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Body size and age structure of a highland population of *Hyla orientalis* BEDRIAGA, 1890, in northern Turkey
(Anura: Hylidae)

Körpergröße und Altersstruktur in einer nordtürkischen Hochlandpopulation von *Hyla orientalis* BEDRIAGA, 1890
(Anura: Hylidae)

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KURZFASSUNG

Unter Verwendung skelettochronologischer Arbeitsmethoden wurden Körpergröße und Altersstruktur in einer Population von *Hyla orientalis* BEDRIAGA, 1890 (n = 41) aus Kantarlı untersucht. Der Ort liegt auf etwa 800 m ü. M. in der Schwarzmeerregion der Türkei. Das höchste festgestellte Alter betrug 8 Jahre bei Weibchen und sieben Jahre bei Männchen. Die Geschlechtsreife trat in dieser Population im Alter von drei (Weibchen) bzw. zwei Jahren (Männchen) ein. Der Unterschied im Alter von Männchen und Weibchen war signifikant (Independent Sample t test, $p < 0.05$, $t = -2.455$). Eine signifikante positive Korrelation zwischen Alter und Kopf-Rumpf-Länge bestand sowohl bei Männchen als auch bei Weibchen. Um den Einfluß der Seehöhe auf Altersstruktur und Körpergröße der Population von Kantarlı aufzuzeigen, wurden die möglichen Wirkungen der Parameter 'Dauer der Aktivitätsperiode' und 'Höhe des Standortes über dem Meer' mit Ergebnissen anderer Studien verglichen.

ABSTRACT

Body size and age structure of a highland population of *Hyla orientalis* BEDRIAGA, 1890 (n = 41) from Kantarlı, located at about 800 m a.s.l. in the Black Sea Region of Turkey, was studied using skeletochronology. The maximum observed longevity was eight years in females and seven in males. Age at maturity in this population was three years in females and two in males. There was a significant difference in age between males and females (Independent Sample t test, $p < 0.05$, $t = -2.455$). A significant positive correlation was found between age and snout-vent-length both in males and females. To demonstrate the role of altitude on age structure and body size of the Kantarlı population, the potential effects of the parameters 'duration of activity period' and 'altitude' were analyzed by comparing present data with data available from the literature.

KEY WORDS

Amphibia: Anura: Hylidae; *Hyla orientalis,* morphology, biology, physiology; skeletochronology, altitude, age, longevity, population ecology, Kantarlı, Turkey

INTRODUCTION

Although the size of an organism is influenced by many features of its environment, the relationship between temperature and body size has captivated generations of biologists (ANGILLETTA et al. 2004). The tendency for organisms to be larger in cooler climates (i.e., BERGMANN's rule) is widely observed in endothermic vertebrates and has been suggested to apply also to some ectothermic vertebrates, such as amphibians (ANGILLETTA et al. 2004; ADAMS & CHURCH 2008). However, its general applicability to ectotherms has been questioned, as many ectotherms have been found to follow the converse to BERGMANN's rule, i.e., decreasing body size with cooler climate (MOUSSEAU 1997; ASHTON 2002; BELK & HOUSTON 2002; LAUGEN et al. 2005; ADAMS & CHURCH 2008).

The environmental factors that are likely to promote variations in physiological activity of amphibians are temperature, humidity and food availability, which may vary seasonally depending on the latitude and altitude of the site (FEDER & BURGGREN 1992; ÖZDEMİR et al. 2012). In temperate regions, studies comparing age, size or growth along a gradient of latitude, altitude or temperature provided convincing evidence of a relationship between life-history traits and environmental variables (Ficetola et al. 2010). In these regions, the length of the cold period has been identified as one of the most important variables in differentiating growth patterns concomitantly with latitudes and altitudes (Caetano & Castanet 1993).

Two species of treefrog are currently recognized to occur in Turkey: *Hyla savignyi* Audouin, 1827 (southeastern parts of Turkey) and *Hyla orientalis* Bedriaga, 1890 ["1889"] (Stöck et al. 2008; Gvozdik et al. 2010; Gül et al. 2012) The range of *H. orientalis*, which was usually referred to as *Hyla arborea* (Linnaeus, 1758) in the previous herpetological literature, includes the Black Sea, Marmara, Aegean, Western Mediterranean, Central Mediterranean and Central Anatolian regions of Turkey.

Only a few demografic studies were conducted on West Palearctic treefrogs (Switzerland: Tester 1990; Germany: Friedl & Klump 1997; Greece: Kyriakopoulou-Sklavounou & Grumiro 2002; Turkey: Özdemir et al. 2012). Most of these refer to *H. arborea*, and there is only one (Özdemir et al. 2012) that definitely deals with *H. orientalis*. Since this study was performed at sea level, no comparative data was available from a highland population of *H. orientalis*.

In this study, mean body size, sexual size dimorphism (SSD) and age-related population structure were analyzed in a highland population (Kantarlı Village, 800 m a.s.l.) of *H. orientalis* occurring in the Black Sea Region of Turkey. The particular aim of the study was to (i) explore the effect of altitude on the population; (ii) assess age at sexual maturity, longevity and the relation of body size to age for this higland population; and (iii) compare the results with data available for lowland populations of *H. orientalis*.

MATERIALS AND METHODS

A total of 41 adult *H. orientalis* (32 males, 9 females) were collected at a permanent pond in a forested area during the breeding season (May) in 2010 from Kantarlı Village (40º58'N, 40º52'E, 800 m a.s.l.) in the Province of Rize, in northeast Turkey. For each individual, sex was determined by examination of external secondary sexual characters, snout-vent length (SVL) was measured to the nearest 0.01 mm using digital callipers (Mitutoyo Corp., Kawasaki, Japan), and the longest toe was clipped, including the first and second phalanges. Toe samples fixed in 10% formalin and stored in 70 % ethanol were subject to histological analysis. After sampling, each individual was released at the place of capture.

The climate of the area is temperate oceanic (submediterranean) (Rivas-Martinez et al. 2003, diagram of Trabzon), there is neither a dry nor a real frost period. Treefrogs are active there from early May to late September.

The age was estimated by skeletochronological methods (Castanet & Smirina 1990; Olgun et al. 2005). After dissection and preparation, the phalanges were washed in running tap water for 24 h, decalcified in 5 % nitric acid for 2 hours and then washed again under running tap water for 12 hours. Cross-sections (18 µm) of the diaphyseal part of each phalanx were obtained using a freezing microtome and stained in Ehrlich's haematoxylin. Sections were submerged in water-based mounting media for observation under a light microscope. Endosteal resorption of the first lines of arrested growth (LAG) was assessed by comparing the diameters of eroded marrow cavities with the diameters of non-eroded marrow cavities in sections from the youngest specimens.

On each section, the numbers of LAGs were assessed by two observers independently and the results compared. Double lines and endosteal resorption did not cause any serious interpretation problems concerning age estimation, and full agreement between the observers was achieved for all samples. The distance between two adjoining LAGs is a good indicator of individual growth in a given year (Kleinenberg & Smirina 1969). Consequently, an obvious decrease in the spacing between two subsequent LAGs ob-

Table 1: Descriptive statistics of life history variables of *H. orientalis* populations of two Turkish localities. SD – Standard deviation; SSD – Sexual size dimorphism present; SVL – Snout-vent length; F – Female; M – Male; Y - Yes.

Tab. 1: Beschreibende Statistiken biologischer Kenngrößen von *H. orientalis* - Populationen zweier Nordtürkischer Fundorte. SD – Standardabweichung; SSD – Geschlechtsdimorphismus in der Körpergröße vorhanden; SVL – Kopf-Rumpf-Länge; F – Weibchen; M – Männchen; Y - Ja.

Locality (altitude) Fundort (Seehöhe)	Sex	n	Mean SVL ±SD (mm) Mittlere SVL ±SD (mm)	Range SVL (min-max) mm Spannweite SVL (min-max) mm	Mean Age ± SD (years) Mittleres Alter ± SD (Jahre)	Age at maturity (years) Alter bei Geschlechtsreife (Jahre)	Longevity (years) Höchstalter (Jahre)	SSD
Kantarlı	M	32	43.56 ± 2.56	36.71- 48.38	4.88 ± 0.83	2	7	Y
(800 m)	F	9	47.02 ± 3.49	40.24 -53.12	5.77 ± 1.39	3	8	Y
Rize	M	66	37.2 ± 3.20	30.0 - 43.8	2.5 ± 0.9	1	5	Y
(26 m)	F	12	41.2 ± 2.50	37.8 - 45.8	3.4 ± 0.9	2	5	Y

served, was taken to mark the age when sexual maturity was achieved (RYSER 1988; YILMAZ et al. 2005).

Student's *t*-test was applied to compare variables between sexes, while Pearson's correlation coefficient was computed to infer the pattern of relationships between SVL and age. Kolmogorov-Smirnov and Levene tests were used to test for differences in distributions and variances, regression analysis was applied to calculate the correlation equation between age and SVL. Data analysis was performed using SPSS 18® statistical software package. Sexual size dimorphism was quantified with the SSD index by LOVICH & GIBBONS (1992).

Results were compared with published data on body size and age patterns from populations of *H. orientalis* in Asia Minor (ÖZDEMİR et al. 2012) and treefrogs of the *H. arborea* group of continental Europe (TESTER 1990; FRIEDL & KLUMP 1997; KYRIAKOPOULOU-SKLAVOUNOU & GRUMIRO 2002).

RESULTS

Both SVL and age showed normal distribution (Kolmogorov-Smirnov test; $p > 0.05$) and homogeneity of variance (Levene test; $p > 0.05$). Descriptive statistics of age and body length are summarized in Table 1.

In all phalangeal cross-sections, LAGs were clearly marked and relatively easy to count, as can be seen in Fig. 1. Endosteal resorption, which created partial erosion of the periosteal bone on the edge of the marrow cavity, was observed in 78.04 % of the individuals from the Kantarlı population. The first (innermost) LAG was partially eroded in 60.97 % of the individuals and completely eroded in 17.07 %. Intersexual differences in body size were female-biased (Standard Deviation Index - SDI: 0.07). Females were significantly larger than males (Independent Sample *t* test; $p < 0.01$, $t = -3.304$). SVL ranged between 36.7 - 48.38 mm (mean: 43.56 ± 2.56) in males and 40.24 - 53.12 mm (mean: 47.02 ± 3.49) in females.

In the Kantarlı population, the oldest individual was an eight years old female. Age ranged between 3 - 8 (mean: 5.77 ±

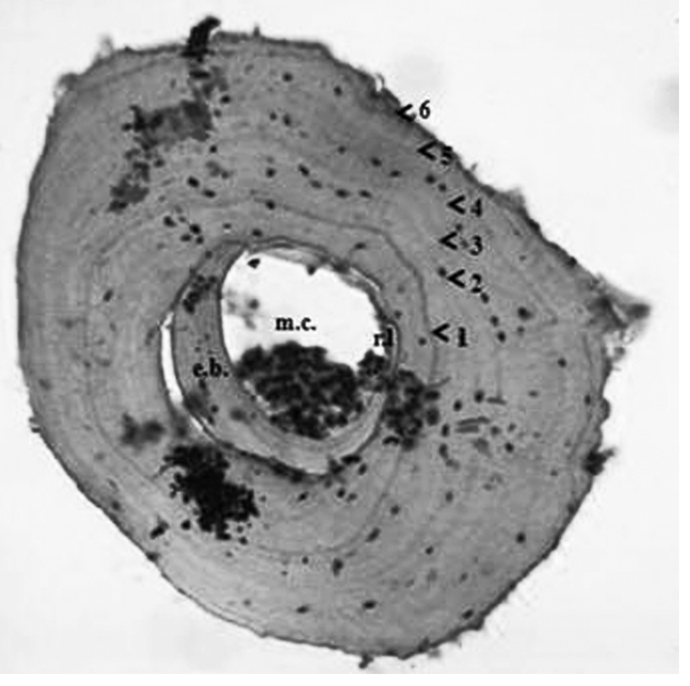

Fig. 1: Cross-section (18 µm thick) at the diaphysis level of a second phalanx of the longest toe of a male *Hyla orientalis* from the Kantarlı population, northern Turkey. The six LAGs are indicated by black arrows; m.c. - marrow cavity; r.l. - resorption line; e.b. - endosteal bone.

Abb 1: Querschnitt (Dicke: 18 µm) auf Höhe der Diaphyse eines zweiten Phalangenknochens der längsten Zehe eines Männchens von *Hyla orientalis* der Population aus Kantarlı (Nordtürkei). Die sechs Linien verminderten Wachstums (LAG) sind durch Pfeile gekennzeichnet; m.c. – Markhöhle; r.l. – Resorptionszone; e.b. – endostaler Knochen.

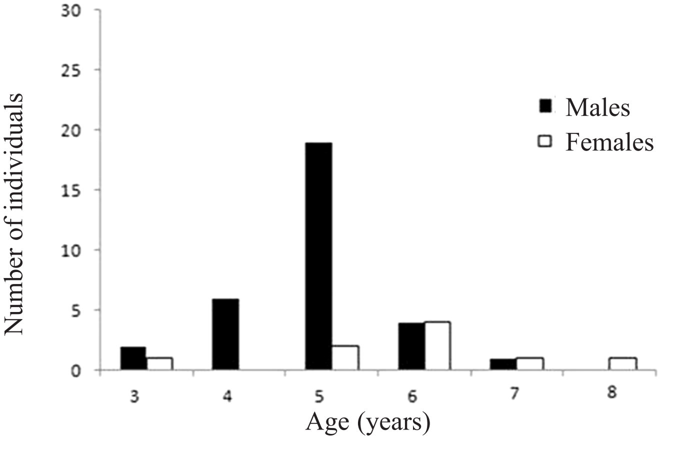

Fig. 2: Age frequency distributions of male and female *Hyla orientalis* from Kantarlı Village, northern Turkey.

Abb. 2: Häufigkeitsverteilung der Altersklassen bei Männchen und Weibchen der *Hyla orientalis* - Population aus Kantarlı (Nordtürkei).

1.39) years in females and 3 - 7 (mean: 4.88 ± 0.83) in males (Fig. 2). There was a significant difference in age between males and females (Independent Sample *t* test; $p < 0.05$, $t = -2.455$). In this population, the youngest breeding females were three years old, whereas some males became sexually active at the age of two.

A significant positive correlation (cubic function) was found between age and SVL both in males ($n = 32$, $r = 0.747$, $p < 0.01$, $y = 43.21 – 4.675 x + 1,44 x^2 – 0.097 x^3$) and females ($n = 9$, $r = 0.943$, $p < 0.01$, $y = 9.63+ 17.10 x – 2.82 x^2 + 0.16 x^3$).

DISCUSSION

This is the first demographic data about a highland population (Kantarlı Village, Rize - 800 m a.s.l.) of *H. orientalis* in northeast Turkey. A previous study (Özdemİr et al. 2012) conducted in about the same region (viz. İslampaşa, Rize – 26 m a.s.l.) analyzed a population of *H. orientalis* (*H. arborea* in Özdemİr et al. 2012) at sea level. Although there is a demographic study from Greece (Kyriakopoulou-Sklavounou & Grumiro 2002), which includes a highland population (population #1: Halkidiki, 0 m a.s.l. , *n* = 24; pop. #2: Lake Kerkini, 38 m a.s.l., *n* = 26; pop. #3: Lake Mikro Prespa, 853 m a.s.l., *n* = 25; pop. #4: Imeros, 0 m a.s.l., *n* = 9), analyses were performed using pooled data. Thus, there is no information about body size and age of *H. orientalis* exclusively from a highland population.

The maximum observed lifespan found in the present study was eight years for females and seven for males, whereas Özdemİr et al. (2012) observed longevities of five years in both sexes of the nearby lowland population. The discrepancy in lifespan may be explained by different altitudes of the habitats, viz. 800 m a.s.l. versus 26 m a.s.l.

Amphibians living in higher latitudes or altitudes go through a longer period of hibernation during which the metabolism of the animals is shut down (Irwin & Lee 2003; Lu et al. 2008), and thereby would expend less energy per year allowing for an extension of their lifespan (Zhang & Lu 2012). Among anuran species, individuals in high-altitude populations lived longer compared to those in low-altitude populations (Zhang & Lu 2012). Analogously in this study, comparing high altitude (this study) and low altitude populations (Özdemİr et al. 2012) of *H. orientalis*, body size and age increased with increasing altitude. This effect is probably associated with the duration of the active period of the animals. Temperatures can affect the ability of amphibians to acquire and process food in many ways. Anurans in cool climates are limited in both their seasonal and daily activity periods by low temperatures (Mullally & Cunningham 1956, Sinsch & Sherif 1989). This circumstance may be reflected by Bergmann's rule, which formulates a well-known ecogeographic pattern that predicts larger body size with increasing latitude or decreasing temperature.

In the present study, *Hyla orientalis* females reached sexual maturity at the age of three, and males two years, whereas the corresponding time spans in a lowland population of the same region were two years for females and one year for males (Özdemİr et al. 2012). The results of this study were also in line with the data from Greek populations (Kyriakopoulou-Sklavounou & Grumiro 2002). A life history theory predicts that delayed maturation and the resulting increase in maturation size may be adaptive in cold environments because fecundity increases with body size (Roff 2002). The meta-analyses in the study of Zhang & Lu (2012) revealed that, in all cases, the overall (grand) mean correlation coefficient was not significant between longevity and latitude, although significantly positive between longevity and altitude.

As previously reported for *Hyla orientalis* (referred to as *H. arborea*) in Turkey (Özdemİr et al. 2012) and Greece (Kyriakopoulou-Sklavounou & Grumiro 2002) but also for *H. arborea* in Germany (Friedl & Klump 1997), the present study found a positive significant correlation between body size (SVL) and age for both sexes, as expected.

Significant differences in SVL were found between sexes. Like previous studies on West Palearctic treefrogs (Tester 1990; Friedl & Klump 1997; Kyriakopoulou-Sklavounou & Grumiro 2002; Özdemir et al. 2012) and e.g., *Hyla annectans* (Jerdon, 1870) (Liao & Lu 2010), results of the present study showed that females grew larger than males (SDI: 0.07).

Endosteal resorption was found in 78.04 % of the highland population individuals versus 76 %, 50 % and 28 %, respectively, in three lowland populations of *H. orientalis* (Özdemir et al. 2012). The extent of endosteal resorption in long bones may be linked to environmental conditions (Smirina 1972). In the study of Caetano &Castanet (1993), less resorption was observed in populations living at low altitudes than in highland populations, even though a converse trend was reported in other cases (Esteban et al. 1996, 1999).

In conclusion, comparison of the present observations with published data suggests that tree frogs inhabiting higher altitudes gain larger mean body size and longer lifespan than their lowland conspecifics, potentially in connexion with lower metabolic rates, shorter activity periods and smaller total energy requirements.

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