



"The reremouse hating light flyeth in the even-tide with breaking
and blenching and swift moving, with full small skin of her wings."

Bartholmew (Berthelet) bk. xii 38.

T
GROWTH AND REPRODUCTION IN THE FRUIT BAT
Epomophorus wahlbergi /

by A
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PREFACE

The experimental work described in this thesis was carried out initially in the Department of Zoology, University of Natal, Pietermaritzburg and subsequently in the Department of Biological Sciences, University of Natal, Durban and the Institute of Natural Resources, University of Natal, Pietermaritzburg, from February 1977 to December 1983 under the supervision of Professor J Hanks.

These studies represent original work by the author and have not been submitted in any form to another University. Where use was made of the work of others it has been duly acknowledged in the text.



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ABSTRACT

This study was carried out on the Natal South Coast between 30° 12' S and 30° 19' S, where 1085 Epomophorous wahlbergi were caught by mist-netting, and a captive breeding colony was kept between June 1977 and February 1982.

Age determination techniques were developed using three criteria; linear growth measurements to obtain the age of animals prior to the attainment of the growth asymptote, tooth eruption to determine the age of animals prior to the attainment of a full permanent dentition, and tooth wear in combination with birth-pulse timing to obtain the age of animals after the growth asymptote and full permanent dentition had been reached. Stevens asymptotic growth curve, performed on forearm length, eye-nose distance and zygomatic width, observations of tooth eruption in cage born bats and linear regression of tooth height data provided the basis for age predictions. Age structure of the population indicated that the greatest percentage of losses occurred between the 5-10 and 10-15 month age group in both sexes. A maximum ecological longevity of nine years is suggested.

A killed sample of 81 males and one live caged male provided the basis for the male reproductive study. Puberty onset occurred at eight months and sexual maturity attainment at 13-17 months. Sperm was present throughout the year and no seasonal variations in testicular or gonadal parameters occurred. However, seasonality was shown in body mass, blood testosterone levels, epaulette hair length, calling and testes position. The unusual and constant presence of spermatocytes/spermatids in the epididymis cauda was observed and meiotic abnormality suggested as an explanation. Epaulettes have an attractive function for females during the mating period, and calling probably acts as a means of year-round territory maintenance. Lek mating is considered possible and a seasonal change in behaviour during the mating season from male groups to individual callers may occur.

Five hundred and fifty three captured and released females and a killed sample of 111 females provided the basis for the female reproductive investigation. Puberty commenced at 2,5 months and sexual maturity was attained at six months. The first proestrus onset took place at a mean age of 5,6 months and first conception at 6,2 months. The species exhibits a seasonally polyoestrous pattern with an extended season. Conceptions occur from May to December, the peak months being May, June and July. Births occur from October to June with the peak birth season in November and December. The majority of females undergo one pregnancy per year terminating in November/December with a small percentage terminating around April.

Primordial, primary, secondary and early vesicular follicles were present in

the ovary year round. The presence of intermediate and late vesicular follicles however followed a seasonal pattern covering April to November. Increased uterine epithelial height and endometrial gland numbers followed a bimodal pattern.

Oestrus can occur in the absence of a male and conception peaks coincided with shortest daylength, lowest rainfall, temperature and humidity. Fruiting and rainfall are suggested as ultimate causes of breeding as the peak in lactation coincided with rainfall and fruiting maxima.

A series of timetables showing variations in the timing of the annual reproductive cycle with one and two pregnancies is presented. A precaution against abortion and preparation for a postpartum pregnancy were used to explain the presence of intermediate and late vesicular follicles in the non-luteal ovary at the beginning and end of pregnancy.

Ovaries and uterine horns showed a functional dextral dominance although anatomically left and right appear symmetrical. Alternation of ovarian function between left and right may occur in those animals undergoing two annual pregnancies. A localized endometrial reaction may occur but was not confirmed. No transovular migration takes place. The corpus luteum reached maximum size at the beginning of pregnancy and was absent at the end. E. wahlbergi was found not to fit into the usual pattern of autumn conceptions and spring births. The species showed a high fecundity rate for a monotocous species and the gestation period was long for its body size.

Parturition, lactation, maternal care and juvenile behaviour were observed in the captive colony. A method for inducing birth, using Prostaglandin E_2 and Oxytocin was applied. Births took place during daylight hours. The labour posture was head down and delivery occurred after three hours of labour. Fetal presentation was head first and placental delivery was delayed until approximately two hours after birth. Placentophagia was observed. The infant is born dorsally furred, with eyes closed and large muzzle, weighing up to 20,3 % of the mother's postpartum mass.

Mother/infant behaviour and juvenile behaviour up to flight initiation is described. Competent flight takes place at a mean age of 81,1 days. Milk is higher in carbohydrate content and lower in protein content than previously studied insectivorous bat milk. A correlation is suggested between head first delivery presentation, delay in placental delivery, appearance of the infant at birth and those species which carry their young in flight instead of leaving them behind in a nursery.

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CHAPTER ONE

INTRODUCTION

1.1 THE ORDER CHIROPTERA

1.1.1 Origins, evolution and palaeontology

All members of the order Chiroptera possess the unique ability among mammals of true powered flight. This has enabled them to utilize a medium unexploited by others of the class. Their success in this is evidenced by the fact that there are 175 genera and 875 species (Jepsen, 1970) second only to rodents in the class Mammalia. Jepsen (1970) remarked: 'no other grade of mammal has ever had so many eccentric and extremely specialized characteristics combined into such a highly successful organism'.

From where did bats originate and by what stages did they become so successfully volant? Despite a lack of evidence in the form of fossil clues, there seems to be little diversity of speculation about these answers. Most of the concerned authors believe that some arboreal insectivore, perhaps a small population group, passed tachytelicly through a gliding phase and quickly perfected the structures for flight.

Jepsen (1970) suggests a three stage theory of evolution. Stage one was the imaginary 'pre-bat'; a small inconspicuous insectivore-like nocturnal inhabitant of ground-bush-tree regions in temperate latitudes. It was omnivorous and by means of small leaps caught its prey in large webbed front feet. In stage two the webbing on the front feet had extended and was used to catch flying prey. Skin flaps joined the fore-limbs to the sides of the body enabling the 'sub-bat' to be briefly sustained in the air by flapping. The hind-foot took over some of the functions formerly performed by the fore-limbs such as grooming, food grasping and holding. Food specialization began, some forms possibly turning to fruit, initially as a source of worms and grubs. With dietary changes new dental types appeared, along with various modifications and developments in limb and tail structures. Fruit bats require freedom of hind-limbs in order to hang by one foot and manoeuvre fruit to the mouth. Reduction of the tail and uropatagium was an obvious modification. Stage three was a continuation in refinement to the present condition and a diversification of habitats, diets and behaviour.

Such theories may suggest answers to the questions 'Where from?' and 'How?', but fossil evidence is available to at least partially answer 'When?'. The single specimen Icaronycteris index, discovered in the

fossil-fish marlstones of the Green River formation, southwest Wyoming, is at present the oldest bat (Jepsen, 1966; 1970). The deposits are lower Eocene which dates the specimen at ± 50 million years. Remarkably complete, the skeleton has provided valuable information for comparative osteology with modern chiropts and clues for speculation on the specimen's mode of flight, diet, sex and age.

I. index was a true flyer and is not considered a linking fossil between a non-volant ancestor and volant descendants. A number of its characteristics are thought primitive and lacking in specialization, but a few are especially characteristic of the present-day Megachiroptera (fruit bats); long nasal bones, palate projected rearwards slightly beyond the posterior molars, ascending process of the dentary broad antero-posteriorly with high rounded superior border and digits one and two both terminating in a claw (Jepsen, 1966). I. index combined features which are widely distributed among living bats, but has been placed by taxonomists in the suborder Microchiroptera. Without more fossil evidence the evolutionary position of this one specimen is uncertain and Jepsen (1970) remarked: 'I. index may have been directly ancestral to all or to some living microbats and megabats, or to none of our contemporary chiropts'.

The origins of modern Megachiropterans may have been much later. The oldest known fossil megabat, Archaeopteropus transiens, is 35 million years old from the Oligocene (Anderson, 1912; Dal Piaz, 1937; Meschinelli, 1903). It is thought to have had a wingspread of about one metre, a long tail, well developed calcars, a long clawed index finger and teeth with pointed cusps.

1.1.2 Classification

The classification of Chiroptera is as follows (Young, 1962):

Class	:	Mammalia
Subclass	:	Theria
Infraclass	:	Eutheria
Cohort	:	Unguiculata
Order	:	Chiroptera

The oldest Eutherian fossils are insectivores from the upper Cretaceous and probably gave rise to all other orders of Eutheria (Matthews, 1969). Those orders which showed the closest affinities to the primitive insectivore stock were grouped by Simpson (1945) under the cohort Unguiculata.

The Chiroptera are characterized by a patagium involving all the digits of the hand except the first, and extending along the sides of the body to include the legs and usually the tail but not the feet. The radius, ulna and particularly the phalanges are elongated and a keeled sternum is present for pectoral muscle attachment. There is fusion of the sternum, clavicle and scapula in many species and the pelvis is rotated so that the acetabulum lies dorsally, positioning the hind-limbs outward and upward. The hind-legs are weak and the feet have five curved, clawed digits. A wing claw is present on the first digit of the fore-limbs and respiration is diaphragmatic.

The order is further divided as follows (Walker, 1975):

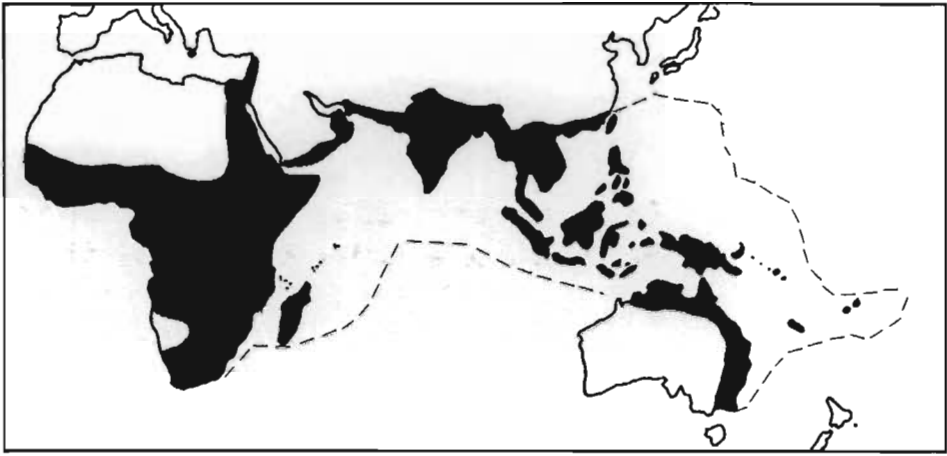
Order : Chiroptera
 Suborder : Megachiroptera
 Family : Pteropodidae
 Suborder : Microchiroptera
 Family : Rhinopomatidae
 Emballonuridae
 Noctilionidae
 Nycteridae
 Rhinolophidae
 Megadermatidae
 Hipposideridae
 Mormoopidae
 Phyllostomatidae
 Desmodontidae
 Nataiidae
 Furipteridae
 Thyropteridae
 Myzopodidae
 Vespertilionidae
 Mystacinidae
 Molossidae

1.1.3 Megachiroptera with reference to South African species

The suborder Megachiroptera has one family Pteropodidae, known as Old World fruit bats or flying foxes. The family has 39 genera and approximately 130 species found in the tropical and subtropical regions of the Old World east to Australia, Samoa and the Caroline Islands (Walker, 1975; (FIGURE 1).

Megachiropteran characteristics are a simple funnel shaped ear with no tragus, large well developed eyes, long papillated tongue, simple teeth

FIGURE 1 Geographic range of the suborder
Megachiroptera (after Kingdon, 1974)



with longitudinally grooved molars, a deeply ridged palate for fruit crushing, wing claw on the second digit (except in Dobsonia, Eonycteris, Neopteryx, Nesonycteris and Notopteryx), a tail which is either short, rudimentary or absent (except Notopteryx) and the uropatagium present only as a narrow band not joining the legs and tail as in the Microchiroptera.

On the African continent there are 12 genera of megabats, comprising 28 species (Meester & Setzer, 1971; Bergmans, 1980) and a further genus of nine species inhabits the tropical Indian Ocean Islands between the East African coast and longitude 65° E (TABLE 1).

Of these 12 genera, nine consisting of 17 species are found in the southern African subcontinent (Angola, Botswana, Malawi, Mozambique, Namibia, Republic of South Africa, Zambia and Zimbabwe), Angola having the greatest diversity of fruit bat fauna with 14 of these species (Meester & Setzer, 1971). In the Republic of South Africa where this research was carried out, two resident genera occur, Epomophorus and Rousettus.

Epomophorus is represented by two species. Epomophorus wahlbergi (Sundevall, 1846) Wahlberg's epauletted bat occurs from the Northern and Eastern Transvaal in the north, along the eastern coastal belt to Plettenberg Bay, Cape Province, in the south (for authority see 1.2.3). Epomophorus crypturus (Peters, 1852) Peters' epauletted bat is recorded from the Eastern Transvaal lowveld (Rautenbach, 1982) and from Natal, South Coast region (Cowles, 1936). (During the course of this study however no specimens of E. crypturus were collected).

Rousettus aegyptiacus (Geoffroy, 1810) the Egyptian fruit bat is found throughout the Republic to the Cape Province, choosing large roomy caves for roosting where the temperature fluctuations are at a minimum (Jacobsen & Du Plessis, 1976).

Eidolon helvum (Kerr, 1792) the straw coloured fruit bat has occasionally been reported as occurring in the Cape Province (Herselman & Hanekom, 1978), Natal (Sapsford, pers. comm.), the Orange Free State and the Transvaal (Roberts, 1951). Kingdon (1974) and Roberts (1951) attribute its presence in South Africa to its migratory habits.

1.1.4 The genus Epomophorus (Bennett, 1836), Genotype Pteropus gambianus (Ogilby, 1835).

The genus Epomophorus of eight species, inhabits Africa south of the Sahara, ranging throughout the woodland savanna regions, but not found at altitudes above 2000 m (Kingdon, 1974).

Two species E. wahlbergi and E. crypturus extend southwards into South

TABLE 1 Megachiropteran species of Africa and the Indian Ocean Islands lying between the East African coast and longitude 65° E (after Meester, Setzer, 1971; Bergman, 1980)

<u>Casinonycteris argynnis</u>	<u>Nanonycteris veldkampi</u>
<u>Eidolon helvum</u>	<u>Plerotes anchietai</u>
<u>Epomophorus angolensis</u>	<u>Pteropus aldabrensis</u>
<u>Epomophorus anurus</u>	<u>Pteropus comorensis</u>
<u>Epomophorus crypturus</u>	<u>Pteropus livingstoni</u>
<u>Epomophorus gambianus</u>	<u>Pteropus niger</u>
<u>Epomophorus labiatus</u>	<u>Pteropus rodricensis</u>
<u>Epomophorus pousarguesi</u>	<u>Pteropus rufus</u>
<u>Epomophorus reii</u>	<u>Pteropus seychellensis</u>
<u>Epomophorus wahlbergi</u>	<u>Pteropus subniger</u>
	<u>Pteropus voeltskowi</u>
<u>Epomops buettikoferi</u>	
<u>Epomops dobsoni</u>	<u>Rousettus aegyptiacus</u>
<u>Epomops franqueti</u>	<u>Rousettus angolensis</u>
	<u>Rousettus lanosus</u>
<u>Hypsighathus monstrosus</u>	
	<u>Scotonycteris ophiodon</u>
<u>Megaglossus woemanni</u>	<u>Scotonycteris zenkeri</u>
<u>Micropteropus grandis</u>	
<u>Micropteropus intermedius</u>	
<u>Micropteropus pusillus</u>	

Africa along the eastern coastal belt, centrally through Zaire and Zambia and across to the west along the northern coastal regions of Angola (Meester & Setzer, 1971).

Epomophorus angolensis (Gray, 1870) is a southwestern species occurring only in southern Angola and Namibia (Meester & Setzer, 1971).

Epomophorus gambianus (Ogilby, 1835) and Epomophorus anurus (Heuglin, 1864) are found across central Africa; E. gambianus particularly from the west; Senegal, Mali to Zaire and Ethiopia and E. anurus particularly from the east; Tanzania, Kenya, Sudan to Zaire (Kingdon, 1974; Meester & Setzer, 1971).

Epomophorus labiatus (Temminck, 1837) is a central eastern species found from Ethiopia, south to Zambia and Malawi (Meester & Setzer, 1971).

Two species, Epomophorus reii (Aellen, 1950) and Epomophorus pousarguesi (Trouessart, 1904) have limited distributions.

E. reii is found only in northern Cameroun (Meester & Setzer, 1971) and E. pousarguesi from the Bangui district of the Central African Empire (Bergmans, 1978a).

1.2 EPOMOPHORUS WAHLBERGI

1.2.1 Taxonomic history

E. wahlbergi was first described by Sundevall in 1846 under the synonym Pteropus wahlbergi from a collection of southern African mammals made by Wahlberg (PLATE 1).

Epomophorus was first proposed as a genus separate from Pteropus because of the backward placing of its wings ('as almost to seem to be placed behind the centre of gravity') and its epaulettes (Bennett, 1836). This proposal was preliminary, based on an example of what is now called E. gambianus, but later accepted as the first description of a new genus. This did not prevent Sundevall in 1846 describing a new and apparently epomophorine species as Pteropus wahlbergi. Peters (1867) was the first to recognize that Sundevall's P. wahlbergi actually belonged to the genus Epomophorus.

Anderson (1912) recognized two subspecies, E. wahlbergi haldemani (Halowell, 1846), occurring in the northern and western parts of the species range (Meester & Setzer, 1971) and E. wahlbergi wahlbergi

PLATE 1 The species Epomophorus wahlbergi



(Sundevall, 1846), present mainly in the southern and eastern parts of the range (see 1.2.3) (Meester & Setzer, 1971). Anderson (1912) traces the history of these two subspecies and presents a list of their synonyms.

1.2.2 Description

The species attains a maximum mass of 150 g and a maximum forearm length of 90 mm. Its colour ranges from grayish brown to light buff brown with characteristic tufts of white hair in front of and behind the ears (PLATE 2). The ears are erect, rounded and hairless and the eyes are large and ranging in colour from golden brown to greenish brown. The maximum wingspread is 500 mm, the patagium stretching between digits two and five. Digits one and two terminate in a wing claw (PLATE 3). A vestigial external tail is present beneath the interfemoral membrane. The feet have five toes bearing long curved claws which act as efficient hooks for hanging from branches.

Sexual dimorphism is apparent, the adult males being larger and heavier than the females and bearing loose eversible pouches, containing long white hairs on the shoulders (PLATE 4). From this the genus gets its common name of 'epauletted fruit bat'. A ruff of darker fur, reddened almost naked throat region and large folded pendulous lips distinguish the males. When reproductively active the testes are scrotal.

The females have one pair of pectoral mammae with nipples situated subaxially.

The skull is elongated, expanding posteriorly with a shallow dentary (PLATE 5). The dental formula is (Grassé, 1955):

$$I \frac{2}{2} \quad C \frac{1}{1} \quad P \frac{2}{3} \quad M \frac{1}{2}$$

The teeth have closed roots and are specialized for carrying fruit in flight and crushing and pulping it to extract the juice. Tiny incisors are present anteriorly, large curved, pointed canines, premolars with sharp longitudinal cusps and positioned posteriorly are the broad, slightly flattened molars. A single palate ridge beyond the tooth row distinguishes this species from others of the genus (Meester & Setzer, 1971). A simple alimentary canal showing little regional differentiation, is consistent with a liquid diet of fruit juices.

1.2.3 Distribution

Information concerning collecting localities and site records obtained from the sources listed below and from material examined in the Kaffrarian Museum, King William's Town and the Natural History Museum, London, enabled

PLATE 2 Epomophorus wahlbergi showing the characteristic tufts of white hair in front of and behind the ears, present in both species



PLATE 3 Epomophorus wahlbergi showing the wing claw
present on digit one



PLATE 4 Epaulette in the male Epomophorus wahlbergi,
consisting of a loose eversible pouch bearing
long white hairs



PLATE 5 Skull and lower jaw of Epomophorus wahlbergi
(x 2)



a distribution map for E. wahlbergi to be compiled (FIGURE 2).

The northernmost points from which material has been collected are Caitoi in the Somali Republic (Funaioli & Lanza, 1968) and Karamoja district of Uganda (Kingdon, 1974). The species occurs in central and southern Kenya (Anderson, 1912; Kingdon, 1974), eastern Uganda (Kingdon, 1974), the southern tip of Burundi (Bergmans, pers. comm.), Tanzania (Anderson, 1912; Kingdon, 1974; Rautenbach, pers. comm.), the northern and eastern regions of Zambia (Ansell, 1960; Bergmans, pers. comm.), Zimbabwe (Smithers & Wilson, 1979), Malawi (Chimimba, pers. comm.), Mozambique (Bergmans, pers. comm.; Rautenbach, pers. comm.), Northern and Eastern Transvaal (Lyster Jameson, 1909; Rautenbach, 1982; Jacobsen, pers. comm.), Swaziland (Rautenbach, pers. comm.) and down the Natal coastal belt (Bergmans, pers. comm.; Bourquin, pers. comm.; Rautenbach, pers. comm.) to the southern limit so far collected, Keurboomsrivier, near Plettenberg Bay in the Cape Province (Herselman, pers. comm.).

The range also extends across southern Zaire (Hayman, Misonne & Verheyen, 1966), to the Angolan coast as far south as the Lucira district (Anderson, 1912; Bergmans, pers. comm.) and northwards through Cabinda (Anderson, 1912), Congo (Bergmans, 1979; Malbrant & McClatchy, 1949), to its northern limit on the west coast, Port Gentil in Gabon (Allen, Lang & Chapin, 1917; Anderson, 1912; Malbrant & McClatchy, 1949).

Bergmans (1979) expressed doubts concerning the identification of the immature specimen from southwest Cameroun (Anderson, 1912). Subsequently he has seen more material from this country, but no specimens of E. wahlbergi have been identified (Bergmans, pers. comm.). For this reason the Cameroun locality has not been incorporated into the distribution map.

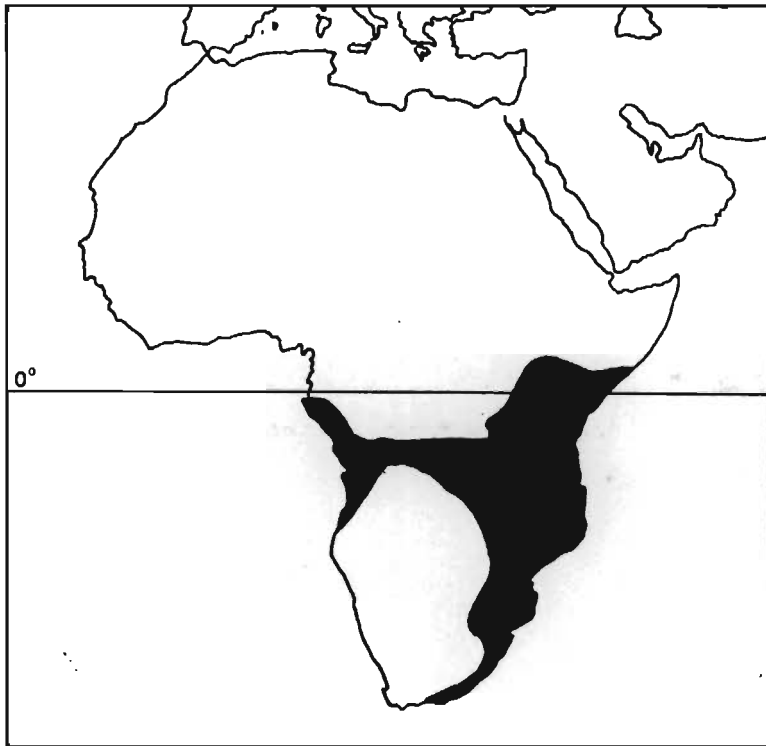
The specimen from northern Zaire, mentioned by Hayman et al. (1966), had been incorrectly identified (Bergmans, pers. comm.). Recent collecting expeditions to the Ivory Coast (Bergmans, Bellier & Vissault, 1974), Nigeria (Happold & Happold, 1978), Central African Empire (Vielliard, 1974), Rio Muni (Jones, 1971), Sudan (Kock, 1969) and Ethiopia (Largen, Kock & Yalden, 1974) have not found this species.

1.2.4 Ecology

1.2.4.1 Habitat

E. wahlbergi is a savanna, woodland and forest margin species (Kingdon, 1974), roosting during the day in a variety of situations. Wickler & Seibt (1976) found them roosting on the midribs of coconut palms (Cocos nucifera) and on the underside

FIGURE 2 Distribution of Epomophorus wahlbergi
(compiled from information sources listed in
1.2.3)



of thatched roofs. Kingdon (1974) mentioned the main ribs of banana leaves (Musa sp.), mango (Mangifera indica) and other thickly foliated trees, as favourite roost sites.

Roost sites seem to be chosen for their ability to provide shelter from wind and rain, a good view of the ground below and inaccessibility to tree climbing predators. In the study area (2.3.1.7) favourite roosts were underneath the upper canopy of large leaved figs (Ficus vogelii), strangler figs (Ficus natalensis), midribs of palm fronds of the Cuban Royal Palm (Roystonea regia) and occasionally in Jacaranda trees (Jacaranda mimosifolia).

1.2.4.2 Behaviour

(a) Roosting

The species roost in groups of three to 100 individuals (Kulzer, 1962), preserving a distinct interpersonal distance (Wickler & Seibt, 1976). Wickler & Seibt reported that they establish principal roosts for many years in suitable areas and have preferences for special places within the roost area. Departure times from, and return to the roost are remarkably constant (Wickler & Seibt, 1976) and observations made during the course of this work concur with this, though departure times vary with time of sunset.

(b) Comfort behaviour

While roosting, the animal hangs motionless by one or both feet, either with the snout clearly visible and the eyes open or closed, or with the snout drawn in to the belly and the face and eyes covered by the wing membrane.

Grooming occurs in bouts of two to three minutes, in which the bat carefully nibbles and licks the fur, thumb claws, ears and wing membranes. The hind-foot acts as a comb raking through the fur.

Frequent stretching of the wings occurs during grooming activity bouts (Wickler & Seibt, 1976). Periodically, the roosting bats have been seen to swing gently from side to side with no other associated movement (Wickler & Seibt, 1976).

(c) Flight

Flight in this Megachiropteran is slow and wing contacts with obstacles occur (Wickler & Seibt, 1976). Kingdon (1974) remarks on their low level of flight and the infrequency with which they rise above the tree canopy. Wickler & Seibt (1976) noted that when taking off, the bats may simply 'drop' from the roost with a slight preparatory opening of their wings. Landing is usually accomplished by simply hooking the wing claws onto the midrib or twig with the last wing stroke. The feet are then swung upwards to take a firm grip while the wing claws relinquish their hold. A few 'steps' with the feet, to find a convenient position, completes the manoeuvre.

(d) Calling

Male Epomophorus and other related genera (Epomops, Micropteropus and Hypsignathus) are known for their habit of emitting loud, metallic calls (Aellen, 1952; Bradbury, 1972; Brosset, 1966; Allen, Lang & Chapin, 1917; Rosevear, 1965). Dobson (1881) described the epomophorine larynx as long, capacious with ossified walls.

Wickler & Seibt (1976) studied the behaviour of male E. wahlbergi associated with calling. Shortly after leaving the roost, the males start calling, often from nearby trees. During continuous calling, the animal may turn from one side to the other with slightly opened, quivering wings, briefly closing with each sound emission. Wickler & Seibt also observed epaulette eversion and hair erection during the process of calling.

1.2.4.3 Feeding

Epomophorus feeds on many wild fruits, particularly of the family Moraceae (Walker, 1975; Wickler & Seibt, 1976) and on soft cultivated fruits, such as mango, guava (Psidium guajava), bananas, peaches (Prunus persica) and pawpaws (Carica papaya) (Kingdon, 1974). Wickler & Seibt (1976) provide a list of indigenous fruits which they saw the species feeding on in Kenya.

The fruit is picked, either by landing next to it, or hovering alongside. It is carried in the mouth to a perch some distance from plucking. During eating the bat hangs by one foot using the other and the wings to guide the food to the mouth. The

juice is extracted by crushing between the teeth, and the skin and seeds spat out. Wickler & Seibt (1976) never observed fruit debris beneath roost sites and assumed that food was never consumed in the roost.

Bergmans (1978b) critically reviewed reports of drinking behaviour in Megachiroptera. Rousselot (1950) related how he saw E. gambianus fly so low over the surface of a river that it could wet its ventral fur and so lick off the water afterwards. The same observations have been made in this study with E. wahlbergi particularly over swimming pools. Bergmans however concludes that there is insufficient evidence from careful observations to state that this behaviour is for the purpose of drinking.

1.2.4.4 Reproduction

The breeding patterns of some African Megachiroptera have been well documented: Eidolon helvum by Fayenuwo & Halstead (1974) and Mutere (1967); Epomops franqueti by Okia (1974 a); Rousettus aegyptiacus by Mutere (1968); but little information is available for the genus Epomophorus. Okia (1974 b) found E. anurus to have two distinct breeding seasons, gestation lasting April to September and October to March in Uganda. O'Shea & Vaughan (1980) found volant young, pregnant and lactant female E. wahlbergi continually evident from November to May in Kenya. In the Congo, Anciaux de Faveaux (1972) concluded a biannual cycle for this species with birth periods in March and in October or November, but did not exclude the possibility of a continuous polyoestrous reproduction.

1.2.5 Economic importance to man

1.2.5.1 The fruit industry

There are numerous reports of damage by bats to fruit crops throughout the Old World (Cheema, Bhat & Naik, 1954; Malzy & Jagord, 1960; Medway, 1965; Rosevear, 1965).

In South Africa damage to litchi orchards (Nephelium litchi) during the brief fruiting season (Jacobsen & Du Plessis, 1976; Porter, pers. comm.) was mainly attributable to Rousettus aegyptiacus and occasioned a study of their ecology in the Eastern Transvaal (Jacobsen & Du Plessis, 1976). Netting in litchi orchards during the course of this study showed both E. wahlbergi and R. aegyptiacus were responsible in the Port

Shepstone area of Natal. Although in Israel extensive damage to commercial fruit crops by bats has caused the government to adopt stringent control measures of Rousettus populations (Stebbing, pers. comm.), Constantine (1970) concluded that the harm done to the fruit industry generally is of little consequence except in isolated instances.

1.2.5.2 Diseases transmitted by fruit bats

Constantine (1970) extensively reviewed the presence and transmission of disease in bats. Megachiroptera in Africa have been found to carry several viruses, of which only one, Yellow Fever Virus found in Eidolon helvum (Williams, Simpson & Shepherd, 1964), in Epomophorus sp. (Andral, Bres, Serie, Casals & Panthier, 1968) and in Rousettus (Simpson, Williams, O'Sullivan, Cunningham & Mutere, 1968) is considered possibly to play a role in the epidemiology of the disease.

There is only one known instance of the presence of rabies in Megachiroptera (Smith, 1967), although in 1980 several specimens of E. wahlbergi in Natal were thought to be carrying the disease (Standing, pers. comm.). The virus was however later identified as Lagos Bat Virus (Standing, pers. comm.) as first described in Nigeria by Boulger & Porterfield (1958).

1.3 OBJECTIVES OF THIS STUDY

The main objective of this study was to investigate aspects of reproduction in colonies of the epauletted fruit bat from the area between the Mkomazi River and Park Rynie on the Natal South Coast. The study was initiated in the light of limited reproductive data being available for the genus Epomophorus in general and E. wahlbergi in particular. The specific objectives were as follows :

- (a) To investigate the breeding cycle in males with particular emphasis on seasonal variations in testes and epididymides size and mass, and blood testosterone levels, and to study variations in epaulette condition and in calling throughout the year.
- (b) To investigate the breeding cycle in females; to describe ovarian development from birth to maturity and throughout the reproductive cycle, to determine time of ovulation and fertilization, length of gestation, time of parturition, and lactation, and to describe

maternal care.

- (c) To determine the age at onset of puberty and attainment of sexual maturity in both sexes and to test the hypothesis that age at puberty in both sexes is related to body growth.
- (d) To determine the proximate and ultimate factors which might influence seasonal breeding.

CHAPTER TWO

STUDY AREA

2.1 INTRODUCTION

2.1.1 Location

This research was carried out in an area situated approximately 50 km southwest of Durban on the upper South Coast of Natal, Republic of South Africa. The region lies between the Mkomazi River to the north ($30^{\circ}12' S$) and Park Rynie to the south ($30^{\circ} 19' S$), extending inland to $30^{\circ} 43' E$ and covering an area of approximately 80 km^2 (FIGURE 3). FIGURE 3 also shows the position of collecting sites in the study area.

Bioclimatically the region is classified as coastal lowland, humid/humid-subhumid, evergreen forests, short forest and thicket with various successional stages, within the seasonal temperature range of Hot-Warm to Warm-Mild (Loxton, Hunting & Associates, 1971; Phillips, 1973).

2.1.2 History

The South Coast was originally inhabited by neoanthropic people engaged in hunting. Traces of their habitation of rock-strewn beaches are abundant in the form of kitchen middens composed of discarded mollusc shells. They used the beaches as harvest grounds for shellfish upon which they subsisted in the arid seasons. Coarse unglazed pottery and awls, belonging to a period prior to the arrival of the Bantu, testify to their existence (Natal Town & Regional Planning Commission, 1974).

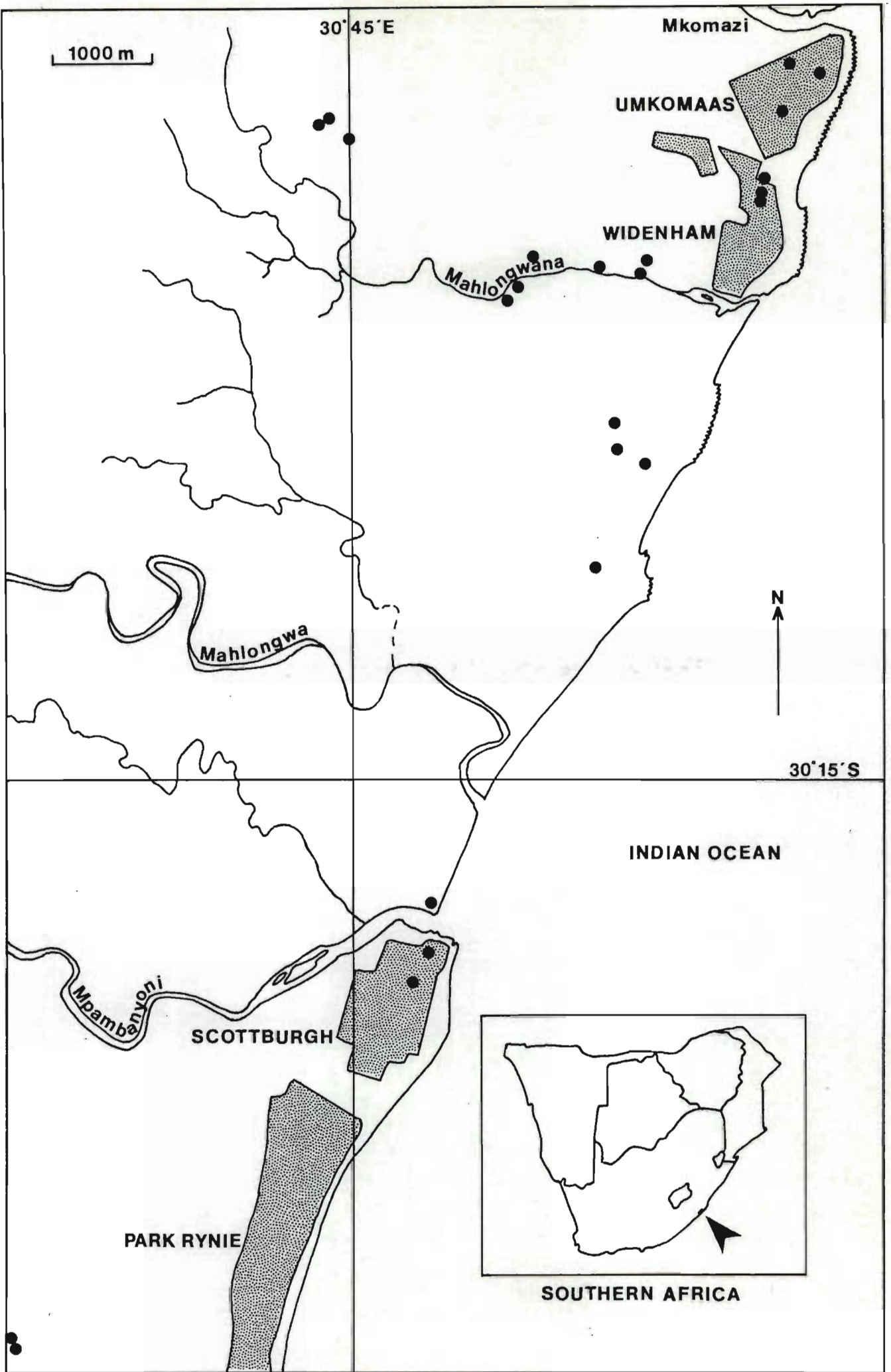
Concrete evidence of the Bushmen habitation is scanty, but it is thought that they dwelled in the region, living on game and indigenous berries and roots. They once roamed over a large area, but as a result of subsequent frequent invasions, gradually disappeared (Natal Town & Regional Planning Commission, 1974).

When the Bantu arrived from 1100 AD onwards, Natal was comparatively empty. Several waves of Bantu tribes migrated from the north to settle in the region, the Nguni being one of the more notable. Their culture revolved around cattle, and crops of maize and millet were grown (Bulpin, 1972; Natal Town & Regional Planning Commission, 1974).

In 1880 - 1882, the Zulus under Shaka invaded Natal, depopulating the land from the Tugela to the Mzimvubu Rivers.

FIGURE 3 The study area on the Natal South Coast, showing its position in relation to Southern Africa

Shaded areas - built-up areas
Black dots - collecting sites



The first significant European settlement occurred in 1824 when traders from the Cape Colony sailed to Port Natal (Durban). Shaka granted them land around the port and they settled there, trading with the natives in ivory, skins and gum. Inevitably this trade expanded and by 1843 the settlement of the South Coast began, after the British annexation of Natal (Natal Town & Regional Planning Commission, 1974).

In the study area, the first land grant was made in 1852 and was that of the farm Clansthal No. 1202, south of Umkomaas. The first township to be surveyed was Scottburgh in 1860 followed by Umkomaas which owes its origin to the navigability of its river. From 1870 to the turn of the century, there was a regular traffic of small coasters plying southward from Durban to Umkomaas, Scottburgh and Port Shepstone (Bulpin, 1972).

The development of the South Coast region followed the usual pattern of early agricultural and mineral utilization. Marble quarrying in Port Shepstone in 1882, led to the further development of harbour facilities. Simultaneously with the realization of these projects, came the emergence of the sugar companies and the subsequent establishment of sugar mills. The next phase in development of the region was the tourist industry, assisted by the arrival of the railway, tarred roads and the increase in private car ownership (Natal Town & Regional Planning Commission, 1974).

2.2 PHYSICAL FEATURES

2.2.1 Geology

The geology of an area plays an important role in determining general topography, drainage patterns, soils and because of the influence of these, vegetation and fauna.

The geology of the South Coast (FIGURE 4) consists of contorted and intensely metamorphosed basement of Archaean schists, gneisses and granites. Younger formations were deposited on top of these and the whole area became flexed to form a very broad asymmetrical anticline (the Natal Monocline), the axis extending from the Mtamvuna River to 16 km south of Melmoth. To the west of the axis, the strata dip at low angles, while to the east the rocks dip with increasing steepness to the sea (Natal Town & Regional Planning Commission, 1974).

Along the axis of the monocline, erosion has exposed granites of the Archaean Era. Towards the coast, the strata dip at a steeper angle than the surface of erosion, exposing successively younger sedimentary rocks in zones parallel to the shore. They disappear under recent coastal sand

FIGURE 4 Geology of the study area compiled from:

A.A. Loudon & Partners on behalf of Zakrewski Associates geological survey for South Coast Freeway for National Transport Commission, (1979).

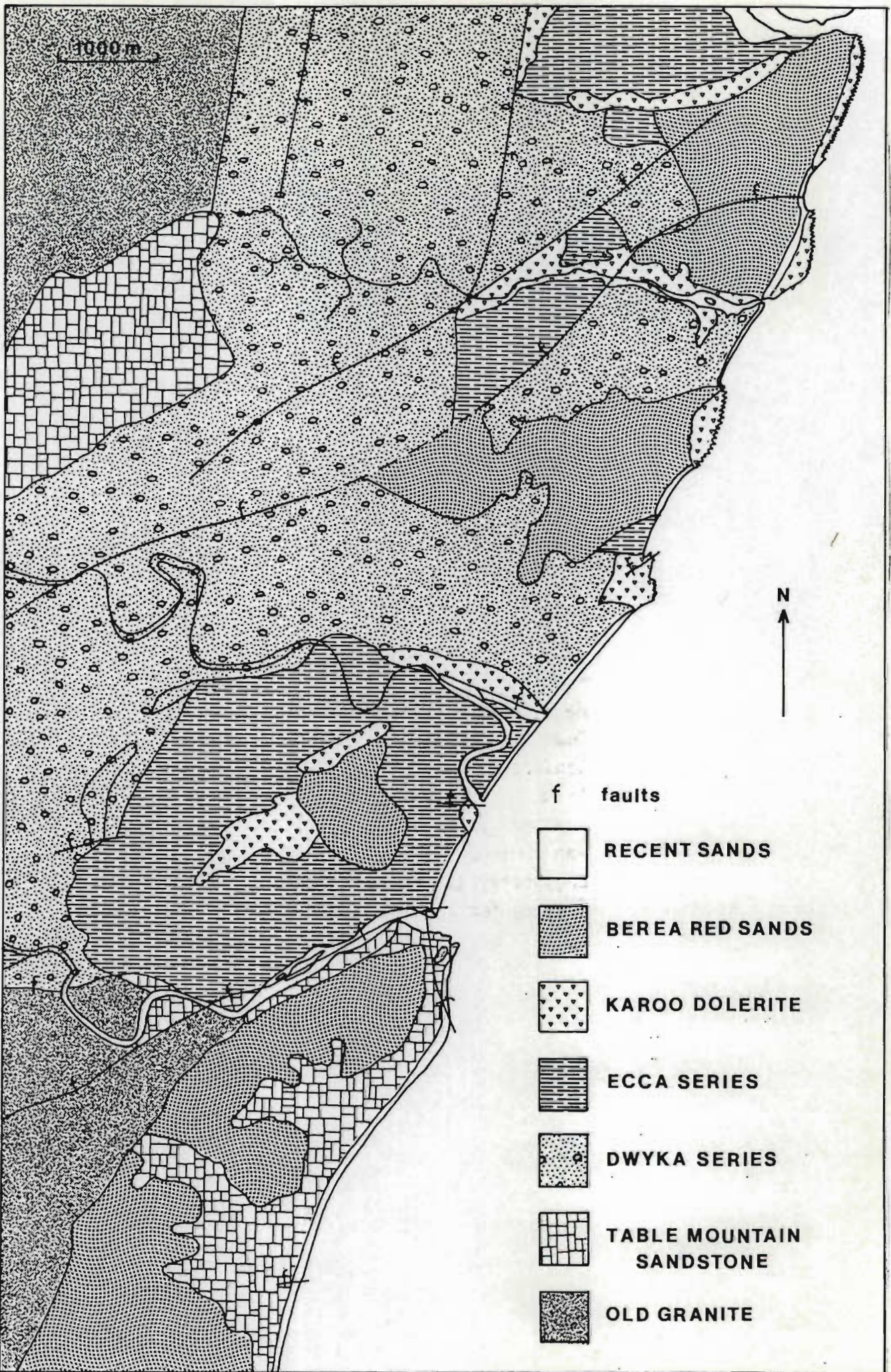
Mason, pers. comm.

Natal Town & Country Planning Commission, (1974).

Natal South Coast, Draft Regional Plan. Natal Town & Regional Planning Report, 29: 1-144.

Segatto, P. (1980) Geology of the Umkomaas Area. Honours Project, Department of Geology, University of Natal, Durban.

Van Niekerk, Kleyn & Edwards, Consulting Engineers, geological survey for South Coast Freeway for National Transport Commission.



deposits and reappear as outcrops along the beach (Natal Town & Regional Planning Commission, 1974).

2.2.1.1 Archaean Complex (2400-3500 million years) (Mountain, 1968)

In the study area the Archaean Complex is represented by the Old Granite, (to distinguish it from granites of other eras). It is mainly migmatite granite, with lesser amounts of granite-gneiss and often has intruded, granitized and engulfed masses of a metamorphosed component of the Archaean Basement, the Primitive Formation (Loxton *et al.*, 1971). The Old Granite is found occurring in the northern and southern inland portions of the study area, approximately west of 30° 44' E.

2.2.1.2 Cape System (350-400 million years) (Mountain, 1968)

In much of the Natal South Coast a massive unconformity of ±2000 million years exists between the granites of the Archaean Complex and the sandstones of the Cape System, which is the youngest system of the Postbushveld-Prekaroo Era (Loxton *et al.*, 1971). The only representative of the Cape System in this area is Table Mountain Sandstone which is found outcropping along the coast south of Scottburgh and in an isolated patch southeast of Umkomaas.

2.2.1.3 Karoo System (150-300 million years) (Mountain, 1968)

The Karoo System comprises four sedimentary series of which the two oldest, Dwyka and Ecca are present in the study area.

(a) The Dwyka Series

The lowermost member of the Karoo System occurring in Natal is the Dwyka Series which occurs in the area mainly as glacially derived tillite but also as light coloured shales and occasionally sandstone. The tillite is dark bluish grey, jointed and consisting of erratics varying in size from 13 mm to 3 m in diameter. It weathers to an ochre yellow coloured soil and is the main rock type in the study area (Loxton *et al.*, 1971).

(b) The Ecca Series

The Ecca Series comprises a thick succession of well-laminated and jointed, dark coloured shales. The largest

outcrop in the area lies between the Mpambinyoni and Mahlongwa Rivers. Other surface outcrops are found between faults upstream of the Mahlongwana River mouth and the south bank of the Mkomazi River.

(c) Karoo dolerite

Igneous intrusions of Karoo age are scattered throughout the study area cutting through all rock successions from the Archaean Basement up to the Ecca shales. They are mainly in the form of inclined sills and the dolerite headlands outcropping between the Mahlongwana and Mahlongwa River mouths are an example of a large sill. The intrusions are frequently found in association with faults and river valleys (Mason, pers. comm.).

2.2.1.4 The Quaternary System (0-3 million years) (Mountain, 1968)

The Quaternary System, mainly represented in the area by Berea Red Sand and Coastal Dunes, lies unconformably on the Karoo Series. The deposits are loose and unlithified occurring only in the immediate vicinity of the coast. The Berea Red Sand is Pleistocene dune sand, calcareous and weathered with a derivation of clay from included feldspar. The colour varies from reddish brown to brown depending on the clay content (Loxton et al., 1971). Younger lighter coloured dunes have developed along the immediate shoreline with little or no clay content, the tallest of these being the Widenham sand dune, south of Umkomaas.

2.2.1.5 Tectonics of the area

The coast area of the Natal South Coast is characterized by extensive gravity faulting of tensional origin of post-Karoo age (0-150 million years) (Loxton et al., 1971).

2.2.2 Soils

The soils of the region draw their basic characteristics from the rocks from which they are derived, but they vary in structure, depth and fertility according to the slope of the land and the climatic conditions under which they were formed. The geological classification for rock types is used therefore as a simplified starting point for the classification of soils (Natal Town & Regional Planning Commission, 1974).

In the study area, soil types correspond very closely to the underlying rocks from which they have formed and for this reason no additional map showing soil type distribution has been included.

2.2.2.1 Soils derived from granite

This is a major soil group on the South Coast and covers approximately 29 % of the region under study. Owing to mineralogical variations in the granite, the resulting soil varies appreciably but in general is highly productive (Beater, 1959).

2.2.2.2 Soils derived from Table Mountain Sandstone

Soils of this category cover 7 % of the study area. They are light greyish, coarse, sandy loams and are considered by Beater (1959) to have poor natural fertility.

2.2.2.3 Soils derived from Dwyka tillite

Tillite is the most abundant rock in the region and soils derived from it occupy approximately 37 % of the area. The soil type is generally associated with poor sugar cane growth despite the fact that it is not infertile. Problems exist with correct tilling as a result of a constant rubble layer formed from the erratics in the weathered tillite (Beater, 1959).

2.2.2.4 Soils derived from Ecca shales

These soils account for about 9 % of the soil cover and are characteristically dark greyish brown to greyish black rubbly loams often found in association with doleritic soils. They are superior to the Dwyka soils in chemical and physical properties, though they show the effect of drought more than any other soil type (Beater, 1959).

2.2.2.5 Soils derived from dolerite

A small part of the region (about 3 %) is influenced by soils derived from the intrusive dolerite, particularly in the upper reaches of the Mahlongwana River and west of Umkomaas. These soils are often extremely fertile chocolate or reddish clay loams, but where leaching has occurred under conditions of high rainfall, fertility is significantly reduced (Beater, 1959; Natal Town & Regional Planning Commission, 1974).

2.2.2.6 Soils derived from Berea Red Sand and alluvium

Berea Red Sands result in poor soils which require intensive fertilization if they are to be used agriculturally (Beater, 1959). They occupy approximately 11 % of the region, and of this about half (51 %) are found in the built-up areas of Umkomaas, Widenham and Scottburgh.

Soils formed from alluvial deposits are notable along the Mahlongwa and Mpambinyoni River valleys but are considered by Beater (1959) to be not very productive as a result of high drainage and consequent lack of moisture in the soil profile. These soils account for about 4 % of the region's topsoil.

2.2.3 Physiography

Faulting, of which there is abundant evidence along the South Coast, has given rise to some rugged topography and erosion is rapid because of the flexed surface of the Natal Monocline. Rivers are short and steep and the resulting vigorous river erosion gives rise to a region of uneven rugged topography with deep valleys. Seaward facing scarps are steeply sloping, a factor seriously limiting land use. The characteristic land form is therefore one of rolling hills with few areas of flat land.

The coastal region, which once presented a smooth land surface was drowned to a depth of 46 m resulting in a very regular coastline (Natal Town & Regional Planning Commission, 1974).

Loxton et al. (1971) classify the study area as 'Coast Lowland' and divide the region into six landscape types :

Coast Dune landscapes

Dissected Coast Granite landscapes

Dissected Coast Dwyka tillite landscapes

Coast Lowland : Ecca shale, dolerite and schist landscape

Table Mountain Sandstone sandy plateaux and weakly dissected region

Landscape dominated by rocky outcrops and litholic soils

2.2.4 Drainage and surface water

Drainage patterns and availability of surface water influence vegetational distribution and in particular members of the Ficus and Syzygium genera (Palmer, 1977) whose fruits form an important part of the diet of E. wahlbergi in the study area.

The general topography of the South Coast is steep and rugged. Over a

distance of about 160 km the country rises from sea level to the top of the Drakensberg, a height of 3 000 m. The ancient peneplain has been uplifted and severely dissected by active back-cutting rivers. The river valleys are therefore youthful, with narrow deep valley bottoms and steep precipitous sides. For most of their courses the stream beds are steep, ranging in falls from five to 10 metres per kilometre; for a few kilometres inland of the coast the grades become flatter (Loxton *et al.*, 1971). Sand bars have closed off many river mouths to form lagoons. The lagoon water is generally mildly saline owing to the proximity of the sea.

The region where this study was carried out is drained by four perennial rivers; Mkomazi, Mahlongwana, Mahlongwa and Mpambanyoni and their tributaries. FIGURE 5 shows the extent of the perennial and seasonal water supply to the area.

The largest of these rivers is the Mkomazi River, 298 km long, mean annual runoff $1\ 072 \times 10^6 \text{ m}^3$ and a mean annual discharge of $30 \text{ m}^3/\text{second}$ (Begg, 1978). The river is usually open to the sea and its closure by sand bar is a rare occurrence (Begg, 1978).

South of the Mkomazi River the Mahlongwana River drains into a lagoon on the coast and is usually blocked off from the sea by a persistent sandbar. The river is 6 km long with an unrecorded but perennial flow (Begg, 1978).

The Mahlongwa River is 23 km in length, mean annual runoff of $14,7 \times 10^6 \text{ m}^3$ and a flow estimate for 1970 of $0,2 \text{ m}^3/\text{second}$ in summer (Begg, 1978). A mangrove flanked lagoon lies on the landward side of the main road. The lagoon is usually closed off to the sea by a sand bar.

The Mpambanyoni River is the southernmost river draining the study area. It is 100 km long, mean annual runoff of $32,1 \times 10^6 \text{ m}^3$ and estimated annual flow of $0,71 \text{ m}^3/\text{second}$. The river opens to the sea at Scottburgh, but has been known to close occasionally in winter (Begg, 1978).

2.2.5 Land use

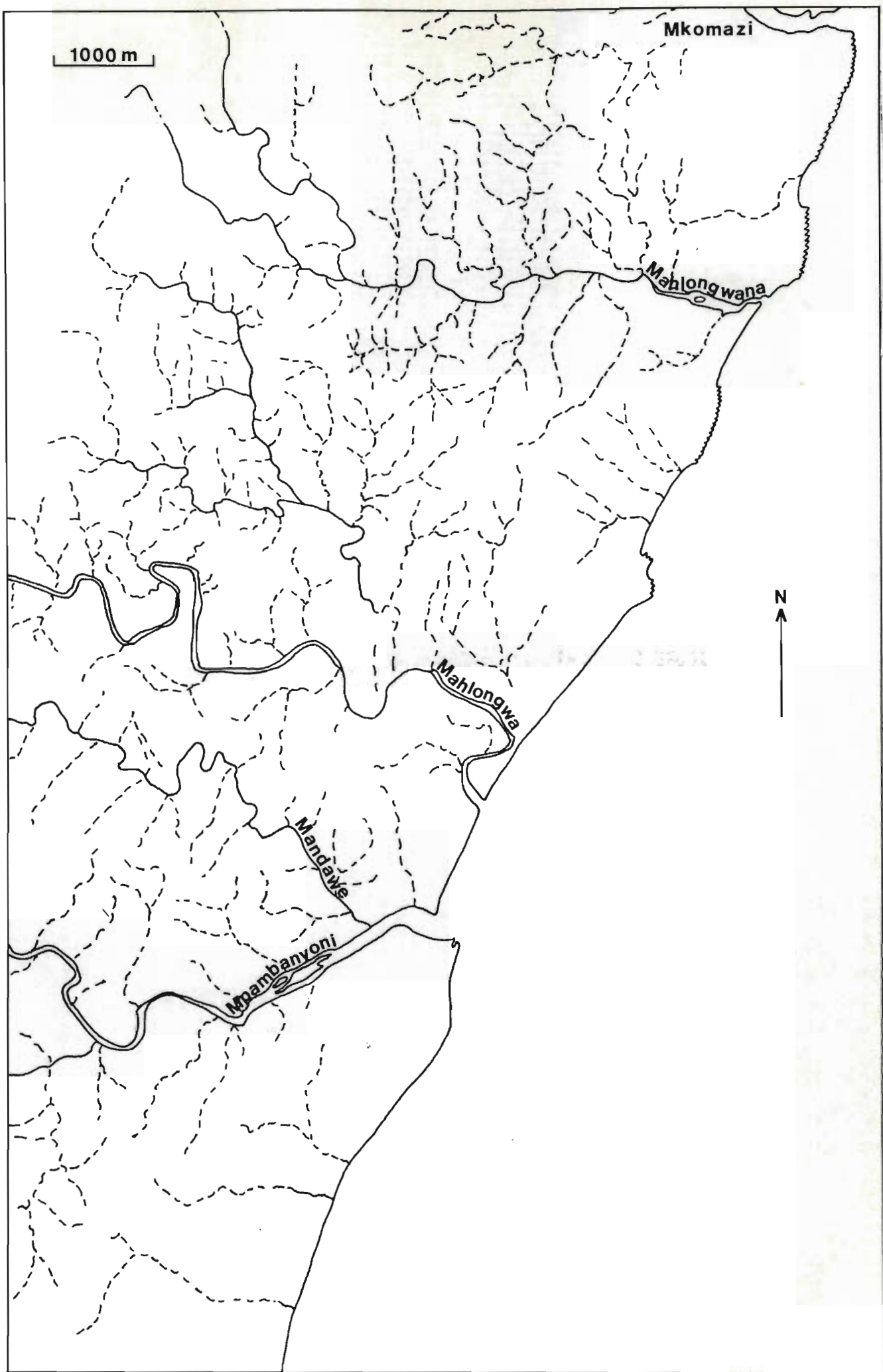
2.2.5.1 Agriculture

The single most important agricultural land use in the region of study is the growing of sugar cane. It is considered by the Natal Town & Regional Planning Commission (1974) to be the most important economic activity along the South Coast.

On a number of small farms in the area, bananas, litchis and pawpaws are grown for local consumption.

FIGURE 5 Drainage patterns and surface water supply in the study area

Solid lines - perennial rivers and streams
Broken lines - seasonal rivers and streams



2.2.5.2 Industry

With the closing of the Renishaw Sugar Mill in 1975, there are now no main industrial centres within the study area. SAICCOR, a major producer of dissolving pulp for overseas markets, lies just outside the area to the north.

2.2.5.3 Tourism

Fine bathing beaches, attractive lagoons and a warm, year-round sunny climate have been important factors in the establishment of the tourist industry on the Natal South Coast.

Within the study area, the need to provide parking shade for such tourist amenities as hotels, restaurants, caravan parks and golf courses, has resulted in the planting of many large canopied trees, particularly the large-leaved fig, Ficus vogelii. The importance of the presence of this species in the study area is referred to in 2.3.1.7 and 2.4.3.1.

2.3 NATURAL HISTORY

2.3.1 Vegetation

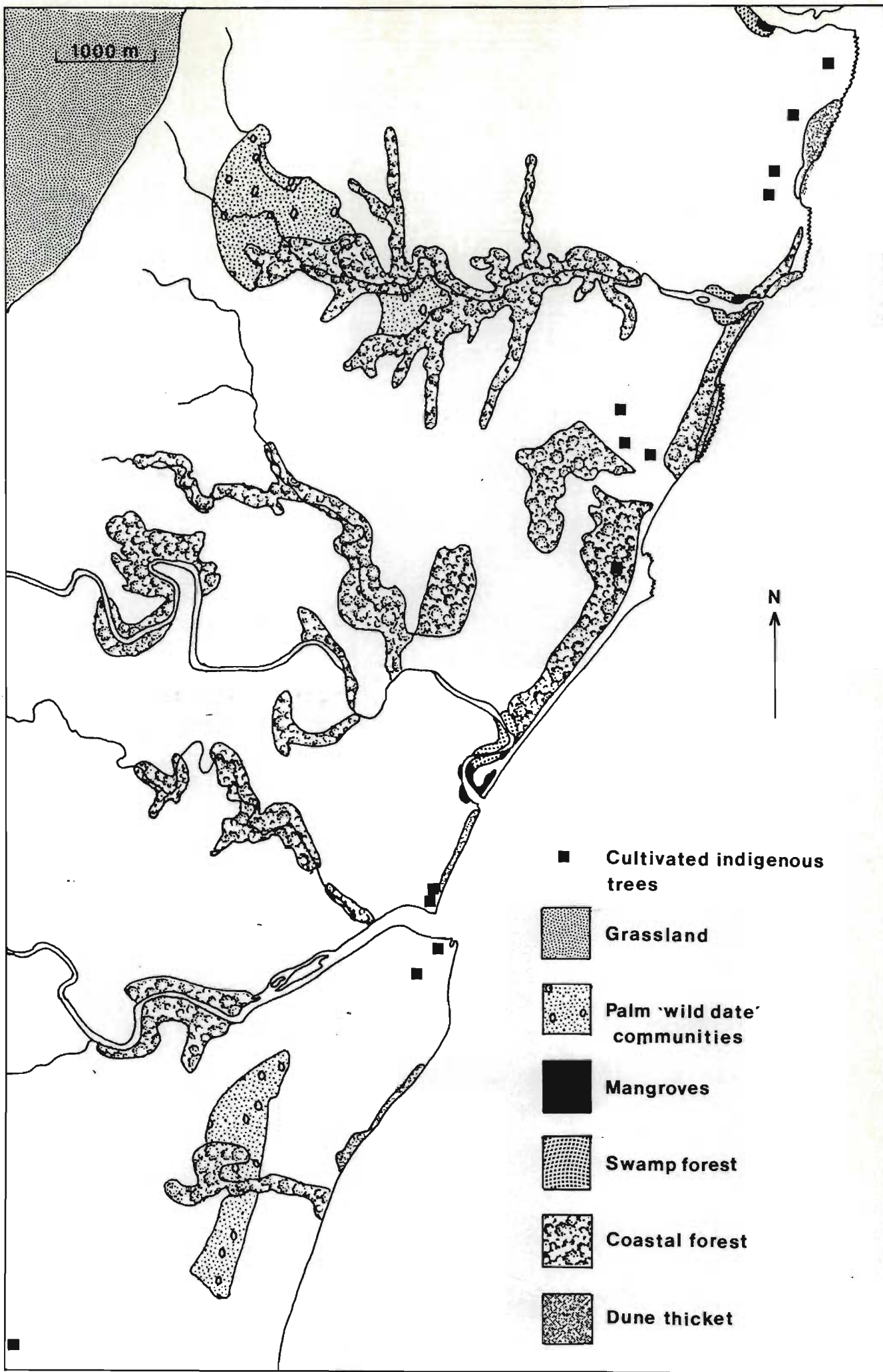
FIGURE 6 shows the vegetation of the study area

2.3.1.1 Dune thicket

The coast dune thicket occurs along the coastal fringe of the study area and is particularly noticeable on the sand dune at Widenham, just north of the Mahlongwana River. The dune forest shows very definite gradation with the following vegetation types typically encountered :

- (1) Salt tolerant succulents in loose sand e.g. Scaevola sp. and Carpobrotus sp.
- (2) A close cover of Gazania sp., Barleria obtusa, Asystasia gangetica and Chrysanthemoides monilifera.
- (3) Low shrubs e.g. Eugenia capensis, Passerina rigida, Carissa macrocarpa and Cynanchum natalitum.
- (4) Tall shrubs e.g. Maytenus heterophylla and lianas

FIGURE 6 Distribution of indigenous vegetation
compiled from aerial survey photographs



e.g. Dalbergia armata.

- (5) Trees of many species e.g. Mimusops caffra, Brachylaena discolor, Euclea natalensis, Harpephyllum caffrum, Protorhus longifolia, Trema orientalis, Tricalysia sonderana, Canthium obovatum, Ficus natalensis, Rhus chirindensis and Strelitzia nicolai.

This gradation is determined by salt spray that blows off the sea, the vegetation closest to the sea being the most salt tolerant. The coast dune thicket has a stabilizing influence on loose sand of the beach dunes and is important in the early colonization of skeletal sand soils (Natal Town & Regional Planning Commission, 1974).

2.3.1.2 Coastal forest

The coast forest extends from the inland fringe of dune thicket to an altitude of approximately 450 m above sea level. The forest is subtropical and consists of low forest and scrub. The trees are widely spaced with spreading crowns and dense shrubby undergrowth with an abundance of lianas. Commonly occurring trees of this type in the study area are Ficus natalensis, Apodytes dimidiata, Chaetacme aristata, Canthium obovatum, Bridelia micrantha, Erythrina lysistemon, Antidesma venosum, Trichilia dregeana, Sapium integerrimum, Euphorbia ingens, Milletia grandis, Croton sylvaticus and Sideroxylon inerme.

The distribution of the forest was never continuous, because of rock outcrops and ravaging by grass fires and today the natural forest only survives in patches, usually where sugar cane cannot be grown (Natal Town & Regional Planning Commission, 1974).

2.3.1.3 Swamp forest

Swamp forest constitutes reeds, sedges, rushes, large woody shrubs and some tree species (Loxton et al., 1971), which in the study area are found a short way upstream of the mouths and lagoons of the Mkomazi, Mahlongwana and Mahlongwa Rivers. Common elements of these communities are Phragmites australis, Typha capensis, sedges such as Caldium sp., Mariscus sp. and Cyperus sp. and among the trees, Syzygium cordatum, Voacanga thouarsii, Macaranga capensis, Hibiscus tiliaceous and Bridelia capensis.

2.3.1.4 Mangrove

A small relict community of the white and red mangroves, Avicennia marina and Rhizophora mucronata, exists on the south bank of the Mkomazi River (Thomas, pers. comm.). A few trees of the black mangrove, Bruguiera gymnorrhiza are growing on the north bank of the Mahlongwana River and a more extensive community of this species is found on both banks of the Mahlongwa River inland of the main road (Begg, 1978).

2.3.1.5 Palm 'wild date' communities

Relict populations of the palm 'wild date' communities exist in the study area inland, west of Umkomaas and south of Scottburgh. They once were extensive from just behind the dunes to well inland in edaphically suitable sites being in the succession towards coastal forest (Loxton et al., 1971). The 'wild date' Phoenix reclinata is associated with small trees and shrubs, such as Euclea sp., Cussonia sp., Ficus sp., Trichilia sp., Maytenis sp. and Strelitzia nicolai set in grassland communities composed of Themeda triandra, Cymbopogon sp. and as a result of heavy grazing pressures, Aristida junciformis and Digitaria sp. (Loxton et al., 1971).

2.3.1.6 Grassland

Cultivation of sugar cane, burning and shifting bantu cultivation have been responsible for the destruction of the original grassland communities. Today Themeda triandra is rare though locally present in the northwest corner of the study area. The replacement of T. triandra by Aristida junciformis, Eragrostis sp. and Cynodon has probably been brought about by veld mismanagement (Loxton et al., 1971).

2.3.1.7 Indigenous vegetation in built-up areas

One of the most important fruiting tree species, in which E. wahlbergi was seen roosting and under which they were frequently captured while feeding, was Ficus vogelii. Although this species is an indigenous forest tree (Moll, 1981; Palmer, 1977) it was never encountered in the forest zones of the study area but commonly occurred as cultivated shade trees in built-up areas such as hotel, restaurant and municipal car parks, caravan parks and camp grounds, private gardens and on farms in cattle corrals. Occasionally isolated examples of these trees were found in sugar cane, probably the only remnants of a once afforested patch. Other important fruit bat food trees which

are found cultivated in built-up areas are Ficus natalensis, Ficus polita, Syzygium cordatum, Carissa macrocarpa and Podocarpus latifolius.

2.3.2 Fauna

The study area is noted for its high species diversity of both its invertebrates (Day, 1974; Dickson & Kroon, 1978; Pinhey, 1965; Smith Meyer, 1974; Williams, 1969) and vertebrates (Bourquin & Sowler, 1980; Crass, 1964; Cyrus & Robson, 1980; De Graaf, 1981; Dorst & Dandelot, 1972; Fitzsimons, 1962; Hanks, 1980; Hughes, 1974; Kingdon, 1974; Meester & Setzer, 1971; Passmore & Carruthers, 1979; Prozesky, 1970; Roberts, 1951; Rowe-Rowe, 1975; Smith, 1953; van der Elst, 1981; Wager, 1961; Zalounis & Cross, 1975).

Data regarding distribution of Chiroptera are available in Bourquin & Sowler (1980), Kingdon (1974), Meester & Setzer (1971) and Sowler (in prep.). A maximum of 17 species could occur in the study area. Of these, the following eight species were captured during the study period :

Megachiroptera	:	<u>Epomophorus wahlbergi</u> <u>Rousettus aegyptiacus</u>
Microchiroptera	:	<u>Nycteris hispida</u> <u>Pipistrellus nanus</u> <u>Scotophilus nigrita</u> <u>Tadarida aegyptiaca</u> <u>Tadarida pumila</u> <u>Taphozous mauritianus</u>

2.4 SEASONALLY VARIABLE ENVIRONMENTAL FACTORS

It was suggested by Baker (1938) that certain environmental factors may act as proximate and ultimate triggers for breeding, in order that offspring may be produced at the most propitious time of the year.

2.4.1 Climatic factors

South Africa lies in the subtropical high pressure belt in which high pressure anticyclonic cells move continuously from west to east. These cells are stronger in winter than in summer. Anticyclones characteristically generate anti-clockwise movements of air in the southern hemisphere, which because it is descending becomes warmer and drier. This pattern is interrupted by eastward moving waves of low

pressure and also by small low pressure cells which circulate round the coast (Natal Town & Regional Planning Commission, 1974).

2.4.1.1 Winter conditions

In winter a strong high pressure centre develops over the Indian Ocean at about 60° E. The effect of this, combined with the high pressure area usually found over land is to produce the settled, pleasant weather which is characteristic of the South Coast region. This pattern is broken occasionally by eastward moving waves of low pressure. These bring deep cold fronts, which sweep across the country on the coast, the southwesterly 'busters', bringing low temperatures, cloud and rain (Natal Town & Regional Planning Commission, 1974).

2.4.1.2 Summer conditions

In summer the Indian Ocean high pressure centre moves eastwards, far away from South Africa. At the same time the pressure systems over the land shift southwards and the customary high pressure cell over the land weakens, sometimes giving way to low pressure thermal conditions. Although the eastward moving low pressure cells frequently pass much further to the south in summer, the smaller low pressure cells which move around the coasts of South Africa penetrate into the South Coast region. These seem to pass with a frequency of about four to six days in summer and bring a southeast wind, cloudy conditions, a temperature drop and light rain (Natal Town & Regional Planning Commission, 1974).

2.4.1.3 Berg winds and land and sea breezes

A phenomenon which has considerable effect on the climate of the South Coast is the Berg wind. These winds blow in a northwesterly direction from the mountains of the interior to the coast. They bring high temperatures and low humidity and are especially frequent in winter, spring and autumn.

The differential heating and cooling rates of the land and sea result in land and sea breezes. They blow off the sea towards the land during the day and off the land towards the sea at night. The moderating effect, due to the proximity of the sea, greatly minimizes temperature extremes.

2.4.1.4 Temperature

FIGURE 7 shows the mean monthly maximum and minimum temperatures over the period of study from June 1977 to February 1982 taken at Scottburgh (Horsfall, pers. comm.). The hottest month was February (mean maximum 28,23° C; mean minimum 23,60° C) and the coldest month was July (mean maximum 22,12° C; mean minimum 15,62° C).

2.4.1.5 Humidity

FIGURE 8 shows the mean maximum and minimum monthly relative humidity in the study area taken over the period of study (Horsfall, pers. comm.). The most humid month was February (mean maximum relative humidity 83,0 %; mean minimum relative humidity 31,6 %).

2.4.1.6 Precipitation

Precipitation was experienced in the study area as rainfall. No snow was recorded. FIGURE 9 shows the range and mean monthly rainfall in mm during the study period. On average the wettest month was February (mean rainfall 128,3 mm) and the wettest month during the study period was February 1978 when 213 mm of rain was recorded. On average the driest month was June with a mean total rainfall of 19,6 mm. The driest month during the study period occurred in June 1970 when 7,0 mm of rain fell (Horsfall, pers. comm.).

2.4.1.7 Prevailing winds

FIGURE 10 shows percentage wind direction per month compiled from daily data over the study period. There are two prevailing winds, northeast and southwest which blow equally throughout the year. From August through to January the southwest wind tends to predominate, changing from February to July to a northeast wind (Horsfall, pers. comm.).

2.4.1.8 Hours of sunshine

The average monthly sunshine hours per day measured over the study period is shown in FIGURE 11. The sunniest month was June (mean daily sunshine hours 7,93) and the cloudiest month, September (mean daily sunshine hours 5,53) (Louis Botha Metereological Office).

2.4.2 Daylength

FIGURE 7 The mean monthly maximum and minimum temperatures over the study period from June 1977 to February 1982 taken at Scottburgh

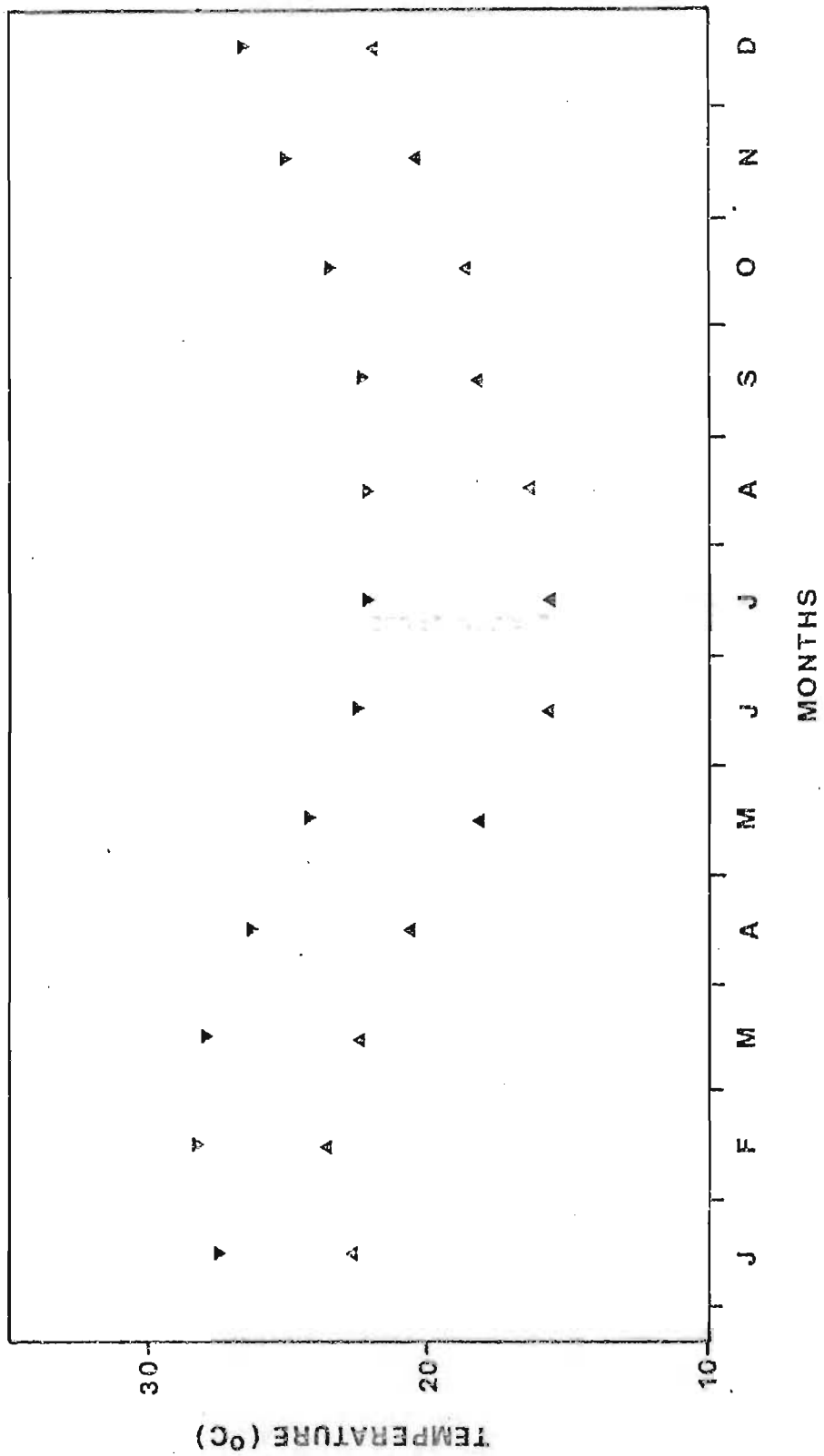


FIGURE 8 The mean monthly maximum and minimum relative humidity over the study period from June 1977 to February 1982 taken at Scottburgh

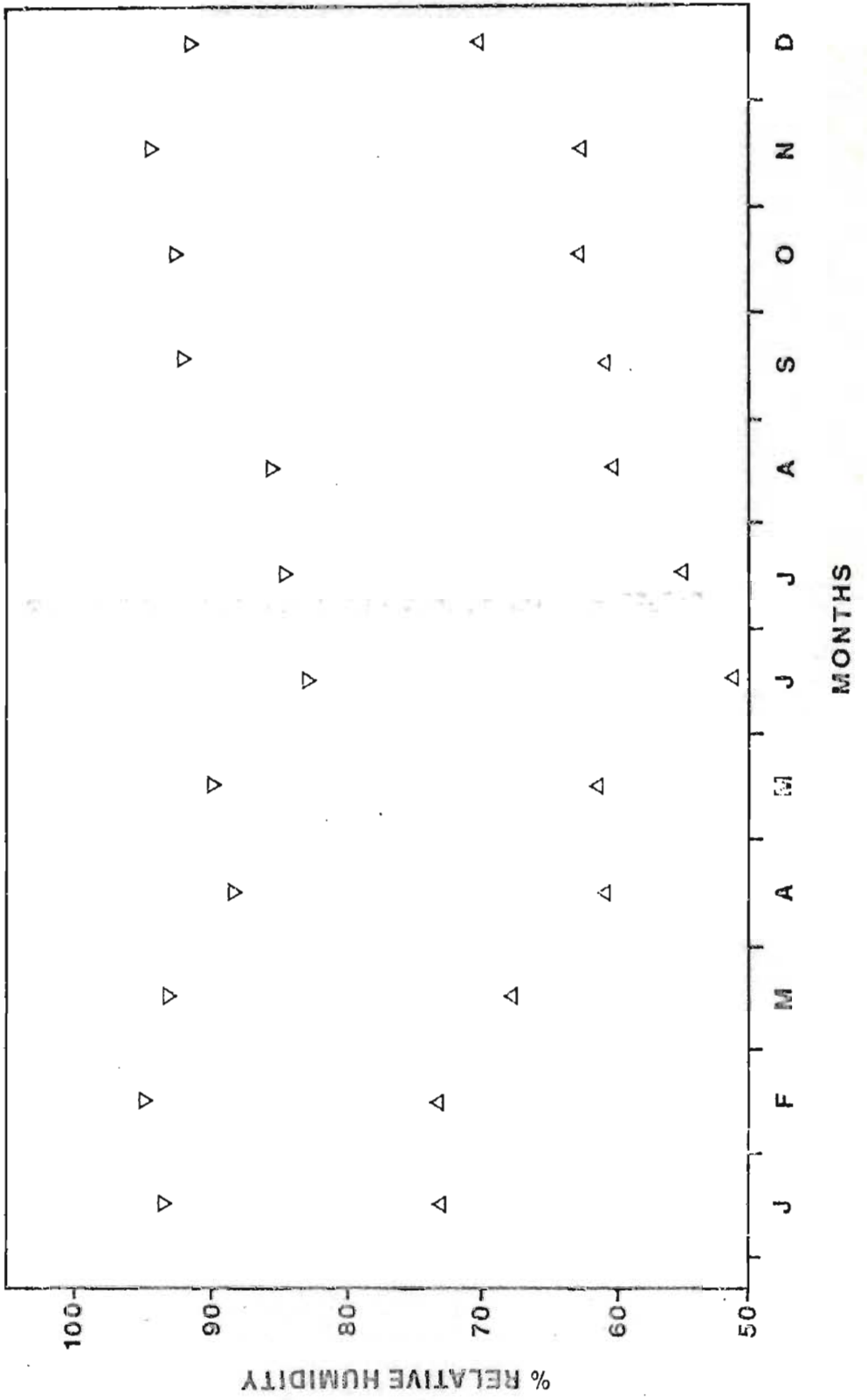


FIGURE 9 The range and mean monthly rainfall in mm taken during the study period from June 1977 to February 1982 at Scottburgh

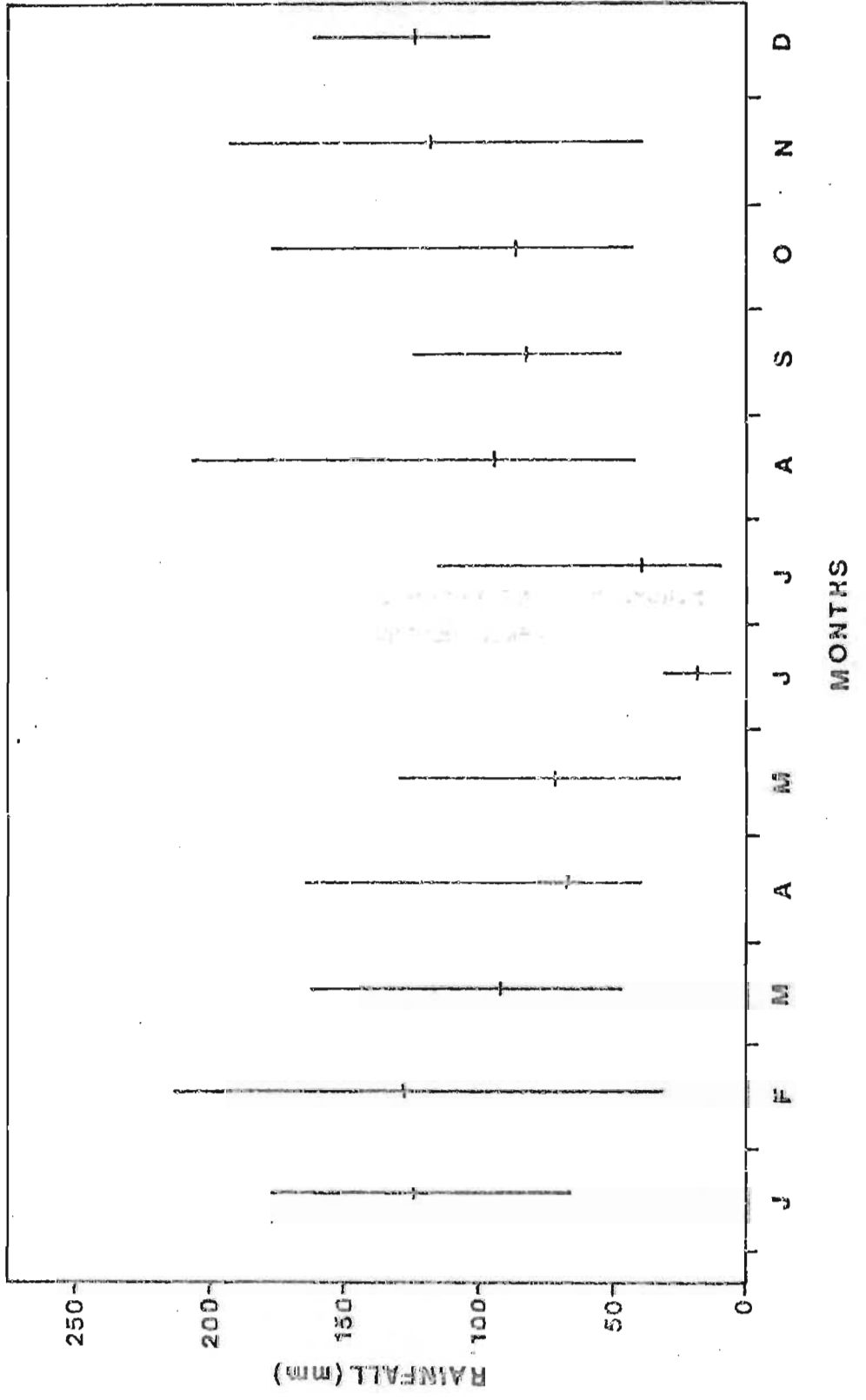


FIGURE 10 Percentage wind direction per month compiled from daily data over the study period from June 1977 to February 1982, taken at Scottburgh

% WIND DIRECTION PER MONTH

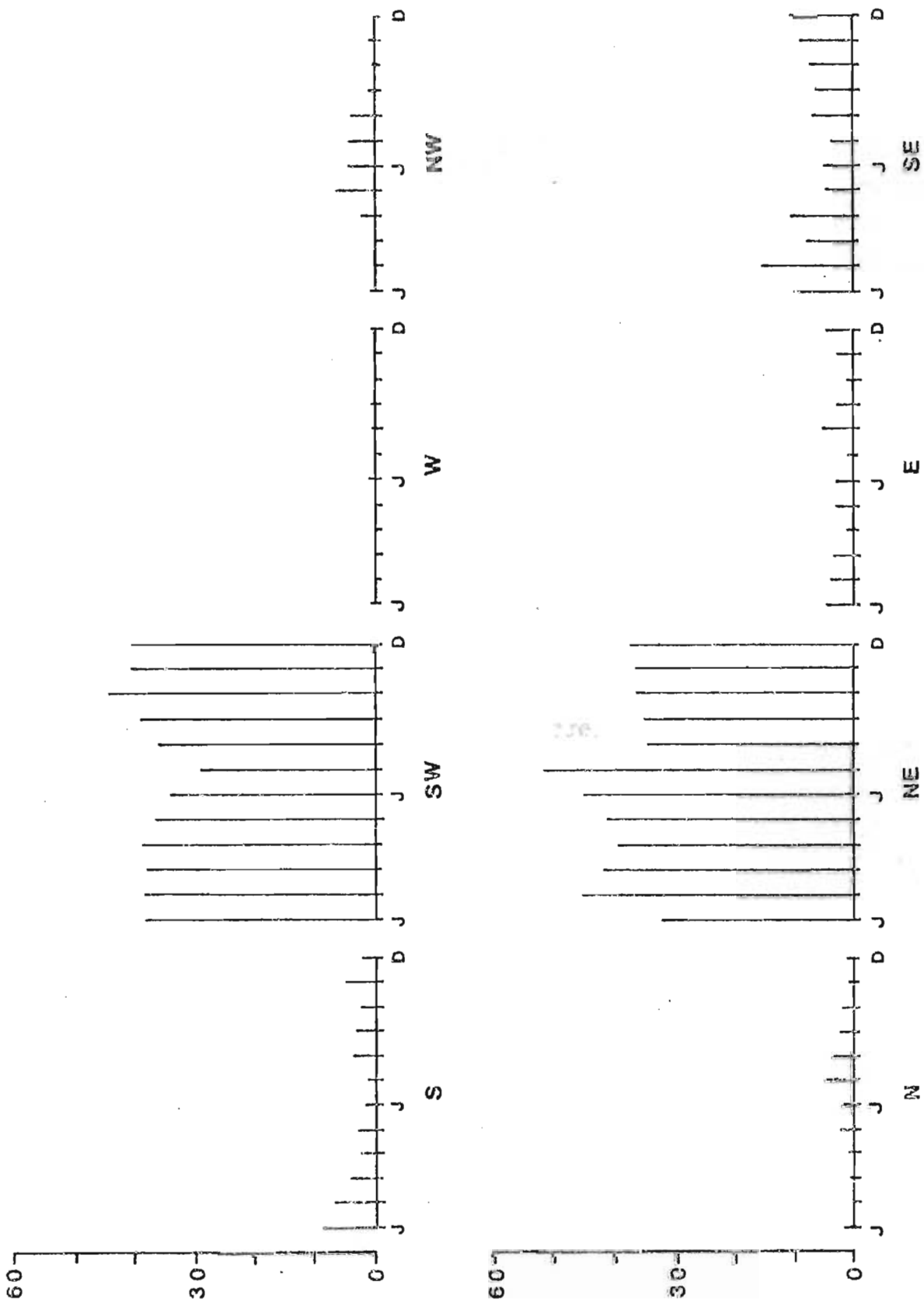
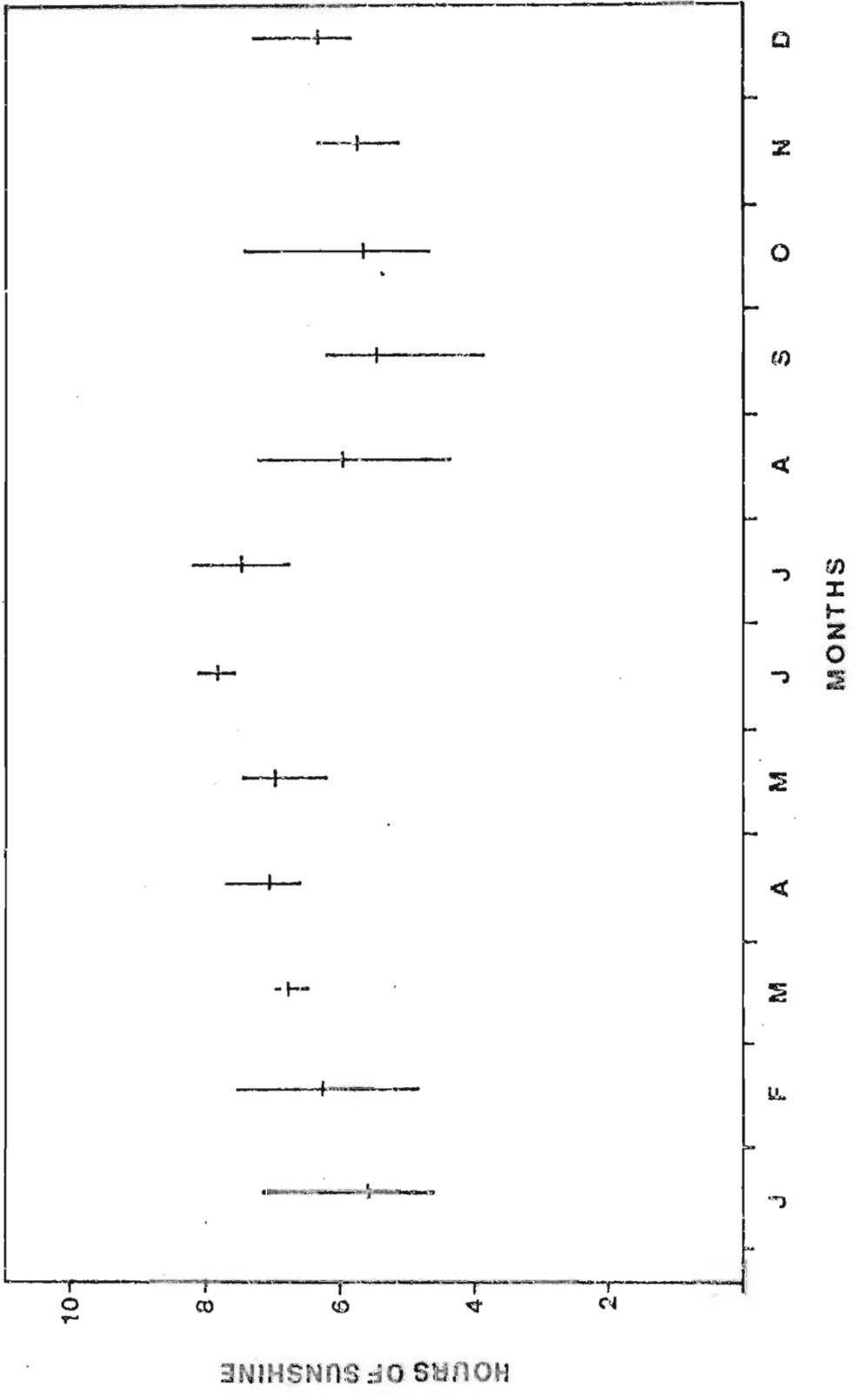


FIGURE 11 The average monthly sunshine hours per day measured over the study period compiled from data supplied by the Louis Botha Metereological Office



Daylength figures were obtained from Louis Botha Metereological Office (30° S) and are shown in FIGURE 12. The shortest days occur from the 15 to 23 of June, having 10,13 hours between sunrise and sunset. The longest days occur from the 23 to 25 December having 14,06 hours between sunrise and sunset. An extra 15 minutes may be added before sunrise and after sunset to obtain total hours of daylight (De Villiers, pers. comm.). The increase and decrease in daylight hours over the year follows a sine wave.

2.4.3 Food availability

Sadleir (1969a) considered food availability and nature of food to be the most important ultimate ecological factor in the timing of seasonal breeding.

In order to assess the availability of bat fruit throughout the year in the study area, 55 individual trees of seven species were examined for the presence of ripe fruit every three weeks for three years. All trees chosen were seen to be fed on by fruit bats when their fruit became ripe.

2.4.3.1 Indigenous fruiting trees

Fifty one indigenous trees comprising five species; Ficus natalensis, Ficus polita, Ficus vogelii, Podocarpus latifolius and Syzygium cordatum were surveyed. FIGURE 13 (broken line) shows the mean percentage of these trees which were in fruit per month over the three years. Although some trees are in fruit in all months of the year, a distinct increase in numbers of trees in fruit is noticeable in January. February, March, May and August were months during which the percentage of indigenous trees in fruit was less than 9 %.

2.4.3.2 Alien and cultivated fruiting trees

Four trees of two species Nephelium litchi and Psidium guajava were also examined. Unlike some of the indigenous fruiting trees, notably F. vogelii and F. natalensis, these trees fruited consistently at the same times each year. N. litchi fruited during January and P. guajava from late March through to late May.

FIGURE 13 (solid line) also shows the mean percentage of all 55 trees, both indigenous and non-indigenous which were in fruit each month.

1. 2. 3. 4. 5. 6. 7. 8. 9. 10. 11. 12. 13. 14. 15. 16. 17. 18. 19. 20. 21. 22. 23. 24. 25. 26. 27. 28. 29. 30. 31. 32. 33. 34. 35. 36. 37. 38. 39. 40. 41. 42. 43. 44. 45. 46. 47. 48. 49. 50. 51. 52. 53. 54. 55. 56. 57. 58. 59. 60. 61. 62. 63. 64. 65. 66. 67. 68. 69. 70. 71. 72. 73. 74. 75. 76. 77. 78. 79. 80. 81. 82. 83. 84. 85. 86. 87. 88. 89. 90. 91. 92. 93. 94. 95. 96. 97. 98. 99. 100.

FIGURE 12 Hours of daylight for 30° S supplied by the Louis Botha Metereological Office

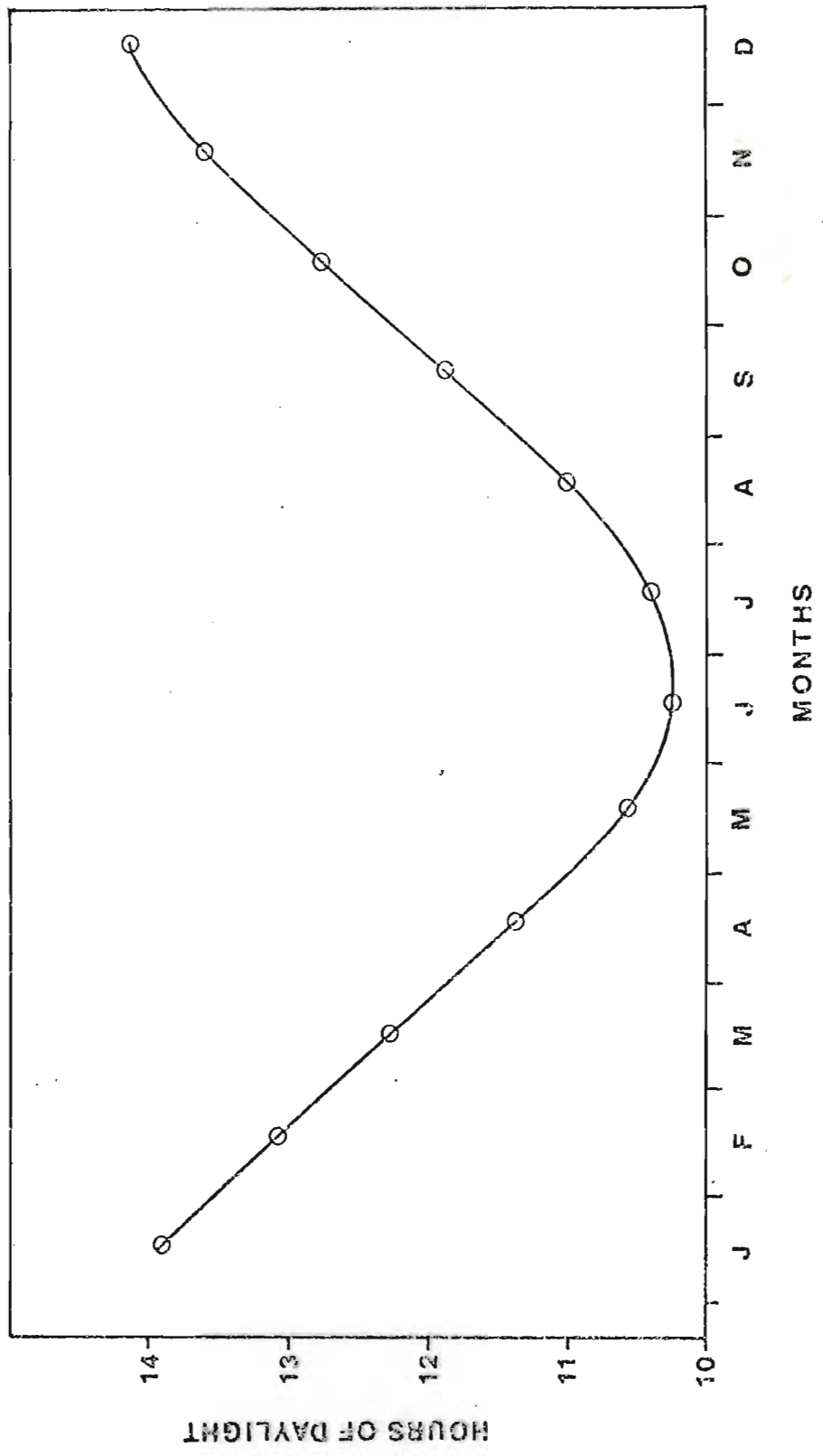
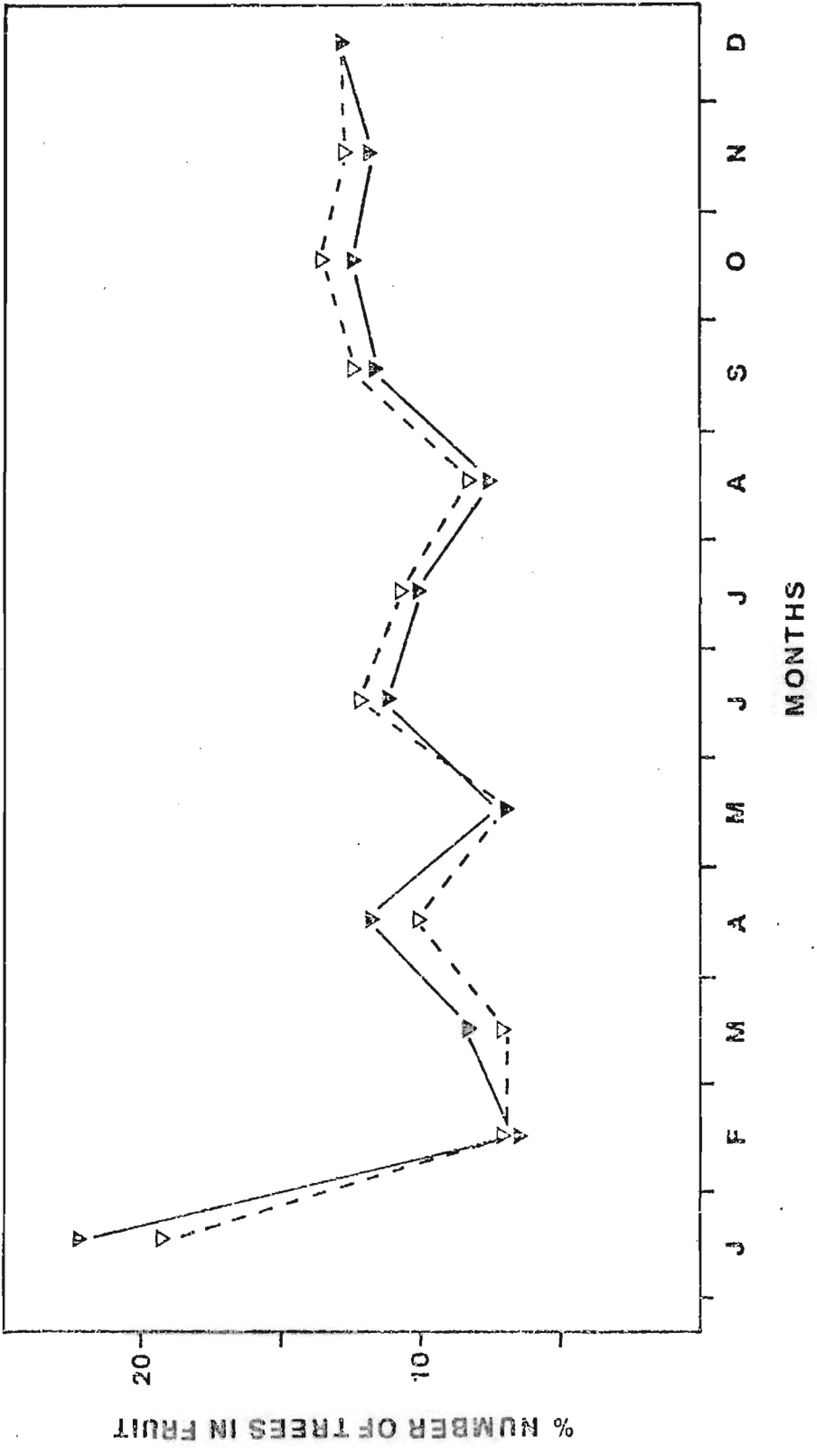


FIGURE 13 Mean percentage of trees in fruit per month observed over three years from January 1979 to February 1982

- Solid line - Mean percentage of all 55 trees, both indigenous and non-indigenous which were in fruit each month
- Broken line - Mean percentage of 51 indigenous trees which were in fruit each month



CHAPTER THREE

AGE DETERMINATION AND GROWTH

3.1 INTRODUCTION

In any investigation into the reproductive biology of a species, valuable information, such as age at puberty, age at maturity, reproductive senescence and life span, cannot be calculated without methods for determining the age of specimens.

Although age determination methods are an essential tool of present day researchers, the techniques, particularly those involving features of dentition, are by no means new. For centuries man has needed to know the age of his domesticated stock in order to assess palatability in food animals and expected duration of active service in draught animals. Indeed, the old saying: 'Never look a gift horse in the mouth' (to ascertain its age) is attributed to St. Jerome of the 5th Century. Much more recently, Girard (1824) described routine age assessment of young domestic animals by stages in eruption and replacement of teeth. Rörig (1905) published data on tooth eruption in the three European deer species, red (Cervus elephus), roe (Capreolus capreolus), and fallow (Dama dama), while Galvayne in the same year suggested the use of the lateral groove in the third upper incisor of the horse (Equus caballus) to estimate age up to 30 years. One of the earliest methods used for bats was estimation of the state of ossification of the hand bones in order to distinguish young bats from old (Barrett-Hamilton, 1910).

Today procedures used in age determination are based upon four major criteria, (a) increase in size (b) structural degradation (c) growth changes and (d) growth rings or incremental lines (Morris, 1972).

(a) Methods based on size increase

In this category the methods are based on the surmise that as the animal ages it gets bigger, at least until maturity. Increase in body mass has been used for age determination in Eptesicus fuscus (Burnett & Kunz, 1982) and Myotis lucifugus (Kunz & Anthony, 1982). Several linear dimensions have been used, including forearm length (Baagøe, 1977a; Burnett & Kunz, 1982; Kunz & Anthony, 1982) and total wing tip length. Condylbasal length and mandible length have all been used by Baagøe (1977a) for comparison with X-ray fusion techniques in studies of three Microchiropteran species.

Morris (1972) remarked that the baculum is likely to show marked changes in size and shape correlated with age and particularly with attainment of sexual maturity. Baagøe (1973) using bacula of two species of Myotis found a correlation with age as determined by the epiphyseal fusion method. However using bacula from Myotis daubentoni, Baagøe (1977b) found so much overlap in the correlation between age and size that he concluded that in this species the baculum was of limited use as an age indicator.

The technique of using dry mass of eye lens, as reviewed by Friend (1968), was tried by Perry (1965) when analysing populations of the guano bat (Tadarida brasiliensis). Perry & Herreid (1969) compared this method with tooth wear in the same species and found a 71,6 % agreement between the two methods for the overall analysis of the age structure of the population.

Baculum mass and size is a convenient method for separating juveniles from adults, but its application is limited to material from dead males. The technique for eye lens mass is most accurate in the period of rapid growth prior to the attainment of adult size, but its main disadvantage, like the baculum method, is that it is applicable only to dead material and material should be collected within a few hours of death.

(b) Methods based on structural degradation

There is an increase in the degeneration of certain mammalian body structures with age, and these changes give rise to the second category of age determination techniques. The degradation of teeth has been used for bats.

Age evaluation based on tooth wear is a much used method and depends on the fact that in most mammalian teeth (except those with open roots), growth ceases once the tooth is fully formed. The teeth are therefore gradually worn from the time they have emerged until the animal dies. Thus the degree of tooth wear is assumed to be proportional to the animal's age.

A subjective assessment of tooth wear was used for bats as early as 1917 by Knud Anderson who distinguished five stages of tooth wear in the W pattern of the upper molars of Rhinolophus rouxi. Sluiter & Bouman (1951) recognized three age categories of Myotis myotis on a purely subjective basis. Later Sluiter (1954) obtained an index of age by measuring the heights of the buccal cones of M_1 and M_2 . Stegeman (1956) was able to distinguish four age groups using wear of M^1 in Myotis lucifugus and also noted a sequence in the wear of

commissures and cusps. Based on maxillary canine wear, Twente (1955) allotted six groupings of tooth wear for three Microchiropteran species but was unable to find canine wear in a fourth species investigated. Hall, Cloutier & Griffin (1957) tested the subjective methods of Stegemen (1956) and Twente (1955) and concluded from their results that these tooth wear methods presented highly unreliable criteria. Davis, Herreid & Short (1962) working with the Mexican free-tailed bat (Tadarida mexicana) and Christian (1953) with the big brown bat (Eptesicus fuscus) used three age classes for analysis on a subjective basis. Later Christian (1956) used occlusal tip width of upper canine as an age indicator in E. fuscus. This procedure was repeated by Clark (1979) for E. fuscus and Myotis lucifugus. Baagøe (1977b) adopted the method of Sluiter (1961a) in measuring the crown height of M_1 in four species of insectivorous bat. He then obtained a tooth wear index by adding the heights of the two cusps on the crown and dividing by the tooth length. When compared with the results from X-ray epiphyseal fusion techniques and pulp cavity decrease, tooth wear showed an overall gross correlation in all four species.

Bradbury (1977) working with the Megachiropteran Hypsignathus monstrosus, assigned specimens to one of four known age classes for bats older than two years, based on eruption of cusps on premolars and degree of wear on molars.

(c) Methods based on growth changes

In the majority of mammalian species, milk teeth develop and are replaced by permanent teeth. The successive loss and eruption of teeth enable relative age to be determined by simple inspection of the mouth to see which stage has been reached, and by knowledge of the eruption sequence. Jeffrey (1978) working on eland (Taurotragus oryx), Smuts, Anderson & Austin (1978) on the African lion (Panthera leo) and Spinage (1967) on the Uganda Defassa waterbuck (Kobus ellipsiprymnus) employed this procedure. A quantitative approach has been used by Grimsdell (1973) for the African buffalo (Syncerus caffer), Ockerse (1959) for the vervet monkey (Cercopithecus pygerythrus) and Sowls & Phelps (1968) for the African bushpig (Potamochoerus porcus) applying known ages of eruption of the permanent teeth and variations in eruption timing. Apart from this present research (Sowler, 1980), no reference has been found to the application of tooth eruption sequence as a means of age determination in bats.

However some information on Chiropteran dental development and descriptions of deciduous dentition is available. Friant (1951)

describes the deciduous dentition of Hipposideros caffer and Matthews (1950) the deciduous dentition and its replacement by the permanent teeth in Nycteris leisleri.

The pulp cavity is gradually diminished with age as secondary dentine is deposited around the walls of the cavity inside the tooth (Klevezal & Kleinenberg, 1969). Morris (1972) suggests this as a guide to age either by X-ray analysis or by sectioning of teeth. Baagøe (1977b) developed a technique for bat teeth in which he suspended the upper canines of the four species used in glycerine and inspected and measured the width of the pulp cavity under a dissecting microscope. He found that the results gave gross overall correlation with other age determination methods.

Based on the observation that ossification of the epiphyses of long bones occur when adult size is attained, unfused epiphyses have been used to indicate growth in a mammal as an approximate guide to age. The technique was described by Weinman & Sicher (1947). In bats the state of ossification of the hand bones is estimated by holding the wing against a light source to see if the epiphyses and diaphyses are fused (Cranbrook & Barrett, 1965; Stebbings, 1968) or by feeling for flexibility in the joints (Rosevear, 1965). These criteria have been used in Myotis myotis (Rybar, 1969), Myotis lucifugus (Davis & Hitchcock, 1965), Rhinolophus hipposideros (Rybar, 1971) and Pipistrellus subflavus (Davis, 1963). A refinement to this method for bats was employed by Baagøe (1977b) using a modified X-ray technique. Five different groups were established based on observed details of the fusion process by X-ray procedure. Burnett & Kunz (1982) working on Eptesicus fuscus measured the length of the fourth metacarpal phalangeal epiphyseal gap and used this as an age determination criterion. Linear dimensions and dentitional techniques provide a more easily applied means of determining the age around which phalangeal epiphyseal fusion occurs.

Quality, length and colour of pelage have been used by many authors to distinguish juvenile, subadult and adult bats (Eisentraut, 1936; Gaisler, 1966; 1971; Mazak, 1963; 1965; Stebbings, 1968).

Nipple size and condition may be used to distinguish between nulliparous and parous bats (Racey, 1974a). These criteria were used by Pearson, Koford & Pearson (1952) for age diagnosis of young female Plecotus rafinesquei until they reached nine months old, by Sluiter (1954, 1961a) for two species of Myotis and by Baagøe (1977b) for Myotis daubentoni.

In male bats of temperate species during the summer months the testes

increase in size in the period of spermatogenesis, enabling immature and mature males to be distinguished on the basis of testis size (Baagøe, 1977b). Sluiter (1961b) observed that the caudae epididymides of Myotis myotis projected conspicuously into the interfemoral membrane and supported these findings with histological data. As this species does not reach sexual maturity until the autumn of the second year, he was able to distinguish hibernating first year males from older ones.

Pearson, Koford & Pearson (1952) distinguished young male Plecotus rafinesquei from adult males throughout their first year on several criteria including testis and epididymis size and thickness of tunica vaginalis.

In this category dentitional development offers a simple and rapid guide to age assessment with few serious drawbacks, particularly when used in combination with tooth wear. Epiphyseal ossification, like the technique using dentitional development can be used for both living and dead animals and has the further advantage of being widely flexible and can be modified to suit a particular species or study. The pelage criterion is limited in its usefulness to comparisons within one geographical population.

Criteria involving sexual characteristics are valuable when supported by other age diagnostic data. By themselves they can indicate only whether the animal has or has not reached the age at which sexual maturity is known to occur.

(d) Methods based on incremental lines or growth rings

All previous methods mentioned relate to relative age. The last of the four categories involves those methods using incremental lines or growth rings which reflect seasonal differences and discontinuities in growth processes. They are therefore helpful guides to absolute age. Klevezal & Kleinenberg (1969) wrote a detailed review of the whole subject of growth layers in teeth and bone in mammals.

Several species of vespertilionid and one species of phyllostomatid bat have been aged by microscopic study of dental tissue (Baagøe, 1977b; Christian, 1956; Klevezal & Kleinenberg, 1969; Linhart, 1973; Lord, Muradali & Lazaro, 1976; Schowalter, Harder & Treichel, 1978). Klevezal & Kleinenberg (1969) also reported that distinct annual layers were formed during the first two to three years in the periosteal zone of bones of three species of vespertilionid bat.

Although some authors have expressed doubt concerning the accuracy of

the method (Phillips, 1971; Phillips & Steinberg, 1976; Phillips, Steinberg & Kunz, 1982) and its practical application (Baagøe, 1977b) for Chiropteran age determination, Morris (1972) believes that age can be accurately assessed for both young and old animals provided practice and care is used in the selection of material and interpretation of results.

In this study techniques based on all four major criteria were attempted; body growth measurements representing increase in size, tooth wear representing structural degradation, tooth eruption sequence, pregnancy palpation, nipple distension, and epaulette development representing growth changes and canine tooth sectioning in order to count the incremental lines in dentine. This last method was however rejected because of difficulty in accurately counting the number of incremental rings and the length of the procedure.

A complex method (described in APPENDIX 1) involving the use of pregnancy palpation, nipple distension, epaulette development methods and knowledge of birth seasons, was used to assess initially the age of bats which were to be used for the development of age determination techniques. A combination of methods, using body growth presented as growth curves, tooth wear and tooth eruption sequence was then selected for routine use. These three criteria were chosen for their application to living specimens (since 85 % of the data used in this research were obtained from live animals) and for their ease of measurement or detection in the field at night.

In addition to providing a supportive age assessment tool, the investigation of body growth and its expression in growth curves also enables comparisons to be made between the age at which sexual maturity is attained and the age at which the growth asymptote is reached.

Growth curves are a useful means of presenting the complete pattern of growth of one part of the body. They may be used to yield predictive theoretical results or to summarize growth data. If the growth curve equation is derived from a theoretical model so that the parameters have physiological meaning, then their values may be useful for theoretical purposes or at least in determining that a good fit is possible, thus adding to the validity of the model. On the other hand a growth curve of no theoretical significance might be fitted merely because of convenience of having the data summarized.

A review of growth curve literature (see APPENDIX II) draws attention to the enormous differences in opinion as to which is the best growth equation to apply. The situation was concisely summarized by Medawar (1945) who stated: 'The universal growth equation is a fiction'.

Beverton & Holt (1957) commented: 'The important question is not whether a universal representation of growth in a mathematical form is possible, but whether a representation can be made that is adequate for a particular purpose'. The solution therefore, would seem to be not to search for one formula to describe all growth forms, but to choose the function which best satisfies the essential requirements of the case in hand.

Having examined the possible growth functions available, it was decided to apply two equations, the Von Bertalanffy and the Stevens asymptotic regression to the E. wahlbergi growth data, with a view to accepting the function most appropriate to the data. These two equations were selected for four main reasons: (i) The data suggest an obvious asymptote; (ii) the parameters in both equations are regarded as having some biological significance (and therefore can be used for predictive purposes); (iii) the existence of a computer program for the Von Bertalanffy function (Hanks, 1972); (iv) the suitability of the Stevens asymptotic regression for easy conversion to a computerized form.

The main purpose of this chapter is to develop foundation techniques for use in the age and growth stage assessment of material examined to investigate the reproductive biology of E. wahlbergi.

3.2 MATERIALS AND METHODS

3.2.1 Age determination by increase in size and body growth

Although methods involving increase in size criteria are simple and easy to apply, their uses and reliability are limited. A diversity of factors affect mass, irrespective of age, leading to possible misinterpretation of results. Linear dimensions were chosen as age assessment criteria in this study because they are less subject to external factors such as seasonal variations and because these measurements are easy to take from live specimens in the field at night. However linear dimensions can also present problems resulting in the limitation of their uses, as was experienced when assessing the extent to which known age data from cage born bats could be used (3.3.1.2).

Linear measurements were used to provide a supportive age determination method for bats up to the age at which the growth asymptote is reached and to describe body growth.

(a) Linear measurements

The following three linear measurements were taken from live

specimens to the nearest 0,1 mm (reproduceability $\pm 0,1$ mm) using a vernier calliper. (Some cage maintained animals provided more than one measurement as they were measured at monthly intervals).

The forearm length was measured from the tip of the elbow to the radio/carpal junction on the inside surface of the fully extended left wing (FIGURE 14). More usually forearm length is measured along the outside of the wing. Experience has shown that this measurement varies with degree of wing flexure. The above technique was used to increase reproduceability and enable comparisons to be made with dry mounted museum material of varying wing flexures. Four hundred and fourteen bats (233 $\sigma\sigma$ and 181 ♀♀) were used to provide 489 forearm measurements for growth analysis.

Eye-nose distance was measured from the anterior corner of the right eye to the outer edge of the right nostril (FIGURE 15). Three hundred and eighty eight bats (223 $\sigma\sigma$ and 165 ♀♀) were used to provide 449 eye-nose measurements for growth analysis.

Zygomatic width was measured across the widest part of the head, just posterior to the eye (FIGURE 16). One hundred and seventy five bats (114 $\sigma\sigma$ and 61 ♀♀) were used to provide 190 zygomatic width measurements for growth analysis.

Although animals were weighed, mass was not used in growth assessment because large fluctuations in mass were observed in caged animals as a result of variation in diet, weather, pregnancy and state of health.

(b) Source of growth data

Bats which were used to provide growth data were initially age assessed by a complex method fully described in APPENDIX I.

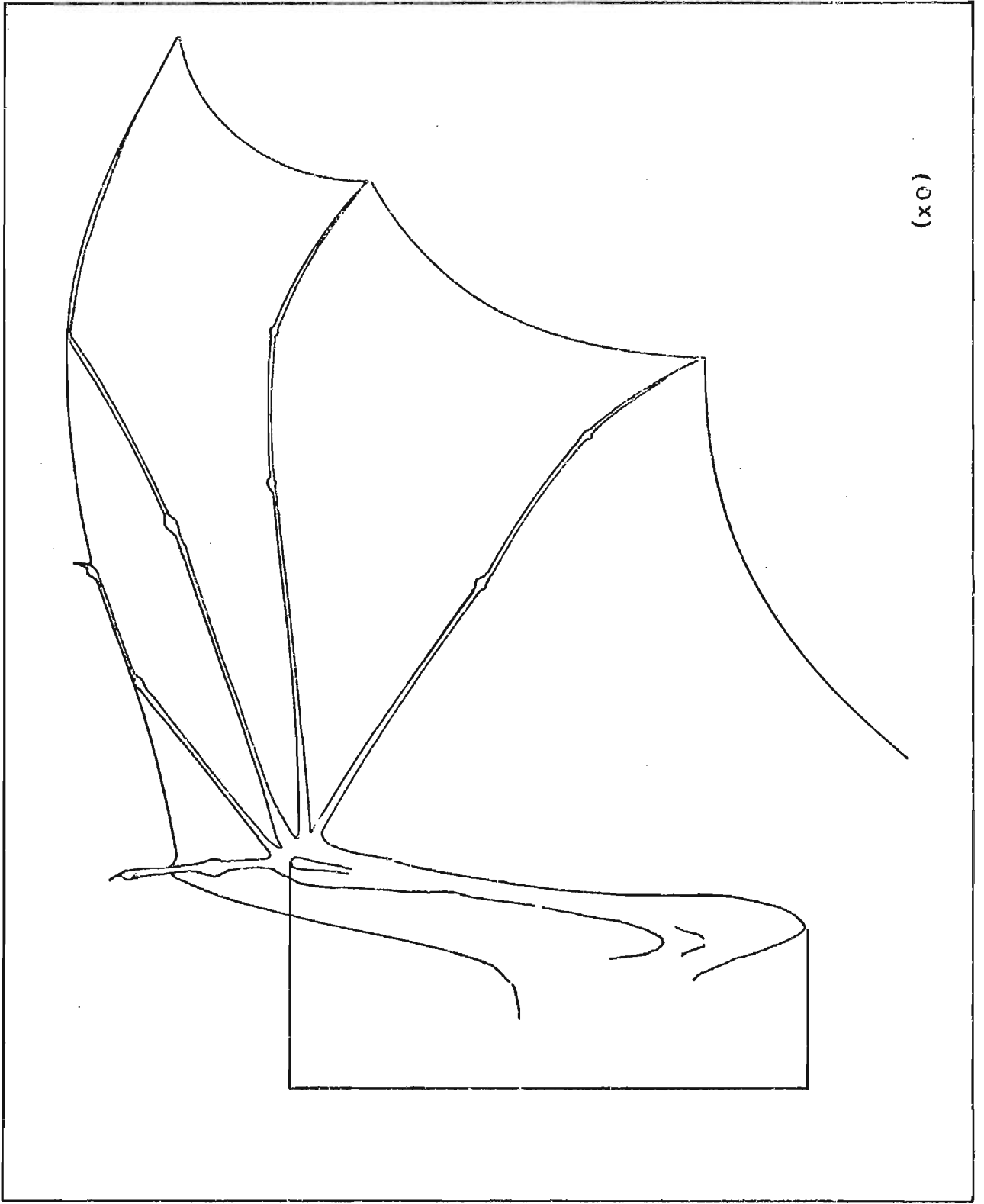
(c) Growth functions

The Von Bertalanffy equation and the Stevens asymptotic regression (APPENDIX II) were applied to forearm length, eye-nose distance and zygomatic width growth data. A computer program as used by Hanks (1972) was employed to fit the Von Bertalanffy function and a new program was written for the Stevens (1951) asymptotic regression (APPENDIX III), providing 95 % confidence limits for the raw data.

3.2.2 Age determination by dentitional criteria

Two dentitional techniques were used to determine age. The first,

FIGURE 14 Inside of wing surface showing the method by which the forearm measurement was taken

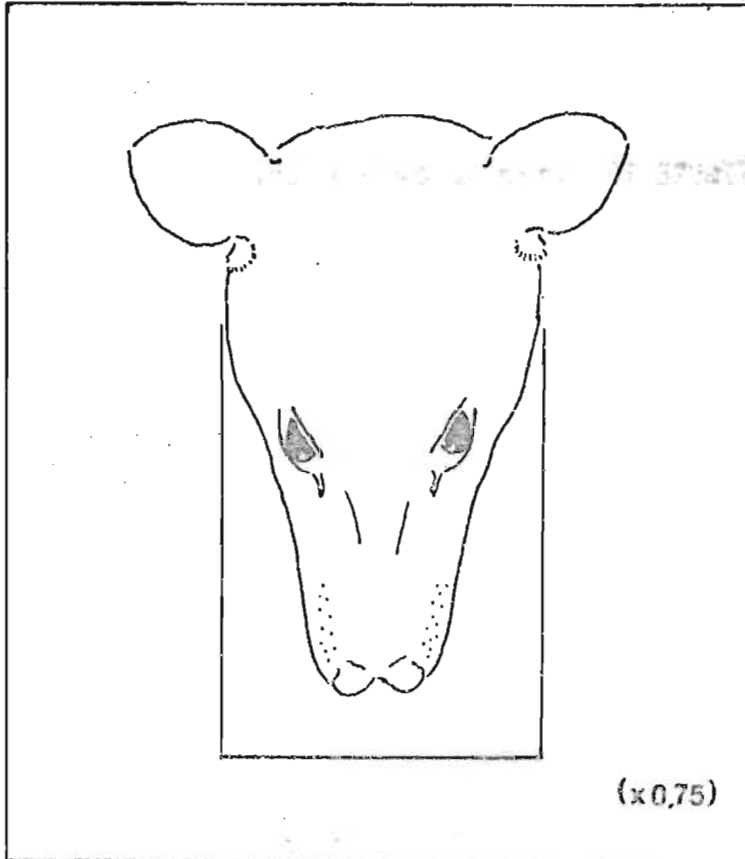
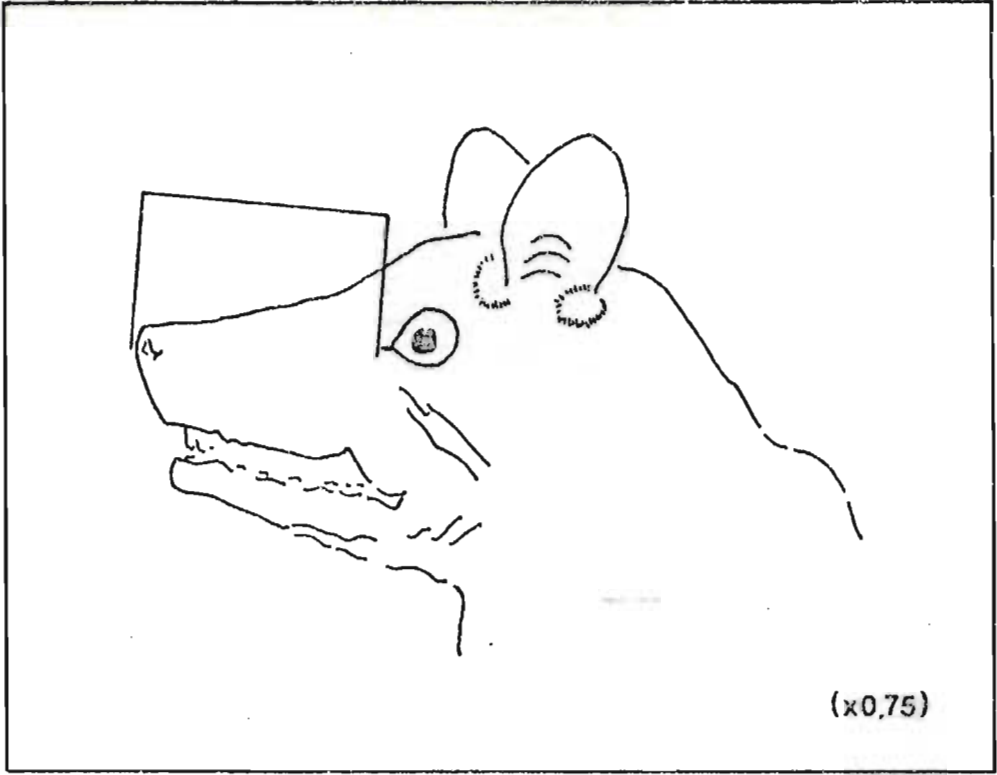


(x0)

FIGURE 15 Head showing the method by which the eye-nose distance measurement was taken

927 944

FIGURE 16 Head showing the method by which the zygomatic width measurement was taken



involving eruption sequence, ages at eruption and variation in eruption timing, was used to develop a method for determining the age of bats from birth until a full permanent dentition had formed. The second, using tooth crown height above the gum as a measure of wear, was used for assessing the age of bats after the permanent teeth had erupted.

3.2.2.1 Age determination by tooth eruption sequence

The method was selected here, for use in the field, because it offered a quick and accurate means of determining the age of young bats. Clear evidence of linear growth retardation in cage born bats from which the eruption sequence had been determined, threw some doubt on the validity of the method. This was later dispelled by favourable comparisons between cage born and wild bat tooth eruption timings (3.3.3).

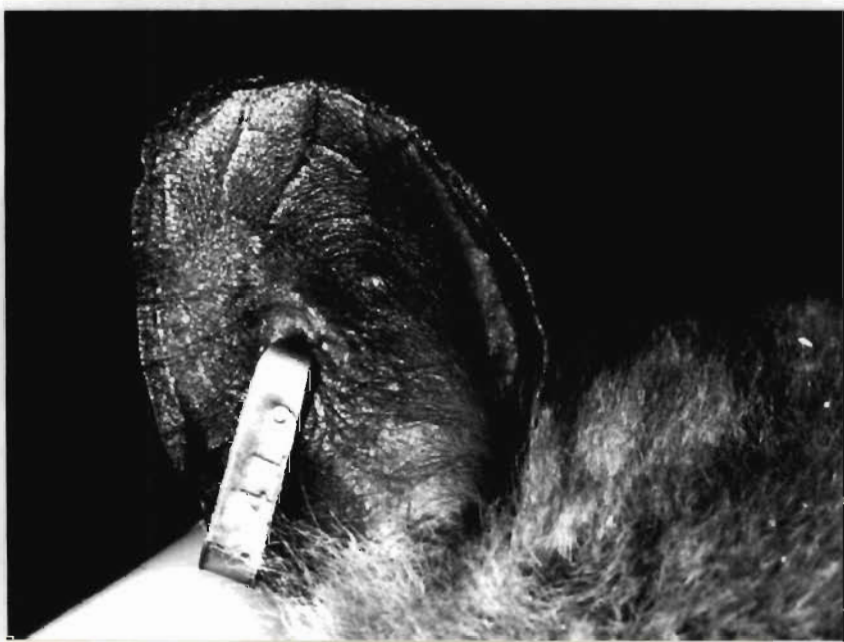
Tooth eruption data were obtained from two captive colonies kept in successive years. The first consisted of eight females, eight infants and one mature male and the second of six females and six infants, to give a total of 14 cage born specimens of precisely known age.

Each infant born in captivity was ear tagged (PLATE 6) at birth and afterwards caught and examined at a minimum of three day and a maximum of 14 day intervals up to a maximum age of 24 weeks. Examination of the right hand side of the mouth was conducted on each infant to detect the presence of teeth as they erupted. As the bats were not caught every day, the day recorded when the tooth was first seen to have erupted is the latest possible age at which eruption could have occurred. For deciduous teeth, the day recorded on which the tooth was last observed is the earliest age after which it could have been shed.

For comparative purposes and in order to confirm the validity of results obtained from caged subjects, part of the eruption sequence in three wild recaptured neonates of known age and seven juveniles of estimated age based on linear body measurements (for definition of neonate and juvenile see APPENDIX I (a) (ii) and (b) (i)) was examined and recorded.

For illustrative purposes and to confirm the relative positions of the deciduous teeth and erupting permanent teeth X-rays, using a dental X-ray machine were taken of adult and infant dry skulls, sagittally sectioned to avoid superimposition of teeth from left and right hand sides. Abbreviations for tooth names are those used by Smuts, Anderson & Austin (1978).

PLATE 6 Ear tag of the type used on one day old
juvenile, used to identify caged bats



3.2.2.2 Age determination by tooth wear

Tooth wear as expressed by tooth height measured from the gum line to the crown was chosen as the only practical method for age assessing live physically mature bats.

The crown heights of two teeth, P_3 and upper C were measured from the anterior gum line to the anterior tooth crown on the right hand side of the mouth (FIGURE 17) using a vernier calliper (reproduceability $\pm 0,1$ mm). (Some cage maintained animals provided more than one measurement as they were measured at monthly intervals).

Three hundred and sixty nine bats (214♂♂ and 155♀♀) were used to provide 547 P_3 tooth height measurements and 266 bats (158♂♂ and 108♀♀) were used to provide 392 upper C tooth height measurements.

(a) Source of tooth height data

Bats which were used to provide tooth height data were initially age assessed by the method fully described in APPENDIX I. As P_3 and upper C start to erupt at 1,0 month, bats younger than this were not used. Bats between 1,0 and 5,0 months were used to determine the age at which tooth emergence ceases and tooth wear alone proceeds. This was found to occur from 5,0 months onwards for both male and female P_3 and upper C.

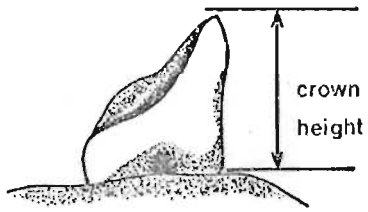
(b) Tooth height/age relationship

Two methods were employed to determine (or confirm) the mathematical relationship between tooth height and age from five months onwards, in order that an age prediction technique based on tooth wear could be developed. The two methods used were a comparison of four regression analyses and a comparison of actual tooth wear rates with theoretical tooth wear rates, obtained from linear regression analysis.

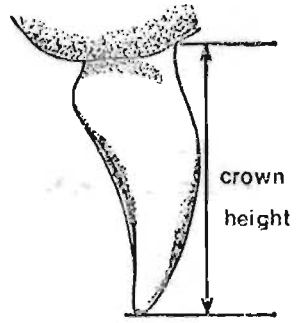
(i) Comparison of four regression analyses

Four regression analyses, linear, exponential, logarithmic and power were applied to the male and female P_3 and upper C heights with age, from 5,0 months onwards, in order to

FIGURE 17 Measurement of P_3 and upper C tooth height above the gum



P₃
(x7)



UC
(x7)

select the best fit (3.3.4).

- (ii) Comparison of actual tooth wear rates with theoretical tooth wear rates, obtained from linear regression analyses.

The results of the comparison between the four regression analyses (i) showed that the best fit to the tooth height/age data was a straight line (3.3.4). In order to corroborate this result, the slope of the fitted straight line was adopted as the theoretical value for tooth wear rate and this compared with an actual value for tooth wear rate, calculated independently as shown below.

The actual tooth wear rate (mm/month) was determined by taking the absolute loss in P_3 and upper C height in bats between captures, divided by the precisely known interval in months. (Intervals between captures of less than three months and bats younger than 5,0 months were not used). This value was calculated for female P_3 using wild recaptured animals and for female upper C using a combination of wild recaptured and cage maintained animals, as insufficient wild recaptures alone were available for this measurement. Too few male animals, either wild or cage maintained were available to perform this test on male P_3 and upper C.

Eighteen wild recaptured females (APPENDIX I (b) (iv)) with widely different tooth heights (3,3 - 1,1 mm) and recaptured over intervals varying from three to 28 months, were used to obtain the actual female P_3 wear rate. Six wild recaptured females and 15 cage maintained females (APPENDIX I (b) (v)), also with widely different tooth heights (4,4 - 2,5 mm) and recaptured over intervals varying from three to 17 months were used to obtain the actual female upper C wear rate.

All the wild recaptured bats which were used for the calculation of actual tooth wear rate were of unknown age and had not been used to provide data for the tooth height/age plots from which the theoretical tooth wear rates were calculated.

- (c) Tooth growth/wear curve

On the basis that a linear relationship exists between

tooth height and age from 5,0 months onwards, and that tooth growth would be asymptotic if wear did not intervene, a biologically significant curve was developed to describe the entire process of tooth development from eruption and growth through to wear. The data which were used to develop the tooth growth/wear curve are described in 3.2.2.2 (a) and in the APPENDIX I. The development of the equation for this curve and the computer program which was designed to execute it are set out in APPENDIX III.

3.3 RESULTS

3.3.1 Body growth

3.3.1.1 Comparison of the Von Bertalanffy function with Stevens asymptotic regression

The Von Bertalanffy function and Stevens asymptotic regression were used to construct the six growth curves; male and female forearm length, eye-nose distance and zygomatic width. The curve fits achieved for the same data sets by the two growth functions were compared by taking the point of greatest divergence between the curves and expressing it as a percentage difference. The greatest difference occurred between the two curves for growth in male eye-nose distance (10,54 %). The mean percentage difference for the three male growth curves was 7,76 %, s.d. = 4,29 and for the three female growth curves 1,14 %, s.d. = 0,38. As would be expected by nature of the form they are designed to assume, very little difference exists between the fits provided by the two growth functions and on this basis alone selection could not be made. However Stevens regression was chosen for application to the growth data in this research because it is a simple and easily applied formula, providing as good a fit as the Von Bertalanffy function without the computation of parameters of dubious biological significance (Hanks, 1972) and derivation (Richards, 1959) and for its useful quantification of growth rate (ρ) suitable for comparative purposes. The Von Bertalanffy equation has been used frequently in the study of African mammals (APPENDIX II) whereas the equally suitable Stevens regression appears to have been overlooked.

3.3.1.2 Stunted growth in cage born bats

Forearm length and eye-nose growth data from cage born and wild bats were compared (FIGURES 18 to 21) in order to assess the extent to which these two sources of data could be combined. (See FIGURE captions for details of numbers of specimens and measurements used in these comparisons). Zygomatic width growth was not compared as this measurement was not available for cage born bats older than 2,5 months.

The differences between the asymptotic growth values were found to be highly significant ($P < 0,001$) when one tailed tests of significance using the z statistic were applied (TABLE 2). Unfortunately, although the sample sizes for cage born bats aged 0 to 2,5 months are five or more, from 3,0 to 8,5 months the sample sizes are reduced to two or three. For this reason an indication of spread (95 % confidence limits or s.d.) of asymptotic growth data in cage born bats has not been possible, but the extremely large z values are highly indicative of great significant difference without the application of this information.

Ideally, therefore cage born bat growth data should not be used in the development of age determination procedures for use in wild populations. However because of a behavioural strategem, age groupings 0,5 to 1,5 months were never caught in the wild and apart from three wild neonates which provided growth data for male forearm length and eye-nose distances at 0 and 0,25 months, no wild specimens were caught in age groupings 0 to 0,25 months. This biased sampling was a consequence of a behavioural strategem in which mothers carry their young with them in flight while feeding for the first week, and thereafter, until the juvenile learns to fly at 2,0 to 2,5 months, leave them hanging in a tree. Mist netting of feeding bats at night therefore failed to capture young bats during this phase of life.

The age at which the cage born and wild growth patterns have distinctly diverged is variable (male forearm length at 2,5 months, male eye-nose distance at 4,0 months, female forearm length at 3,0 months, female eye-nose distance at 5,0 months).

Therefore as a result of stunting in growth, data from cage born bats were not used in the construction of growth curves except to provide a starting point where wild data in age grouping 0 months were either not available or insufficient. This was considered justified because the earliest age at which the cage born and wild growth patterns diverged was at 2,5 months.

FIGURE 18 Comparison of forearm length growth between cage born males (Δ) and wild male bats (\blacktriangle)
 Cage born bat growth curve: Ten animals provided 52 measurements. ---- represents the computer calculated asymptote for cage born bat forearm length as no bats older than 8,5 months were available in this category
 Stevens equation for this curve:

$$y = 69,29 - 36,79 (0,48)^x$$

Wild bat growth curve: Two hundred and thirty three animals provided 276 measurements. Stevens equation for this curve:

$$y = 84,78 - 51,30 (0,59)^x$$

(Vertical line - range, triangle - mean)

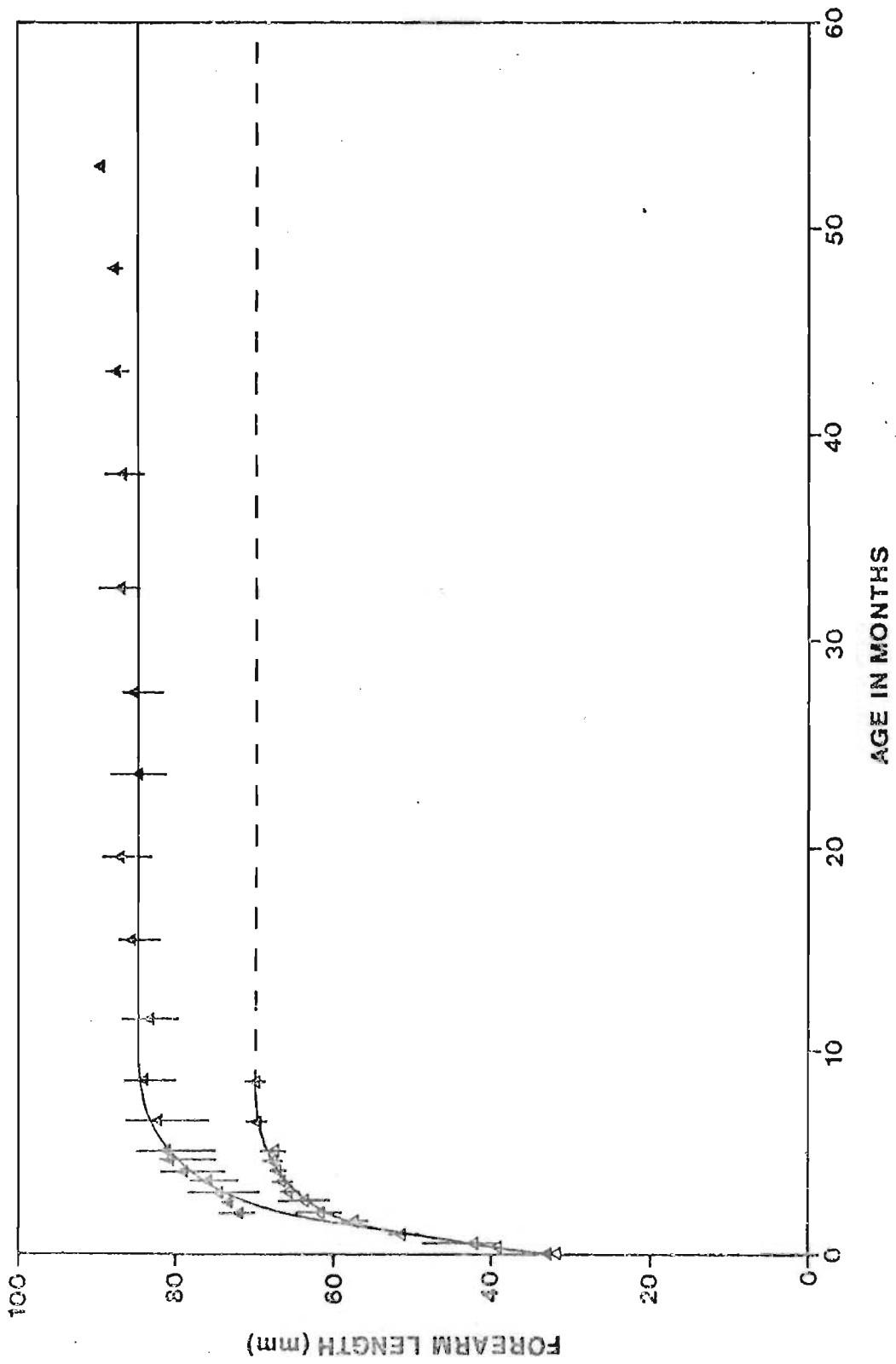


FIGURE 19 Comparison of eye-nose distance growth between cage born males (□) and wild male bats (■)

Cage born bat growth curve: Nine animals provided 61 measurements. ---- represents the computer asymptote for cage born bat eye-nose distance, as no bats older than 8,5 months were available in this category
Stevens equation for this curve:

$$y = 19,55 - 8,46 (0,71)^X$$

Wild bat growth curve: Two hundred and twenty three animals provided 255 measurements. Stevens equation for this curve:

$$y = 25,44 - 13,94 (0,79)^X$$

(Vertical line - range, square - mean)

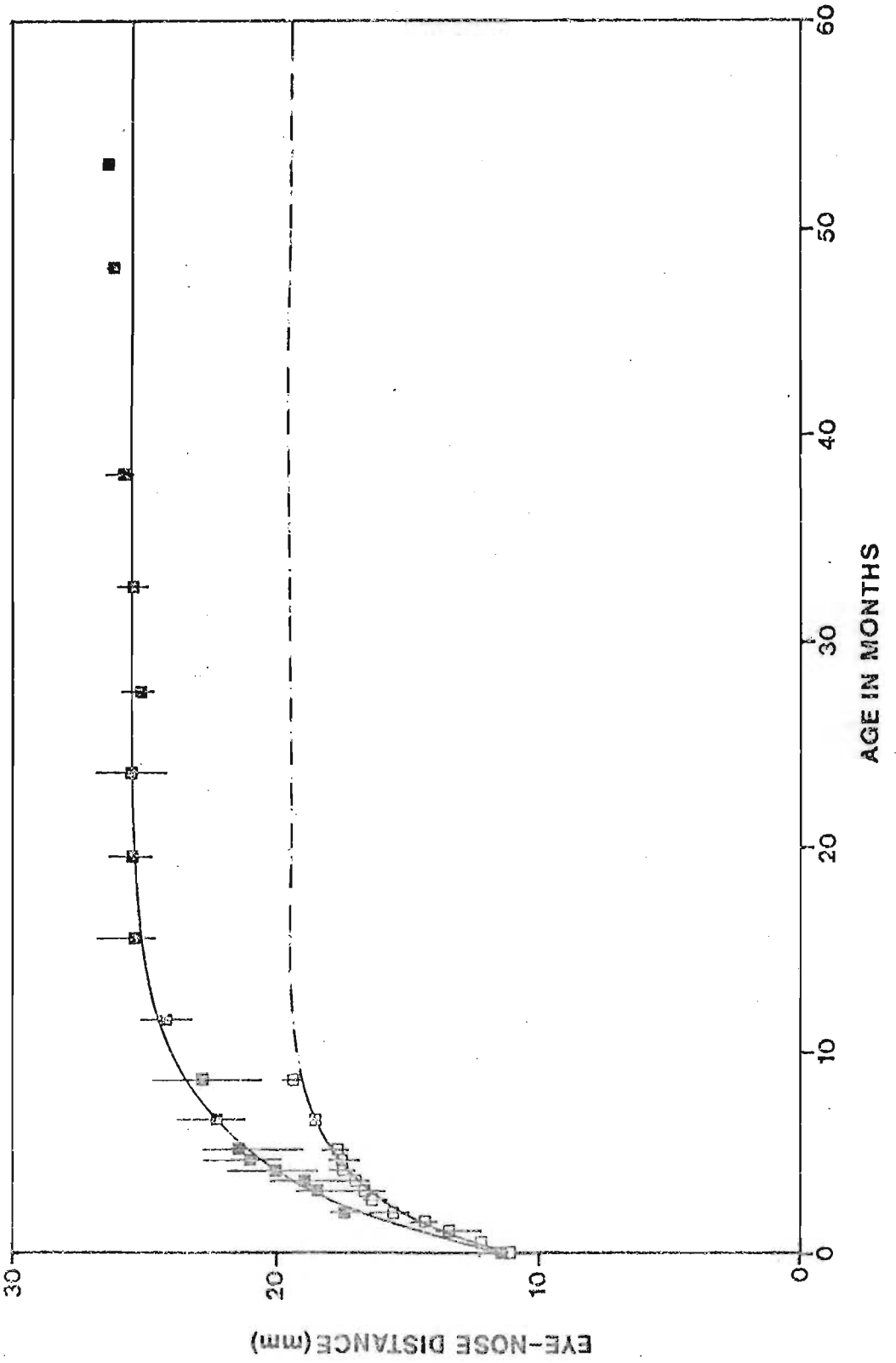


FIGURE 20 Comparison of forearm length between cage born female (Δ) and wild female bats (\blacktriangle)
Cage born bat growth curve: Nine animals provided 53 measurements. ---- represents the computer calculated asymptote for cage born bat forearm length, as no bats older than 5,0 months were available in this category
Stevens equation for this curve:

$$y = 69,26 - 38,42 (0,41)^x$$

Wild bat growth curve: One hundred and eighty one animals provided 213 measurements.
Stevens equation for this curve:

$$y = 80,68 - 48,67 (0,51)^x$$

(Vertical line - range, triangle - mean)

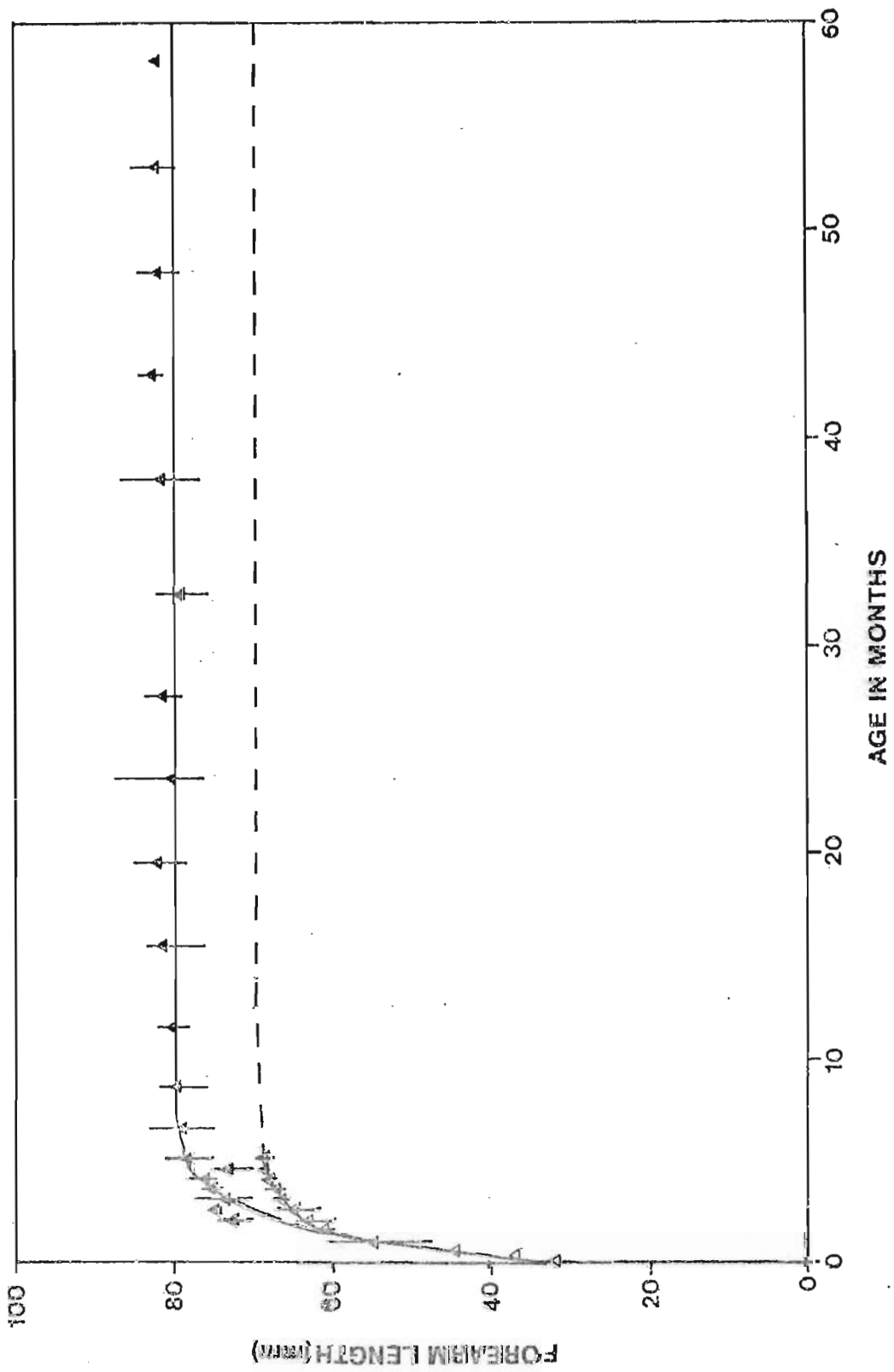


FIGURE 21 Comparison of eye-nose distance between cage born female (□) and wild female bats (■).
 Cage born bat growth curve: Nine animals provided 53 measurements. ---- represents the computer calculated asymptote for cage born eye-nose distance, as no bats older than five months were available in this category
 Stevens equation for this curve:

$$y = 18,35 - 7,76 (0,65)^x$$

Wild bat growth curve: One hundred and sixty five animals provided 194 measurements.
 Stevens equation for this curve:

$$y = 22,32 - 11,52 (0,74)^x$$

(Vertical line - range, square - mean)

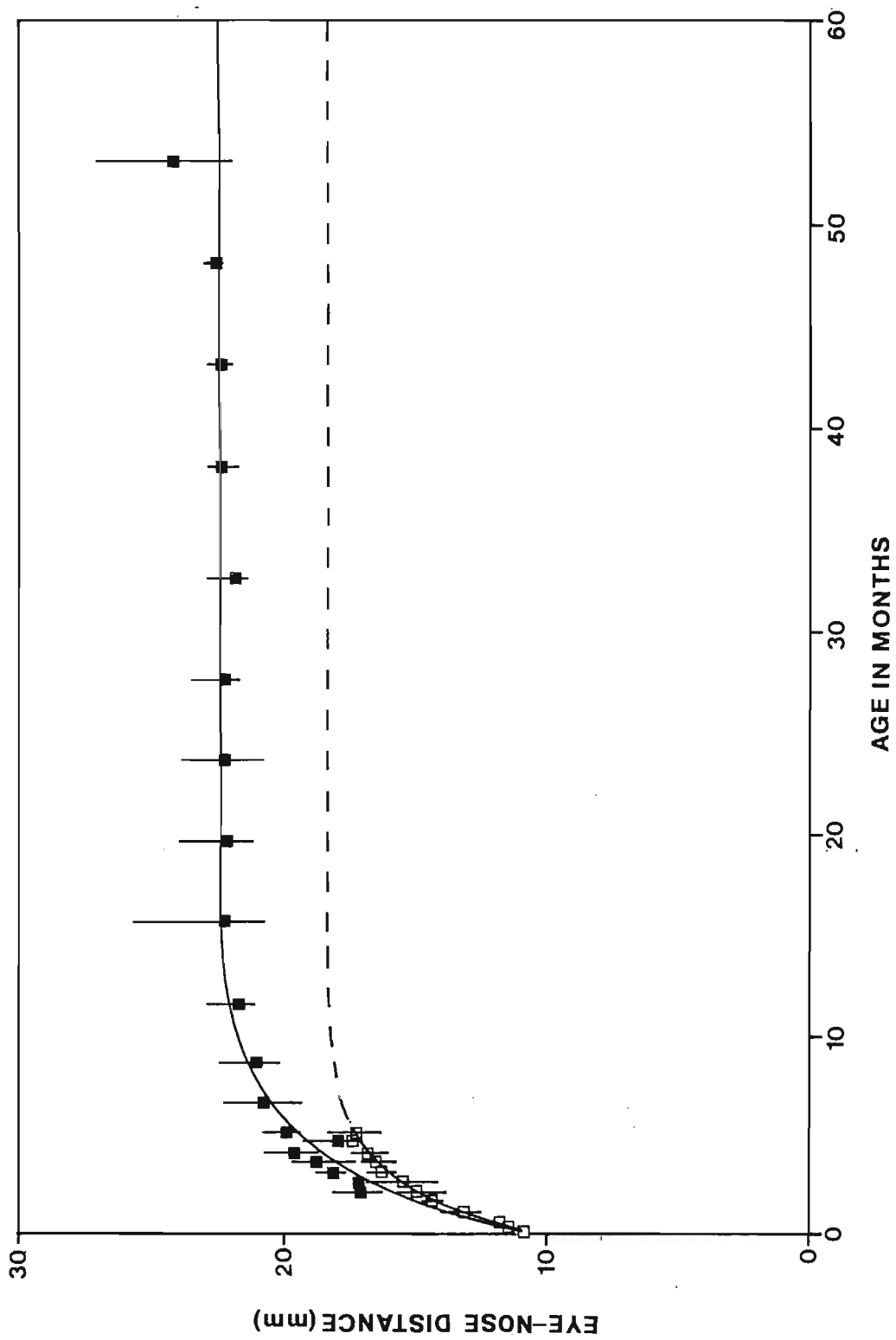


TABLE 2 Comparison of asymptotic growth values
between wild and cage born bats

| Growth measurement | wild n | wild $\bar{x} \pm 1$ s.d.
(mm) | cage born
(mm) | z for
difference | significance |
|--------------------------|--------|-----------------------------------|-------------------|---------------------|--------------|
| Male forearm length | 43 | 84,78 \pm 2,97 | 69,29 | 34,21 | P <0,001 |
| Male eye-nose distance | 36 | 25,44 \pm 0,90 | 19,55 | 39,12 | P <0,001 |
| Female forearm length | 113 | 80,68 \pm 4,40 | 62,26 | 44,52 | P <0,001 |
| Female eye-nose distance | 106 | 22,32 \pm 1,40 | 18,35 | 27,28 | P <0,001 |

(No n or s.d. values are given for cage born bats, because the asymptotic value presented is a computer calculated value based on measurements of bats prior to reaching the asymptote and small numbers (two and three) which had reached the asymptote)

3.3.1.3 Growth curves and relationship of growth stages to dentitional, reproductive and behavioural events

Growth curves for male and female forearm length, eye-nose distance and zygomatic width were constructed using the computer program for Stevens asymptotic regression with 95 % confidence limits for the raw data (FIGURE 22 to 27). Information concerning the numbers of animals used for each curve, the composition of known age and estimated age bats, and the Stevens regression equations for each curve is given in the caption for each FIGURE.

Growth does not occur as smoothly as a mathematically constructed growth curve would suggest (Jenss & Bayley, 1937), nor is maximum size achieved instantaneously or at the same age by the entire population (upper 95 % confidence limit in FIGURES 22 to 27 demonstrate this). For these reasons an exact value for the age at which the asymptote is reached cannot be given, but to enable comparisons to be made, an arbitrary value may be chosen. In this study the asymptotic age is given as the age at which the growth rate first reaches 0,01 % of the asymptotic value (as calculated from Stevens regression) per month.

(a) Male forearm length (FIGURE 22)

Forearm length growth from birth until 3,0 months is rapid, with a mean growth rate of 13,61 mm/month. This phase coincides with the juvenile period (APPENDIX I (a) (i)) during which the young bat is suckled and dentitional loss and eruption occur. At 3,0 months the growth rate starts to decrease, weaning takes place and a full permanent dentition is present. From 3,0 to 11,0 months (attainment of the asymptote of 84,78 mm), the growth rate slows to a mean value of 1,29 mm/month. This phase corresponds to the subadult period (APPENDIX I (b) (ii)) prior to sexual maturity and covers the transitional stage when tooth emergence is replaced by tooth wear.

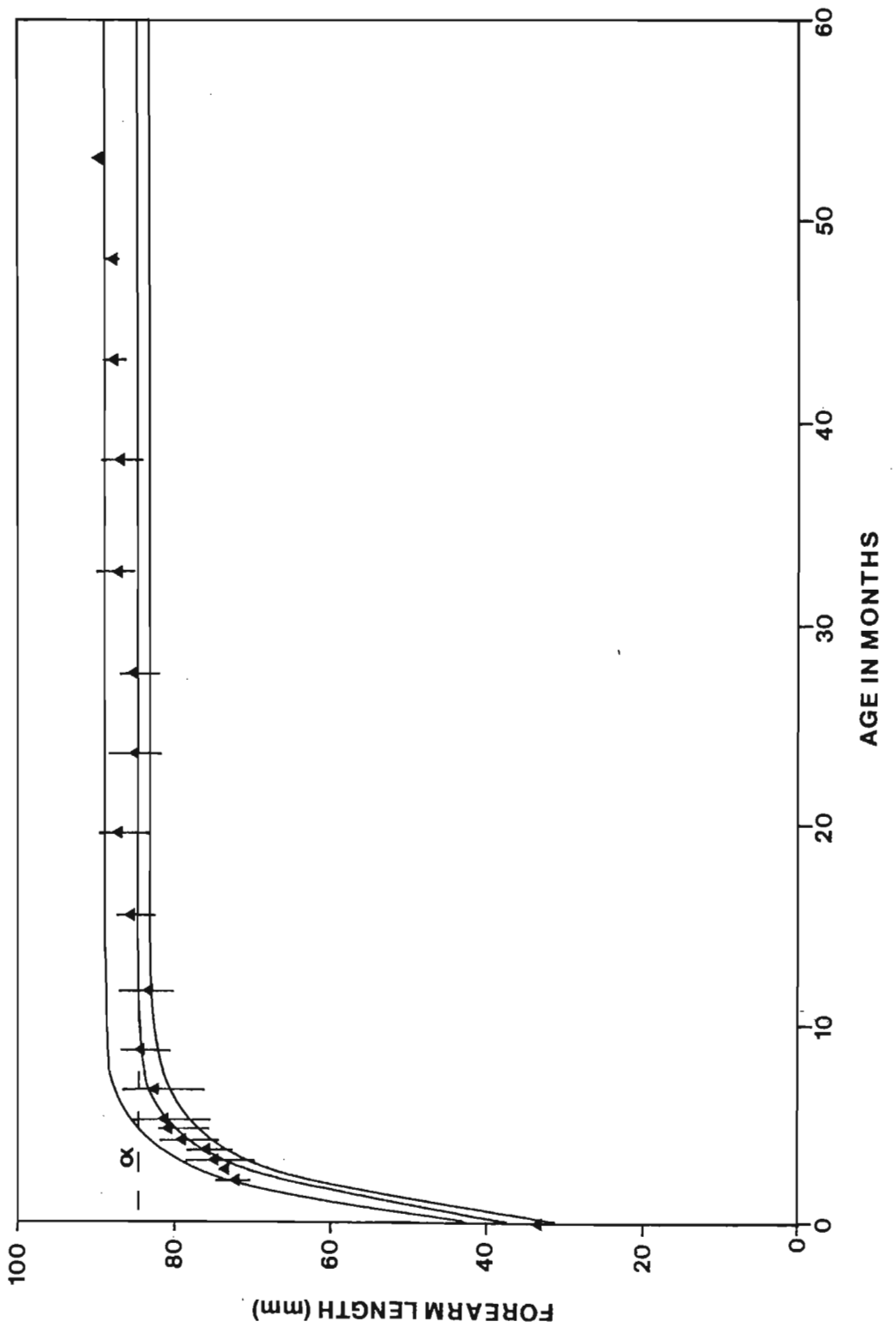
The attainment of sexual maturity, evidenced by epaulette and dark ruff development, testes tubule diameter in excess of 140 μ m and the presence of spermatazoa in the epididymis cauda (4.3.2) occurs between 13,0 and 17,0 months of age. This occurs soon after the attainment of the asymptotic forearm length of 84,78 mm is reached at 11,0 months.

FIGURE 22 Stevens asymptotic regression curve for male forearm length growth with 95 % confidence bands Two hundred and thirty three bats were used (15 known age, 218 estimated age)
Equation for the curve:

$$y = 84,78 - 51,30 (0,59)^x$$

α = asymptote

(Vertical line - range, triangle - mean)



The adult phase of life succeeds this and is characterized by reproductive activity and tooth wear.

(b) Male eye-nose distance (FIGURE 23)

Eye-nose distance has a much slower growth rate than forearm length. The most rapid period of growth occurs from birth up to 7,0 months (1,61 mm/month) and extends from the juvenile into the subadult period of life. From 7,0 months until the eye-nose distance asymptote (25,44 mm) is reached at 20,5 months, the rate of growth decreases to a mean rate of 0,19 mm/month. The asymptotic value is attained well into adult life, as defined by the onset of sexual maturity and attainment of forearm length asymptote.

(c) Male zygomatic width (FIGURE 24)

The growth pattern of this cranial measurement is similar to that for eye-nose distance. The most rapid period of growth occurs from birth to 7,0 months (1,33 mm/month) and also extends throughout the juvenile phase into the subadult.

The growth asymptote of 27,50 mm is reached at 21,0 months after a period of rapidly decreasing growth rate (from 7,0 to 21,0 months; mean growth rate 0,18 mm/month).

(d) Female forearm length (FIGURE 25)

Like the male the most rapid period of forearm growth, from birth to 3,0 months (mean growth rate 14,05 mm/month) coincides with the juvenile phase. From 3,0 to 8,5 months the growth rate decreases to 1,15 mm/month. During this period of slower growth, prior to the attainment of the forearm length asymptote, the female bat, unlike the male reaches sexual maturity and conceives for the first time at 5,0/6,0 months of age.

A female subadult nulliparous bat (APPENDIX I (b) (ii)) is defined as being from 3,0 to 5,0/6,0 months old. At 5,0/6,0 months old she first becomes pregnant and passes from the subadult to the adult stage. Therefore the two aspects of physical maturity, attainment of maximum linear size and sexual maturity do not coincide. Growth in linear dimensions continues to occur into 'adulthood' as defined by the ability to reproduce. After the attainment of the

FIGURE 23 Stevens asymptotic regression curve for male eye-nose distance growth with 95 % confidence bands. Two hundred and twenty three bats were used (12 known age and 211 estimated age). Equation for the curve:

$$y = 25,44 - 13,94 (0,79)^x$$

α = asymptote

(Vertical line - range, square - mean)

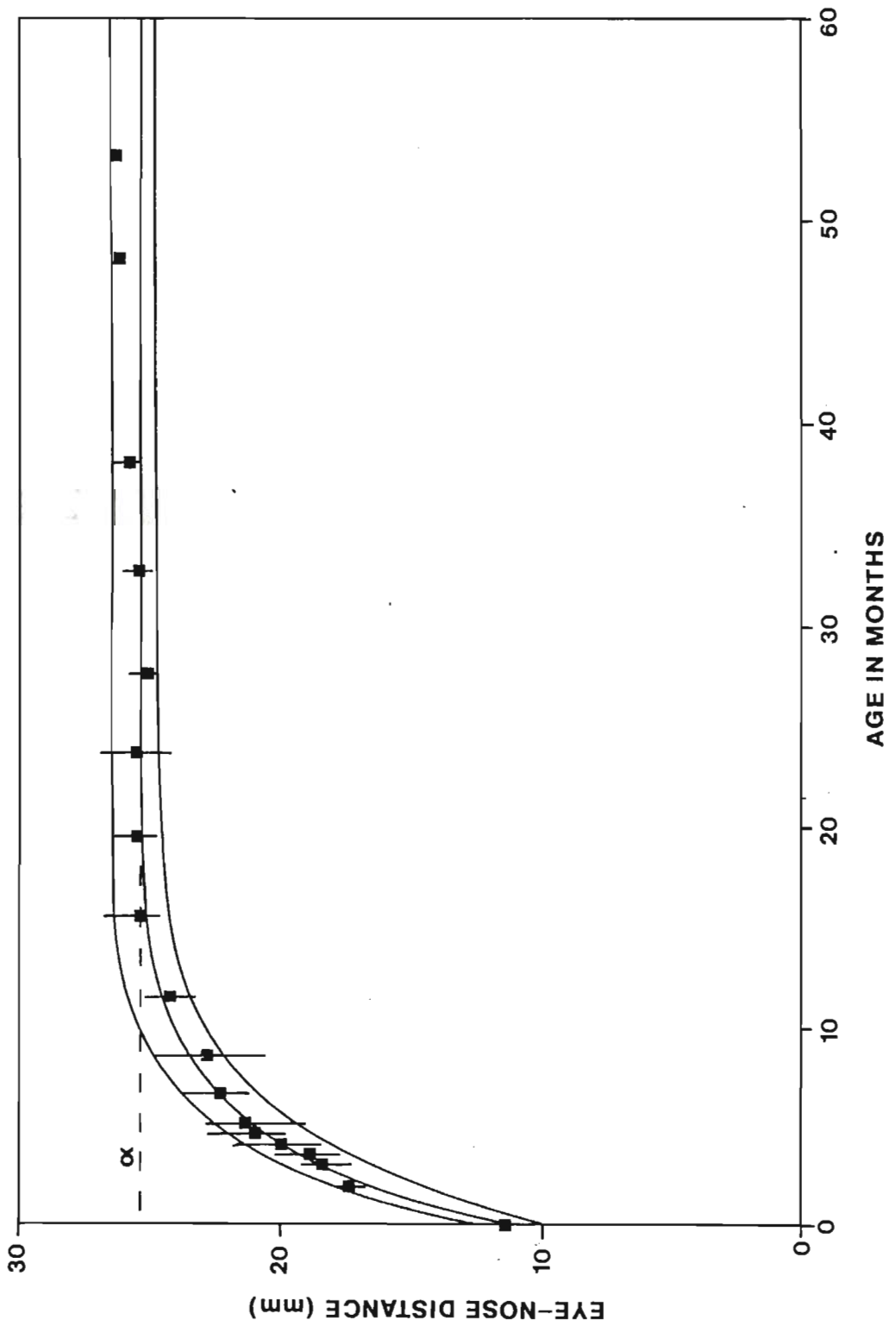


FIGURE 24 Stevens asymptotic regression curve for male zygomatic width growth with 95 % confidence bands One hundred and fourteen bats were used (11 known age and 103 estimated age) Equation for the curve:

$$y = 27,50 - 12,05 (0,81)^x$$

α = asymptotic

(Vertical line - range, circle - mean)

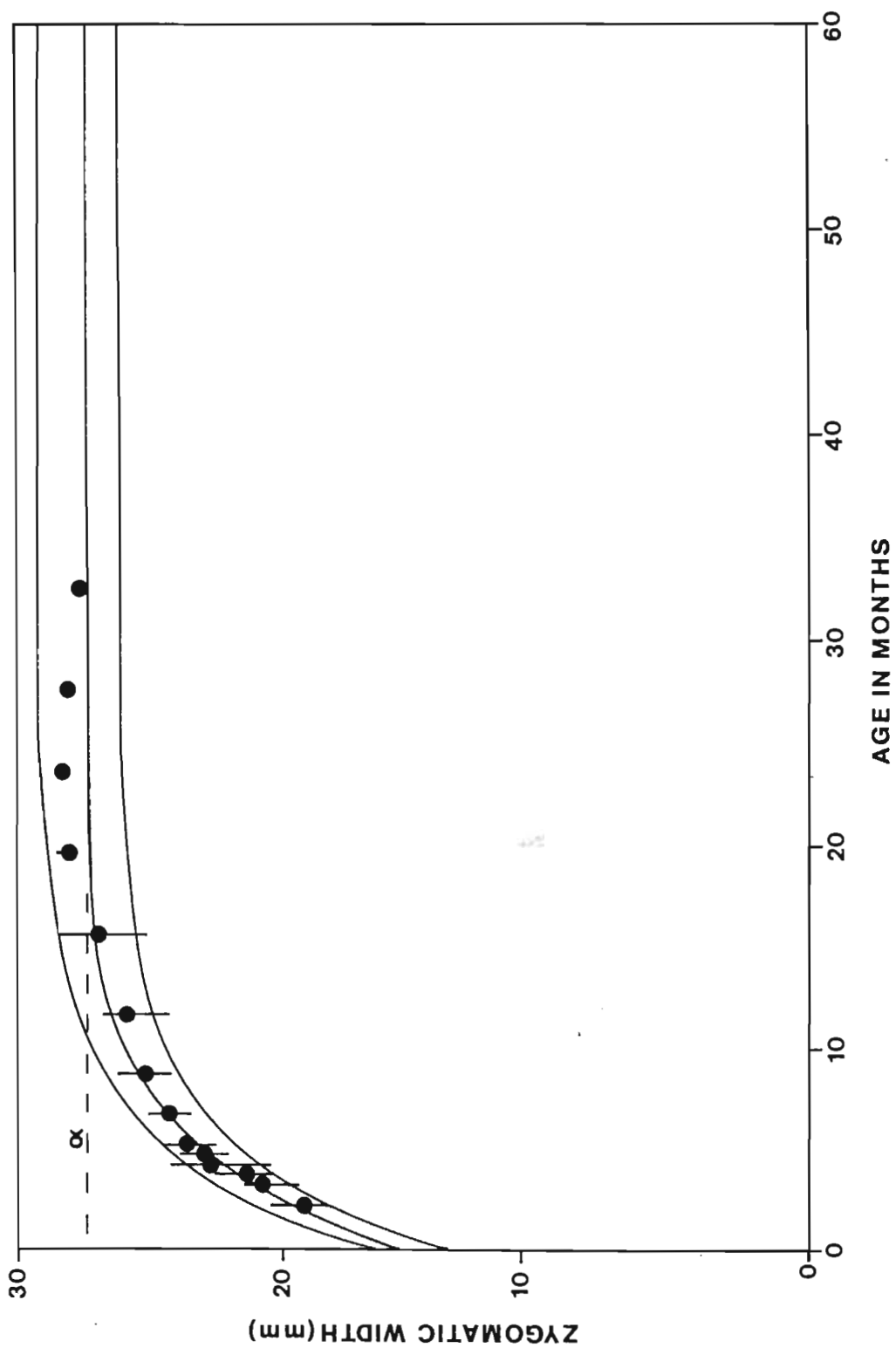
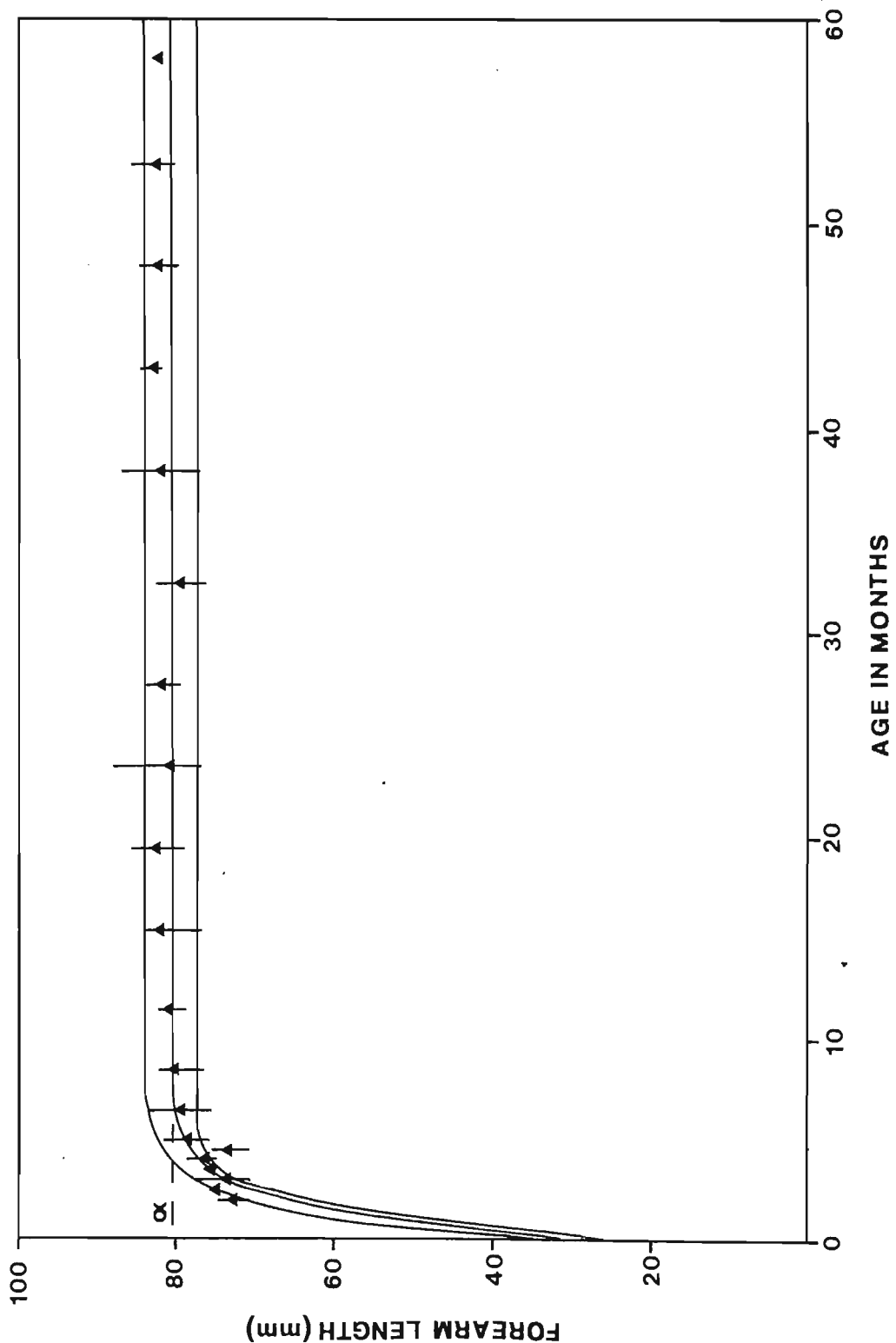


FIGURE 25 Stevens asymptotic regression curve for female forearm length growth with 95 % confidence bands. One hundred and eighty one bats were used (10 known age and 171 estimated age). Equation for the curve:

$$y = 80,68 - 48,67 (0,51)^x$$

α = asymptote

(Vertical line - range, triangle - mean)



forearm length asymptote (mean 80,68 mm) at 8,5 months, the female bat gives birth for the first time when approximately 12 months old.

(e) Female eye-nose distance (FIGURE 26)

As in the male the most rapid period of eye-nose distance growth is from birth to 7,0 months (1,46 mm/month). Thereafter the growth rate slows to a mean rate of 0,12 mm/month until the asymptotic value 22,32 mm is reached at 17,5 months.

(f) Female zygomatic width (FIGURE 27)

The early period of rapid growth occurs in the first seven months of life at a mean growth rate of 1,23 mm/month. The usual pattern of decreasing growth rate takes place from 7,0 to 17,5 months (mean growth rate 0,14 mm/month) when the zygomatic width asymptote of 25,62 mm is reached.

3.3.2 Dentition

3.3.2.1 Description of deciduous dentition

At birth, infants have the following dentition:

$$i \frac{2}{2} \quad c \frac{1}{1} \quad p \frac{1}{1}$$

Between the second and third week, the second deciduous premolars appear in the upper and lower jaw, making a total of 20 teeth (FIGURE 28). In appearance the milk teeth are small and needle sharp. They have shallow roots, are easily dislodged and they are also lightly enamelled (PLATE 7).

3.3.2.2 Description of permanent dentition

The total number of permanent teeth in a normal adult E. wahlbergi is 28 (FIGURE 29):

$$I \frac{2}{2} \quad C \frac{1}{1} \quad P \frac{2}{3} \quad M \frac{1}{2}$$

Of the four upper and lower premolars and the three upper and lower molars present in the theoretical ancestral mammal, the Megachiroptera have lost P_2 , P^2 and M^3 . Epomophorus has in addition lost P^1 , M^2 and M_3 (Grassé, 1955). Compared with the ancestral mammalian condition, the dentition can be represented as follows:

FIGURE 26 Stevens asymptotic regression curve for female eye-nose distance growth with 95 % confidence bands One hundred and sixty five bats were used (nine known age and 156 estimated age) The equation for the curve:

$$y = 22,32 - 11,52 (0,74)^x$$

α = asymptote

(Vertical line - range, square - mean)

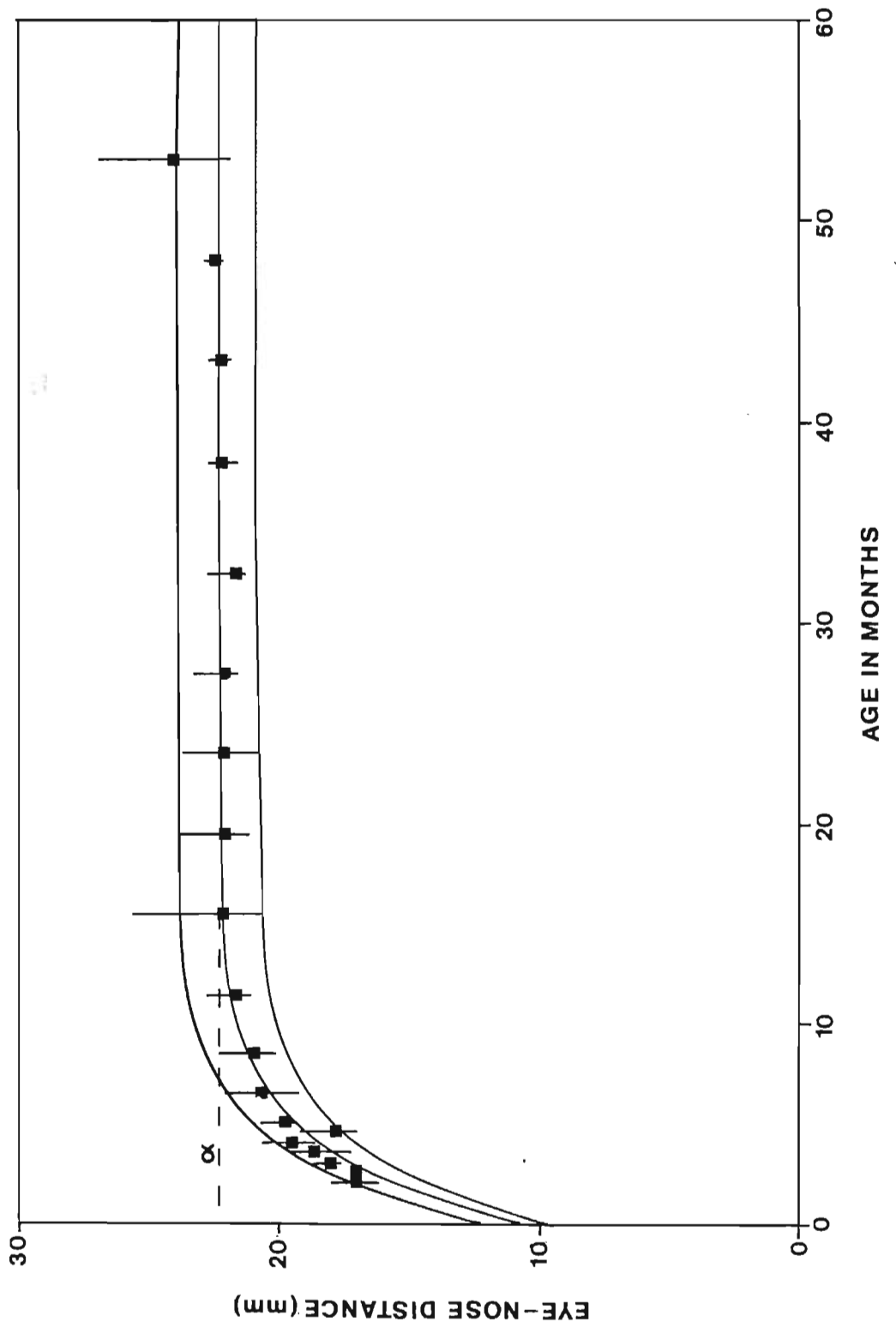


FIGURE 27 Stevens asymptotic regression curve for female zygomatic width growth with 95 % confidence bands Sixty one animals were used (six known age and 55 estimated age) Equation for the curve:

$$y = 25,62 - 10,22 (0,77)^x$$

α = asymptote

(Vertical line - range, circle - mean)

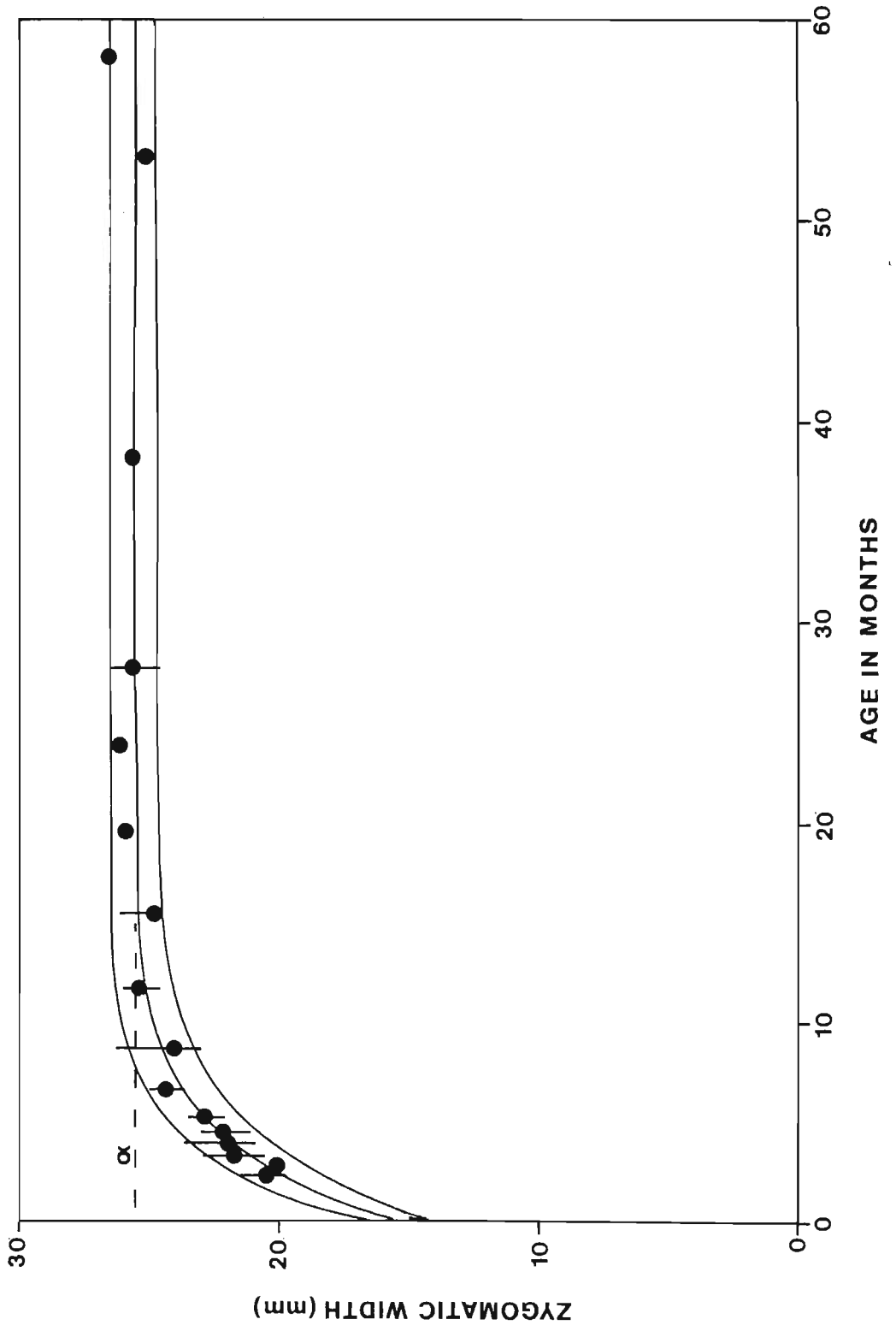


FIGURE 28 Full deciduous dentition in a 14 day old juvenile

PLATE 7 X-ray of 14 day old juvenile, showing deciduous and unerupted permanent teeth

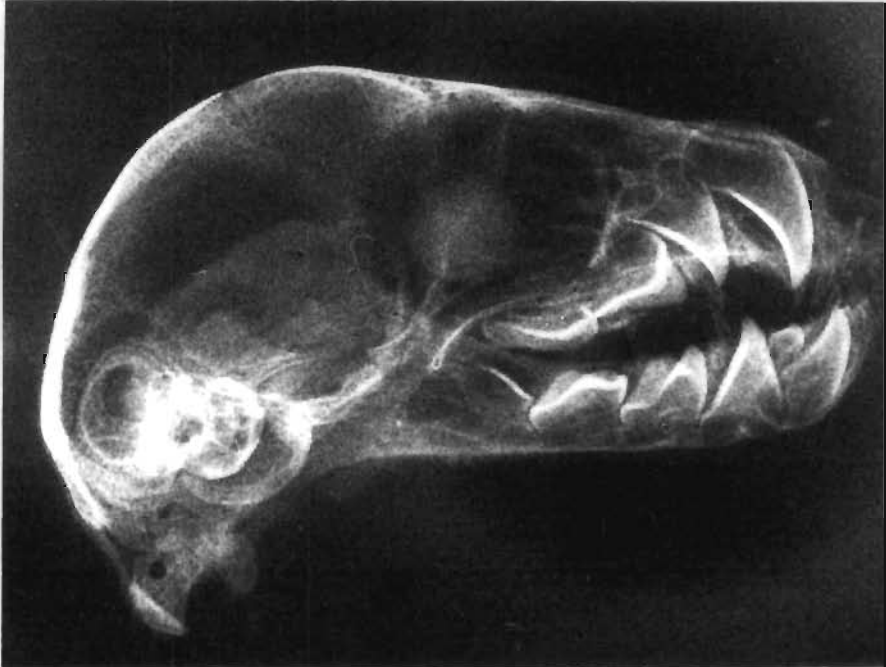
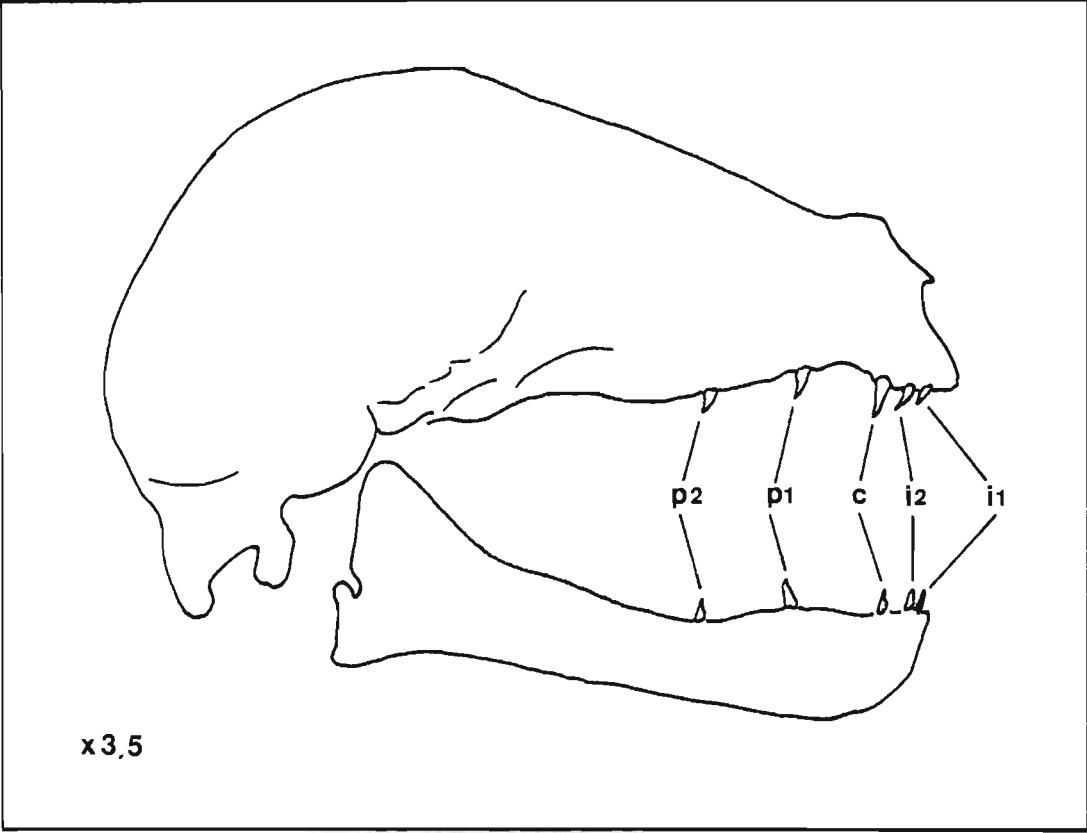
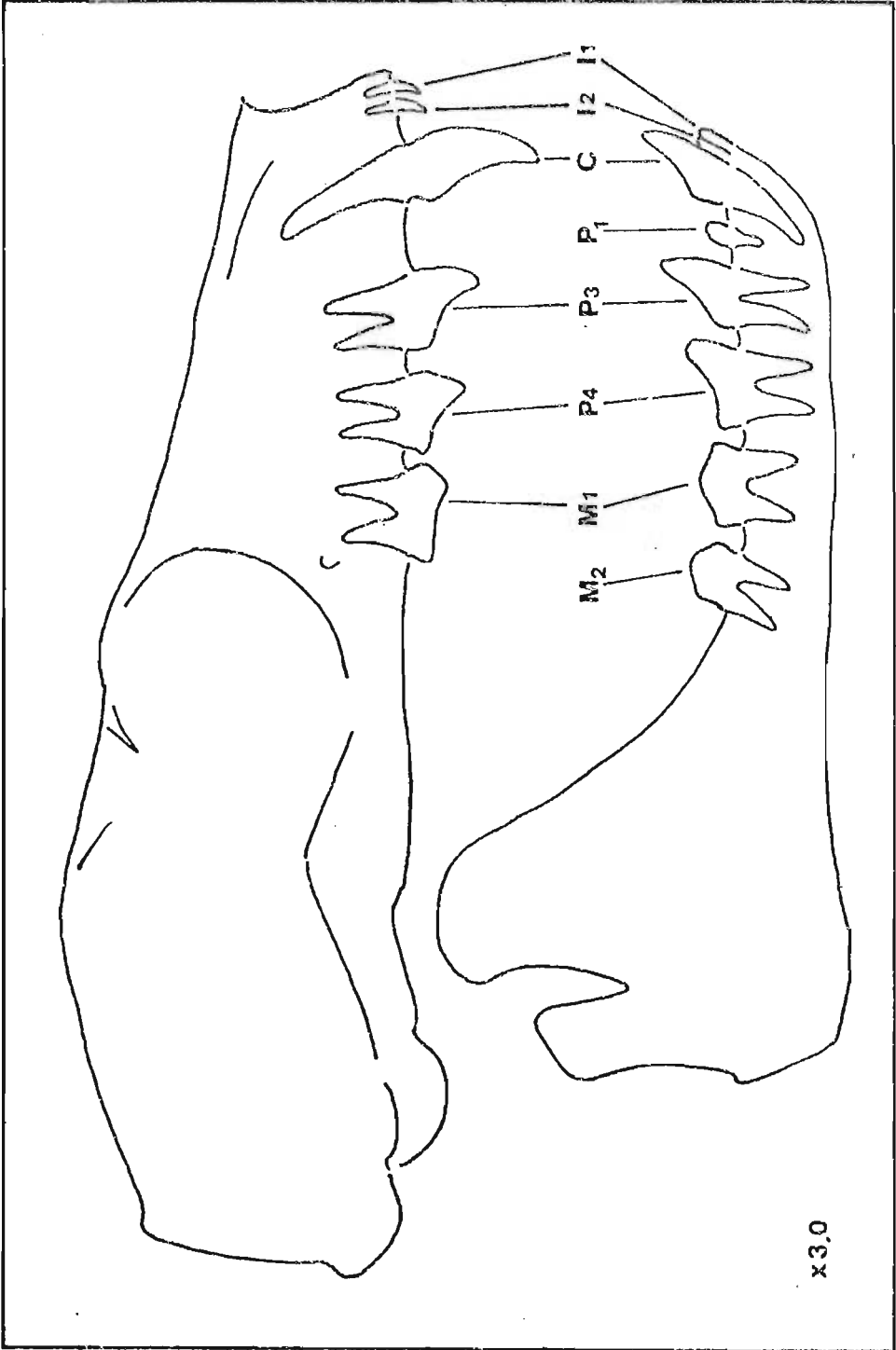


FIGURE 29 Permanent dentition in an adult Epomophorus
wahlbergi



$$I \begin{array}{c} 1 \\ 1 \\ 2 \end{array} \quad C \begin{array}{c} 1 \\ 1 \end{array} \quad P \begin{array}{c} 0 \\ 1 \end{array} \begin{array}{c} 0 \\ 0 \end{array} \begin{array}{c} 3 \\ 3 \end{array} \begin{array}{c} 4 \\ 4 \end{array} \quad M \begin{array}{c} 1 \\ 1 \\ 2 \\ 0 \end{array}$$

The teeth are strongly enamelled (PLATE 8) and the premolars and molars are conspicuously cusped.

3.3.3 Tooth eruption

TABLE 3 summarizes the sequence of loss and eruption of teeth with age and the variations in age at loss of deciduous teeth and eruption of permanent teeth. The overall pattern of loss and eruption varies between individuals. The minimum variation was 12 days for the eruption of lower C, while the maximum was a 67 day variation in loss of i^2 . In general, the teeth of the upper jaw showed a wider range over which each loss/eruption occurred (14-67 days), than those of the lower jaw (12-38 days).

There is some variation in sequence of erupting teeth, but the first and last teeth to erupt are the same in all individuals, upper C and P_3 being the first to appear and M_2 and I^2 being the last to erupt.

The age at which a full permanent dentition was reached varied from 81 to 150 days, with a mean of 106 days. This range was due to the extended presence of i^2 in two out of five juveniles. If the prolonged presence of this tooth is discounted and the eruption of the last tooth (I^2) is counted as heralding the onset of full permanent dentition, then the range is reduced to 81-100 days (12-15 weeks) with a mean of 90 days.

Although a small amount of variation exists, the position of the deciduous teeth in relation to the permanent teeth which erupt alongside is fairly constant. Upper and lower incisors all erupt immediately posterior to the deciduous incisors. Both upper and lower canines appear anterior to the milk canines. P_1 is cut posterior to the deciduous canine, while upper and lower P_3 and upper and lower P_4 erupt on either side of p^1 and p_1 (FIGURE 30 and PLATE 7).

Tooth data from three wild recaptured neonates of known age confirmed that the ages of dentitional loss and eruption found in caged bats at birth and 2,5 to 4,0 months of age were concordant. Seven wild age estimated juveniles further confirmed that dentitional ages were the same as in caged bats of 2,0 to 3,0 months old.

PLATE 8 X-ray of permanent dentition in adult
Epomophorus wahlbergi

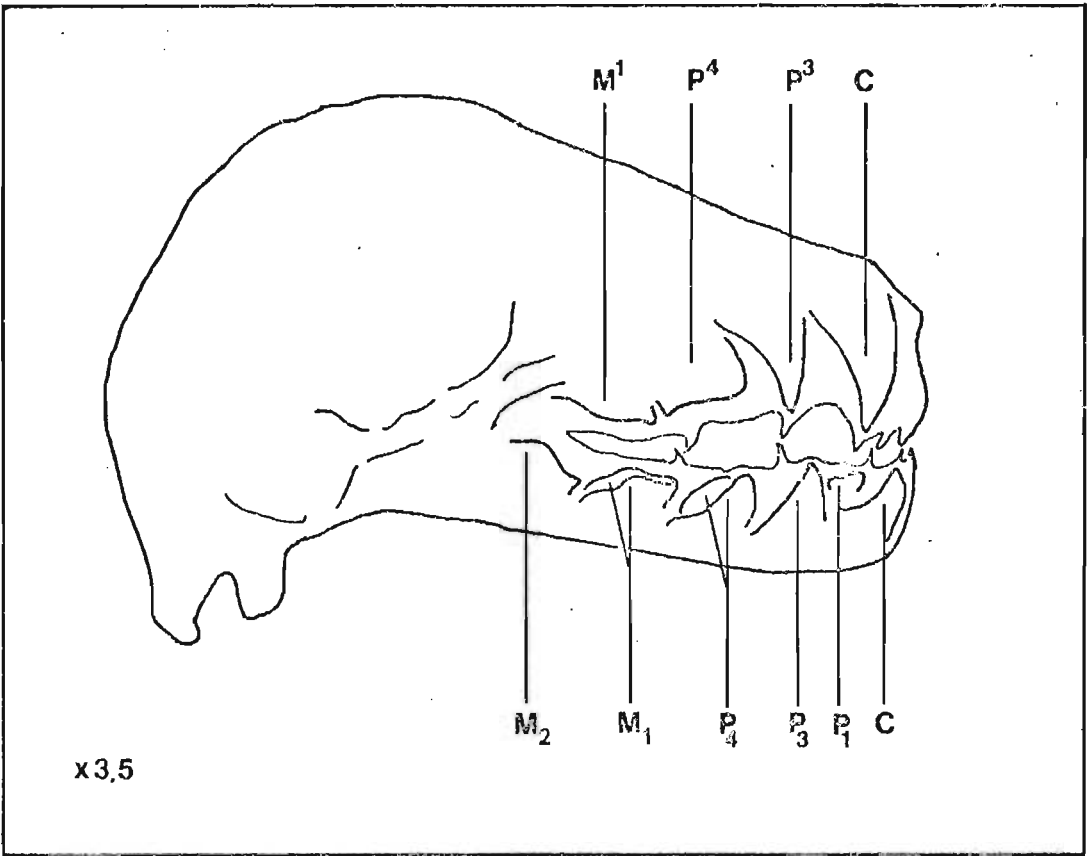


TABLE 3 Table summarizing sequence and range of ages of dentitional loss and eruption in E. wahlbergi

| Lower jaw | | | | | | | | | | | | | Upper jaw | | | | | | | | Age | | | | | |
|--------------------------------------|----------------|----|----------------|----------------|----------------|----------------|----|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----|----------------|----------------|----------------|----------------|----|----------------|----------------|----------------|-------|------|--------|
| i ₁ | i ₂ | c | p ₁ | p ₂ | I ₁ | I ₂ | C | P ₁ | P ₃ | P ₄ | M ₁ | M ₂ | i ¹ | i ² | c | p ¹ | p ² | I ¹ | I ² | C | P ³ | P ⁴ | M ¹ | weeks | days | |
| D | D | D | D | | | | | | | | | | D | D | D | D | | | | | | | | | 1 | 0-7 |
| ↑ | D | D | D | D | | | | | | | | | D | D | D | ↑ | D | | | | | | | | 2 | 8-14 |
| ↑ | D | D | ↑ | ↑ | | | | | | | | | D | D | D | ↑ | ↑ | | | | | | | | 3 | 15-21 |
| ↑ | ↑ | ↑ | ↑ | ↑ | ↑ | | | | | | | | D | D | ↑ | ↑ | ↑ | ↑ | ↑ | | | | | | 4 | 22-28 |
| | ↑ | ↑ | ↑ | ↑ | ↑ | | | | | | | | D | D | ↑ | ↑ | ↑ | ↑ | ↑ | ↑ | | | | | 5 | 29-35 |
| | | | | | | | | | | | | | D | D | ↑ | ↑ | ↑ | ↑ | ↑ | ↑ | | | | | 6 | 36-42 |
| | | | | | | | | | | | | | ↑ | D | ↓ | | | | | | | | | | 7 | 43-49 |
| | | | | | | | | | | | | | ↑ | ↑ | ↓ | | | | | | | | | | 8 | 50-56 |
| | | | | | | | | | | | | | ↑ | ↑ | ↓ | | | | | | | | | | 9 | 57-63 |
| | | | | | | | | | | | | | ↑ | ↑ | ↓ | | | | | | | | | | 10 | 64-70 |
| | | | | | | | | | | | | | ↑ | ↑ | ↓ | | | | | | | | | | 11 | 71-77 |
| | | | | | | | | | | | | | ↑ | ↑ | ↓ | | | | | | | | | | 12 | 78-84 |
| | | | | | | | | | | | | | ↑ | ↑ | ↓ | | | | | | | | | | 13 | 85-91 |
| | | | | | | | | | | | | | ↑ | ↑ | ↓ | | | | | | | | | | 14 | 92-98 |
| | | | | | | | | | | | | | ↑ | ↑ | ↓ | | | | | | | | | | 15 | 99-105 |
| | 10 | 11 | 9 | 12 | 19 | 11 | 9 | 13 | 12 | 13 | 13 | 10 | 5 | 6 | 6 | 9 | 14 | 8 | 6 | 5 | 13 | 14 | 13 | 10 | | |
| age (days) of loss or eruption range | 21 | 32 | 35 | 26 | 25 | 35 | 45 | 36 | 44 | 29 | 35 | 55 | 75 | 57 | 90 | 37 | 26 | 29 | 65 | 90 | 29 | 32 | 32 | 55 | | |
| | 18 | 29 | 22 | 17 | 26 | 24 | 30 | 12 | 35 | 12 | 20 | 38 | 21 | 16 | 67 | 33 | 32 | 19 | 29 | 19 | 14 | 25 | 22 | 39 | | |

D = Deciduous tooth
 P = Permanent tooth
 ↑ = Range over which loss/eruption occurs
 - = Mean age at which loss/eruption occurs

FIGURE 30 14 day old juvenile showing the relationship between deciduous teeth and unerupted permanent teeth (see also PLATE 7)



3.3.4 Tooth wear

(a) Tooth height/age relationship

(i) Comparison of four regression analyses

It was necessary to determine which of four types of regression analysis; linear, exponential, logarithmic or power, would provide the best fit for all four sets of tooth height/age data (male and female P_3 and upper C).

The four regression analyses were carried out on all four sets of data and a χ^2 test of significance was carried out between the four resulting r values (from male and female P_3 and upper C) for each type of regression (TABLE 4). There was no significant difference between them and therefore the r values were pooled in order to provide a comparison between the types of regression analysis. The linear regression gave the best fit to the data.

(ii) Comparison of actual tooth wear rates with theoretical tooth wear rates, obtained from linear regression analysis

Actual tooth wear rates for female P_3 (0,0321 mm/month) and upper C (0,0407 mm/month) were compared with the theoretical tooth wear rates ($\bar{P}_3 = 0,0316$ mm/month, \bar{P} upper C = 0,0449 mm/month) obtained from the slope of the linear regression for tooth height with age, and were found not to be significantly different. (\bar{P}_3 , $t = 0,12$; 17 d.f. $P > 0,05$ n.s.; \bar{P} upper C, $t = 0,98$; 20 d.f. $P > 0,05$ n.s.).

On the basis of these tests a linear relationship was accepted as best describing the relationship of male and female P_3 and upper C tooth height with age, from 5,0 months onwards (FIGURES 31 - 34).

(b) Tooth growth/wear curve

Although the tooth growth/wear equation (APPENDIX III) could be used as an age predictive tool based on tooth height for bats from 1,0 month old, it has not been used in this research, because too few tooth height measurements were taken during the tooth growth or eruption phase. Instead age determination (by dentition) of bats up to 3,0/4,0 months was achieved by tooth eruption sequence and

TABLE 4 Results of χ^2 tests of significant difference between the four sets of tooth height/age data and their pooled r values for the four regression analyses

| Type of regression | χ^2 test of significant difference between r values for σ & φ P ₃ and upper C tooth height/age data | Pooled value for r |
|--------------------|--|--------------------|
| Linear | $\chi^2 = 0,33, 3d.f. n.s. P>0,05$ | 0,97 |
| Exponential | $\chi^2 = 0,62, 3d.f. n.s. P>0,05$ | 0,95 |
| Logarithmic | $\chi^2 = 0,12, 3d.f. n.s. P>0,05$ | 0,88 |
| Power | $\chi^2 = 0,12, 3d.f. n.s. P>0,05$ | 0,84 |

FIGURE 31 Male P_3 wear Linear regression of tooth height with age, with 95 % confidence bands. Equation for the line:

$$y = 3,29 - 0,03 x; \quad r = 0,97; \quad P < 0,001$$

Two hundred and fourteen bats providing 272 measurements were used

(Vertical line - range, circle - mean)

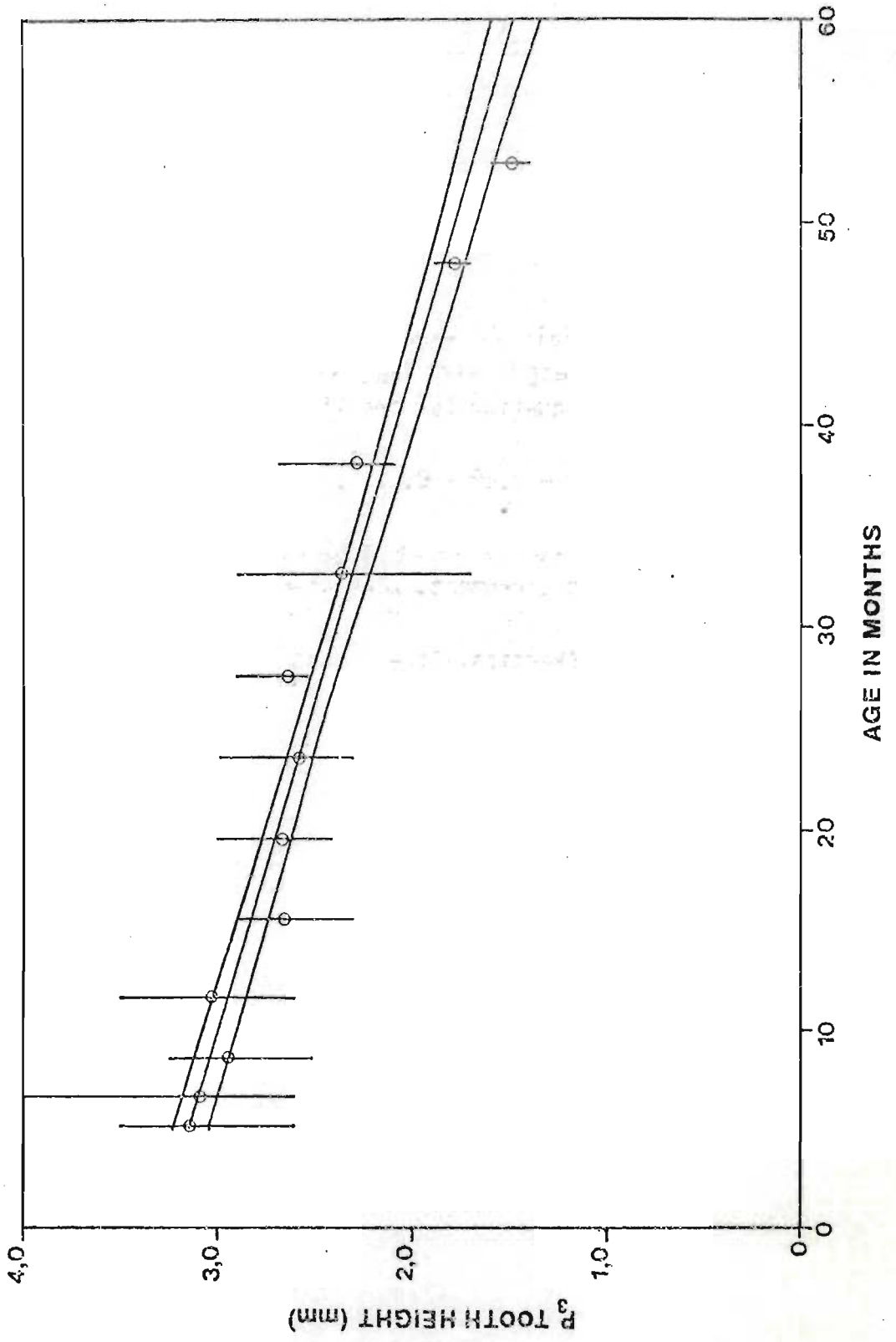


FIGURE 32 Male upper C wear Linear regression of tooth height with age, with 95 % confidence bands Equation for the line:

$$y = 4,83 - 0,03 x; \quad r = 0,97; \quad P < 0,001$$

One hundred and fifty eight bats providing 199 measurements were used

(Vertical line - range, circle - mean)

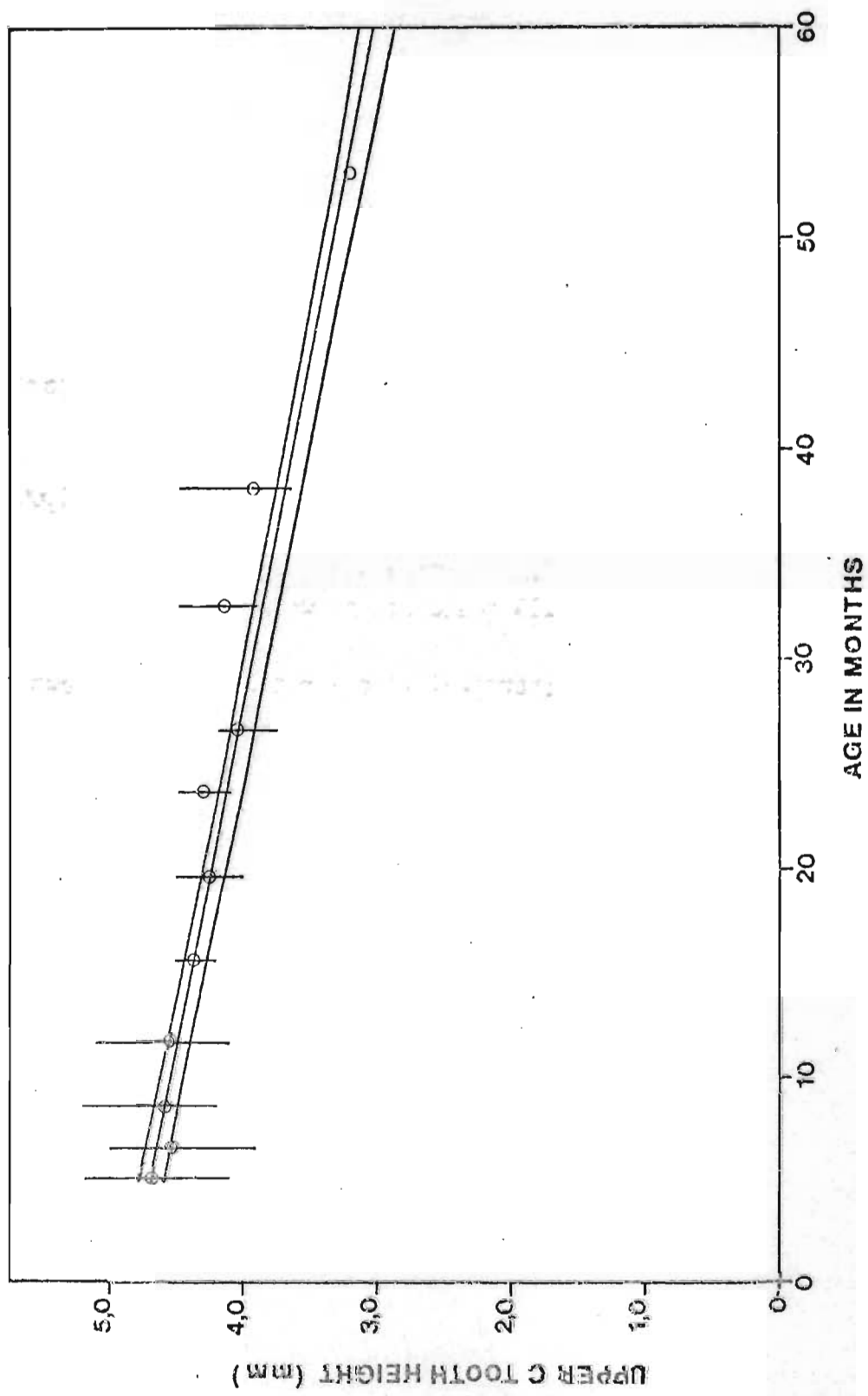


FIGURE 33 Female P₃ wear Linear regression of tooth height with age, with 95 % confidence bands. Equation for the line:

$$y = 3,24 - 0,03 x; \quad r = 0,96; \quad P < 0,001$$

One hundred and fifty five bats providing 275 measurements were used

(Vertical line - range, circle - mean)

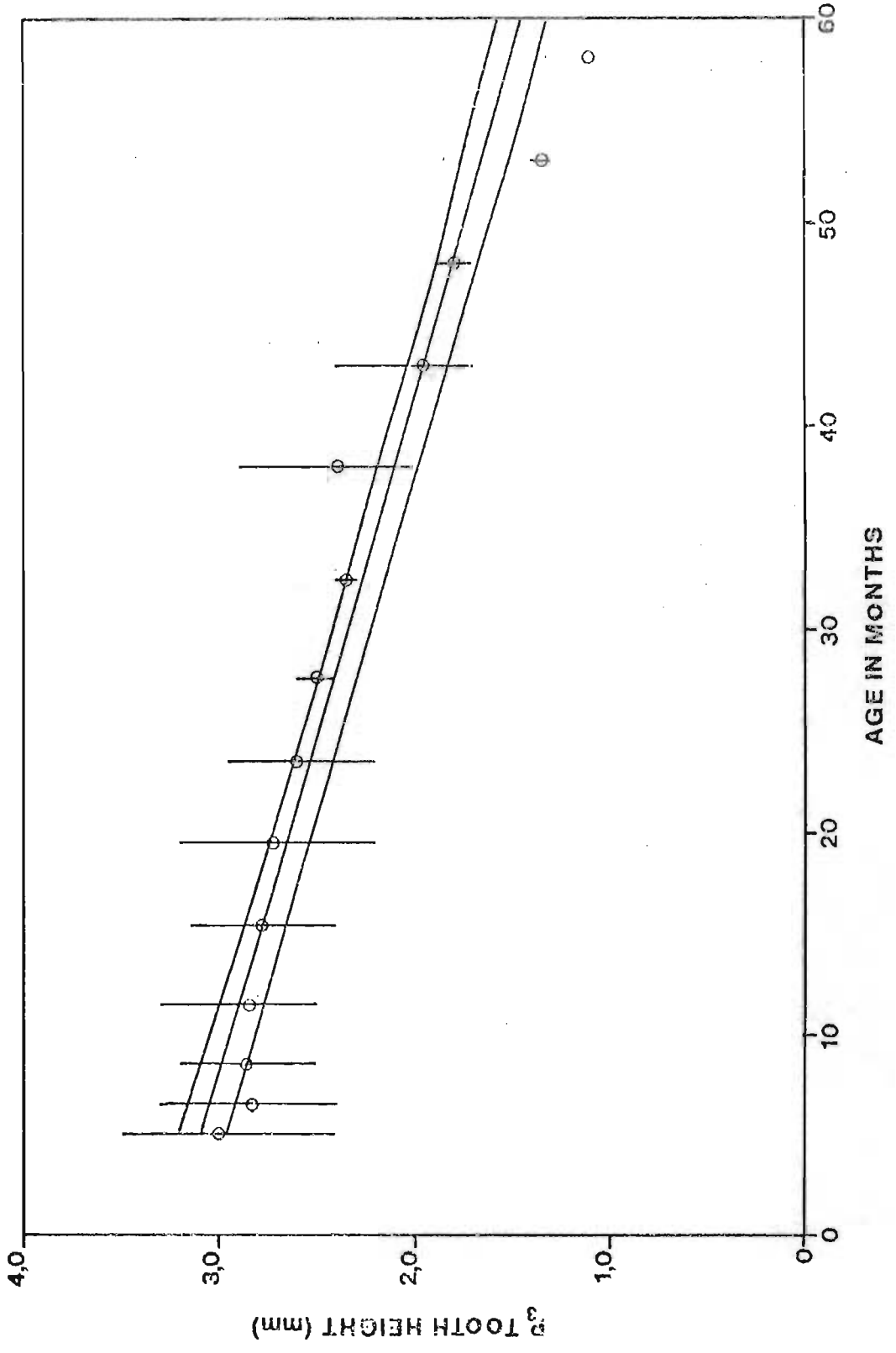


FIGURE 34 Female upper C wear Linear regression of tooth height with age, with 95 % confidence bands Equation for the line:

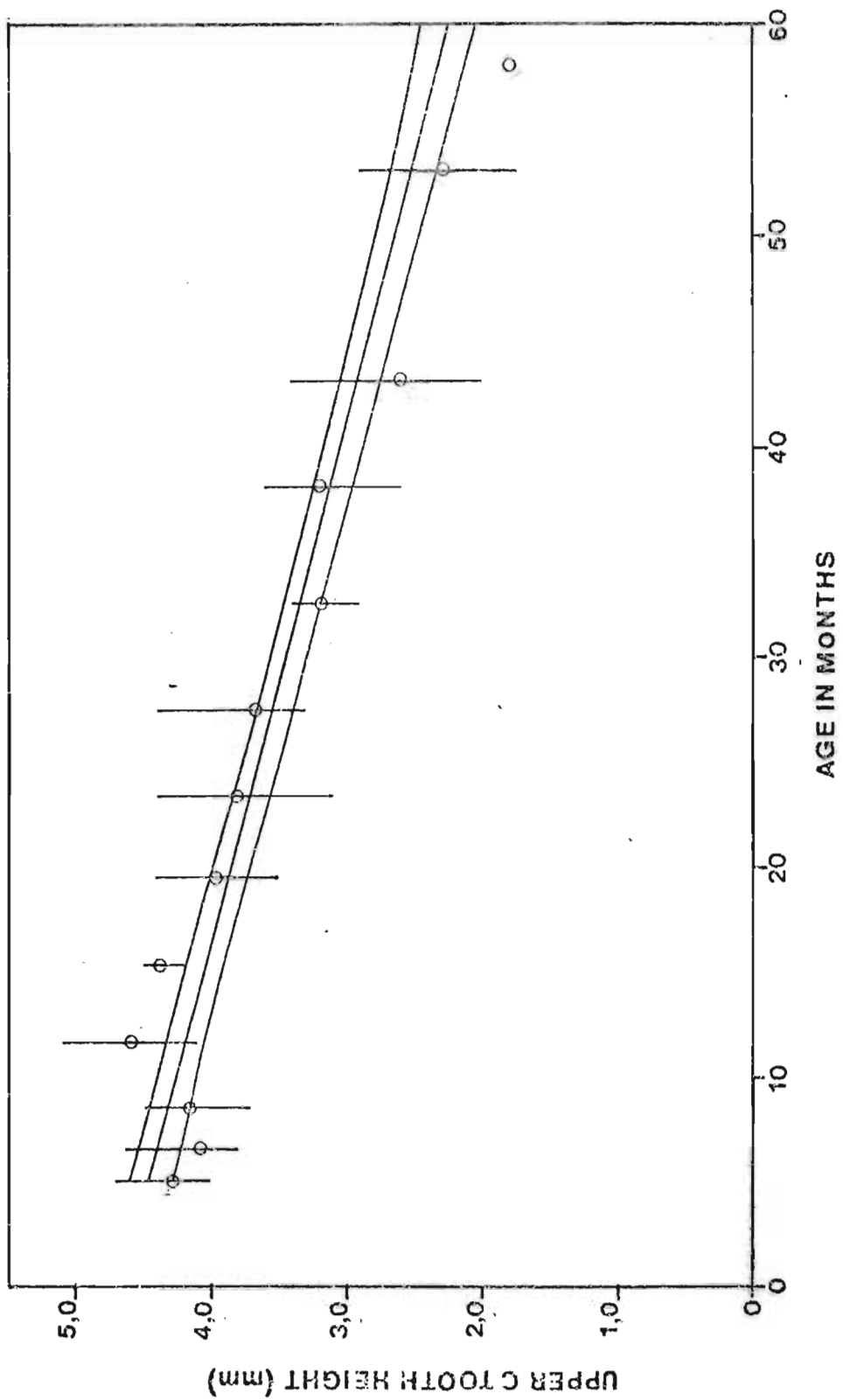
$$y = 4,65 - 0,04 x; r = 0,98; P < 0,001$$

One hundred and eight animals providing 193 measurements were used.

(Vertical line - range, circle - mean)



USE OF JAWSTRENGTH



after 5,0 months by a straight line plot of tooth height on age.

3.3.5 Age determination

TABLES 5 & 6 present a guide to age assessment throughout the life of E. wahlbergi, using a combination of selected growth, dentitional, behavioural and reproductive criteria. In the tooth eruption sequence column, events have been chosen for their consistency in time of appearance and widely spaced ages of eruption.

To determine the age of bats between 0 and 3,0-4,0 months a combination of eruption sequence, linear measurements and behavioural/reproductive events (where applicable) are used. From 5,0-6,0 to 11,0-12,0 months tooth height and linear measurements are used, with an absence of epaulettes in males and the palpable presence of a full term fetus (accompanied by little to no nipple distension) in females to indicate approximately one year of age. From 13,0 to 15,0 months, epaulettes develop in males and after this tooth height is the only criteria available as the linear measurements have either reached or nearly reached their asymptotic values. Using 95 % confidence intervals some overlap in tooth height exists between the tabulated age categories. In practice this means that tooth height at best will provide an age assessment to within five months (for example: σ upper C = 3,10 mm - 5,0 to 10,0 months) and at worst to within 14 months (for example: σ upper C = 2,00 mm - 36,0 to 50,0 months).

However the method which was used in APPENDIX I for initially age estimating the subadult category, can be usefully applied here to bats which have reached the asymptote of growth. By assuming the animal was born in November/January birth pulse and calculating back accordingly, the age estimation can be narrowed. For example, a female caught in July 1980 with $P_3 = 2,5$ mm and upper C = 3,7 mm would be age assessed by TABLE 6 at 16 to 25 months. She could have been born in November/January 1977/1978, 1978/1979 or 1979/1980. The first and last birth periods would give age estimations outside the table calculated range (30-32 and 6-8 months). If she was born in November/January 1978/1979 the age estimation of 18 to 20 months falls within the table calculated range and this would be the age recorded.

3.3.6 Age structure of the population

Using TABLES 5 and 6 and the birth pulse method for narrowing the age estimation range (described above), all bats netted in the study area in 1979 and 1980 (526) were age estimated and used in FIGURE 35 to present the age structure of the population sample. Each age group is represented

TABLE 5 Guide to age determination in male
Epomophorus wahlbergi

| Age
(months) | Forearm
length (mm)
95 % C.I. | Eye-nose
distance (mm)
95 % C.I. | Zygomatic
width (mm)
95 % C.I. | Tooth eruption
sequence | P ₃
height
(mm)
95 % C.I. | upper C
height
(mm)
95 % C.I. | Behavioural/
reproductive
events |
|-----------------|-------------------------------------|--|--------------------------------------|---|---|--|--|
| 0 | 29,2-38,2 | 10,2-12,6 | 13,9-16,6 | Partial decid. dent. | | | Eyes closed, carried
in flight at night
by mother |
| 0,25 | 35,0-44,2 | 10,8-13,4 | 14,5-17,2 | Full decid. dent. | | | Eyes open
Left hanging at night,
not carried by mother |
| 0,5 | 40,9-50,3 | 11,6-14,3 | 15,1-17,7 | | | | |
| 1,0-1,25 | 50,0-63,1 | 12,8-16,3 | 16,1-19,3 | P ₃ and upper C erupt
Lower C erupts | | | Starts to fly
Flies well
Weaned |
| 1,30-1,5 | 53,6-66,6 | 13,4-16,7 | 16,6-19,7 | | | | |
| 1,55-2,0 | 57,1-71,9 | 13,9-18,1 | 17,1-20,6 | M ₂ erupts
I ² erupts, full perm.dent. | | | Develops epaulettes |
| 2,5-3,0 | 67,1-79,0 | 15,9-19,9 | 18,8-22,1 | | | | |
| 3,05-4,0 | 70,5-83,1 | 16,7-21,4 | 19,5-23,3 | | 3,20-3,00 | 4,75-4,55 | |
| 5,0-6,0 | 78,3-86,9 | 19,4-23,4 | 21,7-25,2 | | 3,15-2,95 | 4,70-4,50 | |
| 7,0-8,0 | 81,2-87,7 | 21,2-24,7 | 23,2-26,5 | | 3,10-2,90 | 4,60-4,45 | |
| 9,0-10,0 | 82,3-88,6 | 22,4-25,4 | 24,2-27,4 | | 3,00-2,80 | 4,56-4,40 | |
| 11,0-12,0 | 82,7-88,7 | 23,2-25,9 | 24,0-23,0 | | 2,97-2,75 | 4,53-4,30 | |
| 13,0-15,0 | | 23,7-26,3 | 25,3-28,6 | | 2,83-2,60 | 4,45-4,14 | |
| 16,0-20,0 | | 24,2-26,5 | 25,7-29,0 | | 2,75-2,45 | 4,30-4,00 | |
| 21,0-25,0 | | | 25,0-29,2 | | 2,56-2,30 | 4,15-3,85 | |
| 26,0-30,0 | | | | | 2,42-2,15 | 3,99-3,70 | |
| 31,0-35,0 | | | | | 2,28-2,00 | 3,84-3,55 | |
| 36,0-40,0 | | | | | 2,13-1,85 | 3,70-3,35 | |
| 41,0-45,0 | | | | | 2,00-1,70 | 3,55-3,20 | |
| 46,0-50,0 | | | | | 1,85-1,50 | 3,42-3,05 | |
| 51,0-55,0 | | | | | 1,72-1,35 | 3,30-2,90 | |
| 56,0-60,0 | | | | | | | |

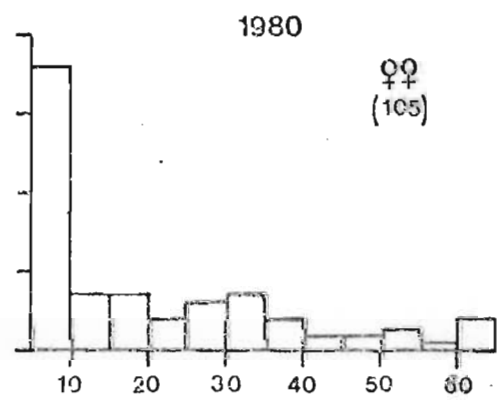
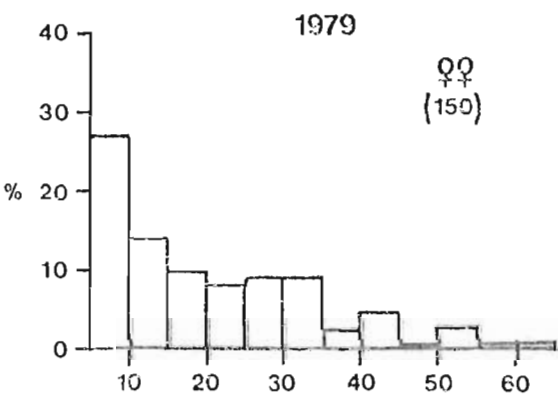
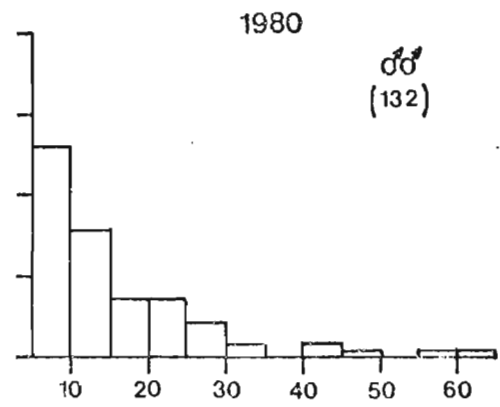
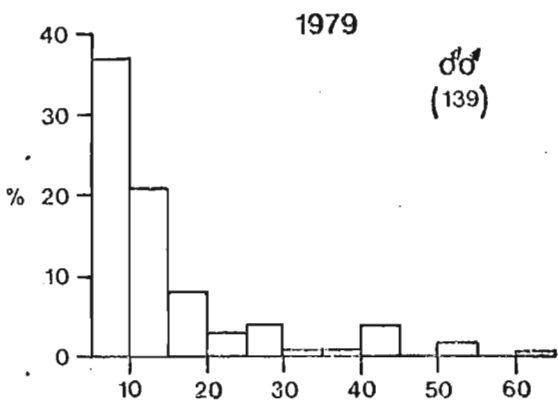
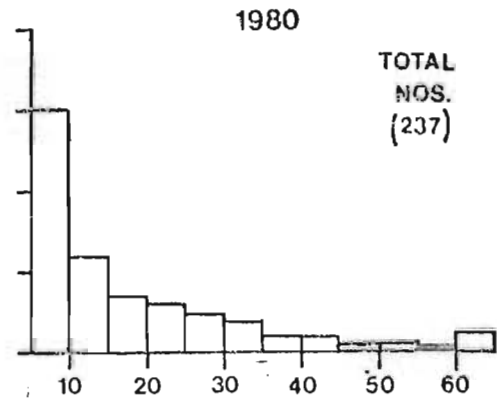
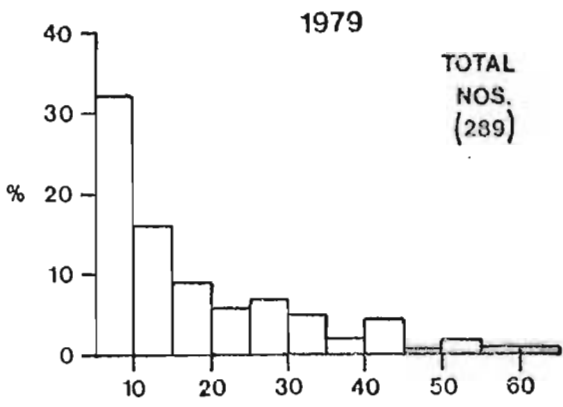
TABLE 6 Guide to age determination in female
Epomophorus wahlbergi

| Age
(months) | Forearm
length (mm)
95 % C.I. | Eye-nose
distance (mm)
95 % C.I. | Zygomatic
width (mm)
95 % C.I. | Tooth eruption
sequence | P ₃
height
(mm)
95 % C.I. | upper C
height
(mm)
95 % C.I. | Behavioural/
reproductive
events |
|-----------------|-------------------------------------|--|--------------------------------------|---|---|--|---|
| 0 | 28,6-35,7 | 9,6-12,3 | 14,1-16,5 | Partial decid. dent. | | | Eyes closed, carried
in flight at night
by mother |
| 0,25 | 36,9-47,5 | 10,3-13,1 | 14,7-17,2 | Full decid. dent. | | | Eyes open |
| 0,5 | 45,3-49,3 | 11,1-13,9 | 15,3-16,5 | | | | Left hanging at night,
not carried by mother. |
| 1,0-1,25 | 56,3-62,7 | 12,4-15,9 | 16,3-18,6 | P ₃ & upper C erupt
Lower C erupts | | | Starts to fly |
| 1,30-1,5 | 59,7-66,3 | 12,9-16,5 | 16,8-20,2 | | | | |
| 1,55-2,0 | 63,2-71,4 | 13,5-17,5 | 17,2-21,2 | M ₂ erupts
I ² erupts, full perm.dent. | | | Flies well |
| 2,5-3,0 | 70,8-77,8 | 15,4-19,2 | 18,8-22,6 | | | | |
| 3,05-4,0 | 72,7-81,2 | 16,1-20,4 | 19,4-23,7 | | 3,20-2,95 | 4,60-4,20 | Weaned |
| 5,0-6,0 | 75,6-83,9 | 18,2-22,0 | 21,4-25,0 | | 3,15-2,90 | 4,50-4,15 | Conceives first time |
| 7,0-8,0 | 76,2-84,6 | 19,4-22,9 | 22,7-25,7 | | | | Pregnant, nipples not
distended |
| 9,0-10,0 | | 20,1-23,4 | 23,4-26,1 | | 3,10-2,85 | 4,45-4,10 | First, full term fetus* |
| 11,0-12,0 | | 20,5-23,6 | 23,9-26,3 | | 3,00-2,80 | 4,35-4,00 | |
| 13,0-15,0 | | 20,7-23,8 | 24,3-26,4 | | 2,95-2,70 | 4,25-3,90 | |
| 16,0-20,0 | | 20,9-23,9 | 24,5-26,5 | | 2,85-2,55 | 4,15-3,70 | |
| 21,0-25,0 | | | | | 2,70-2,35 | 3,95-3,50 | |
| 26,0-30,0 | | | | | 2,55-2,25 | 3,70-3,30 | |
| 31,0-35,0 | | | | | 2,40-2,10 | 3,55-3,10 | |
| 36,0-40,0 | | | | | 2,26-1,95 | 3,35-2,90 | |
| 41,0-45,0 | | | | | 2,10-1,80 | 3,15-2,70 | |
| 46,0-50,0 | | | | | 1,95-1,65 | 2,95-2,50 | |
| 51,0-55,0 | | | | | 1,80-1,45 | 2,80-2,25 | |
| 56,0-60,0 | | | | | 1,65-1,30 | 2,60-2,05 | |

* Fetal zygomatic width at full term = ± 16,0 mm

FIGURE 35 Age structure of the population sample netted in 1979 and 1980, excluding bats aged 0 to 5 months

(This FIGURE uses all bats netted in 1979 and 1980 not just those animals which were chosen to develop the age determination methods)



AGE GROUPS IN MONTHS

as a percentage of the total number of bats caught in that year. From observations of caged animals, bats do not fly well until 2,0 to 2,5 months of age. For this reason the 0,5 months age group has not been incorporated in the FIGURE.

3.3.7 Longevity

In this study E. wahlbergi was not maintained in captivity for longer than two years so an estimate of physiological longevity for the species cannot be made. An estimate of maximum ecological longevity however may be obtained from wild specimens found dead, marked and recaptured, and captured once and age estimated by tooth height and birth pulse.

- (a) Six natural deaths were encountered, of which four were marked animals. These four were estimated at 15-17, 17-19, 30-32 and 37-39 months. Two unbanded bodies were examined and by tooth wear and birth pulse one was estimated to have died at 9-11 months. The second was a female with molars and premolars worn flat with the gum. An estimate of nine years for the age of this bat was made by projecting the regression line (representing the tooth height/age relationship) to its intersection with the age axis.
- (b) The oldest wild recaptured living bats were three in the 46-50 month grouping, three in the 51-55 month grouping and one female estimated to have been 58-60 months old. Stegeman (pers. comm.) reports that an E. wahlbergi female was recaptured 55 months after it had been banded as an adult, which would age estimate this individual at a minimum of 61 months.
- (c) A further nine individuals were netted and estimated by tooth height and birth pulse to be older than 60 months.

From these animals it is difficult to obtain a satisfactory maximum ecological longevity value, but if the oldest animal, (as estimated by tooth height) was correctly aged, an ecological longevity of nine years can be suggested.

3.4 DISCUSSION

3.4.1 Age determination techniques

Taking into account the necessity of having a known age series regardless of the method to be used, undoubtedly the most satisfactory methods of age assessment are those which determine absolute age in units of time for

both young and old. The main technique which provides this, involves the counting of incremental lines in hard structures within the body, reflecting growth discontinuities. Phillips, Steinberg & Kunz (1982) examined secondary dentine and cementum histologically in seven species of insectivorous bats and critically evaluated the usefulness of annular layers as an age determination technique for Chiroptera. They found that the layers were highly variable between and within individuals although a loose correlation existed between the numbers of layers and age. A critical examination of the literature on incremental lines, revealed several possible solutions for this variability and for inconsistencies between their findings and the apparent satisfactory findings of previous authors (Christian, 1956; Linhart, 1973; Lord et al., 1976). Kleveland & Kleinenberg (1969) had warned against the use of tooth sections in which the deposition of dentine was directly influenced by intensive mechanical load, a warning unheeded by Linhart (1973), Lord et al. (1976) and Schowalter et al. (1978). Morris also noted that resorption, hypertrophy, pathology and the subjective nature of the analysis all can affect the accuracy and precision of age determination. Phillips et al. (1982) added to this list selection of teeth, location, angle of section, the quality of optical equipment and the experience of the individual in dental histology.

The technique has many innate problems and because of the difficulty experienced in identifying and accurately counting the lines in the dentine of decalcified and stained sections of upper canines from E. wahlbergi, the method was regrettably rejected as being unsuitable for the species under study. Similarly Phillips et al. (1982) concluded that though data from histological analysis of teeth may yet prove useful in age estimation in some mammals, the technique appears unsuitable for Microchiropteran bats.

Failing methods of absolute age determination, relative age determination techniques must be resorted to. As 85 % of the growth/reproductive data used in this project were obtained from living specimens, the emphasis was placed on techniques applicable to live animals and those easily and quickly accomplished in field situations at night.

Linear body measurements were an obvious choice, although as noted by Baagøe (1977a), growth of wing elements (forearm length), ceases completely, early in life. For this reason cranial measurements were also chosen, skull growth continuing after forearm growth has reached the asymptote.

Relative age determination techniques as with absolute age determination methods, require checking against known age material for accuracy. In this study, as in many others, an attempt was made to confirm linear

growth measurements of wild specimens with those born and maintained in captivity. Morris (1972) cautioned, 'Such a practice, far from serving to validate an age determination method may cast doubts upon it. Captive animals are in every way at the mercy of their captors; good feeding and lack of stress may cause precocious development, lack of exercise or poor denning facilities may result in obesity or chronic retardation'. Comparative growth curves in fact showed significant differences between cage born and wild bat growth. The only alternative is the mark/recapture technique, a method widely used in this project. The validity of this however, presupposes that the methods chosen to mark do not in any way affect the life style of the released bat.

Tooth wear assessed by measurement of tooth height above the gum was chosen because it could easily be carried out on bats captured live, marked and later possibly recaptured, so giving an indication of rate of wear between captures. However a drawback to the use of tooth height measured in live animals is that the possibility of gum recession cannot be taken into account. This would lead to older animals being placed in a younger age category.

As pointed out by Morris (1972), a second problem associated with this method is that tooth wear will vary with the nature of the diet. Thus an age determination technique based on this criteria and developed for a population in one region may be invalid for another population where the diet differs considerably in its abrasive properties. Before using an age determination technique based on tooth wear for a geographically separate population, the tooth wear rate should be determined by the mark/recapture method (3.2.2.2 (b) (ii)) and compared with that in the method to be used.

The main advantage of tooth wear as a technique, is that it covers all ages, once a full permanent dentition has formed. However, the problems of gum recession, seasonal dietary variations, inaccuracies in measurement and individual variations in tooth abrasion resistance are likely to result in errors which reduce both the accuracy and reliability of the method.

For the first four months of life, tooth eruption sequence can be used to determine the age of E. wahlbergi. The main disadvantage of this technique, which is simple, rapid and accurate with few serious drawbacks (Morris, 1972), is that it cannot be used once a full dentition is present. A common compromise and one used here, is to use tooth replacement as an age guide in the younger more numerous age groups, then change over to tooth wear as a guide to older individuals where the tooth series is complete.

In the following chapters of this study, animals providing reproductive

data were age assessed by use of TABLES 5 and 6 and the birth pulse method described in 3.3.5. This ploy was recommended by Morris (1972) as a means of limiting the possible range and so improving the usefulness and accuracy of the age determination method. When a single value for age was required the median value was taken. For example, age estimate = 13,0 to 15,0 months old, value used = 14,0 months. Difficulties in the use of TABLES 5 and 6 occasionally arise when the P_3 and upper C tooth measurements for the same bat indicate two different age categories. In such a case the age group falling between the two is chosen.

Relative means of age determination, by virtue of the variability that exists between and within all populations, can at best only produce a good estimate of age, sufficient in most cases for the intended application of the results.

3.4.2 Stevens asymptotic regression

Although not derived from a physiological process, like the Von Bertalanffy equation, Stevens asymptotic regression has three parameters of biological significance, α being the asymptote of growth, β the total growth achieved and ρ is inversely proportional to the rate of growth. When $\rho = 0$ growth would be represented by a vertical line and when $\rho = 1$ there would be no growth, represented by a horizontal line.

The parameter ρ provides a useful means of easily comparing growth rates of one species with another or different populations of the same species. In wildlife management research ρ values could provide a simply applied mathematical tool for estimating the success of a population in a particular habitat, since growth rates reflect pasture utilization, competition, predator pressures, prey availability, disease, parasitic load and water availability.

The function has been used satisfactorily by Stebbings (pers. comm.) to describe Microchiropteran growth and by Fourie (pers. comm.) for growth in the rock hyrax Procavia capensis. It fitted all sets of E. wahlbergi growth data very closely despite variations in curve flexure needed to describe growth and growth rate decline in the three functions of forearm length, eye-nose distance and zygomatic width. It is a simple easily applied formula, and although it does not attempt to describe metabolism, it is versatile (see APPENDIX II) and has proved totally appropriate to the data in this research.

3.4.3 Age structure of the population

The age structure of the netted sample of the population (assuming that mist-netting is a non-age selective means of catching volant members)

provides an indication of the reproductive problems facing the population. The greatest percentage losses recorded in FIGURE 35, occur between the 5-10 and 10-15 month age groups for both sexes. It is at these ages that young females become pregnant and give birth for the first time. Female losses in this category (5-10 months) therefore not only represent losses in breeding individuals, but also fetal deaths and a greater potential loss to the population than is at first apparent. This loss is seen in both 1979 and 1980 sample figures, suggesting that it is usual and countered by the normal breeding strategy of the species.

3.4.4 Longevity

Two types of longevity are referred to in discussions on life span, maximum physiological longevity and maximum ecological longevity. Maximum physiological longevity is the maximum possible age an animal could attain (Grimsdell, 1973) assuming environmental factors such as predation, starvation or disease do not prematurely terminate life. Such an absolute age maximum would be most likely to occur in captivity. Spingale (1973) discussed the criticism that life span records from captive animals are not relevant to the wild situation. He remarked that captivity cannot prolong life beyond its physiological maximum and this maximum could be reached in the wild, particularly by herbivores. Carnivores however would be unlikely to attain their physiological maximum since they cannot continue to catch prey when senescent decline sets in.

Maximum or potential ecological longevity is the mean maximum age attained by animals in the wild affected by environmental factors of predation, starvation, disease and accident (Krebs, 1972). This value would vary within a species, between one population and another depending upon predation pressures, presence and extent of diseases and food availability.

In the African context the difference between these two types of longevity has been examined for the buffalo (*Syncerus caffer*) by Grimsdell (1973) and for the lion (*Panthera leo*) by Smuts, Anderson & Austin (1978). Grimsdell (1973) reported that the oldest estimated wild buffalo was 22 years, while the oldest captive buffalo died in a zoo at 26 years. The oldest wild lion, as reported by Schaller (1972) is believed to have been 20 to 22 years old and in comparison the oldest recorded captive lion (Flower, 1931) died at 29 years of age.

Cockrum (1956), in a bat longevity review, reported that three methods had been used to determine life span in the Chiroptera; length of life in captive individuals, life expectancy as determined by mortality or survival tables and recovery of marked individuals. He extensively reviews the records of chiroptera obtained by these methods and from them

it would appear that wild Microchiropterans may obtain a maximum ecological longevity varying from 5,0 to 14,5 years according to the species.

Captive Microchiropteran records show a maximum of 24 years for Myotis lucifugus (Griffin & Hitchcock, 1965) and Rhinolophus ferrumequinum (Hooper & Hooper, 1967).

Records from wild Megachiroptera are unavailable, but maximum ages attained by captive fruit bats (representing maximum physiological longevity) are Eidolon helvum - 21 years 10 months (Jones, 1972), Rousettus aegyptiacus - 19 years 9 months (Flower, 1931) and Pteropus giganteus - 17 years 1 month (Flower, 1931). From these values it may be that the estimate of nine years for maximum ecological longevity in E. wahlbergi could be an underestimate, since maximum physiological and ecological longevity are similar in non predatory mammals.

3.5 SUMMARY

Age determination and growth analysis are important supportive studies to the investigation of the reproductive biology of a species.

A complex method, fully described in APPENDIX I was employed to assess initially the age of bats which were used in the development of age determination techniques. In these techniques three criteria were used, linear growth measurements to obtain the age of animals prior to the attainment of the growth asymptote, tooth eruption to determine the age of animals prior to the attainment of a full permanent dentition and tooth wear in combination with birth pulse timing, to obtain the age of animals after the growth asymptote and full permanent dentition had been reached.

Stevens asymptotic growth curves, performed on forearm length, eye-nose distance and zygomatic width data, linear regressions on tooth height data and selected features of tooth eruption provided the basis for age predictions.

The forearm length growth asymptote was reached at 11,0 months in males and 8,5 months in females. Examination of the population age structure revealed that the greatest percentage losses occur between 5-10 and 10-15 month age groups for both sexes. Nine years is suggested as the maximum ecological longevity for the species.

Bat longevity and age determination techniques with particular reference to those used for Chiroptera are reviewed and the implications of the age

structure of the population are discussed.

CHAPTER FOUR

REPRODUCTION IN THE MALE EPOMOPHORUS WAHLBERGI

4.1 INTRODUCTION

Seasonality in sexual activity, regardless of climate and latitude, has been demonstrated in many Chiropteran species (Anciaux de Faveaux, 1978a; Baker & Baker, 1936; Gopalakrishna & Choudhari, 1977; Marshall, 1946; Mutere, 1967; 1973a; 1973b; Okia, 1974a; 1974b). Although seasonality in births is evidence of seasonal reproductive patterns in females, it may or may not be reflected in males.

Three different male reproductive patterns occur in seasonally breeding Chiroptera.

- (a) Full male reproductive seasonality with a period of spermatogenic cessation. This pattern has been widely recorded and discussed in hibernating Microchiroptera of temperate latitudes (Courrier, 1927; Gustafson, 1975; 1979; Herlant, 1967; Oh, 1977; Pearson, Koford & Pearson, 1952; Phillips, 1966; Racey, 1973; 1974a; 1974b; 1979; Rice, 1957; Stebbings, 1966; Wimsatt, 1960a; 1969) and in non-hibernating Microchiroptera of tropical latitudes (Bradshaw, 1961; 1962; Gopalakrishna, 1949; Krutzsch, Watson & Lox, 1976; Krutzsch, 1979; Menzies, 1973; Mutere, 1973a; Ramakrishna, 1951; Ramaswamy, 1961).
- (b) Spermatogenic activity continuous, but seasonally variable in intensity, coincident with fluctuations in testis and epididymal mass. Several tropical Megachiropteran species Pteropus geddiei (Baker & Baker, 1936), Pteropus giganteus (Marshall, 1946), Rousettus aegyptiacus (Mutere, 1968), Eidolon helvum (Mutere, 1967) and Pteropus poliocephalus (Nelson, 1965a) and a tropical Microchiropteran species, Artibeus jamaicensis (Tamsitt & Mejia, 1962; Tamsitt & Valdivieso, 1963; 1965a) exhibit this type of activity. Although no comment is made on spermatogenic activity, a similar seasonal variability in testis mass has been recorded for Epomops franqueti (Okia, 1974a) and Epomophorus anurus (Okia, 1974b).
- (c) Spermatogenic activity continuous with no seasonal variations in intensity or testis mass. This pattern is most common in aseasonal breeders, but Tadarida condylura is a tropical example of a seasonal breeder with unchanged testis mass throughout the year (Mutere, 1973b).

The main aim of this chapter is to describe the basic anatomy of the male reproductive tract in E. wahlbergi and to examine the male reproductive pattern for seasonality, and if present, to determine the extent of its influence and later relate it to the seasonal pattern of births and the female reproductive cycle.

Information on the breeding biology of Megachiropterans reveals that of the species studied, males usually reach maturity in their second year. Bradbury (1977) noted that sexual maturity, as indicated by testicular activity and muzzle enlargement in Hypsignathus monstrosus occurred at 12 to 18 months of age. Gopalakrishna & Choudhari (1977) found that the earliest age at which spermatogenesis took place was 14 months in Rousettus leschenaulti and that many males mated for the first time only at 15 to 20 months. Similarly, Mutere (1968) suggested that male Rousettus aegyptiacus would mate for the first time at 12 to 19 months old.

A further aim of this chapter is to determine if a similar pattern of second year sexual maturity attainment also occurs in E. wahlbergi and in the following chapter examine its significance in relation to the age at which sexual maturity is reached in the female.

4.2 MATERIALS AND METHODS

4.2.1 Reproductive tissues

Eighty one males were sampled for histological preparation of testes and epididymides between February 1979 and February 1982. From subsequent histological examination 47 were adults characterized by the presence of spermatazoa in the epididymis cauda. The youngest in the adult group was estimated to be 13 months and the oldest 46 months. Thirty four males were juveniles and subadults showing no indication of spermatogenic activity and ranging in age from 0,75 to 16 months old.

Mass, forearm length, eye-nose distance, zygomatic width, P_3 and upper C tooth heights were measured in each male sampled and the presence of epaulettes and position of testes noted. Specimens were age estimated by the method described in 3.4.1 (penultimate paragraph) and the median value of a range of values taken to give a single age value.

Both testes were removed from each specimen by severing the spermatic cord level with the epididymis caput and the vas deferens at its point of emergence from the epididymis cauda. Mass of each testis with and without

the epididymis was measured to the nearest 0,01 g using an electronic balance. The maximum length and width of the testis without the epididymis was measured to the nearest 0,05 mm using a calliper vernier.

After removal the epididymis was cut into three sections (FIGURE 36), roughly corresponding to the epididymis caput, corpus and cauda.

Testes and epididymal portions were fixed in 10 % formal saline for a minimum of 48 hours then routinely embedded in paraffin wax (APPENDIX IV). The blocked tissues, which were orientated for transverse sectioning, were roughly sectioned until the broadest portion was exposed. Ten sections at 5 μ m were mounted, the next ten discarded and the following ten mounted. The sections were stained with haematoxylin and eosin (APPENDIX V).

The testes were microscopically examined for the presence or absence of spermatogenesis, and the mean seminiferous tubule diameters obtained using a micrometer eyepiece. The three epididymal regions were examined, (1) for the presence of spermatozoa in the ducts, (2) to obtain the mean maximum and minimum width of the ducts by measuring across the lumen (including the single cell thick epithelial layer) the width being calculated from all ducts present in one histological thin section and (3) to obtain the mean height of the ductal epithelial layer from 30 measurements. In addition, the cellular contents of the epididymis cauda ductal lumina were identified and counted by using 16 squares (4 x 4, 1183,36 μ m²) of a grid squares eyepiece fitted into a x 10 ocular lens and examined through a x100 oil immersion objective lens. The numbers of each cell type were calculated as a percentage of the total numbers of cells present. A mean value of the two testes was used for length, width, seminiferous tubule diameter, epididymal ductal diameter, epididymal ductal epithelial height and epididymal lumen cellular composition, but a combined value was used for testis and epididymis mass.

4.2.2 Body mass measurements

In order to examine seasonal fluctuations in body mass as part of the male seasonality investigations, 196 wild adult males (bearing epaulettes) netted between July 1977 and February 1982, were used to provide the measurements.

4.2.3 Sperm smears

Sperm smears were used to examine, measure and photograph the external appearance of individual spermatozoa. Smears were taken by dabbing the cut surface of the epididymis cauda onto a glass slide, fixing with 100 % methanol and staining with periodic acid-Schiff (PAS).


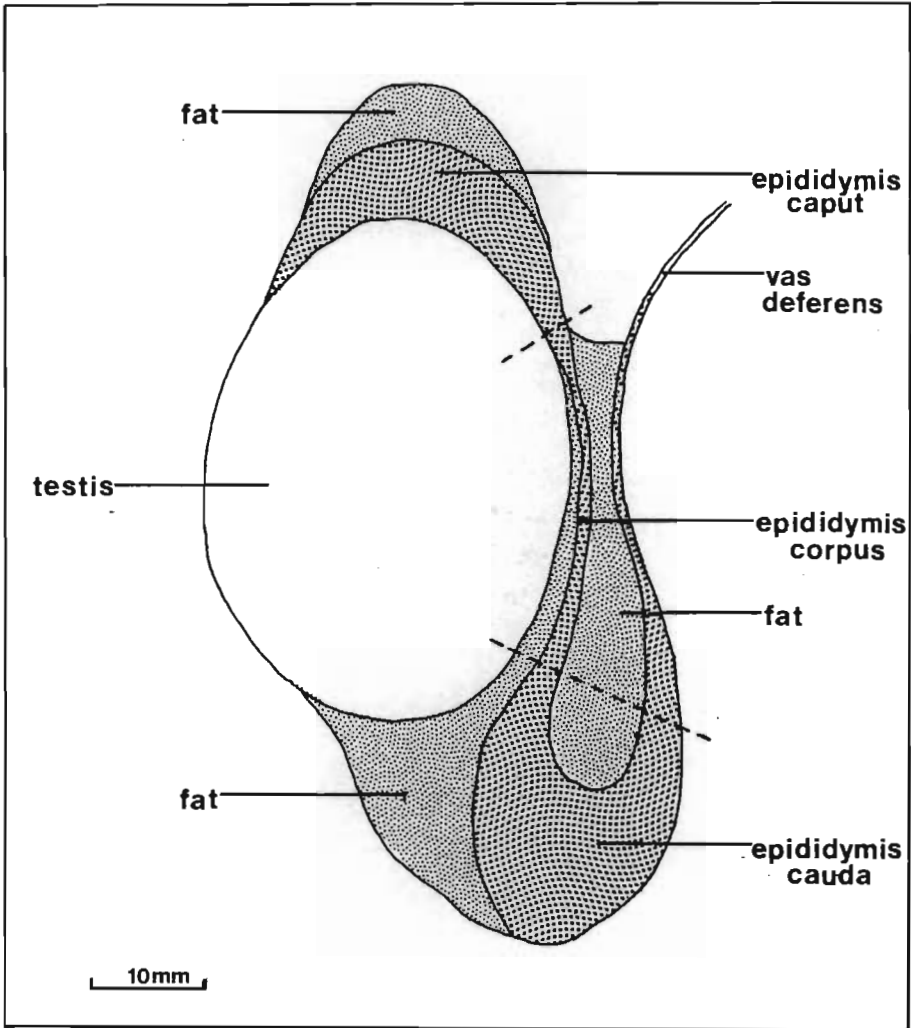


FIGURE 36 Testis and epididymis of E. wahlbergi
The dashed line shows where the epididymis
was cut into three sections corresponding to
the epididymis caput, corpus and cauda



4.2.4 Blood testosterone

Seventy two blood samples of 0,5 ml were taken from males by puncture of the left cephalic vein in the propatagium and collection of the blood droplets in a plastic syringe. The samples were taken over the period May 1980 to February 1982, representing a minimum of three and a maximum of eight samples for each month of the year. Thirty seven samples were taken from the adult males killed for histological examination of reproductive tissues. Thirty five samples were collected at fortnightly intervals from a sexually active, calling caged male. Each sample was placed in a heparinized tube, centrifuged, and a minimum of 0,1 ml of plasma obtained. 1ℓ of sodium nitrite was added and the samples frozen at -15° C until radioimmunoassay of testosterone could be carried out by the method of Dr. R.P. Millar,(pers. comm.).

4.2.5 Epaulettes

The epaulettes from 56 males (46 adult males and 10 subadults) killed for histology, were examined by cutting out the loose skin forming the pouch on the right shoulder. The skin patch was stretched widthways and lengthways by pinning out and the maximum diameter of the epaulette region from where the white hairs grow, was measured. Ten of the white epaulette hairs, extracted from the 'point of crown' within the pouch, were measured and their mean length recorded.

Epaulette appearance and hair length was also examined fortnightly from May 1980 to February 1982 in the caged breeding male.

4.2.6 Calling

Wickler & Seibt (1976) studied male calling of E. wahlbergi in Kenya for two weeks per year over four years. Although they could not comment on seasonality of calling, they concluded from sonic composition and rhythm that the function of the call was to attract females. Studies by Bradbury (1977) showed a breeding season for Hypsignathus monstrosus and a correlation between calling intensity and period of mating.

It was therefore decided to examine male calling in the study area for seasonality. This was studied in three ways:

- a) By noting the dates on which males were heard calling in the area every night between 19h00 and 22h00, from January 1979 to December 1981.
- b) By noting if the caged male had called within each fortnightly period from May 1980 to February 1982.
- c) By recording fifteen minutes of caged male calling, approximately

once a fortnight between 21h00 and 22h00 when calling occurred, during the period March 1981 and February 1982.

The calls were recorded on a UHER 4000 REPORT IC, from 2,5 m directly beneath the caller. Each set of recordings was then played through a Bruel & Kjaer Audio Frequency Spectrometer TYPE 2113 and Level Recorder TYPE 2305, in order to quantify the amplitude of component frequencies in the call and time lapses between successive calls. Frequencies were analysed in jumps of a third of an octave. Thirty successive calls were analysed from each recording.

4.2.7 Caged male

The caged male (E.T. 419) was first captured 11 January 1980 as a subadult male without epaulettes and estimated to be 13 months old. He was recaptured and caged 21 April 1980 as an adult bearing epaulettes and scrotal testes. From then until 14 February 1982, he was routinely caught at two to four week intervals, body mass, forearm length, eye-nose distance, P_3 and upper C tooth height and epaulette hair length measured, blood extracted for testosterone assay and position of testes noted.

4.3 RESULTS

4.3.1 Anatomy and histology of male reproductive system

The anatomical and histological descriptions to follow pertain to sexually mature adults, defined as having spermatazoa in the ducts of the epididymis cauda.

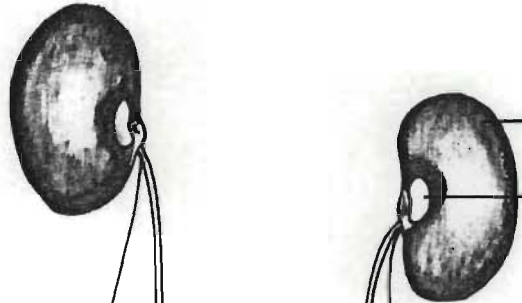
4.3.1.1 Gross anatomy

FIGURE 37 shows the ventral view of the male urinogenital system. The right kidney is situated cranially to the left. The ureters emerge from the medial ventral surface of the kidney adjacent to the prominent adrenal glands. They pass caudally and open into the dorsal surface of the bladder at the level of the neck.

The testes have been observed lying both scrotally and abdominally in mature males. Each testis is ovoid in shape and the epididymis is loosely bound to the tunica albuginea. The epididymis caput forms a cone shaped mass embedded in fat at the cephalic pole of the testis. The epididymis corpus merges into the U-shaped epididymis cauda. The epididymis corpus, cauda and

FIGURE 37 Ventral view of the urinogenital system of
E. wahlbergi

10mm



kidney
adrenal gland

ureter

spermatic cord

seminal vesicle

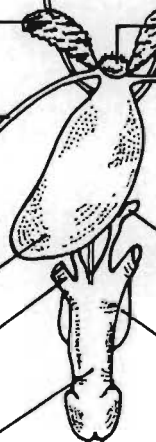
vas deferens

prostate gland

epididymis caput
testis
epididymis cauda

bladder
ischio cavernosus muscle
penis

Cowper's gland
bulbo cavernosus muscle



epididymal end of the vas deferens are encased in adipose tissue, binding them loosely to the medial margin of the testis (FIGURE 36). TABLE 7 presents mean gross testicular and epididymal measurements. The vasa deferentia pass ventrally over the ureters to enter the bladder cranially to them.

The penis is of the vascular type where erection is produced primarily by vascular engorgement of the cavernous tissue. No baculum is present. Two well developed ischio cavernosus muscles attach the ventral surface of the penis to the pelvic girdle. Dorsally the bulbo cavernosus muscle lies between the penis and the rectum.

A small pair of Cowper's glands arise on the lateral region of the penis, close to the prostate glands. The prostate gland is situated on the dorsal surface of the penis and bladder, obscuring the points of junction of the ureters and vasa deferentia with the bladder. A pair of horn shaped seminal vesicles emerge on either side of the prostate gland.

4.3.1.2 Histology of the testis

The testis of a sexually mature male, showing spermatazoa in the seminiferous tubules is illustrated in PLATE 9. The mean seminiferous tubule diameter recorded for adult males was $183,45 \mu\text{m}$ (S.E.M. = $2,95$, range = $145,90 - 229,40 \mu\text{m}$, $n = 47$).

4.3.1.3 Histology of the epididymis caput, corpus and cauda

Transverse sections through the three regions of the epididymis are illustrated in PLATES 10, 11 and 12. TABLE 8 gives the mean maximum and minimum width of the ducts, height of the epithelial layer and percentage of ducts per animal containing no cellular material, in all three epididymal regions. The epididymal ductal widths are greatest in the cauda and smallest in the corpus. The height of the epithelial cell layer surrounding the ducts, decreases from caput to cauda, while the percentage of ducts per animal containing no cellular material decreases from caput to cauda.

4.3.1.4 Cellular contents of the epididymis cauda lumen

Examination of the cellular contents of the epididymis cauda lumen reveal the consistent presence of cells other than mature spermatazoa. Although absolute confirmation of identification can only be made by a detailed electron microscopy study, the

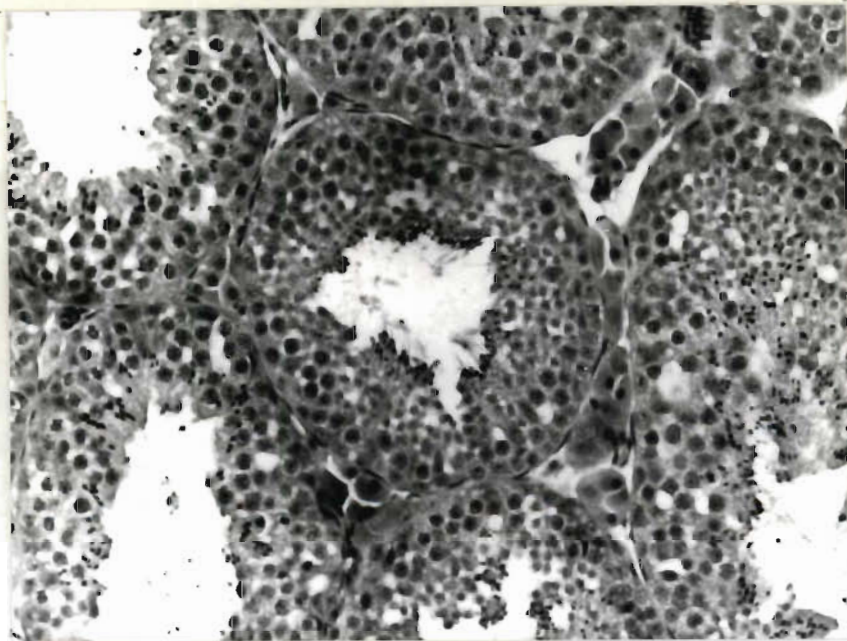
TABLE 7. Gross testicular and epididymal measurements from adult males (single not combined)

| | Testis | | | Epididymis |
|-------------|-------------|-------------|------------|-------------|
| | Mass (g) | Length (mm) | Width (mm) | Mass (g) |
| n | 47 | 47 | 40 | 47 |
| \bar{x} | 0,061 | 5,60 | 4,30 | 0,033 |
| \pm S.E.M | 0,002 | 0,09 | 0,08 | 0,002 |
| Range | 0,035-0,100 | 4,20-7,10 | 3,35-5,10 | 0,010-0,100 |

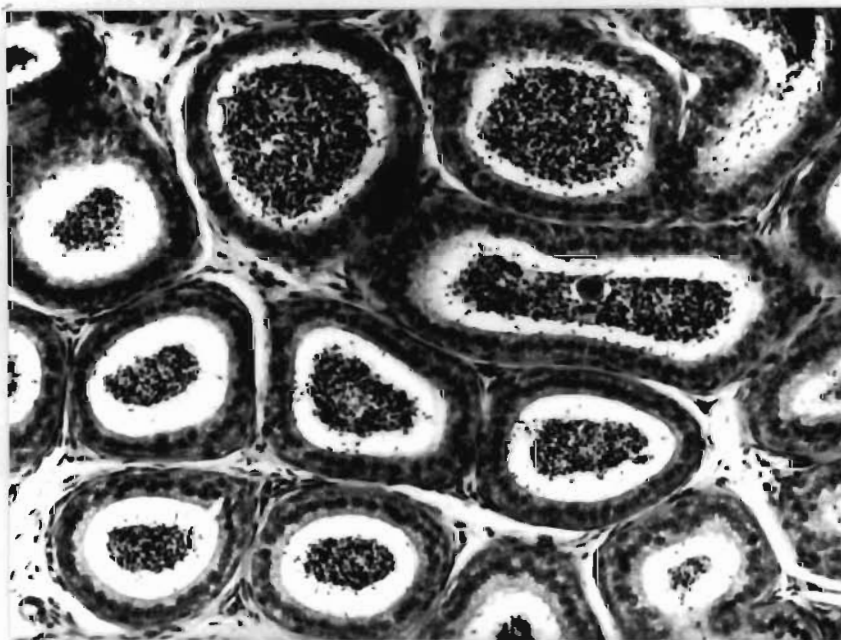
PLATE 9 Seminiferous tubule from testis of mature male (X 250)

PLATE 10 Epididymis caput ducts from mature male (X 250)

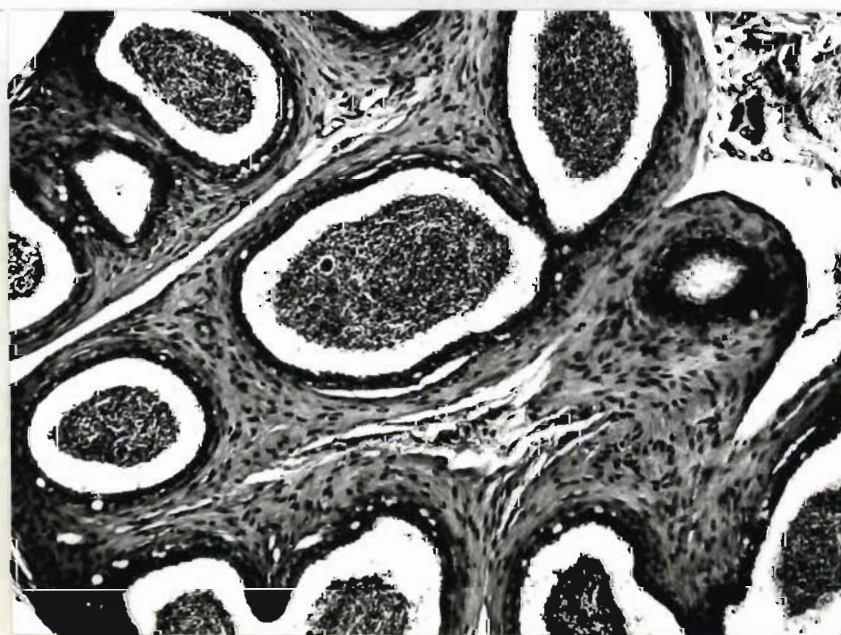
PLATE 11 Epididymis corpus ducts from mature male (X 250)



9



10



11

TABLE 8. Epididymis caput, corpus and cauda measurements
from adult males

Epididymis caput

| | minimum ducal
diameter (μm) | maximum ducal
diameter (μm) | epithelial
height (μm) | % ducts/animal
containing no
cells |
|--------------|---|---|--|--|
| n | 22 | 22 | 22 | 22 |
| \bar{x} | 88,86 | 164,18 | 21,17 | 49,59 |
| \pm S.E.M. | 2,51 | 9,65 | 1,25 | 7,10 |
| Range | 72,0-114,5 | 112,5-327,0 | 11,75-36,2 | 0-100 |

Epididymis corpus

| | minimum ducal
diameter (μm) | maximum ducal
diameter (μm) | epithelial
height (μm) | % ducts/animal
containing no
cells |
|--------------|---|---|--|--|
| n | 22 | 22 | 22 | 22 |
| \bar{x} | 76,95 | 139,27 | 14,61 | 22,18 |
| \pm S.E.M. | 1,91 | 6,71 | 0,63 | 4,26 |
| Range | 62,0-92,5 | 89,0-214,0 | 9,7-21,3 | 14-100 |

Epididymis cauda

| | minimum ducal
diameter (μm) | maximum ducal
diameter (μm) | epithelial
height (μm) | % ducts/animal
containing no
cells |
|--------------|---|---|--|--|
| n | 23 | 23 | 23 | 23 |
| \bar{x} | 124,59 | 271,91 | 12,51 | 10,26 |
| \pm S.E.M. | 4,59 | 15,45 | 0,68 | 4,40 |
| Range | 96,0-180,5 | 153,0-447,0 | 9,16-20,80 | 14-100 |

cells examined under a light microscope, show the appearance characteristic of spermatocytes/spermatids (PLATE 13) and multinucleated spermatids (PLATE 14) (Bedford, pers. comm., Pillay, pers. comm., Racey, pers. comm., Fawcett & Bedford, 1979) and for the purposes of this study are referred to as such. The percentages of the three types of cellular components were: spermatozoa $\bar{x} = 92,51 \%$, S.E.M = 1,78, range = 37,50-100,0 %, n = 47; spermatocytes/spermatids $\bar{x} = 7,46 \%$, S.E.M = 1,78, range = 0-62,50 %, n = 47; multinucleated spermatids $\bar{x} = 0,07 \%$, S.E.M = 0,04, range = 0-1,1 %, n = 47.

4.3.1.5 Spermatozoan measurements

The spermatozoa (PLATE 15) of E. wahlbergi have spatulate heads, giving the overall impression of sperm with two different head widths. TABLE 9 presents the measurements of ten selected spermatozoa from sperm smears.

4.3.2 Age at puberty and sexual maturity

Puberty is a complex process of physiological changes occurring over a time period. It commences with the rapid duplication of spermatogonia by mitosis and terminates with the formation of mature spermatozoa (Austin & Short, 1972). It is accompanied by rapid gonadal growth and the development of external secondary sexual characteristics. Sexual maturity is reached at the end of puberty when mature spermatozoa are present in the epididymis cauda and the animal is capable of successful fertilization. Quantification in terms of age at which this process starts and terminates is difficult to achieve in view of the different starting and finishing points of its component parts and variations within individuals.

This study therefore aims to investigate three aspects of puberty; (a) spermatogenesis, (b) gonadal growth, and (c) development of secondary sexual characteristics, in terms of the range of ages over which they take place.

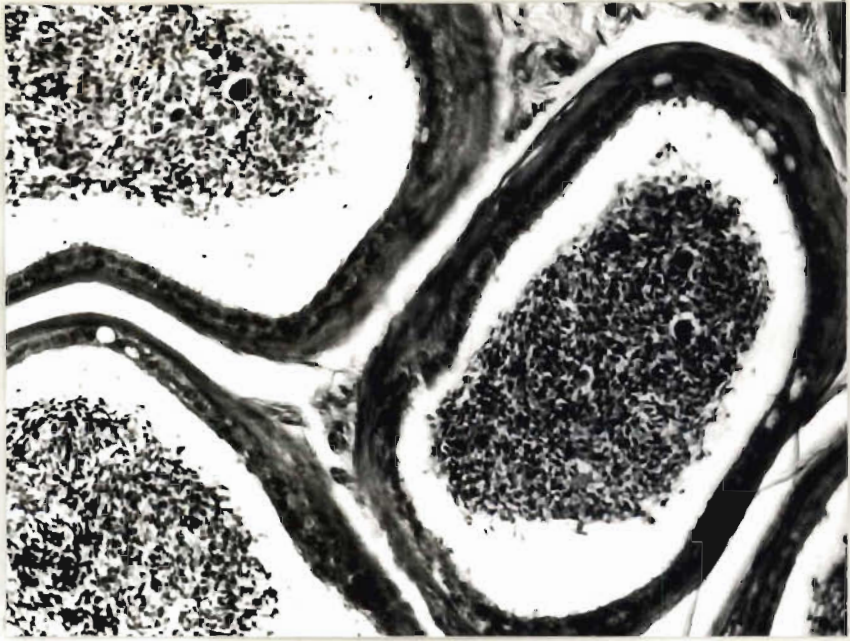
(a) Spermatogenesis

FIGURE 38 shows the percentage and range of percentages of spermatocytes/spermatids (1) and spermatozoa (2) in the epididymis cauda lumen plotted against age. The greatest range for both cell categories, the highest percentage of spermatocytes/spermatids and the lowest percentage of spermatozoa occurs from 13 to 16 months inclusive. This period of enormous ranges, high spermatocyte/spermatid percentages (26 - 65 %) accompanied by low

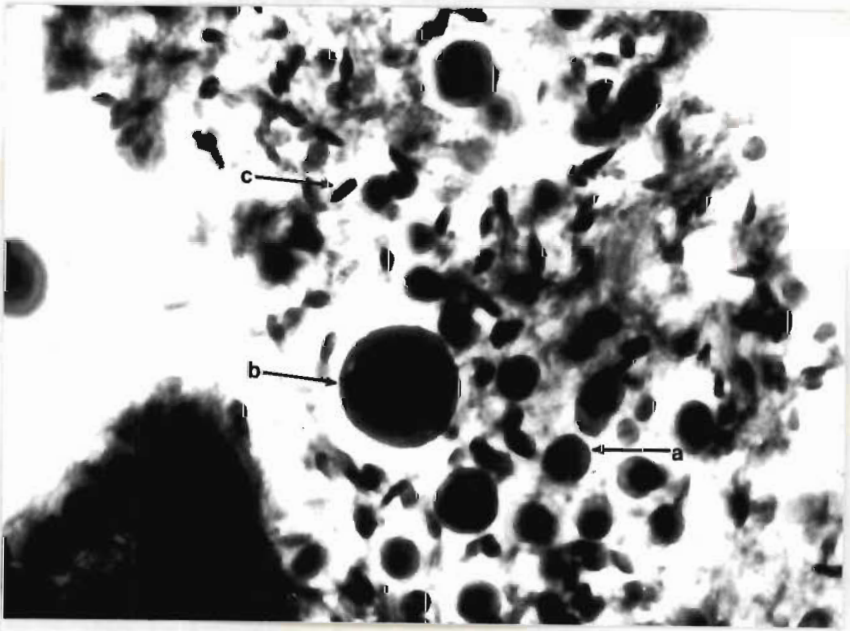
PLATE 12 Epididymis cauda ducts from mature male
(X 300)

PLATE 13 Lumen of epididymis cauda duct from mature
male showing spermatocytes/spermatids
(arrowed - a), multinucleated spermatid
(arrowed - b) and spermatozoa (arrowed - c)
(X 1000)

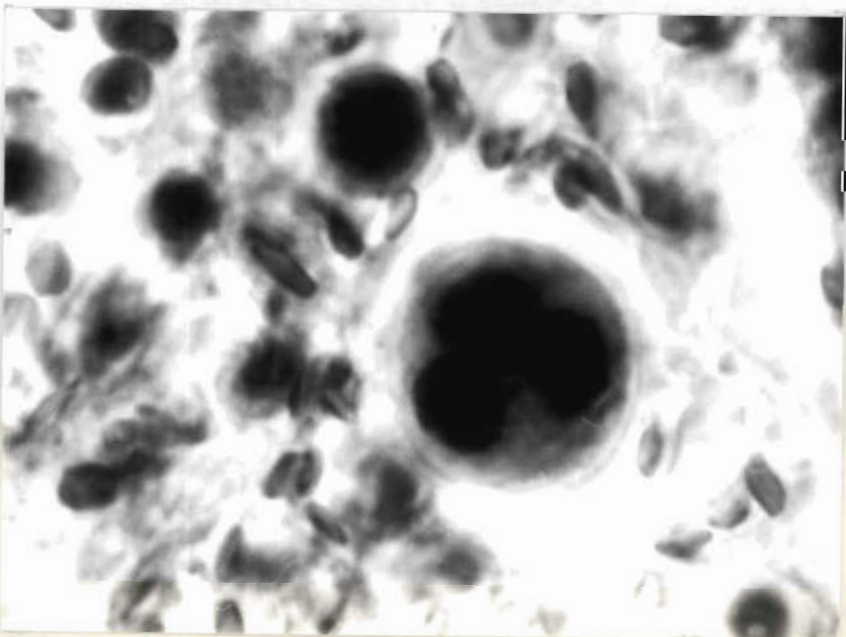
PLATE 14 Multinucleated spermatid
(X 2000)



12



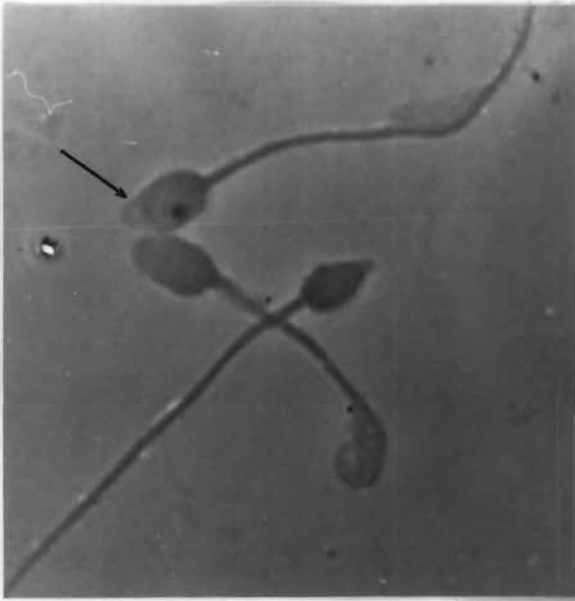
13



14

PLATE 15 Spermatozoa showing acrosome, arrowed in (a)
(X 3000) and midpiece arrowed in (b) (X 2000)

(a)



(b)

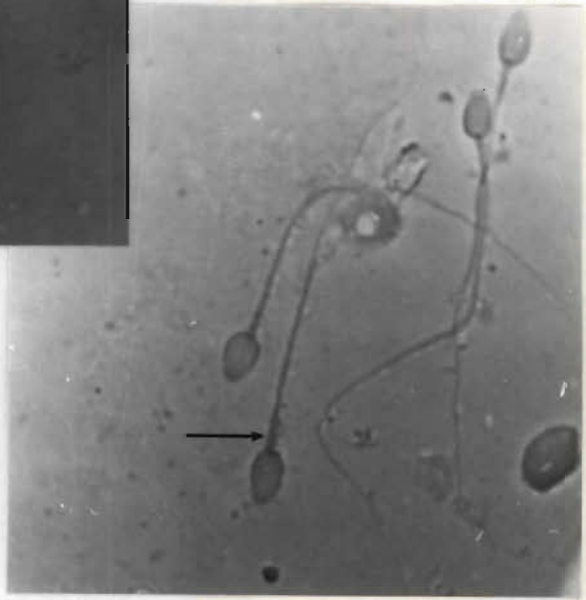
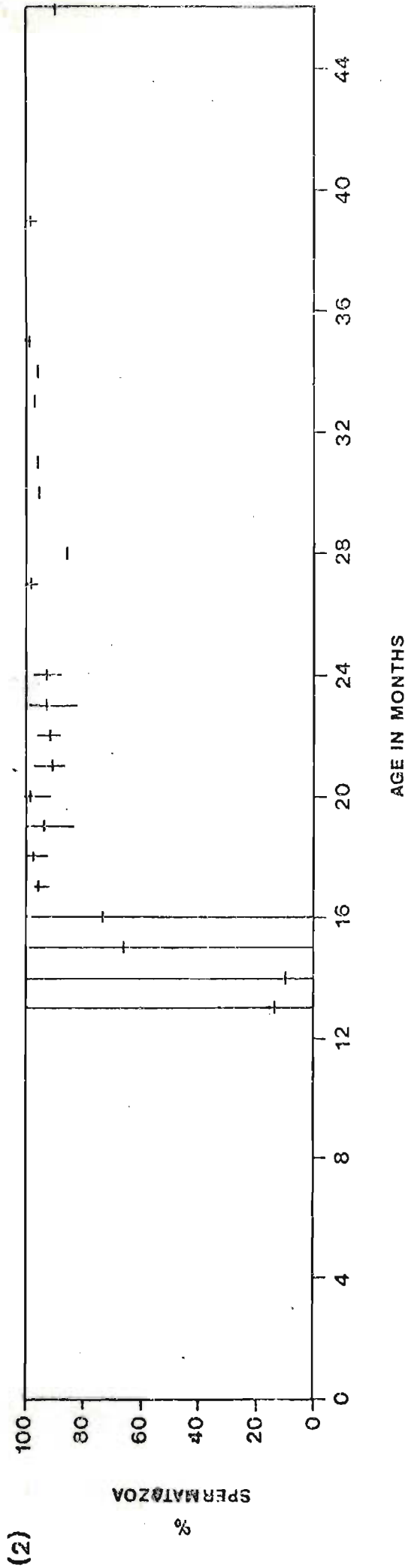
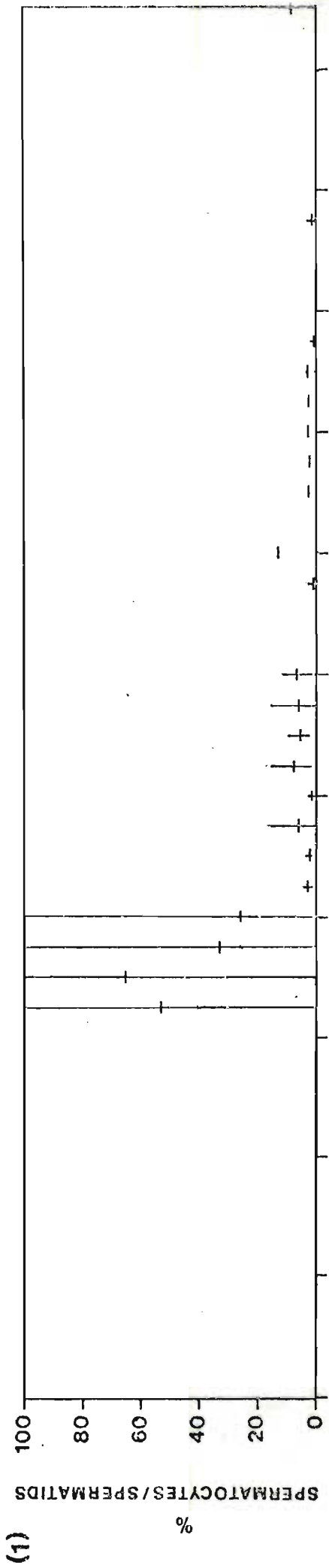


TABLE 9. Spermatozoan measurements (based on ten spermatazoa)

| | \bar{x} (μm) | S.E.M. |
|--------------------|-----------------------------|--------|
| Total length | 21,67 | 0,24 |
| Head length | 3,40 | 0,09 |
| Midpiece length | 2,55 | 0,06 |
| Tail piece | 17,00 | 0,21 |
| Maximum head width | 2,55 | 0,14 |
| Minimum head width | 1,27 | 0,04 |

FIGURE 38 Number of spermatocytes/spermatids (1) and spermatozoa (2), expressed as a percentage of the total number of cells in the epididymis cauda lumen (n = 55)

(Vertical line - range, crossbar - mean)



spermatazoan percentages (9 - 73 %) not only reflects the presence of the youngest males with spermatazoa (13 months old) and the oldest males without spermatazoa (16 months old), but also individuals with low percentages of spermatazoa accompanied by high percentages of spermatocytes/spermatids. From 17 months, all adults show spermatazoa percentages greater than 80 % and spermatocytes/spermatid percentages less than 20 %.

It would appear therefore that 'spermatogenic puberty' in most males takes place during this period of cellular change from 13 to 16 months and that sexual maturity, as determined by the presence of spermatazoa in the epididymis cauda, is attained at the earliest at 13 months and the latest at 17 months.

(b) Gonadial growth

Five aspects of gonadial growth, namely growth in testis mass (including epididymides), in length, in width, in seminiferous tubule diameter and in minimum and maximum diameter of the epididymis cauda ducts are illustrated in FIGURES 39, 40, 41, 42 and 43. In order to determine quantitatively the range of ages over which gonadial growth in these five parameters occurs, an asymptotic value was assigned to each parameter, by calculating the mean size of that parameter from all animals of 17 months and older (the latest age at which sexual maturity was attained). Growth was then considered as taking place from the age at which a size increase of 7 % of the asymptotic value, or more, occurred, up to the age at which the asymptote was reached. The asymptotic values, and age and size at start of gonadial growth are presented in TABLE 10.

'Gonadial growth puberty' therefore commences at eight months for testis mass, length, width and epididymis cauda ducal diameter and at 11 months for seminiferous tubule diameter and continues until the growth asymptotes are reached.

(c) Development of secondary sexual characteristics

The secondary sexual characteristics which were examined were epaulette development and the males' ability to call.

Epaulette development

The youngest males found to have epaulettes containing white hairs, were 13 months old, and as FIGURE 44 shows, epaulette presence in these animals was not always coincident with spermatazoan presence in the epididymis cauda. This implies that epaulettes first appear

FIGURE 39 Growth in combined testis mass (including epididymides) with age (n = 81)

(Vertical line - range; crossbar - mean, broad portion of vertical line - S.E.M.)

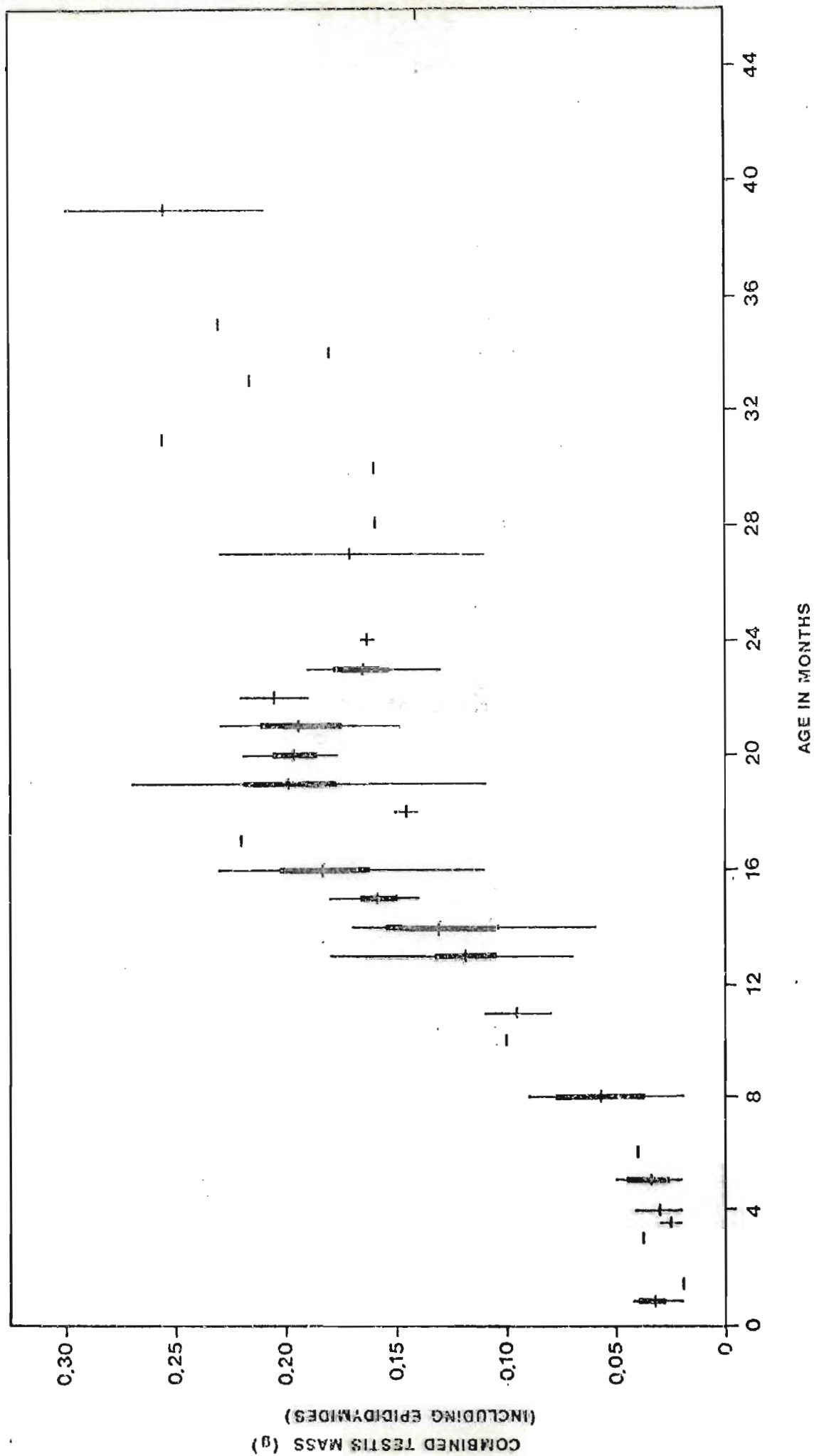


FIGURE 40 Growth in testis length with age (n = 81)

(Vertical line - range, crossbar - mean,
broad portion of vertical line - S.E.M.)

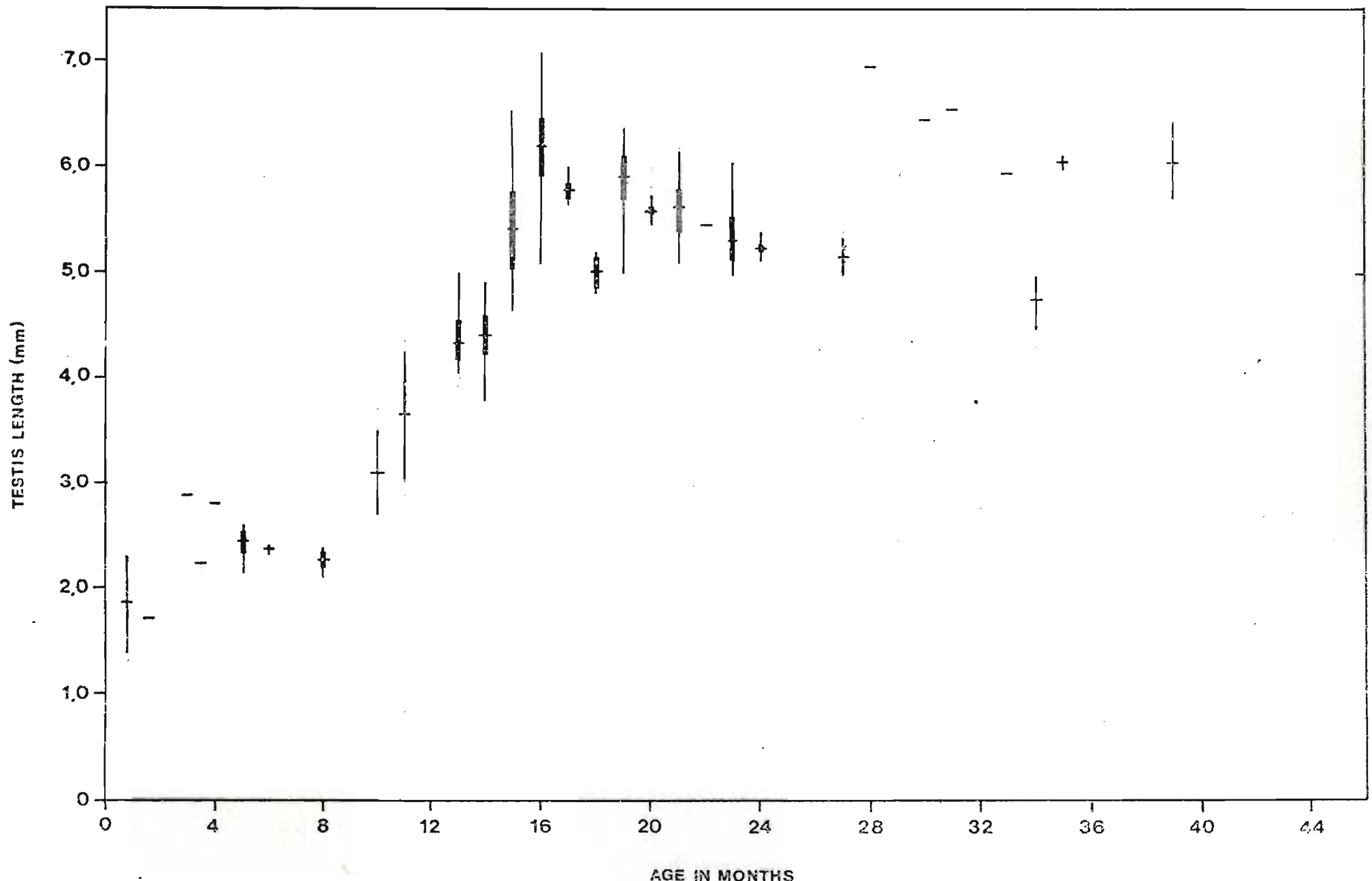


FIGURE 41 Growth in testis width with age (n = 66)

(Vertical line - range, crossbar - mean,
broad portion of vertical line - S.E.M.)

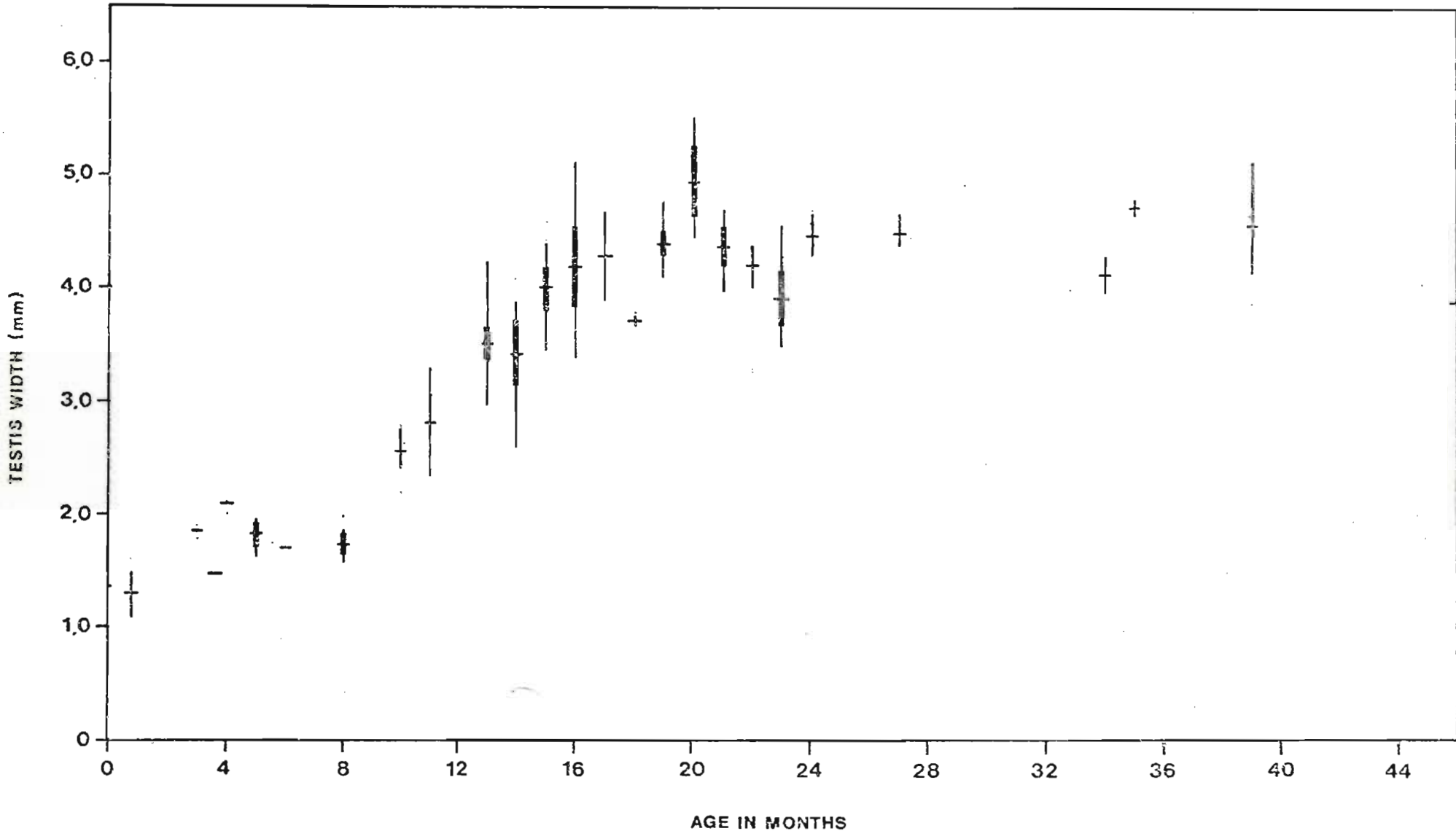


FIGURE 42 Growth in seminiferous tubule diameter with age (n = 81)

(Vertical line - range, crossbar - mean, broad portion vertical line - S.E.M.)

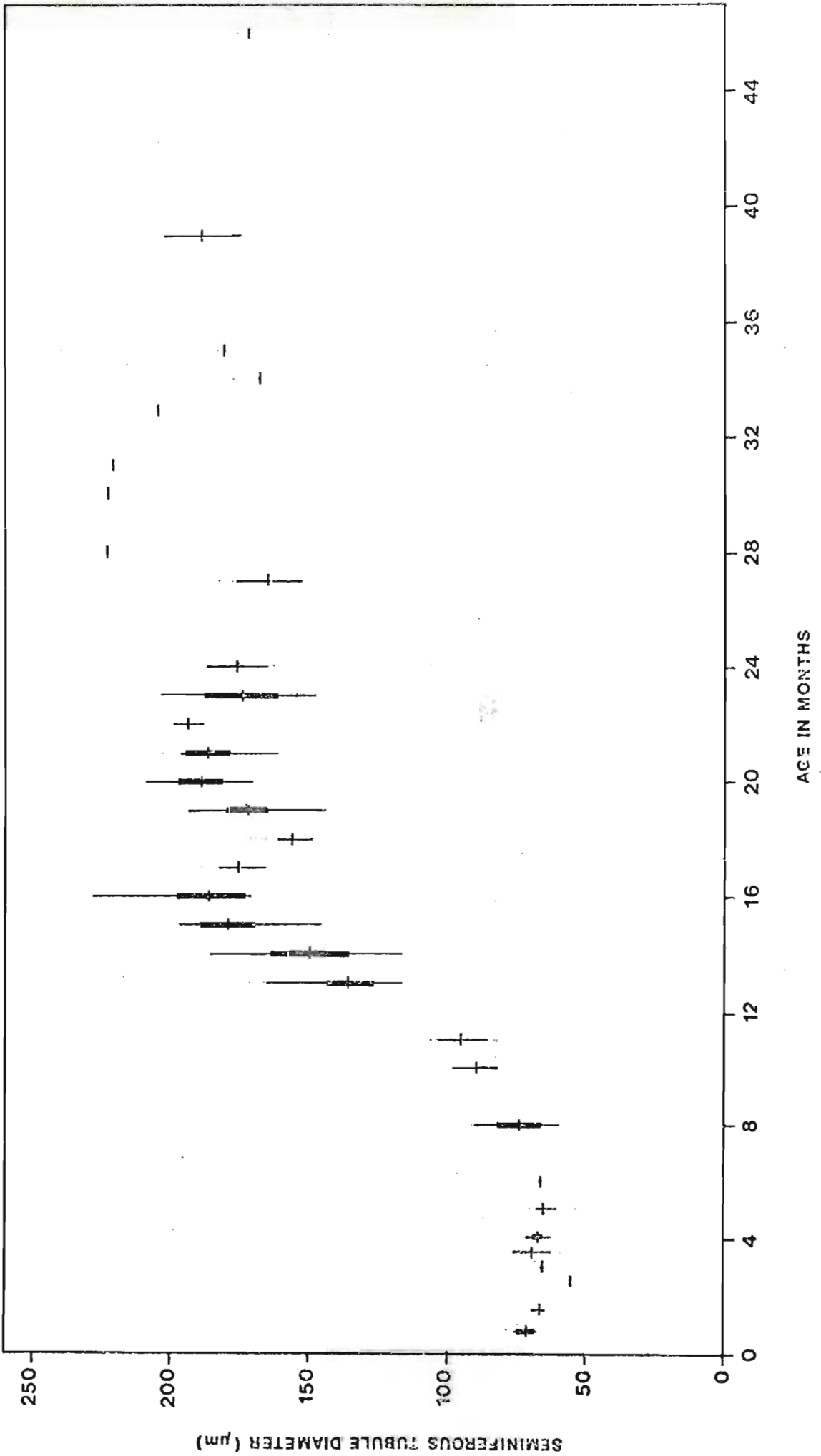


FIGURE 43 Growth in minimum and maximum epididymis caudal diameters with age (n = 42)

(dashed vertical line - range of minimum diameter, crossbar - mean of minimum diameter, solid vertical line - range of maximum diameter, crossbar - mean of maximum diameter)

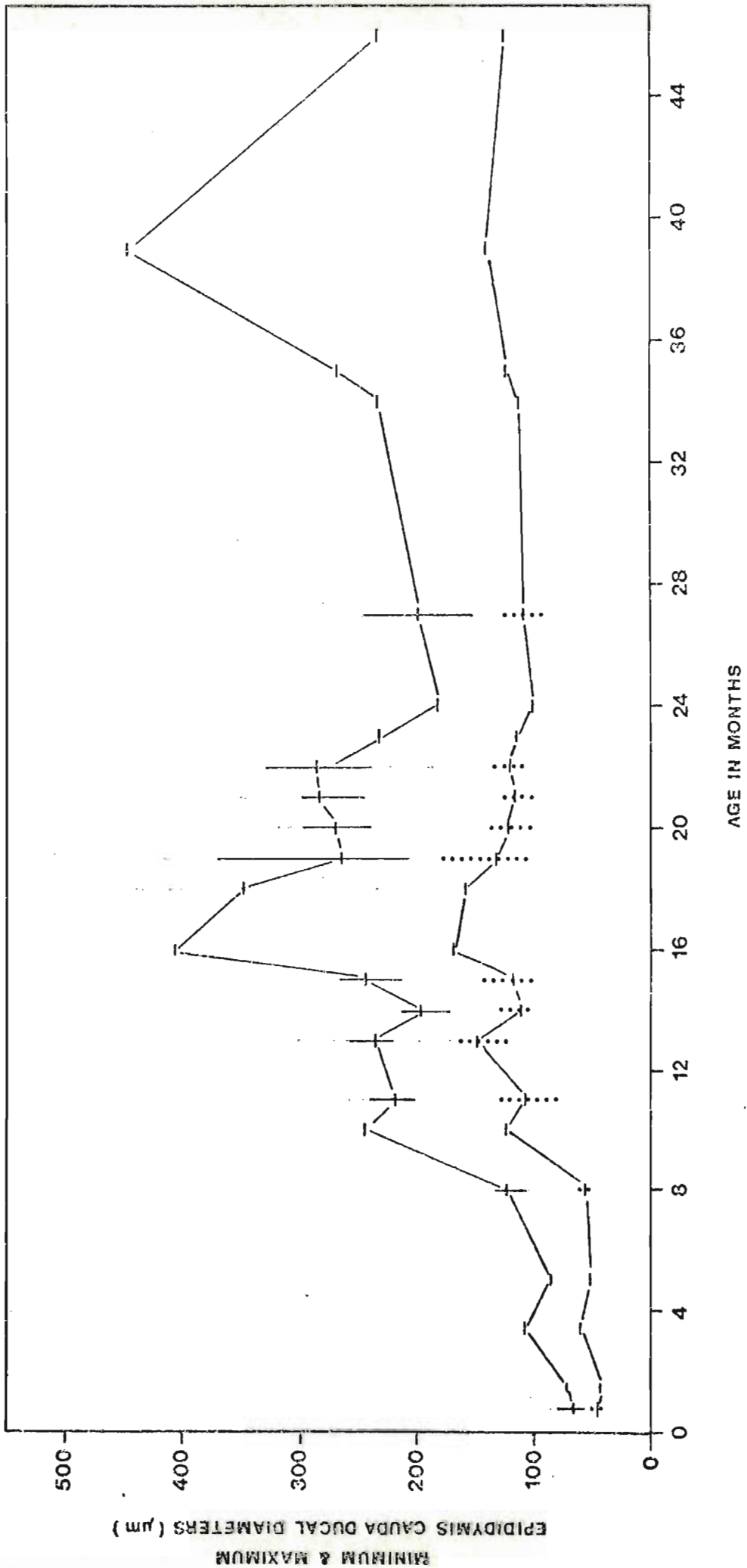


TABLE 10. Gonadial growth - asymptotic values and age and size at start of growth

| | combined
testis
mass
(including
epids.)
(g) | testis
length
(mm) | testis
width
(mm) | seminiferous
tubule diam.
diameter
(μm) | epididymis
cauda ducal
diameter
(μm)
min. max. | |
|---|--|--------------------------|-------------------------|---|---|--------|
| Asymptote | 0,186 | 5,60 | 4,35 | 182,66 | 123,55 | 270,31 |
| \pm S.E.M. | 0,007 | 0,088 | 0,079 | 3,38 | 4,61 | 5,83 |
| Age at start
of growth
(months) | 8,0 | 8,0 | 8,0 | 11,0 | 8,0 | 8,0 |
| \bar{x} size at
start of
growth | 0,057 | 2,28 | 1,72 | 95,40 | 57,5 | 124,75 |

FIGURE 44 Epaulette hair length with age

- males with spermatozoa in the epididymis cauda (n = 46)

- ★ males without spermatozoa in the epididymis cauda (n = 10)

before the attainment of sexual maturity and therefore may be regarded as representing another physiological change which forms part of the pubertal phase.

Calling

The initiation of calling was observed in a caged male whose age was calculated from an earlier recapture. In this single case, calling commenced at 20 months which would suggest that this ability may not be initiated until after the attainment of sexual maturity.

In summary, the sequence and timing of physiological changes associated with puberty in male E. wahlbergi are:

- (1) At eight months - growth in testis size (including epididymides) and epididymis cauda ductal diameter commence. This also represents the age of puberty onset.
- (2) At 11 months - growth in seminiferous tubule diameter commences.
- (3) At 13 months - epaulettes containing long white hairs first develop.
- (4) 13 - 17 months - during this time period the young males in the population first undergo spermatogenesis, exhibit spermatazoa in the epididymis cauda and therefore become sexually mature.
- (5) At 20 months - the possible initiation of male calling.

4.3.3 Age at first mating

This may occur later than the age of sexual maturity attainment. The main mating season occurs from May to July. A male born in November to January will attain sexual maturity at the earliest (13 months old) in December to February, and thus could be 16 to 18 months old before first copulation.

4.3.4 Body mass in relation to puberty and sexual maturity

It is well known that puberty and sexual maturity are more closely related to body mass than to age (Barnett & Coleman, 1959; Crichton, Aitken & Boyne, 1959; Dickerson, Gresham & McCance, 1964; Frisch, 1974; Joubert, 1963; Kennedy, 1969; Kennedy & Mitra, 1963; McCance, 1962; Monteiro & Falconer, 1966; Widdowson & McCance, 1960; Widdowson, Mavor & McCance, 1964).

In order to test this for E. wahlbergi, combined testis mass (including

epididymides) and seminiferous tubule diameter were plotted against body mass (FIGURES 45 & 46). A reverse 'L' shaped distribution of points resulted, indicating two distinct growth patterns; (a) the 'horizontal bar' of the reverse 'L' - greatly increasing body mass with little to no increase in testis mass/seminiferous tubule diameter, and (b) the 'vertical bar' of the reverse 'L' - greatly increasing testis mass/seminiferous tubule diameter with little increase in body mass.

Is the criterion for the transition from type (a) growth to type (b), the attainment of sexual maturity or the onset of one of the physiological changes associated with puberty ?

Non-mature males form part of the 'vertical bar' of the reverse 'L', so sexual maturity was discounted. Puberty was therefore considered. Puberty onset in gonadal growth terms is the commencement of this growth. In order to set a value for testis mass and seminiferous tubule diameter at the onset of 'gonadal growth puberty', the values derived in 4.3.2 (b) and presented in TABLE 10 were used. Thus the mean size at the start of growth was a combined testis mass (including epididymides) of 0,057 g and a seminiferous tubule diameter of 95,40 μm .

All values less than these were designated as representing prepubertal males and values at, or greater than these as representing pubertal or sexually mature males. These values were chosen because they represent not only the start of gonadal growth but also the onset of the earliest of the physiological changes associated with puberty, examined in this study.

An almost horizontal line fitted the prepubertal males (FIGURE 45 (1) and 46 (1)) and an almost vertical line fitted the pubertal and sexually mature animals (FIGURE 45 (2) and 46 (2)). The intersection of these two lines gives a calculated value for testis mass, seminiferous tubule diameter and body mass at the onset of the complex series of pubertal changes. From FIGURE 45 the point of intersection is at a combined testis mass (including epididymides) of 0,041 g and a body mass of 97,5 g. From FIGURE 46, the intersection is at a tubule diameter of 73,0 μm , and a body mass of 100 g.

The horizontal and vertical tendency of the fitted lines indicates a poor correlation between body mass and testis mass/seminiferous tubule diameter. Body mass is not proportional to these criteria, but the onset of puberty coincides with a rapid deceleration in body mass growth and a sudden acceleration in testis mass and seminiferous tubule diameter growth. There is a lack of definition of critical body mass at which sexual maturity occurs, the range being 100 to 120 g.

FIGURE 45 Relationship between testis mass and body mass

● males with spermatozoa in the epididymis cauda (n = 47)

* males without spermatozoa in the epididymis cauda (n = 34)

Dashed line separates prepubertal males from pubertal and sexually mature males at 0,057 g testis mass

(1) Linear regression for prepubertal males
n = 17:

$$y = 0,02 + 0,00021 x; \quad r = 0,37; \quad P > 0,05$$

(2) Linear regression for pubertal and sexually mature males n = 64:

$$x = 92,56 + 117,56 y; \quad r = 0,63; \quad P < 0,001$$

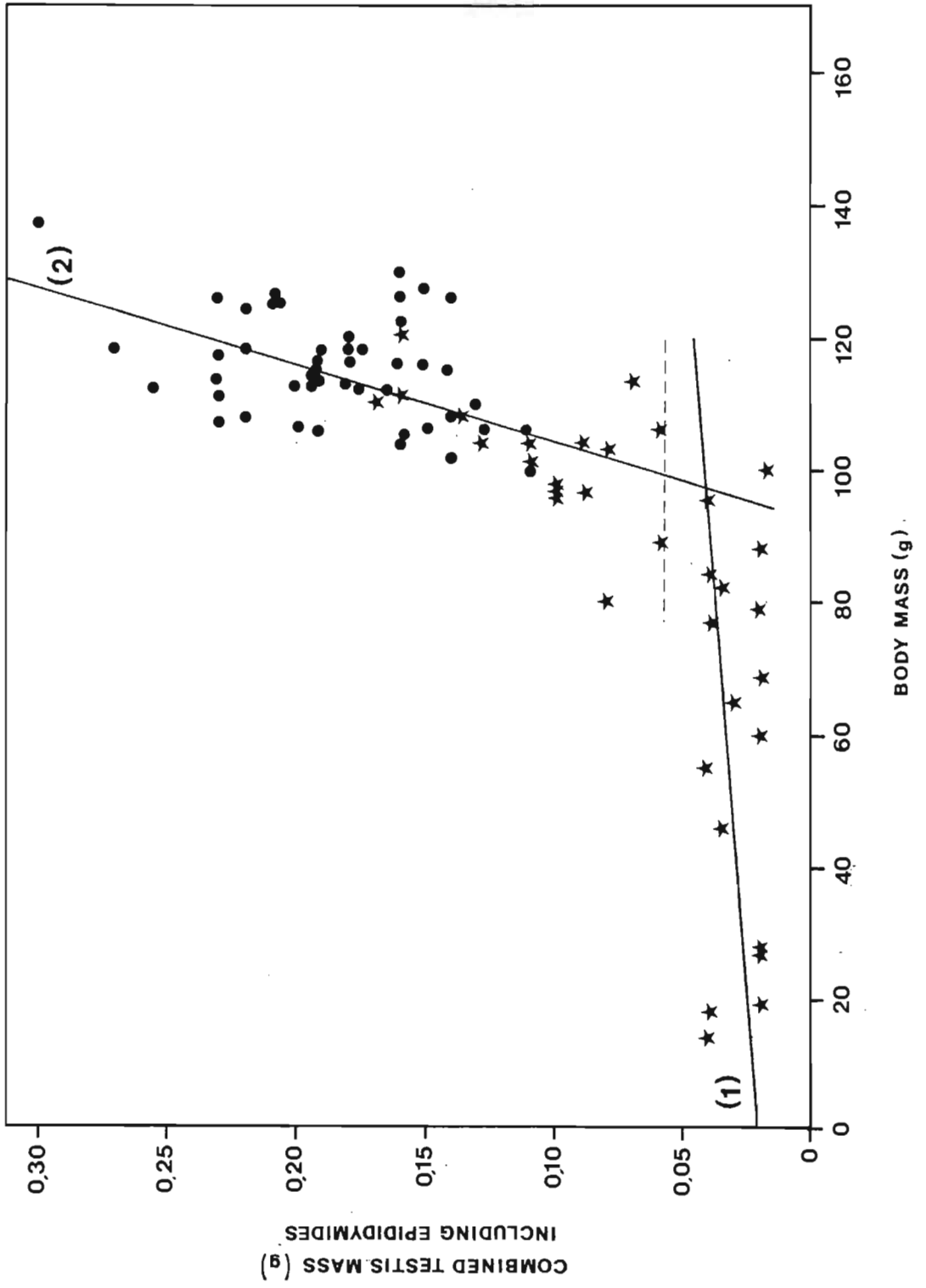


FIGURE 46 Relationship between seminiferous tubule diameter and body mass

● males with spermatozoa in the epididymis cauda n = 47

★ males without spermatozoa in the epididymis cauda n = 34

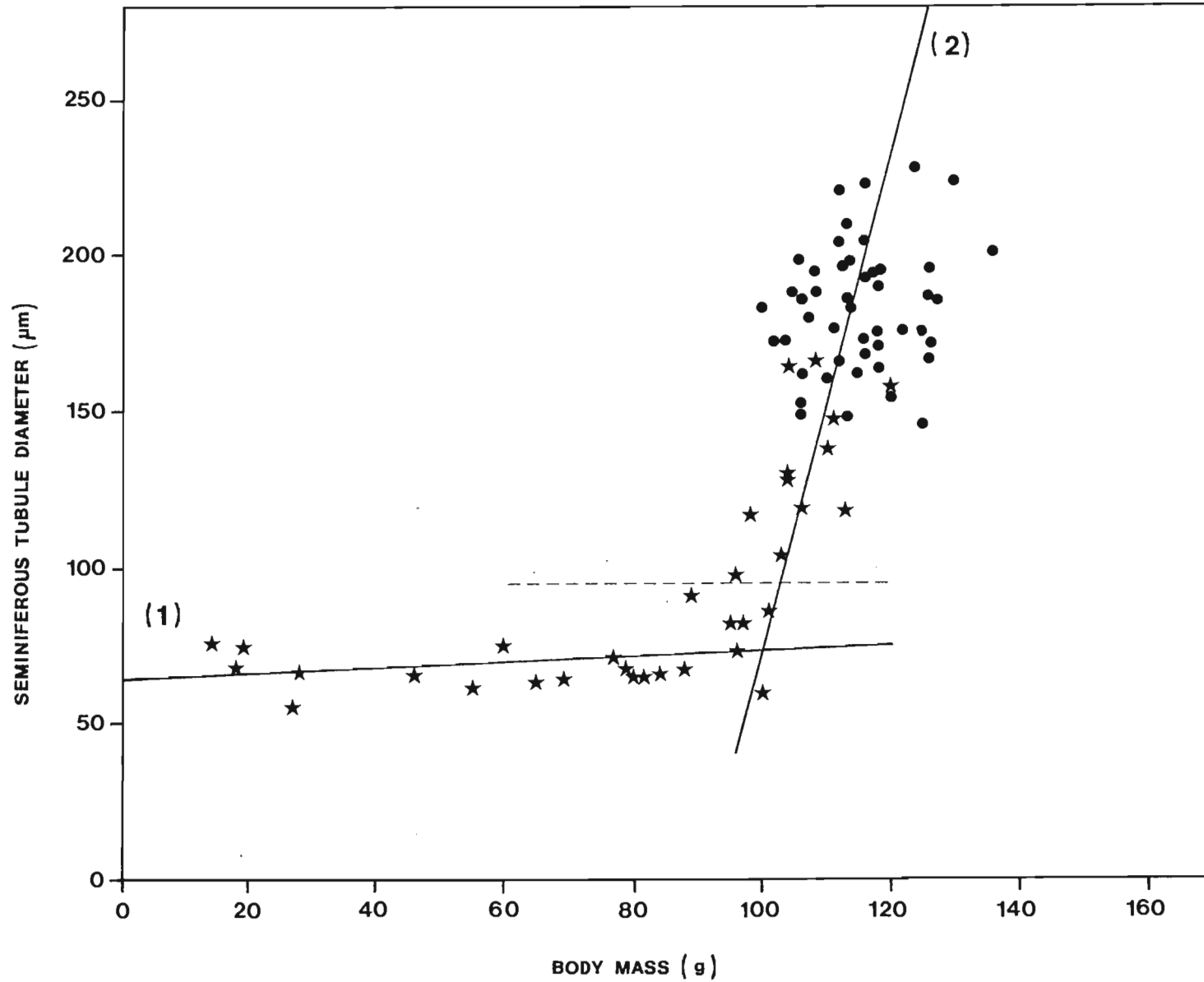
Dashed line separates prepubertal from pubertal and sexually mature males at 95,4 μm seminiferous tubule diameter

(1) Linear regression for prepubertal males n = 22:

$$y = 64,37 + 0,093 x; r = 0,30; P > 0,05$$

(2) Linear regression for pubertal and sexually mature males n = 59:

$$x = 90,45 + 0,133 y; r = 0,45; P < 0,001$$



4.3.5 Forearm length in relation to puberty and sexual maturity

Combined testis mass (including epididymides) and seminiferous tubule diameter were plotted against forearm length (FIGURE 47 & 48). The same reverse 'L' shaped pattern as described for body mass (4.3.4) emerged.

The point of intersection of the two straight lines gives a calculated value for testis/seminiferous tubule diameter and forearm length at commencement of puberty. From FIGURE 47, the point of intersection is at a combined testis mass (including epididymides) of 0,039 g and a forearm length of 83,5 mm. From FIGURE 48, the intersection is at a tubule diameter of 72,0 mm and a forearm length of 85,5 mm. Forearm length is not proportional to testis mass/seminiferous tubule diameter, but the onset of puberty coincides with a sudden deceleration in forearm length growth and an acceleration in testis mass/seminiferous tubule growth. As with body mass, there is a lack of definition of critical forearm length at which sexual maturity occurs, the range being from 81,5 to 88,0 mm.

4.3.6 Examination of adult sex-related criteria for seasonality

By examining monthly quantitative changes in sexually mature males (those with spermatozoa in the epididymis cauda), six sex-related criteria were investigated for seasonality. Although in many cases trends were indicated (a tendency for increased measurements from autumn through winter to spring and decreased measurements during the summer), no statistical significance could be attached to them on a monthly basis.

FIGURE 49 shows the percentage of conceptions and births per month from June 1977 to February 1982. These values were calculated from 214 pregnant females and infants up to three months old and based on the method described in 5.2.3. 84 % of all conceptions occur in May, June and July, with 16 % occurring in August to December. No conceptions were recorded from January to April.

It was decided therefore to analyse the variations in male sex-related criteria in relation to periods of conception, and on this basis the year was divided into two unequal parts, as follows:

Three months (May to July) representing the main mating season;

Nine months (January to April and August to December) representing the period of no or low intensity mating.

The two sets of combined measurements for all parameters examined, were tested for significant differences.

FIGURE 47 Relationship between testis mass and forearm length

● males with spermatozoa in the epididymis cauda n = 47

★ males without spermatozoa in the epididymis cauda n = 34

Dashed line separates prepubertal males from pubertal and sexually mature males at 0,057 g testis mass

(1) Linear regression for prepubertal males n = 17:

$$y = 0,02 + 0,00023 x; r = 0,22; P > 0,05$$

(2) Linear regression for pubertal and sexually mature males n = 64:

$$x = 82,81 + 16,30 y; r = 0,36; 0,01 < P < 0,001$$

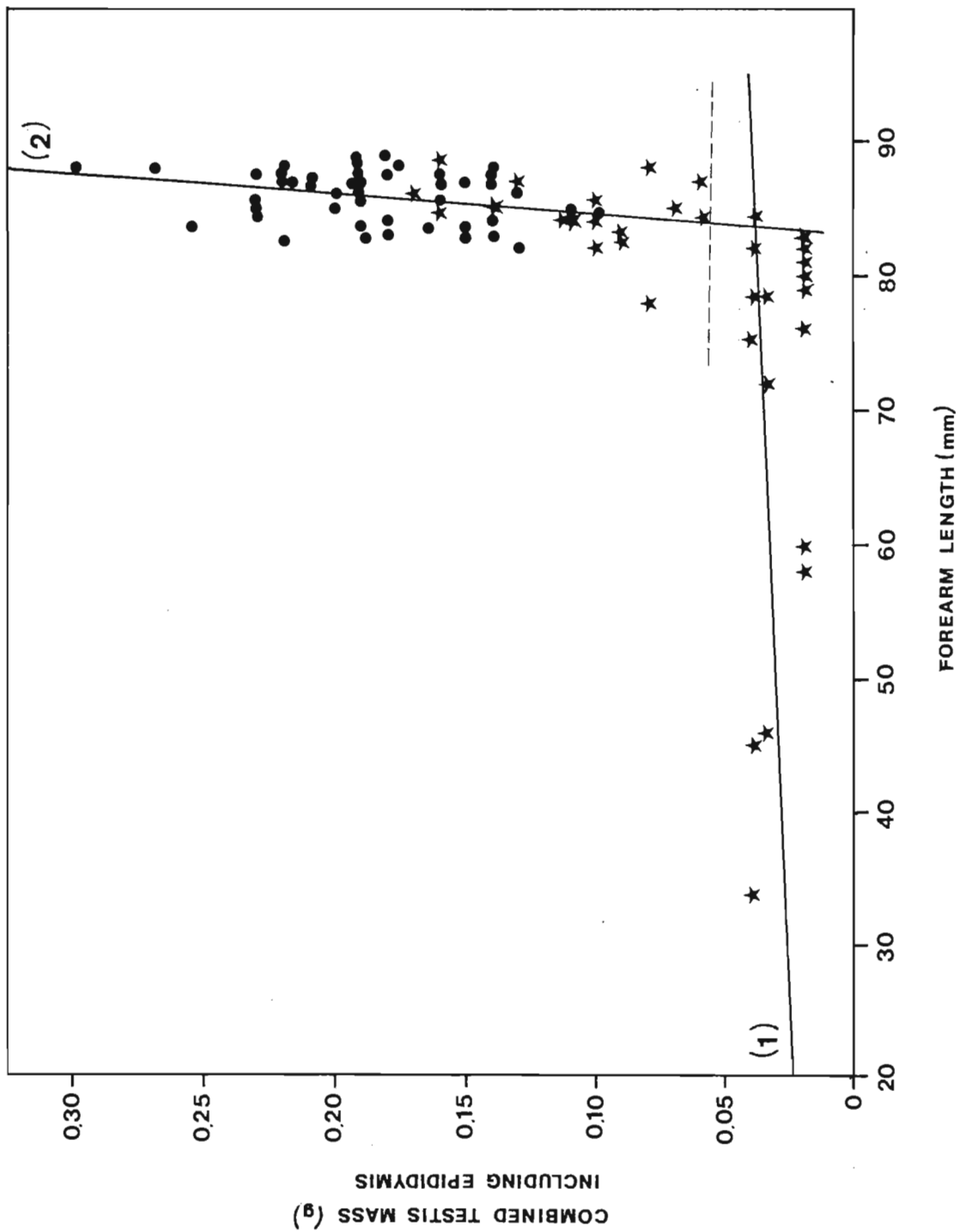


FIGURE 48 Relationship between seminiferous tubule diameter and forearm length

● males with spermatozoa in the epididymis cauda n = 47

★ males without spermatozoa in the epididymis cauda n = 34

Dashed line separates prepubertal males from puberty and sexually mature males at $74,0 \mu\text{m}$ seminiferous tubule diameter

(1) Linear regression for prepubertal males n = 22:

$$y = 64,84 + 0,08 x; \quad r = 0,12; \quad P > 0,05$$

(2) Linear regression for pubertal and sexually mature males n = 59:

$$x = 85,57 + 0,002 y; \quad r = 0,03; \quad 0,05 < P < 0,01$$

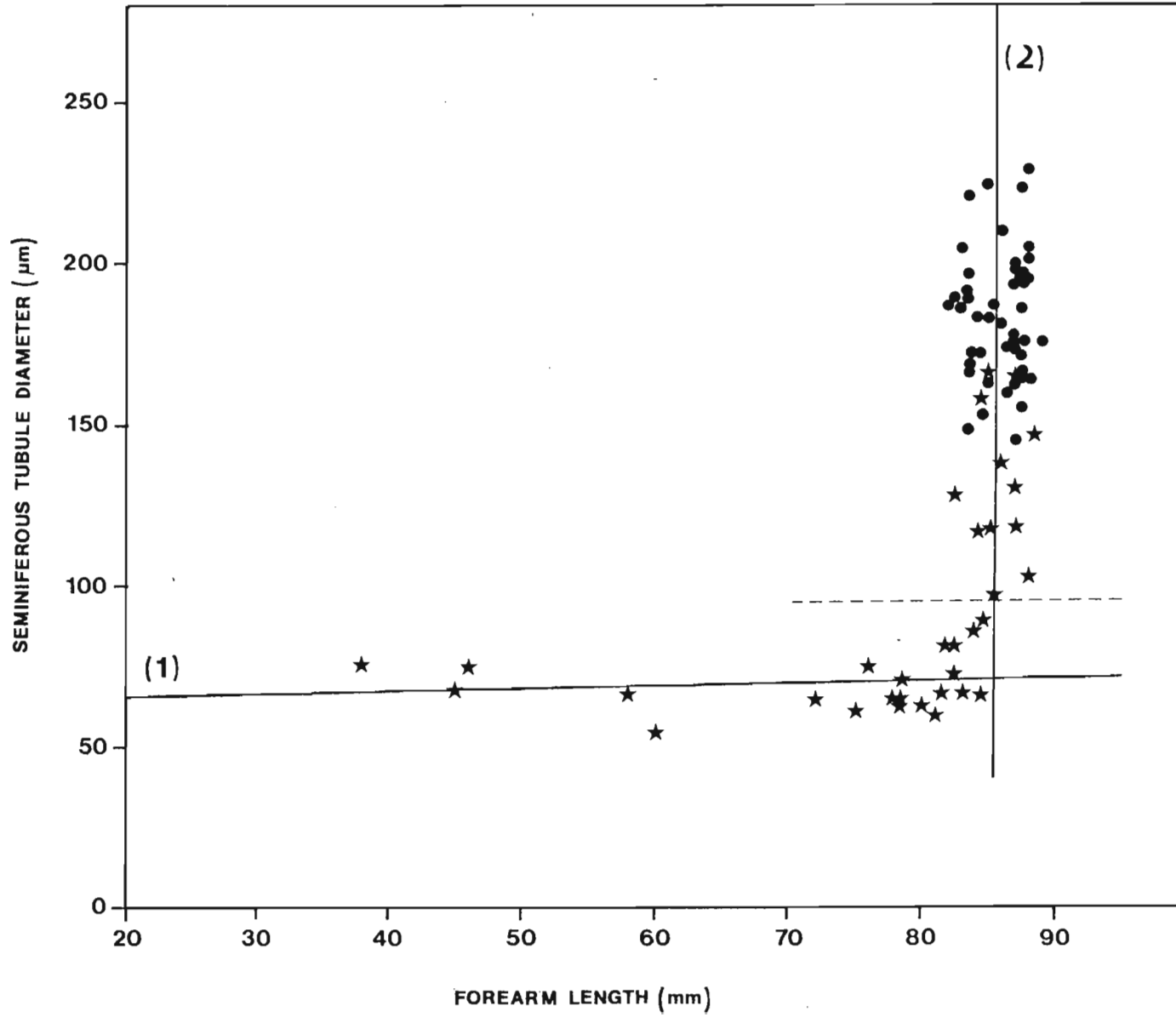
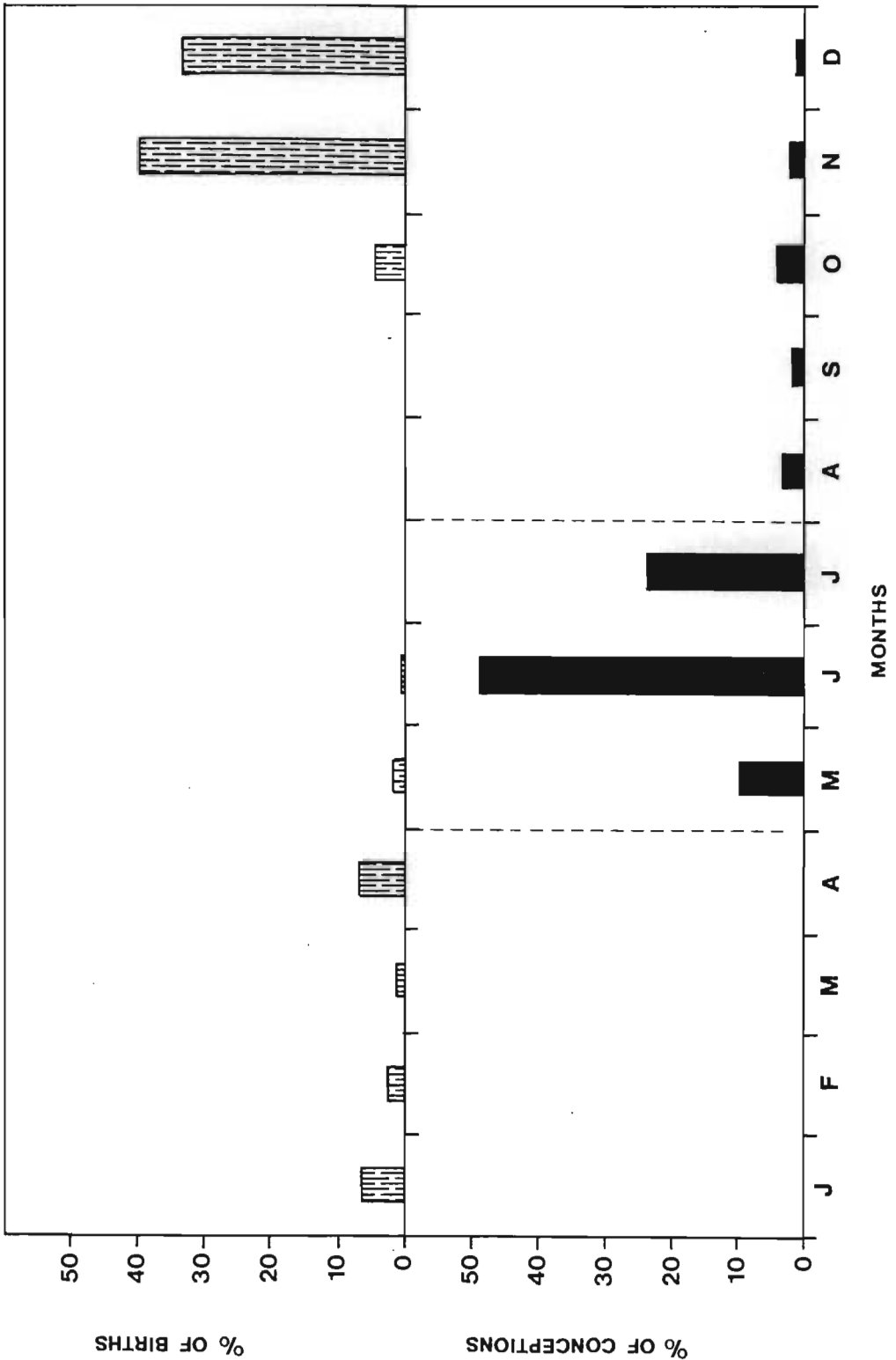


FIGURE 49 Percentage of conceptions and births per month, over 57 months (n = 214)

The vertical dashed lines divide the year into the period May to July and from January to April and August to December.



4.3.6.1 Testis

The five testicular parameters examined for seasonality were combined testis mass (excluding epididymides), testis length, testis width, seminiferous tubule diameter and testis position. TABLE 11 shows that there was no significant difference in any of the parameters between the two year divisions.

4.3.6.2 Epididymis

The nine epididymal parameters examined for seasonality were combined epididymal mass, epididymis caput minimum and maximum ducal diameters, epididymis corpus minimum and maximum ducal diameters, epididymis cauda minimum and maximum ducal diameters, epididymis caput ducal epithelial height, epididymis corpus ducal epithelial height, epididymis cauda ducal epithelial height, percentage of spermatozoa in the epididymis cauda ducal lumen, percentage of spermatocytes/spermatids in the epididymis cauda ducal lumen. TABLE 12 shows that there was no significant difference in any of these parameters between the two year divisions.

4.3.6.3 Body mass

Body mass in 196 wild adult males (with epaulettes) was examined for seasonality (FIGURE 50). From May to July the mean body mass was 129,62 g (S.E.M. = 1,62, range = 115,00 - 147,00 g, n = 34). From the period January to April and August to December, the mean body mass was 124,11 g, (S.E.M. = 0,73, range = 95,00 - 153,00 g, n = 162) and this difference was significant ($z = 3,11$; $0,001 < P < 0,005$).

4.3.6.4 Blood testosterone

Blood testosterone levels were examined for seasonality in 37 sexually mature wild males (FIGURE 51) and in one captive male, and in the wild males were significantly higher in the period May to July ($\bar{x} = 4,71$ nm/l, S.E.M. = 2,071, range = 0,60 - 14,30 nm/l, n = 6) than during the rest of the year ($\bar{x} = 1,18$ nm/l, S.E.M. = 0,136, range = 0,29 - 4,56 nm/l, n = 31), ($t = 3,77$; $P < 0,001$).

Blood testosterone levels in the captive male showed no distinct trends. Large ranges occurred in January, April and September to November, during which months the greatest mean levels of testosterone were recorded.

TABLE 11

Comparison between testicular parameter values for the period May to July and from January to April and August to December

| Testicular parameter | May to July | | | | January to April & August to December | | | | t for difference | significance |
|---|-------------|-----------|--------|---------------|---------------------------------------|-----------|--------|---------------|------------------|------------------|
| | n | \bar{x} | S.E.M. | range | n | \bar{x} | S.E.M. | range | | |
| Combined testis mass (- epids.) (g) | 14 | 0,135 | 0,010 | 0,09-0,20 | 33 | 0,119 | 0,005 | 0,07-0,20 | 1,49 | 0,10<P<0,20 n.s. |
| Testis length (mm) | 14 | 5,79 | 0,151 | 4,80-6,55 | 33 | 5,52 | 0,114 | 4,20-7,10 | 1,30 | P = 0,20 n.s. |
| Testis width (mm) | 11 | 4,29 | 0,133 | 3,70-5,10 | 29 | 4,30 | 0,096 | 3,35-5,10 | 0,08 | P>0,50 n.s. |
| Seminiferous tubule diameter (μ m) | 14 | 180,51 | 6,25 | 145,90-223,07 | 33 | 184,68 | 3,31 | 148,90-229,40 | 0,63 | P>0,50 n.s. |
| Testis position % scrotal | 3 | 69,33 | 19,55 | 33,3-100 | 9 | 71,85 | 10,35 | 16,0-100 | 0,13 | P>0,50 n.s. |

TABLE 12 Comparison between epididymal parameter values for the period
May to July and from January to April and August to December

| | | May to July | | | January to April & August
to December | | | | t for
differ-
ence | significance |
|---|----|-------------|--------|---------------|--|-----------|--------|---------------|--------------------------|------------------|
| Epididymal
parameter | n | \bar{x} | S.E.M. | range | n | \bar{x} | S.E.M. | range | | |
| Combined
epididymal mass (g) | 14 | 0,069 | 0,007 | 0,02-0,10 | 33 | 0,062 | 0,004 | 0,02-0,10 | 1,01 | 0,20<P<0,50 n.s. |
| Epididymis caput
ducal diam.(min.) | 5 | 89,10 | 4,74 | 78,50-104,00 | 17 | 88,79 | 3,01 | 76,00-114,50 | 0,04 | P<0,05 n.s. |
| (μm) (max.) | 5 | 198,40 | 35,24 | 141,00-327,00 | 17 | 154,12 | 6,24 | 112,50-213,00 | 1,90 | 0,05<P<0,10 n.s. |
| Epididymis corpus
ducal diam.(min.) | 5 | 72,90 | 4,32 | 62,00-86,00 | 17 | 78,15 | 2,11 | 63,00-92,50 | 1,10 | 0,20<P<0,50 n.s. |
| (μm) (max.) | 5 | 135,70 | 20,19 | 106,00-214,00 | 17 | 143,26 | 6,46 | 89,00-214,00 | 0,45 | P>0,05 n.s. |
| Epididymis cauda
ducal diam.(min.) | 6 | 137,08 | 11,36 | 112,00-180,50 | 17 | 120,35 | 4,12 | 96,00-168,00 | 1,65 | 0,10<P<0,20 n.s. |
| (μm) (max.) | 6 | 309,75 | 38,70 | 211,50-447,50 | 17 | 256,24 | 14,11 | 153,00-409,00 | 1,55 | 0,10<P<0,20 n.s. |
| Epididymis caput
epithelial height
(μm) | 5 | 19,07 | 1,95 | 11,75-22,40 | 17 | 21,79 | 1,51 | 14,40-36,20 | 0,87 | 0,20<P<0,50 n.s. |
| Epididymis corpus
epithelial height
(μm) | 5 | 12,26 | 0,67 | 9,70-13,50 | 17 | 15,17 | 0,73 | 10,00-21,30 | 1,99 | 0,05<P<0,10 n.s. |
| Epididymis cauda
epithelial height
(μm) | 6 | 11,72 | 1,19 | 9,34-16,80 | 17 | 12,76 | 0,78 | 9,16-20,80 | 0,67 | P>0,05 n.s. |
| % spermatazoa in
epididymis cauda | 14 | 96,09 | 1,14 | 84,20-100,00 | 33 | 90,93 | 2,48 | 37,50-100,00 | 1,32 | 0,10<P<0,20 n.s. |
| % spermatoocytes/
spermatids in
epididymis cauda | 14 | 3,91 | 1,14 | 0-15,58 | 33 | 9,11 | 2,48 | 0-62,50 | 1,33 | 0,10<P<0,20 n.s. |

FIGURE 50 Monthly variations in body mass of wild bats
(n = 196).

(Vertical line - range, crossbar - mean,
broad portion of vertical line - S.E.M.)

The vertical dashed lines divide the year
into the period May to July and from January
to April and August to December.

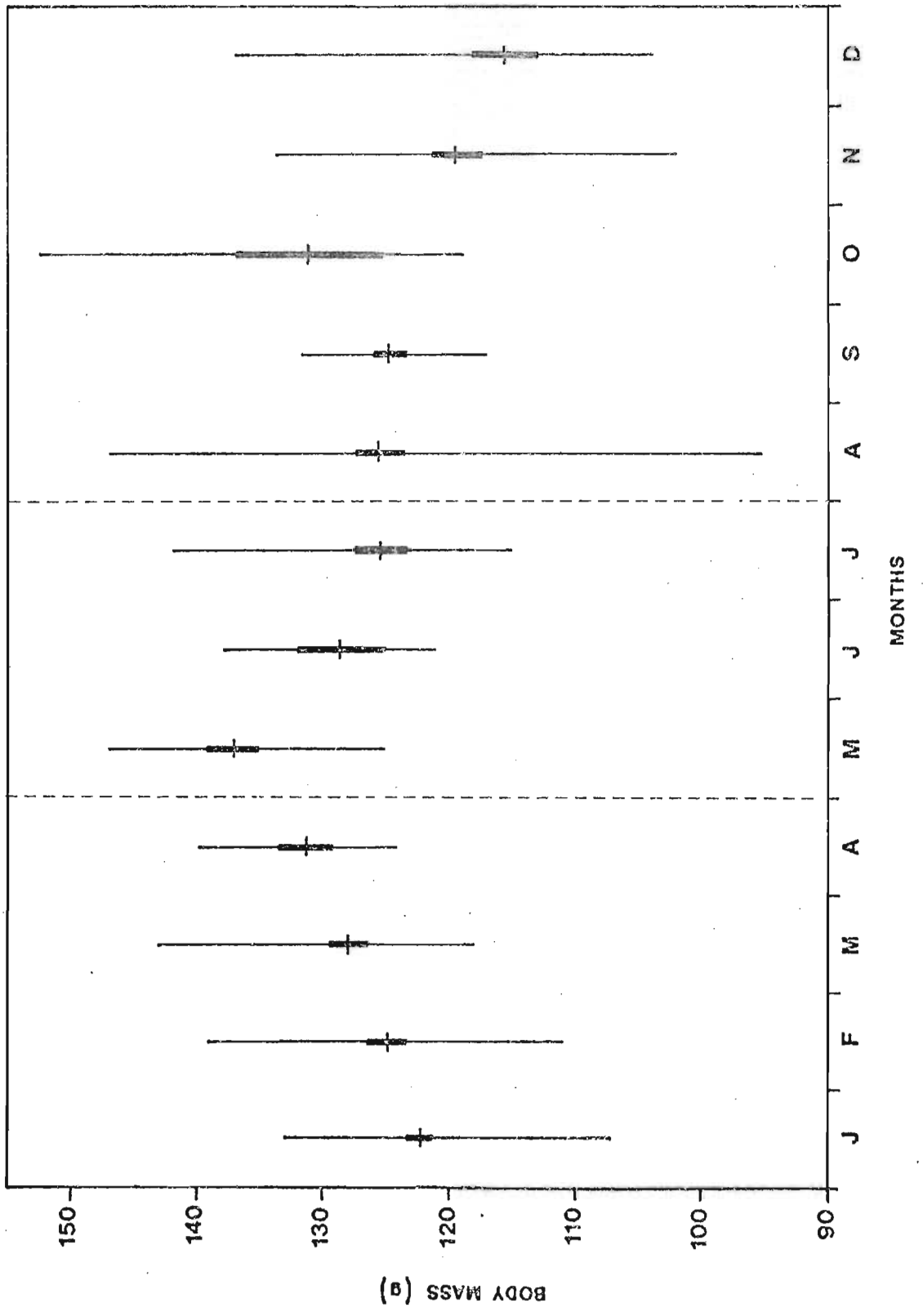
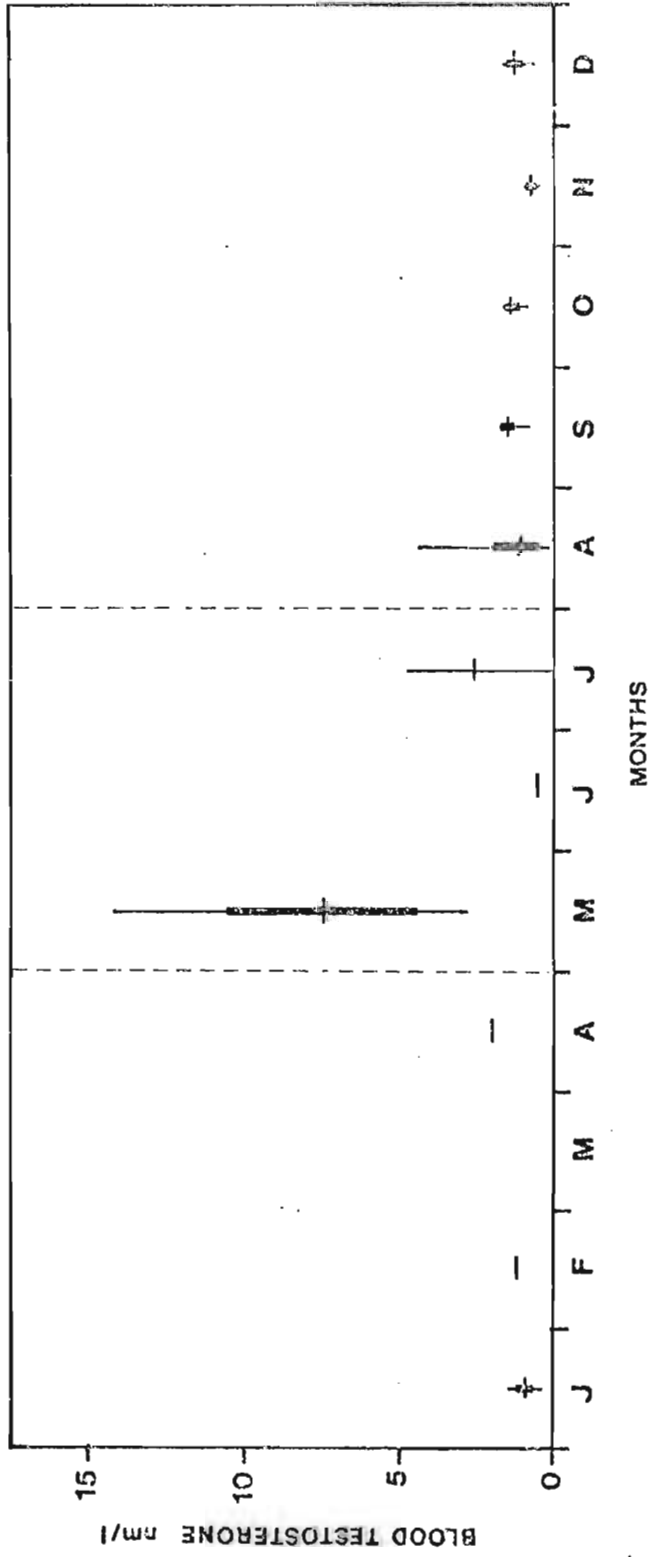


FIGURE 51 Monthly variations in blood testosterone levels in wild males (n = 37)

(Vertical line - range, crossbar - mean, broad portion of vertical line - S.E.M.)

The vertical dashed lines divide the year into the period May to July and from January to April and August to December)



4.3.6.5 Epaulettes

FIGURE 52 shows there was a reduction in epaulette hair length during November to February. This implies a moult and hair regrowth during these months. Mean hair length during the November to February period of moult and regrowth was significantly shorter ($\bar{x} = 7,46$ mm, S.E.M. = 1,21, range = 0 - 13,60 mm, $n = 14$) than for the remainder of the year (covering the May to July mating period) ($\bar{x} = 12,58$, S.E.M. = 0,30, range = 7,70 - 18,70 mm, $n = 32$) ($t = 5,43$; $P < 0,001$).

This moult and regrowth of epaulette hairs was confirmed by observations of two captive males (PLATES 16 & 17 and TABLE 13). In the three cases the process took a minimum of 45 days and a maximum of 78 days to complete.

4.3.6.6 Calling

- (a) FIGURE 53 shows the mean number of nights per month when wild males were heard calling over a three year period (January 1979 to December 1981). Six months (March, May, June, October, November and December) had large ranges over the three year period, with two months (August and September) having significantly fewer calling nights per month ($\bar{x} = 2,67$, S.E.M. = 0,76, range = 1 - 5, $n = 6$) than the remainder of the year ($\bar{x} = 9,17$, S.E.M. = 1,18, range = 0 - 30, $n = 30$), ($t = 2,37$; $0,01 < P < 0,05$).

Using the May to July, and January to April and August to December year divisions, there were significantly more calling nights in May to July ($\bar{x} = 12,67$, S.E.M. = 2,90, range = 2 - 30, $n = 9$), than in the remainder of the year ($\bar{x} = 6,56$, S.E.M. = 0,92, range = 0 - 20, $n = 27$), ($t = 2,58$; $0,01 < P < 0,05$).

- (b) Sound analysis of caged male calling

The calling of E. wahlbergi males is characterized by a sharp metallic donging sound repeated at regular intervals. Frequencies within the call range from 1000 hz to 2500 hz, with 1250 and 1600 hz having the greatest energy output (amplitude). The time intervals between calls varies from 0,90 to 1,35 seconds.

An adult male was captured and caged in April 1980. From

FIGURE 52 Monthly variations in epaulette hair length
(n = 46)

The vertical dashed lines divide the year into the period November to February - period of epaulette hair moult and regrowth, and from March to October - covering the main May to July mating period

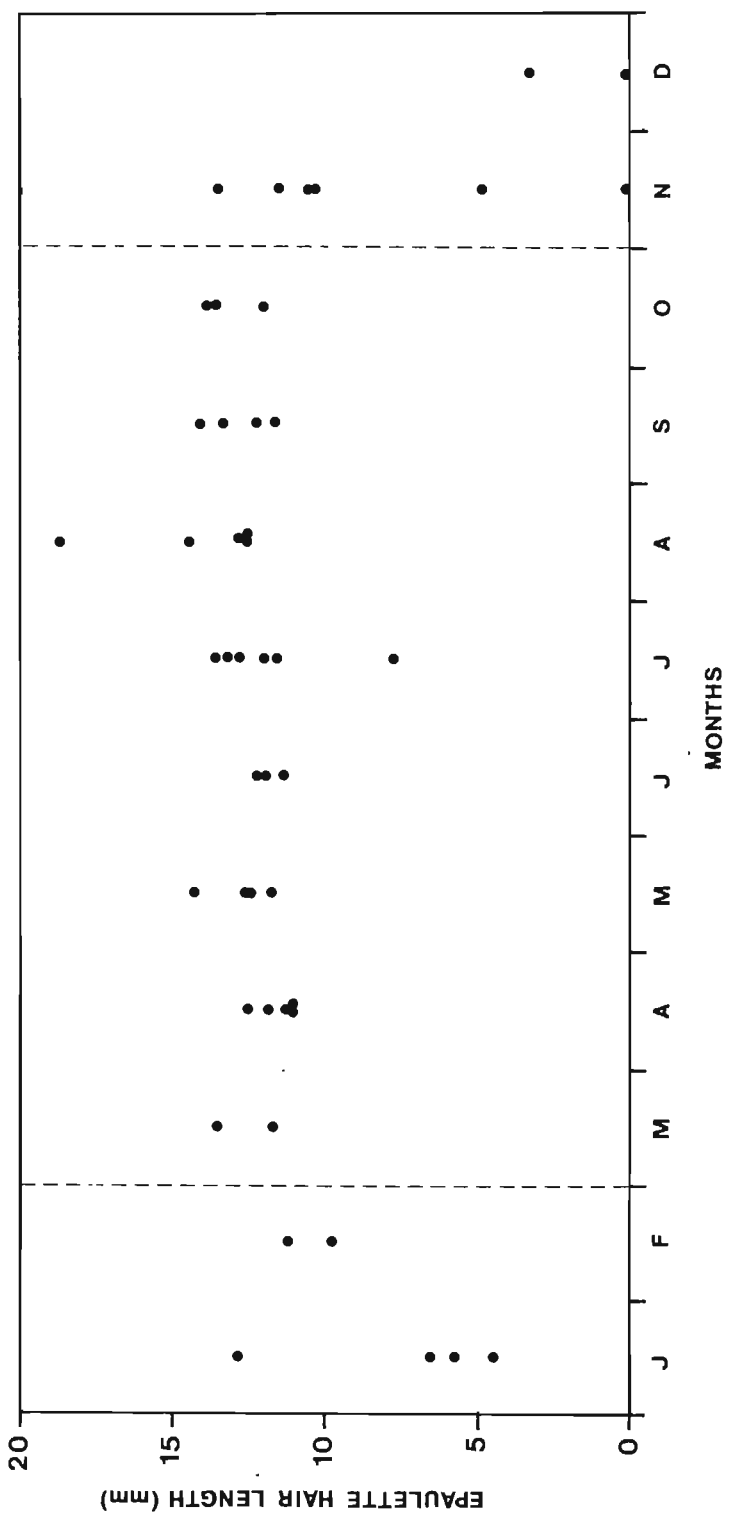


PLATE 16 Epaulette moult in male E. wahlbergi almost complete

PLATE 17 Epaulette hairs regrown



16



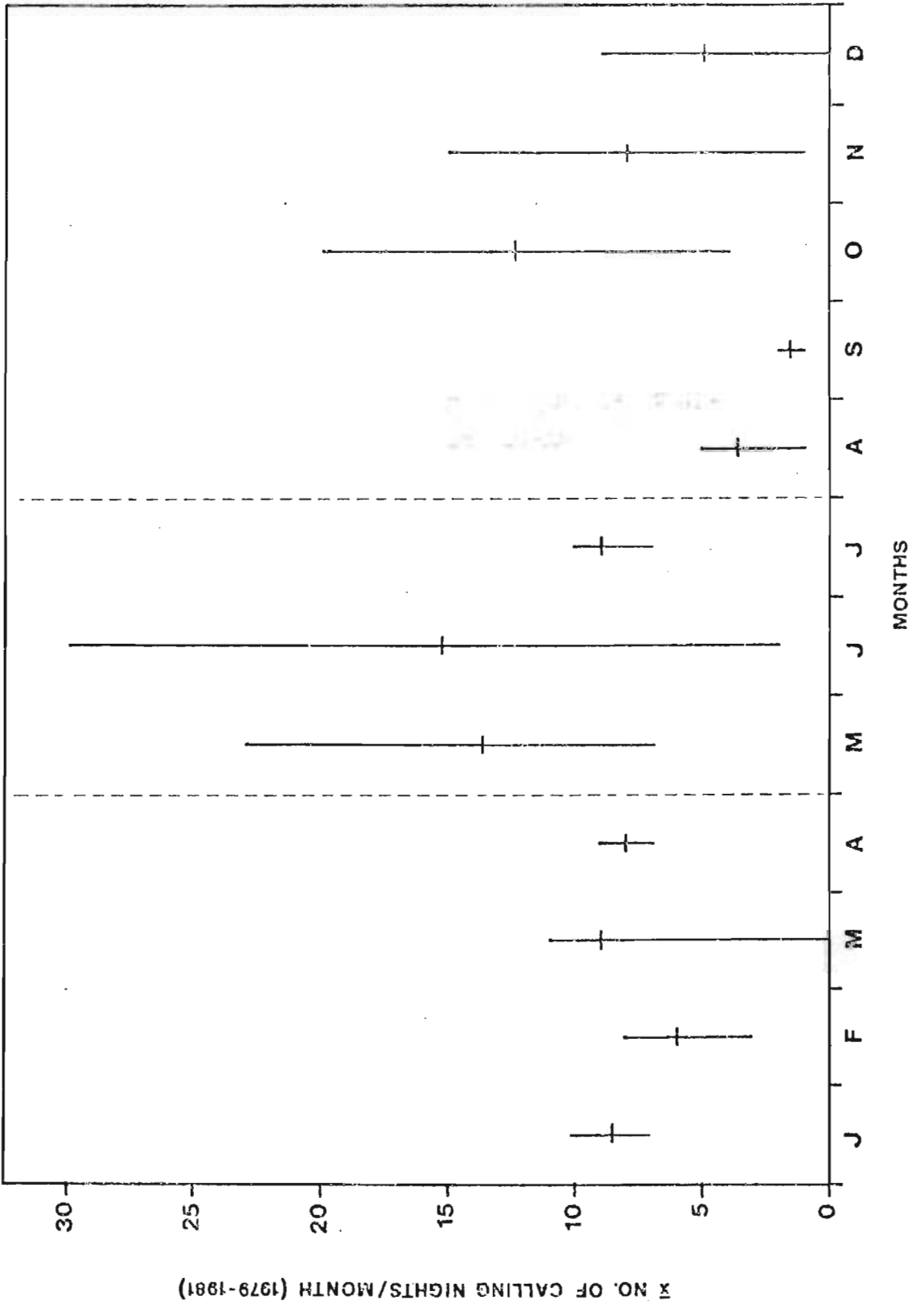
17

TABLE 13. Moulting and regrowth of epaulette hairs
in two captive males

| Male
no. | Year | Dates | | |
|-------------|-------|-------------------------|--------------------|---------------------------|
| | | balding first
starts | epaulettes
bald | hair regrown
(10 mm +) |
| 5-07502 | 78/79 | 27 Nov. | 8 Dec. | 11 Jan. |
| ET 419 | 80/81 | 7 Dec. | 21 Dec. | 10 Feb. |
| ET 419 | 81/82 | 28 Nov. | 13 Dec. | 14 Feb. |

FIGURE 53 Mean number of nights per month when wild males were heard calling in the study area over a three year period (January 1979 to December 1981) (n = 36)

The vertical dashed lines divide the year into the period May to July and from January to April and August to December



then until his release in February 1982, the dates on which he was heard calling were as follows: 1980 - 25 Aug., 2 Nov., 20 Nov., 30 Nov. - 19 Dec., 26 Dec. - 30 Jan., 1981 - 25 Feb. - 1 Mar., 11 Apr. - 5 July, 17 July, 14 Aug., 15 Sept., 1 - 3 Oct., 15 Oct. - 1 Nov.

Eight sound recordings of the calls were made from 27 April 1981 to 23 October 1981. Calling was analysed for seasonality by examining variations in amplitude in five frequencies (1000 hz, 1250 hz, 1600 hz, 2000 hz, 2500 hz) and variations in time intervals between calls (FIGURE 54). Superimposed onto FIGURE 54 are two shaded areas, which represent the period during which the caged male mated with six females. (This was calculated from subsequent pregnancies and birth dates).

No significant difference was found in either amplitude or time intervals of calls between the period during mating and that before and after it.

However four points of interest, drawn from FIGURE 54 may be significant to the onset of copulation and have relevance to future work. (1) In three out of five frequencies, amplitude builds up to a peak just prior to the commencement of copulation. (2) In all five frequencies, amplitude decreases after mating commences. (3) During the mating period the interval between calls lengthens. (4) The 1000 hz frequency is absent from the call in April and early May but appears from the middle of May onwards.

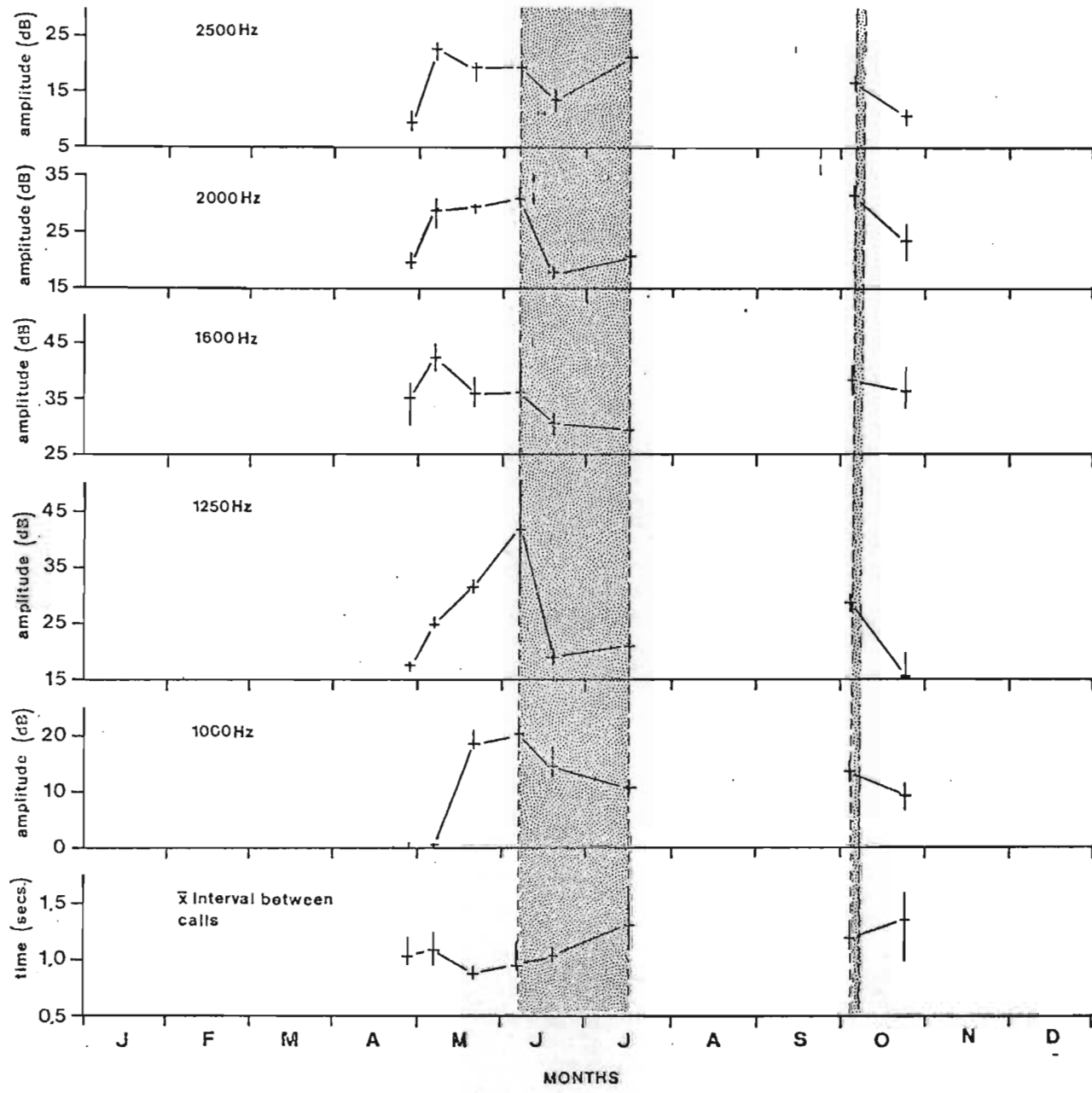
Recordings were not made of the single night calls in August and September or those at the end of February. Without this information meaningful conclusions cannot be drawn from the preceding comments.

(c) Observations of caged male calling

The caged male was observed calling during May 1981. The same corner of the cage was used for calling from, each night. Each call was preceded by partly opened wings, much wing fluttering, formation of the mouth into a long swollen tube and much ear rotation. As the call was emitted the wings closed and the mouth was opened. No epaulette eversion was observed. During calling the females were seen to commence active flying around, and in

FIGURE 54 Monthly variations in amplitude of caged male calling, examined for five frequencies; 1000 Hz, 1250 Hz, 1600 Hz, 2000 Hz, 2500 Hz and time intervals between calls n = 240 calls analysed

(Vertical line - range, crossbar - mean. Shaded portion represents the period during which the caged male was known to have mated)



particular, flying past and hovering close to the caller. One female settled within one metre of him.

4.3.7 An overview of seasonality in a single captive male

FIGURE 55 combines five sex-related parameters measured from one actively breeding caged male, over a maximum period of 23 months, at intervals of two to four weeks. Superimposed onto this figure are two shaded areas during which the caged male mated with six females.

The measurements of body mass, epaulette hair length, testes position, calling and blood testosterone levels obtained only in 1981, were taken into account when investigating the possibility of seasonality in the caged male, because it was during this year that the male was first known to breed.

(a) Body mass

FIGURE 50 and 4.3.6.3 show the monthly variations in body mass of wild males and the significantly greater mean body mass during the May to July mating period than the rest of the year. Despite a diet, constant in both mass and composition, the body mass of the caged male showed remarkably similar fluctuations.

The mean body mass of the caged male for May to July was significantly greater ($\bar{x} = 130,83$ g, S.E.M. = 3,19, range = 116,00 - 138,00 g, $n = 6$) than the mean body mass for the remainder of the year ($\bar{x} = 118,85$ g, S.E.M. = 1,71, range = 103,00 - 136,00 g, $n = 20$), ($t = 3,22$; $0,001 < P < 0,005$) and the May to July mean body mass for the wild males (129,62 g) and caged male (130,83g) showed no significant difference.

The mean body mass of the caged male during his known mating periods (shaded areas in FIGURE 55) ($\bar{x} = 129,20$ g, S.E.M. = 2,52, range = 123,00 - 136,00 g, $n = 5$) was also significantly higher than the mean body mass during the remaining non-copulatory part of the year ($\bar{x} = 119,81$ g, S.E.M. = 1,95, range = 103,00 - 138,00 g, $n = 21$), ($t = 2,14$; $0,01 < P < 0,05$).

(b) Epaulette hair length

The same situation shown for wild males in FIGURE 52 is seen for the caged male in FIGURE 55. Moulting and regrowth of epaulette hair occurs from November to February and is reflected by the significantly shorter mean hair lengths for these months ($\bar{x} = 6,45$ mm, S.E.M. = 1,63, range = 0 - 12,00 mm, $n = 10$) than for the rest of the

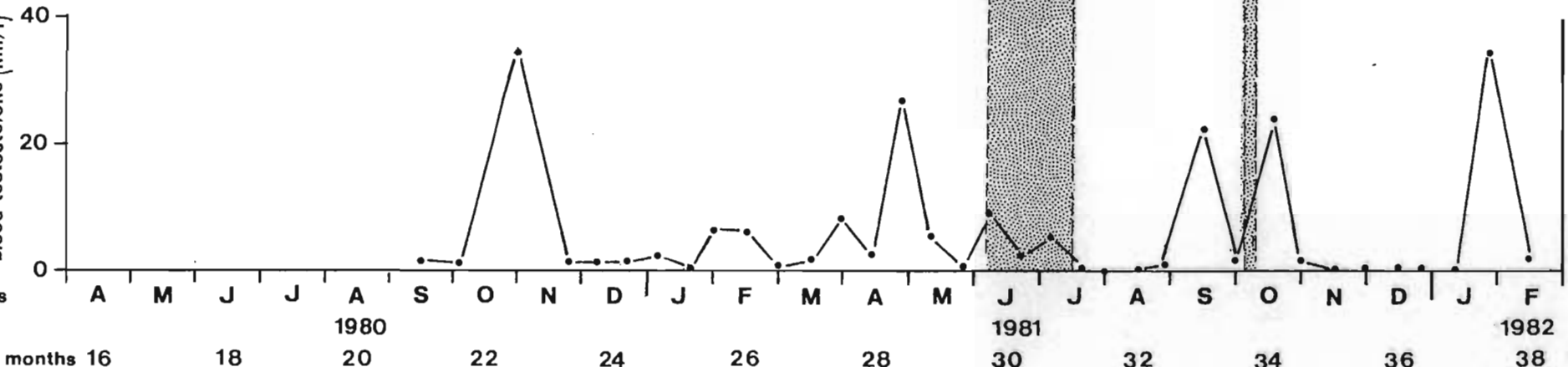
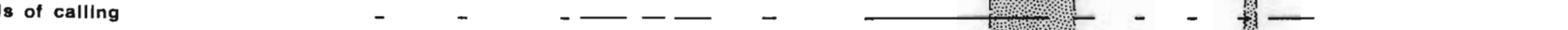
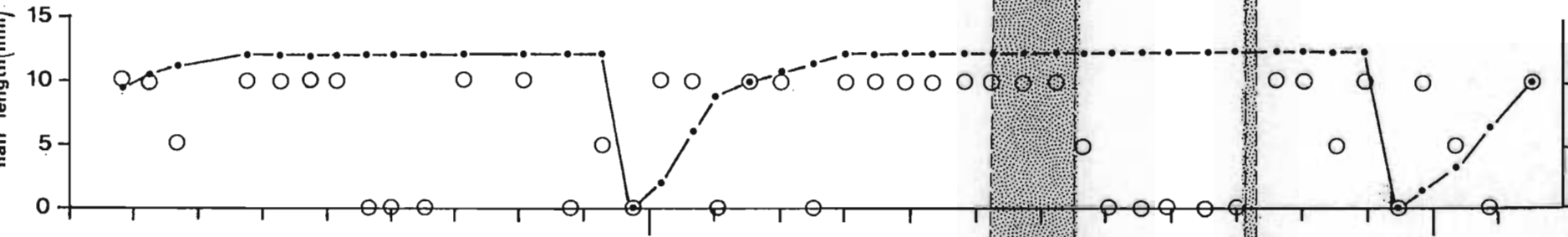
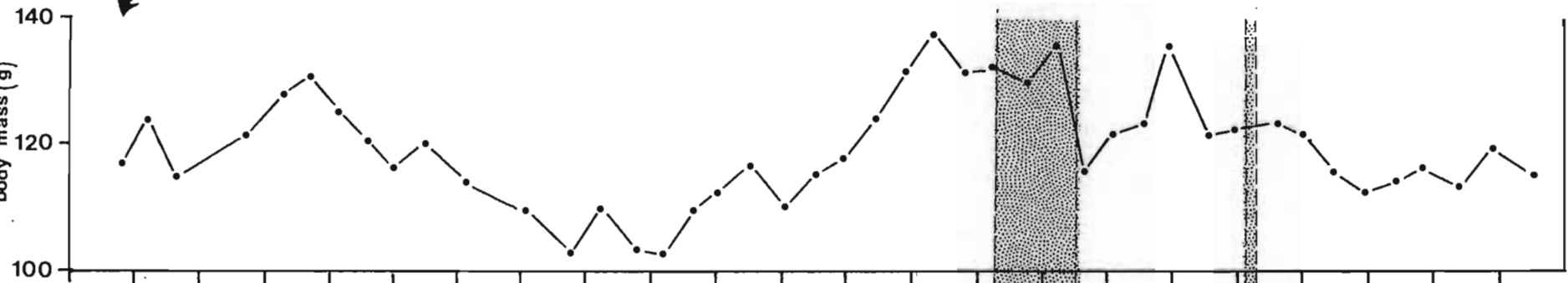
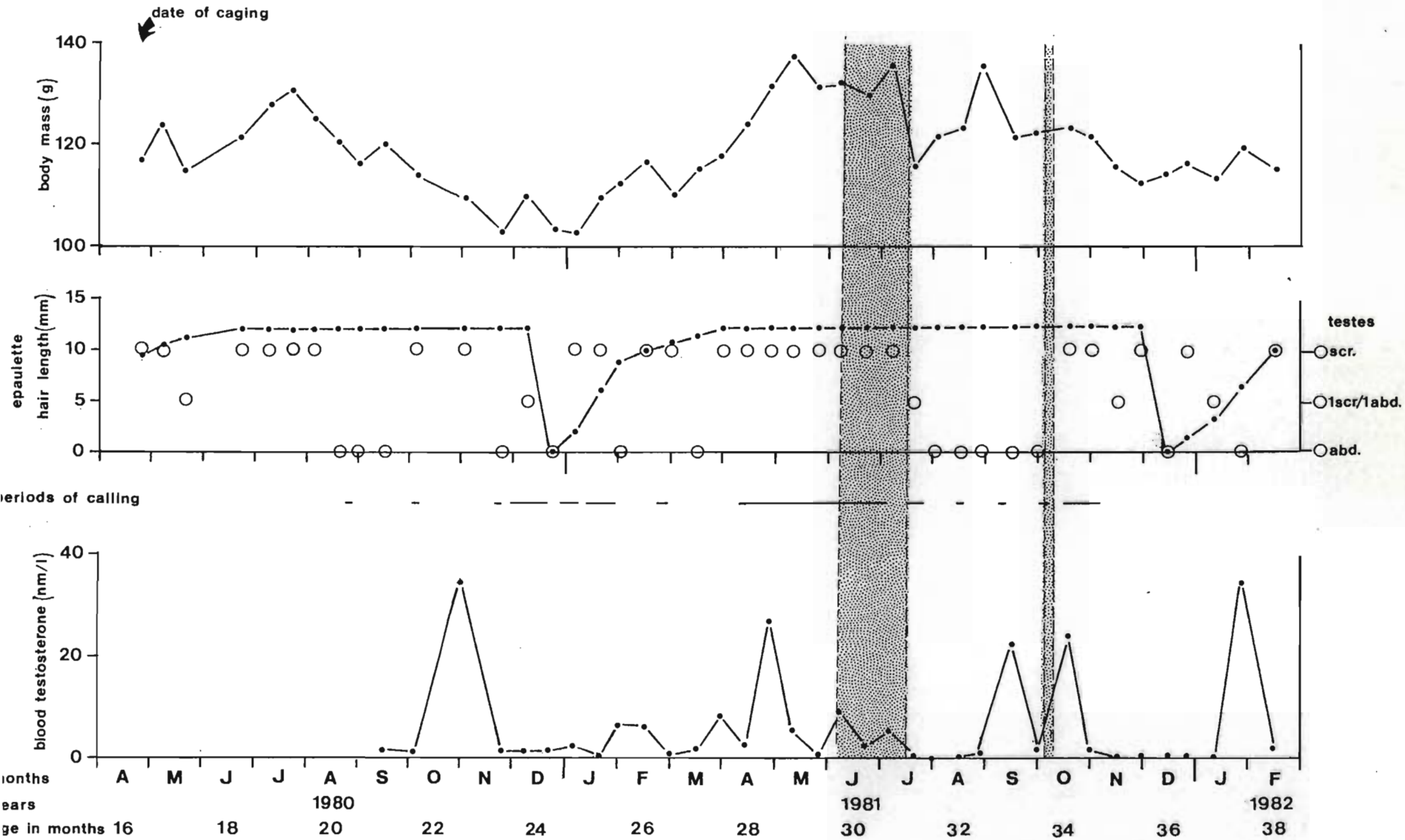
FIGURE 55 Monthly variations in five sex-related parameters measured from one caged male from time of caging to time of release

(The shaded portion represents the period during which the caged male was known to have mated

scr. - testis scrotal

1 scr./1 abd. - one testis scrotal and one testis abdominal

abd. - testes abdominal)



year, incorporating the May to July mating period ($\bar{x} = 11,91$ mm, S.E.M. = 0,06, range = 11,00 - 12,00 mm, $n = 17$), ($t = 3,32$; $0,001 < P < 0,005$).

Once the epaulette hair had attained its maximum length of 12,00 mm in the caged male, this length was maintained until the hair was shed at the end of November, beginning of December. During the caged male's mating periods (shaded portions of FIGURE 55) therefore the shoulder hairs were fully grown.

(c) Testes position

During November to February the caged male's testes were observed to be scrotal on 50 % of examinations. From April up to the end of the caged male's June/July mating period and during the brief October mating period, they were observed to be scrotal on all examinations. However during the period between the two mating phases they became abdominal.

(d) Calling

Periods of calling were closely coincident with periods during which the testes were scrotal. Calling occurred every night during the April to July phase of scrotal testes, and during the intercopulatory phase of abdominal testes, was reduced to occasional nights.

(e) Blood testosterone

As previously mentioned in 4.3.6.4, no distinct trend was evident and mating was not coincident with peaks in recorded testosterone presence in the blood.

Looking at the holistic picture over 23 months, two parameters show distinct seasonal variations, body mass and epaulette hair length. Both significantly increased during the May to July year division and significantly decreased during the remainder of the year. Scrotal testes position and calling seem to be interdependent and both reflect a decrease in activity during the caged male's intercopulatory phase in August and September. Abdominal testes were also found to be coincident with bald epaulettes. Blood testosterone levels in the caged male however showed no evidence of seasonality.

4.3.8 Seasonality in male social behaviour

During netting operations from 1977 to 1982, which were usually carried out by erecting one or two mist nets beneath a large fruiting tree, seen

to be actively fed on by bats, a male catching pattern emerged. FIGURE 56 illustrates this pattern by showing the percentage of adult males (with epaulettes) in the monthly catch, over a 50 month period.

Males formed the smaller percentages of the catch during April to July and in October each year, and the larger percentages during November to March and in August and September. The percentage of males in the catch from May to July ($\bar{x} = 9,30 \%$, S.E.M. = 2,30, range = 0 - 25,00 %, n = 12), is significantly greater than the percentage of males caught during the rest of the year ($\bar{x} = 25,28 \%$, S.E.M. = 3,20, range = 0 - 100 %, n = 38), ($t = 2,67$; $0,01 < P < 0,05$). A possible interpretation of these results in reproductive terms is given in the discussion (4.4.9).

4.4 DISCUSSION

4.4.1 Age at puberty and sexual maturity

When investigating the age of commencement of male sexuality in a seasonally breeding mammal there are three stages to be considered; (a) age at commencement of pubertal changes, (b) age at attainment of sexual maturity and (c) age at first mating. (In an aseasonal breeder (b) and (c) would occur at the same age).

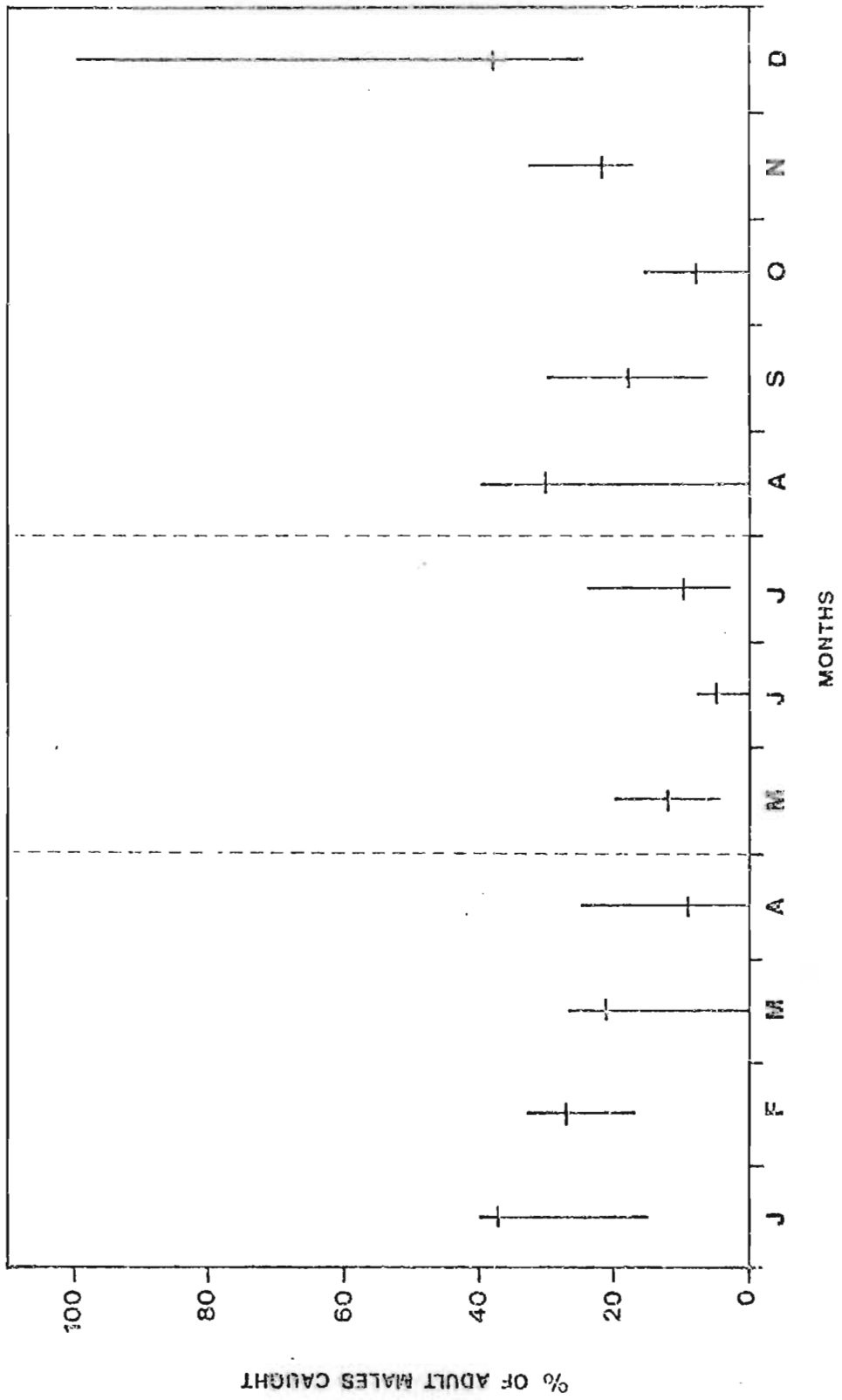
This information has been determined for E. wahlbergi, where (a) occurs at eight months (4.3.2), (b) occurs at 13 to 17 months (4.3.2) and (c) occurs at 16 to 18 months (4.3.3). Sexual maturity attainment and first mating therefore occur in the males' second year.

A review of age at sexual maturity in Microchiropteran species reveals that this is the most common pattern, sexual maturity occurring in the majority of species at 12 to 18 months; Rhinopoma kinneari - 15 months (Anand Kumar, 1965); Nyctalus noctula - 15 months (Cranbrook & Barrett, 1965); Antrozous pallidus - 16 months (Davis, 1969); Rhinolophus hipposideros - 15 months (Gaisler & Titlbach, 1964); Myotis lucifugus and M. grisescens - 15 months (Miller, 1939); Otomops martiensseni - 12 months (Mutere, 1973a); Plecotus rafinesquei - 18 months (Pearson, Koford & Pearson, 1952); Myotis austroriparius - 15 months (Rice, 1957); Myotis myotis - 15 months (Sluiter, 1961b).

Few Microchiropteran species were found that were recorded as becoming sexually mature in their first year; Eptesicus fuscus - three months (Christian, 1956); Scotophilus wroughtoni - under 12 months (Gopalakrishna, 1949, or after their second year;

FIGURE 56 Percentage of adult males in the netted catch per month over 50 months. (Total number of males caught during this period = 196)

Dashed vertical lines divides the year into the period May to July and from January to April and August to December



Rhinolophus ferrumequinum - 3,25 and 4,25 years (Dinale, 1964);
Rhinolophus euryale - 2,35 years (Dinale, 1968).

A review of the Megachiroptera reveals that all species so far studied attain sexual maturity or age at first mating, in their second year; Hypsignathus monstrosus - 12 to 18 months (Bradbury, 1977); Rousettus leschenaulti - 14 to 20 months (Gopalakrishna & Choudhari, 1977); Rousettus aegyptiacus - 12 to 19 months (Mutere, 1968); Pteropus gouldi - 18 months (Nelson, 1965b); Pteropus poliocephalus - 18 months (Nelson, 1965a); Pteropus scapulatus - 18 months (Nelson, 1965a); Pteropus geddiei - 24 months (Sanborn & Nicholson, 1950); Pteropus ornatus - 24 months (Sanborn & Nicholson, 1950).

Epomophorus wahlbergi therefore follows the general Chiropteran trend and does not differ from any of the Megachiropterans so far investigated.

4.4.2 Puberty, sexual maturity and growth

Since the pioneering work of Brody (1945) the characteristics of growth curves of different animals have been analysed in considerable detail. These early studies suggested that the point of inflection of the growth curve (decrease in growth velocity) usually follows soon after the attainment of sexual maturity and that the secretion of hormones associated with sexual maturation is responsible for the changing pattern of growth. However recent work with domesticated animals (Foxcroft, 1980; Joubert, 1963; Russell, 1969) has shown that the relationship between puberty/sexual maturity and growth is not constant and may be retarded or advanced, for example by undernutrition or intense genetic selection for high growth rates.

In E. wahlbergi the onset of pubertal changes and in particular, the start of gonadial growth, at eight months coincides with a rapid deceleration in growth velocity (4.3.4 and 4.3.5) and sexual maturity at 13 to 17 months occurs after the growth curve inflection at the attainment of the forearm length asymptote at 11 months (3.3.1.3). By comparison with domesticated breeds (Foxcroft, 1980) this suggests late maturity in relation to growth. However without comparative data from other fruit bat populations these results cannot be put into proper perspective.

Aspects of body growth have been used to assess population condition (Hanks, 1981). A value for puberty/sexual maturity attainment in relation to growth would not only provide a useful tool for comparing intraspecific population condition, but also a useful means of comparing their reproductive potential. In future work on geographically distant populations of E. wahlbergi this type of investigation would provide valuable condition and reproductive comparisons and may supply an insight

into the trigger mechanisms for the onset of puberty.

Foxcroft (1980) pointed out that there is much evidence from domesticated breeds, particularly those with a seasonal breeding pattern, to suggest that the attainment of a particular stage in growth may not necessarily be the effective stimulus for the attainment of sexual maturity. Photoperiod, temperature, or humidity stimuli may be involved. E. wahlbergi is a seasonal breeder and this study has shown a lack of critical body mass or forearm length at the attainment of sexual maturity. Growth therefore may not be the effective stimulus in this species but further detailed work would be required to determine which (if any) external environmental triggers are involved.

4.4.3 Seasonality in male breeding

Spermatozoa were present in the lumen of the epididymis cauda throughout the year and no significant differences occurred in either testicular or epididymal parameters between the May to June main mating period and the remainder of the year. E.wahlbergi therefore fits into the male reproductive pattern, 'spermatogenic activity continuous with no seasonal variations in intensity or testis mass', discussed in the introduction to this chapter and a pattern more common in aseasonal breeders.

However body mass, epaulette hair length and number of calling nights per month in both wild bats and the caged individual, blood testosterone levels in the wild bats and testes position in the caged bat, all showed evidence of seasonality. Significant differences occurred between the mean values for these parameters in May to July and in the rest of the year. Some seasonality is evidently shown by the male E. wahlbergi but it does not extend to spermatogenic activity or seasonal gonadial variations.

FIGURE 49 shows that 84 % of all conceptions occur in May to July and in consequence 87,3 % of all births occur from October to January. There is some evidence in this figure to suggest a bimodal birth pattern. 16 % of conceptions occur from August to December with a small peak of 4,3 % in October. 12,5 % of births occur from February to June with a peak of 6,2 % in April. This trend is further supported by a secondary October peak (the main peak occurring in May to July) in body mass (FIGURE 50) and in number of calling nights (FIGURE 53). Significantly fewer calling nights occurred in August and September than during the rest of the year. The caged male showed two periods of mating, June/July and October; he exhibited abdominal testes during August and September in both years of captivity and his calling changed from continuous to sporadic from the beginning of July to the middle of October (FIGURE 55).

Bimodal seasonality in births has been recorded for five Megachiropteran

species, Lissonycteris angolensis (Anciaux de Faveaux, 1978a); Hypsignathus monstrosus (Bradbury, 1977); Rousettus leschenaulti (Gopalakrishna & Choudhari, 1977); Rousettus aegyptiacus (Mutere, 1968) and Epomophorus anurus (Okia, 1974b), two of which (R. aegyptiacus and E. anurus) exhibit marked increases in testis mass during the two mating periods. This cannot be shown for E. wahlbergi and the bimodal tendency can only be interpreted as indicating a possible second increase in reproductive activity of the male population during October.

Although some seasonal variability exists in the reproductive state of the male E. wahlbergi, spermatozoa are continuously present and so fertilization is possible throughout the year. The trigger to breeding therefore must be sought not with the male, but in the interaction between the environment and the female physiology.

4.4.4 Spermatocytes and spermatids

The continual presence of spermatocytes and spermatids, including multinucleated spermatids, in the epididymis cauda lumen of male E. wahlbergi is an outstanding and unusual feature of the species.

There are few references to this phenomenon. In man these cell types are associated with infertility. Multinucleated spermatids have been recorded as an abnormality in spermatid development in the human testis of males with fertility disturbances (Holstein & Schirren, 1979). Phadke & Phadke (1961) have recorded the presence of large numbers of cells in the process of spermiogenesis in infertile human semen samples.

In three other mammalian species spermatids have been observed as normally present in the epididymides: Reid and Cleland (1957) recorded spherical spermatids in all zones of the epididymal duct of the white rat (Rattus rattus). Millar (1971) remarked on their presence in the caput epididymis of the seasonally breeding rock hyrax (Procavia capensis). Of relevance to this study, Marshall (1946) recorded the presence of spermatocytes and spermatids among the spermatozoa in the epididymides all the year round and in all specimens of Pteropus giganteus. He remarked on the variability of the numbers of these cells, but observed no seasonal variation. No explanation however was offered for the presence of these cell types in any of the three species.

The findings of this study of the epididymal content of E. wahlbergi, support those of Marshall (1946) for P. giganteus. However a further result of this present work, (and of possible relevance in the search for an explanation for the presence of these cells), are the increased percentages of the cell types (greater than 20 %) in young males prior to or at the attainment of sexual maturity (13 to 16 months old) (FIGURE 38).

It appears that spermatocytes/spermatids are released prematurely from the testes in large percentage numbers when spermatogenesis first starts and in smaller percentage numbers (less than 20 %) in adults 17 months old and over, throughout the year.

A functional explanation for the presence of spermatocytes/spermatids in the epididymis would be difficult to find and one other possible explanation for their presence, a species-related chromosomal aberration resulting in some abnormal meiotic products, could only be confirmed by extensive karyotyping studies.

4.4.5 Testosterone

Blood plasma testosterone levels from wild males showed a significant increase during the May to July period, whereas testosterone levels in the caged male did not show this, were very variable within each month and attained higher levels than in the wild males. These differences can be explained by the sampling methods.

The wild males were kept in an enclosed bag from the time of capture at \pm 22h00, to just prior to blood sampling at \pm 10h00. The time of capture and sampling was constant to within 45 minutes. The time at which blood samples were taken from the caged male however varied between 09h00 and 15h00.

Studies by Rowe, Racey, Lincoln, Ellwood, Lehane & Shenton (1975) and Illius, Haynes & Lamming (1976), where blood samples were taken hourly from man and sheep, show that blood testosterone levels varied by as much as 800 % in man and 1700 % in the sheep within a 24 hour period. In man the greatest testosterone content occurred around midday and the least around midnight.

Consistent time of sampling the wild bats may therefore explain the less varied and more meaningful results.

Illius *et al.* (1976) showed that in the ram, testosterone levels increased with ewe proximity. The caged male was in constant proximity to females, whereas the captured wild males were kept isolated for 12 hours prior to sampling. This could explain the higher testosterone levels measured in the caged male.

4.4.6 Function of epaulettes

Wickler & Seibt (1976) described the pulsing eversion of the shoulder epaulettes of *E. wahlbergi* males during calling. They review previous authors' suggestions as to their function and conclude that the epaulettes

provide either optical or olfactory signals for the attraction of females for mating.

This study has shown that in November and December, after the main mating period and after the small secondary October peak in conceptions, the long white shoulder hairs are shed and the new hairs regrow to their former length by mid January to mid February. This would be consistent with the opinion that the epaulettes have an attractive function for the female during breeding.

4.4.7 Function of calling

Wickler & Seibt (1976) investigated male calling in E. wahlbergi and found the call to belong to the 'location' category of sounds where the sender is advertizing his location. They concluded that calling had the dual function of spacing out members of the same sex and attracting members of the opposite sex.

Attraction of females would imply a reproductive function and this research has shown that the number of calling nights per month are directly related to the May to July breeding period, although calling does occur in every month of the year. It can be concluded from this that calling may have another function besides attraction of females. Year-round maintenance of a territory may be a consideration, although it was beyond the scope of this research to investigate the possibility.

4.4.8 Lek mating

Bradbury (1977) defined a 'lek' as an assembly of males visited by females primarily for mating and compiled a list of four major and six minor criteria fulfilled by lek species. E. wahlbergi meets three of the four major requirements, absence of male parental care, male territories containing no resources, and females have an opportunity to select a male for mating. The species also meet three out of the six minor requirements, strong sexual dimorphism, sexual bimaturism with later maturing males and ritualization of male display.

Because of the partial fulfilment of these criteria, the possibility of lek mating in E. wahlbergi is considered. The lek species examined by Bradbury (1977) was the hammer-headed fruit bat, Hypsignathus monstrosus. E. wahlbergi differs from this species in that no large aggregations of calling males at regular sites were observed and calling, though seasonally correlated with mating did occur in all months of the year.

Single E. wahlbergi males however were noticed returning to the same tree perch to call for several months in succession and adjacent males could be

faintly heard calling nearby. These may be part of a widely dispersed lek or merely loose aggregations of calling males which are defending territories around crucial resources needed by females. Considering the existence of calling throughout the year, territory defence seems more likely.

However the truth may lie in a concluding remark by Bradbury (1977): 'I suspect that subsequent work on other epomophorine species will reveal a gradient of strategies ranging from complete resource defence to true leks as in Hypsignathus.'

4.4.9 Seasonal social behaviour

Herd forming in mature adult males outside the breeding season and dispersal of these herds for individual dominant males to establish territories, is well documented, particularly for gregarious species of Bovidae and Cervidae (Anderson, 1972; Lincoln, Guinness & Short, 1972; Lynch, 1974).

While mist-netting for fruit bats under food trees, it was noticeable that during the summer months, particularly in December and January, large numbers of males (greater than eight) would be caught together in less than an hour. Whereas during the months of mating, males were infrequently caught. The seasonality of the male content of the catch is illustrated in FIGURE 56 and it is shown (4.3.8) that a significantly smaller percentage of males were caught during May to July than during the remainder of the year. A second 'dip' in catch percentage also occurred in October, once again suggesting the possibility of a bimodal mating pattern.

When compared, it is seen that FIGURE 56 inversely parallels FIGURE 53. Low percentage of males in the catch per month coincide with the greater number of calling nights per month, and vice versa.

It would appear that outside the mating periods, when numbers of calling nights per month are low, adult males fly and feed in groups, a situation paralleling the out of rut seasonal herding of impala rams (Anderson, 1972). During the mating periods these groups disperse, individuals become solitary, to call, establish territories and so feed singly instead of in groups.

If this is a correct interpretation of the facts, seasonality associated with mating, which cannot be demonstrated in gonadal terms, may be seen in the changes of behavioural pattern associated with the possible dispersal of male groups into individual callers and territory holders during certain parts of the year.

4.5 SUMMARY

Reproduction in male E. wahlbergi is examined in detail. The gross anatomy and histology of the reproductive system is described. Age at onset of puberty is given as eight months and sexual maturity attainment at 13 to 17 months. There was no critical body mass or forearm length at which sexual maturity occurred, although onset of puberty coincided with a deceleration in body growth velocity.

Seasonality in the male was investigated in relation to mating, by examination of testes, epididymides, body mass, blood testosterone, epaulette hair length, male calling and by a holistic approach, using a caged male. Spermatozoa were present throughout the year and no significant variations in the testicular and epididymal parameters occurred between May to July (the main mating period) and the remainder of the year. Male seasonality in gonadal terms was absent. However, body mass, blood testosterone, epaulette hair length, male calling and in the case of the caged male, testes position, showed seasonal variations, as did the adult male content of the monthly netted catch.

Age at puberty and maturity is discussed in relation to other Chiropteran species, E. wahlbergi following the common Chiropteran pattern of male second year maturity. It is suggested that a value for growth stage at onset of puberty would provide a useful tool for comparing intraspecific population conditions.

The extent of male seasonality and the possibility of a bimodal pattern is discussed. The unusual presence of spermatocytes/spermatids are examined and an explanation regarding meiotic abnormality is offered. Sampling methods are used to explain discrepancies between wild and caged bat testosterone results. The functions of epaulettes and calling are examined and the possibility of lek mating in the species is discussed. Finally, the significantly smaller percentages of adult males in the netted catch during the mating season are interpreted as a seasonal change in behaviour patterns associated with dispersal of male groups into individual callers and territory holders.

CHAPTER FIVE

REPRODUCTION IN THE FEMALE EPOMOPHORUS WAHLBERGI

5.1 INTRODUCTION

The first aim of this chapter is to describe the gross and histological anatomy of the female reproductive tract as a basis for investigating female reproductive patterns in E. wahlbergi.

The second aim is to determine the age at attainment of sexual maturity, which Racey (1974a) defines in the female as being the age at first oestrus. In two Megachiropteran species Hypsignathus monstrosus (Bradbury, 1977) and Rousettus leschenaulti (Gopalakrishna & Choudhari, 1977) this is attained in the first year, at five to seven months of age. In other documented Megachiroptera (Pteropus geddiei, Baker & Baker, 1936; Eonycteris spelaea, Krutzsch & Beck, 1975; Rousettus aegyptiacus, Mutere, 1968; Pteropus gouldi, Nelson 1965b; Pteropus poliocephalus and Pteropus scapulatus, Nelson 1965a), this event occurs in the second year, often at 18 months. Many female Microchiroptera follow a similar second year maturity pattern with the genus Myotis, Nyctalus noctula and Plecotus rafinesquei attaining sexual maturity in their first year at three to six months. Thus in this chapter, an attempt will be made to determine whether E. wahlbergi females fit into the first or second year maturity pattern by investigating the age at first oestrus and at first conception.

The third aim of this chapter is to investigate seasonality and determine the reproductive cycle in female E. wahlbergi resident on the Natal South Coast by examining seasonal variations in numbers and types of ovarian follicles, endometrial and myometrial thickness, numbers of endometrial glands, height of uterine epithelium and percentage frequencies of gravids, lactants, neonates and juveniles in the population. Universally accepted terms exist to describe female reproductive cycle patterns, but examinations of their application in the literature reveals some interpretive differences. In this study the terms used are modified from those applied by Fleming, Hooper & Wilson (1972) and by Jerrett (1979). Their definitions and interpretations are set out below:

(a) Aseasonally polyoestrous

Oestrous cycles occur more than once per year, but are not restricted to any season and occur throughout the year, resulting in births occurring in

all months of the year.

(b) Seasonally polyoestrous

Oestrous cycles occur more than once per year, but are restricted to either one often extended season, or two, or three short seasons (bimodal or trimodal). As a result births occur during one extended season or in two or three short seasons.

(c) Seasonally monoestrous

An oestrous cycle occurs once a year and is confined to a fixed part of the year, resulting in births occurring during one short season.

Megachiroptera inhabit tropical and subtropical regions where climatic seasonal differences are minimal. Despite this, an overview of their reproductive cycle patterns reveals that the majority of species fall into the seasonally polyoestrous and seasonally monoestrous categories. Of the seventeen species looked at in detail, one only (Eonycteris spelaea, Krutzsch & Beck, 1975) is documented as an aseasonal breeder. The remainder of the documented African Megachiroptera (with the exception of Eidolon helvum) and two Asian species have been shown to be seasonally polyoestrous. Six have bimodal patterns (Lissonycteris angolensis, Anciaux de Faveaux, 1978a; Hypsignathus monstrosus, Bradbury, 1977; Rousettus leschenaulti, Gopalakrishna & Choudhari, 1977; Rousettus aegyptiacus, Mutere, 1968 (interpreted by Anciaux de Faveaux, 1978a, as aseasonal); Epomophorus anurus, Okia 1974b (recorded by Herlant, (1953) as aseasonal); Epomops franqueti, Okia 1974a), one has a trimodal pattern (Cynopterus brachyotis, Liat 1970) and two have extended season patterns (Epomophorus wahlbergi, O'Shea & Vaughan, 1980; Epomophorus crypturus, Smithers, 1971). Six species of Pteropus (P. geddiei and P. eotinus, Baker & Baker, 1936; P. giganteus, Marshall, 1946; P. gouldi, P. poliocephalus and P. scapulatus, Nelson, 1965b) and Eidolon helvum (Mutere, 1967) are seasonally monoestrous.

A study incorporating the investigation of seasonality in a species would be incomplete without attempting to examine the triggers or causes of the seasonality. The fourth aim of this chapter therefore is to determine the proximate and ultimate causes of breeding, by examining climatic and nutritional factors that could influence the reproductive cycle. In particular photoperiod is experimentally investigated since the peak in E. wahlbergi conceptions (FIGURE 49) occurs around the shortest daylength in June.

Most mammalian species give birth during discrete periods of the year, separated by intervening periods when no births occur (Sadleir, 1968).

This situation allows the young to grow up in optimal climatic and nutritional conditions, and lactating females to have access to the best food at a time when particular energetic stress is placed upon them. Favourable climatic conditions and abundance of food are considered to be the ultimate causes of breeding (Baker, 1938). The timing of such seasonal births is directly affected by the timing of conception and the length of gestation. Sequentially the timing of conception is dependent on sexual receptivity (oestrus) which in turn is brought about by ovulation. The timing of ovulation and therefore of conception are initiated or modified by external factors such as daylength, temperature, nutrition and social factors (Sadleir, 1972). Those proximate causes of breeding, in collaboration with gestation length, which may be modified by such strategies as sperm storage, delayed implantation and delayed embryonic development, result in births occurring in the most propitious season of the year for mother and infant survival.

Lack of distinct climatic variations presents problems when investigating the proximate and ultimate causes of seasonal breeding in the tropical and subtropical Megachiroptera. Baker & Baker (1936) and Marshall (1946) suggested that decreasing photoperiod may be a controlling factor in the onset of copulation in Pteropus and Baker & Baker (1936) further suggested that varying amounts of ultra violet light may have some importance. Groome (1940) observed that copulation in Pteropus coincided with an increased variety of fruits and suggested that this may act as a copulatory trigger.

Mutere (1967, 1968) correlated birth peaks in Eidolon helvum and Rousettus aegyptiacus with rainfall peaks and as rainfall directly affected fruiting, concluded that rainfall could be an ultimate cause of breeding in these two species in East Africa. Okia (1974b) reached a similar conclusion when examining the possible ultimate causes of the bimodal birth peak in Epomophorus anurus.

Mammalian species exhibit a wide variety of strategies associated with ovaries and uteri during the breeding cycle from ovulation to parturition. A fifth aim of this chapter is to examine selected aspects of the breeding cycle, namely; ovulation, progesterational endometrial reaction, implantation symmetry or asymmetry, the role and length of persistence of the corpus luteum, condition of the non-pregnant uterus and its ovary during pregnancy and gestational length with its possible associated strategies. These aspects have been chosen for consideration in most cases, because of their notable occurrence in Chiropteran species.

Wimsatt (1979) extensively reviewed reproductive asymmetry and unilateral pregnancy in Chiroptera, commenting that this was more frequent in bats than in any other mammalian Order. He listed the 'pteropid pattern' as

involving a non-random alternation of ovulations between right and left ovaries in successive years and the impossibility of ovular transuterine migration because of a septate uterus. He also commented on an unusual characteristic of this pattern; the development of a localized progesterational endometrial reaction at the distal end of the cornu on the ovulating side. This unilateral reaction has been documented for Pteropus giganteus (Marshall, 1949; 1953), Rousettus leschenaulti (Gopalakrishna & Karim, 1971) and Cynopterus sphinx (Ramakrishna, 1950).

The unusual persistence of the corpus luteum through one pregnancy until mid-gestation of the following pregnancy is reported as a unique feature of Rousettus leschenaulti and as a mechanism bringing about the regular alternation of ovulation between the two ovaries in successive cycles (Gopalakrishna & Choudhari, 1977). By comparison, Mossman & Duke (1973) recorded the luteal gland presence in Pteropus vampyrus only until mid-gestation of the pregnancy which initiated its formation.

The order Chiroptera displays three types of delaying tactics designed to modify gestational length and so enable births to take place at the most advantageous time of the year for mother and infant. Hibernating bats, notably of the Vespertilionid and Rhinolophid families, exhibit two types of gestational postponement. Oestrus and copulation are initiated in autumn followed by delayed ovulation and sperm storage through winter, until the spring when ovulation, fertilization and gestation occur. The second pattern involves oestrus, copulation, ovulation and fertilization in autumn, followed by delayed implantation during winter and implantation and gestation in the spring (Oxberry, 1979).

Unexpectedly a Megachropteran species, Eidolon helvum, has been shown to have delayed implantation, resulting in a gestation period from fertilization to birth of ten months (Mutere, 1967). Births are then timed to take place at the onset of heavy rains.

A third pattern, that of delayed embryonic development has been recorded for several Microchiropteran species, Macrotus californicus (Burns & Easley, 1977), Macrotus waterhousii (Burns & Wallace, 1975), Artibeus jamaicensis (Fleming, 1971), Nyctalus stramineus (Wimsatt, 1975) and Nycteris thebaica (Bernard, pers. comm.).

Fecundity of a mammal is measured as the number of live births produced over an interval of age (Caughley, 1977). The final aim of this chapter is to compile a fecundity table which will provide an indication of the reproductive potential of the different age groups within the population and the effects of reproductive senescence.

5.2 MATERIALS AND METHODS

Two samples of the wild population were used in the investigations, a captured and released sample, and a killed sample. The captured and released sample comprised of 553 females netted in the study area between June 1977 and February 1982. Of these, 74 were recaptured once, seven twice and two three times. One hundred and eleven netted females were destructively sampled between April 1978 and March 1982.

In all females, measurements of mass, forearm length, eye-nose distance, zygomatic width, P_3 and upper C tooth heights and observations of dental eruption state were made. The width of the gravid uterine horns in early pregnancy and the fetal cranial breadth in later pregnancy were palpated through the abdominal wall and measured by means of a calliper vernier. Age was estimated by a combination of the use of TABLE 6 and the birth pulse, taking the median of the age range to give a single value.

For convenience of data analysis, each female was also assigned to one of the following eight categories based on reproductive status (see TABLE 14). The first four categories were introduced in APPENDIX 1.

(a) Neonate. Young bat with eyes still closed. (b) Juvenile. Young bat, after eyes have opened and until a full permanent dentition is reached at three months of age. (c) Nulliparous subadult. Young female, after attaining a full permanent dentition and until first palpably pregnant (usually between three and six months old). (d) Primigravid. Young female, pregnant for the first time, distinguishable from older pregnant females by lack of nipple distension (usually between six and twelve months of age). (e) Gravid. Pregnant females in their second or later pregnancies, recognizable by pendulous nipples (usually eighteen months and older). (f) Lactant. Non-pregnant females with enlarged mammary tissues and expressible milk (usually twelve months and older). (g) Gravid and lactant. (h) Postlactant. Non-pregnant and non-lactant females with distended nipples indicating at least one previous pregnancy and lactation.

5.2.1 Killed sample

The vagina, uterine cornua and ovaries with oviducts from each specimen were dissected out and laid onto dental wax. The maximum width and length of both uterine cornua with and without the ovaries were measured to the nearest 0.1 mm using a calliper vernier and the side of implantation (if any) was recorded. In addition the fetuses of gravid females were removed, sexed, and mass, forearm length, cranial breadth and crown-rump measurements were taken. Mammary tissue from the right breast of

TABLE 14 Number of females in each category based on reproductive status, in the captured and released sample, and the killed sample

| Reproductive status | Captured & Released sample | Killed sample | | |
|---------------------|----------------------------|-------------------------|--------------------|--------------------|
| | | Routine monthly samples | Seasonality trials | Ovul. trig. expts. |
| Neonate | 0 | 1 | | |
| Juvenile | 27 | 4 | | |
| Nulliparous | 129 | 12 | 6 | 4 |
| Primigravid | 62 | 17 | 1 | |
| Gravid | 120 | 25 | 3 | |
| Lactant | 70 | 11 | | |
| Gravid & Lactant | 5 | 3 | | |
| Postlactant | 140 | 10 | 6 | 8 |
| TOTAL | 553 | 83 | 16 | 12 |

lactating females was removed and weighed to the nearest 0,01 g using an electronic balance.

Histological material and measurements

The dissected uterine/ovarian systems were further dissevered for histological preparation. The ovaries, complete with ovarian bursa and oviducts were cut from the uterine horns and the uterine horns were excised from the vagina at their point of divergence. The four pieces of reproductive tissue obtained from each female were fixed in 10 % formal saline for a minimum of 48 hours and routinely embedded in paraffin wax (APPENDIX IV). Ovaries were roughly sectioned at 10 μm until the maximum width was almost reached. Ten sections at 5 μm were then mounted, ten discarded, ten mounted, and this procedure was continued until 30 mounted sections had been obtained per ovary. The uterine cornua were roughly sectioned in the transverse plane until the middle of the cornual length had been reached. Ten sections at 5 μm were mounted, ten discarded and ten mounted, giving 20 mounted sections for each uterine horn. (In three individuals complete serial sectioning of ovaries and uterine horns was carried out). Ovarian and uterine sections were stained with haematoxylin and eosin (APPENDIX V) and microscopically examined, using an eye-piece graticule for all measurements.

Ovaries

Five categories of follicle were recognized (after Mossman & Duke, 1973) and identified for counting and measuring, by the following features:

- (a) Primordial: oocyte with single cell thick layer of squamous epithelium.
- (b) Primary: oocyte with single cell thick layer of simple columnar epithelium.
- (c) Secondary: oocyte surrounded by a clear zona pellucida and two or more layers of stratified cuboidal epithelium (granulosa), with no evidence of vesicular fluid.
- (d) Vesicular: oocyte surrounded by granulosa containing fluid filled intercellular spaces. For the purposes of this work three types of vesicular follicle were recognized:
 - (i) Early: granulosa containing one or more small isolated fluid pockets (no single follicular antrum), usually < 300 μm in diameter.
 - (ii) Intermediate: granulosa containing a single follicular antrum

completely surrounding the oocyte and its cumulus oophorus, but without massive antral enlargement, <500 μm in diameter.

(iii) Late: enlarged antrum with eccentrically positioned oocyte and cumulus, >500 μm in diameter.

(e) Atretic: For the purposes of this study, atresia was only counted in secondary and vesicular follicles. Also, it was only recognized as occurring from the midpoint in the process when the cumulus oophorus disintegrates. Follicles showing evidence of this and the successive stages up to the shrivelled remnants of the zona pellucida in the stroma, were counted as atretic.

The ovarian section providing the greatest area was used to count the total number of all five follicle types and to measure the mean maximum diameters of the secondary and vesicular follicles including the theca externa for each ovary.

The area of the corpus luteum of pregnancy when present, was calculated by multiplying together two measurements taken at right angles to each other, one of which was always the greatest width.

Uterine cornua

Five histological uterine measurements were taken, using the section providing the greatest uterine area:

- (a) The minimum and maximum diameters of the uterine cornual lumen.
- (b) The minimum and maximum endometrial thickness including the endometrial epithelial layer.
- (c) The total number of endometrial glands in one section.
- (d) The minimum and maximum myometrial circular muscle thickness.
- (e) The mean height of the epithelial layer taken from 30 measurements. Uterine measurements were not taken from gravid uterine horns.

In the reproductive analysis, follicle numbers per animal are presented as the combined number from both ovaries, whereas all ovarian and uterine measurements are given as the mean value from left and right sides (except in pregnant females where a uterine measurement from the non-gravid uterine horn alone was used).

5.2.1.1 Routine monthly samples

Eighty three females were killed for routine monthly samples to provide information on the anatomy and histology of the reproductive system, age at first oestrus and at first conception, seasonality in ovarian and uterine tissues and the breeding cycle from ovulation to parturition (TABLE 14).

5.2.1.2 Seasonality trials

FIGURE 49 shows that 84,0 % of all conceptions occur in May, June and July. A decrease to 3,3 % occurs in August, 2,3 % in September and a slight increase to 4,2 % in October. No conceptions occurred in January to April. This apparent reproductive seasonality was investigated in more detail by examining four groups of bats (16 in all) (TABLE 14) in cage acclimatized conditions with the following objectives:

Group 1. To determine if there is a significant increase in vesicular follicle development during June.

Six females were used, two postlactant, three nulliparous and one gravid (in order to determine the condition of the ovary of the non-gravid side). All six females were kept in an outdoor cage isolated from male contact (but not sound or sight) for two weeks prior to the start of the period of trial. They were then killed at weekly intervals commencing on 26 May 1981 and finishing on 3 July 1981.

Group 2. To determine if there is a significant reduction in number and size of vesicular follicles during August.

Four females were used, two postlactant, one nulliparous and one primigravid (in order to investigate any similar follicular changes in the ovary of the non-gravid side). All four bats were kept in an outdoor cage, isolated from male contact (but not sound or sight) for a minimum of two months. Three were killed at weekly intervals from 17 August 1981. The fourth (a postlactant) was transferred to the cage with the sexually active male on 9 August 1981 and killed three weeks later. She was postlactant at death.

Group 3. To determine if there is a significant increase in number and size of vesicular follicles during October compared to August and September.

Three females were used, two postlactant and one gravid. All three bats were kept in an outdoor cage isolated from male

contact (but not sound or sight) for a minimum of five months. Two were killed on 19 October 1981. The third (a postlactant) was transferred to the cage containing the male on 9 October 1981 and killed on 2 November 1981. She was gravid at death.

Group 4. To determine if there is a depression in vesicular development during January and February and if conceptions during this time can occur.

Two females, both nulliparous were kept isolated from male contact (but not sight or sound) from July 1980 until 19 February 1981 when they were killed. One other female, a post lactant was captured in June 1981 and kept isolated from male contact until 15 January 1982. She was then transferred to the male cage and killed a month later, on 15 February. She was postlactant at death.

5.2.1.3 Ovulatory trigger experiments

Two experiments were conducted using 12 bats (TABLE 14) to try to determine the proximate causes of breeding and the cues for oestrus onset/ovulation.

Experiment 1 : To determine if the presence of a male is necessary to initiate oestrus onset/ovulation.

A total of seven females, all captured at the end of April 1980, were used for this experiment. Four females, (two postlactant and two nulliparous) were caged together in an indoor sound proofed cage, without a male and with the normal light regime and standard cage food, from 1 May 1980 until they were killed on 23 June 1980. Three females, (two postlactant and one nulliparous) acted as the control. They were held in an outdoor cage with a male from 1 May 1980 until they were killed on 23 June 1980.

Experiment 2 : To determine if short photoperiod initiates oestrus onset/ovulation.

Five females were used, all of which had been captivity acclimatized for a minimum of five months. They were placed in indoor controlled environment rooms (using 'Gro-lux' fluorescent tubes as the light source) on 15 January 1982, where the temperature was at ambient (24° C - 27° C). Calling males could be heard and standard cage food was supplied.

Four females, three postlactant and one nulliparous were subjected to rapidly shortening daylength over 14 days, changing from the January current daylength (05h00 - 19h00) to the mid June daylength (06h45 - 17h00). They were then maintained at this daylength until sampled on 4 March 1982.

A single postlactant female acted as control (a further three control females escaped), being exposed to the current January to March daylengths until sampled on 4 March 1982.

5.2.2 Date of conception determinations

Dates of conception were calculated from two methods of determining fetal age:

- (a) Fetal age determination from live in utero fetuses. A method was devised by which the date of conception could be determined by palpation of the uterus and fetus in live gravid females. This technique greatly extended the usefulness of wild live population data, by increasing the amount of seasonality information available for analysis.

In the derivation of the technique, it was first necessary to determine the length of gestation. This was calculated from 28 cage pregnancies terminating in births, six of which were cage conceived and the date of introduction of the females to the male was known. The length of gestation determined for E. wahlbergi was 160 days ($x = 159,62$ days, range = 146 - 170 days). The mean value was rounded up to 160, for ease of calculations.

Throughout pregnancy the gravid females were caught and palpated at fortnightly intervals. In the early stages of pregnancy the width of the swollen uterine horn was recorded (until it reached a width of 15 - 20 mm). When the fetus head could clearly be felt, the fetal cranial breadth was recorded (usually at a minimum of 9 mm). This was carried out throughout the 28 pregnancies providing 61 uterine width and 71 fetal cranial breadth measurements. The date of birth at the end of each pregnancy was recorded and using 160 days as the length of gestation, the date of conception was calculated. The uterine width and fetal cranial breadth measurements were then plotted against fetus age from conception (FIGURES 57 & 58), and linear regression was used to fit a straight line through the data points.

These FIGURES were then used to determine the fetal ages of palpated

FIGURE 57 The relationship between uterine horn width (mm) and fetal age in days

$$y = -0,22 + 0,159 x; \quad r = 0,913; \quad P < 0,001$$

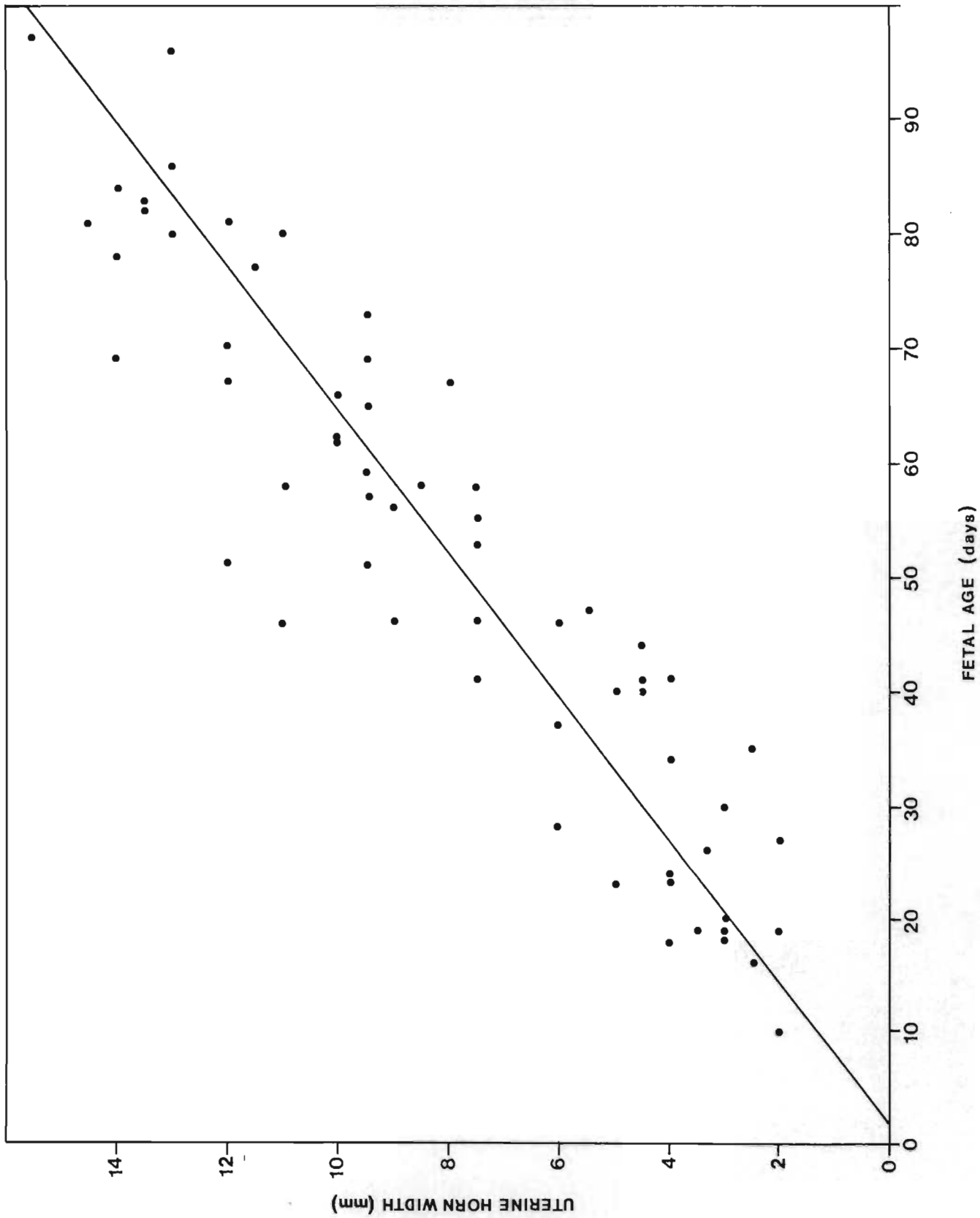
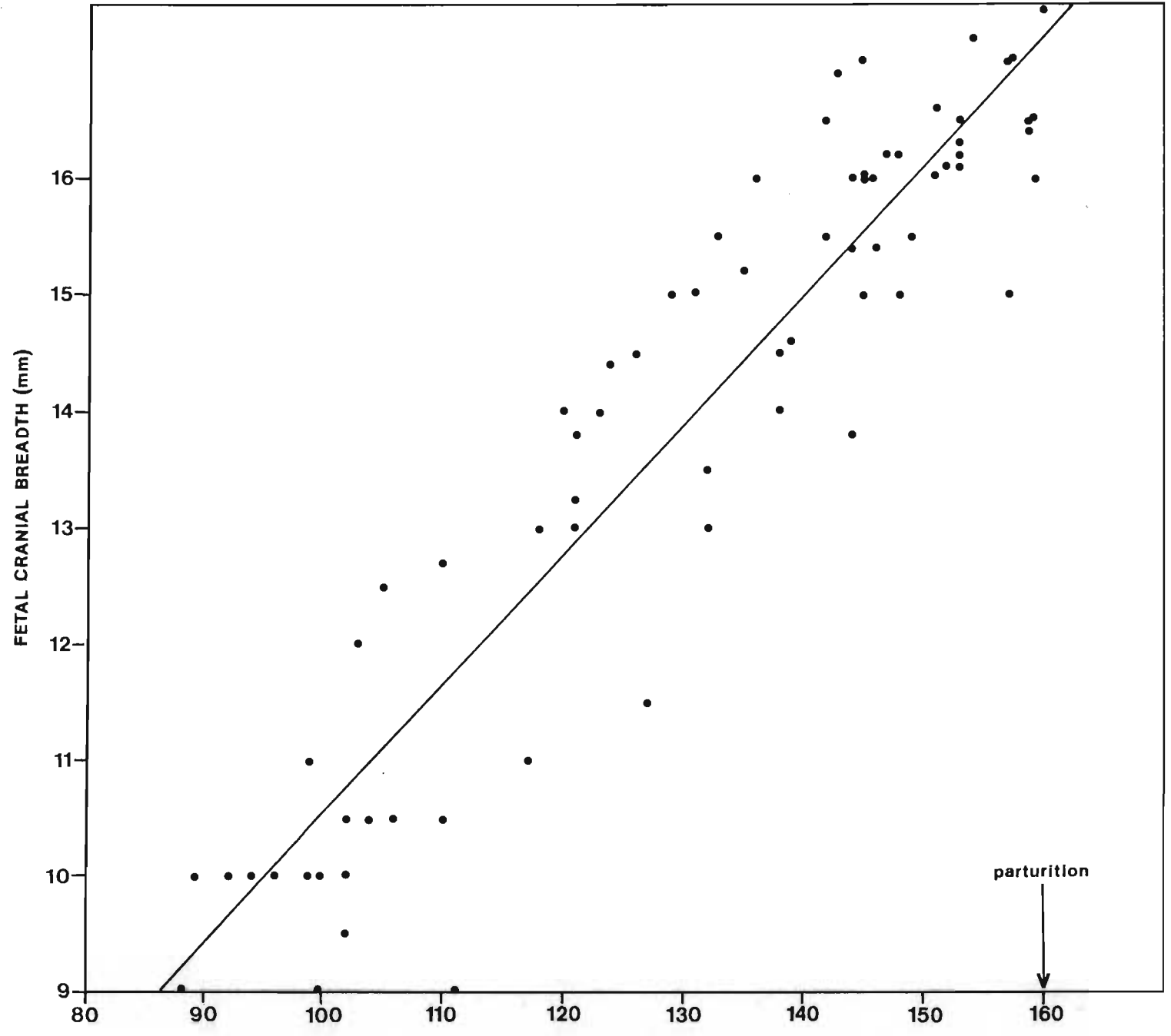


FIGURE 58 The relationship between fetal cranial breadth (mm) and fetal age (days)

$$y = -0,62 + 0,112 x; \quad r = 0,929; \quad P < 0,001$$



fetuses in live gravid bats and so calculate their dates of conception.

The monthly percentages of conceptions and births in FIGURE 49 were calculated by using FIGURES 57 and 58 to give the dates of conception and birth in 214 pregnant females and juveniles.

- (b) Fetal age determination from dead ex utero fetuses. FIGURES 57 and 58 could not be used to calculate fetal age and date of conception in dead fetuses (collected by removal from destructively sampled females), because the FIGURES take into account the thickness of uterine walls, fetal membranes and maternal abdominal thickness, which are all absent from a dead 'cleaned' fetus.

Twenty three fetuses were collected by destructive sampling and their fetal ages were calculated by the Huggett & Widdas (1951) method. The birth mass was calculated from 28 cage births at 15,96 g (S.E.M. = 0,28); the gestation period taken as 160 days, t_0 as 32 and the specific fetal growth velocity 'a' was calculated as 0,0197. The Huggett & Widdas fetal growth curve for E. wahlbergi is shown in FIGURE 59.

5.3 RESULTS

5.3.1 Anatomy and histology of the reproductive system

5.3.1.1 Gross anatomy of the reproductive tract (FIGURE 60)

The left and right ovaries of E. wahlbergi did not differ significantly in size ($P > 0,1$), the mean length in the infant being 1,30 mm, in the juvenile 1,43 mm and in the nulliparous, postlactant and gravid females 2,43 mm. The ovaries are smoothly globose, encased in a thin tunica albuginea and completely enclosed with the oviduct in an ovarian bursa (PLATE 18). The oviduct emerges laterally from the cephalic end of the uterus and curves medially in an unlooped manner around the ovary, terminating in the infundibulum which closely adheres to the medio caudal surface of the ovary. Capillaries forming the anastomosis tubo-ovarica are visible between the ovary and oviduct. The arteria ovarica and the ovarian suspensory ligament emerge from the cephalic pole of the ovary.

The uterus is bicornuate and in nulliparous and postlactant animals there is no difference in length between the left and

FIGURE 59 Huggett & Widdas (1951) fetal growth curves based on gestation length (t) of 160 days, a birth mass of 15,96 g and $t_0 = 0,2 t$

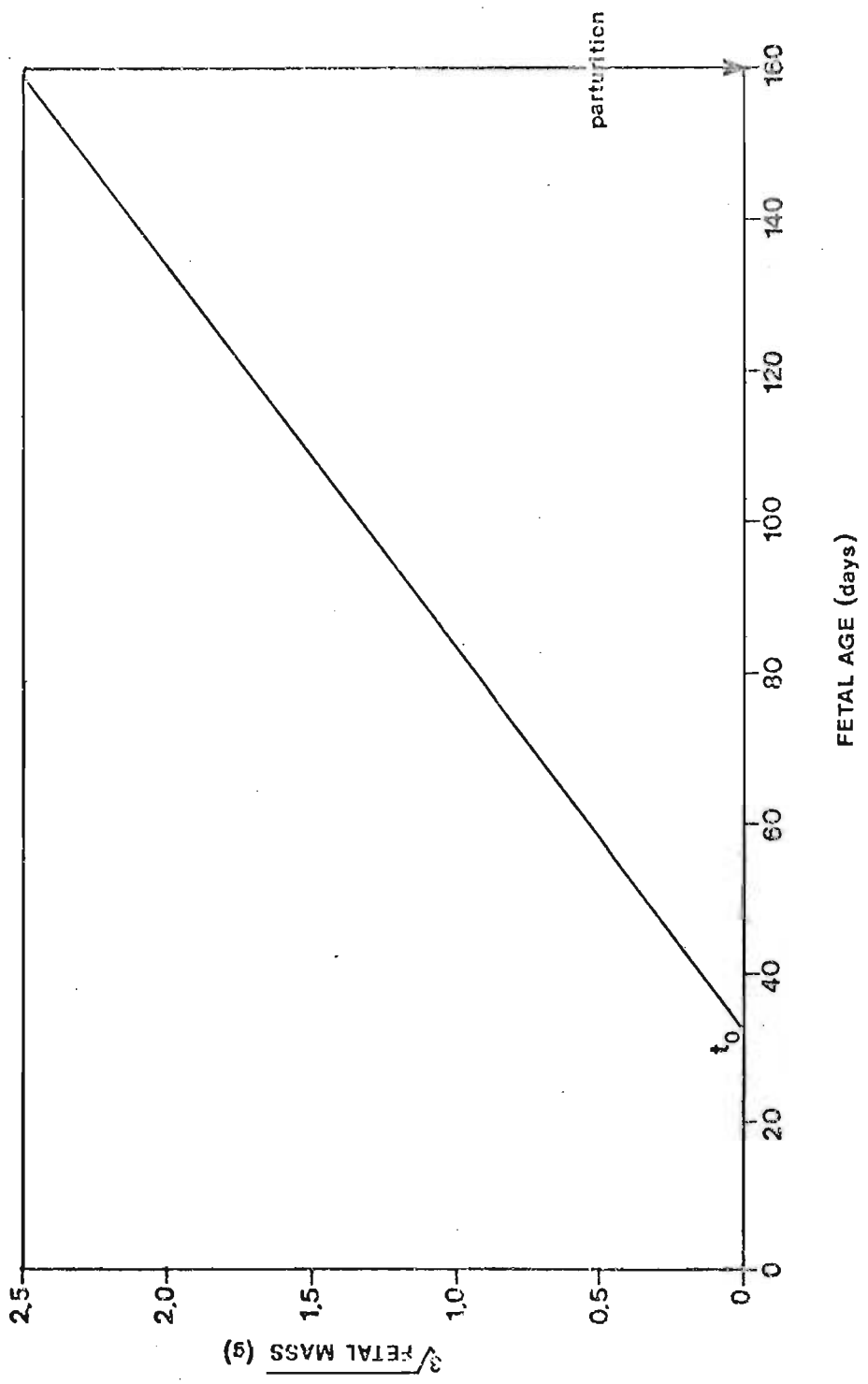
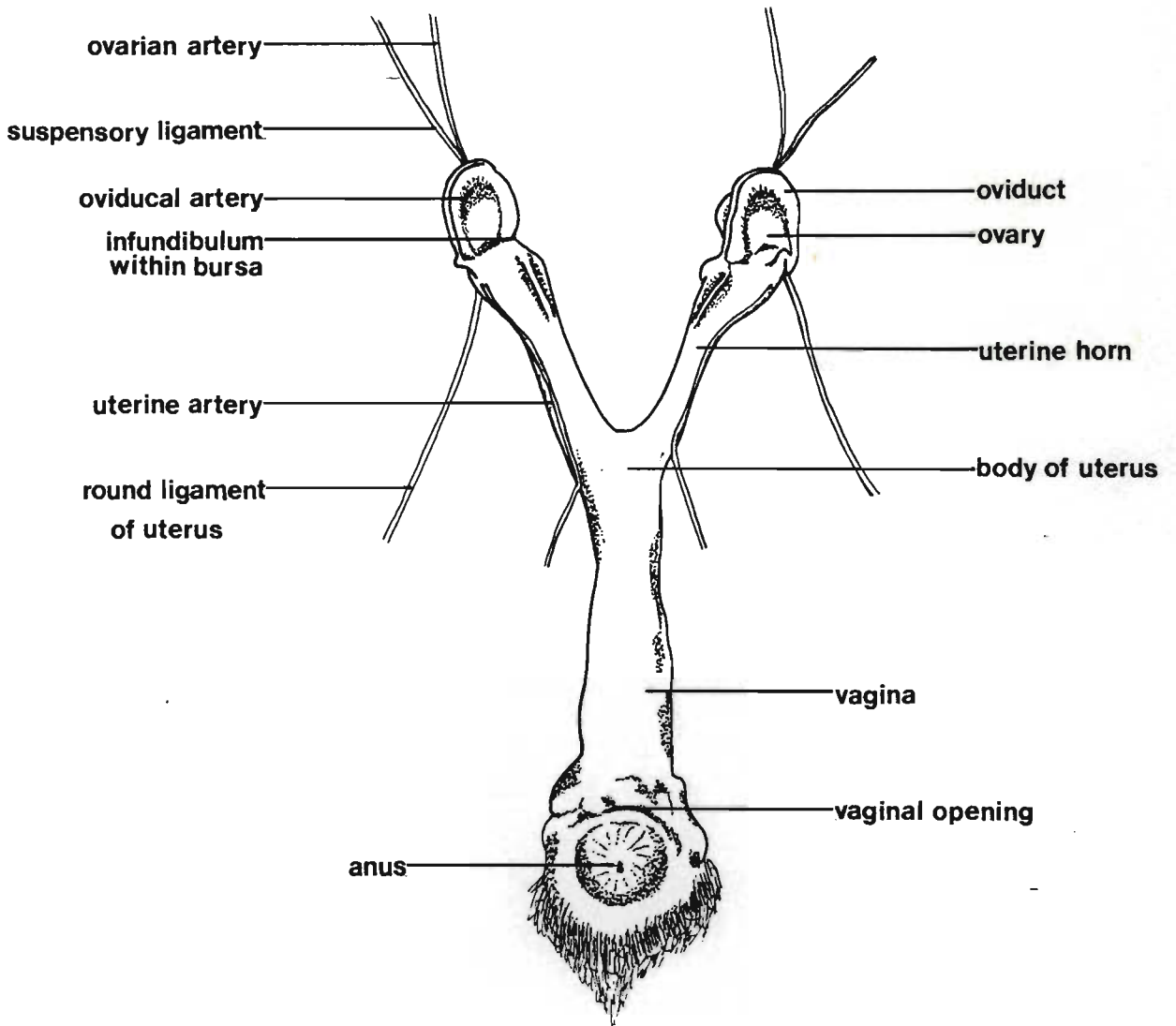


FIGURE 60 Gross anatomy of the female reproductive tract (ventral view)

1mm



right uterine horns ($P > 0,1$). Mean lengths and widths of uterine horns for infants, juveniles, nulliparous and post lactant females are shown in TABLE 15. At the junction of the uterine cornua, within the lumen, a trilobate septum is situated. The arteria uterina is closely applied to the outer lateral surface of the uterine horns up to their cephalic ends where it passes dorsally and merges into the arteria ovarica. The vagina is a thin walled tube with a mean length of 11,4 mm. A slightly pigmented clitoris is present at the vulval exit.

5.3.1.2 Histology of the ovary

Microscopic examination of the ovary confirmed the presence of a complete bursa ovarica, comprising the recurved oviduct and mesosalpinx, and the close proximity of the ovary to the uterine tissue at its caudal pole (PLATE 18). The infundibular opening of the oviduct is capacious and completely enclosed within the bursa, opposite the indented ovarian surface which forms the ovulation pit (PLATE 19).

The main gonadal structures examined were present in the ovarian cortex. PLATE 20 shows the characteristic localized mass of primordial follicles, scattered primary, secondary, early vesicular follicles and a secondary follicle undergoing atresia at the stage of zona pellucida dissolution. PLATE 21 shows a late vesicular follicle occupying a large proportion of the ovarian volume. The mean diameters of the five categories of follicle recognized are given in TABLE 16.

After follicular rupture, a single mass of glandular tissue, the primary corpus luteum forms by enlargement and multiplication of the follicular epithelium (granulosa cells). PLATE 22 shows a developed primary corpus luteum of a 20 day pregnancy, comprising a solid spheroidal mass of large polyhedral gland cells, supplied by sinusoidal blood.

Polyovular follicles have been reported for several mammalian species (Mossman & Duke, 1973) and Van der Merwe (1979) noted their presence in the Microchiropteran Miniopterus schreibersi. A single example of this abnormality was observed in a three month old nulliparous female (PLATE 23).

5.3.1.3 Histology of the uterus

The microscopic structure of the uterus follows the usual mammalian pattern with a glandular endometrium and muscular

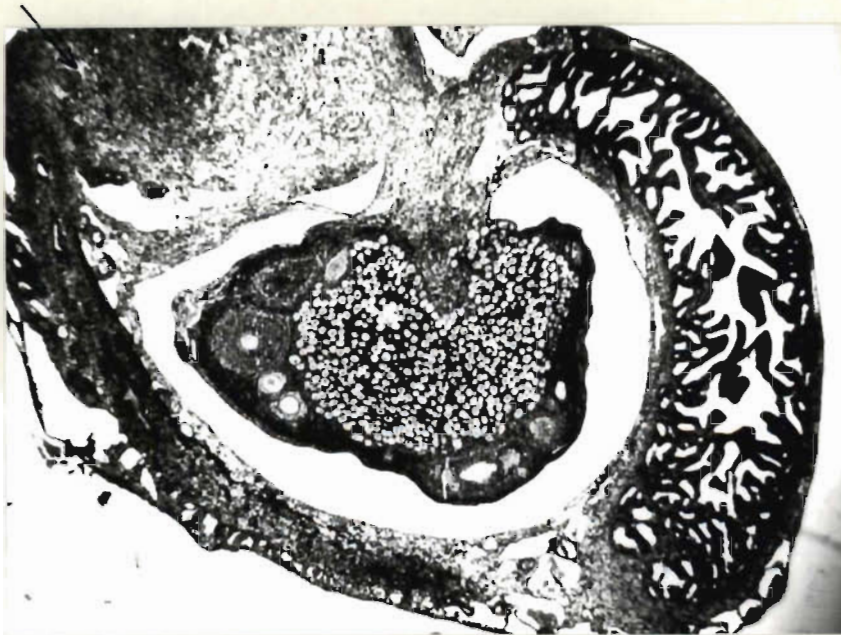
TABLE 15 The mean lengths and widths of uterine horns in infants, juveniles, nulliparous and postlactant females measured in mm

| | uterine length | | | | uterine width | | | |
|-------------|----------------|--------|-----------|----|---------------|--------|-----------|----|
| | \bar{x} | S.E.M. | range | n | \bar{x} | S.E.M. | range | n |
| Infants | 6,65 | | | 1 | 1,00 | | | 1 |
| Juveniles | 4,55 | 0,35 | 4,15-5,25 | 4 | 1,13 | 0,02 | 1,10-1,15 | 4 |
| Nulliparous | 6,13 | 0,46 | 4,50-7,40 | 12 | 1,34 | 0,11 | 1,00-1,70 | 12 |
| Postlactant | 6,73 | 0,49 | 5,60-7,60 | 10 | 1,91 | 0,15 | 1,45-2,38 | 10 |

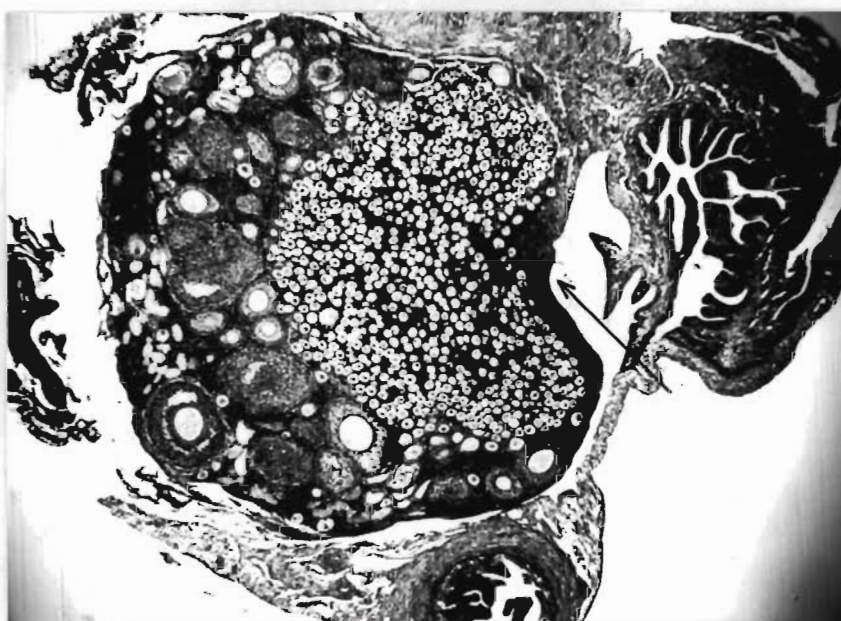
PLATE 18 Sections through the ovary and oviduct, showing complete bursa ovarica and proximity of uterine horn (arrowed) (x 30)

PLATE 19 Infundibular opening opposite ovulation pit (arrowed) within the bursa ovarica (x 30)

PLATE 20 Primordial, primary, secondary, early vesicular follicles and a secondary follicle undergoing atresia (x 30)



18



19



20

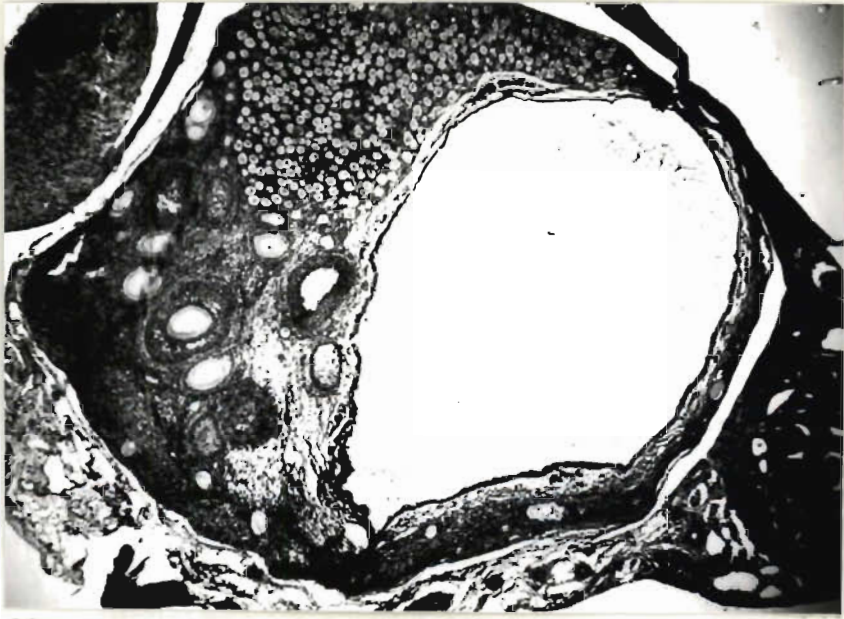
TABLE 16 Mean diameter (μm) of the five categories of follicle recognized

| Type of follicle | \bar{x} | S.E.M | range | n |
|------------------------|-----------|-------|------------|----|
| Primordial | 28,9 | 0,8 | 22 - 37 | 83 |
| Primary | 105,6 | 2,4 | 80 - 126 | 77 |
| Secondary | 146,9 | 2,5 | 90 - 288 | 77 |
| Early vesicular | 210,2 | 3,6 | 105 - 312 | 68 |
| Intermediate vesicular | 291,1 | 11,8 | 168 - 405 | 23 |
| Late vesicular | 916,0 | 141,6 | 555 - 1905 | 10 |

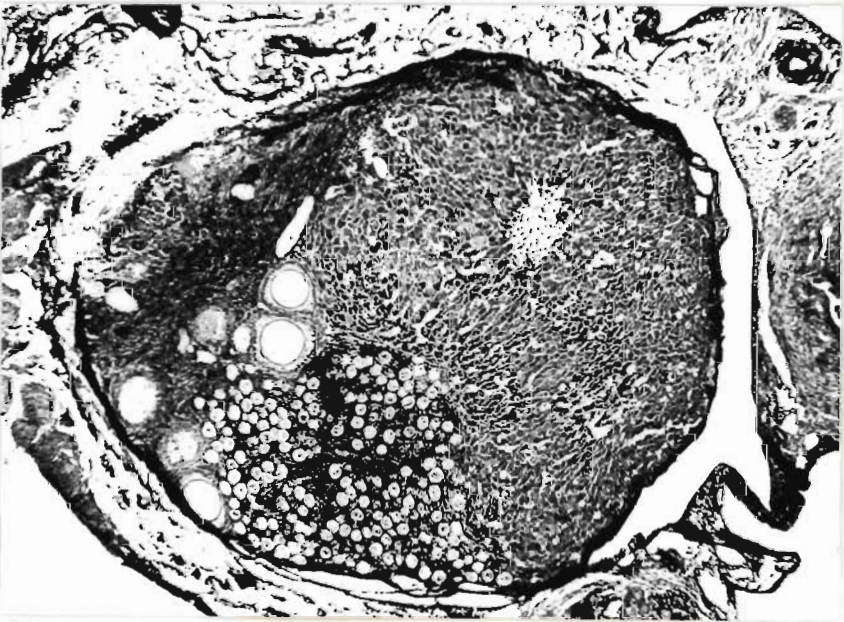
PLATE 21 Late vesicular follicle
(x 30)

PLATE 22 A developed primary corpus luteum
(x 30)

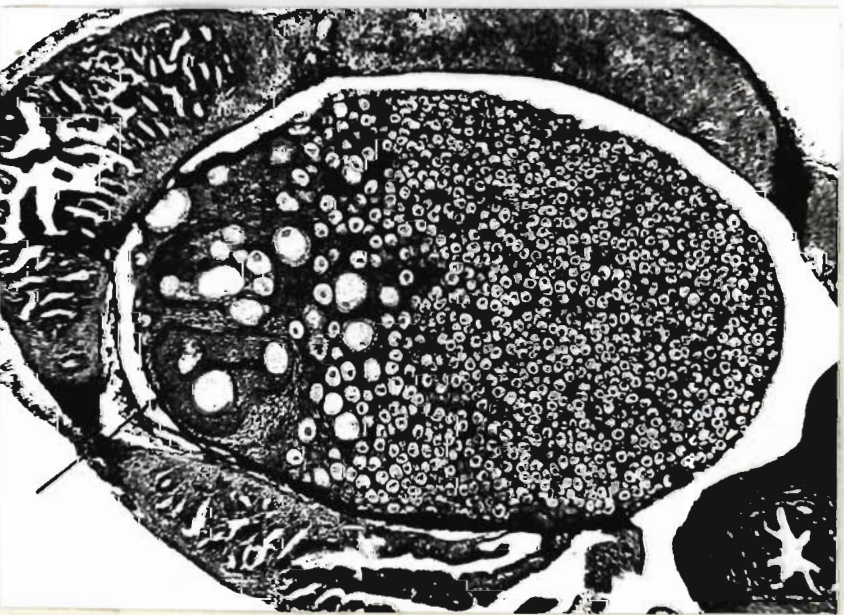
PLATE 23 Polyovular follicles (arrowed)
(x 30)



21



22



23

myometrium of varying thicknesses surrounding a lumen of variable shape and volume.

In pubertal and sexually mature females three conditions of uterine horn were recognized (partly based on those described by Gopalakrishna (1949) for Scotophilus wroughtoni); inactive (PLATE 24); active (PLATE 25) with significantly larger mean maximum lumen diameter ($P < 0,001$), mean maximum endometrial thickness ($P < 0,001$), mean number of endometrial glands per section ($P < 0,001$) and significantly taller epithelial layer ($P < 0,001$) than in the inactive state; postparous (PLATE 26) characterized by the large (often greatly involuted) lumen and significantly fewer endometrial glands per section than in the inactive ($0,001 < P < 0,005$) or active ($P < 0,001$) states. TABLE 17 provides the uterine horn measurements which are useful in distinguishing the three uterine horn conditions.

Probably as a result of the spread of conceptions and births over several months, these conditions were not reflected in significant monthly variations in these uterine horn measurements (except in epithelial height, see 5.3.3.2).

The microscopic structure of the uterine horn changes towards the cephalic end. PLATE 27 shows a section through the body of the horn and PLATE 28 shows a section through the same uterine horn at the cephalic pole. The lumen becomes less well defined and is replaced only by a small concentration of endometrial glands.

5.3.2 Puberty and sexual maturity

When the ovaries contain only primordial follicles, this signifies prepubertal females (PLATE 29) and this stage was found to occur from birth to 2,2 months old. The additional presence of primary follicles with no later stages of follicular development was used to indicate the onset of puberty (PLATE 30). This was observed in only one specimen of 2,5 months of age.

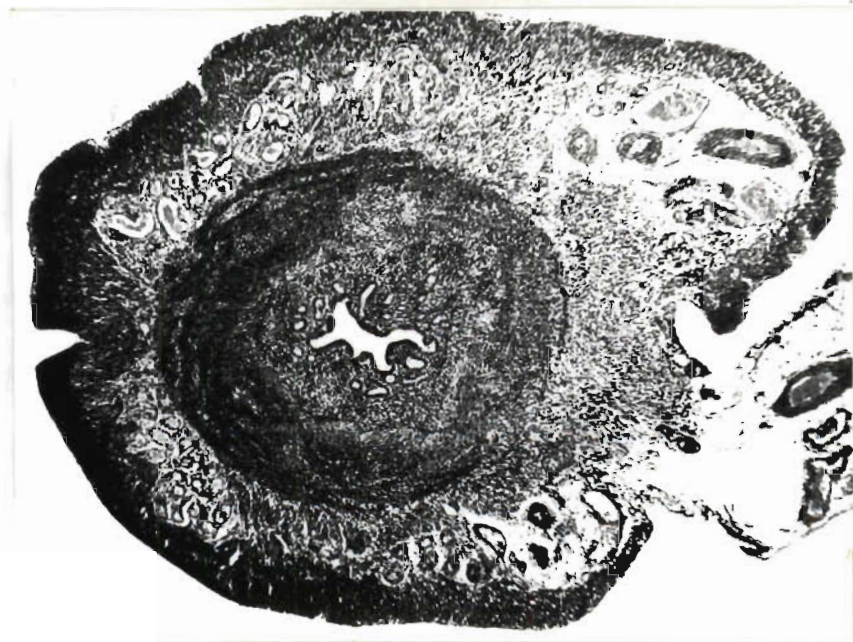
The proestrous phase which marks the onset of the oestrous cycle, occurs when the ovary is first activated by gonadotrophic stimulation resulting in the formation of developing vesicular follicles (Bernard, 1980; Clegg & Clegg, 1963), taken for the purposes of this work as intermediate vesicular follicles. This first took place at a mean age of 5,6 months (S.E.M. = 0,3; range = 5,0 - 6,0 months; $n = 3$).

Racey (1974a) defined sexual maturity as being attained at the start of

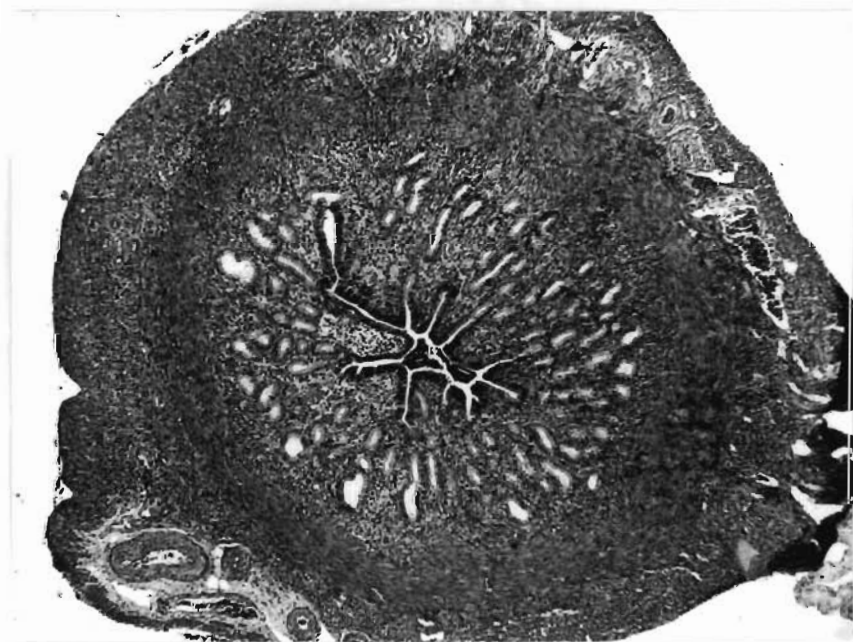
PLATE 24 Inactive uterine horn
(x 40)

PLATE 25 Active uterine horn
(x 40)

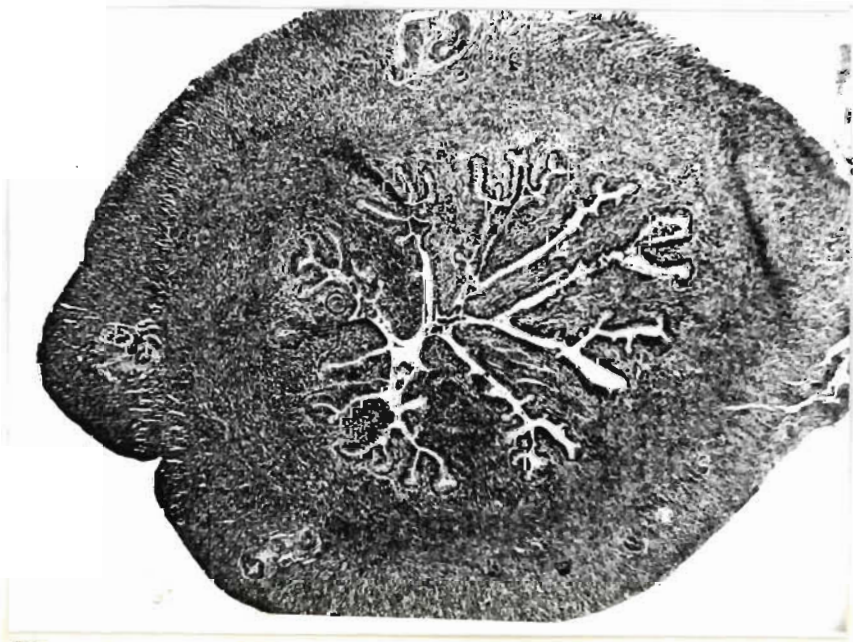
PLATE 26 Immediately postpartous uterine horn
(x 40)



24



25



26

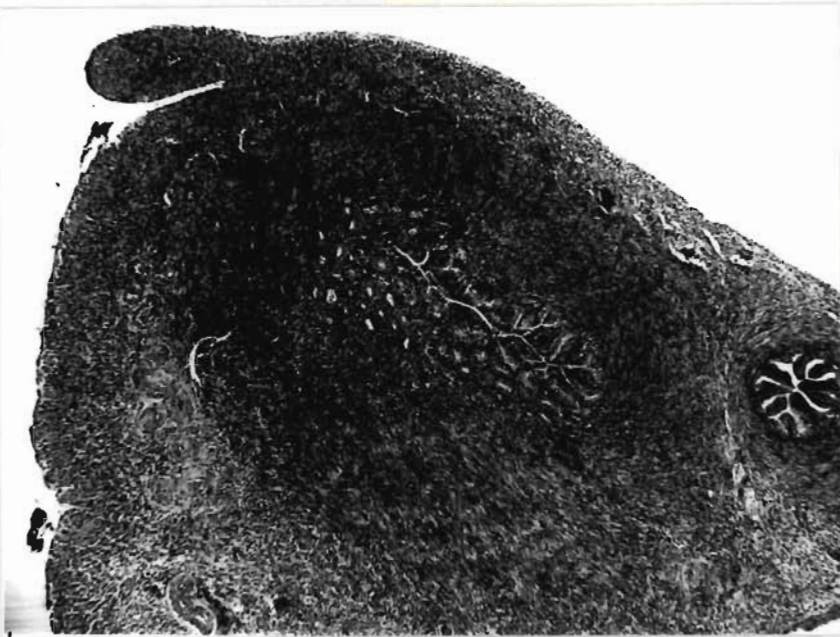
TABLE 17 Uterine measurements for inactive, active and postparous uterine horns

| Uterine horn measurement | Inactive uterine horn | | | | t for difference |
|--|-------------------------|-------|-------------|----|---------------------|
| | \bar{x} | S.E.M | range | n | Inactive/active |
| Max. lumen diameter (μm) | 224,64 | 24,91 | 68-525 | 22 | 6,35 *** |
| Max. endometrial thickness (μm) | 193,18 | 7,63 | 132-292 | 22 | 7,76 *** |
| No. endometrial glands/thin section | 23,86 | 3,09 | 4-64 | 22 | 5,72 *** |
| Height of epithelial layer (μm) | 10,08 | 0,51 | 5,10-15,30 | 22 | 4,78 *** |
| | Active uterine horn | | | | Active/post-parous |
| Max. lumen diameter (μm) | 480,78 | 31,23 | 156-750 | 27 | 2,63 * |
| Max. endometrial thickness (μm) | 395,52 | 22,18 | 206-675 | 27 | 1,37 |
| No. endometrial glands/thin section | 107,89 | 9,19 | 42-200 | 27 | 5,13 *** |
| Height of epithelial layer (μm) | 13,38 | 0,45 | 8,50-16,76 | 27 | 0,88 |
| | Postparous uterine horn | | | | Postparous/inactive |
| Max. lumen diameter (μm) | 672,50 | 71,50 | 525-975 | 6 | 7,12 *** |
| Max. endometrial thickness (μm) | 321,17 | 50,27 | 200-540 | 6 | 4,10 *** |
| No. endometrial glands/thin section | 4,67 | 1,98 | 0-12 | 6 | 3,08 ** |
| Height of epithelial layer (μm) | 14,30 | 0,81 | 11,16-16,43 | 6 | 3,84 ** |

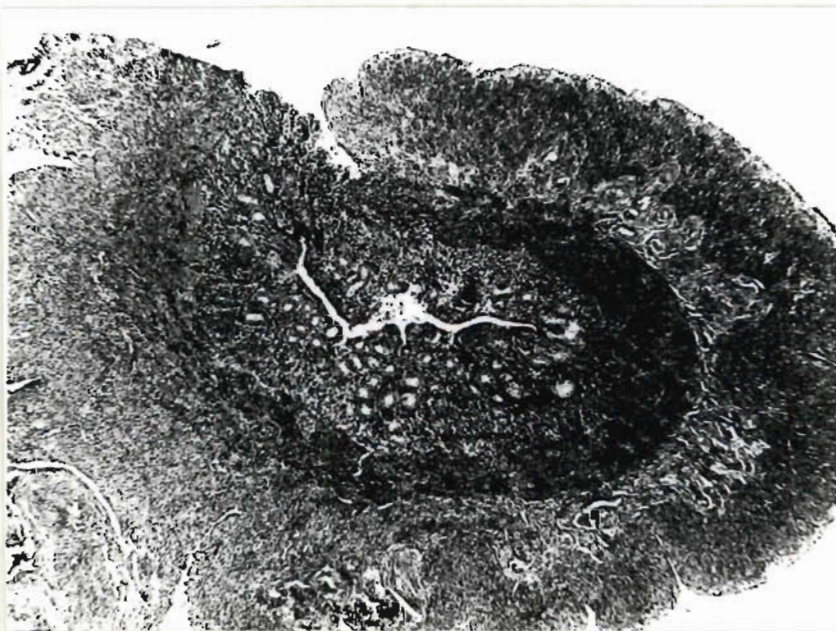
*** $P < 0,001$ ** $0,001 < P < 0,005$ * $0,01 < P < 0,05$

PLATE 27 Section through the body of the uterine horn
(x 40)

PLATE 28 Section through the same uterine horn at its
cephalic pole
(x 40)



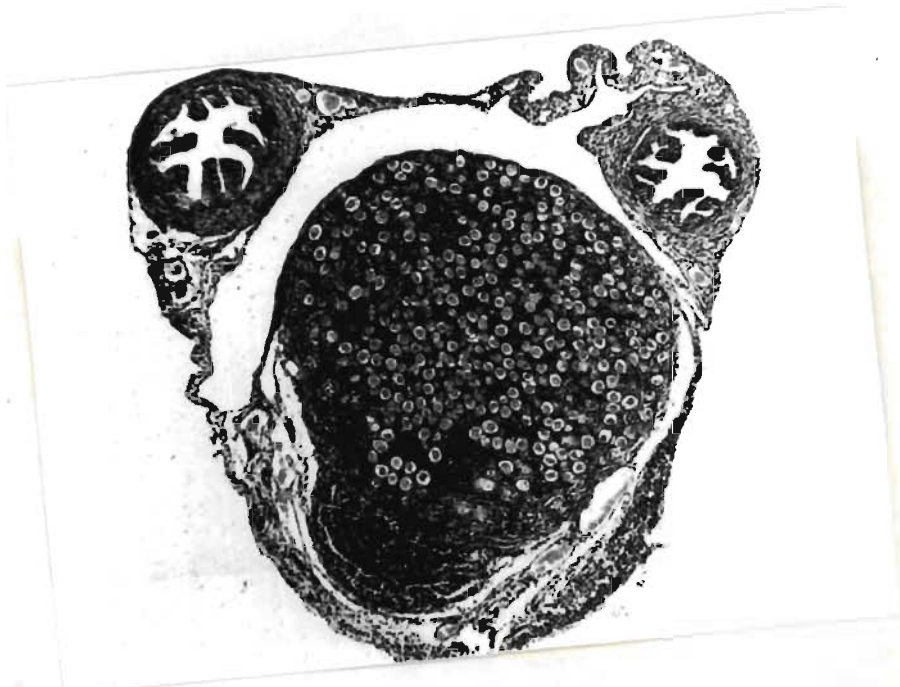
27



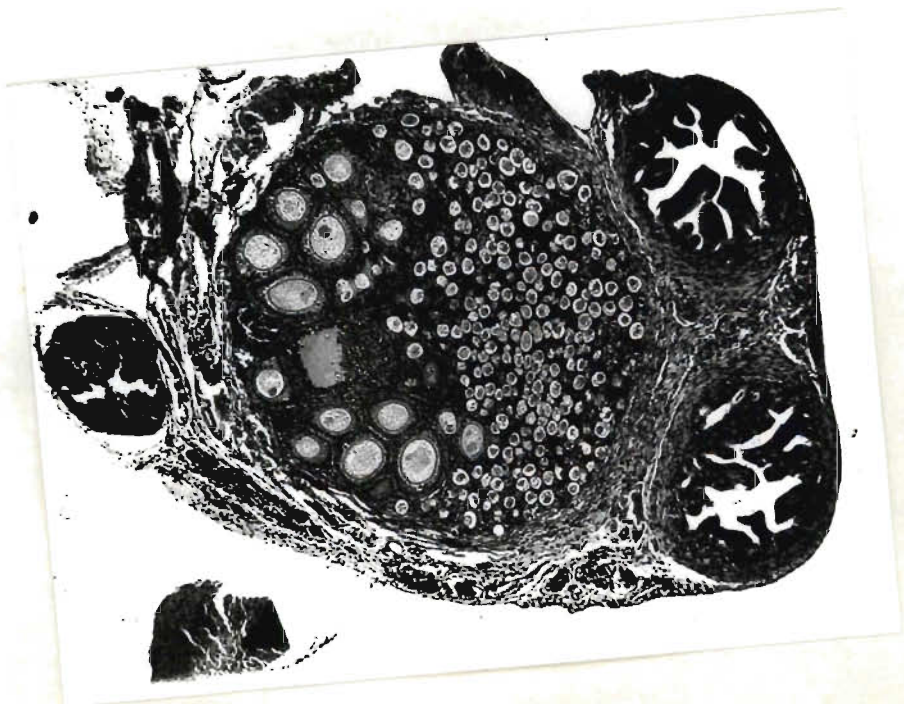
28

PLATE 29 Ovary from a prepubertal female showing presence of primordial follicles only (x 40)

PLATE 30 Ovary from a female at the onset of puberty showing the presence of primordial and primary follicles (x 40)



29



30

first oestrus, which is characterized by the presence of a single late vesicular preovulatory follicle (Bernard, 1980) and taken here as the presence of late vesicular follicles. As this brief phase was missed in the killed sample of nulliparous females, the age at first oestrus and therefore the attainment of sexual maturity can only be estimated as occurring between the mean age at first proestrus (5,6 months) and at first conception (see below, 6,2 months), at approximately six months of age.

Female puberty was taken therefore as occurring from the first presence of primary follicles in the ovary, through the proestrous phase of the oestrous cycle up to the attainment of sexual maturity at first oestrus (2,5 - 6,0 months).

The mean age at first conception was calculated from six primigravid females from the routine monthly killed sample and 21 from the recaptured and released sample, all with pregnancies of 35 days or less (palpated uterine width of 5,5 mm or less). The gestation age of the pregnancy was then subtracted from the females' age to provide age at conception. The mean age at first conception was 6,21 months (S.E.M. = 0,24; range = 3,37-7,83 months; n = 27), (with body mass varying from 55 to 82 g).

The onset of puberty coincides with the latter stages of rapid forearm length growth, while the attainment of sexual maturity occurs before the forearm length asymptote is reached at 8,5 months (3.3.1.3).

5.3.3 Seasonality

FIGURE 49 summarizes reproductive seasonality in the species in terms of births and conceptions from the wild population data. The majority of conceptions (84 %) occur in May to July with the remaining 16 % occurring from August to December. From this FIGURE it can be concluded that the reproductive pattern is of the seasonally polyoestrous type and that although either one extended pregnancy period or two pregnancy periods occur annually, all females do not undergo two successive conceptions/pregnancies each year.

It is clear that a seasonal pattern exists, but several pertinent questions remain unanswered. Is the seasonally polyoestrous pattern of the extended season type with a birth peak in November and a tail to June, or is it of the bimodal type with a main peak in November and a second minor peak in April? What percentage of the female population only undergo the pregnancy terminating around November, only undergo the pregnancy terminating around April (if any) and what percentage actually participate in both pregnancies successively?

Composition of the reproductive status of the wild population, and monthly changes in selected ovarian, uterine and mammary criteria from the routine monthly samples and seasonality trials have been used in an attempt to answer these questions.

5.3.3.1 Reproductive status composition of the wild population

FIGURE 61 shows the monthly percentage of neonates and juveniles of both sexes in the total catch. Most of these animals (99 %) were two to three months of age, because unless carried in flight by the mother, non-volant juveniles were not netted. The main peak in numbers therefore occurred in February. Juveniles were found through until August. No infants or juveniles were caught in September and October.

FIGURE 62 (a) to (e) shows the monthly percentages of nulliparous, primigravid and gravid, lactant, postlactant, and gravid and lactant females in the total female catch.

Nulliparous females range from three months old with a full permanent dentition to approximately six months old (before first conception). The main peak in numbers occurred in April (two months after the main infant/juvenile peak) with a steady decrease until September.

Analysis of pregnancy data reveals that gravid animals could be found in all months of the year except May. Numbers of gravid females increased from June to October when 92,3 % of the total female catch were found to be pregnant. A decrease from 85,0 % to 26,3 % occurred from November to December indicating parturition. Gravid females continued to form a small percentage of the total female catch (4,0 % to 12,5 %) until April.

Lactant females were found in eight months of the year, the greatest percentage (73,7 %) occurring in December. From February to March the percentage decreased sharply from 44,4 % to 6,3 % and from then until June small percentages of lactant females (6,5 % to 11,1 %) were found.

The largest percentage of postlactant females enter the population in March. From June the numbers decrease to October, and in November and December no postlactant females were evident.

FIGURE 61 Monthly percentage of neonates and juveniles of both sexes in the total catch (n = 66)

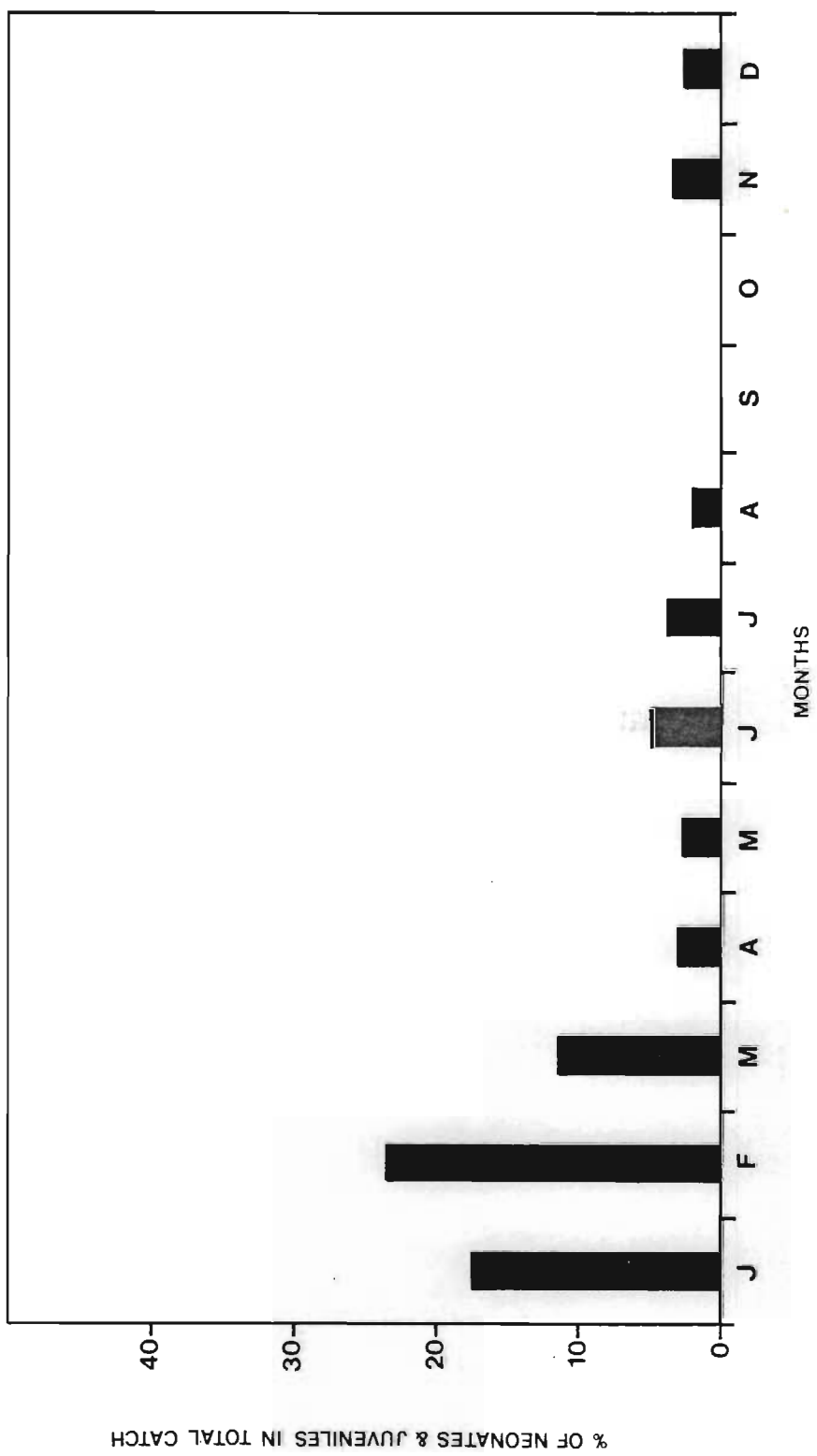
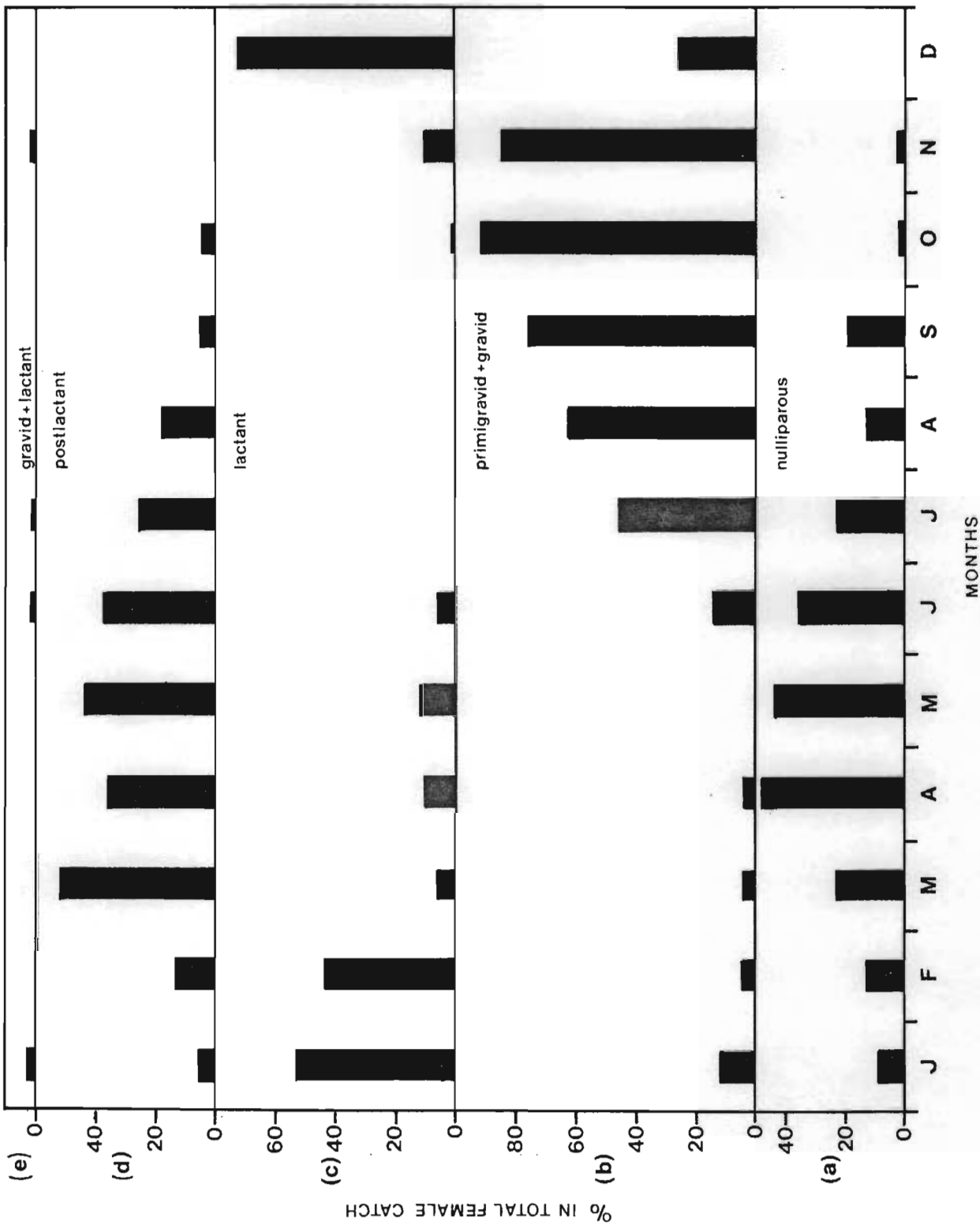


FIGURE 62 Monthly percentage of nulliparous (n = 129) (a), primigravid and gravid (n = 182) (b), lactant (n = 70) (c), postlactant (n = 140) (d) and gravid and lactant (n = 5) (e) females in the captured and released female sample



These results indicate one main breeding season terminating in births in November and December in which most females (92,3 % of the female population) participate. Low intensity breeding continues producing births from January to June involving a small percentage of the female population (approximately 5 % are pregnant in April). Obviously those females giving birth in February and March cannot be undergoing a second successive pregnancy (after giving birth in November or December), but were probably late in conceiving during the main season, perhaps due to a miscarriage. However, births from April to June could be the result of a second successive pregnancy following a post partum conception. Evidence for this is shown in FIGURE 62 (e). Gravid and lactant females were caught in November and January and in June and July, suggesting that a small percentage of females undergo two pregnancies per year. It is impossible to estimate what percentage of the female population may undergo the pregnancy terminating around April.

It would appear therefore that approximately 5 % of females pregnant in January and June are composed partly of 'tail-enders' from the main season and partly of postpartum conceiveres undergoing a second successive pregnancy. The 'tail-enders' giving birth merge into the births of the second pregnancy females. Seasonally polyoestrous with an extended season therefore better describes the female reproductive pattern than truly bimodal.

FIGURE 63 further emphasizes the seasonal breeding trends. Using the mean uterine horn widths and fetal cranial breadths for each month, clear evidence of the main pregnancy period culminating in births in November and December and some evidence of a second pregnancy period up until April, can be seen.

5.3.3.2 Monthly variation in ovarian, uterine and mammary criteria

Ovary

FIGURE 64 shows the monthly variations in primordial, primary and secondary follicle numbers. Primordial and primary follicle numbers show no significant monthly variations, but there is a significant increase in secondary follicle numbers in April, May and June compared to the rest of the year ($z = 3,42$; $P < 0,001$).

FIGURE 65 shows the monthly variations in early, intermediate and late vesicular follicle numbers. There is a significant

FIGURE 63 Monthly palpated uterine horn widths and fetal cranial breadths (n = 187)

(vertical line - range, crossbar - mean, broad portion of vertical line - S.E.M.)

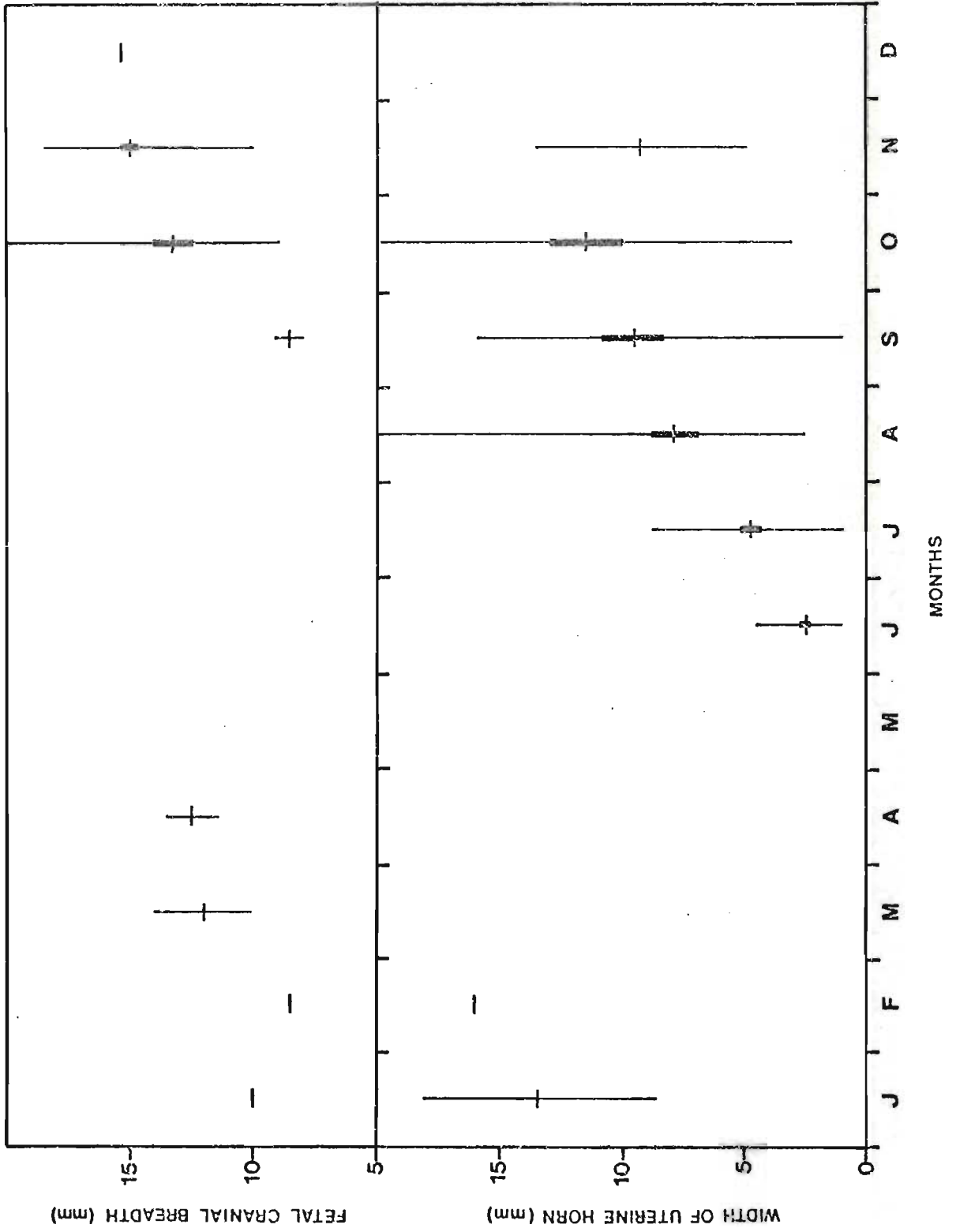


FIGURE 64 Monthly variations in primordial (a), primary (b) and secondary (c) ovarian follicles in the routine monthly samples (n = 78)

(vertical line - range, crossbar - mean, broad portion of vertical line - S.E.M.)

10000
1000
100

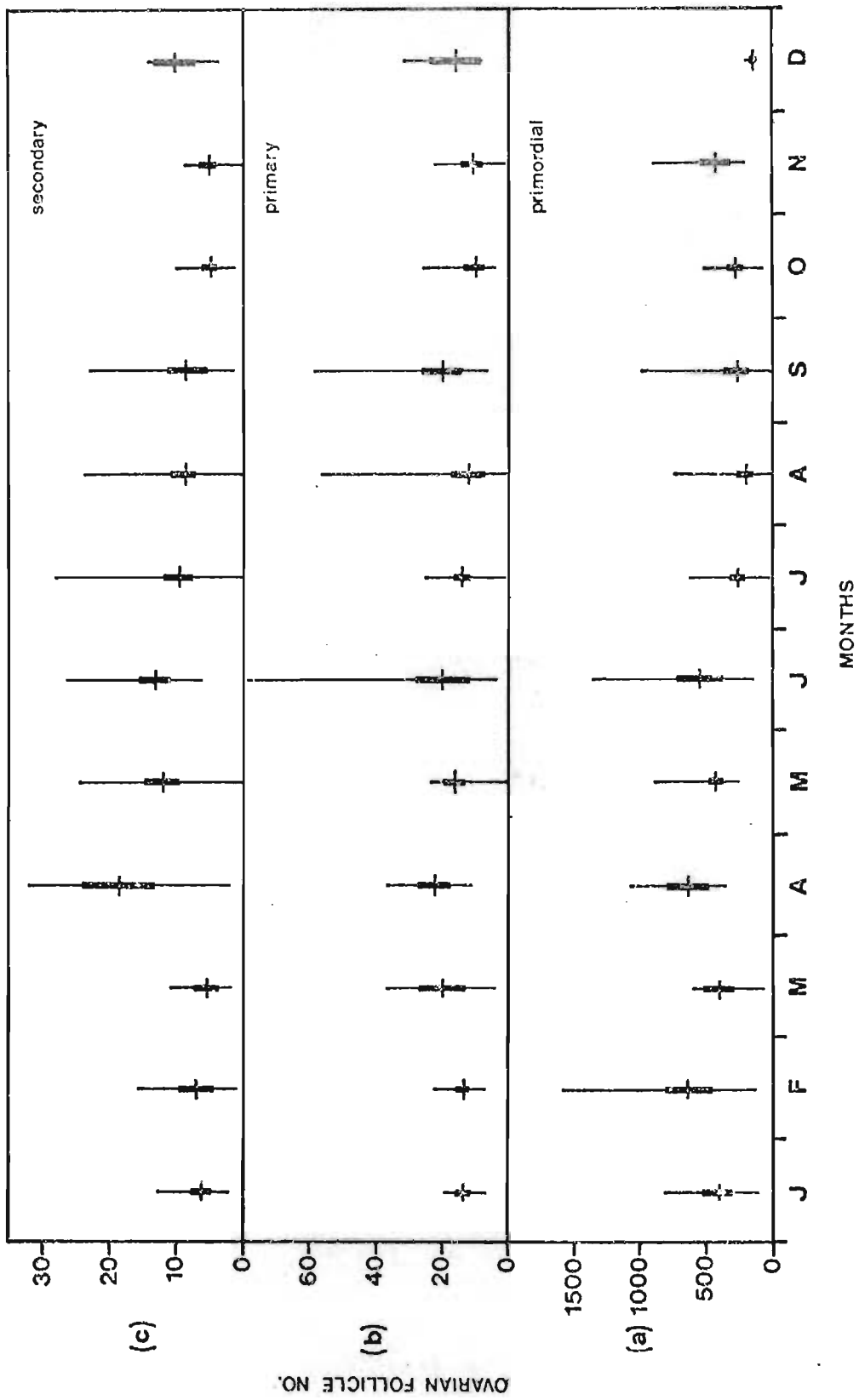
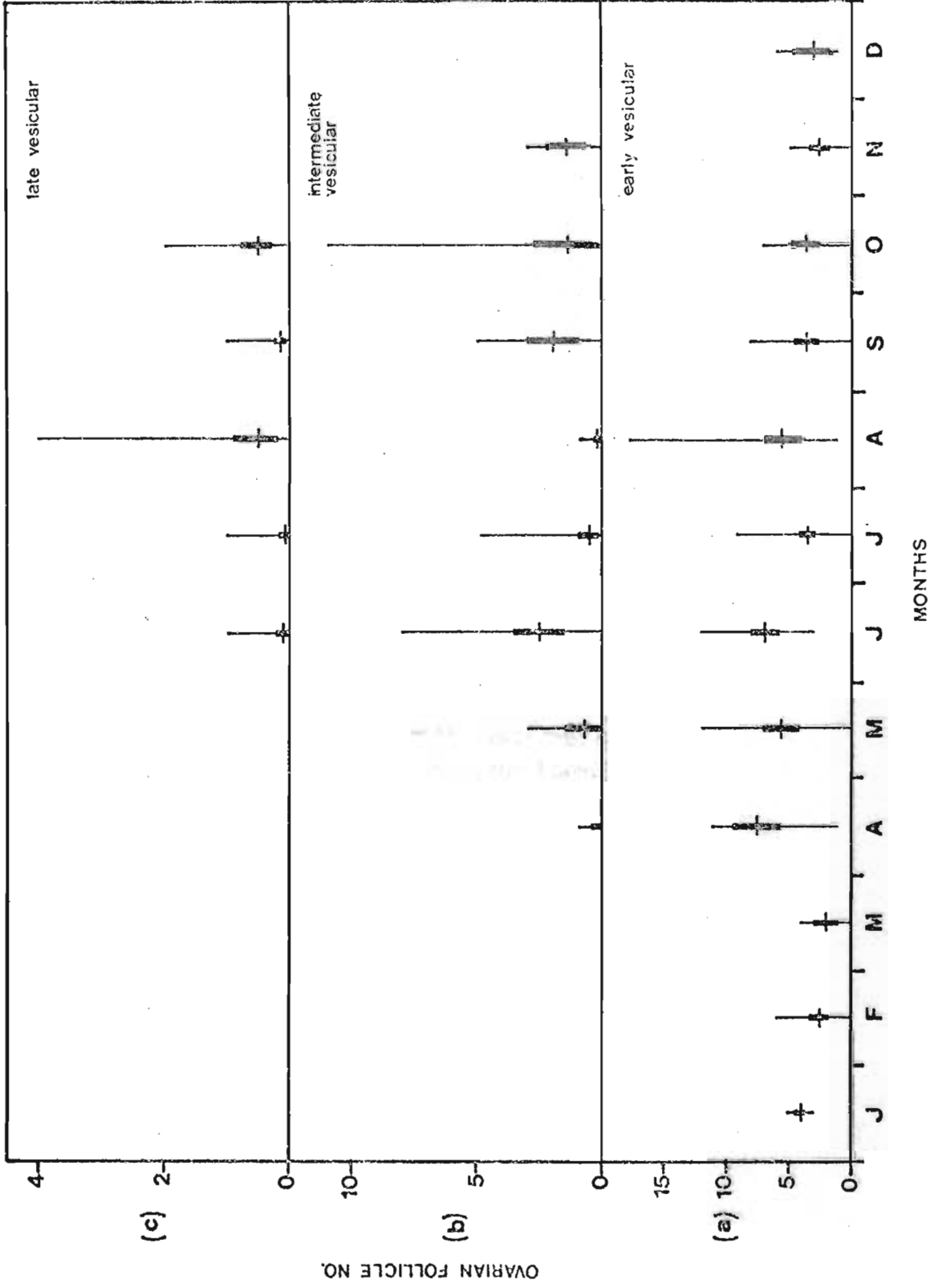


FIGURE 65 Monthly variations in early (a), intermediate (b) and late (c) vesicular follicle numbers in the routine monthly samples (n = 78)

(vertical line - range, crossbar - mean, broad portion of vertical line - S.E.M)



increase in early vesicular follicle numbers in April, May and June ($z = 3,38$; $P < 0,001$). Intermediate vesicular follicles were only found from April to November and late vesicular follicles from June to October (PLATE 31). The prooestrous phase of the oestrous cycle therefore commences in April with the appearance of intermediate vesicular follicles and continues into the oestrous phase in June with the appearance of late vesicular follicles.

Follicle numbers used were from combined left and right ovaries including those from gravid animals. As the majority of females (79 %) which were used to provide the June to November data were pregnant, it can be seen that follicular development does not cease after conception, but continues during pregnancy (PLATE 32). Four gravid females were found to have late vesicular follicles in the ovary of the non-gravid uterine horn. In two of the females these occurred during the first 30 days of pregnancy and were starting to show the first signs of atresia. In the other two females they occurred in the last 50 days of gestation and were in the other ovary which was not responsible for the current pregnancy. This suggests a possible preparation for a postpartum oestrus and conception and is dealt with in further detail in 5.3.5.1.

Mean monthly secondary, early, intermediate and late vesicular follicle sizes were examined for seasonality, but no significant variations were found to occur.

Atresia was also investigated for seasonality (FIGURE 66). The number of atretic follicles was significantly lower during February and March prior to the onset of the oestrous cycle than in the remainder of the year ($z = 4,75$; $P < 0,001$).

Uterus

Lumen diameter, endometrial and myometrial thicknesses showed no significant monthly variations. Uterine epithelial height however showed a bimodal pattern (FIGURE 67) with significantly greater cell heights in May and June (reflecting the active uterine conditions) and in November and December (reflecting the postparous uterine condition ($z = 3,24$; $0,001 < P < 0,005$)). A similar distinctive bimodal pattern was seen in the mean number of endometrial glands per month (FIGURE 68). Uterine horns in July, August, September and in December showed significantly larger numbers of endometrial glands than those during the remainder of the year ($z = 2,60$; $0,005 < P < 0,01$).

PLATE 31 Active ovary sampled in June, showing primary, secondary, early and intermediate vesicular follicles

PLATE 32 Ovary of the non-gravid uterine horn in a pregnant female showing active and atretic early and intermediate vesicular follicles




31



32



FIGURE 66 Monthly variations in atretic follicle numbers in the routine monthly samples (n = 78)

 (vertical line - range, crossbar - mean, broad portion of crossbar - S.E.M.)

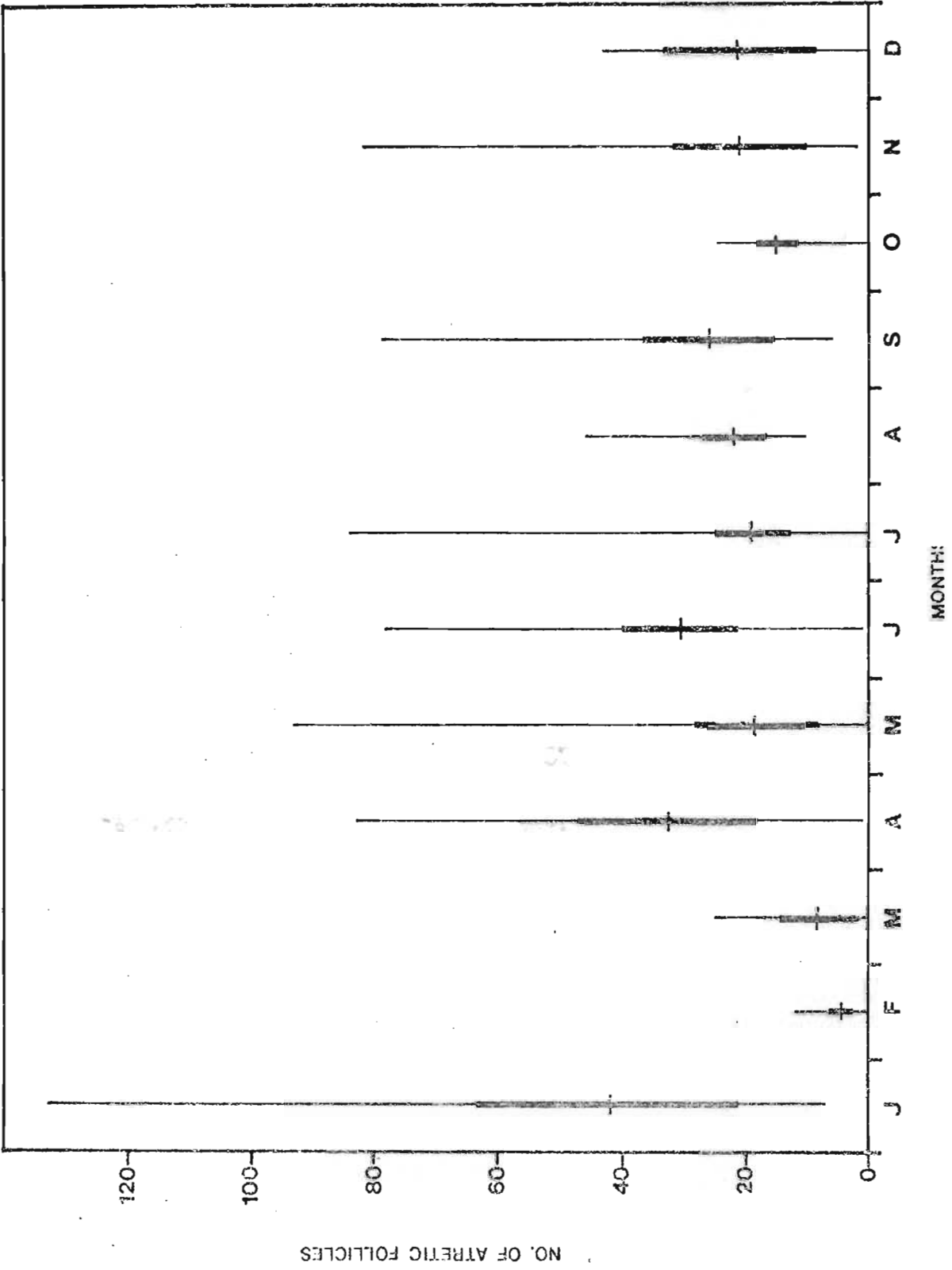


FIGURE 67 Monthly variations in uterine epithelial height in the routine monthly samples (n = 78)

(vertical line - range, crossbar - mean, broad portion of vertical line - S.E.M.)



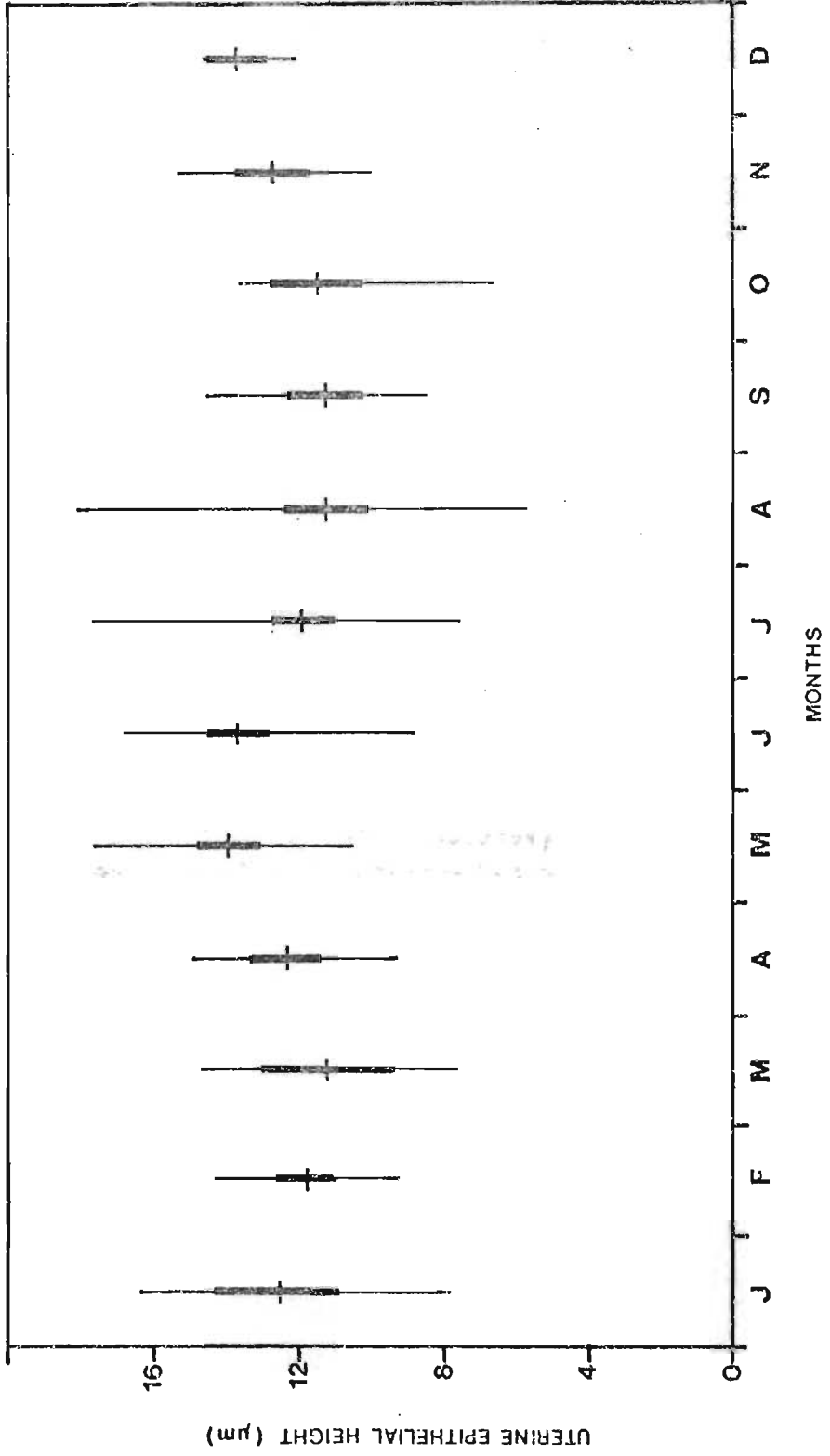
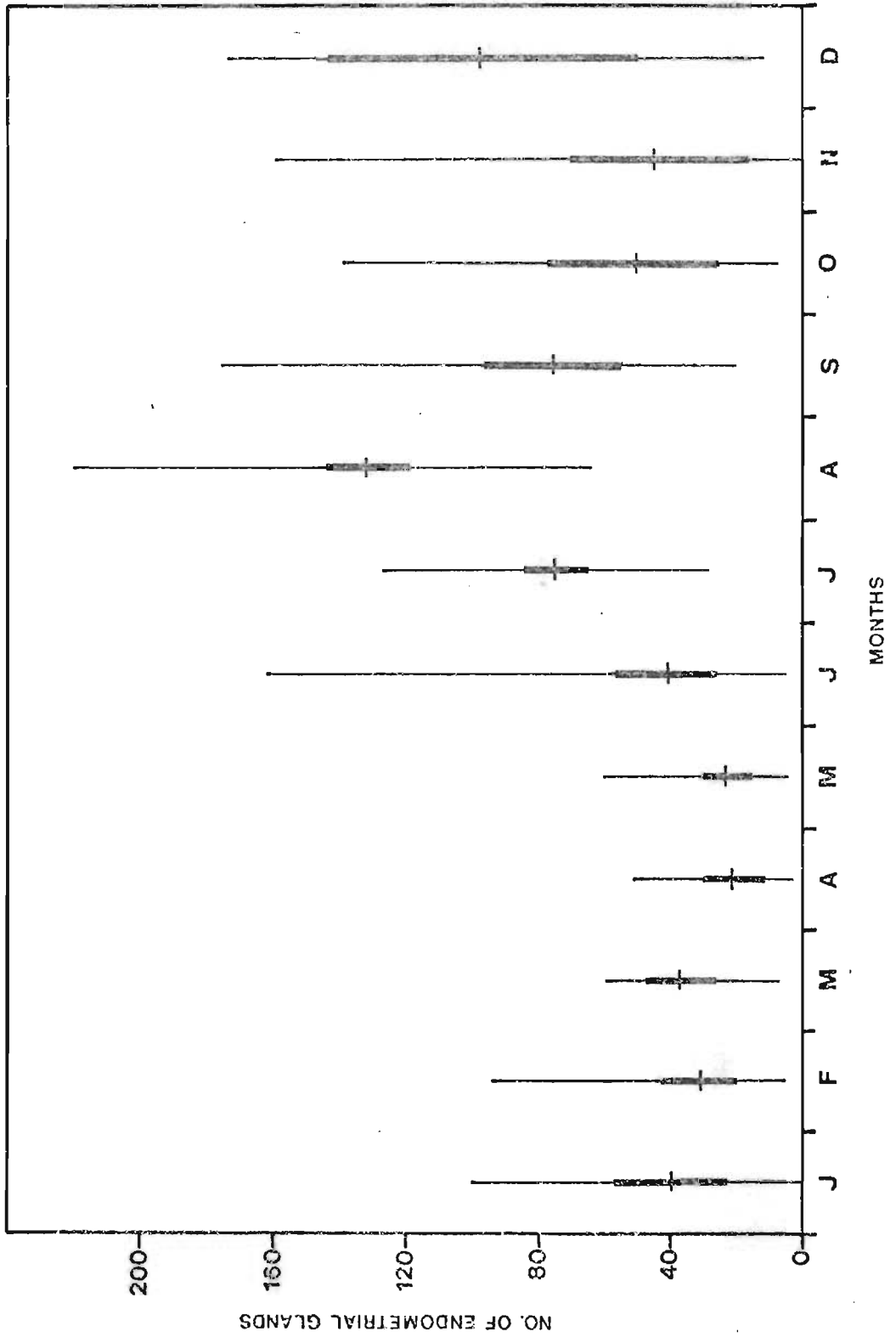




FIGURE 68 Monthly variations in the number of endometrial glands in the uterus of the routine monthly samples (n = 78)



(vertical line - range, crossbar - mean, broad portion of vertical line - S.E.M.)



Mammary tissue

FIGURE 69 shows the mean mammary gland mass per month. The heaviest mammary glands were recorded in December and January and again in May and June, reflecting the data provided by lactant females from the wild population in FIGURE 62.

The histological evidence and that from the wild population fully support a seasonality pattern of a main birth pulse in November/December followed by a low intensity extension until June.

5.3.4 Environmental factors affecting reproduction

The majority of conceptions occur in May to July, preceded by a four month period where no conceptions were found to occur. What factors could act as triggers for the initiation of oestrus/ovulation/mating at this time and so serve as proximate causes of breeding? Likewise the majority of births occur in November and December. Are there nutritional or climatic advantages at this time which may act as the ultimate cause of breeding?

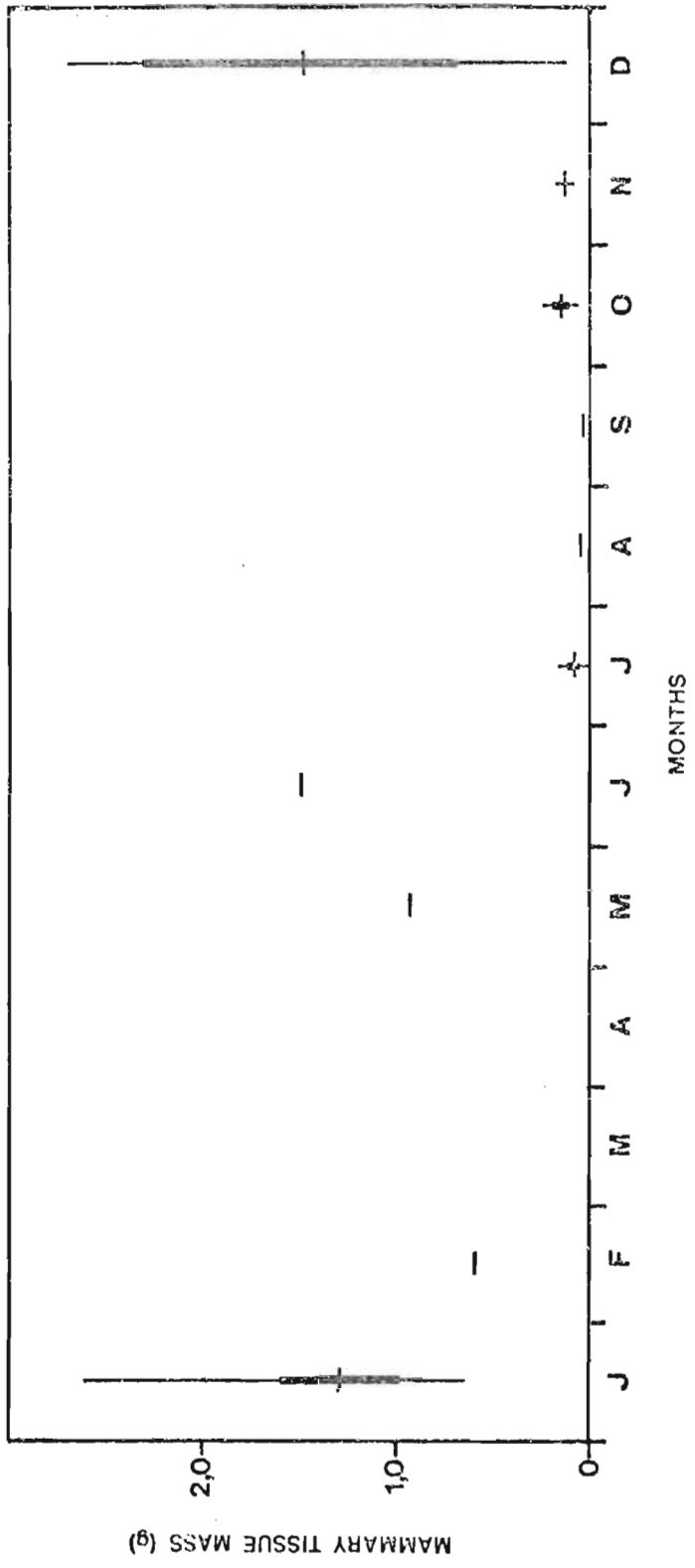
An attempt to answer these questions has been made by using a combination of controlled experimentation and by examination of the circumstantial evidence of climatic and nutritional seasonal variations.

5.3.4.1 Proximate causes of breeding

'Breeding' is a general term and is used to refer to the entire reproductive event series from the onset of the oestrous cycle through to parturition. When dealing with proximate causes of breeding it is the early sequence of these events which are relevant. In this work the histological rather than the behavioural manifestation of breeding was investigated, and the specific histologically identifiable event chosen to represent 'breeding' was the onset of oestrus, recognized by the presence of late vesicular follicles. Two other 'breeding' events, the onset of proestrus and ovulation were rejected, because proestrus is approximately two months removed in time from conception, and ovulation, despite attempts to witness evidence of its occurrence (seasonality trials) was not observed.

FIGURE 69 Monthly variations in the mass of mammary tissue of the routine monthly samples (from one side only) (n = 68)

(vertical line - range, crossbar - mean, broad portion of vertical line - S.E.M.)



Assuming that specific triggers exist for oestrus onset (instead of this event being a simple continuation from proestrus), two possible external triggers were experimentally tested, namely male presence and the short June photoperiod.

Male presence

Captive females caged continuously with an adult male for periods of two years continued to show breeding seasonality. It is unlikely therefore that a simple male presence at fixed times only results in this seasonality. However, it has been shown that male calling is significantly greater in May to July (FIGURE 53) and even though it was not conclusively proved that the sonic quality of the call is different at this time of the year (FIGURE 54), calling or another perhaps pheromonal changes in the male could act as a breeding cue.

Ovulatory triggers, Experiment 1, was designed to test the importance of male presence. Although the occurrence of late vesicular follicles indicating oestrus, was used as the main indicator, the four ovarian/uterine criteria which were found to be significantly greater in June routine killed samples (5.3.3.2, FIGURES 64, 65 and 67) were also examined in the experimental animals.

The five criteria, namely secondary, early vesicular, intermediate vesicular and late vesicular follicle numbers and uterine epithelium height from the experimental bats were compared with both the control animals and the June routine killed sample (TABLE 18). No significant differences in any of the criteria were found to occur. From this it was concluded that E. wahlbergi females could proceed to oestrus in the absence of sight, sound or smell of a male.

Photoperiod

FIGURE 70 shows that the May to July conception peak coincides with daylengths less than eleven hours. This circumstantial evidence suggested that decreasing or short photoperiod may be an obligatory factor and ovulatory trigger. Experiment 2, was designed to test if shortening photoperiod to the minimum June daylength could induce oestrus onset.

The ovarian and uterine criteria measured in the short photoperiod animals, killed in March, were compared with the March routine killed sample combined with the single control

TABLE 18 Five ovarian/uterine criteria from females kept in the absence of a male, compared with those from the control and June routine killed sample. (results of Ovulatory trigger Experiment 1)

| | n | \bar{x} | S.E.M. | range | comparison | t for difference |
|--|----|-----------|--------|-------------|---------------|------------------|
| <u>Secondary follicle no.</u> | | | | | | |
| Experimental♀♀(no male) | 4 | 9,50 | 2,22 | 5-15 | | |
| Control (with male) | 3 | 10,67 | 4,06 | 4-18 | Expt./Control | 0,23 |
| June sample (with male) | 9 | 13,00 | 2,24 | 7-26 | Expt./June | 0,88 |
| <u>Early vesicular follicle no.</u> | | | | | | |
| Experimental♀♀(no male) | 4 | 9,0 | 2,08 | 4-14 | | |
| Control (with male) | 3 | 10,57 | 7,67 | 3-26 | Expt./Control | 0,20 |
| June sample (with male) | 9 | 6,89 | 1,15 | 3-12 | Expt./June | 0,88 |
| <u>Intermediate vesicular follicle no.</u> | | | | | | |
| Experimental♀♀(no male) | 4 | 0,75 | 0,48 | 0-2 | | |
| Control (with male) | 3 | 0,33 | 0,33 | 0-1 | Expt./Control | 0,58 |
| June sample (with male) | 9 | 2,56 | 1,07 | 0-8 | Expt./June | 1,02 |
| <u>Late vesicular follicle no.</u> | | | | | | |
| Experimental♀♀(no male) | 4 | 0,25 | 0,20 | 0-1 | | |
| Control (with male) | 3 | 0,67 | 0,67 | 0-2 | Expt./Control | 0,72 |
| June sample (with male) | 9 | 0,11 | 0,11 | 0-1 | Expt./June | 0,56 |
| <u>Uterine epithelial height (μm)</u> | | | | | | |
| Experimental♀♀(no male) | 4 | 13,88 | 0,79 | 12,64-16,19 | | |
| Control (with male) | 3 | 12,68 | 0,89 | 11,78-14,47 | Expt./Control | 0,85 |
| June sample (with male) | 10 | 13,69 | 0,81 | 8,84-16,83 | Expt./June | 0,13 |

1970
1971
1972

FIGURE 70 Hours of daylight at 30° S (solid curved line) (taken from FIGURE 12) in relation to the monthly percentage of conceptions (bars) (taken from FIGURE 49)



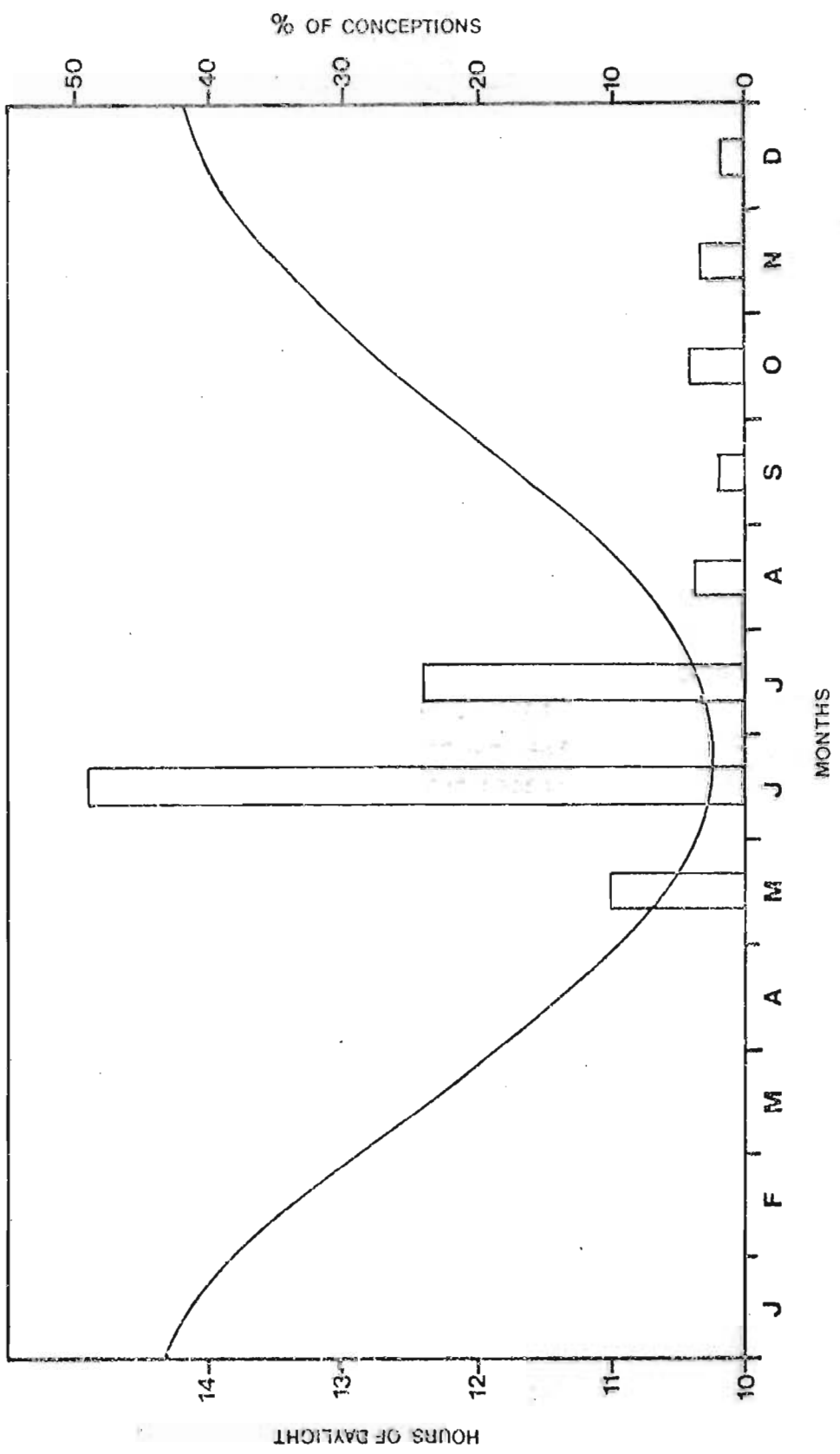


FIGURE 73 Mean monthly rainfall (from FIGURE 9) and monthly percentage of fruiting trees (from FIGURE 13) in relation to the percentage of lactant females per month in the captured and released female sample (from FIGURE 62 (c))

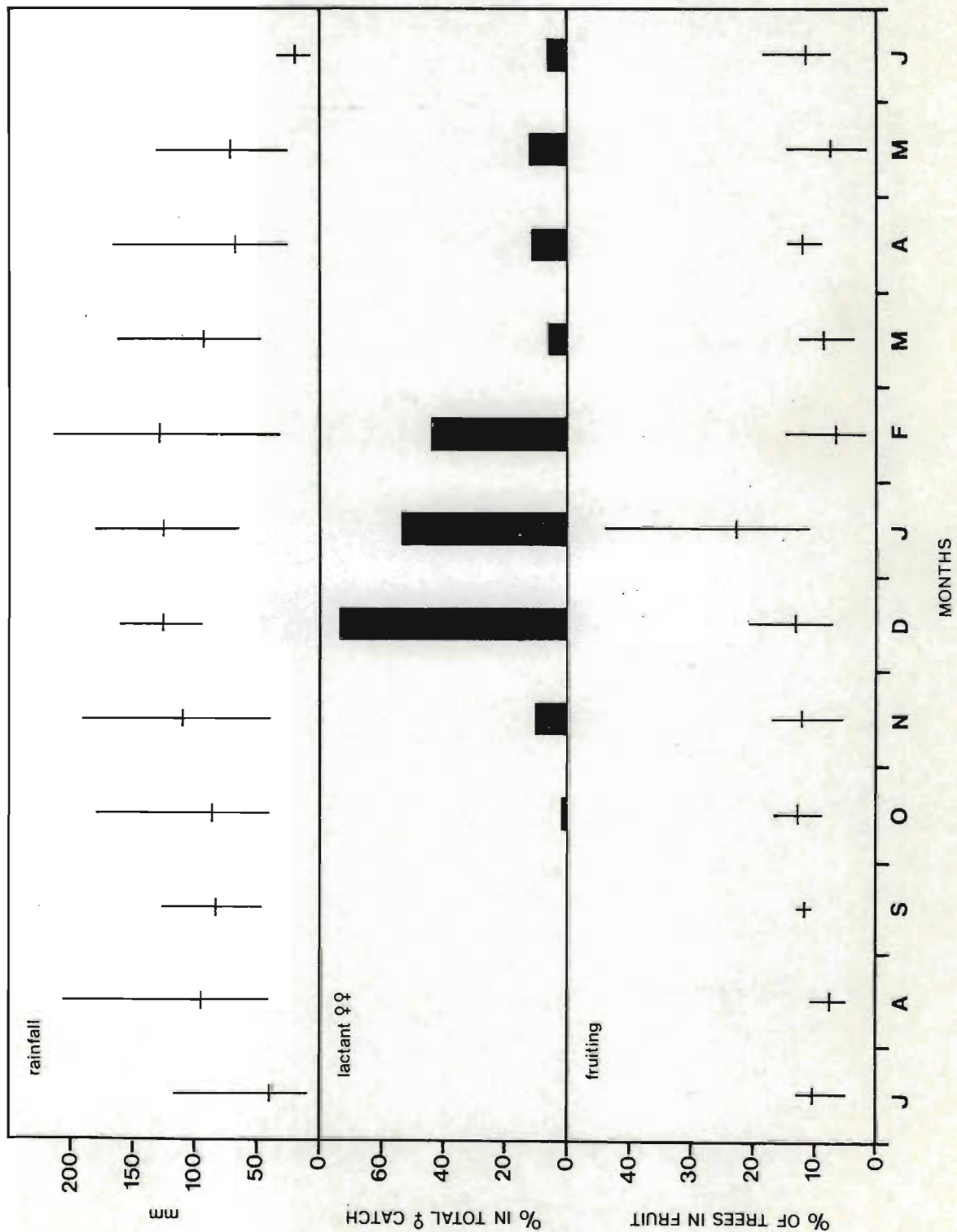


FIGURE 74 Variations in the timing of oestrous cycles and the estimated percentage of the female population participating in each time variation

1 - 9 represents each time variation for the annual cycles

The double horizontal line, divides the annual cycles with a single pregnancy, from those with two pregnancies

Estimate of the % ♀ population
undergoing the 9 cycle timings

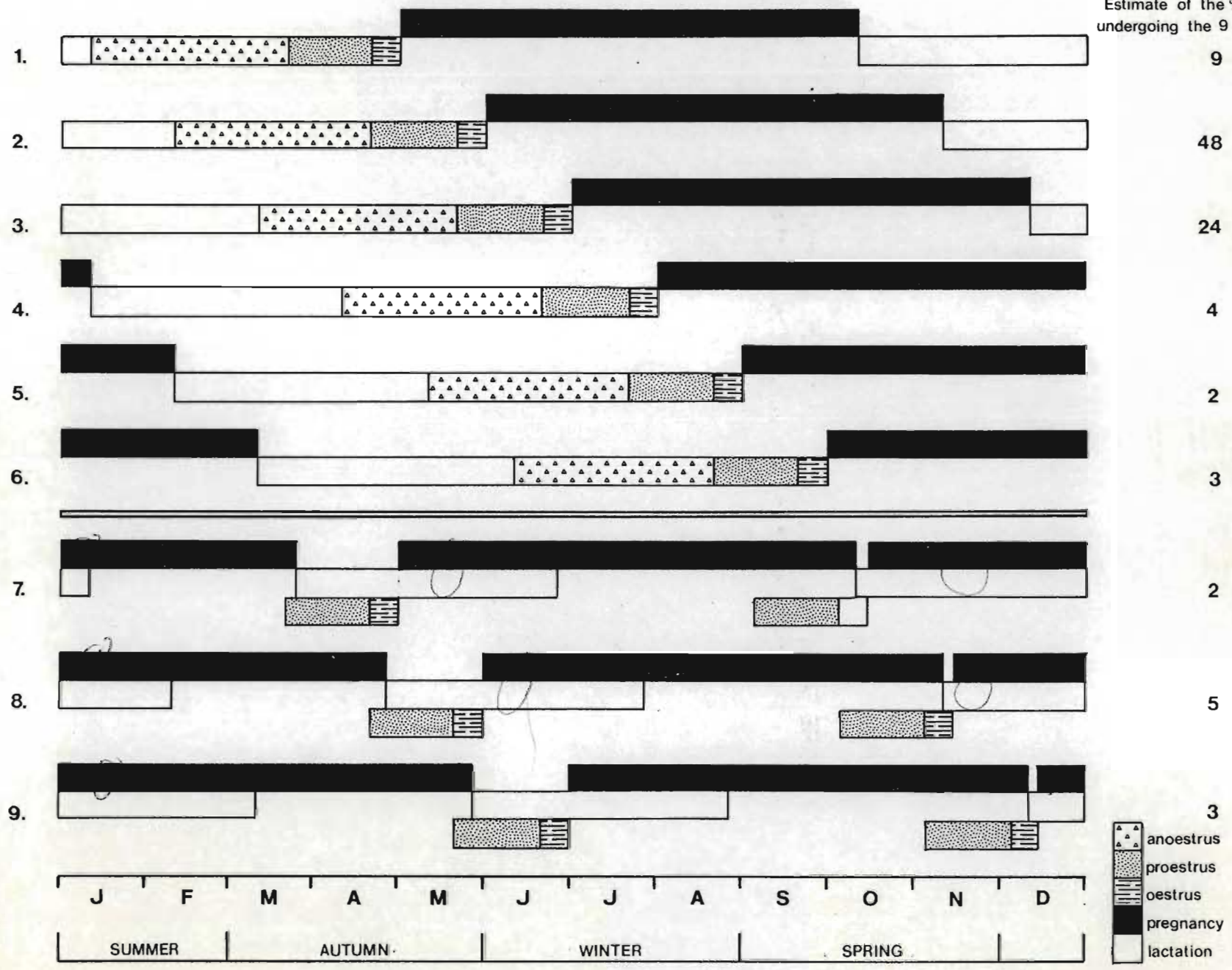


FIGURE 75 Number of intermediate and late vesicular follicles in the non-active ovary in ten day units during pregnancy

(vertical line - range, crossbar - mean, broad portion of vertical line - S.E.M.)

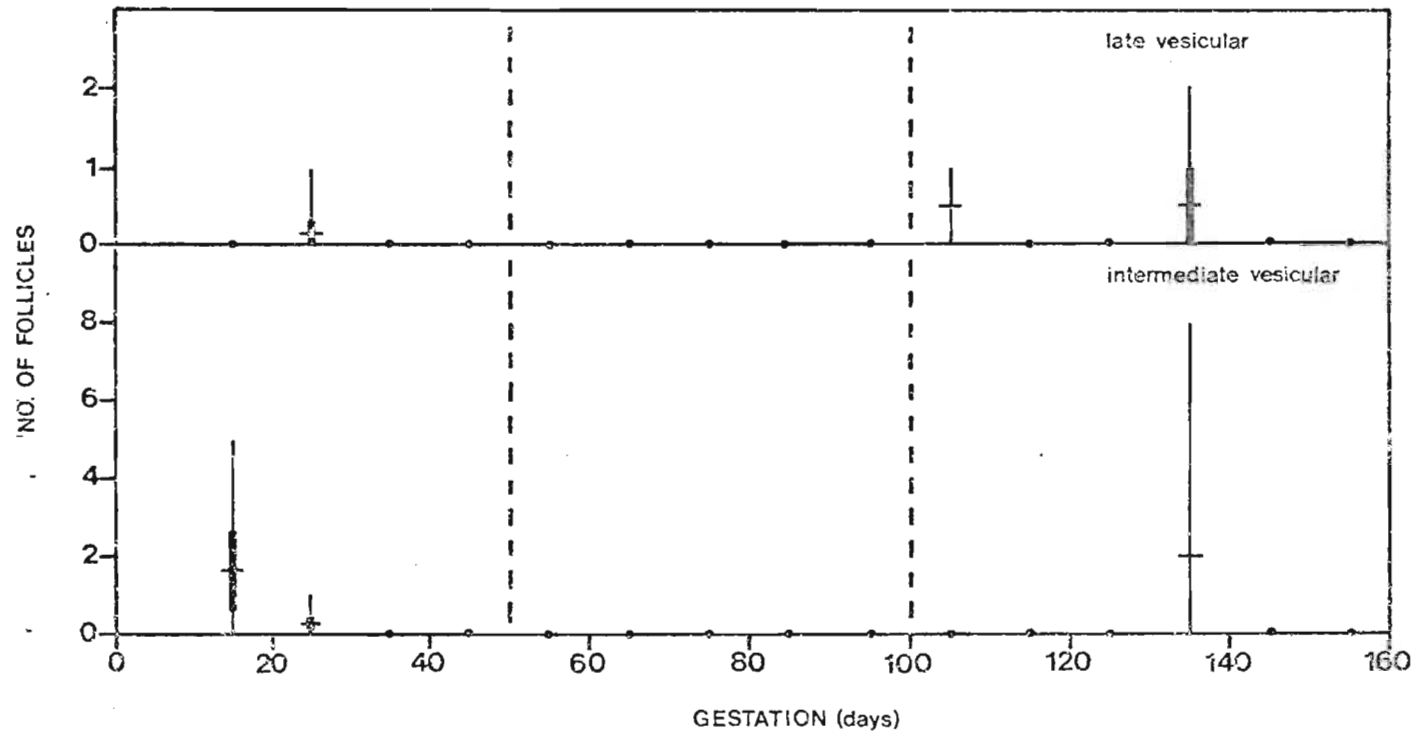


TABLE 20 Number of intermediate and late vesicular follicles occurring in the non-active ovary during the three stages in gestation; 0-50 days, 51-100 days and 101-160 days. Comparison of the mean values for the last third of pregnancy with the mean values obtained from the June killed sample

| Follicle | stage in gestation (days) | n | \bar{x} | S.E.M. | range | comparison with June sample |
|------------------------|---------------------------|----|-----------|--------|-------|-----------------------------|
| | | | | | | t for difference |
| Intermediate vesicular | 0-50 | 23 | 0,48 | 0,27 | 0-5 | 1,26 |
| | 51-100 | 15 | 0 | 0 | 0 | |
| | 101-160 | 11 | 0,80 | 0,80 | 0-8 | |
| Late vesicular | 0-50 | 23 | 0,05 | 0,05 | 0-1 | 2,04 * |
| | 51-100 | 15 | 0 | 0 | 0 | |
| | 101-160 | 11 | 0,30 | 0,21 | 0-2 | |

* $0,05 < P < 0,1$

gestation period (0-50, 51-100 and 101-160 days). In order to examine the possibility that the presence of these follicles are indicative of a proestrus/oestrus period occurring in some animals during the latter stages of the winter/spring pregnancy in preparation for a postpartum oestrus/ovulation and successive conception, the mean follicle numbers for the last third of gestation (101-160 days) were compared with the mean follicle numbers for the month of June, when the main winter oestrus occurs. Two out of 49 pregnant females sampled (4,1 %) exhibited intermediate and late vesicular follicles in the last third of gestation. TABLE 21 shows there was no significant difference between the June values and those for the last third of gestation for the intermediate vesicular follicles ($t = 1,26$; $P > 0,1$) and the late vesicular follicles ($t = 2,04$; $0,05 < P < 0,1$).

FIGURE 74 shows that two patterns of breeding occur. In the first pattern there is one oestrous cycle per year, with one period of pregnancy, lactation and anoestrus (FIGURE 74, 1-6). Conceptions in this pattern may occur from May to October, with the majority in June. A possible explanation for the small percentage of conceptions occurring after the main May to July peak, could be that these animals had aborted in the early stages of pregnancy from a conception during May - July and conceived again. Supportive evidence for this was obtained from two caged animals. One female was palpated as pregnant in July, aborted between 23 August and 15 September (after approximately 75 days of gestation) and was killed on 18 September. This animal was found to be in oestrus with one large late vesicular follicle close to rupture (1905 μm in diameter). A second animal aborted a 153 day fetus on 4 January. She was killed on 9 January and found to have neither intermediate or late vesicular follicles in the ovaries. This is consistent with conceptions occurring from May to December but not from January to April.

In the second pattern of breeding two oestrous cycles per year occur with two periods of pregnancy and lactation (FIGURE 74, 7-9). Conceptions in this pattern may occur from May to July and from October to December. These animals appear to prepare for the successive pregnancy by developing intermediate and late vesicular follicles in the non-active ovary during the latter stages of pregnancy. This is followed by a postpartum oestrus/ovulation.

5.3.5.2 Implantation

In three species of Megachiroptera reviewed by Wimsatt (1979), the uterine cornua and ovaries were physiologically equal but functionally asymmetrical, with ovaries and uterine horns alternating in successive years. Within a sample this resulted in 50 % implantations in the right and 50 % in the left cornu.

Although the left and right uterine horns and ovaries in E. wahlbergi appear equal in size and shape, it was found that out of 49 pregnant females, 69,7 % had a right implantation and 30,3 % had a left implantation and this difference was significant ($\chi^2 = 7,36$; $0,005 < P < 0,01$). However it was also observed that in females undergoing a second successive pregnancy (gravid and lactant females), the new implantation was in the other uterine horn, suggesting an alternation of cornu and ovary in successive pregnancies, in individuals having two per year.

Localized progestational endometrial reaction in the distal end of the uterine horn on the ovulating side has been recorded for Pteropus giganteus (Marshall, 1949; 1953), Rousettus leschenaulti (Gopalakrishna & Karim, 1971) and Cynopterus sphinx (Ramakrishna, 1950). A similar swelling at the distal end of the cornu, adjacent to the ovarian capsule was observed in E. wahlbergi (PLATE 33). By use of TABLE 57 the gestational age of the three earliest pregnancies were calculated at 19, 20 and 20 days. Serial sectioning of the swollen uterine horns showed that implantation had already taken place by these ages. Therefore, although the appearance of the swelling is characteristic of progestational endometrial reaction, it cannot be confirmed in this species because the pregnancies examined were all post implantational.

5.3.5.3 Transuterine ovular migration

Wimsatt (1979) noted that in Pteropids, transuterine migration of the ovum was impossible because of the septate uterus. This was confirmed in E. wahlbergi, where in 100 % of the pregnancies implantation and the corpus luteum were present on the same side.

5.3.5.4 Corpus luteum

The persistence of primary corpora lutea in mammals is variable depending on the extent to which the placenta takes over its hormonal function and at what stage in pregnancy. Mossman &

PLATE 33 Swollen uterine horn, situated at the cranial end of the uterus adjacent to the ovarian bursa, in a gravid female with a 20 day pregnancy
(x 3)



Duke (1973) reported that in four Megchiropteran species the corpus luteum persisted throughout gestation and in two other Microchiropteran species was still present in early lactation. In the one Megachiropteran species examined by them, Pteropus vampyrus, the corpus luteum prevailed until mid gestation. By contrast Gopalakrishna (1969) reported the persistence of the corpus luteum of one pregnancy into mid gestation of the succeeding pregnancy in Rousettus leschenaulti and suggested that this may be a means of ensuring alternation of ovarian function.

In this study no primary corpora lutea were found in non-pregnant animals. The single corpus luteum of pregnancy was visible to the naked eye as a yellow spot on the surface of the ovary, but was not extroverted. FIGURE 76 shows the relationship between luteal size and gestational age. Maximum size occurred during the first month of pregnancy and the corpus luteum could be completely absent from 130 days onwards. It was not found in animals immediately prior to parturition or during lactation.

5.3.5.5 Reproductive strategies

Regular palpation of caged females after first introduction of the male revealed no evidence to suggest that any of the three delaying tactics, delayed ovulation, implantation or embryonic development used by some Chiropteran species, were employed by E. wahlbergi. Constant fetal growth occurred throughout gestation as seen in FIGURES 57 and 58.

5.3.6 Fecundity

TABLE 21 presents a fecundity schedule for E. wahlbergi according to the method of Caughley (1977) suggested for birth-pulse populations. Females caught during the main season of births (November and December) over a four year period (1977 - 1980) have been used. E. wahlbergi is monotocous and a sex ratio at birth of 1 : 1 was assumed to calculate m_x (female births/female).

5.4 DISCUSSION

5.4.1 Anatomy and histology of the reproductive system

Ovarian and uterine characteristics observed in E. wahlbergi were found to

FIGURE 76 The relationship between corpus luteum size (expressed in mm^2) and gestational age (days) (n = 42)

$$y = 1,19 - 8,14 x; \quad r = -0,776; \quad P < 0,001$$

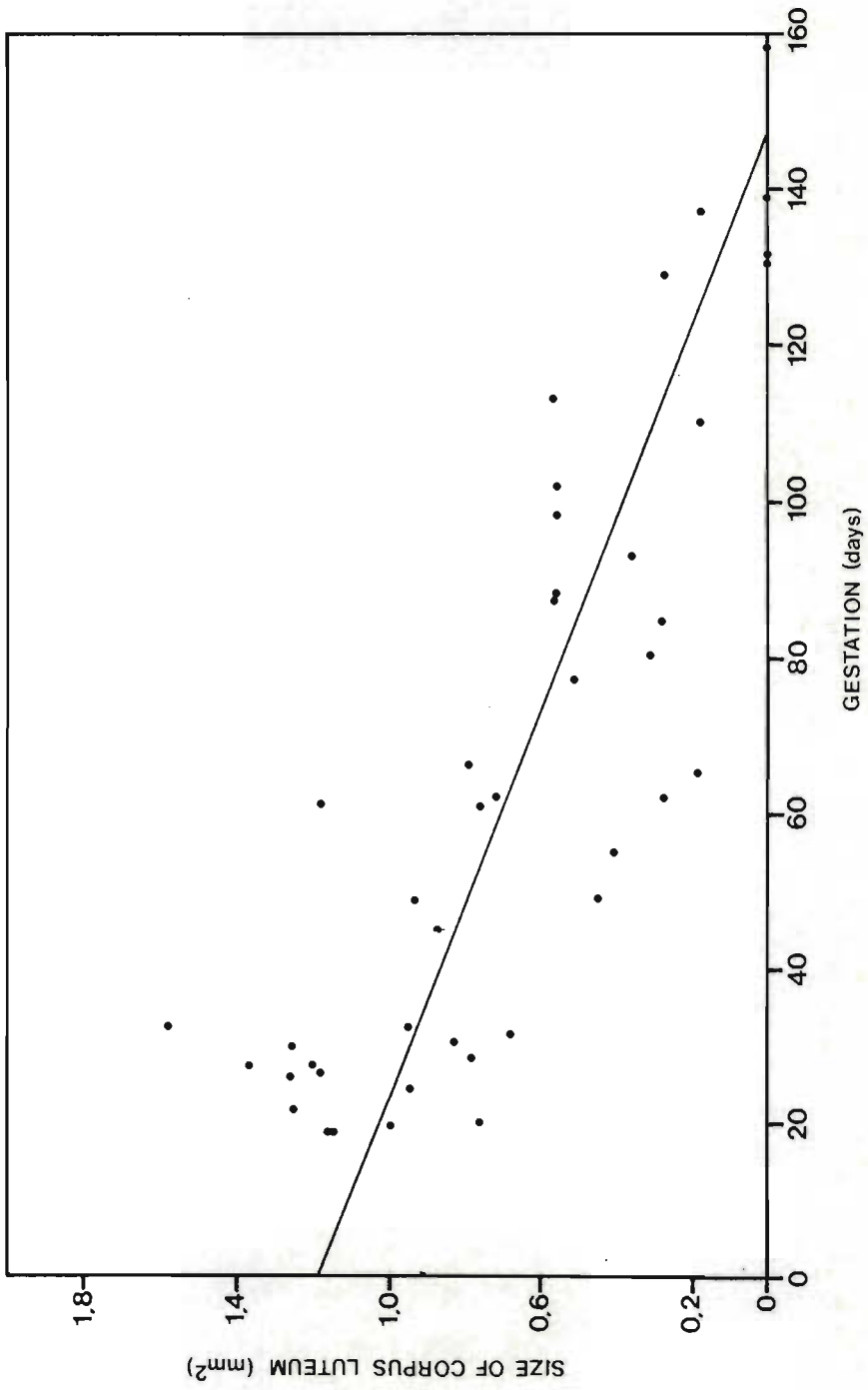


TABLE 21 Fecundity schedule for *E. wahlbergi*, constructed from data from females caught in November and December 1977 to 1980

| Age in years | Sampled number | Number pregnant or lactating | Female births/female
($B_x/2f_x$) |
|--------------|----------------|------------------------------|--|
| x | f_x | B_x | m_x |
| 1 (11-12 m) | 27 | 26 | 0,482 |
| 2 (23-24 m) | 13 | 13 | 0,500 |
| 3 (35-36 m) | 17 | 17 | 0,500 |
| 4 (47-48 m) | 4 | 4 | 0,500 |
| 5 (59-60 m) | 1 | 1 | 0,500 |
| 6 (71-72 m) | 0 | 0 | |
| 7 (83-84 m) | 1 | 1 | 0,500 |

be consistent with those described for other Chiropteran species.

The most notable ovarian feature of E. wahlbergi is the presence of a completely enclosed bursa ovarica with its incorporated simple uncoiled oviduct. Complete bursae ovaricae are common particularly in Insectivoran and Chiropteran orders (Matthews, 1941; Mossman & Duke, 1973). In the Megachiroptera they have been recorded in P. vampyrus (Mossman & Duke, 1973), P. giganteus (Moghe, 1951) and R. leschenaulti (Gopalakrishna & Choudhari, 1977). The ovary of P. giganteus is most similar in structure to E. wahlbergi having an enclosed simple looped oviduct, while that of R. leschenaulti is distinguished by the large slit-like opening in the bursa.

The bicornuate, septate uterus recorded in the study species is characteristic of the Megachiroptera (Wimsatt, 1979) and has been described for several Pteropids (Gopalakrishna & Choudhari, 1977; Grassé, 1955; Moghe, 1951; Mutere, 1968).

5.4.2 Sexual maturity

Sexual maturity, as defined by age at first oestrus, occurs in the first year, at approximately six months of age, 2,5 months before the attainment of the forearm length asymptote. This combined with the seasonal nature of the onset of conceptions and the wide range of ages (3,37 - 7,83 m) and masses (55 - 82 g) at first conception would suggest that sexual maturity is more closely related to the time of year in a birth-pulse situation, than to the age or size of the individual.

Attainment of female sexual maturity before attainment of full size was also noted by Bradbury (1977) for Hypsignathus monstrosus and Gopalakrishna & Choudhari (1977) for Rousettus leschenaulti. In all three cases the female became mature in the first year (5 - 7 m) and the male in the second year. This form of bimaturism has also been documented in several Microchiropteran species (Gopalakrishna & Choudhari, 1977; Racey, 1974a). The significance of this phenomenon apart from being examined in the specific Megachiropteran context as a possible pointer to lek mating (Bradbury, 1977 and 4.4.8), can be viewed in the general Chiropteran context. The monotocous habit is common to the Chiropteran order (Wimsatt, 1979) and is probably related to the weight distribution problems of carrying a developing fetus in flight. With the production of a single young at each parturition, methods to ensure maximum possible fecundity would therefore be advantageous and the sexual maturation of females in their first year, instead of during their second year (after physical maturity attainment) may be a strategy employed by some species to raise the overall productivity of the population.

5.4.3 Seasonality

E. wahlbergi exhibits a seasonally polyoestrous reproductive pattern of the extended season type with births from October to June. A small percentage of the female population undergo a second successive pregnancy immediately following the first.

The seasonally polyoestrous pattern is well documented among the Megachiroptera (excluding those of the monoestrous genera Eidolon and Pteropus) (Jerrett, 1979). Many exhibit clear bimodal patterns (Lissonycteris angloensis, Anciaux de Faveaux, 1978a; Hypsignathus monstrosus, Bradbury, 1977; Rousettus leschenaulti, Gopalakrishna & Choudhari, 1977; Rousettus aegyptiacus, Mutere, 1968; Epomophorus anurus, Okia, 1974b; Epomops franqueti, Okia, 1974a; or trimodal patterns, Cynopterus brachyotis, Liat, 1970). Two African Megachiropteran species, however, both of the genus Epomophorus, have been recorded as having an extended season of births very similar in timing to those recorded in this study for E. wahlbergi. Smithers (1971) noted births from November to February in Zimbabwe for E. crypturus and O'Shea & Vaughan (1980) reported a polyoestrous reproductive pattern for E. wahlbergi with births from November to May in Kenya (02° 18' S). It may be that the seasonally polyoestrous pattern with an extended birth season is characteristic of the study species regardless of its latitude position.

Baker & Baker (1936), Marshall (1946) and Anciaux de Faveaux (1978b) while investigating birth seasonality in equatorial Megachiroptera collated evidence to show that fruit bats with few exceptions, conceived in the autumn and gave birth in the spring, regardless of which hemisphere they lived in. A crossover zone, between 04° 13' N and 01° 32' S within which both austral and boreal reproductive patterns occurred, was designated by Anciaux de Faveaux (1978b) as the biological equator. South of this the austral pattern of March - May conceptions dominated, and north of this the boreal pattern of September - November conceptions dominated. Such evidence would suggest that the majority of species have a common consistent proximate trigger to breeding and several authors have suggested the decreasing autumn photoperiod (Baker & Baker, 1936; Marshall, 1946).

In order to test the universality of autumn conceptions (and therefore the decreasing daylength cue) in the face of recent work, and to see the relative position occupied by E. wahlbergi in this context, the conception timings of 54 Megachiropteran populations (28 species) were plotted against latitude (FIGURE 77). The majority of populations confirm the findings of Baker & Baker (1936) and Marshall (1946) with southern hemisphere conceptions occurring from February to May and northern

FIGURE 77 Conception timings in 54 Megachiropteran populations of 28 species

| Latitude | Species | Months of conception | Reference |
|---|---------------------------------|----------------------|-----------------------------|
| <u>Megachiroptera following the boreal reproductive pattern</u>
(thick solid line) | | | |
| 30° N | <u>Rousettus aegyptiacus</u> | Nov/Dec | Flower, 1932 |
| 30° N | <u>Rousettus aegyptiacus</u> | Sept/Oct | Anderson, 1902 |
| 23° N | <u>Pteropus giganteus</u> | Oct | Tickell, 1843 |
| 21° N | <u>Pteropus giganteus</u> | late Aug/early Sept | Moghe, 1951 |
| 13° N | <u>Rousettus arabicus</u> | Oct | Yerbury & Thomas, 1895 |
| 07° N | <u>Pteropus giganteus</u> | early Dec/early Jan | Marshall, 1946 |
| 07° N | <u>Pteropus melanotus</u> | Sept/Oct | Kloss, 1903 |
| 07° N | <u>Pteropus giganteus</u> | Dec | Phillips, 1924 |
| 06° N | <u>Pteropus ariel</u> | Nov | Gardiner, 1906 |
| 04° 13' N | <u>Lissonycteris angolensis</u> | Sept | Anciaux de Faveaux, 1978(a) |
| 04° N | <u>Eidolon helvum</u> | Sept/Oct | Anderson, 1912 |
| 03° 58' N | <u>Lissonycteris angolensis</u> | Sept | Anciaux de Faveaux, 1978(a) |
| 03° 30' N | <u>Lissonycteris angolensis</u> | Sept | Anciaux de Faveaux, 1978(a) |
| 01° 20' N | <u>Lissonycteris angolensis</u> | Sept | Anciaux de Faveaux, 1978(a) |

FIGURE 77 (continued)

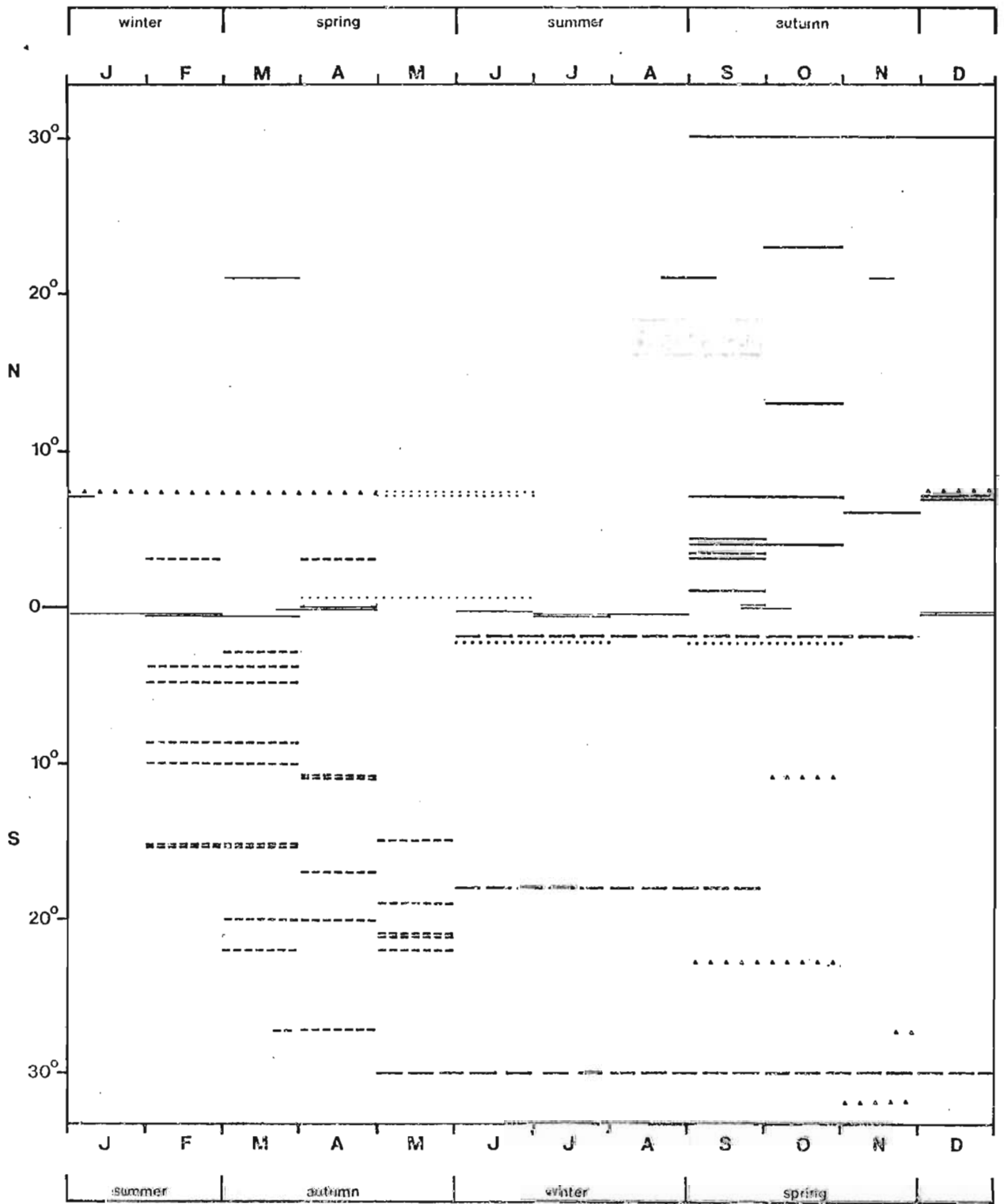
| Latitude | Species | Months of conception | Reference |
|--|---------------------------------|----------------------|-----------------------------|
| <u>Megachiroptera following the austral reproductive pattern</u>
(short dashes) | | | |
| 03° N | <u>Cynopterus brachyotis</u> | April | Thomas & Hartert, 1894 |
| 03° N | <u>Epomops franqueti</u> | Feb | Bates, 1905 |
| 03° S | <u>Macroglossus lagochilus</u> | March | Anderson, 1912 |
| 03° 53' S | <u>Lissonycteris angolensis</u> | Feb/March | Anciaux de Faveaux, 1978(a) |
| 05° 10' S | <u>Lissonycteris angolensis</u> | Feb/March | Anciaux de Faveaux, 1978(a) |
| 08° 40' S | <u>Lissonycteris angolensis</u> | Feb/March | Anciaux de Faveaux, 1978(a) |
| 10° S | <u>Pteropus morio</u> | Feb/March | Anderson, 1912 |
| 11° S | <u>Pteropus gouldi</u> | April | Ratcliffe, 1932 |
| 11° S | <u>Pteropus poliocephalus</u> | April | Ratcliffe, 1932 |
| 15° S | <u>Epomophorus crypturus</u> | May | Loveridge, 1922 |
| 15° 15' S | <u>Pteropus geddiei</u> | Feb/March | Baker & Baker, 1936 |
| 15° 15' S | <u>Pteropus eotinus</u> | Feb/March | Baker & Baker, 1936 |
| 17° S | <u>Pteropus conspicillatus</u> | April | Ratcliffe, 1932 |
| 19° S | <u>Pteropus rodricensis</u> | May | Pook, 1977 |
| 20° S | <u>Pteropus poliocephalus</u> | March/April | Ratcliffe, 1932 |
| 21° S | <u>Pteropus subniger</u> | May | Buffon, 1776 |
| 21° S | <u>Pteropus niger</u> | May | Buffon, 1776 |
| 22° S | <u>Pteropus ornatus</u> | May | Sanborn & Nicholson, 1950 |
| 22° S | <u>Notopterus macdonaldi</u> | March | Sanborn & Nicholson, 1950 |
| 27° 20' S | <u>Pteropus gouldi</u> | April | Nelson, 1965 (b) |
| 27° 20' S | <u>Pteropus poliocephalus</u> | late March | Nelson, 1965 (b) |

FIGURE 77 (continued) Exceptions to autumn conceptions

| Latitude | Species | Months of conception | Reference |
|---|---------------------------------|--|-------------------------------------|
| (a) <u>species with a bimodal cycle</u> (thin solid line) | | | |
| 21° N | <u>Rousettus leschenaulti</u> | mid Nov
& Mar | Gopalskrishna &
Choudhari (1977) |
| 00° 05' N | <u>Epomops franqueti</u> | April
& late Sept | Okia, 1974 (b) |
| 00° 05' N | <u>Epomophorus anurus</u> | late March/April
& late Sept/early
Oct | Okia, 1974 (b) |
| 00° 22' S | <u>Rousettus aegyptiacus</u> | Dec
& June | Mutere, 1968 |
| 00° 24' S | <u>Hypsignathus monstrosus</u> | July/Aug
& Dec/Feb | Bradbury, 1977 |
| 00° 30' S | <u>Lissonycteris angolensis</u> | Feb/March
& July | Anciaux de Faveaux,
1978 (a) |
| (b) <u>populations of Eidolon helvum with delayed implantation</u> (small dots) | | | |
| 07° 24' N | <u>Eidolon helvum</u> | May/June | Fayenuwo & Halstead,
1974 |
| 07° N | <u>Eidolon helvum</u> | May/June | Funnilayo, 1979 |
| 00° 20' N | <u>Eidolon helvum</u> | April/June | Mutere, 1967 |
| (c) <u>populations of Eidolon helvum without delayed implantation but migratory in habit</u> (big dots) | | | |
| 02° 36' S | <u>Eidolon helvum</u> | Sept/Oct | Anciaux de Faveaux,
1978(b) |
| 02° 36' S | <u>Eidolon helvum</u> | June/July | Anciaux de Faveaux,
1978(b) |
| (d) <u>species with extended season of conceptions</u> (long dashes) | | | |
| 02° 18' S | <u>Epomophorus wahlbergi</u> | June/Nov | O'Shea & Vaughan 198 |
| 18° S | <u>Epomophorus crypturus</u> | June/Sept | Smithers, 1971 |
| 30° S | <u>Epomophorus wahlbergi</u> | May/Dec | present study |

FIGURE 77 (continued) Exceptions to autumn conceptions

| Latitude | Species | Months of conception | Reference |
|--|------------------------------|----------------------|------------------|
| (e) <u>paradoxical species</u> (triangles) | | | |
| 07° N | <u>Cynopterus brachyotis</u> | Dec/April | Phillips, 1924 |
| 11° S | <u>Pteropus scapulatus</u> | Oct | Ratcliffe, 1932 |
| 23° S | <u>Pteropus scapulatus</u> | Sept/Oct | Ratcliffe, 1932 |
| 27° 20' S | <u>Pteropus scapulatus</u> | late Nov | Nelson, 1965 (b) |
| 32° S | <u>Pteropus scapulatus</u> | Nov | Ratcliffe, 1932 |



hemisphere conceptions from September to December.

However, five categories of exceptions were noted. (a) Those species with a bimodal cycle which tended to conceive in both spring and autumn, and with the exception of R. leschenaulti, (Gopalakrishna & Choudhari, 1977) occurred within 1° of the equator, (b) Populations of Eidolon helvum which exhibited delayed implantation, but whose births still occurred in the spring. (c) Populations of Eidolon helvum which did not exhibit delayed implantation but whose reproductive pattern was explained by its migratory habit (Anciaux de Faveaux, 1978b). (d) Those species with extended season conceptions, whose conceptions began at the end of autumn and continued into spring/summer. This category included two populations of E. wahlbergi (including the study population) and one population of E. crypturus. (e) The paradoxical species for which no explanation could be found. These were four populations of Pteropus scapulatus and one population of Cynopterus brachyotis.

These five categories of exceptions to the autumn conception pattern may represent seasonal breeders cued either by increasing daylength or by a proximate factor other than daylength.

5.4.4 Environmental factors affecting reproduction

The evolution of seasonal breeding is not surprising since natural selection favours the survival of species whose young are born during the season best suited for meeting the challenges of the environment. What is intriguing is that seasonal breeders have acquired the capacity for successively turning their reproductive systems on and off (Karsch & Foster, 1981). It has long been recognized that the environment has an important role to play in the regulation of reproductive function. Reproduction like all other physiological processes will react to different environmental stimuli and these are frequently employed to trigger off the onset of breeding or bring about its cessation (Gilmore & Cook, 1981).

Proximate triggers

In recent years a great variety of techniques and subject species has been applied to the problems of the environment and its multi-faceted effects on reproduction, with amazingly variable results. Most authors however agree that amongst mammals, changing photoperiod is probably the most important external environmental stimulus in controlling reproductive activity (Herbert, 1977); Karsch & Foster, 1981; Sadleir, 1969b; Tucker, 1981).

It was for this reason that photoperiod was investigated as a proximate

trigger to breeding in E. wahlbergi, particularly as the study population at 30° S is subjected to considerable daylength variations during the year. The results show however that shortening the photoperiod during January to June daylength (when oestrus/ovulation/fertilization occurred in the majority of the population), failed to produce signs of either proestrus or oestrus in the ovaries of the experimental females. The experimental animals were killed in March and their ovaries and uteri were found not to differ significantly with those of the March routine sample (except in uterine epithelial height).

The results of this experiment may be interpreted in one of two ways. Either, photoperiod is the proximate trigger to oestrus onset, but the experimental conditions inadequately represented the natural conditions, or photoperiod is not the effective stimulus in this case.

If photoperiod is the proximate stimulus, the experimental design or technique may have been at fault in one of three spheres.

Unsuitable exposure. The experimental females were killed after two weeks of photoperiod adjustment and five weeks at the shortened daylength. The natural process of gradually increasing daylength after reaching the minimum photoperiod was not simulated. As the maximum number of conceptions occurred during the month of shortest daylength, perhaps the effective trigger is not decreasing photoperiod, or short daylength, but the start of increasing photoperiod, as in 'long day' species.

Light quality. It has been suggested by some authors that the quality of light, its wavelength composition and intensity, may be the triggering factors (Baker & Baker, 1936; Herbert, 1977; Marshall, 1946). Although the light source used in the experiment was chosen for its suitability to biological systems, it may have failed to simulate the required light quality.

Incorrect trigger. It was assumed at the start of experimentation that the trigger to be examined was for oestrus, and therefore the daylength prevalent at the time of maximum conceptions, was used to induce it. However it is possible that oestrus/ovulation builds up from proestrus without a separate cue and it is the photoperiod trigger for this event (decreasing April daylength) which should have been used.

The only further evidence to suggest that photoperiod may not be the proximate stimulus to the onset of breeding in this species, is its inclusion in the category of exceptions to autumnal conceptions (5.4.3 and FIGURE 77).

Conceptions do not occur in the months of January to April. Photoperiod may not be responsible for breeding onset, but a future investigation into the start of decreasing photoperiod as a trigger for breeding cessation, may be worthwhile.

Male presence

E. wahlbergi developed late vesicular follicles in the absence of sight, sound or smell of males, but no evidence that ovulation had occurred was seen. It can be surmised that oestrus will occur in the absence of visual, audible or pheromonal male stimuli but it was not proved that ovulation will occur in the absence of a male. It is possible that evidence of ovulation was not observed because E. wahlbergi is a reflex ovulator, requiring coital stimulation to induce ovulation. It has been suggested that reflex ovulation is prevalent in animals that are widely dispersed, whereas spontaneous ovulation is found in gregarious species (Zarrow & Clark, 1968). Unlike cavern-dwelling Microchiroptera, E. wahlbergi may roost singly or in small numbers of up to twenty (observed in this research), each observing a strict interpersonal distance. Such behaviour is not truly gregarious and may be indicative of a possible reflex ovulator, but only detailed investigation would confirm this.

These ovulatory trigger experiments were preliminary and because of the limited numbers of cage acclimatized animals could not be replicated. The conclusions drawn from the results are therefore tentative and interpretations must be viewed cautiously in the light of the limited nature of the experiments.

Ultimate triggers

Food availability and rainfall

Sadleir (1969a) commented that there can be little doubt that the annual fluctuations in availability and nature of food are the most important ultimate ecological factors in the timing of seasonal breeding. The heavy nutritional demands of lactation (Sadleir, 1969a; Widdowson, 1981) prompted the examination of food availability and the closely related factor of rainfall, in relation to the timing of lactation in E. wahlbergi. During a three year period it was found that the principle months for lactation coincided with the two months having the greatest percentage of fruiting trees and the three months of highest rainfall.

Similar findings have been documented for other Megachiropteran species, implicating both food availability and rainfall as the ultimate factors in

the timing of births (Liat, 1970; Mutere, 1967; 1968; Okia, 1974a & b). The coincidence of birth season with the annual rainfall peak (or wet seasons) have been recorded for Eidolon helvum (Fayenuwo & Halstead, 1974; Mutere, 1967), Rousettus aegyptiacus (Mutere, 1968) and for E. wahlbergi (O'Shea & Vaughan, 1980), confirming the findings of this study.

5.4.5 Breeding cycle

Oestrous cycle

In many mammals the corpus luteum of pregnancy inhibits ovarian follicular development and so suppresses oestrus and ovulation (Herbert, 1977). An unusual feature of the non-luteal ovary of E. wahlbergi during pregnancy, in a small percentage of the female population, is the presence of intermediate and late vesicular follicles at the beginning and towards the end of gestation. This phenomenon, though rare in most mammalian orders, has been observed in the Megachiropteran, Pteropus giganteus (Marshall, 1949) and in a Microchiropteran Miniopterus schreibersi (Bernard, 1980). Marshall (1949) suggested that the significance of 'this continuous egg-formation' during pregnancy, may be that if abortion occurs, ovulation and conception may take place quickly. This theory closely corresponds with that suggested to explain the 'tail end' conceptions after the May to July peak in the study animal and would be the most likely explanation for the developing vesicular follicles during the first third of pregnancy.

The presence of these follicles in the latter stages of pregnancy was interpreted in this research as being evidence of a proestrus/oestrus in preparation for a postpartum oestrus/ovulation/pregnancy. Postpartum pregnancies have been recorded in African pteropid species (Lissonycteris angolensis, Anciaux de Faveaux, 1978a; Epomops franqueti, Okia, 1974a; Epomophorus anurus, Okia, 1974b) and in Asian pteropid species (Rousettus leschenaulti, Gopalakrishna & Choudhari, 1977; Cynopterus sphinx, Ramakrishna, 1947). As in the study species, Gopalakrishna & Choudhari (1977) noted that in R. leschenaulti fewer females participated in the second pregnancy than in the first.

Implantation

E. wahlbergi was found to exhibit a clear ovarian dextral dominance in function despite apparent anatomical symmetry. This was in direct contrast to the 50% - 50% implantational distribution in six other pteropid species (Gopalakrishna, 1964; Marshall, 1946; 1949; Moghe, 1951; Mutere, 1967; 1968; Okia, 1974b; Nelson, 1965a).

Wimsatt reviewed the pteropid pattern as involving a non-random alternation of ovulation between left and right ovaries in successive

years, resulting in equal numbers of implantations in left and right uterine horns within a population. There is evidence to suggest that in the small percentage of E. wahlbergi females undergoing a postpartum pregnancy, this ovarian alternation does occur, but the overall picture is one of dextral dominance. A progesterational endometrial reaction was not confirmed as occurring in E. wahlbergi. It is however listed by Wimsatt (1979) as a characteristic feature of the pteropid pattern and has been described in P. giganteus by Marshall (1949; 1953), in R. leschenaulti by Gopalakrishna & Karim (1971) and mentioned as occurring in E. helvum (Funmilayo, 1979). Marshall (1949) suggested that the uterine modifications which ensured a single implantation taking place as anteriorly as possible, may be an adaption to an arboreal and aerial habit. The anteriorly placed embryo relieves strain on the uterine ligaments when the mother is resting in the head down position, and is closest to the centre of gravity in flight.

Transovular migration

Transovular migration is common in two genera of Chiroptera, Myotis and Miniopterus, but is impossible in the Pteropids because of a uterine septum. This was confirmed in E. wahlbergi, where the gravid uterine horn and the ovary carrying the corpus luteum of pregnancy occurred on the same side in all cases.

Corpus luteum

The pattern of growth and survival of the corpus luteum of pregnancy varies very widely between species. Even in the order Chiroptera there are examples of the corpus luteum being lost at an early stage in pregnancy (Nycteris luteola and Triaenops afer, Matthews, 1941), persisting until midway through gestation (Pteropus vampyrus, Mossman & Duke, 1973), persisting towards the end of gestation (Tadarida brasiliensis, Mossman & Duke, 1973) and even persisting into lactation (Eptesicus fuscus, Mossman & Duke, 1973).

In E. wahlbergi after maximum size was attained in early gestation, the corpus luteum regressed in size until it was absent by the end of gestation. This luteal regression would suggest a gradual take over of endocrine function by the placenta probably starting from an early gestational stage.

Reproductive strategies

Mossman & Duke (1973) remarked on the 'bizarre reproductive phenomena' often displayed by the Chiroptera. Notable in this respect are the variety of gestation length delaying tactics employed, particularly by the

Microchiroptera (Oxberry, 1979; Rasweiler, 1979). One example in a Megachiropteran species has been found to occur differentially in populations of E. helvum. In some populations E. helvum exhibits delayed implantation of about 3-4 months duration and seems designed to ensure that births will occur at the onset of a rainy season during which abundant food supplies are available (Fayenuwo & Halstead, 1974; Funnilayo, 1979; Mutere, 1967). Other populations of the species in tropical Africa are reported as not undergoing delayed implantation and the gestation length is reduced to four months (Anciaux de Faveaux, 1978b).

No form of reproductive delaying strategy has been observed in E. wahlbergi, but if gestation length is compared with mean male forearm length in the 13 species of fruit bat (for which data was available) plotted in FIGURE 78, it will be seen that the two species of Epomophorus (ringed) tend to have a long gestation for their size.

5.4.6 Fecundity

For a monotocous species, E. wahlbergi has a high fecundity rate of $m_x = 0,500$, unvarying from two to seven years of age. Older animals which may have shown reproductive senescence, were not caught during November and December, and as a result no indication of age at reproductive senescence may be made.

A high fecundity rate is especially essential to the survival of the species when only one infant at each parturition is produced and the majority of the female population only undergo one pregnancy per year.

5.5 SUMMARY

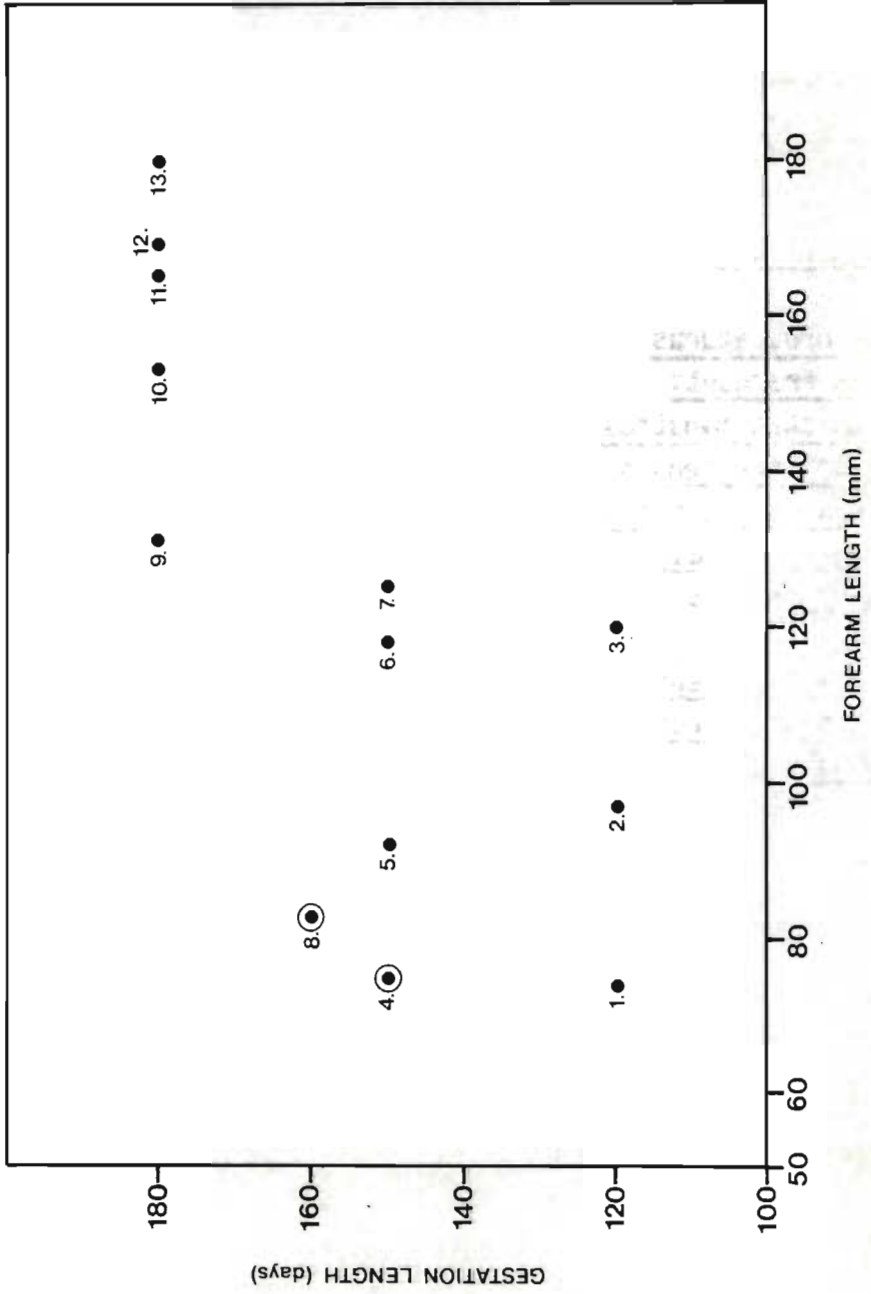
The anatomy and histology of the female reproductive system was investigated. The ovary is completely enclosed in an ovarian bursa and the uterus is bicornuate and septate.

Puberty was found to commence at a mean age of 2,5 months and terminate with sexual maturity at six months. The mean age at proestrus onset was 5,6 months and at first conception 6,2 months.

E. wahlbergi displays a seasonally polyoestrous reproductive pattern with an extended season. Conceptions occur from May to December, the peak months being May, June and July. Births occur from October to June with the peak birth season in November and December. The majority of females undergo one pregnancy per year, terminating around November/December with a small percentage terminating around April.

FIGURE 78 Gestation length in 13 species of fruit bat in relation to mean male forearm length

| Species | Gestation length
(days) | Mean male
forearm
length (mm) | Reference |
|--|-----------------------------|-------------------------------------|--------------------------------|
| 1 <u>Lissoncyteris
anglolensis</u> | 120 | 74 | Anciaux de
Faveaux 1978 (a) |
| 2 <u>Rousettus
aegyptiacus</u> | 120 | 97 | Mutere, 1968 |
| 3 <u>Eidolon helvum</u> | 120 (no delayed
implant) | 120 | Anciaux de
Faveaux, 1978(b) |
| 4 <u>Epomophorus anurus</u> | 150 (ringed) | 75 | Okia, 1974 (b) |
| 5 <u>Epomops franqueti</u> | 150 | 92 | Okia, 1974 (a) |
| 6 <u>Hypsignathus monstrosus</u> | 150 | 118 | Bradbury, 1977 |
| 7 <u>Pteropus rodricensis</u> | 150 | 125 | Pook, 1977 |
| 8 <u>Epomophorus wahlbergi</u> | 160 (ringed) | 83 | present study |
| 9 <u>Pteropus scapulatus</u> | 180 | 131 | Nelson, 1965 (b) |
| 10 <u>Pteropus ornatus</u> | 180 | 153 | Sanborn &
Nicholson, 1950 |
| 11 <u>Pteropus poliocephalus</u> | 180 | 165 | Nelson, 1965 (b) |
| 12 <u>Pteropus giganteus</u> | 180 | 169 | Marshall, 1946 |
| 13 <u>Pteropus gouldi</u> | 180 | 180 | Nelson, 1965 (b) |



Primordial, primary, secondary, and early vesicular follicles were present in the ovaries year round. A significant increase in numbers of early vesicular follicles occurred in April/May/June. Intermediate follicles were only found from April to November and late vesicular follicles from June to October. No seasonal variation in lumen diameter, endometrial or myometrial thickness was recorded. A bimodal pattern of significantly greater uterine epithelial height occurred in May/June and again in November/December. Similarly a bimodal pattern of significantly larger numbers of endometrial glands occurred in July/August/September and again in December.

Proximate triggers for oestrus were examined. It was found oestrus can occur in the absence of a male, but the results of experimentally shortening the photoperiod were inconclusive. Conception peaks coincided with shortest daylength, lowest rainfall, temperatures and humidity. Fruiting and rainfall were suggested as ultimate causes of breeding in E. wahlbergi as the peak lactation period coincided with rainfall and fruiting maxima.

A series of timetables showing variations in the timing of the annual reproductive cycle, with one and two pregnancies, is presented. The presence of intermediate and late vesicular follicles in the non-luteal ovary at the beginning and towards the end of pregnancy was explained as a precaution against an early abortion in the cases of developing follicles in early gestation, and as preparation for a postpartum pregnancy in the case of developing follicles at the end of gestation.

The ovaries and uterine horns were found to show functional dextral dominance with 69,7 % implantations in the right and 30,3 % in the left, although anatomically they appear symmetrical. There was evidence to suggest that alternation of ovarian function between left and right occurred in those animals having two pregnancies per year. A localized progesterational endometrial reaction may occur in the study species but this was not confirmed. No transovular uterine migration occurred.

The corpus luteum reached maximum size at the beginning of gestation and regressed through pregnancy until it was absent by the end of gestation. No gestational delaying strategies were apparent and the fecundity rate was considered high for a monotocous species ($m_x = 0,500$ for all ages except one year).

The occurrence of the phenomena recorded in the study species were compared to those found in other Megachiropteran and Microchiropteran species. A review of the timing of conceptions of 54 populations of fruit bat (from 28 species) between latitudes 35° N and S was carried out

showing a distinct pattern of autumn conceptions and spring births. It was found that the two populations of E. wahlbergi for which there were data, did not fit this pattern.

Comparison of gestation length with size in other pteropids showed that E. wahlbergi has a long gestation period for its size.

CHAPTER SIX

PARTURITION, LACTATION, MATERNAL CARE AND JUVENILE BEHAVIOUR

6.1 INTRODUCTION

In 1960 (b) Wimsatt remarked that the most neglected aspect of reproduction in bats is the process of parturition. He cited 19 references to original descriptions of birth in bats, the majority being in the family Vespertilionidae. Recent examination of the literature has revealed at least 32 accounts of parturition covering 25 species and seven families.

Details of the birth process are varied from species to species, although birth itself seems to occur consistently during the hours of daylight and particularly around noon (Bhatnager, 1978; Bogan, 1972; Fölsch, 1967; Gopalakrishna, Khaparde & Sapkal, 1976; Wickler & Seibt, 1976; Mallinson, pers. comm.). Two fetal presentations at delivery have been observed; head first in 13 species and breech in 12 species. All five species of pteropid so far recorded have shown the head presentation at birth (*R. aegyptiacus*, Kulzer, 1969; *P. poliocephalus*, Nelson, 1965 (b); *Cynopterus sphinx*, Ramakrishna, 1950; *E. wahlbergi*, Wickler & Seibt, 1976; *P. giganteus*, Fölsch, 1967; Mallinson, pers. comm.) as have four phyllostomatids (Blake, 1885; Bhatnagar, 1978; Jones, 1946; Tamsitt & Valdivieso, 1965b; 1966), and one species each of the families Rhinopomatidae, (Anand Kumar, 1965), Hipposideridae, (Ramakrishna, 1950) and Megadermatidae (Gopalakrishna, Khaparde & Sapkal, 1976). One species of vespertilionid (*Nyctalus noctula*, Daniell, 1834; Ryberg, 1947; Whitaker, 1905) has also consistently shown a head first delivery presentation, though in the majority of this family, breech presentation is usual (Wimsatt, 1960b).

Postures adopted during labour are also variable. Three main postures have been reported: head down, hanging vertically in the characteristic resting position with straddled legs (Bhatnagar, 1978; Goguyer & Gruet, 1957; Gopalakrishna, Khaparde & Sapkal, 1976; Jones, 1946; Ramakrishna, 1950; Sheman, 1937; Whitaker, 1905), horizontal with abdomen uppermost and ventrally recurved tail clinging to a horizontal surface by wing claws and feet (Bogan, 1972; Pearson, Koford & Pearson, 1952; Wimsatt, 1960b) and an inverted head upward position, also with ventrally recurved tail, clinging to a vertical surface by wing claws (Wimsatt, 1960b).

Other variable aspects of parturition reported in the Chiroptera are

length of labour and delivery, timing of amnion rupture and placental birth, and the extent of placentophagia. Infant bats are large at birth, some species weighing as much as 20 % of the maternal postpartum mass (Wimsatt, 1960b). The majority are born with eyes closed, including R. aegyptiacus (Kulzer, 1958), though some species have been observed with open eyes at birth, Artibeus jamaicensis (Bhatnagar, 1978), Megadenna lyra (Gopalakrishna, Khaparde & Sapkal, 1976), Artibeus planirostris (Jones, 1945; 1946), Tadarida brasiliensis (Jones, 1945) and the pteropid species, Pteropus poliocephalus (Nelson, 1965b) and Cynopterus sphinx, (Ramakrishna, 1950). Many are naked with fine downy hair on the head and extremities (Wimsatt, 1960b), others are furred on the head, back and forearm and completely naked on the ventrum (Gopalakrishna, Khaparde & Sapkal, 1976; Kulzer, 1969; Nelson, 1965b). The head, feet and trunk are disproportionately large with shortened forearms and small but fully formed wings (Kulzer, 1969; Wimsatt, 1960b).

The first aim of this chapter therefore is to describe the entire parturitional process in E. wahlbergi, covering the aspects of time of day when births occur, length and event sequences in labour and delivery, maternal delivery posture, fetal birth presentation, placentophagia and the appearance of the infant at birth.

After parturition, the second and major phase of maternal behaviour is lactation, when the young are dependent on the mother for nourishment. In Megachiroptera this phase has been reported as lasting 35 to 40 days in Rousettus leschenaulti (Gopalakrishna & Choudhari, 1977), four months in Pteropus poliocephalus (Nelson, 1965b), 23 weeks in P. rodricensis (Pook, 1977), four months in Rousettus sp. and three to four months in Pteropus poliocephalus (Ratcliffe, 1931) and three to four months in Pteropus ornatus (Sanborn & Nicholson, 1950).

Shillito Walser (1977) wrote that lactation can be seen as a series of behaviour patterns which are elicited by the different stages of the developing young and which relate to the way of life of the animal concerned. A further aim of this chapter is to determine the length of the lactation period, the chemical composition of the milk and the approximate age of the young at weaning, and also to describe the mother/juvenile behavioural patterns during this phase.

Nelson (1965b) described the activity stages in the early life of P. poliocephalus. For the first few days the young was rarely seen away from the nipple and up to three weeks old was carried in flight by the mother on feeding expeditions. After this age, although unable to fly the infants were left behind in the roost until flight was initiated at three months of age. Carroll (1981) reported that young P. rodricensis of perhaps six weeks of age were still carried by the mother in flight.

The final aim of this chapter is to describe the stages in juvenile behaviour tracing its decreasing dependence on the mother until flight is initiated and the young bat ceases to go to the mother for protection.

6.2 MATERIALS AND METHODS

6.2.1 Parturition

From November 1978 to December 1980 23 females (cage acclimatized for a minimum period of four months), gave birth in captivity to 28 juveniles, 15 males and 13 females. Five of the mothers gave birth twice in the cage in successive years. The time of day in which delivery took place was recorded in nine of the 28 births and the entire process of parturition was witnessed on four occasions. One of these was a natural birth and three were induced. Detailed observational records were kept and a stopwatch was used to time events. One birth was photographed with a still camera and a second was filmed with a 16 mm cine camera.

Birth induction

Wimsatt (1960b) successfully hastened parturition in Myotis lucifugus with an intra-peritoneal oxytocin injection. In this study however it was decided to examine induction techniques which did not involve either injections or incisions, in order to reduce trauma and so increase the chances of success. A variety of birth induction methods are used in human gynaecology (Philpott, pers. comm.). A human method, suitable for modification for small mammal application, was selected for use here.

Cage acclimatized females which were close to term (judged by palpation of the fetal cranial breadth at 16 mm or more) were chosen. It had been observed in the study that pregnant mothers were always first seen with the new born young around midday, so the induction procedure was initiated between 08h00 and 09h00. A single tablet of 'Prostin E' (Upjohn Limited) was divided into four. One quarter was shaved into a rounded cylindrical suppository, lightly smeared with vaseline and inserted into the vagina. A spray of 'Syntocinon' (Sandoz Limited) was administered onto the nasal membranes. The bat was then removed to a small holding cage within the main cage. The procedure was used successfully on three parous gravid females.

Infant at birth

Infants were photographed, weighed, forearm length, eye-nose distance and

zygomatic width measured, dentition examined and ear tagged immediately after the placenta had been lost.

6.2.2. Lactation and mother/juvenile relations

Eight of the 28 infants born in captivity were reared until weaned. Length of lactation and age of infant at weaning was determined by observation and regular capture of the mother with infant (at a minimum of three and a maximum of fourteen day intervals) in order to determine if milk could be expressed from the nipples. Observations of mother/juvenile behaviour during the lactation phase were made by sitting in the cage for one hour at varying times over a 24 hour period, nocturnal observations being made with a low intensity light.

Milk analysis

Information regarding the chemical composition of bat milk is limited to that provided by Huibregtse (1966) who determined the specific gravity, Kjeldahl protein, carbohydrate (lactose), total solids and ash content of milk obtained from two Microchiropteran species, Leptonycteris sanborni and Tadarida brasiliensis. By comparison with the insectivorous diet of T. brasiliensis, L. Sanborni's diet of pollen nectar and E. wahlbergi's diet of fruit, provide a high carbohydrate intake. Milk analysis was undertaken in order to compare the results with those of Huibregtse (1966) and examine to what extent the diet is reflected in the chemical composition of the milk.

Milk was collected from six caged mothers and seven wild mist-netted mothers. All were treated with 'Syntocinon' nasal spray (Sandoz Limited) to assist milk 'let down', then 'milked' by gently squeezing the nipples. This was done three or four times at twenty minute intervals, allowing the milk to run into a sterile plastic vial. A total of approximately 1,00 ml was collected and stored in a deep freeze at -15°C prior to analysis.

The milk analysis was carried out by Professor G V Quicke of the Department of Biochemistry, University of Natal, Pietermaritzburg and is fully described in Quicke and Sowler (in press). Total solids were estimated by weighing milk-saturated, dried filter paper and fat was estimated by reweighing the filter paper after fat extraction by solvents. Protein was determined by micro-Kjeldahl procedure, followed by direct Nesslerisation of the diluted digest. Carbohydrates were estimated as 'total neutral carbohydrates' using the method of Du Bois, Gilles, Hamilton, Rebers & Smith (1956), and as lactose using the Nelson-Somogyi procedure as modified by Marais, De Wit & Quicke (1966).

6.2.3 Juvenile behaviour

Of the 28 cage born juveniles, 18 lived to at least 30 days old, 13 to at least 60 days old and seven were over three months of age when released. The breeding colony was housed in a free flight aviary (PLATE 34), made of 70 % shade cloth stretched over a wooden pole frame measuring 4 m wide, by 8 m long and 4 m high.

Observations from within the cage were made for an hour in the morning, an hour around noon, an hour at dusk and for 30 minutes three or four times between dusk and midnight.

The young bats were caught for weighing, measuring and tooth examination at a minimum of three day and a maximum of fourteen day intervals. At these times, diet and flight development were assessed. Fruit was offered by hand to determine the earliest age at which it would be readily accepted and eaten. Progressive development of flight ability was tested at each capture by first launching the young bat into the air. If he fell, glided down or level, without gaining altitude, he was regarded as unable to fly. If he flew level and gradually gained altitude he was assessed at the Stage 1 phase of flight (just being able to fly). Once this stage was attained, the young bat was placed on the floor of the cage. If he could just lift off from the ground and slowly gain height he was judged to be at Stage 2. Active, fast eager flight from the hand and an observed readiness to fly when disturbed was regarded as competent flight (Stage 3).

6.3 RESULTS

6.3.1 Parturition

6.3.1.1 Time of day of birth

The mean time of day delivery took place in the natural births was 13h11 (range = 11h00 - 15h20, n = 6), in the induced births 15h10 (range = 12h13 - 17h34, n = 3) and in all nine births, 13h51 (range = 11h00 - 17h34).

6.3.1.2 Labour and Delivery

Posture and behaviour during labour

In all observed cases the usual resting position of head down and hanging by the feet from a branch, was adopted throughout the process of parturition (PLATE 35). Uterine contractions

PLATE 34 Captive breeding colony hanging from corner
of free flight aviary constructed from shade
cloth



PLATE 35 Gravid parous female at term, during labour



were accompanied by slightly opened wings, arched back, upthrusting of the pelvic region and occasionally, closed eyes. During the rest periods between contractions the wings were folded tightly around the body and sometimes a gentle side to side swaying movement was observed. From fifteen minutes before delivery, and between the strong periods of contraction, the vaginal region, the ventral chest fur and the wing membranes were washed repeatedly.

Delivery and fetal head presentation

At full cervical dilation, strong contractions delivered the infant's head (PLATE 36). In all births witnessed, the fetal presentation at delivery was head first. As soon as the head emerged the mother bent upwards and vigorously washed it with her tongue. A brief rest of three to five minutes followed. With a single further contraction, the body was born (PLATE 37) and caught in the mother's partially opened wings.

Throughout the entire labour and delivery period, the parturient females were markedly quiet and oblivious to external distractions.

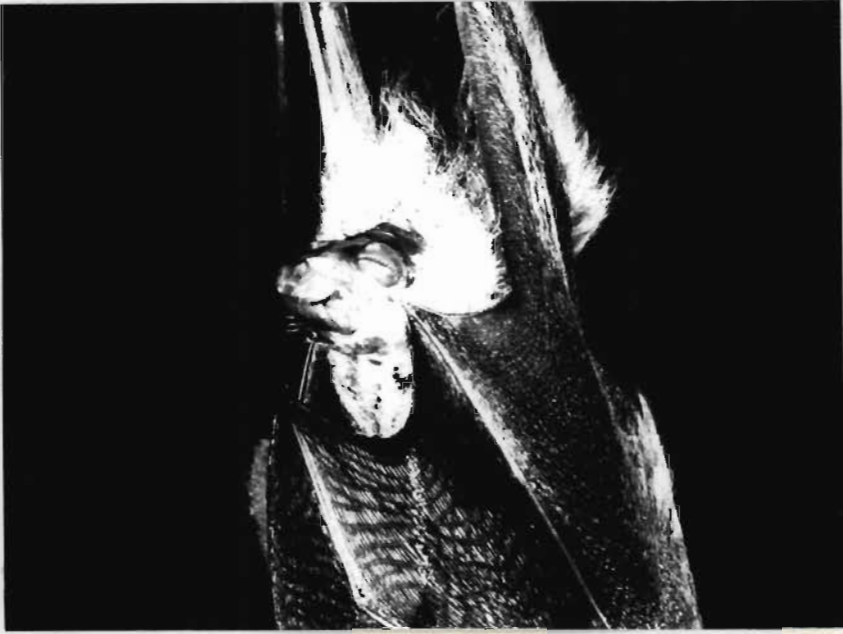
Postpartum behaviour and placental delivery

Immediately following emergence, the new born hung motionless within the mother's circled wings, suspended by the umbilical cord from the vaginal orifice. Immediate and thorough washing ensued (PLATE 38). Within ten minutes of birth, the young, assisted by 'juggling' movements of the mother's wings, gripped the maternal chest fur with feet and wing claws and at the same time was first heard to emit high pitched squeaks. During the hour following birth much washing and 'wing juggling' by the mother was observed (PLATE 39), accompanied by active crawling of the infant (using feet and wings) over the mother's chest and abdomen. This activity was interpreted as searching for the axially situated, pendulous nipples. Within 60 - 90 minutes a nipple was usually located by the blind offspring, (PLATE 40) assisted by maternal wing movements. By sinking its tiny pointed deciduous teeth (3.3.2) into the fleshy teat, it became securely attached to the mother, a hold it could only be made to relinquish by forcing open the jaws. A single discordal placenta was delivered only after nipple attachment was achieved, usually about two hours after birth (see below and TABLE 22).

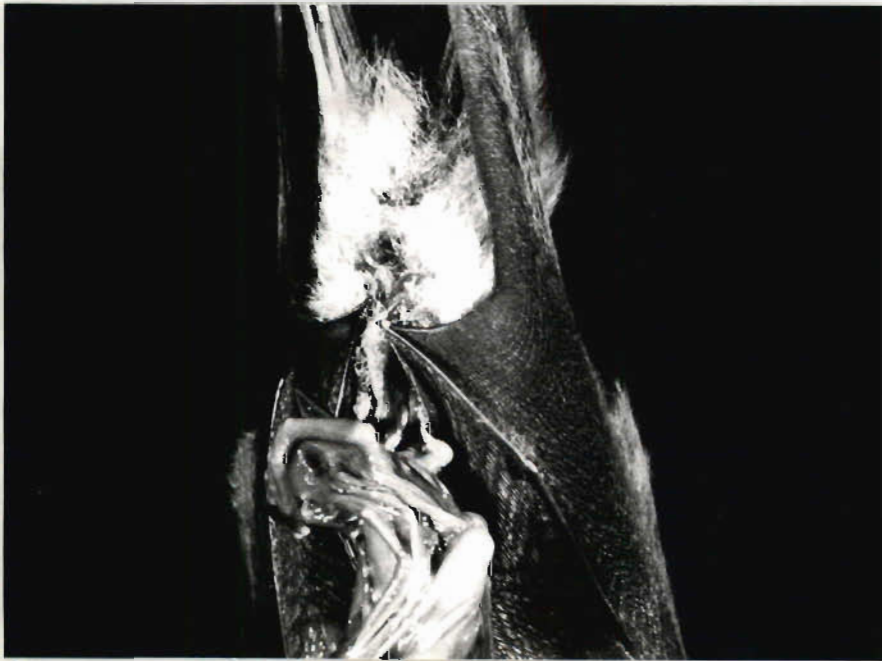
Event sequence and timing of labour and delivery

PLATE 36 E.T. 7864 at head delivery, showing the head first presentation of the fetus, after 165 minutes of labour

PLATE 37 E.T. 7864 at body delivery, three minutes after head delivery



36



37



38



39



PLATE 40 Suckling position adopted by neonate within
two hours of birth

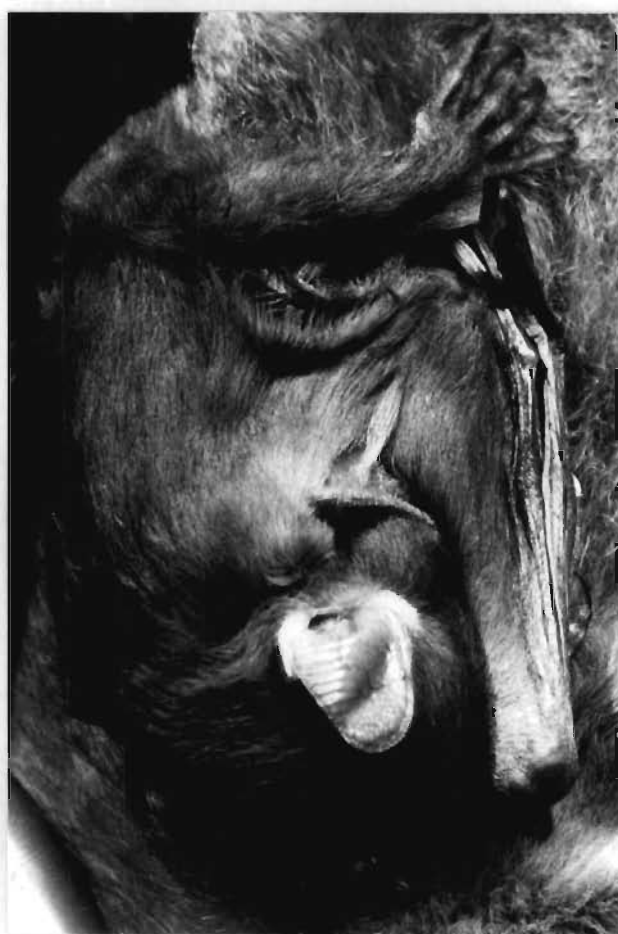


TABLE 22 Timing of labour stages and delivery in three induced parous gravid females and one uninduced primigravid female

| Ear tag no.
of bat | INDUCED | | | | | | UNINDUCED | |
|--|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| | 627 | | 7864 | | 7883 | | 392 | |
| EVENT | time
period
(mins) | accum.
time
(mins) | time
period
(mins) | accum.
time
(mins) | time
period
(mins) | accum.
time
(mins) | time
period
(mins) | accum.
time
(mins) |
| First contraction | | 0 | | 0 | | 0 | | 0 |
| 'First labour
phase'-length
(contractions
3-15 s)
(rests between
5-70 m long) | 140 | 140 | 100 | 100 | 60 | 60 | 225 | 225 |
| 'Second labour
phase'-length
(contractions
30-45 s)
(rests between
50-95 s) | 48 | 188 | 58 | 158 | 25 | 85 | 50 | 275 |
| 'Third labour
phase'-length
(contractions
40-45 s)
(rests between
10 s) | 3 | 191 | 7 | 165 | 5 | 90 | 10 | 285 |
| Head delivery | | 191 | | 165 | | 90 | | 285 |
| Rest before body
delivery | 5 | 196 | 3 | 168 | 4 | 94 | 3 | 288 |
| Body delivery | | 196 | | 168 | | 94 | | 288 |
| Placenta delivery | | 333 | | 270 | | 210 | | 423 |

Accum. = accumulative

TABLE 22 sets out the event sequence, timing of labour phases and infant and placental delivery in the three induced parous gravid females and the uninduced primigravid female. In order to analyse labour and thereby make comparisons between individuals, pre-delivery labour was divided into three phases:

'First labour phase'

This phase is the longest and starts with the first uterine contraction. It is characterized by back-arching contractions of short duration (3 - 15 s), interspersed by long rest periods (5 - 70 mins long), mild rapid vaginal pulsations, gently body swaying and an absence of any washing activity. In one out of the four births, the amnion was seen to rupture during this phase.

'Second labour phase'

The onset of this phase is characterized by the appearance of a rounded protruberant bulge (the fetal head) just beneath the vaginal orifice with each contraction, and its disappearance during the rest phase. Contractions are longer in duration than in the first phase (30 - 45 s) and rests shorter (50 - 95 s). Cervical dilation commences and fur and wing washing is frequent. In three out of four births amnion rupture occurred during this phase.

'Third labour phase'

This phase is of short duration and begins with a fully dilated cervix. Contractions are 40 - 45 seconds long with very short rest periods (10 s) between. Much washing of the vaginal orifice takes place.

The timing of the amnion rupture from first contraction was very variable ranging from 47 - 195 minutes. The duration from first contraction to body delivery varied from 94 - 288 minutes. In all cases the placenta was not delivered straight after the birth of the infant, but from 102 - 137 minutes later. The length of the total parturition process in the four individuals ranged from 210 - 423 minutes.

6.3.1.3 Placentophagia and onset of lactation

After delivery the placenta was immediately grasped in the

mother's mouth, pulped in the same way as fruit is macerated prior to eating, then swallowed. In doing so the umbilical cord was broken from the placenta about 15 mm from its point of emergence from the infant.

The point of lactation onset was determined for one of the four mothers. In this case fluid was first palpated from the nipples four hours after birth of the infant.

6.3.1.4 Infant appearance

PLATE 41 shows an infant four hours after birth. The head, upper forearms and back are covered in thick fine grey fur. The face is naked and pink with closed eyes, a disproportionately large muzzle and soft, floppy, pink ears. The characteristic white ear tufts in front and behind the ears are present at birth. In males a rudimentary shoulder pouch is visible, inverted and containing the soft grey fur of the back. The ventral surface is completely naked and pink. The wings are fully formed, soft and pink in colour but small in proportion to the rest of the body. Wing and feet claws are hard and well developed.

At birth the new born is on average 16,50 % of the mother's post partum mass (S.E.M. = 0,43; range = 12,93 - 20,27 %; n = 28). TABLE 23 presents the mass, forearm length, eye-nose distance and zygomatic width at birth of the 28 young born in captivity (differences between males and females not significant ($0,05 < P < 0,1$)).

The sex ratio at birth was 1 : 0,87, males to females, which did not depart significantly from unity ($P > 0,05$).

6.3.1.5 Induction success

Although birth induction was achieved, out of 16 induction attempts only three were successful. Of these, the time interval between suppository insertion and the first uterine contraction varied between 28 minutes and 150 minutes (\bar{x} = 74 mins). In seven other attempts contractions were initiated between 25 minutes and 170 minutes (\bar{x} = 67 mins later), but labour did not proceed into the second phase.

6.3.2 Lactation

6.3.2.1 Length of lactation and age of infants at weaning

PLATE 41. Neonate four hours after birth showing the furred head, back and forearms and naked chest and abdomen. The characteristic white ear tufts and rudimentary shoulder pouch are visible.



TABLE 23 Mass, forearm length, eye-nose distance and zygomatic width at birth of 28 cage born infants

| | \bar{x} | S.E.M. | range |
|------------------------|-----------|--------|-------------|
| Body mass (g) | 16,05 | 0,37 | 13-19 |
| Forearm length (mm) | 30,92 | 0,68 | 22,55-35,40 |
| Eye-nose distance (mm) | 10,78 | 0,21 | 9,00-12,70 |
| Zygomatic width (mm) | 15,70 | 0,20 | 14,20-17,50 |

Length of lactation was estimated by taking the median value between the 'giving birth to last day milk could be expressed' time interval and the 'giving birth to first day milk could not be expressed' time interval; $\bar{x} = 83,2$ days; S.E.M. = 6,9; range = 60 - 104 days; n = 8.

Similarly age at weaning was estimated by taking the median value between the age the infant was last seen suckling and the age at which the infant was first seen not attempting to suckle from the mother; $\bar{x} = 81,1$ days; S.E.M. = 6,1; range = 60 - 99 days; n = 8. Age at weaning and age at attainment of full permanent dentition ($\bar{x} = 90$ days), were closely comparable.

6.3.2.2 Mother/juvenile relations

The first two weeks after birth

During the first week after birth, the infant remains continuously attached to the nipple by its well developed mouth, its body slung obliquely across the lower abdomen of the mother (PLATE 42). The offspring's wings are folded in under its body and it clings to the mother's long abdominal fur by its thumb wing claws and feet. In this position it is carried at all times, both when the mother is at rest and while flying, including during her nocturnal feeding flights. When she flies the furred back of the young is exposed to the moving air while its naked ventrum is in contact with the mother's body.

During the day the mother hangs quietly with her wings around the infant completely obscuring it from view. Little activity is seen apart from when she maneuvers the suckling from one nipple across to the other by means of her wings. Maternal grooming of the young was seen particularly at these times of maneuver and in the late afternoon.

During this period the eyes of the infant first open (PLATE 43) ($\bar{x} = 10,4$ days; S.E.M. = 0,9; range = 1 - 22 days; n = 22). An increase in diurnal juvenile activity was seen following this event. The young were seen to crawl across the mother's body from one nipple to the other and frequently rearrange their position.

Initiation of 'hanging'

At a mean age of 17,8 days (S.E.M. = 1,9; range = 13 - 27 days;

PLATE 42 Transverse position of the neonate, across the mother's abdomen and attached to the nipple. This posture was observed during the first week and maintained when the mother flew.



n = 18) the infants are first left hung up in the roof of the cage at night (PLATE 44), while the mother flies off to feed. The age for this event in captive juvenile bats is comparable with the age of the oldest wild juvenile (20 days) caught, still carried by the mother in flight at night while feeding.

Increasing juvenile mass will decrease flight maneuverability and with the caged animals it was found that initiation of 'hanging' occurred when the juvenile mass reached a mean 20,5 % of the mother's mass (S.E.M. = 0,7; range = 18,5 - 23,1 %; n = 8).

Detachment of the young by the mother, prior to feeding, was observed twice. The mother partially opens her wings and slightly bends upwards towards the cage roof. Simultaneously the juvenile releases its hold of the mother by its feet and stretches them up to hook into the netting of the roof. The wing claws follow and when a four-point hanging position is achieved, the nipple is relinquished. The mother leaves and the youngster unhooks its wing claws, shakes its wings then wraps them tightly around itself.

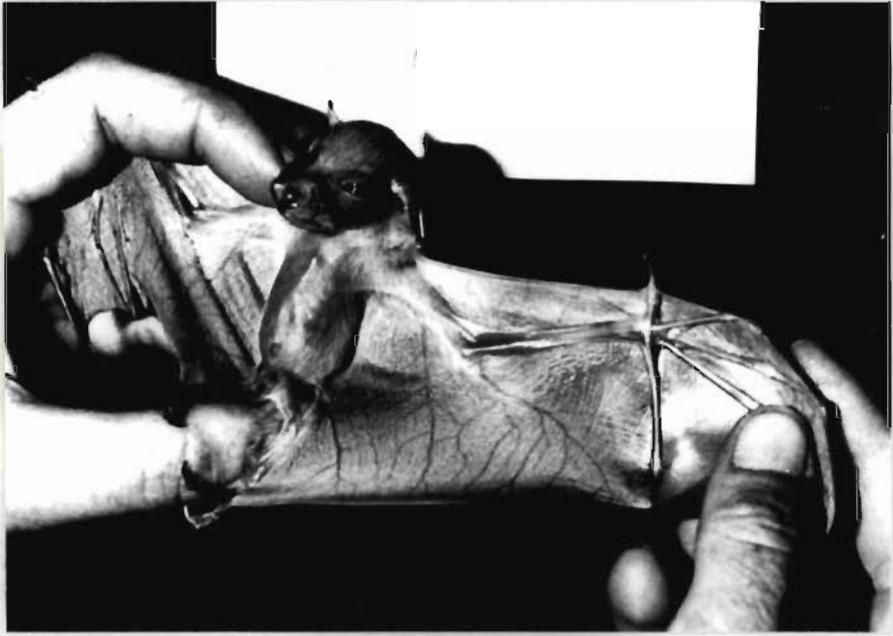
Retrieval of the young, non-volant, hanging bat by the mother after feeding, was frequently witnessed and depending on disturbance, occurs from 15 minutes to two hours after detachment. Without hesitation she flies up to her own infant, lands next to it, places a wing around its body and gently draws it to her by pulling in the wing. The infant responds by stretching its nose downwards and searching for the nipple beneath her forearm. When it has securely grasped the nipple, it hooks into her ventral fur with its wing claws, lets go of the cage roof with its feet and positions itself across her body. She then closes both wings around it.

It is only at night while the mother feeds that the non-volant young is detached and left hanging alone. During the day, until it can fly well, it remains attached to the mother and if disturbed she will fly off carrying the juvenile. One mother was observed carrying a juvenile (not yet able to fly), 34 % of her own body mass, when she was disturbed during the day by a low flying hawk.

On two separate occasions at night, a non-volant, hanging juvenile was seen to 'approach' (by taking steps across the cage roof) a lactant female which was not its own mother. It attempted to grasp her nipple and attach itself, but she rapidly

PLATE 43 Nine day old juvenile with newly opened eyes

PLATE 44 Seventeen day old juvenile, at the age when
first left hanging in the cage at night by
the mother



43



44

rejected it by threatening with an open mouth, 'clacking' noises and flapping wings. It would seem that the mother recognizes her own infant, but the infant cannot recognize its own mother.

Latter stage of the mother/juvenile relationship and independence from the mother

Dependence on the mother lasts from birth until weaning at a mean age of 81,1 days. Flight initiation (6.3.3.1) occurs before weaning, and volant unweaned juveniles, between approximately 60 and 80 days old, were seen flying up to the mother, landing alongside and reaching across to grasp the nipple. The mother responds by folding her wings around the youngster, which retains its foothold on the roof. If disturbed at this stage, mother and infant fly off independently.

After weaning the juvenile no longer approaches the mother to suckle, is not folded in her wings, but may hang independently close by.

6.3.2.3 Milk analysis

TABLE 24 presents the results of E. wahlbergi milk analysis and for comparison the equivalent values for Leptonycteris sanborni and Tadarida brasiliensis (Huibregtse, 1966). Although fats, non-fat solids and total neutral sugars were not analysed by Huibregtse (1966), the biochemical content of E. wahlbergi milk is most similar to the milk of the pollen and nectar feeder, Leptonycteris sanborni.

6.3.3 Juvenile behaviour

6.3.3.1 Flight initiation

Before early flight activity commences, the juveniles show signs of the impending event. During the day they hang by their feet from either their mother's body or from the cage roof and repeatedly stretch and flap their wings. The first short flights were witnessed during the day. The juvenile opens its wings, arches its back and stretches its nose down and back. After a few wing flaps it lets go with its feet and flies three to four metres across the cage roof. Landing is achieved in the same way as the adults accomplish it. It approaches the landing spot, swings its feet forward and up and hooks into the roof with its wing claws first, followed fractionally later by its feet. The wing claws let go and the bat swings head down

TABLE 24 Composition of milk from E. wahlbergi and comparable values
 from the milk of Leptonycteris sanborni and Tadarida
brasiliensis (Huibregtse, 1966)

| Constituent | Replicate values | | mean | Published values for | |
|---|------------------|------|------|----------------------|------------------------|
| | | | | <u>L. sanborni</u> | <u>T. brasiliensis</u> |
| Total solids (g/100 g) | 11,3 | 12,7 | 12,0 | 12,1 | 34,4 |
| Fat (g/100 g) | 2,9 | 4,1 | 3,5 | - | - |
| Non-fat solids (g/100 g) | 8,4 | 8,6 | 8,5 | - | - |
| Protein (N x 6,25)
(g/100 g) | 4,1 | - | 4,1 | 4,4 | 11,1 |
| Reducing sugars as
lactose (g/100 ml) ^a | 4,0 | - | 4,0 | 5,4 | 3,7 |
| Total neutral sugars
(g/100 ml) ^b | 6,6 | - | 6,6 | - | - |

a Mean of four replicate determinations on a single sample

b Mean of three replicate determinations on a single sample

attached only by its feet.

The early phase in flight development was found to occur in the captive bats at a mean age of 53,9 days (S.E.M. = 1,7; range = 45 - 62 days; n = 11). The second phase of flight was achieved at a mean age of 61,8 days (S.E.M. = 1,9; range = 58 - 69 days; n = 7). Active competent flight (Stage 3), as characterized by a willingness to fly when disturbed, was achieved at a mean age of 64,3 days (S.E.M. = 1,2; range = 60 - 70 days; n = 7). By comparison the minimum ages wild juveniles were caught flying independently were 49 - 56 days, the difference probably being due to the observed retarded development in the caged bats, (3.3.1.2).

6.3.3.2 Establishment of the fruit diet

Although as yet unable to fly and therefore feed themselves, the mean age at which captive bats first accepted fruit from the hand was 55,6 days (S.E.M. = 1,9; range = 45 - 61 days; n = 8). However actual self fruit-feeding did not occur until after the Stage 3 phase of flight was attained, at a mean age of 71,8 days (S.E.M. = 4,7; range = 64 - 88; n = 8) and a mean ten days before weaning (PLATE 45).

6.4 DISCUSSION

6.4.1 Parturition

6.4.1.1 Time of day of births

All births witnessed in this study occurred during daylight hours, which is consistent with the results recorded for other Chiroptera (6.1) and in particular for E. wahlbergi in Kenya by Wickler & Seibt (1976). This concurs with the general mammalian pattern of births taking place during the normal period of inactivity, characteristic of the species.

6.4.1.2 Labour and delivery

Vertical, head down labour posture of the mother, and head first delivery presentation of the infant in E. wahlbergi follow the pattern recorded for other pteropids (6.1) and for the New World fruit bats of the family Phyllostomatidae (6.1).

PLATE 45 Seventy five day old juvenile, able to fly
and feed on fruit, but not yet weaned



In order to determine why some Chiropteran species, notably the Old and New World fruit bats, should regularly deliver head first, while others, mainly of the family Vespertilionidae, should deliver breech first, the factors influencing presentation should be examined.

Wimsatt (1960b) suggested that size of fetus, length of umbilicus and quantity of amniotic fluid may affect the turning ability of an advanced fetus. Philpott (pers. comm.) remarked that in man the shape of the uterus determines the position the fetus adopts prior to birth. A further consideration is the size of the head in relation to the diameter of the pelvic girdle (Philpott, pers. comm.). Where the fit of the cranium into the girdle is tight, it is advantageous to the survival of the infant for the head to be presented first in order to prevent trapping of the umbilicus against the pelvis and so cause premature termination of the fetal blood supply before respiration is initiated.

A notable feature of E. wahlbergi at birth, is the large, well developed, muscular muzzle, the significance of which lies in the necessity of the infant to remain attached to the mother's teat. (Without this attachment the infant cannot retain hold in flight and was observed always to be lost). It may be surmised therefore that species which carry their young in flight and are dependent on nipple attachment to retain their young, are born with disproportionately large heads, in order to accommodate the extra muscular development for oral grip.

It is tentatively suggested therefore that these species exhibit a head first delivery presentation to safeguard the infants survival. Evidence for a correlation between carriage of infant in flight and head first delivery is provided by several species; for example Artibeus jamaicensis (Bhatnagar, 1978); Artibeus planirostris (Jones, 1946), Pteropus giganteus (Fölsch, 1967; and own observations), Rousettus aegyptiacus (Kulzer, 1969; and own observations), Pteropus poliocephalus (Nelson, 1965b; Ratcliffe, 1931) and Epomophorus wahlbergi (Wickler & Seibt, 1976; this study).

Conversely it is suggested that those species which do not carry their infants in flight, leaving them behind in a nursery while feeding, have small heads at birth, and, as in 50 % of premature human births (Philpott, pers. comm.) (where the head is small in comparison to the body), breech delivery is easily effected. Evidence for a correlation between nursery care and breech

delivery is provided by three vespertilionid species; Myotis myotis (Jobert, 1872; Kolb, 1977), Plecotus rafinesquei (Pearson, Koford & Pearson, 1952), Myotis lucifugus, (Turner, Shaughnessy & Gould, 1972; Wimsatt, 1945).

A comprehensive investigation of maternal behaviour and neonate cranial breadth in relation to body size, in both 'breech birth' and 'head first birth' species, would be necessary in order to confirm this suggestion.

It was noticed in this study that during parturition E. wahlbergi became markedly quiet compared with the restive and sometimes hostile behaviour encountered while handling non-parturient females. A similar behaviour was commented on by Bogan (1972) while observing birth in Lasiurus cinereus. He offered the explanation that such behaviour might allow a normally solitary tree roosting bat to tolerate the excessive physical contact of the young or, that it might enable the bat to escape predation by remaining quiet at a time when it is ill-equipped to threaten or flee from a predator.

Wimsatt (1960b) remarked that the duration of labour and the number of contractions necessary to expel the fetus, has been recorded by several observers and both vary greatly even within the same species. He cited labour durations of as long as 285 minutes in an exceptional case of a 'weakened' Myotis austroriparius and as short as 1.5 minutes in Tadarida brasiliensis (Sheman, 1937).

It is difficult to compare the length of time parturition took in E. wahlbergi with other species, since many authors do not state from which point in labour they started timing or whether they finished at the birth of the infant or the delivery of the placenta. In Pteropus giganteus (Mallinson, pers. comm.) birth duration is recorded as 33 minutes, terminating at the birth of the infant and apparently starting during the labour phase. Ramakrishna (1950) recorded 135 minutes for Cynopterus sphinx up to the birth of the infant. A lengthy parturition of 255 minutes, including placenta delivery was recorded for Artibeus jamaicensis by Bhatnagar (1978).

In E. wahlbergi parturition from first contraction to placental delivery varied between 210 minutes and 423 minutes. The longer parturition in the uninduced female was probably due to her primigravid condition, rather than to the fact that the other three (all parous) were induced and she was not.

Several authors have noted that the position of the fetus in utero just prior to parturition is transverse (Wimsatt, 1960b). This would appear to be also true in E. wahlbergi judging by the palpated position of the head in pre-labour gravid females. It was observed at the onset of the second labour phase that the fetal head suddenly became visible as a protuberant bulge just beneath the vaginal opening during the contraction. This changing of position from transverse to longitudinal during labour, or just before delivery, has been recorded by other authors (Jones, 1946; Wimsatt, 1960b).

The placenta in all bats is probably deciduate (Wimsatt, 1960b) and most authors record its delivery some time after the emergence of the young, not immediately after it. Gopalakrishna, Khaparde & Sapkal (1976) witnessed the event 24 minutes after infant delivery in Megadema lyra; Fölsch (1967), 30 minutes after, in the fruit bat Pteropus giganteus and Sherman (1937), 30 minutes after, in Tadarida brasiliensis. Time intervals ranging up to 570 minutes (for an unidentified phyllostomatid, Blake, 1885) have been listed for other monotocous Chiropteran species.

In E. wahlbergi the time interval between infant birth and placental delivery fall well within these extremes, ranging from 102 minutes to 137 minutes. The helplessness of the blind infant fruit bat and its inability to hold securely onto its mother prior to its attachment to her nipple, suggested that placental delivery delay might serve a 'lifeline' function as expostulated by Bogan (1972). While the placenta remains undelivered, within the mother, the infant remains attached to her via the umbilical cord. If she is disturbed and has to fly at this stage, even though the infant is unattached to her teat, it will remain with her because of the umbilical connection. Kulzer (1969) noted that only after the infant had attached itself firmly to the nipple, was the placenta extruded, an observation also made in this research. This theory, if correct, leads to the possibility of a tactile stimulus, that of the infant biting into or sucking from the nipple, being responsible for the uterine contractions which deliver the placenta.

6.4.1.3 Placentophagia

Placentophagia was seen on four occasions in this study and has been observed in most Chiroptera in which births were witnessed, including the frugivorous species Pteropus giganteus (Fölsch,

1967), Artibeus planirostris (Jones, 1946), Rousettus aegyptiacus (Kulzer, 1969) and E. wahlbergi (Wickler & Seibt, 1976). This behaviour is prevalent regardless of the non-carnivorous nature of the diet and probably has important nutritional significance, particularly prior to lactation onset and in species whose protein intake is normally low.

6.4.1.4 Infant appearance at birth

Young bats are large at birth, being in some species as much as 28 % of their mother's postpartum mass (Gopalakrishna, Khaparde & Sapkal, 1976). The head, feet and thumb wing claws are disproportionately large, while the forearm and wing membranes are by comparison very small (Wimsatt, 1960b). Many Microchiroptera are born naked with a small amount of hair on the face and extremities (Pearson, Koford & Pearson, 1952; Wimsatt, 1960b). Frugivorous species, including E. wahlbergi in this study, however are born with soft fine grey fur on the back, forearms and head, and naked on the chest, abdomen and wing membranes (Bhatnagar, 1978; Jones, 1946; Kulzer, 1969; Mutere, 1968; Nelson, 1965b).

Back and head furring in the new born is probably an adaptation to being carried in flight, since these are the regions which are exposed to moving air currents. Many of the species born naked are not carried in flight, but are left in large nurseries where body warmth is maintained by a 'body massing' effect.

6.4.1.5 Induction

Prostaglandin E₂ (which is the active ingredient in 'Prostin E') has been used successfully to induce abortions in second and third trimester human pregnancies, as well as parturition at full term (Karim, 1972). In this study however a low success rate of 18,75 % was realized in E. wahlbergi. In those bats where the induction attempt failed, natural birth took place two to eleven days later. This combined with the documented success of prostaglandins to promote delivery regardless of the gestation state, would suggest that induction did not fail just because it was attempted 'too soon'.

The method of Prostaglandin introduction may have been responsible for some failures, since retention of the suppository was a problem in a few individuals. If this was at fault, a greater success rate may be achieved by intravenous prostaglandin injection.

It must also be considered whether the 'Prostin E'/'Syntocinon' treatment actually failed to induce birth and that the three successful cases would have delivered anyway without interference. Certainly the low success rate throws doubt on the effectiveness of this form of induction in E. wahlbergi.

6.4.2 Lactation

6.4.2.1 Lactation length and age of infant at weaning

Lactation length and age at weaning occurred in E. wahlbergi at 2,7 months. By comparison Nelson (1965b) gave age at weaning in Pteropus poliocephalus as four months, while Kulzer (1958) remarked that lactation in captive Rousettus aegyptiacus lasted about six weeks. Mutere (1968) commenting on Kulzer's findings, thought that the period of lactation was probably less in the wild.

6.4.2.2 Mother/juvenile relations

The findings of this study regarding the tenacious grip of the neonate on the mother have been confirmed by other Megachiropteran workers. Kulzer (1969), Nelson (1965b) and Ratcliffe (1932) have all described the firm attachment of the young fruit bat to the mother by means of tiny teeth into the nipple and wing and feet claws into the fur. The youngster with its muscular mouth, deciduous teeth present at birth and well developed claws (Kulzer, 1969) is well adapted to clinging onto the mother during the time it is carried in flight.

After approximately 17 days the non-volant infants of E. wahlbergi are no longer carried at night in flight, but were first seen hanging from the cage roof, while the mother feeds. This behaviour was also observed for wild Pteropus poliocephalus (Nelson, 1965b) and for captive Pteropus rodricensis (Carroll, 1981). In these species 'hanging initiation' was first observed at three weeks of age and Nelson (1965b) noted that P. poliocephalus mothers left their young in well-foliated nurseries.

This behaviour was observed in captive mothers and infants, and because the cage was both a day and night roost as well as a feeding place to them, it was impossible to determine if, in wild individuals, the young would be left hanging in the day roost, or carried closer to the feeding location and left in a night roost.

Why do fruit bats not leave their young behind in a nursery from birth as do some cave-dwelling Microchiropteran species? The probable answer is that cave-dwelling species have an almost predator-proof roost to safely leave their young in. Tree roosts however are open to the sky and to nocturnal aerial and arboreal predators. In the first week the young are blind, and a species which relies largely on its night vision, probably could not be expected to remain motionless if danger threatened. However at a later age the danger to an overweighted flying and feeding mother, probably outweighs the danger to an alert and sighted (if non-volant) hanging young.

In this study it was noted that the mothers appear to recognize their own young, but that the converse was not true. The findings of Nelson (1965b) during recognition experiments with P. poliocephalus confirm this. It was not possible during this work to determine the means by which identification occurs, but researchers working with fruit bats however have suggested olfactory stimuli (Kulzer, 1961; Nelson, 1965b). Turner, Shaughnessy & Gould (1972) and Kolb (1977), both working with Myotis species concluded recognition of nursery infants in cave roosts by mothers was achieved by ultrasonic communication and smell.

6.4.2.3 Milk analysis

The results of milk analysis in E. wahlbergi suggest that diet affects milk composition. This was confirmed by Huibregtse (1966) who found a high carbohydrate and low protein content in the milk of the nectar and pollen feeding bat, Leptonycteris sanborni Compared to the insectivorous bat Tadarida brasiliensis.

6.4.4 Juvenile behaviour

Competent flying was achieved in E. wahlbergi at approximately two months of age ($x = 64,3$ days), self feeding about seven days later ($\bar{x} = 71,8$ days) and total independence of the mother ten days after this ($\bar{x} = 81,1$ days). By comparison P. poliocephalus (Nelson, 1965b) was observed flying and feeding at three months of age and Ratcliffe (1932) noted independence of young Australian pteropids at four months of age.

6.4.5 Subject for further work

This chapter has suggested that features such as delivery presentation and appearance and development of the infant at birth may be related to the

type of maternal care involved. Further investigation of behavioural and anatomical adaptations to the two main modes of maternal care, the 'massed nursery care of cave-dwelling microchiropts' and the 'solitary carried-in-flight-care of arboreal megachiropts' was not within the scope of this work, but would warrant investigation on the grounds of revealing worthwhile comparative reproductive information.

6.5 SUMMARY

Parturition, lactation, maternal care and juvenile behaviour were observed in a captive colony. Birth in E. wahlbergi took place during daylight hours between 11h00 and 17h34. The labour posture was head down and delivery occurred after approximately three hours labour. Delivery presentation was head first and placental delivery occurred about two hours after infant birth. At birth the young are blind, furred on the back, head and forearms, and naked on the abdomen, chest and face. The muzzle is disproportionately large and the new born may weigh up to 20,27 % of the mother's post partum mass. Placentophagia was seen to occur.

Three births were induced by use of prostaglandin E₂ and oxytocin. The total success rate however was low (18,75 %).

The eyes of the young open at about ten days and the mother carries the infant constantly with her until about 17 days after birth. After this the young were hung up from the roof of the cage while the mothers fed, and then were retrieved.

Analysis of the milk revealed a higher carbohydrate and lower protein content than that found in the milk of an insectivorous bat suggesting that diet affects the biochemical composition.

Juveniles become competent fliers at a mean age of 64,3 days and were able to feed for themselves at 71,8 days. They did not become independent of the mother, however until after weaning at a mean age of 81,1 days.

An explanation is offered for the time interval between infant and placental delivery. It is suggested that the umbilical cord acts as a temporary 'lifeline' until attachment to the nipple is achieved. Delivery presentation and appearance of the young at birth are related to the method of care by the mother. Length of parturition, delivery presentation, placentophagia, infant appearance, length of lactation, age at weaning and age at different behavioural stages in the juvenile, are compared with recorded data for other Chiropteran species.

REFERENCES

- AELLEN, V. (1950). Un Megachiroptère nouveau, Epomophoris reii. Revue Suisse de Zoologie. 57: 559-563.
- AELLEN, V. (1952). Contribution de l'étude des Chiroptères du Cameroun. Mémoires Societe Neuchateloise des Sciences Naturelle. 8: 1-121.
- ALLEN, J.A., LANG, H. & CHAPIN, J.P. (1917). The American Museum Congo expedition collection of bats. American Museum of Natural History Bulletin. 37: 405-563.
- ANAND KUMUR, T.C. (1965). Reproduction in the rat tailed bat Rhinopmoma kinneari. Journal of Zoology. 147: 147-155.
- ANCIAUX DE FAVEAUX, M. (1972). Répartition biogéographie et cycles annuels de Chiroptères d'Afrique centrale. Ph.D. Thesis, Paris University.
- ANCIAUX DE FAVEAUX, M. (1978a). Chez les Chiroptères cavernicoles du Shaba at du Rwanda. Mammalia. 42: 458-461.
- ANCIAUX DE FAVEAUX, M. (1978b). Definition de l'equateur biologie en fonction de la reproduction de Chiroptères d'Afrique centrale. Annales Societe Royale Zoologique de Belgique. 107: 78-89.
- ANDERSON, J. (1902). Zoology of Egypt : Mammalia. London: Hugh Rees.
- ANDERSON, J.L. (1972). Seasonal changes in the social organization and distribution of the impala in Hluhluwe Game Reserve, Zululand. Journal of the South African Wildlife Management Association. 2: 16-20.
- ANDERSON, K. (1912). Catalogue of the Chiroptera in the collection of the British Museum, I Megachiroptera. London: Trustees of the British Museum.
- ANDERSON, K. (1917). On the detemination of age in bats. Journal of the Bombay Society of Natural History. 25: 249-259.
- ANDRAL, L., BRES, P., SÉRIÉ, C., CASALS, J. & PANTHIER, R. (1968). Études sur la fièvre jaune en Ethiopie. 3. Étude serologique at virologique de la faune sylvatique. Bulletin of the World Health Organisation. 38: 855-861.
- ANSELL, W.F.H. (1960). Some fruit bats from Northern Rhodesia with the description of a new race of Epomophorus gambianus. Revue de Zoologie et de Botanique Africaines. LXI: 160-166.
- ATTWELL, C.A.M. (1977). Reproduction and population ecology of the blue wildebeeste Connochaetes taurinus taurinus in Zululand. Ph.D. Thesis, University of Natal.
- AUSTIN, C.R. & SHORT, R.V. (1972). Reproductive Patterns. Oxford: Cambridge University Press.
- BAAGØE, H.J. (1973). Taxonomy of two sibling species of bats in Scandinavia, Myotis mystacinus and Myotis brandtii (Chiroptera). Videnskabelige Meddelelser Dansk Naturhistorisk Forening. 130: 191-216.

- BAAGØE, H.J. (1977a). Choice of age criteria and judgement of the attainment of full grown size in bats. Videnskabelige Meddelelser Dansk Naturhistorisk Forening. 140: 93-110.
- BAAGØE, H.J. (1977b). Age determination in bats. Videnskabelige Meddelelser Dansk Naturhistorisk Forening. 140: 53-92.
- BAKER, J.R. (1938). Essays on aspects of evolutionary biology. In Evolution, ed. De Beer, G.R. Oxford: Clarendon Press.
- BAKER, J.R. & BAKER, Z. (1936). The seasons in a tropical rain forest (New Hebrides), Part II Fruit Bats (Pteropidae). Journal of the Linnean Society. 40: 123-141.
- BARNETT, S.A. & COLEMAN, E.M. (1959). Effects of low environmental temperatures on the reproductive cycle of female mice. Journal of Endocrinology. 19: 232-240.
- BARRETT-HAMILTON, G.E.H. (1910). A History of British Mammals I London : Gurney and Jackson.
- BATES, G.L. (1905). Notes on the mammals of southern Cameroons and the Benite. Proceedings of the Zoological Society of London. 1905: 65.
- BEATER, B.E. (1959). Soils of the Sugar Belt. Cape Town : Oxford University Press.
- BEGG, G. (1978). The Estuaries of Natal. Pietermaritzburg : Natal Town and Regional Planning Report, Vol. 41.
- BENNETT, E.T. (1836). On a remarkable species of pteropine bat. Zoological Society of London. Transactions 2: 31-40.
- BERGMANS, W. (1978a). Rediscovery of Epomophorus pousarguesi in Central African Empire. Journal of Natural History. 12: 681-687.
- BERGMANS, W. (1978b). Review of drinking behaviour of African fruit bats. Bulletin of the Carnegie Museum of Natural History. 6: 20-25.
- BERGMANS, W. (1979). Taxonomy and zoogeography of the fruit bats of the People's Republic of Congo, with notes on reproductive biology. Bijdragen tot de Dierkunde. 48: 161-186.
- BERGMANS, W. (1980). A new fruit bat of the genus Myonycteris, Matschie (1899) from eastern Kenya and Tanzania. Zoologische Mededelingen. 5: 171-181.
- BERGMANS, W., BELLIER, L. & VISSAULT, J. (1974). A taxonomic report on a collection of Megachiroptera from the Ivory Coast. Revue Zoologique Africaine. 88: 18-48.
- BERNARD, R.T.F. (1980). Female reproductive anatomy and development of ovarian follicles in Miniopterus schreibersi. South African Journal of Zoology. 15: 111-116.
- BERTALANFFY, L. von. (1938). A quantitative theory of growth. Human Biology. 10: 181-213.

- BERTALANFFY, L. von. (1941). Stoffwechselformen und Wachstumstypen. Biologisches Zentralblatt. 61: 510-532.
- BERTALANFFY, L. von. (1957). Quantitative laws in metabolism and growth. Quarterly Review of Biology. 32: 217-231.
- BERTALANFFY, L. von. (1960). Principles and theory of growth. In Fundamental Aspects of Normal and Malignant Growth, ed. Nowinski, W.W. Amsterdam: Elsevier.
- BEVERTON, R.J. & HOLT, S.J. (1957). On the dynamics of exploited fish populations. Fishery Investigations London. Series 2. 19: 1-533.
- BHATNAGAR, K.P. (1978). Head presentation in Artibeus jamaicensis with some notes on parturition. Mammalia. 42: 359-363.
- BLAKE, H.A. (1885). Notes on the parturition of a West Indian bat. Scientific Proceedings of the Royal Dublin Society. 4: 449-450.
- BOGAN, M.A. (1972). Observations on parturition and development in the hoary bat Lasiurus cinereus. Journal of Mammalogy 53: 611-614.
- BOULGER, L.R. & PORTERFIELD, J.S. (1958). Isolation of a virus from Nigerian fruit bats. Transactions of the Royal Society of Tropical Medicine and Hygiene. 52: 421-424.
- BOURQUIN, O. & SOWLER, S.G. (1980). The vertebrates of Vernon Crookes Nature Reserve I. The Lammergeyer. 28: 20-32.
- BRAACK, H. (1973). Population dynamics of the blue wildebeest Connochaetes taurinus taurinus (Burchell, 1823). Project report submitted to the University of Rhodesia. (Unpublished).
- BRADBURY, J. (1972). The silent symphony: Tuning in on the bat. In: The marvels of animal behaviour. National Geographic Society 1972: 112-125.
- BRADBURY, J. (1977). Lek mating behaviour in the hammer-headed bat. Zeitschrift für Tierpsychologie. 45: 225-255.
- BRADSHAW, G.V.R. (1961). Le cycle de reproduction de Macrotus californicus (Chiroptera, Phyllostomatidae). Mammalia. 25: 117-119.
- BRADSHAW, G.V.R. (1962). Reproductive cycle of the Californian leaf-nosed bat Macrotus californicus. Science. 125: 645-646.
- BRODY, S. (1937). Relativity of physiologic time and physiologic weight. Growth. 1937 61-67.
- BRODY, S. (1945). Bioenergetics and Growth. New York: Reinhold.
- BROOKS, P.M. (1978). Relationship between body condition and age, growth, reproduction and social status in impala and application to management. South African Journal of Wildlife Research. 8: 151-157.
- BROSSET, A. (1966). Les Chiroptères du Haut-Ivindo (Gabon). Biologia Gabon. 2: 47-86.

- BUFFON, G.L.L. Compte de (1766). Histoire Naturelle. Supplement A l'histoire Naturelle Paris Volume 3: 1774-1789.
- BULPIN, T.V. (1972). Natal and the Zulu Country. Cape Town: Books of Africa.
- BUNAK, V.V. (1946). The principal type of growth curve of mammals. Société des Naturalistes de Moscou Bulletin. 51: 41-43.
- BURNETT, C.D. & KUNZ, T.H. (1982). Growth rates and age estimation in Eptesicus fuscus and comparison with Myotis lucifugus. Journal of Mammalogy. 63: 33-41.
- BURNS, J.M. & EASLEY, R.G. (1977). Hormonal control of delayed implantation in the Californian leaf-nosed bat Macrotus californicus. General and Comparative Endocrinology. 32: 163-166.
- BURNS, J.M. & WALLACE, W.E. (1975). Hormonal control of delayed development in Macrotus waterhousii. II Radioimmuno assay of estrone and estradiol 17 β during pregnancy. General and Comparative Endocrinology. 25: 529-533.
- CARMON, J.R., GOLLEY, F.B. & WILLIAMS, R.G. (1963). An analysis of the growth and variability in Peromyscus polionotus. Growth. 27: 247-254.
- CARROLL, J.B. (1981). The wild status and behaviour of the Rodrigues fruit bat Pteropus rodricensis. A report of the 1981 Field Study. The Dodo. 18: 20-29.
- CAUGHLEY, G. (1977). Analysis of Vertebrate Populations. New York: Wiley and Sons.
- CHEEMA, G.S., BHAT, S.S. & NAIK, K.C. (1954). Commercial Fruits of India. New York: Macmillan.
- CHEW, R.M. & BUTTERWORTH, B.B. (1959). Growth and development of Merriam's kangaroo rat. Growth. 23: 75-95.
- CHRISTIAN, J.J. (1953). The natural history of a summer aggregation of Eptesicus fuscus. Naval Medical Research Institute Memoirs. 16: 161-193.
- CHRISTIAN, J.J. (1956). The natural history of a summer aggregation of the big brown bat Eptesicus fuscus fuscus. American Midland Naturalist. 55: 66-95.
- CLARK, D.R. Jnr. (1979). Lead concentrations: Bat vs. terrestrial small mammals collected near a major highway. Journal of Environmental Science & Technology. 13: 338-341.
- CLEGG, A.G. & CLEGG, P.C. (1963). Biology of the Mammal. London: Heinemann.
- CLOERN, J.E. & NICHOLS, F.H. (1978). A Von Bertalanffy growth model with a seasonally varying coefficient. Journal of the Fisheries Research Board of Canada. 35: 1479-1482.
- COCKRUM, E.L. (1956). Homing, movement and longevity in bats. Journal of Mammalogy. 37: 48-57.

- CONDY, P.R. (1980). Postnatal development and growth in southern elephant seals Mirounga leonina. South African Journal of Wildlife Research. 10: 118-122.
- CONSTANTINE, D.G. (1970). Bats in relation to the health, welfare and economy of man. In Biology of Bats, ed. Wimsatt, W. New York: Academic Press.
- COUNT, E.W. (1945). Growth patterns of the human physique, an approach to kineic anthropometry. Human Biology. 15: 1-32.
- COURRIER, R. (1927). Étude sur le déterminisme des caractères sexuels secondaires chez quelques mammifères à activité testiculaire périodique. Archives de Biologie. 37: 173-334.
- COURTIS, S.A. (1932). The Measurement of Growth. Ann Arbor, Michigan: Brumfield and Brumfield.
- COWLES, R.B. (1936). Notes on the mammalian fauna of the Umzumbe Valley, Natal, South Africa. Journal of Mammalogy. 17: 121-130.
- CRANBROOK, The Earl of & BARRETT, H.G. (1965). Observations on noctule bats (Nyctalus noctula) captured while feeding. Proceedings of the Zoological Society of London. 144: 1-24.
- CRASS, R.S. (1964). Freshwater Fishes of Natal. Pietermaritzburg: Shuter & Shooter.
- CRICHTON, J.A., AITKEN, J.N. & BOYNE, A.W. (1959). Effect of plane of nutrition during rearing on growth, production, reproduction and health of dairy cattle. I Growth to 24 months. Animal Production. 1: 145-162.
- CYRUS, D. & ROBSON, N. (1980). Bird Atlas of Natal. Pietermaritzburg: University of Natal Press.
- DAL PIAZ, G. (1937). Mammiferi dell'Oligocene Veneto, Archaeopteropos transiens. Memoire degli Insituti de Geologia e Mineralogia Universita degli studi di Padova. 11: 1-8.
- DANIELL, G. (1834). Observations of the habits of the pipistrelle Vespertilio pipistrellus and the noctule Vespertilio noctula in captivity. Proceedings of the Zoological Society of London. 2: 129-132.
- DAVIDSON, F.A. (1928). Growth and senescence in purebred Jersey cows. Bulletin of the University of Illinois Agricultural Experimental Station. 21: 183-230.
- DAVIS, R. (1969). Growth and development of young pallid bats Antrozous pallidus. Journal of Mammalogy. 50: 729-736.
- DAVIS, R.B., HERREID, C.F. Jnr. & SHORT, H.L. (1962). Mexican free tailed bats in Texas. Ecological Monographs. 32: 311-346.
- DAVIS, W.H. (1963). Ageing bats in winter. Transactions of the Kentucky Academy of Science. 24: 28-30.
- DAVIS, W.H. & HITCHCOCK, H.B. (1965). Biology and migration of the bat Myotis lucifugus in New England. Journal of Mammalogy. 46: 296-313.

- DAY, J.H. (1974). A Guide to the Marine Life on South African Shores. Cape Town: A.A. Balkema.
- DE GRAAF, G. (1981). The Rodents of Southern Africa. Durban: Butterworth.
- DEMING, J. (1957). Application of the Gompertz curve to the observed pattern of growth in length of 48 individual boys and girls during the adolescent cycle of growth. Human Biology. 29: 83-132.
- DICKERSON, J.W.T., GRESHAM, G.A. & McCANCE, R.A. (1964). Effects of undernutrition and rehabilitation on the development of the reproductive organs: Pigs. Journal of Endocrinology 29: 111-118.
- DICKSON, C.G.C. & KROON, D.M. (1978). Pennington's Butterflies of Southern Africa. Johannesburg: A.D. Donker.
- DINALE, G. (1964). Studi sur Chirotteri italiani. II Il raggiungimento della maturità sessuale in Rhinolophus ferrumequinum. Atti Societa Italiana di Scienze Naturali. 103: 141-153.
- DINALE, G. (1968). Studi sur Chirotteri italiani. VII Sul raggiungimento della maturità sessuale nei Chirotteri europei ed in particolare nei Rhinolophidae. Archivio Zoologico Italiano. 53: 51-71.
- DOBSON, G.E. (1881). On the structure of the pharynx, larynx and the hyoid bones in Epomophori with remarks on its relation to the habits of these animals. Journal of Zoology 1881: 685-693.
- DORST, J. & DANDELLOT, P. (1972). A Field Guide to the Larger Mammals of Africa. London: Collins.
- DUBOIS, M., GILLES, K.A., HAMILTON, J.K., REBERS, P.A. & SMITH, F. (1956). Colorimetric method for determination of sugars and related substances. Analytical Chemistry. 28: 350-356.
- EISENTRAUT, M. (1936). Zur Fortpflanzungsbiologie de Fledermäuse. Zeitschrift für Morphologie und Oekologie der Tiere. 31: 27-63.
- FABENS, A.J. (1965). Properties and fitting of the Von Bertalanffy growth curve. Growth. 29: 265-289.
- FAIRALL, N. (1980). Growth and age determination in the hyrax. South African Journal of Wildlife Research. 15: 16-21.
- FAWCETT, D.W. & BEDFORD, J.M. (1979). The Spermatazoa. Baltimore: Urban and Schwarzenburg.
- FAYENUWO, J.O. & HALSTEAD, L.B. (1974). Breeding cycle of the straw-coloured fruit bat Eidolon helvum at Ife-Ife, Nigeria. Journal of Mammalogy. 55: 453-454.
- FITZSIMONS, V.F.M. (1962). Snakes of Southern Africa. Cape Town: Purnell.
- FLEMING, T.H. (1971). Artibeus jamaicensis: delayed embryonic development in a neotropical bat. Science, New York. 17: 402-404.
- FLEMING, T.H., HOOPER, E. & WILSON, D. (1972). Three Central American bat

- communities, structure, reproductive cycle and movement patterns. Ecology. 53: 555-569.
- FLOWER, S.S. (1931). Contributions to our knowledge of the duration of life in vertebrate animals. V Mammals. Proceedings of the Zoological Society of London. 1931: 145-235.
- FLOWER, S.S. (1932). Notes on the recent mammals of Egypt with a list of the species recorded from that kingdom. Proceedings of the Zoological Society of London. 1932: 369.
- FÖLSCH, Von D. (1967). Vor und Nachgeburtphasen bei drei Flughundgeburten Pteropus giganteus (Brunnich, 1782) Zeitschrift für Säugetierkunde. 32: 375-377.
- FOXCROFT, G.R. (1980). Growth and breeding performance in animals and birds. In Studies in Agricultural and Food Science, Growth in Animals, ed. Lawrence, T.L.J. Boston: Butterworth.
- FRIANT, M. (1951). La dentition temporaire dite lactéale de la Rousette Chiroptère frugivore Comptes Rendus Hebdomadaires des Séances Academie des Sciences Paris. 233: 890-892.
- FRIEND, M. (1968). The lens technique. Transactions of the 33rd North American Wildlife and Natural Resources Conference. 33: 279-298.
- FRISCH, R.E. (1974). Critical weight at menarche, initiation of adolescent growth spurt and control of puberty. In Control of Onset of Puberty, ed. Grumbach, M.M., Grave, G.D. & Mayer, F.E. London: Wiley and Sons.
- FUNAIOLI, V. & LANZA, B. (1968). On some bats from Somalia. In Ricerche sulla Fauna della Somalia. Monitore Zoologico Italiano. (N.S.) 2 (Supplement): 199-202.
- FUNMILAYO, O. (1979). Ecology of the straw-coloured fruit bat in Nigeria. Revue Zoologique Africaine. 93: 589-600.
- GAISLER, J. (1966). Reproduction in the lesser horseshoe bat (Rhinolophus hipposideros hipposideros). Bijdragen tot der Dierkunde. 35: 45-64.
- GAISLER, J. (1971). Vergleichende Studie über das Haarkleid der Fledetiere (Chiroptera). Acta Scientiarum Naturalium Academiae Scientiarum acae Brno 5: 1-44.
- GAISLER, J. & TITLBACH, M. (1964). The male sexual cycle in the lesser horseshoe bat (Rhinolophus hipposideros hipposideros). Vestník Československa Zoologicka Spolecnost. 28: 268-277.
- GALVAYNE, S. (1905). The XXth Century Book of the Horse. London: Robert Atkinson.
- GARDINER, J.S. (1906). The Fauna and Geography of the Maldive and Laccadive Archipelagoes. Cambridge: Cambridge University Press.
- GILMORE, D. & COOK, B. (1981). Environmental Factors in Mammal Reproduction. London: MacMillan.

- GIRARD, N.F. (1824). Mémoire sur les Moyens de Reconnaître l'Age du Cheval. ed. Girard. M. Paris: Gueffier.
- GOGUYER, G. & GRUET, M. (1957). Observation d'une parturition chez Myotis emarginatus. Mammalia. 21: 97-110.
- GOMPERTZ, B. (1825). On the nature of the function expressive of the law of human mortality. Philosophical Transactions of the Royal Society. 115: 513-585.
- GOPALAKRISHNA, A. (1949). Studies on the embryology of Microchiroptera. Indian Academy of Science Proceedings. 30: 17-46.
- GOPALAKRISHNA, A. (1964). Post partum pregnancy in the Indian fruitbat Rousettus leschenaulti. Current Science 18: 558-559.
- GOPALAKRISHNA, A. (1969). Unusual persistence of the corpus luteum in the Indian fruit bat Rousettus leschenaulti. Current Science. 16: 388-389.
- GOPALAKRISHNA, A. & CHOUDHARI, P.N. (1977). Breeding habits and associated phenomena in some Indian bats. Part I. Rousettus leschenaulti. Journal of the Bombay Natural History Society. 74: 1-16.
- GOPALAKRISHNA, A. & KARIM, K.B. (1971). Localised progesterational reaction in the uterus of the Indian fruit bat Rousettus leschenaulti. Current Science. 40: 490-491.
- GOPALAKRISHNA, A., KHAPARDE, M.S. & SAPKAL, V.M. (1976). Parturition in the Indian false vampire bat Megaderma lyra lyra. Journal of the Bombay Natural History Society. 73: 464-467.
- GRAHAM, M. (1933). Report on the North Sea Cod. Minister of Agriculture and Fisheries. Fishery Investigation London. 13: 1-160.
- GRAHAM, M. (1956). Sea Fisheries - their investigation in the United Kingdom. London: Arnold.
- GRASSÉ, P. (1955). Ordre des Chiroptères. In Traité de Zoologie, Anatomie Systemique Biologie. Mammifères. XVIII. Paris: Masson et Cie.
- GRIFFIN, D.R. & HITCHCOCK, H.B. (1965). Probable 24-year longevity records for Myotis lucifugus. Journal of Mammalogy. 46: 332.
- GRIMSDELL, J.J.R. (1973). Age determination of the African buffalo. East African Wildlife Journal. 11: 31-53.
- GRIMSDELL, J.J.R. & BELL, R.H.V. (1972). Population growth of red lechwe (Kobus leche leche) in the Busanga plain, Zambia. East African Wildlife Journal. 10: 117-122.
- GROOME, J.R. (1940). The seasonal modification of the interstitial tissue of the testis of the fruit bat (Pteropus). Journal of Zoology. 110: 37-42.
- GULIAND, J.A. & HOLT, S.J. (1959). Estimation of growth parameters data at unequal time intervals. Journal du Conseil International pour l'Exploration de la Mer. 25: 47-49.
- GUSTAFSON, A.W. (1975). A study of the annual male reproductive cycle in a

- hibernating vespertilioned bat (Myotis lucifugus lucifugus) with emphasis on the structure and function of the interstitial cells of Leydig. Ph.D. Thesis, Cornell University.
- GUSTAFSON, A.W. (1979). Male reproductive patterns in hibernating bats. Journal of Reproduction and Fertility. 56: 317-331.
- HALL, J.S., CLOUTIER, R.J. & GRIFFIN, D.R. (1957). Longevity and notes on tooth wear in bats. Journal of Mammalogy. 38: 407-409.
- HANCOCK, D.A. (1963). Marking experiments with the commercial whelk (Buccinum undatum). International Commission for the North West Atlantic Fisheries. (Special Publication). 4: 176-187.
- HANKS, J. (1972). Growth of the African elephant. East African Wildlife Journal. 10: 251-272.
- HANKS, J. (1980). Mammals of Southern Africa. Johannesburg: McGraw Hill.
- HANKS, J. (1981). Characterization of population condition. In Dynamics of Large Mammal Populations, ed. Forbes, C.W. London: Wiley and Sons.
- HAPPOLD, D.C.D. & HAPPOLD, M. (1978). The fruit bats of western Nigeria. Nigerian Field. 43: 30-37, 72-77, 121-127.
- HAYMAN, R.W., MISONNE, X. & VERHEYEN, W. (1966). The bats of the Congo and of Rwanda and Burundi. Annales Musée Royale de l'Afrique Centrale. 154: 1-105.
- HERBERT, J. (1977). External factors and ovarian activity in mammals. In The Ovary II Physiology, ed. Zuckerman Prof. Lord & Weir, S.J. New York: Academic Press.
- HERLANT, M. (1967). Action de la gonadotrophine FSH sur le tube séminifère de la Chauvre-Souris hibernante. Comptes Rendus Hebdomadaires des Séances Académie des Sciences Paris. 264: 2483-2486.
- HERSELMAN, J.G. & HANEKOM, D.J. (1978). Die simbool van 'n land en gelukkige lewe. African Wildlife. 32: 17-19.
- HIORNS, R.W. (1965). The fitting of growth and allied curves of the asymptotic regression type by Steven's method. Tracts for Computers. 28.
- HOLSTEIN, A.F. & SCHIRREN, (1979). Abnormalities in spermatazoa development. In The Spermatazoa, eds. Fawcett, D.W. & Bedford, J.M. Baltimore: Urban and Schwarzenberg.
- HOOPER, J.H.D. & HOOPER, W.M. (1967). The longevity of Rhinolophid bats in Britain. Nature (London). 216: 1133.
- HOWELLS, W.W. & HANKS, J. (1975). Body growth of the impala Aepyceros melampus in Wankie National Park. Journal of the South African Wildlife Management Association. 5: 95-98.
- HUGGETT, A. St. G. & WIDDAS, W.F. (1951). The relationship between mammalian foetal weight and conception age. Journal of Physiology. 114: 306-317.
- HUGHES, G.R. (1974). The sea turtles of south east Africa. Report of the

Oceanographic Research Institute Durban. 36: 1-96.

- HUIBREGTSE, J. (1966). Some chemical and physical properties of bat milk. Journal of Mammalogy. 47: 551-554.
- ILLIUS, A.W., HAYNES, N.B. & LAMMING, G.E. (1976). Effects of ewe proximity on peripheral plasma testosterone levels and behaviour in the ram. Journal of Reproduction and Fertility. 48: 25-32.
- ISRAELSON, W.J. (1969). Description and modes of analysis of human growth. In Symposia of the Society for the Study of Human Growth, ed. Tanner, J.M. New York: Pergamon.
- JACOBSEN, N.H.G. & DU PLESSIS, E. (1976). Observations on the ecology and biology of the Cape fruit bat Rousettus aegyptiacus in the Eastern Transvaal. South African Journal of Science. 72: 270-273.
- JEFFREY, R.C.V. (1978). Age determination, growth and condition of a population of eland Taurotragus oryx under semi intensive management at Coleford Reserve. M.Sc. Thesis, University of Natal.
- JEFFREY, R.C.V. & HANKS, J. (1981). Age determination of eland Taurotragus oryx oryx (Pallas, 1766) in the Natal Highveld. South African Journal of Zoology. 16: 113-122.
- JENSS, R.M. & BAYLEY, N. (1937). A mathematical method for studying growth in children. Human Biology. 9: 556-563.
- JEPSEN, G.L. (1966). Early Eocene bat from Wyoming. Science. 154: 1333-1339.
- JEPSEN, G.L. (1970). Bat origins and evolution. In Biology of Bats, ed. Wimsatt, W. Vol. 1. New York: Academic Press.
- JERRETT, D.P. (1979). Female reproductive patterns in non hibernating bats. Journal of Reproduction and Fertility. 56: 369-378.
- JOBERT, C. (1872). Études d'anatomie comparée sur les organes du toucher chez divers mammifères, oiseaux, poissons et insects. Annales des Science Naturelle, Zoologie et Biologie Animale 16: 1-162.
- JONES, C. (1971). The bats of Rio Muni, West Africa. Journal of Mammalogy. 52: 121-140.
- JONES, M.L. (1972). Unpublished. In Biology Data Book, eds. Altmann, P.L. & Dittmer, D.S. Bethesda, Maryland: Federation of American Societies for Experimental Biology.
- JONES, T.S. (1945). Unusual state of birth of a bat. Nature. 156: 365.
- JONES, T.S. (1946). Parturition in a West Indian fruit bat (Phyllostomatidae). Journal of Mammalogy. 27: 327-330.
- JOUBERT, D.M. (1963). Puberty in female farm animals. Animal Breeding Abstracts. 31: 295-306.
- KARIM, S.M.M. (1972). Physiological role of prostaglandins in the control of parturition and menstruation. Journal of Reproduction and Fertility.

Supplement 16: 105-119.

- KARSCH, F.J. & FOSTER, D.L. (1981). Environmental control of seasonal breeding: a common final control mechanism governing seasonal breeding and sexual maturation. In Environmental Factors in Mammal Reproduction, ed. Gilmore, D & Cook, B. London: MacMillan.
- KENNEDY, G.C. (1969). Interactions between feeding behaviour and hormones during growth. Annals of the New York Academy of Science. 157: 1049-1061.
- KENNEDY, G.C. & MITRA, J. (1963). Hypothalamic control of energy balance in the reproductive cycle of the rat. Journal of Physiology. 166: 395-406.
- KETCHEN, K.S. & FORRESTER, C.R. (1966). Population dynamics of the petrale sole Eopsetta jordini in waters off western Canada. Bulletin of the Fisheries Research Board of Canada. 153: 195.
- KINGDON, J. (1974). East African Mammals. Vol. IIA London: Academic Press.
- KLEVEZAL, G.A. & KLEINENBERG, S.E. (1969). Age determination of mammals by layered structures in teeth and bone. Translated from Akademiya Nauk S.S.S.R. Moscow: 144 pp. 1967. Fisheries Research Board of Canada. Translation Series. no. 1024: 142pp.
- KLOSS, C.B. (1903). In the Andamans and Nicobars. London: John Murray.
- KNIGHT, W. (1968). Asymptotic growth. An example of nonsense disguised as mathematics. Journal of the Fisheries Research Board of Canada. 25: 1303-1307.
- KOCK, D. (1969). Die Fledermaus - Fauna des Sudan. Abhandlungen Senckenbergische Naturforschende Gesellschaft. 521: 1-238.
- KOLB, A. von (1977). Wie erkennen sich Mutter und Junges des Mausohrs Myotis myotis bei der Rückkehr vom Jagdflug wieder ? Zeitschrift für Tierpsychologie. 44: 423-431.
- KREBS, C.J. (1972). Ecology, the Experimental Analysis of Distribution and Abundance. New York: Harper and Row.
- KRUTZSCH, P.H. (1979). Male reproductive patterns in non hibernating bats. Journal of Reproduction and Fertility. 56: 333-344.
- KRUTZSCH, P.H. & BECK, A.J. (1975). Reproductive biology of the pteropid bat Eonycteris spelaea. Anatomical Records. 181: 401.
- KRUTZSCH, P.H., WATSON, R.H. & LOX, C.D. (1976). Reproductive biology of the male leaf-nosed bat Macrotus waterhousii in south western United States. Anatomical Records. 184: 611-636.
- KULZER, E. (1958). Untersuchungen über die Biologie von Flughunden der Gattung Rousettus. Zeitschrift für Morphologie und Oekologie der Tiere. 47: 374-402.
- KULZER, E. (1961). Über die Biologie der Nil - Flughunde (Rousettus aegyptiacus). Natur und Volk. 91: 219-228.

- KULZER, E. (1962). Fledermäuse aus Tanganyika. Zeitschrift für Säugtierkunde. 27: 164-181.
- KULZER, E. (1969). African fruit eating cave bats. African Wildlife. 23: 39-46, 129-137.
- KUNZ, T.H. (1974). Reproduction, growth and mortality of the vespertilionid bat Eptesicus fuscus in Kansas. Journal of Mammalogy. 55: 1-13.
- KUNZ, T.H. & ANTHONY, E.L.P. (1982). Age estimation and postnatal growth rates of the bat Myotis lucifugus. Journal of Mammalogy. 63: 23-32.
- LACKEY, J.A. (1967). Growth and development of Dipodomys stephensi. Journal of Mammalogy. 48: 624-632.
- LAIRD, A.K. (1967). The evolution of the human growth curve. Growth. 31: 345-355.
- LAIRD, A.K., TYLER, S.A. & BARTON, A.D. (1965). Dynamics of normal growth. Growth. 29: 233-248.
- LARGEN, J.J., KOCK, D. & YALDEN, D.W. (1974). Catalogue of the mammals of Ethiopia. I Chiroptera. Monitore Zoologico Italiano. Supplement 5: 221-298.
- LIAT, L.B. (1970). Food habits and breeding cycle of the Malaysian fruit eating bat Cynopterus brachyotis. Journal of Mammalogy. 51: 174-177.
- LINCOLN, G.A., GUINNESS, F. & SHORT, R.V. (1972). The way in which testosterone controls the social and sexual behaviour in the red deer stag (Cervus elephus). Hormones and Behaviour. New York. 3: 375-396.
- LINHART, S.B. (1973). Age determination and occurrence of incremental lines in the dental cementum of the common vampire bat (Desmodus rotundus). Journal of Mammalogy. 54: 493-496.
- LINZEY, D.A. & LINZEY, A.V. (1967). Growth and development of the golden mouse Ochrotomys nuttalli nuttalli. Journal of Mammalogy. 48: 445-458.
- LORD, R.D., MURADALI, F. & LAZARO, L. (1976). Age composition of the vampire bats (Desmodus rotundus) in northern Argentina and southern Brazil. Journal of Mammalogy. 57: 573-575.
- LOVERIDGE, A. (1922). Notes on East African mammals (other than horned ungulates) collected or kept in captivity 1915-1919. Part 2. Journal of the East African and Ugandan Natural History Society. XVII: 39.
- LOXTON, HUNTING & ASSOCIATES, (1971). Report on a survey of the South Coast (European owned land). 1-110.
- LYNCH, C.D. (1974). A behavioural study of blesbok, Damaliscus dorcus phillipsi with special reference to territoriality. Memoirs van die Nasionale Museum. No. 8. Bloemfontein.
- LYNE, A.G. & VERHAGEN, A.M.W. (1957). Growth of the marsupial Trichosurus vulpecula and a comparison with some higher mammals. Growth. 21: 167-195.

- LYSTER JAMESON, H. (1909). On a collection of mammals from South Africa. Annals and Magazine of Natural History. 8: 455-474.
- MALBRANT, R. & McCLATCHY, A. (1949). Faune de l'equateur Africain Francais II Mammifères. Encyclopedie Biologique 36. Paris.
- MALZY, P. & JAGORD, H. (1960). A propos des rousettes. Notes Africaines. 87: 89-92.
- MARAIS, J.P., De WIT, J.L. & QUICKE, G.V. (1966). A critical examination of the Nelson-Sonogyni method for the determination of reducing sugars. Analytical Biochemistry. 15: 373-381.
- MARSHALL, A.J. (1946). The breeding cycle of an equatorial bat (Pteropus giganteus) of Ceylon. Linnean Society of London Proceedings. 159: 103-111.
- MARSHALL, A.J. (1949). Pregestational changes in the giant fruit bat Pteropus giganteus with special reference to an asymmetric endometrial reaction. Linnean Society of London Proceedings. 161: 2-37.
- MARSHALL, A.J. (1953). The unilateral endometrial reaction in the giant fruit bat. Journal of Endocrinology. 9: 42-44.
- MATTHEWS, L.H. (1941). Notes on the genitalia and reproduction of some African bats. Proceedings of the Zoological Society of London. 111B: 289-346.
- MATTHEWS, L.H. (1950). La dentition de lait chez Nycteris leisleri. Mammalia. 40: 11-13.
- MATTHEWS, L.H. (1969). The Life of Mammals. London: Weidenfeld and Nicholson.
- MAZAK, V.I. (1963). Hair growth in the large mouse-eared bat Myotis myotis (Mammalia, Chiroptera) during its prenatal and early postnatal life. Vestnik Ceskoslovenska Zoologicka Spolecnost. 27: 234-242.
- MAZAK, V.I. (1965). Changes in pelage of Myotis myotis Borkhausen 1797. (Mammalia, Chiroptera). Vestnik Ceskoslovenska Zoologicka Spolecnost. 29: 368-376.
- MCCANCE, R.A. (1962). Food, growth and time. Lancet. 2: 671-675.
- MEDAWAR, P.B. (1940). Growth, growth energy and ageing of the chicken's heart. Proceedings of the Royal Society of London Biological Sciences. 129: 332-355.
- MEDAWAR, P.B. (1945). In Essays in Growth and Form, eds. Le Gros Clark, W.E. & Medawar, P.B. Oxford: Oxford University Press.
- MEDWAY, Lord (1965). Mammals of Borneo. Singapore: Malaysian Branch of the Royal Asiatic Society.
- MEESTER, J. & SETZER, H.W. (1971). The Mammals of Africa. An Identification Manual. Washington: Smithsonian Institution Press.
- MELTON, D.A. (1978). Ecology of waterbuck Kobus ellipsiprymnus (Ogibly, 1833)

in the Umfolozi Game Reserve. D.Sc. Thesis, University of Pretoria.

- MENZIES, J.I. (1973). A study of leaf-nosed bats (Hipposideros caffer and Rhinolophus landeri) in a cave in northern Nigeria. Journal of Mammalogy. 54: 930-945.
- MERRELL, M. (1931). The relationship of erect body length to supine body length. Human Biology. 4: 262-271.
- MESCHINELLI, L. (1903). Un nuovo chiroterro fossile (Archaeopteropterus transiens, Mesch.) della ligniti di Monteviale. Atti Istituto Veneto di Scienze, Lettere ed Arti. 62: 1329-1344.
- MILLAR, R.P. (1971). Degradation of spermatazoa in the epididymis of a seasonally breeding mammal, the rock hyrax, Procavia capensis. Journal of Reproduction and Fertility. 30: 447-450.
- MILLER, R.E. (1939). The reproductive cycle in male bats of the species Myotis lucifugus lucifugus and M. grisescens. Journal of Morphology. 64: 267-295.
- MOGHE, M.A. (1951). Development and placentation of the Indian fruit bat Pteropus giganteus giganteus. Journal of Zoology. 121: 703-721.
- MOLL, E. (1981). Trees of Natal. Cape Town: University of Cape Town Eco-lab Trust Fund.
- MONTEIRO, L.S. & FALCONER, D.S. (1966). Compensatory growth and sexual maturity in mice. Animal Production. 8: 179-192.
- MORRIS, P. (1972). A review of mammalian age determination methods. Mammal Review. 2: 69-104.
- MOSSMAN, W.H. & DUKE, K.L. (1973). Comparative Morphology of the Mammalian Ovary. Wisconsin: University of Wisconsin Press.
- MOUNTAIN, E.D. (1968). Geology of Southern Africa. Cape Town: Books of Africa.
- MUNRO, J.L. (1982). Estimation of the parameters of the Von Bertalanffy Growth equation from recapture data at variable time intervals. Journal du Conseil International pour l'Exploration de la Mer. 42: 199-200.
- MUTERE, F.A. (1967). The breeding biology of equatorial vertebrates: reproduction of the fruit bat Eidolon helvum at latitude 0° 20' N. Journal of Zoology. 153: 153-161.
- MUTERE, F.A. (1968). The breeding biology of the fruit bat Rousettus aegyptiacus living at 0° 22' S. Acta Tropica. 25: 97-108.
- MUTERE, F.A. (1973a). A comparative study of reproduction in two populations of the insectivorous bat Otomops martiensseni at latitude 1° 5' S and 2°30' S. Journal of Zoology. 171: 79-92.
- MUTERE, F.A. (1973b). Reproduction in two species of equatorial free-tailed bat. East African Wildlife Journal. 11: 271-280.
- NATAL TOWN AND REGIONAL PLANNING COMMISSION, (1974). Natal South Coast Draft

Regional Plan. Natal Town and Regional Planning Report. 29: 1-144.

- NEEDHAM, A.E. (1964). The Growth Process in Animals. London: Pitman and Son.
- NELSON, J.E. (1965a). Movement of Australian flying foxes. Australian Journal of Zoology. 13: 53-73.
- NELSON, J.E. (1965b). Behaviour of Australian Pteropodidae. Animal Behaviour. 13: 544-557.
- OCKERSE, T. (1959). The eruption sequence and eruption times of the teeth of the vervet monkey. Journal of the Dental Association of South Africa. 14: 422-424.
- OIL, Y.K. (1977). Periodic changes of the testis and ductus epididymis in Korean hibernating bats. Korean Journal of Zoology. 20: 67-76.
- OKIA, N.O. (1974a). Breeding in Franquet's bat Epomops franqueti in Uganda. Journal of Mammalogy. 55: 462-465.
- OKIA, N.O. (1974b). The breeding pattern of the eastern epauletted bat Epomophorus anurus in Uganda. Journal of Reproduction and Fertility. 37: 27-31.
- O'SHEA, T.J. & VAUGHAN, T.A. (1980). Ecological observations on an East African bat community. Mammalia. 44: 485-496.
- OXBERRY, B.A. (1979). Female reproductive patterns in hibernating bats. Journal of Reproduction and Fertility. 56: 359-367.
- PALMER, E. (1977). A Field Guide to the Trees of Southern Africa. Johannesburg: Collins.
- PASSMORE, N.I. & CARRUTHERS, P.C. (1979). South African Frogs. Johannesburg: University of the Witwatersrand.
- PEARL, R. (1925). The Biology of Population Growth. New York: Alfred Knopf.
- PEARL, R. & REED, L.J. (1925). Skew growth curves. Proceedings of the National Academy of Science Washington D.C. 11: 16-22.
- PEARSON, O.P., KOFORD, M.R. & PEARSON, A.K. (1952). Reproduction in the lump nosed bat (Corynorhinus rafinesquei) in California. Journal of Mammalogy. 33: 272-320.
- PERRY, A.E. (1965). Population analysis of the guano bat Tadarida brasiliensis mexicana (Saussure) using the lens weight method of age determination. Ph. D. Thesis, Oklahoma State University.
- PERRY, A.E. & HERREID, C.F. (1969). Comparison of tooth wear and lens weight methods of age determination in the guano bat Tadarida brasiliensis brasiliensis. Journal of Mammalogy. 50: 357-360.
- PETERS, W.C.H. (1852). Naturwissenschaftliche Reise nach Mossambique. Zoologie: I Saughthiere. Berlin: George Reimer.
- PETERS, W.C.H. (1867). Hr. W. Peters gab die Fortsetzung und den Schufseiner

Übersicht der Flederhunde. Monatsberichte der Königlischen Preussischen Akademie der Wissenschaften zu Berlin. 1867: 869.

- PHADKE, A.M. & PHADKE, G.M. (1961). Occurrence of macrophage cells in the semen and the epididymis in cases of male infertility. Journal of Reproduction and Fertility. 2: 400-403.
- PHILLIPS, B.F. & CAMPBELL, N.A. (1968). A new method of fitting the Von Bertalanffy growth curve using data on the whelk Dicathais. Growth. 32: 317-329.
- PHILLIPS, C.J. (1971). The dentition of glossophagine bats: development, morphological characteristics, variation, pathology and evolution. Miscellaneous Publications of the Museum of Natural History of the University of Kansas. 54: 1-138.
- PHILLIPS, C.J. & STEINBERG, B. (1976). Histological and scanning electron microscopic studies of tooth structure and thegosis in the common vampire bat Desmodus rotundus. Occasional Papers of the Museum of Texas Tech. University. 42: 1-12.
- PHILLIPS, C.J., STEINBERG, B. & KUNZ, T.H. (1982). Dentin, cementum and age determination in bats. A critical evaluation. Journal of Mammalogy. 63: 197-207.
- PHILLIPS, G.L. (1966). The ecology of the big brown bat (Chiroptera, Vespertilionidae) in north eastern Kansas. American Midland Naturalist. 75: 168-198.
- PHILLIPS, J. (1973). The Agricultural and Related Development of the Tugela Basin and its Influent Surrounds. Pietermaritzburg: Natal Town and Regional Planning Report no. 19.
- PHILLIPS, W.W.A. (1924). A guide to the mammals of Ceylon. Ceylon Journal of Science B. 13: 4-15.
- PINHEY, E. (1965). Butterflies of Southern Africa. Johannesburg: Nelson.
- POOK, A.G. (1977). Breeding of the Rodrigues fruit bat Pteropus rodricensis at Jersey Wildlife Zoological Park. The Dodo. 14: 30-33.
- PROZESKY, O.P.M. (1970). A Field Guide to the Birds of Southern Africa. London: Collins.
- QUICKE, G.V. & SOWLER, S.G. (in press). Tentative analysis of milk from the fruit bat Epomophorus wahlbergi.
- RACEY, P.A. (1973). The viability of spermatazoa after prolonged storage by male and female European bats. Peridicum Biologorum. 75: 201-205.
- RACEY, P.A. (1974a). Ageing and assessment of reproductive status of the pipistrelle bat Pipistrellus pipistrellus. Journal of Zoology. 173: 264-271.
- RACEY, P.A. (1974b). The reproductive cycle in male noctule bats Nyctalus noctula. Journal of Reproduction and Fertility. 41: 169-182.

- RACEY, P.A. (1979). The prolonged storage and survival of spermatazoa in Chiroptera. Journal of Reproduction and Fertility. 56: 391-402.
- RAMAKRISHNA, P.A. (1947). Post partum oestrus in the short-nosed fruit bat Cynopterus sphynx sphynx. Current Science. 16: 186.
- RAMAKRISHNA, P.A. (1950). Parturition in certain Indian bats. Journal of Mammalogy. 31: 274-278.
- RAMAKRISHNA, P.A. (1951). Studies on the reproduction of bats. I- Some aspects of reproduction in the oriental vampires Lyroderma lyra lyra and Megadema spasma. Journal of the University of Mysore. 12: 107-118.
- RAMASWAMY, K.R. (1961). Studies on the sex cycle of the Indian vampire bat Megadema (Lyroderma) lyra lyra. Proceedings of the National Institute of Sciences of India. 27: 287-307.
- RASWEILER, J.J. (1979). Early embryonic development and implantation in bats. Journal of Reproduction and Fertility. 56: 403-416.
- RATCLIFFE, F.N. (1931). The flying fox (Pteropus) in Australia. Commonwealth Scientific and Industrial Research Organization of Australian Bulletin. 53: 6-81.
- RATCLIFFE, F.N. (1932). Notes on the fruit bats of Australia. Journal of Animal Ecology. 1: 33-37.
- RAUTENBACH, I.L. (1982). Mammals of the Transvaal. Ecoplan Monograph no. I. Pretoria: Ecoplan.
- REID, B.L. & CLELAND, K.W. (1957). The structure and function of the epididymis. I the histology of the rat epididymis. Australian Journal of Zoology. 5: 223-243.
- RICE, D.W. (1957). Life history and ecology of Myotis austroriparius in Florida. Journal of Mammalogy. 38: 15-32.
- RICHARDS, F.J. (1959). A flexible growth function for empirical use. Journal of Experimental Botany. 10: 290-300.
- ROBERTS, A. (1951). The Mammals of South Africa. Johannesburg: Trustees of The Mammals of South Africa book fund.
- ROBERTSON, T.B. (1923). The Chemical Basis of Growth and Senescence. Philadelphia: Lippincott.
- ROFF, D.A. (1980). A motion for the retirement of the Von Bertalanffy function. Canadian Journal of Fishery and Aquatic Science. 37: 127-129.
- RÖRIG, A. (1905). Das Wachstum des Schadels von Capreolus vulgaris, Cervus elephus und Dama vulgaris. Anatomischer Anzeiger. 26: 17-25
- ROSEVEAR, D.R. (1965). The Bats of West Africa. London: Trustees of the British Museum of Natural History.
- ROUBICEK, C.B., PAHNISH, O.F. & TAYLOR, R.L. (1964). The growth curve of the rat in relation to adult size and temperature. Growth. 20: 233-241.

- ROUSSELOT, R. (1950). Les rousettes du Soudan (Animaux nuisibles). Comptes Rendus conference Internationale des Africanistes de L'Ouest 1st. 1: 233-238.
- ROWE, P.H., RACEY, P.A., LINCOLN, G.A., ELLWOOD, M., LEHANE, J. & SHENTON, J.C. (1975). The temporal relationship between the secretion of luteinizing hormones and testosterone in man. Journal of Endocrinology. 64: 17-26.
- ROWE-ROWE, D.T. (1975). Biology of Natal Mustelids. M.Sc. Thesis, University of Natal.
- RUSSELL, T.S. (1969). Mathematical models of growth. In Animal growth and Nutrition, ed. Hafez, E.S.E. & Dyer, I.A. Philadelphia: Lea and Febiger.
- RYBAR, P. (1969). A contribution to the study of bones in the bat Myotis myotis in regard to the age determination. Acta Musei Regina egrade censis. SA X: 89-101.
- RYBAR, P. (1971). On the problems of the practical use of the ossification of bones as age criteria in the bats. Prace a Studie Priir Paradubice. 3: 97-121.
- RYBERG, O. (1947). Studies on bats and bat parasites. Stockholm: Svensk Naturvetenskap.
- SADLEIR, R.M.F.S. (1968). Reproductive responses to the environment in mammals. Journal of Psychosomatic Research. 12: 3-9.
- SADLEIR, R.M.F.S. (1969a). The role of nutrition in the reproduction of wild mammals. Journal of Reproduction and Fertility. Supplement 6: 39-48.
- SADLEIR, R.M.F.S. (1969b). Ecology of Reproduction in Wild and Domesticated Mammals. London: Methuen and Co.
- SADLEIR, R.M.F.S. (1972). Environmental effects. In Reproductive Patterns, ed. Austin, C.R. & Short, R.V. Oxford: Cambridge University Press.
- SANBORN, C.C. & NICHOLSON, A.J. (1950). Bats from New Caledonia, the Solomon Islands and New Hebrides. Fieldiana Zoology. 31: 313-338.
- SCHALLER, G.B. (1972). The Serengeti Lion. Chicago: University of Chicago Press.
- SCHOWALTER, D.B., HARDER, L.D. & TREICHEL, B.H. (1978). Age composition of some vespertilionid bats as determined by dental annuli. Canadian Journal of Zoology. 56: 355-358.
- SHERMAN, H.B. (1937). Breeding habits of the free-tailed bat. Journal of Mammalogy. 18: 176-187.
- SHERRY, B.Y. (1978). Growth of elephants in the Gonarezhou National Park, south eastern Rhodesia. South African Journal of Wildlife Research. 8: 49-58.
- SHILLITO WALSER, E. (1977). Maternal behaviour in mammals. Symposia of the Zoological Society of London. 41: 313-331.
- SIKOV, M.R. & THOMAS, J.M. (1970). Prenatal growth of the rat. Growth. 34:

1-14.

- SIMPSON, D.I.H., WILLIAMS, M.C., O'SULLIVAN, J.P., CUNNINGHAM, J.C. & MUTERE, F.A. (1968). Studies on arboviruses and bats (Chiroptera) in East Africa. II Isolation and haenagglutination inhibition studies on bats collected in Kenya and throughout Uganda. Annals of Tropical Medicine and Parasitology. 62: 432-440.
- SIMPSON, G.G. (1945). The principles of classification and a classification of mammals. Bulletin of the American Museum of Natural History. 85: 1-350.
- SINCLAIR, A.R.E. (1977). The African Buffalo. Chicago: University of Chicago Press.
- SLUITER, J.W. & BOUMAN, M. (1951). Sexual maturity on bats of the genus Myotis. I Size and histology of the reproductive organs during hibernation in connection with age and wear of teeth in female Myotis myotis and Myotis emarginatus. Proceedings Koninklijke Nederlandse Akademie Wetenschappen Amsterdam C 5: 595-601.
- SLUITER, J.W. (1954). Sexual maturity in bats of genus Myotis. II females of M. mystacinus and supplementary data on female M. myotis and M. emarginatus. Proceedings Koninklijke Nederlandse Akademie Wetenschappen Amsterdam C 57: 696-700.
- SLUITER, J.W. (1961a). Abrasion of teeth in connection with age in the bat Myotis myotis. Proceedings Koninklijke Nederlandse Akademie Wetenschappen Amsterdam C 64: 424-434.
- SLUITER, J.W. (1961b). Sexual maturity in the males of the bat Myotis myotis. Proceedings Koninklijke Nederlandse Akademie Wetenschappen Amsterdam C 64: 243-249.
- SMITH, J.L.B. (1953). The Sea Fishes of Southern Africa. Johannesburg: Central News Agency.
- SMITH, P.C. (1967). Isolation of rabies virus from fruit bat in Thailand. Nature. 216: 384.
- SMITHERS, R.H.N. (1971). The Mammals of Botswana. Salisbury: The Trustees of the National Museum of Rhodesia.
- SMITHERS, R.H.N. & WILSON, V.J. (1979). Check List and Atlas of the Mammals of Zimbabwe Rhodesia. Salisbury: The Trustees of the National Museum and Monuments of Zimbabwe Rhodesia.
- SMITH MEYER, M.K.P. (1974). A revision of the tetranychidae of Africa (Acari) with a key to the genera of the world. Pretoria: Department of Agriculture and Technical Services.
- SMUTS, G.L. (1974). Growth, reproduction and population characteristics of the Burchell's Zebra (Equus burchelli antiquorum) in the Kruger National Park. D.Sc. Thesis, University of Pretoria.
- SMUTS, G.L., ANDERSON, J.L. & AUSTIN, J.C. (1978). Age determination of the African lion. Journal of Zoology. 185: 115-146.

- SOWLER, S.G. (1980). Tooth eruption in known age specimens of Epomophorus wahlbergi. South African Journal of Wildlife Research. 10: 112-117.
- SOWLER, S.G. (in prep.). Bats of Natal.
- SOWLS, L.K. & PHELPS, R.J. (1968). Observation of the African bush pig Potamochoerus porcus. Zoologica. 53: 75-83.
- SPINAGE, C.A. (1967). Ageing of the Uganda Defassa waterbuck. East African Wildlife Journal. 5: 1-17.
- SPINAGE, C.A. (1973). A review of the age determination of mammals by means of teeth, with special reference to Africa. East African Wildlife Journal. 11: 165-187.
- STEBBINGS, R.E. (1966). A population study of bats of the genus Plecotus. Journal of Zoology. 150: 53-75.
- STEBBINGS, R.E. (1968). Measurements, composition and behaviour of a large colony of the bat Pipistrellus pipistrellus. Journal of Zoology. 156: 15-33.
- STEGEMAN, L.C. (1956). Tooth development and wear in Myotis. Journal of Mammalogy. 37: 58-63.
- STEVENS, W.L. (1951). Asymptotic regression. Biometrics. 7: 247-267.
- STEYN, D. (1980). Reproduction in the hyrax Procavia capensis in relation to environmental factors. M.Sc. Thesis, University of Natal.
- SUNDEVALL, C.J. (1846). Nya Mammalia från Sydaafrika. Pteropus wahlbergi. Ofversigt af Kongl. Vetenskaps - Akademiens Fordhandlingar, Stockholm. 3: 118.
- TAMSITT, J.R. & MEJIA, C.A. (1962). The reproductive status of a population of the neotropical bat Artibeus jamaicensis at Providencia. Caribbean Journal of Science. 2: 139-144.
- TAMSITT, J.R. & VALDIVIESO, D. (1963). Reproductive cycle of the big fruit eating bat Artibeus lituratus. Nature. 198: 104.
- TAMSITT, J.R. & VALDIVIESO, D. (1965a). The male reproductive cycle of the bat Artibeus lituratus. American Midland Naturalist. 73: 150-160.
- TAMSITT, J.R. & VALDIVIESO, D. (1965b). Reproduction in the female big fruit eating bat Artibeus lituratus palmarium in Colombia. Caribbean Journal of Science. 5: 157-166.
- TAMSITT, J.R. & VALDIVIESO, D. (1966). Parturition in the red fig eating bat Stenodermia rufum. Journal of Mammalogy. 47: 352-353.
- THOMAS, O & HARTERT, E. (1894). First glimpses of the zoology of the Natuna Islands. III list of the first collection of mammals from the Natuna Islands. Novitates Zoologicae. 1: 652-660.
- TICKELL, S.R. (1843). Remarks on Pteropus edulis (Geoffroy). Calcutta Journal of Natural History. 3: 29-36.

- TROUCESSART, E.L. (1904). Catalogue Mammalium tam viventium quam fossilium. Novo edito (prima completa). Berlin: Friedländer und Sohn.
- TUCKER, H.A. (1981). Photoperiod control of hormones, growth and lactation in cattle. In Environmental factors in mammal reproduction, ed. Gilmore D. & Cook, B. London: MacMillan.
- TURNER, D., SHAUGNESSY, A. & GOULD, E. (1972). Individual recognition between mother and infant bats (*Myotis*). Animal Orientation and Navigation, NASA special publication. 262: 365-371.
- TWENTE, J.W. (1955). Aspects of a population study of cavern dwelling bats. Journal of Mammalogy. 36: 379-390.
- VAN DER ELST, R. (1981). A Guide to the Common Sea Fishes of Southern Africa. Cape Town: Struik.
- VAN DER MERWE, M. (1979). Growth of ovarian follicles in the Natal clinging bat. South African Journal of Zoology. 14: 111-117.
- VIELLIARD, J. (1974). Les Chiroptères du Tchad. Revue Suisse de Zoologie. 81: 975-991.
- WALFORD, L.A. (1946). A new graphic method of describing growth in animals. Biological Bulletin. 90: 141-147.
- WALKER, E.P. (1975). Mammals of the World. Baltimore: John Hopkins.
- WAGER, V.A. (1961). The Frogs that inhabit the Wild Life Sanctuaries of Natal. Durban: Wildlife Protection and Conservation Society of Southern Africa.
- WEINMAN, J.P. & SICHER, H. (1947). Bone and Bones. Fundamentals of Bone Biology. St. Louis: Mosby.
- WEISS, P. & KAVANAU, J.L. (1957). A model of growth and growth control in mathematical terms. Journal of General Physiology. 41: 1-47.
- WELCH, Q.B. (1970). Fitting growth and research data. Growth. 34: 293-312.
- WEYMOUTH, F.W., McMILLAN, H.C. & RICH, W.H. (1931). Latitude and relative growth of the razor clam. Journal of Experimental Biology. 8: 228-249.
- WEYMOUTH, F.W. & THOMPSON, (1930). The age and growth of the Pacific coccle. (*Cardium corbis*) Bulletin of the Bureau of Fisheries. 46: 633-641.
- WHITE, G.C. & BRISBIN, I.L. Jnr. (1980). Estimation and comparison of parameters in stochastic growth models for barn owls. Growth. 44: 97-111.
- WHITE, G.C. & RATTI, J.T. (1977). Estimating and testing of parameters in Richard's growth model for western grebes. Growth. 41: 315-323.
- WHITAKER, A. (1905). Notes on the breeding habits of bats. Naturalist London. 1905: 325.
- WICKLER, W. . & SEIBT, U. (1976). Field studies of the African fruit bat *Epomophorus wahlbergi* with special reference to male calling. Zeitschrift

für Tierpsychologie. 40: 345-376.

- WIDDOWSON, E.M. (1981). Role of nutrition in mammalian reproduction. In Environmental Factors in Mammal Reproduction, ed. Gilmore, D. & Cook, B. London: MacMillan.
- WIDDOWSON, E.M., MAVOR, W.O. & McCANCE, R.A. (1964). Effects of undernutrition and rehabilitation in the development of the reproductive organs: Rats. Journal of Endocrinology. 29: 119-126.
- WIDDOWSON, E.M. & McCANCE, R.A. (1960). Some effects of accelerating growth. I General somatic development. Proceedings of the Royal Society. B 152: 188.
- WILLIAMS, J.G. (1969). A Field Guide to the Butterflies of Africa. London: Collins.
- WILLIAMS, M.C., SIMPSON, D.I.H. & SHEPHERD, R.C. (1964). Bats and arboviruses in East Africa. Nature. 203: 670.
- WIMSATT, W.A. (1945). Notes on breeding behaviour, pregnancy and parturition in some vespertilionid bats of eastern United States. Journal of Mammalogy. 26: 23-33.
- WIMSATT, W.A. (1960a). Some problems of reproduction in relation to hibernation in bats. Bulletin of the Museum of Comparative Zoology Harvard. 124: 249-267.
- WIMSATT, W.A. (1960b). An analysis of parturition in Chiroptera, including new observations on Myotis lucifugus lucifugus. Journal of Mammalogy. 41: 183-200.
- WIMSATT, W.A. (1969). Some interrelations of reproduction and hibernation in bats. Symposia of the Society for Experimental Biology. 23: 511-549.
- WIMSATT, W.A. (1975). Some comparative aspects of implantation. Biology of Reproduction. 12: 1-40.
- WIMSATT, W.A. (1979). Reproductive asymmetry and unilateral pregnancy in Chiroptera. Journal of Reproduction and Fertility. 56: 345-347.
- WRIGHT, S. (1926). Book Review. Journal of the American Statistical Association. 21: 493-497.
- YERBURY, J.W. & THOMAS, O. (1895). On the mammals of Aden. Proceedings of the Zoological Society of London. 1895: 542.
- YOUNG, J.Z. (1962). The Life of Vertebrates. Oxford: Clarendon Press.
- ZALOUMIS, E.A. & CROSS, R. (1975). Antelope of Southern Africa. Durban: Wildlife Society.
- ZARROW, M.Y. & CLARK, J.H. (1968). Ovulation following vaginal stimulation in a spontaneous ovulator and its implications. Journal of Endocrinology. 40: 343-352.
- ZERBE, G.O. (1979). A new non parametric technique for constructing percentiles and normal ranges for growth curves determined from

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APPENDIX I

Techniques used to assess the ages of bats used to provide
growth and tooth height data for the development of age
determination methods

Of the 1085 bats caught during this study, 414 were chosen to provide data for the establishment of age determination procedures. They were chosen because their age was either known or could be estimated from recaptures, from age dependent features such as lack of epaulettes and pregnancy in primigravid females, or from captivity.

These bats were classed under the categories listed below, and according to these categories were assigned to the mean ages listed in TABLE 1, APPENDIX 1.

(a) KNOWN AGE SPECIMENS

- (i) Cage born bats. These bats were caught and tagged on the day of birth and thereafter cage maintained until 24 weeks old. At a minimum of three days and a maximum of 14 days, they were caught and examined. This category provided narrow range age data in the 0 to 5,0 month age groupings of TABLE 1, APPENDIX 1. Ranges of four to 10 days for each age grouping reflect the coalition of data from several known age bats whose ages were around the mean age value, in order to provide sample sizes of two or more.
- (ii) Wild neonates. Neonate is used to refer to newly born bats whose eyes have not yet opened. Observations from cage born bats have shown that neonates are between one and four days old. This category provided data in the 0 age grouping of TABLE 1, APPENDIX 1.
- (iii) Recaptured neonates. These are young bats captured as neonates, ear tagged and later recaptured and are of known age. They provided data for the 0 to 8,5 age groupings. (TABLE 1, APPENDIX 1).

(b) APPROXIMATE AGE SPECIMENS

- (i) Juveniles. Juveniles are bats from birth to 3,0/4,0 months, their age being calculated on the basis of deciduous tooth loss and permanent tooth eruption as

described in 3.2.2.1 and 3.3.3. At 3,0/4,0 months a full permanent dentition is attained. The mean age of loss and eruption of teeth (TABLE 3) was used to give a single age value and these bats were used to provide data in the 0 to 3,5 month age groupings of TABLE 1, APPENDIX 1.

- (ii) Subadult males and females. Males with full permanent dentition, no epaulette development, abdominal testes, and mass and forearm (100 g, 84,0 mm) were classed as subadult. Information from recaptured males showed that they first develop epaulettes at 13 months old. On this basis the male subadult category lies between 3,0/4,0 and 13,0 months.

Females with full permanent dentition, not pregnant and showing no nipple enlargement were classed as nulliparous. Information from recaptures showed that females first conceive at 5,0/6,0 months old. On this basis the female subadult nulliparous category lies between 3,0/4,0 and 5,0/6,0 months.

The age range of subadults was further narrowed by employing the following information:

Over a period of 57 months, it was found that 81,7 % of all births occurred from November to January, while 9,48 % occurred from March to May (FIGURE 49 shows percentage of conceptions and births per month). By taking the month of capture into account and working back to the main birth season in most cases (or to the minor March/May season in a few cases where the linear measurements were inconsistent with those born in November/January period), the age was estimated to within two or three months. For the purposes of this study it was assumed that growth of the November/January born bats was at the same rate as those born in the March/May birth season.

This category provided age data in the age groupings 3,5 to 11,5 months of TABLE 1, APPENDIX 1.

- (iii) Primigravid bats. The method for narrowing the estimated age range used in the previous category was extended to estimate the ages of females pregnant for the first time. (Primigravid bats are palpably pregnant, yet have little or no nipple distension). These females are between 5,0/6,0 and 12,0 months and their age was estimated by working back

to the birth season. The category provided age data in the 5,0 to 11,5 age groupings of TABLE 1, APPENDIX 1.

- (iv) Recaptured bats. For recaptured bats, their age on first capture was estimated as described above in (b) (i), and (ii) and (iii) and by adding the time interval between subsequent captures to this, their age was estimated. This category provided data in the 6,5 to 58,0 month age groupings of TABLE 1, APPENDIX 1.

Paucity of data for ages older than 9,0 months, resulted in some age groupings of necessity covering a wider age period. For example, the age grouping 12,5 months, had a range of 10 to 13 months.

- (v) Cage maintained bats. These bats were wild captures or recaptures, their age estimated as previously described then caged for up to two years. They provided data in the age groupings 11,5 to 58,0 months of TABLE 1, APPENDIX 1.

Numbers of bats used and the number of measurements they provided from each of the categories listed above ((a) (i) - (iii) and (b) (i)-(iv)) are given in TABLES 2 and 3, APPENDIX 1.

APPENDIX 1 TABLE 1 The chronological age groupings into which the animals from the age assessment categories (described in APPENDIX 1) were divided

| Age groupings
(mean age in months) | Range of ages
within age
grouping | Age groupings
(mean age in months) | Range of ages
within age
grouping |
|---------------------------------------|---|---------------------------------------|---|
| 0 (birth) | 0 - 4 days | 8,5 | 8 - 9 months |
| 0,25 | 7 - 10 days | 11,5 | 10 - 13 months |
| 0,5 | 13 - 16 days | 15,5 | 14 - 17 months |
| 1,0 | 28 - 32 days | 19,5 | 18 - 21 months |
| 1,5 | 42 - 46 days | 23,5 | 22 - 25 months |
| 2,0 | 56 - 63 days | 27,5 | 26 - 29 months |
| 2,5 | 70 - 75 days | 32,5 | 30 - 35 months |
| 3,0 | 84 - 91 days | 38,0 | 36 - 40 months |
| 3,5 | 98 - 105 days | 43,0 | 41 - 45 months |
| 4,0 | 112 - 120 days | 48,0 | 46 - 50 months |
| 4,5 | 126 - 135 days | 53,0 | 51 - 55 months |
| 5,0 | 140 - 150 days | 58,0 | 56 - 60 months |
| 6,5 | 6 - 7 months | | |

APPENDIX 1, TABLE 2

Numbers of bats and measurements used from the age assessment categories

| Age assessment categories | Linear measurements | | | | | | | | | | | | | | | | | |
|---------------------------|---------------------|-----------------|--------------|-----------------|--------------|-----------------|-------------------|-----------------|--------------|-----------------|--------------|-----------------|-----------------|-----------------|--------------|-----------------|--------------|-----------------|
| | Forearm length | | | | | | Eye-nose distance | | | | | | Zygomatic width | | | | | |
| | Total | | Male | | Female | | Total | | Male | | Female | | Total | | Male | | Female | |
| | nos. of bats | nos. of m'ments | nos. of bats | nos. of m'ments | nos. of bats | nos. of m'ments | nos. of bats | nos. of m'ments | nos. of bats | nos. of m'ments | nos. of bats | nos. of m'ments | nos. of bats | nos. of m'ments | nos. of bats | nos. of m'ments | nos. of bats | nos. of m'ments |
| (a) (i) | 19 | 19
(115) | 10 | 10
(62) | 9 | 9
(53) | 18 | 18
(114) | 9 | 9
(61) | 9 | 9
(53) | 13 | 13 | 7 | 7 | 6 | 6 |
| (a) (ii) | 3 | 3 | 3 | 3 | 0 | 0 | 3 | 3 | 3 | 3 | 0 | 0 | 2 | 2 | 2 | 2 | 0 | 0 |
| (a) (iii) | 3 | 3 | 2 | 2 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 2 | 2 | 0 | 0 |
| (b) (i) | 79 | 79 | 51 | 51 | 28 | 28 | 77 | 77 | 51 | 51 | 26 | 26 | 40 | 40 | 23 | 23 | 17 | 17 |
| (b) (ii) | 154 | 154 | 124 | 124 | 30 | 30 | 148 | 148 | 241 | 24 | 24 | 24 | 71 | 71 | 63 | 63 | 8 | 8 |
| (b) (iii) | 34 | 34 | | | 34 | 34 | 29 | 29 | | | 29 | 29 | 12 | 12 | | | 12 | 12 |
| (b) (iv) | 85 | 108 | 40 | 55 | 45 | 53 | 76 | 94 | 33 | 44 | 43 | 50 | 31 | 41 | 16 | 22 | 15 | 19 |
| (b) (v) | 37 | 89 | 3 | 31 | 34 | 58 | 37 | 80 | 3 | 24 | 34 | 56 | 4 | 9 | 1 | 2 | 3 | 7 |
| TOTALS | 414 | 489 | 233 | 276 | 181 | 213 | 388 | 449 | 223 | 255 | 165 | 194 | 175 | 190 | 114 | 121 | 61 | 69 |

() number of measurements of cage born bats used in FIGURES 18 to 21

APPENDIX 1, TABLE 3

Numbers of bats and measurements used from the age assessment categories

| Age assessment categories | Tooth height measurements | | | | | | | | | | | |
|---------------------------|-----------------------------|-----------------|--------------|-----------------|--------------|-----------------|----------------------|-----------------|--------------|-----------------|--------------|-----------------|
| | P ₃ tooth height | | | | | | upper C tooth height | | | | | |
| | Total | | Male | | Female | | Total | | Male | | Female | |
| | nos. of bats | nos. of m'ments | nos. of bats | nos. of m'ments | nos. of bats | nos. of m'ments | nos. of bats | nos. of m'ments | nos. of bats | nos. of m'ments | nos. of bats | nos. of m'ments |
| (a) (i) | 8 | 46 | 5 | 23 | 3 | 23 | 5 | 19 | 3 | 15 | 2 | 4 |
| (a) (iii) | 3 | 3 | 2 | 2 | 1 | 1 | 2 | 2 | 2 | 2 | 0 | 0 |
| (b) (i) | 77 | 77 | 51 | 51 | 26 | 26 | 47 | 47 | 30 | 30 | 17 | 17 |
| (b) (ii) | 145 | 145 | 121 | 121 | 24 | 24 | 107 | 107 | 95 | 95 | 12 | 12 |
| (b) (iii) | 27 | 27 | | | 27 | 27 | 14 | 14 | | | 14 | 14 |
| (b) (iv) | 73 | 89 | 32 | 43 | 41 | 46 | 60 | 70 | 26 | 33 | 34 | 37 |
| (b) (v) | 36 | 160 | 3 | 32 | 33 | 128 | 31 | 133 | 2 | 24 | 29 | 109 |
| TOTALS | 369 | 547 | 214 | 272 | 155 | 275 | 266 | 392 | 158 | 199 | 108 | 193 |

APPENDIX II

Growth curves

While undertaking a critical analysis of the Von Bertalanffy (1938) growth equation for application to bat growth data, it became apparent that no recent attempt had been made to review growth curve literature and that such an undertaking would be of value to this research.

Both mathematicians and biologists over the last fifty years have developed, adapted and modified a variety of formulae designed to describe organic growth. Three early growth equations, whose mathematical properties permit the most rational application to the normal growth of organisms have provided the basis for most subsequent computations.

They are : The Gompertzian function (Gompertz, 1825)

$$w_t = W_\infty e^{-be^{-kt}} \quad (1)$$

Brody's function (Brody, 1937; 1945)
(Also known as the monomolecular function)

$$w_t = W_\infty (1 - e^{-kt}) \quad (2)$$

The logistic function (Robertson, 1923)
(Also known as the autocatalytic function)

$$w_t = W_\infty / (1 + e^{-kt}) \quad (3)$$

where:

w_t = size at time t

W_∞ = the ultimate limiting value; the size asymptote

b = a constant reflecting the choice of the zero of time

e = the exponential or natural logarithm

k = the rate constant which determines the spread of the curve along the time axis

t = time (age of specimens)

These three functions can be used to give a graphical presentation of growth as a curve.

The Gompertzian function

A growth curve can be defined as a mathematical relationship between the size of an animal and time (Fabens, 1965). The growth curve constructed from the Gompertzian equation (1) is asymmetrical, inflecting at $w_t = W_\infty/e$ and is based on the concept of exponential growth rate decay with time. It was originally developed by Gompertz in 1825 in connection with human mortality studies. Since then the relationship was discovered independently by Wright (1926) and later by Weymouth, McMillian & Rich (1931) and Weymouth & Thompson (1930) who developed it for the studies on the growth of molluscs and gave it a physiological interpretation.

Only a few studies have appeared in which the Gompertz function has been fitted to actual growth measurements (Curtis, 1932; Davidson, 1928; Deming, 1957; Laird, 1967; Weymouth et al., 1931; Weymouth & Thompson, 1930).

Laird, Tylor & Barton (1965) state that in the growth of normal organisms the Gompertz equation fails to fit the last part of the growth curve, because, as noted by Weymouth et al. (1931), in their study of the razor clam (*Siliqua patula*), there is a tendency for 'growth to be maintained at a higher rate than the first part of the curve would lead one to predict'. In order to overcome this difficulty, Laird et al. (1965) derived a modified form of the Gompertz function, supplemented by an arithmetic growth curve which fits the accretionary growth displayed by many animals at early maturity. Sikov & Thomas (1970) used Laird's reformulation of the classical Gompertz growth curve to produce a satisfactory fit to prenatal growth data from the rat.

Although it has been shown that many sets of growth data do not fit this type of curve (Lyne & Verhagen, 1957; Needham, 1964; Richards, 1959) some logical and experimental support has been found for it (Deming, 1957; Medawar, 1940).

Brody's function

Brody's function (2) was developed by Brody in 1937 and later modified by him in 1945. It has been popular with biologists for some time and has been used particularly in the analysis of rodent growth. Chew & Butterworth (1959), Lackey (1967), Linzey & Linzey (1967) and Roubicek, Pahnish & Taylor (1964) used the arith-log method proposed by Brody (1945) in which measurement values were plotted on the log scale and age on the arithmetic scale. From these linear sections, Instantaneous Growth Rates (IGR) were calculated:

$$k \text{ (IGR)} = \frac{W_2 e - W_1 e}{t_2 - t_1} \quad (4)$$

where:

k = the instantaneous percentage rate of growth for the unit of time in which t_1 and t_2 are expressed

$W_2 e$ and $W_1 e$ = natural logarithms of the measurements made at t_1 and t_2

t_1 and t_2 = time at which measurements W_1 and W_2 were made

Laird et al. (1965) have rejected Brody's function stating:

'The monomolecular equation can be disposed of at once; we can observe that growth is fitted, even in the post natal period, only beyond the inflection point, the earlier data points trailing off to the left of the curve'.

The logistic function

The logistic equation (3) produces a curve which is symmetrical around its point of inflection and its relative growth rate declines linearly with increasing size. It has been used from a theoretical standpoint (Robertson, 1923) and as a convenient empirical curve for describing growth (Merrell, 1931), especially the growth of populations (Grimsdell & Bell, 1972; Laird et al., 1965; Pearl, 1925).

Sikov & Thomas (1970) fitted rat prenatal growth data to both the symmetrical logistic (3) and the Gompertzian equations (1) and found that the logistic gave the better fit for the observed specific growth rate over the range of gestation studied, as well as giving fitted curves with

a smaller residual sum of squares than any comparable Gompertz curve.

Laird *et al.* (1965) also compared the Gompertz with the logistic function using both to compute age changes in the specific growth rate from one set of raw data. They found that the Gompertz equation offered the most economical description of the observed changes.

In the field of human biology, a simple logarithmic equation has been used to describe preadolescent growth (Israelson, 1960). An exponential equation has been used by Jenss & Bayley (1937) to describe the first six years of life. Count (1945) however wished to describe human growth from conception to adulthood. He therefore divided growth into three phases and for the first two phases used logarithmic expressions of the type used by Israelson (1960). For the third phase, involving growth to the asymptote, he applied a complex form of the logistic, the skew logistic.

Prior to Count's work, Pearl & Reed (1925) successfully employed the skew logistic to describe growth in a variety of plant and animal material.

A further type of logistic has been used in growth studies. Carmon, Golley & Williams (1963) applied the reciprocal of the regression equation to obtain the logistic function:

$$y = \frac{1}{\alpha + \beta \rho^t} \quad (5)$$

where:

α = the asymptotic parameter

β = changes in y as t passes from 0 to ∞

ρ = the factor by which the deviation of y from the asymptotic value is reduced every time a step is taken along the x axis

This was used in a detailed report to describe growth and development in deer mice (*Peromyscus maniculatus*).

The Von Bertalanffy function

Several growth models have been devised which fit into the category of having parameters of physiological significance. The best known and most frequently used of these is the Von Bertalanffy function (Von Bertalanffy, 1938; 1941; 1957; 1960). In commenting on this function Needham (1964)

remarked:

'On the combined scores of biological rationale, ability to fit actual results and abilities to confirm and predict other facts about growth and metabolism, the relation of Von Bertalanffy is of outstanding value.'

The Von Bertalanffy growth model is based on the concept that growth occurs when and to the extent that anabolism exceeds catabolism, the anabolic factors acting in proportion to volume. In deriving the equation Von Bertalanffy started from the allometric relation during growth, between an animal's metabolic rate and its mass, claiming that the slope of the allometric line (m) may have three possible values, thus recognizing among animals three metabolic types.

The general form of this equation in current use is that proposed by Beverton & Holt (1957).

$$l_t = L_\infty \left(1 - e^{-k(t-t_0)} \right) \quad \text{for linear growth} \quad (6)$$

$$w_t = W_\infty \left(1 - e^{-k(t-t_0)^3} \right) \quad \text{for growth in mass} \quad (7)$$

where:

- L_∞, W_∞ = asymptotic mass and length, the maximum that an animal can attain under given conditions
- k = coefficient of catabolism, a constant representing the catabolism of body materials per unit mass (length) and time
- e = exponential of natural logarithm
- t = age of animals
- t_0 = theoretical age at which the animal would have zero mass (length) with the same growth pattern as that observed in later life

The equation has been used to fit growth curves for a great variety of species, including whelk (*Dicathais aegrota*) by Phillips & Campbell (1968), bivalve mollusc (*Macoma balthica*) by Cloern & Nichols (1978), *Lates calcariser* by Munro (1982), plaice (*Pleuronectes platessa*) by Beverton & Holt (1957) and Graham (1956), cod (*Gadus callarias*), North Sea sole (*Solea vulgaris*) and haddock (*Melanogrammus aeglefinus*) by Beverton

& Holt (1957), flat headed sole (Hippoglossoides elassodon) by Cloern & Nichols (1978), elephant (Loxodonta africana) by Hanks (1972) and Sherry (1978), buffalo (Synceros caffer) by Sinclair (1977), Burchell's zebra (Equus burchelli) by Smuts (1974), impala (Aepyceros melampus) by Brooks (1978) and Howells & Hanks (1975), eland (Taurotragus oryx) by Jeffrey & Hanks (1981), blue wildebeeste (Connochaetes taurinus) by Braack (1973) and by Attwell (1977), waterbuck (Kobus ellipsiprymnus) by Melton (1978), lion (Panthera leo) by Smuts, Anderson & Austin (1978), Southern elephant seal (Mirounga leonina) by Condy (1980), Eptesicus fuscus by Kunz (1974) and rock dassie (Procavia capensis) by Fairall (1980) and Steyn (1980).

Several authors have modified the Von Bertalanffy function to suit species with seasonal growth patterns and data obtained at irregular time intervals. Cloern & Nichols (1978) incorporated a time varying coefficient and significantly improved the capability of the Von Bertalanffy equation to describe seasonal growth in Macoma balthica and Hippoglossoides elassodon. Gulland & Holt (1959) and Munro (1982) both described methods for estimating the parameters in the equation from data obtained at variable time intervals.

Popular though the Von Bertalanffy function has proved to be, it has been severely criticized by some authors. Hanks (1972) examined the two parameters, k and t_0 . k the constant of catabolism was only shown to have any physiological significance in starving animals of short life span. Hanks remarked that in an animal with a long life span, it is possible k might be physiologically significant over a short time period, under certain prescribed nutritional conditions, but not over a period of 60 years of varying nutritional status, as in the case of the elephant. t_0 represents a theoretical age at which the animal would have a zero mass with the same growth pattern as that observed in later life. Clearly, in practice t_0 is artificial, as the adult pattern is never found at the earliest age. He therefore concluded that there is little biological significance in the parameters contained in the Von Bertalanffy equation.

Knight (1968) noted the popular use of the Von Bertalanffy curve and proceeded to examine the validity of the parameter L_∞ . He cited references (Graham, 1933; Ketchen & Forrester, 1966) in which truncated growth data (that is data terminating just before or just at the point of which maximum size is reached) was used for calculating the asymptote. L_∞ calculated in this manner would be meaningless and the data unsuited to a Von Bertalanffy curve. Knight concluded by warning of the danger of forcing any growth curve on inappropriate data.

Roff (1980) went further and published a paper proposing the retirement of the Von Bertalanffy function on the grounds of extreme difficulty in fitting the equation in a statistically satisfactory manner.

Richards (1959) examined the Von Bertalanffy growth function and found that the derivation of the growth rate (k) contained assumptions and approximations which cast doubt upon its theoretical validity. In particular, Von Bertalanffy rejected any values for the slope of the allometric line (m), over one. Richards remarks that if m is assessed from the growth data instead of from 'dubiously relevant metabolic studies', the Von Bertalanffy function would find its chief application with values of m greater than one. He felt that values of m much exceeding unity are necessary if the general function is to have wide empirical application. On this basis, he derived a function from the Von Bertalanffy equation containing four parameters instead of three; L_{∞}/L_{∞} , t_0 , k and m . This extended form of the equation was applied by Richards (1959) to plant growth and later successfully used on animal data (White & Brisbin, 1980; White & Ratti, 1977).

The Weiss and Kavanau growth model

A second important growth model based on physiological concepts is that developed by Weiss & Kavanau (1957). It is based on the principle of growth regulation by negative feedback and is supported by much experimental evidence including compensatory growth after injury and growth regulation by organ extracts. This method however has not found popularity and there are few references to its practical application, the probable reason being the extreme complexity of the methods used for determining the parameters.

The Walford plot

A graphical method for fitting exponential growth curves was developed by Walford (1946) resulting in a linear transformation of the usual growth curves. He showed its application for two molluscan species, four species of marine fish and three mammal species. The method was used to describe growth of the commercial whelk (Buccinum undulatum) by Hancock (1963). Since then the technique has been used widely in fisheries research (Phillips & Campbell, 1968).

The polynomial regression

A frequently used but purely empirical approach, which can adequately present the growth data in a summarized form, but has no pretence towards growth predictions, is the application of the polynomial regression formula. Welch (1970) and Zerbe (1979) both adapted the high degree polynomial to describe human growth. Bunak (1946) and Roff (1980) advocated the use of parabolic functions. Lyne & Verhagen (1957), who failed to find a good fit for their marsupial growth data, used a

combination of a parabola for the early part of the growth curve and an exponential for the asymptotic section. Beverton & Holt (1957) suggested the use of high degree polynomials in such cases where the Von Bertalanffy function was unsatisfactory.

Stevens (1951) adequately summarized the situation: 'Although the field of application of the polynomial regression formula is extremely wide, there yet exists a large number of regression problems for which they are unsuitable, either because the polynomial curve in fact does not provide an adequate graduation of the data or because the curve is capable of taking a form which must be rejected intuitively. Perhaps the most frequently encountered type of problem for which a polynomial regression is clearly unsuitable is one in which the value of the dependent variable y , steadily approaches an unknown asymptotic value, as x passes to infinity'.

Stevens asymptotic regression

Where organic growth proceeds towards an asymptote, as does mammalian growth, the asymptotic regression formula, as suggested by Stevens (1951) can be employed.

$$y = \alpha + \beta \rho^x \quad (8)$$

where:

y = the size of the animal

α = the asymptotic value of y

β = the change in y when x passes from 0 to $+\infty$

ρ = the factor by which the deviation of y from its asymptotic value is reduced every time a step is taken along the axis of x

x = the age of the animal

Although not derived from a physiological process, the three parameters have biological significance, α being the asymptote of growth, β the total growth achieved and ρ the rate of growth.

It is in fact a universal equation which is used in every branch of science. It is Newton's Law of Cooling, Mitscherlich's Law describing the response to a fertilizer and equally useful for representing the growth of an organism from birth to maturity.

In 1951 computer development was in its infancy and the problem of using this method of curve fitting was the enormous arithmetic labour involved in determining the parameters α , β and ρ . With the inception of early computer methods Rothampstead Experimental Station drew up a program for the application of this formula.

Following a paper by Hiorns (1965) in which techniques for fitting Stevens asymptotic regression were put forward, Phillips & Campbell (1968) applied the method to the whelk (Dicathais aegrota) data. Its use for mammalian growth seems to have been overlooked, though it has been used to describe growth in some species of insectivorous bats (Stebbing, pers. comm.).

APPENDIX III

Growth, and tooth growth/wear curves derived from an asymptotic function: $y = \alpha + \beta \rho^x$

Introduction

Stevens (1951) has shown the basis of a method to fit the equation:

$$y = \alpha + \beta \rho^x \quad (0 < \rho < 1) \quad (1)$$

to an appropriate set of data. Because of the large amount of computation necessary in the process, and the non-availability, at that stage, of computers, certain averaging of the data, with its resultant loss of generality, was required. Presumably a sample of any number of observations could have been handled; however, reduction to an effective set of five, six or seven averaged points was required so that a reasonable value of ρ could be estimated and thereby a covariance matrix evaluated. An inversion process yielded values for the parameters α and β as well as a correction factor to be applied to the original estimate of ρ . The fitted curve was thus dependent on these three parameters as evaluated, and no further modification was contemplated.

The degree to which the initial estimate of ρ affected its final assay was not discussed by Stevens (1951). Considering that the initial estimate was obtained via the formula:

$$\text{Estimate of } \rho = \frac{y_1 - y_n}{y_0 - y_{n-1}} \quad (2)$$

where the values of y are ordered into a descending set, and where each y represents an averaged set of ordinates over a specific range of x values, its value has a significant bearing on the parameters of the fitted curve. The calculated assay would be dependent upon the choice of the division, into equally sized intervals of the x axis and could theoretically vary considerably if outliers, that could feasibly occur, were included in the averaged value of y_0 or y_1 (or for that matter y_n or y_{n-1}).

Development of growth program

Given the facility of a modern digital computer, the least squares calculation of the curve as described by Stevens (1951) becomes quite simple. It is however, possible to project the method into a series of iterative fittings, each stage of which provides an improved regression line based on some

criterion of 'goodness of fit'. Further, computation can now include all data values without averaging or grouping being necessary.

Thus, from the second iteration onwards, the value of ρ as calculated at the previous stage, becomes the starting point for the following attempt. The 'goodness of fit' was based on the SSD (sum of squares of differences) between each observed y value and its prediction from the fitted line. The process is programmed to terminate when an iteration fails to improve on the SSD as provided by its predecessor, and will occur when no significant change is effected in the value of ρ . An arbitrary value between 0 and 1 can be assumed for the first value of ρ . The process will converge on the value of ρ which minimizes the SSD irrespective of the starting point, the only limitation being the greater number of iterations necessary if the initial estimate varies considerably from its final value. Experience has shown that the majority of growth relationships for which (1) is an appropriate formula will yield a 'best fit' value for ρ in the range 0,4 - 0,8. It was therefore considered appropriate to allow in the program for a standard initial estimate of ρ at 0,7, with convergence when taking place in some six to eight iterations for five place decimal accuracy to be ensured.

The relationship (1) exhibits a number of interesting properties, the physical interpretation of which assists in the understanding of the attributes being measured. The intercept on the y axis (that is at $x = 0$, or at birth day) yields the physical size at birth of the characteristic being measured. Thus, given that observations commenced sometime after birth, a reasonable estimate of 'value' at birth can be made from the fitted equation. The sum of α and β (where β is inevitably negative) yields this value. β (absolute value) yields total growth between birth and maturity, α gives the asymptotic or adult size of the characteristic being measured, and ρ can be considered as the growth factor (FIGURE 1, APPENDIX III).

TABLE 1 (APPENDIX III) presents the percentage of growth at age x after birth, depending upon different ρ values (growth factors). A high value of ρ predicts slow growth and a low value extremely rapid growth. The relationship may be redefined as:

$$y = \alpha + \beta (1 - \rho_*)^x \quad (3)$$

without loss of generality. In this form low ρ_* would predict slow growth and high ρ_* would predict fast growth. On this basis it will be observed that α and β relate to size while growth is completely defined by ρ .

Since in the relationship (1), y still has low positive values over certain ranges of negative x in the proximity of the origin, and since the value, $x = 0$ is the day of birth, one can consider this region as relating to the gestation period. However, fetal growth displays a concave form, and since the

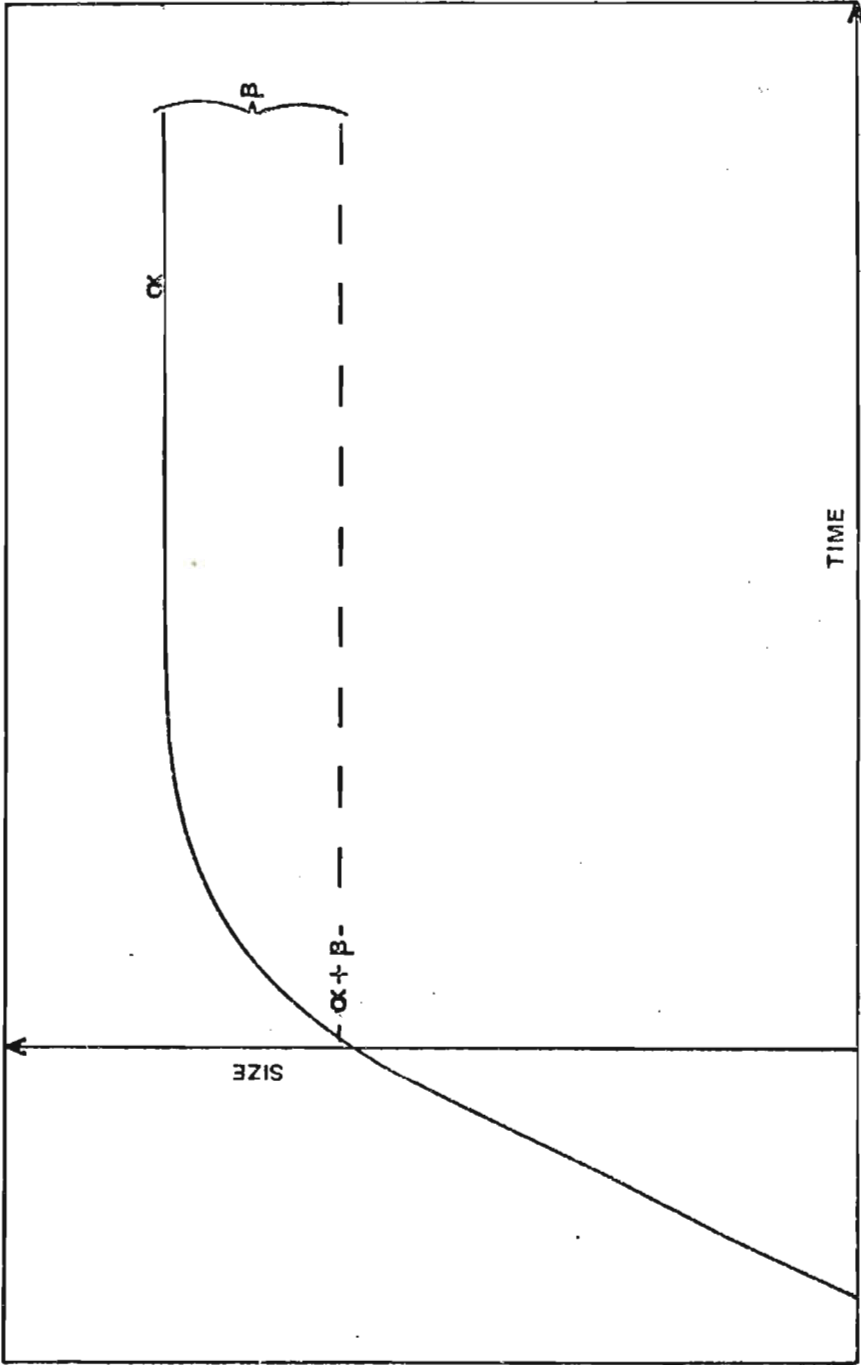


TABLE 1 APPENDIX III

Percentage of growth at age x after birth, depending on different ρ values

| ρ | Age x in months | | | | | | | | | | | | | | | | |
|--------|-----------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|------|------|------|------|------|------|
| | 0,5 | 1,0 | 2,0 | 3,0 | 4,0 | 5,0 | 6,0 | 7,0 | 8,0 | 9,0 | 10,0 | 11,0 | 12,0 | 15,0 | 20,0 | 25,0 | 30,0 |
| 0,9 | 5 | 10 | 19 | 27 | 34 | 41 | 47 | 52 | 57 | 62 | 65 | 69 | 72 | 79 | 88 | 93 | 96 |
| 0,8 | 11 | 20 | 36 | 49 | 59 | 67 | 74 | 79 | 83 | 87 | 89 | 91 | 93 | 96 | 99 | | |
| 0,7 | 16 | 30 | 51 | 65 | 76 | 83 | 88 | 92 | 94 | 96 | 97 | 98 | 99 | | | | |
| 0,6 | 23 | 40 | 64 | 78 | 87 | 92 | 95 | 98 | 99 | | | | | | | | |
| 0,5 | 30 | 50 | 75 | 87 | 94 | 97 | 98 | 99 | | | | | | | | | |
| 0,4 | 37 | 60 | 84 | 94 | 97 | 99 | | | | | | | | | | | |
| 0,3 | 45 | 70 | 90 | 97 | 99 | | | | | | | | | | | | |
| 0,2 | 55 | 80 | 96 | 99 | | | | | | | | | | | | | |
| 0,1 | 68 | 90 | 99 | | | | | | | | | | | | | | |

relationship (1) is nearly linear in this region, its use for the gestation period would be inappropriate.

Development of tooth growth/wear program

In growth measurements and subsequent curve fitting it is commonly assumed that some asymptote, which reflects the ultimate size of the characteristic being measured, exists. Teeth, however, once the adult diet has been adopted, are subject to wear. Assuming that growth of the tooth was still continuing at the onset of wear, an appropriate graph and mathematically-fitted curve would portray a more rapid deceleration of the growth an observable turning point at which stage growth and wear were equal. Finally a downward slope, becoming more pronounced until growth was no longer present and wear was the sole influencing factor, can be seen.

From normal observation, growth follows a curvature which can be described by the relationship (1). Wear, if constant abrasion of the teeth occurs, could be considered as linear after the stage at which suckling ceases. A short period undoubtedly exists when suckling and initiation of the adult diet coincide. However since in the case of small mammals, this duration is probably short in relation to the frequency of measurements being taken, no specific modelling of this event is feasible.

Ignoring this special case, an assumption of linearity of wear, from its onset until adulthood, is made. It is probable that as senescence sets in, wear will accelerate due to poor condition. Again as this was beyond the scope of this work it was omitted.

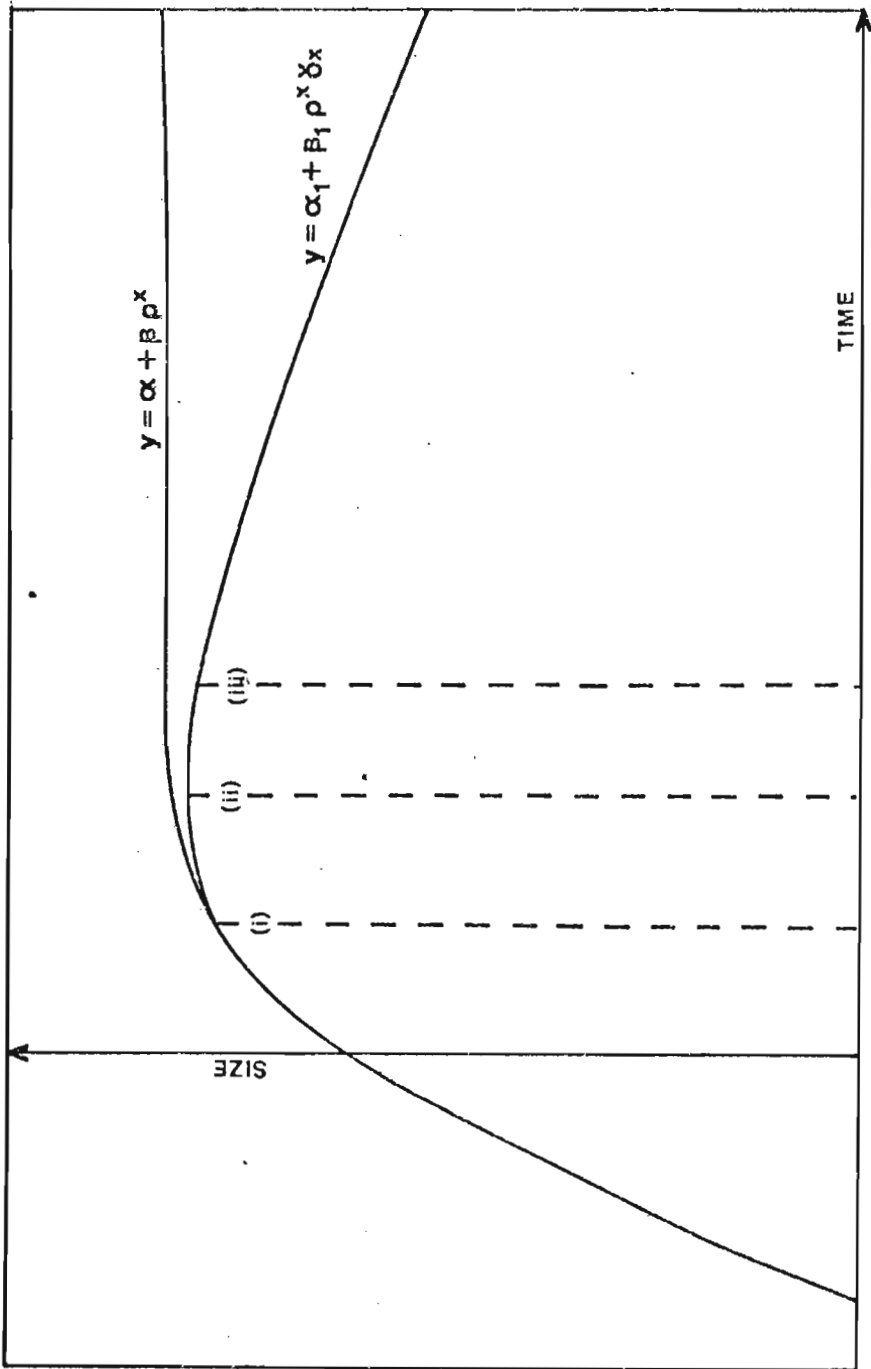
An equation:

$$y = \alpha_1 + \beta_1 \rho + \gamma x \quad (4)$$

has, on the above hypothesis been fitted to incorporate in one relationship, both the factors of growth and wear. FIGURE 2 (APPENDIX III), illustrates the similarities of and differences between the original growth curve and the wear influence factor. It will be noted from FIGURE 2 (APPENDIX III) that the growth/wear turning point falls below the asymptote of the usual growth. This is to be expected since wear is presumed to set in prior to adulthood at which stage the asymptote is reached, if wear is absent.

The constants α and α_1 differ slightly from one another as do β and β_1 and ρ and ρ_1 . The effect of γx on the fit of the wear curve is the cause of this slight divergence of the two curves in the region of the y axis.

In FIGURE 2 (APPENDIX III) there exists a point between (i) and (ii) when wear will be seen to mathematically take effect, causing an actual divergence of the



wear curve from the assumed growth line.

In an experiment to verify this, very close agreement was obtained from statistically fitted data. Within the region where physical wear was not expected, the curves followed one another and the divergence due to wear could be clearly predicted from the equations at the point when it was expected to occur.

In order to fit the equation:

$$y = \alpha_1 + \gamma x + \beta_1 (\rho_1)^x \quad (5)$$

an extension to the theory provided in Stevens (1951) paper had to be made. A 4 x 4 covariance matrix (in the notation of Stevens, 1951) was evolved as follows:

$$\begin{bmatrix} n & \Sigma(r^x) & \Sigma(x) & \Sigma(x r^{x-1}) \\ \Sigma(r^x) & \Sigma(r^{2x}) & \Sigma(x r^x) & \Sigma(x r^{2x-1}) \\ \Sigma(x) & \Sigma(x r^x) & \Sigma(x^2) & \Sigma(x^2 r^{x-1}) \\ \Sigma(x r^{x-1}) & \Sigma(x r^{2x-1}) & \Sigma(x^2 r^{x-1}) & \Sigma(x^2 r^{2x-2}) \end{bmatrix} \quad (6)$$

where r is the estimate of ρ , while the right hand side is accordingly altered to the column vector:

$$(\Sigma(y) \quad \Sigma(y r^x) \quad \Sigma(x y) \quad \Sigma(x y r^{x-1})) \quad (7)$$

The method of fit was similar to the standard growth curve (FIGURE 2 (i) APPENDIX III). An iterative procedure, based on changing values of ρ was used to obtain the minimum SSD of observed and predicted values.

APPENDIX IV

Dehydration and wax embedding schedule

| Step | Solution | Time | Process |
|------|--|------------|--------------|
| 1 | 50 % alcohol | 2 hours | dehydration |
| 2 | 70 % alcohol | 2 hours | |
| 3 | 80 % alcohol | 2 hours | |
| 4 | 90 % alcohol | 2 hours | |
| 5 | 95 % alcohol | 2 hours | |
| 6 | 95 % alcohol | 2 hours | |
| 7 | 100 % alcohol | 2 hours | |
| 8 | 100 % alcohol | 3 hours | |
| 9 | Xylene | 2 hours | clearing |
| 10 | Xylene | 3 hours | |
| 11 | Wax bath | 2 hours | infiltration |
| 12 | Wax bath | 2 hours | |
| 13 | Vacuum infiltration
at 4,5 - 6,6 kg | 10 minutes | |

APPENDIX V

Soft tissue staining schedule

| Step | Solution | Time
(minutes) |
|------|----------------------|-------------------|
| 1 | Xylene | 5 |
| 2 | Xylene | 5 |
| 3 | 100 % alcohol | 5 |
| 4 | 90 % alcohol | 3 |
| 5 | 70 % alcohol | 0,5 |
| 6 | Running tap water | 5 |
| 7 | Mayer's haematoxylin | 5-15 |
| 8 | Running tap water | 5 |
| 9 | 1 % alcoholic eosin | 6-10 |
| 10 | 95 % alcohol | 1 |
| 11 | 95 % alcohol | 1 |
| 12 | 100 % alcohol | 5 |
| 13 | Xylene | 5 |