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Impact of various ecological parameters on the life-history characteristics of *Bufotes viridis sitibundus* from Turkey

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Abstract

In this study, we used the skeletochronology method to estimate various growth parameters, such as age structure, minimum and maximum life span, age of sexual maturity, and the relationship between body size and weight of eight different populations of the variable toad, *Bufotes viridis sitibundus*, in Turkey. Further, we determined the relationship between these parameters and ecologic factors using the partial Mantel test. A significant difference was found among the populations with respect to age, body size, and body weight in both males and females. On average, the maximum life span was recorded as 10 years for males and 11 years for females. In the studied populations, the average age of sexual maturity ranged between 2 and 4 years for both sexes. Sexual dimorphism in terms of snout-vent length (SVL) was not observed between males and females in all the populations. Toads from the higher altitudes tended to be significantly larger, older, and heavier than those from lower altitudes. We concluded that altitude and temperature have an impact on the growth rate, body size, and body mass.

KEYWORDS

age structure, bone growth, demography, ecological factors, skeletochronology

1 | INTRODUCTION

With the increase in global warming, the importance of studies investigation the impact of climate on animal populations has also increased (Ashton, 2002). Climate change can alter the distribution of organisms by causing longitudinal, latitudinal, or altitudinal shifts and has a notable effect on the geographic ranges of animal species (Pearson & Dawson, 2003; Raxworthy et al., 2008). Climate change is hypothesized to have a larger impact on ectothermic vertebrate animals than that on endothermic vertebrate animals because there is a close association between body and environmental temperatures in the former (Cunningham, Rissler, Buckley, & Urban, 2016; Winter et al., 2016). In most ectotherms which exhibit indefinite growth, life-history traits show distinct and specific geographical variations which are consistent with variations in the environment (Morrison & Hero, 2003; Rosso, Castellano, & Giacoma, 2005).

Since amphibians are found in both terrestrial and aquatic habitats, changes in these habitats directly affect them (Hecnar, 1995; Mahaney, 1994; Shi, 2000; Watt & Oldham, 1995). The skin of amphibians is highly permeable, and, consequently, water is essential for maintaining the moisture levels of the skin and for performing respiration (Shi, 2000; Watt & Oldham, 1995), making amphibians more sensitive to the effects of water pollution (Blaustein et al., 1994; Vitt, Caldwell, Wilbur, & Smith, 1990). The permeable nature of the skin causes it to interact more with chemicals in water (Hecnar, 1995; Mahaney, 1994). Toxic substances and contaminated materials are either absorbed through the skin or

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ingested (Baker & Waights, 1993; Berger, 1989; Cooke, 1981). Chemicals, such as nitrite and ammonium, in water may influence important variables such as the distribution, abundance, and breeding area selection of amphibians (Ensabella, Loriga, Formichetti, Isotti, & Sorace, 2003; Griffis-Kyle & Ritchie, 2007).

Bergmann's rule is one of the most well-known rules for the eco-geographical variation of body size in vertebrates mostly living in colder climates, and it states that body size increases with an increase in latitude or altitude (Bergmann, 1847). Although, Bergmann's rule was originally stated for endothermic animals (Ashton, 2002), some ectothermic animals, such as arthropods (Blanckenhorn & Demont, 2004) and reptiles (Ashton, 2001; Ashton & Feldman, 2003), have been shown to follow this rule. Among ectotherms, the relevance of Bergmann's rule is particularly complex and controversial for amphibians. As a matter of fact, some amphibian taxa follow the Bergmann's rule (Ashton, 2004; Ficetola et al., 2010), some follow the converse (Cvetković, Tomašević, Ficetola, Crnobrnja-Isailović, & Miaud, 2009; Ma, Lu, & Merilä, 2009), while some others do not exhibit any latitudinal or altitudinal clines (Adams & Church, 2008).

Life-history traits are often associated with patterns of growth, development, reproductive investment, and survival and can be studied at the level of individual animals, populations, and species (Liao, Luo, Lou, Lu, & Jehle, 2016). Differences in abiotic factors, including temperature, quantity and quality of food, and humidity, lead to variations in life-history traits between populations (Liao, Liu, & Merilä, 2015; Liao & Lu, 2012; Morrison & Hero, 2003; Reniers, Brendonck, Roberts, Verlinden, & Vanschoenwinkel, 2015). In addition, body size may be affected by the length of the activity period. For example, those that exhibit a longer annual activity period have a faster annual growth rate than those that exhibit a shorter annual activity period, in individual animals of the same age (Guarino, Di Già, & Sindaco, 2008; Miaud, Âtant, & Elmberg, 1999). However, the average body length of a population depends not only on the annual growth rate (which is related to the duration of the activity period) but also on the average age of the population. Without controlling for age, it has been shown that animals from populations who have a longer active season are often larger than those with a shorter active season (e.g., Angilletta & Sears, 2004; Belk & Houston, 2002; Blanckenhorn & Demont, 2004; Sears, 2005).

The taxa *Bufo* was re-named as *Bufotes* by Stöck et al. (2006), and the new taxonomic name of the Anatolian and Russian populations, previously considered to be a distinct species, was *Bufotes sitibundus* (Dufresnes et al., 2019) which was later designated as two subspecies, *Bufotes viridis viridis* and *Bufotes viridis sitibundus*, respectively (Speybroeck et al., 2020). The variable toad, *B. v. sitibundus* (Pallas, 1769), shows a widespread geographical distribution, ranging from the Anatolian part of Turkey to Georgia, in the west, and Armenia in the east and from eastern Russia to Jordan and Saudi Arabia, in the north, and Iran, in the south direction (Dufresnes et al., 2019). The length of *B. v. sitibundus* can be up to 9 cm, and they enter water bodies only during their relatively long breeding periods (from January to June) (Altunışık, 2019).

Herein, we reveal various demographic life-history traits, such as age structure, age of sexual maturity, lifespan, growth, and survival rate, and investigated the effects of some ecologic factors, such as altitude, temperature, and water quality, on the growth rate of the *B. v. sitibundus* in eight populations from different regions of Turkey. In this study, we aim to assess the importance of life-history variations in order to improve conservation strategies for sustaining healthy amphibian populations.

2 | MATERIAL AND METHODS

2.1 | Species and study areas

To increase the power of the data on size and age-related life-history traits from the eight distinct populations, we used both new data, collected by us, and previously published data (Altunışık & Özdemir, 2015). A total of 308 (189 ♂♂ and 119 ♀♀) *B. v. sitibundus* adults were sampled from various regions of Turkey between 2012 and 2013 (*Table* 1, *Figure* 1). Experimental protocols involving animals were approved by the local ethics committee at Recep Tayyip Erdoğan University (approval reference number: 2012/4). External secondary sexual characteristics (e.g., a nuptial pad on the first finger in males) were used to determine the sex of the animal. The snout-vent length (SVL) was measured to the nearest 0.01 mm using a digital compass Vernier caliper (Mitutoyo Corp., Kawasaki, Japan), and the body mass was weighed to the nearest 0.01 g using an analytical balance (Shimadzu AW 320AW, Japan). The longest toe (fourth), including the first and second phalanges, of the right hind limb was removed in all specimens, and the bones of this toe were fixed in 10% formalin and preserved in 70% ethanol solution for subsequent age analysis. After the completion of the sampling procedures, all toads were released at the capture site.

2.2 | Age determination

Skeletochronology has been used by many researchers, since it does not require animals to be sacrificed and

TABLE 1 Coordinates, sample sizes, altitude, and activity period for each population

Locality	Coordinates	Altitude (m)	N	Capture date	Activity period
Kestanelik/Sakarya	40°41′N 30°15′E	58	30	29.04.2013	April–November
İshakçelebi/ Manisa	36°35′N 31°52′E	150	29	18.06.2013	April–November
Seydiköy/Çankırı	40°31′N 33°29′E	960	57	20.07.2012	May–October
Yeniköy/Antalya	36°25′N 32°19′E	480	30	27.05.2012	April–November
Yeşilbağlar/Adana	36°28′N 36°13′E	13	52	10.04.2013	March–November
Ulubağ/Şanlıurfa	37°09′N 38°53′E	490	40	06.04.2013	April–November
Söğütlü/Iğdır	39°55′N 44°04′E	858	37	01.06.2012	April–November
Liman/Rize	41°02′N 40°30′E	7	41	02.05.2012	April–October



FIGURE 1 (a) Distribution map of *B.v. sitibundus* and (b) localities sampled in this study

saves time (Altunışık, 2018a; Arısoy & Başkale, 2019; Bülbül et al., 2018,b; Erismis, 2018; Lyapkov, Kidov, Stepankova, Afrin, & Litvinchuk, 2020; Özdemir et al., 2012; Sinsch, 2015). Skeletochronological analysis involves counting the dark-stained rings, which represent the lines of arrested growth (LAG) that occur as a consequence of reduced bone growth in the winter season. Our analysis followed the standard methods described by Smirina (1994) and modified by Altunışık and Özdemir (2015). The fourth toe, comprising the first and

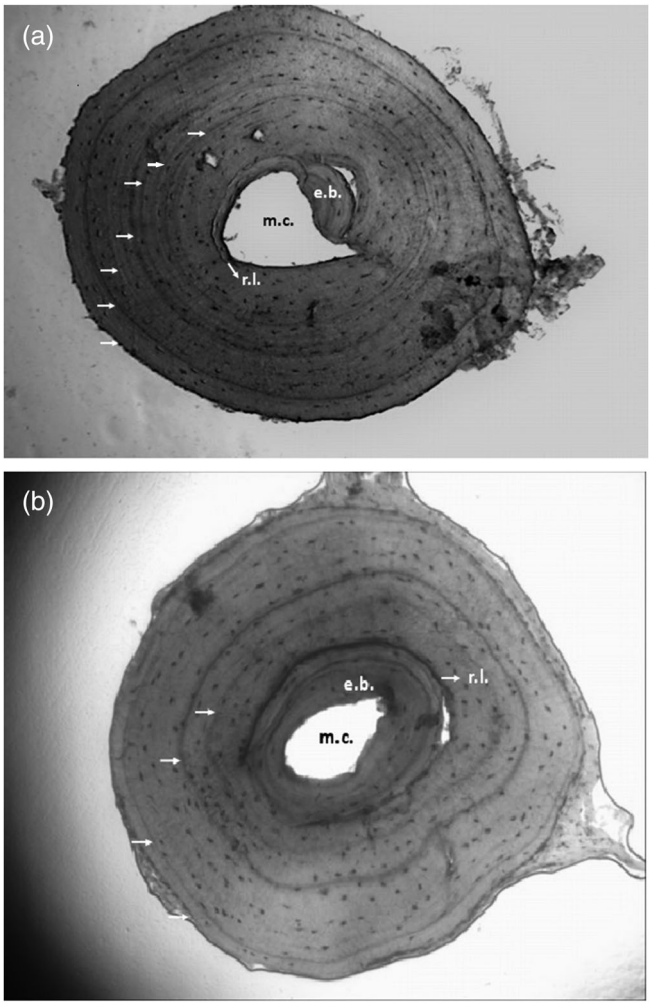


FIGURE 2 (a) Cross sections, at the diaphysis level, of a phalanx from a 7-year old animal from the Ulubağ population (b) and 4-year old animals from the Liman population. (m.c., marrow cavity; e.b., endosteal bone; r.l., resorption line). The LAGs are indicated by white arrows

second phalanges of the right hind limb, was clipped using a surgical scissor. Stored phalanges were decalcified in 5% nitric acid (HNO_3), for about 2 h, washed overnight in distilled water, and used for histological analysis.

Phalangeal cross sections of 16 μm thickness were obtained on a cryostat (Thermo Shandon, Germany), and these sections were kept in Erlich's hematoxylin dye for 15 min. After this, sections that had a small medullary cavity were selected and placed in a water-based mounting medium. The stained cross sections were examined under a BX51 light microscope (Olympus, Japan) at ×10 and ×20 magnification, and imaged using a camera (PVC 100c, Pixera) connected to the microscope. Two independent authors (A. Altunışık and N. Özdemir) examined the images and counted the number of LAGs.

2.3 | Environmental parameters and statistical analysis

Life-history traits (age and body size), environmental parameters (temperature, altitude latitude, mean temperature, and mean precipitation), and water quality parameters (pH, ammonium, aluminum, iron, nitrite, conductivity, and water temperature) of each sampled habitat were analyzed.

Climate data from the year 1954 to the year 2013 were obtained from the Turkish Meteorological Administration (http://www.dmi.gov.tr). Since the climate data varied between the eight localities, the effects of these parameters on growth and age were tested. Similarly, data on water quality parameters of the habitat, temperature, altitude, and precipitation were also collected. Eigenvalues of temperature and altitude values, which were higher than 1.000, were obtained using the principal component analysis and Kaiser-Meyer-Olkin test ($p > .5$), and explained about 83% of the total variance, and thus have a large impact on the different populations. The effect of these factors on SVL, age, and growth rate were determined using the partial Mantel test, which helps correlate ecological and geographical data to morphometric data.

Kolmogorov–Smirnov and Levene tests were used to test the normality and homogeneity of the variances, respectively. Since, the data showed a normal distribution ($p > .05$), we used the parametric tests ANCOVA and ANOVA to test for statistical significance. Sexual size dimorphism (SSD) was quantified using the sexual dimorphism index (SDI) by Lovich and Gibbons (1992). According to this formula, the mean SVL of the bigger sex is divided into the mean SVL of the smaller sex and subtracted by 1 (SVLbig/SVLsmall −1) to give an approximate measure of the sexual size dimorphism.

We calculated the survival rates with the following formula generated by Robson and Chapman (1961).

$$Sr = \frac{T}{(R + T - 1)}$$

According to this formula, a constant survival rate is assumed for all sampled individuals and age groups, and wherein T = N1 + 2 N2 + 3 N3 + ..., R = Σ Ni, Ni is the number of individuals in the age group, and Sr refers to the finite annual survival rate.

The adult life expectancy is a measure of the expected longevity of individuals that have reached sexual maturity (ESP) and was calculated based on Seber's (1973) formula:

TABLE 2 Comparison of age, size, and growth parameters of *B. v. sitibundus* in eight populations and cognate species: a comparison with existing literature

Species	Locality	Sex	N	Mean SVL (mm)	Asymptotic size (mm)	Max age (years)	AM (years)	Mean age (years)	Mean weight (g)	k	ESP	Sr	References
B. v. sitibundus	Kestanelik, Sakarya, Turkey	M	23	63.97	85.41	7	2	3.08	30.59	0.32	3.68	0.69	Present study
		F	7	67.94	91.18	8	3	4.28	36.06	0.21	4.17	0.73	
	İshakçelebi. Manisa, Turkey	M	19	65.26	71.41	6	2	3.05	32.51	0.42	3.72	0.69	
		F	10	68.17	79.27	5	3	3.30	32.65	0.29	4.28	0.74	
	Yeniköy. Antalya, Turkey	M	25	75.42	87.76	8	3	4.24	43.68	0.22	4.88	0.77	
		F	5	79.26	93.12	8	4	5.80	46.46	0.16	4.75	0.76	
	Söğütlü. Iğdır, Turkey	M	14	52.94	67.13	5	2–3	3,00	17.04	0.55	3.65	0.68	
		F	18	51.12	70.12	5	2–3	2,50	15.56	0.45	3.09	0.61	
	Liman, Rize. Turkey	M	23	58.02	78.15	5	2–3	3.13	23.15	0.39	3.72	0.69	
		F	15	58.28	83.17	6	2–3	3.60	20.49	0.27	3.21	0.63	
	Ulubağ. Şanlıurfa. Turkey	M	28	64.67	80.14	6	3	3.71	30.69	0.35	5.06	0.78	
		F	12	67.52	87.10	8	3	4.50	34.73	0.24	3.80	0.70	
	Yeşilbağlar. Adana, Turkey	M	29	56.11	72.18	6	2–3	3.51	19.66	0.37	4.14	0.73	Altunışık & Özdemir, 2015
		F	23	54.18	76.80	6	2–3	4.08	19.09	0.24	3.68	0.69	
	Seydiköy. Çankırı, Turkey	M	29	69.42	89.67	10	3–4	6.42	42.73	0.23	5.06	0.78	
		F	28	70.41	96.58	11	3–4	6.51	44.83	0.19	5.21	0.79	
	Lorestan, Zagros, Iran	M	26	72.65	-	6	2–3	3.27	-	-	-	-	Ashkavandi, Gharzi, & Abbasi, 2012
		F	18	72.50	-	8	3–4	4.40	-	-	-	-	
	Giresun island, Giresun, Turkey	M	15	77.11	82.01	6	2–3	4.66	43.36	0.59	-	-	Kutrup, Cakir, Colak, Bulbul, & Karaoglu, 2011
		F	41	79.46	87.92	8	2–3	4.70	48.58	0.50	-	-	
	Giresun province, Giresun, Turkey	M	39	66.12	72.90	8	2–3	4.76	24.04	0.49	-	-	
		F	5	69.24	-	7	2–3	2.60	30.30	-	-	-	
		M	25			6		3.69					

(Continues)

TABLE 2 (Continued)

Species	Locality	Sex	N	Mean SVL (mm)	Asymptotic size (mm)	Max age (years)	AM (years)	Mean age (years)	Mean weight (g)	k	ESP	Sr	References
	Borjomi canyon, Georgia	F	11			10		4.91					Gokhelashvili &
	Armenia	M				5	2–3						Ledentsov & Melkumyan, 1986
		F				5	2–4						
B. viridis	Urmitz, Koblenz, Germany	M	113			6	1						Sinsch, Leskovar, Drobig, König, & Grosse, 2007
		F	42			6	1						
	Queis, Landsberg, Germany	M	167			6	1						
		F	34			7	1						
	Halle botanical garden, Halle Germany	M	65			9	3						
		F	22			11	3						
	Zaschwitz, Saxonia-Anhalt, Germany	M	29			9	2						
		F	29			9	3						
	Zucchea, Turin, Italy	M	369	61.80		11	4	7.00	20.36				Castellano, Rosso, Doglio, & Giacoma, 1999
		F	-										
	Portoscuso, Sardinia, Italy	M	109	83.12		11	4	6.20	50.75				
		F	-										
		F											

Abbreviation: AM, age at maturity; ESP, adult life expectancy; k, growth coefficient, Sr, survival rate.

$$\mathrm{ESP} = 0.5 + \frac{1}{1 - Sr}$$

Wherein, Sr represents the survivorship rate. Data analysis was conducted using the SPSS 21 statistical software package (IBM SPSS Statistics for Windows).

3 | RESULTS

3.1 | Body size and mass

We found significant differences among the eight populations in terms of body size and mass, for both females (ANCOVA: body size: $F_{7,058} = 21.194$; $p < .0001$; mass: $F_{7,498} = = 19.85$, $p < .0001$) and males (ANCOVA: body size: $F_{7,057} = 30.216$; $p < .0001$, mass: $F_{7,488} = = 32.428$, $p < .0001$).

When body size was evaluated collectively and the effect of age was controlled for, sexual dimorphism was not observed in the variable toad (ANCOVA: $F_{1,002} = 1.81$; $p > .05$). When body size was analyzed within each population, females were larger than males in six of the eight studied populations, that is Kestanelik, İshakçelebi, Seydiköy, Yeniköy, Ulubağ, and Liman. In the rest, that is Yeşilbağlar and Söğütlü, males were larger than females (Table 2, Figure 3).

In terms of body mass, there was no statistically significant difference between males and females of the eight populations (independent sample *t* test, t: 2.644 *df*: 306; $p > .05$). Similar to body size measurements, females in five of these populations (Seydiköy, Ulubağ, Kestanelik, İshakçelebi, Yeniköy) and males in three (Yeşilbağlar, Söğütlü, Liman) were, on average, heavier than the opposite sex. When the effect of age was controlled for, there was a significant difference in body mass between sexes, with males being heavier than females in all eight populations (ANCOVA: $F_{1,065} = 4.588$, $p < .05$).

3.2 | Age determination

A significant difference was detected among populations in the average age for both females (ANCOVA: $F_{7,321} = 21.645$; $p < .0001$) and males (ANCOVA: $F_{7,334} = 16.78$; $p < .0001$). Animals from different populations attained sexual maturity at diverse ages (2–-4 years), as shown in Table 2. Survivorship rate and potential reproductive lifespan were computed for the eight populations and are given in Table 2. A periosteal growth zone and thin hematoxylinophilic LAG, which are a measure of the number of winter seasons experienced by the animal, were present in all cross sections (Figures 2 and 3). Collectively, the maximum lifespan was 10 years for males and 11 years for females

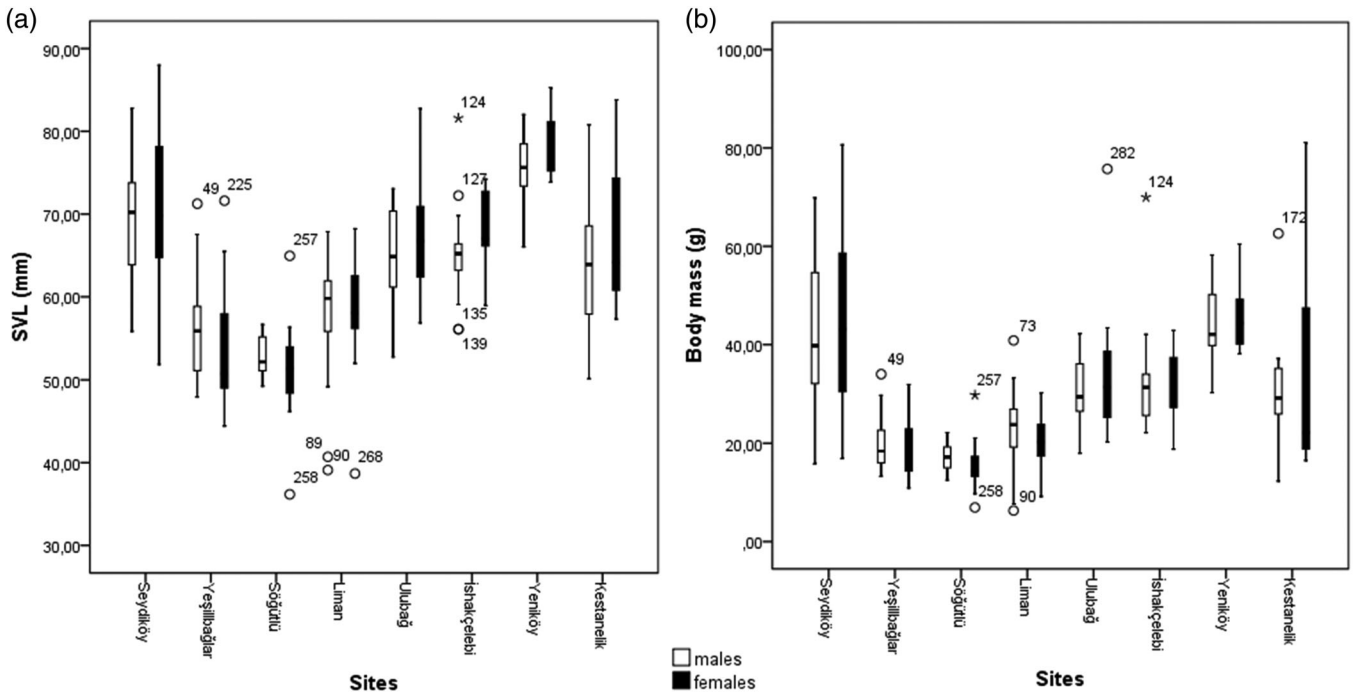


FIGURE 3 Body size and mass distributions of *B.v. sitibundus*. (a) Body size, (b) Body mass

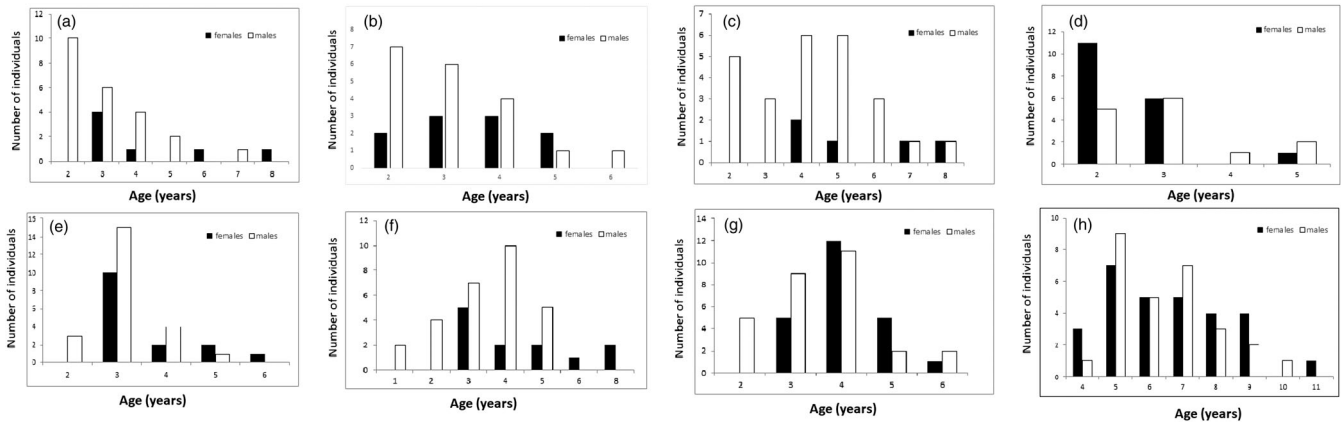


FIGURE 4 Age distributions of *B.v. sitibundus* from the eight populations. (a) Kestanelik, (b) İshakçelebi, (c) Seydiköy, (d) Yeniköy, (e) Yeşilbağlar, (f) Ulubağ, (g) Söğütlü, (h) Liman

TABLE 3 Results of some ecological and chemical parameters of water for each sampled habitat

Locality	MTDAP (°C)	MPDAP (kg/m^2)	pH	Conductivity (µs/cm)	Nitrite (mg/L)	Aluminium (mg/L)	Amonium (mg/L)	Iron (mg/L)
Seydiköy/ Çankırı	19.50	28.52	6.7	228	0.01	0.02	0.04	0.07
Söğütlü/Iğdır	18.40	24.15	8.3	262	0.80	1.20	3.52	4.10
Liman/Rize	18.50	170.44	7.4	256	0.60	0.08	1.18	0.90
İshakçelebi/ Manisa	21.30	34.36	7.3	510	0.06	0.01	0.03	0.06
Kestanelik/ Sakarya	18.08	59.22	6.8	352	0.06	0.08	0.18	0.05
Yeşilbağlar/ Adana	21.97	36.53	7.2	412	0.06	0.04	0.07	0.08
Ulubağ/Şanlıurfa	23.63	19.55	6.4	272	0.04	0.09	0.60	0.05
Yeniköy/ Antalya	22.27	41.62	6.9	342	0.04	0.05	0.04	0.03

Abbreviations: MPDAP, mean precipitation during activity period; MTDAP, mean temperature during activity period.

(Figure 4), and the age range was 2–10 years (mean: 3.89 ± 0.12) in males and 2–11 years (mean: 4.44 ± 0.18) in females (Table 2).

3.3 | Environmental and water quality parameters

The altitude and temperature were found to be closely related to each other (Mantel test, r = ,384, $p < .05$). Thus, the relationship of these factors with age, SVL, body mass, and growth rate were analyzed using the partial Mantel test. The partial Mantel test showed that altitude and temperature has an impact on SVL, body mass, and growth rate (k) (Partial Mantel Test, SVL: $r = .384$, $p < .01$; body mass: $r = .390$, $p < .05$; k: $r = .403$, $p < .05$), but did not have a significant effect on the average age (partial Mantel Test, $r = .287$, $p > .05$).

A weak but significant relationship was found between altitude and SVL, age, and body mass (Pearson correlation, altitude-SVL: $r = .376$, $p < .01$; altitude-age: $r = .267$, $p < .01$; altitude body mass: $r = .415$, $p < .01$). Animals at higher altitudes have a higher average body size, age, and body mass than those living at lower altitudes. The results of the cubic regression equations showed that there was a significant relationship between altitude and SVL ($y = 1.73 + 0.01x\text{-}3.62x^2 + 2.362x^3$, $F_3 = 61.84$, $R^2 = .379$, $p < .001$), altitude and age ($y = 0.487 + 0.01x + 4.023x^2 + 3.04x^3$, $F_3 = 29.033$, $R^2 = .223$, $p < .001$), and altitude and body mass ($y = 1.235 + 0.004x\text{-}1.097x^2 + 7.282x^3$, $F_3 = 68.72$ $R^2 = .404$, $p < .001$). The ecological and chemical

parameters of the water sampled from each habitat are given in Table 3.

4 | DISCUSSION

In this study, 308 variable toads from eight different populations in Turkey were investigated using a skeletochronological method to determine the age and growth rate. Previous studies investigating the age structure and morphology of variable toad populations are limited (Ashkavandi et al., 2012; Castellano et al., 1999; Gokhelashvili & Tarkhnishvili, 1994; Kutrup et al., 2011; Ledentsov & Melkumyan, 1986; Sinsch et al., 2007). The findings of this study demonstrate that the different populations of *B. v. sitibundus* show both similarities and differences with respect to various aspects (Table 2).

An 11-year-old female toad belonging to the Seydiköy population accounted for the maximum lifespan among the eight populations. Similarly, Castellano et al. (1999) and Sinsch et al. (2007) have also reported an 11-year-old male and female toad in their studied populations. Animals from the Turkish, Italian, and German populations of *Bufotes viridis* sensu *lato* were also found to live for a maximum of 11 years (Table 2). The minimum longevity for the variable toad has been reported to be 5 years by Ledentsov and Melkumyan (1986). We also arrived on a similar number in this study (Söğütlü population). In other populations (Ashkavandi et al., 2012; Gokhelashvili & Tarkhnishvili, 1994; Kutrup et al., 2011) the longevity varied between and 6–10 years. The Italian (Zucchea) population had the highest mean age of 7.00 years, while the Turkish (Söğütlü) population had the lowest mean age of 2.50 years (Table 2).

Age of sexual maturity is greatly influenced by other life-history traits, such as differences in climatic conditions or the length of the larval stage (Duellmann & Trueb, 1986). Differences in environmental temperature, due to differences in geography and altitude, are the main source of variations in the length of the larval period and metamorphosis period of various amphibian populations (Berven & Gill, 1983; Miaud & Guyetant, 1998; Morrison & Hero, 2003). Amphibians living in a higher latitude or altitude grow slower and reach maturity later than those living in a hotter climate or lower altitude (Hemelaar, 1988; Ryser, 1988). For example, *Lithobates clamitans* living at high altitudes have a longer larval period and a larger size at metamorphosis than those at low altitudes (Wells, 2007). Various researchers have reported that males reach sexual maturity a year earlier than females (Altunışık, 2018b; Özdemir et al., 2012; Sinsch et al., 2007; Smirina, 1994). Males and females have also been reported to simultaneously attain sexual maturity (Altunışık, 2018a; Arısoy & Başkale, 2019; Leskovar, Sanuy, Oromi, & Sinsch, 2006). Similar to the Georgian population (Gokhelashvili & Tarkhnishvili, 1994), males reached maturity 1 year earlier than females (2–3 years vs. 3–4-years) in our studied populations. It has also been reported that females reach sexual maturity at 2–4 years of age and males at 2–3 years of age in the Armenian population of *Bufotes viridis* sensu *lato* (Ledentsov & Melkumyan, 1986). In the population from Germany, females have been reported to reach sexual maturity at 2–3 years of age and males at 1–3 years age (Sinsch et al., 2007). In contrast, males and females in the Iranian and Turkish populations simultaneously reached sexual maturity at 2–3 years of age (Ashkavandi et al., 2012; Kutrup et al., 2011). Overall, in *Bufotes viridis* sensu *lato*, the minimum age of sexual maturity was found to be 1 year in populations from Germany, and the maximum age of sexual maturity was found to be 4 years in Turkish, Armenian, and Italian populations (Table 2).

Body size difference between the sexes of the same species aids in the understanding of important biological intuitions (Denoël, Ivanović, Dzukić, & Kalezić, 2009). Selection mechanisms create a difference in the body sizes of females and males, since different sizes confer competitive advantages in terms of ecology (feeding strategies and habitat use), reproduction, and sexual selection (size-dependent mate selection and intraspecific challenge) (Blanckenhorn, 2005). According to the ontogenetic perspective of SSD evolution, SSD occurs during developmental growth, before the maturation of the animal, in species that exhibit definite growth (Hasumi, 2010). However, in species with indefinite growth, such as amphibians, a sharp decrease in growth rate occurs at the beginning of breeding season. In this case, sexual maturity depicts an exchange between fertility, development time, and life span (Day & Taylor, 1997; Hasumi, 2010; Heino & Kaitala, 1999). Collectively, in all the studied populations, we found no statistically significant difference in the SVL of males and females. However, in the vast majority of populations, such as Seydiköy, Ulubağ, Kestanelik, İshakçelebi, Yeniköy, and Liman, females had a longer average SVL than males. Only in the Yeşilbağlar and Söğütlü populations, the average SVL of males was larger than that of females. It has been reported that in 90% of anurans, females are larger than males (Shine, 1979). Findings from studies of *Rana temporaria* (Miaud et al., 1999), *Hyla arborea* (Özdemir et al., 2012), and *Bufo viridis* (Sinsch et al., 2007) support this trend. In this study, although females generally have higher SVL, this difference is not statistically significant. Similarly, in two populations of *B. v. sitibundus* (mentioned as *B. viridis*) from Turkey, the

researchers emphasized that females have a longer SVL than that of males, in both mainland and island populations, but this difference was not statistically significant (Kutrup et al., 2011). In a study of *B. v. sitibundus* (referred to as *B. viridis*) in Iran, there was no significant difference between females and males, although the males tended to be larger than the female (Ashkavandi et al., 2012). The Yeşilbağlar and the Söğütlü populations in this study are similar to the populations from Iran, with a longer average SVL in males than that in females. In terms of average SVL, the Portuscoso (Italy) and Söğütlü (Turkey) populations stand out as the populations with the longest and shortest SVLs, respectively (Table 2). Parallel results have also been reported for other frog species. For example, Guarino, Lunardi, Carlomagno, and Mazzotti (2003) found no significant difference in SVL between males and females of *Rana latastei*, but emphasized that females tend to be larger than males. The three main hypotheses that explain the evolution of sexual dimorphism are sexual selection, interspecific food competition, and the female productivity hypothesis (Gvoždík & Boukal, 1998). Since *B. v. sitibundus* lives sympatrically with *Hyla orientalis* in the Yeşilbağlar biotope and with *H. savignyi* in the Söğütlü biotope, competition for food between males may have tipped sexual dimorphism in favor of males, supporting the second hypothesis. In other biotopes, we did not find any species that lived sympatrically with the variable toad. Although we did not test the female productivity hypothesis, we believe that sexual or fecundity selection may explain the larger body size of females in the remaining six populations.

There are many factors that affect body size in amphibians including time and duration of metamorphosis, growth rate before and after maturation, age of maturity, and life span (Augert & Joly, 1993; Friedl & Klump, 1997; Hemelaar, 1988; Moravec, 1990). In anurans, these factors vary with variations in environmental factors and body size, which in turn depends on macro-ecological parameters. In cold climates, the body size of anurans is expected to be larger as a consequence of an inverse relationship with climatic factors, such as temperature and daylight, that affect individual thermoregulation (Hutchinson & Dupre, 1992). According to Bergmann's rule, animals living in regions of hotter temperatures generally mature earlier and are smaller (Angilletta & Sears, 2004; James, 1970; Walters & Hassall, 2006). Ambient temperature changes are likely responsible for most of the variations, in various amphibian species, associated with the geography and altitude (Berven & Gill, 1983; Miaud & Guyetant, 1998; Morrison & Hero, 2003). The effect of altitude on life-history characteristics has been demonstrated in various amphibian species (Altunışık & Özdemir, 2015; Baraquet, Otero, Valetti, Grenat, & Martino, 2018; Cvetković et al., 2009; Ma et al., 2009; Maletzky et al., 2004; Najbar, Konowalik, Halupka, Najbar, & Ogielska, 2020; Üzüm & Olgun, 2009; Zhang & Lu, 2012). In one of these studies (Ma et al., 2009), variation in body size of the Tibetan frog (*Nanorana parker*) depending on the elevational gradient was investigated in six populations. They showed that the average body size decreases with an increase in altitude, complying with the reverse Bergmann rule, and they also showed that average age increases with an increase in altitude. They explained their findings using two different scenarios. In the first case, although the species followed the Bergmann rule, the effect of age on body size exceeded the effect of growth rate. In other words, when average body size increases with a decrease temperature, age, and not growth rate, is the main determinant of body size differences between populations. In the second case, contrary to Bergmann's rule, growth rate determines the variations in body size (Ma et al., 2009).

In this study, we examined *B. v. sitibundus* populations from different altitudes and showed that animals from higher altitudes were larger than those from lower altitudes. In addition, we found a statistically significant effect of temperature and altitude on body size (partial Mantel test). While the Seydiköy and the Yeşilbağlar populations follow the Bergmann's rule, the Yeniköy, Söğütlü, and the Liman populations were found to follow the reverse Bergmann's rule. Animals from the Yeniköy population, with the highest average size, lagged behind the Seydiköy population in terms of average age. When the growth rates were analyzed, it was seen that toads from the Yeniköy population grew at a slower rate than the toads of the Seydiköy population (Table 1). This supports the second explanation provided by Ma et al., 2009. In other words, the growth rate, exhibited by animals that follow the reverse Bergmann's rule, have a larger impact on the body size than age. A similar situation is also observed in the Söğütlü population, which has the lowest average body size, however, the growth rate in the Söğütlü population is higher than that in other populations. Growth rate and body length is known to be inversely related to each other; thus, a high growth rate may lead to a decrease in the average body length.

Amphibians are threatened in habitats shared with people. They are exposed to water pollution during the egg and larval periods, which occur in aquatic environments. Research on amphibian populations has shown a correlation between population decline and proximity to regions of agriculture (Bishop, Mahony, Struger, Ng, & Pettit, 1999; Davidson, 2004; Joly, Miaud, Lehmann, & Grolet, 2001). The effect of nitrate toxicity on aquatic animals increases in direct proportion with concentration

and duration of exposure (Camargo, Alonso, & Salamanca, 2005). Nitrate exposure in adult salamanders has been shown to affect reproduction by altering the time of appearance of secondary sexual characters and shortening body length (Secondi, Hinot, Djalout, Sourice, & Jadas-Hecart, 2009). However, nitrate exposure affects swimming and feeding in larvae and have complex effects on aquatic ecosystems by altering productivity and hunter-hunter relationships (Smith, Temple, Dingfelder, & Vaala, 2006; reviewed in Miaud, Oromi, Navarro, & Sanuy, 2011). In addition, many elements such as aluminum, iron, cadmium, and zinc have toxic effects on amphibians. Some environmental variables affect the ion exchange rate between amphibians and their environment (Wells, 2007). Low pH (high acidity) adversely affects osmoregulation in amphibian larvae by inhibiting sodium ion loss, from the skin, and active sodium ion uptake (Freda & Dunson, 1985; McDonald, Ozog, & Simons, 1984). According to the water analysis, the Söğütlü biotope has the highest pH. In addition, this biotope also has high amounts of ammonium, aluminum, iron, and nitrite. When we estimated the average age of this population, which lives in a habitat that falls in the category of "very dirty water" according to the water quality standards, it was seen that females have an average age of 2.50 years, whereas males have an average age of 3.00 years. The Söğütlü biotope experiences a high rate of pollution, due to expulsion of industrial wastes with high amounts of heavy metals such as aluminum and iron, and it also has high levels of ammonium and nitrite, which are toxic to amphibians. Accordingly, this population have a reduced average body size. Following the Söğütlü biotope, the second highest pH and levels of ammonium and iron were detected in the Rize biotope. This biotope is located close to the Rize Port and is likely to be affected by freight transport. The lowest pH value was measured in the Ulubağ biotope, which is located near an agricultural region. A possible explanation for this is that the pesticides used in agriculture may have affected the pH levels in the biotope. The Seydiköy and Yeniköy biotopes were the cleanest according to water quality standards and have the highest values in terms of average body size, weight, and age. Although water quality parameters did not statistically differ between biotopes, habitat quality may have positively affected the life-history characteristics of these populations. Since better water quality is associated with older frogs (i.e., possibly healthier populations), habitat quality parameters are an important indicator for the sustainability of healthy amphibian populations.

Ecological factors and habitat quality are among the possible causes of differences between populations. In order to better understand the causes of these differences, the effects of ecological factors (water and soil parameters, etc.) on the variable toad at the metamorphosis stage should be examined histopathologically, and the acute and chronic exposure to pollutants should also be investigated.

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AUTHOR CONTRIBUTIONS

Abdullah Altunışık: Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Resources, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization, Supervision, Project Administration, Funding Acquisition. **Serkan Gül:** Investigation; methodology; writing-review and editing. **Nurhayat Özdemir:** Data curation; formal analysis; investigation; resources; supervision; validation; writing-original draft; writing-review and editing.

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