Chapter 6: Identification of leaf cuticles in scats to establish diet of *Dendrolagus lumholtzi.*

6.0 INTRODUCTION

Few researchers would dispute that feeding and food selection are key ecological processes (Stephens and Krebs 1986, Hart *et al* 2002) and that knowledge of an animal's food choice is important. However, there has been great debate over which technique is optimal for describing or quantifying animal diets (Hart *et al* 2002). Estimates of dietary intake can be made using either visual observations of feeding or through other techniques investigating the ingested material. Unfortunately accurate feeding observations are often difficult with arboreal folivores.

There have been several studies on the identification of digested food items of mammals and birds from *post mortem* stomach contents (Recchia and Read 1989, Martin *et al* 1996), from induced vomiting (Radke and Fydendall 1974, Prys-Jones *et al* 1974, Gales and Burton 1988) or by stomach pumping or flushing (Randall and Davison 1981, Gales 1987). Unfortunately all of these techniques are invasive and are stressful for animals in the wild, especially those that are difficult to catch.

An alternate technique, identifying incompletely digested food particles in the droppings or scats, is much less invasive and a plausible technique for use in herbivores. This technique has been used successfully for many different mammal species (Tullis *et al* 1982, Dickman 1995). In herbivores it is possible to identify leaf and stem epidermis or cuticle in scats of all plant species eaten, as these plant parts are not easily digested (Storr 1961). Each plant species has a unique cuticular pattern and can be identified to species by characters such as size, shape and alignment of cells, stomates and trichomes (Storr 1961, Christophel and Rowett 1996).

Though, this technique provides proof of inclusion in the diet, it is not fully quantitative as some food particles can be digested more quickly than others (Hart *et al* 2002). The use of microscopic analysis of faecal material for diet

determination assumes that all plant cuticle fragments remaining in the faeces have the same probability of being detected. Further, it is assumed that the proportions of all plant material consumed will be accurately represented in the faecal pellets (Ellis *et al* 1999). Jones (2000) investigated the use of this technique with captive Matschies tree-kangaroo s (*D. matschiei*) and did find that all of the species known to be eaten by individuals were found in the faeces.

Storr (1961, 1964) first used this technique on Quokkas (*Setonix brahyurus*) in Australia. It was found it to be both qualitative and quantitative as there was little or no digestion of the leaf and stem epidermis (Storr 1961). Since then, this technique has been more widely used with marsupials, such as koalas (Ellis *et al* 1999, Sullivan *et al* 2003), yellow-footed rock wallabies (Copley and Robinson 1983, Lapidge 2000), Northern Nailtail wallabies (Ingleby *et al* 1989), grey kangaroos (Norbury 1988), brushtail possums (How and Hillcox 2000), and as a dietary comparison between wombats and grey kangaroos (Woolnough and Johnson 2000).

Little is known about the diet of Lumholtz's tree-kangaroos except for observations from previous studies (Proctor-Grey 1985, Newell, 1999, this study see chapter 5) and from locals and researchers from the Atherton Tablelands (TKMG unpublished data).

Dendrolagus lumholtzi is considered a generalist folivore, with their diet consisting primarily of rainforest leaves, with a small percentage of fruit and flowers (Procter-Gray 1985, Flannery *et al* 1996, Newell 1999). However, this study suggests that *D. lumholtzi* is not simply a generalist, as it selects particular rainforest species more than expected (Section 5.2.10).

Observations of feeding are difficult with tree-kangaroos as they tend to cease feeding when being observed or they may be eating something other than the tree species they are in at the time, such as a vine in the canopy of that tree. Feeding observations can also be difficult due to the fact that Lumholtz's tree-kangaroos are often very high in the canopy, up to 30-40m. Moreover, the thick rainforest vegetation and the decreased visibility whilst using spotlights hamper

night observations. Additionally, misidentification of the tree species from sightings could also occur.

Using microscopic faecal analysis to identify the cuticle features of leaf species eaten is one way to resolve these problems. This technique can be used to confirm tree species choice by *D. lumholtzi,* as well as being quantified and used to compare use and availability of dietary species.

This study was undertaken to confirm the diet of *D. lumholtzi* by the use of leaf cuticles in faecal material and to establish the preference of individual animals for certain species and compare this to the availability of these tree species within their home range area.

6.1 METHODS

6.1.1 Collection of scats

Scats were collected from 8 individual *D. lumholtzi* during capture for radio tracking for home range analysis (Section 4.2). Only one scat per animal was used for cuticle analysis.

6.1.2 Reference slides

A reference collection of 179 local rainforest floral species of leaf cuticles, established by Jones (2000) was used to identify the fragments to species level. This reference collection was expanded during this study to include an additional 19 tree, vine and weed species from observations of use by *D. lumholtzi* during the home range study (Section 5.2) and from observations of others (Proctor-Gray 1985, Newell 1999, TKMG unpublished data), now consisting of 198 species in total, to ensure all possible species were included. *D. lumholtzi* is known to eat only the petiole of *Litsea leefeana* at times (pers. obs.) and has been observed eating some fruit (TKMG unpublished data) so additional reference slides of petioles, fruit and flowers of some species were also prepared. The “Leaf and Cuticle atlas of Australian leafy Lauraceae” (Christophel and Rowett 1996) was also referred to for identification of this group and for the description of cuticular characters. Local botanists identified the plant species for the reference collection (R. Jensen, T. Irvine and P. Dellow).

Reference slides were prepared by the technique used by Christophel and Rowett (1996) and Jones (2000), using a 1cm^2 piece cut from the edge of the identified leaf and boiled in 35% hydrogen peroxide (H_2O_2) until the upper (adaxial) and lower epidermis (abaxial) layers separate to expose the cuticle (approximately 8-10 hours). The layers were then laid flat, cleaned of debris with a fine camel hair brush in water, stained with gentian violet and mounted on a slide with phenol glycerin jelly. When the jelly has set and excess has been removed with a scalpel and warm water, the edge of the coverslip was ringed with clear nail polish to retard dehydration of the preparation.

Petioles were prepared by cutting them into lengths of approximately 2cm and splitting longitudinally. Fruit and flowers of some species (those observed being consumed by *D. lumholtzi*) were also prepared to examine whether they would be identifiable by cuticle features. Young leaves were also prepared to compare with older leaves of the same species.

If the leaves dissolved completely during this preparation it was assumed that they would also be digested fully and therefore not present in the faeces.

6.1.3 Preparation of slides from faecal material

One scat per animal was prepared for microscopic analysis by the technique used by Jones (2000). Each scat was placed individually into 50ml beakers with 35% hydrogen peroxide and boiled until the chlorophyll was dissolved and the fragments appeared clear (usually about 8 hours).

Each sample was then rinsed through a fine sieve (150µm plankton mesh) with distilled water and then stained with gentian violet for 15 mins. The stain was then rinsed out through repeated washing through the sieve with distilled water. The samples were then mounted on a 50mm x 50mm slide with glycerine. Four slides were prepared for each animal (scat).

The slides were examined under a microscope at 40x magnification. Twenty-five fragments from each slide, a total of 100 for each animal, were randomly selected using random numbers and the measuring increments on the microscope stage. Each fragment was photographed using a Kodak DX4330

digital camera through the microscope eyepiece, cross-referenced with the reference slides and photographs, and identified to species using cuticular features. The frequency of each species was recorded for each animal.

Unidentifiable fragments or other unidentifiable plant parts were recorded and photographed. Fragments were deemed to unidentifiable if they were obscured by other fragments, too small, or did not have any stomata present. In dicotyledons the stomata are usually only present on the abaxial side (under side) of the leaf. Fragments are difficult to distinguish to species without stomata characters to identify them, but if there were sufficient abaxial (lower) pieces on a slide to identify a species were present and the adaxial was distinguishable from other species then these were also included. Adaxial fragments were deemed unidentifiable if no clearly associated abaxial pieces of this species were present. Unidentifiable fragments were not replaced.

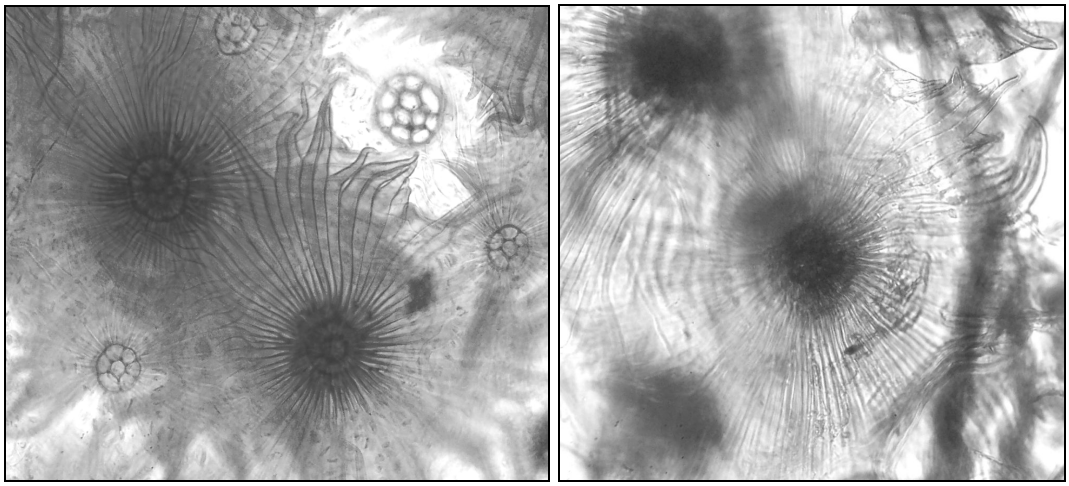

Figure 6.1. *Elaeagnus triflora*, a) reference slide showing flower-like trichomes b) fragment from scat sample.

6.2 RESULTS

Sixteen species were represented within the scat samples (Table 6.1). The average number of species per scat was 6.0 ± 0.5 (SE) (range 4 – 8). *Alphitonia petriei* was the only species found in all samples (Table 6.1). The average number of unidentifiable fragments was 11 ± 0.01% (SE) (range 6 – 17%).

Figure 6.1 shows the mean proportion (± SE) of each floral species for all of the scat samples. *Litsea leefeana, Guioa lasioneura* and the weed species *Lantana camara* were found in 75% (6 out of 8) of the samples.

There were three vines species found in the scat samples, *Cissus hypoglauca*, *Elaeagnus triflora* and *Ripogonum album.*

Table 6.1: Proportion of plant species fragments found in scat samples.

Tree species	Colin	Alex	Errol	Eve	Lisa	MJ	Carlia	Simone	Mean	SE
Alphitonia petriei	0.07	0.56	0.05	0.14	0.02	0.11	0.27	0.08	0.16	0.06
Cardwellia sublimis	0	0	0	0	0	0.14	0	0.02	0.02	0.02
Cissus hypoglauca	0	0	0	0	0.31	0	0	0	0.04	0.04
Elaeagnus triflora	0.03	0	0	0.08	0	0	0.12	0.03	0.03	0.02
Flindersia bourjotiana	0	0	0.11	0	0	0	0	0	0.01	0.01
Flindersia brayleyana	0	0	0	0	0	0	0	0.55	0.07	0.07
Flindersia pimenteliana	0	0	0	0.01	0	0	0.05	0	0.01	0.01
Glochidion hylandii	0.23	0	0	0	0	0.13	0	0	0.05	0.03
Guioa lasioneura	0.17	0.03	0	0.11	0.06	0	0.08	0	0.06	0.02
Lantana camara	0.29	0.11	0.08	0.06	0.35	0	0.03	0	0.12	0.05
Litsea leefeana	0.06	0.01	0.02	0.19	0	0	0.05	0.04	0.05	0.02
Neolitsea dealbata	0	0	0.11	0	0	0.51	0.02	0	0.08	0.06
Polycias elegans	0	0.01	0.29	0	0	0	0.05	0	0.04	0.04
Ripogonum album	0	0.19	0	0	0	0	0	0.19	0.05	0.03
Sloanea australis	0	0	0.17	0	0	0	0	0	0.02	0.02
Xanthostemon whitei	0	0	0	0.32	0	0	0	0	0.04	0.04
Unknown	0.15	0.09	0.17	0.09	0.08	0.11	0.06	0.09	0.11	0.01

Although in small numbers, petioles of five species and the young leaves of *Litsea leefeana* were found in the scat samples (Table 6.2).

Table 6.2: Proportion of petioles and young leaves found in scat samples.

Petioles/new leaves	Colin	Alex	Errol	Eve	Lisa	MJ	Carlia	Simone	Mean	SE
Alphitonia petriei	0	0	0	0	0.01	0	0.03	0.01	0.006	0.004
Cissus hypoglauca	0	0	0	0	0.02	0	0	0	0.003	0.003
Litsea leefeana petioles	0	0.01	0.01	0.02	0	0	0.01	0.02	0.009	0.003
Litsea leefeana new leaves	0.04	0	0	0	0	0	0	0	0.005	0.005
Neolitsea dealbata	0	0	0.06	0	0	0	0	0	0.008	0.008
Polycias elegans	0	0	0.04	0	0	0	0	0	0.005	0.005

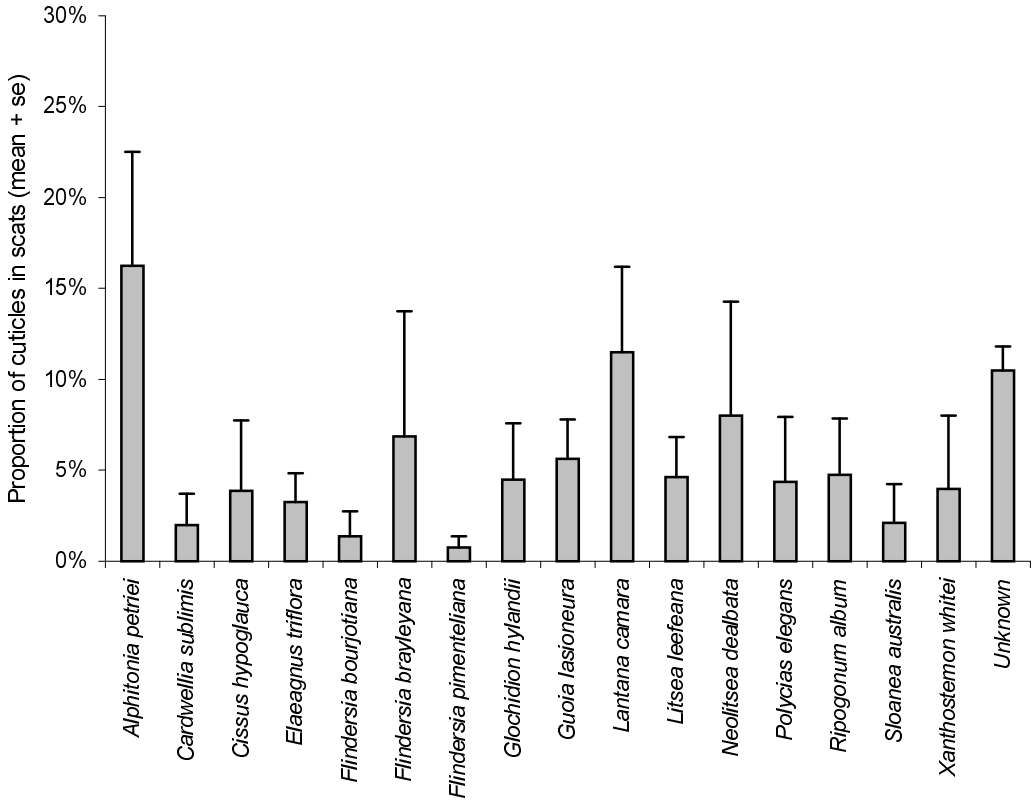

Figure 6.2: Mean proportion of fragments in scats for each tree species.

6.3 DISCUSSION

All of the species found in the scat samples were recorded as used by *D. lumholtzi* in the radio tracking study (Section 5.2.10) except for five species: *Neolitsea dealbata, Polyscias elegans, Ripogonum album*, *Xanthostemon whitei* and *Cardwellia sublimis*. However, use of *Cardwellia sublimis, Neolitsea dealbata, Polyscias elegans* and *Ripogonum album* by *D. lumholtzi* has been previously recorded (pers. obs., TKMG unpublished data). This is the first record for *Xanthostemon whitei* although the congener *Xanthostemon chrysanthus* has been recorded previously (TKMG unpublished data). *Xanthostemon whitei* was only found in one sample although it was in high numbers (32% of the fragments in the scat).

Alphitonia petriei was the most consistently recorded species in the scats. This supports the conclusions from the vegetation analysis (Section 5.2.10) that *Alphitonia petriei* is an important resource for *D. lumholtzi*. Similarly, *Litsea*

leefeana, Flindersia brayleyana and *Guioa lasioneura* were among the most commonly utilised tree species in direct observations (Section 5.2.10), even though *Guioa lasioneura* was not utilised in greater proportion than its occurrence in the forest. Finding *Guioa lasioneura* well represented in the scats indicates that it is an important resource for *D. lumholtzi*. This is an example of where a species is so abundant and usage is less than available (Section 5.2.10), that the conclusion that *Guioa lasioneura* is not important as a resource is misleading (see Section 5.0, Johnson 1980). Furthermore, some species were found in the scats for individual animals, which were not utilised during feeding observations (Table 5.2 and Table 6.1). For example "Simone" was not observed utilising *Flindersia brayleyana* (Table 5.2), but there were cuticles of this species in her scats (Table 6.2). This shows that the technique is very useful in confirming diet for animals in addition to observations.

Five species found in the scats were species that were also used by *D. lumholtzi* in 5b rainforest: *Flindersia brayleyana*, *Litsea leefeana*, *Neolitsea dealbata, Elaeagnus triflora* (vine) and *Lantana camara* (weed) (Procter-Gray 1984, 1985, Newell 1999b).

Lantana camara is a weed species that *D. lumholtzi* has been observed eating on various occasions (TKMG unpublished data, pers. obs.), including the earlier studies by Procter-Gray (1984, 1985) and Newell (1999) and this species was found in 75% of the samples, confirming that *D. lumholtzi* do feed on this species in this study site also, although they were not recorded as using it in the radio-tracking study.

There were three vines species found in the scat samples, *Cissus hypoglauca*, *Elaeagnus triflora* and *Ripogonum album*, and these are all vines species that have been observed being used by *D. lumholtzi* (Procter-Gray 1984, 1985, Newell 1999, TKMG unpublished data) confirming that vines are also important to *D. lumholtzi*. *Cissus hypoglauca* accounted for 31% of the scat sample for one female (Lisa) who inhabitat the riparian zone on a spring in the study site. This animal was observed in *Ficus crassipes* on a regular basis and was found to be selecting it more than expected (Section 5.2.11). This individual tree had a large amount of *Cissus hypoglauca* in its canopy and it was proposed that the

presence of this vine was the reason for the preferred choice of this tree as the cuticles of the fig were not apparent in the scat samples.

From observations in the wild and in captivity, *D. lumholtzi* appear to favour the young leaves of some species (pers. obs.) and also the petioles of at least two tree species (*Litsea leefeana* and *Mischocarpus lachnocarpus*: pers. obs.). The presence of fragments of petioles and some young leaves in *D. lumholtzi* scats samples confirm that they do, although not in high abundances. However, these plant parts may be more readily digested and hence not always be present in the scats. Other arboreal folivores such as leaf-eating monkeys (Hladik 1978, McKey 1978, Glander 1978) consume large numbers of young leaves and petioles and is believed to be because the young leaves and petioles contain more nutrients and minerals (Hladik 1978).

It has been suggested that *Dendrolagus* spp occasionally eat animal protein in the form of eggs or birds (Betz 2001, Johnson *et al* 2002). There was no evidence of animal protein, feathers, insect exoskeleton or eggshell in any of the faecal samples from this study.

Some important points to consider when using this technique to sample diet, is that one pellet is only a snapshot of their diet, it would be preferable to take samples of the same animals over a period of time especially for seasonal differences in diet. Furthermore, due to possibly long retention times in folivores, the scats found under a tree would not necessarily have cuticles representing that particular tree species. The species present would more likely be from between a few days to possibly a week earlier (Jones 2000). Jones found in captive *D. matschiei* that retention times varied on average from 4.8 – 7.8 days range 0 -13 days). Moreover, not all cuticles would survive digestion and therefore would not be present in the faeces, especially the less robust leaves (Storr 1961). These species would then be under represented in the samples if found at all.

6.4 CONCLUSIONS

This study has shown that the use of microscopic faecal analysis using plant cuticles is a practical way to confirm the diet of tree-kangaroos in the wild as it has been found in other marsupial species (Copley and Robinson 1983, Norbury 1988, Ingleby *et al* 1989, Ellis *et al* 1999, How and Hillcox 2000, Lapidge 2000, Moore and Foley 2000, Woolnough and Johnson 2000, Sullivan *et al* 2003) and can confirm the use of tree species otherwise found to be not preferred from habitat selection studies, such as *Guioa lasioneura*. Furthermore, the ingestion of vine species, which is difficult to observe in the wild, can be confirmed using this technique.

This technique has been found to be a practical way to access and confirm the diet of *D. lumholtzi* and will also prove invaluable for dietary analysis in areas where tree-kangaroos are seldom seen in the wild. Furthermore, it gives supplementary information on preferred floral species, especially when that species is in high abundances such as *Guioa lasioneura*, as preference is reflected in selection, which can only occur when a component is relatively scarce (Johnson 1980, Manly *et al* 2002). Moreover, it provides additional information on diet for individual animals of floral species that were not observed being utilised.

Because the scat analysis represents a specific and short-term sample of *D. lumholtzi* diet it can also be used to increase our understanding of seasonal change in food resources by sampling over different seasons. The increased knowledge of preferred species using this technique will assist in the management and the restoration of tree-kangaroo habitat in the future.

Chapter 7: Gross morphology and capacities of the gastrointestinal tract of *Dendrolagus lumholtzi,* an arboreal folivore.

7.0 INTRODUCTION

Optimal foraging theory predicts that the foraging behaviour of an animal (including mating, territory maintenance and predator avoidance behaviours) that provides it with the greatest fitness is that which maximises the net rate of energy or nutrient intake (Townsend and Hughes 1981, Hume 1989). Similarly, optimal digestion theory predicts that the digestive strategy of an animal that provides it with the greatest fitness is that which maximises the net rate of energy or nutrient release from ingested food (Sibly 1981, Sibly and Callow 1986, Hume 1989). Optimal digestion time will vary among food types, being longer for poor-quality (high fibre) foods than for high quality (low fibre) food types. Therefore, animals that eat poorer-quality foods should have larger digestive tracts so that more food is being processed at any one time (Hume 1989). Finally, for any given rate of food intake, an animal should maximise retention of food to maximise the rate of obtaining energy (Sibly 1981, Sibly and Callow 1986, Hume 1989).

Herbivorous animals have the additional complexity of processing the tough plant material in their diet. Plant material is broadly composed of cell contents, cell walls, plant exudates and anti-herbivore secondary compounds (Hume 1989). These components have varying degrees of digestibility and herbivores have a variety of digestive tract modifications to deal with these. Possession of large numbers of symbiotic microorganisms is a central feature in the digestion of structural carbohydrates of plants by herbivores. In addition, these microorganisms also synthesise amino acids and vitamins essential to the host animal (Stevens and Hume 1995).

To achieve this microbial degradation herbivorous marsupials, like eutherians, have modified digestive tracts with either an expanded foregut or hindgut fermentation chamber housing the microbial symbionts (Bauchop 1978, Richardson 1980). In the marsupials, the Vombatoidea (wombat and koala) and

the Phlangeroidea (possums) are hindgut fermenters with a modified caecum and large intestine for microbial fermentation, whereas the Macropodoidea (kangaroos, wallabies and rat kangaroos) are all foregut fermenters with modified stomachs (Richardson 1980). Consequently, tree-kangaroos, the only arboreal folivorous kangaroos, are foregut fermenters, as opposed to the other hindgut fermenting marsupial arboreal folivores. Other foregut fermenting arboreal folivores are found among the colobine primates (leaf eating monkeys) and the sloths (Cork 1996).

Foregut fermentation, in contrast to hindgut fermentation, subjects food to microbial attack before exposure to intestinal enzyme or microbial action (Hume 1982). Therefore, the main advantage of the forestomach fermentation is that after leaving the stomach, the fermentation products, including bacteria, can be digested and absorbed in the functional sites of the small intestine (Bauchop 1978). Another benefit is that the digesta can be delayed for longer periods than in caecum or caecum-colon fermentation systems, giving maximum time for microbial digestion of the fibre (Van Soest 1982).

Although foregut fermentation is best developed in the Ruminantia, it has probably evolved independently in the sloth, leaf eating colobine monkeys and in the macropodoid marsupials (Bauchop 1978), where the forestomach consists grossly of one or more diverticulae or sacs (Hume 1989). Early workers classed these animals as either ruminants or ruminant-like animals (Richardson 1980). However, in macropodoids the modification is primarily an expansion of the forestomach or cardiac region of the stomach as the primary site for microbial fermentation of plant tissue (Richardson 1980; Hume 1982), as opposed to a complex expansion of the oesophagus and portions of the stomach in the true ruminants (Stevens and Hume 1995). Furthermore, although macropods do regurgitate their food occasionally, a process called “merycism”, they do not ruminate (Hume 1989).

The earliest ancestral form of macropodoid marsupial, which split from arboreal rainforest diprotodontids by the mid-Miocene (Flannery 1989), is believed to have had simple dentition and gut morphology (Freudenberger *et al* 1989). The extant omnivorous musky rat-kangaroo (*Hypsiprymnodon moschatus*), a

Potoroid, which also has simple dentition and gut morphology is believed to be similar to this early macropod (Flannery 1989, Freudenberger *et al* 1989).

As the Australian climate became increasingly arid, in the late Miocene and early Pliocene, new nutritional niches appeared and a diversity of morphological and physiological adaptations occurred (Freudenberger *et al* 1989). This diversity ranges from the small Potoroids (0.5 - 3kg) which seek diets rich in nutrients such as plant roots, fungi and invertebrates; to the large grazing kangaroos (>20kg) which exploit poor quality but abundant grasslands (Freudenberger *et al* 1989). Their dietary diversity is evident in their gut morphology. Potoroids have a largely sacciform forestomach, whereas the large grazing Macropodids tend to have the most reduced sacciform but most expanded tubiform forestomach (Hume 1982, 1999, Freudenberger *et al* 1989), with a trend toward increasing relative size of the tubiform forestomach with increasing body size (Freudenberger *et al* 1989) and increasing reliance on grazing rather than browsing. It has been suggested that an expanded tubular forestomach allows macropods to eat more poor quality forage than ruminants of similar size (Hume 1984).

Tree-kangaroos are thought to have returned to the trees in the late Miocene when others members of the family were moving out to the grasslands (Flannery 1989), and appear to fit intermediately between these two classes of gut morphology, with both a large sacciform forestomach and a long tubiform forestomach (Figure 7.1). This is similar to other browsing species of the forest, such as pademelons (*Thylogale* spp.), where the sacciform forestomach constitutes about 50% of the total stomach weight, and probably true for the ancestral macropodids (Freudenberger *et al* 1989). The foregut-fermenting folivorous monkeys (Colobines) also tend to have an intermediate grade or browser type gut morphology (Hume 1989).

The large sacciform morphology of browsers maximises retention of digesta for fermentation and results in high digestibilities of plant cell walls (Freudenberger *et al* 1989). Freudenberger *et al* (1989) propose that this is a derived feature to facilitate the rapid passage of less digestible plant material. On the other hand, a more tubiform morphology, such as in the large grazing kangaroos, leads to a

less than maximal retention of digesta for fermentation (Hume 1989) with a greater flow rate, allowing a greater amount of plant material to be processed faster.

Foregut fermentation employing a sacciform/tubiform forestomach (as in tree-kangaroos, tree sloths and colobus monkeys) is postulated to be optimal for mixing leaf/fruit and leaf/seed diets that are available in many tropical forests, assuming it allows high-fibre meals to be retained for microbial fermentation while permitting, low-fibre meals to pass rapidly to the hindstomach for acid/enzymic digestion (Cork 1996).

Relatively little is known about tree-kangaroo gut morphology and physiology. There have been some descriptions on the morphology of the gastrointestinal tract in a few species of tree-kangaroos (Owen 1852, Flannery *et al* 1996, Hume 1999). However, there are few quantitative data or comparisons to other kangaroo species in relation to size and capacities of the different digestive regions.

This chapter describes the morphology and capacity of the gastrointestinal tract in *Dendrolagus lumholtzi* and compares it to studies on other macropod species. The differences between species and other arboreal folivores are discussed.

7.1 METHODS

This study was based on 21 adult road and dog killed *D. lumholtzi* specimens, and an additional two pouch young 557g and 590g in body weight, collected over a two year period (2001 – 2003) on the Atherton Tablelands in Far North Queensland. When possible, specimens were dissected fresh but most were frozen for processing at a later date. Two specimens were collected in earlier years by other researchers and had been stored frozen prior to processing.

Each carcass was weighed and measured, before the entire gastrointestinal tract from the sub-diaphragmatic oesophagus to rectum was removed via a mid-ventral incision, with as little disturbance of gut content as possible (as in Lentle *et al* 1998). The mesenteric attachments were cut to allow uncoiling of

the intestines and the entire gut was laid out on a board with a 10cm x 10cm grid (Dellow 1982) and photographed (Figures 7.1 and 7.2).

The gastrointestinal compartments were tied off and separated as follows: the stomach, from the commencement of the sub-diaphragmatic oesophagus, to the site of attachment to the small intestine at the pylorus; the small intestine, from the site of attachment to the pylorus, to the site of attachment with the caeco-colonic junction; the caecum, from the site of attachment at the junction of the colon and the small intestine; the colon, from the site of attachment at the caeco-colonic junction to the rectum (as in Lentle *et al* 1998, Freudenberger 1992). The different regions of the forestomach, sacciform and tubiform, were not separated in this study for measurements as the ventral fold that separates the regions was not externally visible in *D. lumholtzi* specimens and it was not possible to precisely determine their junction. However, for the purpose of morphological descriptions, approximate sizes of each region could be measured using the grid when the gut was laid out.

The length of each segment was measured (± 1.0mm) with a plastic tape. The stomach was measured by placing the tape around the outside or longest route round the gastric wall (greater curvature) from the pylorus to the end of the blind sac of the sacciform stomach; and also by measuring along the inside or shortest route round the gastric wall (lesser curvature) from the pylorus to the blind sac of the sacciform stomach along the taenia. The length of the intra-abdominal oesophagus was also measured on six specimens.

Each gut compartment was weighed (wet weight ± 1.0g) and then samples of digesta were taken as follows: sacciform forestomach region, tubiform forestomach region, hind stomach region, proximal and distal small intestine, proximal and distal colon and caecum. Digesta was then thoroughly washed out of each segment using a hose, excess water removed by squeezing and blotted dry with tissue (as in Lentle *et al* 1998, Freudenberger 1992). Each segment was then re-weighed (± 1.0g) giving total wet weight of regions of the digestive tract and total wet weight of digesta was estimated from the difference (as in Lentle *et al* 1998, Freudenberger 1992). Caecum and colon (large intestine) weights were combined to allow comparisons to other studies.

Additional photographs were taken of the inside of the stomach of several specimens after the stomach was inverted to show gastric sulcus (Figures 7.1 and 7.2).

The term “capacity” refers to the total wet weight of digesta contained in a defined region. Gut capacity was defined as the wet mass of total digesta from the entire gastrointestinal tract as a proportion of body mass.

Male and female measurements were compared using Student’s T-tests. The 557g and 590g male pouch young were excluded from these analyses and resulting tables, as the intestinal tract in these animals were not fully developed. Correlations between body weight (kgs) and wet gut contents (g); and body weight (kgs) and gut tissue weight (g) were also investigated using Statistica for Windows V4.5 (Softstat Inc. 1993).

7.2 RESULTS

7.2.1 Morphology

The external and internal features of the gastrointestinal tract of both adult and pouch young *D. lumholtzi* are shown in Figures 7.1 and 7.2.

The mean (± SE) length of intra-abdominal oesophagus in *D. lumholtzi* is 7.2 ± 0.4cm (*n* = 6). The oesophagus enters the stomach, at an entry point called the cardia, at approximately one-third of the way along the lesser curvature of the stomach, into the sacciform forestomach near the tubiform-sacciform junction (Figure 7.1). The cardia opens directly into the gastric sulcus.

D. lumholtzi has a well developed gastric sulcus or ventricular groove, in adults (Figures 7.1c, 7.1d, 7.1f) and the two pouch young examined (Figure 7.2b). The gastric sulcus runs from the cardia along the lesser curvature of the stomach almost all the way to the hindstomach.

D. lumholtzi has a relatively large sacculated forestomach compared to other kangaroos (Figures 7.1b, 7.1e, 7.6), a large tubiform forestomach and a smaller hindgut. Although the different regions of the stomach were not separated in this study, it can be seen in the figures that the sacciform (~ 30cm) and tubiform

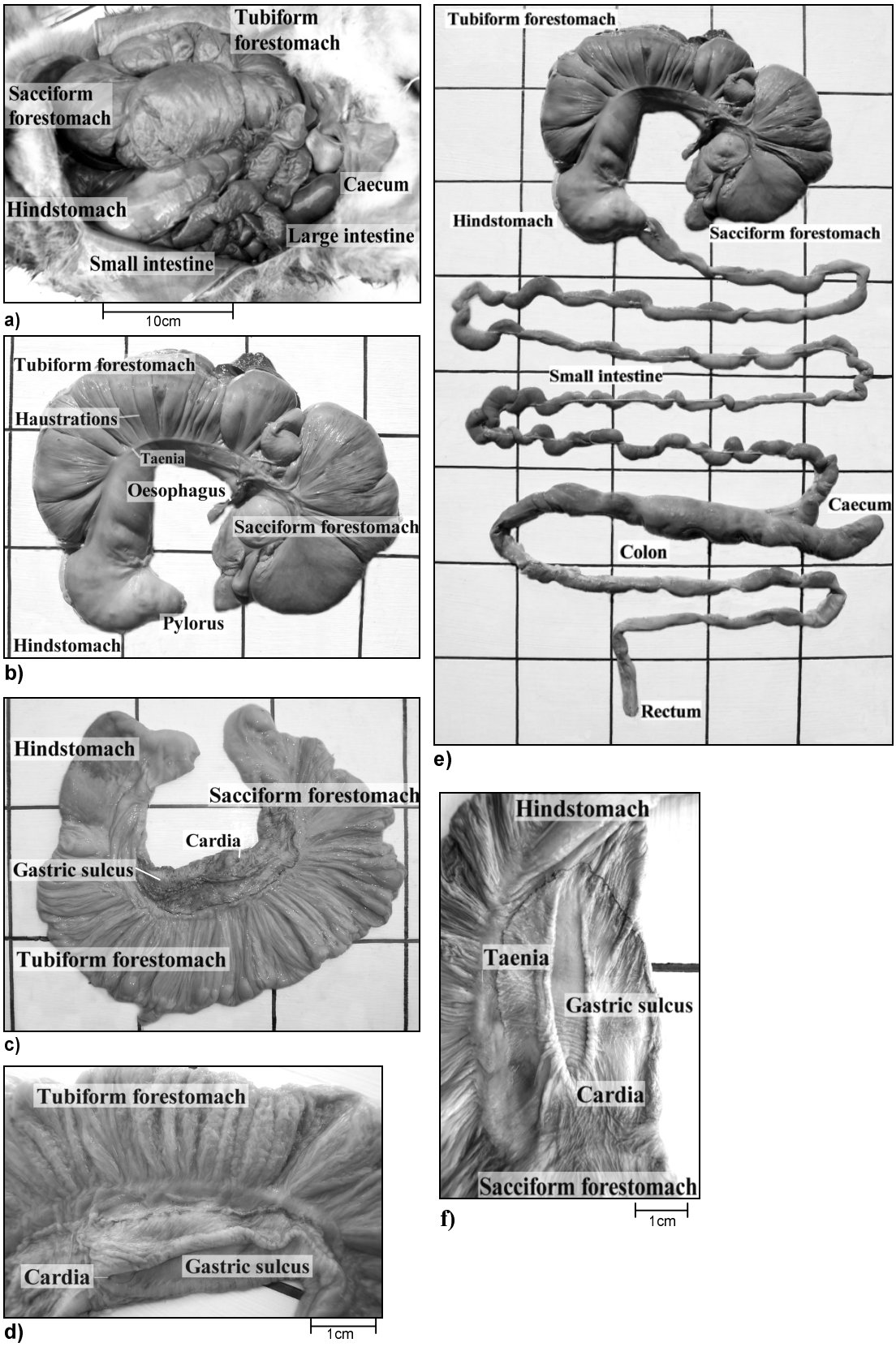

Figure 7.1. Photos of the gastrointestinal tract of an adult *Dendrolagus lumholtzi* (TK13).
a) Dissected body showing position of gut *in situ*. b) Stomach. c) internal view of stomach. d) close-up of gastric sulcus. e) entire digestive tract dissected free from mesenteric attachments and uncoiled to show sections clearly. f) internal view of gastric sulcus.
Scale: 10cm x 10cm grid in background. Scale shown where grid cannot be seen.

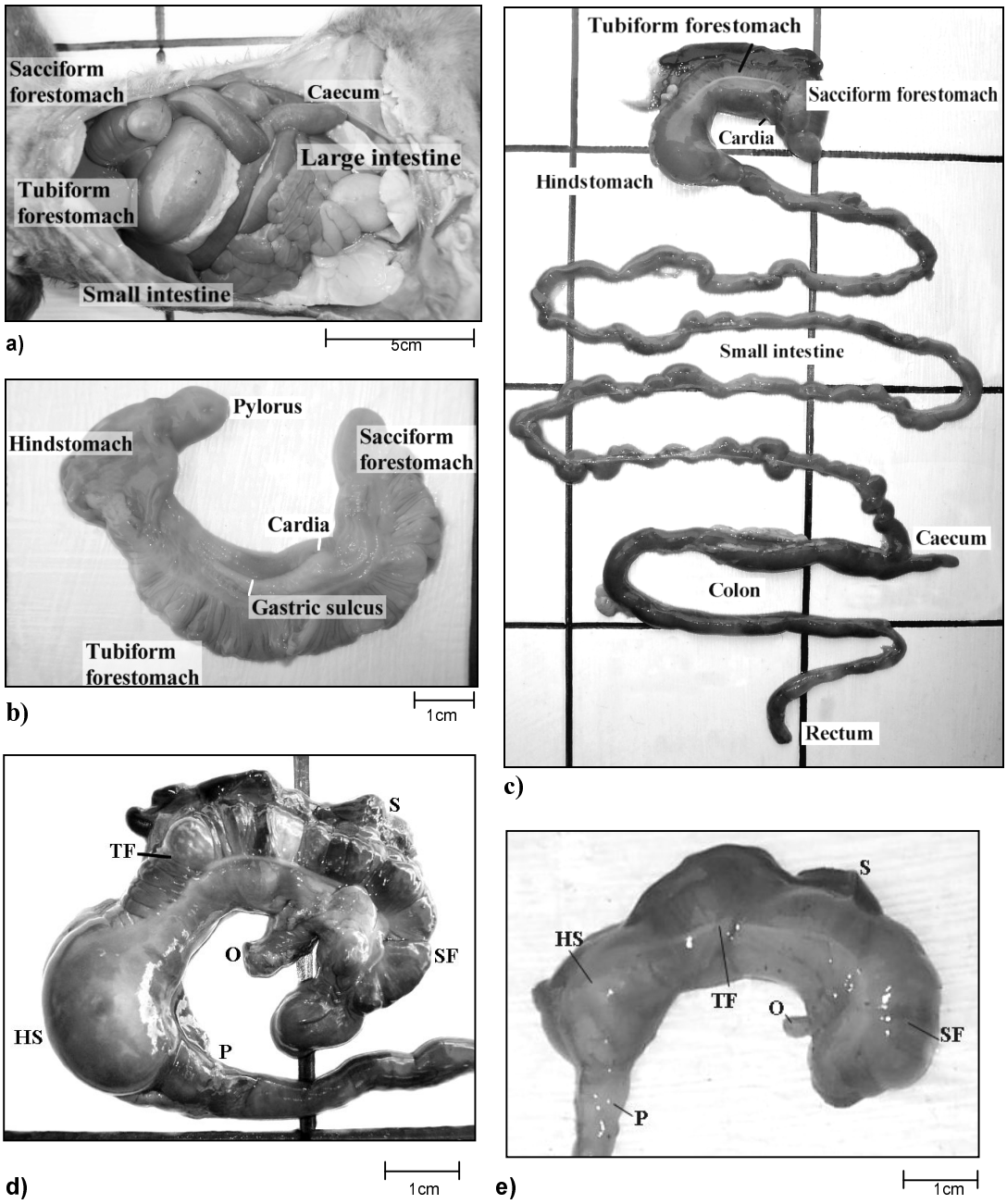

Figure 7.2. Photos of the gastrointestinal tract of *D. lumholtzi* pouch young.
a) Dissected body of 557g joey showing position of guts in situ. b) Internal view of stomach of 557g joey showing the gastric sulcus. c) Entire digestive tract of 557g joey, dissected free from mesenteric attachments and uncoiled to show sections clearly. d) stomach of 590g male pouch young e) 142g joey stomach.
SF, sacciform forestomach; TF, tubiform forestomach; HS, hindstomach; P, pylorus; O, oesophagus (cardia); S, spleen
Note that the haustrations in the tubiform or sacciform forestomach regions and the taenia are not obvious at 142g and 557g, the gastric sulcus is well developed and the joey has lots of fat tissue in the abdomen (a).
Scale: 10cm x 10cm grid in background. Scale is shown where grid cannot be seen.

(~ 35cm) foreguts are almost the same size, with the tubiform being only slightly longer (Figure 7.1b). However, the hindgut is smaller (~ 15cm) as in other kangaroos (Langer *et al* 1980). The hindgut is large in the pouch young (Figures 7.2c-e).

As with all macropods, the major external features of the forestomach are the taeniae and associated haustrations (Figure 7.1). The hindstomach does not possess these and is relatively smooth. The musculature of the forestomach wall is differentiated into several bands of longitudinal muscle, the taeniae, one on either side. Semi-lunar folds extend between the taeniae, creating the haustrations, which give the stomach its "colon-like" appearance (Owen 1868, Hume 1982, Freudenberger *et al* 1989). These taeniae and haustrations are not well developed in *D. lumholtzi* pouch young of 142g and 557g body weight (Figure 7.2a-c,e), are more developed in the 590g pouch young (Figure 7.2d), but are well developed by the time they are 2kg in body weight.

The small intestine (245.7 ± 9.5cm, n = 12) is longer than large intestine (121.3 ± 7.0cm, n = 12) (Table 7.1). The large intestine is relatively short and the caecum is small (9.9 ± 0.5cm, n = 12) and not haustrated. The mean values of dimensions for the different regions of the gut for males and females are shown in Table 7.1.

Table 7.1. Dimensions of each region of the gastrointestinal tract of male and female *D. lumholtzi*. Values are the means ± standard errors and ranges. *n* in parentheses is the number of specimens.

Lengths (cm)	Male mean (*n* = 9)	Male range (*n* = 9)	Female mean (*n* = 3)	Female range (*n* = 3)	Female and male mean (*n* = 12)
Stomach inside (Lesser curvature)	45.2 ± 2.5	38.0 - 60.0	42.2 ± 6.1	30.0 - 49.0	44.5 ± 2.3
Stomach outside (greater curvature)	79.7 ± 4.3	61.0 - 101.0	80.7 ± 2.03	77.0 - 84.0	79.9 ± 3.2
Small intestine	253.6 ± 11.5	225.0 - 340.0	222.0 ± 3.8	215.0 - 228.0	245.7 ± 9.5
Large intestine	113.6 ± 8.0	82.0 - 151.0	104.7 ± 14.3	77.0 - 125.0	111.3 ± 6.8
Caecum	10.4 ± 0.6	7.5 - 13.0	8.7 ± 0.9	7.0 - 10.0	10.0 ± 0.5
Oesophagus	7.2 ± 0.4	5.7 - 8.5			7.2 ± 0.4

7.2.2 Capacities

The capacities (total wet weight of digesta) of the different regions of the gut, along with the capacities as a percentage of body weight and total wet weight of each region for males and females are shown in Table 7.2.

The mean total wet tissue weight of the gut was 330.6 ± 19.3g, with 331.0 ± 22.0g for males and 330.1 ± 35.8g in females (Table 7.2). There was a strong positive correlation between wet tissue weights to body weight (r = 0.84, P = <0.001, Figure 7. 4).

Total gut contents as percentage of body weight was 16.5 ± 1.3% for males and 20.1 ± 1.9% for females, with a range up to 26.8%. There was a strong positive correlation between body weight and wet gut contents (total digesta) (r = 0.69, P = 0.0006, Figure 7.3).

The capacity of the stomach as a percentage of body weight was 13.1 ± 1.1% for males and 16.7 ± 1.7% for females, with an overall mean of 14.6 ± 1.0% (Table 7.2).

The stomach was the largest region of the gut (80.6 ± 0.9%) and significantly larger in females than males (79% for males and 82.8% for females; t = 2.3, df = 19, P = 0.03, Table 7.2). There was a strong positive correlation between body weight and wet stomach contents (r = 0.7, P = 0.001, Figure 7.5).

The large intestine had greater capacity than the small intestine (13.8% large and 5.6% small, P = < 0.0005, Table 7.2) and the large intestine and caecum, as a percentage of total gut contents, was larger in males than females; 16% males, 10.9% females (t = -3.0, df = 19, P = 0.007, Table 7.2). There was no significant difference with the sexes in any other measurement.

Table 7.2: Capacities of regions of the gastrointestinal tract of male and female *D. lumholtzi*. All weights refer to the wet weight of digesta contents (g) or wet weight of tissue (g). Values are the means ± standard errors and ranges. *n* in parentheses is the number of specimens.

	Male mean (*n* = 12)	Male range (*n* = 12)	Female mean (*n* = 9)	Female range (*n* = 9)	Male and female mean (*n* = 21)
Body weight (kgs)	6.5 ± 0.4	3.3 - 9.2	5.4 ± 3.3	2.0 - 7.6	6.02 ± 0.4
Stomach contents (g)	831.8 ± 73.7	490.0 - 1259.0	904.3 ± 162.1	381.0 – 1723.0	862.9 ± 79.2
Stomach contents (% of body weight)	13.1 ± 1.1	8.0 - 19.7	16.7 ± 1.7	8.9 - 22.7	14.6 ± 1.0
Small intestine contents (g)	53.7 ± 8.0	12.0 - 114.0	69.7 ± 17.2	19.0 - 179.0	60.5 ± 8.6
Large intestine contents (g)	142.5 ± 16.5	54.0 - 229.0	91.7 ± 21.0	22.0 - 211.0	120.7 ± 13.9
Caecum contents (g)	27.5 ± 2.2	14.0 - 41.0	22.1 ± 4.7	6.0 - 46.0	25.2 ± 2.4
Total gut contents (g)	1055.5 ± 93.1	609.0 - 1538.0	1087.8 ± 190.2	473.0 - 2033.0	1069.3 ± 94.5
Total gut contents (% of body weight)	16.5 ± 1.3	10.1 - 23.8	20.1 ± 1.9	10.4 - 26.8	18.0 ± 1.2
Relative capacities (% of total gut contents)					
Stomach	79.0 ± 1.3	73.3 - 85.5	82.8 ± 0.8	78.8 - 86.4	80.6 ± 0.9
Small intestine	5.0 ± 0.5	2.0 - 8.3	6.3 ± 1.0	3.4 - 12.1	5.56 ± 0.5
Large intestine + caecum	16.0 ± 1.0	10.9 - 21.4	10.9 ± 1.5	2.2 - 15.4	13.8 ± 1.0
Wet tissue weight (g)					
Stomach	193.3 ± 14.7	86.0 - 279.0	195.0 ± 21.6	85.0 - 299.0	194.0 ± 12.1
Small intestine	78.8 ± 5.6	48.0 - 123.0	82.8 ± 9.7	51.0 - 129.0	80.5 ± 5.1
Large intestine	56.1 ± 3.6	23.0 - 71.0	49.0 ± 6.4	24.0 - 83.0	53.1 ± 3.4
Caecum	2.8 ± 0.3	1.0 - 4.0	3.3 ± 0.6	2.0 - 8.0	3.1 ± 0.3
Total Wet Tissue weight (g)	331.0 ± 22.0	158.0 – 476.0	330.1 ± 35.8	167.0 – 505.0	330.6 ± 19.3

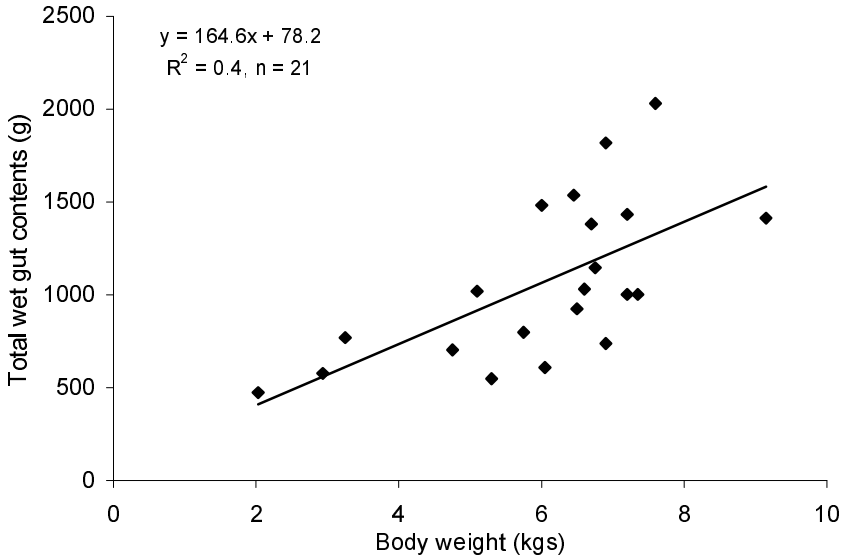

Figure 7.3 Total wet mass of the gut contents as a function of body mass for *D. lumholtzi*.

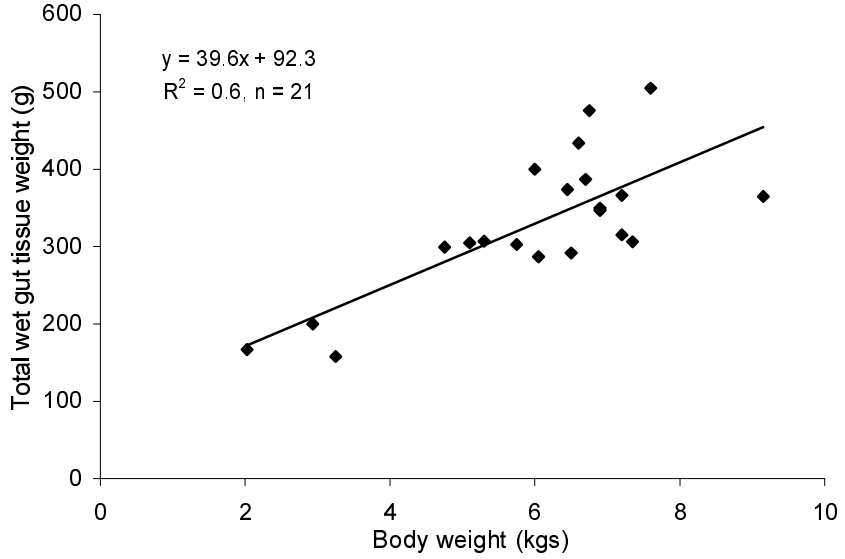

Figure 7.4 Total wet mass of the gut tissue as a function of body mass for *D. lumholtzi*.

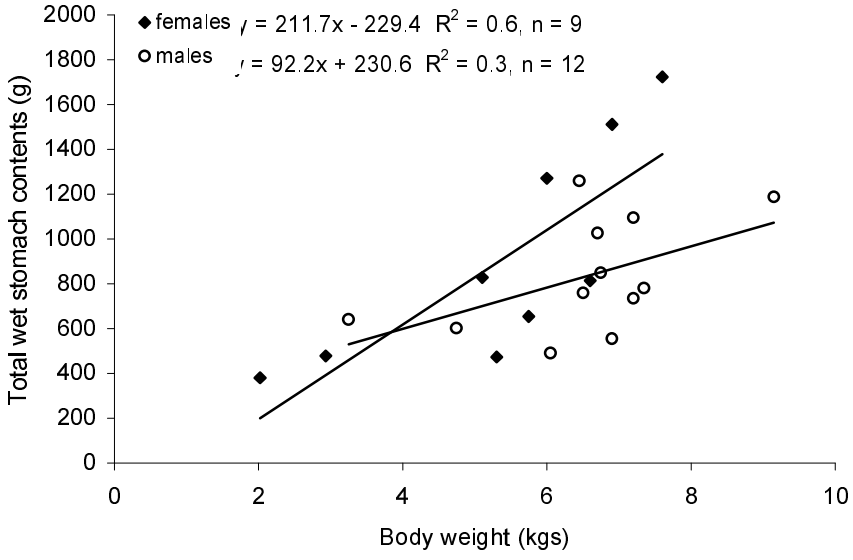

Figure 7.5 Total wet mass of the stomach contents as a function of body mass for *D. lumholtzi*.

7.3 DISCUSSION

7.3.1 Morphology and capacities

Langer *et al* (1980) and Hume (1999) have described the gross morphology of macropod stomachs. They concluded that, although the gross structure of the macropod stomach had generally been considered similar among species that there are marked differences between the species that they examined related to diet (Langer *et al* 1980, Hume 1999). This study has shown that this is also true for *D. lumholtzi*. The gross morphology of the gastrointestinal tract of *D. lumholtzi* is similar to that of other browsing macropods and other foregut fermenting folivores such as the colobine monkeys and sloths, with a larger sacciform forestomach and greater stomach capacities than grazing macropodids (Figure 7.6).

As with other mammals, the oesophagus enters the stomach on the lesser curvature and this entry point is called the cardia. The cardia opens directly into the gastric sulcus in *D. lumholtzi*. This ventricular groove extends from the cardia to the non-sacculated region along the lesser curvature, and its length and definition varying with different macropod species (Bauchop 1978). In the ruminant forestomach a reticular groove, similar to the gastric sulcus in macropods, connects the cardia with the reticulo-omasal orifice. Contraction of its muscular walls forms a tube which allows milk in suckled young to bypass the reticulum and rumen, where it would be fermented, and to pass directly into the abomasum to allow for efficient utilisation of milk (Langer *et al* 1980, Hume 1999, Van Soest 1982). The stimulus for closure of the sulcus is the suckling reflex.

The gastric sulcus is absent in the red-necked pademelon *Thylogale thetis* (Langer *et al* 1980), red-legged pademelon *T. stigmatica* and the little rock wallaby *Peradorcas concinna* (Hume and Dellow 1980). It is also reduced in during ontogenetic development in *M. giganteus* (Langer *et al* 1980). However, both adult and pouch young *D. lumholtzi* examined in this study had well developed gastric sulci. The gastric sulcus is also present in other arboreal folivores such as colobine monkeys and sloths (Cork 1996).

The presence of this groove in most species of Macropodoids may be an example of convergent evolution between marsupials and eutherians. Whether the function of the groove is similar in the two groups remains to be determined (Richardson 1980). However, it has been postulated that this may function, not only to bypass milk while suckling, but also to assist the caudal movement of liquid digesta, and other material that needs no fermentation such as cell contents, to directly to the hind stomach (Hume 1982, 1999). This is supported by the belief that a well-defined ventricular groove allows ingested liquids to pass directly from the oesophagus to the middle compartment of the stomach in the colobine monkeys (Bauchop 1978). This is also supported by radiographic studies by Langer *et al* (1980), where the gastric sulcus has been reported to facilitate the movement of more liquid digesta caudally along the lesser curvature of the tubiform forestomach, directing it into the hindstomach. The presence of a well developed gastric sulcus in *D. lumholtzi* would be important for suckling young after they begin to eat leaves. *D. lumholtzi* young at foot continue suckling for up to 240 days after permanent emergence from the pouch (Johnson and Delean 2003). Furthermore, in adults, a well developed gastric sulcus would be beneficial by bypassing fermentative digestion of liquid digesta, directing it to the hindstomach allowing rapid digestion of cell contents. It is plausible that the reduction in the gastric sulcus in *M. giganteus* and other large grazing macropodids is due to the increased flow of digesta in their larger tubiform stomach and therefore it would be unnecessary to bypass liquid digesta.

It has been suggested that in many species of Macropodidae that the two regions of the forestomach, the sacciform and tubiform, are separated by a permanent ventral fold (Langer *et al* 1980). This ventral fold was not visible in *D. lumholtzi*, therefore any separation and subsequent measurement of these sections would have been somewhat subjective. Hume (1982) suggested that it cannot be seen in eviscerated specimens, which are invariably dissected free from mesenteric attachment and partially uncoiled during preparation. He also suggests that it is possible that the ventral fold described by Langer *et al* (1980) may be an artefact of the preparation *in situ* caused by collapse of parts of the stomach wall not held firmly by mesentery (Hume 1982).

Even though the three regions were not separated in this study, it can be seen in the photographs (Figures 7.1b and 7.1e) that the sacciform and tubiform stomachs can be roughly separated at the region caudally to the position of the oesophagus (cardia) and are approximately the same size. In comparison, *Macropus robustus robustus* has a sacciform stomach that is relatively larger than *M. giganteus* (Dellow 1982) and in *T. thetis* the sacciform stomach is much larger in proportion to the total forestomach (Langer *et al* 1980) (see Figures 1-4 in Dellow 1982).

Larger grazing kangaroos have larger tubiform forestomachs, and browsers, such as pademelons and tree-kangaroos, have larger sacciform forestomachs (Freudenberger *et al* 1989). It has been suggested that a larger tubiform forestomach allows for a greater flow rate and a larger quantity of low quality, high fibre plant material to be processed (Freudenberger *et al* 1989). Whereas a large sacciform forestomach allows the accumulation of digesta and slow rate of passage essential for extensive fermentation of plant materials (Bauchop 1978). Another benefit of a large sacciform stomach and the symbiotic bacteria within, allows folivores to detoxify fruits, leaves and seeds containing a range of potentially toxic ingredients, including cyanide and strychnine, before the toxins are absorbed (Janzen 1978, Coley 1983, Waterman *et al* 1988, Dasilva 1992). Some gut microbes are capable of metabolising toxic plant compounds (Barry and Blaney 1987) and there is evidence that tannin-protein complexes can be broken down by microbial action (Milton 1978, Foley and Hume 1987). A larger sacciform stomach in relation to the tubiform stomach is also found in colobine monkeys (Hill 1952, Bauchop 1978, Stevens and Hume 1995) and sloths (Montgomery and Sunquist 1978, Bauchop 1978) (Figure 7.6).

Although the forestomach of sloths resembles that of ruminants in being mainly sacciform, those of colobine primates and tree-kangaroos are different from ruminants in having both tubiform and sacciform components with the sacciform component being offset from the main flow of digesta (Cork 1996). A similar arrangement of sacciform and tubiform stomach components in potoroine marsupials (rat kangaroos) is postulated to allow variable flow of dietary fibre and the opportunity for much of the digesta, especially components

such as cell contents dissolved or suspended in the fluid fraction, to bypass fermentative digestion (Cork 1996, Hume and Carlisle 1985). This is enhanced by the presence of a gastric sulcus in sloths (Montgomery and Sunquist 1978, Bauchop 1978), colobine monkeys (Hill 1952, Bauchop 1978, Stevens and Hume 1995) and tree-kangaroos, which also assists in the bypass of liquid digesta.

Table 7.3: Comparison of the relative capacities and dimensions of regions of the gastrointestinal tract of *D. lumholtzi;* and *Thylogale thetis, Macropus eugenii* and *Macropus giganteus* (Dellow and Hume 1982). All weights refer to the wet weight of digesta contents or wet weight of tissue. Values are the means ± standard errors and ranges. *n* in parentheses is the number of specimens.

	D.lumholtzi (*n* = 21)	*T. thetis* Browser	*M. eugenii* Grazer	*M. giganteus* Grazer
Body weight (kgs)	6.0 ± 0.4	5.9 ± 1.0	4.5 ± 0.3	19.1 ± 0.7
Total stomach contents (g)	862.9 ± 79.2	448.0 ± 86.0	279.0 ± 23.0	1924.0 ± 198.0
Total stomach contents (% of body weight)	14.6 ± 1.0	7.6 ± 0.6	6.4 ± 0.7	10.0 ± 0.7
Relative capacities (% of total gut contents)				
Stomach	80.6 ± 0.9	76.0 ± 3.0	76.0 ± 4.0	80.0 ± 1.0
Small intestine	5.6 ± 0.5	8.0 ± 1.0	9.0 ± 1.0	9.0 ± 1.0
Large intestine + caecum	13.8 ± 1.0	17.0 ± 1.0	15.0 ± 2.0	11.0 ± 1.0
Lengths (cm)				
Small intestine	245.7 ± 9.5	254.0 ± 23.0	271.0 ± 14.0	407.0 ± 28.0
Large intestine + caecum	121.3 ± 7.0	90.0 ± 11.0	100.0 ± 3.0	165.0 ± 17.0
Caecum	10.0 ± 0.5	12.0 ± 1.0	10.0 ± 1.0	30.0 ± 2.0

Dendrolagus lumholtzi has a similar average body weight to both *Thylogale thetis*, a rainforest dwelling browser, and *Macropus eugenii*, a dry country grazer (Dellow and Hume 1982: Table 7.3). However, the total stomach contents in *D. lumholtzi* were much greater at 862.9g vs 448g and 279g respectively (Table 7.3). The much larger *M. giganteus* has over double the absolute stomach contents (1924 ± 198g) of *D. lumholtzi*, but substantially less as a proportion of body weight (Table 7.3).

The stomach of *D. lumholtzi* is very large and when full can weigh up to 23% of its body weight and total gut contents up to 26% of the body weight, a proportion similar to ruminant (15%: Bauchop 1978, Table 7.4), and other

ruminant-like animals, especially other arboreal folivores such as the colobine monkeys (10.5 - 20.6% of the total body weight: Ohwaki *et al* 1974), the langur monkey (17% of body weight: Bauchop and Martucci 1968) and sloths (*Bradypus* sp: 20-30% of the body weight: Montgomery and Sunquist 1978, Bauchop 1978). However, this is approximately twice the size of other species of kangaroos previously studied, even other "browsers", such as *T. thetis*, which had much smaller stomach capacities of less than 10% of their total body weight (Langer *et al* 1980, Dellow and Hume 1982). Therefore, *D. lumholtzi* has a larger sacciform forestomach and greater total stomach capacity than other macropods, including other browsers, and is more similar to that of other arboreal foregut fermenting folivores.

Table 7.4. A comparison of the relative capacities and dimensions of regions of the gastrointestinal tract of *D. lumholtzi*, other kangaroo species, ruminants and arboreal folivores. All weights refer to the wet weight of digesta contents or wet weight of tissue. Values are the means ± standard errors and ranges. *n* in parentheses is the number of specimens.

	Body weight (kgs)	Total stomach contents (% of body weight)	Total gut contents (% of body weight)	References
Dendrolagus lumholtzi (n = 21)	6.0 ± 0.4	14.6 ± 1.0	18.0 ± 1.2	This study
Thylogale thetis (n = 4)	5.9 ± 1.0	7.6 ± 0.6		Dellow and Hume 1982
Macropus eugenii (n = 4)	4.5 ± 0.3	6.4 ± 0.7		"
M. giganteus (n = 4)	19.1 ± 0.7	10.0 ± 0.7		"
M. robustus robustus (n = 16)	21.0 ± 1.4	9.2 ± 0.4*	13.2 ± 0.5	"
Colobine monkey	8.0[F]	10.5 - 20.6		Bauchop 1978
Langur monkey	5.4	17.4		Bauchop & Martucci 1968
Sloth	4.7	15.3		Hartman 1959
Goat	32.1 ± 2.2	14.9 ± 1	20.4 ± 1.5	Freudenberger 1992
Sheep	58.0	15.6	19.1	Parra 1978
Cattle	273.0 – 546.0	10.0-18.0	13.0-24.0	Parra 1978

Both the sacciform and tubiform forestomachs have haustrations, which would assist with the mixing and fermentation of the gastric contents (Richardson 1980). These haustrations were not well developed in the *D. lumholtzi* pouch young examined in this study, except in the 590g joey where they were beginning to form. This is the approximate size that tree-kangaroo pouch young begin to eat foliage (Dabek 1991).

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a)

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b)

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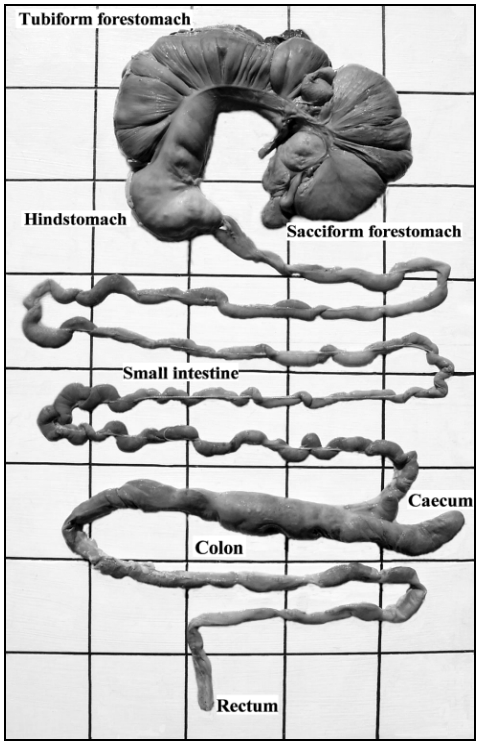
c)



d)

Figure 7.6. Comparison of different gastrointestinal tracts. a) Colobus monkey, *Colobus abyssinicus* b) Eastern Grey kangaroo, *Macropus giganteus* c) sloth, *Bradypus tridactylus* d) Lumholtz's tree-kangaroo, *D. lumholtzi.* Scale 10cm grid. a–c). Taken from Stevens and Hume 1995.

The hindstomach of macropods, as described by Hume (1978) and Langer *et al* (1980), consists of the gastric pouch (the site of hydrochloric acid secretion) and the pyloric region. The hindstomach of *D. lumholtzi* is smooth with no haustrations, as in other kangaroos.

The small and large intestines in *D. lumholtzi* are both shorter than those of ruminants (Hume 1982) and to those of larger kangaroos (Table 7.3). It has been suggested that with increasing fermentation in the foregut, such as in tree-kangaroos, herbivores will minimise the proportion of the gut (i.e. the small intestine), which is used in enzymatic digestion (Penry and Jumars 1987). Lentle *et al* (1989) suggested that the colon is generally longer in grazers than browsers, such as tree-kangaroos. The caecum in *D. lumholtzi* is relatively small compared with that of other macropods, simple and tubular with no haustrations (Dellow 1982). The caecum has also been found to be longer in grazing macropods (Lentle *et al* 1989). The increase in length of the colon and caecum in grazers (Osawa 1987) or macropods with diets of low nutrient quality (Lentle *et al* 1998) is believed to provide additional capacity of the hindgut for microbial digestion of plant cell walls (Freudenberger *et al* 1989). This increase in hindgut activity would be beneficial for grazing macropods with their larger tubiform forestomach with increased passage of digesta and less time for fermentation in this region. In contrast tree-kangaroos with a larger sacciform stomach for increased storage of digesta for fermentation, do not require as long a colon and caecum as the grazers, although some microbial digestion would still occur in these regions. The large intestine of the sloth is also short and the caecum is absent (Bauchop 1978).

There was some sexual variation in relative capacities of gut regions in *D. lumholtzi* (Table 7.2, Figure 7.5). Female *D. lumholtzi* had larger relative capacities of the stomach than did males and male *D. lumholtzi* had larger colon and caecums than the females. Lentle *et al* (1998) found significant differences in the allometric scaling of the colon tissue between the sexes in the Tammar wallaby (*M. eugenii*). Moreover, they found other sexual dimorphism in gut morphology, with females having relatively heavier walls in foregut and hindgut and relatively longer colons. Similar sex differences in colon length

have been reported in rabbits and were attributed to an increase in digestive capacity in breeding females at this time (Sibly *et al* 1990). Although female *D. lumholtzi* were found to have shorter colons than males in this study, females did show significantly larger stomach in relation to the rest of the gut, which may also be attributed to an increase in digestive strategy for breeding.

7.4 CONCLUSIONS

Understanding the gastrointestinal morphology of tree-kangaroos is important in relation to how they meet the optimal digestive strategy. Optimal digestive strategies and optimal foraging strategies are linked, and should be considered together when studying an animal's ecology (Hume 1989).

The large stomach capacity and larger sacciform region in *Dendrolagus lumholtzi* is more similar to other foregut fermenting arboreal folivores (e.g. the Colobine monkeys and the three-toed sloth) than to other macropod species previously studied (even other browsing species). This is consistent with the suggestion that a large sacciform forestomach is beneficial for foregut fermenting arboreal folivores to efficiently meet their optimal digestion by maximising retention of tough plant material, while still allowing low fibre or fluid contents to pass through to the hindgut for digestion via the gastric sulcus.

On the other hand, a more tubiform morphology, such as the grazing kangaroos, leads to a less than maximal retention of digesta for fermentation with a greater flow rate, allowing for a greater amount of plant material to be processed faster. Grazers can essentially eat a large amount of grass, as there is not many plant defences in grass. However, leaf eaters cannot eat large amounts of leaves as they have a whole varied set of problems to deal with, such as the potentially high levels of toxins in leaves. If folivores were to consume as much as grazers, they run the risk of poisoning themselves. Therefore, they need to be able to digest as much as possible of the leaves they eat, instead of consuming more, to get the same nutrition from them.

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Chapter 8: Ageing Lumholtz's tree-kangaroos (*Dendrolagus lumholtzi*), using premolar tooth wear and annual rings in the cementum of molars.

8.0 INTRODUCTION

An accurate method for assessing the age of an individual animal provides a valuable insight into many aspects of a species biology (Morris 1978) and is an important part of any study of the population dynamics of a species (Caughley 1977, Inns 1982). Accurate age data are vital in establishing such characters as the rate of growth, onset of sexual maturity, periodicity of reproduction or life span of a species (Klevezal and Kleinenberg 1967), population viability analysis and many other parameters essential to the formulation of wildlife management programmes (Morris 1978). Reliable age data can also be used to construct life-tables for the estimation of age-specific mortality (Inns 1982). Furthermore, this information may be important in explaining the distribution of individuals of differing age in habitat of varying quality (Martin 1996). Age determination is also very important for any study on the fluctuations of populations (Klevezal and Kleinenberg 1967).

Age determination is also indispensable to many studies of comparative anatomy, morphology and taxonomy, since without this information we cannot determine the uniformity of comparable data or the character of variability associated with age (Klevezal and Kleinenberg 1967).

Mammals can be aged by a variety of techniques (Caughley 1977, Inns 1982, see Section 2.7). Common techniques for aging in macropods use a variety of different body measurements (Sadlier 1963, Shield 1968, Sharman *et al* 1964, Murphy and Smith 1970, Maynes 1972, Poole *et al* 1982, 1985, Inns 1982, Blanshard 1990, Johnson and Vernes 1994, Johnson and Delean 2003), tooth eruption (Caughley 1965, Sharman *et al* 1964, Ealey 1967, Maynes 1972, Driessen and Hocking 1996), molar progression (Kirkpatrick 1964, 1965, Hughes 1965 Sharman *et al* 1964, Dubzinski *et al* 1977, Dawson 1995, Lentle

et al 2003b) and tooth wear (Logan and Sanson 2002, Lentle *et al* 1998, 2003a, 2003c).

Tree-kangaroo dentition is similar to koalas in that they have a premolar and four molars in a flat tooth row and their teeth erupt completely early in the animal's maturity and in a relatively short period of time (Flannery *et al* 1986, Martin 1981). Tree-kangaroos have no molar progression, as found in many other macropods (Flannery *et al* 1996). Tree-kangaroos do, however have two deciduous premolars that are replaced by a large secator premolar of the same size by the approximate age of 2 or 3 years (Figure 8.1).

Therefore, as tree-kangaroos do not have molar progression and body measurements and tooth eruption are only relevant up to a certain age (Inns 1982), the techniques for aging tree-kangaroos are limited.

Tooth wear is a widely used and successful indication of age in koalas (Martin 1981, Lanyon and Sanson 1986, Gordon 1991, Logan and Sanson 2002). Continual wear of the biting and grinding surfaces of the teeth occurs (Gordon 1991, Martin *et al* 1999) and continues until the enamel of the cutting ridges and cusps is worn away completely (Martin *et al* 1999). The underlying dentine is then exposed and the characteristic wear patterns that appear can be used to assign age classes or indices of age to animals.

Until this study, there have been no previous attempts to age Lumholtz's tree-kangaroos, *Dendrolagus lumholtzi,* except in pouch young in captivity (Johnson and Delean 2003).

This study examined the suitability of developing an aging index for *D. lumholtzi* based on both tooth eruption and replacement for young animals and tooth wear of the upper premolars similar to that used to age koalas (Martin 1981, Gordon 1991, Martin *et al* 1999).

For a comparison, and to test if tooth wear corresponds with independent measures of age, the tooth wear index was compared with analysis of tooth cementum annuli from dead tree-kangaroos, largely road-kills.

8.0.1 Tooth cementum annuli

Annuli in cementum and dentine layers of teeth have been used to age many different mammals (Laws 1952, Klevezal and Kleinenberg 1967, Gasaway *et al* 1978, Fancy 1980, McCullough and Beier 1986, Cool *et al* 1994, Azorit *et al* 2002a, 2002b), including marsupials (Pekelharing 1970, Clout 1982). Over 40 species of mammals have been aged by this method since Laws (1952) first showed that internal dentine and cementum annulations were correlated with age.

Although early attempts made with marsupials were unsuccessful (Kingsmill 1962, Catt 1979, Inns 1982), recent improvements to the preparation and staining methods has resulted in an accurate and frequently used technique to age or to develop an age index for many species (Fancy 1980, Azorit *et al* 2002a). In fact, tooth cementum is currently the most widely used tissue for mammal aging (Fancy 1980, Azorit *et al* 2002a).

Tree-kangaroos have limited longitudinal growth of their teeth (Flannery *et al* 1996), and do not appear to have any clear dentine growth lines (pers. obs.), so the cementum layers are the most suitable tissue for aging in *D. lumholtzi*.

8.0.2 Tree-kangaroo tooth eruption

Like other macropods, tree-kangaroos do have a limited number of deciduous teeth (Flannery *et al* 1996) and a peculiar form of tooth replacement. As an animal matures, a permanent sectorial premolar (P3) ejects both a deciduous blade-like premolar (P1) and a molar-shaped tooth (P2) (Flannery *et al* 1996). This characteristic, where - one permanent tooth replaces two deciduous ones - is unique to kangaroos (Flannery *et al* 1996). It is interesting that the two deciduous teeth form a functioning unit similar in shape and size to the premolar that replaces them (Flannery *et al* 1996). In fact, the premolar replacing the deciduous ones sits directly beneath the two deciduous ones, fitting precisely under their roots (Figure 8.1).

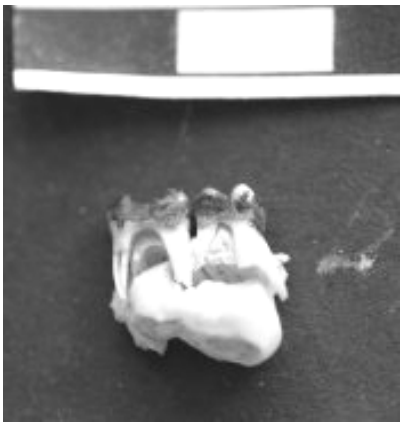


Figure 8.1. Permanent premolar (P3) under deciduous premolars in young joey.

The eruption of P3 replacing the deciduous premolars tends to be delayed until the tooth row is complete in tree-kangaroos, whereas in other species of macropods this takes place before the eruption of M5 (Tate 1948, Groves 1982). This premolar replacement is believed to occur by the time the animal reaches 3 years of age in *D. lumholtzi* (pers. obs). After this age there are no new teeth, just continuing wear of the biting and grinding surfaces of the existing teeth (Flannery *et al* 1986).

8.1 METHODS

8.1.1 Specimen collection and preparation

37 specimens of *Dendrolagus lumholtzi* were collected, from road kills or death whilst in captivity, over a period of two years (2002-2004) on the Atherton Tablelands in far north Queensland. This included one known-age individual that was born in captivity and died at 17 years of age.

Body weight and body measurements were recorded for each animal and the head removed. Each head was then macerated in an individual plastic container filled with water. The skulls were cleaned with water after a period of one month to remove any remaining tissue and dried in the sun. No chemicals were used in this process. Skulls were then kept dry in individual archival boxes. Many incisor teeth became loose and fell out of the tooth socket during the maceration process. All loose teeth were collected and placed in the original tooth socket in skull, although some of the very small canines were lost.

8.1.2 Tooth wear

All skulls were photographed and right upper premolar tooth wear was described. A series of skulls representing the full spectrum of wear observed were arranged in increasing premolar tooth wear. They were then divided into eight distinguishable classes on the basis of this wear, similar to those described for koalas (Martin 1981). Drawings and descriptions were made of each class for use classifying tooth wear in the field (Figure 8.5). Tooth wear was described on upper RHS P3 for consistency and due to its accessibility in live animals.

Tooth wear was described before teeth were sent away for sectioning for cementum annuli analysis.

8.1.3 Tooth cementum annuli

The right lower 1st molar (M1) tooth was removed from each dry skull and sent away for sectioning and staining. M1 was chosen, as it is the first molar to emerge and was used successfully by Clout (1982) to age possums. Canines, which are used in many mammals (Fancy 1980, Cool *et al* 1994, Azorit *et al* 2002a), are not suitable for use with tree-kangaroos due to their small size and the fact that most are lost during preparation of skull.

The most suitable region to observe incremental lines clearly was believed to be the longitudinal cut of the interradicular pad under the crown of the molar (Clout 1982, Azorit *et al* 2002a). However, it is best to examine all areas along the root for comparison.

8.1.4 Tooth preparation

Dry teeth were prepared and sectioned by the Histopathology Laboratory of the Veterinary Pathology Diagnostic Services, Faculty of Science, at the University of Sydney. Each tooth was placed into processing cassettes and soaked in 10% buffered formalin for 2 hours then transferred to a decalcifying solution consisting of 10% formic acid in 10% buffered formalin (100ml concentrated formic acid and 900ml of 10% buffered formalin). Teeth were kept overnight in this solution. Teeth were then transferred into RDO (rapid decalcifying solution, Lomb Scientific) and left until sufficiently decalcified, but only for up to 4 hours.

The teeth were cut into two longitudinal pieces between the roots giving one perfectly flat piece from tip to root and a second piece of the area just between the roots (Figure 8.2).

Teeth were returned to the initial decalcifying solution overnight to complete the decalcification of the cut surface. The teeth were washed in running water to remove surface calcium and were then processed in an automatic tissue processor (Shandon Excelsior) over a long period of 30 hours (normal processing time is 12 hours). Teeth were then embedded in Paraplast Tissue embedding wax. Blocks were trimmed of excess wax and a series of at least ten sections were cut from each tooth on a conventional microtome at 10μm and placed onto Selleys Aquadhere coated slides. Slides were dried overnight at 56°C.

Slides were de-waxed through changes of xylol, absolute ethanol, 95% and 70% ethanol and then washed in water. They were then stained in Whitlock's haematoxylin for 13 minutes and then washed in running tap water. The slides were placed in Scott's blueing solution for 2 minutes and washed in running tap water for a following 2 minutes. They were then dehydrated through 2 changes each of 70%, 95% and 100% ethanol. Slides were placed in a 50% mixture of absolute ethanol and xylol. A coverslip was then placed on each slide using DPX synthetic resin.

8.1.5 Counting cementum annuli

The series of sections for each tooth were examined under a binocular microscope in transmitted light at 10x or 40x magnification.

In all tooth sections broad, lightly stained bands containing cementocytes, alternating with narrow, darkly stained bands could be seen in the cementum (Figure 8.5) using transmitted light. These correspond to the "Opaque" and "Translucent " layers described by Klevezal and Kleinenberg (1967) and Pekelharing (1970). Pekelharing (1970) counted the broad opaque layers when aging possums, but I followed the standard procedure described by Klevelzal and Kleinenberg (1967) and counted the narrow dark stained bands, commonly called incremental lines. In some sections "accessory bands" (Klevelzal and

Kleinenberg 1967) were also visible. Comparison of sections from the same tooth was helpful in distinguishing between true bands and accessory bands, which were generally faint and discontinuous.

All animals were included in the analysis except for the 140g pouch young as it had no premolars or molars emerged and therefore, the teeth would not have any cementum at this age. The two 500gm animals were also studied for annuli to establish if any lines are set down before the age of 1 year.

8.1.6 Validation and analysis

Only one animal in the collection was of known age. She was born in captivity at Queensland Parks and Wildlife Service Pallarenda Research Station and died at 17 years of age. The sections from this animal form a single point of validation of the technique in *D. lumholtzi*.

There were also two 500g *D. lumholtzi* pouch young in the collection. At this body size they are estimated to be approximately 5-6 months of age (Johnson and Delean 2003). These animals, therefore, provide a confirmation of cementum annuli deposition before one year.

Tooth wear classes were compared to the number of cementum annual rings by bivariate analysis (Spearman's Rank coefficient) using SPSS.

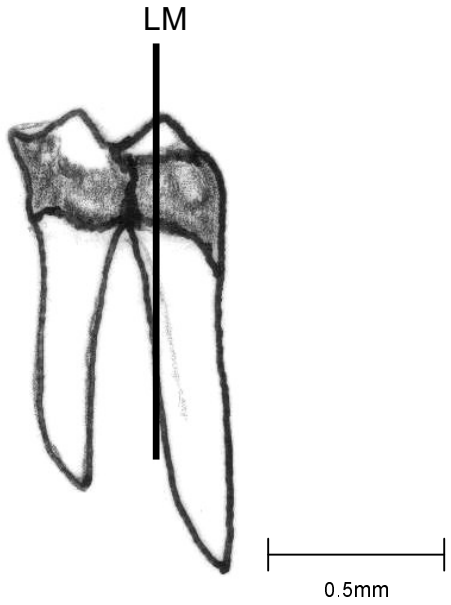


Figure 8.2. Cutting plane of the Molar (M1).
LM is the longitudinal cut of the inter-radicular pad made prior to embedding tooth in paraffin wax for sectioning. A series of sections were taken from either side of cut.

8.1.7 Morphological measurements

Morphological measurements such as body weight (kg), body length (mm), tail length (mm), head length (mm) and width (mm), pes (hind foot) length (mm), were recorded for all animals.

Head and hind foot length (pes length) (mm) were used to estimate age for the smaller, younger animals using Johnson and Delean's (2003) polynomial growth equation. There were nine animals that could be used for this analysis, ranging from 0.142g to 7.35kgs (Table 8.2).

This growth equation was used to calculate tooth eruption ages, as a comparison to tooth wear and tooth cementum annuli in these younger animals, but was not used for adults as the precision diminishes with age (Johnson and Delean 2003). The only adult that was included was a young adult with deciduous premolar teeth but 7.35kg body weight so that the deciduous premolar replacement age could be estimated and compared to the number of cementum annuli.

8.2 RESULTS

8.2.1 Tooth wear

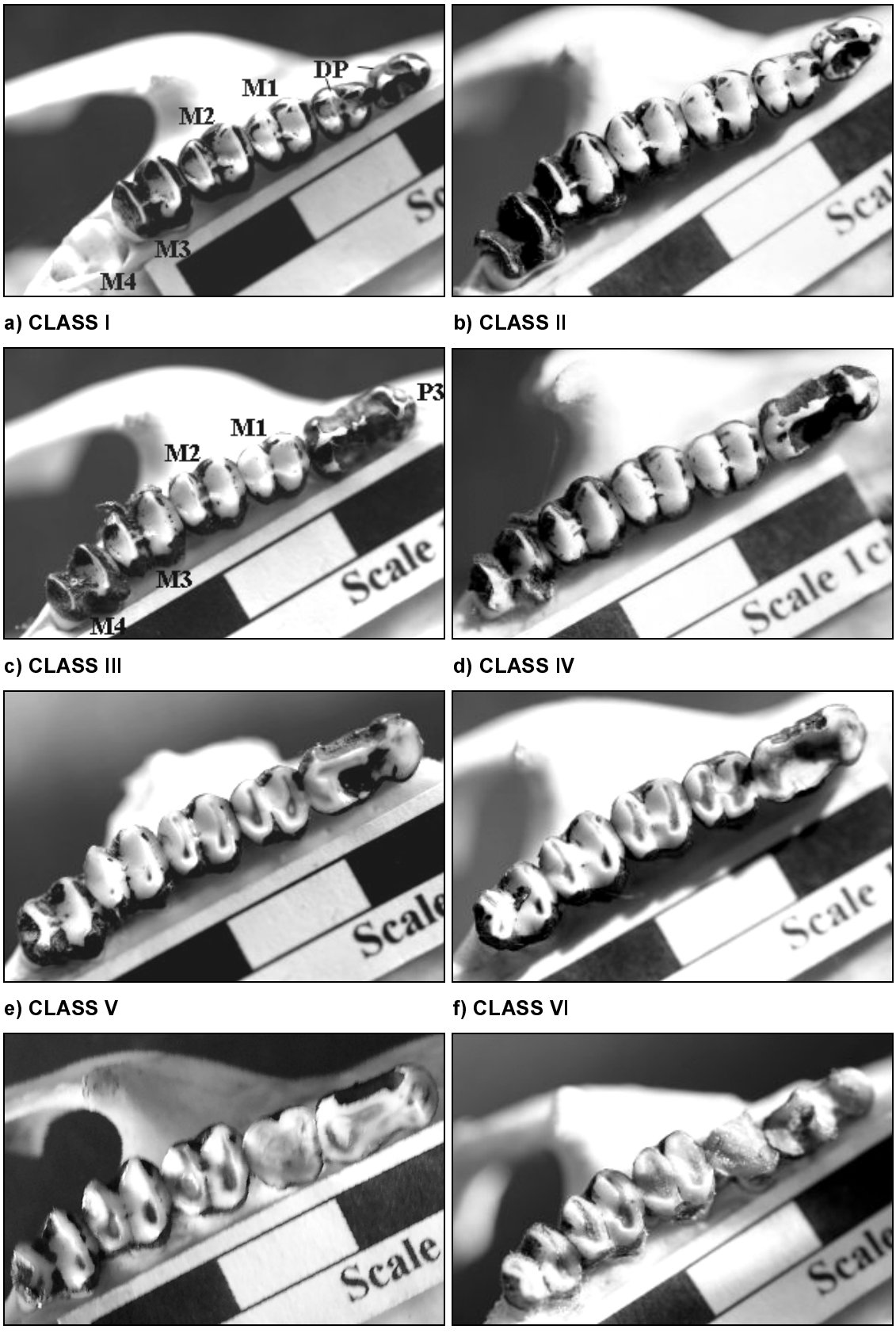
Wear of the premolars begins at an early age in *D. lumholtzi*, with some wear even on the deciduous premolars before complete eruption of all of the molars (Figure 8.3a). All molars examined had a slight amount of wear on the tips of the cusps. This continued in the P3 after the eruption of this tooth (Class III, Figure 8.3c).

There was more wear on the posterior end of the P3 resulting in rounding and polishing of the posterior prominence and a slight rounding and polishing of the anterior prominence (Class IV, Figure 8.3d). The wear of the enamel increases until the entire longitudinal crest was worn resulting in an island of worn enamel and a flattening of the anterior and posterior prominences results (Class V, Figure 8.3e). This continues with complete wear of the enamel and the formation of a cavity or basin by Class VI (Figure 8.3f). At this stage there may

be a sharp ridge on the check (buccal) side (Figure 8.3f). After this stage the tooth was worn flat with little cusps remaining (Class VII, Figure 8.3g). At Class VIII (Figure 8.3h) there was wasting between the anterior and posterior roots. At this age all of the molars had cavities in them.

The last 2 classes were more difficult to distinguish; hence the wear of the 1st molar (M1) was also taken into account (Figures 8.3g, 8.3h, 8.4g, 8.4h). Class VI the 1st molar (M1) has some exposed dentine but there were still cusps visible. By Class VII M1 was flat with no cusps remaining and by Class VIII M1 was worn to half its original height (Figure 8.3h).

There was more wear on the lingual (tongue) side of the P3 than the buccal (cheek) side, sharpening the longitudinal crest, eventually grinding down to a basin on the lingual side (Figure 8.3h-i). The molars begin to wear down to the dentine by age Class V starting at M1 and then to all other molars with increasing age (Figure 8.3e-i).



a) CLASS I

b) CLASS II

c) CLASS III

d) CLASS IV

e) CLASS V

f) CLASS VI

g) CLASS VII

h) CLASS VIII

Figure 8.3. Photographs of upper tooth row of *D. lumholtzi* showing tooth wear classes. Anterior of tooth row to right. Top of page is check side, bottom tongue (lingual) side of mouth. Teeth labelled on a) and c) DP, deciduous premolars; P3, premolar; M1 – M4, molars.

Tooth wear was uneven with differential wear on either side of jaw. There appeared to be more wear on LHS than RHS. However, RHS was used as for tooth wear for consistency with other studies.

Classes I and II may be difficult to tell apart in the field on live animals, as it is awkward to see the back molars. However, there was some wear on the deciduous premolars and at this age body measurements can also be used to confirm age (Johnson and Delean 2003). Head or pes length would be more precise measurements for age as body mass would vary with individuals with better conditions. For example, one animal in this study was 7.35kg but still had deciduous premolars. Hence head length would be more precise measurement to use than body mass as this animal was obviously in prime condition.

The differential wear of the upper premolar allowed the establishment of tooth wear classes. Eight tooth wear classes resulted, which were similar to the seven used on koalas (Martin 1981). The 37 specimens were then placed in these classes based on their tooth wear (Tables 8.1 and 8.2).

Table 8.1. Key to 8 tooth wear classes in *D. lumholtzi* (Figures 8.3 and 8.4).

Class		Description
I.	***Young animals less than 4 molars***	Only slight wear of deciduous P1 and P2. Slight wear on molar crests (Figures 8.3a and 8.4a).
II.	***Young animals with 2 deciduous premolars and all 4 molars***	Showing wear on deciduous premolars and all molars (Figures 8.3b and 8.4b).
III.	***Young animals with P3 emerged and all molars***	With only slight wear restricted to facets on the crests of **P3 with polishing of tips** without breaching the enamel (Figures 8.3c and 8.4c).
IV.	***"I shaped wear" on P3.***	*Moderate wear on P3 cusps.* **Not quite forming an island** (Figures 8.3d and 8.4d). Rounding of either posterior or both ends of P3, not breaching the enamel. Slight wear on tips of cusps of all molars.
V.	***"C shaped wear" on P3.***	Enamel breached continuously with ***dentine exposed on P3 leaving an island*** of enamel in the centre of the tooth surface (Figures 8.3e and 8.4e). M1 entire longitudinal ridge with exposed dentine. Dentine exposed on most teeth to some extent.
VI.	***"Basin" on P3.***	*Dentine exposed on entire P3* with all enamel worn away from occlusal surface **forming a basin** or cavity (Figures 8.3f and 8.4f). Sharp ridge may be remaining on buccal side of P3. Dentine exposed to some degree on all molars.
VII.	***"Flat tooth" P3 and M1***	P3 worn down to almost flat surface (Figures 8.3g and 8.4g). Flat occlusal surface on M1, beginning to form a dish.
VIII.	***Extreme wear P3 and M1***	P3 half worn away with wasting between anterior and posterior roots and M1 worn down to half (Figure 8.3h and 8.4h). Front upper incisors also very worn.

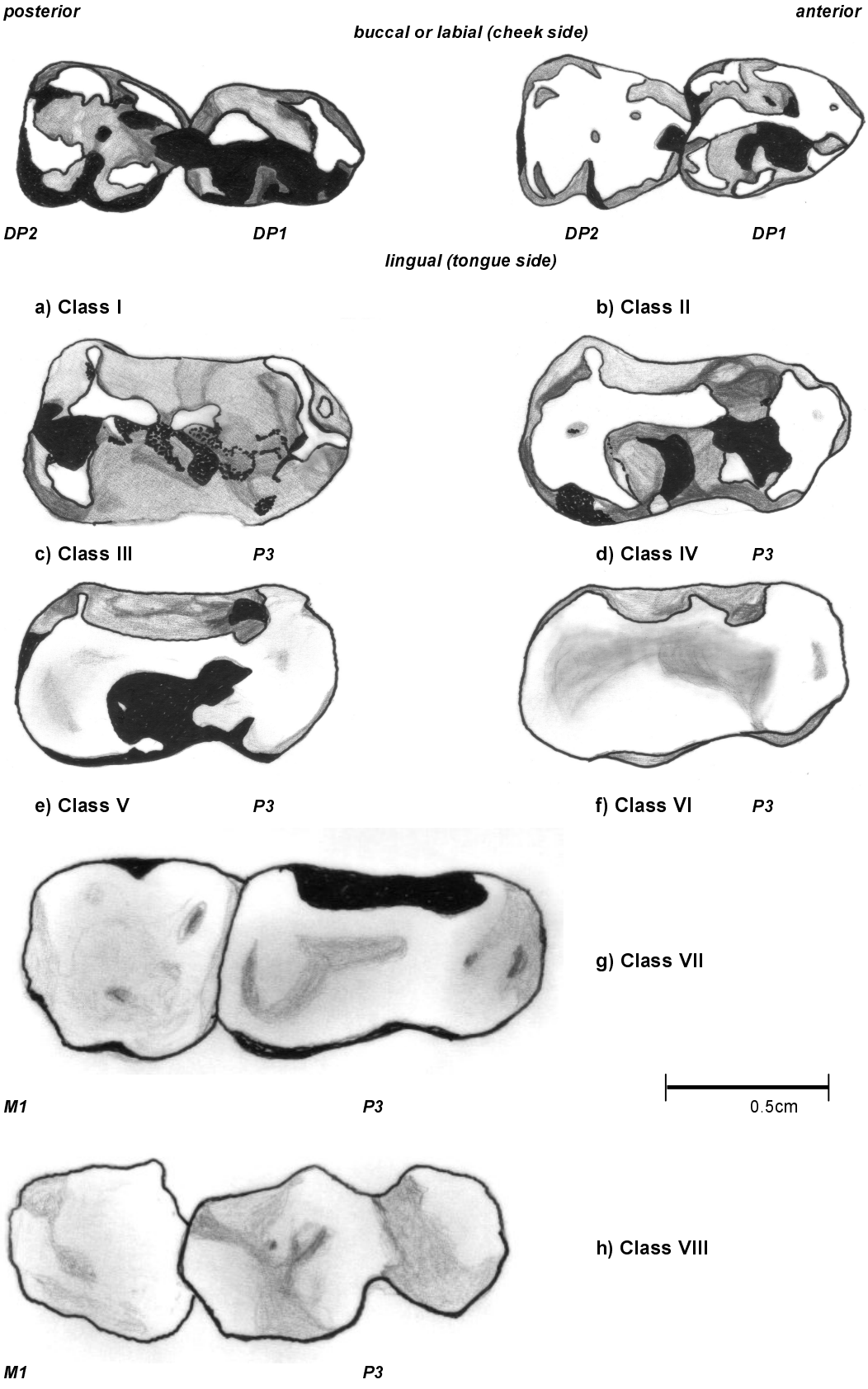


Figure 8.4. Illustrations of each of the eight tooth wear classes. White areas show enamel wear; black areas are plaque, grey areas are not worn except on Class VI where it shows basin forming "Cavity". a-b) deciduous premolars, c-f) P3 premolars, g-h) P3 and M1.

8.2.2 Tooth cementum annuli

On all of the slides, the cementum lines were clear and relatively easy to count. Each layer is laid onto the outside of the earlier layer. However, in animals with many lines (presumably older animals) it was difficult to count lines at the root cushion and the lines were closer together. Consequently, it was found that it is best to check along root as well for comparison.

In the pouch young (< 600g) that were believed to be less than one year of age, there was a line already set down and this identified as the dentine-cementum junction. Therefore for all other animals this line was not counted.

The 17-year-old animal had 17 cementum lines (Figure 8.6h). Therefore, it seems likely that cementum annuli correspond with age in years of the known animal and correspond with the tooth wear index (Figure 8.5). That is Class 1 had 1 line and Class II had 2 and so on up to Class VI with 7-8 lines, Class VII with 9-10 and Class VIII with 14-17 lines (Table 8.1, Figure 8.5). This is a strong relationship (n = 37, r_s = 0.97, P = <0.01) therefore, these lines probably do represent years.

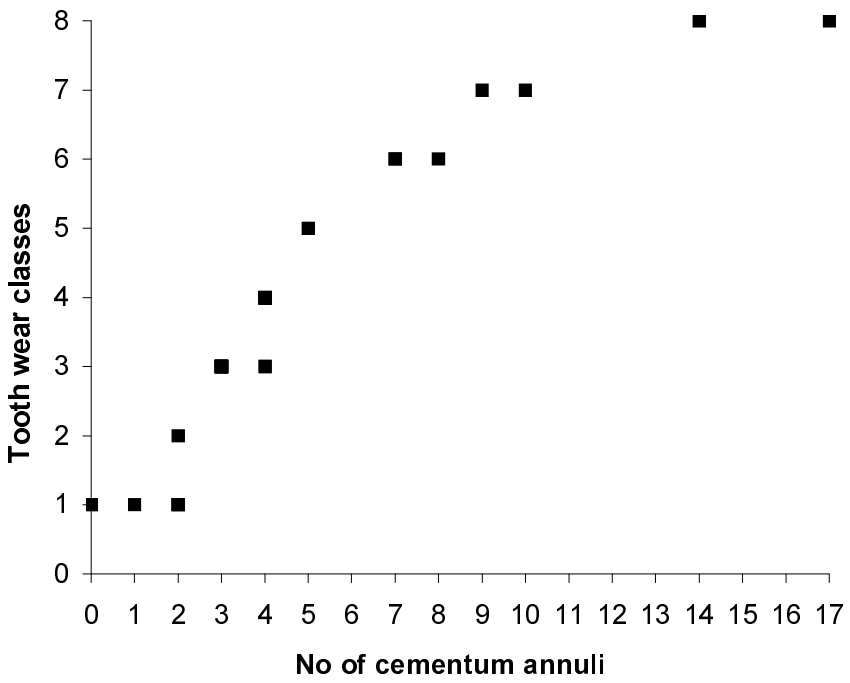


Figure 8.5. Tooth wear classes in relation to the number of cementum annuli. (n = 37, r_s = 0.97, P = <0.01)

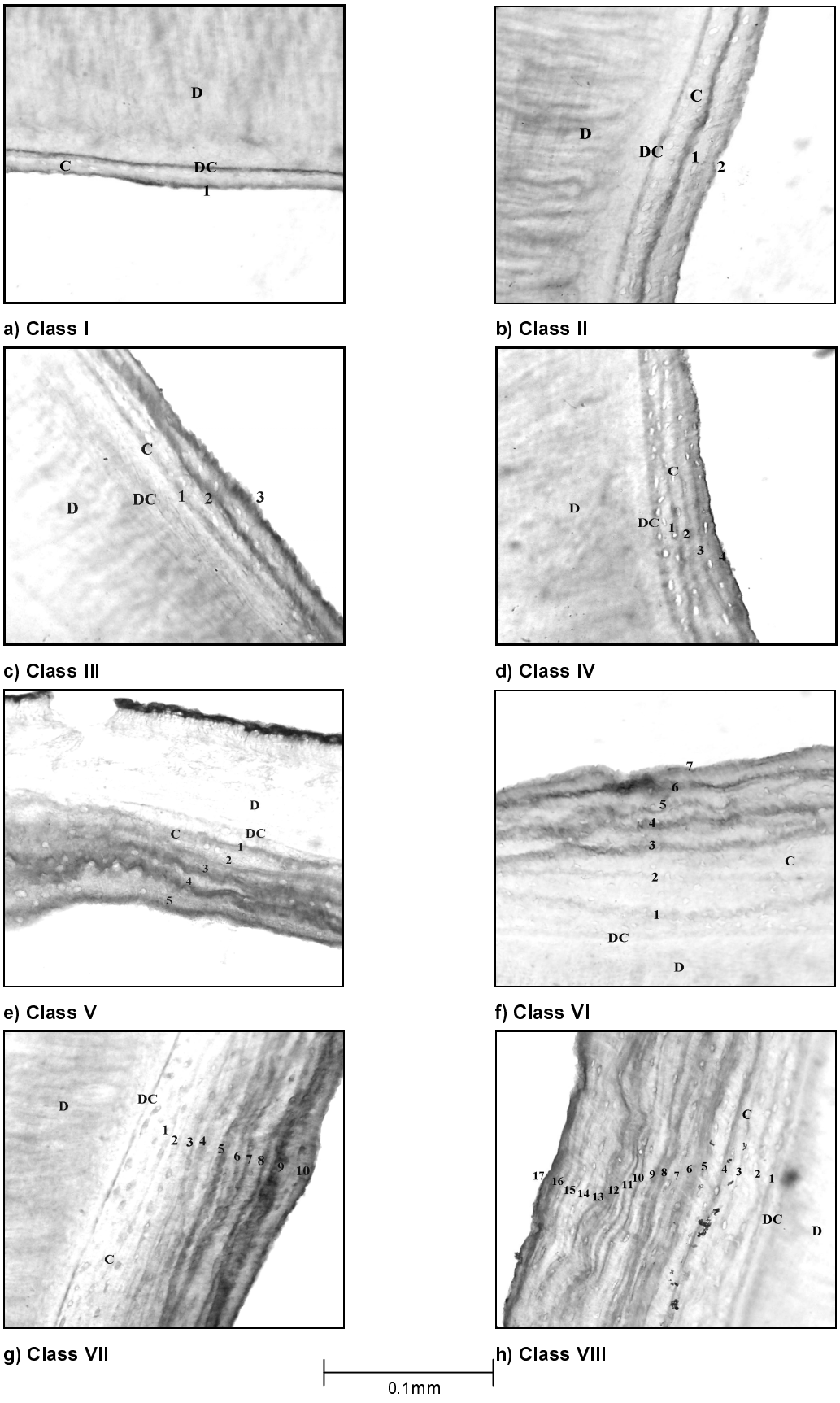


Figure 8.6. Photographs of cementum annuli for each class. Incremental lines are numbered on each photograph. Class VIII was of known age of 17 years old. D, dentine; DC, dentine-cementum junction; C, cementum.

8.2.3 Body measurements

As illustrated in table 8.2, there is a lot of variation in age using head or pes lengths for animals in the wild, especially as the animals get bigger. It can be seen that the age predictor error increases with body size.

Also the results (Table 8.2) suggest that a 3kg animal is younger than a 2kg animal. If we examine the tooth eruption in these two animals the 3 kg animal had M4 erupted and the 2 kg animals had their M3 erupted but not M4. Therefore, this would suggest that the 3 kg animal is older.

Body weight can not be used to age animals precisely as there is too much variation, especially once the animals are over 5 kgs in body mass, although there is a significant relationship between body weight and tooth wear classes ($P = <0.01$, $r_s = 0.64$, Figure 8.7).

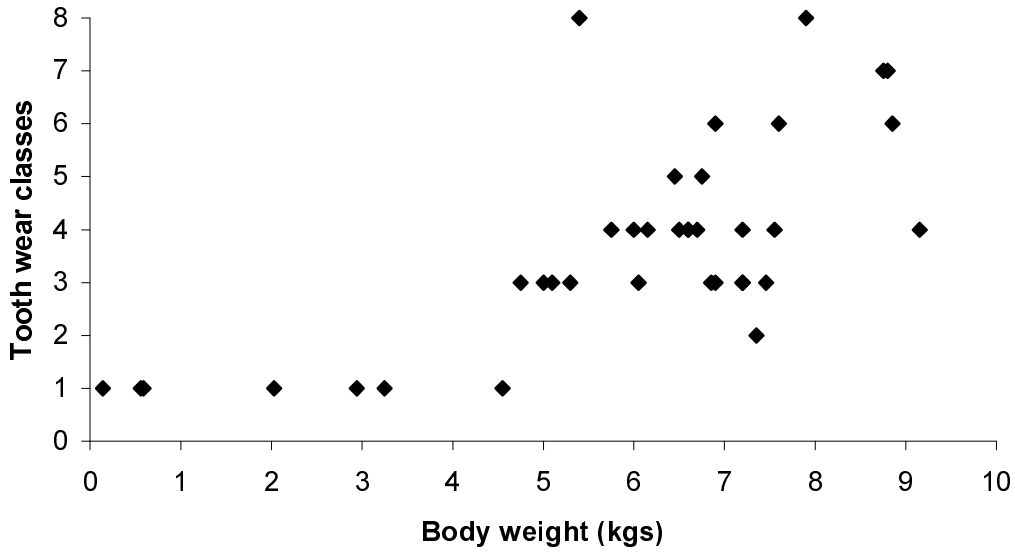


Figure 8.7. Tooth wear classes as a function of body weight (kgs) for *D. lumholtzi*. ($n = 37$, $r_s = 0.64$, $P = <0.01$).

Table 8.2. Age estimation using head and pes lengths using Johnson and Delean's (2003) "polynomial growth equation" for animals in this study. PE predictor error in days.

Animal	Sex	Tooth wear class	Weight (kgs)	Head length (mm)	Age (days) - Head	*PE (days) - Head*	Pes length (mm)	Age (days) - Pes	*PE (days) - Pes*
TK31	F	1	0.142	50.3	126.0	*6.1*	42	134.5	*8.1*
TK20	M	1	0.557	68.5	187.2	*10.7*	74	203.2	*12.1*
TK27	M	1	0.59	67	181.9	*10.0*	76	207.8	*12.9*
TK16	F	1	2.025	98.5	318.6	*42.0*	93.5	256.2	*24.2*
TK19	F	1	2.935	99	321.4	*42.9*	111	324.7	*46.4*
TK24	M	1	3.25	95	299.9	*36.2*	100	278.8	*31.0*
TK5	F	1	4.55	105	357.1	*54.8*	115	344.2	*53.3*
TK65	M	3	6.85	120	468.6	*94.9*	136.2	478.2	*104.5*
TK8	M	2	7.35	115	427.3	*79.7*	139	500.4	*113.3*

8.2.4 Tooth eruption

The deciduous premolars have erupted by the time *D. lumholtzi* is 500gm and first 2 incisors and M1, which is approximately 5-6 months of age (Johnson and Delean 2003).

Unfortunately, there were no animals in this study with M2 erupting (between 500g and 2kgs).

M3 is erupting by the time *D. lumholtzi* reaches 2kgs and fully erupted by 2.9kgs (or before the age of 12 months). M4 starts to erupt by 4.5kg, which corresponds with Class I at approximately 12-24mths of age.

P3 erupts at an estimated age of 2-3 years in *D. lumholtzi*, after M4.

8.3 DISCUSSION

8.3.1 Tooth wear

All indices of age are subject to error (Caughley 1977). Some are worse than others. Indices that change by annual quanta give the most accurate

estimates, particularly for adult animals, but they are not foolproof (Caughley 1977).

It has been suggested that tooth wear as an age indicator has two main sources of error: one being the variation of wear between individuals or populations; the other is misinterpretation of tooth wear classes by the observer (Winter 1980). The first would be dependant upon the size of the study area and the variation in the diet of the individuals (Winter 1980, Gordon 1991). The second may be minimised by using a single trained observer (Winter 1980).

In this study the first error was reduced by the fact that all of the animals used for the determination of tooth wear were from all over the region of the Atherton Tablelands as they were mostly road kills, providing us with a variety of individuals to examine from different populations. The second error was reduced by one researcher (the author) performing the readings.

Although tooth wear can vary between animals and populations depending on what the animals are eating and the animal's condition, it still remains a reasonable, quick and easy way of categorising animals in age classes. The classes proposed here are the most differentiated to the eye for ease of identification in the field by any researcher or veterinarian.

8.3.2 Tooth cementum annuli

Some earlier attempts of aging marsupials using cementum annuli were unsuccessful (Kingsmill 1962, Catt 1979, Inns 1982). However, the recent improvements in the preparation of teeth and staining methods have made this technique viable and more reliable than in the past. In this study, the identification of cementum layers in the molars of *D. lumholtzi* was successful.

The longitudinal sections of the interradicular pad between the roots of the molar were found to be most suitable for distinguishing cementum incremental lines clearly in *D. lumholtzi*. This was probably due to the significant width of growth layers and the sharpness of thin lines and the easiness to distinguish accessory lines (Azorit *et al* 2002a). However, this proved more difficult with older animals as the lines were closer together and some were more faint. This

is why it is important to have a series of sections cut from the same tooth and sections including the root. This allows the observer to check other areas and compare the number of lines and assists in determining accessary lines. In this study, it was found that for the older animals it was actually easier to count lines at the root zone (Figure 8.5).

There was a good correspondence between the incremental lines and age in years as shown by the 17-year-old animal and the young animals aged from body measurements. Although there was only one old animal of known age, cementum lines have been shown to correspond with age in years in many other mammals (Laws 1952, Klevezal and Kleinenberg 1967, Pekelharing 1970, Gasaway *et al* 1978, Fancy 1980, Clout 1982, McCullough and Beier 1986, Cool *et al* 1994, Azorit *et al* 2002a, 2002b).

The technique of using cementum annuli to age animals is invasive, therefore it is not suitable for use with live animals. Although it is more accurate than tooth wear it was only used here to establish an association with the tooth wear classes. As there is a close relationship between the numbers of these lines and the tooth wear classes proposed, it is concluded that the tooth wear classes are suitable for use for estimation of absolute age of *D. lumholtzi* (within a few years error) in both the wild and in captivity.

8.3.3 Body measurements and aging

This study has supported the view (Inns 1982, Johnson and Deleans 2003) that once an animal reaches maturity, or a certain size, body measurements cannot be used successfully to age animals, including Lumholtz's tree-kangaroos.

Body weight will also vary with factors such as the animal's condition, and consequently cannot be used to estimate age accurately either, as illustrated by the animal that was 7.35kg but still had deciduous premolars.

In addition, pouch young growth estimations developed on captive animals should be used cautiously when studying animals in the field, as there could be differences in growth rates (Inns 1982). This could only be confirmed with growth rates measured in the field, which could prove difficult with *D. lumholtzi*

as they are difficult to catch and would have to be captured on a regular basis for repeated measurements of pouch young.

8.3.4 Tooth eruption stages

It appears that tooth eruption in *D. lumholtzi* is much earlier than for other kangaroos. For example, in *Macropus eugenii* the incisors and M1 have only just erupted as the young are permanently leaving the pouch at 245-270 days (Inns 1982). In this study the M1 and the incisors of *D. lumholtzi* have emerged at the age when they are beginning to venture from the pouch, at approximately 5-6 months of age. Furthermore, M3 and M4 are emerging when *D. lumholtzi* are between 10-18 months of age, similar to koalas (Martin 1981, Martin *et al* 1999). However, P3 erupts at a mean age of 1049 days for *M. eugenii* (Inns 1982), which corresponds with the estimations for *D. lumholtzi* here with P3 erupting before the age of 3 years.

Variation also occurs in tooth eruption within populations of a species and between captive animals and those in the wild (Inns 1982). This along, with the fact that *D. lumholtzi* have complete tooth eruption by the age of 3 years makes tooth eruption an unsuitable technique for aging this species.

8.4 CONCLUSIONS

Being able to place tree-kangaroos in relative age classes will enable us to identify age specific fecundity and mortality for modelling populations, especially issues such as the relative age of road kill and dog kill animals. It was previously believed that most of the Lumholtz's tree-kangaroos that are killed on roads are young males. In fact, the road-kill specimens collected during this study were from a variety of age classes, including females with pouch young and joeys at foot.

In addition, assessment of the age structure of a population is also important for management purposes, particularly for understanding the dynamics of a population and undertaking population viability analysis.

This tooth wear index will also assist veterinarians or rescuers of injured animals to identify whether the animal is old and if its condition may be age related. In veterinary medicine it is useful to have some idea of the animal's age in both the diagnosis of an illness and the prognosis of any treatment.

This study has shown that we can not use body measurements or tooth eruption to age *D. lumholtzi* once an animal reaches approximately 10 months of age and that using tooth wear classes is the easiest and most reliable technique to age tree-kangaroos. Furthermore, as tooth wear classes correspond strongly with cementum annuli and cementum annuli correspond with years, we can suggest age in years for each tooth wear class.

Chapter 9: Conclusions and further research

9.0 CONCLUSIONS

Our previous knowledge of *D. lumholtzi* ecology and use of habitat was limited, except for a few earlier studies (Procter-Gray 1985, Newell 1999), which were both undertaken on the same spatially restricted rainforest type. We know that *D. lumholtzi* are primarily found in privately owned rainforest fragments on the Atherton and Evelyn Tablelands, and they are territorial with relatively small non-overlapping home ranges and have strong site fidelity. *D. lumholtzi* are threatened by habitat fragmentation, dog and road kills when moving between fragments. These rainforest fragments are not totally protected from clearing and their preservation and restoration are crucial to the long-term conservation of *D. lumholtzi*.

This study was designed to investigate *D. lumholtzi* use of habitat in another more abundant rainforest type, building on and testing results from previous studies. It also aimed to enhance our understanding of the ecology and biology of *D. lumholtzi* to enable us to reassess the conservation status and population viability of the species in the future. To do this we need to know more information about their habitat use in differing habitats, their diet and gastrointestinal morphology in relation to diet and habitat, along with the ability to age them to enable us to construct life tables and determine age specific mortality and fecundity. This study has progressed a great deal of the way to enabling us to do this. However, a population analysis was not performed during this study, as we still require more data on dispersal rates between fragments and environmental stochasticity, which were beyond the scope of this study, before an accurate and meaningful population viability analysis can be performed.

Previous studies suggest that *D. lumholtzi* are generalist folivores but that they do show individual preferences to tree species. There have also been several explanations of the observed distributions of *D. lumholtzi* that relate to habitat selection, e.g. that they prefer regrowth forest to mature forest.

There was no overall difference in spatial organisation of *D. lumholtzi* in the two different rainforest types (Section 4.2.2), apart from variances in female home range sizes within one rainforest type. This variation in home range sizes cannot be explained by differences in body weight. Understanding the basis of variation in range size is important in understanding the population dynamics of tree-kangaroos as the reproductive output of a population is determined by female density and abundance. The focus of activity by *D. lumholtzi* can not determined by single or multiple structural and/or floristic characteristics of the habitat tested in this study, even though individual animals each had preferences for specific species within their range. Therefore, the habitat characters used in this study cannot be used to model *D. lumholtzi* variation in spatial or habitat use.

Although *D. lumholtzi* select a variety of species they are not broad generalists as such, only using 22% of the tree species available to them, with clear preferences for only a few species. There is also considerable individual variation in tree species preference. The reasons for these specific choices are currently unclear but it is likely that *D. lumholtzi* are choosing trees for foliage characters such as nutritional content or lack of plant defences, not for the species at a taxonomic level. These behaviours would be consistent with tree choice in other arboreal folivores, such as koalas, sloths and leaf eating monkeys (Glander 1977, 1978, Hladik 1978, Milton 1978, Moore and Foley 2000, Umapathy and Kumar 2000). Because intra-specific variation in chemical composition, and consequent palatability can be as great as inter-specific variation in these characteristics (Lawler *et al* 1998), the high species diversity and low abundance of individuals within canopy species found in the rainforest habitat of *D. lumholtzi* could lead to apparently idiosyncratic preferences for tree species even if choices were being made on a common and mechanistic basis.

The gastrointestinal morphology of *D. lumholtzi* shares a number of features with other foregut fermenting folivores. Compared to other macropodids, *D. lumholtzi* has a large sacciform forestomach and a large overall stomach capacity, and more similar in size and morphology to that of other arboreal

foregut fermenting folivores, such as colobine monkeys. It is likely that these characteristics are adaptive for its diet of rainforest leaves.

Lumholtz's tree-kangaroos can be simply aged using a tooth wear index developed during this study. Aging is essential for establishing demographics, such as age specific mortality and fecundity of populations, currently unknown in *D. lumholtzi*. Age data improves population viability analysis and other analyses, and contributes to a better understanding of population dynamics and subsequent conservation and management outcomes.

This study has highlighted that not only one rainforest type is important to *D. lumholtzi* and that more emphasis should be made on the preservation and restoration of all rainforest types. Furthermore, it is vital that all rainforest fragments including riparian zones, regrowth and corridors and stepping stones, should be conserved, rehabilitated and areas replanted as *D. lumholtzi* habitat, as they are important to the species' long term survival.

9.1 FUTURE DIRECTIONS

This study has sought to understand the factors controlling the spatial and habitat use of *D. lumholtzi* in a rainforest fragment of varying habitat types. Although it has rejected a number of previous suggestions of characteristics determining their habitat use, our understanding of their habitat and/or tree species or individual choices is still unclear. Furthermore, our knowledge of the population dynamics and the effects of fragmentation on *D. lumholtzi* remains incomplete. The following paragraphs briefly outline some of areas of research that would assist in our understanding of these questions.

9.1.1 Dietary ecology

Although we do have numerous observations of tree use by *D. lumholtzi* from different areas on the Atherton Tablelands (Tree Kangaroo and Mammal Group unpublished data), few are confirmed feeding observations. A better understanding of their dietary ecology could be established by leaf cuticle analysis of scats. Collecting multiple faecal samples from several areas within a fragment or from different fragments, over varying times of the year and using

cuticle analysis would enable us to determine seasonal differences in floral species selection.

9.1.2 Chemical basis of diet selection

The results of this study have suggested that foliar characters such as plant defences or levels of nutrients may drive individual tree choice by *D. lumholtzi,* as they do in other arboreal folivores such as koalas (Cork and Foley 1991, 1997, Lawler et al 1998, Moore and Foley 2000) and folivorous monkeys (Glander 1978, Umapathy and Kumar 2000).

An understanding of the role of foliar chemistry in the ecology of *D. lumholtzi* requires further and directed experimental work to determine which components actually determine intake and preference. Some captive studies may be possible, as *D. lumholtzi* are held in captivity in far north Queensland. However, this research would ideally be directed by radio telemetry studies of the intra- and inter-specific food preferences, and the ways these affect habitat preferences and range sizes of *D. lumholtzi*.

9.1.3 Effects of habitat fragmentation and large scale patterns of habitat use

Our current understanding on the effects of habitat fragmentation on *D. lumholtzi* is also limited. Their habitat is highly fragmented and this is generally considered to be an important threatening process to *D. lumholtzi.* Substantial numbers of Lumholtz's tree-kangaroos are killed on the roads and by dogs every year (250 road kills and 31 dog kills recorded over the last decade: Tree Kangaroo and Mammal group 2000) whilst moving between fragments. It was previously believed that most of the tree-kangaroos killed on the roads were young males (Tree Kangaroo and Mammal Group 2000). However, this study has found that there are many adults also being killed, including females with pouch young. We can now accurately age *D. lumholtzi* road and dog kills enabling us to continue to monitor these mortalities and model the impacts of these losses upon *D. lumholtzi* populations.

9.1.4 Population viability and conservation status of Dendrolagus lumholtzi

The ability to age tree-kangaroos, using the tooth wear index established during this study, will allow us to construct life tables and perform analyses on the populations such as population viability analysis. Furthermore, this technique can now be utilised by other researchers studying the more endangered species of tree-kangaroos in Papua New Guinea.

It is important to point out that any information acquired on the ecology of the Australian species will assist in the studies of the New Guinea species, which are much more difficult to study due to the remoteness and difficult terrain in which they live.

However, as already mentioned, there are still some areas of the demographics of *D. lumholtzi* that we need to research in order to preform an reliable population viability analysis of this species or to accurately reassess their conservation status. These include the dispersal rates between fragments, their population sizes in differing habitats including continuous forest and environmental stochasticity.