Age estimation of the Gray Four Eyed Opossum, *Philander opossum* (Didelphimorphia : Didelphidae)

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Summary. – Variation of body mass, size of head and body, tail, and developmental traits were observed during the life of *Philander opossum* reared in laboratory in order to establish a method for age estimation. The morphometric data were fitted empirically to a exponential curve, for the pre-weaning phase, and to a logarithmic curve, for the post-weaning phase. External morphological traits, in the pre-weaning phase, and dentition stages, in the post-weaning phase, were transformed in discrete units and multiple regressions were calculated as empirical estimators of age for each phase. These methods had acceptable experimental errors and can be used to estimate age in field studies.

Résumé. – Des variations du poids corporel, de la taille du corps et de la queue, et des caractéristiques du développement ont été observées au cours de la vie de sarigues, *Philander opossum*, élevées en laboratoire, afin de mettre au point des méthodes pour estimer leur âge. Les données morphométriques ont été ajustées empiriquement, à des courbes exponentielles pour la phase pré-sevrage, et à des courbes logarithmiques pour la phase post-sevrage. Les caractéristiques morphologiques externes pour la phase pré-sevrage, et les stades de dentition pour la phase post-sevrage, ont été transformés en unités discrètes, et des régressions multiples ont été calculées pour estimer l'âge de chaque phase. Les erreurs expérimentales sont acceptables et ces méthodes peuvent être utilisées pour estimer l'âge sur le terrain.

INTRODUCTION

The age determination of field collected mammals is very important for detailed studies on population dynamics. It can be used for the determination of breeding season, recruitment, age at puberty, and other demographic parameters used in life tables.

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It is also necessary in wildlife management and conservation (Leberg *et al.* 1989, Morris 1972, Larson and Taber 1987).

Methods of age determination that have been used chiefly for medium and large mammals involve the use of sequence of tooth eruption and wear (Severinghaus 1949, Low and Cowan 1963, Lockard 1972, Chapman *et al.* 1985), annual deposition of cementum and dentine rings (Laws 1952, Fancy 1980, Driscoll and Jones 1985, McCullough and Beier 1986, Van Jaarsveld *et al.* 1987), growth of horn rings (Geist 1966), weight of eye lens (Dudzinski and Mykytowicz 1961) and level of epiphyseal closure (Washburn 1946). Yet, most of these methods requires sacrifice of the animals.

An important method for field studies, which avoids sacrifice, consists in comparing body measurement and morphological development of field animals to those of known age living in captivity (Poole *et al.* 1982a). Marsupials are excellent for such studies, as their young stay attached to the mother's teats during lactation. This allows age estimation by using growth data associated with morphological changes.

The present paper shows the results of the calculation of quantitative parameters from morphological data on *Philander opossum* (L.) (Didelphidae, Didelphimorphia) to estimate absolute age of field animals.

MATERIAL AND METHODS

Growth. – Data on the growth of head and body length (HB), tail length (T) and body mass (M) were obtained for 61 young from 14 litters before weaning and 44 animals from 11 litters after weaning. The total number of measurements taken before and after the weaning were respectively 931 and 656 for head and body, 772 and 570 for tail and 149 and 656 for body mass. Animals were measured and weighed from birth to eruption of the 4^{th} molar. Measurements were taken routinely on three specific days of the week, throughout the growth of the animals.

Before day 6, measurements of crown/rump length were only taken. After this period, standard measurements (to the nearest 0.1 mm) were taken from the young attached to the teats, using a vernier caliper. The young were not individually identified until weaning. When it became possible to release the young from the teats, measurements were taken from the animal laying on a surface. The distances from the rhinarium to the beginning of the first tail vertebra, and from there to the tip of the tail were measured with a millimeter rule. The animals were weighed to the nearest 0.1 g.

Data were analysed separately for the pre-weaning and the post-weaning phases, considering the different methods of measurement used and the distinctive growth pattern shown in each phase.

Growth curves were fitted by an empirical method, plotting data against time on a Cartesian plane. The empirical models were chosen by simple visual inspection : an exponential model for the phase before the weaning, and a logarithmic one for the phase after the weaning.

Development. – Observations of changes in external morphological traits were made simultaneously with measurements for 19 litters, from birth until adult form have been reached. Numerical values were attributed for each stage of development (Table 1).

Sequence of tooth eruption and functionality was another trait used to estimate age. As for morphological traits, numerical values were attributed to each stage of dental development : 1 - absent ; 2 - present, but not functional ; 3 - functional. The teeth observed were ; 3[rd] decidual premolar (dp3), 3[rd] permanent premolar (P3) and all permanent molars (M1, M2, M3 and M4) of the lower and the upper jaws.

Stepwise multiple regression was used to predict age from either morphological development or tooth eruption. In both equations, time was considered as the dependent variable. The resulting growth and development equations were used for age estimation of *Philander opossum*. The equations were tested by estimating the age of a group of animals of known age born in captivity, which had not been used in the calculation of the equations. The associated error of the method was obtained using experimental errors of age estimates. The associated error formula is :

$$E = \sqrt{\Sigma r_i^2 / N - 1}$$

Where :

r_i = experimental error in the ith age estimate.

N = number of age estimates.

The experimental error for the pre-weaning phase was calculated as the difference between real age and estimated age using the data selected for the test.

The experimental error for the post-weaning phase was calculated following the method proposed by Inns (1982).

TABLE 1. – Stages of morphological development characteristics of pouch young *P. opossum*. The attributed values are used to calculate an empirical multiple linear regression for age estimation.

Character	state	values
Vibrissae	absent	1
	papillae	2
	present	3
Underfur	absent	1
	on head	2
	dorsal	3
	ventral	4
Mouth	closed	1
	open	2
External ear	absent/attached	1
	free	2
	open	3
	erect	4
Eyelid	absent	1
	present	2
Eyes	closed	1
	open	2
Pigmentation	absent	1
	snout	2
	hind foot	3
	tail	4
	ear	5
Tail movements	absent	1
	present	2
Scrotum/marsupium	undefined	1
	defined	2

RESULTS

Growth during the pre-weaning phase. – This phase includes all the observations made from birth to weaning, which occurred at the day 76.

Coefficients and standard errors of the exponential equation calculated are in Table 2.

The variances for these equations were : HB = 18.2, TA = 18.6 and M = 37.3. The coefficients of determination for the three variables HB, TA and M were, respectively, 0.973, 0.961 and 0.965, being, for HB, F = 29854.1, GL_{res} = 817 ; for TA, F = 19012.4, GL_{res} = 770 and for M, F = 4083.4, GL_{res} = 147. All the analyses had $P < 0.00005$.

The associated errors of the equations are in Table 3.

The equations for age estimation, in days, are :

$$t = (\log_e HB - 3.01)/0.024$$

Where : t = estimated age in days ; HB = head-body length in millimetres ;

$$t = (\log_e TA - 1.73)/0.04$$

Where : t = estimated age in days ; TA = tail length in millimetres ;

$$t = (\log_e M + 1.61)/0.071$$

Where : t = estimated age in days ; M = body mass in grams.

Growth during the post-weaning phase. – This phase includes all observations made from the first day after weaning to a maximum of 452 days of life.

Growth after weaning was analysed until eruption of the 4th superior molar. This eruption occurs between day 207 and day 261, and concurs with an increased disper-

TABLE 2. – Coefficients and associated standard errors of growth curves of *P. opossum*, in pre and post weaning phase. HB - head-body length. TA - tail length. M - body mass.

		Pre-weaning		Post-weaning	
		Coefficient	Standard error	Coefficient	Standard error
HB	slope	0.024	1.36E-04	116	2.03
	intercept	3.01	5.93E.03	- 378	9.96
TA	slope	0.04	2.92E-04	137	3.17
	intercept	1.73	1.28E.01	- 477	15.58
M	slope	0.071	1.11E-03	225	4.88
	intercept	1.61	7.00E.02	- 961	23.76

TABLE 3. – Associated errors of the equations calculated for age estimation of *P. opossum*. HB - head-body length. TA - tail length. M - body mass. MD - morphological development. TE - tooth eruption.

	Growth			Development	
	HB	TA	W	MD	TE
pre weaning	2.6	3.1	3.4	5.0	-
post weaning	14.1	14.1	10.5	-	9.2

sion of the growth data. Yet, the last curve inflexion occurs at this period, when growth becomes asymptotic.

Coefficients and standard errors of the logarithmic equations calculated are in Table 2;

The variances of the equations were : HB = 18.2, TA = 18.6 and M = 37.3. The coefficients of determination for the variables HB, TA and M were, respectively, 0.89, 0.83 and 0.84, obtaining for HB, F = 3271.5, GL_{res} = 407 ; for TA, F = 1876.4, GL_{res} = 379 and for M, F = 2137.8, GL_{res} = 408. All the analyses had $P < 0.00005$.

The associated errors of the equations are in Table 3.

The equations for estimation of the age in days are :

$\log_e t = (HB + 378)/116$

Where : t = estimated age in days ; HB = head-body length in millimetres ;

$\log_e t = (TA + 477)/137$

Where : t = estimated age in days ; TA = tail length in millimetres ;

$\log_e t = (M + 961)/225.$

Where : t = estimated age in days ; M = body mass in grams.

Development during the pre weaning phase : external morphological traits. – The observations were made from birth until the offspring showed all the external traits visible in an adult. This occured around day 70. Nineteen breeds were observed, with as total of 85 young.

Coefficients and standard error of the multiple regression are in Table 4. The coefficient of determination is 0.97. The analysis of the variance (ANOVA) had F = 895.8, GL_{res} = 150 and $P < 0.05$.

TABLE 4. – Coefficients and standard errors of the multiple linear regression calculated from morphological traits (pre-weaning phase) and tooth eruption stages (post-weaning phase) of *P. opossum*. P_3 - 3rd lower permanent premolar, M_2 - 2nd lower molar, M_3 - 3rd lower molar, M_4 - 4th lower molar, M^1 - 1st upper molar, M^2 - 2nd upper molar and M^3 - 3rd upper molar.

Character	Coefficient	Standard
Pre-weaning phase		
Underfur	3.86	0.72
Vibrissae	7.43	0.74
External ear	3.45	0.64
Tail movements	3.37	0.47
Eyelid	6.15	1.08
Eyes	3.11	1.42
Constant	- 22.4	1.71
Post-weaning phase		
M_3	31.6	3.01
M^3	33.5	3.35
M_2	14.7	3.03
M_4	25.8	3.27
M^2	16.9	2.53
P_3	12.6	2.64
M^1	15.1	3.62
Constant	59.9	1.35

The equation for estimating age in days is :
$t = -22{,}4 + 3.86Und + 7.43Vib + 3.45Ear + 3.37Tai + 6.15Lid + 3.11Eye.$
Where : *Vib* = vibrissae ; *Und* = underfur ; *Eye* = eyes ; *Ear* = external ear ; *Lid* = eyelid ; *Tai* = tail movements ; *t* = estimated age in days.

Developments during the post-weaning phase : sequence of tooth eruption and functionality. – The observations began when the animals could be detached from the teat. The mouth was opened for checking of teeth presence, until all the teeth were present. The last tooth observation coincided with the last growth measurement.

The regression calculated including the fourth superior molar has a coefficient of determination of 0.91. The analysis of variance had F = 1265.1, GL_{res} = 610 and P < 0.05. However, the inclusion of this tooth creates problems for the estimates. Therefore, a fitting was searched excluding this molar. Coefficients and standard errors in Table 4 were obtained by stepwise multiple linear regression. The coefficient of determination of the equation is 0.97. ANOVA had F = 1908.1, GL_{res} = 414 and P < 0.05. The associated errors are in Table 3.

The equation for estimating age in days is :
$t = 59.9 + 31.6M_3 + 33.5M^3 + 14.7M_2 + 25.8M_4 + 16.9M^2 + 12.6P_3 + 15.1M^1.$
Where : F_3 = 3rd lower permanent premolar ; M_2 = 2nd lower molar ; M_3 3rd lower molar ; M_4 = 4th lower molar ; M^i = 1st upper molar ; M^2 = 2nd upper moral ; M^3 = 3rd upper moral ; *t* = estimated age in days.

DISCUSSION

Growth patterns established under laboratory conditions, as a method for age estimation in wild mammals, assume similar growth rates for laboratory and field animals. Sadleir (1963) found that females' nourishment in *Macropus robustus* is never poor enough to delay growth of pouch young. Sharman *et al.* (1964) proposed that growth of pouch young in the kangaroo *Megaleia rufa* is an all or nothing strategy : only females in good conditions keep lactating young. Results of these studies showed that litters with substandard growth rates are not viable, permitting comparison with optimum growth rates of laboratory reared young. Shield and Woolley (1961), studying *Setonix brachyurus*, concluded that relative growth rates are similar for field and laboratory animals. Delaney and De'ath (1990) did not find significant differences between growth curves of laboratory (Close and Bell 1990) and field captured *Petrogale assimilis*. Ealey (1967) studying *M. robustus* and Inns (1982) studying *M. eugenii* showed that periods of harshness can delay growth of young about to be weaned. This suggests that the condition of the female can be important in adverse environmental circumstances. Inns (1982) pointed out that for normal rainy seasons, age determination of fied marsupials using growth curves obtained in laboratory is accurate for pouch young. Therefore, there are several pieces of evidence in the literature that show that growth patterns established from laboratory animals are useful for age estimation in pre-weaning phase.

The error estimates for several proposed methods of marsupial age estimation during the pre-weaning phase are very dissimilar. Inns (1982) obtained for *M. eugenii* an average error varying between 5 and 15 days. Murphy and Smith (1970) obtained for head, hind leg and foot of *Protemnodon eugenii* a maximum average error of 9 days, representing 5 % of weaning age. The maximum associated error for development of morphological traits during the pre-weaning phase of *P. opossum* in this study is 5 days, less than 10 % of weaning age. The remaining equations produced estimates with even smaller variances. Thus, the methods proposed in this study are comparatively efficient for evaluating pouch young age.

During the post-weaning phase, high growth rates of pouch young are gradually replaced by lower growth rates until stabilization. However, it was seen that the young *P. opossum* doubles its size and increases ten times its body mass between the end of the weaning phase and growth stabilization. These results are similar to those found by Poole *et al.* (1982b) for *Macropus fuliginosus*. When growth decreases, the methods for age determination relying upon body measurements become less efficient (Poole *et al.* 1982a).

The stabilization of growth in *P. opossum* is followed by an increase in the variance of size, getting the curve fitting difficult. This increase coincides with the eruption of the 4th molar. Therefore, it is pratical to use this tooth as a marker of the limit of body growth. The sequence of tooth eruption (Newsome *et al.* 1977, Kirkpatrick 1964), tooth wear (Winter 1980) and deposition of cementum rings (Pekelharing 1970) are well known methods for age estimation. Rose (1989) considers age estimation based on dentition as the most accurate for age determination during the post weaning phase. This accuracy is reduced for the last tooth since its eruption is slower. Cowan and White (1989) evaluated as satisfactory age estimation by tooth wear, and they emphasize the convenience of this method in the field, avoiding sacrifice. Tyndale-Biscoe and Mackenzie (1976) developed an age classification system for *Didelphis marsupialis*. The method relies on the presence and functionality of maxilary teeth from the 3rd decidual premolar to the fourth molar. It is a good method for relative age estimation of Didelphidae (Bergallo and Cerqueira in press, Cerqueira 1984, Fonseca and Cerqueira 1991, Tribe 1990).

After weaning, the best age estimate for *P. opossum* has been obtained with the dentition traits until eclosion of the fourth upper molar. This occurred on average at day 234 (SD = 15 days). Functionality of this tooth occurred on average at day 375 days (SD = 51). The interval between the two stages is nearly 150 days, its utilization being unpractical. Thus, animals captured with the 4th molar ecloding or not functional are between day 219 and 324. Those with the 4th molar functional are older then 324 days.

The applicability in the field of growth curves for the period after weaning was tested by Read (1986, apud Read 1987), who showed that for seasons with normal temperature and rain, growth rates are similar for *Planigale tenuirostris*. He considers as well that growth rates can be optimized in laboratory animals that are not exposed to rough environmental conditions. Anyway, growth curves for laboratory reared animals are approximations that provide useful age estimates for demographic studies.

The maximum error given by the age estimation method for *P. opossum* in this work is 14 days. This error may be considered unimportant for the determination of a marsupial reproductive season. Yet, *P. opossum* shows a life expectancy of at least 400 days in captive conditions, making the error given by the method also acceptable in the elaboration of age classes.

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