Acta Herpetologica 13(2): 147-154, 2018
DOI: 10.13128/Acta_Herpetol-21026

Age structures and growth parameters of the Levantine frog, *Pelophylax bedriagae*, at different localities in Denizli, Turkey

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Submitted on: 2017, 26th July; revised on: 2018, 1st January; accepted on: 2018, 20th September
Editor: Rocco Tiberti

Abstract. Skeletochronology is a reliable tool for assessing several parameters in amphibian populations. We used skeletochronology to determine the age structure, growth rate, age at first reproduction, and longevity of Levantine frog *Pelophylax bedriagae* populations from different localities in Denizli, Turkey. All examined individuals (N = 161) exhibited Lines of Arrested Growth in the bone cross-sections. Age structure and age at first reproduction were similar among localities and sexes, while longevity and growth rates showed significant variation among localities. Obtained results were compared with literature data on age-related and grow parameters in *Pelophylax bedriagae* and cognate species.

Keywords. Skeletochronology, Levantine frog, longevity, growth rate, sexual maturity.

INTRODUCTION

Turkish water frogs were long considered as a single species, and *Pelophylax bedriagae* and *Pelophylax caralitanus* were previously considered to be subspecies of *Pelophylax ridibundus* (Sinsch and Schneider, 1999). However, both subspecies have been raised to species level, based on morphometric, molecular and bioacoustic evidence (Schneider and Sinsch, 1999; Sinsch and Schneider, 1999; Jdeidi et al., 2001; Plötner et al., 2001; Akın et al., 2010a, 2010b). The Levantine frog is a highly opportunistic amphibian, widely distributed in the eastern Mediterranean region, including several Greek islands close to the coast of Turkey (Papenfuss et al., 2009). In Turkey, it is present along the Aegean coast and the southern part of the Anatolian highlands, from sea level to 2100 m (Özeti and Yılmaz, 1994). It is highly aquatic and inhabits a wide variety of running and standing water habitats, e.g., puddles, shallow ponds, large lakes, large rivers, mountain streams. The Levantine frog has been listed in the Least Concern category of the IUCN Red List of Threatened Species because of its wide distribution and presumed large populations, but habitat loss and its commercial use as food could threaten natural populations (Papenfuss et al., 2009). The Levantine frog also spread outside its native range by means of several introductions (e.g., for aquaculture), threatening the genetic integrity of native water frogs (e.g., Plötner et al., 2015).

The aim of the present study is to describe the age structure and individual growth patterns of Levantine frog populations from distinct areas of Turkey. We used skeletochronology for estimating the age of amphibians and studying age-related parameters in four frog populations (e.g., mean longevity, growth rate, age at sexual maturity; Castanet et al., 1993; Smirina, 1994; Guarino et al., 1999, 2003; Esteban and Sanchiz, 2000). Skeletochronology can be applied on amphibian phalanges, which can be sampled without animal culling and without affecting amphibian populations and endangered species (Smirina, 1994; Khonsue et al., 2000; Guarino and Erişmiş, 2008; Guarino et al., 2011; Bionda et al., 2015).

ISSN 1827-9635 (print)
ISSN 1827-9643 (online)

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MATERIAL AND METHODS

Study sites

The field study was conducted during the 2015-2017 breeding seasons at four distinct localities at different altitudes in Denizli, Turkey.

Süleymanlı Lake, Buldan, Denizli province, Turkey (38°03'N, 28°46'E; 1163 m a.s.l.) is a natural lake which covers 0.5 km^2 and attains a maximum depth of 2 m. The lake is surrounded by coniferous tree (red pine *Pinus brutia*), willow trees (*Salix alba*) and reeds (*Phragmites australis*). The water surface is covered by aquatic vegetation (i.e., *Myriophyllum spicatum*).

Vali Recep Yazıcıoğlu Dam, Denizli, Turkey (37°46'N, 29°07' E; 328 m a.s.l) is a reservoir located in the city centre of Denizli. It is fed by underground water and by the Gökpınar creek. This reservoir covers approximately 2 km^2 and is surrounded by sparsely distributed trees such as *Salix alba* and *Juniperus* sp. Aquatic vegetation is absent, but some vegetation patches are present in the littoral zone.

Acıgöl Lake, Denizli, Turkey (37°47'N, 29°51'E; 839 m a.s.l.) is included in a natural wetland surrounded by agricultural land. The lake covers approximately 47 km^2 but it reaches approximately 100 km^2 if the surrounding wetlands are included. The littoral area and the water surface are covered by aquatic vegetation.

Ornaz Valley, Denizli province, Turkey (37°46'N, 28°58'E; 834 m a.s.l.) is crossed by a slow-flowing stream, which starts at Karcı Mountain and continues to Başkarcı. Along the river, there are many ponds that form natural amphibian breeding areas rich in aquatic and riparian vegetation.

Field studies and morphometric measurements

The Levantine frogs were captured by 2-3 people with a dip net or by hand after sunset using flashlights. Snout-Vent Length (SVL) was measured with a dial calliper (accuracy: 0.02 mm) and body mass (W) with a precision balance (accuracy: 0.1 g). Sex was determined based on secondary sexual characteristics of males, i.e. presence of metatarsal tubercules on the first finger of the front feet and vocal sac. The Sexual Dimorphism Index (SDI) was calculated as SDI = [(mean length of the larger sex / mean length of the smaller sex) - 1] according to the method of Lovich and Gibbons (1992). We collected bone samples only from breeding individuals, i.e. calling males, females responding to the males, and already paired individuals. We assumed that the age at first reproduction (Age at Maturity: AM) is the lowest age recorded among the breeding individuals.

The longest digit of the hind foot was cut off, fixed in 70% ethanol, and then processed according to the routine skeletochronological procedure (e.g., Castanet et al., 1993; Castanet and Smirina 1990; Smirina 1994). Following tissue removal, the bones were washed in running water for 12-14 hours, decalcified for 3-5 hours in 5% nitric acid and then placed in distilled water overnight. The bones were dehydrated using graded ethanol series and then cleared in xylene, before embedding in paraffin. Using a rotary microtome, we obtained 16-μm-thick cross-sections from the central region of the diaphysis. Bone sections were stained with haematoxylin and eosin and analysed under a light microscope. The age of the frog was determined by two of the authors who independently counted the number of Lines of Arrested Growth (LAGs) present in each of the bone sections. With regard to first LAG resorption, we distinguished between "completely resorbed" (the first LAG was not observable in bone cross-section), "partially resorbed" (only some parts of the first LAG were visible in bone cross-section), and "not resorbed" (the first LAG well visible in bone cross-section).

Statistical analyses

Length and weight data were normally distributed (Kolmogorov–Smirnov D test, all P > 0.05), thus allowing comparisons using parametric tests. We used a t-test for independent samples to compare the lengths and weights of male and female frogs. Length–weight relationships were estimated separately for each sex and locality. Growth rate was estimated according to the von Bertalanffy equation (von Bertalanffy, 1938):

$$SVL_t = SVL_{max} - (SVL_{max} - SVL_{met})e^{-k(t-tmet)} \quad [1]$$

where SVL_t is the average body length at age t; SVL_{max} is the asymptotic maximum body length; SVL_{met} is the body length at metamorphosis that was calculated on newly metamorphosed individuals at the end of the summer (fixed to mean 26.3±3.22 mm); k is the body growth rate coefficient (year^{-1}); t_{met} is age at metamorphosis (0.3 years). The parameters SVL_{max} and k and their asymptotic confidence intervals (CI) were estimated by nonlinear regression. All statistical analyses were conducted using SPSS ver. 20.0 (SPSS 2011).

RESULTS

A total of 161 individuals (80♂, 81♀) were examined. SVL and body mass were significantly higher in females than in males (Table 1), but not among the sampling localities (females: $F_{3,77} = 3.324$, $P > 0.05$; males: $F_{3,76} = 2.326$, $P > 0.05$). Consistently the sexual dimorphism index was equal to 0.13 for all studied populations, i.e. female Levantine frogs are larger and heavier than males.

All the examined bones sampled from adult frogs had well-defined LAGs with visible layers (Fig. 1). The first LAG was partially resorbed in 22.3% of females and 19.2% of males and completely resorbed in 8.8% females and 6.4% males. The age structure of the Levantine frogs from the different sampling sites is summarized in Table 1. The average age varied between 4.6 and 6.5 years among the populations, most individuals were between 4-8 years, and a few individuals reached a maximum age of 12 years (Fig. 2). The age of males and females did not show significant differences (Table 1). The sex-specific age structure did not differ significantly among localities

Table 1. Student t tests comparisons of mean Snout-Vent Length (SVL), body mass and age in male and female Levantine frogs from four localities in Denizli, Turkey.

Location	Parameters	Gender	N	Min	Max	Mean	Std. Error Mean	t	df	Sig.
Vali Recep Yazıcıoğlu Dam	SVL (mm)	Female	30	51.0	86.0	71.20	1.726	3.242	50	0.002**
		Male	22	43.6	72.3	63.01	1.774			
	Body mass (g)	Female	30	12.9	71.3	38.59	2.751	4.001	50	0.001**
		Male	22	11.6	34.5	24.49	1.679			
	Age	Female	30	2	9	5.20	0.334	1.198	50	0.237
		Male	22	2	9	4.59	0.382			
Süleymanlı Lake	SVL (mm)	Female	24	57.0	104.9	76.63	2.194	3.079	47	0.003**
		Male	25	45.0	84.2	68.01	1.760			
	Body mass (g)	Female	24	12.6	94.4	46.05	3.710	2.354	47	0.023*
		Male	25	11.6	75.9	35.29	3.075			
	Age	Female	24	2	12	6.46	0.474	0.025	47	0.981
		Male	25	2	11	6.44	0.575			
Acıgöl Lake	SVL (mm)	Female	15	45.0	90	71.93	2.872	2.511	30	0.018*
		Male	17	44.8	80.7	63.09	2.127			
	Body mass (g)	Female	15	11.3	84.4	37.59	4.540	1.971	30	0.058
		Male	17	8.7	60.4	26.98	3.095			
	Age	Female	15	2	9	5.13	2.232	-0.648	30	0.522
		Male	17	2	9	5.59	1.734			
Ornaz Valley	SVL (mm)	Female	14	50.4	103.0	81.14	4.635	2.168	26	0.039*
		Male	14	50.0	96.0	69.49	2.718			
	Body mass (g)	Female	14	32.4	94.5	67.39	5.462	4.011	26	0.000**
		Male	14	24.3	74.3	41.36	3.504			
	Age	Female	14	2	12	7.21	0.903	1.596	26	0.122
		Male	14	2	11	5.50	0.571			
Pooled localities	SVL (mm)	Female	81	45.0	104.7	74.31	1.337	5.027	159	0.000**
		Male	80	43.6	94.0	65.78	1.040			
	Body mass (g)	Female	81	11.3	94.4	45.33	2.243	4.889	159	0.000**
		Male	80	8.7	75.9	31.92	1.568			
	Age	Female	81	2	12	5.79	0.264	0.375	159	0.708
		Male	80	2	12	5.65	0.265			

*P< 0.05, **P < 0.01.

(females: $F_{3,77} = 2.406$, $P > 0.05$ and males: $F_{3,76} = 2.351$, $P > 0.05$). The age at first reproduction was two years in all localities and in both sexes. The asymptotic SVL (SVL_{max}) was estimated as 88.1±1.68 mm (CI = 85.08-91.58) for males and 91.5±1.57 mm (CI = 88.62-94.58) for females, while growth coefficient (k) was estimated as 0.239 ± 0.0761 (CI = 0.196-0.255) and 0.346 ± 0.0924 (CI = 0.3145-0.7162) respectively (Fig. 3).

DISCUSSION

Our findings showed that counting LAGs on the phalanges of *P. bedriagae* was relatively simple because the growth arrest was clear during the winter and the degree of endosteal resorption was low. We did not observe double LAGs in any sample. The lack of double lines, which indicate a double annual growth cycle (e.g., due to aestivation period; Guarino and Erişmiş, 2008), or partly resorbed LAGs, suggests that these frogs experience an uninterrupted and regular annual bone growth cycle.

As observed in other cognate species, female Levantine frogs grow larger than males (Sinsch and Schneider, 1999; Erişmiş, 2005; Erişmiş and Chinsamy, 2010; Çiçek et al., 2011; Erişmiş, 2011; Gül et al., 2011; Table 2). Many amphibians show sexual size dimorphism, and females are larger than males in 90% of anuran species (Shine, 1979). Sexual size dimorphism can be explained in terms of differences in the age structure between the sexes in breeding populations (Monnet and Cherry 2002), or by adaptive differences in the grow rates

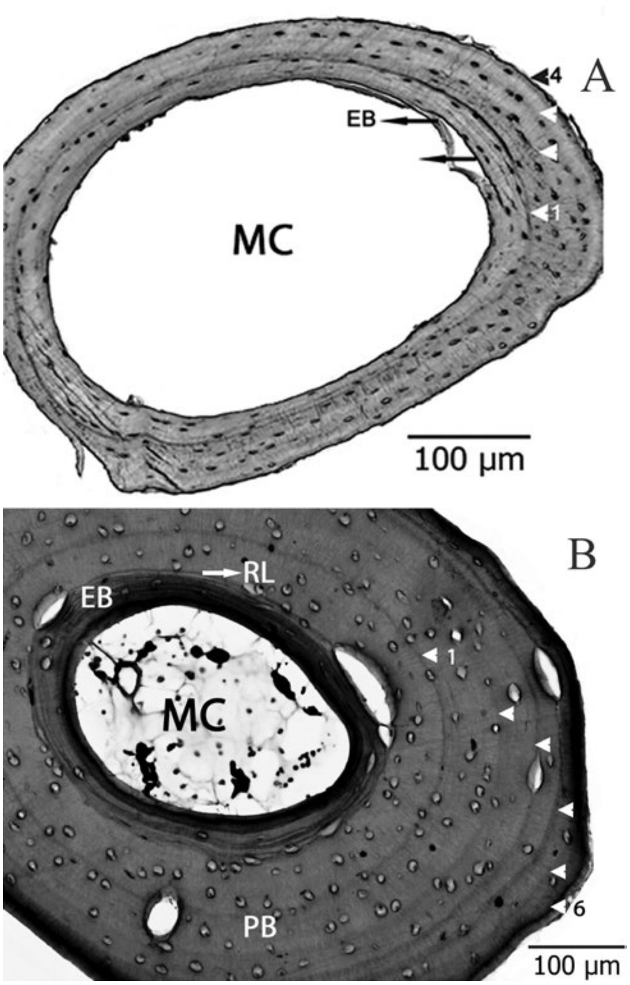

Fig. 1. Phalangeal cross-sections (16 µm thick) of Levantine frog. The white arrows show LAGs in the periosteal bone: (A) Male: 4 LAGs with 65 mm SVL, (B) Female, 6 LAGs with 79.5 mm SVL. EB = Endosteal bone, PB= Periosteal bone, MC =Marrow cavity, RL=Resorption line.

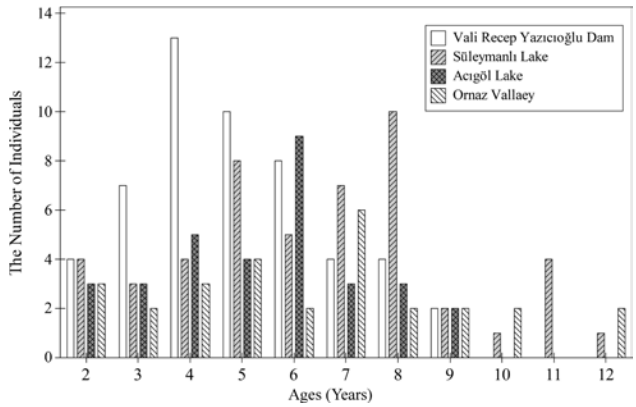

Fig. 2. Age distribution of four Levantine frog reproductive populations from different localities in Denizli, Turkey.

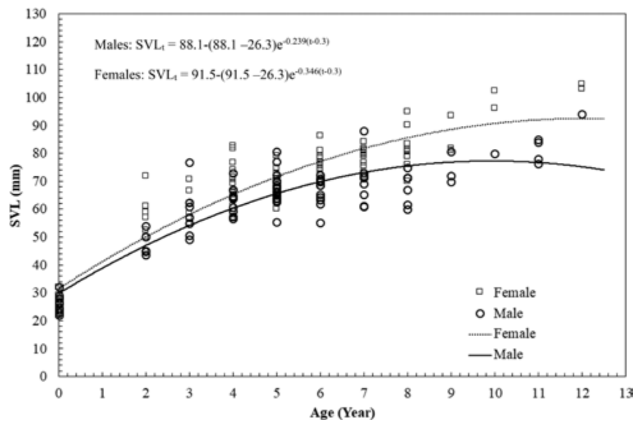

Fig. 3. Von Bertalanffy growth curve (age-length relationship) for Levantine frogs from different localities in Denizli, Turkey.

between sexes (as observed in *P. bedriagae*; present study). Indeed, larger females are able to produce more and larger eggs (Crump, 1974; Crump and Kaplan, 1979; Berven, 1988; Kupfer et al., 2004; Haddad and Prado, 2005; Gunzburger, 2006) and males may prefer larger females (Dittrich et al., 2018).

Body size parameters were comparable with previous studies performed on other populations of Levantine frog (Çiçek et al., 2011) and on cognate species, such as *P. ridibundus* (Kyriakopoulou-Sklavounou et al., 2008; Gül et al., 2011; Erişmiş, 2011; Ashkavandi et al., 2012) and *P. caralitanus* (Erişmiş and Chinsamy, 2010; Başkale et al., 2017; Erişmiş, 2018) (Table 2). The age composition of amphibian populations may depend on several genetic and environmental factors, e.g., climate, trophic resources, habitat quality, and interspecific interactions (Miaud et al., 2001; Morrison and Hero, 2003; Üzüm et al., 2011; Gümüş Özcan and Üzüm, 2015). Although our sampling habitats were quite different in terms of altitude, surface area, and habitat quality, all the studied populations had a similar age structure. However, different age structures can be retrieved in literature. Çiçek et al. (2011) and İsmail and Çiçek (2017) determined that the mean age of a Levantine frog population from Sülüklü Lake, Manisa, Turkey was much lower than what we observed (Table 2), possibly due to anthropogenic pressures. Similarly, also the age structure of the other cognate species may present high degrees of variation (Table 2). Compared to the present study (max longevity = 12 years), Çiçek et al. (2011) and İsmail and Çiçek (2017) found a lower longevity in *P. bedriagae* possibly due to anthropogenic stressors. Considering cognate species, the maximum ages can reach 11 years (range = 5-11 years) and age at maturity ranged from 1-4 years in different parts of their range (Table 2).

Table 2. Body size and grow parameters in *P. bedriagae* and cognate species: a comparison with existing literature. AM: age at sexual maturity; k: von Bertalanffy growth coefficient.

Species	Locality	Sex	N	Mean SVL (mm)	Max SVL (mm)	Mean age (years)	Max age (years)	AM (years)	k	References
P. bedriagae	Vali Recep Yazıcıoğlu Dam, Denizli, Turkey	M	30	63.01	80.17	4.59	9	2	0.18	Present study
		F	22	71.20	83.75	5.20	9	2	0.27	
	Süleymanlı Lake, Denizli, Turkey	M	24	68.01	73.80	6.44	11	2	0.37	
		F	25	76.63	82.67	6.46	12	2	0.22	
	Acıgöl Lake, Denizli, Turkey	M	15	63.09	70.04	5.59	9	2	0.37	
		F	17	71.93	80.79	5.13	9	2	0.24	
	Ornaz Valley, Denizli, Turkey	M	14	69.49	78.64	5.9	12	2	0.19	
		F	14	81.14	96.41	6.8	12	2	0.33	
	Pooled localities	M	80	65.78	88.1	5.65	12	2	0.24	
		F	81	74.31	91.5	5.79	12	2	0.35	
	Sülüklü Lake Manisa, Turkey	M	14	56.1	73.2	2.5	4	2	0.30	Çiçek et al., 2011
		F	22	64.5	92.4	2.95	5	2		
		M	51	59.80	120	3.45	7	2	0.22	İsmail and Çiçek, 2017
		F	76	59.78	137	4.33	9	2	0.36	
P. ridibundus	Yıldızlı Stream, Trabzon, Turkey	M	38	64.58		3.90	7	3-4		Yılmaz et al., 2005
		F	11	76.64		3.72	6	3-4		
	Lake Vistonis, Lagos, Greece	M	52	69.03	93.4	2.96	5	1	0.57	Kyriakopoulou-Sklavounou et al., 2008
		F	56	82.38	107.5	3.73	5	1	0.54	
	Milicz Ponds Reserve, Stawno, Polland	M	32	72.2	90	3.7	6	2	0.76	Socha and Maria, 2010
		F	38	79.8	102.3	4.4	7	3	0.59	
	Artvin (Borçka, Lake Karagöl), Turkey	M	20	72.96		5.15	8	2		Gül et al., 2011
		F	25	63.49		4.20	7	2		
	Dörtyol, Hatay, Turkey	M	20	64.70		5.50	11	4		
		F	19	76.74		5.58	7	3		
	Verkhne-Tagil Reservoir, Tagil and Vogulka rivers, Middle Urals.	M	-	-	-	-	-	-	-	Ivanova et al., 2011
		F		92.8	116	5.4	9	2		
	The Reftinskii Reservoir Reft River, Middle Urals.	M	-	-	-	-	-	-	-	
		F	26	112.9	132	4.4	8	2		
	The north of Lorestan Province, central Zagros, Iran	M	26	71.14		6.43	11	3		Ashkavandi et al., 2012
		F	14	74.05		4.5	7	3		
P. caralitanus	Beysehir Lake, Turkey	M	38	75.56	109	5.01	9	3-4	0.18	Erişmiş and Chinsamy 2010
		F	51	92.05	126.24	6.01	10	3-4	0.16	
		M	96	90.41	111.35	5.63	9	3		Erişmiş, 2018
		F	73	98.29	126.50	6.33	10	3		
	Karamık Lake, Turkey	M	66	82.33	99.48	4.86	7	3		
		F	76	88.36	111.63	5.30	8	3		
	Işıklı Lake, Turkey	M	49	73.06	93.68	3.69	6	2		
		F	47	82.54	106.72	4.80	8	2		
	Eğirdir Lake, Turkey	M	90	86.44	110.12	5.42	8	3		
		F	97	94.52	120.28	6.21	10	3		

Local climatic conditions can determine the activity period of amphibians and can be a key factor controlling the activity and reproductive periods and population parameters in temperate species of anurans (Stebbins and Cohen, 1995; Kyriakopoulou-Sklavounou, 2000). For example, the Bergmann's rule (Bergmann, 1847) indicates that the individuals living in a cold climate tend to be larger than individuals inhabiting warmer climates (i.e. Miaud et al., 2001; Khonsue et al., 2001; but see Ashton (2002) for several exceptions to this general role). Altitude variation causes steep environmental gradients at small geographic scale (e.g., temperature and humidity), but altitude had limited effects on the parameters of our study populations. Erişmiş (2018) observed that the *P. caralitanus* from the Lake District of Anatolia exposed to warmer temperatures had longer growth seasons, but smaller growth rates. Our results may depend on an unusually weak link between local climatic conditions and altitude. Alternatively, they could indicate that *P. bedriagae* could have stable population parameters across its range, e.g., Gokhelashvili and Tarkhnishvili (1994) stated that *P. ridibundus* (formerly known as *Rana ridibunda*) from Georgia showed a constant age of maturation (after two hibernations) as a species-specific trait not depending on local environmental factors.

Our calculated grow rate in the present study is similar to literature values for *P. bedriagae* (e.g., 0.30; Çiçek et al., 2011) and is lower than the values reported for most European *Pelophylax* species (Table 2). Previous studies on *P. ridibundus* have shown significant correlations between SVL and age for both sexes (Yılmaz et al., 2005; Kyriakopoulou-Sklavounou et al., 2008) as commonly reported for the genus *Pelophylax* (Erişmiş et al., 2009; Esteban et al., 1996, 1999). In the present study, we found that the growth coefficients were higher in females than in males, and that the growth curves reached a plateau at an age of 3-4 years. In addition, the sizes at the same ages were highly variable among individuals, so that the size ranges of different age classes overlapped. These results show that body size is not an accurate indicator of the age of *P. bedriagae*, as already observed for other amphibians (Halliday and Verrell, 1988).

ACKNOWLEDGEMENTS

The permissions for field work, handling the frogs and laboratory studies were issued by the Animal Ethics Committee of Pamukkale University (Protocol No: PAUHDEK-2012/033), the Ministry of Forestry and Water Affairs, the General Directorate of Nature Conservation and Natural Parks and the Turkish Ministry of Food, Agriculture and Livestock. We would like to thank to Pamukkale University Scientific Research Projects Unit – BAP (2012FBE029) for their support during this study.

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