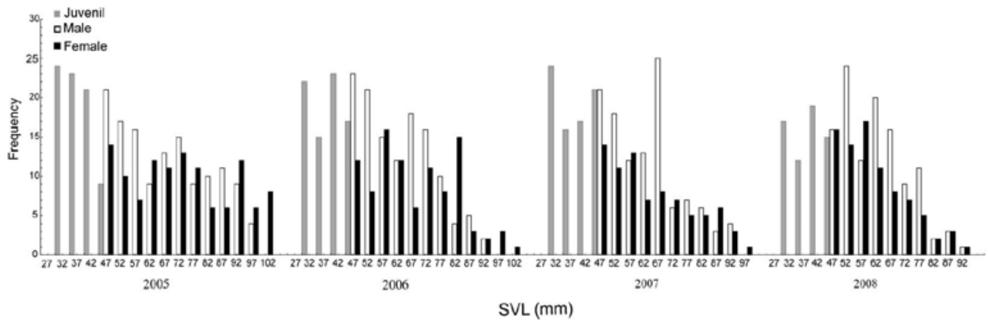


Figure 3. SVL size distribution of *Pelophylax ridibundus* specimens captured per year between 2005 - 2008.

In the study carried out between 8 - 10 September 2007, a total of 273 frogs (115 males, 80 females and 78 subadults) were marked and 18 individuals were recaptured (Table 1). According to the calculated data on day 2 of BE, population size was 725 (95%CI: 671 - 778). On day 3, the estimated population size was 1138 (95% CI, 1070-1205) for BE. Accordingly, mean ± SD of the estimated population on day 2 and day 3 was 931 ± 292 (95%CI: 870 - 991). The survival rate (Fig. 2) and the population gain were estimated to be 0.88 and 0.42, respectively. Population density of the all captured frogs was 1.65 individuals/100 m^2 and the mean rate was 6.5 per capita/hr. Distribution of size classes revealed that samples consisted mainly of 71.4% adults (Fig. 3). The sex-ratio of all captured adult frogs was significantly biased in favor of males (1.43 males: 1 female, $\chi 2= 6.28$ df=1, $P<0.001$). This year of the study, mean ± SD of adult SVL was 63.34 ± 4.29 SD mm in males (n= 115, range from 42.16 to 93.38 mm) and 71.02 ± 5.72 SD mm in females (N= 80 range from 44.22 to 97.08 mm). The SVL means did not differ significantly between the sexes (t-test, $t_{194}= 9.25$, $P> 0.05$), but females are slightly bigger than males.

In the study carried out between 23 - 25 August 2008, a total of 250 specimens (110 males, 72 females, and 68 subadults) were marked and 18 individuals of those were captured (Table 1). While population size with regard to BE was estimated at 667 (95%CI: 474 - 565) on 2 days, according to calculated data on the third day it was 426 (95%CI: 384 - 468). Mean ± SD of the estimated population size was 546 ± 120 (95%CI: 499 - 593). The survival rate and the population gain were estimated to be 0.55 and -0.15 respectively (Fig. 2). Population density of the all captured frogs was 1.51 individuals/100 m^2 and the mean encounter rate was 5.95 per capita/hr. Distribution of size classes revealed that samples consisted mainly of 72.8 % adults (Fig. 3). The sex-ratio of all captured adult frogs was significantly biased in favor of males (1.48 males : 1 female, χ^2= 9.43, df = 1, P< 0.001). The mean ± SD of adult SVL was 62.73 ± 3.09 mm in males (n= 110 range from 43.52 to 89.72 mm) and 70.21 ± 4. 69 mm in females (n= 72 range from 44.86 to 91.30 mm). The SVL means did not differ significantly between the sexes (t-test, t_{181} = 9.25, P>0.05).

Change in population size (ΔN)

The mean ± SD of ΔN (the change in abundance within population) were calcutated in -0.081 ± 0.015 for 2005 year, -0.094 ± 0.002 for 2006, -0.11 ± 0.02 for 2007, and -0.19 ± 0.03 for 2008 and -0.05 ± 0.01 according to data of 1999. The mean ± SD of ΔN showed significant differences between 1999 and the years studied in the present study (one-way ANOVA = 4.6, df=9, P<0.05) and these results indicated that the population of *P. ridibundus* in lake has declined significantly (Fig. 2).

Relationships between age - SVL and reproductive characteristics

Cross-sectioned phalanx of *P. ridibundus* were nearly circular in shape. In adults, the diaphysis of the phalanges was composed of two compact, concentric bone layers: an outer, broad layer of periosteal bone (PB), and an inner, narrow layer of endosteal (EB).

The PB was mainly formed of parallel-fibered bone. In all frogs, well-defined lines of arrested growth (LAG) were visible in the periosteal bone and were relatively easy to count in order to assess individual age (Fig. 4). To validate the skeletochronological method we analyzed our data on the basis of reproductive activity of the species and the period during which frogs were collected.

The annual reproductive activity period of *P. ridibundus* extends approximately from the middle of March through the middle of September. During the study, the earliest initial egg-laying was observed at night on May 10. Larval development was quite rapid, enabling the tadpoles to metamorphose after 40 - 52 days from hatching. The 21 newly metamorphosed froglets captured at the end of July were on average 21.58 ± 4.49 (range from 15.32 to 32.10 mm). One LAG was found in the periosteal bone of collected subadult individuals (n=25 range from 34.78 to 39.32 mm). These findings indicate that the first LAG is formed approximately during hibernation between the middle of November and the end of March of the following year. The outer edge of periosteal bone was considered as a LAG because the frogs were collected at the end of their breeding season.

It is assumed that one zone and one LAG corresponded to growth during a single year, with the LAGs representing periods of hibernation (Paton et al. 1991, Esteban et al. 2002). Thus, applying skeletochronology, the ages of all the individuals were assessed directly from the thin sections of the bones without the need for back-calculations (Sagor et al. 1998). The bone wall of the phalanx was made up of wide zonal regions of parallel-fibred bone tissue that alternated with distinct lines of arrested growth (LAG). Some sections exhibited very closely adjacent LAG, called "double LAG" (Fig. 4A).

The mean ± SD of adult SVL was 63.72 ± 4.58 mm and range from 42.52 to 89.72 mm for males (n=32) and 77.25 ± 3.62 mm and range from 44. 86 to 102.86 mm for females (N= 36). The SVL mean differed significantly between the sexes (t-test, t_{67}= 3.70, P<0.05 as well as SVL distribution, the Mann - Whitney U-test, U= 534 t-test, P<0.05).

Age ranged from 2 to 7 years for males and from 2 to 8 years for females, and did not significantly differ between the sexes (mean ± SD 4.82 ± 1.08 for males and 5.73 ± 1.06 for females (t-test, t_{67}= 2.63, P>0.05 as well as age distribution, the Mann-Whitney U-test, U= 742, P>0.05 and U= 629, P>0.05). SVL was significantly correlated with age for both sexes (Pearson's correlation, r=0.66, df=31, P<0.001 for males, r=0.73, df=35, P<0.001 for females).

Age and SVL data (available for males and females) were fitted to the von Bertanlanffy model and both asymptotic SVL (SVL_{max} ± CI) and growth coefficient (K ± CI) were very similar between the sexes ($SVL_{max-males}$ = 93.25 ± 2.9, $SVL_{max-females}$ = 104.56±3.59 and K_{males}= 0.6 ± 0.04, $K_{females}$ = 0.9 ± 0.02). Growth curves (Fig. 5A) showed a similar shape in males and females, but the growth coefficient (K) was slightly greater in $K_{females}$ than in K_{males}. Age- growth rate (Fig. 5B) was higher in females from metamorphosis until 2-3 years of age, and it was rather similar (or slightly lower in males than that of females) during the rest of life. Although the slow down in growth rate of female begins at 2 - 3 years of age, this occurs when males are 2 years old. In summary, inferred growth rate reached a maximum during the juvenile phase. It was higher in females than in males from metamorphosis until 2 - 3 years of age. Furthermore, inferred growth rate decreased clearly in both sexes at an age of 3 - 4 years old, i.e. before sexual maturity.

The mean SVL±SD was 66.52 ± 2.81 mm (N: 11) and range from 57.40 to 85.12 mm for males in in amplexus and 81.47 ± 3.62 mm (N: 11) and range from 68. 16 to 97.34 mm for females in amplexus. There was a significant positive corelation between SVL of female and males in amplexus (Pearson's correlation r =0.71, df=21, P<0.001). The minimum and maximum recorded values of egg number (fecundity) were 1255 eggs for a 62.48 mm SVL and three-year-old frog and 2610 eggs for a 98.46 mm SVL and eight year-old frog from mid-

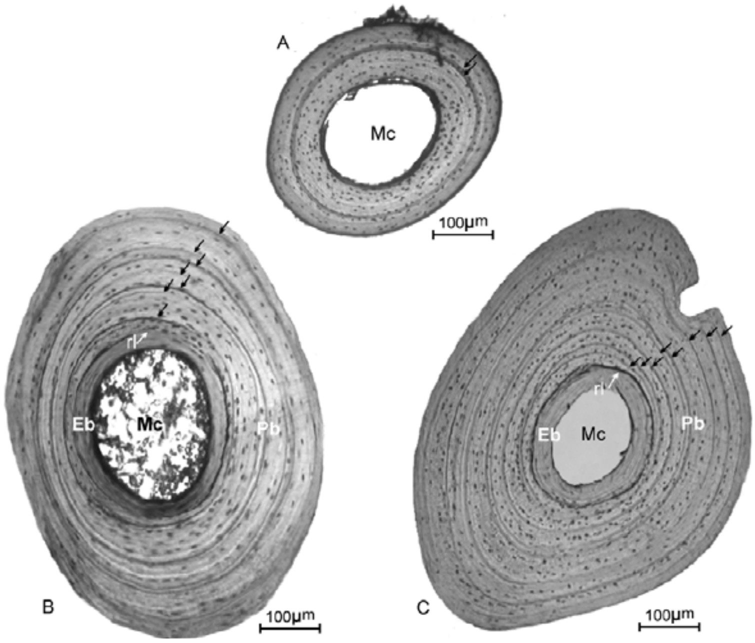
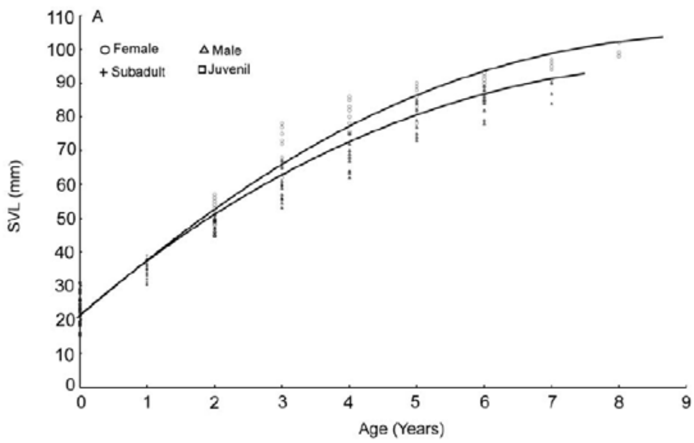


Figure 4. Haematoxylin-stained cross sections of *Pelophylax ridibundus* phalanges Mc=marrow cavity. Arrows indicate line of arrested growth (LAGs), Eb=endosteal bone, Pb=Periosteal bone, rl= resorption line, a.Subadult, 37.32 mm, double LAGs, b.Male, 89.16 mm, seven LAGs, c.Female, 93.76 mm, eight LAGs.

Table 2. The influence of age and SVL on reproductive characteristics of females.

Character	Factor	Df	MS	F	P
Fecundity	Age	5	93053.29	52.78	< 0.000
	SVL	1	313238.88	93.73	< 0.000
	SVL-Age	5	72298.34	2.47	0.008
Eggs Diameter	Age	5	0.624	12.391	< 0.000
	SVL	1	0.883	51.213	< 0.000
	SVL-Age	5	0.008	3.25	0.009
Reproductive effort	Age	5	0.00306	0.98	0.745
	SVL	1	0.00016	3.1	0.082
	SVL-Age	5	0.00058	0.614	0.069



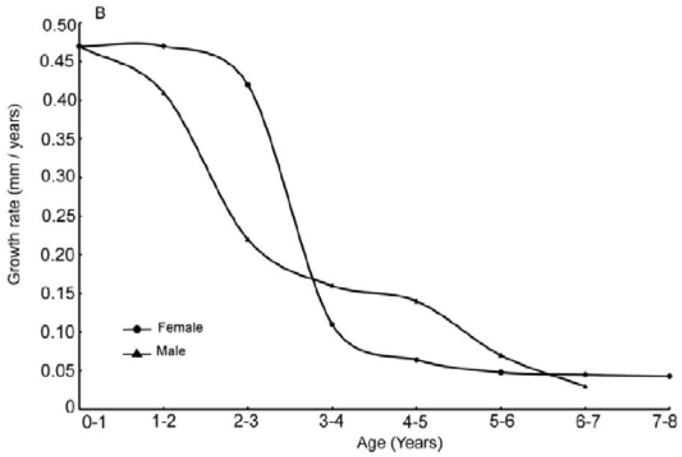


Figure 5. (A) Relationship between SVL and age classes, Growth curve follow the von Bertalanffy model. **(B)** Age-specific growth curve for males and females of *Pelophylax ridibundus*).

May to end-July. The mean ± SD of the eggs diameter calculated in 1.62 ± 0.49 mm varied from 1.3 mm to 2.55 mm. In the estimation of the statisical significance among the data on body length and reproductive characteristics in the age groups, ANCOVA (factors »age«) was used. The factor »age« had six levels (3 - 8) SVL. There was a significant relationship among the SVL, fecundity, and egg diameter compared to factor »age«. On the other hand, there was no significant relation among SVL and reproductive effort when compared to factor »age«. ANCOVA revealed a significant effect of the interaction of age and SVL, the interaction effect on fecundity became non-significant, also indicating that these characteristics are influenced by body length rather than age. A significant positive correlation with SVL was revealed for fecundity and egg diameters, $r=0.58$, $P<0.001$ and $r=0.26$, $P<0.001$, respectively. Additionally, there was a moderate positive correlation ($r=0.20$ $P>0.001$) between fecundity and egg diameters, but not between reproductive effort and age groups ($r=-0.18$ $P>0.001$).

Ecological Observations

Adult males move into the breeding areas in early spring, and call during April and September. The males call from small chambers in moss or other soft vegetation at the edges of the lake. The frogs have three call types, an advertisement call, threat call, and courtship call. The advertisement call and courtship call are used to attract females to the males calling site, whereas the threat call serves as a warning to other males. Stimulating the males to give threat calls has been recommended as a reliable procedure for monitoring the number of calling males. Females only enter the bogs briefly to lay their eggs in the terrestrial oviposition site, and then leave the breeding site. The males continue calling for a number of weeks, presumably to continue mating.

Observations made between the years 2005 - 2008 suggest that Eurasian marsh frogs use two distinct habitat types: a breeding season habitat associated with lake covered with star duckweed (*Lemna trisulca*), sanicle-leaved water crowfoot (*Ranunculus saniculifolius*), swamp knotweed (*Polygonum amphibium*) and wet tussock grasslands and wet heath, and a terrestrial non-breeding habitat consisting of woodland and tall heath adjacent to the breeding area (dominant vegetation surrounding the lake) including black willow (*Salix nigra*), perennial pepperweed (*Lepidium latifolium*), great yellowcress (*Rorippa amphibia*), salt cedar (*Tamarix parviflora*), pale smartweed (*Polygonum lapathifolium*), water plantain (*Alisma plantago-aquatica*), narrow-leaf water-plantain (*Alisma gramineum*), and flowering rush (*Butomus umbellatus*).

The breeding locations are the most sensitive and crucial aspect of the reproduction activity of the Eurasian marsh frog. Because of the lack of suitable breeding area in the region studied, protection of the area around this lake and seepage is essential to keep it as a long-term persistence breeding site. The fauna of the lake is comprised of the following vertebrates: Amphibians: Syrian spadefoot toad (*Pelobates syriacus*), and Green toad (*Bufo viridis*); Reptiles: European pond turtle (*Emys orbicularis*), Grass snake (*Natrix natrix*); Birds: Hoopoe (*Upupa epops*), White stork (*Ciconia ciconia*), Greylang goose (*Anser anser*), Gadwall (*Anas strepera*); and Mammals: Mouse-eared bat (*Myotis myotis)*, Water shrew (*Neomys teres*), Anatolian vole (*Microtus anatolicus*). Possible predators of the species inhabiting the investigated area largely destroy the juveniles trapped in the shallow parts of the lake. Flocks of birds gathering over the lake prey on vulnerable individuals in shallow waters.

Discussion

Although numerous studies of population size, structure, and dynamics of amphibians have been conducted since the 1960s (e.g.,Turner 1960, Pope & Matthews 2001, Richter & Seigel 2002, Watson et al. 2003), few have considered long-term changes in population structure and density (Tocher et al. 1997, Meyer et al. 1998, Sas et al. 2006). Until now, no previous study documented long-term monitoring of any population of *Pelophylax ridibundus.* The preliminary study made during 1999 (REF) indicated that the eurasian marsh frog was abundant within its limited geographic range (Kaya & Erismis 2001). However, by 2005 it was apparent that the species had declined considerably in abundance. Monitoring throughout a three year period (2006- 2007- 2008) has indicated that populations continued to decline and these results suggest that the remaining population is faced with a continued downward trend. Although the eurasian marsh frogs showed a significant tendency towards population decrease, the mean values of ΔN were slightly negative (-0.05) in 1999. However between the years 2005-2008 it was found that this population in Akören Lake averaged a significantly faster decrease in population size according to mean ΔN (-0.13 ± 0.08 SD) than in the year 1999 (Fig. 2). The species has disappeared from 78 % of the sites in which it formerly occurred (Table 1).

Fluctuations in population size are a common phenomenon in amphibians and can be caused by competition, predation, density (Tocher et al. 1997, 2006, Meyer et al. 1998, Sas et al. 2008, Yu et al. 2009). In addition to these explanations, population growth usually depends on the number of females available for reproduction rather than the number of males. Studies carried out on different anuran species have also revealed that males either outnumber females during the breeding season or exist in equal numbers (Vences et al. 1999). However, females outnumbering males is a predictable result under normal conditions (Sperling et al. 1996, Ryser 1988, 1996, Richter & Seigel 2002). Thus, these results indicate a dire problem regarding population size, since there is clearly a male-biased sex ratio. The observed sex ratio is expected to be 1:1 (female : male). In the present study, it is observed that The male biased sex ratios (female to male < 1:1) represents the entire population. The base study on this region (Kaya & Erişmiş 2001) reported that the population size of Akören Lake numbered 3532 individuvals, with a ratio of 3 females to 1 male. Thus the present study has shown that numbers of both mature males and female of *P. ridibundus* in Akören lake had decreased each year with resulting lower numbers than that of previous years (Table 1, Fig. 2).

As far as SVL is concerned, anthropogenic activities have negative/positive effect on amphibian populations (Demaynadier and Hunter, 1998; Sireika and Stasaitis, 1999). The present study found that past anthropogenic activities had negative effect on body size of *P. ridibundus.* Since the commercial collectors prefer collecting larger sized individuals, harvesting of the species could lead to significant changes in age and body sizes of the Eurasian marsh frog population of Akören Lake (Fig. 3). Man-made activities and conversion of habitat have rendered a direct and indirect effect on habitat quality affecting the population size of this frog. Interestingly, our study has documented significant changes in average body size between 1999 - 2008 years which may be attributed directly to human effects.

It is assumed that amphibians show indeterminate growth and that body size and ages are strongly correlated in adult amphibians (Duellman & Trueb 1994), although Halliday (1988) questioned this assumption by reviewing the data available not only for amphibians but also for reptiles. In fact, among anurans, skeletochronologically studied positive correlation between body size and age was generally reported for both sexes or only for females but not for males (Esteban et al. 2002, Guarino et al. 2003), and more rarely only for males (Cherry & Francillon 1992). Therefore, these results showing significant correlations between age and body size in both sexes of *P. ridibundus* in Akören Lake are similar to those of most anurans studied so far.

Like many amphibian species (Gibbons & McCarthy 1984, Yılmaz et al. 2005, Tsiora & Kyriakopoulou 2002, Kyriakopoulou et al. 2007) in *P. ridibundus* male and female growth rates are high until sexual maturity and decrease thereafter. Generally, this growth pattern is explained by the fact that individuals mainly use energy for somatic growth before sexual maturity (Jorgensen 1992). After sexual maturity is attained, among amphibians it appears to be great variation in growth rates of the two sexes. In fact, in some amphibian species growth rates tend to be higher in female than males (Ryser 1988, Tsiora & Kyriakopoulou 2002), while in other species the contrary is observed (Leclair et al. 2005), this contributing to determine sexual size dimorphism in adults. In *P. ridibundus*, von Bertalanffy model indicates that growth rates of females are slightly higher than those of males after sexual maturity. For the *P. ridibundus* population of Akören Lake, males started breeding at 2 and females at 3 years of age, and thus breeding males were younger than females on average. These values of first breeding may be viewed as moderate when compared with published results of the other anuran species (2 yrs in males and 2-3 yrs in females: e.g., Yılmaz et al. 2005, Kyriakopoulou et al. 2007). The fact that males tend to be younger than females at maturity has been reported in various anuran species (e.g., Cherry & Francillon-Vieillot 1992, Ryser 1996, Marquez et al. 1997, Kyriakopoulou et al. 2007). Males would breed as soon as they mature reproductively whereas females would not attempt to breed until reaching a critical size (Leclair & Castanet 1987, Cherry & Francillon-Vieillot 1992). This delayed maturity in females must have resulted from the individual investing its energy resources for egg production (Guarino et al. 2003). This study has found that the oldest females were 8 years old while the males were 7 years older.

Finally, the progressive decline of this species has been observed in several fragmented areas. From our study it is evident that the eurasian marsh frogs population in Akören Lake faces three primary threats: (1) destruction of habitats and microhabitats, especially frog breeding habitats or areas adjacent to the habitat, (2) vulnerability by predators such as snakes, birds, mammals (3) commercial harvesting by humans. In Turkey, the estimation of abundance and the detection of threats for populations of eurasian marsh frog need to have planned studies with long-term monitoring, especially since this species is collected and exported indiscriminately. As a result of this trade, populations are dramatically decreasing and this species will thus soon become endangered.

Conclusion

It is considered that age and size differences found between populations of *P.ridibundus* might have resulted from some biological factors such as food availability and interspecific competition that are different between populations. But direct and indirect effects of environmental pollution, commercial collectors, detrimental landscaping and global climate change are more difficult to quantify in many instances and will be more difficult to change in the short term monitoring of species. Therefore long-term monitoring of species such as the eurasian marsh frog is essential and must be aided by the establishment of standard methods and techniques. Furthermore, the current study has contributed to establish management actions for this species conservation, and demonstrates that population age structure data may provide valuable insights into demographic variability within and between populations and species. This may have important implications for interpretation of population declines, and conservation and management responses. The current study reports that skeletochronology provides a valuable insight into the structure of declining amphibian population. From all these results it appears that SVL remained a significant predictor of fecundity and eggs diameter. Interspecific competition in 26 - August National Park may have been strengthened through artificial modification of the areas inhabited by frogs. Thus, it appears that ecological studies are needed to assess how habitat modification by humans affect the populations of *P. ridibundus* and other frog species in 26- August National Park.

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