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Population dynamics of amphibian community in Lake Çıldır, Northeastern Anatolia

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Knowledge on life history traits and population dynamics of amphibians from northeastern Anatolia is limited. This results in a heavy reliance on published data regarding the European and Caucasian counterparts of the Anatolian populations in local conservation studies. The present study evaluated the phenology, age structure, population size, and population structure of the amphibian community in Lake Çıldır, northeastern Anatolia, Turkey. The site was surveyed between April 2017 and July 2019, detecting the presence of *Bufo verrucosissimus*, *Bufotes viridis sitibundus*, *Pelophylax ridibundus,* and *Rana macrocnemis.* The estimated population size ranged from 553 to 789 individuals for *P. ridibundus* and from 404 to 655 individuals for *R. macrocnemis*. The estimated mean capture probability was 0.09-0.10 for *P. ridibundus* and 0.08-0.11 for *R. macrocnemis*. The mean survival rates were estimated at 0.40-0.60 for *P. ridibundus* and 0.24-0.56 for *R. macrocnemis*. A male-biased sex ratio was observed for all species. The mean age was estimated at 5.28 years (2-9) for *P. ridibundus*, 4.32 years (2-6) for *R. macrocnemis*, and 5.55 years (3-9) for *B. viridis sitibundus*. Depending on weather conditions, the amphibian species emerged from brumation in the second half of April, and the breeding period took place between the end of April and June. The metamorphosis was attained in 55-125 days for *P. ridibundus*, 44-124 days for *R. macrocnemis*, and 56-128 days for *B. viridis sitibundus*, depending on weather conditions. The amphibian community of Lake Çıldır comprises healthy frog and toad populations, whose primary threats appear to be weather fluctuations and climate change. In contrast to their western Anatolian conspecifics, the studied amphibians suffered limited habitat loss and degradation, rendering them effective candidates as indicator species for climate change studies.

Key words: amphibian community; Caucasus; life history; population dynamics; skeletochronology.

Amphibians are considered indicator species for wetland habitat quality in various ecosystems, ranging from tropical rainforests to arid deserts (Stebbins & Cohen, 1995). However, amphibian populations are globally threatened, with numerous species facing the risk of extinction. The main factors driving these population declines are habitat loss and destruction (Fisher & Shaffer, 1996; Davidson *et al*., 2001; Marsh & Trenham, 2001). Knowledge about the underlying causes of extinction is imperative to undertake measures to implement effective solutions

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for the critical issues (GASCON *et al*., 2007). Therefore, it is of pivotal concern in ecology and conservation biology to understand the mechanisms that drive population dynamics (CAYUELA *et al*., 2020). Researchers have recently focused on mechanisms at different scales to synchronize population dynamics (RANTA *et al*., 1995; BJØRNSTAD *et al*., 1999; LIEBHOLD *et al*., 2004; KOENIG & LIEBHOLD, 2016). Estimating the fecundity, survival, and age-related variation in demographic parameters is crucial for managing and protecting natural populations (SANDERCOCK, 2006).

The foremost threat to amphibian species in Turkey is habitat loss, degradation, and fragmentation (ÇIÇEK & CUMHURIYET, 2007; YAŞAR *et al*., 2021). Urbanization, infrastructure development, mining operations, agricultural expansion, and forestry activities cause substantial damage to natural habitats and pose a significant threat to the survival of amphibian species (ÇIÇEK & CUMHURIYET, 2017). In Turkey, the Marmara, Aegean, and Mediterranean regions face the highest levels of human-mediated pressure, with published data on amphibian population densities and urbanization rates showing a significant negative correlation (AKSU, 2011; YAŞAR *et al*., 2021). The degradation of wetlands is primarily caused by agricultural irrigation activities, draining of swamps, construction of dams, intensive fishing practices, and pollution (WWF, 2008). Also, intensive forestry activities and improper afforestation can significantly disrupt forest ecosystems surrounding the wetlands, adversely affecting the resident species. Moreover, the introduction of non-native fish species, overgrazing, and excessive exploitation for pet trade or human consumption also cause great harm to amphibian populations (ÇIÇEK & CUMHURIYET, 2017; YAŞAR *et al*., 2021). Therefore, it is crucial to collect more data on the life history and ecology of amphibians in Turkey to aid the development of effective management strategies for conservation efforts.

The Caucasus Ecoregion covers an area of approximately 580 000 km^2 and lies within the borders of Armenia, Azerbaijan, Georgia, Russia, and Turkey (northeastern Anatolia) (ZAZANASHVILI & MALLON, 2009). Twenty-eight out of 77 reptiles and four out of 14 amphibians in the Caucasus are endemic to the region (TUNIYEV *et al*., 2020). Northeastern Anatolia is renowned for its rich herpetofauna (TUNIYEV *et al*., 2020) and harbors nine amphibian species (YAŞAR *et al*., 2021). Located in the southwest of the Lower Caucasus Ecoregion, Lake Çıldır is characterized by its alpine meadow landscape. In this study, four species of anurans comprising the amphibian community around Lake Çıldır (i.e. *Bufo verrucosissimus*, *Bufotes viridis sitibundus*, the *Pelophylax ridibundus* group, and *Rana macrocnemis*) were investigated for population structure (sex ratio, size distribution), age structure (age at sexual maturity, mean age, growth rate), and population dynamics (population size, density, survival rate, capture probability).

Materials and Methods

Study site

Lake Çıldır [41.0425° N, 43.2552778° E, 1959 m a.s.l.] covers a surface area of 123 km^2 and is located at the border between Ardahan and Kars provinces. It is the sec-

ond-largest freshwater lake in the region, bordered by Mount Akbaba in the east and Mount Kısır in the west. The lake basin itself spans approximately 640 km² and is surrounded by rural settlements (ZENGIN *et al*., 2012). Although climate stress is the main factor affecting the lake, human activities in the region, including agriculture and animal husbandry, also have an impact on it. Around 12-13 streams primarily fed by snowmelt contribute to the lake's water inflow. These streams include Gölbelen, Kındırga, and Gölebakan in the western part of the basin, Gülyüzü in the southwest, the one that emerges from Doğruyol Village in the northeast, and the ones that pass through Taşbaşı-Taşköprü villages and Göldalı-Çanaksu villages in the south and east, respectively (ULUTAŞ, 2011). The climate in the vicinity of the lake is classified as warm-summer humid continental climate (Dfb) according to the Köppen-Geiger system (BECK *et al*. 2018). The region experiences short, mild summers and cold winters at higher elevations. The average annual temperature in Ardahan is 11.9°C and varies between 6.3 and 17.8°C throughout the year. The average yearly precipitation in the province is 391.9 mm (TURKISH METEOROLOGY STATION, 2022).

Fieldwork

Field surveys were conducted during breeding season in spring and post-breeding season in summer, spanning from April 2017 to June 2019. The surveys were carried out at regular intervals over a period of three years, with yearly fieldwork amounting to a total of 25 days. Additionally, to investigate the life cycle and phenology of the amphibian species, four sites were visited at least two days per month between April and November, from 2017 to 2018. Firstly, random routes were selected on a map to assess the presence or absence of amphibian species in and around the lake. Then, visual encountering surveys (HEYER *et al*., 1994) were conducted along these routes in two-observer teams.

Four permanent sites that effectively represent different types of macrohabitats were selected to evaluate the amphibian community from the lake. These sites included the banks of streams that feed the lake (St.2_Memba [Lat. = 40.962150, Long. = 43.297433, 1970 m a.s.l.], St.4_Gölebakan [Lat. = 41.056721, Long. = 43.148675, 1963 m a.s.l.]), a marshy area (St.3_Gölbelen [Lat. = 41.074679, Long. = 43.137906, 1971 m a.s.l.]) and a small pond (St.1_Lake Lavaş [Lat. = 40.938736, Long. = 43.264685, 1994 m a.s.l.]) (Fig. 1). Mark-recapture surveys were carried out at these stations by a team of two researchers over a period of three years. Visible Implant Elastomer tags (Northwest Marine Technology Inc.) were used to mark individuals for identification. Anestol (5% lidocaine) ointment was applied to provide anesthesia and Furacin (0.2% nitrofurazon) and Bactroban (2% mupirosin) ointment to prevent infection (ÇIÇEK *et al*., 2011a).

The snout-urostyle length [SUL, mm] was measured using a digital caliper (Wert brand, China) to the nearest 0.01 mm. Additionally, the weight (W, gr) was measured using a digital scale with 0.1 g sensitivity. Following the completion of measurements, the marked individuals were released back into their natural environ-

Figure 1: General view of Lake Çıldır and sampling stations. Adapted from Google Earth.

ment within an hour of capture. Secondary sex characteristics were examined to determine the sex of individuals. For each species, sex ratios (juvenile-adult, female-male) and the average length and weight values were calculated separately. Chi-squared (expected / observed) and Student's t-test were used to compare the body size between sexes, and one-way analysis of variance (ANOVA) was used to compare the phenology, age structure, population size, and population structure. Statistical analyses were performed using the software PAST (vers. 4.02, HAMMER *et al*., 2001) at a 95% confidence interval, and means were given alongside their standard deviations.

Age estimation

To determine the age of anuran populations, a total of 121 *P. ridibundus* group (11 juveniles, 68 males, 42 females), 76 *R. macrocnemis* (8 juveniles, 30 males, 38 females), and 21 *B. viridis sitibundus* (13 ♂♂, 8 ♀♀) were randomly selected. Age estimation was achieved based on the skeletochronological analysis of the second phalanx of the fourth toe taken from the right hindlimb of each individual. The phalanges were cut using surgical scissors and preserved in Eppendorf tubes filled with 70% ethanol. In the laboratory, layers of skin and muscle were removed from the bone, and cross-sections with a thickness of 10-15 µm were prepared using standard histological techniques. Phalangeal cross-sections were stained with Ehrlich's hematoxylin for 10-15 minutes, and lines of arrested growth (LAGs) were separately counted by both authors (O.C. and K.Ç.), who were blinded to sample identifiers to avoid observer bias. To estimate the age at sexual maturity, field observations such as the sizes of individuals engaged in am-

plexus, and the presence of secondary sex characteristics associated with sexual maturity were recorded. According to KLEINEBERG AND SMIRINA (1969), the first incidence of a markedly narrow distance between the two outermost LAGs indicates that sexual maturity is attained. The non-parametric Mann-Whitney U test was used to compare age structure between the sexes. The relationship between size and age was tested using the VON BERTALANFFY (1938) growth formula modified by HEMELAAR (1985): $SUL_t = SUL_{max} - (SUL_{max} - SUL_{met}) e^{-k(t-tmet)}$, where SUL_t is the mean length at age t (mm), SUL_{max} the maximum length (mm), SUL_{met} the mean metamorphosis length (mm), t the growing season (year), t_{met} the intersection with time axis (metamorphosis time, year), and k the growth coefficient.

Population estimation

The calculation of population sizes and relevant parameters, including population size (*N*), capture probability (*p*), and survival rate (ϕ), was carried out in two different ways, depending on whether the populations are open (year) or closed (season). For the samplings that were carried out during four consecutive days in spring and summer, the populations were considered closed, assuming that no individuals joined (birth and immigration) or left (death and emigration) the populations for that short period. Using the CAPTURE (OTIS *et al*., 1978; WHITE *et al*., 1982) module of the MARK program (COOCH & WHITE, 2022), we calculated *N*, *p*, and their confidence intervals according to the model (h, heterogeneity).

The sampling studies at the stations included sufficient evaluation to make the calculations for the years 2017 and 2018. When evaluating the annual data of 2017 and 2018, populations were considered open, assuming that some individuals joined or left the population between samplings campaigns. Pollock's Robust Design formula "Huggins p and c" (HUGGINS, 1989) estimator was used to evaluate the annual data (from eight 8 times per year). This estimator takes into account both the capture probability (*p*) and recapture probability (*c*) and assumes them to be equal, representing a Markov model for the parameters of temporary migration. The time -dependent survival rate (ϕ) and capture probability (*p*) model {ϕ(t), p(t)} was estimated based on the model with the lowest value of the Akaike information criterion (AICc). The low value of AICc indicates the consistency of the model (COOCH & WHITE, 2022). All analyses were performed using the program MARK (COOCH & WHITE, 2022).

RESULTS

Morphology

Four amphibian species [*Pelophylax ridibundus* (Pallas, 1771) group; *Rana macrocnemis* Boulenger, 1885; *Bufotes viridis sitibundus* (Pallas, 1771); *Bufo verrucosissimus* (Pallas, 1814)] were found in suitable habitats in and around Lake Çıldır. Females of two species were significantly larger (Student's t-test; $t = 6{,}098$; $P \leq 0.001$ for *P. ridibundus* group, $t = 7.806$; $P \leq 0.001$ for *R. macrocnemis*) and heavier ($t = 4.991$; $P \leq 0.001$ for *P. ridibundus* group, $t = 13.570$; $P \leq 0.001$ for *R. macrocnemis*) than the males (Table 1). The SUL values varied slightly among stations (One-way ANOVA; $F_{2,301}$ =

Table 1: Body length data (in mm) for each species, age class and sex. Data are presented as mean ± SD values, with ranges between parentheses.

Species	Juveniles	Males	Females
B. verrucosissimus	-	66.8 ± 8.3 (58.3-77.5)	
B. viridis sitibundus	39.7 ± 7.46 (31-48.3)	79.5 ± 8.2 (58.6-89.4)	77.0 ± 5.5 (68.5-88.7)
P. ridibundus group	42.7 ± 3.1 (35.9-47.2)	63.3 ± 8.4 (46.6-89.2)	72.8 ± 14.1 (48.1-104.9)
R. macrocnemis	38.2 ± 3.4 (30.4-41.8)	54.3 ± 6.0 (47.3-73.4)	60.0 ± 5.0 (48.4-69.9)

Table 2: Sex ratios, expressed as the quotient between male and female abundance, per study site for the two sampled ranid species. Results of the chi-squared (X^2) analyses to test significant deviations from the balanced sex ratio are shown.

Species	St.1_Lake Lavaş	St.2_Memba	St.3_Gölbelen	St.4_Gölebakan	Overall
P. ridibundus group	1.4 $X^2 = 6.39$ P = 0.12	1.2 $X^2 = 2.54$ P = 0.45	1.1 $X^2 = 9.14$ P = 0.03	1.2 $X^2 = 1.32$ P = 0.47	1.2 $X^2 = 2.70$ P = 0.45
R. macrocnemis	1.3 $X^2 = 5.12$ P = 0.5	1.3 $X^2 = 2.62$ P = 0.21	1.3 $X^2 = 2.04$ P = 0.27	1.5 $X^2 = 5.81$ P = 0.02	1.2 $X^2 = 2.35$ P = 0.45

2.977; P ≤ 0.032 for *P. ridibundus* group, $F_{2,296} = 0.607$; P ≤ 0.611 for *R. macrocnemis*). In *B. viridis sitibundus*, both sexes were found to have similar SUL values (t = 1.446; P ≤ 0.153) and weight (t = 1.901; P ≤ 0.062), and the SUL values varied slightly among stations (t = 2.209; P ≤ 0.032).

Population structure and dynamics

In 2017, the distribution of age classes and sexes in Lake Çıldır populations of *P. ridibundus* group was determined to be 23% juveniles, 45% males, and 32% females. In 2018, the percentages of these groups were 19%, 41%, and 40%, respectively. In *R. macrocnemis*, the distribution of classes in 2017 was 20% juveniles, 47% males, and 33% females, and in 2018 it was 20% juveniles, 42% males, and 38% females. The statistical analysis revealed that, although a male-biased sex ratio was observed in the two ranid populations, the differences were generally non-significant (Table 2). Populations of *B. viridis sitibundus* included 7% juveniles, 52% males, and 41% females. Regarding Data collected on *B. verrucosissimus* the available data did not allow to determine age or sex class distribution, but the available information reflected a balanced sex ratio.

Only four (two males and two females) individuals of *B. verrucosissimus* were observed during the entire study period. The total number of *B. viridis sitibundus* found in field surveys was 155 (five juveniles, 86 males, 64 females). Due to the low recapture rate in this species (<5%), the number of captured individuals was designated as the population size, and the parameters could not be calculated. Regarding the two

Table 3: Seasonal estimated population size (*N*) with its standard error (SE) and capture probability (*p*). Data include mean estimate and range for each parameter, species, year and location.

Species	Year	Station	*N*	SE	*p*
P. ridibundus group	2017	St.1_Lake Lavaş	435 (313-657)	87.57 (39.18-135.96)	0.11 (0.07-0.15)
		St.2_Memba	328 (241-481)	59.16 (8.56-59.76)	0.11 (0.11-0.11)
		St.3_Gölbelen	385 (374-476)	72.44 (67.43-77.45)	0.10 (0.10-0.10)
		St.4_Gölebakan	314 (215-499)	69.80 (60.14-79.46)	0.09 (0.09-0.10)
	2018	St.1_Lake Lavaş	625 (513-788)	69.00 (60.45-77.55)	0.12 (0.11-0.15)
		St.2_Memba	466 (382-591)	52.40 (42.54-62.26)	0.14 (0.13-0.16)
		St.3_Gölbelen	469 (380-602)	55.73 (52.88-58.59)	0.13 (0.13-0.13)
		St.4_Gölebakan	405 (318-544)	56.34 (52.36-60.32)	0.12 (0.12-0.13)
R. macrocnemis	2017	St.1_Lake Lavaş	331 (234-506)	67.08 (49.42-84.74)	0.10 (0.08-0.12)
		St.2_Memba	466 (301-779)	116.79 (77.13-156.45)	0.07 (0.06-0.09)
		St.3_Gölbelen	357 (231-604)	90.88 (72.68-109.09)	0.08 (0.07-0.09)
		St.4_Gölebakan	338 (254-482)	56.67 (46.82-66.53)	0.12 (0.11-0.13)
	2018	St.1_Lake Lavaş	461 (382-579)	49.58 (40.87-58.29)	0.14 (0.13-0.16)
		St.2_Memba	408 (326-535)	52.25 (50.04-54.46)	0.13 (0.13-0.13)
		St.3_Gölbelen	261 (213-340)	31.63 (28.25-35.02)	0.17 (0.16-0.18)
		St.4_Gölebakan	436 (356-557)	50.53 (49.83-51.23)	0.14 (0.14-0.14)

Table 4: Population parameters calculated by Pollock's Robust Design: capture probability (*p*), survival rate (ϕ), temporary migration rate (γ), overall population size (*N*) and density (in individuals per hectare). Data include mean estimate and range for each parameter, species and location.

Species	Station	*p*	ϕ	γ	*N*	Density
P. ridibundus group	St.1_Lake Lavaş	0.10 (0.06-0.17)	0.45 (0.07-1)	0.80 (0.07-1)	789 (341-1188)	28.17
	St.2_Memba	0.10 (0.06-0.13)	0.60 (0.06-1)	0.81 (0.12-1)	624 (364-1066)	135.65
	St.3_Gölbelen	0.09 (0.06-0.12)	0.66 (0.02-1)	0.84 (0.07-1)	665 (361-995)	29.55
	St.4_Gölebakan	0.09 (0.06-0.12)	0.40 (0.02-0.58)	0.77 (0.00-1)	553 (273-907)	138.25
R. macrocnemis	St.1_Lake Lavaş	0.10 (0.07-0.16)	0.24 (0.00-0.69)	0.73 (0.28-1)	579 (290-1004)	20.67
	St.2_Memba	0.08 (0.05-0.13)	0.56 (0.00-0.99)	0.73 (0.28-1)	655 (363-1367)	142.39
	St.3_Gölbelen	0.11 (0.05-0.17)	0.40 (0.00-0.77)	0.60 (0.03-1)	404 (272-657)	17.95
	St.4_Gölebakan	0.11 (0.08-0.14)	0.55 (0.05-0.80)	0.80 (0.02-1)	573 (336-870)	143.25

ranids, during the study period between 2017-2018, 2715 individuals (558 juveniles, 1158 males, and 999 females) of *P. ridibundus* group and 2400 individuals (496 juveniles, 1045 males, and 859 females) of *R. macrocnemis* were marked. The number of recaptured individuals within the two years was 784 (98 juveniles, 374 males, 312 females) for *P. ridibundus* group and 714 (74 juveniles, 384 males, 256 females) for *R. macrocnemis*. The seasonal population size of *P. ridibundus group* in the studied stations was calculated as 436-1003 individuals in spring (breeding season) and 383-919 in summer. For *R. macrocnemis*, the population size ranged between 342 and 677 individuals in spring (breeding season) and between 399 and 851 individuals in sum-

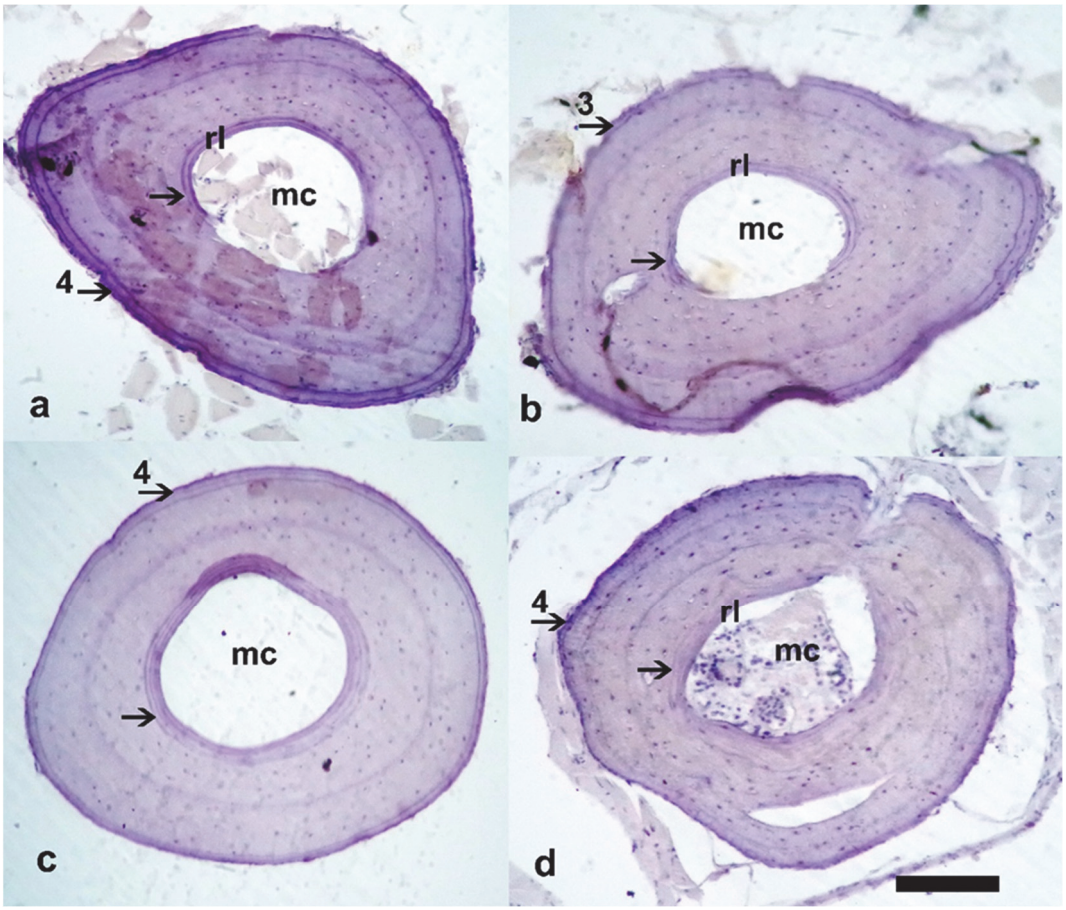

Figure 2: Phalangeal cross-sections of amphibians from Lake Çıldır (Northeastern Anatolia). (a) 4 LAGs male *P. ridibundus* group (SUL = 71.54 mm), (b) 3 LAGs female *P. ridibundus* group (84.72 mm), (c) 4 LAGs female *R. macrocnemis* (52.18 mm), (d) 4 LAGs male *B. viridis sitibundus* (73.54 mm). mc: marrow cavity, rl: resorption line. Arrows point to lines of arrested growth and resorption lines. Bar = 100 μm.

mer (Table 3).

According to Pollock's Robust Design formula, the capture probability (p) of *P. ridibundus* group at studied stations was 0.06-0.17, with a survival rate (ϕ) of 0.02-1, migration rate (γ) of 0-1 and overall population size of 553-789 individuals. For *R. macrocnemis*, a capture probability of 0.05-0.17, average survival rate (ϕ) of < 0.1-0.99, mean temporary migration rate (γ) of 0.02-1, and mean overall population size of 404-655 individuals were estimated (Table 4). When the two species were compared, it was determined that the population of *P. ridibundus* group outnumbered *R. macrocnemis* at stations St.1_Lake Lavaş and St.3_Gölbelen. In contrast, the latter outnumbered the former at St.2_Memba and St.4_Gölebakan.

Table 5: Number of individual amphibians per age class (in years, represented in roman numerals) captured during the study in Lake Çıldır.

Species	Gender	II	III	IV	V	VI	VII	VIII	IX
P. ridibundus group	Males	4	5	12	11	9	7	2	
	Females	2	3	4	5	7	4	2	3
	Overall	6	8	16	16	16	11	4	3
R. macrocnemis	Males	3	7	8	7	9			
	Females	3	9	7	8	8			
	Overall	6	16	15	15	17			
B. viridis sitibundus	Males		2	5	6	4	3	3	2
	Females			3	4	6	6		
	Overall		2	8	10	10	9	3	2

Age structure

The ages of 80 individuals (50 males, 30 females) of *P. ridibundus* group, 69 individuals (34 males, 35 females) of *R. macrocnemis*, and 44 individuals of *B. viridis sitibundus* (25 males, 19 females) were estimated using skeletochronology. Basophilic arrested growth lines (LAGs) were observed in all phalangeal cross-sections (Fig. 2).

For *P. ridibundus* group, the mean age was estimated at 5.27 years (median = 5; SD = 1.36; range 2-9); specifically, 5.28 years in males (median = 5; SD = 1.28; range 2-8) and 5.26 years in females (median = 5; SD = 1.51; range 2-9) (Table 5). In *R. macrocnemis*, the mean age was estimated at 4.32 years (median = 4; SD = 1.16; range 2-6); specifically, 4.53 years in males (median = 5; SD = 0.992; range 2-6) and 4.11 years in females (median = 4; SD = 1.28; range 2-6). For *B. viridis sitibundus*, the mean age was estimated at 5.55 years (median = 5; SD = 1.21; range 3-9); specifically, 5.72 years in males (median = 6; SD = 1.43; range 3-9) and 5.32 years (median = 5; SD = 0.82; range 4-7) in females. In any of the three species the life expectancy significantly differed between sexes (Mann-Whitney tests; *P. ridibundus*: U = 704.50, P = 0.640; *R. macrocnemis*: U = 460.0, P = 0.095; *B. viridis sitibundus*: U= 185.0, P = 0.192).

According to the von Bertalanffy growth formula, *P. ridibundus* group males had an SUL_{max} of 93.61 mm and a growth coefficient (k) of 0.30, and females had an SUL_{max} of 104.92 mm and a k of 1.79. In *R. macrocnemis* males, SUL_{max} was 67.22 mm and k was 0.05, and in females SUL_{max} was 66.29 mm and k was 0.62. In *B. viridis sitibundus*, SUL_{max} was 88.25 mm and k was 0.02 in males, and SUL_{max} was 86.86 mm and k was 0.83 in females.

Phenology

Depending on seasonal conditions, data on the life cycle of amphibian species inhabiting the Lake Çıldır area were collected during field studies carried out from late April to late September (Table 6). The lake and surrounding wetlands froze up by the end of October, and the breakup did not occur until late April. Reproductive activity started in mid-April in tandem with the thawing of ice and continued until late May or early June.

Water temperature in April was measured at 11.5°C at its coldest. The move-

Table 6: Information about breeding phenology of amphibian community from Lake Çıldır.

Year	Event	*P. ridibundus* group	*R. macronemis*	*B. viridis sitibundus*
2017	Emerging from brumation	3rd week of April	3rd week of April	4th week of April
	Beginning of breeding	28.04.2017	30.04.2017	03.05.2017
	End of breeding	02.06.2017	30.05.2017	03.06.2017
	Breeding duration (days)	35	31	31
	Hatching (days)	6-32	12-36	18-38
	Metamorphosis (days)	65-92	60-88	56-100
2018	Emerging from brumation	4th week of April	4th week of April	1st week of May
	Beginning of breeding	30.04.2018	01.05.2018	06.05.2018
	End of breeding	04.06.2018	03.06.2018	11.06.2018
	Breeding duration (days)	34	34	36
	Hatching (days)	8-25	10-35	15-36
	Metamorphosis (days)	55-88	44-98	58-105
2019	Emerging from brumation	3rd week of April	3rd week of April	1st week of May
	Beginning of breeding	29.04.2019	28.04.2019	07.05.2019
	End of breeding	04.06.2019	31.05.2019	12.06.2019
	Breeding duration (days)	36	33	36
	Hatching (days)	9-24	14-28	19-38
	Metamorphosis (days)	62-125	58-124	62-128

ments of anurans observed during this period were quite sluggish. The breeding season reached its peak in early May. During this month, a staggering abundance of anurans was observed in and around the lake, with advertisement calls of *P. ridibundus* group being heard intensely. Numerous individuals engaged in amplexus were observed and egg clutches began to appear in the area. Afterward, tadpoles started to emerge in mid-May. Advertisement calls decreased by the end of May, and newly emerged metamorphs were encountered starting from the second week of June. All reproductive activities subsided in late June. Studied anurans had their metamorphosis completed between 44 to 128 days. In September, all species gradually entered brumation as air and water temperatures fell. No active individuals were observed by the end of that month.

Recent metamorphs at Gosner Stage 46 had an average SUL of 24.85 mm in *P. ridibundus* group, 20.67 in *R. macrocnemis,* and 22.56 mm in *B. viridis sitibundus*. Among these species, *B. viridis sitibundus* (56-128 days) took the longest time to complete its metamorphosis. Whereas full development took 44-124 days for *R. macrocnemis* and 55-125 days for *P. ridibundus* group. All species were observed to lay their eggs in stagnant pools or in the shallows of slow-flowing waters at all surveyed stations. The spawns were observed from late April to mid-June.

Discussion

Population structure and dynamics

Water frogs (*Pelophylax* spp.) are generally abundant in Anatolia from sea level to 3000 m a.s.l. and inhabit a wide range of freshwater habitats including rivers, streams, lakes, ponds, and fountains (Başoğlu *et al*., 1994; Yaşar *et al*., 2021; Am-

PHIBIAWEB, 2022). The first study on the population size of Anatolian water frogs (*Pelophylax* spp.) in Turkey was a stock determination study carried out by BARAN *et al*. (1992). Another study determined the sizes of two *P. ridibundus* group populations in the Aegean to range between 53 and 301 individuals, with a capture probability of 0.16 and survival rate of 0.30 (BAŞKALE & KAYA, 2012). Other studies calculated *P. ridibundus* group population sizes at 3274 individuals (KAYA & ERIŞMIŞ, 2001) in Lake Akören (Afyonkarahisar) and at 7222 individuals / km² (BARAN *et al*., 1992) in Lake Çivril (Denizli). The population size of *P. ridibundus* group in Lake Sülüklü (Manisa) was reported to be 1585 individuals, with a female-biased sex ratio (0.9), capture probability of 0.05-0.07, and mean survival rate of 0.51 (BAŞKALE, 2009). Eight years later, the same population was calculated at 6346 individuals (5595-7199), with a capture probability and survival rate of 0.24 and 0.72, respectively. Another population of *P. ridibundus* group was estimated at 14 733 individuals with a female-biased sex ratio (0.56) in Lake Yayla (Buldan, Denizli) (AYAZ *et al*., 2007). The density of Caucasian populations of water frogs (*P. ridibundus* group) was found to vary between a few and several thousand individuals (GOKHELASHVILI & TARKNISHIVILI, 1994). The present study estimated the *P. ridibundus* group population size at 553-789 individuals, and the density was calculated to lie between 28 and 138 individuals per hectare.

The spatial distribution and abundance of *Rana macrocnemis* vary according to the geographical region and altitude (TARKHNISHVILI & GOKHELASHVILI, 1999; ÇIÇEK, 2009; ÇIÇEK *et al*., 2011a). The number of adult females was reported to vary between 200 and 400 at altitudes of 1000 m a.s.l. and above in the Digmistkali Canyon (Tbilisi, Georgia) and Nedzura Canyon in the Greater and Lesser Caucasus (TARKHNISHVILI & GOKHELASHVILI, 1999). In Uludağ (Bursa), the population of *R. macrocnemis* consisted of 14% juveniles, 47% males, and 39% females, and a male-biased sex ratio was generally observed (ÇIÇEK, 2009; ÇIÇEK *et al*., 2011a). According to numerous studies on Ranids, sex ratios can be balanced, male-biased, or female-biased (COMBES, 1968; VENCES *et al*., 1999; KAYA & ERIŞMIŞ, 2001; AYAZ *et al*., 2007; ÇIÇEK *et al*., 2011a; İSMAIL & ÇIÇEK, 2017).

Age structure

The age structure of *P. ridibundus* group varies among populations across its wide geographical range (Table 7). The age of a northern Anatolian (Trabzon) population was estimated between 2 and 7 years, with a mean of 4 years (YILMAZ *et al*., 2005). In a Central Anatolian population, the age at sexual maturity was determined as 2-3 years and the mean age was estimated at 5 -6 years (ERIŞMIŞ, 2011). The oldest age attained by frogs of the genus *Pelophylax* (*P. esculentus* and *P. lessonae*) was reported to be 12 years in the Caucasus (ALEXANDROVA & KOTOVA, 1986; SMIRINA, 1994) and 10 years in Europe (COGĂLNICENU & MIAUD, 2003). The life expectancy of *Pelophylax* spp. was found to increase in northern or high-altitude populations and decrease in southern or low-altitude populations (SMIRINA, 1994). Some studies suggested that females generally had a higher life expectancy than males

Table 7: Comparison of the age structure of populations of the studied species based on published literature.

Species	Age (years)	Location	References
P. ridibundus group	2-7	Trabzon, Turkey	YILMAZ *et al*., 2005
	2-8	Karagöl, Artvin, Turkey	GÜL *et al*., 2011
	4-11	Dörtyol, Antakya, Turkey	GÜL *et al*., 2011
	5-6	Afyon, Turkey	ERIŞMIŞ, 2011
	3-4	Georgia	GOKHELASHVILI & TARKNISHIVILI, 1994
	4-11	Russia	ALEXANDROVA & KOTOVA, 1986
	2-9	Lake Çıldır, Ardahan, Turkey	Present study
R. macrocnemis	3-5	Trabzon, Turkey	KUTRUP *et al*., 2011
	3.6-4.4	Georgia	TARKHNISHVILI & GOKHELASHVILI, 1999
	2-6	Uludağ, Bursa, Turley	ÇIÇEK, 2009
	2-6	Lake Çıldır, Ardahan, Turkey	Present study
B. viridis sitibundus	4-11	Adana and Çankırı, Turkey	ALTUNIŞIK & ÖZDEMIR, 2015
	max. 11	Germany	SINSCH *et al*., 2007
	max. 11	Italy	CASTELLANO *et al*., 1999
	2-10	Borjomi Canyon, Georgia	GOKHELASHVILI & TARKHNISHVILI, 1994
	5-8	Armenia	LEDENTSOV & MELKUMYAN, 1986
	2-8	Giresun, Turkey	KUTRUP *et al*., 2011
	3-9	Lake Çıldır, Ardahan, Turkey	Present study

(TSIORA & KYRIAKOPOULOU-SKLAVOUNOU, 2002; SOCHA & OGIELSKA, 2010; ÇIÇEK *et al*., 2011b; GÜL *et al*., 2011). For the genus *Pelophylax*, the growth coefficient has been reported to vary between 0.22 and 0.76 (TSIORA & KYRIAKOPOULOU-SKLAVOUNOU, 2002; COGĂLNICENU & MIAUD, 2003; SOCHA & OGIELSKA, 2010; ÇIÇEK *et al*., 2011b; ISMAIL & ÇIÇEK, 2017).

Research on the age structure of *R. macrocnemis* populations at high altitudes showed that the mean age was 3-5 years for both sexes, depending on the elevation in northern Anatolia (KUTRUP *et al*., 2011). The mean age of the population around Kura River (Georgia) was estimated at 3 years for males and 3.6 years for females (GOKHELASHVILI & TARKHNISHVILI, 1994), whereas the mean age of different populations in other regions of Georgia was estimated between 3.6 and 4.4 years, among which the oldest age attained was found to be 7 years (TARKHNISHVILI & GOKHELASHVILI, 1999). The mean age in populations of *R. macrocnemis* in Uludağ (Bursa) was estimated at 5 years for both sexes, and sexual maturity was reported to be attained at 2 to 6 years of age (ÇIÇEK, 2009).

In *B. viridis sitibundus*, the age of the oldest individuals was estimated at 11 years in females and 10 years in males (ALTUNIŞIK & ÖZDEMIR, 2015). The oldest age attained in Europe was reported as 11 years (SINSCH *et al*., 2007). In a study on *B. viridis sitibundus* in Georgia (Borjomi Gorge, 900-1200 m a.s.l.), the reported age range was 3-10 years in females and 2-6 years in males (GOKHELASHVILI & TARKHNISHVILI, 1994). In Armenian and Iranian populations, the longevity was

determined as 5 and 8 years, respectively (LEDENTSOV & MELKUMYAN, 1986; ASHKAVANDI *et al*., 2012).

Phenology

Brumation in *P. ridibundus* group lasts from September-October to early June in the north and from November-December to January-February in the south (GASC *et al*., 1997). Reproduction begins a few days after frogs emerge from brumation (KUZMIN, 1999). The time it takes for the completion of metamorphosis primarily depends on the weather conditions, as well as the characteristics and latitude of the habitat, but it generally occurs between April and November (BAŞOĞLU *et al*., 1994). Sexual maturity was determined to be attained at 1 to 4 years of age, and longevity was reported at 5-12 years (TARKHNISHVILI & GOKHELASHVILI, 1999).

In the Caucasus, *R. macrocnemis* is one of the most widespread and abundant amphibians; its population densities can reach several hundred to several thousand individuals per hectare (ISHCHENKO & MOLOV, 1979). Brumation begins in late September or early November and lasts until sometime between February and May, depending on the altitude (TARKHNISHVILI & GOKHELASHVILI, 1999). The breeding season can last from February to late August, depending on the weather and altitude, but reproductive activities generally peak in April and May. The completion of metamorphosis can occur from late May to early October but generally takes place in June or July. In different populations of *R. macrocnemis*, sexual maturity was reported to be reached at 2-3 years and longevity was estimated at 5-12 years (KUZMIN, 1999).

B. viridis sitibundus is mostly active at dusk and night and spends daylight hours hidden in natural or artificial shelters. Spawning occurs in a wide variety of aquatic habitats, including ponds, swamps, lakes, stream and river edges, reservoirs, and ditches; with water usually no deeper than 50 cm (TARKHNISHVILI & GOKHELASHVILI, 1999). Metamorphosis can be completed in spring through summer, depending on the latitude and altitude. The longevity of different Caucasian populations was estimated at 7-10 years. The breeding season generally takes place between February and July, with both the onset and the length differing across regions. The southern regions possess the longest breeding season (about 170 days) and the shortest period of embryonic and larval development (about 21-25 days) (HEMMER *et al*., 1978).

Bufo verrucosissimus generally shows a lower abundance than the other studied species; however, in ideal conditions, up to 70 specimens can be found along each 100-meter stretch of pond shores, or 200 individuals per hectare on land (KUZMIN, 1999). *Bufo verrucosissimus* spends overwintering period individually or communally, starting from September to early November and lasting until March to June, depending on altitude and latitude (GASC *et al*., 1997). Breeding occurs between March and June, with peak activity in late April and May (NIKOLSKY, 1936). Embryonic and larval development usually takes 1.5 to 2.5 months to be completed (KUZMIN, 1995). When the data on this species were evaluated, it was determined that the populations from the study area had life cycles

similar to shown by previous studies.

Since Lake Çıldır is far from human settlements and agricultural activities are limited in the area, there is no intense human pressure on the amphibian populations. Instead, fluctuations in seasonal temperature and precipitation regimes are the principal pressures affecting the amphibian community, significantly upsetting their life cycle and fecundity. Premature drying of vernal pools during the breeding season or their unexpected freezing due to an increase in diurnal temperature variation can greatly impact the reproductive success of amphibians. The amphibian community that inhabits the Lake Çıldır area comprises healthy frog and toad populations, whose main threats are changes in annual temperature and precipitation regime due to the effects of climate change. Therefore, Lake Çıldır anurans are evaluated to be effective candidates as indicator species for future studies on the effects of climate change and can be used as data providers on the status of amphibian populations in the region and beyond.

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