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Dried eye lens weight as an indicator of age in *Rousettus aegyptiacus*: Comparison with some other tools of age determination in bats (Chiroptera: Pteropodidae)

Sohail SOLIMAN* & Ayat TAHA

Department of Zoology, Faculty of Science, Ain Shams University, Abbasiya, Cairo, 11566, Egypt
* corresponding author: sohailsoliman@hotmail.com

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Abstract. The present study deals with the use of the dried eye lens weight as an indicator of age in the Egyptian fruit bat. The accuracy of this tool is checked by comparison with some traditional tools used for age determination in bats; i.e., forearm length, and the third and fifth digit lengths. Fruit bats in the present study were collected over a period of thirteen months extending from June 2017 to June 2018, inclusive, from the Giza Governorate, Egypt. The whole collection of bats thus includes all possible age categories; i.e., young, juveniles, adults, and old adults. The dried eye lenses proved to increase in weight for a longer time of the bat's life span compared with the other measurements of the body. The use of this weight is thus more suitable for classifying collected bat samples into more relative age classes.

Key words. Age determination, Egyptian fruit bat, eye lens.

INTRODUCTION

Age estimation is important for determining the growth rate, onset of sexual maturity, reproductive cycle, longevity, and behavioral strategies in bats and other mammals (Elangovan et al. 2002). Several methods have been used for age determination, either absolute (chronological) or relative (biological), in wild animals (Morris 1972). Body mass, length of the forearm, and total epiphyseal gap are important body measurements that were used for estimating the age of young individuals of various bat species. This was carried out with the help of capture-mark-recapture data of known-aged individuals (Baptista et al. 2000, Cheng & Lee 2002, Isaac & Marimuthu 1996, Kunz & Anthony 1982, Kunz & Robson 1995, Rajan & Marimuthu 1999). It was indicated, however, that the forearm length was the most accurate and reliable variable for estimating age in bats during the early linear growth period, while the epiphyseal gap length was best for estimating age in later stages of postnatal growth (Burnett & Kunz 1982, de Paz 1986, Hoying & Kunz 1998, Isaac & Marimuthu 1996, Kunz & Anthony 1982, Kunz & Robson 1995, Stern & Kunz 1998). The lengths of the third and fifth digits were also used for determining relative age in microchiropteran bats (Soliman et al. 2015).

A common tool that was frequently used for the determination of age in mammals was the weight of the dried eye lenses (Morris 1972). The latter author suggested that this weight was a good tool for estimating the chronological age of individuals under consideration if calibrated

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with the dried eye lens weights of individuals of known age. As far as we know, the dried eye lens weight was used as an indicator of age in bats only by Perry (1965) and Perry & Herreid (1969) who applied this tool in age studies of the guano bats, *Tadarida brasiliensis mexicana*, in western Oklahoma, USA.

In the present paper, the use of the dried eye lens weight is applied in age studies of the Egyptian fruit bats from Egypt. The accuracy of this technique, as an indicator of age, is examined by comparing it with other traditional indicators of age in bats; i.e., forearm length, and the third and fifth digit lengths.

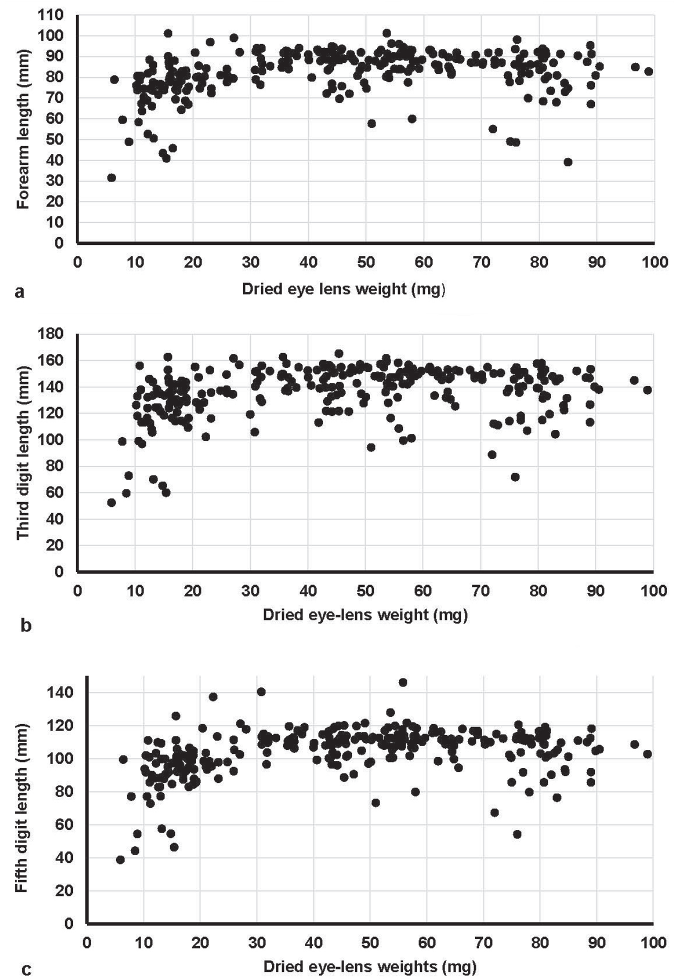

Fig. 1. A scatter diagram of different body measurements at different dried eye lens weights of both sexes of the Egyptian fruit bat; a – forearm length, b – third digit length, c – fifth digit length.

MATERIAL AND METHODS

A total of 271 individuals of the Egyptian fruit bat (114 males and 157 females) were collected from a cave in the Aquarium Grotto Garden in Zamalek, Giza Governorate, Cairo. The bats were collected monthly during the period extending from June 2017 to June 2018, inclusive. The monthly collected bats were transported alive to the laboratory at the Department of Zoology, Faculty of Science, Ain Shams University, Cairo, where they were euthanized with chloroform, given code numbers, weighed and sexed. The animal handling was in accordance with the ethical standards of the Helsinki Declaration of 1975 as revised in 1983. The experimental protocol was approved by the Ain Shams University Research Ethics Committee.

The following measurements (in millimeters) were taken for each specimen by a digital caliper as indicated by Dietz (2005): forearm length: the arm was measured from the elbow to the wrist; lengths of the third and the fifth digits: the digits were measured from the wrist to the tip of finger.

Skulls of collected bats were skinned, given the same code numbers of respective individuals, and preserved in 10% formaldehyde for at least one week to harden the eye lenses. The skulls were then removed from the fixative and eyeballs were extracted from each skull using a forceps. The lenses were extracted from the eyeballs by forceps and rolled a few times on a filter paper to remove excess surface moisture (Lord 1959). The lenses were dried at 100 °C to a constant weight in an oven. After drying, the lenses were immediately placed inside a desiccator to prevent rehydration and weighed to the nearest 0.1 mg.

Numerical data are reported as means±SEM. GraphPad Prism (version 5.0, GraphPad Software, San Diego, CA) was used for all statistical analyses. Data were analyzed using regression analysis by SPSS/PASW statistics software version 18.0.

RESULTS

Three different body measurements; namely, forearm length, third and fifth digit lengths, as well as individual dried eye lens weight were all used as indicators of the relative age of collected individuals. A comparison was made between each of the first three body measurements and the dried eye lens weight, as an indicator of age, to determine the accuracy limits of each of these measurements in determining the relative age of individuals of this bat species.

The forearm, the third and fifth digits noticeably increased in length up to the given values of dried eye lens weight after which they did not show parallel increase with further increase in the dried eye lens weight (Fig. 1). For quantitative comparison between each of these body measurements and dried eye lens weight, males and females were divided into 10 relative age groups based on their dried eye lens weight. The mean lengths of these three body measurements were calculated for each relative age group.

The forearm steadily increased in mean length with the increase in the dried eye lens weight up to the fourth group (30–40 mg) in males, and group number 6 (50–60 mg) in females. In both sexes, the mean forearm length did not show regular increase in higher relative age groups (Table 1, Fig. 1a). The relationship between these two parameters is represented by the following equations: in males, $Y=24.446+34.59\log X$ (1), $R^2=0.689$; in females, $Y=41.44+24.84\log X$ (2), $R^2=0.7173$; where (X) represents the dried eye lens weight in milligrams and (Y) represents forearm length in millimeters.

The mean third digit length generally increased with the increase in the dried eye lens weight up to the third group (20–30 mg) in males and group number 5 (40–50 mg) in females. In both sexes, the third digit did not show regular increase in the mean length in higher age groups (Table 1, Fig. 1b). The relationship between these two parameters is represented by the following equations: in males, $Y=73.11+40.10\log X$ (3), $R^2=0.72$; in females, $Y=64.12+42.61\log X$ (4), $R^2=0.785$; where (X) represents the dried eye lens weight in milligrams and (Y) represents the third digit length in millimeters.

Table 1. Mean lengths of the forearm, third and fifth digits in different relative age groups (based on dried eye lens weight) of both sexes of the Egyptian fruit bat. Mean is followed by ±S.E., range in (parentheses), and number of specimens; W – dried eye lens weight category

W [mg]	forearm length [mm]		third digital length [mm]		fifth digital length [mm]	
	males	females	males	females	males	females
<10	49.3±7.4 (31.5–67.9) 4	50.6±5.7 (39.1–57.6) 3	85.7±14.3 (52.5–114.2) 4	80.9±10.7 (59.6–94.2) 3	63.7±10.6 (38.8–85.7) 4	61.7± 8.8 (44.3–73.4) 3
10–20	76.4±1.9 (45.7–90.0) 5	73.1±2.0 (40.9–101.0) 12	128.3±3.3 (70.2–153.0) 5	121.2±3.5 (60.1–162.7) 12	95.4±2.1 (57.9–111.3) 5	90.5±2.5 (46.3–125.9) 12
20–30	85.7±2.3 (74.5–96.9) 25	81.2±2.1 (72.3–98.9) 31	146.3±3.5 (128.4–156.8) 25	130.7±4.4 (102.4–161.7) 31	108.2±2.8 (95.4–118.5) 25	102.4±4.4 (87.9–137.4) 31
30–40	88.5±1.2 (83.0–92.7) 12	86.9±1.7 (76.2–94.1) 9	139.4±6.2 (105.8–162.7) 12	139.6±4.4 (88.3–154.9) 9	111.5±4.8 (77.3–140.5) 12	107.8±2.5 (84.1–119) 9
40–50	86.0±1.8 (72.2–94.5) 10	88.9±2.5 (69.6–144.1) 13	144.2±3.6 (121.9–165.4) 10	143.4±2.3 (113.1–157.4) 13	111.1±2.3 (96.6–121.5) 10	110.0±2.6 (88.7–154.2) 13
50–60	89.1±1.2 (79.9–96.2) 12	90.1±3.1 (59.9–152.1) 26	145.3±3.0 (116.5–158.3) 12	141.9±3.4 (99.9–161.9) 26	112.7±1.3 (100.5–119.8) 12	114.1±3.1 (79.9–152.1) 26
60–70	88.2±1.4 (81.3–93.1) 14	86.6±1.7 (78.8–78.8) 24	148.2±2.3 (136.3–155.1) 14	144.1±2.9 (125.5–153.5) 24	114±1.7 (107.9–118.4) 14	107.2±2.1 (94.5–115.6) 24
70–80	84.9±2.8 (59.5–98.1) 8	84.6±1.3 (77.6–92.1) 11	139.8±5.5 (98.8–157.8) 8	137.9±3.6 (111.1–150.5) 11	106.5±3.9 (77.1–120.6) 8	115.1±4.9 (100.7–154.6) 11
80–90	88.9±1.3 (79.1–95.3) 11	80.1±2.3 (68.4–93.5) 13	147.5±2.2 (131.5–158.0) 11	133.6±3.5 (115.0–153.6) 13	111.5±1.7 (100.8–119.2) 11	99.9±2.6 (85.7–114.1) 13
90–100	84.3±0.7 (82.7–85.2) 13	85.1±0.2 (84.9–85.2) 15	140.3±2.3 (137.7–145.0) 13	141.6±3.4 (138.2–145.0) 15	105.0±1.7 (102.7–108.6) 13	107.2±1.5 (105.7–108.6) 15

The mean fifth digit length steadily increased with the increase in the dried eye lens weight up to the fourth group (30–40 mg) in males and group number 6 (50–60 mg) in females. In both sexes, the mean fifth digit length did not show regular increase in higher relative age groups (Table 1, Fig. 1c).

The relationship between these two parameters is represented by the following equations: in males, Y=53.05+32.20 log X (5), R^2=0.717; in females, Y=47.51+34.21 LogX (6), R^2=0.749; where (X) represents the dried eye lens weight in milligrams and (Y) represents the fifth digit length in millimeters.

DISCUSSION

The present study, as far as we know, is the first one to use the dried eye lens weight in age studies of the Egyptian species of bats. Bats are long-lived mammals compared to other non-flying mammals with similar body sizes (Austad & Fischer 1991, Wilkinson & South 2002), since they survive up to 8–10 years in the wild (Kwiecinski & Griffiths 1999). According to the same authors, maximum life spans of 22 and 25 years were recorded for both sexes of wild and captive Egyptian fruit bats, respectively.

The estimation of accurate ages of individual bats collected from the wild is not, however, an easy task. It depends on permanent marking of bats at birth and recapturing them at known time intervals, which is a time-consuming process (Kunz & Anthony 1982, Baptista et al. 2000, Brunet-Rossinni & Wilkinson 2009, Isaac & Marimuthu 1996, Kunz & Robson 1995, Rajan & Marimuthu 1999). The determination of the relative or biological age of individual bats is thus an alternative approach that is widely used in ecological studies.

Brunet-Rossinni & Wilkinson (2009) classified the existing techniques for estimating age into two groups; one for estimating age of juveniles and the other for estimating age in adults. Linear growth of long bones, epiphyseal-diaphyseal fusion, and body mass and pelage coloration are used for estimating age in juveniles. Tooth wear, incremental dentine and cementum lines, size of pulp cavity, and population-level method are used for the determination of age in adults. The latter authors added that none of such methods gave knowledge of the exact chronological age of individual bats. Several other methods have also been used for the determination of relative age in bats such as forearm and wing digit length (Soliman et al. 2015), the length of the total epiphyseal gap (Baptista et al. 2000, Brunet-Rossinni & Wilkinson 2009), and the dried eye lens weight (Perry & Herreid 1969).

Body mass proved to be an inaccurate tool for estimating age of growing bats (Kunz 1987) because it is affected by several factors such as food availability, energy costs, and water flux (Hoying & Kunz 1998, Kunz 1987, Stern & Kunz 1998). Kunz & Stern (1995), on the other hand, considered body mass as an ideal tool for interspecific comparison of postnatal growth rates of species from different regions.

In the present study, length of the forearm, third digit, and fifth digit were separately compared with the corresponding dried eye lens weight in the Egyptian fruit bat. Such comparisons have indicated that these length values stopped growing at a relatively low age of individual bats compared to the growth in eye lens weight. This could be attributed to the fact that bats which reach adult size have little changes in markers of age (Brunet-Rossinni & Wilkinson 2009). Dried eye lens weight is thus recommended to be used for classifying individuals of both sexes of this bat species into relative age groups. The use of this tool allows for the classification of collected bats into a greater number of age classes covering a wide range of ages; a fact that was earlier confirmed by Perry & Herreid (1969).

The dried eye lens weight was widely used in age studies of wild mammals such as rabbits (Dudzinski & Mykytowycz 1961, Lord 1959, Rongstad 1966), dogs (Hockwin et al. 1979), Columbian black-tailed deer (Connolly et al. 1969), woodchucks (Davis 1964, Nuckle & Bergeron 1983), and guano bats (Perry & Herreid 1969).

The preferability of the use of eye lens in classifying mammal populations into accurate relative age groups is based on several facts. First, by continuous proliferation of new lens fibers, lens weight increases continuously with increasing age (Lord 1959, Tanikawa 1993). Second, the eye lens accumulates an insoluble albuminoid throughout an individual life time (Dische et al. 1956), and third, the eye lens lacks blood vessels and is, therefore, unaffected by many physiological variations which take place in the body (Dapson & Irland 1972). The use of eye lenses is usually confined to classification of individuals into age classes rather than to determination of their chronological age since they lack fine resolution in old-aged individuals (Dapson & Irland 1972). Eye lens weight could, however, be used as a good indicator of chronological age if calibrated with eye lenses extracted from animals of known age (Morris 1972).

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