One hundred and forty days in the life of a flying-fox tooth-fairy: estimating the age of pups using tooth eruption and replacement

Anja Divljan, Kerryn Parry-Jones and Glenda M. Wardle

Institute of Wildlife Research, School of Biological Sciences, Heydon-Laurence Building A08, University of Sydney, Sydney, New South Wales 2006, Australia

ABSTRACT

Age can be an important predictor of an individual's survival or reproductive fate and therefore methods for determining ages of wild animals are of general interest. Bats are assigned to age classes based on morphological measurements (e.g. forearm measurement and tooth wear); or to a chronological year based on annual cementum rings in teeth. However, for infants, only morphometric techniques are available, and individual variation can lead to less reliable predictions of age from these measurements. Here we describe the sequences and timing of tooth emergence and replacement in the Grey-headed Flying-fox (*Pteropus poliocephalus*) and evaluate the usefulness of the method for ageing pups.

Tooth eruption and replacement were assessed visually and at least four stages of growth were described for each permanent tooth for 20 known-age, mother-reared pups that were monitored weekly from October to February. Forearm measurements and mass were also recorded. To test the reliability of the method we aged 30 additional pups. The ages derived from tooth eruption were compared to ages derived from the traditional method using forearm and weight measurements. Our results indicate that the tooth eruption technique is more reliable in estimating age in flying-foxes up to 140 days old. Further research should compare rates and patterns of tooth eruption in hand-raised to mother-raised pups and use a larger sample size to look for any sex differences. Accurate ages for pups will contribute to determining the age-specific mortality rates for this vulnerable species.

Key words: flying-fox, tooth eruption, ageing, *Pteropus*

Introduction

Increasing pressure on colony sites, and feeding grounds, as forested habitat has been cleared for development and agriculture, is believed to be the primary cause of the decline in Grey-headed Flying-fox (*Pteropus poliocephalus*) numbers (Tidemann 1999; Parry-Jones 2000). This, coupled with mortalities from human-induced causes and mass mortalities from extreme heat, results in the loss of juveniles, and more importantly breeding adults. Death of mature females is particularly significant since they are lost from the breeding pool, but is also associated with orphaning of their dependent young. The altricial nature of the species implies that orphaned pups are destined to die of starvation and/or exposure to predators and other environmental factors. Improved conservation outcomes and animal welfare benefits are possible if orphaned pups are rescued and hand-reared, using established protocols (e.g. George 1990; Parry-Jones and Parry-Jones 2003). However, a general lack of information on how to accurately age flying-fox pups hinders the monitoring of the pup's progress needed to ensure the wellbeing of the animal in care. For instance, the stunted growth of an animal may not be recognised due to the mistaken belief that the pup is younger (and therefore smaller); this missed opportunity to address the nutritional needs could result in a potential non-flier.

In addition, establishing the accurate age of the pups can be useful in research, particularly demographic studies that seek to understand how the population dynamics may be responding to altered environments. Life history parameters such as age at weaning, age at flying and age at reproductive maturity can be established through accurate ageing of the pups. Similarly, knowing the ages of juveniles born in any specific year, and/or at any colony site, could aid studies on the duration, timing and differences between the birthing seasons. Assessments of juvenile age groups at risk of dying from particular mortality sources (e.g. extreme temperatures) would also benefit from accurate ageing technique. Behavioural studies focusing on social interactions and specialised feeding behaviour are also dependant on an accurate estimation of age (Kunz and Anthony 1982).

Physical size is often used as an indicator of age, particularly in developing individuals, as the general increase in size of the young, measured by the overall body length or some part of it, usually corresponds to its age. In bats the length of the forearm (radius) is routinely measured and taken as an indication of a pup's age (Davis 1969; Kunz and Anthony 1982). The advantage of the method is that it can be measured with readily-available callipers, is repeatable (both within and between observers), is non-invasive and easily performed with only simple restraining of the animal (Anthony 1988). However, Morris (1972) acknowledged that bone measurements can be unreliable due to individual variation and as such should be used as relative age determinants. Furthermore, sexual dimorphism in size among pups may (A. Divljan, personal observation) or may not (Burnett and Kunz 1982) be apparent. Therefore,

Pp. 97–105 in *The Biology and Conservation of Australasian Bats*, edited by Bradley Law, Peggy Eby, Daniel Lunney and Lindy Lumsden. Royal Zoological Society of NSW, Mosman, NSW, Australia. 2011.

most measurements of size are not sufficient to age a young pup and alternative methods are required. Body mass of pups can be a useful indicator of an individual's general health, or nutritional status, but it is a poor age-estimator, unless used in conjunction with other ageing criteria (Anthony 1988).

The chronology of tooth emergence and replacement can also be used to age juveniles (Morris 1972; Lawrence et al. 1982; Phillips-Conroy and Jolly 1988). Patterns of tooth eruption and replacement in juveniles have been related to age and/or body size in several microbat species (Anthony 1988; and references therein), however, current knowledge on flying-fox dentition is limited. Hall and Richards (2000) reported that pups are born with deciduous (milk) needle-like teeth, but the number and position of the teeth are not documented. Thus, like all mammals, flying-foxes have diphyodont dentition, with a set of deciduous teeth being replaced with permanent dentition by the time pups are about three months old and able to fly (Nelson 1965). The teeth are replaced sequentially, at set intervals with increasing age, and a simple visual inspection of presence/absence, and the appearance of each individual tooth (both deciduous and permanent) will in theory determine the age of the pup. The technique is usually fast, non-invasive, repeatable and potentially applicable at any stage of a pup's life. Despite this, the order and time of tooth replacement have not been readily available in flying-foxes to date.

In two birthing seasons (2005 and 2006) we took the opportunity to examine captive, known-age, juveniles over a period of time and to examine tooth replacement and growth. The aims of this paper are 1) to provide a detailed record of flying-fox dentition and 2) to develop a novel method of ageing juvenile flying-foxes up to 140 days of their life. To evaluate the effectiveness of this tooth-eruption technique, we compare our findings to current ageing methods based on the forearm lengths of pups.

Methods

Collection of animals

Twenty known-age juveniles were used in this study. Eleven individuals were born in the 2005/2006 season, and a further 9 were 2006/2007 season pups. All individuals were mother-reared and lived under similar conditions at the Wambina Flying-fox Education and Research Centre, Matcham, NSW (33°24'S, 151°25'E). Maternal bats were fed daily on a seasonal fruit diet and protein supplement and water was always available in the enclosures. The pups were monitored weekly between October and February each season, and their continuous growth records were obtained. However, due to the different birth dates of the pups, the ages of individuals on the recording days were variable. Dental profiles for individual pups were recorded including: tooth presence/absence, size, and general appearance of each tooth on the right side of both the upper and lower jaws. Additionally, we documented the position of each tooth with respect to the gum line, thereby creating the following categories: tooth is (A) not visible, (B) deep under the gum line (in some instances seen on the lateral side of the gums), and (C) just under the gum surface. A total of 179 observations were made for the 20 juveniles (8 females and 12 males; 6–10 observations per animal). A further 7 individuals whose dates of birth were known to within 3 days were also available. Thirty additional pups, of which 16 were of known age, contributed to the data collection, however, these were not assessed on a weekly basis.

Forearm length and body mass of each pup were recorded to assess them against age, and also monitor the wellbeing and growth progress of the pups. Forearms were measured with Vernier callipers, accurate to the nearest 0.05 mm, while the body mass was recorded on a spring balance (0–500 g).

Data analyses

In longitudinal studies where each individual is tracked from birth, the date of tooth emergence can be determined with precision due to the consistency of days available to record measurements. In these studies variability associated with age of emergence is primarily a result of inter-animal variability. However, the data used in this study were cross-sectional, which meant that the tooth eruption events were necessarily recorded on particular dates, resulting in irregular days between observations for pups born on different days. Thus the average (mean or median) age of each tooth eruption could not be directly determined from the cross-sectional sample because individuals either did, or did not have the tooth, with the actual age of transition unknown in most cases. Still, the nature of the data was such that the observations from at least one individual were available for most of the 140 days, with the largest gap between two successive observations being 3 days.

We described the median age of tooth eruption as the mid point between the age of the oldest animal in the unbroken series not to have the tooth, and the youngest animal in the unbroken series in which the tooth was above the gum line (Phillips-Conroy and Jolly 1988). The dispersion was measured as the range between the youngest animal to have the tooth and the oldest that was still missing the tooth, and it was dependant on the sample size. We, therefore, reported the sample size both in terms of the number of observations, as well as the actual animals within this range. Variation in age of each tooth eruption is shown as the range of overlap expressed as a percentage of the mid point age. The ages of the first and last tooth eruptions in the entire sample were also recorded. Similarly, we tabulated the times of loss of deciduous teeth using the same principle in reverse (i.e. the median age of tooth loss was taken as the mid point between the age of the oldest animal in an unbroken series to have a tooth and the youngest animals in the series which had lost the tooth). The categories of each tooth position and size were then added to the eruption and tooth loss times to create a key for ageing flying-fox pups. The eruption times of deciduous teeth were additionally recorded from 6 pups that died at birth or shortly thereafter. The forearm measurements of 20 animals were plotted against their age to establish the growth curves for the mother-reared juveniles living under captive conditions. In the preliminary data assessment, body mass was found to be less accurate than forearm length for age determination and was not subsequently used.

Do-it-yourself: assessing the levels of tooth eruption and replacement in flying-fox pups

One of the aims of this study is to encourage the uptake of this ageing technique by flying-fox carer groups. We have, therefore, included this section describing how to examine the pup's teeth. Successful tooth assessment in flying-fox pups is a relatively easy process. The animal is temporarily immobilised by being wrapped in a cloth, exposing only its head. One hand is used to gently hold the wrapped animal in a horizontal position, ensuring that the pup can firmly hold onto the cloth with its legs for security. With the other hand one can open the jaw by pressing both cheeks at the posterior end of the mouth (closer to the point of articulation of the mandible with the temporal bones) with the thumb and the index finger. When opening the mouth, the pup is likely to pull its head backwards, so to restrict its movement it is best to keep the animal against a firm surface (placing it on your lap is optimal). To keep its mouth open and avoid potential bite injury from the pup's reflexive reaction to close the jaw, the cheek should be pushed in slightly, so that the pup can feel it between its molar teeth. Both upper and lower jaw teeth are then examined in terms of their presence/absence, size, appearance, and general position with respect to the gum-line. There is no apparent difference between the left and the right side of the jaw, thus recording only one side is sufficient. The procedure is fast, taking only a few minutes, and should be conducted under well lit conditions.

Results

Number of teeth and their emergence sequence

Grey-headed Flying-foxes have 20 deciduous and 34 permanent teeth (Table 1). Pups are born with some milk teeth (incisors and canines), and the full complement of deciduous dentition was obtained by 9 days in cases where present. Permanent premolar and molar teeth were first visible deep under the surface on the lateral side of the jaw (Figure 1). They moved in the three-dimensional space (medially and upwards) before their tips were seen just under the surface of the gum line. The duration of that movement varied between teeth, seen by the differences in time between classes A, B and C (Table 3). The permanent dentition began to emerge with canines, approximately midway through the first month (Table 2). The eruption sequence was C P^3 P^4 I^1 M^1 I^2 M^2 for the maxilla, and C P_3 [P_4 P_1] [I_2 I_1] M_1 M_2 M_3 for the mandible (Table 2). We observed no cases in which this sequence was out of order. Emergence of the same tooth occurred at the same time in the right and left sides of the skull, however equivalent teeth erupted earlier in the mandible. First incisors (I^1 and I_1) were the exception, and appeared to erupt roughly at the same time. Permanent tooth eruption was complete by 140 days and there was no apparent difference in the tooth emergence and replacement between males and females, although this was not statistically examined due to small numbers of individuals.

A tabular key for ageing flying-fox pups based on the sequence of eruption of the teeth is presented in Table 3. The measurements of teeth were approximated to the nearest 0.5 of a millimetre due to the limited time a pup could hold its mouth open. Therefore, it is the pattern of increase in size of each tooth that should be considered rather than the absolute size, which should serve as a guideline. All additional animals (*n*=33) fell within the correct age groups, based on the predictions using tooth eruption. Also it should be noted that the accuracy of the key decreases at approximately 105 days as all teeth, except the last molars in both jaws, are at their full size, leaving age to be determined from M^2 and M_3 only.

Table 1. Dental formula for both sets of flying-fox teeth. Capital letters denote permanent teeth, and lower case indicates deciduous teeth; incisors (I or i), premolars (P or p), canines (C or c) and molars (M). P^1 is a rudimentary tooth and is often missing, therefore it has not been looked at in this study. In small number of cases there are two sets of P_1 teeth.

Teeth	Dental formula	Total number of teeth
Deciduous	i-2/2, C-1/1, p-2/2	20
Permanent	I-2/2, C-1/1, P-3/3, M-2/3	34

Forearm measurements

A range of ages at different forearm lengths was observed (Figure 2). Variation generally increased with the size of the forearms (partially due to the cumulative effect of the

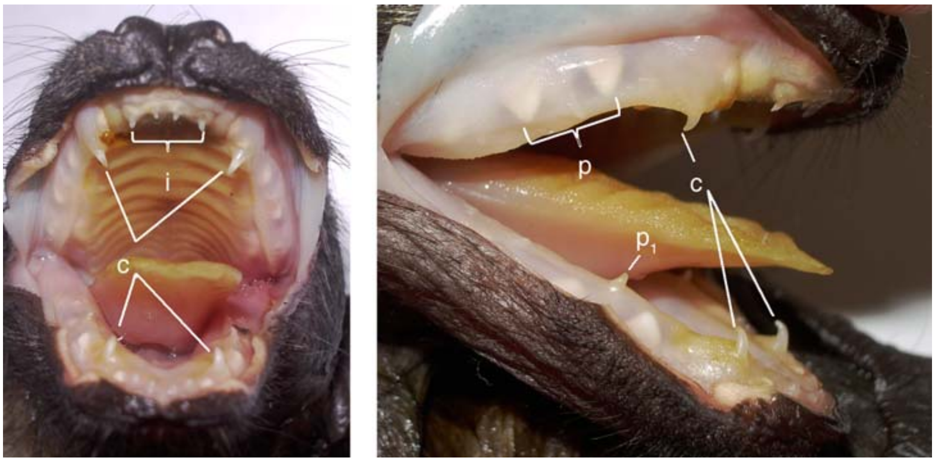

Figure 1 Frontal and lateral view of a five-day-old deceased *P. poliocephalus* pup. Deciduous, hooked teeth (i, c, p_1) are visible above the surface. Remaining deciduous teeth (p_2, p^1 and p^2) have not erupted yet. Permanent premolar dentition (P) is visible on the lateral side of the jaw, still under the gum surface.

increase in the sample size). Growth curves for forearm lengths of two individuals of the same age (a male and a female) were used to demonstrate the potential initial difference in size, which was apparent throughout the monitoring time (Figure 3). Pups were divided into nominal 5-day intervals (age classes) to assess variation in forearms for these age classes (Table 4). A large overlap in the forearm size was observed between the intervals, suggesting that forearm lengths were not appropriate for predicting pup's age to within 5 days (Figure 4, Table 4).

Discussion

This is the first study to fully document the sequence of tooth emergence and replacement of the deciduous teeth by the permanent teeth in the Grey-headed Flying-fox. Hall and Richards (2000) suggested that pups are born with deciduous teeth, but it appears that this is only partially the case (A. Divljan, personal observation; George 1990). All 20 deciduous teeth appear sharp, medially recurved and have a single fine root. This design is primarily adapted for gripping to the mother's nipples and to the fur, particularly during flight (Nelson 1965). Considerable variability in the presence and replacement of these hooked teeth was observed, however most of the deciduous teeth had disappeared by the age of 80 days. We agree with the suggestion of Olsson (2000) that this variability renders deciduous teeth unsuitable for ageing *P. conspicillatus* pups, but we found that the information is useful in combination with permanent tooth eruption. All temporary incisors are found in the position of the permanent incisors and consequently these first teeth have to fall out prior to the eruption of permanent teeth, thereby affecting the timing of the incisor eruption.

Table 2. (a) Age at loss of deciduous dentition. range: age of the youngest animal to have lost the tooth and oldest animal that still had the tooth; %variation: range expressed as a percentage of the mid point; *n* individuals: number of individuals within the range of the overlap (some individuals were observed more than once). (b) Age at tooth eruption for permanent dentition. range: age of the youngest animal to have had the tooth and the oldest animal that did not have the tooth. Other variables are same as the above. Teeth are ordered in the sequence of loss (deciduous) or eruption (permanent).

(a)	tooth	mid-point	range	% variation	*n* observations	*n* individuals	first age of tooth loss	last age of tooth loss
Maxilla	i^1	48	48 and 50	4.2	3	3	48	62
	i^2	62	58–67	14.5	17	13	58	67
	c	70	64–75	15.7	27	19	52	82
	p^1	35	34–35	2.9	7	7	27	39
	p^2	only 2 of the 20 bats have them						
Mandible	i_1	48	47–48	2.1	3	3	41	48
	i_2	41	39–41	4.9	8	8	35	61
	c	63	57–69	19.0	27	18	52	82
	p_1	51	50–51	2.0	2	2	35	63
	p_2	only 2 of the 20 bats have them						
(b)	**tooth**	**mid-point**	**range**	**%variation**	***n* observations**	***n* individuals**	**first age of eruption**	**last age of eruption**
Maxilla	C	20	19–21	10.0	5	5	19	25
	P^3	44	43 and 45	4.5	2	2	43	45
	P^4	48	47–48	2.1	3	3	48	54
	I^1	52	50–52	3.8	7	7	50	56
	M^1	60	56–62	10.0	15	13	52	62
	I^2	62	58–67	14.5	17	13	58	67
	M^2	119	117–120	2.5	6	6	117	124
Mandible	C	19	17–21	21.1	10	10	17	21
	P_3	27	24–30	22.2	10	10	24	32
	P_4	41	38–41	7.3	9	9	38	48
	P_1	41	40–41	2.4	6	6	34	43
	I_2	52	50–52	3.8	7	7	50	56
	I_1	52	50–52	3.8	7	7	50	56
	M_1	58	56–61	8.6	11	11	52	61
	M_2	69	62–75	18.8	32	20	62	80
	M_3	120	120	0.0	1	1	120	131

Table 3. A tabular key for ageing Grey-headed Flying-fox pups. Read together, the data in each column (age class) give information about the appearance of the pup's jaw at any particular age class. For instance an animal without deciduous teeth, fully grown permanent teeth, and M^2 visible deep under the surface and no M_3 is approximately 100–104 days old. Dark boxes denote time of tooth eruption; lighter boxes represent the period of change of each tooth (from the time of eruption to when it is lost – in case of deciduous teeth, or the time it reaches it's full size – permanent teeth); letters (A,B,C) stand for "no tooth visible", "tooth is deep under the gum surface", "tooth is just under the gum surface", respectively; numbers represent the approximate size (mm) of the tooth at the time; where there is a blank space the reading is the same as the most immediate reading to the left. In instances where permanent canines are 1, 2–3, and 3–4 mm long they are smaller than, same as, and slightly larger than the deciduous canines. No changes occur at 90–94, and 105–109 age classes.

Months							1						2						3						4				
Age in days		0–4	4–9	10–14	15–19	20–24	25–29	30–34	35–39	40–44	45–49	50–54	55–59	60–64	65–69	70–74	75–79	80–84	85–89	90–94	95–99	100–104	105–109	110–114	115–119	120–124	125–129	130–134	135–139
	Tooth																												
Maxilla	i^1	■	▒	▒	▒	▒	▒	▒	▒	▒	▒ A																		
	i^2	■	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒ A															
	c	■	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒ A													
	p^1	A	■	▒	▒	▒	▒	▒	▒ A																				
	p^2	A	■	▒	▒	▒	▒ A																						
	C	A	B	C		■	▒	▒	▒ 1	▒ 2–3	▒ 3–5	▒	▒	▒	▒	▒ 5–6	▒	▒	▒ 6.5–8										
	P^3	A	B				C			■	▒	▒ 1	▒ 2–3	▒	▒	▒	▒ 3–3.5	▒	▒	▒	▒ 4								
	P^4	A	B				C				■	▒	▒	▒ 1–2	▒	▒	▒ 2.5–3												
	I^1	A								B	C	■	▒ 1																
	M^1	A							B			C		■	▒	▒	▒ 1–1.5	▒	▒	▒	▒ 1.5–2.5								
	I^2	A										B	C	■	▒	▒ 1													
	M^2	A																				B		C	■	▒	▒	▒	▒ 1
Mandible	i_1	■	▒	▒	▒	▒	▒	▒	▒	▒	▒ A																		
	i_2	■	▒	▒	▒	▒	▒	▒	▒	▒ A																			
	c	■	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒ A															
	p_1	A	■	▒	▒	▒	▒	▒	▒ A																				
	p_2	A	■	▒	▒	▒ A																							
	C	A	B	C	■	▒	▒	▒	▒ 1	▒ 2–3	▒	▒ 3–4	▒	▒ 4–4.5	▒	▒	▒	▒	▒	▒	▒	▒ 5–6.5							
	P_3	A	B		B	C	■	▒	▒ 1	▒	▒ 2–2.5	▒	▒	▒	▒ 2.5–3.5	▒	▒	▒ 3.5–4.5											
	P_4	A			B			C		■	▒	▒ 1	▒	▒ 1.5–2	▒	▒	▒ 2.5–3.5												
	P_1	A			B			C		■	▒	▒	▒	▒ 1															
	I_2	A							B	C		■	▒ 1																
	I_1	A							B	C		■	▒	▒ 1															
	M_1	A								B	C		■	▒	▒ 1–1.5														
	M_2	A									B	C			■	▒	▒	▒	▒	▒	▒ 1–1.5								
	M_3	A																						B	C	■	▒	▒	▒ 1

Other deciduous teeth are generally located in the spaces between the permanent teeth (c is behind the C, p1 is between P2 and P3 and p2 is between M1 and M2) and persist for some time during the growth of permanent teeth. Thus the permanent molars are effectively without a primary precursor and have to erupt in precise positions behind the last erupted tooth (Marks and Schroeder 1996). This could explain the directional pattern of molar eruption from anterior to the posterior end of both jaws.

It appears that the adult dentition is obtained prior to individuals attaining their full adult size and mass. However, by this stage they have reached about 90% of their forearm size and 75% of their full weight. The timing when the complete permanent dentition is obtained (5 months), therefore, coincides with the proposed time of weaning (between 4–6 months) (Nelson 1965). Age at which the juveniles attain their full adult dentition (approximately 140 days) was the same as that

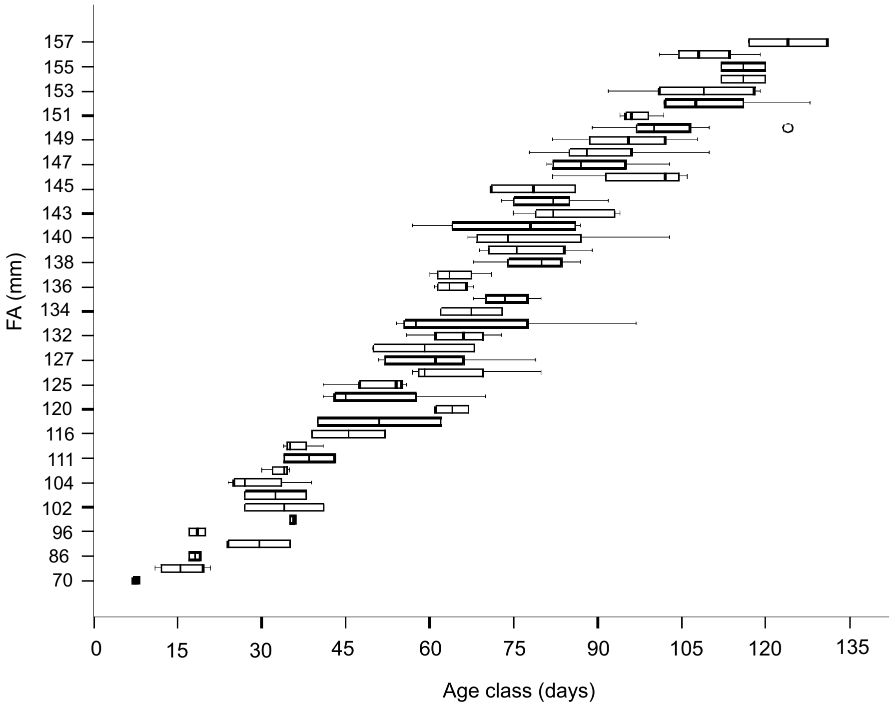

Figure 2 Multiple forearm (FA) measurements for 20 flying-foxes up to 135 days old. All juveniles were born to non-releasable mothers in captivity (housed under identical conditions), eliminating some variation associated with the growth conditions. Individual variation in the growth of pups is indicated by the length of boxplots (for example: an animal with a forearm of 140 mm was between 67 and 103 days old). Outliers are indicated by open circles (○); the horizontal lines inside the boxes represent the medians; single measurements are not presented.

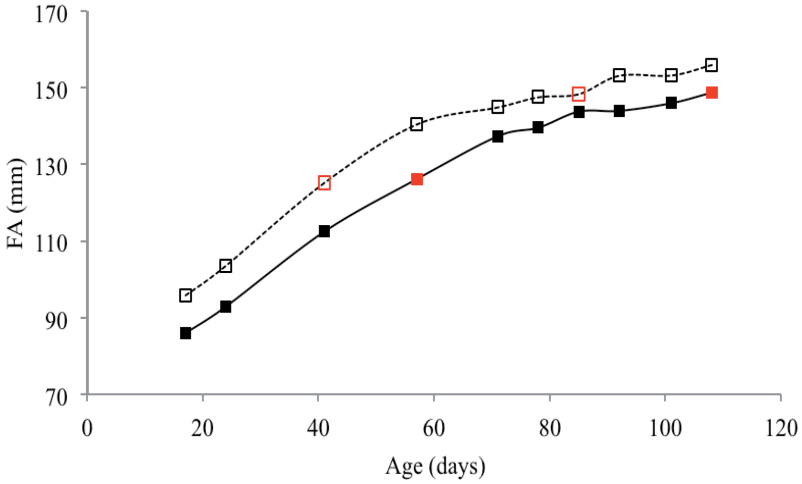

Figure 3. Comparison of forearm growth rates for two individuals, male (solid line) and female (dashed line), born in captivity on the same day to non-releasable mothers. The initial difference in forearm length at birth (not measured) was carried through to the last day (108) of recording. Red data points show the difference in age at which the animals reached two (126 and 148 mm) forearm lengths.

found in *P. conspicillatus* (Olsson 2000). Similarly, the timing and the sequences of the eruption of individual teeth were comparable between the species, suggesting that this ageing method might not be restricted to the Grey-headed Flying-fox, but applicable across the genus.

Table 4. Summary of the forearm measurements for twenty flying-foxes divided into 5-day age intervals. All animals are represented in more than one age class. The table provides supplementary information to Figure 4, the number of observations (*n*) for each age class is provided in addition to the mean and standard deviation.

Age class	*n*	Minimum	Maximum	Mean	SD
5–9	5	70.1	83.9	74.88	5.77
10–14	4	71.9	82.2	77.87	4.96
15–19	7	79.0	95.8	85.67	5.87
20–24	5	82.0	103.7	93.16	7.83
25–29	7	89.2	107.9	100.47	6.57
30–34	5	105.8	112.0	108.94	2.71
35–39	8	93.1	116.0	104.12	7.47
40–44	7	102.0	125.3	114.85	7.76
45–49	4	107.4	128.6	119.59	9.01
50–54	9	108.9	132.9	125.11	7.69
55–59	7	124.8	140.5	130.73	5.60
60–64	10	117.3	137.4	130.87	7.52
65–69	12	120.2	140.4	134.58	6.20
70–74	11	122.7	145.0	135.91	7.30
75–79	8	127.5	147.6	139.75	6.29
80–84	10	125.7	149.0	141.71	7.11
85–89	12	137.5	150.0	144.68	4.09
90–94	5	142.6	153.3	146.84	5.07
95–99	9	132.7	151.5	146.42	6.04
100–104	12	140.4	155.9	149.67	4.27
105–109	5	146.2	156.0	151.86	4.30
110–114	6	148.5	154.7	152.02	2.57
115–119	5	152.4	157.4	154.41	2.21
120–124	4	149.6	158.6	154.35	3.73
125–129	1			152.30	
130–134	1			157.40	

In our preliminary study we found the forearm length to be a better predictor of age than the mass of the pup, due to large variation in mass (Davis 1969; Kunz and Anthony 1982). Anthony (1988) suggested that the this individual variation in mass could be a result of the energy utilisation by juvenile pups. Juveniles are likely to differ in energy intake (the amount of milk provided by their mothers) as well as energy expenditure (energy used for maintenance and thermoregulation), rendering the body mass inadequate for accurate ageing. The forearms too became progressively more inaccurate due to overlapping ranges with the increasing size of the pup. In support, Anthony (1988) suggested that measuring the length of the long bones is well suited for ageing juveniles only during the short initial linear phase of growth. The age can be predicted to within 1–2 days using the species-specific linear regression equations generated from known-age bats (Kunz and Anthony 1982). However, these studies omitted to mention that both the body mass and the forearm measurements are likely to differ between pups at birth. Hayssen and Kunz (1996) demonstrated a strong relationship between the mass and size of the pups and their mothers in megachiropterans. The condition of the mother might, therefore, partially determine any variation between individuals at birth. Further differences between pups are likely to be introduced through the duration and rates of growth during this early growth phase. These are driven by genetic and nurturing components respectively. Pups raised under different condition, and/or fed different food (i.e. milk) types, for example, have different rates of growth (K. Parry-Jones, unpublished data). Thus, a linear regression equation should theoretically be specified for each juvenile group.

This study improves the accuracy of age estimation of juveniles to within 5 days. The method is effective as it uses a combination of recorded discreet/qualitative characters (namely the sequence and pattern of tooth eruption), in contrast with remeasuring of a single continuous/quantitative variable of size (i.e. the forearm length). The close relationship between chronological age and timing of the eruption of teeth therefore provides an improved method for estimating the age of Grey-headed Flying-fox pups. The eruption sequence provided in this paper and the associated age range for the eruption of each tooth provide a reasonable index for ageing juveniles of unknown birth date. The speed and repeatability of the method and lack of the necessity for any specific equipment make the technique advantageous, particularly in remote field research. Further studies are required on individuals from the wild to compare the timing of tooth replacement between the captive and wild populations. Additionally, forearm measurements and body mass of the pup should still be recorded as indicators of the approximate age, development and wellbeing of the juveniles. Tooth eruption appears to have a reduced individual variation at birth. All juveniles were born with same dentition, although the eruption and loss of deciduous teeth varied greatly thereafter. Similarly, no sexual dimorphism was observed, contrary to the size measurements where females appear to reach their smaller adult size at a faster growth rate (Vardon and Tidemann 1998). However, sexual dimorphism in the timing of tooth eruption and replacement cannot be excluded at present. Setchell and Wickings (2004), for example, showed that in mandrills females have an earlier eruption pattern as well as a different pattern to males. Due to the limited timescale at which the eruption and replacement occur in flying-foxes (months *vs*. years in primates), we suspect that any adjustments to the current key with respect to potential sexual dimorphism would be minimal. Nonetheless this ageing technique, like any other, could potentially suffer from the genetic and environmental variation. Pups raised by two different carers and releasable female bats fitted well with the current age predictions; however it is possible that more individuals are needed to recognise a potential difference. Wild primates, for instance, are likely to have a delayed tooth eruption when compared to captive individuals (Phillips-Conroy and Jolly 1988; Kahumbu and Eley 1991). Thus, if similar patterns occur in flying-foxes, ages obtained in this study could be underestimates of those occurring in the wild.

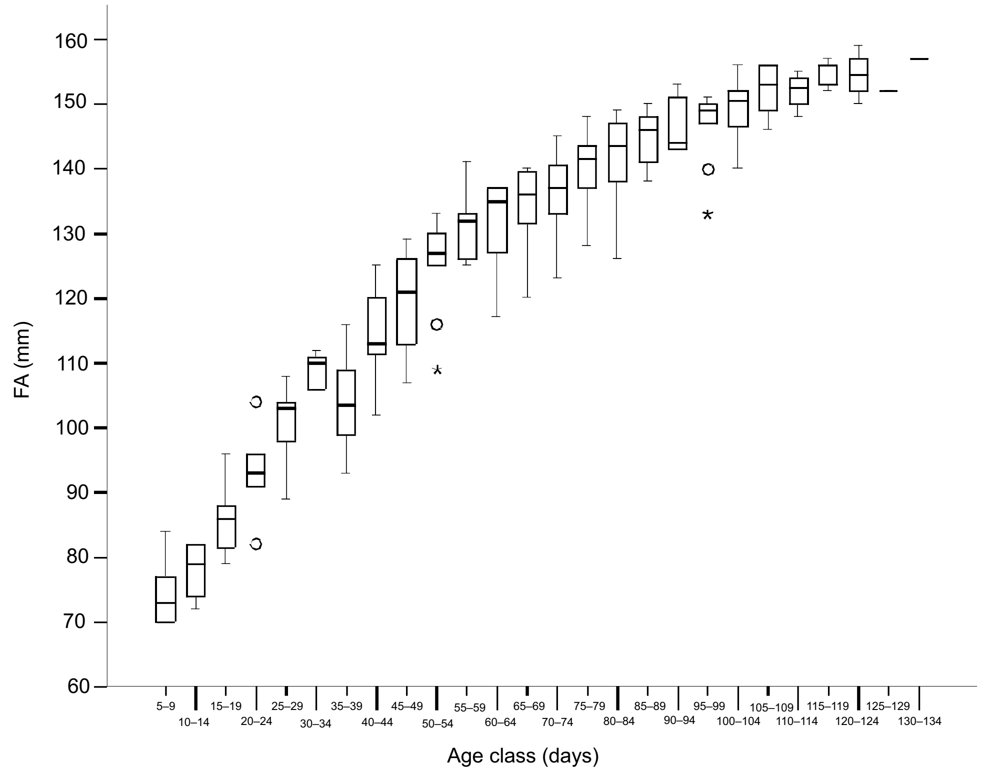

Figure 4. Forearm measurements for the twenty flying-foxes sorted by age class (sexes are combined due to small sample size). Outliers are shown with open circles and extreme values with stars. Horizontal lines inside the boxes indicate medians. There is a general increase in forearm measurements with age class, but the overlap between classes is substantial. Last two classes had one measurement each.

Acknowledgements

We would like to thank the Wildlife Animal Rescue and Care Society (Wildlife Arc), Mandi Griffith and Lillian McDermott for providing us with their pups. Special thanks also to Jan Bazel for helping us in the recording of the data.

References

Anthony, E. L. P. 1988. Age determination in bats. Pp 47–58 in: *Ecological and Behavioral Methods for the Study of Bats.* edited by T.H. Kunz, Smithsonian Institution Press: Washington, D.C., USA.

Burnett, C. D. and Kunz, T. H. 1982. Growth rates and age estimation in *Eptesicus fuscus* and comparison with *Myotis lucifugus*. *Journal of Mammalogy* 63: 33–41.

Davis, R. 1969. Growth and development of young pallid bats, *Antrozous pallidus*. *Journal of Mammalogy* 50: 729–736.

George, H. 1990. *The Hand-rearing and Management of Captive Grey-headed Flying-foxes*. Copyright Helen George 1990. Kangaroo Valley, New South Wales.

Hall, L. S. and Richards, G. 2000. *Flying Foxes: Fruit and Blossom Bats of Australia*. University of New South Wales Press, Sydney, New South Wales.

Hayssen, V. and Kunz, T. H. 1996. Allometry of litter mass in bats: maternal size, wing morphology, and phylogeny. *Journal of Mammalogy* 77: 476–490.

Kahumbu, P. and Eley, R. M. 1991. Teeth emergence in wild olive baboons in Kenya and formulation of a dental schedule for aging wild baboon populations. *American Journal of Primatology* 23: 1–9.

Kunz, T. H. and Anthony, E. L. P. 1982. Age estimation and post-natal growth in the bat *Myotis lucifugus*. *Journal of Mammalogy* 63: 23–32.

Lawrence, W. A., Coelho, A. M., Jr. and Relethford, J. H. 1982. Sequence and age of eruption of deciduous dentition in the baboon (*Papio* sp). *American Journal of Primatology* 2: 295–300.

Marks, S. C., Jr. and Schroeder, H. E. 1996. Tooth eruption: theories and facts. *The Anatomical Record* 245: 374–393.

Morris, P. 1972. A review of mammalian age determination methods. *Mammal Review* 2: 69–104.

Nelson, J. E. 1965. Behaviour of Australian Pteropodidae (Megachiroptera). *Animal Behaviour* 13: 544–557.

Olsson, A. R. 2000. Normal haematological and serum biochemical values and the effects of anaesthesia and parasites in the spectacled flying fox, *Pteropus conspicillatus*. Master of Science Thesis. James Cook University, Townsville, Queensland.

Parry-Jones, G. & Parry-Jones, K. 2003. *Flying-fox Training Manual*. Wildlife Animal Rescue and Care Society Inc., Matcham, New South Wales.

Parry-Jones, K. 2000. Historical declines since the early 1900s, and current mortality factors and abundance of the Grey-headed Flying-foxes in NSW. Pp 56–65 in: *Proceedings of a Workshop to Assess the Status of the Grey-headed Flying-fox in New South Wales.* edited by G. Richards, At: <http://batcall.csu.edu.au/abs/ghff/ghffproceedings.pdf>. Accessed: 05 May 2007.

Phillips-Conroy, J. E. and Jolly, C. J. 1988. Dental eruption schedules of wild and captive baboons. *American Journal of Primatology* 18: 553–579.

Setchell, J. M. and Wickings, E. J. 2004. Sequences and timing of dental eruption in semi-free-ranging mandrills (*Mandrillus sphinx*). *Folia Primatologica* 75: 121–132.

Tidemann, C. R. 1999. Biology and management of the grey-headed flying-fox, *Pteropus poliocephalus*. *Acta Chiropterologica* 1: 151–164.

Vardon, M. J. and Tidemann, C. R. 1998. Reproduction, growth and maturity in the black flying-fox, *Pteropus alecto* (Megachiroptera: Pteropodidae). *Australian Journal of Zoology* 46: 329–344.