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The Biology and Management of Australasian Carnivorous Marsupials

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Maintenance and Breeding of the Numbat

(Myrmecobius fasciatus) in Captivity

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Introduction

Until about fifteen years ago, the future of the numbat (Mynnecobius fasciatus) was thought to be reasonably secure. Despite the huge contraction of its range which followed European settlement of Australia, populations persisted in forests and remnant woodlands of south-western Australia (Calaby, 1960). By the late 1970s, however, attrition of woodland remnants in the cereal-growing region east of Perth (the southern wheat-belt) and an increase in the introduced red fox (Vulpes vulpes) population in this region combined to reduce further the species' numbers and range (Christensen, 1980; Connell and Friend, 1985; Friend, 1987; 1990) until it appeared that extinction might be imminent. A four-month survey of all known numbat habitat conducted in 1979-80 resulted in only two sightings of numbats (Turner and Borthwick, 1980).

Although there were a number of rare and endangered mammals in Western Australia whose basic biology was not well understood, and which were in urgent need of research aimed at their conservation, most either had reasonably secure populations on offshore islands or were breeding well in captivity. Neither condition applied to the numbat. The Western Australian State Government therefore gave priority to a study to determine ways to manage numbat populations and habitats to ensure its conservation. This study was initiated in 1981 and concentrated on the ecology and management of the numbat population at Dryandra Forest, 170km south-east of Perth, one of the few strongholds of the species. As numbats had not been bred successfully in captivity, a simultaneous program was implemented to develop techniques for captive breeding so as to provide a further safeguard in the event of another major decline. The additional funds necessary to support this project were provided by the World Wide Fund for Nature Australia (WWFA) in September 1983. The findings of the project were documented in a report to WWFA in February 1988 (Friend and Whitford, 1988).

Aims of the Captive Breeding Project

The major obstacle to keeping numbats in captivity is the provision of appropriate food. As the numbat's natural diet consists almost entirely of termites (Calaby, 1960), the maintenance of a large number of captive animals on this food alone, which must be collected from the bush, would be prohibitively expensive. Despite considerable effort, attempts to produce termites in artificial colonies have not been successful at the scale necessary to meet the requirements of this program (Dr. J.A.L. Watson, CSIRO Division of Entomology, personal communication). The specific aims of this project, therefore, were: 1) to establish conditions of captivity under which numbats will breed, and 2) to develop an artificial diet on which numbats will thrive, breed and raise young in captivity. Replacement of termites with a diet consisting of more readily available ingredients was clearly vital to the success of the project.

History of Numbats in Captivity

The first record of numbats in captivity is attributable to Sir George Grey, an early Governor of South Australia, who reported that his wife had seen several in captivity being maintained on sugar and milk (Gould, 1863). There have been other short-lived attempts to keep numbats on

termites and substitute diets, including the well-documented effort of Fleay (1942; 1949) who flew a young female numbat from Western Australia to Healesville Sanctuary in Victoria where it was kept on termites for two months before dying suddenly.

The most successful record of keeping numbats in captivity before the present project is that of the Taronga Zoo (Sydney), where the species was kept continuously for a period of more than eight years (Strahan, 1978). The individual held longest in captivity was a female, brought in from the wild as an adult, which survived in captivity for five years and three months. Taronga Zoo's numbats were maintained on a diet of termites. They bred on two occasions but the young did not survive more than a few weeks. The mother of both litters was the above mentioned long-lived female and the young were produced in her first two breeding seasons in captivity.

At Dryandra, where numbats have been studied intensively, most females give birth during the second half of January (Friend, in lit.). The first litter born at the Taronga Zoo was recorded on 21 January 1969, but the second was not recorded until 20 April 1970 (Taronga Zoo records). The second year's litter was unusually late and the female's reproductive physiology may have been out of phase with that of the wild population. Alternatively, it is possible that the birth went unnoticed for some time and actually occurred closer to January. Collins (1973) suggested that the loss of these litters might have been due to physical interference by the male which was kept continuously with the female. Collins observed the male sniffing the female's pouch area while the young were attached eliciting an annoyed response from the female. This is a plausible explanation as numbats are solitary for most of the year (Calaby, 1960; Friend, unpublished). The confinement of two animals together may therefore promote interactions which do not normally occur in the wild.

The death of the last two remaining numbats at Taronga followed a change of accommodation from a small enclosure, exposed to sunlight for much of the day, to a display facility situated on a south-facing (shady) slope provided with a running stream; the result being a shady, moist en-

vironment at all times of the year. Basking, an apparently important thermoregulatory mechanism in numbats, probably was restricted in the new quarters. The death of the numbats was attributed to thrush, a fungal infection (R. Strahan, personal communication).

The history of previous attempts to keep and breed numbats in captivity gave reason for optimism. First, there seemed to be no major difficulty in maintaining numbats as long as they were fed termites and were given good access to sunlight. Second, mating and birth had occurred in captivity, albeit only on a diet of termites. Third, numbats had been maintained on non-termite diets, so it was obviously possible to provide them with alternative food which they would accept. The challenge was to find an artificial maintenance diet on which they would breed.

Termites as food

Compared to other invertebrates, termite workers and soldiers tend to be low in fat and high in ash and about equal in total nitrogen (Redford and Dorea, 1984). However, fat content is higher in reproductive castes, being maximal in alates at release when total lipids may exceed 50% dry weight (La Fage and Nutting, 1978). Nasutitermes exitiosus workers (the species used extensively in this project) contain only 5-15% lipid on a dry weight basis (1-3% lipid on a wet weight basis (Moore, 1969; B.P. Moore, personal communication)). The ratio of unsaturated to saturated fats in this species is also unusually high for insects (Moore, 1969).

Specialist termite-eaters feed mainly on workers, as they are the most abundant caste and lack the defense mechanisms of soldiers. Most termite eaters also ingest large quantities of soil as they feed and their diet is consequently relatively low in caloric content (McNab, 1984). These constraints also apply to specialist anteaters. As a possible consequence, most ant- and termite-eating mammals have low metabolic rates. McNab (1984) suggested that the adaptive advantage of ant- and termite-eating was in lack of competition with other mammals rather than to high nutritive value of the diet. The suggestion that termites are not a particularly rich source of

nutrition is supported by the observation that numbat pouch young grow very slowly (Calaby, 1960).

Artificial diet

Many diets developed to maintain ant- and termite-eaters in captivity have been based on meat, eggs and milk, supplemented with vitamins, minerals, formic acid and other minor ingredients. Some published examples of ant- and termite-eater diets are given below:

Aardvark (Sampsell, 1969): Dry dog food, Pablum, Mellins food (milk modifier), honey, vitamin-mineral supplement, condensed milk, eggs.

Aardwolf (Spinelli, 1970): Meat, bone meal and milk.

Tamandua (Merritt, 1975): Meat mixture (horsemeat and mink developer chow), canned milk and water, vitamins, Gevral protein powder, Paltone powder (vitamins and minerals), Tome powder (protein and vitamins).

Pangolin (Menzies, 1962): Ground meat, bran, milk, ants eggs or formic acid.

Armadillos (Merritt, 1973): Ground horsemeat, mink developer chow, eggs, cod-liver oil, honey, molasses and vitamins.

Echidna (Collins, 1973): Philadelphia Zoo: Milk, egg; St Louis Zoo: Pablum, egg, milk, salt, calcium, minerals; Taronga Zoo, Sydney: Meat, carrot, blood, egg, milk, Lactogen, clay, lettuce, formic acid.

Echidna, aardvark and aardwolf (Young, 1966) Rawminced meat, pre-cooked cereal, skim milk, whey powder, corn meal, yeast, calcium phosphate and vitamins.

Successful breeding of ant- and termite-eaters has been rare (however see Spinelli, 1970; Young, 1973). Thus, while many individuals have been maintained on these diets, there is little information on their adequacy for growing young or lactating females. As Griffiths (1978) showed that the growth rates of young echidnas fed on an egg-milk custard supplemented with termites was greater than those fed on termites alone, we decided to try it with the numbats, and were given valuable assistance by Dr. Griffiths. This diet was subsequently modified by using a low-lactose

milk powder, Digestelact (Sharpe Laboratories, Sydney) (Green et al., 1985) to reduce the danger of scouring as a result of lactose intolerance. [The enzyme lactase, which is responsible for the breakdown of lactose, is not produced by all mammal species, and in some appears only in post-juveniles (van Reenen, 1986). There is a danger that intestinal scouring will occur as a result of lactose intolerance, a condition frequently seen in marsupials (Stephens et al., 1974; Oglesby, 1981)].

Breeding in the wild

Like most dasyurids, to which it is closely related (Friend, 1989), the numbat is a strictly seasonal breeder and most young at Dryandra appeared on the females' nipples in the latter half of January (Friend, unpublished). On the basis of published information on dasyurid reproduction (Lee et al., 1982), we anticipated that the gestation period of the numbat would not exceed four weeks and that mating would begin shortly before Christmas. This hypothesis was also supported by the observation at Dryandra that numbat testes size changed dramatically during the course of the year, reaching maximum size in November-December (Figure 1). Furthermore, male presternal glands, believed to be used for scentmarking (Calaby, 1960), also showed an annual cycle of activity in wild animals (Friend, unpublished) with maximum secretion occurring between October and January.

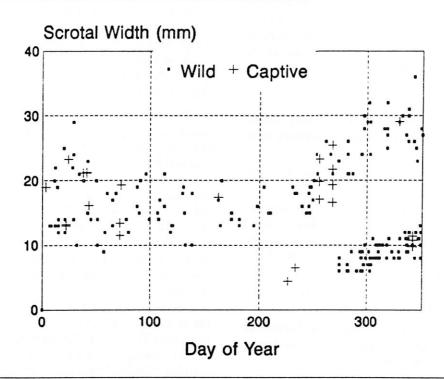
Field studies also established that female numbats breed in their first year. The development of radio-collared young indicated that first-year males did not display marked testicular growth over the summer and autumn (Figure 1) and their pre-sternal glands displayed minimal activity during the breeding season. Plans for captive breeding, therefore, involved all available females, but only males in their second year or older.

Methods

Source of Animals

At the beginning of the program, there were no numbats in captivity. Individuals were taken from the wild at Dryandra Forest, 170km south-east of Perth, 200km from the breeding facility at Woodvale. One male (identified as 1M) and one female

Figure 1. Comparison of Scrotal Widths of Wild and Captive Male Numbats Measured Throughout the Year.



(2F) with four attached young (two females, 3F and 4F and two males, 5M and 6M) were captured and taken into captivity in June 1984. Between November 1985 and October 1987, three more adult males (16M, 22M and 23M) and one adult female (17F) with four attached young (two females 18F and 19F, two males 20M and 21M) were brought into the colony. During that same period, the original wild-caught male and female (1M and 2F), and a captive-reared male and female (5M and 10F) were released into the wild. In November 1986, all captive numbats were transferred to the Perth Zoo, and kept under similar conditions to those at Woodvale. In October 1988, two females (15F and 19F) and two males (13M and 20M) were transferred to Woodvale until February 1989, then four males (13M, 20M, 21M and 26M) and five females (15F, 18F, 19F, 24F and 25F) were moved to Woodvale in November 1989 and kept there, with a subadult male (34M) taken from the wild in December, until February 1990. See Appendix 1 for provenance of individual numbats.

Enclosures

One line of four and another line of six enclosures, each measuring 5m x 3m and at least 2m high, were constructed of 32mm and 25mm (inside) diameter galvanized steel pipe; walls and roofs were made of 25mm x 12.5mm mesh, 1.3mm diameter welded galvanized wire. The mesh forming the walls continued to 1m below ground level on all outer walls and to 0.6m between enclosures. Hollow logs and nest boxes were provided, but most numbats dug sleeping burrows in the sandy substrate within three months. These burrows consisted of simple shafts sloping down to a nest chamber, so the likelihood of escape by digging out was minimal.

Husbandry

As numbats are solitary in the wild (Calaby 1960), they were kept in individual enclosures, except during the breeding season; young were kept with their mothers for a short time after weaning. Animals were weighed and measured (cranial length, cranial width at ear and eye, pes

length, male scrotal width) regularly. Coat condition, activity of male pre-sternal gland and appearance of female mammary area were also noted.

Provision of termites

The termites fed to numbats in early stages of the project, and later to supplement the diet, were obtained in two ways. First, sections of Nasutiternes exitiosus mounds were harvested and placed in plastic trash bins where they were kept moist with lids tightly shut. A piece of palatable wood (karri: Eucalyptus diversicolor) placed on the mound attracted the termites which were then easily separated. Second, 20 liter oil drums packed with pine (Pinus radiata) laths and sealed at the top were set in the ground in suitable bushland areas. These drums were soon invaded by the termite Coptoternes acinacifornis raffrayi which could be collected and stored until needed.

Artificial Diet

In the initial stages of the project (June-October, 1984) the two adult numbats 1M and 2F were fed termites exclusively (mostly Nasutitermes exitiosus). When female 2F's four young were weaned, we had an insufficient supply of termites and an artificial diet custard was formulated. This artificial diet has been used subsequently. The custard was based on an artificial diet devised by Dr. Mervyn Griffiths to feed juvenile and lactating echidnas (Tachyglossus aculeatus) (Griffiths 1978; Green et al. 1985). We added calcium carbonate and a multi-vitamin supplement to the custard; sand was also added to aid maceration of termites after ingestion (Calaby 1960).

The formula used to feed numbats in the present study consisted of 6 eggs (55g each with shell), 154g Digestelact (Sharpe Laboratories, Sydney), 680 ml water, 1/2 teaspoon calcium carbonate powder and 1/4 teaspoon SA-37 vitamin supplement (Brisfarm).

The egg/milk mixture was cooked until set and allowed to cool before being fed. Each of the twice daily feedings consisted of 45g of custard, to which one teaspoon of dry sterilized sand and 5g of termites were added (despite the addition of termites, this formulation is referred to here as the "artificial diet").

Although wild numbats rarely have access to free water in the wild, drinking water was provided in the enclosures. Numbats maintained on the artificial diet were observed drinking water in summer.

Breeding Groups And Diet

1985 breeding season

In the first breeding season the captive group comprised one adult male (1M), one adult female (2F), two one-year old females (3F and 4F) and two one-year old males (5M and 6M) (i.e. only one male and three females were of breeding age).

The four young brought in with 2F in July 1984 were removed from their mother in October 1984 with the males being separated from the females on 12 December. The females (3F and 4F) were placed in cages on either side of the adult male (1M) while their mother (2F) was placed in the remaining cage of that four cage unit.

We tested the relative value of artificial and natural diets for breeding by monitoring the growth of young (see below) raised on different diets. Female 2F was maintained on termites (mainly Nasutitermes exitiosus, but supplemented from time to time with Coptotermes acinacifornis raffrayi until 7 December 1984 and between January 3, 1985 and January 6, 1986. The two younger females (3F and 4F) were fed the artificial diet continuously from soon after their weaning in September 1984.

1986 breeding season

After male 1M mated successfully with all three females in the first breeding season, we decided it was undesirable to allow him to continue breeding in the group. Consequently, he was released as part of WWFA Project ("Re-establishment of the Numbat in areas of its former occurrence") at Boyagin Nature Reserve, 40km from Dryandra, on November 25, 1985 after being fitted with a radio-collar. One of the two second-year males (5M) was also released there.

Adult male 16M was brought in from Dryandra Forest on December 6, 1985 so in the second breeding season (January 1986), the potential breeding group comprised two males (6M and 16M) and eight females (2F, 3F, 4F, 8F, 9F, 10F, 12F and 15F). In order to house all these in-

dividuals separately at Woodvale, the four firstyear males (7M, 11M, 13M and 14M) were moved to the Perth Zoo on December 23.

One adult male was housed in each set of cages so that he could have access to the females in the adjoining cages. Thus, 16M could be introduced to 2F and 10F, who were kept in the same cage, or with 3F, 4F or 12F. In the other set of cages, 6M could be given access to 8F, 9F or 15F. All animals were maintained on the artificial diet.

1987 and 1988 breeding seasons

After the 1986 breeding season, two females (2F and 10F) were released at Boyagin Nature Reserve after being fitted with radio-collars and all the remaining numbats at Woodvale were transferred to the Perth Zoo in December 1986. Husbandry techniques at the Perth Zoo were essentially the same although the enclosures were of various sizes, some being larger and others smaller than those at Woodvale. In January 1987, the breeding group consisted of three females (3F, 9F and 15F) and five males (6M, 7M, 11M, 13M and 16M) and pairings were carried out under constant observation. No births occurred.

In 1987, a female (17F) with four attached young (18F, 19F, 20M and 21M) was brought in from the wild in May and two adult males (22M and 23M) were wild caught in early November. In January 1988 the breeding group comprised six females (3F, 9F, 15F, 17F, 18F and 19F) and five males (6M, 13M, 16M, 22M and 23M). Again, all animals were kept on the artificial diet but no births occurred.

1989 breeding season

As there had been no breeding since January 1985, four numbats (13M, 20M, 15F and 19F) were moved from the Perth Zoo to Woodvale for the 1989 breeding season. Their diet consisted of 50% termites instead of the usual 10% in order to determine whether the lack of breeding was in some way related to a deficiency in the artificial diet. The remaining numbats (21M, 23M, 17F, and 18F) held at the Perth Zoo continued to be maintained on the standard artificial diet.

1990 breeding season

To test this hypothesis further, the ten numbats held at Woodvale for the 1990 breeding season were split into two groups and each fed either the high termite diet consisting of 50% termites (three females (15F, 19F, 25F) and two males (26M and the subadult 34M)) or the low termite diet consisting of 10% termites (two females (18F and 24F) and three males (13M, 20M and 21M)).

Estrus Detection

Each January, urine was collected from every female we could catch. However, it was not always possible to catch the females for fear of causing them undue stress. Captured animals were placed in a holding box or cage with a clean floor or a raised wire mesh floor with a clean tray underneath. A urine sample was generally obtained within one to three hours after which animals were returned to their enclosures. A subsample of urine was fixed and stained with Shorr's solution to detect epithelial cell cornification (Close, 1983). In the 1989 and 1990 breeding seasons, three additional sub-samples were examined microscopically for squamous epithelial cells (Woolley 1971). At the first appearance of these cells, most were nucleated, but on the next and subsequent days of estrus, many were enucleated (cornified). The onset of behavioral estrus was indicated when high numbers of cornified epithelial cells were first present. Once the estrous flush commenced, the concentration of epithelial cells was so high that the urine appeared cloudy to the naked eye. This monitoring procedure was used sparingly in the first year, but samples were collected daily from as many females as possible between 8 and 17 January 1986 and from 1 January until mating was confirmed during the 1989 and 1990 breeding

Assessment of sperm production

We assessed sperm production by microscopically examining occasional urine samples. While spermatorrhoea indicates spermatogenesis, the absence of spermatorrhoea cannot be taken as proof of infertility.

Breeding Protocol

Just prior to the 1984 breeding season, the male was given access to a female for a short time each day, under observation, commencing on December 25. In subsequent years, females were introduced into cages with males, using the technique described below, when estrus was detected.

Mating was confirmed either by direct observation or by detection of sperm in the female's urine at the next collection. When mating was confirmed, the animals were separated and pouch checks were carried out daily except when it was not possible to catch the female concerned.

Growth of Young

The growth of young attached to the nipple was monitored by regular measurement of cranial width at the ear and pes length. These measurements were compared with those obtained from numbats in the wild.

Milk Collection and Analysis

On 29 October 1985, near the weaning of her young, milk was collected from female 4F and the constituent fatty acids in the triglyceride fraction determined by methods described in Griffiths et al. (1988). This numbat had been maintained on the artificial diet for almost a year. Samples of numbat milk collected at Dryandra Forest earlier in October and some of the custard as fed to the captive numbats were analyzed by the same methods.

Results

Palatability of the artificial diet

The numbats could pick up the stiff custard mixture relatively easily with their long, slender tongues. They also tended to scratch at it with their forepaws, first pulling it out of the bowl, then licking up individual lumps. The custard was tried first in October 1984, when the captiveraised young of 2F (3F, 4F, 5M and 6M) were newly weaned and, at this stage, the young numbats took to the custard more readily than the adults. Their mother was not interested in it at first and only ate it sparingly even when given no choice. The young male 6M stopped eating the custard after a week and lost an alarming amount of weight. He was subsequently isolated and fed on termites for 10 days before being put back with his siblings. When 2F was taken off termites in late 1985 she was kept with one of her young (10F) to encourage her to eat through competition for food. This strategy worked well, and no significant diet acceptance problems were subsequently experienced.

The weights of the sixteen numbats in the captive colony during 1984 and 1985 are shown in Figure 2. Non-reproductive adults maintained their weight on a daily ration of 80g of custard (as determined before the addition of termites and sand) and lactating females would eat up to 120g each day.

Estrus Detection and Breeding

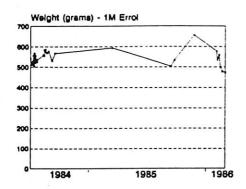
1985 breeding season

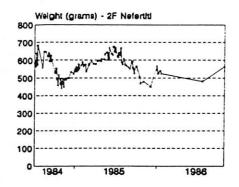
On December 25, the doors between the cages of 1M, 3F and 4F were opened. Although the male investigated the females occasionally, no mating was observed. The male was subsequently given access to one or other females for a short time in the late afternoon on most days, always under observation.

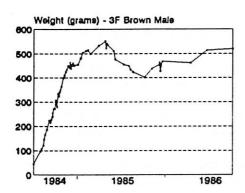
Table 1 shows the results of urine monitoring. On 6 January, cornified epithelial cells were first seen in the urine of 3F. She mated with 1M late in the afternoon of the same day. A few cornified epithelial cells were present in 4F's urine again on 6 January but were much more prevalent the next day. Mating occurred when male 1M was placed in 4F's cage on 7 January. Beginning on the day after mating, both 3F and 4F were isolated from other animals. The male 1M was let into 2F's cage daily, and mating occurred on 14 January. She was subsequently isolated until 22 January, when the male was introduced again; when he approached her, 2F growled and drove him off, so we again isolated her.

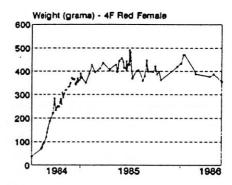
The first two matings (with 3F and 4F) lasted approximately two hours, while the third (with 2F) lasted 35 minutes. Mating followed a typical dasyurid pattern: the male initially used a neck grip, but subsequently only if the femlae struggled or tried to get away; short periods of thrusting were followed by periods of inactivity. All matings occurred in the open, although nestboxes were available.

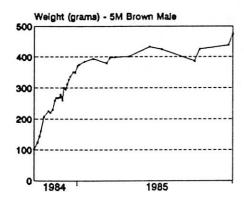
Figure 2. Weights of Individual Numbats in Captivity During the Period July 1984 - December 1986.











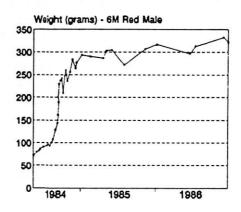
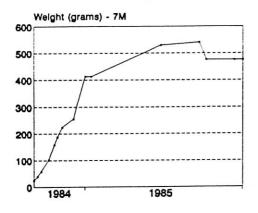
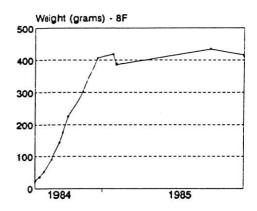
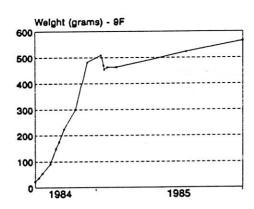
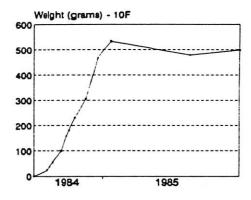


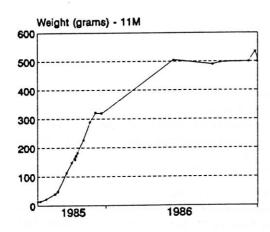
Figure 2 (continued).











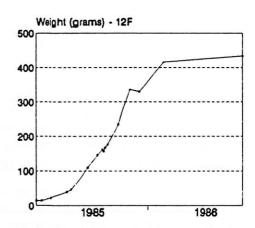
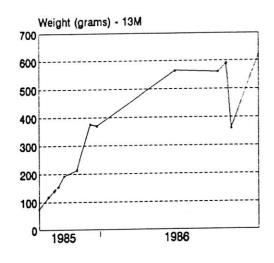
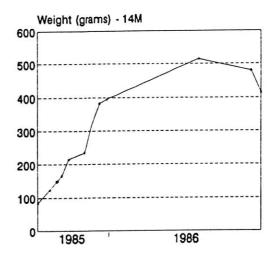
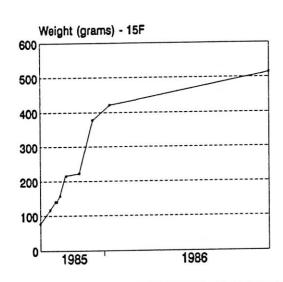
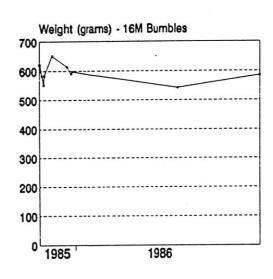


Figure 2 (continued).









Pouches of all three females showed development consistent with pregnancy. A swelling developed across the female's abdomen anterior to the mammary area and swollen areas also appeared on the inside thighs. Thus the exposed teats were surrounded by swollen skin. During the later stages of its development, the interior of this depression was moist and the skin around the teats assumed a granular appearance. The

anterior swelling observed in some animals at this stage is so large that it formed an engorged flap covering the mammary area. By early January, teats of all females were tiny (1-2mm long) and bright pink, even those of 2F which as late as November had been 10mm long and pale in color.

On January 20, three young were found on 3F's nipples; three young were found on 4F's nipples on January 21, but one disappeared four days

later; four young were found in 2F's pouch on January 28. The gestation periods (interval between last mating and birth) for all three litters was 14 days.

1986 breeding season

On 7 January 1986, 4F was introduced to 16M. Some interaction occurred while the animals were not being observed, because the

Table 1. Results of Urine Screening for Cornified Epithelial Cells and Reproductive Status, January, 1985.

Date	2F	3F	4F
2/1			
3/1		-	•
4/1		•	
6/1		+ (mated)	X
7/1		+	+ (mated)
8/1			
9/1			
10/1			
11/1			
12/1			
13/1			
14/1	(mated)		
15/1			
16/1			
17/1			
18/1			
19/1			
20/1		3 young	
21/1			3 young
22/1			
23/1			
24/1			
25/1			
26/1 27/1			
28/1	4		
20/1	4 young		

- = No epithelial cells.

x = Nucleated epithelial cells.

+ = Cornified epithelial cells. Blank indicates no urine collected. hair on the female's neck and rump were later seen to be wet and ruffled. On the same day, each of the females was introduced to 6M; his response was to chase each female around the cage for several minutes without mating. Over the next month, various pairings were made, some animals even being left together overnight, but no matings were observed. Male 6M con-

Table 2. Results of Urine and Reproductive Screening January and February, 1986.

Date	2F	3F	4F	8F	9F	10F	12F	15F
8/1	•	•	+					
9/1	-	•	-		ļ			
10/1	•	•						
11/1								
12/1			i					
13/1	-							
14/1	+		į		l			
15/1	+				-			
16/1	+			-	+			
17/1	-	-		+	-			
18/1								
19/1								
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21/1						1		
22/1						1		
23/1						- 1		
24/1		PD						
25/1								
26/1								
27/1								
28/1	PD							
29/1	PD			PD	PD	1		
30/1						1		
31/1						1		
1/2						- 1		
2/2						i		
3/2						PD		
4/2			2.1					
5/2								
6/2								PD

Legend is same as for Table 1; PD = pouch development recorded. tinued chasing females until 3 February when his urine was found to contain large numbers of sperm. Scrotal measurements of both males showed that their testes diminished in size by early to mid February. The results of urine screening and observations of pouch condition are shown in Table 2. At no time were sperm detected in the urine of females screened.

1987 and 1988 breeding seasons

Mating was observed on several occasions in both years, but no young were produced.

1989 breeding season

Females 15F and 19F entered estrus on 15 and 23 January respectively and males were introduced to them on the afternoon of the day of the flush of epithelial cells and on several subsequent days. Sperm were never found in either female's urine but on February 6 a pouch check revealed young on 19F's nipples. No young were produced by 15F or by the females being fed the artificial diet at the Perth Zoo (17F and 18F). Results of urine and reproductive condition checks are shown in Table 3.

1990 breeding season

Obvious estrus was not recorded in 24F or 25F during the monitoring period and female 24F's urine was full of immature epithelial cells on February 8 indicating post-estrus. Female 15F apparently came into estrus on 12 January. Male 20M was given access to her cage from January 6-15 but no sperm were subsequently found in her urine although few samples were collected as the two animals disappeared underground from January 6-10. Male 13M had access to female 19F's cage January 13-18 January. This female came into estrus on January 19 and 13M was again admitted to her cage that afternoon. The next day, we collected a thick cloacal discharge containing masses of sperm. Female 18F shed epithelial cells on January 6, 11 and 12, but no cornified cells were seen. On each day of January 6 and January 9-12 male 13M and female 18F were allowed together in the afternoon but no sperm were found the female's urine. On January 21 and 22, male 13M was again admitted to female 18F's cage, after he had mated with female 19F. On January 22, the epithelial cell content of female 18F's urine rose but no sperm were found and on January 23 her urine was full of cornified squamous epithelial cells and many sperm. Pouch checks revealed no young on 15F,

Table 3. Results of Urine and Reproductive Screening January and February, 1989.

Date	15F	19F
2/1	•	
3/1	•	-
4/1		
5/1	+	+ MF
6/1	+	
7/1	+ MF	
8/1		
9/1	+	
10/1	+	
11/1		-
12/1	+	+
13/1	+	+
14/1	+	+
15/1	+++	+
16/1	+ + +	+
17/1	+	+
18/1		
19/1	+	+
20/1	+ ML	
21/1		
22/1		
23/1	+	+++
24/1		+++
25/1		
26/1	+	+++
27/1		+
28/1	PD	+
29/1	PD	+ ML
30/1		
31/1		
1/2		
2/2		
3/2		No Young
4/2		
5/2		1724
6/2		4 Young
7/2		

- = No squamous epithelial cells present.

+ = Some squamous cells present.

+ + + = Many squamous cells present.

MF = First day with male.

ML = Last day with male.

Table 4. Results of Urine and Reproductive Screening January and February, 1990.

Date	15F	18F	19 F	24F	25 F
2/1	•		•		
3/1				•	
4/1	-				
6/1	MF	+++	MF	+ MF	
7/1		+			
8/1		+			-
9/1			•		+
10/1		+			+ MF
11/1		+++	+		+
12/1	+++	+++		+	+
13/1			MF	+	-
14/1				+	+
15/1	• _	+	+	•	+
16/1		+		•	-
17/1	+	+			•
18/1	•	+	+	+	-
19/1	-PD	+	+++	-	
20/1	-PD	+	+ + + M	L	-
21/1	-PD	+	sperm	+	•
22/1	-PD	+ML		-	-
23/1	-PD	+++	+++		•
		sperm	sperm		
24/1		1.50	+++		-
25/1	-PD MI	.+++	+++		
26/1		+++			• 14
27/1	PD				
28/1					
29/1	-PD		PD		
			no young		
30/1					
31/1					
1/2					
2/2					
3/2					
4/2		PD			
5/2	PD	PD			
		no youn	g		
6/2			-		
7/2					
8/2		4 young	3 young	+ + + M	L
12/2		,	,		PDML

^{- =} Few or no squamous epithelial cells.

24F or 25F, but on 8 February, both 18F and 19F had young. Results of urine and reproductive condition checks are shown in Table 4.

Growth of young and the effect of diet

As breeding occurred in all three females in January 1985, the artificial diet was clearly adequate to that stage. It may have been significant that only female 2F, the female on the termite diet, produced a full litter of four young. In fact, one of female 4F's three young was lost within the first four days. With a sample size of only three individuals it is impossible to draw firm conclusions, but it is possible that the artificial diet was responsible for the lower fecundity of 3F and 4F.

Figure 3 compares the growth of captive young during the period of nipple attachment with that of wild caught young measured in 1982-1987. Cranial width was used to monitor the size of the young as it is easily measured during the period of attachment. The most important conclusion from Figure 3 is that the growth of the captiveborn young, irrespective of diet, was within the range of that found in the wild. Differences in the growth rate of different captive born litters was more easily attributable to differences between mothers than differences in diet. The cranial width growth rate of female 4F's young began falling behind the others in late October after their mother began acting aggressively towards them. Male 11M lost part of his tail in one of these aggressive encounters and the young were separated from their mother and placed with female 2F although they had not yet been weaned. Although her young had recently been weaned, female 2F female still had milk and suckled female 4F's young for approximately another week.

Figure 4 shows the important fatty acids constituents of the artificial diet and of the milk of wild numbats and a captive numbat maintained on the artificial diet. A nutritional assay of termites (Nasutitermes exitiosus) is also given. The most striking result of these analyses is that the main fatty acid components of the mother's diet

^{+ =} Some squamous epithelial cells present

^{+ + + =} Many squamous cells present.

PD = Pouch development recorded.

MF = First day with male

ML = Last day with male

Figure 3. Cranial Widths of Numbats in the Wild and Captivity Measured Throughout the Year.

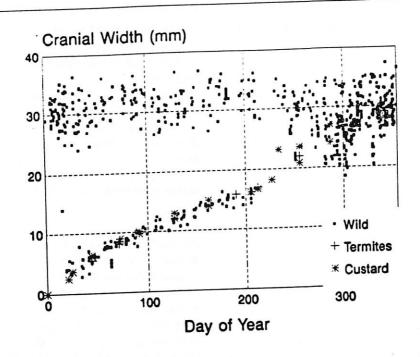
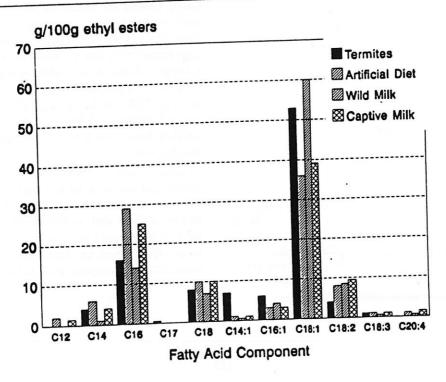


Figure 4. Component Fatty Acids of the Triglyceride Fraction of Termites, Artificial Diet, Wild Numbat Milk and Captive Numbat Milk.



are passed on to the young in the milk (Griffiths et al., 1988). The milk produced by females eating the custard is correspondingly lower in oleic acid and higher in palmitic acid than the milk produced by females eating termites.

Observations on the Lactation Period in Captivity

While many details of development in pouch young had already been studied in the field (Friend and Burrows, 1983), we were able to follow development more closely in captive animals. Changes in pigmentation and growth of hair have been described by Friend and Burrows (1983). We were able to sex two of the litters on 1 April. At this stage, female 2F had four young (1 male, 3 females), female 3F had three young (2 males, 1 female) and female 4F had two (1 male, 1 female). On July 5, 169 days after parturition, female 3F left her young in the burrow of her outside enclosure during the day for the first time. By July 9 (day 172), female 2F's young were found on different teats, indicating that they were releasing the nipples at night. On July 12, female 2F was brought inside and kept in a large glassfronted box (1.6m X 0.7m X 0.7m) to more closely monitor development of the young. Female 4F had already been brought inside and placed in another box on June 25 after her newly constructed burrow collapsed. She first came out of her nest box without her young on 24 July(day 180). At this time, the young still had their eyes closed, but their mouths were open and they were making a chirping sound. By 31 July these young were quite mobile within the nest box. Although female 2F's young continued to reattach to different nipples, she always emerged from her nest box with them attached, despite the fact that the eyes of one were open on 31 July.

On August 2 we marked two of the three females in female 2F's litter by clipping different hindfoot nails. Female 4F's two young were not marked as they were a male and a female. One of female 2F's young was the first to emerge from the nest on August 10 (day 205). All of female 3F's young were out of the nest on August 21 (day 224) but female 4F's did not emerge on their own until September 4 (day 230). At this stage they did not move around much, but spent a lot of time basking under the light or in the sun. They quickly

became more active and on September 23 female 2F's young first ate termites at 249 days of age while female 3F's young first ate termites on 1 October (257 days of age). Weaning occurred before the end of October and all young were soon transferred to the artificial diet.

At weaning, the adult females' nipples were 10mm long, pale and flaccid. By mid-December, they had shrunk and were tiny (1-2mm) and bright pink. The weight records of these three females (Figure 2) show fluctuations in female body weight during the mating and lactation. After reaching a minimum just before weaning their young, the female's weight increases as the breeding period approaches. At this stage there is a slight weight decline, presumably as energy is expended during estrus and mating. Once young appear, the combined weight of mother and young increases steadily to a maximum in July, when the young are deposited in the burrow. At this time the mothers' are at their peak weights. Maternal weight declines rapidly in late lactation and by November the female's weight has declined to its minimum. In the case of female 2F, lactation accounted for a loss of 30% in body weight between July and December.

Discussion

Comparison between termites and artificial diet: Proximate constituents

The proximate constituents of termites have not been determined previously because of difficulties in reaching a meaningful estimate of the amount of available nitrogen. The usual method of estimating dietary protein is by determining total nitrogen by acid hydrolysis and multiplying by a constant (usually 6.25) to estimate total protein. The exoskeleton of insects contains a significant amount of nitrogen, but it is unavailable to insectivores because it is bound up in indigestible chitin. Acid digestion releases the nitrogen from the chitin so total nitrogen assays on insects tend to overestimate available nitrogen. In attempting to determine the amino acids available to echidnas in a diet of Nasutitermes exitiosus. Griffiths (1968) used the enzyme pronase, a potent proteolytic enzyme of bacterial origin which is inactive on chitin, to perform the initial digest of the termite protein. His amino

acid estimates suggested 37% protein by dry weight. In contrast, Griffiths' (1965) estimate of total nitrogen in *N. exitiosus* using acid digestion (8.5% dry weight of N) gives 53% protein.

Other published data permit estimation of the proximate constituents of *N. exitiosus*. The fat content given by Moore (1969), 1-3% of wet weight, included seasonal fluctuations (values were lower in winter), so 2% might be a realistic average for the species. According to Griffiths (1978: p.82) the water content of *N. exitiosus* is 74%. The ash content of workers is about 5% of dry weight (Abensperg-Traun, unpublished data). Carbohydrate content may be calculated approximately by subtraction, if the total nitrogen figure is used. A comparison of the proximate constituents of a diet of *N. exitiosus* workers, and the artificial diet is shown in Table 5.

This comparison shows that much more protein is available to the numbats in the artificial diet than in termites. It also shows that the artificial diet is much higher in fat than N. exitiosus

Table 5. Proximate Constituents of Termites and the Artificial Diet.

	N. exi Work		Artificial Diet		
	%wwb	%dwb	%wwb	%dwb	
Water	74		81	i da in la	
Protein					
- Total	14	53	7	36	
- Useable	10	37	7	34	
Fat	2	8	6	32	
Carbohydrate	9	34	6	27	
Ash	1	5	1	5	

wwb = Wet weight basis

dwb = Dry weight basis

Values for Nasutitermes exitiosus workers calculated from data in Griffiths 1968; 1978.

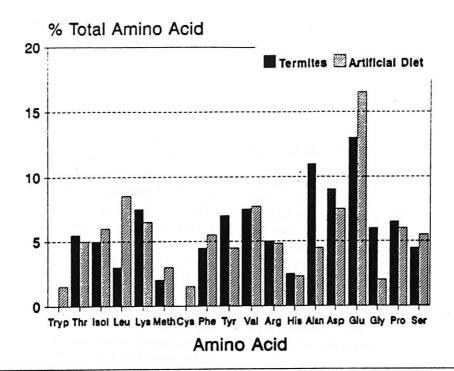
workers. Two factors will work to minimize these differences, however. The significant proportion of unusable protein in termites increases the relative importance of fat in the wild diet to numbats. Numbats feeding in the wild frequently encounter alates and pre-alates, which generally have a much higher fat content than workers or soldiers (La Fage and Nutting, 1978). For example, Christensen et al. (1984) found a single numbat scat to contain the remains of 161 alates of one species of termite.

Amino acids

It is also possible to compare the amino acid composition of the protein in termites with that in the artificial diet. Griffiths (1968) presented amino acid assays for pronase digests of *N. exitiosus* workers which we converted to weights of amino acids per gram (dry weight) of termite. We then calculated each amino acid component as a percentage of total amino acid detected. Corresponding values were calculated for the artificial diet (Figure 4).

The proportions of most individual amino acids present in the artificial diet are surprisingly close to those in termites (Figure 5). Only glycine and alanine are present in significantly lower proportions in the artificial diet. Both are considered non-essential amino acids in animals: alanine can be synthesized by transamination from a-keto acids, which arise in the citric acid cycle and glycine can be synthesized from the amino acid serine (White et al., 1978).

Cystine appears to be virtually absent from available termite protein (Griffiths, 1968). Later work (Griffiths, 1978) showed this sulphur-containing amino acid to be present in acid hydrolysates of N. exitiosus. Insect exoskeleton contains virtually no sulphur (Gilmour, 1965), so it appears that the cystine detected was in the termite flesh. Levels of sulphur-containing amino acids (cystine and methionine) are low in the artificial diet, but these correspond to low levels in the natural diet (Figure 4). The main requirement for sulphur, for the manufacture of keratin in hair and claws, can apparently be met by these low levels in the diet.



Leucine is present in the artificial diet in about twice the proportion found in termite protein. Massive imbalances involving high proportions of leucine can be toxic through the build-up of its breakdown products, but only at several orders of magnitude greater than the difference shown here (Harper, 1964).

Lipid Fatty Acids

The proportions of different fatty acids in termites and in the artificial diet are shown with those in the two milks in Figure 4. The levels of fatty acids of the lipids in termites (*N. exitiosus* various castes) are taken from Griffiths et al. (1984). The main difference between the two diets is in the relative proportions of palmitic (C16) and oleic (C18:1) acids. Moore (1969) remarked on the high degree of unsaturation present in termite fats. In *N. exitiosus*, this is due to the high level of oleic acid in the triglyceride fraction. The effect on the numbat of a more saturated lipid profile in the artificial diet is discussed later with respect to numbat milk.

Cholesterol

There are no data available on the cholesterol content of termites. Given the high levels present in chicken's eggs, and hence in this artificial diet, deleterious effects of high dietary cholesterol might be expected in captive colonies maintained on this diet.

Effect of Diet on Milk Lipid Constituents

Judging from the similarity in growth rates of young captive numbats fed on different diets young numbats in the wild (Figure 3) the difference in milk lipid constituents apparently has no effect on the growth and development of young. The high levels of saturated fats in the artificial diet may be partly responsible for less than optimal reproduction.

Gestation length

The first year's results gave strong evidence that the gestation period of the numbat is about 14 days. In 1989 and 1990, pouches were not checked daily, so gestation period was only approximated. Gestation ranges were determined as follows:

1985	2F	12d,18 h - 13d,18h
1985	3F	12d,16.5h - 13d,16.5h
1985	4F	12d,22h - 13d,23h
1989	19 F	9d - 32d
1990	18F	14d - 17d
1990	19 F	9d - 19d

The appearance of cornified epithelial cells coincided with the first receptiveness of the female to the male. More detailed information was collected in the second year, when breeding did not occur (Table 2). Despite the lack of births, most females showed strong swelling around the outside of the mammary area ("pouch development"). These swellings were present for less than a week. The duplication in unmated females of some of the external changes associated with pregnancy (i.e. "pseudopregnancy") has been noted in dasyurids (Woolley, 1971), a group to which the numbat is closely related.

Possible Inhibition of Female Reproduction

During the captive breeding project between 1984 and 1990, the maximum number of litters that could have been produced from the pairings made was 32. However, only six litters were produced, a success rate of less than 20%. During the project to February 1990, the females that had young were as follows:

ID	Age	Origin	Yrs in Capt.	Diet
2F	2	WC	0.5	Termites
3F	1	CR	0.5	Artificial
4F	1	CR	0.5	Artificial
19F	2	CR	1.5	50/50
	3	CR	2.5	Artificial
19F	3	CR	2.5	50/50
	2F 3F 4F 19F 18F	2F 2 3F 1 4F 1 19F 2 18F 3	2F 2 WC 3F 1 CR 4F 1 CR 19F 2 CR 18F 3 CR	ID Age Origin Capt. 2F 2 WC 0.5 3F 1 CR 0.5 4F 1 CR 0.5 19F 2 CR 1.5 18F 3 CR 2.5

WC = Wild-caught CR = Captive-reared

It is interesting that while captive-bred females were involved in 13 (40%) of the pairings, none produced young. In addition, the oldest females

to produce young were only three years old, although a female numbat has been kept in captivity for five years and three months (Strahan 1978; Taronga Zoo records).

The female 2F, which had bred in captivity in January 1985 but not in January 1986, was released on 2 December 1986. Although by this time, she had been kept for almost a year on the artificial diet, she produced young in late January 1987, which had undoubtedly been conceived in the wild. This means that if diet had produced an effect inhibitory to breeding, it was a rapidly reversible effect.

Possible Inhibition of Male Reproduction

The lack of breeding, and probably of mating in the 1986 breeding season raises the possibility that the males used were not fertile; the evidence, however, does not support this suggestion. Firstly, all males used in breeding have displayed swollen and secreting pre sternal glands during the season at which these were active in the wild population (October to February). Secondly, measurements of scrotal width in the colony males showed that their testes were becoming enlarged in phase with the wild population (Figure 1). In addition, the region between the cloaca and the base of the tail was greatly swollen in all males, a seasonal condition observed in the wild (Friend, unpublished). Examination of urine for sperm was carried out on only two occasions during the second breeding season. On 10 January 1986, urine from 6M was examined and found to contain only a few sperm. On 3 February, however, both 6M and 1M were found to have high numbers of sperm in their urine.

If the impediment to mating concerned the males rather than the females, it is more likely to have been a behavioral than a physiological problem. In fact, there was some indication of a behavioral disorder in 6M as his response to being introduced into a cage with a female was to chase her. This contrasted with the behavior of other males when introduced, which either ignored the female or quietly but persistently investigated her cloacal area. While 16M, the male brought in to replace 1M, did not display this unusual behavior, he rarely showed much interest in the females when caged with them. It is likely that the time allowed for his adjustment to captivity

before the breeding season, about four weeks, was insufficient. He was seen out of his box much less than the other numbats during his first six months in captivity.

By the time 1M was brought back in from the wild, the testes of all the males had gone into decline, and it may well have been too late in the season.

Clearly, it is dangerous to rely on a small number of animals of either sex in captive breeding projects. If only one or two individuals of either sex are available, unforeseen problems even with one animal may result in failure, and in an annual breeding species, loss of a year's work.

During the project to February 1990, the males that sired young were as follows:

			Age		Yrs. i	n
Year	ID	Litter	s (years	s)Origin	Capt.	Diet
1985	1M	3	2	WC	0.5	Artificial
1989	13M	1	4	CB	4	Artificial
1990	13M	2	5	CB	5	Artificial

WC = Wild-caught CB = Captive-born

Although it is difficult to draw conclusions from such sparse data, it appears that age, diet and provenance are not important in determining male breeding success. However, it is clear that certain males have repeated success. Only two males (out of 10 used) featured in the six successful pairings, comprising only three of the 22 male/seasons from 1985 to 1990. The temptation to over-use a successful male and so produce a high degree of inbreeding is clear. Captive breeding programs for numbats should not, therefore, be based on small numbers of animals.

Significance of results

Major advances in numbat husbandry made through this study include:

- 1) the breeding and raising of young in captivity
- 2) the development of techniques for acquiring and keeping a large supply of live termites

- 3) the first successful use of an artificial diet to maintain numbats in captivity
- 4) the documentation of reproductive events through the close observation of individuals in captivity.

While numbats had produced young in captivity before, their survival had been very short, presumably because of the conditions under which the mother was kept. The high level of attention to the animals which was possible in this project through having one person full-time to look after them was no doubt significant in the survival of the young. The availability of sufficient space to house all animals individually, more closely resembling the natural solitary habits of the species, might also have enhanced

Even in Perth, termite availability is not guaranteed at certain times of year. However, with a little planning, by setting up traps for Coptotermes and making the most efficient use of Nasutitermes mounds, it is possible to have access to a constant large supply of live termites with the investment of a few hours each week. These techniques will not work in all parts of Australia, but could be adapted to suit the local species in the regions of several capital cities.

The ultimate solution to feeding insectivorous animals in captivity is to develop a diet composed of readily-available ingredients. While the custard diet developed during this project satisfies many of the criteria for a termite substitute, there is still some doubt that it supplies everything needed by numbats, since female numbats fed on it for long periods have not bred. It is clear that further dietary manipulation is necessary, firstly to establish if non-breeding animals will breed again in captivity on a termite diet, and secondly whether other formulations, possibly even closer in composition to termites, will allow breeding.

The indication that estrus can be accurately monitored in numbats is most significant for further research. It means that it should be possible to discover the reason for any future lack of breeding by following closely the changes in both males and females which occur at that time of year.

While the problems involved in breeding numbats in captivity are by no means solved, there is now a strong base upon which to carry out further research. Furthermore, there is now a group of animals in captivity which may be used in this research. Maintenance techniques have been passed on to staff at Perth Zoo, and are being further developed by them. Several zoos have expressed interest in being involved in an expanded research and captive breeding program. It is likely, however, that the research component will be beyond the resources of most Australian zoos, and will require the input of expertise and possibly funds from other institutions.

Acknowledgments

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Appendix 1. Identification and Provenance of Individuals

Code	Birthdate	Born	Raised	Mother	Father	Name (W = Wild; C = Captive
1M	Pre-1984	w	w	?	?	Errol
2F	Pre-1984	W	W	?	?	Nefertiti
3F	Jan 1984	w	C	2F	?	Brown Female
4F	Jan 1984	W	0000000000	2F	?	Red Female
5M	Jan 1984	W	Č	2F	?	Brown Male
6M	Jan 1984	W	Č	2F	1M	
7M	28/1/85	Ĉ	Č	2F	1M	
8F	28/1/85	Č	č	2F	1M	
9 F	28/1/85	č	č	2F	1M	
10F	28/1/85	č	Č	4F	1M	
13M	20/1/85	č	č	3F	1M	
14M	20/1/85	č	č	3F	1M	
15F	20/1/85	000000	č	3F	1M	
16M	Pre-1985	w	w	?	?	Bumbles
17F	Pre-1986	ŵ	W	?	?	Zoe
18F	Jan 1986	ŵ	Ċ	17 F	?	
19F	Jan 1986	w	CCC	17F	?	
20M	Jan 1986	w	Č	17F	?	
21M	Jan 1986	ŵ	w	17F	?	
22M	Pre 1986	ŵ	ŵ	?	?	Sooty
23M	Pre 1986			'n	•	Winston
24F	6/2/89	č	Ċ	19 F	13M	
25F	6/2/89	č	č	19 F	13M	
26M		č	č	19F	13M	
27	4/2/90	č	č	19 F	13M	
28	4/2/90	č	č	19 F	13M	
29	4/2/90	č	č	19F	13M	
30	4/2/90	č	č	19F	13M	
31	4/2/90	≱ 0000000000	\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\	19 F	13M	
32	4/2/90	č	č	19 F	13M	
33	4/2/90	č	č	19 F	13M	
33 34M	Jan 1989	w	w	?	?	Hook
34IVI	Jan 1909	**	**	•		