

**Aspects of digestion and physiology in Wahlberg's
epauletted fruit bat (*Epomophorus wahlbergi*)**

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ABSTRACT

Degradation of forest environments in South Africa have greatly affected both the plants and animals that interact with these plants. It is therefore important to understand plant-animal interactions in forest ecosystems in an effort to develop strategies for forest conservation. Animal dispersal can assist in the restoration of forest plant diversity and consequently animal diversity. Wahlberg's epauletted fruit bat (*Epomophorus wahlbergi*) occurs in the eastern part of South Africa and is known to feed on fruit, nectar, pollen and flowers. It is an important disperser of various indigenous and alien invasive forest fruit species. Little is known about the benefits these bats get from feeding on fruit. Furthermore, the buccal cavity and lingual adaptations of fruit bats in Southern Africa are unknown. This study investigated morphological and physiological adaptations for frugivory in *E. wahlbergi*.

In particular, the palatal and lingual morphological structures of *E. wahlbergi* were examined by light microscopy and scanning electron microscopy. The results of this study showed that the morphological structures of tongue papillae in *E. wahlbergi* were similar to that of other fruit and nectar feeding bats. The structural arrangement of tongue papillae in *E. wahlbergi* probably allows food to move from the anterior part of the tongue and collect at the median line of the posterior part of the tongue, and thereafter move down to the pharynx. These bats had an elongated tongue, wide, flattened molar teeth, and a hard papillae structure observed on the upper palate at the posterior end of the buccal cavity. This papillae structure has not been described previously; and it appears that it is with this structure, together with the palatal ridges, and teeth that *E. wahlbergi* crushes fleshy fruit to extract the juices when feeding. Consequently palatal and lingual structures of *E. wahlbergi* showed morphological adaptations for efficiently feeding on fleshy fruit and nectar.

South African indigenous and exotic fleshy fruits available to *E. wahlbergi* are found to be generally hexose dominant. These bats are suggested to have high glucose intake irrespective of sugar concentration to power their high energy demands due to flight. High glucose intake could result in increased blood plasma glucose levels which are detrimental to mammals of small body size. This study investigated the diel variations in blood plasma glucose concentrations of *E. wahlbergi*. *Epomophorus wahlbergi*'s blood plasma glucose concentration was lower (5.24 ± 0.38 mmol/l) at 18h00 before feeding and increased during/after feeding (8.19 ± 1.24 mmol/l), however bats appeared to regulate it within limits. Their range in concentrations was higher than the normal mammalian blood plasma glucose concentrations range. Consequently these bats appear to regulate their blood plasma glucose concentration although at a range higher than normal mammalian levels and thus reduce the negative consequences associated with hyperglycemia. The data obtained provide a baseline for comparison with free-ranging *E. wahlbergi*.

Proteins are important in a diet to provide the required nitrogen and amino acids necessary for maintaining body tissues. Fruits, however, appear to have energy-rich but protein-limited foods. Frugivores that feed exclusively on fruit may therefore have difficulties in maintaining their protein requirements since fruits are generally high in sugar content but low in protein content. The importance of protein in the diet of *E. wahlbergi* was determined by measuring diet intake at varying levels of protein in the laboratory. *Epomophorus wahlbergi* were offered equicaloric 15 % glucose solutions with varying protein concentrations (2.58, 5.68, 7.23 g soy protein/kg H₂O) and a solution with no protein. This was repeated using 15 % sucrose instead of glucose solutions. *Epomophorus wahlbergi*'s volumetric intake of the respective glucose and sucrose solutions varied among individual bats, with total volumetric intake highest

for the solution with no protein (control) and lowest at 2.58 g/kg soy protein concentration solution for glucose and 5.68 and 7.23 g/kg soy protein concentration for sucrose. These bats appeared to prefer sugar solutions without or low protein, and their daily protein intake was relatively low. This suggests they have low-protein requirements, and this relates to their characteristic low-protein fruit available in the wild.

Feeding and digestive efficiency has been widely studied in frugivorous and/or nectarivorous birds but relatively few studies have been done on bats, particularly African bats. Feeding on a liquid nectar diet and fruit juices could cause physiological challenges for nectarivores and/or frugivores as they have to balance water and energy intake from this liquid diet. Mammalian kidneys have to eliminate salts and nitrogenous wastes, conserve water during water restriction as well as excrete it when ingested in excess. Morphological renal characters are known to be reliable indicators of urinary concentrating abilities in mammals. Short digesta residence time is a digestive trait that is known to be associated with frugivory and this may be a problem since digestive efficiency is a function of the length of digesta retention time. Histological sections of *E. wahlbergi* kidneys and small intestines were examined under microscopy to determine water regulation and specialization for sugar absorption respectively. Cortex and medulla length measurements were taken to calculate the medulla: cortex ratio (M/C) and the percent medullary thickness (PMT). The observed M/C ratio and PMT of *E. wahlbergi* is typical of a mesic species. *Epomophorus wahlbergi* feeds on a watery diet and does not need to concentrate urine; therefore the medulla and cortex were more or less the same size. Analysis of the histological sections showed that the kidney structure of these bats allows efficient water regulation in a mesic environment. The microvilli of *E. wahlbergi* intestine were relatively long

with a large surface area thus serves the purpose for efficient digestion and absorption of sugars in these bats.

Future studies need to be done to determine the mechanisms by which these bats regulate their blood plasma glucose levels, and also determine intake on a selection of fruit in order to broaden research to adaptation of these bats on low-protein diets. This study contributes to a greater understanding of the physiological and morphological mechanisms that may affect fruit intake and consequently dispersal. It will contribute to a greater understanding of plant-animal interactions in southern Africa.

PREFACE

The data described in this thesis were collected in Pietermaritzburg, Republic of South Africa from February 2011 to August 2012. Experimental work was carried out while registered at the School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg, under the supervision of Professor Colleen T. Downs.

This thesis, submitted for the degree of Master of Science in the College of Agriculture, Science and Engineering, University of KwaZulu-Natal, Pietermaritzburg campus, represents original work by the author and has not otherwise been submitted in any form for any degree or diploma to any University. Where use has been made of the work of others, it is duly acknowledged in the text.



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August 2012

I certify that the above statement is correct and as the candidate's supervisor I have approved this thesis for submission.



.....
Professor Colleen T. Downs

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DECLARATION 1 - PLAGIARISM

I, Babalwa R. Mqokeli, declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. This thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
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DECLARATION 2 - PUBLICATIONS

DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this thesis.

Publication 1

BR Mqokeli & CT Downs

Palatal and lingual adaptations for frugivory and nectarivory in the Wahlberg's epauletted fruit bat (*Epomophorus wahlbergi*). *Zoomorphology* In press.

Author contributions:

BRM conceived paper with CTD. BRM collected and analysed data, and wrote the paper. CTD contributed valuable comments to the manuscript.

Publication 2

BR Mqokeli & CT Downs

Blood plasma glucose regulation in Wahlberg's epauletted fruit bat.

Author contributions:

BRM conceived paper with CTD. BRM collected and analysed data, and wrote the paper. CTD contributed valuable comments to the manuscript.

Publication 3

BR Mqokeli & CT Downs

Is protein composition in the diet of Wahlberg's epauletted fruit bat important?

Author contributions:

BRM conceived paper with CTD. BRM collected and analysed data, and wrote the paper. CTD contributed valuable comments to the manuscript.

Publication 4

BR Mqokeli & CT Downs

Effects of a nectar and fruit diet on the kidney and small intestine morphology of

Wahlberg's epauletted fruit bat (*Epomophorus wahlbergi*)

Author contributions:

BRM conceived paper with CTD. BRM collected and analysed data, and wrote the paper. CTD contributed valuable comments to the manuscript.



Signed:

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Chapter 1

INTRODUCTION

Anthropogenic effects have severely fragmented the indigenous forest habitat of South Africa and thus affecting its biodiversity (Lawes et al., 2000; Chapman et al., 2006). Forest fragmentation affects plants and animals that interact with these plants (Lawes et al., 2000; Chapman et al., 2006). It is therefore important to understand and evaluate plant-animal interactions in forest ecosystems in order to develop management strategies for forest conservation (Saunders et al., 1991; Kirika et al., 2008). Animal seed dispersal can, within a period of time, assist in the restoration of forest plant diversity on degraded lands which then helps minimize restoration costs (Wunderle, 1997). Plants bearing fruits attract frugivores to a site and the seeds deposited underneath the fruiting plants indicate a diversity of plant species (Wunderle, 1997).

Frugivorous bats play an important role in plant and fruit establishment, in that they affect seed and fruit set thereby increasing the reproductive success of their food plants (Fleming and Sosa, 1994; Whittaker and Jones, 1994; Herrera, 1999). These bats are effective pollinators and seed dispersers of many plant species (Fleming and Sosa, 1994; Whittaker and Jones, 1994; Herrera, 1999; Medellin and Gaona, 1999; Hodgkinson et al., 2003; Corlett, 2011). Old-World fruit bats have been found to disperse small seeds hundreds of kilometers and may thus be of key importance in maintaining forest ecosystem structure (Shilton et al., 1999; Herrera, 1999). In island ecosystems, in particular, they play an important role in forest regeneration, whereby fruit bats act as seed dispersers and are probably the most important pollinators (Cox et al., 1991). Hodgkinson et al., (2003) suggested that 13.7% of trees are partially dependent on fruit bats for seed dispersal and/or pollination within a hectare of old-growth forest. This, therefore, means

that a decline in fruit bat abundance and diversity could affect the reproductive biology of a large percentage of forest plants (Hodgkinson et al., 2003). New and Old-World fruit bats are also important in the dispersal of early and late successional fig *Ficus* (Hamman and Curio, 1999; Muscarella and Fleming, 2007). Some studies showed that these bats are prospective dispersers of more seeds than local bird species (Medellin and Gaona, 1999; Jordaan et al., 2012).

Wahlberg's epauletted fruit bat (*Epomophorus wahlbergi*) (Fig. 1) is an important disperser of various indigenous forest fruit species and more recently of invasive exotic species (Monadjem et al., 2010; Jordaan et al., 2012). Little is known about the benefits these bats get from feeding on fruit, particularly in terms of water and glucose regulation, as well as protein intake. Furthermore, little is described about the morphological dietary adaptations they have for fruit digestion.

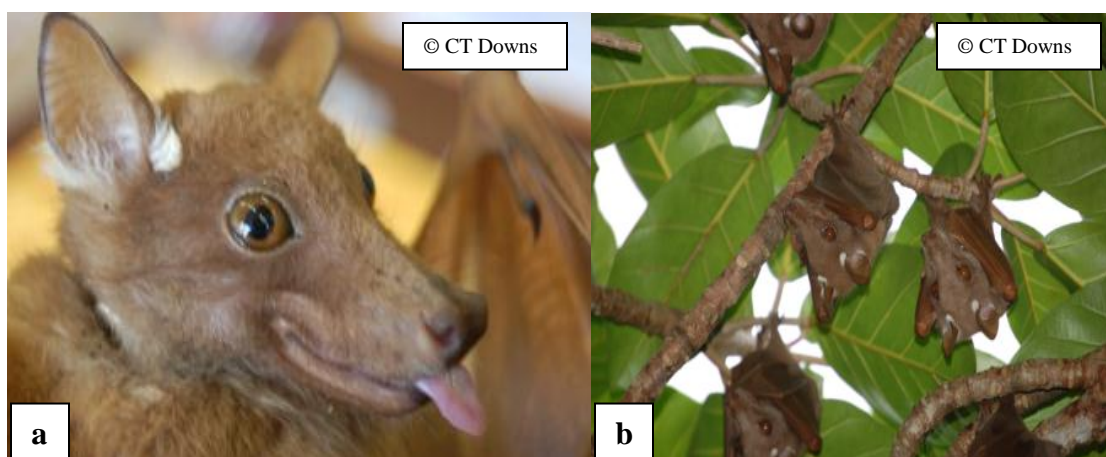


Fig. 1 *Epomophorus wahlbergi* where a. is a lateral head view showing the white epaulettes and tongue; and b. is a group roosting in a *Ficus* tree.

There has long been an interest on jaw and teeth structure of mammals, specifically fruit bats and how these structures link to their diet (Freeman, 1988). Lucas (1979) believes that studying the design of jaws and teeth is in fact studying different apparatuses responsible for

breaking up different foods. It is by taking into account and understanding the important mechanical properties of the different foods, may the mechanical breakdown (dentition and mastication) of foods be understood (Lucas, 1979). Bats that have strong skulls and teeth are said to feed on the not-so-ripe fruits, while those with the least strong skulls and teeth could feed on soft, ripe or overripe fruit (Freeman, 1988). Fruit is different from prey in that it does not struggle to be free and thus fruit bats may not require canine teeth responsible for grabbing struggling prey (Freeman, 1988). These teeth, however, may be required to pluck and transport the relatively large fruit as compared to the size of bat's head (Freeman, 1988).

Schondube et al., (2001) suggested that evolutionary shifts in diets of species are accompanied by changes in physiology which enable use of the new diet while limiting use of the ancestral diet. Fruit bats have a widened tongue used to squeeze fruit against the hard palate thus releasing fruit juices (Morrison, 1980). Morphological studies on the tongue structures of Old-World fruit bats suggested that these bats are highly adapted to fruit and nectar diets and are able to change between a fruit and nectar diet when their preferred food is not available (Birt et al., 1997). Tongue and papillae structures of these bats aid their important role in pollination and dispersal (Birt et al., 1997). Bat species from this study possessed filiform papillae at the tip which is thought to increase the surface area for nectar collection (Birt et al., 1997). The filiform papillae increase the gripping of food on the tongue surface, thus efficiently moving food towards the pharynx (Birt et al., 1997). The Egyptian fruit bat's (*Rousettus aegyptiacus*) arrangement of the mechanical filiform papillae and gustatory papillae is regarded as being useful for the efficient uptake of semi liquid food and also an adaptation to a fruit diet (Jackowiak et al., 2009). The buccal cavity adaptations of fruit bats in southern Africa are unknown.

Frugivores and nectarivores feed on sugars that are made up of varying concentrations of sucrose and hexose (glucose and fructose) sugars, with the latter being generally high in fruits eaten by New World bats (Baker et al., 1998). These sugars are highly suitable for providing the energy required for an animals' costly movement, such as flight, in that these sugars are rapidly digested or absorbed (Suarez et al., 1990). Fruit bats ingest large amounts of sugar which they are able to absorb in a short period of time thus facing a glucose challenge (Brunet-Rossinni and Austad, 2004). Kelm et al., (2011) stated that fruit bats would require a constant supply of these sugars to meet their energetically costly flight movement, thus resulting in increased blood glucose concentrations with increasing glucose intake. Increased blood glucose concentrations are detrimental to mammals of small body size (Kjeld and Olafsson, 2008) and the inability to regulate glucose results in increased blood glucose concentration and ultimately diabetic complications (Kawahito et al., 2009). The Egyptian fruit bat has an endocrine structure that is different from other mammals; it has a high percentage of pancreatic endocrine tissue (Michelmore et al., 1998). This suggested the secretion of regulatory hormones, thus maintaining blood glucose concentrations in a narrow range (Protzek et al., 2010). Glucose intake of *E. wahlbergi* is generally high irrespective of sugar concentration (Downs et al., 2012) and the regulation of blood glucose concentrations is important due to negative effects that high glucose concentrations have on health (Suh et al., 2007).

Fruits appear to have energy-rich but protein-limited foods for their frugivores (Witmer, 1998a; Jordaan and Downs, 2012; Wilson and Downs, 2012) and nectarivorous bats mostly consume a diet low in both fat and proteins but rich in simple carbohydrates (Roces et al., 1993). Certain fruits may provide fruit bats with the necessary nitrogen requirements to grow and reproduce (Herbst, 1986). Thomas (1984) argued that fruit intake of Old-World fruit bats is

determined by the protein content rather than energy content. Korine et al., (1996) however, suggested that energy rather than nitrogen is the limiting nutritional factor and that the Egyptian fruit bat can maintain its nitrogen requirement on a natural fruit diet. Tsahar et al., (2005) also suggested that nectarivorous birds have a low nitrogen requirement in their diet due to a physiological adaptation of these birds to a low-protein diet. When bats ingest only the fluid, leaving out the fiber, this allows transit times as low as 20 min; therefore enabling bats to process 1.4 to 2.5 times their body weight of low-protein foods daily (Thomas, 1984). They are able to take up more to compensate for the low protein thus avoiding a deficiency in the required amount of protein if protein is limiting (Thomas, 1984). On protein-supplemented diets, however, bats reduce intake; and adjusting fruit intake as a response to protein amounts appears to result in high energy intakes for bats (Thomas, 1984). New and Old-World fruit bats share convergent physiological adaptations to compensate for the lack of nitrogen in their diet (Voigt et al., 2011). Feeding on a wide variety of fruits, containing varying amounts of protein, may provide wild bats with adequate protein on an entirely fruit diet (Courts, 1998). Herrera et al., (2001a; b; 2002) found that frugivorous bats derive most or all of their dietary protein from fruits; their diet did not differ with season or with sexual activity (lactating or pregnant females) (Herrera et al., 2001a; b). Fruit pulp from a variety of fruits eaten by a neotropical frugivorous bat contains all the essential amino acids, and these bats select certain fruit species thus may not need to supplement their diets with insects (Herbst, 1986). Potential sources of protein for fruit bats are fruits, leaves and pollen (Thomas, 1984; Kunz and Diaz, 1995; Herrera et al., 2002). When bats probe on flowers for nectar; pollen a major source of proteins may attach to their papillae and be swallowed thus obtaining proteins as well while feeding (Birt et al., 1997). *Epomophorus wahlbergi* feeds on fruits of a variety of cultivated and indigenous tree species

(Monadjem et al., 2010) and the fruits available to these bats and other frugivores are found to be low in protein content (Witmer, 1998a; Wilson and Downs, 2012).

Feeding and digestive efficiency has been widely studied in frugivorous and/or nectarivorous birds (Jackson et al., 1998; Witmer, 1998a; b; McWhorter and Martinez del Rio, 2000; Brown and Downs, 2003; Brown et al., 2010) but relatively few studies have been done on bats, particularly African bats (Herrera, 1999; Voigt and Speakman, 2007; Herrera and Mancina, 2008; Downs et al., 2012). Specialist nectarivorous birds such as hummingbirds and sunbirds as well as nectarivorous or frugivorous bats feed on the nectar of flowers (Beuchat et al., 1990; Brown et al., 2010; Dumont, 2003). Balancing water and energy from this diet could cause physiological challenges for this bird as it has to extract energy and nutrients from dilute nectar and also excrete large amounts of water from this food source (Beuchat et al., 1990; Brown et al., 2010). Fruit pulp sugars are mainly made up of sucrose and hexose (glucose and fructose) sugars with the sugar composition and concentration varying among different plant species (Baker et al., 1998). Studies on Pallas' long-tongued bats (*Glossophaga soricina*) show that intake rate is constrained at dilute sucrose solutions (Herrera and Mancina, 2008). The nectar intake rate of these bats appears to be limited by the load of excess water in dilute solutions (Herrera and Mancina, 2008). It is possible, however, that excess water can be eliminated through evaporation, with higher amounts evaporated during flight in free-ranging bats (Herrera and Mancina, 2008), or alternatively with increased ambient temperature (Marom et al., 2006).

The Egyptian fruit bat's kidney is able to cope with the water load when fed fruits of high water content thus regulating the excess water by excreting large volumes of dilute urine (Arad and Korine, 1993). The kidneys function of water control is necessary to regulate body mass during flight (Arad and Korine, 1993). Arad and Korine (1993) suggested that the Egyptian fruit

bat has a kidney that is well adapted to regulate water budgets during water restriction; therefore the fruit bat is able to cope with seasonal changes and variations in fruit supply. Frugivorous bats possess a kidney that is made up of two parts (medulla and cortex), whereas those of other feeding habits are made up of three parts (cortex, inner medulla and outer medulla) (Studier et al., 1983). Frugivorous bats have undivided medullae in their kidneys (Studier et al., 1983). Kidneys with reduced relative medullary thickness, a large cortex and an undivided medulla are features that have been suggested to allow nectarivorous and/or frugivorous bats to filter large amounts of water from their diet while conserving electrolytes (Studier et al., 1983; Schondube et al., 2001).

The microvilli of Wahlberg's epauletted fruit bat intestine and other fruit bats are long with a large surface area (Makanya et al., 1997). This serves the purpose for nutrient absorption, increasing the availability of membrane digestive enzymes and providing a larger surface for digestion to occur (Makanya et al., 2001). Short digesta residence time is a digestive trait that is known to be associated with frugivory and this may be a problem since digestive efficiency is a function of the length of digesta retention time. Some bird species, however, are observed to show relatively constant uptake of carbohydrates along the length of the intestine thus compensating for the short retention times (Karasov and Levey, 1990, McWilliams and Karasov, 2001). A rapid passage rate of food in fruit bats, that is, the ability to consume, digest and excrete food in a short time, allows them to fly easily without the burden of a full gastrointestinal tract increasing their weight (Tedman and Hall, 1985; Caviedes-Vidal et al., 2007; Tracy et al., 2007). There is relatively little known on the sugar absorption and water regulation of fruit bats in southern Africa.

Wahlberg's epauletted fruit bat occurs in the eastern part of South Africa and is known to feed on fruit, nectar, pollen and flowers (Monadjem et al., 2010). This fruit bat feeds on fruits of a variety of cultivated and indigenous tree species, as well as invasive exotic fleshy fruits (Skinner and Chimimba, 2005; Monadjem et al., 2010). Old-World fruit bats assist to promote regeneration of late successional plant species (Muscarella and Fleming, 2007). Fruit bats such as the flying fox (*Pteropus niger*) are long distance seed-dispersers and thus play an important role in maintaining plant diversity in heavily fragmented landscapes (Nyhagen et al., 2005). Muscarella and Fleming (2007) concluded that frugivores facilitate tropical forest regeneration and assist in maintaining species diversity by introducing seeds to disturbed areas. Therefore, the community and population structure of these forests would probably be different if frugivorous bats were absent (Muscarella and Fleming, 2007).

This study aimed to investigate 1) morphological structures adaptation to dietary intake; 2) regulation of plasma glucose concentration; 3) aspects of protein intake and digestion and 4) aspects of water balance of Wahlberg's epauletted fruit bat. I predicted that for Wahlberg's epauletted fruit bat 1) buccal and lingual morphological structures facilitate efficient fruit and nectar feeding and digestion; 2) these bats are able to regulate plasma glucose concentration; 3) protein composition of the diet affects the bats intake and digestion; 4) kidney structure of the bats allows efficient water regulation; and 5) their intestinal morphology enables efficient absorption of sugars.

This thesis is presented as chapters prepared for submission to respective Journals and some repetition was unavoidable as a consequence:

Chapter 2. Palatal and lingual adaptations for frugivory and nectarivory in the Wahlberg's epauletted fruit bat (*Epomophorus wahlbergi*).

Chapter 3. Blood plasma glucose regulation in Wahlberg's epauletted fruit bat.

Chapter 4. Is protein composition in the diet of Wahlberg's epauletted fruit bat important?

Chapter 5. Effects of a nectar and fruit diet on the kidney and small intestine morphology of Wahlberg's epauletted fruit bat (*Epomophorus wahlbergi*).

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Chapter 2

Palatal and lingual adaptations for frugivory and nectarivory in the Wahlberg's epauletted fruit bat (*Epomophorus wahlbergi*)

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Abstract Wahlberg's epauletted fruit bat (*Epomophorus wahlbergi*) feed on fleshy fruit and nectar of flowers and have an important role in pollination and seed dispersal. It was expected that their buccal morphological structures are adapted to this type of feeding. Consequently, buccal cavity and lingual structures of *E. wahlbergi* were examined by extended focus light microscopy (LM) and scanning electron microscopy (SEM). Morphology of the tongue of *E. wahlbergi* was similar to that of other fruit- and nectar-feeding bats. The elongated tongue of these bats possessed filiform and conical papillae as mechanical papillae and fungiform and circumvallate papillae as gustatory papillae that varied in distribution. *Epomophorus wahlbergi* had five palatal ridges and one post-dental palatal ridge, and relatively wide, flattened molar teeth. A hard, papillae structure at the posterior end of the upper palate of the upper plate, which has not been previously described, was observed. It appears that this structure works together with the palatal ridges and teeth, so that the bats crush fleshy fruits during feeding and extract the juices before discarding the pulp. Consequently, lingual and particularly palatal structures of *E. wahlbergi* show morphological adaptations for efficiently feeding on fleshy fruit and nectar.

Keywords: Buccal cavity · Lingual · Palatal ridge · Mechanical papillae · Gustatory papillae

Introduction

Nectarivores and frugivores are effective pollinators and seed dispersers of many plant species (Fleming and Sosa 1994; Hodgkison et al. 2003; Matias et al. 2010; Corlett 2011; Fleming and Kress 2011). Frugivorous bats affect seed and fruit set, thereby increasing the reproductive success of the plant species they feed on (Fleming and Sosa 1994; Whittaker and Jones 1994; Herrera 1999). Due to their mobility, feeding habits, species richness and high biomass, fruit bats are important seed dispersers in tropical ecosystems (Dumont 2003) in that they have a significant role in the distribution of tropical plants (Schober 1984; Hodgkison et al. 2003; Muscarella and Fleming 2007; Fleming and Kress 2011). New and Old World frugivorous bats are known to pollinate approximately 528 species of flowering plants (Fleming et al. 2009). The tongue and its papillae structures support their ability to feed on nectar and fruit (Birt et al. 1997).

There has long been an interest in jaw and teeth structure of mammals and their adaptations for a specific diet, with several specifically investigating fruit bats (Freeman 1988; 1995; Dumont 2003; Dumont and O'Neal 2004). It is believed that the recognized features in the teeth of frugivores are due to the physical properties of fruit they eat. Fruit bats that have strong skulls and teeth are said to feed on the less ripe fruits, while the others feed on soft, ripe fruit (Freeman 1988; Dumont and O'Neal 2004). While fruit bats may not require canine teeth for grabbing struggling prey, they may be required to hold and transport the relatively large fruit as compared with the size of bat's head (Freeman 1988). Freeman (1995) suggested that the canines play a more important role in nectar feeding than the incisors do, and that the gaps observed between canines allow free movement of nectar to the mouth.

Nectarivorous bats use their elongate tongue to collect nectar down the tubes of flowers which vary in length (Freeman 1995). Schondube et al. (2001) suggested that evolutionary shifts in diets of species are accompanied by changes in physiology, which enable the use of a new diet while limiting the use of the ancestral diet. Morphological studies on the tongue structure of Old World fruit bats suggest that these bats are highly adapted to fruit and nectar diets and are able to change between a fruit and nectar diet when their preferred food is not available (Morrison 1980; Birt et al. 1997).

Old World fruit bat species possess filiform papillae at the tip of the tongue, which is thought to increase the surface area for nectar collection (Birt et al. 1997). Filiform papillae increase the gripping of food on the tongue surface, thus efficiently moving food towards the pharynx (Birt et al. 1997). The tongue tip filiform papillae of the little red flying fox (*Pteropus scapulatus*, Peters 1862) are longer, denser and more finely pointed, and cover a larger surface area of the front portion, which suggests it is more structurally suited for a nectar diet (Birt et al. 1997). Bats feeding on fruit also have a large tongue surface area, but generally lack tongue tip filiform papillae, and possess tridentate papillae (Birt et al. 1997). Tridentate papillae are thought to be valuable when piercing through the skin of soft fruits (Birt et al. 1997). Morrison(1980) suggested that fruit bats have a widened tongue and consequently larger tongue surface area that is used to squeeze fruit against the hard upper palate thus releasing fruit juices. The Egyptian fruit bat's (*Rousettus aegyptiacus*, Geoffroy 1810) arrangement of mechanical filiform papillae and gustatory papillae is regarded as being useful for the efficient uptake of semiliquid food and also an adaptation to a fruit diet (Jackowiak et al. 2009).

Wahlberg's epauletted fruit bat (*Epomophorus wahlbergi*, Sundevall 1846) occurs in the eastern part of southern Africa and feed on fruit, nectar, pollen and flowers (Monadjem et al. 2010). They have an important role in pollination and seed dispersal (Monadjem et al. 2010; Jordaan et al. 2012) and it was therefore expected that their morphological structures are adapted to this type of feeding. Buccal cavity and lingual structure of fruit bats in Southern Africa and their adaptations are poorly described, and consequently, the primary objective of this study was to detail their adaptations for frugivory and nectarivory in *E. wahlbergi*.

Materials and methods

Epomophorus wahlbergi were caught in Pietermaritzburg, South Africa in late September and November 2009 and kept in outside aviaries for a range of experiments with permits from Ezemvelo KZN Wildlife (Downs et al. 2012; Jordaan et al. 2012). As part of another study, eight bats were euthanized. Digital photographs were taken immediately of the buccal cavity in situ and thereafter specimens were obtained for buccal and whole tongue morphological investigations ($n = 8$). Specimens were frozen until further analyses.

For the buccal cavity of *E. wahlbergi*, measurements of the lower and upper cavity, and tongue were taken using a divider caliper (Faber-Castell 174233) and a ruler. Buccal cavity and tongue observations were made using Leica MZ 16 Stereomicroscope (Leica Microsystems, Switzerland) and digital images were obtained.

Dorsal surface of the tongues and the structure at the posterior of the upper palate of the mouth were viewed at higher resolution using a scanning electron microscope (SEM) (ZEISS

EVO LS15, Germany). Sections of *E. wahlbergi* individual's tongues were observed from the anterior apex, the mid-region, and the posterior region of the tongue, respectively. Tongue sections and upper palate structure were fixed in 3% buffered glutaraldehyde and then washed in 0.05 cacodylate buffer. These were then dried in a sequence of alcohol (30 - 100%) and then critical point dried using CO₂ (Critical Point Dryer HCP-2, HITACHI, Japan). The respective samples were mounted on aluminum stubs covered with carbon tape and sputter-coated with golden palladium (Sputter Coater E5100, POLARON, England). Sections were then observed using SEM at a voltage of 5.00 kV and images were electronically captured. Descriptive statistics were used to analyse the data (Statistica v7, Tulsa, OK).

Results

All eight *E. wahlbergi* studied had an elongated, muscular tongue of 4.4 ± 0.33 cm (mean \pm SD) long and 1.1 ± 0.4 cm (mean \pm SD) wide (Fig. 1). The anterior tip of the tongue of each was narrower compared with the broader posterior part of the tongue. The anterior tip of the tongues was 0.2 ± 0.05 cm thick with a width of 0.7 ± 0.10 cm (mean \pm SD). The mid-region of the tongues had a thickness of 0.6 ± 0.05 cm and a width of 1 ± 0.20 cm (mean \pm SD). The posterior regions of the tongues were 0.7 ± 0.00 cm thick and 1.5 ± 0.10 cm wide. Observations of *E. wahlbergi* feeding in captivity showed that they were able to elongate and extend the tongue beyond the buccal cavity (pers. obs.)

The dorsal surface of *E. wahlbergi* tongues possessed two types of mechanical papillae, filiform and conical papillae, and two types of gustatory papillae, fungiform and circumvallate papillae, which varied in distribution. Filiform papillae (Fig. 2) were highly abundant and

occurred on the entire surface of the tongue. These papillae were posteriorly directed, with the small filiform papillae situated at the anterior tip of the tongue (Fig. 3). Larger filiform papillae were present as a band posterior to these in the anterior to mid-regions of the tongue (Fig. 4). Small filiform papillae with fungiform papillae occurred on either side of the giant filiform papillae band (Fig. 5a, b).

The posterior part of the *E. wahlbergi* tongue (behind the giant filiform papillae) had elongated basket-like filiform papillae (Fig. 6). These had large projections and occupied most of the posterior region of the tongue (Fig. 6). Fungiform papillae occurred among these filiform papillae. The elongated basket-like filiform papillae were symmetrically arranged with respect to the axis of the tongue. The filiform papillae transformed into conical papillae on the left and right side of the posterior base of the tongue and pointed inwards (Fig. 6b). Short conical papillae occurred on the midportion of the posterior region of the tongue (Fig. 7). Fungiform papillae were scattered among the filiform papillae, except in areas of giant filiform and conical papillae. At the posterior end of the tongue occurred a triangular arrangement of three circumvallate papillae directed toward the pharynx surrounded by a deep groove (Fig. 8).

The upper palate of *E. wahlbergi* had five palatal ridges and one post-dental palatal ridge (that is, a ridge on the palate behind the last molar) (Fig. 9). These bats had relatively wide, flattened molar teeth compared with the other teeth. Their dentition had incisors 2/2, canines 1/1, premolars 2/3 and molars 1/2 with a total of 28 teeth. The teeth were previously described elsewhere in detail (Monadjem et al. 2010). The incisors appeared to be reduced in size. Small gaps were observed between the adjacent teeth, and those between the incisors appeared to be

narrow compared with those of the molars. These bats had relatively long, narrow palates (across the molars), and jaw length and width of 3.0 ± 0.05 and 1.6 ± 0.01 cm (mean \pm SD), respectively.

All *E. wahlbergi* ($n = 8$) examined possessed a papillae-type structure on the upper palate at the posterior end of the buccal cavity (Fig. 10a, b, 11). This structure appeared to be hard or bony-like. A small section of its posterior end appeared to be flat and thereafter raised to a hump-like structure towards its anterior part. The structures' anterior end had approximately four rows of small bristles on its surface.

Discussion

Epomophorus wahlbergi is known to feed on a variety of fleshy fruits, nectar, flowers and pollen (Monadjem et al. 2010). Morphological adaptations of bats' tongues in response to different food types and eating patterns are known (Emura et al. 2002). Frugivorous bats use their elongated protruding tongue to eat both fruit and nectar (Freeman 1998). When feeding on fruit, such as banana (*Musa acuminata*), the tongue moves forward to compress the bolus against the upper palatal ridges (Freeman 1998). Similarly, *E. wahlbergi* appear to have elongated, muscular tongues for efficient feeding on nectar and fruit.

The morphological structure of tongue papillae in *E. wahlbergi* was similar to that described for other mammals such as dogs, cats and rats (Ojima et al. 1996; 2000; Makiyama et al. 1998; Ojima 2001; Essawy 2008). The morphological structure of tongue papillae in these mammals also suggests the role of filiform papillae in food and liquid transport toward the pharynx and that of fungiform papillae in gustatory sensations (Ojima et al. 2000; Ojima 2001;

Essawy 2008). It appears, however, that taste can no longer be decided by the presence of papillae alone, and that biochemical analyses are required for taste or sweet sensing determination (Zhao et al. 2010).

The posteriorly and symmetrically directed filiform papillae, together with the inner directed conical papillae, arrangement of *E. wahlbergi* was similar to that described for the large flying fox (*Pteropus vampyrus*) and Egyptian fruit bat (Emura et al. 2002; Jackowiak et al. 2009); which suggested that this arrangement probably allows food to move from the anterior part of the tongue and collect at the median line of the posterior region of the tongue, and thereafter move down to the pharynx (Jackowiak et al. 2009). The tilted direction of papillae toward the tongue root facilitates food retention (Iwasaki 2002). The crowded filiform papillae are thought to increase the surface area of the tongue when feeding on a liquid diet (nectar and fruit juice) and the tridentate giant filiform papillae are useful when piercing through the skin of soft fruits (Birt et al. 1997). Occurrence of fungiform papillae over a broad range of the surface area of the *E. wahlbergi* tongue indicates that the tongue is important for feeding as well as tasting (Iwasaki 2002). The presence and arrangement of fungiform papillae in *E. wahlbergi* was similar to that described for other fruit bats (Emura et al. 2002; Jackowiak et al. 2009; Trzcielińska-Lorych et al. 2009). Jackowiak et al. (2009) described the occurrence of fungiform papillae among the posterior filiform papillae as a means to increase taste perception in this area before food is swallowed. In addition to capturing and manipulating foods, which are functions of the lingual papillae, the fungiform and circumvallate papillae facilitate taste of food (Kilinc et al. 2010).

Abayomi et al. (2009) stated that the conical and crown-like structure of papillae observed in fruit bats suggests their function in food retention when flying. The papillae projections may be for gripping on food and scraping on fruits when feeding. The high abundance and increase in surface area of these enlarged papillae are most likely to assist in oral absorption (Abayomi et al. 2009) and efficient transport of food, particularly a liquid diet, toward the pharynx and consequently increasing uptake.

The relatively widened, flat molar teeth of *E. wahlbergi* allow them to crush fruits (Freeman 1995). These bats use the tongue together with the palate ridge on the roof of the mouth to press and crush the hard fruit (Fenton 1983) and ultimately suck the juices out from the fruit that they eat (Morrison 1980; Fenton 1983; Skinner and Chimimba 2005; Monadjem et al. 2010). This type of feeding allows them to ingest nutritional substances and spit the remainder out without adding unnecessary weight while flying (Novick 1969). The reduction in the size of incisors for these bats is similar to that described by Carstens et al. (2002) for Glossophaginae. Incisors of these bats were observed to have moved laterally, leaving an obvious gap between the front teeth causing an increase in the amount of space between the canine teeth. Freeman (1995) considered this increase in space as an adaptation for better efficiency when feeding, allowing the elongated tongue to move freely.

The hard papillae structure on the upper palate at the posterior end of the buccal cavity of *E. wahlbergi* could have a mechanical function, that is, it may be important to assist the bats crushing hard fruits or provide a firm surface to press fruit pulp against and maximize fruit juice extraction. The bristle-like structures on the anterior lingual surface of the hard papillae structure may be useful in scraping and piercing through the skin of fruits. This structure could be

responsible for efficient grinding of fruit pulp and ultimately maximizing fruit juice extraction together with the palatal ridges. This structure may also aid in holding the crushed fruit while squeezing it against the palate, so that none passes down the throat, that is, control of the food in the mouth while feeding often upside-down.

In conclusion, the buccal cavity and tongue surface structure of *E. wahlbergi* appear to facilitate their nectar- and fruit-eating habits. The distribution and type of mechanical filiform papillae as well as gustatory papillae on the tongue surface were similar to those described for other fruit bats. We assume that the hard papillar structure on the posterior upper palate has a mechanical function for crushing fruits and for consequent maximizing the uptake of juice from these fruits. Therefore, the buccal and lingual structures of *E. wahlbergi* are adapted for efficiently feeding on a fruit and nectar diet.

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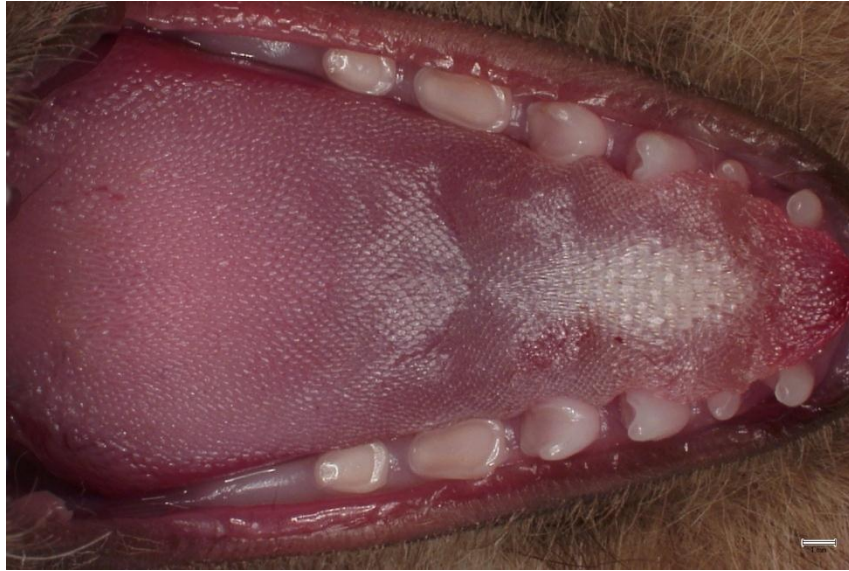


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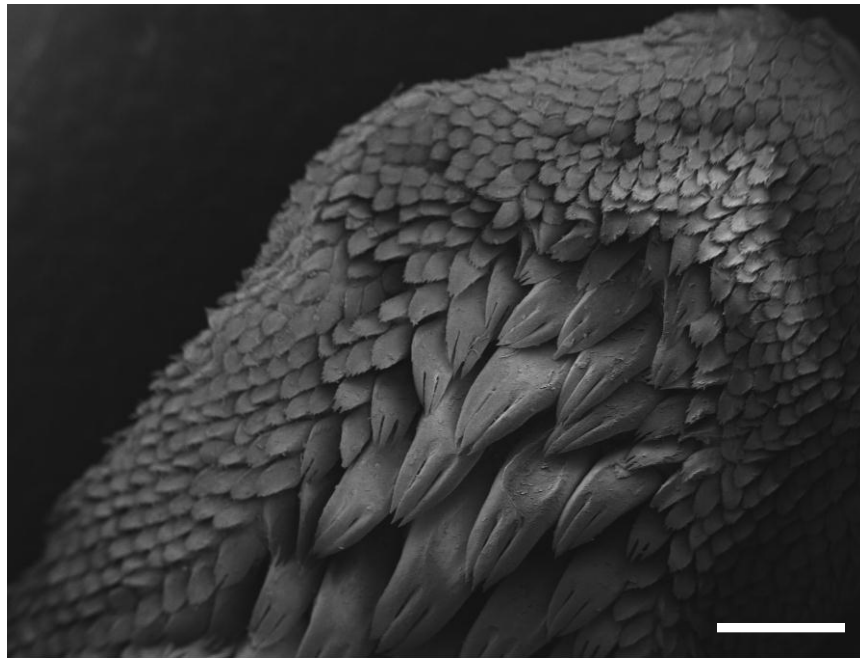


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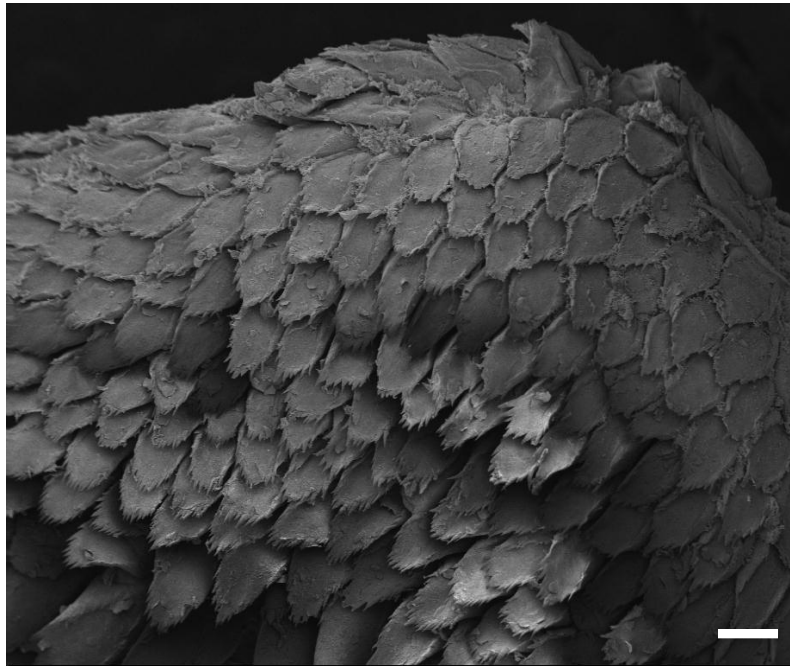


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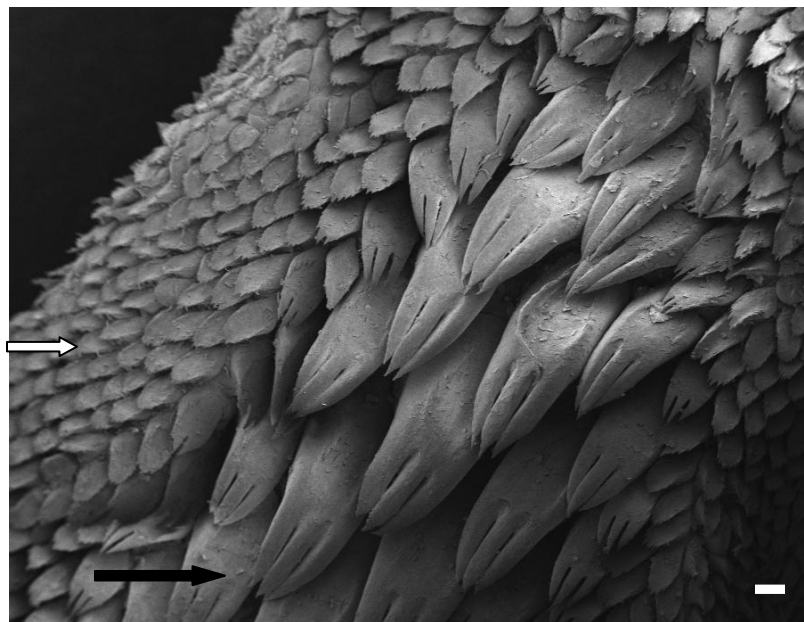


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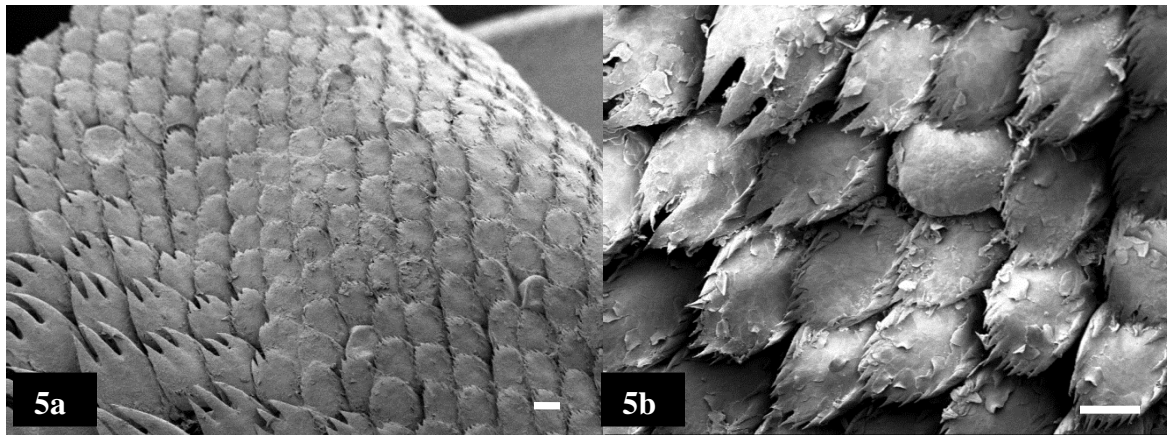


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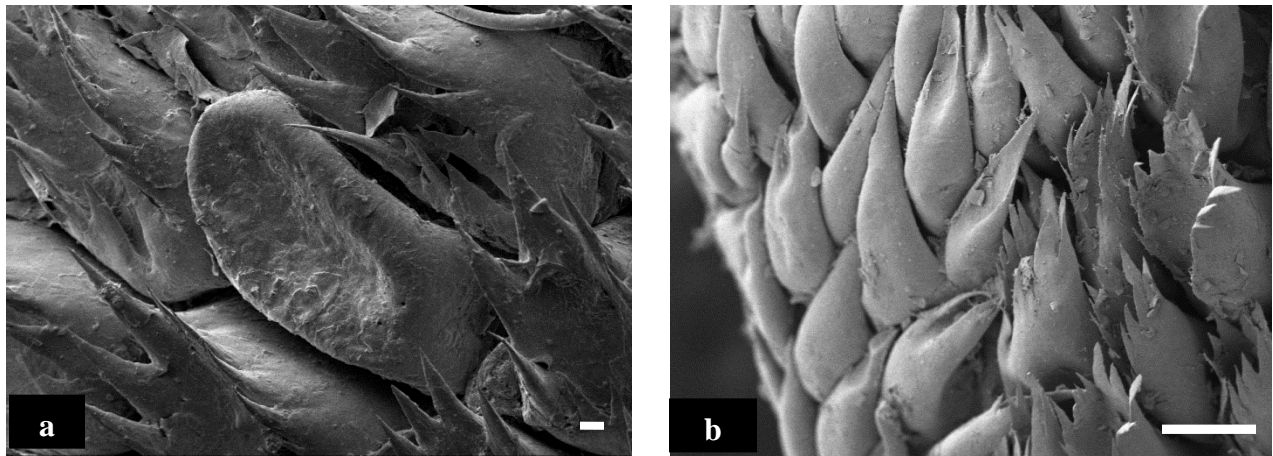


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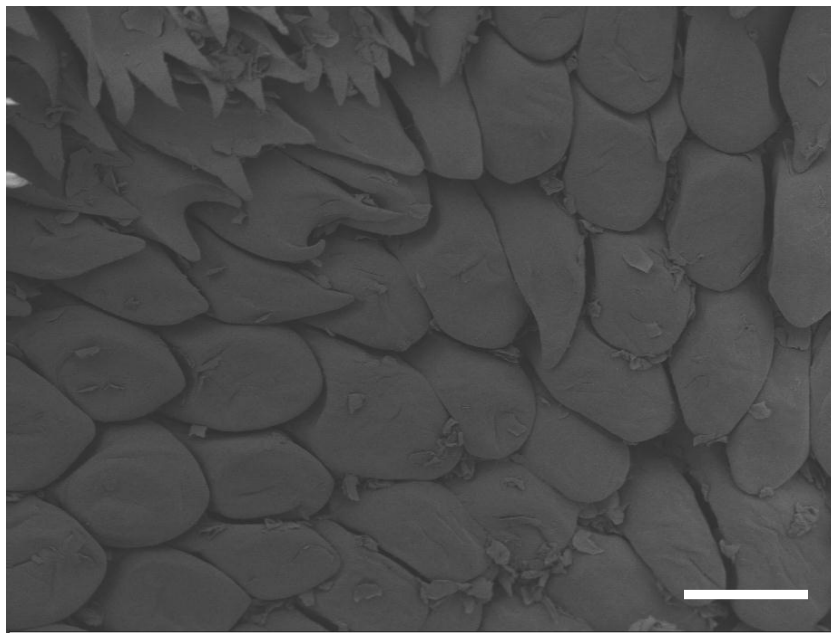


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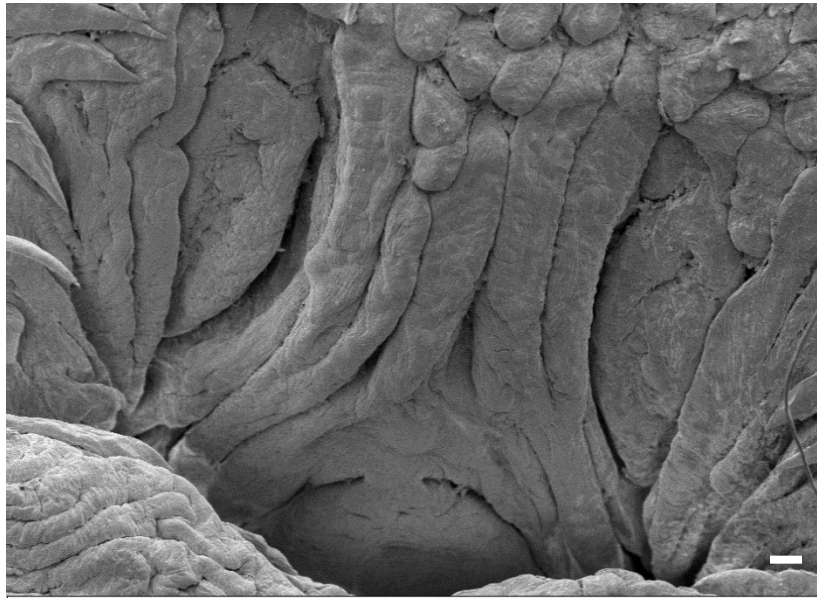


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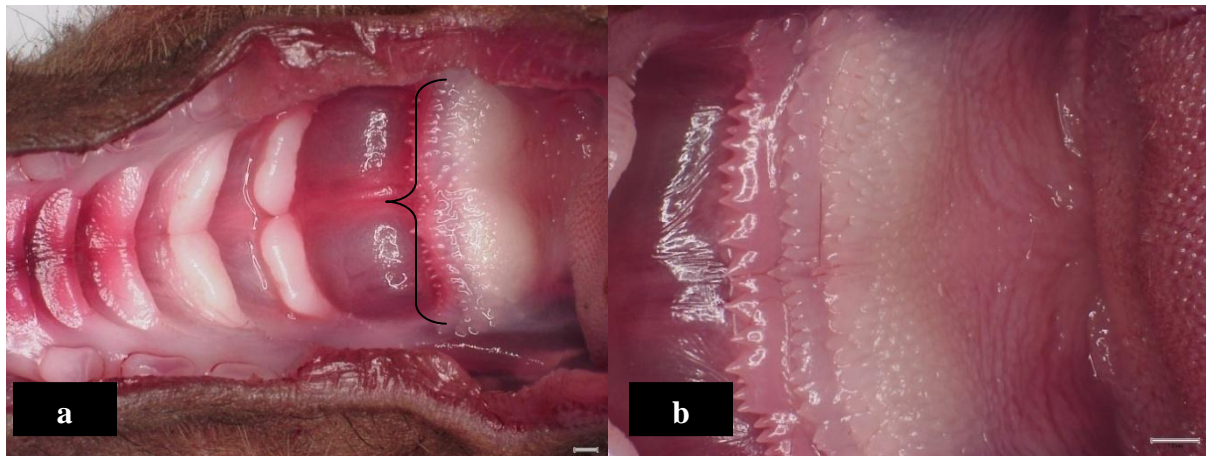


Fig. 10 Ventral view of the upper palate and buccal cavity of *E. wahlbergi* observed using LM; **a** Left parenthesis indicates the bony-like papillae structure on the posterior part of the upper palate of the buccal cavity. **b** A higher magnification of the bony-like papillae structure. (Scale bar 1mm).

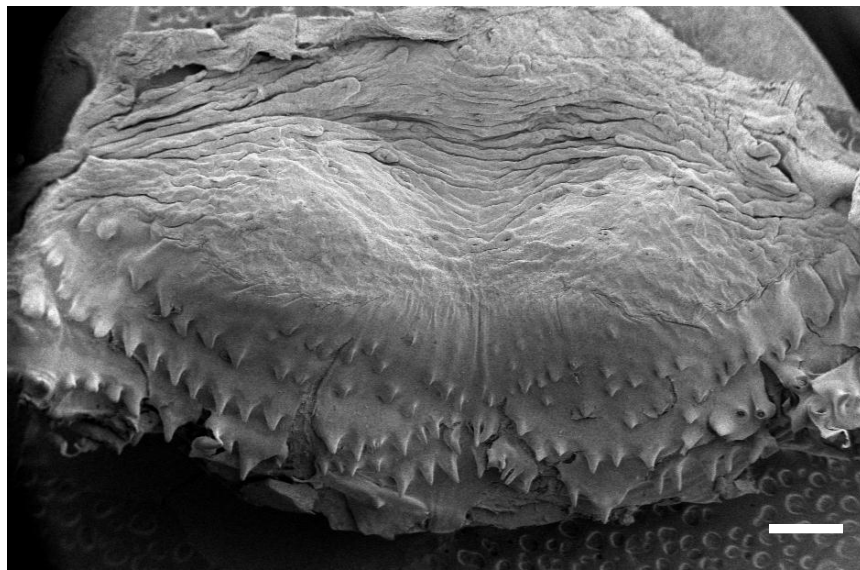


Fig. 11 Dorsal view of the surface of the bony-like papillae structure from the posterior part of the upper palate of *E. wahlbergi* observed using SEM. (Scale bar 1mm).

Chapter 3

Blood plasma glucose regulation in Wahlberg's epauletted fruit bat.

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ABSTRACT

Frugivores feed on fruits and nectars that contain different types of sugars in different proportions, which provide these animals with energy. Wahlberg's epauletted fruit bat (*Epomophorus wahlbergi*) has a high glucose intake irrespective of sugar concentration of nectar. It is not known how these bats regulate their blood plasma glucose concentrations in order to avoid the negative effects associated with hyperglycemia. Fruit bats have a high amount of sugar intake in a short period of time which could cause a glucose challenge and it is therefore necessary to determine whether these bats are able to regulate their blood plasma glucose concentrations within normal concentrations. This study investigated the diel variations in blood plasma glucose concentrations of *E. wahlbergi*. *Epomophorus wahlbergi*'s blood plasma glucose concentration was lower (5.24 ± 0.38 mmol/l) at 18h00 before feeding and increased during/after feeding (8.19 ± 1.24 mmol/l), however bats appeared to regulate it within limits. Their range in concentrations was higher than the normal mammalian blood plasma glucose concentrations range. Consequently these bats appear to regulate their blood plasma glucose concentration, although at a range higher than normal mammalian levels, and thus reduce the negative consequences associated with hyperglycemia.

Key words: frugivore, plasma glucose, glucose regulation, nectar, fruit

INTRODUCTION

Frugivorous and nectarivorous bats and birds pollinate flowers and disperse fruit seeds, thus contributing to the reproductive success of the plants they feed on (Herrera 1999; Medellín & Gaona 1999; Ingle 2003). Nectarivorous and frugivorous bats have high energy needs due to the cost of flight (Tracy *et al.* 2007; Voigt & Speakman 2007), and sugar is a good source of energy as it is easily absorbed and digested (Suarez *et al.* 1990; Voigt & Speakman 2007). Fruit juices are made up of glucose, sucrose and fructose sugars (Baker *et al.* 1998; Wilson & Downs 2012) and these sugars are a key biochemical reward for the frugivores that feed on them (Ribá-Hernández *et al.* 2003).

Wahlberg's epauletted fruit bats (*Epomophorus wahlbergi*) feed on a wide range of indigenous and exotic fleshy fruits and nectars of flowers (Skinner & Chimimba 2005; Monadjem *et al.* 2010). The bats obtain juices and nutrients from the fruits they feed on and discard the pulp (Skinner & Chimimba 2005; Monadjem *et al.* 2010). South African indigenous and invasive exotic fleshy fruits are mainly hexose dominant (Jordaan & Downs 2012; Wilson & Downs, 2012). *Epomophorus wahlbergi* have a positive effect on invasive exotic fruit germination (Jordaan *et al.* 2012) which would suggest that these bats may preferentially feed on these fruits as they have higher sugar content (Jordaan & Downs 2012).

The evolution of nectarivorous bats to specialize on a sugar-rich nectar diet was due to high energetic demands, such as flight (Kelm *et al.* 2011). For nectarivorous bats to directly fuel these high energetic costs exclusively on exogenous sugars, these bats would require a constant and sufficiently high supply of this sugar via the bloodstream (Kelm *et al.* 2011). For example,

glucose intake of *E. wahlbergi* is generally high irrespective of sugar concentration (Coleman & Downs 2012; Downs *et al.* 2012).

Nectarivores feed on a sugar-rich nectar diet (Lepczyk *et al.* 2000) during which large amounts of glucose are ingested (Kelm *et al.* 2011). While sugar-rich diets can be beneficial to nectarivores (Lepczyk *et al.* 2000), it is not known how they avoid the negative effects of high glucose concentration (Beuchat & Chong 1998). Some nectarivores show an increase in blood plasma glucose after feeding on fructose and glucose diets (Martinez del Rio & Stevens 1989), while others have diel changes in blood plasma glucose concentration (Downs *et al.* 2010; Lobban *et al.* 2010). Above normal blood plasma glucose concentrations are considered harmful, especially to mammals of small body size (Kjeld & Olafsson 2008), as this ultimately causes diabetic complications (Kawahito *et al.* 2009).

Directly after feeding, blood plasma glucose concentrations, increase with the amount of glucose ingested in resting bats and decrease with increasing flight time in active bats (Kelm *et al.* 2011). However, most frugivorous bats appear to regulate blood plasma glucose concentrations within normal mammalian limits (Widmaier & Kunz 1993; Heard & Whittier 1997; Korine *et al.* 1999). It is expected that insulin has a typical role in regulating blood glucose levels as in most mammals (Sherwood *et al.* 2005; Braun & Sweazea 2008). We hypothesized that *E. wahlbergi* regulate blood plasma glucose concentrations within the normal mammalian range irrespective of dietary intake. However, we predicted that they would have higher blood plasma glucose concentrations post-feeding although within the normal range. We therefore investigated blood plasma glucose regulation of *E. wahlbergi* at six hourly intervals to determine the effect of dietary intake. Furthermore relatively few haematological studies have been conducted on Chiropteran species despite their species richness and relevance for zoonotic

diseases (McLaughlin *et al.* 2007; Schinnerl *et al.* 2011 and references therein). Most of these studies have focussed on aspects of their erythrocytes or leucocytes. A recent hematological survey of common neotropical bat species from Costa Rica investigated white blood cell parameters (Schinnerl *et al.* 2011), however no plasma glucose levels were recorded. It was hoped the current laboratory study would provide baseline data to compare with free-ranging *E. wahlbergi*.

MATERIALS & METHODS

Epomophorus wahlbergi were caught in mist-nets in Pietermaritzburg (29°38'S, 30°25'E) in late September and November 2009. Bats were collected under permit from Ezemvelo KZN Wildlife. Males and females were housed in separate outdoor aviaries (4.1 x 2.4 x 2 m) at the animal house, University of KwaZulu-Natal, Pietermaritzburg campus. They were fed a maintenance diet of nectar (20% glucose, sucrose and fructose) in a nectar feeder, a selection of chopped fresh fruit (pear, banana and apple) and a nutritional supplement (Lory Life, AVICO, California, USA) provided each evening. Water in a nectar feeder was provided ad lib.

Bats had been in captivity for several months before they were transferred to a 25°C constant environment room with a 12 L: 12 D cycle and placed individually in cages (77 x 52 x 81 cm) for two weeks before blood sampling. They were able to fly to a lesser extent in these cages compared with the outside aviaries. Fresh fruit (apple, pear and banana) was provided each evening at 18h00 when the dark phase began. Water was available in a nectar feeder. Blood plasma glucose concentration and body mass of all the bats were determined every second day at one of the following times per day: 12h00, 18h00, 24h00 and 06h00. Each bat (n = 7) was caught by hand and blood immediately obtained. The alternate wing of each was used each two days.

The brachial vein was lightly swabbed with cotton-wool dampened with surgical spirits then punctured using a 0.33mm gauge needle. Accu-Chek Performa glucose control test strips (Roche Diagnostic, Mannheim, Germany) were used to obtain blood drops from the point of puncture. Following this, glucose concentrations were measured in mmol/l (accurate to 0.1) using an Accu-Chek Performa Blood Glucose Meter (Roche Diagnostic, Mannheim, Germany) which had been calibrated. A cotton-wool swab was held on the point of puncture to stop bleeding. No haemostasis was observed. Bats were then weighed using an electronic scale (± 0.01 g, Adam Equipment, South Africa) and returned to their respective cages.

Blood plasma glucose concentrations and body mass of the bats were analysed against time using a generalized linear model repeated measures analysis of variance (RMANOVA), followed by a post-hoc Tukey HSD test. Data were analysed using STATISTICA, version 7 (Statsoft, Tulsa, USA).

RESULTS & DISCUSSION

Body masses of *E. wahlbergi* differed significantly at the respective blood sampling times (RMANOVA, $F_{(3, 18)} = 8.455$, $P = 0.001$) (Fig. 1). The lowest mean body mass (\pm S.E) was at 18h00 (99.69 ± 5.55 g) while the highest mean body mass was at 24h00 (105.05 ± 5.75 g). There was a significant difference in body mass between the 12h00 and 18h00 sampling times (Post hoc Tukeys' HSD test, $P = 0.009$), and between the 24h00 and 18h00 sampling times (Post hoc Tukeys' HSD test, $P = 0.000$). At 24h00 all bats had fed on the fruit provided and at 06h00 little fruit remained. The increase in body mass at 24h00 was about 6%. Some fruit bats are known to eat their body mass equivalent in a night (van der Westhuysen 1976).

Blood plasma glucose concentration of *E. wahlbergi* also differed significantly with time (RMANOVA, $F_{(3, 18)} = 3.5802$, $P = 0.034$). Their blood plasma glucose concentrations ranged from 5.24 ± 0.38 mmol/l at 18h00 to 8.19 ± 1.24 mmol/l (mean \pm S.E) at 24h00 (Fig. 2). There was a significant difference between the 24h00 and 18h00 sampling time (Post hoc Tukeys' HSD test, $p = 0.033$). The normal mammalian range of blood plasma glucose concentrations is about 3.6 - 5.6 mmol/l although some species show lower or higher values (Widmaier & Kunz 1993; Opazo *et al.* 2004; Boily *et al.* 2006). Consequently, *E. wahlbergi* blood plasma glucose concentrations were generally higher than the upper limit of the normal mammalian blood plasma glucose range. However, they were lower than concentrations measured in most bird species (Braun & Sweazea 2008; Downs *et al.* 2010; Lobban *et al.* 2010). The bats showed the greatest individual variation in blood plasma glucose concentrations at 24h00 possibly because of varying feeding and digestion rates (Fig. 2).

Epomophorus wahlbergi's blood plasma glucose concentrations varied with time and food availability. They had the lowest mean blood plasma glucose concentration of 5.24 mmol/l at 18h00 when they had rested and fasted for 12h. The mean blood plasma glucose concentrations increased to 8.19 mmol/l at midnight after bats had been feeding. South African indigenous and invasive exotic fruits are generally hexose dominant (Jordaan & Downs 2012; Wilson & Downs 2012) which would thus explain the increase in blood glucose concentrations of *E. wahlbergi* after consuming fruits. Nectar feeding birds also show an increase in blood glucose concentrations after feeding on a hexose diet (Martinez del Rio & Stevens 1989).

Generally bats regulate blood plasma glucose concentrations within limits and it is independent of diet intake or energy use during flight, that is, glucose concentrations are kept fairly constant during food intake as well as during physical activities (Kjeld & Olafsson 2008).

Pteropus hypomelanus, *P. vampyrus*, and *R. aegyptiacus* in captivity have diel changes in blood glucose concentration, however these concentrations were within the normal range for mammals (Widmaier & Kunz 1993; Korine *et al.* 1999). Similarly Heard and Whittier (Heard and Whittier, 1997) found these *Pteropus* species and *P. rodricensis* had blood glucose concentrations within the normal mammalian range. Two frugivorous bats (*R. aegyptiacus* and great fruit-eating bats (*Artibeus lituratus*)) have a high percentage of pancreatic tissue (Micheltmore *et al.* 1998; Protzek *et al.* 2010). This is suggested to allow these bats to control the high intake of glucose during absorption and thus maintain blood plasma glucose concentrations within limits (Micheltmore *et al.* 1998; Protzek *et al.* 2010). In a study of *R. aegyptiacus* this was not evident as its blood plasma glucose concentrations were observed to be greater than 40 mmol/l after glucose uptake (Keegan 1977; Micheltmore *et al.* 1998). However, this was not after natural feeding but during an oral glucose tolerance test (Keegan 1977; Micheltmore *et al.* 1998). The comparative international standard for assessing blood plasma glucose concentrations regulation is a Glucose Tolerance Test, whereby animals are fasted for at least 12h then provided with a known amount of glucose, and blood plasma glucose concentrations are measured at intervals of 15min for 2h or until they return to fasting levels (Opazo *et al.* 2004). However, this method does not show the normal diel variation in blood plasma glucose concentrations of animals. When feeding on fruit, blood plasma glucose concentrations of *E. wahlbergi* had diel variations. However, these blood plasma glucose concentrations appeared to be within limits irrespective of feeding and nocturnal activity despite being higher than the normal mammalian range.

In conclusion, *E. wahlbergi* showed diel variations in blood plasma glucose concentrations with an increase in concentrations during or directly after feeding. Their concentrations were, however, maintained within limits, although higher than the typical normal

mammalian blood plasma glucose range. Consequently these bats regulated their blood plasma glucose concentrations despite their energy intake, thus reducing the negative consequences associated with hyperglycemia. We suggest that further research is needed to determine the hormonal control with which these fruit bats regulate their blood plasma glucose concentrations. In addition comparison with free-ranging *E. wahlbergi* will give insight into diel variations in plasma glucose concentrations of non-captive bats.

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Fig. 1. Mean (\pm SE) body mass (g) of *Epomophorus wahlbergi* (n = 7) at the respective blood plasma glucose sampling times.

Fig. 2. Changes in mean (\pm SE) blood plasma glucose concentrations (mmol/l) of *Epomophorus wahlbergi* (n = 7) at the respective blood plasma glucose sampling times.

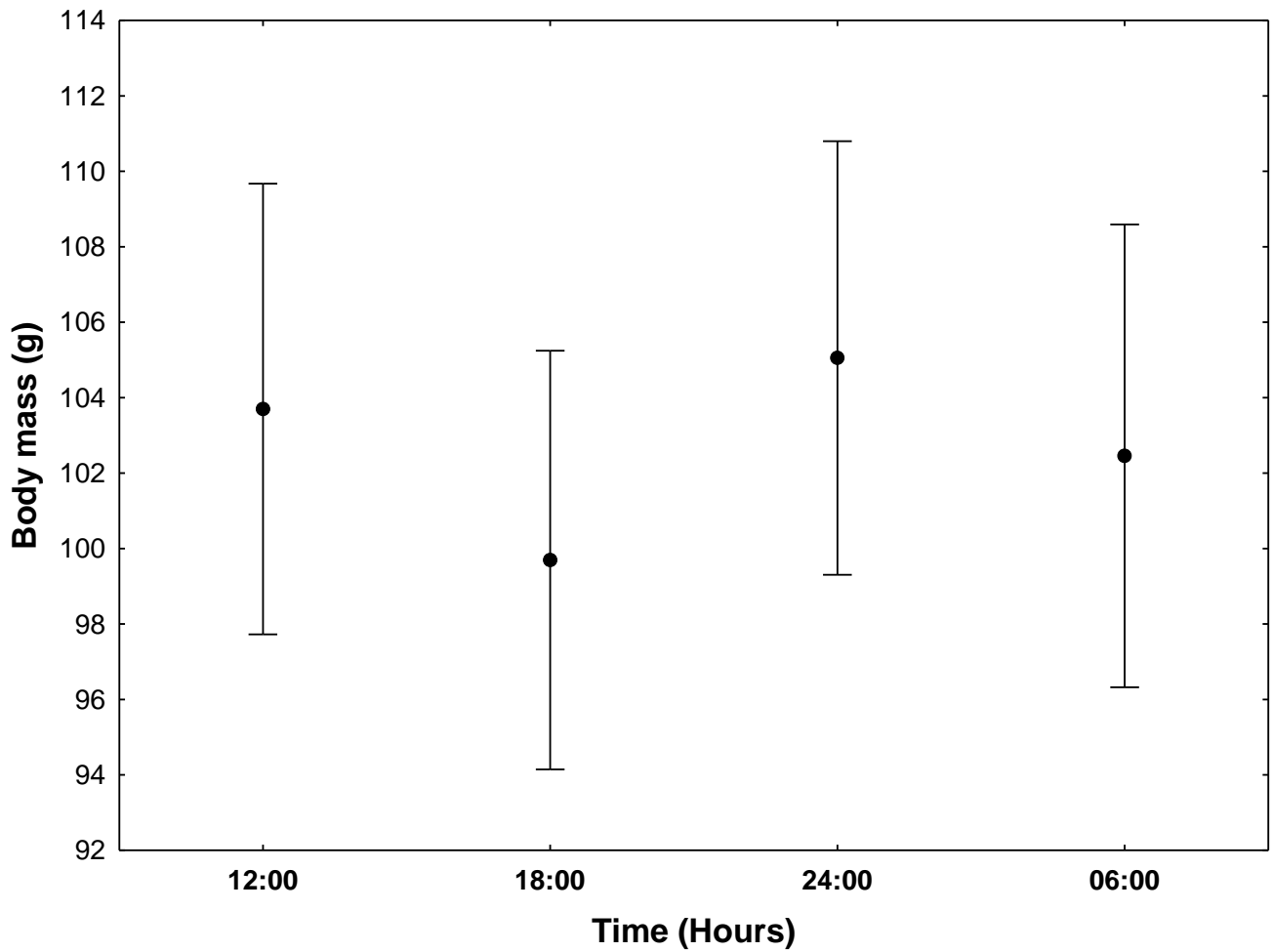


Fig. 1. Mean body mass (\pm SE) (g) of *Epomophorus wahlbergi* ($n = 7$) at the respective blood plasma glucose sampling times.

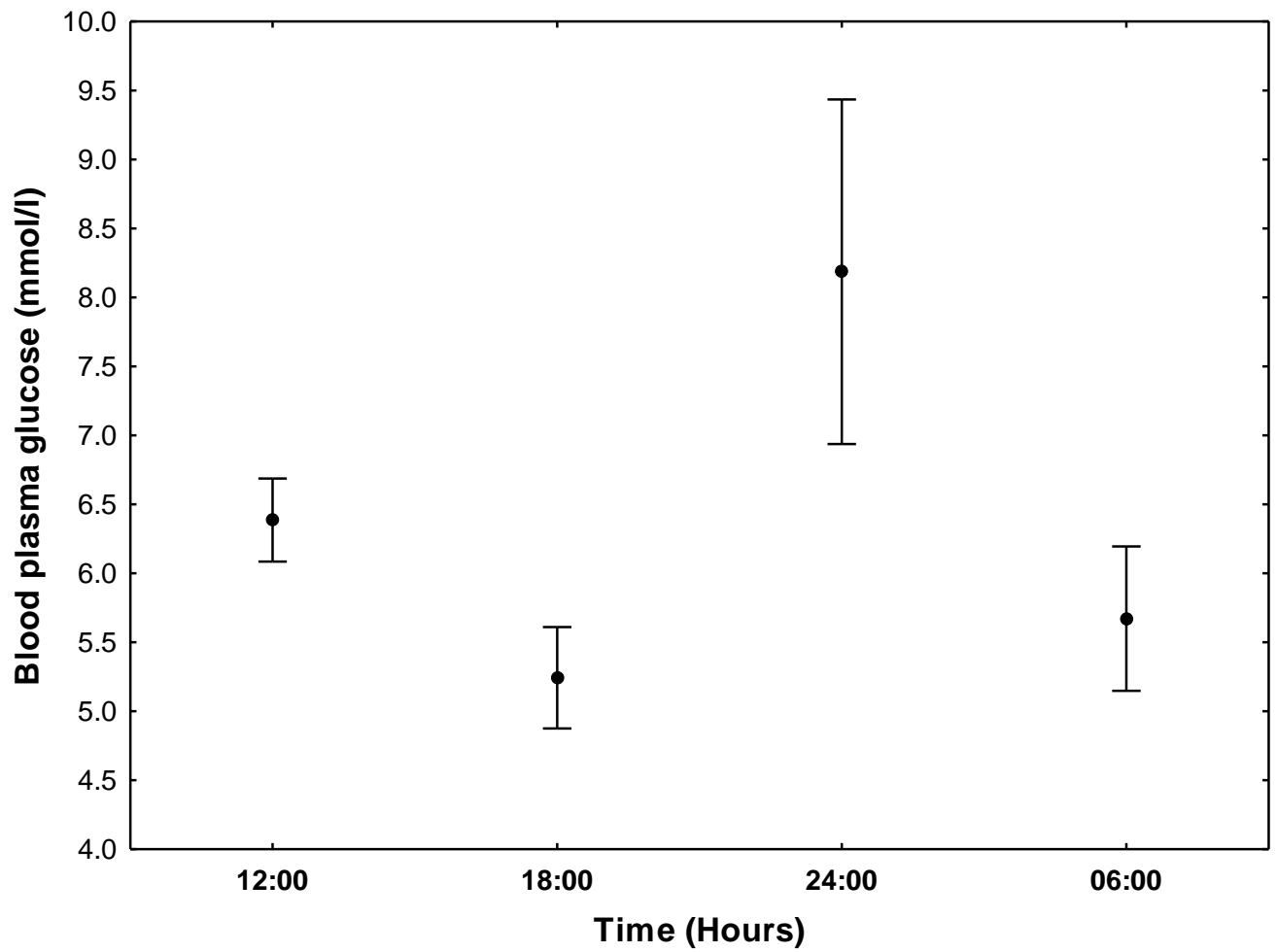


Fig. 2. Changes in mean (\pm SE) blood plasma glucose concentrations (mmol/l) of *Epomophorus walbergi* ($n = 7$) at the respective blood plasma glucose sampling times.

Chapter 4

Is protein composition in the diet of Wahlberg's epauletted fruit bat important?

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ABSTRACT

Wahlberg's epauletted fruit bat *Epomophorus wahlbergi* mainly feed on fruit of a range of tree species. Frugivores that feed exclusively on fruit may have difficulties in maintaining their protein requirements since fruits are generally high in sugar content but low in protein content. Some studies have found that fruit bats obtain most of their food protein when feeding on a variety of fruits. We investigated if *E. wahlbergi* prefer nectar solutions with protein. They were offered equicaloric 15 % glucose solutions with varying protein concentrations (2.58, 5.68, 7.23 g soy protein/kg H₂O) and a solution with no protein. This was repeated using 15 % sucrose instead of glucose solutions. *Epomophorus wahlbergi*'s volumetric intake of the respective glucose and sucrose solutions varied among individual bats, with total volumetric intake highest for the solution with no protein (control) and lowest at 2.58 g/kg soy protein concentration solution for glucose and 5.68 and 7.23 g/kg soy protein concentration for sucrose. These bats appeared to prefer sugar solutions without or low protein, and their daily protein intake was

relatively low. This suggests they have low-protein requirements, and this relates to their characteristic low-protein fruit available in the wild.

Key words: Frugivory, sugar solutions, protein intake, protein requirements

INTRODUCTION

Proteins are essential in a diet to provide the required nitrogen and amino acids necessary for maintaining body tissues (Morrison 1980). Wahlberg's epauletted fruit bat (*Epomophorus wahlbergi*) occurs in the eastern part of southern Africa and is known to feed on fruit, nectar, pollen and flowers. This fruit bat feeds on fruits of a variety of cultivated, indigenous and alien invasive fruiting tree species (Monadjem *et al.* 2010; Jordaan *et al.* 2012). Fruits appear to have low protein for the frugivores that feed on them (Witmer 1998; Wilson and Downs 2012), in particular most southern African fruit are relatively low in protein content and so a poor source of nitrogen for *E. wahlbergi*. Frugivores that solely depend on fruit as the main source of food may therefore have challenges in meeting their protein requirements (Stellar 1986; Law 1992; Korine *et al.* 1996). It was thus suggested that for fruit bats to solely depend on fruits as a main diet, a sufficient amount of nitrogen supply is therefore an important aspect (Korine *et al.* 1996). Certain fruits, however, may provide these frugivores with the required nitrogen to grow and reproduce as it contains all the essential amino acids (Herbst 1986). Morrison (1980) suggested that since mammals need approximately 37 mg of protein per kcal of basal metabolism, then therefore the Jamaican fruit bat (*Artibeus jamaicensis*) needs approximately 0.33 g of protein per day. Fruit bats have transit times as fast as 20 min, thus taking up more of their liquid diet to compensate for the low protein in their diet, therefore allowing the bat to avoid a deficiency in

the required amount of protein if protein is limiting (Thomas 1984). When feeding on nectar, on the other hand, most of the pollen attaches to the bat and is ingested, and ultimately becomes a reliable source of nitrogen or protein for the bat (Howell & Hodgkin 1979; Law 1992; Birt *et al.* 1997; Altringham 2011).

A study of nectarivorous birds showed that these birds are physiologically adapted to a low protein diet and therefore have a low nitrogen requirement (Tsahar *et al.* 2005). Previous studies, (Herbst 1986; Delorme & Thomas 1996; Korine *et al.* 1996; Herrera *et al.* 2002), found that non-reproductive fruit bats meet all of their protein requirements when feeding on a variety of fruits. Generally these bats did not supplement their diet with insects to obtain the required protein. Studies done by Herrera *et al.* (2001a; 2001b) found that frugivorous bats obtain most, if not all, of their food protein from fruits, without changing their diet with neither season nor sexual period. However, some species like the grey-headed flying fox (*Pteropus poliocephalus*) increase fruit ingestion to meet their nitrogen requirement (Steller 1986). Other species such as Egyptian fruits bats (*Rousettus aegypticus*) deliberately feed on insects such as garden fruit chaffer beetles (*Pachnoda sinuata*) as a source of protein (Barclay *et al.* 2006).

Due to the high energy demands of flight (Voigt & Winter 1999; Tracy *et al.* 2007; Voigt & Speakman 2007), bats must be able to discriminate foods based on their energy and nutritional rewards. Korine *et al.* (1996) found that certain fruit species are adequate to supply the nitrogen requirements of fruit bats, while some are inadequate to supply their energy needs. The Egyptian fruit bat requires more dry matter to supply its energy requirements than that to maintain its nitrogen balance. Therefore, suggesting that energy rather than protein is the limiting nutritional factor in the diet of these fruit bats (Korine *et al.* 1996).

This study investigated protein intake and preference in Wahlberg's epauletted fruit bat. We hypothesized that the protein composition of diet affects their nectar intake and preference. We predicted that they would prefer nectar diets of higher protein content and have higher dietary intake with increasing protein composition to meet their protein requirements.

MATERIAL AND METHODS

Study animals

Wahlberg's epauletted fruit bats ($n = 7$) were captured in mist-nets in Pietermaritzburg, South Africa in late September and November 2009 and in July 2011. They were captured under permit from Ezemvelo KZN Wildlife. They were kept at the animal house in UKZN Pietermaritzburg, with males and females in separate outdoor aviaries (4.1 x 2.4 x 2 m). They were fed a maintenance diet of a selection of chopped fruit (apple, banana, pear and paw-paw) on suspended trays and nectar (20% glucose, sucrose and fructose) in a nectar feeder, provided each evening in the aviaries. Bats had been in captivity for several months before they were transferred to a 25 ± 1.0 °C constant environment room with a 12L: 12D and placed individually in cages (77 x 52 x 81 cm). They were kept for 3 weeks to adjust them to feeding on liquid nectar solutions. Each evening bats were provided a maintenance diet of nectar (20% glucose, sucrose and fructose) in a nectar feeder and a selection of fruit. Three weeks prior to the experiments an additional feeder with the sugar solution and protein was provided. This additional feeder contained a protein concentration of 7.23 g soy protein/kg H₂O (which fulfills *R. aegyptiacus* protein requirements (Korine *et al.* 2006)).

Experimental trials

Bats ($n = 7$) continued to be fed as before prior to each experiment. Body mass of bats were obtained 2h before each feeding trial and each trial lasted one night. In the first and second experiments protein concentration preference of bats was investigated using one particular sugar type offered in modified 50mL glass burette tubes. Bats were offered a choice of four randomly positioned equicaloric 15 % glucose or 15 % sucrose solutions (Coleman & Downs 2012) with varying protein concentrations (2.58, 5.68, 7.23 g soy protein/kg H_2O) and a solution with no protein, which served as a control, on separate nights respectively from 18h00 to 06h00. Each hour amount drunk of the respective solutions was recorded.

Trials were conducted every second night and the bats were provided with a sugar solution containing a particular protein content in calibrated burettes per cage at 18h00 with the onset of the scotophase. Amount of sugar solution initially in each burette was recorded and thereafter recorded hourly until 06:00 to determine whether the bats feed constantly. Bats were then removed, weighed and returned to their respective cages.

Statistical Analysis

Comparisons between initial and final body mass, food intake, total protein intake on the different diets were done using Repeated-Measures Analysis of Variance (RMANOVA) with STATISTICA (Statsoft, version 7, Tulsa, OK, USA). Food intake data was transformed using arcsine (Coleman & Downs 2012). Comparisons between the protein solutions for both glucose and sucrose were done using Friedman ANOVA and Kendall Coefficient of Concordance with STATISTICA (Statsoft, version 7, Tulsa, OK, USA).

RESULTS

There was no significant difference in body mass of *E. wahlbergi* between glucose and sucrose diet treatments (RMANOVA: $F_{(1, 5)} = 6.29$, $p = 0.05$). The initial mean body mass for glucose was 111.98 ± 6.02 g (Mean \pm S.E) and the final mean body mass was 112.11 ± 6.59 g. The mean change in body mass for glucose was 0.13 ± 1.13 g. The initial mean body mass for sucrose was 108.78 ± 6.09 g and the final mean body mass was 112.46 ± 5.78 g. The mean change in body mass for sucrose was 3.68 ± 0.44 g (Fig. 1). On the respective sucrose and glucose treatments the bats had a choice of the 4 solutions (control no protein; 2.58, 5.68 and 7.23 protein treatments g soy protein/kg H₂O, see methods). Consequently only one body mass was obtained per treatment (Fig. 1). The variance is a consequence of bats maintaining body mass on the sucrose compared with the glucose treatment.

Epomophorus wahlbergi's volumetric intake of the respective glucose and sucrose solutions varied among individual bats. Total volumetric intake was highest at the solution with no protein (control) and lowest at 2.58 g/kg soy protein concentration solution for glucose, and and at 5.68 and 7.23 g/kg soy protein concentration sucrose (Fig. 2). Generally intake of the protein solutions was low. There was no significant difference between intake on glucose compared with sucrose solutions (RMANOVA: $F_{(3, 15)} = 1.06$, $p = 0.40$; Post hoc Tukeys' HSD test, $p > 0.05$) (Fig. 2). However, total volumetric intake of the different glucose solutions showed a significant difference (RMANOVA: $F_{(3, 15)} = 4.53$, $p = 0.01$) (Fig. 2). There was a significant difference between the glucose control solution and the 2.58 g/kg soy protein concentration glucose solution (Post hoc Tukeys' HSD test, $p = 0.01$), and between the glucose control solution and the 7.23 g/kg soy protein concentration glucose solution (Post hoc Tukeys' HSD test, $p = 0.04$). Although volumetric intake was higher for the control sucrose solution than

the other protein sucrose solutions, there was no significant difference among the diet treatments (RMANOVA: $F_{(3, 15)} = 1.02$, $p = 0.41$; Post hoc Tukeys' HSD test, $p > 0.05$) (Fig. 2).

There was no significant difference in protein intake at the glucose protein solutions (ANOVA Chi^2 ($N = 6$, $df = 3$) = 2.55, $p = 0.46$) and the sucrose protein solutions (ANOVA Chi^2 ($N = 6$, $df = 3$) = 2.50, $p = 0.47$). *Epomophorus wahlbergi* showed no significant difference in total protein intake between the glucose and sucrose protein solutions (RMANOVA: $F_{(1, 5)} = 1.60$, $p = 0.26$) (Fig. 3). Consequently these bats took hardly any protein in.

DISCUSSION

Contrary to expectations, *E. wahlbergi* generally had a higher intake of sugar solutions with no protein compared with different protein concentration sugar solutions offered concurrently, and generally showed reduced volumetric intake with increased protein concentration. There was significant reduced consumption of only the 2.58g/kg and 7.23g/kg protein+glucose solutions relative to the controls. Differences in consumption of the controls and the 5.68g/kg protein+glucose solutions were not significant, nor were those among the various sucrose solutions.

Previously it was suggested that the low-nitrogen requirements of nectarivores and frugivores have evolved due to their feeding habits, allowing them to survive on their nectar and fruit diet (Tsahar *et al.* 2006). Consequently nectarivorous and frugivorous birds have adapted to low-nitrogen requirements as a result of the characteristics of their diet rather than the birds physiological characteristics (Tsahar *et al.* 2006). Although *E. walbergi* fed on the protein concentration solutions, intake was low and there was no significant difference in intake among the protein concentration solutions for both glucose and sucrose diets. Increased intake of sugar

solutions is suggested to be a regulatory mechanism to compensate for the reduced protein content where in the wild it would ultimately increase fruit intake and as a consequence protein intake would have been achieved (Thomas 1984).

Old-world fruit bats, feeding exclusively on fruits, may supply their protein requirements when feeding on a wide variety of fruit which contain different amounts of protein (Courts 1998). There was no significant difference among the diet treatments on the sucrose solution. This indicates that the bats feed on what is available to supply their energy requirements more than their protein requirement, which indicates that energy rather than protein is the important nutritional requirement in the diet of fruit bats (Korine *et al.* 1996). A concern is that the lower intake on the sugar + protein solutions may reflect taste aversion by the bats to the soy protein, however the sugars would have masked this to a degree.

Epomophorus wahlbergi generally had a low protein intake suggesting that these bats have a low daily protein requirement. A study of South African indigenous and invasive exotic fruit showed that the protein content of fruit pulp was generally low for all fruit species studied (Jordaan & Downs 2012; Wilson & Downs 2012). Fruits consumed by the short-tailed fruit bat (*Carollia perspicillata*) contain sufficient protein to maintain non-reproductive bats, but not enough for those that are lactating (Herbst 1986). Bats used in the current study were non-lactating and therefore the lacking or low amount of protein in their diet may be sufficient for these bats to survive. The protein intake of *E. wahlbergi* was similar to the suggested 0.33 g of protein per day for *A. jamaicensis* although the latter are smaller (about 60g) (Morrison 1980). Using the equation predicted for maintenance nitrogen requirements in eutherians (Robbins 1993), a 100g fruit bat requires 103.50 mg N while a 120 g fruit bat requires 118.66 mg N. These values are much greater than the protein intake of *E. wahlbergi* in the current study. Korine *et*

al. (1996) suggested that a positive nitrogen balance may indicate protein storage or muscle building. Our findings suggest that *E. wahlbergi* may have low-protein requirements because these bats lack relatively big muscle build up (pers. obs. and obs. when dissected Chapter 5). Bats that feed on insects are termed ‘high energy’ bats due to their highly agile flight and have higher muscle mass as compared with lower flight muscle mass of nectarivorous and frugivorous bats (Bullen & McKenzie 2004). Nectarivorous and frugivorous bats do not perform the highly agile flight activity necessary to capture prey and thus do not require excess muscle as they forage at intermediate speeds (Bullen & McKenzie 2004). We were unable to test the possibility that the bats stored proteins in their muscles during the period of feeding before the trials began, and then used these during the trials, which may explain the lack of preference for the protein solutions. However, even the maintenance diet is relatively low in protein content.

Intake of the control glucose solution was generally higher than the control sucrose solution. A previous study showed a similar trend of sugar intake by *E. wahlbergi* (Downs *et al.* 2012). It has been suggested that this is due to physiological constraints of sucrose digestion (McWhorter & Martinez del Rio 2000). Despite this, body mass of *E. wahlbergi* did not change significantly between glucose and sucrose diet treatments. This suggests that these bats do not store energy as fat but rather digest and absorb their sugary intake to power their high energy needs (Suarez *et al.* 1990; Voigt & Speakman 2007).

In conclusion, *E. wahlbergi* appeared to prefer sugar solutions without or low protein, and their daily protein intake was relatively low. This suggests they have low-protein requirements, and this relates to their characteristic low-protein fruit available in the wild. Future studies need to determine intake on a selection of fruit with varying energy and protein content in

order to understand their adaptation to low-protein diets. In addition little is known about whether they obtain their nitrogen requirements from an alternative source.

ACKNOWLEDGEMENTS

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Fig. 1. Mean change in body mass of *E. wahlbergi* (n = 7) during the respective glucose- and sucrose-protein diet trials.

Fig. 2. Transformed total volumetric intake per day of *E. wahlbergi* (n = 7) between the respective glucose- and sucrose-protein diet treatments (control no protein; 2.58, 5.68 and 7.23 protein treatments g soy protein/kg H₂O, see methods). Data reported as mean \pm S.E.

Fig. 3. Mean total protein intake per day of *E. wahlbergi* (n = 7) on the respective glucose- and sucrose-protein concentration solutions.

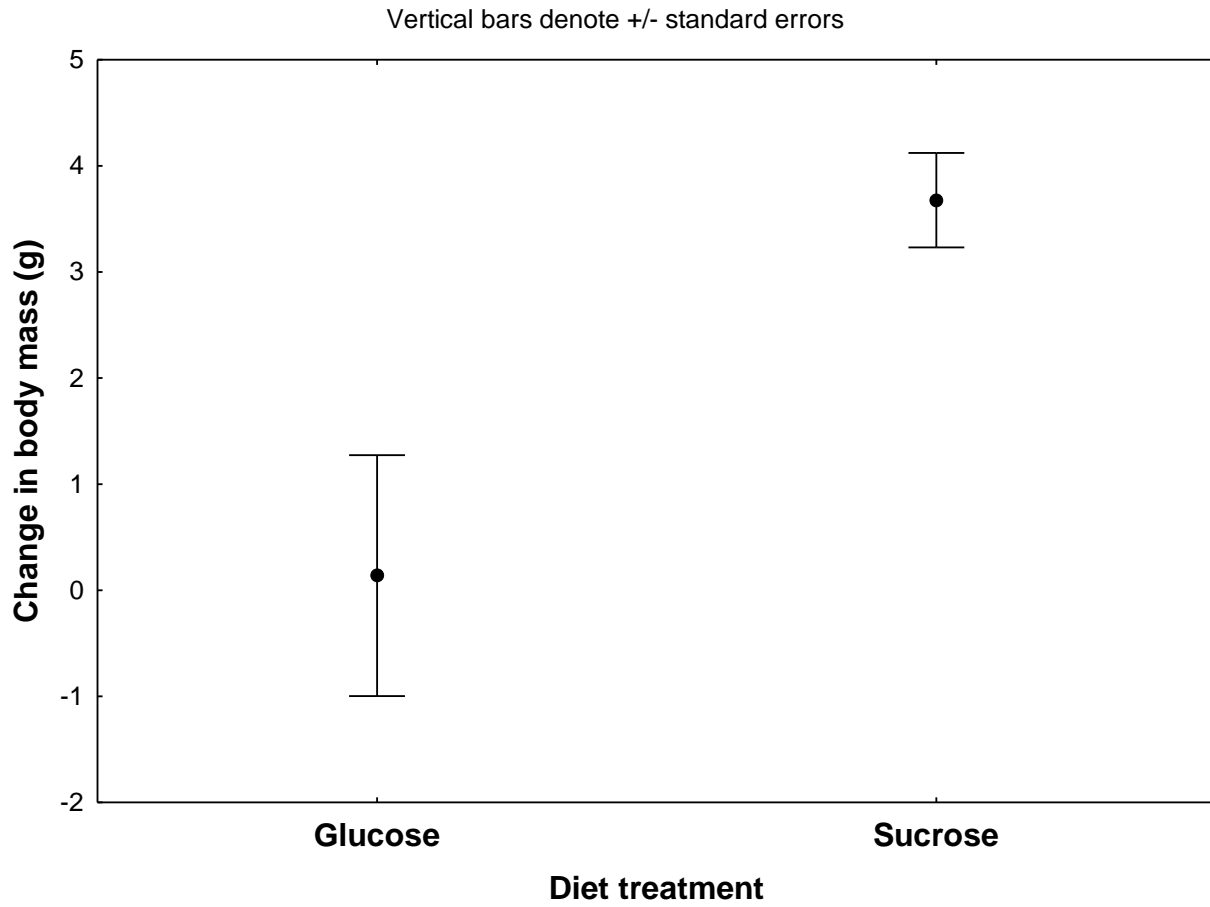


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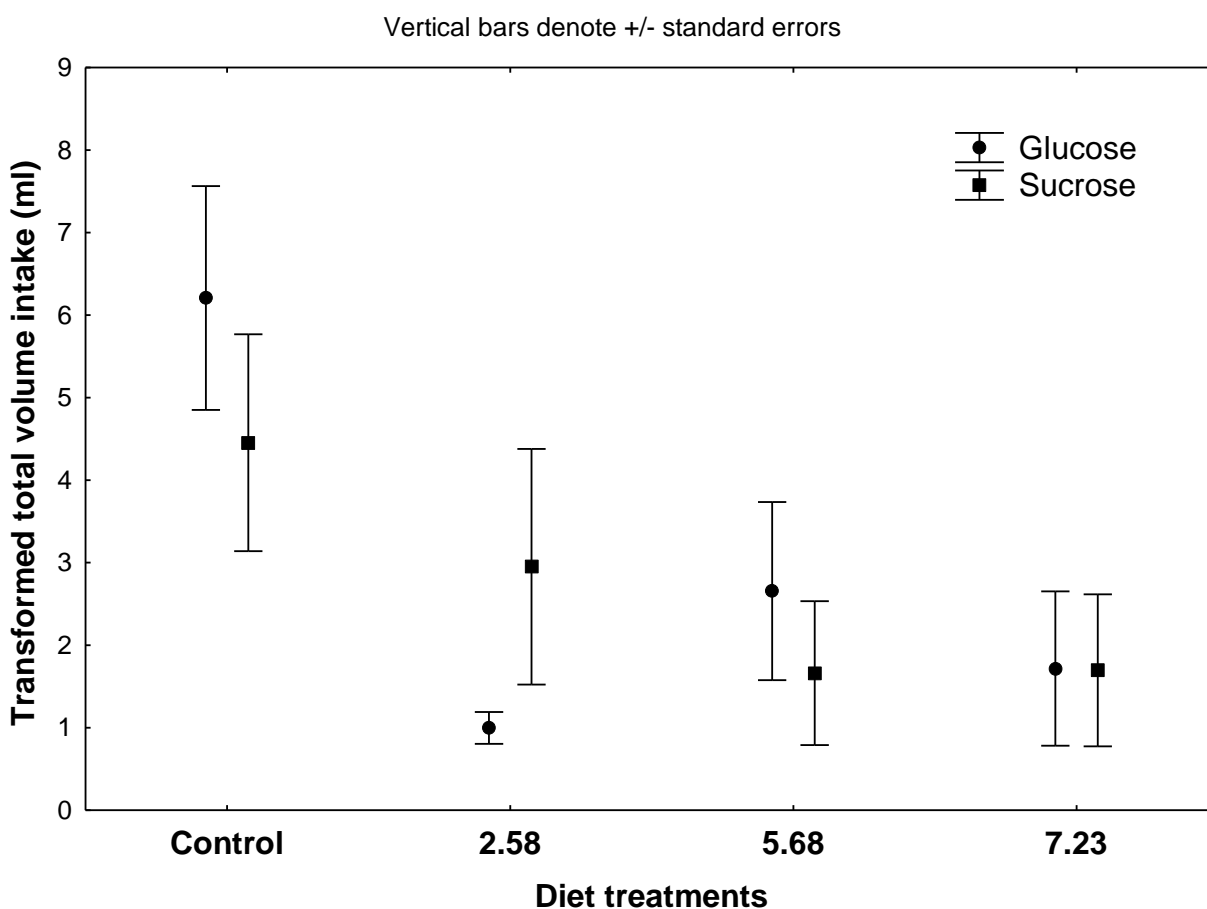


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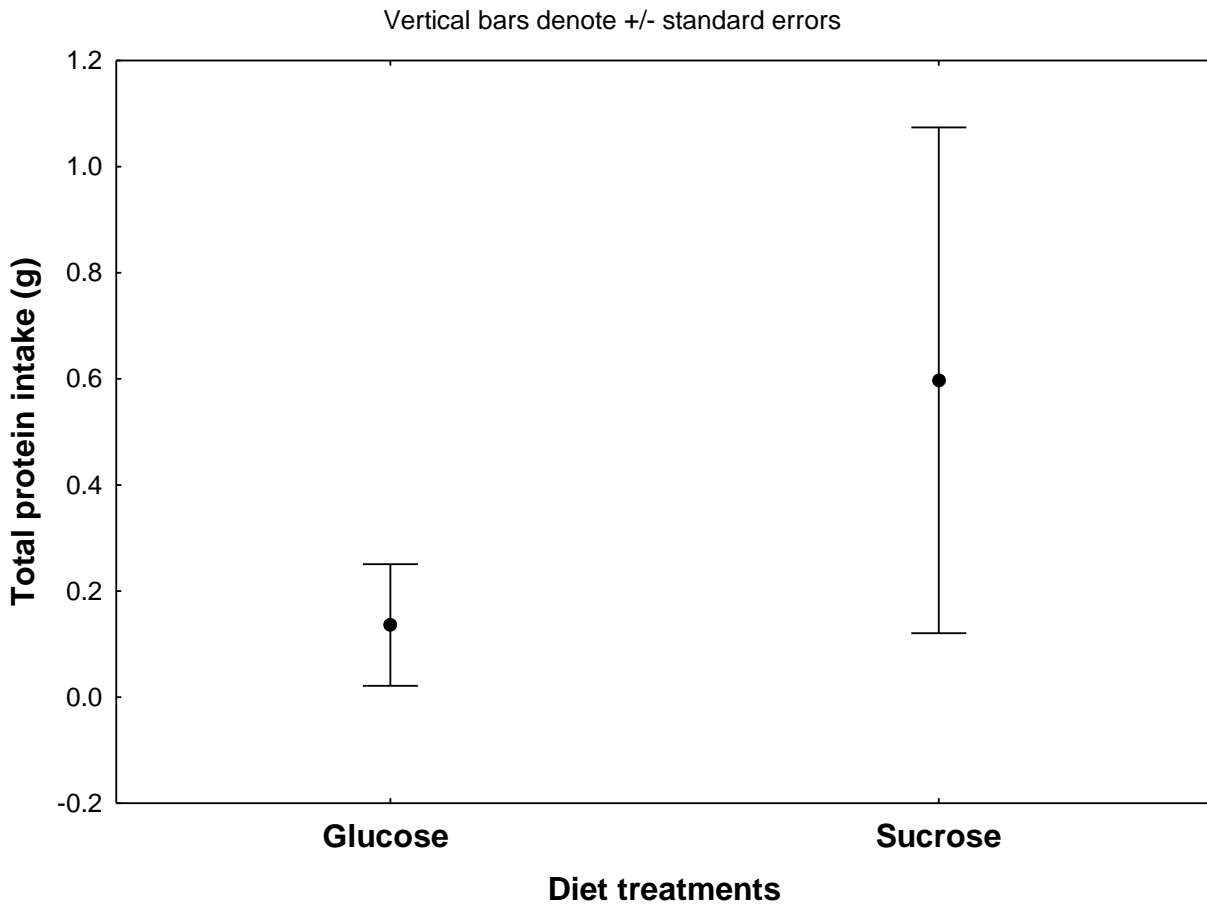


Fig. 3. Mean total protein intake per day of *E. wahlbergi* ($n = 7$) on the respective glucose- and sucrose-protein concentration solutions.

Chapter 5

Effects of a nectar and fruit diet on the kidney and small intestine morphology of

Wahlberg's epauletted fruit bat (*Epomophorus wahlbergi*)

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Abstract

Frugivorous bats consume fruit and nectar juices. This type of feeding results in sugar and dietary water intake which could cause physiological challenges for nectarivores and/or frugivores as they have to balance water and energy intake from this liquid diet. Kidneys are responsible for eliminating nitrogenous wastes whilst maintaining water balance during feeding. Histological sections of kidneys and the small intestine were observed under light microscopy to determine renal and intestinal morphology of *Epomophorus wahlbergi*, respectively. Cortex and medulla length measurements were taken to calculate the medulla: cortex ratio (M/C) and the percent medullary thickness (PMT). The cortex and medulla of *E. wahlbergi* were observed to be relatively similar in size. Morphological renal characters are reliable indicators of urinary concentrating abilities in mammals. Frugivorous bats have lower percent medullary thickness and higher percent cortex, suggesting the bats lack of urine concentrating ability. *E. wahlbergi* feeds on a watery diet and does not need to concentrate urine; therefore the medulla and cortex are more or less the same size. The small intestine structure of these bats is similar to that

described by Makanya et al., (2001), exhibiting morphological structures that allow efficient absorption of sugars during feeding.

Key words: nectar, fruit, kidney, water balance, small intestine, absorption

Introduction

Old-world fruit bats feed on fruit and nectar of flowers, with many depending on nectar as a primary source of food (Dumont, 2003). Interestingly, frugivorous bats usually do not digest the fruits but only get the juices out before spitting out the pulp as ‘spats’ (Morrison, 1980); consequently they are ‘nectarivores’. Feeding on nectar or fruit results in increased sugar intake as well dietary water intake (Martinez del Rio et al., 2001). Nectarivores thus have to ingest and excrete large water volumes when feeding on this watery nectar diet (Lotz and Martinez del Rio, 2004). Balancing water and energy from this diet could cause physiological challenges for nectarivorous birds as they have to extract energy and nutrients from dilute nectar and also excrete large amounts of water from this food source (Beuchat et al., 1990; Lotz and Martinez del Rio, 2004; Brown et al., 2010). Palestine sunbirds’ (*Nectarinia osea*) energy intake is not limited by water processing as these birds are able to regulate their absorbed dietary water whilst maintaining energy intake (McWhorter et al., 2004).

Mammalian kidneys have to eliminate salts and nitrogenous wastes, conserve water during water restriction as well as excreting it when ingested in excess (Yokota et al., 1985). It is suggested that there are connections between ecological features such as diet and habitat, and body mass, renal morphology and physiology (Casotti et al., 2006). Frugivorous bats possess a kidney that is made up of two parts (medulla and cortex), whereas those of other feeding habits are made up of three parts (cortex, inner medulla and outer medulla) (Studier et al., 1983).

Kidneys with reduced relative medullary thickness, a large cortex and an undivided medulla are features that have been suggested to allow nectarivorous and/or frugivorous bats to filter large amounts of water from their diet while conserving electrolytes (Studier et al., 1983; Schondube et al., 2001).

Renal morphological characters such as relative medullary thickness (RMT), percentage medullary thickness (PMT) and medulla to cortex (M/C) ratio have been used as reliable indicators of urine concentrating ability in animals (Sperber, 1944; Geluso, 1978; Studier 1983; Downs and Perrin, 1991; Arad and Korine, 1993; Schondube et al., 2001; Casotti et al., 2006). Nectarivorous and frugivorous bats have decreased RMT and PMT, and increased percent cortex (Casotti et al., 2006).

The Egyptian fruit bat's (*Rousettus aegyptiacus*) kidney is able to cope with the water load when fed fruits of high water content thus regulating the excess water by excreting large volumes of dilute urine (Arad and Korine, 1993). The kidney's function to cope with high water influx is necessary to regulate body mass during flight (Arad and Korine, 1993).

There is known to be a rapid rate of digestion, with relation to carbohydrate-rich meals, in frugivorous and nectarivorous bats (Winter, 1998). Short digesta residence time is a digestive trait that is known to be associated with frugivory (Morrison, 1980; Tedman and Hall, 1985; Tracy et al., 2007) and this may be a problem since digestive efficiency is a function of the length of digesta retention time (Morrison, 1980; Tedman and Hall, 1985). Bats have shorter intestines, less small intestinal surface area and less intestinal tissue compared to other similar sized non-flying mammals (Keegan and Modinger, 1979; Caviedes-Vidal et al., 2007), suggested to be a weight saving mechanism during flight (Tracy et al., 2007). The Egyptian fruit bat and great fruit-eating bat (*Artibeus lituratus*), however, have relatively higher paracellular absorption

(Caviedes-Vidal et al., 2007). The intestines of frugivorous bats possess microvilli that are long with a large surface area (Makanya et al., 1997). This serves the purpose for nutrient absorption, increasing the availability of membrane digestive enzymes and providing a larger surface for digestion to occur; consequently maximizing food exposure to absorptive cells in order to balance the short exposure due to rapid digestion rate (Tedman and Hall, 1985; Makanya et al., 1997; Makanya et al., 2001).

Wahlberg's epauletted fruit bat (*Epomophorus wahlbergi*) occurs in the eastern part of southern Africa feeds on a variety of fruit including cultivated, indigenous and alien invasive fruiting tree species, as well as nectar, pollen and flowers (Monadjem *et al.* 2010; Jordaan *et al.* 2012). These fruits generally are high in water content for the frugivores that feed on them (Witmer 1998; Wilson and Downs 2012). Histological sections of kidneys and the small intestine were observed under light microscopy to determine renal and intestinal morphology of *E. wahlbergi*, respectively. As morphological renal characters are reliable indicators of urinary concentrating abilities in mammals, it was expected that these frugivorous bats would have lower percent medullary thickness and higher percent cortex as efficient urine concentrating ability is required. It was expected that the small intestine structure of these bats is similar to that described by Makanya et al., (2001), exhibiting morphological structures that allow efficient absorption of sugars during feeding.

Materials and Methods

Epomophorus wahlbergi were caught in Pietermaritzburg, South Africa in late September and November 2009 with permits from Ezemvelo KZN Wildlife. The UKZN Ethics Committee granted ethical clearance for use of the bats. The bats were kept in outside aviaries for a range of

experiments. Eight were euthenased, four males with a mass range of 126.11-135.37 g and four females with a mass range of 92.2-101.54 g, for use in this study. Kidneys and intestines were obtained from these bats and kidneys were cut mid-sagittally. Kidney and intestine organs were fixed in Bouin's solution for 24h before being stored in 70% ethanol. Histological sections were prepared by Ampath laboratories, stained with Ehrlich's haematoxylin stain.

Kidney and small intestine sections were viewed using Leica MZ 16 Stereomicroscope (Leica Microsystems, Switzerland) and Olympus AX 70 fluorescent microscope (Olympus, Tokyo) for higher magnification, and images were obtained. Cortex and medulla lengths of the kidneys were determined using a calibrated Soft Imaging System (SIS v3.2) analysis software (GmbH, Germany). Measurements in millimeters were used to calculate the percent medullary thickness (PMT). $PMT = 100 \times (\text{Medullary width}) / (\text{cortical} + \text{medullary width})$ (Heisinger and Breitenbach, 1969). The small intestine sections were viewed using fluorescent light microscopy to determine intestinal morphology. Descriptive statistics were used to analyse the data (Statistica v7, Tulsa, OK).

Results

Epomophorus wahlbergi possessed kidneys with renal medullae that are not subdivided into inner and outer zones (Fig. 1 & 2). From observation, the medulla and cortex of the kidneys of *E. wahlbergi* were relatively similar in size (Fig. 1). Mean medulla: cortex (M/C) ratio was $1:1 \pm 0.06$ (Mean \pm S.E, n = 8). Mean percent medullary thickness (PMT) was $48.11 \pm 1.48 \%$ (Mean \pm S.E, n = 8).

The intestinal structure of *E. wahlbergi* was similar to that described by Makanya et al. (2001). The small intestine's long, the caecum is absent, and the large intestine is short and

almost impossible to differentiate from the small intestine. There appeared to be a slight increase in diameter through the length of the large intestine. The inner surface of the small intestine of *E. wahlbergi* had densely packed and long microvilli (Fig. 3). The intestine of *E. wahlbergi* was simple and not very muscular, indicated by the relatively small muscularis externa (Fig 3, 4 & 5).

Discussion

The kidney structure of *E. wahlbergi* was observed to be made up of the cortex and medulla; the medulla was undivided. Insectivorous bats possess a three part kidney (cortex and divided medulla, whereas undivided renal medullae in kidneys is a renal characteristic observed in frugivorous bats (Studier et al., 1983). Nectarivorous and frugivorous bats have lower percent medullary thickness and higher percent cortex (Casotti et al., 2006) while insectivorous bats possess thick medullae (Studier et al., 1983). Increased medullary thickness is a feature observed in species that have the ability to concentrate urine and consequently minimise water loss (Geluso, 1978; Downs and Perrin, 1991). The loops of Henle and collecting ducts found in the medulla are responsible for urine concentration (Herrera et al., 2001). Species that do not produce concentrated urine have thinner or less thick medulla (Geluso, 1978).

Mammals that have a high protein intake from their diet are known to produce more concentrated urine than those of other diets (Carpenter, 1969; Studier and Wilson, 1983). A study on South African indigenous and invasive exotic fruit showed that the protein content of fruit pulp was generally low for all fruit species studied (Jordaan and Downs, 2012; Wilson and Downs, 2012). *Epomophorus wahlbergi* feeds on a mainly liquid diet (Monadjem et al., 2010) and does not need to concentrate urine; therefore the medulla and cortex are more or less the same size. Animals that shifted to nectar and fruit diets have decreased percentage of renal

medulla and increased percentage of renal cortex (Casotti et al., 2006). This structural arrangement is suggested to be an adaptation for excreting large volumes of water ingested from a nectar diet (Carpenter, 1969). Insectivorous bats and other mammals inhabiting arid regions possess renal structures adapted for water conservation (Geluso, 1978; Downs, 1996). The observed M/C ratio and PMT of *E. wahlbergi* is typical of a mesic species. The kidneys of fruit bats function to elevate frequent urination, producing large volumes of dilute urine and thus avoiding internal flooding by the high dietary water input (Arad and Korine, 1993).

The microvilli of *E. wahlbergi*'s intestine were long with a large surface area thus serves the purpose for nutrient absorption (Tedman and Hall, 1985; Makanya et al., 1997; Makanya et al., 2001). Even though digestion is rapid through the intestinal tract of bats (Winter, 1998), absorption of sugars in the intestine is three to four times faster than in the rat intestine (Keegan, 1977). Surface areas are regarded as important for noting differences in digestive and absorptive functions with regards to species, weights of each region indicate the amount of muscle, and consequently physical activity (Chivers and Hladik, 1980). *Epomophorus wahlbergi* consumes liquid nectar and juices of fruit and thus does not require the mechanisms to push food down; therefore the stomach and small intestine need not be muscular to force food down. A fermenting cavity, such as the caecum, may also not be necessary since frugivorous bats usually do not digest the fruits but only get the juices out before spitting out the pulp as 'spats'. The small intestine structure was observed to be similar to that described by Makanya et al. (1997; 2001) indicating efficient digestion and absorption of sugars in these bats.

In conclusion, analysis of the histological sections showed that the kidney structure of these bats is adapted for efficient water regulation. The microvilli of *E. wahlbergi*'s intestine were long with a large surface area thus serves the purpose for nutrient absorption.

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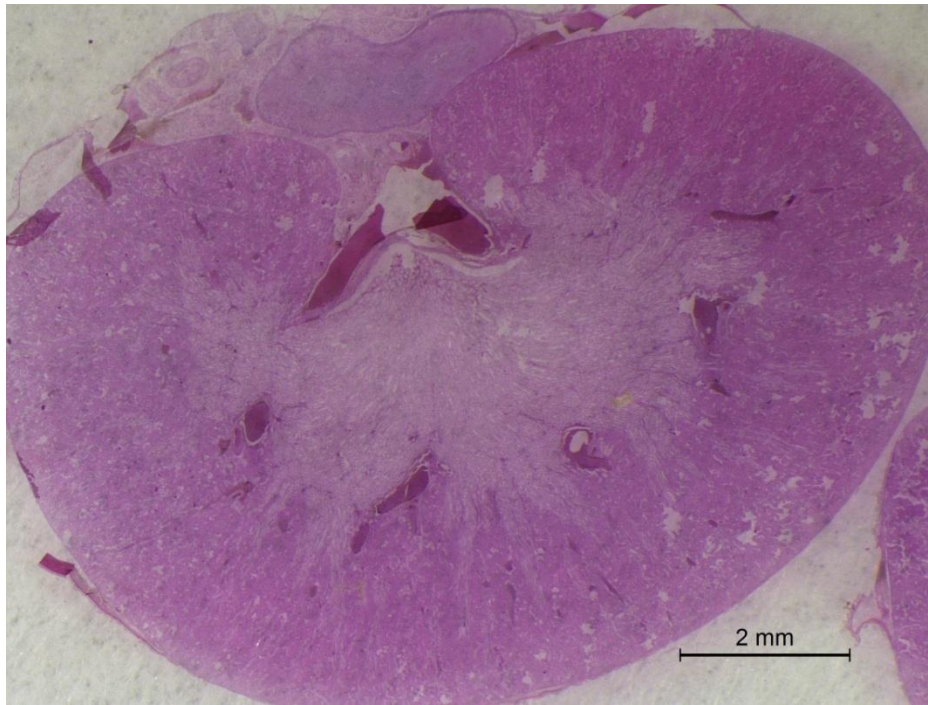


Fig. 1. The kidney structure of *Epomophorus wahlbergi* using light microscope (LM).

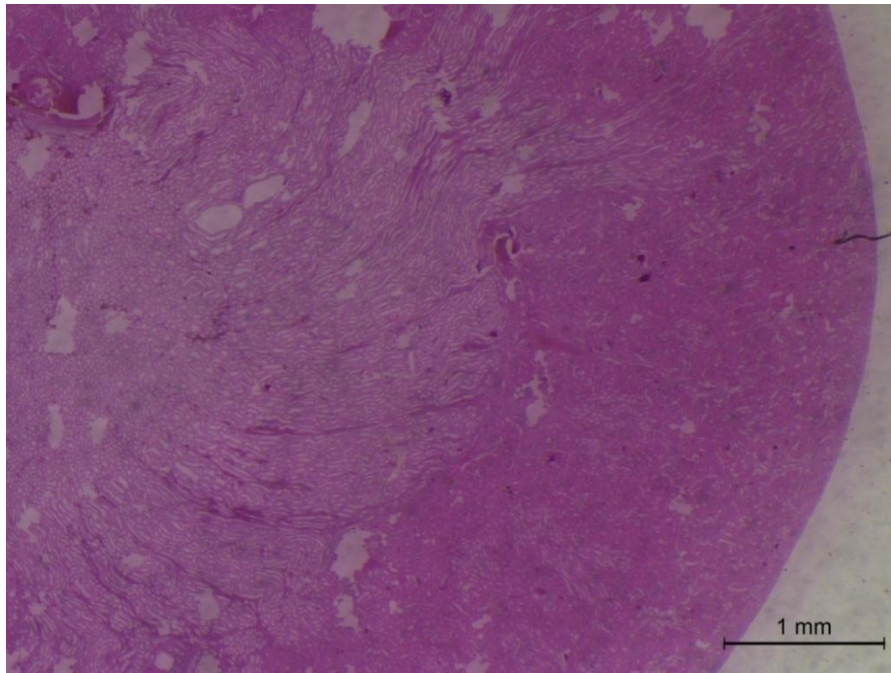


Fig. 2. The kidney structure of *Epomophorus wahlbergi* at higher magnification showing the cortex and medulla. Observed using light microscope (LM).



Fig. 3. Light microscope image showing a villi of the small intestine of *Epomophorus wahlbergi* observed using the fluorescent light microscope.

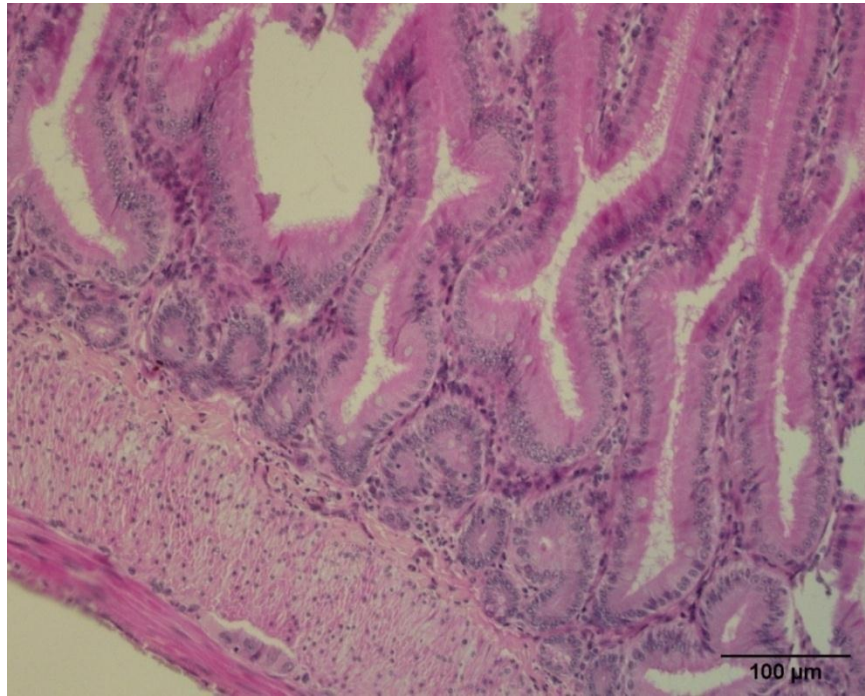


Fig. 4. Structure of the small intestine of *Epomophorus wahlbergi* observed using fluorescent light microscopy.

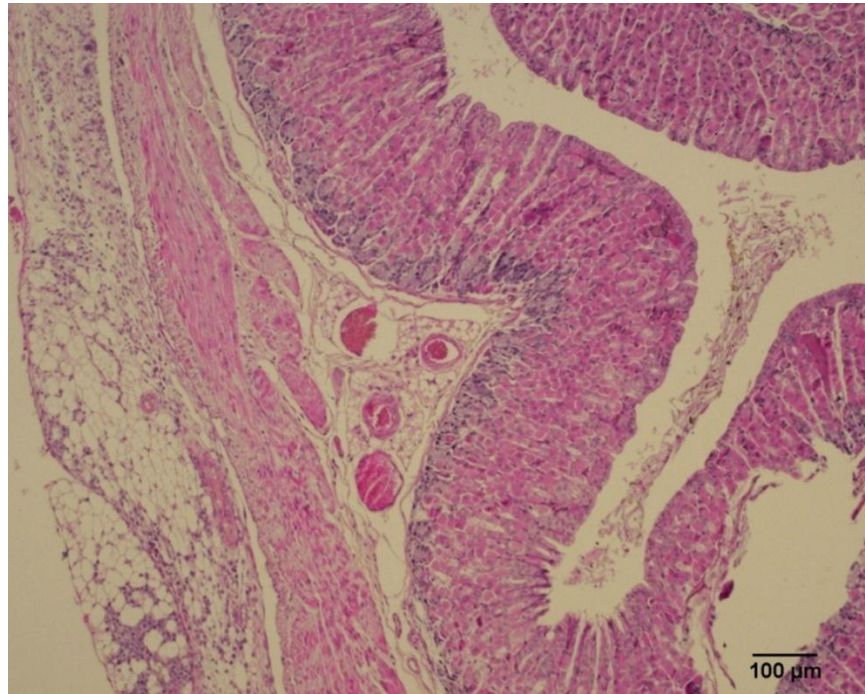


Fig. 5. Structure of the small intestine of *Epomophorus wahlbergi* observed at higher magnification using fluorescent light microscopy.

Chapter 6

CONCLUSION

Forest mammals are globally threatened due to habitat fragmentation, degradation, and other factors; thus making it important to conserve these habitats (Chapman et al., 2006; Altringham, 2011). In order to understand and develop better management strategies for forest conservation, we first need to evaluate and understand the mechanisms involved in plant-animal interactions in forest ecosystems (Saunders et al., 1991; Kirika et al., 2008).

Interactions between frugivores and the plants they feed on are of key importance in maintaining forest ecosystem structure (Herrera, 1999; Hamman and Curio, 1999; Shilton et al., 1999). Seed dispersal plays an essential role in forest regeneration and restoration (Wunderle, 1997), nectarivores and frugivores are effective pollinators and seed dispersers of many plant species (Fleming and Sosa, 1994; Hodgkinson et al., 2003; Matias et al., 2010; Corlett 2011; Fleming and Kress 2011). Frugivorous bats affect seed and fruit set thereby increasing the reproductive success of the plant species they feed on (Fleming and Sosa 1994; Whittaker and Jones 1994; Herrera, 1999). It is therefore important to understand the feeding ecology and physiology of frugivores in order to increase our knowledge on fruit-frugivore interactions. This thesis investigated feeding as well as physiology of a frugivorous bat, Wahlberg's epauletted fruit bat (*Epomophorus wahlbergi*). These bats have an important role in pollination and seed dispersal of various indigenous forest fruit species and invasive exotic species (Monadjem et al., 2010; Jordaan et al., 2012).

Animal-dispersed fruits reward their dispersers by fleshy fruit pulp (Lepczyk et al., 2000). However, frugivorous bats do not swallow the fruit pulp, but remove the fruit juices before spitting out the pulp as 'spats'. Many studies have been interested in morphological

structures of mammals and their adaptations for a specific diet, with several particularly interested in fruit bats (Freeman 1988; 1995; Dumont and O'Neal 2004; Morrison 1980; Birt et al., 1997).

Morphological studies on the mouth and tongue structures of Old-World fruit bats suggest that these bats are highly adapted to fruit and nectar diets (Morrison 1980; Freeman 1995; Birt et al., 1997; Abayomi et al., 2009). Fruit bats possess canine teeth which may be necessary to pluck and transport fruit (Freeman 1988) as well as widened, flat molar teeth to crush fruit (Freeman 1995). They use their elongated tongue to feed on both nectar and fruit (Freeman 1998). Frugivorous bat species have filiform papillae that cover a large surface area of the anterior portion of the tongue which allows the collection of nectar and fruit juices when feeding (Morrison 1980; Birt et al., 1997; Iwasaki 2002; Abayomi et al., 2009; Jackowiak et al., 2009). The posteriorly and symmetrically directed filiform papillae, together with the inner directed conical papillae, arrangement is suggested to probably allow food to move from the anterior part of the tongue and collect at the median line of the posterior region of the tongue, and thereafter move down to the pharynx (Emura et al. 2002; Jackowiak et al. 2009). The presence of fungiform papillae indicates the tongue's importance in taste determination (Iwasaki 2002). The morphological structure of tongue in *E. wahlbergi* was similar to that described for other mammals (Ojima et al. 1996; Makiyama et al. 1998; Ojima et al. 2000; Ojima 2001; Essawy 2008). The hard papillae structure on the posterior upper palate of *E. wahlbergi* is suggested to have a mechanical function, that is, crushing fruit pulp and ultimately maximizing fruit juice extraction (Chapter 2). Therefore, lingual and palatal structures of *E. wahlbergi* appear to be morphologically adapted to efficiently feed on a fruit and nectar diet (Chapter 2).

Fruit and nectar feeding bats have high energy demands because of the cost of flight (Tracy et al., 2007; Voigt and Speakman, 2007), and sugar is a good fuel because it is easily digested and absorbed thus providing these bats with energy (Suarez et al., 1990; Voigt and Speakman, 2007). South African indigenous and invasive exotic fruit available to frugivores are generally hexose dominant (Jordaan and Downs, 2012; Wilson and Downs, 2012). *Epomophorus wahlbergi* obtain juices and nutrients from the fruits they feed on (Skinner and Chimimba, 2005; Monadjem et al., 2012) and these bats are suggested to have high glucose intake irrespective of sugar concentration (Coleman and Downs, 2012; Downs et al., 2012). Nectarivores ingest large amounts of glucose when feeding (Kelm et al., 2011) and it is not known how they escape the negative effects of high glucose concentration (Beuchat and Chong, 1998). *Epomophorus wahlbergi*'s blood plasma glucose concentration was lower (5.24 ± 0.38 mmol/l) at 18h00 before feeding and increased during/after feeding (8.19 ± 1.24 mmol/l), however bats appeared to regulate it within limits (Chapter 3). Their range in concentrations was higher than the normal mammalian blood plasma glucose concentrations range. Consequently these bats appear to regulate their blood plasma glucose concentration, although at a range higher than normal mammalian levels, and thus reduce the negative consequences associated with hyperglycemia (Chapter 3).

Although soluble sugars are a key biochemical reward in fruits (Riba-Hernandez et al., 2003), fruits appear to have low protein content for the frugivores that feed on them (Witmer, 1998; Wilson and Downs, 2012). Proteins are important in a diet to provide nitrogen and amino acids required for maintaining body tissues (Morrison, 1980). Frugivores may therefore have challenges in meeting their protein requirements (Korine et al., 1996) due to their low-protein diet (fruit) (Witmer, 1998; Wilson and Downs, 2012). Nectarivorous and frugivorous birds,

however, are known to have adapted to low-protein requirements due to the characteristic nature of their diet (Tsahar et al., 2006). Frugivorous bats are also suggested to meet their protein requirements when feeding on a variety of fruits (Herbst, 1986; Delorme and Thomas, 1996; Korine et al., 1996; Herrera et al., 2002). Contrary to expectations, *E. wahlbergi* volumetric intake of the respective glucose and sucrose solutions varied among individual bats, with total volumetric intake highest for the solution with no protein (control) and lowest at 2.58 g/kg soy protein concentration solution for glucose and 5.68 and 7.23 g/kg soy protein concentration for sucrose (Chapter 4). These bats appeared to prefer sugar solutions without or low protein, and their daily protein intake was relatively low. This suggests they have low-protein requirements, and this relates to their characteristic low-protein fruit available in the wild (Chapter 4). These bats consume high amounts of the liquid diet to maximise energy gain and ultimately power their high energy needs (Downs et al., 2012).

Epomophorus wahlbergi feeds on fruit and nectar (Monadjem et al., 2010), interestingly, these and other frugivorous bats do not digest the fruits but only get the juices out before spitting out the pulp as ‘spats’ (Morrison, 1980). While it is important to understand fruit and/or nectar intake and preference, it is also essential to evaluate how these bats process this liquid diet. Feeding on a liquid nectar diet and fruit juices could cause physiological challenges for nectarivores and/or frugivores as they have to balance water and energy intake from this liquid diet (Beuchat et al., 1990; Lotz and Martinez del Rio, 2004; Brown et al., 2010). Frugivorous bats possess a kidney that is made up of two parts (medulla and cortex) (Studier et al., 1983). Kidneys with reduced relative medullary thickness, a large cortex and an undivided medulla are features that have been suggested to allow nectarivorous and/or frugivorous bats to filter large

amounts of water from their diet while conserving electrolytes (Studier et al., 1983; Schondube et al., 2001).

Frugivorous and nectarivorous bats are also known to have a rapid rate of digestion, with relation to sugar-rich meals (Winter, 1998). These bats' intestines possess microvilli that are long with a large surface area (Makanya et al., 1997). This assists the purpose for nutrient absorption, increasing the availability of membrane digestive enzymes and providing a larger surface for digestion to occur; consequently maximizing food exposure to absorptive cells in order to balance the short exposure due to rapid digestion rate (Tedman and Hall, 1985; Makanya et al., 1997; 2001).

The kidney structure of *E. wahlbergi* was observed to be made up of the cortex and medulla; the medulla was undivided (Chapter 5). The medulla and cortex of these bats are more or less the same size (Chapter 5). *Epomophorus wahlbergi* feeds on a liquid diet and does not need to concentrate urine; therefore the medulla and cortex are more or less the same size as there is no need for increased medullary size required for urine concentrating ability. The kidney structure of *E. wahlbergi* allows for efficient water removal when feeding on a watery diet (Chapter 5). The small intestine structure was observed to be similar to that described by Makanya et al. (1997; 2001) indicating efficient digestion and absorption of sugars in these bats (Chapter 5). The morphology of these structures is thus adapted to process large amounts of water from their diet while efficiently absorbing the required sugars for energy.

The results obtained in this study were useful in determining fruit and nectar feeding ecology of Wahlberg's epauletted fruit bat in the laboratory, as well as morphological adaptations of these bats to this type of diet; further studies are however needed. The mechanisms by which these bats regulate their blood plasma glucose levels need to be

determined. Future studies that determine intake on a selection of fruit with varying energy and protein content in order to develop comprehensive research to adaptation of these bats on low-protein diets are required. Radio tracking and determining feeding of individuals in the wild may also provide greater insight. This and possible future studies plays a role in understanding plant-animal interactions in an effort to maintain forest ecosystems.

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