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Seasonal variations of sexual size dimorphism in two Mediterranean bat species from Tunisia: the Kuhl's pipistrelle (*Pipistrellus kuhlii*) and the Isabelline serotine (*Eptesicus isabellinus*)

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Abstract. Body mass variations of *Pipistrellus kuhlii* and *Eptesicus isabellinus* were studied in the Bou Hedma National Park (central Tunisia) from June 2010 to June 2011. In both species, adult females were significantly heavier than males throughout the year. Seasonal variations were larger in adult females, body mass peaking during late pregnancy. After a secondary peak in September-October observed in both sexes in *E. isabellinus*, in males only in *P. kuhlii*, body mass reached a minimum value in winter when activity is low. Significant differences were also noticed between years in June for both species. Such pattern of seasonal variations of sexual size dimorphism is typical of hibernating vespertilionids.

Key words: body mass, northwest Africa, pregnancy, Vespertlionidae, winter

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Introduction

Sexual Size Dimorphism (SSD) is generally biased towards males in mammals and occasionally towards females (Ralls 1976, Clutton-Brock 1989, Hughes et al. 1995, Weckerly 1998), mainly in some families of bats (Myers 1978, McNab & Armstrong 2001, McPherson & Chenoweth 2012). In fact most species of Pteropodidae, Emballonuridae, Molossidae, Mormoopidae, Noctilionidae and Phylostomidae do not depart from the general mammalian model (Eisenberg & Redford 1999). On the contrary in Rhinolophidae and almost Vespertilionidae females are usually larger than males (Myers 1978, Williams & Findley 1979). Many hypotheses have been proposed to explain this reversed SSD including adaptive response to challenges of flight and/or to metabolic demand during pregnancy and lactation, differential niche utilisation or big mother hypothesis (Myers 1978, Williams & Findley 1979).

In bats, SSD is usually evidenced by measuring forearm or various skull lengths and widths (e.g. Williams & Findley 1979, Bornholdt et al. 2008, Lisón et al. 2014, Šrámek & Benda 2014, Wu et al. 2014, Stevens & Platt 2015). Body mass is also sensitive to SSD (e.g. Bruce & Wiebers 1970, Jonasson & Willis 2011, Rughetti & Toffoli 2014), but is less studied due to several sources of variation which are often considered as biases: amount of food recently eaten by the animal, reproductive status (pregnancy and lactation for females, mating activity for males), seasonal fat accumulation and consumption and geographic size variation (Bruce & Wiebers 1970, Ralls 1976, Speakman & Racey 1986, Sorz et al. 2001, Welbergen 2010, Jonasson & Willis 2011, Rughetti & Toffoli 2014).

In temperate areas the annual cycle of bats includes a more or less long period of hibernation. During that period the small Daubenton's bats, *Myotis daubentonii*, can loose more than one-third of body mass (Harrje 1999) from the peak recorded in September and October when bats store fat reserves (Krulin & Sealander 1972, Polskey & Sealander 1979) to the minimum observed in April due to their consumption (Jonasson & Willis 2011). In females, body mass shows a secondary peak at the end of pregnancy in June. Such considerable seasonal variations of body mass have been reported from several species in Europe and North America (e.g. Ransome 1990, Kunz et al. 1998, Pandurska-Whitcher & Shanov 2003, Encarnação et al. 2004, 2006, Rughetti & Toffoli 2014). Additionally, body mass which may increase with age after the first year (Ransome 1968) exhibits daily variations (Studier et al. 1970, Studier & Ewing 1971, Vergari & Dondini 1997, Šuba et al. 2011), and also less recorded inter-annual variations (Ransome 1990).

In the Mediterranean region, bat hibernation is usually shorter than in more northern areas, and some species can be active throughout the year (Weber 1955, Lewis & Harrison 1962, Gaisler 1983-1984, Gaisler & Kowalski 1986, Carmel & Safriel 1998, Arlettaz et al. 2000, Lanza 2012, Dalhoumi et al. 2015). So, we recorded noticeable winter bat activity in central Tunisia (Dalhoumi et al. 2015). The most active species during that period, and along the year, were the small-sized Kuhl's pipistrelle, *Pipistrellus kuhlii*, beyond the medium-sized Isabelline serotine, *Eptesicus isabellinus*, two southern species in the Western Palaearctic. They were also the most captured species, which allowed us 1) to monitor the seasonal changes in body mass including the influence of the reproductive status of females, 2) to compare the variations between sexes, and 3) to check some inter-annual differences. We expected a SSD biased towards females, mainly during the reproductive period and a maximum body mass before the winter period followed by a low decrease in response to the low but constant flight and foraging activity.

Material and Methods

From June 2010 to June 2011, bats were mist-netted over water bodies of the Bou Hedma National Park: the Bordj basin (34°28′28.8″ N, 09°37′58.2″ E), the Nouh basin (34°29′36.9″ N, 09°38′39.2″ E) and the Bou Hedma wadi (34°29′40.7″ N, 09°39′23.3″ E). Each site was monitored once a month with a single mist-net (12 × 2.5 m; mesh: 16 × 16 mm) under favourable climate conditions, starting 15 minutes after sunset and lasting three hours. Netted specimens were identified in the field following Dietz & von Helversen (2004), Dietz (2005), Dietz et al. (2007) and Aulagnier et al. (2009), sexed, aged (subadult *vs.* adult based on the detection of epiphyseal cartilage, Anthony 1988), assessed for reproductive status, adult females only (non-reproductive, pregnant, lactating), weighed using a Pesola balance (Switzerland; ± 0.25 g), and immediately released. Capture and handling were operated under licence delivered by the Direction Générale de la Forêt (Ministère de l'Agriculture) and CRDA of Sidi Bouzid.

Due to the low number of captures during winter months, sex and seasonal variations of body mass were analyzed for adults of each species on a bimonthly basis from July 2010 to June 2011 using a two-way ANOVA, after verifying the normality of distributions, followed by Tukey multiple comparisons. The effect of female reproductive status was studied by computing a one-way ANOVA, and Tukey multiple comparisons on data collected from April to July. The winter loss of body mass was calculated for *E. isabellinus* only by comparing data recorded in October and February. At last, the annual influence on body mass was investigated by comparing data recorded in both months of June using a two-way ANOVA on sex and year.

Results

During the 13 months of the study a total of 492 bats belonging to seven species were mist-netted, including 121 *P. kuhlii* (111 at Bordj basin, 6 at Nouh basin and 4 at Bou Hedma wadi) and 349 *E. isabellinus* (186 at Bordj basin, 156 at Nouh basin and 7 at Bou Hedma wadi). The highest numbers of captures were recorded in June 2011 (30 *P. kuhlii* and 73 *E. isabellinus*), and the lowest in December and January (2 and 1 captures respectively for both moths).

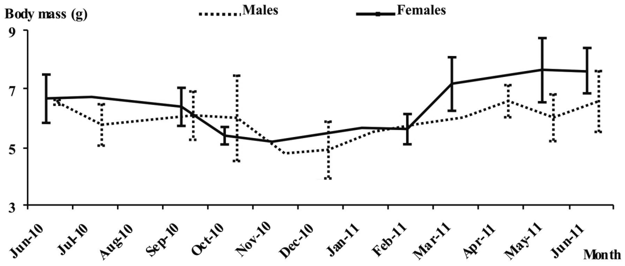

Fig. 1. Monthly variations of body mass of *P. kuhlii* adult males and females in the Bou Hedma National Park (June 2010-June 2011).

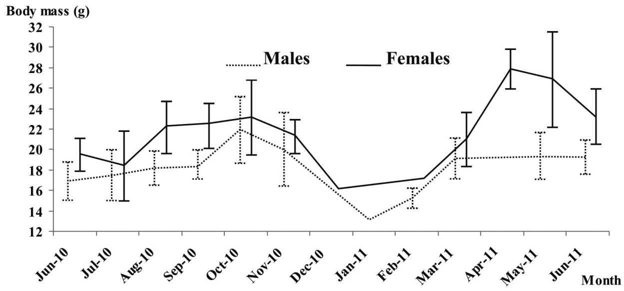

Fig. 2. Monthly variations of body mass of *E. isabellinus* adult males and females in the Bou Hedma National Park (June 2010-June 2011).

Table 1. Body mass of *P. kuhlii* and *E. isabellinus* in the Bou Hedma National Park (June 2010-June 2011). Table shows mean ± standard deviation (minimum-maximum).

Species	Sex	Male		Female			
	Age/Status	Subadult	Adult	Subadults	Non-reproductive adult	Pregnant	Lactating
P. kuhlii	Number	4	29	8	17	17	23
	Body Mass (g)	5.03 ± 0.33 (4.7-5.4)	5.95 ± 0.91 (4.2-7.7)	4.41 ± 0.80 (3.5-5.4)	7.2 ± 0.81 (5.3-7.9)	8.32 ± 0.52 (7.2-9.0)	7.12 ± 0.50 (6.0-7.9)
E. isabellinus	Number		111		67	74	22
	Body Mass (g)	-	18.19 ± 2.59 (12.75-25.5)	-	20.51 ± 2.86 (14.5-28.5)	27.38 ± 2.51 (21.5-32.0)	22.96 ± 2.02 (19-26.25)

P. kuhlii

The interaction sex*bi-month was not significant ($F_{5/67} = 1.48$; $p = 0.207$), the annual variation of body mass was quite similar for both sexes, with the highest values from April to September and the lowest from December to February (Fig. 1). The bi-monthly variation is highly significant ($F_{5/67} = 5.26$; $p < 0.001$). Adult females were heavier than adult males ($F_{1/67} = 4.80$; $p = 0.032$; Table 1), only during the reproductive period (April-July). Female body mass peaked during pregnancy, pregnant females being heavier than lactating females, in turn heavier than non reproductive females at the same period (6.35 ± 0.78 g for non-reproductive females in May-July; $F_{2/47} = 42.48$; $p < 0.001$). At last, when comparing data collected in June, the interaction sex*year was significant ($F_{1/39} = 4.25$; $p = 0.046$), a difference that can be related to the larger number of pregnant females captured in June 2011.

E. isabellinus

The interaction sex*bi-month was significant ($F_{5/263} = 4.87$; $p < 0.001$), the annual variation of body mass was highly different between sexes (Fig. 2). Adult females were heavier than adult males throughout the year ($F_{1/263} = 30.02$; $p < 0.001$; Table 1), their body mass peaked during pregnancy which extended from April to June. During the reproductive period, pregnant females were heavier than lactating females (Table 1), in turn heavier than non reproductive females (19.21 ± 2.32 g for non-reproductive females in May-July; $F_{2/131} = 127.20$; $p < 0.001$). Females exhibited a secondary peak in October (22.68 ± 3.61 g) when male body mass also peaked (21.45 ± 3.34 g) before declining to reach the lowest values in January-February (16.75 ± 0.35 g for females, 14.69 ± 1.13 g for males). This winter loss was 26.1 % for females and 30.9 % for males. At last, when comparing data collected in June, effects of both sex and year were significant ($F_{1/97} = 41.75$; $p < 0.001$; $F_{1/97} = 31.95$; $p < 0.001$ respectively), and not the interaction ($F_{1/97} = 3.23$; $p = 0.075$). The significant difference for females can be attributed to the large number of pregnant females in 2011 due to a later parturition, but the difference was interestingly significant for males too (16.91 ± 1.55 g in 2010 *vs.* 18.75 ± 1.61 g in 2011).

Discussion

Despite a noticeable winter activity of both *P. kuhlii* and *E. isabellinus* in the Bou Hedma National Park, central Tunisia (Dalhoumi et al. 2015), the low number of captures in that season weaken some of our results. From spring to early autumn they are more reliable, but unfortunately we only performed a one-year study when it was clear by comparing two months of June that there can be inter-annual variations, as it was reported by Ransome (1990) after the long-term study of a colony of greater horseshoe bat, *Rhinolophus ferrumequinum*.

In both studied species, females were heavier than males, a result that was previously reported for *P. kuhlii* in Libya (Hanák & Elgadi 1984, Benda unpublished data), Algeria (Kowalski & Rzebik-Kowalska 1991), Saudi Arabia (Alagaili 2008) and Italy (Lanza 2012), and for *E. isabellinus* in Algeria (Kowalski & Rzebik-Kowalska 1991) and Libya (Benda unpublished data) (Appendix 1). Such SSD biased towards females is typical of Vespertilionidae (Myers 1978, Williams & Findley 1979).

Seasonal variations in body mass were roughly similar for both *P. kuhlii* and *E. isabellinus*. From the lowest values recorded in late winter, bats regained in spring to reach a maximum during pregnancy in late spring for females, in early autumn for males when females were also heavy. The increase can be related to foraging activity, females foraging significantly longer than males in relation to reproductive condition and energy demand in Daubenton's bats (Encarnação & Dietz 2006). This pattern was softened in *P. kuhlii*, particularly in males. The summer decrease in both sexes and particularly in males was previously observed in Saudi Arabian *P. kuhlii* by Alagaili (2008) who suggested the impact of moult.

Spring peak of female body mass is linked to pregnancy which occurred from April to June for both species, with the exception of one *P. kuhlii* in March and one *E. isabellinus* in August, a timing previously reported from Tunisia, Israel, Iraq, southern Turkey and Saudi Arabia for *P. kuhlii* (Baker et al. 1974, Barak & Yom-Tov 1991, Harrison & Bates 1991, Aşan Baydemür & Albayrak 2006, Alagaili 2008), from Algeria for *E. isabellinus* (Kowalski & Rzebik-Kowalska 1991). Such peak of female body mass during pregnancy was observed in other bat species such as the Schreibers's long-fingered bat, *Miniopterus schreibersii*, and the greater mouse-eared myotis, *Myotis myotis* (Serra-Cobo 1989, Pandurska-Whitcher & Shanov 2003).

Contrary to *P. kuhlii*, *E. isabellinus* body mass exhibited a secondary peak in early autumn, a pattern that was observed by Pandurska-Whitcher & Shanov (2003) for females of some cave-dwelling bat species (*Rhinolophus ferrumequinum*, lesser horsehoe bat, *R. hipposideros* and long-fingered myotis, *Myotis capaccinii*) in Bulgaria. Nevertheless female serotines should allocate energy to increase mating opportunities (two females with vaginal secretions were captured in October, Dalhoumi et al. 2015). Krzanowski (1961, 1977) related the maximum autumn weight of temperate insectivorous bats to the maximal insect abundance when sperm production reaches a peak and females are in oestrus (Racey & Tam 1974, Encarnação et al. 2004). Following Lisón et al. (2015) an additional study of diet of the species should be undertaken as this body mass increase could be related with prey captures.

Autumn increase of male body mass can be related to mating pattern (Racey 1976, Thomas et al. 1979, Speakman & Racey 1986, Speakman & Thomas 2003) as much as accumulating reserves for wintering (Ewing et al. 1970, Thomas et al. 1990, Whitaker et al. 1997, Kokurewicz 2004). Sharifi et al. (2004) related the increase of testis mass to the development of spermatogenic cells of Western Iranian *P. kuhlii* in late summer and early autumn. On the other hand the gain in body mass before the hibernating period was reported for males of several bat species (Lundberg et al. 1983, Lehnert 1993, Kunz et al. 1998, Pandurska-Whitcher & Shanov 2003, Rughetti & Toffoli 2014). In the little brown myotis, *Myotis lucifugus*, Kunz et al. (1998) signalled a significant relationships between lean dry mass, fat mass and body mass during the pre-hibernation period. This is clearly the time for fat deposit prior to the onset of food shortage (Kunz et al. 1998, Speakman & Rowland 1999, Encarnação et al. 2004). In the Bou Hedma area, both activity and body mass of this species peaked in September, which coincides with the mating season in the southern range (Qumsiyeh 1996, Alagaili 2008). The subsequent decrease of body mass, already reported by Barak & Yom-Tov (1991), could be attributable to the increase in sexual activity, which affects foraging. This loss during mating was reported in several species such as the brown-long-eared bat, *Plecotus auritus* (Entwistle et al. 1998), *M. lucifugus* (Kunz et al. 1998) or *M. daubentonii* (Encarnação et al. 2004). In male *Myotis myotis*, paired males are lighter than single males, suggesting that mating induces an additional effort (Lisón et al. 2014).

In winter, both species and sexes lose weight to reach the lowest values. During that season, in response to weather conditions and shortage of the prey availability bats of temperate areas are known to use their fat reserves for sustaining the energy requirement of basal metabolism (Speakman & Rowland 1999). Winter loss reaches 22 % of body mass in *Plecotus auritus*, 29 % in the grey long-eared bat, *P. austriacus* (Stebbings 1970) and even 40 % in *Myotis daubentonii* (Harrje 1994, Encarnação et al. 2004). In the cave myotis, *Myotis velifer*, this loss is more pronounced in females (22 %) than in males (20 %) (Caire & Loucks 2010). We recorded a reversed result for *E. isabellinus* in the Bou Hedma national park where the loss of body mass reached more than 30 % in males and 26 % in females. Whether the size of the bat or the quite mild temperature explain this pattern should be investigated. Unfortunately our data did not allow estimating the winter loss of body mass in *P. kuhlii* for a comparison.

At last, as Ransome (1990) we report a significant difference between years that cannot be related only to a shifting of reproductive period as it was also evidenced for *E. isabellinus* males. This result could likely be explained by a differential food availability related to different weather conditions; unfortunately we have no data to test this hypothesis.

Conclusion

Body mass is under several influences, from daily variations due to a strong foraging pattern to inter-annual fluctuations. In order to limit some sources of variation, we sampled bats during a short period of time after sunset over only one year. We were not able to control for age of animals, which is impossible to record without marking them and conducting long-term studies. Our results show a significant seasonal pattern within SSD. The winter activity of both *P. kuhlii* and *E. isabellinus* did not counterbalance a loss of body mass that was similar to hibernating bat species in more northern temperate regions. It is unfortunate that the low number of captures during our study prevented a comparison in the area with true hibernators such as horseshoe bats or Saharan species such as the lesser rat-tailed bat, *Rhinopoma cystops*, or the Hemprich's desert bat, *Otonycteris hemprichii*. In this connection, we did not succeed to find data for SSD in most of European bat species. Indeed, body mass should deserve more studies at a larger scale at least because it is the main tool for evaluating body condition of bats.

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Appendix 1. Body mass of both sexes of the two Mediterranean species in several northwest African countries, m – male; f – female; n – number of specimens. [a]Hanák & Elgadi 1984, [b]Kowalski & Rzebik-Kowalska 1991, [c]Benda pers. com.

Pipistrellus kuhlii	sex	n	mean ± SD		
Tunisia[a]	m	6	5.2	(4.9-6.0)	
Algeria[b]	m	7	5.5	(4.8-6.1)	
	f	11	6.3	(4.2-8.0)	
Libya[a]	m	6	6.2	(6.0-6.8)	
	f	22	8.3	(6.5-9.0)	
Libya[c]	m	24	5.46 ± 0.51		$p < 0.01$
(7-28.05)	f	25	6.30 ± 0.91		
Egypt[c]	m	6	5.72 ± 0.72		$p = 0.87$
	f	3	5.80 ± 0.56		
Morocco[c]	m	10	5.25 ± 0.29		$p = 0.28$
(25.08-9.10)	f	8	5.50 ± 0.64		
Eptesicus isabellinus					
Algeria[b]	m	12	18.7	(15.3-22.5)	
	f	8	23.4	(15.5-27.0)	
Libya[c]	m	10	18.03 ± 2.14		$p < 0.05$
(10-28.05)	f	4	20.87 ± 2.23		
Morocco[c]	m	6	18.78 ± 1.61		$p = 0.18$
(26.08-5.10)	f	12	20.31 ± 2.37		

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