

# “Diverse weights and diverse measures”: factors affecting the post-natal growth of the Grey-headed Flying-fox *Pteropus poliocephalus* and implications for ageing juvenile flying-foxes

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Determining the population dynamics of the vulnerable Grey-headed Flying-fox, *Pteropus poliocephalus* requires accurate methods of estimating the age of wild animals. Traditionally, wild juvenile *P. poliocephalus* have been aged by comparing their forearm measurements to those of known-aged captive bred juveniles. To determine the degree of plasticity in the forearm growth rate and hence the reliability of this method, the forearm growth of two groups of captive-bred flying-foxes whose mothers were fed different diets was compared. The difference in diet, a protein supplement of pollen rather than milk powder, made no significant difference in the average length of the forearm at birth, but there were significant differences in the subsequent growth of each growth parameter tested, with the pollen supplemented maternal diet providing faster post-natal growth than the milk supplemented maternal diet. Maternal diet was also significantly correlated with a difference in the sex ratio with a majority of females being produced on the milk supplemented maternal diet and a majority of males being produced on the pollen supplemented maternal diet. There was no significant difference in the post-natal forearm growth of males and females independent of their mother's diet. The sigmoidal curve that best described the average post-natal growth rate of mother-reared *P. poliocephalus* was the Logistic Model Function. As the diet of wild flying-foxes varies from year to year it is likely that the growth rates of wild mother-reared flying-foxes vary from year to year along with the age of competent flight and independence. The plasticity in the forearm growth rate indicates that estimating the age of wild juvenile flying-foxes using growth rates of captive mother reared juveniles is unlikely to be an accurate method of ageing wild juvenile flying-foxes and other methods of ageing these animals should be investigated.

**Key words** *Pteropus*, flying-foxes, sex ratio, age determination, growth, lactation, diet, life history.

## Introduction

Population studies on wild animals are reliant on accurate methods of ageing specific individuals (Morris 1972, Kunz and Anthony 1982). In the absence of reliable techniques, ages are often estimated and if these are incorrect then the understanding of the species as a whole can be compromised (Morris 1972). The post-natal growth of animals is an important life history trait (Case 1978), however, there has been little investigation of the post-natal growth of the Grey-headed Flying-fox *Pteropus poliocephalus* (Nelson 1965a; Welbergen 2004) and none of variations in growth that can be caused, for example, by differing environments. For practical purposes in the absence of field data, it is generally assumed that wild animals will have similar growth characteristics to flying-foxes born in captivity. Hence wild juvenile bats are most commonly aged by both scientists and rehabilitators by a measurement of the forearm or radius (Anthony 1988) compared to a standard curve based on the forearm growth of known aged captive juvenile flying-foxes (Williams and George 1983). This method assumes that *P. poliocephalus* growth rates are independent of their lifestyle: that is, wild and captive animals are born with similarly sized forearms and that they grow at a similar rate. These assumptions

require testing as environmental factors can alter growth rates (Morris 1972).

The Grey-headed Flying-fox *P. poliocephalus* is listed as vulnerable in NSW (Threatened Species Conservation Act 1995) and under Federal Legislation (Environment Protection and Biodiversity Act 1999). It is an endemic member of the genus *Pteropus* (Mickleburgh *et al.* 1992) and it is found along the eastern coast of Australia from Bundaberg in Queensland to Melbourne in Victoria (Hall and Richards 2000). While traditionally existing in large numbers (Ratcliffe 1931) its vulnerability is related to its life history, which is at the slow end of the slow-fast continuum (Read and Harvey 1989).

A “slow” characteristic of the life history of *P. poliocephalus* is the large investment that is put into individual young. The species has a low reproductive rate (Jones *et al.* 2003) where the majority of females do not reproduce until they are three years old (Divljan 2008) and have a high level of post-natal care. Females are pregnant for six months (Nelson 1965a) and have only one baby a year (Ratcliffe 1931, Nelson 1965a, McIlwee and Martin 2002). It is generally believed that juveniles do not fly or attempt to forage under three months, but the lack of information on their post-natal development has resulted in less certainty

as the animal ages: they are weaned between four and six months old (Nelson 1965a), and they “reach maturity” at a forearm size ranging from 145 mm (Nelson 1965b) to 153.5 mm (Welbergen 2004).

Ideally the growth and development of wild juveniles is determined from longitudinal studies in which individuals in a particular cohort are measured at regular intervals (Kunz and Robson 1995). These data can be used to generate an average growth curve appropriate for the species and the curve can be fitted to a sigmoidal equation so that subsequent wild animals can be aged by reference to either a generated curve or to the appropriate equation (Zullinger *et al.* 1984). However, in contrast to microchiropterans, flying-foxes are difficult to study longitudinally because of their roosting behaviour, their mobility and the difficulties involved in recapturing specific animals. Hence cross-sectional studies, where a number of animals are collected at a similar time have provided most of the scientific data on the growth rates of wild juvenile *P. poliocephalus* (Ratcliffe 1931, Nelson 1965b, Welbergen 2004, Divljan 2008), but such methods have their limitations (Hoying and Kunz 1998) and are not suited to investigate factors affecting post-natal growth (Kunz and Robson 1995).

An intrinsic factor that can give variations in post-natal growth is the gender of the animal. From cross-sectional studies of flying-foxes, it is generally accepted that adult *P. poliocephalus* are dimorphic. Nelson (1965a) reported no difference between the sexes with regard to forearm size, but a degree of dimorphism was found in relation to weight. More recently Welbergen (2004) found dimorphic differences in certain morphometric characteristics including a significant difference in the length of the average forearm, with adult males being larger than adult females. However cross-sectional studies did not find significant differences between the forearm growth of juvenile males and females. (Nelson 1965b, Welbergen 2004).

Diet is an environmental factor that can cause variations in the growth rates of mammals (Altmann and Alberts 1987). Not only that, milk composition and quality can vary depending on the maternal diet and variations in maternal diet have been linked to varying growth rates in the offspring (Ofstedal 1984, Del Prado *et al.* 1997). Well fed captive Tasmanian Bettong *Bettongia gaimardi* mothers produced young that grew faster than the young of wild animals or the young of captive animals on a poorer maternal diet (Rose *et al.* 2003) and similar results were obtained with wild and captive Tasmanian pademelons *Thylogale billardieri* (Rose and Flowers 2005).

Within the chiropteran order, differences in post-natal growth across species have been attributed to differences in maternal diet (Kunz and Stern 1995). However, within a species the milk quality of nursing mothers can vary according to their diet. *Pteropus poliocephalus* produces milk that is significantly different in composition depending on whether the animals are captive or living in the wild (Messer and Parry-Jones 1997, see Table 1), but the effect of a difference in the maternal diet on the growth rates of juvenile *P. poliocephalus* has not previously been investigated.

In the absence of data on the post-natal growth of wild *P. poliocephalus*, the growth rates of captive juveniles are used to indicate the factors that are likely to affect those of the wild flying-foxes. Hence the four aims of this study are to document the post-natal growth of captive, mother-reared juvenile flying-foxes, investigate the degree of plasticity in the growth rate of their forearms as a result of sexual dimorphism and/or their mother's diet, to determine a possible sigmoidal equation for their growth, and to determine if the current method of ageing wild flying-foxes, by comparing their forearms to those of juveniles born to captive mothers is effective.

## Methods

### Data Collection

The data was obtained from the historical records of weekly measurements of animals being cared for by The Wildlife Animal Rescue and Care Society Inc. (The ARC), a native animal rehabilitation society located on the Central Coast of New South Wales (NSW). The records cover a period of 14 years and document various changes in the husbandry of mother-reared and hand-reared flying-foxes within the organization. Within this period two groups of mother-reared juvenile flying-foxes were identified as having been raised while their mothers were fed different diets. As the data are not the result of scientific manipulation there is no opportunity to alter variables or make further investigations or measurements that are not held within the existing records.

The two groups were composed of juvenile flying-foxes that were born to captive flying-foxes during 1996 and 1997. In 1996 the lactating females were fed *ad lib* diced mixed fruit (approximately 350g/animal) supplemented with generic brand commercial cream milk powder (approximately 5g/animal). The milk collected from these females was tested as part of another study (Messer and Parry-Jones 1997) and this composition is shown as the “captive” milk in Table 1. Their offspring are the Milk Group. In 1997 the lactating flying-fox diet given above was altered by the substitution of 5g of powdered Myrtaceae pollen for the milk powder. The pollen was supplied in bulk by a local bee-keeper and samples were viewed microscopically and identified at the time as being Myrtaceae pollen by the author. The pollen was not analysed and none survives to be analysed, but the average composition of the pollen of some Myrtaceous species known to be eaten by wild flying-foxes is given in Table 2. The offspring of the females given the pollen supplement make up the Pollen Group. Apart from the introduction of pollen to the diet there was no apparent

**Table 1.** The composition of wild and captive flying-fox milk. No changes in composition were found in the milk from captive females over the period of lactation (Messer & Parry-Jones 1997).

Milk	Protein %	Carbohydrate %	Fat %
Wild Females*	3.6	6.4	2.2
Captive Females*	2.6	6.1	1.9

\*Messer & Parry-Jones 1997

difference in the husbandry of the flying-foxes over the two years. There were 15 mothers that had offspring in both groups.

### Data Analysis

The forearms of the juvenile flying-foxes were measured by the same person in a similar manner over the two years of the study. Vernier calipers used to measure the forearms measured accurately to two decimal places, but measurements to only one decimal place were recorded.

The growth of all the individuals in the Milk Group was graphed. So that comparisons could be made between groups, specific parameters were chosen to indicate the shape of the curve.

SPSS 15 was used for all statistical analyses. Specifically, if the Levene's Test for Equality of Variances was not significant ( $p > 0.05$ ), then the Independent T Test was used to calculate the significance of any difference related to gender or to maternal diet on the means of the parameters of forearm growth.

The offspring of the 15 female flying-foxes that were common to both groups were tested independently to determine if their response to a change in maternal diet was the same as that for the total number of animals tested in each group.

A similar number of males and females were born to the captive animals in the two years where the maternal diet differed; however, the ratio of males to females in each year was uneven (Table 3). To determine if the uneven proportion of males and females in the two years was significant, the data were analysed using Pearson's Chi Square and the Phi coefficient and Cramer's V analyses. To determine the effect of gender on growth, equal numbers of males and females in each group were randomly selected from each mother-reared group and reassigned into categories according to their sex.

Growth curves for each group, using the average values for the chosen parameters, were fitted to sigmoidal equations using Curve Expert (version 1.3), which uses the Levenberg-Marquardt method to solve nonlinear regressions. Five sigmoidal equations; Gompertz Relation, Logistic Model, Richards Model, the MMF Model and the Weibull Model were tested to find the best fit for the average data from both groups.

### Results

The post-partum forearm growth of all flying-foxes in the Milk Group is graphed in Figure 1. The composite curve shows a steep linear incline to approximately 120 mm at which point the gradient decreases and continues to decrease as it moves towards the asymptote (Figure 1), the term used for the horizontal line on a growth curve when an animal stops growing; that is, when it reaches adult size. The forearm growth of three flying-foxes are identified on the graph: these are the offspring of Wookie and Terri, fast growing animals with slightly different shaped growth curves and the offspring of Leah that was the slowest growing juvenile of the Milk Group.

Five parameters of the growth curve were chosen; the "intercept" parameter where the linear gradient is extended to the y-axis to give an approximation of the size of the forearm at birth (the intercept value underestimated the birth size by an average of 1.5 mm (range 0.4–3.3 mm) in the 13 cases where birth data were available, as a result of slow growth for a day or so after birth by some individuals, for example Leah's offspring (Figure 1); the initial linear slope of the forearm growth curve (using the gradient of the line from the intercept parameter until 120 mm was reached); the number of days taken for the forearm to reach 120 mm (a length of forearm within the initial gradient); 130 mm (approximately when the curve bends away from the initial gradient); and 140 mm (approximately when the

**Table 2.** The composition of pollen and fruit (Stace 1996, Wills et al. 1987) eaten in the wild by *P. poliocephalus* (Parry-Jones 1993).

Name	Protein (%)	Carbohydrates (%)	Fats (%)
Pollen*			
<i>Banksia ericifolia</i>	30.9	–	–
<i>Corymbia maculate</i>	27.8	–	–
<i>Eucalyptus tereticornis</i>	26.6	–	–
<i>Eucalyptus pilularis</i>	21.8	–	–
<i>Melaleuca quinquenervia</i>	22.7	–	–
Fruit**			
Apple	0.3	11.9	0.1
Fig	1.4	8.1	0.3
Nectarine	1.1	9.2	0.1
Peach	1.0	6.6	0.1
Pear	0.3	12.2	0.1
Plum	0.6	6.1	0.1

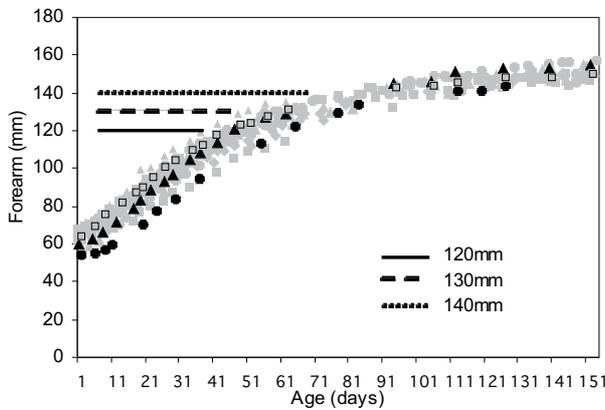
\* Data from Stace 1996

\*\* Data from Wills et al. 1987

**Table 3.** The number of males and females in 1996 (the Milk Group) and 1997 (Pollen Group). Brackets indicate the number of males and females born to the mothers that were common to both groups.

Sex	1996 (Milk Group)	1997 (Pollen Group)	Total
Males	7 (5)	14 (10)	21 (15)
Females	16 (10)	8 (5)	24 (15)
Total	23 (15)	22 (15)	45 (30)

curve starts to approach the asymptote). These three forearm lengths were chosen as they describe the changing gradient of the growth curve in the data for most individuals (Figure 1).



**Figure 1.** Forearm growth data for young born to all 23 Milk Group females are graphed in grey. Specific growth curves of juveniles born to Wookie (h), Terri (t) and Leah (h) are shown. Parameters used to describe the growth curves are the x intercept (birth forearm), the steep initial gradient and the age of the juvenile when the forearm is 120 mm, 130 mm and 140 mm. The age of the fastest (Wookie's) and slowest growing juvenile (Leah's) at a forearm length of 120 mm is 37 and 67 days, respectively and at a forearm length of 140 mm is 65 and 118 days old, respectively.

The average values for the parameters for each group are given in Table 4. The milestone length parameters divide the growth curve into three sections: birth to 120 mm; 120–130 mm; and 130–140 mm, with the gradients of each section giving the average forearm growth per day. Based on these parameters and the gradients between them, average growth charts for both Groups were produced (Table 5).

**Individual Differences**

There was a wide range of forearm lengths in the juveniles at birth and in the post-natal growth rate of juveniles within each group of captive mothers fed the same diet.

In both the Milk and Pollen Groups, the average lengths of the forearms of juvenile flying-foxes at birth were not significantly different ( $p < 0.05$ ). By intercept values, the Milk Group juveniles had a mean birth forearm of 63.27 mm (+/- 1.037 mm) and the Pollen Group animals had an average birth forearm of 63.56 mm (+/- 0.91 mm) (Table 6), however, there was a considerable range in forearm birth lengths in both Groups and juvenile flying foxes were recorded with forearms that ranged in length from 55 mm to 73 mm (Figure 2).

Individual flying-foxes grew at different rates within each Group. In the Milk Group, female juveniles, the offspring of Wookie and Leah, provided both the fastest and

slowest forearm growth rates (Figure 1). Wookie's offspring was 37 days old at a forearm length of 120 mm, while Leah's was 67 days of age at that length. By the time forearm lengths reached 140 mm there was 52 days difference in age. (The animals were 65 and 118 days old respectively).

**Table 5.** Growth Charts derived from Table 4 of the Milk Group and the Pollen Group Hand-reared Flying-foxes

Age (weeks)	Milk Group Average forearm (mm)	Pollen Group Average forearm (mm)
0	63.3	63.6
1	71.3	72.5
2	79.3	81.5
3	87.2	90.4
4	95.2	99.4
5	103.2	108.4
6	111.2	117.3
7	119.1	122.0
8	123.3	126.7
9	127.4	131.4
10	131.6	135.7
11	134.4	140.1
12	137.2	
13	140.0	

**Gender Based Differences**

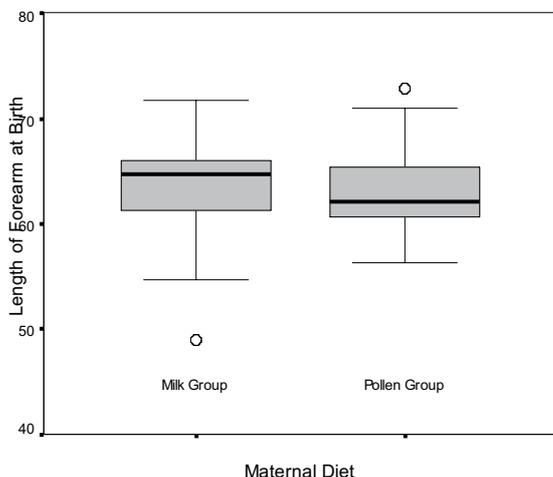
Although approximately equal numbers of males and females (21 males: 24 females) were born to the captive flying-foxes during the two years, there was a disproportionate number of females born in the first year (16 out of 23 – milk supplement), whereas males predominated in the second year (14 out of 22 – pollen supplement). This was different from the expected ratio of equal numbers of males and females born in each year (Table 3). The number of males and females born to captive females in each year was compared to the expected ratio and the Pearson's Chi-Square gave a significant association between the sex of the animal and its year of birth ( $\chi^2 = 4.980$ ;  $df = 1$ ;  $P = 0.026$ ). This relationship is confirmed by the Phi coefficient and Cramer's V analysis ( $\phi = -0.333$ ;  $p < 0.05$ , Cramer's V = 0.333;  $P = 0.026$ ).

When the offspring from the 15 mothers that were common to both groups were considered, there were similar numbers of males and females born to this subgroup (15:15), but once again the male: female ratio was reversed from one year to the next; with more females being born in the first year (10 out of 15) and more males in the second (10 out of 15). In this smaller group the trend is not significant when tested by Pearson's Chi-Square

**Table 4.** Differences between forearm growth of the Milk Group and 2: the time it took to reach the 3 milestones of 120 mm, 130 mm and 140 mm and the growth per day (the gradient of the curve) at each stage.

	Days to 120 mm	Growth rate to 120 mm	Days to 130 mm	Growth 120–130 mm	Days to 140 mm	Growth 130–140 mm
Milk Group	50	1.14 mm/day	67	0.59 mm/day	92	0.40 mm/day
Pollen Group	44	1.28 mm/day	59	0.67 mm/day	75	0.62 mm/day

When equal number of males and females from each year were compared using the Independent T test no significant difference was found in any of the growth parameters between males and females (Table 7). Hence the determination of a juvenile's sex at conception appears to be related to its mother's diet, but its subsequent post-natal forearm growth is not affected by its gender.



**Figure 2.** Box Plots of the estimated length of the forearm at birth in the Milk and Pollen Groups based on the growth curve's intercept with the Y axis. The horizontal lines on the Box Plot give the range of values in each group with values between the 25<sup>th</sup> and 75<sup>th</sup> percentile forming the box and the thick black horizontal line, the median. Individual outlier values (more than 1.5 box lengths from the box) are shown by small circles.

### Diet Based Differences

The substitution of pollen for the milk powder given as a protein supplement in the captive maternal flying-fox diet made a significant difference to the growth rates of the offspring: the forearms of the Pollen Group juveniles grew significantly faster than the Milk Group.

The results of the Independent T-test on the effect of the diet on forearm growth are given in Table 6. The means of the Milk and Pollen Group were found to be significantly different in 4 of the 5 parameters measured. As stated above, the size of the forearm at birth, as given by the intercept value, was not significantly correlated with the diet of the captive mothers in the Milk and Pollen Groups, but all the other parameters showed significance between the two Groups; individuals in the Pollen Group had a significantly steeper gradient of growth (Figure 3) and reached 120 mm, 130 mm and 140 mm significantly before their counterparts in Group 1. In particular, there was 17 days difference in the average age of juveniles from the Milk and Pollen Groups at a forearm length of 140 mm (Table 4) and the two Groups had ranges that only partially overlapped (Figure 4).

When the mean forearm growth of the 15 offspring from mothers that were common to both Groups were compared using the Independent T-test, these smaller groups exhibited similar trends which were significant for the mean age of the juveniles at forearm lengths of 130 mm and 140 mm (Table 8).

**Table 6.** Independent T-test results on the effect of diet on forearm growth rates in captive born juvenile flying-foxes. All differences between Groups 1 and 2 are significant ( $p < 0.05$ ) except that of the intercept (the estimated forearm at birth).

	Group	N	Mean	Std. Deviation	Std. Error Mean	Sig. 2 tailed
Slope	1	23	1.17	0.157	0.032	$p < 0.05^*$
	2	22	1.29	0.164	0.035	
Intercept	1	23	63.37	4.975	1.037	ns
	2	22	63.56	4.266	0.909	
FA120 mm	1	23	49.74	7.350	1.533	$p < 0.05^*$
	2	22	43.86	6.777	1.445	
FA130 mm	1	22	66.50	8.517	1.816	$p < 0.005^*$
	2	22	59.32	7.074	1.508	
FA140 mm	1	22	91.45	10.774	2.297	$p < 0.005^*$
	2	22	75.41	7.379	1.573	

**Table 7.** Independent T-test results for the effect of gender on forearm growth rates in captive born juvenile flying-foxes. None of the parameters is significantly different.

	Group	N	Mean	Std. Deviation	Std. Error Mean	Sig. 2 tailed
Slope	M	15	1.29	0.164	0.042	ns
	F	15	1.26	0.084	0.021	
Intercept	M	15	63.17	4.491	1.160	ns
	F	15	62.47	5.308	1.371	
FA120 mm	M	15	45.07	7.096	1.832	ns
	F	15	47.80	6.689	1.737	
FA130 mm	M	15	61.40	10.473	2.704	ns
	F	15	65.07	8.084	2.087	
FA140 mm	M	15	81.53	16.256	4.197	ns
	F	15	86.33	13.468	3.477	

### Sigmoidal Equations

There were differences in the shape of the growth curves of individual flying-foxes within each Group (Figure 1), however, the two best fit equations that described the average forearm growth for the two Groups were the Logistic Function and the Richards Model that is derived from it. Mathematically these equations generate related sigmoidal curves as the Richards Model equation reduces to the Logistic curve when one of its coefficients is given a value of minus one (Grosjean 2001). The equation that best fits the growth curve is one where the correlation coefficient (r) is the closest to 1 and the standard error (S) is closest to 0. The Richards Model is the equation of best fit for the Milk Group (S = 0.66, r = 0.99) and the second best for the Pollen Group, whereas the Logistic Function gives the best fit for the Pollen Group and the second best for the Milk Group (Figure 5). The Logistic Function gave a more realistic value for the forearm growth asymptote of the two curves than the Richards Model (150 mm and 152 mm in comparison with 142 mm and 143 mm) and so on biological grounds it was chosen as equation of choice.

The Logistic Function has the equation of:

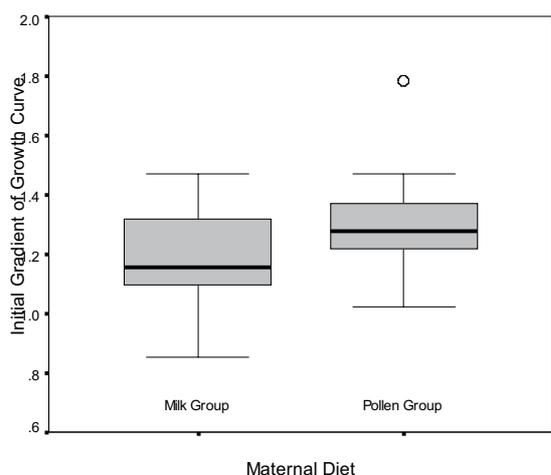
$$Y(t) = Ya/1 + be^{-cx}$$

When  $Y(t)$  = length of forearm at time “t”,  $Ya$  = average length of the forearm at the asymptote (maturity),  $b$  is a constant and  $c$  = the “maturity index” a Function of the ratio of the maximum growth rate to mature size (at the asymptote) (Grosjean 2001). The numerical values for these coefficients are given in Table 9.

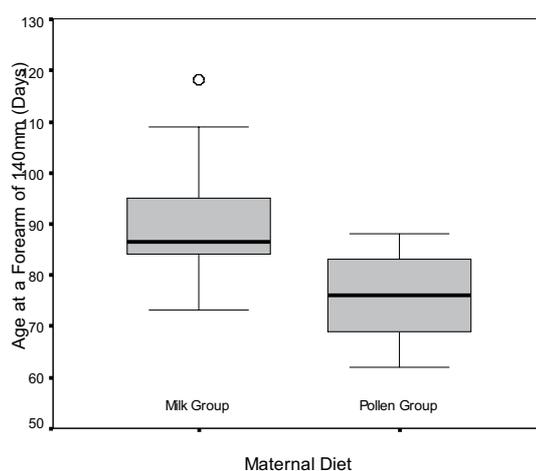
### Discussion

The traditional method of ageing wild juvenile *P. poliocephalus* by comparing the length of their forearms to that of captive bred juveniles of known age is not accurate. While the average forearm length of neonates found in this study was fairly constant and independent of maternal diet (Figure 2) there was a wide range of forearm lengths included in each average value. This inherent variation between individuals increased with differences in the maternal diet and it is possible that other environmental factors also affect the growth of wild and captive individual animals. The extent of these individual differences were such that a high level of error is inevitable in determining the age of a particular wild flying-fox by comparing its forearm with an average determined from captive animals.

Neonates from both mother-reared groups had average forearms (Figure 2) that were not significantly different



**Figure 3.** Box plots of the initial gradients of the growth curves for the offspring of the Milk and Pollen Group.

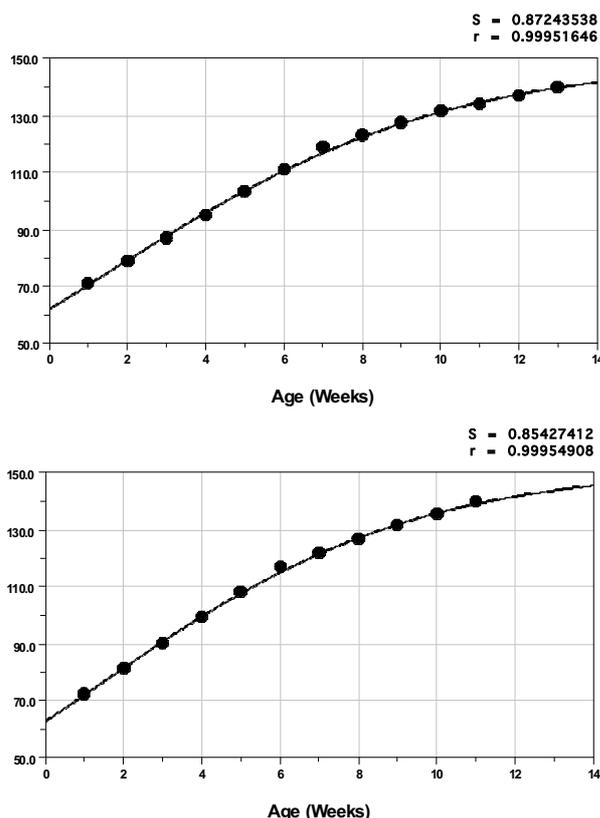


**Figure 4.** Box plots of the ages at which animals from the Milk and Pollen Group reached forearm lengths of 140 mm.

**Table 8.** Independent T-test results on the effect of diet on forearm growth rates in captive born juvenile flying-foxes when the mothers are common to both groups. The differences between Groups 1 and 2 are significant ( $p < 0.05$ ) at the age at a forearm length of 130 mm and 140 mm.

	Group	N	Mean	Std. Deviation	Std. Error Mean	Sig. 2 tailed
Slope	1	15	1.21	0.125	0.033	ns
	2	15	1.31	0.148	0.038	
Intercept	1	15	64.16	4.570	1.180	ns
	2	15	64.34	4.838	1.249	
FA 120 mm	1	15	48.00	5.868	1.515	ns
	2	15	43.67	6.747	1.742	
FA 130 mm	1	15	67.73	8.988	2.321	$p < 0.005^*$
	2	15	56.87	7.846	2.026	
FA 140 mm	1	15	93.20	9.901	2.556	$p < 0.005^*$
	2	15	72.67	9.574	2.472	

from each other, hence it is likely that maternal diet does not affect the growth rate *in utero*, however it does affect post-natal growth. Captive mothers on the diet supplemented by Myrtaceae pollen had offspring whose forearms reached the milestone lengths of 120 mm, 130 mm and 140 mm significantly earlier than the offspring of the females fed fruit and milk powder (Table 6) and the magnitude of the difference was such that the juveniles from the Pollen Group were 17 days younger at an average forearm length of 140mm than were the juveniles from the Milk Group (Table 4).



**Figure 5.** Average growth curves for the Milk Group and the Pollen Group derived from the data in Table 5 (*r* is the correlation coefficient and *S* is the standard error). Data from the Milk Group (top) and the Pollen Group (bottom) were fitted to the Logistic Function and the parameters for both graphs are given in Table 9.

**Table 9.** Coefficients and Goodness of Fit for the Logistic Model Equations for the two Groups.

Coefficients	Milk Group	Pollen Group
Ya	149.7426	152.1025
B	1.399293	1.409228
C	0.231145	0.248225

The maternal diet, which affects the health and condition of the mother and the growth rate of her offspring, may also influence the determination of its sex. The ratio of females to males born to the captive flying-foxes varied significantly with maternal diet: the pollen enriched diet that produced the faster growing juveniles also produced significantly more males. The pollen enriched diet had been given to the flying-foxes well before the start of the

mating season and so it could have influenced the condition of the mother at conception. On fruit and a milk supplement the flying-foxes produced more females than males and the effect was reversed in the following year when pollen was substituted for milk powder (Table 3). These results support the Trivers-Williard hypothesis that females in good condition in dimorphic and polygynous species will produce more males than females: if the mothers are in good condition then their offspring will be big and sexually successful and so maximize the mother's chance of passing on her genes (Isaac *et al.* 2005, Cameron 2004, Rosenfeld and Roberts 2004). While maternal diet may influence the sex ratio and although sexual dimorphism has been found in adult flying-foxes (Welbergen 2004, Divljan 2008) juvenile *P. poliocephalus* are not dimorphic in their forearm sizes (Nelson 1965b, Welbergen 2004) and the difference in growth rates between the Milk and Pollen Groups is not caused by the gender of the offspring: the sex of a juvenile does not have an effect on either the size of the forearm at birth or its subsequent growth (Table 7).

In a study of bats across a number of genera, Kunz and Stern (1995) found that a maternal diet higher in protein is reflected in higher protein in the milk and results in faster juvenile growth rates. However, in this study the difference in the growth rates of juvenile flying-foxes appears to be related to the quality of the protein in the maternal diet of *P. poliocephalus* rather than its quantity. The chemical composition of the two supplements is given in Table 10. The Myrtaceae pollen supplement is assumed to have a protein value derived from an average protein composition of the Myrtaceae pollens given in Table 2. If this assumption is correct then the milk supplement given to the Milk Group has a higher protein composition than the supplement given to the Pollen Group, yet results in lower growth rates. Both supplements (Table 10) have protein compositions well above the maintenance nitrogen requirement for non-lactating *P. poliocephalus* of 457mg.kg.d<sup>-0.75</sup> (Steller 1986) and based on the growth rates of the juveniles, the lower amount of protein given by the pollen supplement provided adequate protein for lactating flying-foxes. If both supplements give adequate protein for lactating flying-foxes, then it appears that additional protein in the maternal diet is not as important in increasing juvenile growth rates, as the nutrients associated with the protein-containing food. The difference in post-natal growth rates could be related to specific amino acids (Herbst 1986, Stace 1996) or more likely to the availability of specific nutrients such as calcium in the maternal milk (Barclay 1995, Stern *et al.* 1997). In a New World fruit bat it has been found that "Among measured nutrients, calcium is the limiting nutritional factor that determines mass of milk needed by suckling young during postnatal growth" (Studier and Kunz 1995 p1)). However, little is know

**Table 10.** Protein available in supplement and per animal/day

Supplement	Protein (%)	Protein/animal/day
Milk powder	32.8 g	1.6 g
Myrtaceae Pollen	24.7 g	1.2 g

about the chemistry of lactation in flying-foxes and further research is needed to determine the reasons why the post-natal growth rate increases with the addition of pollen to the maternal diet.

The growth of living organisms can be modeled mathematically and the post-natal growth of mammals is generally modeled using sigmoidal functions which can be used to ascertain the approximate age and to estimate the future growth of wild juveniles that may be infrequently handled (Zullinger *et al.* 1984). The Logistic Function has been found to be the most appropriate expression to describe the growth of a microchiropteran (Kunz and Robson 1995) and it is also the most appropriate equation to describe the post-natal growth curve of *P. poliocephalus*. The Logistic Function can be used to determine the asymptotic forearm length ( $Y_a$ ): i.e. the theoretical point at which the post-natal growth rate is zero. An average of both Groups gives a length of 151 mm (the Milk Group is 150 mm and the Pollen Group is 152 mm) (Table 9). This is similar to some results found in cross-sectional studies of wild *P. poliocephalus* where the asymptote has been calculated to be 153.5 mm (Welbergen 2004) and 145 mm (Nelson 1965b). The range of values suggests that different growth rates may exist in the wild with the lower value perhaps illustrating the effect of “the uncertainties and constraints of living in the wild” (p131, Kunz & Stern 1995).

In studies of microchiropterans the mathematically derived asymptote indicates the age of maturity and the adult size of the forearm (Kunz & Stern 1995), however, with flying-foxes this terminology is misleading. In flying-foxes the growth curve for the average forearm length flattens and approaches an asymptote in the first year of life. However, in a similar manner to the weight curve of microchiropterans (Kunz & Stern 1995), the forearm continues to lengthen until it reaches its adult length and stops growing, probably at sexual maturity at 18 months of age (Nelson 1965a). This further growth is not predicted by the Logistic Function among other growth equations and is the reason for apparent contradictions in the literature, such as the average forearm length of wild adult *P. poliocephalus* being 162.5 mm (Divljan 2008), yet the length at which the flying-fox “reaches maturity”, that is reaches its mathematical asymptote, being 153.4 mm (Welbergen 2004 p3–59).

Maternal diet causes variations in the duration of time required for juveniles to reach specific wing lengths. It also affects their development, because the size of a bat is linked to its ability to fly (Barclay 1995). There are aerodynamic constraints on the shape and strength of the wing (Kunz 1973, Swartz *et al.* 1992, Hayssen and Kunz 1996) and so the overall shape and size of a bat’s wing is more important than its age in determining the onset of flight. The average growth curve of *P. poliocephalus* and the developmental sequence of the mother-reared juveniles is similar to the generalized model of post-natal growth of bats discussed in Kunz and Stern (1995) where it is stated that competent flight is not achievable before the wing is at least 90% the size of the asymptote (Barclay 1996). As stated earlier, the average mathematically derived asymptote for the two groups is 151 mm (Table 9), but the average length of the average adult forearm in the wild has been measured as 162.5 mm (Divljan 2008). If

the mathematically derived asymptote is the length of forearm when competent flight is possible, then this is achieved at an average length of 136 mm (90% of 151mm). However, it is more likely that competent flight is linked to the actual wing size of the adult rather than a hypothetical asymptote and that competent flight occurs at an average forearm length of 146 mm (90% of 162.5mm).

The smallest volant young found by Welbergen (2004) had a forearm of 124 mm, but being able to fly does not indicate foraging independence and the end of weaning (Koehler and Barclay 2000). Nelson (1965a) reported that flying-foxes were not weaned until they were 4–6 months old and Kunz and Stern (1995 p.124) state that, in general, bats “provide their young with milk until they achieve at least 90% of adult wing dimensions”. If this applies to megachiropterans, then *P. poliocephalus* juveniles are dependent on their mothers until they reach an average forearm length of 146 mm. This forearm was achieved at the age of 107 days (more than 3 months) by the Milk Group and 85 days (two and a half months) by the Pollen Group, ages that are consistent with the lower range of length of lactation proposed by Nelson (1965a) and in agreement with his report (Nelson 1965b) that flying-foxes could forage at the age of three months.

The diet of wild flying-foxes is likely to affect the growth rates of their offspring. Wild *P. poliocephalus* are sequential specialists (Marshall 1983; Parry-Jones and Augee 1991) foraging on a wide range of foods, in a hierarchy of preference (Parry-Jones & Augee 2001): blossom from Myrtaceous and Proteaceous species form the top layer of this hierarchy, while cultivated fruit is found towards its base (Gopalan 2006). The nutritional composition of some pollens and fruit eaten by *P. poliocephalus* is shown in Table 2 and there is a large difference in the amount of protein available to flying-foxes on a pollen diet to that available on a fruit diet. In addition, less than 70% of the protein in fruit is ingested (Steller 1986), while droppings can be composed of 100% pollen grains and can have a large percentage of their nitrogen rich contents extracted during digestion (Parry-Jones 1993). If blossom is available, then the flying-fox diet is extremely high in protein, however Australian blossom producing trees are erratic in their flowering sequences and at times, in some months or years, little blossom is recorded (Parry-Jones & Augee 2001).

It has been suggested that “the nutritional conditions in captivity may push post-natal growth rates to their maximum” (Kunz and Stern 1995 p.131). Certainly captive animal populations have been shown to be larger than their wild equivalents (Altmann and Alberts 1987), but this generalization depends on the quality of the captive food and the availability and quality of the food in the wild (Hoying and Kunz 1998). The plasticity of the post-natal forearm growth in *P. poliocephalus* could result in rapid growth when there is high blossom availability during the lactating season and juveniles may be capable of sustained flight at a far younger age than captive mother-reared or wild living flying-foxes in a poor season. But alternatively, a poor lactation season with low blossom availability could result in a cohort of stunted juveniles that would take longer to mature and are dependent on their mothers for longer.

Kunz and Stern (1995 p.134) suggested that maternal milk composition would “prove to be” important in the post-natal growth rate of bats. In this study, maternal diet, and hence the quality of maternal milk, has been shown to be a major cause of variation in the post-natal growth of juvenile *P. poliocephalus*. However, this is not the only source of variation as there are considerable individual differences in juveniles whose mothers are fed similar diets. The plasticity of the forearm growth rate means that the method of estimating the age of wild

juveniles using the length of their forearm compared to a standard curve generated by the growth rates of captive bred juveniles is inaccurate. The forearm growth rate of *P. poliocephalus* varies according to intrinsic factors (such as inheritance and maternal condition) as well as extrinsic factors (such as maternal diet). So for accuracy and reliability in ageing juvenile flying-foxes, efforts should be made to find a method of ageing juvenile flying-foxes that is less affected by such factors.

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