ASPECTS OF THE THERMAL PHYSIOLOGY AND FRUIT DIGESTION OF KNYSNA (*TAURACO CORYTHAIX*) AND PURPLE-CRESTED (*GALLIREX PORPHYREOLOPHUS*) TURACOS

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ABSTRACT

Chapter 2: Avian frugivores have been somewhat poorly studied with regards to the effects that different fruit sugar types and concentrations have on their digestive efficiencies. Digestion of sugars in equicaloric and equimolar artificial fruit of different sugar types at varying concentrations and molarities were therefore investigated in two relatively large South African frugivores, the Knysna (*Tauraco corythaix*) and the Purple-crested (*Gallirex porphyreolophus*) Turaco. Artificial fruits of 6.6, 12.4 and 22% sucrose and glucose respectively and artificial fruits of 0.42, 0.83 and 1.66 mol L^{-1} sucrose and glucose respectively were used to determine daily food and energy intake, digestive efficiencies and digestive transit times. Digestive transit times of both Turaco species were slower with an increase in sugar concentration and molarity, irrespective of sugar type. Food intake of both Turaco species increased with decreasing concentration and molarity, irrespective of sugar type suggesting compensatory mechanisms for energy requirements. Apparent assimilation efficiencies of both Turaco species ranged from 61.4-90.0% and 60.2-92.4% for equicaloric and equimolar artificial fruit diets respectively. Consequently these two frugivores appear to be tolerant of sugar type and would be expected to select fruits based on energy yields. Future studies of the composition of indigenous forest fruit sugars may give insight into food preferences of the Turaco species and their role as potential seed dispersers of fruiting tree species.

Chapter 3: The effects that different fruit sugar types and concentrations have on food preferences of avian frugivores have been relatively poorly studied. Although it has been recently advocated that preference is based on equicaloric energy it is also important to note whether preferences change as energy content changes. Therefore, sugar preferences of equicaloric and equimolar artificial fruit of different sugar types at varying concentrations and molarities were investigated in two relatively large South African frugivores, Knysna (*Tauraco corythaix*) and Purple-crested (*Gallirex porphyreolophus*) Turacos. Artificial fruits of 6.6, 12.4 and 22% sucrose and glucose respectively and artificial fruits of 0.42, 0.83 and 1.66 mol L^{-1} sucrose and glucose respectively were used to determine sugar preferences. Knysna Turacos preferred the sucrose to the glucose equicaloric artificial fruit diet at low concentrations whereas Purple-crested Turacos showed no preference for either diet. Both Turacos species

preferred the sucrose equimolar artificial fruit diet to the glucose at low concentrations. At high concentrations neither species showed a preference for either equicaloric or equimolar artificial fruit diets. This suggests that energy requirements influence food preferences more than sugar type and that birds will select fruit that is higher in energy irrespective of sugar type. This complements an earlier study on digestion of sugars at varying concentrations of differing equicaloric and equimolar artificial fruit sugar types. It again emphasizes the need for future studies looking at the composition of indigenous forest fruit sugars in order to obtain insight into the role of these avian frugivores as potential seed dispersers of fruiting tree species.

Chapter 4: Avian frugivores may select fruit based on their seed loads and pulp to seed ratios and this may have important implications for their role as seed dispersal agents. Consequently the effect of different seed packaging was investigated in two relatively large South African frugivores, Knysna (*Tauraco corythaix*) and Purple-crested (*Gallirex porphyreolophus*) Turacos. Small-seeded artificial fruits containing on average five black plastic beads (2.24 mm diameter) and large-seeded artificial fruits containing one average one single black plastic bead (3.85 mm diameter) were used to investigate transit rates and food preference. Total seed volume in the two fruits was on average approximately equal (29.42 mm^3 and 29.88 mm^3 for small- and large-seeded fruits, respectively). Bead transit rates ranged from 38-45 min for Knysna Turacos and 36-50 min for Purple-crested Turacos with faster transit rates on the small-seeded diet. Pulp transit rates ranged from 25-39 min for Purple-crested Turacos and 34-40 min for Knysna Turacos. Purple-crested Turacos had significantly shorter pulp transit rates on the large-seeded than the small-seeded diet whereas Knysna Turacos had no significant difference between diets. Knysna Turacos preferred the small-seeded to the large-seeded artificial fruits whereas Purple-crested Turacos showed no preference for either diet. Further studies on avian dispersers will contribute to a greater understanding of their evolutionary relationship with fruiting plants. In particular, future studies establishing suitable seed sizes and volumes for use in determining the effect of seed packaging on larger birds are required as well as studies looking at seed packaging of indigenous forest fruit and the effects of seed packaging on digestion and food preference in indigenous avian frugivores may give insight into the role of avian frugivores as fruit consumers and seed dispersers.

Chapter 5: Fleshy-fruited plants and frugivores share a mutualistic relationship with plants offering the frugivores a reward of fleshy fruit pulp in order to disperse their seeds. In return frugivores receive a food source and consequently a source of energy. However all fruits are not equal in terms of available energy and consequently the digestion efficiency of six indigenous tree species was investigated in an avian frugivore, the Knysna Turaco (*Tauraco corythaix*). It was predicted that the Turacos would process these indigenous fruits efficiently and have fast transit rates and high intake rates irrespective of species. Fruits were fed to the Turacos in separate trials to determine daily food and energy intake, digestive efficiencies and digestive transit times. Digestive transit times of Knysna Turacos (c. 12-28 min) fall within the range of published examples for other frugivorous birds. Apparent assimilation efficiencies of the Turacos ranged from c. 15-84% and were generally lower than those observed in other avian frugivores. Future studies looking at the nutritional composition of indigenous forest fruit may provide insight into the Knysna Turacos digestive efficiency and consequently their food preferences and role as potential seed dispersers of fruiting tree species.

Chapter 6: Seed dispersal plays an important role in the persistence, regeneration and maintenance of plant communities. It is therefore not surprising that much attention has been paid to the germination potential of seeds ingested by frugivorous animals. Consequently the aim of this study was to determine what effect ingestion of seeds by Knysna Turacos (*Tauraco corythaix*) has on the germination rate and germination percentage of indigenous South African tree species. Fruits from twelve tree species were fed to the Turacos in separate trials and seed retention times were determined as it has been suggested that a longer seed retention time may increase germination rates. At the end of each trial, seeds were extracted from excreta of individual birds and planted in trays containing potting soil. Germination was recorded daily until 14 days post of no germination. Knysna Turacos seed retention times were in the range of those obtained by other studies with *Ficus sur* seeds having the fastest retention times (12.4 ± 0.8 min) and *Celtis africana* the slowest (34.6 ± 5.6 min). Mean percentage germination of ingested seeds was not significantly different to pulp removed and whole fruit seeds indicating that ingestion by Knysna Turacos did not enhance percentage germination through either seed coat abrasion or pulp removal. Seed ingestion by Knysna Turacos did not influence the rate at which seeds germinated in 75 % of the tree species while it did for ingested *F. lutea*, *F. natalensis* and *F. sur* seeds which germinated significantly sooner than whole fruit seeds. Future studies relating the composition of indigenous forest fruits to food preferences of Knysna Turacos may give insight into their role as potential seed dispersers of indigenous fruiting tree species.

Chapter 7: Frugivorous animals play a major role in dispersing tropical, and to a lesser extent, temperate tree species. In order to attract potential seed dispersers, plants generally offer a reward of fleshy fruit pulp. Criteria for fruit choice by avian frugivores are influenced by a number of non-nutritive (e.g. fruit size and colour) factors; and nutritional composition of the fruit. There is a paucity of nutritional composition and other fruit trait data of indigenous South African fruit. This information is necessary in order to determine which frugivores are likely to ingest which fruits and consequently act as potential seed dispersal agents. This information would provide us with an understanding of the inter-relationships between indigenous fruit and frugivores in South Africa. Consequently nutritional composition was investigated in various indigenous fruit species that avian frugivores feed on. Fruits were collected from 38 indigenous tree species found in KwaZulu-Natal afromontane and coastal forests. Pulp was freeze-dried to constant mass and then analyzed for sugar, lipid and protein content; and for water content determination. Fruit width in this study ranged from 4 mm (*Searsia rehmanniana* and *Trema orientalis*) to 40 mm (*Annona senegalensis, Ficus sur* and *Xylotheca kraussiana*). Of the fruits examined in this study 29% were black and 43% red when ripe. Most (84%) fruit species analysed for sugar content were hexose dominant with 50% being fructose and 34% being glucose dominant. Only 16% of the fruit species analysed were sucrose dominant. Fruits in this study were generally observed to be high (mean: 68.1 ± 3.3%; n = 30) in water content; and low in protein and lipid content respectively (mean: 8.2 ± 0.5%; 9.3 ± 2.2%; n = 30) indicating that these fruits species could be considered as nutrient-dilute. Future studies need to determine the nutritional composition of the remaining indigenous South Africa fruit in order to develop a comprehensive database as well as examining non-nutritive factors. Together this information would play a key role in understanding fruit preferences and consequently seed dispersal and would therefore be useful in developing management plans for forest conservation.

Chapter 8: Many seasonal thermoregulation studies have been conducted on Holarctic birds that live in predictable, highly seasonal climates with severe winters. However, relatively few studies have been conducted on their southern hemisphere Afrotropical counterparts that encounter less predictable climates with milder winters. These latter birds are expected to conserve energy in winter by down-regulating their metabolic rates. Therefore in this study, metabolic rate was measured during summer and winter in Knysna Turaco, *Tauraco corythaix* (Musophagiformes, Musophagidae) (c. 310 g), a non-passerine, in order to test whether there is energy conservation in winter. No overall significant differences in resting metabolic rates over a range of ambient temperatures were observed between winter and summer. However, whole-organism basal metabolic rates were 18.5 % higher ($p = 0.005$) in winter than in summer (210.83 ± 15.97 vs. 186.70 ± 10.52 O_2 h^{-1}). Knysna Turacos had broad thermoneutral zones ranging from 20-28 ^{o}C in winter and 10-30 ^{o}C in summer. These results suggest that Knysna Turacos show seasonal thermoregulatory responses that represent cold defense rather than energy conservation, which is contrary to what was expected.

Keywords: sugar digestion, sugar preference, seed packaging, basal metabolic rate (BMR), resting metabolic rate (RMR), phenotypic flexibility, indigenous fruit, germination percentage, germination rate, fruit sugars, protein, lipids, fruit size, fruit colour, Knysna Turaco, Purple-crested Turaco.

PREFACE

The data described in this thesis were collected in the Republic of South Africa from June 2008 to October 2010. Experimental work was carried out while registered at the School of Biological and Conservation Sciences, University of KwaZulu-Natal, Pietermaritzburg, under the supervision of Professor Colleen T. Downs.

This thesis, submitted for the degree of Doctor of Philosophy in the Faculty of Science and Agriculture, University of KwaZulu-Natal, Pietermaritzburg, 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.

………………………….
Amy-Leigh Wilson
November 2010

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

Downs

…………………………..
Professor Colleen T. Downs
Supervisor
November 2010

FACULTY OF SCIENCE AND AGRICULTURE

DECLARATION 1 - PLAGIARISM

I, Amy-Leigh Wilson, 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.

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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

A-L Wilson and CT Downs. Digestive efficiency of Knysna and Purple-crested Turacos fed varying concentrations of equicaloric and equimolar artificial fruits

Author contributions:

CTD conceived paper. A-LW collected and analyzed data, and wrote the paper. CTD contributed valuable comments to the manuscript.

Publication 2

A-L Wilson and CT Downs. Food preferences of Knysna and Purple-crested Turacos fed varying concentrations of equicaloric and equimolar artificial fruit

Author contributions:

CTD conceived paper. A-LW collected and analyzed data, and wrote the paper. CTD contributed valuable comments to the manuscript.

Publication 3

A-L Wilson and CT Downs. The effect of seed packaging on digestion and food preference by Purple-crested (*Gallirex porphyreolophus*) and Knysna (*Tauraco corythaix*) Turacos

Author contributions:

A-LW conceived paper with CTD. A-LW collected and analyzed data, and wrote paper. CTD contributed valuable comments to the manuscript.

Publication 4

A-L Wilson and CT Downs. Digestive efficiency of Knysna (*Tauraco corythaix*) Turacos fed on South African indigenous fruits

Author contributions:

CTD conceived paper. A-LW collected and analyzed data, and wrote the paper. CTD contributed valuable comments to the manuscript.

Publication 5

A-L Wilson and CT Downs. Effect of ingestion by Knysna Turacos (*Tauraco corythaix*) on germination success of fruit of indigenous South African tree species

Author contributions:

CTD conceived paper. A-LW collected and analyzed data, and wrote the paper. CTD contributed valuable comments to the manuscript.

Publication 6

A-L Wilson and CT Downs. Fruit nutritional composition of indigenous South African tree species

Author contributions:

CTD conceived paper. A-LW collected and analyzed data, and wrote the paper. CTD contributed valuable comments to the manuscript.

Publication 7

A-L Wilson, M Brown and CT Downs. Seasonal variation in metabolic rate of a relatively large frugivore, the Knysna Turaco (*Tauraco corythaix*)

Author contributions:

CTD conceived paper. A-LW collected and analyzed data, and wrote the paper. CTD and MB contributed valuable comments to the manuscript.

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November 2010

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In memory of my father

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"I am the voice of the voiceless,
Through me the dumb shall speak,
Till the deaf world's ear be made to hear
The wrongs of the wordless weak

From street, from cage, from kennel,
From stable and zoo, the wail
Of my tortured kin proclaims the sin
Of the mighty against the frail.

Oh, shame on the mothers of mortals
Who have not stooped to teach
Of the sorrow that lies in dear, dumb eyes,
The sorrow that has no speech.

And I am my brother's keeper,
And I shall fight his fight;
And speak the word for beast and bird
Till the world shall set things right.

- Ella Wheeler Wilcox, 1850-1919

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Chapter 1

INTRODUCTION

Indigenous forest is the smallest biome represented in South Africa, however, it supports a large proportion of South Africa's biodiversity (Cooper 1985, Geldenhuys 1989, Geldenhuys and MacDevette 1989, Rutherford and Westfall 1994, Mucina and Rutherford 2006). Anthropogenic changes have resulted in indigenous forests becoming severely fragmented and this is generally accepted to have a negative effect on South Africa's biodiversity (Cooper 1985, Geldenhuys 1989, Low and Rebello 1996, Lawes et al. 2000). Furthermore, fragmentation is likely to affect both the forest flora as well as the fauna with which these plants interact. It is thus essential that we gain an understanding of the mechanisms which govern plant-animal interactions in forest ecosystems in order to develop management plans for forest conservation (Kirika et al. 2008).

Many plants throughout the world use a reward of fleshy fruits to attract frugivorous animals in order to aid seed dispersal (Howe 1986). The pulp from these fruits provides frugivores with a key source of energy and it is the relationship between frugivores and fruit selection and preference that is a central question in foraging ecology (Lepczyk et al. 2000). Some frugivores have been found to digest or damage a large number of the seeds that they consume (Hulme 2002, Traveset and Verdú 2002) while many other frugivores seem to increase both the number of seeds that germinate and the rate at which they germinate (Krefting and Roe 1949, van der Pijl 1982).

The amount of available energy in different food types has been found to determine the amount of food that an individual will consume (Slansky and Wheeler 1992, Ayala-Berdon et al. 2008). Individuals will usually reduce their food intake as energy levels increase and this inverse relationship has often been attributed to compensatory feeding (Montgomery and Baumgardt 1965, Simpson et al. 1989, Slansky and Wheeler 1992, Ayala-Berdon et al. 2008).

There are many theories as to how birds select their food. It has been suggested that hummingbirds prefer sucrose-rich nectar while passerines prefer hexose-dominant nectar (Baker and Baker 1983) whereas Lotz and Schondube (2006) suggest that birds will generally prefer sucrose to hexose at high equicaloric concentrations and will prefer hexose sugars to sucrose at low equicaloric concentrations. Johnson and Nicolson (2008) however, recommend that a more useful distinction would be between specialist and generalist bird pollination systems with specialist bird pollinated plants producing nectar with high sucrose proportions and generalist bird pollinated plants producing nectar with low sucrose proportions. Another theory is that relatively small specialist nectarivores base their preferences on energy yields (Downs and Perrin 1996).

However, it has been suggested that experimental procedures may influence the results obtained from choice tests (Brown et al. 2008). While many studies make use of solutions that are equivalent by weight (Lotz and Nicolson 1996, Blem et al. 2000) or equimolar solutions that have approximately double the energy in a sucrose solution than in an equimolar hexose solution (Downs 1997, Downs 2000); it is recommended that choice tests use equicaloric sugar solutions as these are energetically equivalent (Fleming et al. 2004).

A number of factors such as fruit colour, size (Moermond and Denslow 1983), ease of harvest (Moermond and Denslow 1983), pulp mass (Johnson et al. 1985), sugar concentration (Levey 1987), seed size (Sorenson 1984), seed mass (Stanley and Lill 2002), pulp to seed ratio (Herrera 1981, Howe and Vande Kerckhove 1981) and secondary compounds (Murphy 1994) may also influence fruit choice. However the bird’s digestive ability and the fruit’s nutritional value are considered to be important factors (Worthington 1989, Martínez del Rio and Restrepo 1993, Johnson et al. 1985, Izhaki 1992, Fuentes 1994, Stanley and Lill 2002). Sugar types and concentrations in nectar have recently been explained by the plants associations with either specialist or generalist nectar-feeding birds (Johnson and Nicolson 2008). The sugars and sugar concentrations found in fruits, however, are not so easily explained and have traditionally been classified according to a dichotomy of high investment fruits (nutrient-dense) and low investment (nutrient-dilute) fruits (McKey 1975; Howe and Estabrook 1977; Howe and Smallwood 1982; Izhaki 1993). Nutrient-dense fruits are considered to be variable in protein, relatively high in lipids, and low in water and carbohydrates (Izhaki 1993). Conversely, nutrient-dilute fruits are considered to be low in fibre and protein, high in water, and have fewer carbohydrates than nutrient-dense fruits (Snow 1981; Herrera 1982; Izhaki 1993). As most fruits are considered to be nutrient-dilute (Levey 1987) avian frugivores need to select only nutrient-dense fruits or they would need to consume large amounts of nutrient-dilute fruits in order to obtain sufficient energy (Worthington 1989).

Fruits often contain large amounts of seeds that frugivorous birds cannot digest (Levey and Grajal 1991, Stanley and Lill 2002). These seeds therefore form a ballast load in the gut and impose costs on the birds (Levey and Grajal 1991, Stanley and Lill 2002). Firstly, it makes flight costly as the bird's mass is increased (Norberg 1990, Levey and Grajal 1991); and secondly, the volume of the gut decreases due to the indigestible bulk and therefore limits rates of food ingestion and digestion (McKey 1975, Levey and Grajal 1991, Murray et al. 1993). Avian frugivores may select fruit based on their pulp to seed ratios and seed loads and this may have important implications for their role as seed dispersal agents (Howe and Richter 1982, Howe 1986).

While it is important to understand fruit digestion and preferences based on sugar and seed loads it is also vital that we consider how the energy requirements of avian frugivores may differ seasonally. Birds are considered to be homeothermic endotherms meaning that by adjusting their metabolism they are able to maintain a constant body temperature over a wide range of ambient temperatures (Chaui-Berlinck et al. 2002).

Phenotypic flexibility is defined as a reversible variation within a characteristic of a single individual which may be a result of both unpredictable and predictable changes in environmental conditions (Debat and David 2001, Piersma and Drent 2003). This ability to suitably alter ones basal metabolic rate (BMR- defined as the minimum rate of energy expenditure by a non-growing, post-absorptive, non-reproductive homeotherm that is in its thermoneutral zone in the inactive phase of the circadian cycle (Brody 1945, Tieleman and Williams 2000, Dawson 2003, McKechnie and Wolf 2004)) in response to seasonal changes in ambient temperatures is a key component of thermoregulation (Dawson 2003, Smit et al. 2008).

Birds are able to improve their cold defenses by increasing their BMR which increases the threshold of thermogenesis (Dawson and O'Connor 1996, Swanson and Olmstead 1999). This enables birds to survive harsh winters (Dawson and O'Connor 1996, Swanson and Olmstead 1999). However, having a higher BMR means that birds then require more energy which is not always possible to sustain in winter when food resources are scarce (Dawson 2003). In order to conserve energy, birds therefore need to lower their BMR (Dawson 2003).

While most thermoregulatory studies have been conducted on Holarctic bird species (Bech 1980, Swanson and Weinacht 1997, Møller et al. 2004) there are relatively few seasonal studies conducted on Southern hemisphere Afrotropical bird taxa (but see Smit et al. 2008, Chamane and Downs 2009, Nzama et al. 2010). Afrotropical areas experience milder winters with more unpredictable climates that are as a result of El Niño Southern Oscillations (ENSO) (Stone et al. 1996, Jury and Nkosi 2000). It has been suggested that in winter the seasonal metabolic responses of Afrotropical endotherms will be one of energy conservation (Lovegrove and Smith 2003).

Birds that are able to adapt to a rapidly changing environment may have selective advantages over those that cannot (DeWitt et al. 1998, Piersma and Drent 2003). It is therefore crucial to understand the effects that changing environmental conditions have on phenotypic flexibility (Møller et al. 2004).

This study aimed to investigate (1) the transit times, digestion and preference of different sugar types at varying concentrations and molarities; (2) the effects of different seed loads on digestion and preference in two non-passerine avian frugivores, the Knysna (*Tauraco corythaix*) and the Purple-crested (*Gallirex porphyreolophus*) Turaco (Musophagiformes: Musophagidae); (3) the digestion efficiency, transit rates, the germination rate and germination percentage of indigenous South African tree species when ingested by Knysna Turacos; (4) the nutritional composition and other non-nutritive traits (e.g. fruit colour and size) were investigated in 38 indigenous fruit species; and (5) the effects of seasonal changes in ambient temperature on BMR and RMR of captive-bred Knysna Turacos.

It was predicted that both Turaco species would 1) have slower transit rates as concentration and molarity increased; 2) maintain their body mass on all diet treatments; 3) reduce their intake rates as sugar concentration increased, irrespective of sugar type, as they would regulate their energy intake; 4) have high apparent assimilation efficiency, irrespective of sugar type; 5) display no preference for either equicaloric glucose or equicaloric sucrose irrespective of concentration; 6) would display a preference for equimolar sucrose to equimolar glucose based on differences in energy yields; 7) have shorter mean retention times for seeds than for pulp; 8) have shorter mean retention times for large seeds than for small seeds; 9) consume more of the large-seeded fruits than the small-seeded fruits; and 10) would prefer large-seeded fruits to small-seeded fruits in the preference tests. It was also predicted that Knysna Turacos would 11) process the indigenous fruits efficiently; 12) have fast transit rates; 13) have high intake rates irrespective of fruit species; and 14) conserve energy as was predicted for Afrotropical endotherms (Lovegrove and Smith 2003) and BMR and RMR would therefore be lower in winter than in summer. Knysna Turacos are relatively large (c. 310g) fairly common forest (Oatley 1997) frugivores that are endemic (Rowan 1983) to South Africa (du Plessis and Dean 2005a). Similarly, Purple-crested Turacos are relatively large (c. 300g) locally common frugivores with part of their distribution occurring in lowland forests in eastern South Africa (du Plessis and Dean 2005b). While both species are typically resident, they may locally track fruit in response to availability (Rowan 1983). Knysna and Purple-crested Turacos were chosen as both species are relatively large and fairly common and little is known about sugar digestion, fruit preferences and thermoregulation in large frugivores in South Africa.

This thesis is presented as chapters for submission to Journals:

Chapter 2. Digestive efficiency of Knysna and Purple-crested Turacos fed varying concentrations of equicaloric and equimolar artificial fruits. Formatted for the Journal of Experimental Biology. 2011. 214: 607-612.

Chapter 3. Food preferences of Knysna and Purple-crested Turacos fed varying concentrations of equicaloric and equimolar artificial fruit. Formatted for the Journal of Experimental Biology. 2011. 214: 613-618.

Chapter 4. The effect of seed packaging on digestion and food preference by Purple-crested (*Gallirex porphyreolophus*) and Knysna (*Tauraco corythaix*) Turacos. Formatted for the Journal of Ornithology. In Press. DOI 10.1007/s10336-010-0564-2

Chapter 5. Digestive efficiency of Knysna (*Tauraco corythaix*) Turacos fed on South African indigenous fruits. Formatted for the Journal of Ornithology.

Chapter 6. Effect of ingestion by Knysna Turacos (*Tauraco corythaix*) on germination success of fruit of indigenous South African tree species. Formatted for the Journal of Oecologia.

Chapter 7. Fruit nutritional composition of indigenous South African tree species. Formatted for the Journal of Plant Ecology.

Chapter 8. Seasonal variation in metabolic rate of a relatively large frugivore, the Knysna Turaco (*Tauraco corythaix*). Formatted for the Journal of Thermal Biology. In Press.

With a final concluding chapter.

As chapters have been prepared as stand alone manuscripts, some overlap and repetition between chapters has been unavoidable.

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Chapter 2

DIGESTIVE EFFICIENCY OF KNYSNA AND PURPLE-CRESTED TURACOS FED VARYING CONCENTRATIONS OF EQUICALORIC AND EQUIMOLAR ARTIFICIAL FRUITS

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Avian frugivores have been somewhat poorly studied with regards to the effects that different fruit sugar types and concentrations have on their digestive efficiencies. Digestion of sugars in equicaloric and equimolar artificial fruit of different sugar types at varying concentrations and molarities were therefore investigated in two relatively large South African frugivores, the Knysna (*Tauraco corythaix*) and the Purple-crested (*Gallirex porphyreolophus*) Turaco. Artificial fruits of 6.6, 12.4 and 22% sucrose and glucose respectively and artificial fruits of 0.42, 0.83 and 1.66 mol L^{-1} sucrose and glucose respectively were used to determine daily food and energy intake, digestive efficiencies and digestive transit times. Digestive transit times of both Turaco species were slower with an increase in sugar concentration and molarity, irrespective of sugar type. Food intake of both Turaco species increased with decreasing concentration and molarity, irrespective of sugar type suggesting compensatory mechanisms for energy requirements. Apparent assimilation efficiencies of both Turaco species ranged from 61.4-90.0% and 60.2-92.4% for equicaloric and equimolar artificial fruit diets respectively. Consequently these two frugivores appear to be tolerant of sugar type and would be expected to select fruits based on energy yields. Future studies of the composition of indigenous forest fruit sugars may give insight into food preferences of the Turaco species and their role as potential seed dispersers of fruiting tree species.

Key words: sugar type, sugar digestion, frugivory, Knysna Turaco, Purple-crested Turaco, artificial fruits, forest fruit.

Introduction

The amount of energy available in the different food types will influence the amount of food consumed by the individual (Ayala-Berdon et al., 2008) with intake levels usually being reduced as levels of available energy increase (Slansky and Wheeler, 1992). This 'intake response' (Castle and Wunder, 1995) has been well studied in nectarivorous birds (Collins, 1981; López-Calleja et al., 1997; McWhorter and Martínez del Rio, 2000), but remains poorly understood in frugivorous birds (but see Levey and Martínez del Rio, 1999). This inverse relationship between nutritional levels and food intake has often been attributed to compensatory feeding (Simpson et al., 1989) where the amount of food eaten is regulated to maintain a constant amount of assimilated energy (Montgomery and Baumgardt, 1965; Slansky and Wheeler, 1992).

Many studies have examined the effects of sugar type and concentration on food preferences, digesta transit times, digestive efficiencies, and food intake in nectarivorous birds (Tamm and Gass, 1986; Downs and Perrin, 1996; Downs, 2000; Schondube and Martínez del Rio, 2003; Brown et al., 2008; Fleming et al., 2008). Digesta transit times are important as they may give an indication of how well nutrients are absorbed by the gut (Witmer, 1998a). Avian frugivores, in contrast to avian nectarivores, have been poorly studied with regards to the effects that different fruit sugar types, concentrations and molarities have on their digestive efficiency (Levey and Martínez del Rio, 1999; Levey and Martínez del Rio, 2001; Lotz and Schondube, 2006). While fruit choice by avian frugivores may be influenced by a number of non-nutritive factors such as fruit colour, size, and secondary compounds (Murphy, 1994), the bird's digestive ability and the fruit's nutritional value are considered to be important factors (Martínez del Rio and Restrepo, 1993; Johnson et al., 1985; Izhaki, 1992; Fuentes, 1994). Indeed, Worthington (1989) suggests that digestion is likely to be the most important restriction placed on avian frugivores.

Fleshy fruits are roughly regarded as being either nutrient-dense or nutrient-dilute (Izhaki, 1993). Nutrient-dense fruits are considered to be relatively high in lipids, low in water and carbohydrates and variable in protein (Izhaki, 1993) whereas nutrient-dilute fruits are high in water, have fewer carbohydrates than lipid-rich fruits and are low in fibre and protein (Snow, 1981; Herrera, 1982; Izhaki, 1993). Most fruits are nutrient-dilute (Levey, 1987) therefore, avian frugivores need to consume large amounts or only select those fruits that are nutrient-rich (Worthington, 1989).

Fruit choice by some avian frugivores may be affected by their ability to digest the different sugars efficiently (Avery et al., 1999). Several studies have shown that some families from the Sturnid-Muscicapid lineage are unable to digest sucrose efficiently (Schuler, 1983; Martínez del Rio, 1990; Martínez del Rio and Restrepo, 1993; Levey and Martínez del Rio, 2001). This is explained by a lack of the enzyme sucrase in these species, without which sucrose cannot be hydrolysed into glucose and fructose, which can be easily assimilated (Martínez del Rio et al., 1988; Martínez del Rio et al., 1989; Martínez del Rio and Stevens, 1989; Karasov and Levey, 1990, Malcarney et al., 1994). Even those birds that do possess sucrase may prefer hexose sugars in choice tests as they may not be able to digest sucrose efficiently enough (Martínez del Rio et al., 1992; Avery et al., 1995).

A clear gap in scientific research are studies conducted on the sugar content of fruits that are consumed by most non-passerine frugivorous birds (e.g. pigeons, hornbills, trogons and turacos) (Snow, 1981; Levey and Martínez del Rio, 2001; Lotz and Schondube, 2006). Some orders such as the African turacos (Musophagiformes) consist exclusively of frugivores (Kissling et al., 2009) and play an important role in plant reproduction and as seed dispersal agents (Herrera, 2002). Consequently the sugar preferences and the digestion of different sugar types at varying concentrations and molarities were investigated in two non-passerine relatively large avian frugivores, Knysna (*Tauraco corythaix*) and Purple-crested (*Gallirex porphyreolophus*) Turaco (Musophagiformes: Musophagidae).

The aim of the study was to determine if digestion efficiency was affected by sugar concentration and type. Equicaloric artificial fruit were first used and then the experiments were repeated using equimolar artificial fruits to show the possible importance of energy in foraging decisions. It was hypothesized that sugar type and concentration would affect digestion in the two species of Turacos. It was predicted that both species would 1) have slower digesta transit times as concentration and molarity increased; 2) reduce their intake times as sugar concentration increased, irrespective of sugar type, as suggested by Simpson et al. (1989)'s compensatory feeding hypothesis; and 3) have high apparent assimilation efficiency, irrespective of sugar type, as shown by Martínez del Rio (1990), Lotz and Nicolson (1996), Jackson et al. (1998) and Witmer (1999).

Knysna Turacos are relatively large (c. 310g) fairly common forest (Oatley, 1997) frugivores that are endemic (Rowan, 1983) to South Africa (du Plessis and Dean, 2005a). Similarly, Purple-crested Turacos are relatively large (c. 300g) locally common frugivores with part of their distribution occurring in lowland forests in eastern South Africa (du Plessis and Dean, 2005b). While both species are typically resident, they may locally track fruit in response to availability (Rowan, 1983).

Due to a lack of knowledge of South African fruit sugars, we were unable to devise an artificial fruit diet that reflected typical ranges of the nutritional composition of indigenous, bird-dispersed fruits in South Africa. In this study, we therefore used the same artificial glucose fruit diet (Table 1) as Witmer (1998a) and our artificial diet consequently has similar sugar and protein content to bird-dispersed sugary fruits in the United States (Witmer, 1998a). This artificial diet controlled for the effects of seeds and secondary compounds that are known to affect digestion (Bairlein, 1996; Cipollini and Levey, 1997; Fukui, 2003). A second artificial diet was made in which equicaloric sucrose replaced glucose and a third artificial diet was made in which equimolar sucrose replaced glucose (Table 1).

Materials and Methods

Bird Capture and Maintenance

One Purple-crested Turaco was borrowed from Mr R. Poley and four captive-bred Purple-crested and nine captive-bred Knysna Turacos were sourced from Mr. M.C. Weber under permit from Ezemvelo KZN Wildlife (permit number: 1781/2008). The birds were housed in pairs (with the exception of the loaned bird that was housed individually) in outside aviaries (1 x 2.12 x 2.66 m) for one week before experiments were conducted. A maintenance diet was fed daily. The maintenance diet consisted of a choice of mealworms (*Tenebrio molitor* larvae), pears, paw-paws, apples, carrots, bananas, oranges and/or grapes. All fruit either had their skin removed (paw-paws, bananas and oranges) and cut into c. 1 x 1 x 1 cm cubes or was grated. A mixture of Aviplus Softbill/Mynah pellets and crumble (Avi-products, Durban, South Africa) was added to the maintenance diets. Water was provided *ad libitum*.

Experiments

The birds were moved indoors for one week before any trials were conducted. During the experiments, birds were housed in individual cages (42.7 x 43 x 59.3 cm) in a constant environment room with a 12 L: 12 D photoperiod at 25 ± 1°C. To ensure a post-absorptive state at the beginning of each experiment any uneaten food was removed at 18h00 previously and no food was available overnight as birds did not feed at night.

Bird’s body mass (g) was measured daily 30-40 min prior to the light phase (06h00) and again in the evening (18h00). All food items were measured to 0.01 g and the bird’s body mass was measured to 0.5 g.

Digesta transit times and digestive efficiency of sucrose and glucose diet treatments were investigated using three equicaloric concentrations: low (6.6%), medium (12.4%) and high (22%) respectively; and then three equimolar concentrations: low (0.42 mol L^{-1}), medium (0.83 mol L^{-1}) and high (1.66 mol L^{-1}) respectively following the methods of Witmer (1998a).

For each diet treatment, artificial fruits were made by mixing all the relevant ingredients into 1 L of boiling water (Witmer, 1998a). Thereafter, the mixture was left to solidify, stirring all the while to ensure that the ingredients were dispersed evenly throughout the mixture. All dietary treatments were analysed for energy content (Animal Science, University of KwaZulu-Natal).

Digesta transit times and digestive efficiency of artificial fruit diets

On the experimental days, birds were provided with a weighed amount of food at 06h00. Each experimental diet was dyed with red food colourant (Moirs, R.S.A.) and the time that the birds’ first ate and the first appearance of the dye in their excreta recorded to determine digesta transit times. Each experiment was run for 12 h (from 06h00 to 18h00). Controls of each experimental artificial diet were used to estimate evaporative moisture loss from uneaten food. Birds were fed the maintenance diet between experiments and not fed the artificial diet on any two consecutive days. Excreta and food samples were oven dried at 60^{o}C to constant mass.

For each bird, overall daily food intake (DFI measured as wet weight) was determined for the respective diet treatments. These were quantified by subtracting the mass of the food remaining from the amount given and accounting for desiccation. The DFI was converted to daily gross energy intake (GEI) by accounting for the respective fraction of water content and multiplying by the energy content for dry weight of each diet type. Daily excreta energy loss (EE) was calculated as the mass of dried excreta produced multiplied by its dry weight energy value. Daily energy assimilated (DEA) was calculated as GEI subtracting EE. Daily apparent energy assimilation efficiency (AE) was calculated as DEA divided by GEI, and was then squareroot transformed. Finally each respective calculation was converted per gram individual body mass by dividing by the mean of initial and final body mass on the day of experimentation for comparisons. The above protocol was repeated for each diet treatment and each bird species.

Analyses

As the same birds were used repeatedly, digesta transit time, body mass, GEI, DEA and food intake rates were compared with a Generalised Linear Model Repeated Measures of Analysis of Variance (GLM RMANOVA) and Post-hoc Tukey tests, using STATISTICA version 7 (Statsoft, Tulsa, USA). Mean ± SE was reported for all values.

Results

Equicaloric digesta transit times

There were no significant differences in digestive transit times of Knysna Turacos between the sucrose and the glucose artificial diet treatments at each concentration ($F_{(2, 16)} = 0.93$, $p = 0.415$). Digesta transit times were slower with an increase in glucose and sucrose concentration, and showed the greatest variability with glucose 6.6% equicaloric artificial fruit diet (Table 2). The glucose 6.6% artificial fruit diet had the fastest digesta transit time (31.0 ± 2.8 min, n = 9) while the sucrose 22% artificial fruit diet had the slowest digesta transit time (44.4 ± 1.8 min, n = 9) (Table 2).

Similarly there were no significant differences in digestive transit times of Purple-crested Turacos between the sucrose and the glucose artificial diet treatments at each concentration ($F_{(2, 8)} = 2.93$, $p = 0.108$). In addition digesta transit times were slower with an increase in glucose and sucrose concentration, and unlike Knysna Turacos they showed the greatest variability at the sucrose 12.4% artificial fruit diet (Table 2). In contrast to Knysna Turacos, the sucrose 6.6% artificial fruit diet had the fastest digesta transit time (20.6 ± 1.6 min, n = 5). Similar to Knysna Turacos the sucrose 22% artificial fruit diet had the slowest digesta transit time (41.1 ± 2.3 min, n = 5) (Table 2).

Equimolar digesta transit times

There were no significant differences in digestive transit times of Knysna Turacos between the sucrose and the glucose artificial diet treatments at each concentration ($F_{(2, 16)} = 0.88$, $p = 0.435$). Again digesta transit times were slower with an increase in glucose and sucrose molarity but showed the greatest variability with sucrose 1.66 mol L^{-1} artificial fruit diet (Table 2). The glucose 0.42 mol L^{-1} artificial fruit diet had the fastest digesta transit time (31.0 ± 8.4 min, n = 9) while the sucrose 0.83 mol L^{-1} artificial fruit diet had the slowest digesta transit time (65.5 ± 5.2 min, n = 9) (Table 2).

Similarly, there were no significant differences in digestive transit times of Purple-crested Turacos between the sucrose and the glucose artificial diet treatments at each concentration ($F_{(2, 8)} = 0.54$, $p = 0.322$). Digesta transit times were slower with an increase in glucose and sucrose molarity and the greatest variability of digesta transit times was with sucrose 0.42 mol L^{-1} artificial fruit diet (Table 2). The glucose 0.42 mol L^{-1} artificial fruit diet had the fastest digesta transit time (24.6 ± 1.3 min, n = 5) while the sucrose 1.66 mol L^{-1} artificial fruit diet had the slowest digesta transit time (50.8 ± 4.5 min, n = 5) (Table 2).

Body mass

There were no significant differences in mean body mass of either Knysna Turacos or Purple-crested Turacos when fed the six respective artificial equicaloric fruit diets

($F_{(2, 16)} = 0.464$, $p = 0.64$; and $F_{(2, 8)} = 1.64$, $p = 0.253$). Similarly there were no significant differences in mean body mass of either Knysna Turacos or Purple-crested Turacos when fed the six respective artificial equimolar fruit diets ($F_{(2, 16)} = 0.77$, $p = 0.481$; and $F_{(2, 8)} = 4.50$, $p = 0.05$).

Equicaloric food intake and assimilation

Knysna Turacos ingested significantly different amounts of the six respective artificial equicaloric fruit diets ($F_{(2, 16)} = 6.63$, $p = 0.008$, Table 2), with greater amounts ingested at lower concentrations. There were significant differences between the glucose and sucrose diet treatments in terms of GEI ($F_{(2, 16)} = 11.55$, $p < 0.001$, Table 2). There were also significant differences between the glucose and the sucrose diets in terms of energy loss in the excreta ($F_{(2, 16)} = 3.65$, $p = 0.049$, Table 2). As a consequence of GEI and EE, DEA was significantly different in the glucose and the sucrose diet treatments ($F_{(2, 16)} = 4.56$, $p = 0.027$, Table 2). Apparent energy assimilated (AE) was low for all six diet treatments and there were no significant difference between the treatments ($F_{(2, 16)} = 1.86$, $p = 0.188$, Table 2).

Similarly Purple-crested Turacos ingested significantly different amounts of the six equicaloric artificial fruit diets ($F_{(2, 8)} = 12.81$, $p = 0.003$, Table 2), with greater amounts ingested at the lower concentrations. There were significant differences between the glucose and sucrose diet treatments in terms of GEI ($F_{(2, 8)} = 26.20$, $p = 0.003$, Table 2). There were no significant differences between the glucose and the sucrose diets in terms of energy loss in the excreta ($F_{(2, 8)} = 0.73$, $p = 0.509$, Table 2). Again as a consequence of GEI and EE, DEA was significantly different between the glucose and the sucrose diet treatments ($F_{(2, 8)} = 58.90$, $p < 0.001$, Table 2). AE was low for all six diet treatments and there were significant differences between the treatments ($F_{(2, 8)} = 19.24$, $p = 0.0005$, Table 2).

Equimolar food intake and assimilation

Knysna Turacos ingested significantly different amounts of the six respective equimolar artificial fruit diets ($F_{(2, 16)} = 21.06$, $p < 0.0001$, Table 2), with greater amounts ingested at the lower molarities. There were no significant differences between the glucose and sucrose diet treatments in terms of GEI ($F_{(2, 16)}$ = 5.03, p = 0.202, Table 2). There were no significant differences between the glucose and the sucrose diets in terms of energy loss in the excreta ($F_{(2, 16)}$ = 1.84, p = 0.191, Table 2). As a consequence of GEI and EE, DEA was significantly different in the glucose and the sucrose diet treatments ($F_{(2, 16)}$ = 5.10, p = 0.020, Table 2). AE was low for all six diet treatments and there were significant differences between the treatments ($F_{(2, 16)}$ = 3.96, p = 0.040, Table 2).

In contrast Purple-crested Turacos did not ingest significantly different amounts of the six equimolar artificial fruit diets ($F_{(2, 8)}$ = 3.58, p = 0.077, Table 2). There were significant differences between the glucose and sucrose diet treatments in terms of GEI ($F_{(2, 8)}$ = 7.03, p = 0.017, Table 2). There were also significant differences between the glucose and the sucrose diets in terms of energy loss in the excreta ($F_{(2, 8)}$ = 7.23, p = 0.016, Table 2). As a consequence of GEI and EE, DEA was significantly different between the glucose and the sucrose diet treatments ($F_{(2, 8)}$ = 5.88, p = 0.027, Table 2). AE was low for all six diet treatments but there were no significant differences in apparent energy assimilation efficiency between the treatments ($F_{(2, 8)}$ = 1.80, p = 0.227, Table 2).

Discussion

Irrespective of sugar type, both Knysna and Purple-crested Turacos ingested greater amounts of the artificial diets at low sugar concentrations than at high sugar concentrations. They also ingested greater amounts of the artificial diets at low sugar molarity than at high sugar molarity. These results are similar to those obtained for some nectarivores such as sunbirds (e.g. Lotz and Nicolson, 1999, Köhler et al., 2008) and hummingbirds (e.g. López-Calleja et al., 1997). This suggests that increased intake rates at low concentrations and low molarities are necessary to meet their energy requirements as suggested by the compensatory feeding hypothesis (Simpson et al., 1989).

This emphasises the fact that when comparing food intake of different concentrations or molarities of one sugar type, energy requirements rather than preference may determine the amount eaten (Wellmann and Downs, 2009). Indeed, Knysna and Purple-crested Turacos maintained their mean body mass when fed on all six equicaloric artificial diet treatments and on all six equimolar artificial diet treatments indicating that they obtained adequate energy from all the treatments (Downs, 2008). The results obtained from the present study showed a higher energy intake of low equicaloric glucose concentrations than of high equicaloric sucrose concentrations for Knysna Turacos which are similar to the results of Wellman and Downs (2009). Interestingly, Purple-crested Turacos showed a higher energy intake of high equicaloric sucrose concentrations than of low equicaloric glucose concentrations.

The results obtained from the present study showed a higher energy intake of medium equimolar sucrose concentrations compared with high equimolar glucose concentrations for Knysna Turacos. This suggests that they are making a choice based on sugar type rather than concentration. Interestingly, Purple-crested Turacos showed a higher energy intake of high equimolar sucrose concentrations than of medium equimolar sucrose concentrations. This suggests that they are making a choice based on energy levels rather than sugar type.

Apparent assimilation efficiency may be defined as how well a particular diet is digested (Witmer, 1998b). Knysna Turacos showed no significant differences in the apparent assimilation efficiencies on any of the six equicaloric artificial diet treatments whereas Purple-crested Turacos did exhibit significant differences between the equicaloric treatments. In contrast, Knysna Turacos did exhibit significant differences in the apparent assimilation efficiencies between the six equimolar artificial diet treatments whereas Purple-crested Turacos showed no significant differences between the equimolar treatments. Apparent assimilation efficiencies obtained in this study were lower than those obtained for typical nectarivorous species (see Martínez del Rio, 1990; Lotz and Nicolson, 1996; Jackson et al., 1998) and those obtained by Witmer (1999) for three frugivorous American thrush species (American Robin *Turdus migratorius*, Wood Thrush *Hylocichla mustelina*, and Gray-cheeked Thrush *Catharus minimus*) and Cedar Waxwings (*Bombycilla cedrorum*) which ranged from 91-99% (Witmer, 1999). Worthington (1989) also obtained high assimilation efficiencies ranging from 86-97.6% in two Manakin species (*Manacus vitellinus* and *Pipra mentalis*). Our results are more consistent with those obtained by Wellmann and Downs (2009) who obtained apparent assimilation efficiencies of 79.3-85.6% on artificial equicaloric glucose fruit and 69.0-78.4% on artificial equicaloric sucrose fruit for Cape white-eyes (*Zosterops virens*).

Food that is allowed to stay in the gut for a longer period may result in an increase in the absorption of nutrients (Klasing, 1998). Digestive transit times therefore may indicate how well nutrients are absorbed by the gut (Witmer, 1998a). For both Turaco species, digesta transit times increased with increasing concentration and molarity for the glucose and the sucrose artificial diets. While both species experienced the slowest digesta transit times on high equicaloric sucrose concentrations, digesta transit times were the shortest on low equicaloric concentrations of glucose for Knysna Turacos and low equicaloric concentrations of sucrose for Purple-crested Turacos. Digesta transit times for the equimolar artificial diets were the slowest on medium sucrose molarity and high sucrose molarity for Knysna and Purple-crested Turacos respectively. Both species experienced the shortest digesta transit times on low glucose molarity. These results are similar to those of Wellman and Downs (2009) who experienced similar trends for the glucose equicaloric artificial diet. However, digesta transit times were measured on fasted birds and may therefore not be representative of typical digesta transit times as birds usually have food in their gut (Levey and Martínez del Rio, 1999).

Knysna Turacos ingested more of the low equicaloric glucose diet while Purple-crested Turacos ingested more of the low equicaloric sucrose diet and these diets had the shortest digesta transit times respectively. Similarly both Knysna and Purple-crested Turacos ingested more of the low equimolar glucose diet and these diets had the shortest digesta transit times respectively. Similar results were obtained by Witmer (1998a) who suggested that birds would have to have a higher intake of dilute food to increase energy intake and to meet energy requirements as these foods would be processed more swiftly through the gut. Witmer (1998a) suggests that birds may be able to compensate for low sugar concentration by being able to process low sugar fruits more swiftly. This has disadvantages in that more time would then be required to find, ingest and digest these fruits (Witmer, 1998a).

Our results show that both Turaco species had slower digesta transit times as concentration and molarity increased; and suggest that both Turaco species would be able to subsist on either sucrose or glucose rich fruits as, regardless of sugar type, both species were able to maintain body weight on all six artificial fruit diets. Our results also show that both Turaco species were observed to regulate their energy intake with higher intake rates occurring on more dilute sugar concentrations and while apparent assimilation efficiencies obtained for both Turaco species were lower than most nectar and fruit studies they still fell within the range of published examples. Consequently we accept our predictions.

In summary these two frugivores appear to be tolerant of sugar type. Future studies looking at the composition of indigenous forest fruit sugars may give insight into food preferences of the Turaco species and their role as potential seed dispersers of fruiting tree species.

List of symbols and abbreviations

DFI- daily food intake
GEI- gross energy intake
EE- excreta energy loss
DEA- daily energy assimilated
AE- apparent energy assimilation efficiency

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Table 1 Composition of three equicaloric and three equimolar artificial fruit diets used in the diet treatments

	Diet Composition (g)		
Ingredients	Low	Medium	High
D-glucose	75	150	300
Equicaloric sucrose	71.2	142.4	284.8
Equimolar sucrose	142.47	284.93	569.87
Water	1000	1000	1000
Wheat bran	50	50	50
Agar	10	10	10
Sodium chloride	0.75	0.75	0.75
Dicalcium phosphate	0.80	0.80	0.80
Vitamin supplement	0.75	0.75	0.75

Table 2 Mean ± SE of body mass, transit time, amount eaten, gross energy intake, excreta energy, daily energy assimilated and apparent assimilation efficiency of Knysna and Purple-crested Turacos when fed artificial fruit diets of low (6.6 % and 0.83 mol L^{-1}), medium (12.4 % and 0.83 mol L^{-1}) and high (22 % and 1.66 mol L^{-1}) glucose and sucrose concentrations. Treatments with letters in common are not significantly different at the 5 % level

	Equicaloric (%)						**Equimolar (mol L^{-1})**					
	Glucose			**Sucrose**			**Glucose**			**Sucrose**		
Knysna Turaco	**6.6**	**12.4**	**22**	**6.6**	**12.4**	**22**	**0.42**	**0.83**	**1.66**	**0.42**	**0.83**	**1.66**
Body mass (g)	265.1 ± 9.8^{a}	259.3 ± 7.4^{a}	258.0 ± 7.4^{a}	257.9 ± 6.1^{a}	259.1 ± 7.7^{a}	255.7 ± 7.7^{a}	265.1 ± 9.8^{a}	259.3 ± 7.4^{a}	258.0 ± 7.4^{a}	258.3 ± 7.6^{a}	258.9 ± 8.4^{a}	259.2 ± 8.7^{a}
Transit time (min)	31.0 ± 2.8^{a}	43.4 ± 2.4^{b}	44.3 ± 1.8^{b}	312.4 ± 2.6^{a}	38.7 ± 1.9^{ab}	44.4 ± 1^{b}	31.0 ± 2.8^{a}	43.4 ± 2.4^{a}	44.3 ± 1.8^{a}	44.6 ± 3.6^{a}	65.5 ± 5.2^{b}	57.0 ± 7.4^{ab}
Amount eaten (g g^{-1}BM)	0.5 ± 0.03^{c}	0.3 ± 0.01^{a}	0.2 ± 0.01^{b}	0.4 ± 0.03^{d}	0.3 ± 0.01^{a}	0.2 ± 0.01^{b}	0.5 ± 0.03^{c}	0.3 ± 0.01^{a}	0.2 ± 0.01^{b}	0.3 ± 0.02^{a}	0.3 ± 0.04^{a}	0.2 ± 0.01^{b}
Gross energy intake (kJ g^{-1}BM)	1.3 ± 0.1^{a}	1.2 ± 0.04^{a}	0.9 ± 0.03^{b}	1.2 ± 0.1^{b}	1.1 ± 0.03^{c}	0.5 ± 0.03^{d}	1.3 ± 0.1^{ab}	1.2 ± 0.04^{a}	0.9 ± 0.03^{ab}	1.2 ± 0.1^{a}	1.6 ± 0.2^{b}	1.3 ± 0.1^{a}
Excreta energy (kJ g^{-1}BM)	0.2 ± 0.03^{bc}	0.2 ± 0.02^{c}	0.1 ± 0.02^{ac}	0.3 ± 0.03^{b}	0.2 ± 0.02^{bc}	0.1 ± 0.01^{a}	0.2 ± 0.02^{a}	0.2 ± 0.02^{ab}	0.1 ± 0.02^{bc}	0.2 ± 0.02^{a}	0.2 ± 0.02^{abc}	0.1 ± 0.01^{c}
Daily energy assimilated (kJ g^{-1}BM)	1.0 ± 0.1^{a}	0.9 ± 0.04^{ab}	0.8 ± 0.03^{bcd}	0.8 ± 0.1^{ac}	0.9 ± 0.03^{ad}	0.4 ± 0.03^{e}	1.0 ± 0.1^{ab}	0.9 ± 0.04^{b}	0.8 ± 0.03^{b}	0.9 ± 0.1^{b}	1.5 ± 0.2^{a}	1.2 ± 0.1^{ab}
Apparent assimilation efficiency (%)	80.8 ± 2.6^{ab}	80.8 ± 1.7^{ab}	85.3 ± 2.1^{b}	71.5 ± 1.5^{a}	79.4 ± 1.5^{ab}	78.8 ± 1.4^{ab}	80.8 ± 2.6^{bc}	80.8 ± 1.7^{bc}	85.3 ± 2.1^{ab}	78.4 ± 1.6^{b}	89.2 ± 1.5^{ac}	92.3 ± 0.9^{a}
Purple-crested Turaco												
Body mass (g)	252.6 ± 9.4^{a}	253.8 ± 8.4^{a}	252.8 ± 7.9^{a}	254.1 ± 10.0^{a}	254.2 ± 8.0^{a}	252.0 ± 7.8^{a}	252.6 ± 9.4^{ab}	253.8 ± 8.4^{a}	252.8 ± 7.9^{ab}	253.3 ± 8.2^{a}	252.5 ± 6.4^{ab}	249.0 ± 6.1^{b}
Transit time (min)	24.6 ± 1.3^{a}	27.5 ± 4.6^{acd}	36.8 ± 3.2^{bc}	20.6 ± 1.6^{a}	30.2 ± 5.4^{cd}	41.1 ± 2.3^{b}	24.6 ± 1.3^{a}	27.5 ± 4.6^{ab}	36.8 ± 3.2^{c}	35.8 ± 4.6^{bc}	49.3 ± 3.1^{d}	50.8 ± 4.5^{d}
Amount eaten (g g^{-1}BM)	0.5 ± 0.03^{c}	0.3 ± 0.01^{a}	0.2 ± 0.01^{b}	0.4 ± 0.03^{d}	0.3 ± 0.01^{a}	0.2 ± 0.01^{b}	0.5 ± 0.03^{c}	0.3 ± 0.01^{a}	0.2 ± 0.01^{b}	0.3 ± 0.02^{a}	0.3 ± 0.04^{a}	0.2 ± 0.01^{b}
Gross energy intake (kJ g^{-1}BM)	1.0 ± 0.02^{a}	1.3 ± 0.1^{a}	1.3 ± 0.1^{a}	1.7 ± 0.1^{b}	1.2 ± 0.1^{a}	1.7 ± 0.1^{b}	1.0 ± 0.03^{b}	1.3 ± 0.1^{ab}	1.3 ± 0.1^{ab}	1.1 ± 0.1^{b}	1.0 ± 0.1^{b}	1.8 ± 0.2^{a}
Excreta energy (kJ g^{-1}BM)	0.4 ± 0.02^{ad}	0.2 ± 0.02^{bc}	0.1 ± 0.02^{b}	0.5 ± 0.1^{a}	0.3 ± 0.03^{cde}	0.2 ± 0.1^{be}	0.4 ± 0.02^{a}	0.2 ± 0.02^{bc}	0.1 ± 0.02^{b}	0.4 ± 0.1^{a}	0.2 ± 0.1^{bc}	$0.3\pm0.02a^{c}$
Daily energy assimilated (kJ g^{-1}BM)	0.6 ± 0.02^{b}	1.1 ± 0.1^{a}	1.1 ± 0.1^{a}	1.1 ± 0.05^{a}	0.9 ± 0.04^{c}	1.5 ± 0.1^{d}	$0.6+0.02^{b}$	1.1 ± 0.1^{ab}	1.1 ± 0.1^{ab}	0.7 ± 0.1^{b}	0.7 ± 0.04^{bc}	1.5 ± 0.2^{a}
Apparent assimilation efficiency (%)	61.4 ± 1.0^{b}	82.9 ± 0.7^{a}	90.0 ± 0.7^{a}	68.5 ± 1.7^{c}	75.5 ± 1.4^{d}	88.5 ± 2.0^{a}	61.4 ± 1.0^{a}	82.9 ± 0.7^{bc}	90.0 ± 0.7^{b}	60.2 ± 3.8^{a}	77.7 ± 5.1^{cd}	82.4 ± 2.8^{bd}

Chapter 3

FOOD PREFERENCES OF KNYSNA AND PURPLE-CRESTED TURACOS FED VARYING CONCENTRATIONS OF EQUICALORIC AND EQUIMOLAR ARTIFICIAL FRUIT

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The effects that different fruit sugar types and concentrations have on food preferences of avian frugivores have been relatively poorly studied. Although it has been recently advocated that preference is based on equicaloric energy it is also important to note whether preferences change as energy content changes. Therefore, sugar preferences of equicaloric and equimolar artificial fruit of different sugar types at varying concentrations and molarities were investigated in two relatively large South African frugivores, Knysna (*Tauraco corythaix*) and Purple-crested (*Gallirex porphyreolophus*) Turacos. Artificial fruits of 6.6, 12.4 and 22% sucrose and glucose respectively and artificial fruits of 0.42, 0.83 and 1.66 mol L^{-1} sucrose and glucose respectively were used to determine sugar preferences. Knysna Turacos preferred the sucrose to the glucose equicaloric artificial fruit diet at low concentrations whereas Purple-crested Turacos showed no preference for either diet. Both Turacos species preferred the sucrose equimolar artificial fruit diet to the glucose at low concentrations. At high concentrations neither species showed a preference for either equicaloric or equimolar artificial fruit diets. This suggests that energy requirements influence food preferences more than sugar type and that birds will select fruit that is higher in energy irrespective of sugar type. This complements an earlier study on digestion of sugars at varying concentrations of differing equicaloric and equimolar artificial fruit sugar types. It again emphasizes the need for future studies looking at the composition of indigenous forest fruit sugars in order to obtain insight into the role of these avian frugivores as potential seed dispersers of fruiting tree species.

Key words: sugar type, sugar preference, energy requirements, frugivory, Knysna Turaco, Purple-crested Turaco, artificial fruits.

Introduction

Many different animals show preferences for certain food types (Ayala-Berdon et al., 2008). The amount of food consumed by the animal is usually reduced as levels of available energy are increased (Collins, 1981; Slansky and Wheeler, 1992). Studies of this “intake response” (Castle and Wunder, 1995) have mostly focused on nectarivorous birds (Collins, 1981; López-Calleja et al., 1997; McWhorter and Martínez del Rio, 2000) but these issues have seldom been addressed in frugivores (but see Levey and Martínez del Rio, 1999).

According to Baker and Baker (1983), hummingbird-pollinated plants produce nectar that is rich in sucrose while passerine-pollinated plants produce hexose-dominant nectar. Some choice tests have shown that several hummingbird species prefer sucrose to glucose or fructose (Hainsworth and Wolf, 1976; Martínez del Rio, 1990a) whereas some passerine species prefer glucose and fructose to sucrose (Martínez del Rio and Stevens, 1989; Martínez del Rio et al., 1989). However, Lotz and Schondube (2006) suggest that birds will generally prefer hexose sugars to sucrose at low equicaloric concentrations but will prefer sucrose to hexose at high equicaloric concentrations. A possible explanation for this switch at high concentrations is that sucrose nectar contains double the energy content per unit of osmotic concentration than hexose nectar (Beuchat et al., 1990). This reduced osmotic concentration may increase post-ingestional intestinal energy delivery rate by increasing gastric emptying rate (Karasov and Cork, 1994; Schondube and Martínez del Rio, 2003). If the delivery of high osmotic concentrated solutions is not regulated birds may experience osmotic diarrhoea (Lotz and Schondube, 2006). Sucrotic birds may therefore lessen their osmotic stress by choosing sucrose rather than hexose at high concentrations but at low concentrations this effect may have less consequence (Lotz and Schondube, 2006). Johnson and Nicolson (2008) however, recommend that a more useful distinction would be between specialist and generalist bird pollination systems. They suggest that generalist bird pollinated plants are characterised by large volumes of nectar (probably as a result of occasional avian nectarivores having a much large body size) with low concentrations and low sucrose proportions while specialist bird pollinated plants are characterised by smaller volumes of nectar with higher concentrations and high sucrose proportions. It has also been suggested that preferences are based on energy yield for relatively small specialist nectarivores (Downs and Perrin, 1996).

However, experimental procedure may affect the results of choice tests (Brown et al., 2008). Previous studies have used solutions that are equicaloric (Fleming et al., 2004), equimolar (Downs, 1997; Downs, 2000) or solutions that are equivalent by weight (Lotz and Nicolson, 1996; Blem et al., 2000). Equimolar solutions offer equal amounts of molecules per solution however a sucrose solution at a particular concentration has approximately double the energy of an equimolar hexose solution (Hixon, 1980; Schoener, 1983; Downs and Perrin, 1996; Downs, 1997). Sucrose solutions that are equivalent in sugar weight to a hexose sugar solution have approximately 5% more energy available (Fleming et al., 2004). It has therefore been suggested that choice tests use sugar solutions that are energetically equivalent (Fleming et al., 2004).

Most non-passerine frugivorous birds (e.g. pigeons, hornbills, trogons and turacos) (Snow, 1981; Levey and Martínez del Rio, 2001; Lotz and Schondube, 2006) have been poorly studied with regards to the sugar content of fruits that they consume. Musophagiformes play a key role in plant reproduction and as seed dispersal agents (Herrera, 2002) as they consist exclusively of frugivores (Kissling et al., 2009). The sugar preferences of different sugar types at varying concentrations and molarities were therefore investigated in two non-passerine relatively large avian frugivores, Knysna (*Tauraco corythaix*) and Purple-crested (*Gallirex porphyreolophus*) Turaco (Musophagiformes: Musophagidae). Both Knysna (c. 310g) and Purple-crested Turacos

(c. 300g) are forest frugivores (Rowan, 1983; Oatley, 1997; du Plessis and Dean, 2005a; du Plessis and Dean, 2005b).

The aim of the study was to determine if food preference was affected by sugar type and concentration. Initially equicaloric artificial fruit were used, however, to show the possible importance of energy in foraging decisions the experiments were repeated using equimolar artificial fruit. It was hypothesized that sugar type and concentration would affect food preferences in the two species of Turacos. It was predicted that both species would 1) display no preference for either equicaloric glucose or equicaloric sucrose irrespective of concentration; and 2) would display a preference for equimolar sucrose to equimolar glucose based on differences in energy yields.

Materials and Methods

Bird Capture and Maintenance

Four captive-bred Purple-crested Turacos and nine captive-bred Knysna and were sourced from Mr. M.C. Weber under permit from Ezemvelo KZN Wildlife (permit number: 1781/2008). One Purple-crested Turaco was kindly lent to us from Mr R. Poley. With the exception of the loaned bird who was housed individually, the birds were housed in pairs in outside aviaries (1 x 2.12 x 2.66 m) for one week before experiments were conducted. Birds were fed a maintenance diet daily. The maintenance diet given was a choice of apples, mealworms (*Tenebrio molitor* larvae), bananas, pears, oranges, carrots, paw-paws, and/or grapes. The skin was removed from the oranges, bananas and paw-paws and was then cut up into c. 1 x 1 x 1 cm cubes. The other fruit was grated. A mixture of Aviplus crumble and Softbill/Mynah pellets (Avi-products, Durban, South Africa) was added to the maintenance diets. Water was provided *ad libitum*.

Food choice experiments

Birds were moved indoors for one week before any trials were conducted and were housed in individual cages (42.7 x 43 x 59.3 cm) in a constant environment room with a 12 L: 12 D photoperiod at 25 ± 1°C. Prior to 06h00, clean plastic trays that were the same size as the cage's base were placed under each cage. To ensure that birds were in a post-absorptive state at the beginning of each experiment any uneaten food was removed at

18h00 previously and no food was available overnight as birds did not feed at night. Water was provided *ad libitum*.

Food preferences (determined by the intake rates) of sucrose and glucose diet treatments were investigated for both equicaloric and equimolar artificial diets at low (6.6% and 0.42 mol L^{-1} respectively) and high (22% and 1.66 mol L^{-1} respectively) concentrations and molarities. Artificial fruits were made by mixing all the relevant ingredients (see Table 1 for the composition of equicaloric artificial fruit diets and Table 2 for the composition of equimolar artificial fruit diets) into 1 L of boiling water (Witmer, 1998). The mixture was then stirred continuously to ensure that the ingredients were dispersed evenly while it solidified. Once the mixture had hardened the artificial fruit was cut into c. 1 x 1 x 1 cm cubes to ensure that the birds could swallow the fruits whole. All dietary treatments were analysed for energy content using a bomb calorimeter (Animal Science, University of KwaZulu-Natal). The sample was placed in the bomb and oxygen was then admitted under pressure. The water temperature in the bomb calorimeter was recorded and then the sample was ignited electrically. The water temperature was recorded again once equilibrium had been reached. The temperature rise and the specific heats and weights of the water and the bomb were used to calculate the quantity of the heat produced (McDonald et al. 1995).

On the experimental day, bird's body mass (g) was measured at 06h00 (30-40 min prior to the light phase) and 18h00 to the nearest 0.5 g using a digital scale (Adam®) and birds were each provided with a set amount of weighed food (measured to 0.01 g) of each diet treatment. The food trays were placed on opposite sides of the cage with a perch halfway between the trays. To ensure that no depletion effects occurred, each bird was provided with sufficient food of each diet treatment than could be eaten by a bird on a given day. Experiments were run for 12 h (from 06h00 to 18h00). Tables 1 and 2 summarize the trials conducted on individuals of each species for both equicaloric and equimolar food choice experiments respectively. For half of the birds, food trays A and B were placed on the right and left hand sides of the cage (respectively); and left and right (respectively) for the remainder of the birds in order to avoid possible spatial bias. Separate medium sucrose and medium glucose trials (see Tables 1 and 2) were run in order to test for possible spatial bias. Evaporative moisture loss from the uneaten food was estimated using controls of each experimental artificial diet. Maintenance diet was fed to the birds between experiments and each specific experimental diet was incorporated into the maintenance diet one day prior to that experiment being conducted. The birds were not fed the artificial diet on any two consecutive days.

For each bird, hourly intake rates and overall daily food intake (DFI measured as wet weight) of each diet treatment were determined. These were quantified by subtracting the mass of the food left over from the amount given. Evaporative water loss was taken into account. The DFI was converted to daily gross energy intake (GEI) by accounting for the respective fraction of water content and multiplying by the energy content for dry weight of each diet type. Finally each respective calculation was converted per gram individual body mass by dividing by the mean of initial and final body mass on the day of experimentation for comparisons. The above protocol was repeated for each bird species and each diet treatment.

Analyses

As the same birds were used repeatedly, food preference was compared with a Generalised Linear Model Repeated Measures of Analysis of Variance (GLM RMANOVA) and Post-hoc Tukey tests, using STATISTICA version 7 (Statsoft, Tulsa, USA). Mean ± SE was reported for all values.

Results

Body mass

Body mass of Knysna Turacos ranged from 259.31 ± 7.35 g to 260.28 ± 9.05 g when fed the glucose 12.4% control and the glucose and sucrose 6.6% experimental diet respectively; and from 257.89 ± 8.74 g to 261.06 ± 8.17 g when fed the glucose and sucrose 1.66 mol L^{-1} experimental diet and the glucose and sucrose 0.42 mol L^{-1} experimental diet respectively.

Body mass of Purple-crested Turacos ranged from 248.30 ± 7.47 g to 254.40 ± 8.07 g when fed the glucose and sucrose 6.6% experimental diet and the sucrose 12.4% control respectively; and from 248.20 ± 6.45 g to 254.00 ± 8.36 g when fed the glucose and sucrose 0.42 mol L^{-1} experimental diets and the glucose 0.83 mol L^{-1} control.

Equicaloric food preference

Knysna Turacos did not exhibit any side bias for either equicaloric glucose or sucrose ($F_{(1, 8)}$ = 0.98, p = 0.352; $F_{(1, 8)}$ = 7.96, p = 0.225 respectively). Similarly Purple-crested Turacos also did not exhibit any side bias for either equicaloric glucose or sucrose ($F_{(1, 4)}$ = 2.91, p = 0.163; $F_{(1, 4)}$ = 12.20, p = 0.251 respectively).

In terms of hourly intake rates, Knysna Turacos significantly preferred the 6.6% sucrose to the 6.6% glucose artificial fruit diet ($F_{(11, 88)}$ = 3.16, p = 0.001) and they consistently ingested more of the 6.6% sucrose artificial fruit diet every hour over a 12 h period (Fig. 1a). In contrast, Purple-crested Turacos did not significantly prefer the 6.6% sucrose to the 6.6% glucose artificial fruit diet ($F_{(11, 44)}$ = 1.73, p = 0.098, Fig. 1b).

Knysna Turacos ingested significantly different total amounts of the two 6.6% artificial diet treatments and had significant differences between the two 6.6% treatments in terms of GEI (Table 3). In contrast, there were no significant differences between the total amounts of the two 6.6% treatments eaten by Purple-crested Turacos and the Purple-crested Turacos had no significant differences between the treatments in terms of GEI (Table 3).

In terms of hourly intake rates, at high concentrations Knysna Turacos did not significantly prefer the 22% sucrose to the 22% glucose artificial fruit diet ($F_{(11, 88)}$ = 0.61, p = 0.815, Fig. 2a). Similarly, Purple-crested Turacos did not significantly prefer the 22% sucrose to the 22% glucose artificial fruit diet ($F_{(11, 44)}$ = 0.93, p = 0.518, Fig. 2b).

There were no significant differences between the total amounts eaten of the 22% diet treatments by either Knysna or Purple-crested Turacos and both species also had no significant differences between the two 22% treatments in terms of GEI (Table 3).

Equimolar food preference

Knysna Turacos did not exhibit any side bias for either equimolar glucose or sucrose ($F_{(1,8)}$ = 0.97, p = 0.352; $F_{(1,8)}$ = 6.05, p = 0.393 respectively). Similarly Purple-crested Turacos also did not exhibit any side bias for either equicaloric glucose or sucrose ($F_{(1, 4)}$ = 0.24, p = 0.648; $F_{(1, 4)}$ = 1.07, p = 0.359 respectively).

In terms of hourly intake rates, at lower concentrations Knysna Turacos did not significantly prefer the 0.42 mol L^{-1} sucrose to the 0.42 mol L^{-1} glucose artificial fruit diet ($F_{(11, 88)}$ = 1.16, p = 0.330, Fig. 3a). In contrast, Purple-crested Turacos significantly preferred the 0.42 mol L^{-1} sucrose to the 0.42 mol L^{-1} glucose artificial fruit diet ($F_{(11, 44)}$ = 3.15, p = 0.003, Fig. 3b).

Knysna and Purple-crested Turacos both ingested significantly different total amounts of the two 0.42 mol L^{-1} artificial diet treatments and had significant differences between the two 0.42 mol L^{-1} treatments in terms of GEI (Table 3).

In terms of hourly intake rates, at high concentrations Knysna Turacos did not significantly prefer the 1.66 mol L^{-1} sucrose to the 1.66 mol L^{-1} glucose artificial fruit diet ($F_{(11, 88)}$ = 0.80, p = 0.634, Fig. 4a). Similarly, Purple-crested Turacos did not significantly prefer the 1.66 mol L^{-1} sucrose to the 1.66 mol L^{-1} glucose artificial fruit diet ($F_{(11, 44)}$ = 1.31, p = 0.247, Fig. 4b).

There were no significant differences between the total amounts of the two 1.66 mol L^{-1} artificial diet treatments eaten by both Knysna and Purple-crested Turacos (Table 3). Knysna Turacos had no significant differences between the two 1.66 mol L^{-1} treatments in terms of GEI (Table 3). In contrast, Purple-crested significantly differed between the two 1.66 mol L^{-1} treatments in terms of GEI (Table 3).

Discussion

These relatively large frugivorous birds generally had higher food intake rates in the mornings with intake rates decreasing as the day progressed, irrespective of sugar type. This is likely as a result of them having relatively large ventriculus which allows a greater initial food intake (Wilson, 2009). This also shows that birds increase their food intake initially to achieve their energy requirements.

Knysna Turacos preferred sucrose to glucose equicaloric artificial fruit diet at low concentrations whereas Purple-crested Turacos showed no preference for either diet. In contrast Purple-crested Turacos preferred the sucrose equimolar artificial fruit diet to the glucose at low concentrations whereas Knysna Turacos showed no preference for either fruit diet. At high concentrations neither species showed a preference for either sugar in equicaloric or equimolar artificial fruit diets. These results for equicaloric differ from those obtained for some nectarivores such as White-bellied Sunbirds

Cinnyris talatala (Fleming et al., 2004), Cinnamon-bellied Flowerpiercers *Diglossa baritula* and Magnificent Hummingbirds *Eugenes fulgens* (Schondube and Martínez del Rio, 2003), New Holland Honeyeaters *Phylidonyris novaehollandiae*, Rainbow Lorikeets *Trichoglossus haematonotus* and Red Wattlebirds *Anthochaera carunculata* (Fleming et al., 2008) which preferred hexose sugars at low concentrations and sucrose at high concentrations. However our results are similar to those obtained by Stiles (1976), who found that in choice tests four species of hummingbirds (*Calypte anna*, *Selasphorus rufus*, *Archilocus alexandri* and *Thalurania furcata*) all preferred sucrose over equicaloric glucose. In their studies Hainsworth and Wolf (1976) and Martínez del Rio (1990b) also found that hummingbird species prefer sucrose over glucose or fructose. Our results also correspond to those of Downs and Perrin (1996) who found that three nectarivorous birds species (Gurney's Sugarbird *Promerops gurneyi*, Malachite Sunbird *Nectarinia famosa*, and Black Sunbird *Nectarinia amethystina*) all preferred sucrose at low equimolar concentrations, and showed no preference at high equimolar concentrations. Downs and Perrin (1996) suggest that energy rewards may play less of a role as sugar concentrations increase and therefore the birds may not exhibit a preference for either sugar at high concentrations (Lloyd, 1989; Stromberg and Johnson, 1990). In the present study this preference for energy yield would not apply for the equicaloric artificial fruits as the energy yield for sucrose and glucose at a particular concentration should be equal. The preference results obtained in this study support this idea. Although Knysna Turacos showed no significant preference for equimolar sucrose over equimolar glucose at low molarity, which would have been expected in terms of energy rewards (see Downs and Perrin, 1996; Downs, 1997), they ingested more sucrose than glucose in total.

Our results showed that while Purple-crested Turacos had no preference for either diet, while Knysna Turacos preferred sucrose to glucose equicaloric artificial fruit diet at low concentrations, and at high equicaloric concentrations neither species displayed a preference for either sugar. Our results also showed that while Knysna Turacos had no preference for either sugar type, Purple-crested Turacos preferred sucrose to glucose equimolar artificial fruit diet at low concentrations, and at high equimolar concentrations neither species displayed a preference for either sugar. We therefore reject our predictions.

The result of this study concurs with the conclusions obtained in a previous study (See Wilson and Downs, in prep). While both Knysna and Purple-crested Turacos are able to subsist on fruits that are rich in either sucrose or glucose further studies of the sugar composition in indigenous fruits are needed in order to obtain insight into the role of avian frugivores as seed dispersers.

List of symbols and abbreviations

DFI- daily food intake
GEI- gross energy intake

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Fig. 2 Amount of each 22% artificial fruit diet eaten (mean ± SE) per hour by a) Knysna Turacos, and b) Purple-crested Turacos

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Fig. 4 Amount of each 1.66 mol L^{-1} artificial diet eaten (mean ± SE) per hour by a) Knysna Turacos, and b) Purple-crested Turacos

Table 1 Summary of experimental equicaloric food choice diet treatments

	Tray A	**Tray B**
	Sugar type and concentration	**Sugar type and concentration**
Trial 1	Low sucrose	Low glucose
Trial 2	High sucrose	High glucose
Control 1	Medium sucrose	Medium sucrose
Control 2	Medium glucose	Medium glucose

Table 2 Summary of experimental equimolar food choice diet treatments

	Tray A	**Tray B**
	Sugar type and molarity	**Sugar type and molarity**
Trial 1	Low sucrose	Low glucose
Trial 2	High sucrose	High glucose
Control 1	Medium sucrose	Medium sucrose
Control 2	Medium glucose	Medium glucose

Table 3 Total amount eaten and gross energy intake of each artificial fruit diet by Knysna and Purple-crested Turacos during the choice experiments

Knysna Turacos

	Total amount eaten (g g^{-1}BM)						GEI (kJ g^{-1}BM)				
	Sucrose	**Glucose**	**F**	**p**	**n**		**Sucrose**	**Glucose**	**F**	**p**	**n**
6.6%	0.4 ± 0.1	0.1 ± 0.03	9.35	0.016	9	6.6%	1.0 ± 0.1	0.3 ± 0.1	10.49	0.012	9
22%	0.2 ± 0.03	0.1 ± 0.2	2.74	0.136	9	22%	0.5 ± 0.1	0.7 ± 0.2	0.37	0.562	9
0.42 mol L^{-1}	0.2 ± 0.02	0.1 ± 0.02	21.24	0.002	9	0.42 mol L^{-1}	1.0 ± 0.1	0.1 ± 0.1	48.62	< 0.001	9
1.66 mol L^{-1}	0.1 ± 0.03	0.1 ± 0.03	0.72	0.421	9	1.66 mol L^{-1}	1.1 ± 0.2	0.5 ± 0.2	2.23	0.174	9

Purple-crested Turacos

	Total amount eaten (g g-1BM)						GEI (kJ g^{-1}BM)				
	Sucrose	**Glucose**	**F**	**p**	**n**		**Sucrose**	**Glucose**	**F**	**p**	**n**
6.6%	0.2 ± 0.04	0.2 ± 0.03	0.36	0.58	5	6.6%	0.7 ± 0.1	0.7 ± 0.1	0.01	0.928	5
22%	0.2 ± 0.02	0.1 ± 0.03	0.58	0.49	5	22%	1.1 ± 0.2	0.7 ± 0.2	1.48	0.291	5
0.42 mol L^{-1}	0.3 ± 0.02	0.1 ± 0.01	57.12	0.002	5	0.42 mol L^{-1}	0.9 ± 0.1	0.3 ± 0.02	66.48	0.001	5
1.66 mol L^{-1}	0.1 ± 0.001	0.1 ± 0.01	1.51	0.286	5	1.66 mol L^{-1}	0.9 ± 0.1	0.6 ± 0.1	11.49	0.028	5

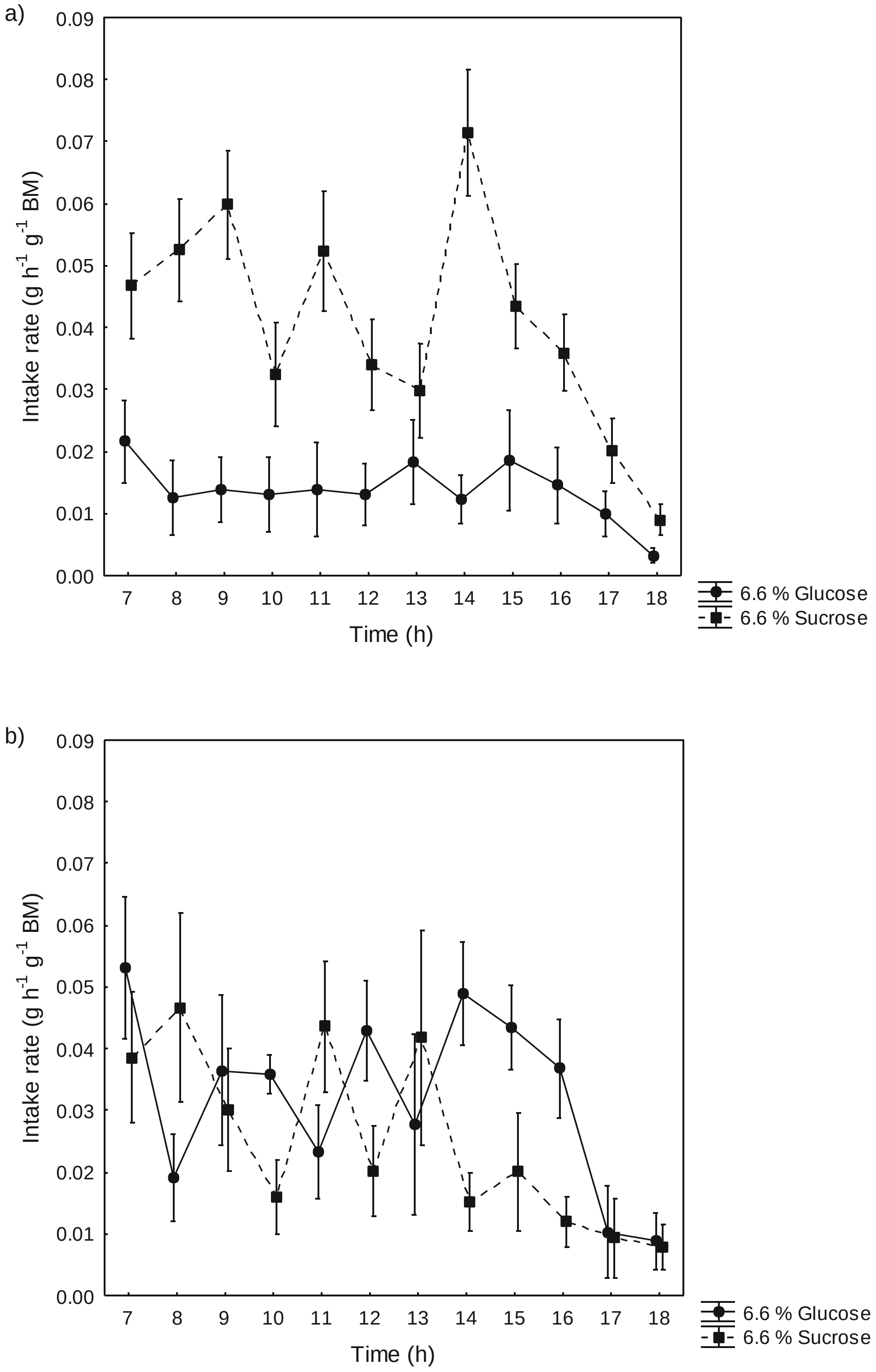

Fig. 1 Amount of each 6.6% artificial diet eaten (mean ± SE) per hour by a) Knysna Turacos, and b) Purple-crested Turacos

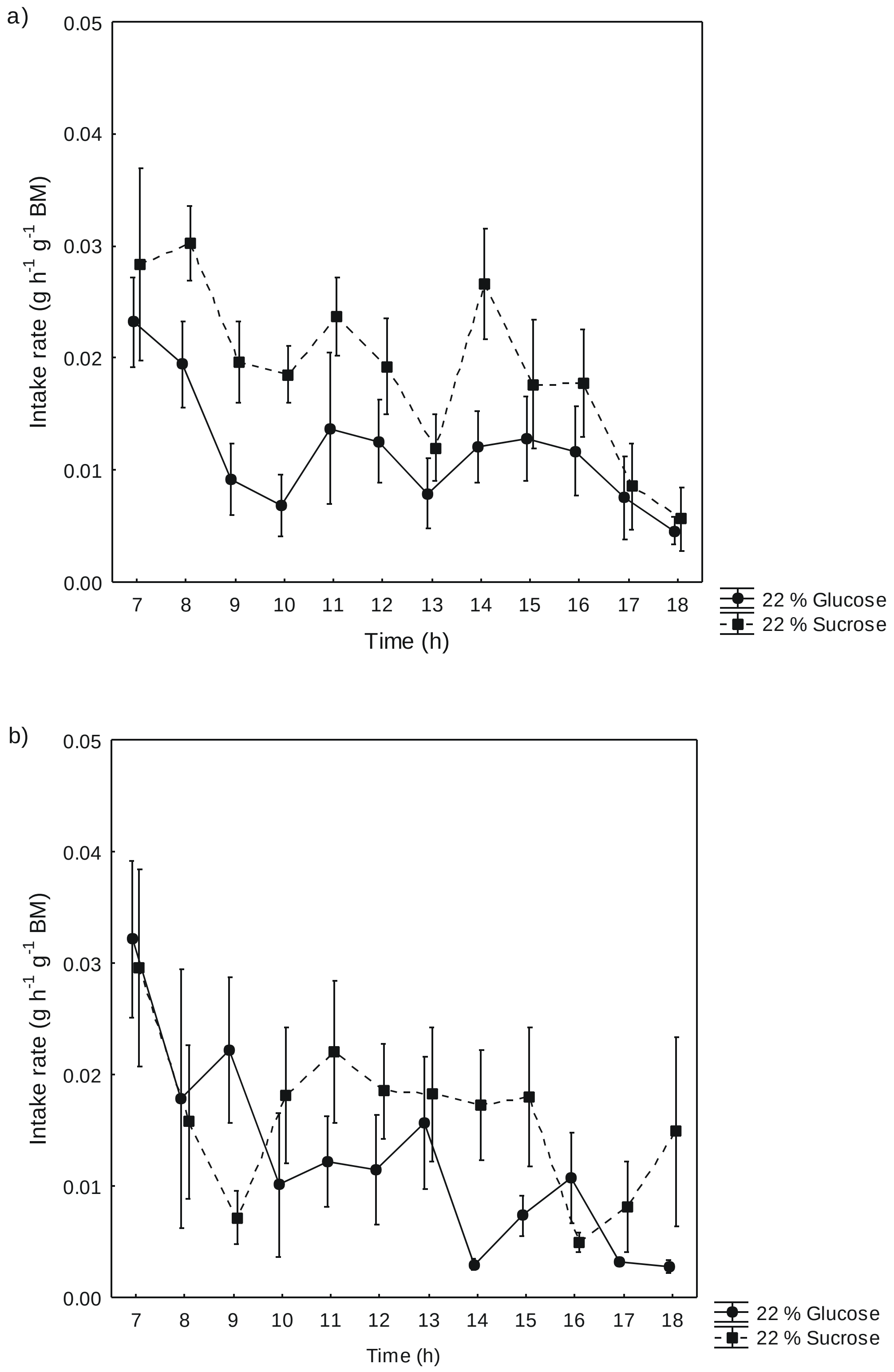

Fig. 2 Amount of each 22 % artificial fruit diet eaten (mean ± SE) per hour by a) Knysna Turacos, and b) Purple-crested Turacos

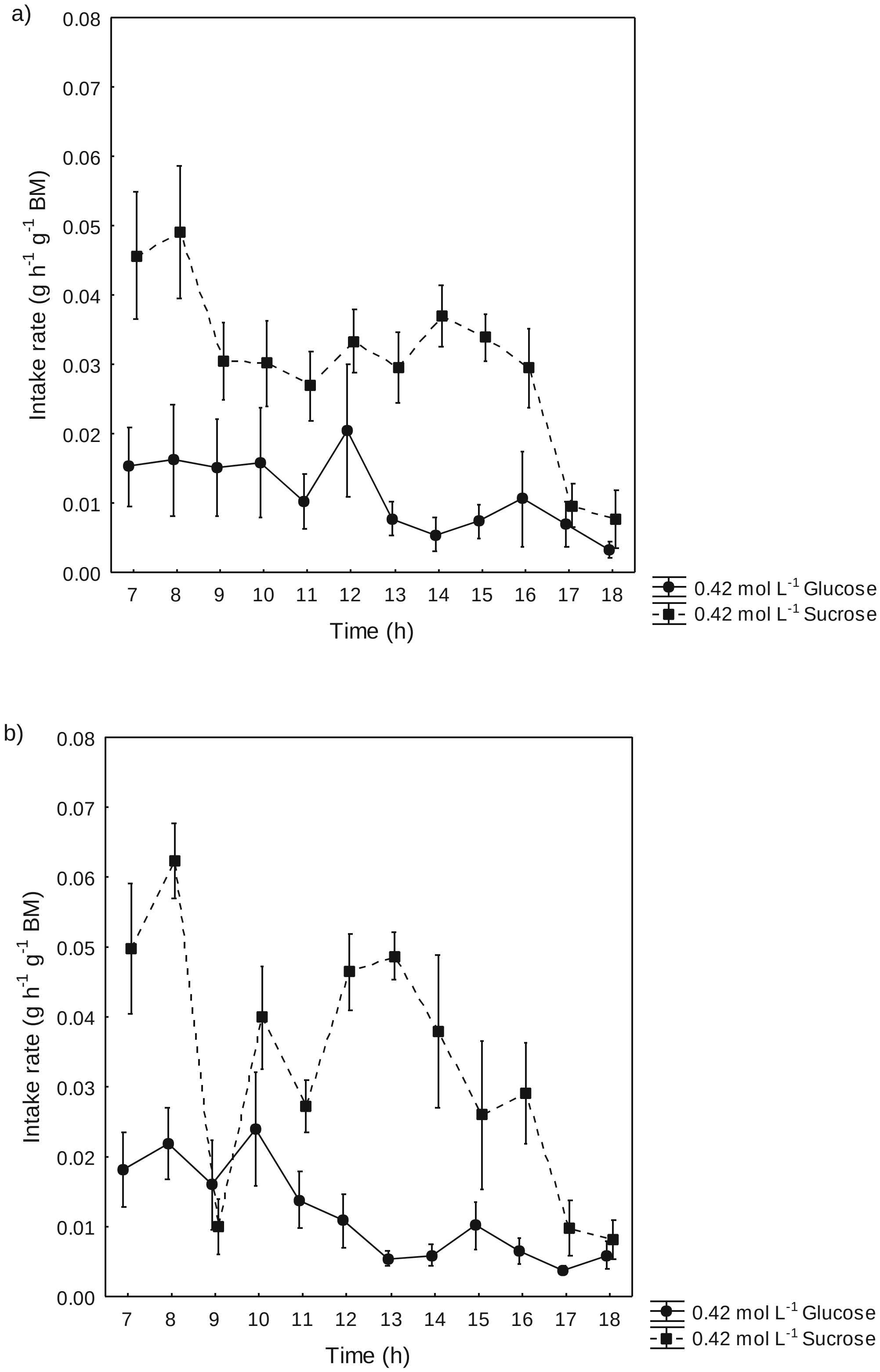

Fig. 3 Amount of each 0.42 mol L^{-1} artificial diet eaten (mean ± SE) per hour by a) Knysna Turacos, and b) Purple-crested Turacos

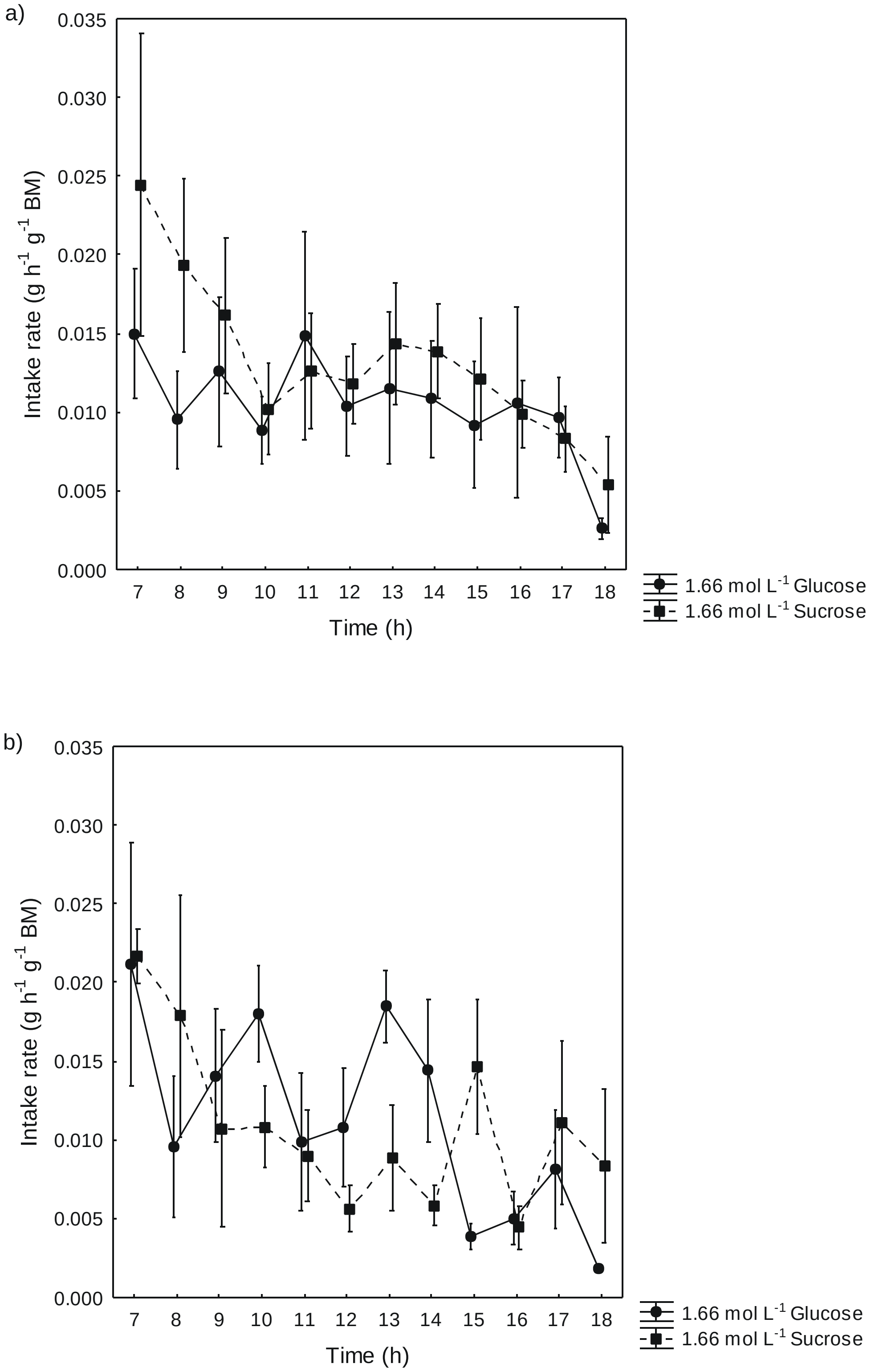

Fig. 4 Amount of each 1.66 mol L^{-1} artificial diet eaten (mean ± SE) per hour by a) Knysna Turacos, and b) Purple-crested Turacos

Chapter 4

THE EFFECT OF SEED PACKAGING ON DIGESTION AND FOOD PREFERENCE BY PURPLE-CRESTED (*GALLIREX PORPHYREOLOPHUS*) AND KNYSNA (*TAURACO CORYTHAIX*) TURACOS

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Avian frugivores may select fruit based on their seed loads and pulp to seed ratios and this may have important implications for their role as seed dispersal agents. Consequently the effect of different seed packaging was investigated in two relatively large South African frugivores, Knysna (*Tauraco corythaix*) and Purple-crested (*Gallirex porphyreolophus*) Turacos. Small-seeded artificial fruits containing on average five black plastic beads (2.24 mm diameter) and large-seeded artificial fruits containing one average one single black plastic bead (3.85 mm diameter) were used to investigate transit rates and food preference. Total seed volume in the two fruits was on average approximately equal (29.42 mm^3 and 29.88 mm^3 for small- and large-seeded fruits, respectively). Bead transit rates ranged from 38-45 min for Knysna Turacos and 36-50 min for Purple-crested Turacos with faster transit rates on the small-seeded diet. Pulp transit rates ranged from 25-39 min for Purple-crested Turacos and 34-40 min for Knysna Turacos. Purple-crested Turacos had significantly shorter pulp transit rates on the large-seeded than the small-seeded diet whereas Knysna Turacos had no significant difference between diets. Knysna Turacos preferred the small-seeded to the large-seeded artificial fruits whereas Purple-crested Turacos showed no preference for either diet. Further studies on avian dispersers will contribute to a greater understanding of their evolutionary relationship with fruiting plants. In particular, future studies establishing suitable seed sizes and volumes for use in determining the effect of seed packaging on larger birds are required as well as studies looking at seed packaging of indigenous forest fruit and the effects of seed packaging on digestion and food preference in indigenous avian frugivores may give insight into the role of avian frugivores as fruit consumers and seed dispersers**.**

Keywords: seed packaging, frugivory, Knysna Turaco, Purple-crested Turaco, artificial fruits.

Introduction

The rate of digestion is considered to be an important limitation in the foraging ecology of frugivorous birds (Worthington 1989; Stanley and Lill 2002a). While their diets are known to be extremely varied (Worthington 1989; Stanley and Lill 2002a), several factors such as size of the fruit (Moermond and Denslow 1983), ease of harvest (Moermond and Denslow 1983), pulp mass (Johnson et al. 1985), sugar concentration (Levey 1987a), seed size (Sorenson 1984), seed mass (Stanley and Lill 2002a) and pulp to seed ratio (Herrera 1981; Howe and Vande Kerckhove 1981) may affect fruit selection.

Fruits are considered to be nutrient-dilute and contain large amounts of seeds (Hulme 1971; Morton 1973; Stiles 1980; Moermond and Denslow 1985; Herrera 1987; Worthington 1989). Because frugivorous birds cannot digest these seeds a ballast load forms in the gut (Levey and Grajal 1991; Stanley and Lill 2002a). This build up is thought to create three major costs (Stanley and Lill 2002a). Firstly, it makes flight costly as the bird’s mass is increased (Norberg 1990; Levey and Grajal 1991); secondly, the volume of the gut decreases due to the indigestible bulk and therefore limits rates of food ingestion and digestion (McKey 1975; Levey and Grajal 1991; Murray et al. 1993); and thirdly birds are likely to require more energy in order to separate pulp from the seeds when in the gut and also when transporting them through the gut (Levey and

Duke 1992). Seeds therefore need to be processed efficiently in order to reduce these costs (Levey and Grajal 1991).

The feeding rate of nectarivorous hummingbirds has been shown to be limited by how long it takes for them to empty their crops (Diamond et al. 1986). Frugivorous birds are expected to be process-rate limited due to them consuming bulky diets (Walsberg 1975; Sorensen 1984; Tedman and Hall 1985; Stanley and Lill 2002a). Process-rate limitation occurs when the rate of food intake is limited by the length of time food takes to pass through the gut (Walsberg 1975; Sorensen 1984; Tedman and Hall 1985; Stanley and Lill 2002a). Avian frugivores have developed ways of processing fruit quickly in order to reduce this cost of ingesting bulky seeds (Courtney and Sallabanks 1992; Levey and Duke 1992). Mashers separate the seeds from the pulp and then discard them (Sorensen 1984; Johnson et al. 1985; Levey 1987b; Murray 1988; Worthington 1989, Levey and Grajal 1991; Symes and Downs 2001). Included in this category are those frugivores that remove the seeds from the pulp while still at the parent plant (eg. Herrera 1984a). While this method of separating the seeds from the pulp has advantages in that pulp is processed and digested more rapidly (Sorensen 1984; Johnson et al. 1985; Levey 1987b; Murray 1988; Worthington 1989, Levey and Grajal 1991), some disadvantages are that handling time is increased (Levey 1987b) and it is only possible for fairly large seeds (Levey and Grajal 1991).

Gulpers ingest the whole fruit and then separate the seeds from the pulp in their digestive system (Levey 1987b). These bird species that ingest and transport small seeds through the entire gut are expected to have the highest energetic cost of seed processing (Stanley and Lill 2002a). In order to process fruit quickly these bird species pass digestible and indigestible parts of the diet at different rates (Björnhag and Sperber 1977; Hörnicke and Björnhag 1980). It has been suggested that it would be more beneficial to the birds to pass indigestible seeds before digestible pulp (Levey and Grajal 1991). As well as voiding seeds by defecation, gulpers may also eliminate them by regurgitation (Sorensen 1984; Johnson et al. 1985; Levey 1987b; Murray 1988; Worthington 1989; Levey and Grajal 1991; Fukui 2003). Large seeds (>4 mm) are more frequently regurgitated than small seeds (<2 mm) (Levey 1987b).

While some avian frugivores prefer small seed loads, seed packaging may also affect their preference (Howe and Vande Kerckhove 1981; Levey and Grajal 1991;

Stanley and Lill 2002b). It has been found that some Neotropical frugivores prefer fruit with small seeds and a high pulp to seed ratio (Howe and Vande Kerckhove 1980) whereas other birds, such as Cedar Waxwings (*Bombycilla cedrorum*), prefer fruit with seeds of a size that can be regurgitated (Levey and Grajal 1991).

It has been suggested that fruits may be selected based on their seed loads and pulp to seed ratios which may determine the effectiveness of avian frugivores as seed dispersal agents (Howe and Richter 1982; Howe 1986). As most studies have focused on smaller avian frugivores (eg. Sorenson 1984; Levey and Grajal 1991; Stanley and Lill 2002a; Fukui 2003) the effect of different seed packaging was therefore investigated in two non-passerine avian frugivores, the Knysna (*Tauraco corythaix*) and the Purple-crested (*Gallirex porphyreolophus*) Turaco (Musophagiformes: Musophagidae). These two bird species were chosen as both species are relatively large (c. 310 g and c. 300 g for Knysna and Purple-crested respectively) frugivores and are limited by gape size.

Knysna Turacos are southern African endemic forest frugivores (Rowan 1983; Oatley 1997; du Plessis and Dean 2005a). Purple-crested Turacos are locally common frugivores with part of their distribution occurring in lowland forests in eastern South Africa (du Plessis and Dean 2005b). While both species are normally resident, they may track the availability of fruit (Rowan 1983). Both Knysna and Purple-crested Turacos have been recorded to feed on a large number of fruit species ranging in size from: e.g. large (25 x 13 mm) *Harpephyllum caffrum* fruits with a single large seed (in the case of Knysna Turacos (Pooley 2003; du Plessis and Dean 2005a) and large (20 x 10 mm) *Syzygium cordatum* fruits usually with a single large seed (in the case of Purple-crested Turacos (Pooley 2003; du Plessis and Dean 2005b)) to smaller fig species e.g. *Ficus natalensis* (7-13 mm) (Pooley 2003; du Plessis and Dean 2005a; du Plessis and Dean 2005b) with c. 169 small seeds (pers. obs,) in the case of both bird species.

It was hypothesized that seed packaging would affect transit rates and food preference in the two species of Turacos. It was predicted that both species would 1) have shorter retention times for seeds than for pulp; and 2) have shorter retention times for large seeds than for small seeds (see Stanley and Lill 2002a). It was also predicted that both species would 3) consume more of the large-seeded fruits than the small-seeded fruits; and 4) would prefer large-seeded fruits to small-seeded fruits in the preference tests as the large seeds could potentially be regurgitated thereby reducing the energetic cost of seed processing.

Artificial fruits were used to control for the effects of secondary compounds that are known to affect digestion (Murray et al. 1994; Bairlein 1996; Cipollini and Levey 1997; Fukui 2003).

Materials and Methods

Bird Capture and Maintenance

Nine captive-bred Knysna and four captive-bred Purple-crested Turacos were sourced from Mr. M.C. Weber under permit from Ezemvelo KZN Wildlife (permit number: 1781/2008) and one Purple-crested Turaco was borrowed from Mr R. Poley. The birds were housed in pairs (with the exception of the loaned bird who was housed individually) in outside aviaries (1 x 2.12 x 2.66 m) for one week before experiments were conducted. Daily birds were fed a maintenance diet of a choice of mealworms (*Tenebrio molitor* larvae), apples, pears, carrots, paw-paws, bananas, oranges and/or grapes. All fruit was either grated or had the skin removed (paw-paws, bananas and oranges) and cut into c. 1 x 1 x 1 cm cubes. A mixture of Aviplus Softbill/Mynah pellets and crumble (Avi-products, Durban, South Africa) was added to the maintenance diets. Water was provided *ad libitum*.

Methods

During the experiments, birds were moved indoors and housed in individual cages (42.7 x 43 x 59.3 cm) in a constant environment (CE) room with a 12 L: 12 D photoperiod at 25 ± 1°C. The birds were acclimated for one week before any trials were conducted. Clean plastic trays that were the same size as the cage's base were placed under each cage prior to 06h00. Any uneaten food was removed at 18h00 the previous evening and no food was available overnight to ensure a post-absorptive state at the beginning of each experiment and as birds did not feed at night. Water was provided *ad libitum*.

Bird's body mass (g) was measured daily 30-40 min prior to the light phase (06h00) and again in the evening (18h00). Bird's body mass was determined by placing the empty cage onto a digital scale (Adam®) before the experiment was conducted and then reweighing the empty cage minus the bird at the end of the experiment. The bird's body mass was measured to 0.5 g and all food items were measured to 0.01 g.

Two types of artificial fruits were used to investigate transit times and food preference of the turacos: (1) small-seeded fruits, containing on average five black plastic beads (2.24 mm diameter), and (2) large-seeded fruits, containing on average one single black plastic bead (3.85 mm diameter). We chose these bead sizes and numbers so that total seed volume in the two fruits would on average be approximately equal (29.42 mm^3 and 29.88 mm^3 for small- and large-seeded fruits, respectively). Hence, we were able to test effects of seed size on transit rates, amount eaten and preference, while controlling for seed volume and the ratio of pulp to seed. These seed sizes fell into the range of those eaten naturally by both Turaco species. The beads were made of smooth plastic and were found to pass harmlessly through the gastrointestinal tract of both Turaco species. Birds were not observed to pick around the beads.

Artificial fruits were made according to Witmer (1998) by mixing all the ingredients (see Table 1) into 1 L of boiling water. Red food colourant (Moirs, R.S.A.) was then added to ensure that the birds could not see the beads inside. Thereafter, the mixture was poured into a tray of 1 cm depth and the beads were then added. The mixture was then left to solidify stirring all the while to ensure that the ingredients and the beads were dispersed evenly throughout the mixture. Once the mixture had set it was cut into c. 1 x 1 x 1 cm cubes to ensure that the birds could swallow the fruits whole. This fruit size fell into the range of those eaten naturally by both Turaco species.

Transit rates of pulp and beads and food intake

On the experimental days, each bird was provided with a set amount of weighed artificial food at 06h00. Time that the birds' first ate and the first appearance of the dye in their excreta and the first appearance of beads in the excreta was recorded in order to determine transit rates of both pulp and beads. Each experiment (using either small- or large-seeded fruits respectively) was run for 12 h and food was weighed hourly to determine hourly food intake. Total amount eaten over 12 h was also recorded and the birds were only given artificial fruit diets over these hours. Controls of each experimental diet were used to estimate the evaporative moisture loss from the uneaten food. Birds were fed the maintenance diet between experiments and not fed the artificial diet on any two consecutive days.

For each bird, hourly intake rates of each diet treatment were determined as well as overall daily food intake (DFI measured as wet weight) for the respective diet treatments. These were quantified by subtracting the mass of the food remaining from the amount given and accounting for desiccation. These were then converted per gram individual body mass by dividing by the mean of initial and final body mass on the day of experimentation.

Food choice experiments

On the experimental day, each bird was provided with a set amount of weighed (approximately equal amounts for each) artificial food of each diet treatment placed on opposite sides of the cage with a perch halfway between the food trays. Experiments were run for 12 h (from 06h00 to 18h00) and food bowls were continually topped up throughout the experiment with additional food that had been kept in the CE room. Food trays A and B were placed on the right and left hand sides of the cage (respectively) for half of the test subjects and left and right (respectively) for the remainder to avoid possible spatial bias i.e. a randomised block design. To test for possible spatial bias, separate trials using large and small beads were run individually. Controls of each experimental diet were used to estimate the evaporative moisture loss from the uneaten food. Food preferences were determined by intake rates. Again birds were fed the maintenance diet between experiments and not fed artificial diets on any two consecutive days.

For each bird, hourly intake rates of each diet treatment were recorded (in order to determine whether dietary preferences changed with time) as well as overall daily food intake (DFI measured as wet weight) for the respective treatments. These were quantified by subtracting the mass of the food remaining from the amount given and accounting for desiccation. These were then converted per gram individual body mass by dividing by the mean of initial and final body mass on the day of experimentation. The above protocol was repeated for each diet treatment and each bird species.

Analyses

As the same birds were used repeatedly, excreta transit rates, bead transit rates, food intake rates and food preferences were compared with a Generalised Linear Model Repeated Measures of Analysis of Variance (GLM RMANOVA) and Post-hoc Tukey tests, using STATISTICA version 7 (Statsoft, Tulsa, USA). Normality of data was established prior to analysis.

Results

Pulp transit rates

There were no significant differences in pulp transit rates of Knysna Turacos when fed the two artificial fruit diets (GLM RMANOVA, $F_{(1,\ 8)}$ = 4.43, p = 0.069, Fig.1), although the small-seeded artificial fruit diet had a shorter transit rate (mean ± SE) (34.3 ± 4.0 min, n = 9) than the large-seeded artificial fruit diet (mean ± SE) (40.1 ± 3.5 min, n = 9).

However, there were significant differences in pulp transit rates of Purple-crested Turacos when fed the two artificial fruit diets (GLM RMANOVA, $F_{(1,\ 4)}$ = 15.09, p = 0.018, Fig. 1). The large-seeded artificial fruit diet had a significantly shorter transit rate (mean ± SE) (24.5 ± 2.3 min, n = 5) than the small-seeded artificial fruit diet (mean ± SE) (47.1 ± 5.1 min, n = 5).

Bead transit rates

Bead transit rates of Knysna Turacos were not significantly different between the two artificial fruit diets (GLM RMANOVA, $F_{(1,\ 8)}$ = 4.98, p = 0.056, Fig. 2), although the small-seeded artificial fruit diet had a shorter transit rate (mean ± SE) (37.5 ± 2.4 min, n = 9) than the large-seeded artificial fruit diet (mean ± SE) (44.9 ± 3.8 min, n = 9).

Similarly there were no significant differences in bead transit rates of Purple-crested Turacos when fed the two artificial fruit diets (GLM RMANOVA, $F_{(1,\ 4)}$ = 6.38, p = 0.065, Fig. 2), although the small-seeded artificial fruit diet had a shorter transit rate (mean ± SE) (35.5 ± 4.8 min, n = 5) than the large-seeded artificial fruit diet (mean ± SE) (49.8 ± 4.8 min, n = 5).

Food intake

Knysna Turacos ingested significantly different amounts of the two artificial fruit diets respectively (GLM RMANOVA, $F_{(1,\ 8)} = 41.93$, $p < 0.001$, Fig. 3). The highest food intake occurred on the small-seeded diet (mean ± SE) (0.4 ± 0.02 g g^{-1}BM, n = 9) and the lowest food intake occurred on the large-seeded diet (mean ± SE) (0.2 ± 0.01 g g^{-1}BM, n = 9).

Similarly Purple-crested Turacos ingested significantly different amounts of the two artificial fruit diets (GLM RMANOVA, $F_{(1,\ 4)} = 41.83$, $p = 0.003$, Fig. 3). The highest food intake occurred on the small-seeded diet (mean ± SE) (0.3 ± 0.01 g g^{-1}BM, n = 5) and the lowest food intake occurred on the large-seeded diet (mean ± SE) (0.2 ± 0.01 g g^{-1}BM, n = 5).

Food choice experiments

Knysna Turacos significantly preferred the small-seeded artificial fruit diet to the large-seeded artificial fruit diet in terms of amount eaten per hour (GLM RMANOVA, $F_{(11,\ 88)} = 1.98$, $p = 0.040$, Fig. 4a). In contrast, Purple-crested Turacos did not significantly prefer the small-seeded artificial fruit diet to the large-seeded artificial fruit diet in terms of amount eaten per hour (GLM RMANOVA, $F_{(11,\ 44)} = 0.62$, $p = 0.806$, Fig. 4b).

Knysna Turacos did not exhibit any side bias for either the small- or the large-seeded diet (GLM RMANOVA, $F_{(1,\ 8)} = 1.51$, $p = 0.254$; $F_{(1,\ 8)} = 1.67$, $p = 0.232$ respectively). There were significant differences between the total amounts of the two artificial diet treatments eaten by the Knysna Turacos (GLM RMANOVA, $F_{(1,\ 8)} = 9.77$, $p = 0.014$, Fig 5). Similarly, Purple-crested Turacos also did not exhibit any side bias for either the small- or the large-seeded diet (GLM RMANOVA, $F_{(1,\ 4)} = 0.0003$, $p = 0.987$; $F_{(1,\ 4)} = 0.17$, $p = 0.697$ respectively). In contrast, there were no significant differences between the total amounts of the two artificial diet treatments eaten by the Purple-crested Turacos (GLM RMANOVA, $F_{(1,\ 4)} = 0.20$, $p = 0.677$, Fig 5).

Discussion

When considering avian frugivores as seed dispersers, it is important to recognize the relationship between seed size and retention time (Fukui 2003). Different sized seeds may have different transit rates (Stanley and Lill 2002a). Faster transit rates may result in more fruits and consequently more seeds being consumed and this therefore leads to seeds being dispersed more frequently (Levey and Grajal 1991). However, faster transit rates may also result in shorter dispersal distances (Stanley and Lill 2002a).

Although bead transit rates for both Turaco species were faster when consuming the small-seeded diet than when consuming the large-seeded diet, these were not significantly different. These results differ to those obtained by Stanley and Lill (2002a) who found that Silvereyes (*Zosterops lateralis*) had faster seed transit times on large-seeded fruit than on small-seeded fruit of equivalent total volume; Levey and Grajal (1991) who found that Cedar Waxwings (*Bombycilla cedrorum*) consumed and defecated large-seeded artificial fruit faster than small-seeded fruit and Fukui (2003) who found that Brown-eared Bulbuls (*Hypsipetes amaurotis*) also defecated large seeds faster than small seeds. Our bead transit rates ranged from 38-45 min for Knysna Turacos and 36-50 min for Purple-crested Turacos. These results are similar to those obtained by Sorenson (1984) for European Blackbirds (*Turdus merula*) (25-50 min); Herrera (1984b) and Johnson et al. (1985) for temperate frugivores (20-90 min); and Levey (1986) for tropical frugivores (9-107 min). However, transit times were measured on fasted birds and may therefore not be representative of typical transit times as birds usually have food in their gut (Levey and Martínez del Rio 1999). Both Turaco species were observed to be gulpers and therefore could be expected to void seeds rapidly, possibly through regurgitation, in order to overcome any gut limitation. However, during these experiments Turacos were not observed to regurgitate any beads.

Purple-crested Turacos had significantly shorter pulp transit rates on the large-seeded than on the small-seeded diet. In contrast, Knysna Turacos had no significant differences on either diet. Pulp transit rates were shorter than bead transit rates for both diets and for both Turaco species. Ours results differ from those obtained by Levey and Grajal (1991) who found that Cedar Waxwings (*Bombycilla cedrorum*) pass through seeds faster than pulp, and pass through large seeds faster than small seeds. They suggest that the nonocclusive peristaltic contractions that move the seeds ahead of the pulp may also explain why in their study large seeds were defecated by the waxwings faster than small seeds.

Both Turaco species ingested significantly more of the small-seeded than the large-seeded diet. These results differ to those obtained by Stanley and Lill (2002a) who found that Silvereyes (*Zosterops lateralis*) consumed more large-seeded than small-seeded fruit of equivalent total seed volume. Knysna Turacos preferred the small-seeded to the large-seeded artificial fruits. In contrast, Purple-crested Turacos showed no preference for either diet. These results differ from those obtained by Murray et al. (1993) who found that American Robins (*Turdus migratorius*) preferred large-seeded over small-seed fruit of equivalent total seed volume as the large seeds could be voided quickly by regurgitation. Our results are similar to those obtained by Willson (1994) who found that American Robins showed no preference for fruits containing one or six *Rubus spectabilis* seeds; and by Willson and Comet (1993) who found that Northwestern Crows (*Corvus caurinus*) showed no preference for artificial fruit with low seed loads.

Our results show that both Turacos species had shorter retention times for pulp than for beads; had generally shorter retention times for small beads than for large beads although they were not significantly different; and consumed more of the small-seeded fruits than the large-seeded fruits. Our results also show that Knysna Turacos prefer small-seeded fruits to large-seeded fruits while Purple-crested Turacos displayed no preference. Consequently we reject our predictions. Despite our bird-appropriate fruit and seed size, it is possible that the length of trials may have had an effect. However, birds had been fed artificial fruit with the maintenance diet.

Although our results were not consistent with some other studies done, they still show that these two Turaco species are capable of making foraging decisions based on seed packaging. The consequences of these foraging decisions may affect plant survival in terms of seedling dispersal (Howe 1986) and may also have implications for the birds in terms of costs associated with foraging (Stanley and Lill 2002b). However, factors such as seed loads and seed-to-pulp ratios may only be important in terms of fruit choices and gut passage rates when dealing with extreme seed sizes e.g. seeds that are too large to swallow and seeds that are too small to have an affect on digestion or are too small for the birds to eat around). Further studies on avian dispersers will contribute to a greater understanding of their evolutionary relationship with fruiting plants. In particular, future studies establishing suitable seed sizes and volumes for use in determining the effect of seed packaging on larger birds are required as well as studies looking at seed packaging of indigenous forest fruit and the effects of seed packaging on digestion and food preference in indigenous avian frugivores may give insight into the role of avian frugivores as fruit consumers and seed dispersers**.**

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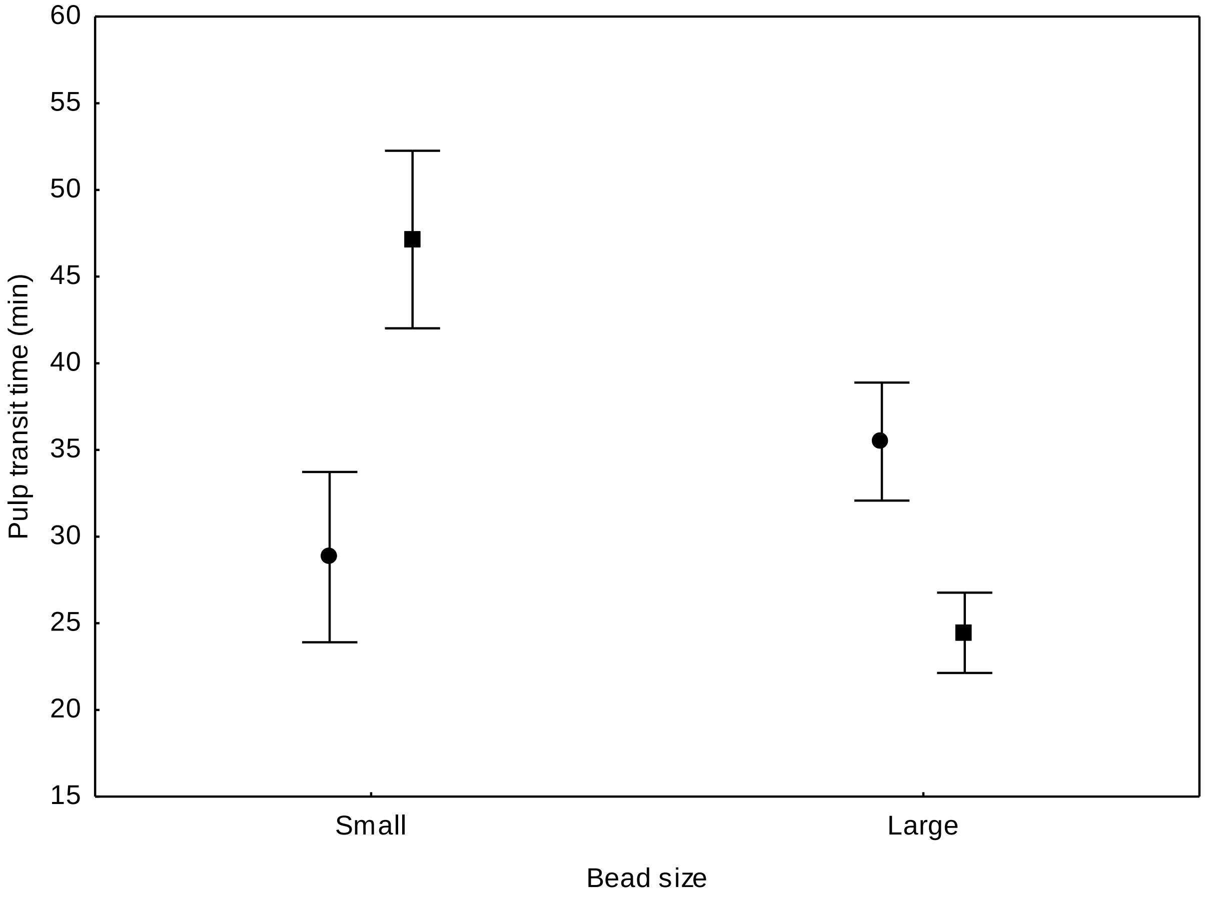
Table 1 Composition of the artificial fruit diet

Ingredients	Diet Composition (g)
D-glucose	150
Sucrose	142.4
Water	1000
Wheat bran	50
Agar	10
Sodium chloride	0.75
Dicalcium phosphate	0.80
Vitamin supplement	0.75



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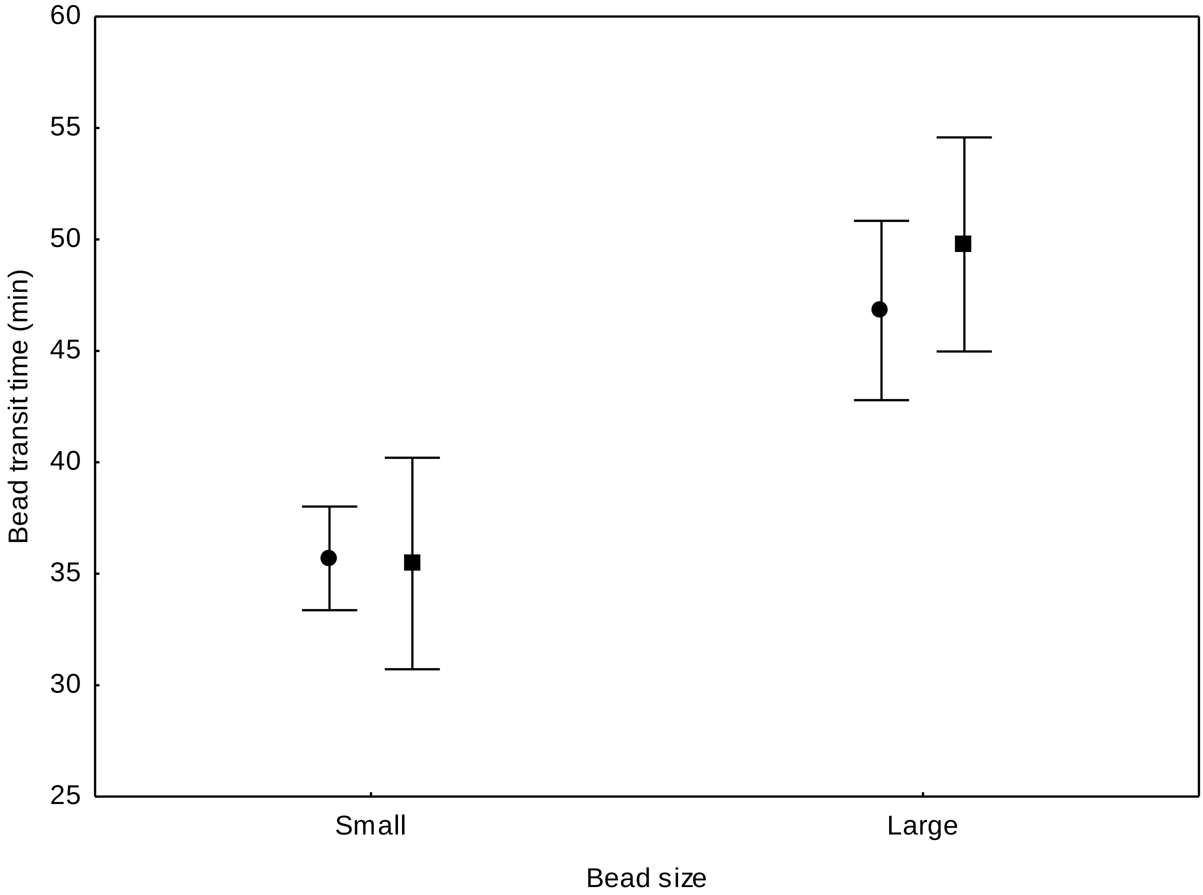

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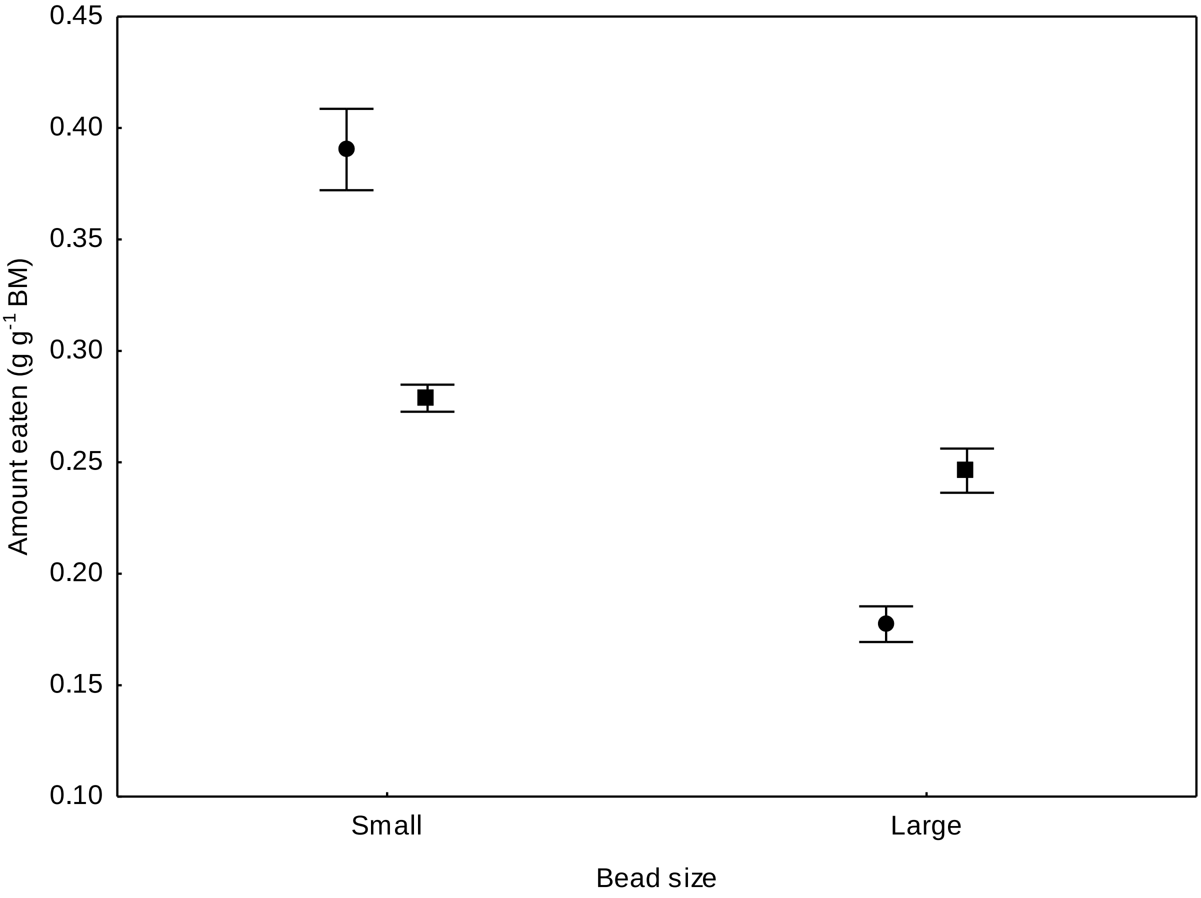

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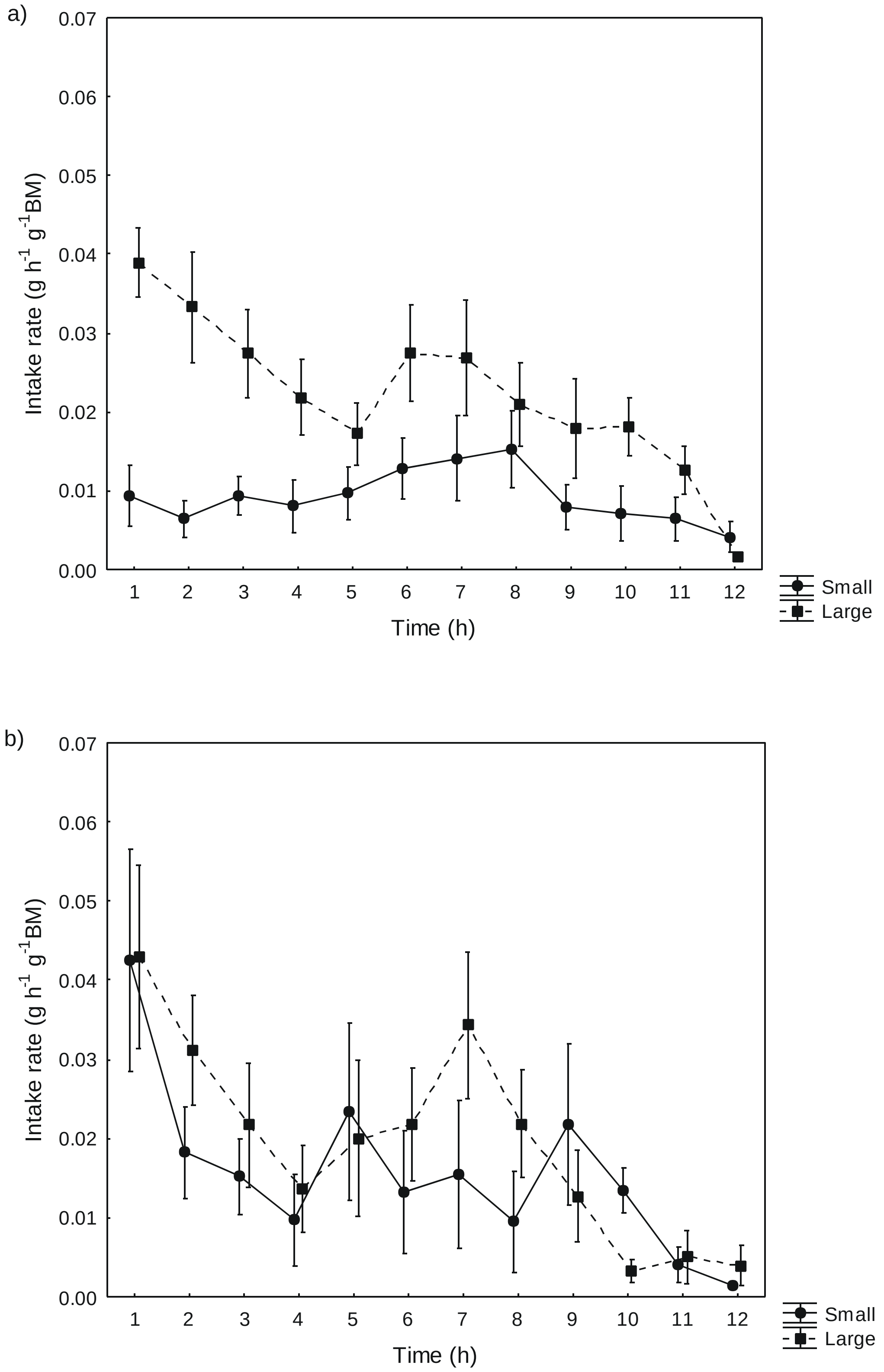

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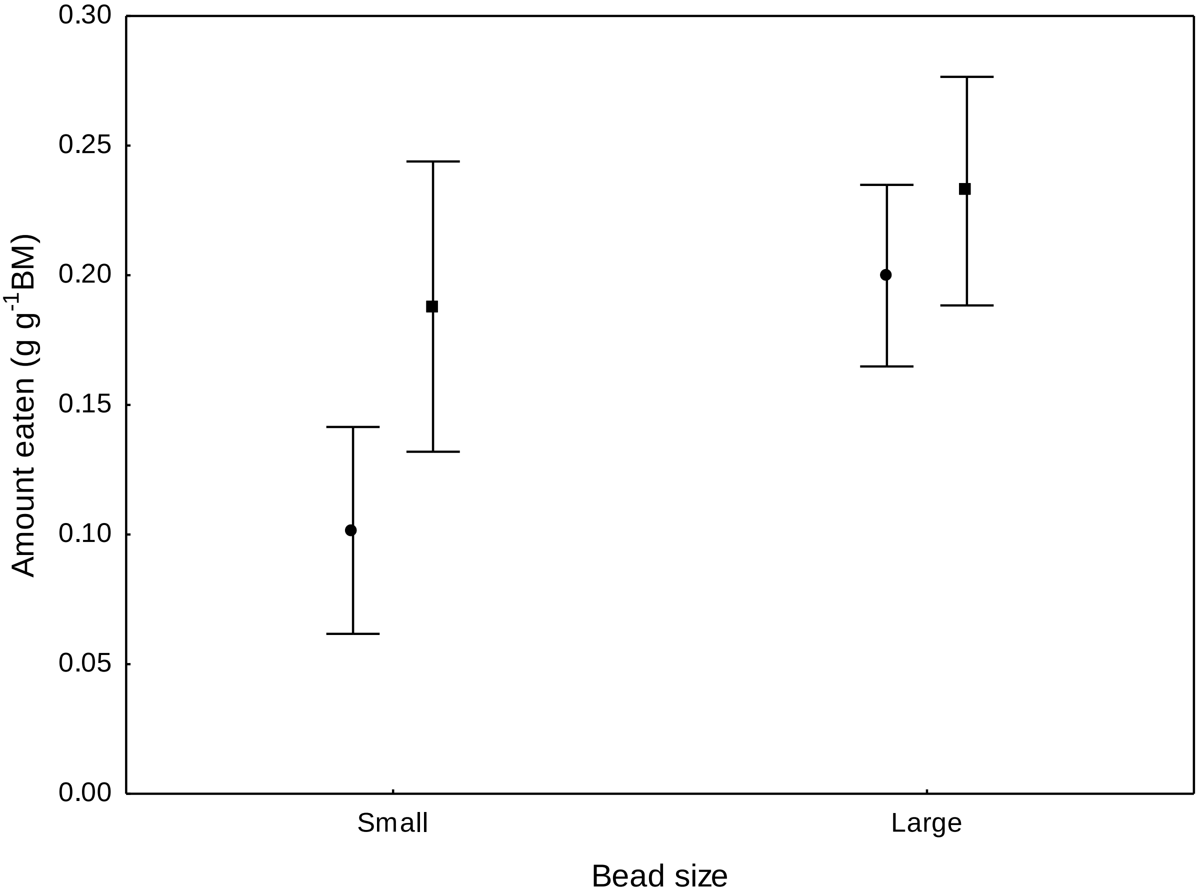

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Chapter 5

FOOD INTAKE RATES, DIGESTIVE EFFICIENCY, AND TRANSIT TIMES OF KNYSNA (*TAURACO CORYTHAIX*) TURACOS FED SOUTH AFRICAN INDIGENOUS FRUIT

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Fleshy-fruited plants and frugivores share a mutualistic relationship with plants offering the frugivores a reward of fleshy fruit pulp in order to disperse their seeds. In return frugivores receive a food source and consequently a source of energy. However all fruits are not equal in terms of available energy and consequently the digestion efficiency of six indigenous tree species was investigated in an avian frugivore, the Knysna Turaco (*Tauraco corythaix*). It was predicted that the Turacos would process these indigenous fruits efficiently and have fast transit rates and high intake rates irrespective of species. Fruits were fed to the Turacos in separate trials to determine daily food and energy intake, digestive efficiencies and digestive transit times. Digestive transit times of Knysna Turacos (c. 12-28 min) fall within the range of published examples for other frugivorous birds. Apparent assimilation efficiencies of the Turacos ranged from c. 15-84% and were generally lower than those observed in other avian frugivores. Future studies looking at the nutritional composition of indigenous forest fruit may provide insight into the Knysna Turacos digestive efficiency

and consequently their food preferences and role as potential seed dispersers of fruiting tree species.

Key words: fruit digestion, transit times, Knysna Turaco, indigenous fruit

Introduction

In order to attract frugivores as dispersers, many plants offer a reward of fleshy fruit pulp (Van der Pijl 1982; Jordano 1995; Lepczyk et al. 2000). This mutualistic relationship entails the plants dispersing their seeds and in return frugivores receive a food source and consequently a source of energy (Van der Pijl 1982; Jordano 1995; Lepczyk et al. 2000; Linnebjerg et al. 2009). However, all fruits are not equal in terms of available energy (Worthington 1989) and amount of energy available in the different food types influences amount of food consumed by the individual (Ayala-Berdon et al. 2008). Amount eaten usually decreases as the amount of available energy increases (Slansky and Wheeler 1992; Downs 2008). Factors such as the ability of the frugivore to efficiently digest the fruit as well as the nutritional composition of the fruit pulp can influence the frugivores ability to meet their energetic demands (Levey and Karasov 1989; Martinez del Rio and Restrepo 1993; Fuentes 1994; Afik and Karasov 1995; Lepczyk et al. 2000; Downs 2008) and optimal foraging theory predicts that frugivores will select the most profitable fruits in order to maximize energy intake (Stephens and Krebs 1986; Lepczyk et al. 2000; Downs 2008).

Fruit choice by some avian frugivores may be affected by the frugivore’s ability to digest the different sugars efficiently (Avery et al. 1999) and some species (e.g. from the Sturnid-Muscicapid lineage) are unable to digest sucrose efficiently as they lack the enzyme sucrase (Schuler 1983; Martínez del Rio 1990; Martínez del Rio and Restrepo 1993; Levey and Martínez del Rio 2001).

How well a frugivore is able to digest and absorb its food depends on a number of factors such as gut transit times, intake rates, gut size and assimilation, as well as the fruit’s nutrient content (Sibley 1981; Worthington 1989; Afik and Karasov 1995; Wellmann and Downs 2009). Fleshy fruits are considered as being either nutrient-dilute or nutrient-dense (Izhaki 1993). Nutrient-dilute fruits have fewer carbohydrates than lipid-rich fruits, are high in water and are low in fibre and protein (Snow 1981; Herrera

1982; Izhaki 1993) whereas nutrient-dense fruits are considered to be low in water and carbohydrates, relatively lipid-rich and variable in protein (Izhaki 1993). Most avian dispersed fruits are considered to be nutrient-dilute (Levey 1987; Levey and Grajal 1991) and avian frugivores would therefore need to either select only nutrient-rich fruits or consume large amounts of nutrient-dilute fruits in order to meet their energy requirements (Worthington 1989; Martinez del Rio and Restrepo 1993).

Frugivores generally have short transit times and small gut volumes (Martinez del Rio et al. 1989; Karasov and Levey 1990; Levey and Karasov 1989; Levey and Karasov 1994). Transit times may be affected by several factors such as pulp to seed ratios (Herrera 1981; Howe and Vande Kerckhove 1981), and nutrient concentrations (Izhaki 1993). Frugivores also tend to ingest high amounts of energy however they are considered to assimilate nutrients inefficiently (Martinez del Rio et al. 1989; Karasov and Levey 1990; Levey and Karasov 1989; Levey and Karasov 1994). Different fruit species vary in the amount of energy available to a frugivorous species (Brown and Downs 2003) and the profitability of the fruit depends on how much time and effort is spent in digesting it versus the pulp's nutritional rewards (Schoener 1971).

Despite the obvious role that fruit pulp plays as an energy source for frugivorous species, little is known about the digestive efficiency of large non-passerine avian frugivores, such as the African Turacos, when fed on different fruit species. This information is important as most previous studies have focused on small (passerine) birds and there could be interesting allometric relationships between avian body size and food processing.

Knysna Turacos (*Tauraco corythaix*) (Musophagiformes: Musophagidae) are fairly common large (c. 310 g) forest frugivores (Oatley 1997) that are endemic to South Africa (Rowan 1983; du Plessis and Dean 2005). Consequently, digestion efficiencies, transit rates, intake rates and daily total energy intake of Knysna Turacos when fed fruit of indigenous tree species were investigated. It was predicted that the Turacos would have high assimilation efficiencies, and have fast transit rates and high intake rates irrespective of fruit species.

Materials and Methods

Bird Capture and Maintenance

Under permit from Ezemvelo KZN Wildlife (permit number: 1781/2008), six captive-bred Knysna Turacos were sourced from Mr. M.C. Weber. Before experiments were conducted birds were housed in pairs outside in aviaries measuring 1 x 2.12 x 2.66 m.

They were fed a maintenance diet daily which consisted of mealworms (*Tenebrio molitor* larvae) and a choice of several fruit (e.g. carrots, oranges, paw-paws, pears, apples, bananas, and/or grapes). Paw-paws, oranges and bananas had their skin removed and the fruit was then cut into c. 1 x 1 x 1 cm cubes. All other fruit was grated and a mixture of Aviplus Softbill/Mynah crumble and pellets (Avi-products, Durban, South Africa) was added to the maintenance diets daily. Water was provided *ad libitum*.

Experiments

Birds were individually housed in cages (42.7 x 43 x 59.3 cm) in a constant environment room with a 12 L: 12 D photoperiod at 25 ± 1°C and acclimated for one week before any trials were conducted. No food was available overnight (from 18h00 onwards) to ensure a post-absorptive state at the beginning of each experiment and as birds did not feed at night. A post-absorptive state was necessary in order to accurately determine digesta transit times and digestive efficiencies. Water was provided *ad libitum*. Birds were fed the maintenance diet for at least three days between trials.

Birds were weighed twice daily (30-40 min prior to the start of the trial at 06h00 and again at the end of the trial at 18h00). All food items were measured to 0.01 g and the bird's body mass was measured to 0.5 g.

Digesta transit times and digestive efficiency of indigenous fruit diets

Fruits were collected from a range of indigenous tree species (n = 6) (Table 1) found in KwaZulu-Natal afromontane and coastal forests. Fruits were used within 48 h of collection and were included into maintenance diets at least one day prior to the experimental day.

On each experimental day, all birds were fed a specific fruit species and fruits were presented whole. Trials were run from 06h00-18h00 and each fruit species was only present for one day. Fruits were weighed at the start and end of each trial. The time that the birds' first ingested the fruit and the first appearance of excreta was recorded to determine digesta transit times. The criteria for what constituted excreta was the appearance of either pulp or seeds, though these usually appeared together. A tray of whole fruit was placed in the same room and weighed at the start and end of each trial in order to control for evaporative moisture loss of uneaten food. Excreta and fruit samples (excluding seeds) were oven dried at 60 ^{o}C to constant mass and later analysed for energy content using a bomb calorimeter (Animal Science, University of KwaZulu-Natal).

For each bird, overall daily food intake (DFI measured as wet weight) was determined for the respective diet treatments. These were quantified by subtracting the mass of the food remaining from the amount given and accounting for desiccation. The DFI was converted to daily gross energy intake (GEI) by accounting for the respective fraction of water content and multiplying by the energy content for dry weight of each diet type. Daily excreta energy loss (EE) was calculated as the mass of dried excreta produced multiplied by its dry weight energy value. Daily energy assimilated (DEA) was calculated as GEI subtracting EE. Daily apparent energy assimilation efficiency (AE) was calculated as DEA divided by GEI. The values were squareroot transformed for statistical analysis. Finally each respective calculation was converted per gram individual body mass by dividing by the mean of initial and final body mass on the day of experimentation for comparisons. The above protocol was repeated for each fruit species.

Analyses

As the same birds were used repeatedly, digesta transit time, GEI, DEA and food intake rates were compared with a Generalised Linear Model Repeated Measures Analysis of Variance (GLM RMANOVA) and Post-hoc Tukey tests, using STATISTICA version 7 (Statsoft, Tulsa, USA). Mean ± SE was reported for all values.

Results

Fruit traits

Fruits used in this study varied in a number of features such as fruit size, seed size, and mean number of seeds per fruit (Table 1). *Ficus sur* fruits were the largest in size (20-40 mm diameter) and had the greatest number of seeds per fruit (828.0 ± 50.6). *Clerodendrum glabrum*, *Ficus petersii* and *Solanum giganteum* fruits were the smallest

(10 mm diameter) used in the study. *Ficus lutea*, *F. petersii* and *F. sur* seeds were the smallest (1.5 x 1 mm) used in the study.

Digesta transit times

Knysna Turacos had significantly different digesta transit times when fed the six indigenous fruit diets ($F_{(5, 25)} = 3.66$, $p = 0.013$). *Ficus sur* had the fastest digesta transit time (12.4 ± 0.8 min, n = 6) while *Mimusops caffra* had the slowest digesta transit time (28.3 ± 2.8 min, n = 6) (Table 2).

Daily food intake and assimilation

Knysna Turacos ingested significantly different amounts of the respective fruit species in terms of wet weight ($F_{(5, 25)} = 12.41$, $p < 0.001$, Table 2). They ingested the greatest amount of *Syzygium cordatum* (0.70 ± 0.05 g g^{-1}BM, n = 6) and the lowest amount of *F. petersii* (0.27 ± 0.02 g g^{-1}BM, n = 6). There were significant differences between the respective fruit species in terms of GEI for the Knysna Turacos ($F_{(5, 25)} = 11.92$, $p < 0.001$, Table 2) with the highest GEI on *S. cordatum* (1.83 ± 0.14 kJ g^{-1}BM, n = 6) and the lowest on *F. petersii* (0.71 ± 0.06 kJ g^{-1}BM, n = 6). There were also significant differences in excreta energy loss in Knysna Turacos between the respective fruit species ($F_{(5, 25)} = 33.31$, $p < 0.001$, Table 2) with the highest excreta energy loss on *F. lutea* (1.31 ± 0.27 kJ g^{-1}BM, n = 6) and the lowest on *F. sur* (0.42 ± 0.04 kJ g^{-1}BM, n = 6). As a consequence of GEI and EE, DEA of Knysna Turacos was significantly different between the respective fruit species ($F_{(5, 25)} = 23.04$, $p < 0.001$, Table 2) with the highest DEI on *S. cordatum* (1.55 ± 0.12 kJ g^{-1}BM, n = 6) and the lowest on *F. petersii* (0.10 ± 0.02 kJ g^{-1}BM, n = 6). Apparent energy assimilated of Knysna Turacos was significantly different between the respective fruit species ($F_{(5, 25)} = 48.94$, $p < 0.001$, Table 2) with the highest AE on *S. cordatum* (84.53 ± 1.29 %, n = 6) and the lowest on *F. petersii* (15.46 ± 4.79 %, n = 6).

Discussion

Digestive transit times may give an indication of how efficiently nutrients are absorbed in the gut (Klasing 1998; Witmer 1998a). The level of nutrient absorption is expected to increase as the length of time the food spends in the gut increases (Klasing 1998;

Witmer 1998a). In order to process fruit rapidly, frugivores tend to have short gut passage times (Levey and Karasov 1989) and this may be an adaptation to low sugar concentrations found in some fruit diets (Brown and Downs 2003). Digestive transit times of Knysna Turacos ranged from c. 13-28 min and, despite being much larger frugivores, were similar to those obtained by Worthington (1989) who found fruit epidermis transit rates of c. 21-22 min for Golden-collared Manakins, *Manacus vitellinus*, and Red-capped Manakins, *Pipra mentalis*, respectively. Our results were also similar to those obtained by Linnebjerg et al. (2009) who found transit times of c.16 mins for *Ligustrum robustum* and c.13 min for *Clidemia hirta* fruit when ingested by Red-whiskered Bulbuls, *Pycnonotus jocosus*.

Murphy et al. (1993) suggests that transit times will decrease as the number of fruits eaten increases. This implies that the Turacos should have experienced the slowest transit times on the *Syzygium cordatum* diet, as intake rates were the highest on this fruit diet. However there were no clear trends when we consider the inverse of Murphy et al. (1993)'s idea as intake rates on both *Ficus petersii* and *Ficus sur* diets were the lowest observed but the transit times varied from one of the slowest to one of the fastest respectively. When considering transit times observed in this study it is important to note that trials were conducted on overnight fasted birds and may therefore not be typical of digesta transit times of wild Turacos as these birds would usually have food in their gut (Levey and Martínez del Rio 1999).

Witmer (1998b) defines apparent assimilation efficiency as how well a bird is able to digest a particular diet. Knysna Turacos had relatively low apparent assimilation efficiencies (< 50% for 67% of the tree species used in the current study). Apparent assimilation efficiencies obtained in this study were lower than those of three frugivorous American thrush species (American Robin *Turdus migratorius*, Wood Thrush *Hylocichla mustelina*, and Gray-cheeked Thrush *Catharus minimus*) and Cedar Waxwings (*Bombycilla cedrorum*), which ranged from 91-99% (Witmer 1999). Similarly, results from the current study were also lower than those obtained by Worthington (1989) for two Manakin species (*M. vitellinus* and *P. mentalis*), which ranged from 86-97.6%; and Witmer (1998b) for five frugivorous species (c. 96%) when fed whole chokecherry (*Prunus virginiana*) fruit. A low sugar concentration in the fruit may cause low assimilation efficiencies, as will high protein levels (Castro et al. 1989;

Witmer 1999). However, an increase in the lipid content of the fruit may increase assimilation efficiencies (Castro et al. 1989). However, further studies of the nutritional content of the fruit used in the current study would need to be conducted in order to either accept or reject these assumptions.

Optimal foraging theory predicts that frugivores will select fruit that is the most profitable in terms of energy intake (Stephens and Krebs 1986; Lepczyk et al. 2000; Downs 2008). Knysna Turacos were observed to have the highest intake rates in terms of wet weight on the *S. cordatum* diet where they had the highest gross energy intake, highest daily energy assimilation and highest apparent energy assimilation; and conversely the lowest intake rates in terms of wet weight on the *F. petersii* diet on which they had the lowest gross energy intake, lowest daily energy assimilation and lowest apparent energy assimilation. This indicates that in this study, *S. cordatum* fruit are a profitable food source in terms of energy and *F. petersii* the lowest for Knysna Turacos, suggesting that in the wild they would select *S. cordatum* fruit over *F. petersii* fruit.

Our results show that Knysna Turacos had digesta transit times that fell into the range of previous studies and consequently we accept this prediction. It was predicted that Knysna Turacos would have high assimilation efficiencies however it was observed that they had lower apparent assimilation efficiencies than those observed in other avian frugivores. Consequently we reject this prediction. Our results also show that they are have greater intake rates in terms of wet weight on fruit which they are able to assimilate efficiently and consequently we accept this prediction.

Future studies looking at the nutritional composition of indigenous forest fruit may give insight into the Knysna Turacos digestive efficiency and consequently their food preferences and role as potential seed dispersers of fruiting tree species.

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Table 1. Fruit characteristics of indigenous tree species used in the study

Tree species	***Family**	***Fruiting period**	***Colour when ripe**	***Fruit size**	**#Fruit Size Rank**	***Seed size**	**^Seed Size Rank**	**Mean ± SE number of seeds**
Clerodendrum glabrum	Verbenaceae	Feb-July	Yellowish white	10 mm diam	1	8 x 2 mm	3	1.0 ± 0.0 (5)
Ficus lutea	Moraceae	June-Oct	Yellowish brown	15-30 mm diam	3	1.5 x 1 mm	1	124.0 ± 17.3 (5)
Ficus petersii	Moraceae	Aug-Dec	Red	10 mm diam	1	1.5 x 1 mm	1	94.6 ± 9.0 (5)
Ficus sur	Moraceae	All year	Orange-red	20-40 mm diam	4	1.5 x 1 mm	1	828.0 ± 50.6 (5)
Mimusops caffra	Sapotaceae	All year	Red	20 x 15 mm	2	15 x 10 mm	4	1.0 ± 0.0 (5)
Solanum giganteum	Solanaceae	Feb-July	Shiny red	10 mm diam	1	4 x 3 mm	2	12.2 ± 1.5 (5)

Note: numbers in parentheses are sample sizes

#1 = smallest; 4 = largest; ^1 = smallest; 4 = largest

(*Griffiths 2006; Boon 2010)

Table 2 Mean ± SE of transit time, amount eaten, gross energy intake, excreta energy, daily energy assimilated and apparent assimilation efficiency of Knysna Turacos (n = 6) when fed fruits from six indigenous tree species. Treatments with letters in common are not significantly different at the 5 % level

	Tree species					
	Clerodendrum glabrum	***Ficus lutea***	***Ficus petersii***	***Ficus sur***	***Mimusops caffra***	***Syzygium cordatum***
Transit time (min)	22.7 ± 4.5^{ab}	21.5 ± 1.2^{ab}	22.6 ± 2.7^{ab}	12.4 ± 0.8^{b}	28.3 ± 2.8^{a}	22.6 ± 2.4^{ab}
Amount eaten (g g^{-1}BM)	0.6 ± 0.04^{a}	0.6 ± 0.04^{a}	0.3 ± 0.02^{b}	0.3 ± 0.02^{b}	0.6 ± 0.1^{a}	0.7 ± 0.1^{a}
Gross energy intake (kJ g^{-1}BM)	1.5 ± 0.1^{a}	1.6 ± 0.1^{a}	0.7 ± 0.1^{b}	0.8 ± 0.1^{b}	1.7 ± 0.3^{a}	1.8 ± 0.1^{a}
Excreta energy (kJ g^{-1}BM)	0.9 ± 0.1^{a}	1.3 ± 0.1^{d}	0.6 ± 0.1^{c}	0.4 ± 0.04^{bc}	0.7 ± 0.1^{ac}	0.3 ± 0.04^{b}
Daily energy assimilated (kJ g^{-1}BM)	0.6 ± 0.1^{ab}	0.3 ± 0.04^{bd}	0.1 ± 0.02^{cd}	0.3 ± 0.1^{bc}	1.0 ± 0.3^{a}	1.5 ± 0.12^{e}
Apparent assimilation efficiency (%)	40.2 ± 2.6^{c}	18.2 ± 2.5^{b}	15.5 ± 4.8^{b}	43.1 ± 8.0^{ac}	56.4 ± 4.2^{a}	84.5 ± 1.3^{d}

Chapter 6

EFFECT OF INGESTION BY KNYSNA TURACOS (*TAURACO CORYTHAIX*) ON GERMINATION SUCCESS OF FRUIT OF INDIGENOUS SOUTH AFRICAN TREE SPECIES

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Seed dispersal plays an important role in the persistence, regeneration and maintenance of plant communities. It is therefore not surprising that much attention has been paid to the germination potential of seeds ingested by frugivorous animals. Consequently the aim of this study was to determine what effect ingestion of seeds by Knysna Turacos (*Tauraco corythaix*) has on the germination rate and germination percentage of indigenous South African tree species. Fruits from twelve tree species were fed to the Turacos in separate trials and seed retention times were determined as it has been suggested that a longer seed retention time may increase germination rates. At the end of each trial, seeds were extracted from excreta of individual birds and planted in trays containing potting soil. Germination was recorded daily until 14 days post of no germination. Knysna Turacos seed retention times were in the range of those obtained by other studies with *Ficus sur* seeds having the fastest retention times (12.4 ± 0.8 min) and *Celtis africana* the slowest (34.6 ± 5.6 min). Mean percentage germination of ingested seeds was not significantly different to pulp removed and whole fruit seeds indicating that ingestion by Knysna Turacos did not enhance percentage germination through either seed coat abrasion or pulp removal. Seed ingestion by Knysna Turacos did not influence the rate at which seeds germinated in 75 % of the tree species while it did for ingested *F. lutea*, *F. natalensis* and *F. sur* seeds which germinated significantly sooner than whole fruit seeds. Future studies relating the composition of indigenous forest fruits to food preferences of Knysna Turacos may give insight into their role as potential seed dispersers of indigenous fruiting tree species.

Key words: Knysna Turacos, indigenous trees, seed retention, germination percentage, germination rate

Introduction

Seed dispersal plays a vital role in the persistence, regeneration and maintenance of plant communities (Howe and Smallwood 1982; Nathan and Muller-Landau 2000; Chave et al. 2002; Herrera 2003; Bascompte and Jordano 2007). Up to 90 % of tropical and between 30-50 % of temperate tree species rely on frugivorous animals to disperse their seeds (Howe and Smallwood 1982; Aizen et al. 2002; Herrera 2003). It is therefore not surprising that much attention has been paid to the germination potential of seeds ingested by frugivorous animals (reviewed in Traveset 1998). According to Ridley (1930), ornithochory is a process of seed dispersal by avian frugivores where fruit pulp is consumed and viable seeds are regurgitated or defecated. The ingestion of seeds by frugivores is assumed to enhance and increase germination success and rate (defined as the speed at which seeds germinate (Traveset et al. 2001)) respectively (Krefting and Roe 1949; Turcek 1963; Van der Pijl 1972; 1982; Traveset and Verdú 2002).

Many studies have found that seeds ingested by frugivores experience higher germination success compared with that of non-ingested seeds (Holthuijzen and Sharik 1985; Lieberman and Lieberman 1986; Barnea et al. 1990; Izhaki and Safriel 1990; Barnea et al. 1991; Clergeau 1992; Ellison et al. 1993; Murray et al. 1994). The removal of the pulp has been suggested to enhance germination as germination inhibitors may be present in the pulp (Mayer and Poljakoff-Mayber 1975, 1989; Cipollini and Levey 1997). Pulp removed seeds may also have reduced risks of predation and/or microbial attacks (Evenari 1949; Rick and Bowman 1961; Ketring 1973; Mayer and Poljakoff-Mayber 1975; Temple 1977; Herrera 1984; Izhaki and Safriel 1990; Barnea et al. 1991;

Witmer and Cheke 1991). Modification of the seed coat structure (either chemically or mechanically) due to ingestion has also been suggested to enhance germination (Agami and Waisel 1986, 1988; Barnea et al. 1990; Izhaki and Safriel 1990; Yagihashi et al. 1998).

Seed ingestion by frugivores has also been shown to decrease (Livingston 1972; Smith 1975; Valido and Nogales 1994; Nogales et al. 1995; Crossland and Vander Kloet 1996) or have no significant effect on germination success (Brunner et al. 1976; McDiarmid et al. 1977; Salomonson 1978; Lieberman et al. 1979; Howe and Vande Kerckhove 1981; Stocker and Irvin 1983; Lieberman and Lieberman 1986; Barnea et al. 1992; Clout and Tilley 1992).

Studies have shown that ingestion of seeds can increase (Swank 1944; Rick and Bowman 1961; Noble 1975) or decrease (McDiarmid et al. 1977; Ellison et al. 1993; Nogales et al. 1995) the rate of germination and can cause a total loss of seed viability (Hudler et al. 1979). Some plant species, however, are completely reliant on ingestion by frugivorous animals for germination (Noble 1975).

The effect of ingestion on germination success differs between both plant and animal species and may be as a result of factors such as differing gut retention times and the resulting level of scarification of the endocarp; and the composition of the seed coat (Krefting and Roe 1949; Glyphis et al. 1981; Holthuijzen and Sharik 1985; Lieberman and Lieberman 1986; Barnea et al. 1990; Izhaki and Safriel 1990; Barnea et al. 1991; Traveset 1998; Yagihashi et al. 1998, Traveset et al. 2001).

Seed retention time (SRT) can be defined as the time spent passing though the bird's digestive system i.e. the time from when the bird ingests a fruit to the time when the seed is defecated or regurgitated (Fukui 2003). It has been suggested that a longer SRT is more beneficial in that the level of scarification of the seed coat is increased and subsequently germination rates are enhanced (Barnea et al. 1991; Fukui 2003). This however, may be an oversimplification of this relationship. Different seed sizes have different retention times with small seeds tending to have longer SRTs (Stanley and Lill 2002; Traveset and Verdú 2002; Fukui 2003). Ingested small seeds are therefore more likely to have an increased germination success (Traveset and Verdú 2002).

Knysna Turacos (*Tauraco corythaix*) (Musophagiformes: Musophagidae) are relatively large (c. 310 g) fairly common forest frugivores (Oatley 1997) that are endemic to South Africa (Rowan 1983, du Plessis and Dean 2005). They may track fruit locally in response to availability (Rowan 1983).

The aim of this study was to determine what effect ingestion of seeds by Knysna Turacos has on the percentage germination and rate of germination of indigenous South African tree species and whether differences were due to scarification of the seed coat or removal of the seeds from the pulp. It was predicted that percent germination and rate of germination of ingested seeds would be greater for most of the indigenous species than for pulp removed and whole fruit seeds. Seed retention time was also measured in order to determine the potential effect on germination success.

Materials and Methods

Bird Capture and Maintenance

Six captive-bred Knysna Turacos were sourced from Mr. M.C. Weber under permit from Ezemvelo KZN Wildlife (permit number: 1781/2008). Before experiments were conducted the birds were housed in pairs in outside aviaries (1 x 2.12 x 2.66 m).

Birds were fed a maintenance diet (a choice of mealworms (*Tenebrio molitor* larvae), carrots, apples, paw-paws, pears, bananas, oranges and/or grapes) daily. Skin was removed from paw-paws, oranges and bananas and the fruit was then cut into c. 1 x 1 x 1 cm cubes. The other fruit was grated. A mixture of Aviplus Softbill/Mynah crumble and pellets (Avi-products, Durban, South Africa) was added to the maintenance diets daily. Water was provided *ad libitum*.

Plant species

Fruits from a range of indigenous tree species (n = 12) (Table 1) were used. Trees were sourced from the KwaZulu-Natal coastal and afromontane indigenous forests and fruits were used within 48 h of collection. The availability of fruit limited the choice of tree species used in this study.

Experiments

Birds were moved indoors for two days before any trials were conducted. Here they were housed in individual cages (42.7 x 43 x 59.3 cm) in a constant environment room with a 12 L: 12 D photoperiod at 25 ± 1°C. To ensure a post-absorptive state at the beginning of each experiment, any uneaten food was removed at 18h00 and no food was available overnight. Clean plastic trays, the same size as the cage's base, were placed under each cage prior to 06h00. Water was provided *ad libitum*.

Seed retention time

Indigenous fruit for each specific trial were added to the birds' maintenance diet one day prior to each experimental day. On the experimental days, birds were provided with whole fruits of a particular tree species. The length of the trials for each particular fruit species varied from 6-12 h depending on fruit availability and the amount eaten. The time that the birds' first ingested the fruit including the seed and the first appearance of seeds in their excreta was recorded to determine SRTs. Excreta were collected from the plastic trays at the end of each trial.

Germination success

Seeds were extracted from the respective individual birds' excreta from each fruit treatment and planted in separate trays (265 x 180 x 75mm) containing potting soil within 24 h after the feeding trial experiment (Table 2). Regurgitated seeds were easily identifiable as they were a different colour to the defecated seeds or, in some cases, still had little bits of pulp still attached. Seeds were covered with a potting soil layer c. 0.5 cm deep. Trays were housed in a shade house and watered daily. Germination was considered as when the seedlings first broke through the soil surface and were visible. Germination was recorded daily for 90 days. If 100 % germination had not occurred by day 90, trials were continued for a further 14 days. If germination was still taking place after day 104, trials were continued until 14 days of no germination occurred. Counted seedlings were removed. Controls of whole fruits and manually pulp removed seeds were planted concurrently and in the same manner. These controls were used in order to determine what effect the removal of pulp and/or the scarification of the seeds has on germination success. Five fresh fruits of each species were dissected to determine the mean number of seeds in the whole fruits in order to determine germination percentages.

Analyses

The cumulative percentage germination was determined for each tree species. Average number of seeds per whole fruit for each tree species was used to calculate germination percentage. Seed retention and the time taken from the time of sowing until first seedling emergence for each plant species was analyzed using Kruskal-Wallis ANOVA tests. Kruskal-Wallis ANOVA tests were also used to determine if there were significant differences between ingested, regurgitated (where applicable), pulp removed and seeds in whole fruits. The relationship between seed size and retention time was analyzed using a simple linear regression. All analyses were conducted using STATISTICA (Statsoft, Tulsa, version 7, USA). Mean ± SE was reported for all values and Bonferroni adjustments were made.

Results

Fruit traits

Fruits used in this study varied in a number of features such as colour when ripe, fruit size, seed size, and mean number of seeds per fruit (Table 1).

Seed retention time

The SRTs varied significantly between the different tree species (Kruskal-Wallis ANOVA $H_{11} = 24.862$, $n = 72$, $p < 0.01$) (Fig. 1). *Ficus sur* seeds had the fastest retention times (12.4 ± 0.8 min) while *Celtis africana* was slowest (34.6 ± 5.6 min). The relationship between seed size and retention time was not significant ($r^2 = 0.167$, $F = 3.21$, $p = 0.104$, $df = 10$).

Germination percentage

The percentage germination of ingested seeds varied significantly between the tree species (Kruskal-Wallis ANOVA $H_{11} = 53.934$, $n = 72$, $p< 0.01$) (Fig. 2). Mean percentage germination of ingested seeds ranged from 12.0 ± 3.6 (*Bridelia micrantha*) to 94.5 ± 4.6 % (*Rhamnus prinoides*), which was not significantly different to pulp removed and whole fruit seeds (Table 3; Figs. 3 and 4).

The percentage germination of regurgitated seeds varied significantly between the tree species (Kruskal-Wallis ANOVA $H_2 = 8.424$, $n = 15$, $p = 0.015$) (Fig. 5). Mean percentage germination of regurgitated seeds ranged from 4.6 ± 2.5 (*R. prinoides*) to 94.2 ± 5.8 % (*Syzygium cordatum*), which was not significantly different to ingested, pulp removed and whole fruit seeds (Table 4; Figs. 3 and 4).

Mean time to seedling emergence

Seedling emergence of ingested seeds varied significantly among the tree species (Kruskal-Wallis ANOVA H_{11} = 58.871, n = 72, $p < 0.01$) (Fig. 6a). Mean seedling emergence of ingested seeds ranged from 12.3 ± 0.7 (*B. micrantha*) to 57.7 ± 7.1 days (*C. africana*), which was not significantly different to pulp removed seeds for 75 % of the tree species (Table 5; Figs. 3 and 4). However, in the case of *F. lutea*, *F. natalensis* and *F. sur*, it was significantly higher than mean seedling emergence of whole fruit seeds (Table 5; Figs. 3 and 4).

Seedling emergence of regurgitated seeds did not vary significantly between the tree species (Kruskal-Wallis ANOVA H_2 = 3.425, n = 15, p = 0.180) (Fig. 6b). Mean seedling emergence of regurgitated seeds ranged from 12.0 ± 0.0 (*B. micrantha*) to 22.0 ± 11.4 days (*R. prinoides*), which was not significantly different to ingested and pulp removed seeds (Table 6; Figs. 3 and 4). However, in the case of *B. micrantha*, it was significantly higher than mean seedling emergence of whole fruit seeds (Table 6; Figs. 3 and 4).

Discussion

Different sized seeds are expected to have different retention times (Stanley and Lill 2002; Fukui 2003) with small, light seeds generally retained longer than large, heavy seeds (Garber 1986; Levey and Grajal 1991; Gardener et al. 1993). This was contrary to the current study where small *Ficus sur* seeds had the fastest (12.36 ± 0.84 min) and the larger *Celtis africana* seeds the slowest (34.55 ± 5.63 min) retention times although this relationship was not found to be significant. Retention times have been suggested to decrease as the number of fruit eaten increases (Murphy et al. 1993). This could explain the inconsistent results obtained in this study, as *C. africana* fruits were smaller and contained less pulp than *F. sur* fruits. Seed retention times in the current study were similar to those obtained by Barnea et al. (1991) for blackbirds, *Turdus merula*, (15-74 min) and bulbuls, *Pycnonotus xanthopygos*, (12-27 min); Linnebjerg et al. (2009) for red-whiskered bulbuls, *Pycnonotus jocosus*, (13-68 min); and by Fukui (2003) for brown-eared bulbuls, *Hypsipetes amaurotis*, (2-123 min).

In order to increase their ingestion rates, frugivorous birds tend to have short gut passage times (Levey 1991), but these may vary for various reasons. However, a longer SRT may be more beneficial to plant fitness in terms of increased germination and longer dispersal distances (Barnea et al. 1991; Fukui 2003) suggesting that, in this study, *C. africana* seeds would have highest germination percentage and potential dispersal and *F. sur* seeds the lowest. This, however, was not found as *Bridelia micrantha* seeds had the highest and *Rhamnus prinoides* the lowest germination percentage with *C. africana* and *F. sur* experiencing germination percentages between this, i.e. *Bridelia micrantha* and *R. prinoides* had similar retention times.

Ingestion by birds has been suggested to enhance seed germination by either depulping the seeds (Mayer and Poljakoff-Mayber 1989, Robertson et al. 2006) or by seed coat abrasion (Agami and Waisel 1986, Traveset 1998). Interestingly, ingestion by Knysna Turacos did not have any significant effect on seed germination percentage. Percentage germination of seeds ingested by the Turacos was not significantly different to that of pulp removed seeds, suggesting that seed coat abrasion was not influencing germination as in other studies (Evenari 1949; Agami and Waisel 1988; Barnea et al. 1990, 1991); and to that of whole fruit seeds, suggesting that the removal of the pulp from the seeds did not enhance percentage germination.

Seed ingestion by Knysna Turacos did not influence the rate at which seeds germinated in all tree species except ingested *F. lutea*, *F. natalensis* and *F. sur* seeds which germinated significantly sooner than whole fruit seeds. A faster germination rate may be beneficial in terms of an advantage in seedling size and therefore a greater chance of survival and also in terms of reducing the risk of the seeds being predated (Traveset et al. 2001). These results are similar to those obtained by Lieberman and Lieberman (1986); Izhaki and Safriel (1990); Barnea et al. (1991); and Traveset and Willson (1997) who found no significant effect on rate of germination of seeds ingested by various avian frugivores.

In conclusion, Knysna Turacos had seed retention rates that were in the range of those obtained by other studies for other frugivores and plant species. Furthermore their ingestion of seeds did not generally enhance percentage germination; nor the rate of germination except for ingested *F. lutea*, *F. natalensis* and *F. sur* seeds which experienced a faster germination rate than that of whole fruit seeds. Consequently we reject our predictions for all tree species except for *F. lutea*, *F. natalensis* and *F. sur*. Future studies relating the composition of indigenous forest fruits to food preferences of Knysna Turacos may give insight into their role as potential seed dispersers of indigenous fruiting tree species.

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Fig. 6 Day of first seedling emergence for indigenous seeds a) ingested and b) regurgitated by Knysna Turacos. (Boxes indicate the 25 and 75 % quartiles; the solid black squares the medians; and the bars the 10 and 90 % values. Treatments with letters in common were not significantly different at the 5 % level.)

Table 1. Fruit characteristics of indigenous tree species used in the study

Tree species	*Family	*Fruiting period	*Colour when ripe	*Fruit size	$^{\#}$Fruit Size Rank	*Seed size	$^{\wedge}$Seed Size Rank	Mean SE number of seeds
Bridelia micrantha	Euphorbiaceae	Nov-April	Black	10 x 7 mm	3	5 x 3 mm	4	1.0 ± 0.0 (5)
Celtis africana	Celtidaceae	Oct-April	Brownish-yellow	8 mm diam	2	7 x 5 mm	6	1.0 ± 0.0 (5)
Clerodendrum glabrum	Verbenaceae	Feb-July	Yellowish white	10 mm diam	4	8 x 2 mm	7	1.0 ± 0.0 (5)
Ficus lutea	Moraceae	June-Oct	Yellowish brown	15-30 mm diam	9	1.5 x 1 mm	1	124.0 ± 17.3 (5)
Ficus natalensis	Moraceae	All year	Red-brown	10-20 mm diam	7	1.5 x 1 mm	1	168.8 ± 35.7 (5)
Ficus petersii	Moraceae	Aug-Dec	Red	10 mm diam	4	1.5 x 1 mm	1	94.6 ± 9.0 (5)
Ficus sur	Moraceae	All year	Orange-red	20-40 mm diam	10	1.5 x 1 mm	1	828.0 ± 50.6 (5)
Grewia occidentalis	Tiliaceae	Jan-July	Reddish purple	25 mm diam	8	7 x 5 mm	6	2.3 ± 1.7 (5)
Mimusops caffra	Sapotaceae	All year	Red	20 x 15 mm	6	15 x 10 mm	8	1.0 ± 0.0 (5)
Rhamnus prinoides	Rhamnaceae	Jan-Aug	Purplish black	6 mm diam	1	4 x 4 mm	1	3.0 ± 0.0 (5)
Solanum giganteum	Solanaceae	Feb-July	Shiny red	10 mm diam	4	4 x 3 mm	2	12.2 ± 1.5 (5)
Syzygium cordatum	Myrtaceae	Oct-June	Deep purple	20 x 10 mm	5	7 x 4 mm	5	1.0 ± 0.0 (5)

Note: numbers in parentheses are sample sizes

$^{\#}$1 = smallest; 10 = largest; $^{\wedge}$1 = smallest; 8 = largest

(*Griffiths 2006; Boon 2010)

Table 2 Number of seeds planted in soil trays for each indigenous tree species fed to Knysna Turacos

Tree species	Ingested/ Regurgitated	Number of seeds per tray	Number of trays	Number of pulp removed seeds in control	Number of whole fruits in control
Bridelia micrantha	Ingested Regurgitated	167 20	6	410	410 (1.0 ± 0.0)
Celtis africana	Ingested	100	6	360	360 (1.0 ± 0.0)
Clerodendrum glabrum	Ingested	40	6	40	40 (1.0 ± 0.0)
Ficus lutea	Ingested	50	6	50	1 (124.0 ± 17.3)
Ficus natalensis	Ingested	30	6	30	1 (168.8 ± 35.7)
Ficus sur	Ingested	50	6	50	1 (828.0 ± 50.6)
Ficus petersii	Ingested	50	6	50	1 (94.6 ± 9.0)
Grewia occidentalis	Ingested	24	6	24	11 (2.3 ± 1.7)
Rhamnus prinoides	Ingested Regurgitated	50 36	6 3*	140	47 (3.0 ± 0.0)
Solanum giganteum	Ingested	30	6	30	3 (12.2 ± 1.5)
Syzygium cordatum	Ingested Regurgitated	30 20	6	170	170 (1.0 ± 0.0)

Note: numbers in parentheses are mean ± SE number of seeds per fruit (n = 5)

*Only 3 Knysna Turacos regurgitated *R. prinoides* seeds

Table 3 Statistical outputs comparing percentage germination of ingested seeds with pulp removed and whole fruit seeds from feeding experiments

Tree species	Germination percentage Pulp removed seeds compared with ingested seeds				Germination percentage Whole fruit seeds compared with ingested seeds			
	H_1	n	p		H_1	n	p	
Bridelia micrantha	1.000	7	0.317	NS	2.250	7	0.134	NS
Celtis africana	2.333	7	0.127	NS	2.333	7	0.127	NS
Clerodendrum glabrum	2.291	7	0.130	NS	0.255	7	0.614	NS
Ficus lutea	1.077	7	0.299	NS	2.291	7	0.130	NS
Ficus natalensis	0.259	7	0.611	NS	2.333	7	0.127	NS
Ficus sur	2.291	7	0.130	NS	2.291	7	0.130	NS
Ficus petersii	0.255	7	0.614	NS	2.291	7	0.130	NS
Grewia occidentalis	0.065	7	0.799	NS	1.620	7	0.203	NS
Mimusops caffra	0.255	7	0.614	NS	2.291	7	0.130	NS
Rhamnus prinoides	1.018	7	0.313	NS	1.620	7	0.203	NS
Solanum giganteum	2.333	7	0.126	NS	1.037	7	0.309	NS
Syzygium cordatum	0.388	7	0.533	NS	1.217	7	0.270	NS

Table 4 Statistical outputs comparing percentage germination of regurgitated seeds with ingested, pulp removed and whole fruit seeds

Tree species	Germination percentage				Germination percentage				Germination percentage			
	Ingested seeds compared with regurgitated seeds				Pulp removed seeds compared with regurgitated seeds				Whole fruit seeds compared with regurgitated seeds			
	H_1	n	p		H_1	n	p		H_1	n	p	
Bridelia micrantha	0.103	12	0.748	NS	0.259	7	0.611	NS	2.333	7	0.127	NS
Rhamnus prinoides	0.831	9	0.362	NS	0.200	4	0.655	NS	0.889	4	0.346	NS
Syzygium cordatum	0.707	12	0.401	NS	0.167	7	0.683	NS	3.500	7	0.061	NS

Table 5 Statistical outputs comparing day of first seedling emergence of ingested seeds with pulp removed and whole fruit

Tree species	Days of first seedling emergence				Days of first seedling emergence			
	Pulp removed seeds compared with ingested seeds				Whole fruit seeds compared with ingested seeds			
	H_1	n	p		H_1	n	p	
Bridelia micrantha	0.167	7	0.683	NS	3.500	7	0.061	NS
Celtis africana	0.255	7	0.614	NS	0.255	7	0.614	NS
Clerodendrum glabrum	0.000	7	1.000	NS	1.750	7	0.186	NS
Ficus lutea	0.000	7	1.000	NS	6.000	7	0.014	S
Ficus natalensis	0.000	7	1.000	NS	6.000	7	0.014	S
Ficus sur	0.000	7	1.000	NS	6.000	7	0.014	S
Ficus petersii	2.291	7	0.130	NS	2.291	7	0.130	NS
Grewia occidentalis	0.000	7	1.000	NS	1.944	7	0.163	NS
Mimusops caffra	2.333	7	0.127	NS	2.333	7	0.127	NS
Rhamnus prinoides	0.064	7	0.801	NS	1.591	7	0.207	NS
Solanum giganteum	0.167	7	0.683	NS	3.500	7	0.061	NS
Syzygium cordatum	0.400	7	0.527	NS	2.800	7	0.094	NS

Note: NS = not significant; S = significant for $p < 0.05$

Table 6 Statistical outputs comparing day of first seedling emergence of regurgitated seeds with ingested, pulp removed and whole fruit seeds

Tree species	Days of first seedling emergence				Days of first seedling emergence				Days of first seedling emergence			
	Ingested seeds compared with regurgitated seeds				Pulp removed seeds compared with regurgitated seeds				Whole fruit seeds compared with regurgitated seeds			
	H_1	n	p		H_1	n	p		H_1	n	p	
Bridelia micrantha	1.000	12	0.317	NS	0.000	7	1.000	NS	6.000	7	0.014	S
Rhamnus prinoides	0.068	9	0.795	NS	0.200	4	0.655	NS	0.889	4	0.346	NS
Syzygium cordatum	0.584	12	0.445	NS	0.000	7	1.000	NS	1.944	7	0.163	NS

Note: NS = not significant; S = significant for $p < 0.05$

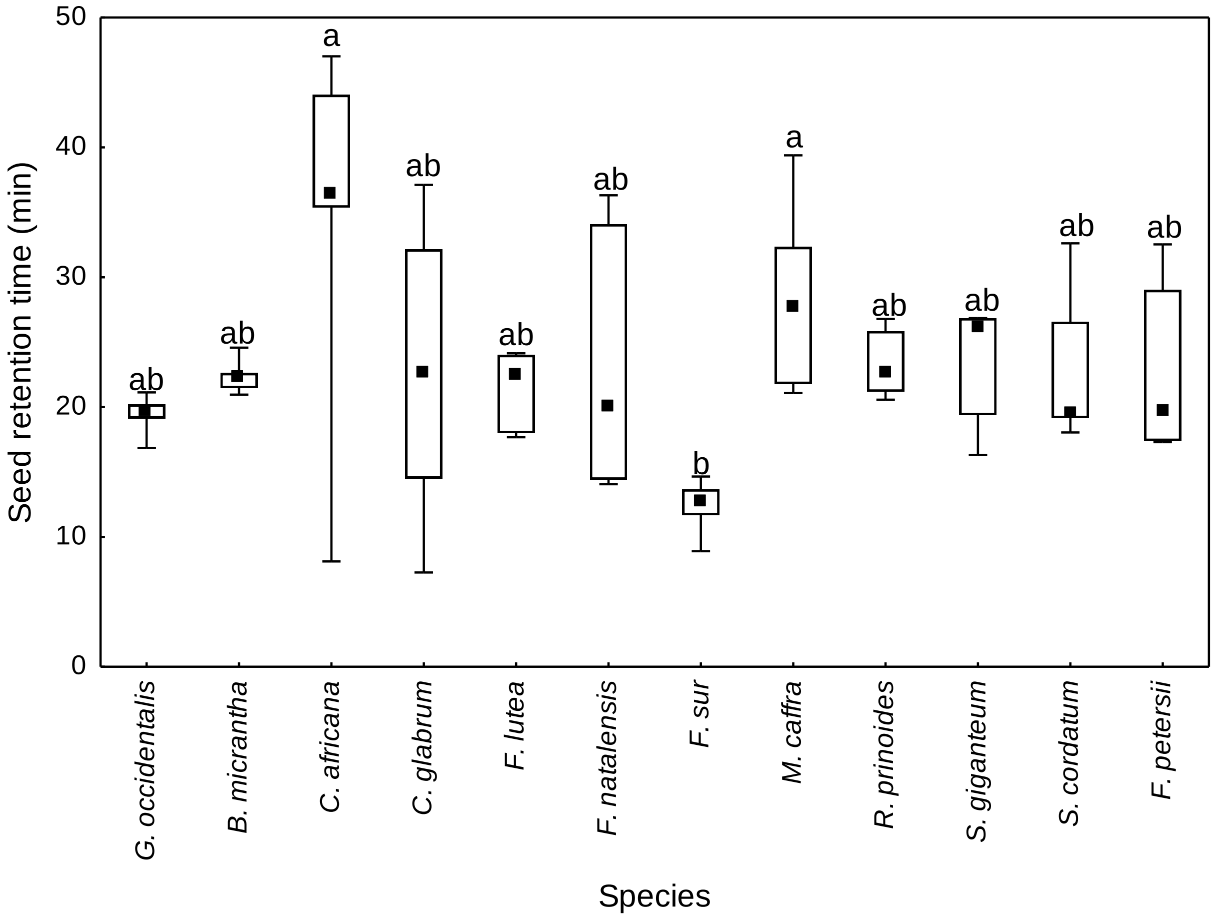

Fig. 1 Seed retention time for fruit of indigenous tree species eaten by Knysna Turacos (n = 6). (Boxes indicate the 25 and 75 % quartiles; solid black squares the medians; and bars the 10 and 90 % values. Treatments with letters in common were not significantly different at the 5 % level. See Table 1 for tree species details.)

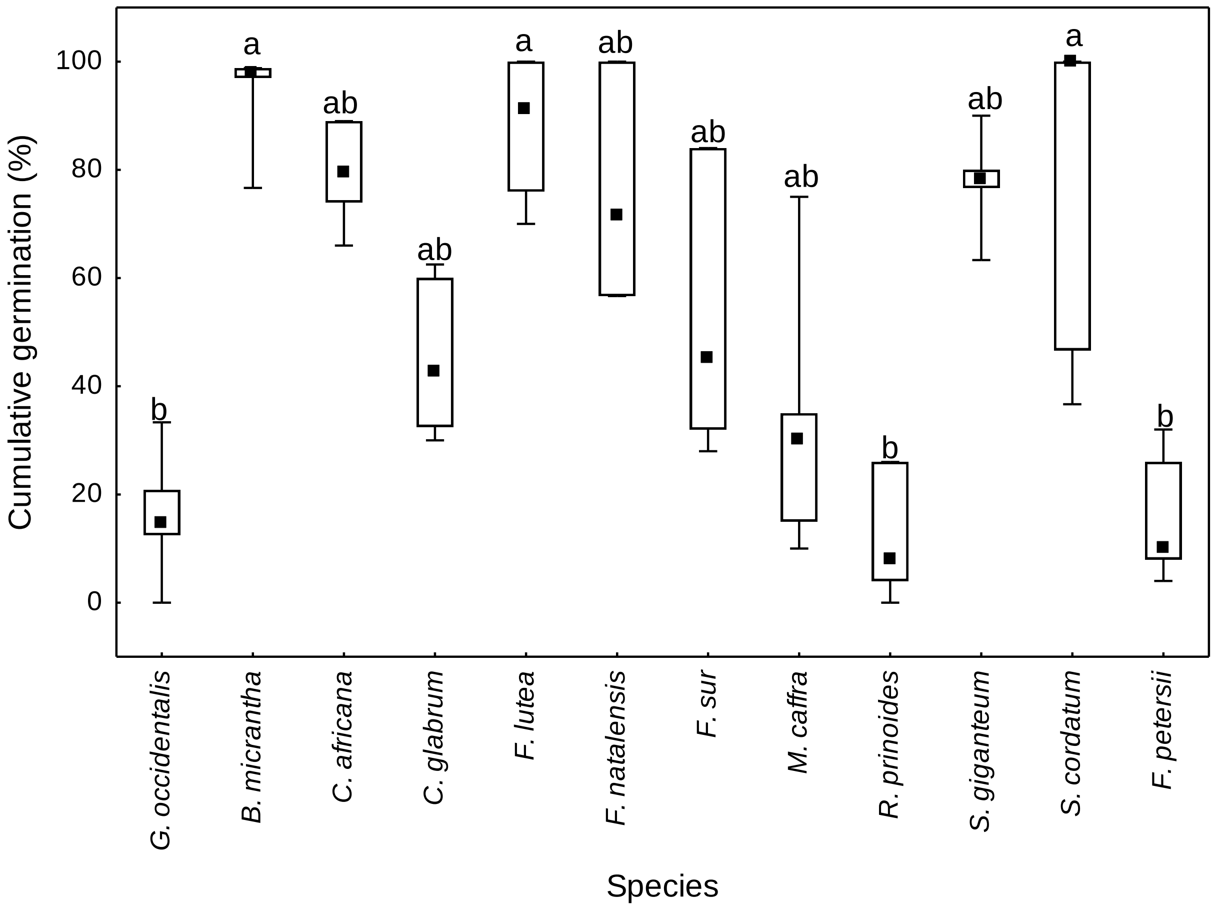

Fig. 2 Cumulative percentage germination for indigenous seeds ingested by Knysna Turacos. (Boxes indicate the 25 and 75 % quartiles; solid black squares the medians; and bars the 10 and 90 % values. Treatments with letters in common were not significantly different at the 5 % level.)

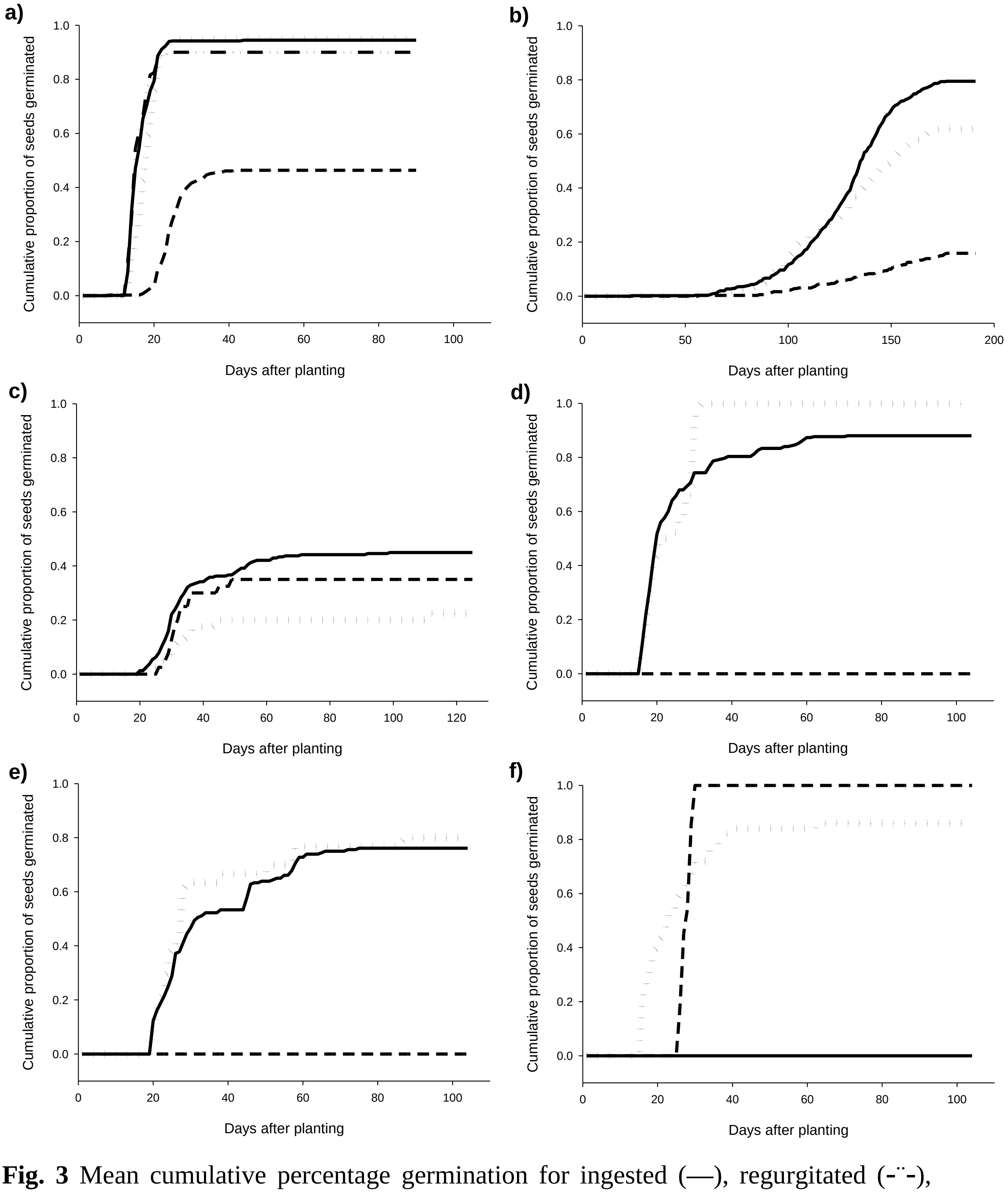

Fig. 3 Mean cumulative percentage germination for ingested (—), regurgitated (-¨-), pulp removed (˙˙˙) and whole fruit (---) seeds from a) *B. micrantha* after 90 days; b) *C. africana* after 191 days; c) *C. glabrum* after 125 days; d) *F. lutea* after 104 days; e) *F. natalensis* after 104 days; and f) *F. sur* after 104 days

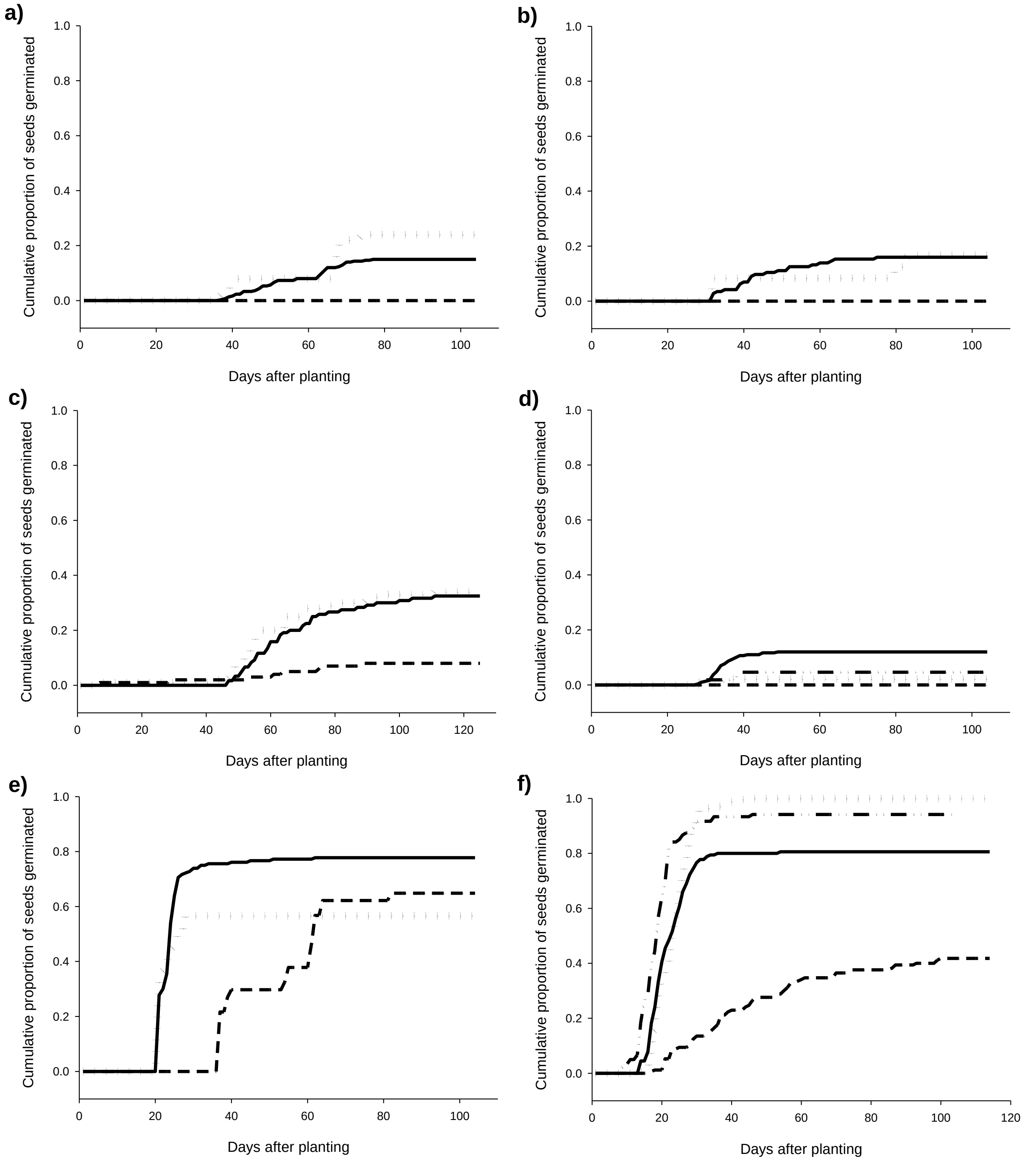

Fig. 4 Mean cumulative percentage germination for ingested (—), regurgitated (-¨-), pulp removed (¨¨) and whole fruit (---) seeds from a) *F. petersii* after 104 days; b) *G. occidentalis* after 104 days; c) *M. caffra* after 125 days; d) *R. prinoides* after 104 days; e) *S. giganteum* after 104 days; and f) *S. cordatum* after 114 (ingested, pulp removed and whole fruit) and 104 (regurgitated) days

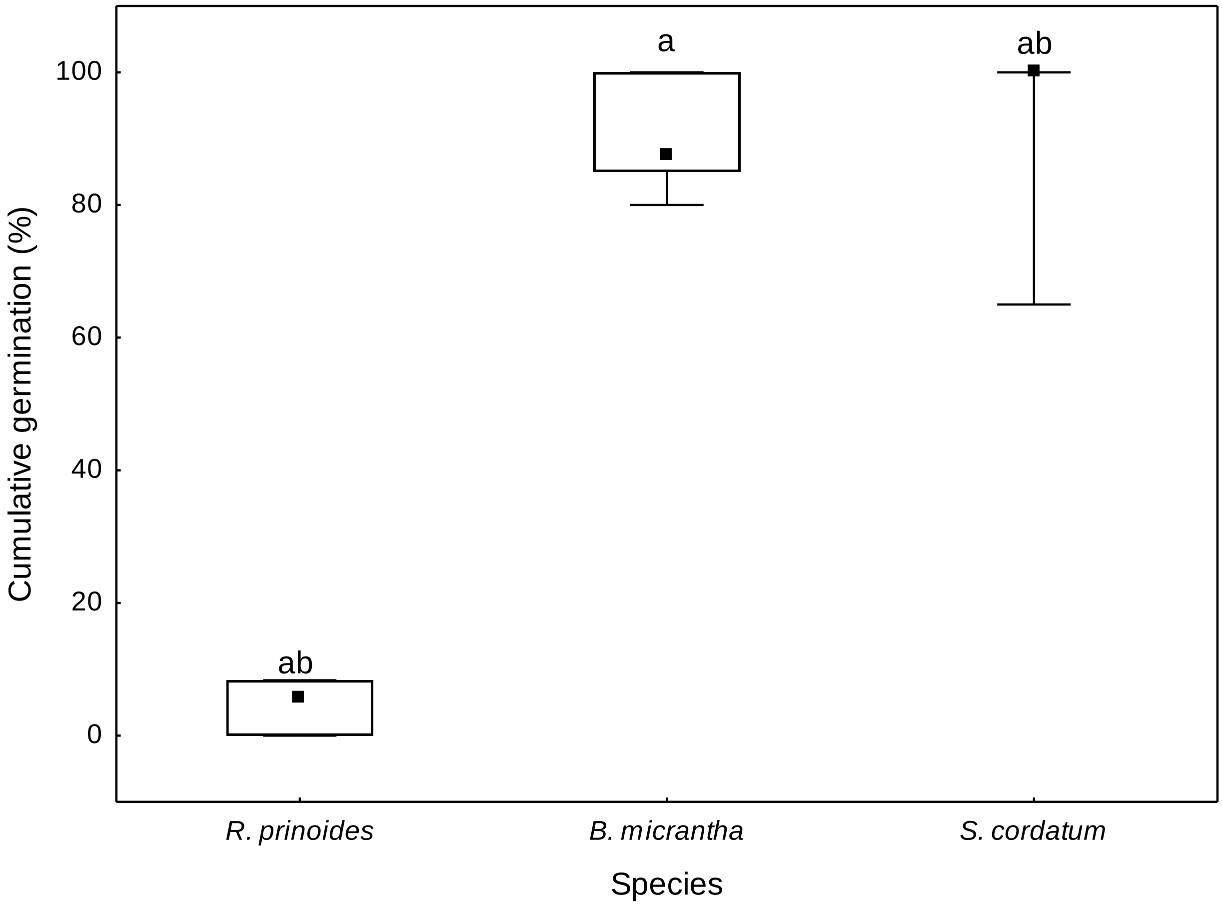

Fig. 5 Cumulative percentage germination for indigenous seeds regurgitated by Knysna Turacos. (Boxes indicate the 25 and 75 % quartiles; solid black squares the medians; and bars the 10 and 90 % values. Treatments with letters in common were not significantly different at the 5 % level.)

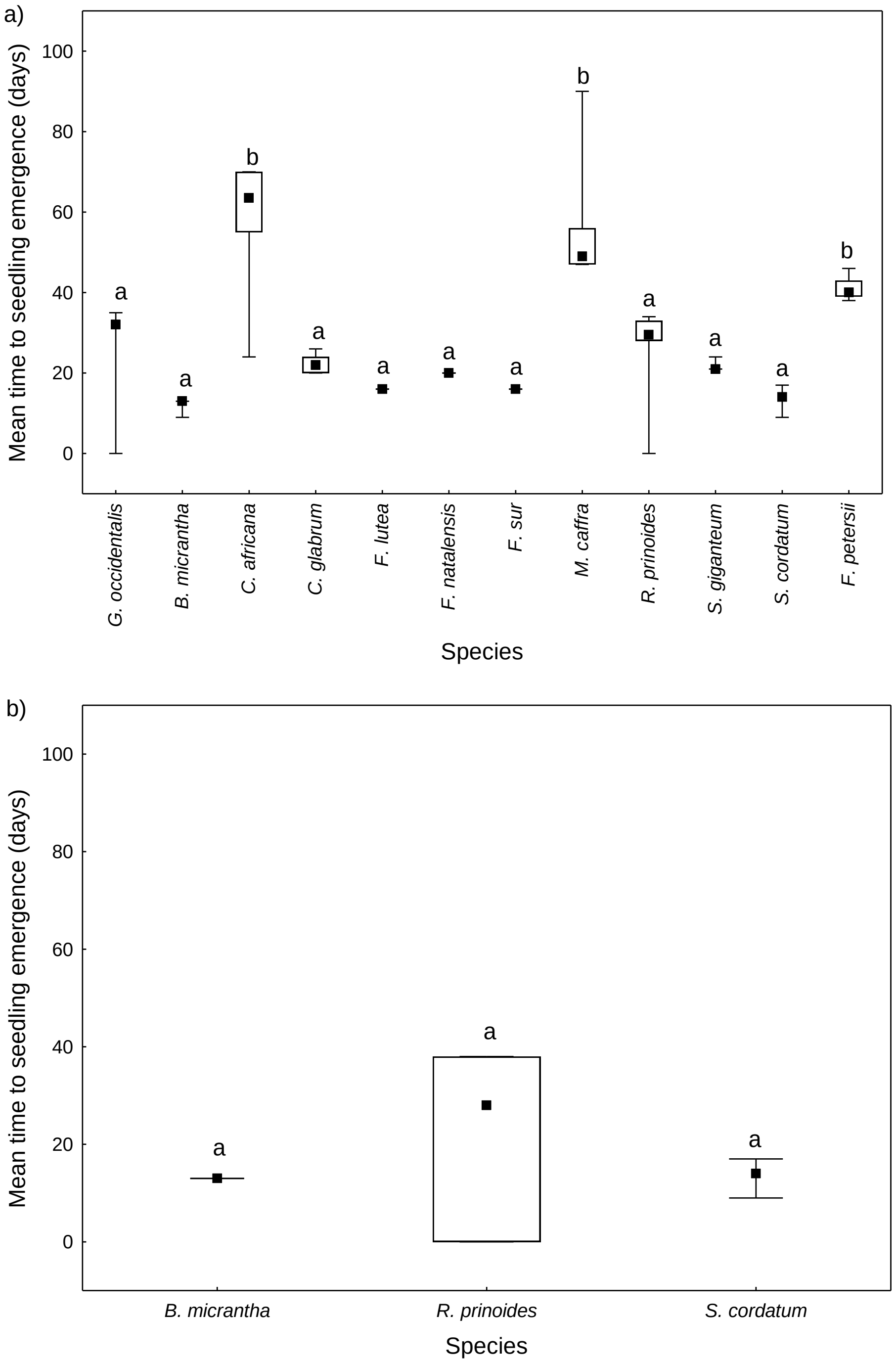

Fig. 6 Day of first seedling emergence for indigenous seeds a) ingested and b) regurgitated by Knysna Turacos. (Boxes indicate the 25 and 75 % quartiles; solid black squares the medians; and bars the 10 and 90 % values. Treatments with letters in common were not significantly different at the 5 % level.)

Chapter 7

FRUIT NUTRITIONAL COMPOSITION AND NON-NUTRITIVE TRAITS OF INDIGENOUS SOUTH AFRICAN TREE SPECIES

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Frugivorous animals play a major role in dispersing tropical, and to a lesser extent, temperate tree species. In order to attract potential seed dispersers, plants generally offer a reward of fleshy fruit pulp. Criteria for fruit choice by avian frugivores are influenced by a number of non-nutritive (e.g. fruit size and colour) factors; and nutritional composition of the fruit. There is a paucity of nutritional composition and other fruit trait data of indigenous South African fruit. This information is necessary in order to determine which frugivores are likely to ingest which fruits and consequently act as potential seed dispersal agents. This information would provide us with an understanding of the inter-relationships between indigenous fruit and frugivores in South Africa. Consequently nutritional composition was investigated in various indigenous fruit species that avian frugivores feed on. Fruits were collected from 38 indigenous tree species found in KwaZulu-Natal afromontane and coastal forests. Pulp was freeze-dried to constant mass and then analyzed for sugar, lipid and protein content; and for water content determination. Fruit width in this study ranged from 4 mm (*Searsia rehmanniana* and *Trema orientalis*) to 40 mm (*Annona senegalensis, Ficus sur* and *Xylotheca kraussiana*). Of the fruits examined in this study 29% were black and

43% red when ripe. Most (84%) fruit species analysed for sugar content were hexose dominant with 50% being fructose and 34% being glucose dominant. Only 16% of the fruit species analysed were sucrose dominant. Fruits in this study were generally observed to be high (mean: 68.1 ± 3.3%; n = 30) in water content; and low in protein and lipid content respectively (mean: 8.2 ± 0.5%; 9.3 ± 2.2%; n = 30) indicating that these fruits species could be considered as nutrient-dilute. Future studies need to determine the nutritional composition of the remaining indigenous South Africa fruit in order to develop a comprehensive database as well as examining non-nutritive factors. Together this information would play a key role in understanding fruit preferences and consequently seed dispersal and would therefore be useful in developing management plans for forest conservation.

Key words: indigenous fruit, fruit sugars, protein, lipids, fruit size, fruit colour

Introduction

A large proportion of South Africa's biodiversity is supported by indigenous forests (Mucina and Rutherford 2006). However indigenous forest is the smallest biome found in South Africa and this biome has become severely fragmented due to anthropogenic activities (Cooper 1985; Geldenhuys 1989; Low and Rebello 1996; Eeley et al. 1999; Lawes et al. 2000; Chapman et al. 2006). Fragmentation is likely to negatively affect the forest flora as well as the fauna with which these plants interact (Cooper 1985; Geldenhuys 1989; Low and Rebello 1996; Lawes et al. 2000; Chapman et al. 2006). It is thus essential that we gain an understanding of the mechanisms that govern plant-animal interactions in forest ecosystems in order to develop management plans for forest conservation (Kirika et al. 2008).

Dispersal of seeds is essential in maintaining and renewing plant communities (Howe and Smallwood 1982; Chave et al. 2002; Herrera 2003). Frugivorous animals play a role in dispersing up to 90% of tropical and between 30-50% of temperate tree species (Howe and Smallwood 1982; Herrera 2003). In order to attract potential seed dispersers, plants offer a reward of fleshy fruit pulp (Howe 1986).

Criteria for fruit choice by avian frugivores may be influenced by a number of non-nutritive factors such as fruit colour, size, and secondary compounds (Wheelwright

1985; Sallabanks 1993; Murphy 1994; Jordano 2000). Width of avian frugivores' gape limits the size of the fruit that can be ingested intact (Martin 1985; Wheelwright 1985; Jordano 2000) although feeding behavior may further influence this as some birds are 'gulpers' meaning that they swallow their fruit whole and therefore cannot ingest fruits larger than the width of their gape (Levey 1987, Symes and Downs 2001).

Birds usually consume black or red fruits (Ridley 1930; Willson et al. 1989; Burns 2005) although fruit colour as assessed by humans is likely to be different to fruit colour assessed by birds (Schaefer et al. 2006). Bird's eyes are well adapted to see red colours as their retinas contain four cone types (Bowmaker 1998; Jacobs and Deegan 1999). Green colour of unripe fruit is believed to indicate that the fruits are inedible (Willson and Whelan 1990). However, few studies have examined whether green fruits are avoided (but see Willson and Whelan 1990) and other colours preferred (but see Willson and Comet 1993; Avery et al. 1995). Unripe fruit generally contains less sugars and more toxic secondary compounds than ripe fruit, which makes them less nutritious and palatable to frugivores (Herrera 1982; Willson and Whelan 1990).

Bird's digestive ability and fruit's nutritional value are considered to be the most important factors that influence fruit choice (Worthington 1989; Martínez del Rio and Restrepo 1993; Johnson et al. 1985; Izhaki 1992; Fuentes 1994). Sugar types and concentrations in nectar have recently been explained by the plants' associations with either specialist or generalist nectar-feeding birds (Johnson and Nicolson 2008, Brown et al. 2010a; b). The sugars and sugar concentrations found in fruits, however, are not so easily explained and have traditionally been classified according to a dichotomy of high investment fruits (nutrient-dense) and low investment (nutrient-dilute) fruits although it has been suggested that fruits instead appear to be arranged along a continuum of nutrient values (McKey 1975; Howe and Estabrook 1977; Howe and Smallwood 1982; Izhaki 1993) although it has been suggested that fruit. Nutrient-dense fruits are considered to be variable in protein, relatively high in lipids, and low in water and carbohydrates (Izhaki 1993). Conversely, nutrient-dilute fruits are considered to be low in fibre and protein, high in water, and have fewer carbohydrates than nutrient-dense fruits (Snow 1981; Herrera 1982; Izhaki 1993).

Fruit pulp varies in both sugar composition and concentration and this should reflect food preferences by frugivores (Martínez del Rio and Stevens 1989). Fruit pulp consists of three main sugars; the disaccharide sucrose and the hexose monosaccharides glucose and fructose (Baker and Baker 1983a; b; Baker et al. 1998; Nicolson 2002; Lotz and Schondube 2006). The ability of the avian frugivores to efficiently digest the different sugars may affect fruit choice (Avery et al. 1999) and several studies have shown that some bird species (e.g. some families from the Sturnid-Muscicapid lineage) avoid sucrose-rich fruits as they lack the enzyme sucrase and are therefore unable to efficiently digest sucrose (Schuler 1983; Martínez del Rio 1990; Martínez del Rio and Restrepo 1993; Levey and Martínez del Rio 2001). Even those birds that do possess sucrase may prefer hexose sugars in choice tests as they may not be able to digest sucrose efficiently enough (Martínez del Rio et al. 1992; Avery et al. 1995). Birds may also prefer glucose and fructose sugars over sucrose as their fast gut passage rates prevent sucrose being hydrolysed and absorbed efficiently (Karasov and Levey 1990; Martínez del Rio and Restrepo 1993).

As there is a paucity of data on the nutritional composition and other fruit traits of indigenous South African fruit (but see Lawes 1990; Gaynor 1994; Wirminghaus et al. 2002; Voigt 2004) we are uncertain whether South African fruits follow similar trends observed in studies done in other parts of the world. This information is necessary in order to determine potential seed dispersal agents. This information would provide us with an understanding of the inter-relationships between indigenous fruit and frugivores in South Africa. Consequently nutritional composition and other non-nutritive traits (e.g. fruit colour and size) were investigated in 38 indigenous fruit species.

Materials and Methods

Collection

Ripe fruits were collected during 2010 as fruiting occurred from a range of indigenous tree species (n = 38) (Table 1) found in KwaZulu-Natal afromontane and coastal forests. Fruits were frozen immediately after collection until they could be freeze-dried. Pulp was freeze-dried to constant mass and water content determined, and then milled to a powder for nutritional composition analyses. Data on fruit size and colour were obtained from Griffiths (2006) and Boon (2010).

Fruit sugars

Sugar concentrations were determined according to Liu et al. (1999). Freeze-dried material (0.05 to 0.10 g) was mixed with 10 mL 80 % (v/v) ethanol and homogenized for 1 min. Thereafter, the mixture was incubated in an 80 ^{o}C water bath for 60 min to extract the soluble sugars. Subsequently the mixture was kept at 4 ^{o}C overnight. After centrifugation at 12000 g for 15 min at 4 ^{o}C, the supernatant was filtered through glass wool and taken to dryness in a vacuum concentrator. Dried samples were resuspended in 2 mL ultra-pure water, filtered through a 0.45 μm nylon filter and analyzed using an isocratic HPLC system (LC – 20AT; Shimadzu Corp., Kyoto, Japan) equipped with a refractive index detector (RID-10A; Shimadzu Corp.) and a 300 mm _ 7.8 mm Rezex RCM–Monosaccharide column (8 ìm pore size; Phenomenex®, Torrance, CA, USA). Concentration of individual sugars in fruits was then determined by comparison with authentic sugar standards.

Fruit lipids and proteins

Fruit lipids were extracted using a Buchi 810 Soxhlett fat extractor (according to the Soxhlett procedure). Fat was extracted from the sample by the solvent petroleum ether and percentage fat was calculated based on the gravimetric analysis (AOAC Official Method 920.39). Fruit protein was analyzed in a LECO Truspec Nitrogen Analyser using the Dumas Combustion method (AOAC Official Method 990.03). Two assumptions were made in calculating protein content based on nitrogen content. Firstly, it was assumed that all the nitrogen of the food was present as protein, and secondly that all of the food protein contained 160 g n/kg(McDonald et al. 1995).

Analyses

A sample size of one was used for % water content, % protein content, and % lipid content. Due to missing values, only traits from 19 fruit species (Table 2) were compared using Principle Components Analysis (PCA), using STATISTICA version 7 (Statsoft, Tulsa, USA). Descriptive statistics were also used and means are presented ± 1 SE.

Results

Out of the fruit used, *Trichilia dregeana*, *Annona senegalensis*, and *Xylotheca kraussiana* were the largest in diameter (c. 50mm, 40mm, and 40mm respectively), and *A. senegalensis* and *Carissa macrocarpa* had the heaviest wet mass (including seeds) (means: 25.07 ± 0.73g and 14.23 ± 1.32g respectively) (Table 1).

Most fruit (84%) were hexose dominant with 50% being fructose and 34% being glucose dominant (Table 1). Only 16% of the fruit species analysed for sugar content were sucrose dominant (Table 1). Percentage water content was generally high for all fruit species (mean: 68.1 ± 3.3%; n = 30) (Table 1) and ranged from 4.1% in *Podocarpus henkelii* to 87.8% in *F. sur*. Percentage protein and percentage lipid content of the fruit pulp was generally low for all fruit species (mean: 8.2 ± 0.5%; n = 30 and mean: 9.3 ± 2.2%; n = 30 respectively) (Table 1). Percentage protein content ranged from 3.0-17.4% in *Kraussia floribunda* and *Trema orientalis* respectively; and percentage lipid content of the fruit pulp ranged from 0.6-50.2% in *Cordia ovalis* and *Trichilia emetica* respectively (Table 1).

The PCA analysis of fruit traits resulted in 15.27% and 10.07% of the total variance being explained by factor 1 and factor 2 respectively (Fig. 1). Sucrose (+0.04), glucose (+0.44), fructose (+0.43), and water (+0.21) were positively correlated with factor 1. Protein (-0.36) and fat (-0.41) were negatively correlated with factor 1. Sucrose (+0.60) was also positively correlated with factor 2. Glucose (-0.23), fructose (-0.08), protein (-0.31), fat (-0.16), and water (-0.21) were negatively correlated with factor 2.

Discussion

Out of the 38 indigenous South Africa fruits analysed in this study, 84% were hexose dominant and 16% sucrose dominant. Our results were similar to those of Baker et al. (1998), where most New and Old World fruits were hexose-dominant with only a small percentage of the fruits being sucrose-dominant.

Mean percentage protein content of fruit (dry weight, 8.2 ± 0.5%; n = 30) obtained in this study was higher than those obtained in other studies that ranged from 4.6-7.0% (Johnson et al. 1985; Herrera 1987; Sakai and Carpenter 1990; Eriksson and Ehrlén 1991; Izhaki 1992; Pizo 2002). However, most of these fruit are northern hemisphere species. Furthermore, controlling for phylogeny would be an important part for further comparison. Mean percentage protein and percentage lipid (dry weight, 9.3 ± 2.2%; n = 30) content in fruit in the present study were lower than those obtained by Voigt et al. (2004) for some South African indigenous fruit tree species. Voigt et al. (2004) found concentrations of c.14% (n = 26) and c.25% (n = 30) for protein and lipid content respectively. Unfortunately individual fruit species values are not presented in Voigt et al. (2004) to allow further comparison.

Mean percentage lipid content of South African fruits in the current study was lower than *Cornus racemosa* (21.3-32.5%, n = 3) (Borowicz and Stephenson 1985) but higher than *Cornus amomum* (5.4-6.6%; n = 3) (Borowicz and Stephenson 1985) fruits. Percentage lipid content for *Rhamnus prinoides* (6.2%; Table 1) was similar to that of *Rhamnus alaternus* (5.7 ± 3.5%, mean ± SD; Izhaki et al. 2002). Percentage protein content (10.8%; Table 1) for *Rhamnus prinoides* was however, much higher than *Rhamnus alaternus* (1.2 ± 0.4%, mean ± SD; Izhaki et al. 2002). Percentage water content (81.1%; Table 1) for *Rhamnus prinoides* was higher than *Rhamnus alaternus* (68.4 ± 2.6%, mean ± SD; Izhaki et al. 2002). Mean percentage water content was similar to those obtained by Borowicz and Stephenson (1985), Izhaki (1992) and Fukui (2003), who obtained percentage water content that ranged from 48.3-79.4%.

Only three species examined in this study for protein and lipid content have previously been studied (Table 3). Protein and lipid content for *Mimusops obovata* (5.2% and 5.2% respectively) and *Mimusops caffra* (5.7% and 6.8% respectively) was similar to those obtained by Gaynor (1994) and Lawes (1990) respectively (Table 3). However, protein (8.5%) and lipid (2.6%) content for *Grewia occidentalis* were lower than those obtained by Lawes (1990) with values of 14.7% and 4.4% respectively (Table 3).

Previous studies (Wellmann and Downs 2009; Wilson and Downs (in press. a; b) have used artificial fruits to determine sugar preferences and digestive efficiencies of some South African frugivores (e.g. Cape white-eyes, *Zosterops virens*; Knysna Turacos, *Tauraco corythaix*; and Purple-crested Turacos, *Gallirex porphyreolophus*). However these fruits were made according to Witmer (1998)'s artificial fruit composition that approximated the sugar concentration of North American fruit species. Our current results suggest that indigenous South African fruits have lower sugar concentrations than North American fruit (Table 1), however this trend might shift as further fruit species are sampled. Mean percentage protein content of South African fruits in the present study was higher than that of North American fruits (3.2%, Witmer 1996; 1998). Consequently Witmer (1998)'s artificial fruit composition is not representative of what South African fruit are providing in general. However, it does fall in the range and is useful if only sugar digestion is being investigated.

Fruit colour is known to influence fruit selection by avian frugivores (Murphy 1994; Burns 2005; Schaefer et al. 2008). Of the fruits examined in this study 29% were black and 43% red when ripe (Table 1). Birds are better adapted to see red colours (Bowmaker 1998; Jacobs and Deegan 1999) and consume black or red fruits (Ridley 1930; Willson et al. 1989; Burns 2005). Birds are believed to associate green with unpalatibility and therefore a lower nutritional reward (Herrera 1982; Willson and Whelan 1990). It has been suggested the colour of ripe fruit has co-evolved with colour vision in birds (Osorio and Vorobyev 1996).

The size of the gape width of an avian frugivore limits the size of the fruit that can be ingested intact (Martin 1985; Wheelwright 1985; Jordano 1987; Jordano 2000; Symes and Downs 2001). It is generally assumed that large frugivores can disperse a wider range of fruit and seed sizes than smaller frugivores, however, avian frugivores process fruits in different ways (Levey 1987). Gulpers ingest fruits whole, while mashers crush the fruit first and then ingest the pulp only (Levey 1987). In both of these gape width is a limiting factor (Martin 1985; Wheelwright 1985; Jordano 2000). Indeed, Avery et al. (1993) found that handling time (defined as the time from picking up a food item to the time of ingestion; Hedge et al. 1991) increased as fruit size increased. They suggest that avian frugivores should prefer the largest fruit possible that they can ingest without incurring handling costs.

Fruit width in this study ranged from 4 mm (*Searsia rehmanniana* and *T. orientalis*) to 40 mm (*A. senegalensis*, *F. sur* and *X. kraussiana*) (Table 1). In Africa avian frugivores play a more important role in seed dispersal than primates (Holbrook and Smith 2000; Bleher and Böhning-Gaese 2001). Studies on the gape width of South African avian frugivores are limited but Knysna (*Tauraco corythaix*) (c. 310g) and Purple-crested (*Gallirex porphyreolophus*) Turacos (c. 300g) (du Plessis and Dean 2005a; b) have gape widths of 25mm and 15mm respectively (Wilson and Downs unpublished data). Besides for some hornbill species, such as the Trumpeter Hornbill

(*Bycanistes bucinator*) (567-721g) (Sanft 1960; Kemp 1995), Knysna and Purple-crested Turacos are two of the larges South African avian forest frugivores (du Plessis and Dean 2005a; b). Potentially 16% and 47% of the fruits respectively examined in this study (Table 1) are too large to be ingested whole by these two species as both swallow their fruit whole (pers. obs.). Although some other bird species will peck at larger fruits and ingest seeds in this way (Voigt et al. 2004). Both these Turaco species are forest inhabitants (du Plessis and Dean 2005a; b) and are therefore threatened by forest fragmentation (Low and Rebello 1996; Lawes et al. 2000). It is important that management plans for forest conservation are developed in order to protect plant-animal interactions in forest ecosystems in the long-term (Kirika et al. 2008; Kankam and Oduro 2009).

In conclusion, fruits are generally considered as being either nutrient-dilute or nutrient-dense (Izhaki 1993). Fruits in this study were generally observed to be high (>50%) in water content (except for *Podocarpus henkelii*), and low (<10%) in protein and lipid content indicating that these fruits species could be considered as nutrient-dilute. Avian frugivores would therefore need to consume large amounts of these fruit species in order to obtain sufficient energy (Worthington 1989). Future studies need to determine the nutritional composition of the remaining indigenous South Africa fruit in order to develop a comprehensive database as well as examining non-nutritive factors (e.g. fruit colour, seed-to-pulp ratio, seed size, fruit size, secondary compounds, micro-nutrients etc.).

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Table 1. Fruit characteristics of indigenous tree species used in the study

Tree Species	*Colour when ripe	*Fruit size	Fruit mass (g)	Sucrose (mg/g)	Glucose (mg/g)	Fructose (mg/g)	Sucrose :Hexose ratio	% Water content	% Protein content	% Lipid content
Annona senegalensis	Yellowish-orange	50 x 40 mm	25.07±0.73 (8)	5.93	36.49	43.75	1:13.54	68.21	8.88	10.00
Antidesma venosum	Purplish-black	7 x 5 mm	0.08±0.007 (10)	2.13	28.55	34.53	1:29.55	63.61	7.85	6.33
Apodytes dimidiata	Black/red	8 mm	0.30±0.017 (10)	4.25	13.55	8.81	1:5.26	58.99	8.02	27.66
Bridelia micrantha	Black	10 x 7 mm	0.40±0.02 (10)	2.14	33.51	40.13	1:34.34	70.84	7.58	2.25
Canthium inerme	Brown	15 x 10 mm	0.90±0.10 (10)	4.01	53.49	58.81	1:28.02	58.46	7.10	6.10
Carissa macrocarpa	Red	50 x 30 mm	14.23±1.32 (10)	13.05	56.12	59.26	1:8.84	82.54	4.41	7.25
Celtis africana	Yellow	8 mm diam	0.20±0.008 (10)	43.39	3.13	42.37	1:1.05	51.75	7.73	5.00
Clerodendrum glabrum	Yellowish-white	10 mm diam	0.61±0.05 (10)	9.79	54.98	36.52	1:9.34	81.33	8.37	3.47
Commiphora neglecta	Red	14 mm diam		1.90	0.27	0.42	1:0.36	77.46		
Cordia ovalis	Orange-red	20 mm	1.13±0.12 (10)	11.47	33.23	34.94	1:5.95	67.20	10.64	0.62
Ficus ingens	Purple	13 mm diam		0.44	3.74	3.54	1:16.52			
Ficus lutea	Yellow to brown	15-30 mm diam	3.02±0.17 (10)		44.23	32.96		82.79	5.47	2.21
Ficus natalensis	Red-brown	10-20 mm diam	0.66±0.02 (10)	2.20	22.49	20.36	1:19.44	87.12	8.32	3.40
Ficus petersii	Reddish	13 mm	4.75±0.20 (10)		60.50	46.68		81.60	5.39	2.29
Ficus sur	Pink-red with pale spots	20-40 mm diam	12.02±0.82 (10)	4.27	23.58	25.32	1:11.45	87.77	8.37	5.88
Ficus trichopoda	Red	10-20 mm diam	4.25±0.85 (10)		54.78	40.22		83.18	6.72	2.37
Grewia occidentalis	Reddish purple to brown	25 mm diam	0.75±0.10 (10)						8.53	2.58
Harpephyllum caffrum	Red	30 x 17 mm	4.97±0.53 (10)	61.73	12.32	6.35	1:0.30	68.50	4.75	1.42
Kraussia floribunda	Purple to black	8 mm diam	0.41±0.02 (10)						2.99	5.26

Note: numbers in parentheses are sample sizes
(*Griffiths 2006; Boon 2010)

Table 1. (*Continued*)

Tree Species	*Colour when ripe	*Fruit size	Fruit mass (g)	Sucrose (mg/g)	Glucose (mg/g)	Fructose (mg/g)	Sucrose :Hexose ratio	% Water content	% Protein content	% Lipid content
Maesa lanceolata	Cream	6 mm diam	0.07±0.004 (10)	3.35	22.15	25.57	1:14.26	83.70	12.01	11.94
Mimusops caffra	Red	25 mm diam	1.92±0.21 (10)	4.40	33.65	45.98	1:18.09	68.18	5.65	6.76
Mimusops obovata	Orange-red	35 x 20 mm	1.20±0.06 (10)						5.19	5.16
Peddiea africana	Purplish-black	10 x 7 mm		4.48	25.44	36.06	1:13.72	75.30	13.70	19.60
Podocarpus henkelii	Olive-green	25 mm diam		17.71	5.03	10.19	1:0.86	4.05		
Rauvolfia caffra	Black	15 mm diam	1.87±0.20 (10)	2.53	37.25	40.69	1:30.82	59.59	6.84	10.70
Rhamnus prinoides	Purplish-black	6 mm diam	0.25±0.02 (10)	6.33	24.52	19.55	1:6.97	81.10	10.81	6.23
Rothmannia globosa	Brown	25 mm diam		1.52	0.59	4.06	1:3.06			
Searsia rehmanniana	Yellowish	4 mm diam	0.02±0.001 (10)					78.04	9.37	2.09
Solanum gigantea	Shiny red	10 mm diam	0.24±0.02 (10)		16.76	18.84		79.91	10.28	0.73
Syzygium cordatum	Deep purple	18 x 9 mm	2.46±0.10 (10)						5.91	0.72
Tarenna junodii	Purplish-black	8 mm diam		0.03	0.23	0.52	1:24.63			
Trema orientalis	Black	4 mm diam	0.03±0.004 (10)	2.22	2.98	3.29	1:2.82	66.25	17.43	33.49
Tricalysia lanceolata	Black	6-8 mm diam	0.09±0.014 (10)	7.24	26.13	29.10	1:7.63	73.28	9.53	1.51
Trichilia dregeana	Brown	50 mm diam	1.48±0.04 (10)	5.02	9.81	9.44	1:3.84	41.84	10.34	36.13
Trichilia emetica	Pale brown	25 mm diam	3.07±0.15 (10)						8.37	50.22
Vitex ferruginea	Purplish-black	21 mm diam		9.24	7.86	4.81	1:1.37	44.52		
Ximenia americana	Orange	20-25 mm diam			16.47	1.72		41.18		
Xylotheca kraussiana	Yellow	40 mm		1.37	3.88	5.20	1:6.62	73.95		

Note: numbers in parentheses are sample sizes
(*Griffiths 2006; Boon 2010)

Table 2. Codes for 19 indigenous tree species used in the Principle Components Analysis

Code	**Species name**
AS	*Annona senegalensis*
AV	*Antidesma venosum*
AD	*Apodytes dimidiata*
BM	*Bridelia micrantha*
CI	*Canthium inerme*
CM	*Carissa macrocarpa*
CA	*Celtis africana*
CG	*Clerodendrum glabrum*
CO	*Cordia ovalis*
FN	*Ficus natalensis*
FS	*Ficus sur*
HC	*Harpephyllum caffrum*
ML	*Maesa lanceolata*
MC	*Mimusops caffra*
RC	*Rauvolfia caffra*
RP	*Rhamnus prinoides*
TO	*Trema orientalis*
TL	*Tricalysia lanceolata*
TD	*Trichilia dregeana*

Table 3. Nutritional content of indigenous tree species

Species	Water (%)	Protein (%)	Lipid (%)	Reference
Acacia karroo		28.10	7.20	Lawes (1990)
Acacia nigrescens		21.50	2.00	Gaynor (1994)
Bridelia cathartica		6.03	1.31	Gaynor (1994)
Calodendrum capense		14.73-15.97	37.38	Wirminghaus et al. (2002)
Capparis brassii		8.30	4.20	Gaynor (1994)
Cassine transvaalensis (*Elaeodendron transvaalense*)	80.02	10.71	3.36	Gaynor (1994)
Cladostemon kirkii		6.79	3.79	Gaynor (1994)
Diospyros inhacaensis		10.86	2.60	Lawes (1990)
Diospyros natalensis		2.73	2.07	Lawes (1990)
Dovyalis caffra		3.84	5.75	Gaynor (1994)
Dovyalis longispina		15.80	3.21	Lawes (1990)
Euclea natalensis		4.45	1.79	Lawes (1990)
Euclea shimperii (*Euclea daphnoides*)		5.03	7.00	Gaynor (1994)
Ficus glumosa	78.32	7.62	1.89	Gaynor (1994)
Ficus ingens	80.30	5.70	7.40	Gaynor (1994)
Ficus soldanella (*Ficus abutilifolia*)	75.20	13.41	14.27	Gaynor (1994)
Ficus sycamorus		5.75	9.24	Gaynor (1994)
Grewia monticola		10.37	1.51	Gaynor (1994)
Grewia occidentalis		14.65	4.44	Lawes (1990)

Note: New names according to Boon (2010) are in parentheses

Table 3. (*Continued*)

Species	Water (%)	Protein (%)	Lipid (%)	Reference
Mimusops caffra		6.56	7.19	Lawes (1990)
Mimusops obovata		5.45	4.52	Gaynor (1994)
Olea africana (*Olea europaea* subspecies *africana*)		4.25	3.74	Gaynor (1994)
Olea woodiana		16.96	9.20	Lawes (1990)
Podocarpus falcatus (*Afrocarpus falcatus*)	64.8+1.21	4.15	20.12	Wirminghaus et al. (2002)
Podocarpus henkelii		6.18-6.61	2.34-3.1	Wirminghaus et al. (2002)
Podocarpus latifolius	61.64+1.36	8.36-10.18	2.87-3.95	Wirminghaus et al. (2002)
Rhus natalensis (*Searsia natalensis*)		8.38	5.93	Lawes (1990)
Schutia myrtina		6.97	4.92	Lawes (1990)
Scutia myrtina		8.23-8.37	10.83	Wirminghaus et al. (2002)
Sclerocarya caffra (*Sclerocarya birrea* subspecies *caffra*)		6.90	6.60	Gaynor (1994)
Scolopia zeyheri		7.81	4.89	Lawes (1990)
Sideroxylon inerme		8.29	24.11	Gaynor (1994)
Sideroxylon inerme		10.35	9.83	Lawes (1990)
Strychnos madagascariensis		4.73	2.68	Gaynor (1994)
Strychnos madagascariensis		9.21	0.78	Lawes (1990)
Strychnos usambarensis		8.06	2.82	Gaynor (1994)
Tricalysia sonderiana		9.66	2.82	Lawes (1990)
Vangueria esculenta		3.10	1.39	Gaynor (1994)
Ziziphus mucronata	71.15	20.82	3.83	Gaynor (1994)

Note: New names according to Boon (2010) are in parentheses

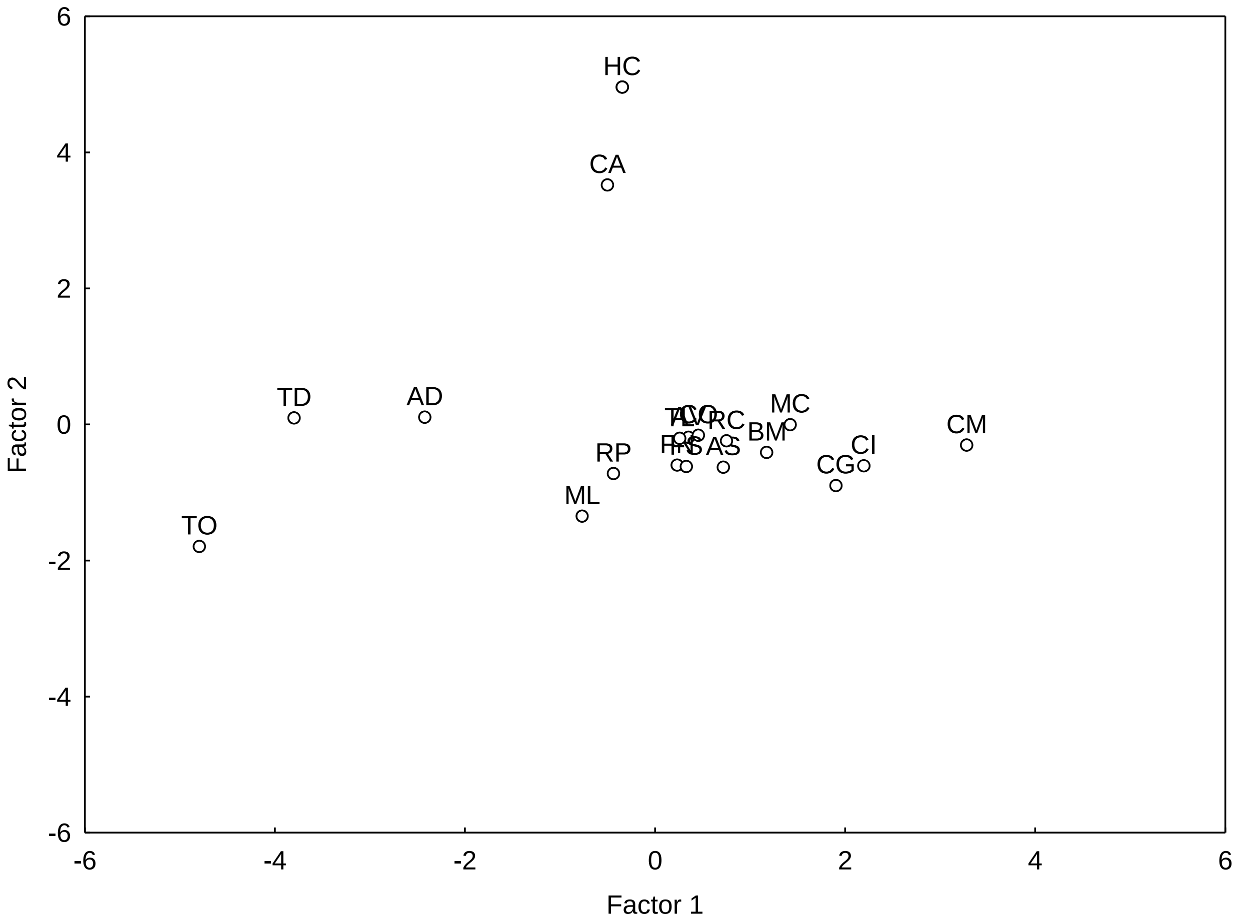

Fig 1. Principal component analysis of 19 indigenous fruit species from KwaZulu-Natal, South Africa

Chapter 8

SEASONAL VARIATION IN METABOLIC RATE OF A MEDIUM-SIZED FRUGIVORE, THE KNYSNA TURACO (*TAURACO CORYTHAIX*)

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Many seasonal thermoregulation studies have been conducted on Holarctic birds that live in predictable, highly seasonal climates with severe winters. However, relatively few studies have been conducted on their southern hemisphere Afrotropical counterparts that encounter less predictable climates with milder winters. These latter birds are expected to conserve energy in winter by down-regulating their metabolic rates. Therefore in this study, metabolic rate was measured during summer and winter in Knysna Turaco, *Tauraco corythaix* (Musophagiformes, Musophagidae) (c. 310 g), a non-passerine, in order to test whether there is energy conservation in winter. No overall significant differences in resting metabolic rates over a range of ambient temperatures were observed between winter and summer. However, whole-organism basal metabolic rates were 18.5 % higher ($p = 0.005$) in winter than in summer (210.83 ± 15.97 vs. 186.70 ± 10.52 O_2 h^{-1}). Knysna Turacos had broad thermoneutral zones ranging from 20-28 ^{o}C in winter and 10-30 ^{o}C in summer. These results suggest that Knysna Turacos show seasonal thermoregulatory responses that represent cold defense rather than energy conservation, which is contrary to what was expected.

Keywords: Basal metabolic rate (BMR); Knysna Turaco; Phenotypic flexibility; Phenotypic plasticity; metabolic rate

Introduction

Various endotherms exhibit seasonal flexibility of metabolic thermoregulation (Cooper and Swanson, 1994; Liknes et al., 2002; Dawson, 2003; Nzama et al., 2010; Smit & McKechnie, 2010). Birds are able to change both their physiology and morphology within as short a period as a few days (Piersma and Lindström, 1997), and by adjusting their metabolism, they are able to maintain a constant body temperature over a wide range of ambient temperatures (Chaui-Berlinck et al., 2002).

Phenotypic flexibility is defined as a reversible variation in a character of a single individual, which can represent a response to predictable and/or unpredictable changes in environmental conditions (Piersma and Drent, 2003). This ability to alter physiological traits suitably, such as basal metabolic rate (BMR), in response to seasonal changes in ambient temperatures is a key component of thermoregulation (Dawson, 2003; Smit et al., 2008). Here we define minimum resting metabolic rate at a particular ambient temperature (RMR_{Ta} or sometimes referred to as standard metabolic rate in the older literature) as the minimum metabolic rate of a resting, post-absorptive bird at a particular ambient temperature. Basal metabolic rate (BMR) is defined as the lowest RMR_{Ta} or minimum rate of energy expenditure by a non-growing, post-absorptive, non-reproductive homeotherm that is in its thermoneutral zone in the inactive phase of the circadian cycle (Brody, 1945; IUPS Thermal Commission, 2001).

Some birds survive harsh winters by increasing their BMR as one facet of the acclimatization process which increases the threshold of thermogenesis (Dawson and O’Connor, 1996; Swanson and Olmstead, 1999). An elevated BMR increases energy use within the thermoneutral zone (TNZ, defined as ‘the range of ambient temperature at which temperature regulation is achieved only by control of sensible heat loss, without regulatory changes in metabolic heat production or evaporative heat loss’ (IUPS Thermal Commission, 2001)). Birds in cold winter climates are almost always exposed to temperatures below the TNZ resulting in increased thermoregulatory costs that increase the energy demand on the bird. Consequently, a disadvantage of this increased energy demand is that it is not always possible to achieve in winter as food is often scarce (Dawson, 2003).

Although avian BMR and RMR_{Ta} were formerly regarded as specific, or fixed for a particular temperature, many species exhibit seasonal or acclimation differences (Liknes and Swanson, 1996; Maddocks and Geiser, 2000; Dawson, 2003; Bush et al., 2008a, b; Smit et al., 2008; Lindsay et al., 2009a, b; Nzama et al, 2010; Smit and McKechnie, 2010) (Table 1). While some small birds have been shown to decrease their RMR_{Ta} and BMR in winter (Maddocks and Geiser, 2000), many small boreal birds (< 200 g) that encounter severe winters have been found to increase their BMR and RMR_{Ta} in adjusting to cold (Dawson, 2003). Some medium-sized birds (200-1000g) have been shown to exhibit no seasonal flexibility or only slight decreases or increases in BMR (Swanson and Weinacht, 1997; Dawson, 2003). However, McKechnie (2008) suggests that current evidence does not support an effect of body size on the seasonal magnitude of changes in BMR or RMR_{Ta}.

Most thermoregulatory studies have been conducted on Holarctic birds (Bech, 1980; Swanson and Weinacht, 1997). The Holarctic region, which includes much of Eurasia and North America, has a predictable, highly seasonal climate characterized in the north by persistent subfreezing temperatures in winter (Bech, 1980; Stone et al., 1996; Swanson and Weinacht, 1997; Cooper, 2002). Relatively few seasonal studies have been conducted on Afrotropical or other southern hemisphere birds (Bush et al., 2008a; Smit et al., 2008, Chamane and Downs, 2009; Nzama et al., 2010; Smit and McKechnie, 2010) (Table 1). Generally southern hemisphere areas, especially in the afrotropics, experience milder winters with more unpredictable climates particularly as a result of El Niño Southern Oscillations (ENSO) (Stone et al., 1996; Jury and Nkosi, 2000; Holmgren at al., 2001). It has been suggested that in winter the seasonal metabolic responses of Afrotropical endotherms will be one of energy conservation (Lovegrove and Smith, 2003). Some studies (e.g. Smit and McKechnie, 2010) have found winter reductions in BMR for several species of southern African birds. Other studies observed no change (Bush et al., 2008a) or an increased BMR in winter (Smit et al., 2008; Chamane and Downs, 2009; Nzama et al., 2010) (Table 1).

In view of the differing patterns of seasonal metabolic responses among southern African birds, we have undertaken a further study of these responses in a medium-sized frugivore, the Knysna Turaco, *Tauraco corythaix* (Musophagiformes, Musophagidae) having a mean mass of approximately 310 g (du Plessis and Dean, 2005). It was predicted that Knysna Turacos would conserve energy as expected for Afrotropical endotherms; and that BMR and RMR_{Ta} would be lower in winter than in summer.

Knysna Turacos are endemic to South Africa (Oatley, 1997) and they occupy riverine, coastal and mistbelt Afromontane forest (Rowan, 1983; du Plessis and Dean, 2005). They are typically resident in such habitats, but they may locally track fruit in response to availability (Rowan, 1983).

Materials and Methods

Procurement and Maintenance

Captive-bred Knysna Turacos were sourced from M.C. Weber under permit from Ezemvelo KZN Wildlife (permit number: 1781/2008). The birds were housed in pairs in outside aviaries (1 x 2.12 x 2.66 m) (Latitude: 29.37^{o}; Longitude: 30.24^{o}) and acclimated for three to nine months before the experiments were conducted. Birds were fed a daily maintenance diet of a choice of mealworms (*Tenebrio molitor* larvae), apples, pears, carrots, paw-paws, bananas, oranges and/or grapes. All fruit was either grated or had the skin removed (paw-paws, bananas and oranges) and cut into c. 1 x 1 x 1 cm cubes. A mixture of Aviplus Softbill/Mynah pellets and crumble (Avi-products, Durban, South Africa) was added to the maintenance diets. Water was provided *ad libitum*.

Due to their rapid transit rates (Wilson, 2009) birds were allowed to feed during the day prior to the oxygen consumption (VO_2) measurements but were deprived of food during trials. Metabolic trials lasted the entire night and thus it was assumed that birds were post-absorptive and that RMR_{Ta} was measured. In addition, RMR_{Ta} was recorded throughout the night, rather than just for a few hours, to ensure minimum levels were reached. Birds were housed in outdoor aviaries for the duration of the study.

Measurement of ambient temperature (outside aviaries)

Ambient temperature was obtained from the South African Weather Service (SAWS) for Pietermaritzburg (Climate Number: 0239698.5; Latitude: 29.63^{o}; Longitude: 30.40^{o};

Height: 673 m) for summer 2008-2009 (December-January) and winter 2010 (June-August).

Measurement of metabolic rate

Metabolic rate was measured indirectly by quantifying oxygen consumption (VO_2) using an oxygen analyser (Ametek S-3A/1, Pittsburgh, PA, USA) and computerised system as described in Lindsay et al. (2009a) Excurrent air was passed through a water condenser (a copper tube in which air was cooled to approximately 3°C, or below dew point) to remove water vapour, and soda lime, to remove Carbon Dioxide (CO_2). The equation $VO_2 = V_E (FI_{O2} – FE_{O2})/ (1 – FI_{O2})$ (VO_2 = metabolic rate (ml O_2. h min^{-1}); V_E = flow rate (ml. min^{-1}); FI_{O2} = incurrent fractional O_2 concentration; and FE_{O2} = excurrent fractional O_2 concentration) was used to calculate metabolic rate (Withers, 1977). The system was switched on at least 3h prior to the start of the respirometry trials. Birds were caught, weighed and then placed in individual respirometry chambers (35 x 20 x 25 cm^3) made of clear Perspex® and containing a wooden perch. Respirometry chambers were placed in a soundproof Conviron® (Winnipeg, Manitoba, Canada) cabinet (1 m^3) that controlled T_a ± 0.1^o C and with 12L: 12D with scotophase from 18:00 – 06:00. Conviron® cabinet temperature (T_a) was measured using thermistor probes calibrated with a standard mercury thermometer (0.05^o C) in a water bath at temperatures between 5 and 45^o C. Birds were placed in respirometers between 15:00-16:00 and the first 2h of readings discarded. Air flow was controlled using a computerized open flow-through system as in Lindsay et al. (2009a). Atmospheric air was pumped in and dried using silica gel, before reaching the Conviron® cabinet. Flow rate was measured on excurrent air and was maintained at a level that ensured <1 % change in oxygen concentration (between 2.300 and 2.800 l. min^{-1} in summer and 1.500 and 1.800 l. min^{-1} in winter). Fractional concentration of O_2 in each chamber was measured every six min and consequently VO_2 was recorded digitally every six min., corrected for standard temperature and pressure. Thus ten readings per individual were recorded on an hourly basis. Measurements of the various parameters for each chamber (T_a, flow rate and fractional O_2 concentrations) were recorded at the end of each 45 s sampling interval, so as to allow sufficient time for the flushing of air from the previous channel from the ducting between relay valves and the subsample tubing. See Lindsay et al. (2009a) for detailed description of methods. At 07:00 the following morning birds were removed from the chambers, weighed and returned to their outside aviaries where food and water were provided *ad libitum*. The exception was at 33^o C when birds were monitored carefully and removed at approximately 21:00 because of high evaporative water loss and to avoid possible thermal stress.

Each VO_2 trial was conducted at a different T_a in a randomly determined order (20, 5, 15, 30, 10, 28, 25 and 33^o C). Lowest mean hourly VO_2 for each Turaco at each T_a was taken as the RMR_{Ta} (Smit et al., 2008; Lindsay et al., 2009a). Nine birds were used for the summer trials (12 January 2009 - 30 January 2009) and eight birds were used for the winter trials (2 August 2010 - 20 August 2010). In summer the scotophase period was from 18:30-05:30 while in winter it was from 17:30-06:30 so the scotophase period used in the measurements overlapped with these.

Statistical analyses

Data were analysed using STATISTICA version 7 (Statsoft Inc, Version 7, Tulsa, USA). As the same birds were used repeatedly, Generalized Linear Models Repeated Measures Analysis of Variance (GLM RMANOVA) and Post-hoc tests were used to compare whole-organism metabolic rates, and body mass at each temperature, and between temperatures. BMR was calculated by taking the lowest mean hourly RMR_{Ta} per individual. The TNZ was determined using Post-hoc Scheffé tests to determine over what range minimum RMR_{Ta} did not differ significantly. Data were presented as mean ± SE of the individuals measured (n).

Results

Ambient temperature

Mean monthly ambient temperatures ranged from 27.0 ± 0.8^o C to 28.3 ± 1.0^o C in summer (January 2008 and December 2009 respectively) and 23.4 ± 0.9^o C to 26.0 ± 1.1^o C in winter (June and August 2010 respectively). Minimum and maximum temperatures varied daily and ranged from 17.3 ± 0.4^o C to 28.4 ± 1.0^o C in summer and 8.9 ± 1.2^o C to 23.8 ± 1.6^o C in winter (Fig. 1).

Body mass

The body masses of individual Turacos did not differ significantly between summer and winter trials at the different ambient temperatures (GLM RMANOVA, $F_{7,49} = 1.66$, $p = 0.141$, Fig. 2). Body mass of these birds ranged from 266.76 ± 6.68 to 296.47 ± 6.98g in winter compared with 286.48 ± 5.50 to 308.91 ± 4.52g in summer.

Metabolic rate

No overall significant difference in RMR_{Ta} (VO_{2min}) existed between summer and winter (GLM RMANOVA, $F_{7,49} = 1.62$, $p = 0.153$) (Fig. 3). Post-hoc Scheffé tests ($p < 0.05$) indicated relatively broad TNZ ranges in summer (10-30^{o} C) and in winter (20-28^{o} C). Lowest mean minimum VO_2, which was taken as BMR, occurred at 25°C in both winter and summer. On whole-organism and mass specific bases, the winter values at this temperature (210.83 ± 15.97 O_2 h^{-1} and 0.77 ± 0.05 ml O_2 g^{-1} h^{-1}) are 18.5% higher than the summer values (186.70 ± 10.52 O_2 h^{-1} and 0.65 ± 0.04 ml O_2 g^{-1} h^{-1}) and the difference is significant (T-test, $t = 3.997$, $df = 7$, $p = 0.005$).

In both winter and summer trials, all the Turacos decreased their VO_2 with the onset of the scotophase and only restored it to a daytime level about an hour before the beginning of the photophase. The decrease in with the onset of the scotophase was also observed in summer and winter birds tested at 33°C, but their early removal from this Ta prevented detection of the reversal of this change just preceding the photophase.

The time during scotophase required to reach a minimum RMR_{Ta} varied among individuals and Ta's as well as between seasons (Table 2). It ranged from 1 h (from 18:00 to 19:00 at 5°C in summer) to 10 h (from 18:00 to 04:00 at 20° and 30°C in winter and summer, respectively). Most birds required at least 4 h to reach minimum RMR_{Ta}. Once metabolism of an individual reached its lower level, little further variation occurred until the increase shortly before the beginning of photophase.

Discussion

Afrotropical areas, particularly southern hemisphere ones, are generally accepted to experience milder winters, but more unpredictable climates than Holarctic areas (Stone et al., 1996; Jury and Nkosi, 2000; Holmgren at al., 2001) and this trend can clearly be observed in the temperature data obtained from SAWS in the current study. Mean monthly temperatures for summer and winter were similar however daily minimum and maximum temperatures showed a much greater variation in temperature, especially in the winter months.

Birds that inhabit Afrotropical regions are expected to lower their BMR in winter to conserve energy (Lovegrove and Smith, 2003). In the case of Knysna Turacos, however, BMR increased significantly after winter acclimatisation although no significant differences in RMR_{Ta} between winter and summer trials were observed. These trends are likely to result from increased support costs for thermogenic tissues and energy processing tissues feeding fuels to thermogenic tissues and are more commonly associated with northern latitude birds, which use an increased RMR_{Ta} and BMR during winter as a cold defense mechanism (Swanson and Weinacht, 1997; Dawson 2003).

Knysna Turacos showed an increase of 18.5 % in BMR in winter. Our results are similar to those of Chamane and Downs (2009) and Nzama et al. (2010) (Table 1) who also found an increase in BMR in winter in Afrotropical southern hemisphere birds. Many studies have found that some medium-sized (200-1000 g) non-passerines show no seasonal difference in BMR (Wijnandts, 1984; Swanson and Weinacht, 1997; Dawson 2003) and that some Holarctic bird species increase BMR and alter RMR during winter (Liknes et al., 2002; McKechnie et al., 2007). Australian Silvereyes (*Zosterops lateralis*) and Australian White-browed Scrubwrens (*Sericornis frontalis*) had higher metabolic rates in summer than in winter (Ambrose and Bradshaw, 1988; Maddocks and Geiser, 2000). Similar to the current study, Zheng et al. (2008) found that Chinese Bulbuls (*Pycnonotus sinensis*) increased BMR in winter. Their explanation for an increased BMR in winter is that the birds need to make metabolic and/or morphological changes in order to meet extra energy demands during severe winters. Another alternative is that the activity organs increase in size resulting in an increased BMR (Zheng et al., 2008). Some studies support this idea as BMR of several passerine species was found to be linked with an increase in the muscle mass of the heart and breast (Swanson, 1991; O’Connor, 1995; Cooper, 2002).

In contrast to the current study, Bush et al. (2008a) (Table 1) found that while Rock Kestrels *Falco rupicolus* showed no significant seasonal difference in BMR, they decreased RMR_{Ta} in winter indicating that the birds were conserving energy during winter periods. Similarly several other Afrotropical southern hemisphere species exhibited reduced BMR in winter (Smit and McKechnie 2010) (Table 1). This trend toward energy conservation is what is expected in birds from Afrotropical regions (Lovegrove and Smith, 2003). However, Knysna Turacos were observed to significantly increase their BMR presumably to support cold defense (Dawson, 2003). Discerning possible factors for the differing trends in seasonal changes in BMR of Afrotropical southern hemisphere bird species may be possible with further data collection from a range of species of differing body mass and feeding guilds.

Some species are thought to increase body mass in winter as a mechanism to cope with colder ambient temperatures and increased energy demands (Zheng et al., 2008). However, body mass of the individual Knysna Turacos did not differ significantly between summer and winter trials. This is also the case for Rock Kestrels and Southern White Faced Scops Owls (Table 1; Bush et al., 2008a; Smit et al. 2008). Our results for the Knysna Turaco differ from those of Zheng et al. (2008), who recorded an increase in body mass in Chinese Bulbuls during winter.

Generally birds that have a wider TNZ are better adapted to cold environments (Schmidt-Nielsen, 1997). However, the persistence of other species having narrower zones of TNZ in severe winter climates argues against regarding the former as better adapted. Having a broad TNZ is just one way of dealing with cold. Knysna Turacos had a very broad TNZ extending from 20 to 28^{o} C in winter and 10 to 30^{o} C in summer. These results are similar to those obtained by Bush et al. (2008a) (Table 1) and McNab (2001).

In summary, Knysna Turacos exhibited no significant difference in RMR_{Ta} between summer and winter; significantly increased BMR in winter and they had a very broad TNZ in both winter and summer indicating a seasonal thermoregulatory response of improved cold defense rather than that of energy conservation which is what was expected for an southern hemisphere Afrotropical bird species that inhabits an area with an unpredictable climate. An explanation for this could involve the Knysna Turaco's although considered medium-sized has a relatively large size compared with most other South African forest birds, and the fact that it, like other forest species, may locally track fruit in response to availability (Rowan, 1983). This Turaco’s diet includes a wide variety of fruit species (Courtenay-Latimer, 1942; Gill, 1952; Jubb, 1965; Rowan, 1983) some of which fruit throughout the year and so potentially there are decreased effects of food resource availability and therefore conservation of energy is not a key factor.

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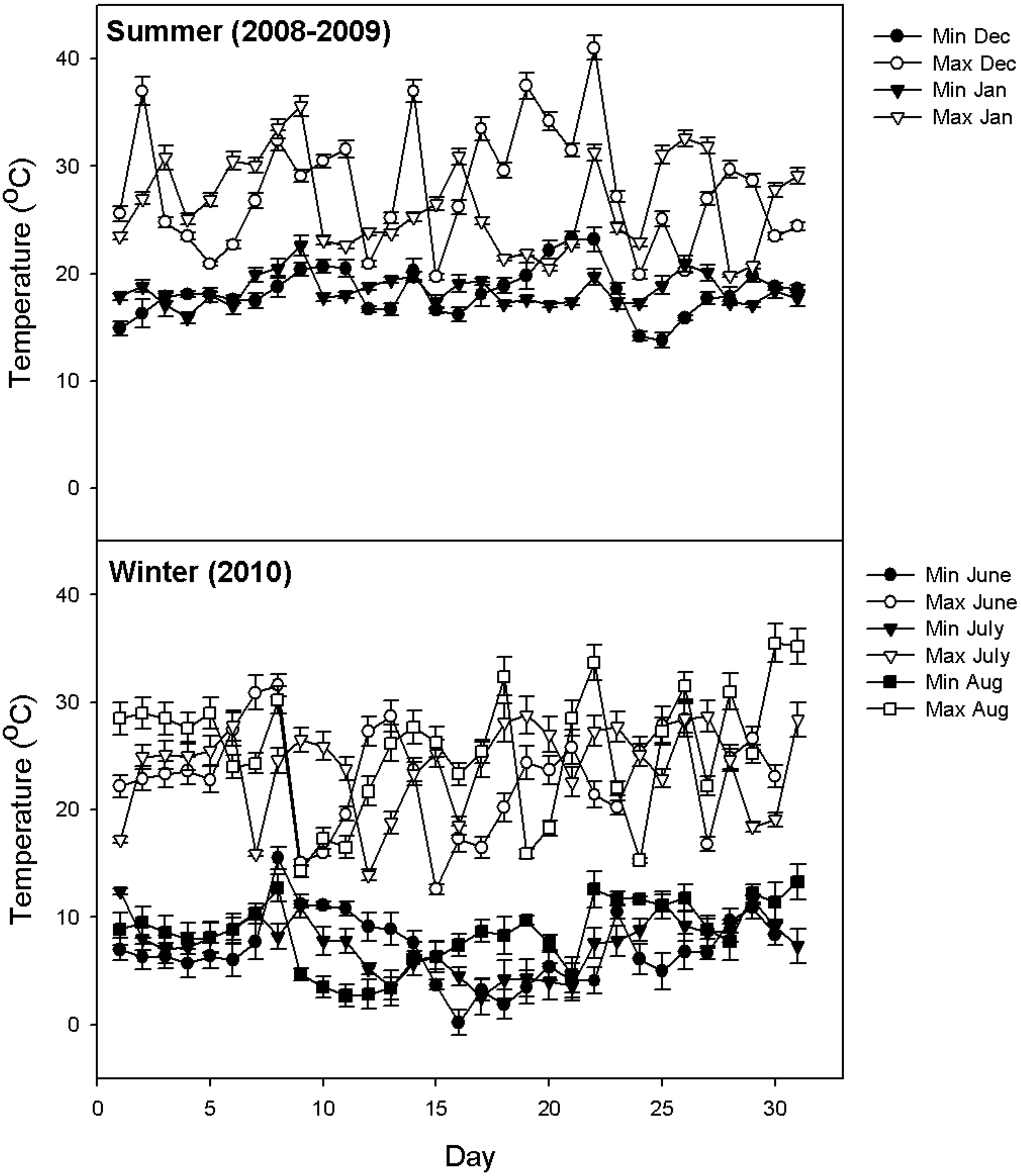
Table 1. Summary of seasonal variation in metabolism of southern African bird species

Species	**Summer body mass (g)**	**Winter body mass (g)**	**Summer BMR (ml O_2 g^{-1} h^{-1})**	**Winter BMR (ml O_2 g^{-1} h^{-1})**	**Summer TNZ (^{o}C)**	**Winter TNZ (^{o}C)**	**Reference**
Rock kestrel (*Falco rupicolis*)	170-248	170-248	1.38 ± 0.071	1.21 ± 0.048	20-33	15-30	Bush et al. 2008
Amethyst sunbird[1] (*Chalcomitra amethystina*)	14-15	14-15	3.50 ± 0.213	5.71 ± 0.402	20-33	10-33	Lindsay et al. 2009a, b
Amethyst sunbird[2] (*Chalcomitra amethystina*)	15.5-16.5	14-15.5	3.47 ± 0.216	2.46 ± 0.299	20-33	25-30	Lindsay et al. 2009a, b
Amethyst sunbird[3] (*Chalcomitra amethystina*)	15-16	14-15	3.29 ± 0.232	3.49 ± 0.312	15-33	20-33	Lindsay et al. 2009a, b
House sparrow (*Passer domesticus*)	22.5-25.5	22-25.5	2.02 ± 0.10	4.44 ± 0.30	25-28	25-28	Nzama et al. 2010
Southern White-faced Scops-owl (*Ptilopsis granti*)	180.1-253.2	180.1-253.2	0.6 ± 0.002	0.6 ± 0.002	20-32	28-32	Smit et al. 2008
Red-winged Starling (*Onychognathus morio*)	124-134	142-132	1.812 ± 0.107	2.326 ± 0.220	15-30	30	Chamane and Downs 2009
African Scops-owl (*Otus senegalensis*)	62.2 ± 4.1	55.4 ± 3.7	6.584 ± 1.330	5.045 ± 0.617			Smit and McKechnie 2010
Pearl-spotted owlet (*Glaucidium perlatum*)	66.4 ± 4.8	70.3 ± 5.7	7.881 ± 1.142	5.496 ± 1.132			Smit and McKechnie 2010
Fork-tailed drongo (*Dicrurus adsimilis*)	44.8 ± 2.6	42.6 ± 4.0	9.425 ± 2.035	6.164 ± 1.215			Smit and McKechnie 2010
Crimson-breasted shrike (*Laniarius atrococcineus*)	43.0 ± 2.8	41.0 ± 2.3	9.210 ± 1.444	6.520 ± 1.109			Smit and McKechnie 2010
White-browed sparrow-weaver (*Plocepasser mahali mahali*)	40.6 ± 2.5	40.6 ± 3.4	8.573 ± 1.347	7.097 ± 0.636			Smit and McKechnie 2010

Note:[1]Underberg population; [2]Howick population; [3]Oribi Gorge population

Table 2. Time in hours at which lowest oxygen consumption (RMR_{Ta}) was measured in each individual Knysna Turaco at each temperature

Season	Individual	Time (h)							
		5 °C	10 °C	15 °C	20 °C	25 °C	28 °C	30 °C	33 °C
Summer	1	19:00	19:00	19:00	22:00	20:00	21:00	20:00	20:00
Winter	1	23:00	19:00	22:00	22:00	20:00	21:00	04:00	20:00
Summer	2	19:00	20:00	19:00	22:00	19:00	20:00	04:00	19:00
Winter	2	19:00	22:00	22:00	04:00	03:00	21:00	03:00	20:00
Summer	3	19:00	19:00	19:00	19:00	20:00	20:00	22:00	20:00
Winter	3	19:00	19:00	21:00	19:00	21:00	20:00	24:00	20:00
Summer	4	19:00	19:00	19:00	19:00	20:00	20:00	04:00	19:00
Winter	4	19:00	22:00	20:00	19:00	22:00	20:00	24:00	20:00
Summer	5	22:00	19:00	23:00	19:00	20:00	20:00	02:00	20:00
Winter	5	22:00	23:00	01:00	19:00	20:00	20:00	22:00	20:00
Summer	6	22:00	02:00	19:00	20:00	19:00	22:00	22:00	20:00
Winter	6	01:00	02:00	23:00	22:00	20:00	21:00	02:00	20:00
Summer	7	19:00	19:00	19:00	19:00	19:00	21:00	20:00	20:00
Winter	7	23:00	19:00	19:00	24:00	02:00	24:00	24:00	20:00
Summer	8	24:00	19:00	21:00	20:00	19:00	19:00	20:00	20:00
Winter	8	23:00	19:00	19:00	23:00	01:00	20:00	24:00	20:00
Summer	9	24:00	24:00	24:00	19:00	20:00	21:00	19:00	20:00



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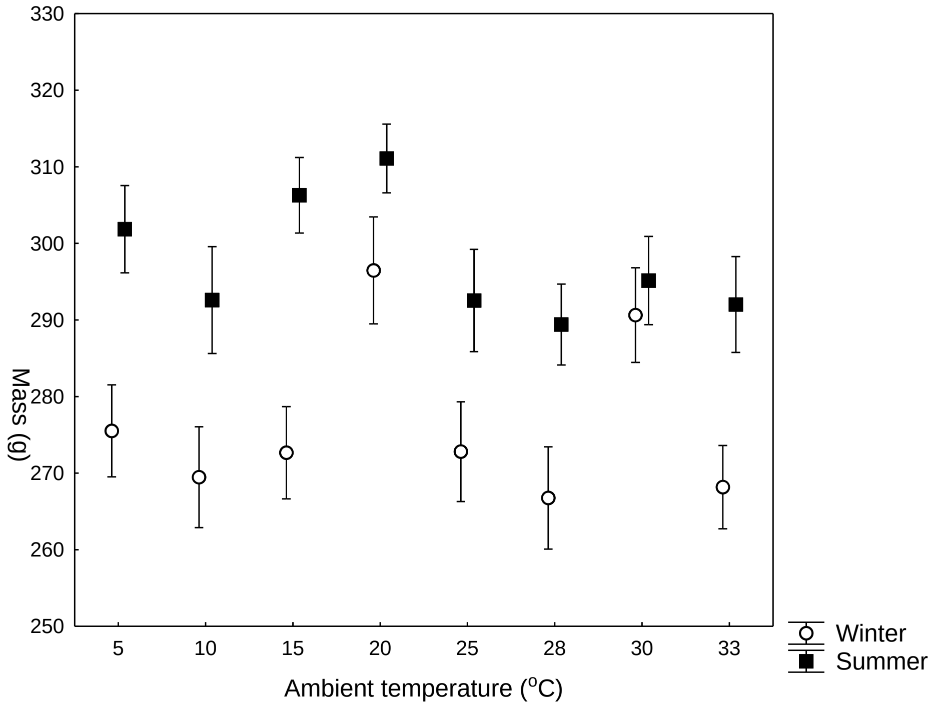

Fig. 2. Change in body mass (g) of Knysna Turacos between winter and summer trials at the different T_as

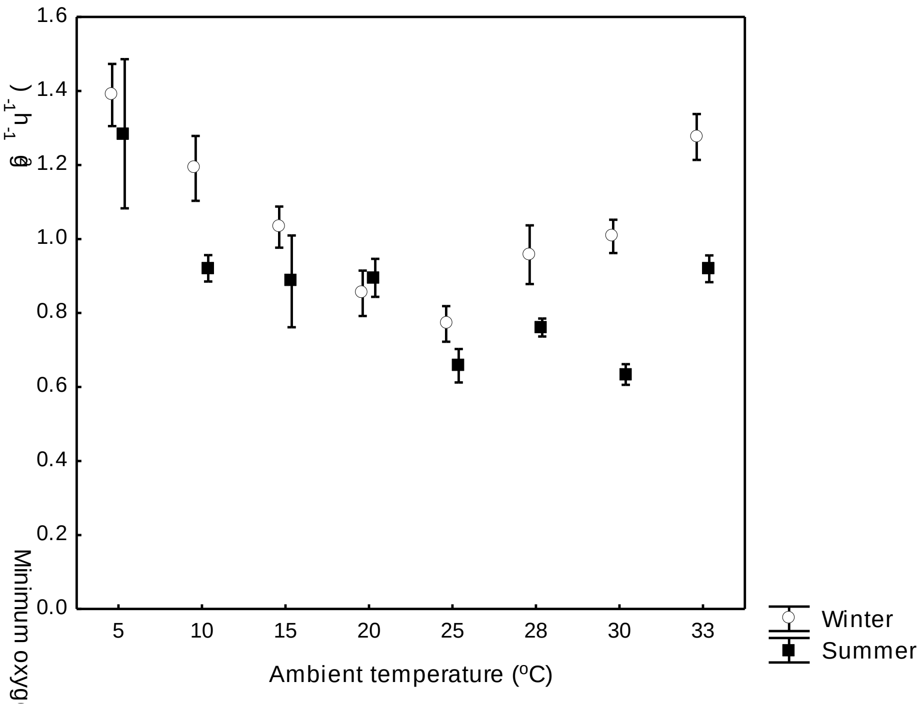

Fig 3. Seasonal variation in mean minimum oxygen consumption (RMR$_{Ta}$) in Knysna Turacos

Chapter 9

CONCLUSION

Indigenous South African forests support a large proportion of the country's biodiversity (Cooper 1985, Geldenhuys 1989, Geldenhuys and MacDevette 1989, Rutherford and Westfall 1994, Mucina and Rutherford 2006). Unfortunately, this biome (the smallest found in South Africa), is becoming increasingly fragmented as a result of anthropogenic activities (Cooper 1985, Geldenhuys 1989, Low and Rebello 1996, Eeley et al. 1999; Lawes et al. 2000, Chapman et al. 2006). This fragmentation is likely to negatively affect both the forest flora as well as the fauna with which these plants interact (Cooper 1985, Geldenhuys 1989, Low and Rebello 1996, Lawes et al. 2000, Chapman et al. 2006). In order to gain a better understanding of how to conserve our forest ecosystems, we first need to understand the mechanisms that govern plant-animal interactions (Kirika et al. 2008). Plant-animal interactions have interested scientists since the time of Charles Darwin (Levey et al. 2002). However, fields such as pollination biology (e.g. Darwin 1877) and herbivory (e.g. Ehrlich and Raven 1964) initially attracted the most attention (Levey et al. 2002); and frugivory only started to attract real attention after work by Janzen (1970), Connell (1971), Snow (1971), McKey (1975) finally popularised this field.

Interactions between plants and frugivores play a vital role in various aspects of forest communities (Silva et al. 2002). Seed dispersal is essential in the maintenance and renewal of plant communities, and frugivores play a role in dispersing between 30-50% of temperate, and up to 90% of tropical tree species (Howe and Smallwood 1982, Nathan and Muller-Landau 2000, Aizen et al. 2002, Chave et al. 2002, Herrera 2003). An understanding of fruit selection and fruit preference among frugivores is therefore essential if we want to gain an understanding of the relationships between frugivores and plant species. This thesis therefore examined the mechanisms of fruit choice and fruit preference by two common forest avian frugivores, the Knysna Turaco and Purple-crested Turaco.

In order to attract potential seed dispersers, plants offer a reward of fleshy fruit pulp which is consumed by a large variety of animals (Herrera and Jordano 1981, Howe 1986, Lepczyk et al. 2000). However all fruit pulp is not equal in terms of energy

(Ayala-Berdon et al. 2008). The amount of available energy will therefore influence how much of the fruit a frugivore will consume (Ayala-Berdon et al. 2008). Frugivores will generally consume less as energy levels increase (Montgomery and Baumgardt 1965, Slansky and Wheeler 1992). It is thought that the amount of food eaten is regulated to maintain a constant amount of assimilated energy (Montgomery and Baumgardt 1965, Slansky and Wheeler 1992). Knysna and Purple-crested Turacos were found to select fruit based on energy content and were observed to regulate their energy intake with higher intake rates occurring on more dilute sugar concentrations (Chapter 2).

Fruit choice by avian frugivores may be influenced by a number of factors such as pulp mass (Johnson et al. 1985), pulp to seed ratio (Herrera 1981, Howe and Vande Kerckhove 1981), seed mass (Stanley and Lill 2002a), sugar concentration (Levey 1987a), fruit size (Moermond and Denslow 1985), fruit colour (Murphy 1994), and seed size (Sorenson 1984). The ability of avian frugivores to efficiently digest different sugars may also affect fruit choice (Avery et al. 1999). Some species (e.g. some families from the Sturnid-Muscicapid lineage) are unable to digest sucrose efficiently and may therefore prefer hexose sugars in choice tests (Schuler 1983, Martínez del Rio and Stevens 1989, Martínez del Rio et al. 1989, Martínez del Rio 1990, Martínez del Rio and Restrepo 1993, Levey and Martínez del Rio 2001). Even birds with sucrase may prefer hexose sugars in choice tests as they may not be able to digest sucrose efficiently enough (Martínez del Rio et al. 1992, Avery et al. 1995). In this study, both the Turaco species appear to be tolerant of sugar type and were able to maintain body weight on all six artificial fruit diets (Chapters 2 and 3) therefore indicating that they would be able to subsist on either sucrose or glucose rich fruits.

However, Brown et al. (2008) suggest that experimental procedure may affect the results of choice tests. Equicaloric (Fleming et al. 2004) and equimolar (Downs 1997, Downs 2000) solutions, and solutions that are equivalent by weight (Lotz and Nicolson, 1996, Blem et al. 2000) have all been used previously. Fleming et al. (2004) suggests that energetically equivalent solutions should be used in choice tests.

Most fruits are considered to be nutrient-dilute (high in water, have fewer carbohydrates than lipid-rich fruits and are low in fibre and protein) (Snow 1981, Herrera 1982, Izhaki 1993) and to contain large amounts of seeds (Hulme 1971, Morton

1973, Stiles 1980, Moermond and Denslow 1985, Herrera 1987, Worthington 1989). Frugivorous birds cannot digest seeds and therefore a ballast load forms in the gut which is costly to the birds in terms of decreasing gut volume; increasing bird mass and increasing energy requirements in order to separate the pulp and seeds in the gut (McKey 1975, Norberg 1990, Levey and Grajal 1991, Levey and Duke 1992, Murray et al. 1993, Stanley and Lill 2002a).

Avian frugivores have developed strategies in order to process fruit quickly and thereby reduce the cost of ingesting bulky seeds (Levey and Duke 1992). Mashers separate the seeds from the pulp before ingestion and then discard them (Sorensen 1984, Johnson et al. 1985, Levey 1987b, Murray 1988, Worthington 1989, Levey and Grajal 1991, Symes and Downs 2001). This allows the pulp to be processed quickly however, disadvantages are an increased handling time and this method can only be used for fairly large seeds (Sorensen 1984, Johnson et al. 1985, Levey 1987b, Murray 1988, Worthington 1989, Levey and Grajal 1991). Gulpers ingest the fruit whole and then separate the seeds from the pulp in their digestive system, passing digestible and indigestible parts of the diet at different rates (Björnhag and Sperber 1977, Hörnicke and Björnhag 1980, Levey 1987b). Knysna and Purple-crested Turacos were found to have shorter mean retention times for pulp than for seeds (Chapter 4) indicating that they have the potential to disperse seeds over relatively large distances.

Gulpers may also regurgitate seeds (Sorensen 1984, Johnson et al. 1985, Levey 1987b, Murray 1988, Worthington 1989, Levey and Grajal 1991, Fukui 2003). It has been suggested that fruits may be selected based on their seed loads and pulp to seed ratios which may determine the effectiveness of avian frugivores as seed dispersal agents (Howe and Richter 1982, Howe 1986). Knysna Turacos preferred small-seeded fruits to large-seeded fruits (Chapter 4) indicating that in the wild they would probably disperse more small than large seeds. Purple-crested Turacos displayed no preference for either seed size (Chapter 4) indicating that in the wild they would probably disperse both small and large seeds.

While the use of artificial fruits makes it easier to study food preferences, digestion and the effect of seed packaging, it is essential that we consider these factors in relation to natural indigenous fruits. Indigenous fruits are not equal in terms of available energy, and factors such as the digestive ability of the frugivore as well as the nutritional composition of the fruit pulp can influence the frugivores ability to meet their energetic demands (Levey and Karasov 1989, Worthington 1989, Martinez del Rio and Restrepo 1993, Fuentes 1994, Afik and Karasov 1995, Lepczyk et al. 2000, Brown and Downs 2003, Downs 2008). As we have stated before, the dispersal of seeds is crucial to the persistence, regeneration and maintenance of plant communities (Howe and Smallwood 1982, Nathan and Muller-Landau 2000, Chave et al. 2002, Herrera 2003, Bascompte and Jordano 2007). Therefore much attention has been paid to the germination potential of seeds ingested by frugivorous animals (reviewed in Traveset 1998).

Many studies have shown that germination success and the rate of germination increases for ingested seeds (Krefting and Roe 1949, Turcek 1963, Van der Pijl 1972, 1982, Holthuijzen and Sharik 1985, Lieberman and Lieberman 1986, Barnea et al. 1990, Izhaki and Safriel 1990, Barnea et al. 1991, Clergeau 1992, Ellison et al. 1993, Murray et al. 1994, Traveset and Verdú 2002). It has been suggested that the removal of the pulp and/or seed coat scarification are responsible for this enhanced germination success (Evenari 1949, Rick and Bowman 1961, Ketring 1973, Mayer and Poljakoff-Mayber 1975, Temple 1977, Herrera 1984, Agami and Waisel 1986, 1988, Mayer and Poljakoff-Mayber; 1989, Barnea et al. 1990, Izhaki and Safriel 1990, Barnea et al. 1991, Witmer and Cheke 1991, Cipollini and Levey 1997, Yagihashi et al. 1998).

However, some studies have shown ingestion to decrease (Livingston 1972, Smith 1975, Valido and Nogales 1994, Nogales et al. 1995, Crossland and Vander Kloet 1996) or have no significant effect on germination success (Brunner et al. 1976, McDiarmid et al. 1977, Salomonson 1978, Lieberman et al. 1979, Howe and Vande Kerckhove 1981, Stocker and Irvin 1983, Lieberman and Lieberman 1986, Barnea et al. 1992, Clout and Tilley 1992). A decreased germination success indicates that the frugivores could not be considered valid dispersal agents (Figueroa and Castro 2002) whereas no significant effect on germination success indicates that the frugivores are acting only as dispersal agents (Barnea et al. 1990). Seed retention times are important as they give an indication of the potential seed dispersal distance ability of a frugivore (Fukui 2003). Digestive transit times of indigenous fruit ingested by Knysna Turacos ranged from c. 12-35 min (Chapters 5 and 6) indicating that they have the potential to disperse seeds over relatively large distances. Knysna Turacos experienced apparent assimilation efficiencies of c. 15-84% (Chapter 5) indicating that they efficiently digest the indigenous fruit used in this study. Mean percentage germination of ingested seeds was not significantly different to pulp removed and whole fruit seeds (Chapter 6) indicating that ingestion by Knysna Turacos did not enhance percentage germination through either seed coat abrasion or pulp removal. Seed ingestion by Knysna Turacos did not influence the rate at which seeds germinated in 75 % of the tree species used in this study (Chapter 6).

Fruit consumed by birds are usually red or black in colour (Ridley 1930, Willson et al. 1989, Burns 2005) and this trend was observed in the indigenous South African fruit used in this study (29% black and 43% red) (Chapter 7). Fruits are generally classified as either nutrient-dense (variable in protein, relatively high in lipids, and low in water and carbohydrates) or nutrient-dilute (low in fibre and protein, high in water, and have fewer carbohydrates than nutrient-dense fruits) (Snow 1981, Herrera 1982, Izhaki 1993). Indigenous fruits in this study were generally observed to be high in water content; and low in protein and lipid content respectively and could therefore be classified as nutrient-dilute (Chapter 7). Avian frugivores in South Africa would therefore need to select only nutrient-dense fruits or would need to consume large amounts of nutrient-dilute fruits in order to obtain sufficient energy (Worthington 1989). Sucrose and hexose (glucose and fructose) sugars are the main sugars found in fruit pulp (Baker and Baker 1983a, 1983b, Baker et al. 1998, Nicolson 2002, Lotz and Schondube 2006) and the ability of the avian frugivores to efficiently digest the different sugars may affect fruit choice (Avery et al. 1999). 84% of the indigenous fruit species analysed for sugar content in this study were hexose dominant with 50% being fructose and 34% being glucose dominant (Chapter 7). Only 16% of the fruit species analysed for sugar content were sucrose dominant (Chapter 7).

While it is important to understand fruit digestion and preferences based on nutritional content and seed loads, it is also vital that we consider how the energy requirements of avian frugivores may differ seasonally especially as global warming is likely to cause noticeable differences in ambient temperatures (Gates 1993, Gibson et al. 2010). As a result of climate change, species may 1) move and track suitable conditions; 2) adapt to a new climatic condition either physiological, behaviourally or genetically;

or 3) go extinct if a species can not move or adapt to new environmental conditions (Holt 1990, Martínez-Meyer et al. 2004, Peterson et al. 2005).

Various endotherms have been shown to experience seasonal flexibility of metabolic thermoregulation (Cooper and Swanson 1994, Liknes et al. 2002, Dawson 2003, Nzama et al. 2010, Smit and McKechnie 2010). By increasing their basal metabolic rate (BMR), some birds are able to survive harsh winters as their threshold of thermogenesis and thereby their cold defense levels also increases (Dawson and O'Connor 1996, Swanson and Olmstead 1999).

Although BMR and minimum resting metabolic rate at a particular ambient temperature (RMR_{Ta}) were formerly regarded as specific, or fixed for a particular temperature, many bird species have been shown to exhibit seasonal or acclimation differences (Weathers and Caccamise 1978, Liknes and Swanson 1996, Maddocks and Geiser 2000, Dawson 2003, Bush et al. 2008a, 2008b, Smit et al. 2008, Lindsay et al. 2009a, 2009b, Nzama et al. 2010, Smith and McKechnie 2010). Knysna Turacos were found to have no significant difference in RMR_{Ta} between summer and winter; however they significantly increased their BMR in winter (Chapter 8). Knysna Turacos were also found to have a very broad thermoneutral zone in both winter and summer (Chapter 8) indicating a seasonal thermoregulatory response of improved cold defense rather than that of energy conservation which is what was expected for an southern hemisphere Afrotropical bird species that inhabits an area with an unpredictable climate.

While the results obtained in this study may be useful in determining fruit preferences and potential seed dispersing abilities of both Turacos species as well as how Knysna Turacos may be affected by global warming, further studies are needed. The nutritional composition, as well as non-nutritive factors, of the remaining indigenous forest fruit sugars needs to be determined in order to give insight into the role of these avian frugivores as potential seed dispersers of fruiting tree species. Future studies establishing suitable seed sizes and volumes for use in determining the effect of seed loads on larger birds are also required. Due to time constraints, metabolic measurements in the current study were only undertaken during one winter and one summer season so ideally future studies should rerun this experiment with repeated seasonal data.

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Errata

The following errors were noticed by examiners and are highlighted here as these papers are published:

Chapter 2

Results

Equicaloric food intake and assimilation

Should read: Apparent energy assimilated (AE) was high for all six diet treatments and there were no significant difference between the treatments ($F_{(2, 16)}$ = 1.86, p = 0.188, Table 2).

Should read: AE was high for all six diet treatments and there were significant differences between the treatments ($F_{(2, 8)}$ = 19.24, p = 0.0005, Table 2).

Equimolar food intake and assimilation

Should read: AE was high for all six diet treatments and there were significant differences between the treatments ($F_{(2, 16)}$ = 3.96, p = 0.040, Table 2).

Should read: AE was high for all six diet treatments but there were no significant differences in apparent energy assimilation efficiency between the treatments ($F_{(2, 8)}$ = 1.80, p = 0.227, Table 2).

Discussion

It is important to note that, in the case of the Purple-crested Turacos, a lack of significant differences between the equimolar treatments may be due to sample size rather than a biological phenomenon.

Chapter 3

Discussion

Should read: In contrast, both Knysna and Purple-crested Turacos preferred the sucrose equimolar artificial fruit diet to the glucose at low concentrations.

Deleted: Although Knysna Turacos showed no significant preference for equimolar sucrose over equimolar glucose at low molarity, which would have been expected in terms of energy rewards (see Downs and Perrin, 1996; Downs, 1997), they ingested more sucrose than glucose in total.

Should read: Our results also showed that both Knysna and Purple-crested Turacos preferred sucrose to glucose equimolar artificial fruit diet at low concentrations, and at high equimolar concentrations neither species displayed a preference for either sugar.

Should read: We therefore reject our first prediction. We also reject our second prediction at high equimolar concentrations but at low equimolar concentrations we accept our second prediction.

Chapter 4

Results

Should read: Knysna Turacos significantly preferred the large-seeded artificial fruit diet to the small-seeded artificial fruit diet in terms of amount eaten per hour (GLM RMANOVA, $F_{(11, 88)} = 1.98$, $p = 0.040$, Fig. 4a).

Discussion

Should read: Both Turaco species ingested significantly more of the small-seeded than the large-seeded diet. Knysna Turacos preferred the large-seeded to the small-seeded artificial fruits. In contrast, Purple-crested Turacos showed no preference for either diet. The results obtained for Knysna Tuarcos are similar to those obtained by Murray et al. (1993) who found that American Robins (*Turdus migratorius*) preferred large-seeded over small-seed fruit of equivalent total seed volume as the large seeds could be voided quickly by regurgitation. They are also similar to those obtained by Stanley and Lill (2002a) who found that Silvereyes (*Zosterops lateralis*) consumed more large-seeded than small-seeded fruit of equivalent total seed volume. Our results for the Purple-crested Turacos are similar to those obtained by Willson (1994) who found that American Robins showed no preference for fruits containing one or six *Rubus* *spectabilis* seeds; and by Willson and Comet (1993) who found that Northwestern Crows (*Corvus caurinus*) showed no preference for artificial fruit with low seed loads.

Should read: Our results also show that Knysna Turacos prefer large-seeded fruits to small-seeded fruits while Purple-crested Turacos displayed no preference.

Appendix 1

PRELIMINARY DESCRIPTION OF THE GASTROINTESTINAL STRUCTURE AND MORPHOLOGY OF A CAPTIVE-BRED KNYSNA (*TAURACO CORYTHAIX*) AND A CAPTIVE-BRED PURPLE-CRESTED (*GALLIREX PORPHYREOLOPHUS*) TURACO

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Prepared as a short note

Introduction

Knowledge of the structure of the gastrointestinal tract of bird species is essential as it determines food transit rates and maximum food intake (Mbatha et al. 2002). A bird's digestive tract usually consists of a beak, mouth, pharynx, oesophagus, crop, proventriculus, ventriculus, intestine, ceca, rectum, cloaca, and vent (Klasing 1998). However the gross morphology and anatomy of bird digestive tracts is not uniform for all bird species (Klasing 1998).

Nectarivorous and frugivorous birds have been shown to have short, simple digestive tracts as their food is easily digestible (Klasing 1998). The presence of a crop enables the bird to store food and therefore birds do not need to eat continuously throughout the day (Klasing 1998). Avian nectarivores generally have an expandable crop which may form while feeding or it may be a permanent bulge of the oesophagus (Farner 1970, Gill 1989). Hummingbirds have relatively large crops (Carpenter et al. 1991; Bednekoff and Houston 1994) while sunbirds seem to not have a crop (Mbatha et al. 2002). Black-capped Lorys (*Lorius lory*) which are nectarivorous parrots, were found to have well-developed, expandable crops with thin, smooth muscle walls (Forshaw 1989, Mbatha et al. 2002) whereas Hoatzins (*Opisthocomus hoazin*) which are obligate folivorous birds were found to have a large muscular crop with two chambers (Grajal 1995). There are few studies looking at the gut structure and morphology of avian frugivores (see Downs et al. 2000). We therefore investigated the gut morphology in two non-passerine relatively large avian frugivores, the Knysna (*Tauraco corythaix*) and the Purple-crested (*Gallirex porphyreolophus*) Turaco.

Knysna Turacos are relatively large (c. 310g) fairly common South African endemic forest frugivores (Rowan 1983, Oatley 1997, du Plessis and Dean 2005a). Similarly, Purple-crested Turacos are relatively large (c. 300g) locally common frugivores that occur in lowland forests in eastern South Africa (du Plessis and Dean 2005b). While both species are typically resident, they may locally track fruit in response to availability (Rowan 1983).

Methods and Study Species

We dissected one captive-bred Knysna and one captive-bred Purple-crested Turaco that were obtained from the University of KwaZulu Natal. The Knysna Turaco died as a result of being egg-bound and it was unknown why the Purple-crested Turaco died. Both specimens had been frozen immediately after death until they were dissected. Body measurements and gastrointestinal tract (GIT) dimensions were made with vernier calipers and a steel ruler (± 0.5 mm).

Results and Discussion

Both Turaco species were found to have a relatively small gape (Table 1) which explains the consumption of relatively small fruits. The gape sizes obtained for Knysna Turacos (Table 1) were consistent with those found by Downs et al. (unpublished database) and Fry et al. (1988) who found a mean culmen length of 23.5 mm. Similarly the gape sizes obtained for Purple-crested Turacos (Table 1) were consistent with those found by Fry et al. (1988) who found a mean culmen length of 24.9 mm.

The structure of the GIT of both the Knysna and Purple-crested Turaco consisted of an oesophagus leading into a well-developed proventriculus (Figs.1 and 2).

Neither species were observed to have a crop. The proventriculus led to the ventriculus which *in situ* was prominent lying to the left side of the abdomen when viewed ventrally (Figs.1 and 2). The Purple-crested had a shorter small intestine than the Knysna Turaco (Table 1) and both species had a gall bladder. The GIT of the frugivorous Speckled Mousebirds (*Colius striatus*), was found to be similar to that of both Turaco species and the mousebirds were also found not to have a crop (Downs et al. 2000). The ventriculus of the Speckled Mousebird was observed to have extensive convolutions while the ventriculus of the Purple-crested Turacos had few convolutions (Downs et al. 2000).

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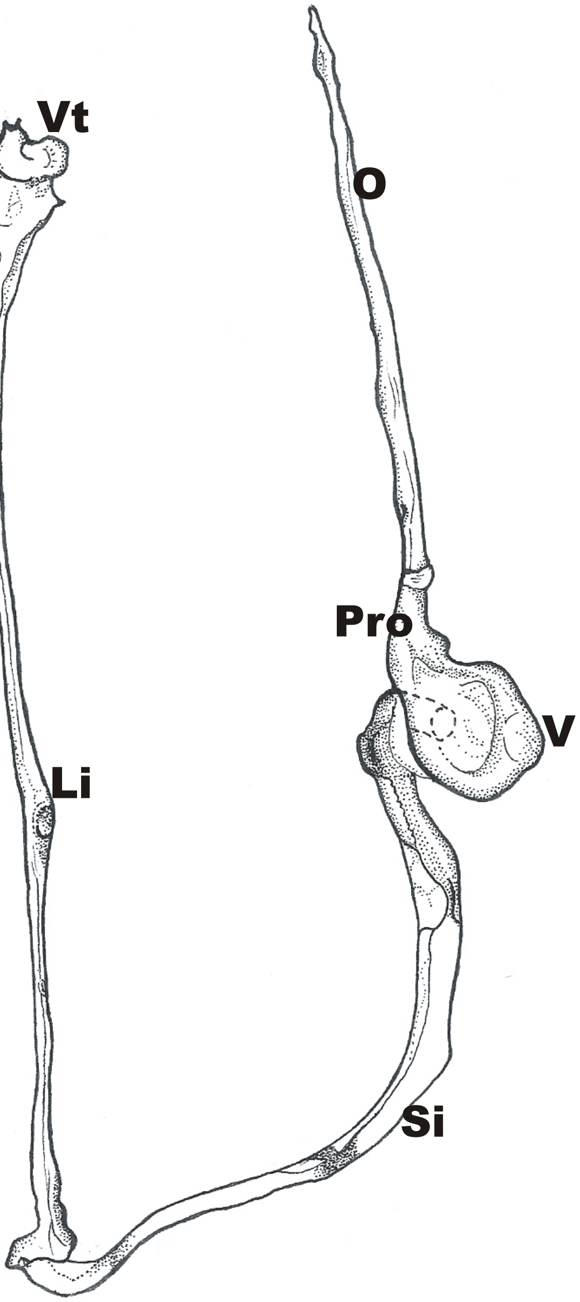
Table 1 Body, organ and gut dimensions of *Tauraco corythaix* and *Gallirex porphyreolophus*. (Sample size is indicated by the parentheses).

Fig. 1 The gastrointestinal tract and associated digestive organs of *Tauraco corythaix* with the exception of the bill, oral cavity; spleen; and liver; O = oesophagus; Pro = proventriculus; V = ventriculus; Si = small intestine; Li = large intestine; and Vt = vent (magnification = 0.8 x)

Fig. 2 The gastrointestinal tract and associated digestive organs of *Gallirex porphyreolophus* with the exception of the bill, oral cavity; spleen; and liver; O = oesophagus; Pro = proventriculus; V = ventriculus; Si = small intestine; Li = large intestine; and Vt = vent (magnification = 0.6 x)

Table 1. Body, organ and gut dimensions of *Tauraco corythaix* and *Gallirex porphyreolophus*. (Sample size is indicated by the parentheses).

Parameter	*Tauraco corythaix*	*Gallirex porphyreolophus*
Body mass (g)	246.5 (1)	233.8 (1)
Gape length (mm)	21 (1)	27 (1)
Gape breadth (mm)	25 (1)	15 (1)
Oesophagus length (mm)	100 (1)	76 (1)
Proventriculus length (mm)	19 (1)	59 (1)
Ventriculus length (mm)	24 (1)	27 (1)
Ventriculus breadth (mm)	24 (1)	27 (1)
Small intestine length (mm)	149 (1)	109 (1)
Large intestine length (mm)	205 (1)	371 (1)
Total GIT length (mm)	497 (1)	642 (1)
Total GIT mass (g)	11.9 (1)	9.9 (1)
Liver mass (g)	6.6 (1)	38.9 (1)
Heart mass (g)	1.7 (1)	2.8 (1)



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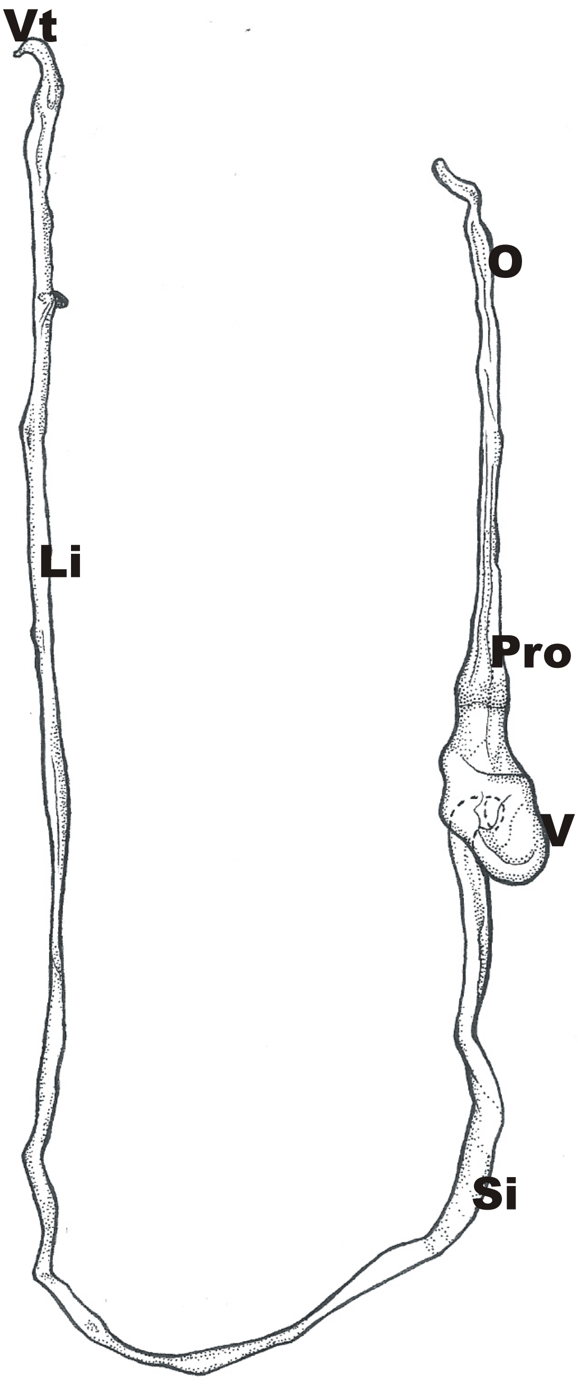

Fig. 2 The gastrointestinal tract and associated digestive organs of *Gallirex porphyreolophus* with the exception of the bill, oral cavity; spleen; and liver; O = oesophagus; Pro = proventriculus; V = ventriculus; Si = small intestine; Li = large intestine; and Vt = vent (magnification = 0.6 x)