

Aspects of fruit digestion and selection in selected southern African avian frugivores

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ABSTRACT

Fruit-frugivore interactions play a central role in maintaining the structure and diversity of ecosystems through their effects on seed dispersal. Because fruit selection and thus fruit removal represents the first stage in the seed dispersal process, understanding factors affecting fruit selection can play an important role in the formulation of sound conservation efforts especially in light of the on-going habitat change. However, to date, the research is inconclusive as to factors affecting fruit selection as results have revealed inconsistent and controversial with a huge variation in fruit selection patterns between species and within individuals of the same species. The huge inter- and intra-specific variation in fruit selection patterns precludes any generalizations on fruit selection notwithstanding the research effort addressing the issue. One factor that could be attributed to this pattern is that most studies on fruit selection patterns in frugivorous birds have studied fruit attributes or physiological adaptations of frugivores to fruits in isolation. Because fruit profitability is affected by both pre- and post-ingestional factors, studying these factors in isolation deprives us of fresh insights into the fruit-frugivore interaction. Therefore by relating the physiological aspects of frugivores to fruit characteristics, this study aims to provide a link between the physiology of birds and their feeding ecology. Cape white-eyes (*Zosterops virens*), red-winged starlings (*Onychognathus morio*) and speckled mousebirds (*Colius striatus*) were used for this study.

In the first part of this thesis, transit times and digestive efficiencies of birds fed equicaloric glucose and sucrose artificial fruit diets of varying concentrations were determined. Three concentrations were used: low (6.6%), medium (12.4%) and high (22%). Digesta transit times of birds increased with an increase in concentration for all diets but were generally higher on glucose diets. This finding has been widely reported in

other similar studies. The increase in digesta transit times with an increase in sugar concentration may be due to high nutrient density on high concentration diets which require a longer processing time. Intake rates, on the other hand, decreased with an increase in sugar concentration. The inverse relationship between food intake and nutrient levels has often been attributed to compensatory feeding which posits that birds respond to nutrient dilution by increasing intake to allow a constant flux of assimilated energy. Indeed, speckled mousebirds and Cape white-eyes maintained a constant assimilated energy intake on sucrose diets by modulating food intake rates. The apparent assimilation efficiencies of glucose diets for all species were comparable and typical of those found in other frugivorous birds. However, red-winged starlings displayed low assimilation efficiencies for sucrose diets and lost significant body mass on all sucrose diets. The lack of significant sucrase activity in this species was attributed to this finding. This study showed the importance of digestive physiology in explaining fruit selection patterns in frugivorous birds.

The second part of this thesis assessed the deterrence effects of tannins which are ubiquitous secondary compounds in plant material and are known for their ability to bind to protein which reduces nitrogen availability in the diet. In this study, birds were fed artificial fruit diets containing varying levels of tannins (0%, 2.5% and 5%) in paired choice tests. It was predicted that tannins would have no effect at low concentrations but at higher concentrations would act as deterrents although the levels at which they would become deterrents would differ among species. Red-winged starlings preferred the control diet, were indifferent to the medium tannin diet and were deterred by the high tannin diet whereas speckled mousebirds and Cape white-eyes were not deterred at all concentrations. The discrepancy in the results was attributed to differences in taste sensitivity, tolerance levels and detoxification mechanisms of secondary compounds between species.

Occasional geophagy and consuming a broad diet were also implicated in producing the results obtained. Plant secondary compounds in fruits are diverse and their effects are similarly diverse and there is a possibility that different groups of secondary compounds generate disparate effects. Similar studies on other types of secondary compounds may thus contribute towards a broader understanding of the role of secondary compounds in mediating fruit-frugivore interactions. Overall, this study showed how diet affects ability to handle secondary compounds in fruits.

The third part of this thesis addressed the influence of ethanol concentration on fruit selection in frugivorous birds. Because ethanol is ubiquitous in fruits and its concentration is positively correlated to fruit sugars, it has been suggested that because frugivores could use its odour to locate fruiting plants, they should select fruit with high ethanol concentrations. The aim of this study was to test this hypothesis by determining whether frugivorous birds show a preference for fruit laden with alcohol at levels equivalent to those of over-ripe fruits. Birds were provided with two artificial fruit diets in pairwise choice tests: an experimental diet containing 1% ethanol and an artificial fruit diet with no ethanol. For all species, there were no significant differences in the amount of fruit consumed between the two food types. These findings provided corroborating evidence to the suggestion that birds are unlikely to prefer over-ripe fruits compared to ripe fruits due to the negative impacts associated with ingesting ethanol at high concentrations. However, it could be possible that the lack of preference observed in this study was because the ethanol concentrations used were too low to be detected. Overall, this study suggests that at high concentrations, ethanol in fruits acts as a deterrent rather than an attractant.

The last part of this thesis was conducted to determine the use of taste and olfaction by red-winged starlings in making foraging decisions. To determine whether they use taste in fruit selection, they were concurrently offered a control artificial fruit diet with another artificial fruit diet flavoured with different concentrations of ethanol and various fruit essences. To test whether they were able to use olfaction to locate food, they were provided with two choices: artificial fruit suspended over either banana and orange fruit essences and ethanol of varying concentrations or a control artificial fruit (without essence). It was hypothesized that (1) red-winged starlings have tasting ability which helps them in selecting fruits to feed on and that (2) red-winged starlings use olfaction to locate food. Results were consistent with the first hypothesis but inconsistent with the second one. Thus, red-winged starlings use taste when selecting fruits to feed on but do not use olfaction to locate fruit sources. The latter was unexpected because birds with olfactory bulb sizes similar to theirs exhibit advanced olfactory abilities. It was speculated that the lack of olfactory abilities in starlings could be attributed to the fact that this study was conducted outside of this species' breeding season when olfactory abilities were likely to be lowest and also to that olfactory abilities may not have been important at the scale investigated in this study. Overall, the study showed that the sense of taste in birds plays an important role in making foraging decisions than currently appreciated.

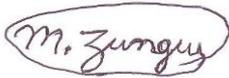
Overall, this thesis demonstrated the value of relating physiological attributes of frugivores to fruit attributes in acquiring deeper understanding of fruit-frugivore interactions. One particular advantage of the methodology employed in this study was that it controlled for covariance among fruit characters and also removed the confounding effects of seed size and secondary compound composition. The shortcoming of this approach is related to the applicability of the results to the field conditions. Therefore an

approach combining laboratory and field observations may produce results that may be important in informing us about how to manage our ecosystems.

PREFACE

The data described in this thesis were collected in Pietermaritzburg, Republic of South Africa from February 2012 to November 2014. Experimental work was carried out while registered at the School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg, under the supervision of Professor Colleen T. Downs.

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



Manqoba M. Zungu

November 2014

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



.....

Professor Colleen T. Downs

Supervisor

November 2014

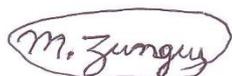
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DECLARATION 1 - PLAGIARISM

I, Manqoba M. Zungu, declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. This thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
 - a. Their words have been re-written but the general information attributed to them has been referenced
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5. This thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the References sections.

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DECLARATION 2 - PUBLICATIONS

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

Publication 1

Manqoba M. Zungu· Amy-Leigh Wilson· Colleen T. Downs

Digestive efficiencies and digesta transit times of Cape White-eyes (*Zosterops virens*), Red-winged Starlings (*Onychognathus morio*) and Speckled Mousebirds (*Colius striatus*) on equicaloric glucose and sucrose artificial fruit diets.

Author contributions:

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

Publication 2

Manqoba M. Zungu· Colleen T. Downs

Effects of tannins on fruit selection in three southern African frugivorous birds.

Author contributions:

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

Publication 3

Manqoba M. Zungu· Colleen T. Downs

The role of ethanol in fruit selection by frugivorous birds.

Author contributions:

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

Publication 4

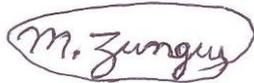
Manqoba M. Zungu · Lorinda A. Hart · Colleen T. Downs

The use of taste and smell in making foraging decisions in frugivorous birds: an experimental test using Red-winged Starlings (*Onychognathus morio*).

Author contributions:

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

Signed:

A handwritten signature in cursive script that reads "M. Zungu". The signature is enclosed within a hand-drawn oval border.

Manqoba M. Zungu

November 2014

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“It’s not about how hard you push along the way, it is about having something in you to finish” - Michael Jordan

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DEDICATION

I dedicate this thesis to my family for bearing with me during the period I was committed towards this work.

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CHAPTER 1

Introduction

1.1 Plant-animal interactions

Because plants are immobile organisms that depend on other organisms during the dispersive stages of their life cycle, mutualisms between plants and animals are common in nature (Vazquez and Aizen, 2004). The mutualistic relationship between plants and animals plays a central role in structuring plant communities. For example, up to 90% of tropical plant species use vertebrates as mechanisms of seed transport (Howe and Smallwood, 1982; Jordano, 2000). In ecological systems, plants rarely interact with a single animal species but interact with a diverse assemblage of mutualists and antagonists (Strauss and Irwin, 2004). Therefore studying plant-animal interactions may be the key towards understanding ecological systems and how to manage them (Vazquez and Aizen, 2004).

Most studies on plant-animal interactions have concentrated on specialized interactions and on small groups of species without considering community-wide interactions (Strauss and Irwin, 2004; Bascompte and Jordano, 2006). Because interactions are subject to spatial and temporal variation in selection pressures, pair-wise coevolutionary studies may not suffice in uncovering selection pressures driving plant-animal interactions, highlighting the need for community-wide studies spanning a wide range of spatial and temporal scales in order to draw conclusions about them (Herrera, 1998; Levey and Benkman, 1999; Strauss and Irwin, 2004; Bascompte and Jordano, 2006). Recent studies have shown that even systems that were traditionally considered obligate in nature, they may not be as obligate as previously thought (Waser et al., 1996). Therefore generalizations in ecological interactions

are the rule rather than an exception especially when considering food webs as a whole, an attribute which should be considered in studies on plant-animal interactions (Zamora, 2000).

Ecologists have long viewed interactions among species as a network whereby nodes represent species with links between pairs representing interspecific interactions (Vazquez, 2005). Most mutualistic networks are nested (i.e. generalists interact with other generalists) resulting in a few species responsible for most of the interactions, suggesting that interactions of a few species in a community may drive the whole community (Jordano, 1987*a*; Bascompte and Jordano, 2006). Therefore studying network structure can provide novel information towards understanding ecological and evolutionary processes. For example, the presence of asymmetric networks suggest low probability for tight coevolution in plant and seed disperser interactions (Jordano 1987*a*; Jordano et al., 2003; Vazquez and Aizen, 2004) and the occurrence of nested networks suggest that they are highly vulnerable to the extinction of species with more interactions and robust to the extinction of species with few links (Vazquez et al., 2009).

1.2 Fruit-frugivore interactions

The earliest evidence of fruit-frugivore interactions dates back at least to the Early Tertiary (Tiffney, 2004). The fruit-frugivore interaction is mutualistic in nature: plants get their seeds moved and animals obtain a nutritional reward in exchange for moving the seeds (Howe and Smallwood, 1982; Herrera, 2002; Levin et al., 2003). Vertebrate dispersal has evolved many times in fleshy-fruited plant lineages suggesting that it has been advantageous on numerous occasions in the evolutionary history of plants (Herrera, 2002). Likewise, frugivorous diets have evolved on several occasions in avian and mammalian lineages over time (Fleming and Kress, 2011). When spread among plant growth forms, fruit-frugivore

interactions are more common among trees than shrubs and consequently, their importance decreases from forests to herbaceous formations (Herrera, 2002).

Earlier conceptual developments of the fruit-frugivore interaction were centred on tight, coevolved interactions (Ridley, 1930; Snow, 1971; McKey, 1975; Howe, 1977; Howe and Estabrook, 1977), probably because they were heavily influenced by studies on plant-pollinator interactions (Wheelwright and Orians, 1982; Herrera, 2002). The central theme was that dispersers differ in their ability to serve as dispersal agents and consequently, mutual dependence between plants with one effective disperser or a group of effective dispersers would ensue (McKey, 1975; Howe, 1977; Howe and Estabrook, 1977). However, due to a wealth of knowledge that has been accumulated over the years, it has been concluded that tight, paired coevolutionary interactions are unlikely (Howe, 1984*a*; Herrera, 1985*a*; Herrera, 1986; Jordano, 1987*a*; 1995*a*; Herrera, 1998; Levey and Benkman, 1999; Jordano et al., 2003). The current consensus is that the coevolution between the two groups is loose (diffuse coevolution, Howe, 1984*a*; Herrera, 1985*a*; Herrera, 1986; Jordano, 1987*a*; 1995*a*).

1.3 Seed dispersal

Seed dispersal is the last step in plant reproduction but the first stage in plant recruitment: it sets the template for plant spatial distribution (Herrera et al., 1994; Jordano, 2000; Nathan and Muller-Landau, 2000; Rey and Alcantara, 2000). Although seed dispersal is mediated by a variety of abiotic and biotic vectors, animal-mediated dispersal has received the most attention and is the most researched (Jordano, 2000; Levin et al., 2003). According to Binggeli (1996), about half (50%) of fleshy-fruited plants globally depend on seed dispersal services by vertebrates. However, the prevalence of animal-mediated seed dispersal differs with climatic zones/regions: it accounts for 30–40% of plants in temperate forests,

50% in Mediterranean scrublands, 80% in tropical woodlands, 80–82% in African and Australian rainforests and 70–94% in Neotropical trees and shrubs (Jordano, 2000).

Because plants invest immensely in structures attracting and advertising fruits to vertebrates, seed dispersal should be beneficial (Howe and Smallwood, 1982; Wheelwright and Janson, 1985). The basic premise regarding the role of seed dispersal is that it promotes germination and establishment as it reduces density-dependent mortality factors near the parent plant (Jordano, 2000; Herrera, 2002). According to the Janzen-Connell model, distance- and density-responsive seed and seedling enemies maintain diversity in plant communities by disallowing recruitment of seedlings in the vicinity of conspecific adults which gives space for other species in the community to colonize (Clark and Clark, 1984; Schupp, 1992). This model was received favourably by ecologists long before being subjected to empirical testing (Howe and Smallwood, 1982). A plethora of empirical studies have accumulated over the years with most supporting it (e.g. Augspurger, 1983; 1984, Fragoso et al., 2003) although some are contrary to it. For example, Schupp (1988; 1992) found that seed predation away from the parent plant was higher than beneath the parent plant in *Faramaea occidentalis*. As a result, survival of seeds increased with adult density in contrast to the Janzen-Connell model (Schupp, 1992). The low seed predation rate under the parent plant was attributed to high densities of seeds at the population-level scale satiating predators resulting in many seeds escaping predation. This argued caution in the interpretation of empirical studies and suggested that density-dependent effects could be scale-dependent (Schupp, 1992).

1.4 Birds as dispersal agents

Birds are dominant both in number and diversity in many forest ecosystems and as a result, the majority of plant species in these ecosystems depend upon their dispersal services

(Armesto et al., 1987; Armesto and Rozzi, 1989; Fleming and Kress, 2011). Because of their high mobility, birds serve as mobile links that connect landscape patches of different levels of degradation and habitat conditions (Whitney et al., 1998; Holbrook and Smith, 2000; Garcia et al., 2010; Lenz et al., 2011). For example, hornbills (*Ceratogymna* spp.), the largest seed dispersers in tropical rainforests across Africa and Asia (Kitamura, 2011; Lenz et al., 2011), have been reported to move over a distance of 290 km, the longest distance ever recorded for an avian seed disperser (Holbrook et al., 2002). Furthermore, European jays (*Garrulus glandarius*) have been reported to carry seeds for more than 20 km at a time, and are thought to be responsible for the incredibly fast post-glacial expansion of trees in the Northern Hemisphere (Sekercioglu, 2006). Such long distance movements are of high conservation importance especially in light of the on-going large-scale fragmentation of tropical forests which is threatening the interdependency and connectedness of these ecosystems, resulting in biodiversity loss (Wunderle, 1997; Holbrook et al., 2002).

Perhaps the most impressive display of the dispersal prowess of birds is their ability to transport seeds over oceanic barriers. For example, between 1999 and 2008, Surtsey Island has been colonized by 69 plant species with about 75% of dispersal due to birds (Magnusson et al., 2009). Birds not only colonize this island but are also responsible for the transfer of nutrients from sea to land, suggesting that they drive the whole succession process (Ellis, 2005; Magnusson et al., 2009). The less appreciated contribution of avian seed dispersers is allowing colonization in degraded areas (Sekercioglu, 2006). Seed dissemination by birds is important in enhancing recolonization by native plant species in degraded areas and current efforts are directed towards preserving small fragments of native vegetation to serve as foci of regeneration by attracting seed dispersers (Robinson and Handel, 1993; Wunderle, 1997; McCarthy et al., 2002).

The efficiency of birds as seed dispersal agents however declines with seed size. Most birds have small mouth parts and thus cannot disperse large seeds (Wheelwright, 1985; Levey 1987*a*; Sekercioglu, 2006). Large-seeded plants in that case will depend on few large frugivores such as hornbills, which in most cases occur at low densities (Holbrook and Smith, 2000; Holbrook et al., 2002) and/or are subjected to direct persecution by humans as they are the most preferred by hunters (Silva and Tabarelli, 2000; Link and Di Fiore, 2006). Trees that depend on large-bodied dispersers are likely to suffer from loss of dispersers and such losses may be detrimental to the plants and in turn may affect species that depend on them (Silva and Tabarelli, 2000). The high degree of inter-connectedness in tropical forests therefore renders them highly susceptible to human perturbation (Howe, 1984*b*). Because large seeds are oftentimes protected by tough husks making them inappropriate for dispersal by birds, primates tend to disperse them more effectively (Link and Di Fiore, 2006; Galetti et al., 2001).

1.5 Factors affecting fruit selection

1.5.1. Fruit abundance

Since fruit removal represents the first step of the seed dispersal process, it plays a pivotal role in plant fitness (Izhaki, 2002*a*). Several factors affect fruit removal rates in plants but the factor that has received the most attention is fruit abundance (Howe and Estabrook, 1977; Jordano, 1995*b*). The fruit-crop hypothesis posits that plants that produce superabundant fruits should attract a wide range of potential dispersers resulting in high removal which could translate into more effective seed dispersal (Izhaki, 2002*a*). Indeed, a number of studies have found empirical support for this hypothesis in a wide range of ecosystems (Howe and Vande Kerckhove, 1981; Howe and DeSteven, 1979; Martin, 1985*a*; Jordano, 1987*a*; Sallabanks, 1993; Murray, 1987; Ortiz-Pulido and Rico-Gray, 2000;

Blendinger et al., 2008) although there are exceptions (Laska and Stiles 1994; Izhaki 2002a). Fruit removal rates; however, seem to be subject to spatio-temporal variability in year-to-year total fruit production by the whole population (Herrera, 1998). For example, high fruit production by the individual plant results in more visits if fruit production by the whole population is low (Ortiz-Pulido and Rico-Gray, 2000). This highlights the difficulty in distinguishing general patterns in fruit removal rates as they are subject to multiple factors which vary spatially and temporally (Izhaki, 2002a) and points to the need for long-term studies to understand the ecological and evolutionary implications of the variation (Laska and Stiles, 1994; Herrera, 1998).

1.5.2 Fruit colour

The influence of colour on fruit selection in vertebrate-dispersed fruits has been a source of interest since the times of Darwin but selection pressures driving fruit colour diversity remain elusive (Willson and Whelan, 1990; Nakanishi, 1996). Black and red are the most common ripe fruit displays in bird-dispersed plants (Willson and Melampy, 1983; Knight and Siegfried, 1983; Wheelwright and Janson, 1985). It has been proposed that the dominance of colourful fruit displays is to increase the colour contrast between fruits and background foliage, thus increasing fruit conspicuousness and ultimately fruit removal (Wheelwright and Janson, 1985; Cazetta et al., 2009). A corollary to this hypothesis is that red and black fruits produce the strongest contrast between fruit and the background foliage and thus should be preferred by most avian frugivores (Knight and Siegfried, 1983; Nakanishi, 1996; Puckey et al., 1996). However, studies have found that birds show huge variability in fruit colour preference between and even within species, casting doubt on the adaptive function of colourful fruit displays (McPherson, 1987; Willson, 1994; Cazetta et al., 2009). Furthermore, studies have shown that fruit colour is not as important as contrast between fruits and background foliage in explaining fruit colour preferences in birds

(Schmidt et al., 2004; Schaefer et al., 2006). However, Honkavaara et al. (2004) suggested that fruit colour could be more important than background contrast at short distances. Therefore the importance of colour in driving fruit selection patterns in avian frugivores could depend on the spatial scale. One major shortcoming of studies on fruit colour selection in birds is that they classified colour from an anthropogenic context (Schmidt et al., 2004). The limitation of this approach is that bird vision extends beyond the visible region into the ultra-violet (UV) which potentially allows them to distinguish colour better than humans (Vorobyev et al., 1998). Therefore the integration of contrast between fruits and secondary structures, and an objective classification of fruit colour with the aid of the state-of-the-art spectrometric devices may yield more insights into the factors affecting fruit colour choices in birds and could ultimately explain avian influence on fruit colour diversity (Schmidt et al., 2004).

1.5.3 Fruit size

Among factors that affect fruit selection in frugivorous birds, fruit size has arguably produced the most consistent results suggesting that frugivorous birds are effective selective agents on fruit size (Alcantara and Rey, 2003). A study which took phylogenetic affinity into account showed that among 16 fruit traits studied, the only ones which corresponded to seed disperser type were those related to fruit size (e.g. fruit diameter and fruit length, Jordano, 1995*b*). As a consequence, a number of studies have found a significant correlation between bird size/gape width and fruit size (Herrera, 1985*b*; Wheelwright, 1985; Armesto et al., 1987; Mazer and Wheelwright, 1993). This seemingly consistent trend has been attributed to the gape width of frugivores setting an upper limit on the size of fruit that can be eaten and consequently dispersed effectively (i.e. gape limitation, Herrera, 1985*b*; Wheelwright, 1985; Levey 1987*a*). As a result, frugivorous birds have been found to select small fruits over large fruits in both captive and field studies (McPherson, 1987; Avery et al., 1993; Rey et al., 1997;

Alcantara and Rey, 2003). Martin (1985*b*) contended that large fruits are taken up until they increase handling time to the point where costs of eating fruits outweigh benefits. As a result, fruits larger than the gape width of frugivores tend to be dropped near/under the parent tree (Herrera, 1985; Martin, 1985*b*; Levey, 1987*a*; Rey et al., 1997).

1.5.4. Seed mass

Bird-dispersed fruits are bulky, low in lipids and proteins and have high seed mass (30–50% of total dry fruit mass, Moermond and Denslow, 1985; Herrera, 1987; Corlett, 1996). The high seed mass introduces significant costs to frugivorous birds (Levey and Grajal, 1991; Martinez del Rio and Restrepo, 1993; Stanley and Lill, 2002*a*; *b*). Firstly, it increases bird body mass resulting in increased energy expenditure for flight (Levey and Grajal, 1991). Secondly, a high seed mass increases handling costs which increases overall foraging costs (Levey, 1987*a*). Thirdly, it limits the rate of food intake and the rate of nutrient assimilation by reducing gut space (Levey and Grajal, 1991; Martinez del Rio and Restrepo, 1993; Murray et al., 1993). Because seeds represent an indigestible material to birds which accumulates in the gut without providing any energetic benefits (Levey, 1987*a*; Levey and Grajal, 1991; Murray et al., 1993; Stanley and Lill, 2002*b*), fast processing of seeds is of high importance for them to maintain a positive energy balance (Karasov and Levey, 1990; Levey and Duke, 1992; Courtney and Sallabanks, 1992; Levey and Karasov, 1994).

Due to the abovementioned costs, frugivorous birds face a conundrum on how to minimize costs associated with seed ingestion while maximizing energy intake from pulp (Martinez del Rio and Restrepo, 1993; Murray et al., 1993; Stanley and Lill, 2002*a*). Some frugivorous birds crush fruits in the bills and discard/drop seed before ingesting fruit pulp, minimizing the probability of ballast formation in the gut (Levey, 1987*a*). Others regurgitate the seed before it reaches the intestine where digestion and absorption of nutrients takes place

(Howe and Vande Kerckhove, 1981; Johnson et al., 1985; Levey, 1987a; Stanley and Lill, 2002a). The latter has been shown to result in high energy intake because the reduction in gut limitation increases overall pulp consumption (Sorensen, 1984; Murray et al., 1993). However, regurgitation and dropping of seeds are only possible for relatively large seeds (Levey and Grajal, 1991; Martinez del Rio and Restrepo, 1993); therefore small seeds are passed through the gut and defecated (Levey, 1987a). To overcome the limitation of gut space occupation by seeds, cedar waxwings (*Bombycilla cedrorum*) separate seeds and pulp in the gut and process them separately (Levey and Grajal, 1991). Following pulp processing, seeds are flushed quickly through the gut resulting in short retention time for seeds than fruit pulp (Levey and Duke, 1992). Australian silvereyes (*Zosterops lateralis*), when offered small and large seeds of similar volume; pass large seeds through the gut quicker than small seeds (Stanley and Lill, 2002a). As a result, they prefer large-seeded fruits when offered with small-seeded fruits (Stanley and Lill 2002a; b). Therefore fast passage rates of fruits in birds could be seen as an adaptive response to a bulky, nutrient-dilute diet (Levey and Karasov, 1994).

1.5.5. Fruit nutritional content

The interaction between frugivores and plants whose seeds they disperse is assumed to be based on the nutritional content of fruit (Izhaki, 1992). Fruits dispersed by frugivores are typically rich in carbohydrates but poor in lipids and protein (Herrera, 1987; Corlett, 1996; Witmer, 1998). McKey (1975) suggested that plants producing high investment (lipid-rich) fruits should have prolonged phenologies and be visited by a limited suite of specialist frugivores. On the other hand, low investment carbohydrate-rich fruits should be produced superabundantly and attract a diverse array of generalist frugivores (McKey, 1975; Howe and Estabrook, 1977). Despite the significant interest generated by the hypothesis in fruit-frugivore research, the majority of studies failed to find a relationship between fruit

nutritional content and frugivore preference patterns (Borowicz and Stephenson, 1985; Herrera, 1987; Martinez del Rio and Restrepo, 1993). This was the case even in preference tests conducted under controlled laboratory settings (Sorensen 1984; Johnson et al., 1985; McPherson, 1987).

The lack of a significant relationship between fruit nutritional traits and frugivore preferences has been attributed to two factors: the inappropriateness of the approach used to determine the fruit nutritional composition and the possibility that fruit selection patterns of frugivores may not be based on fruit nutritional content (Martinez del Rio and Restrepo, 1993). Most nutritional analyses reported in literature were based on the proximal nutrient analysis which analyses the nutritional content based on the fractional composition of digestible and indigestible cell contents in food (Karasov and Martinez del Rio, 2007). Although the approach is useful in nutritional analyses for herbivores, its applicability for frugivores is questionable because they do not depend on microbes but on specific enzymes and nutrient transport pathways to assimilate food (Alpers, 1987; Karasov and Martinez del Rio, 2007). Furthermore, the analysis is too coarse because it does not go beyond the broad nutrient classes (Martinez del Rio and Restrepo, 1993) but frugivores have been shown to select diets based on the specific composition of amino acids or lipids (Schaefer et al., 2003a; Pierce and McWilliams, 2005). Clearly, the lack of appropriate and fine-scale assessment of fruit nutritional composition combined with the fact that fruit size (Mack, 1993) and the ability of the frugivore in question to digest and assimilate nutrients can override fruit nutritional composition (Martinez del Rio et al., 1988; 1989; Levey and Martinez del Rio, 2001) complicates the search on how the fruit nutritional content affects fruit selection.

1.5.6 Sugar type

It has been generally agreed that the selection of a particular sugar should be related to an organism's ability to digest it (Lotz and Schondube, 2006). Pioneering work of Martinez del Rio and colleagues (Martinez del Rio et al., 1988; 1989; Martinez del Rio and Stevens, 1989; Martinez del Rio and Karasov, 1990; Martinez del Rio et al., 1992) has shown that certain species of birds show a complete lack of sucrase, the enzyme responsible for sucrose hydrolysis. As a result, birds lacking this enzyme show aversion to sucrose as it causes osmotic imbalance in the gut (Martinez del Rio et al., 1988; 1989). However, even species with significant sucrose activity prefer hexose over sucrose. For example cedar waxwings (*Bombycilla cedrorum*), which show a significant sucrase activity, prefer hexose solutions (Martinez del Rio et al., 1989). This has been attributed to the fact that sucrose hydrolysis is a two-step process, thus the fast passage rate observed in frugivores does not allow for sufficient exposure time of digesta to digestive enzymes (Martinez del Rio et al., 1992).

Earlier studies on sugar selection by birds suggested a hummingbird-passerine dichotomy based on the data by Baker and Baker (1983) on the sugar composition of nectar in a large sample of plants. According to this dichotomy, hummingbirds were assumed to possess high sucrase activity and thus expected to prefer sucrose-dominant nectar while passerines, due to a lack of sucrase, were expected to prefer hexose-dominant nectar (Baker and Baker, 1983; Martinez del Rio et al., 1992). However, subsequent studies have led to the abolition of this dichotomy as studies leading to its acceptance were shown to suffer from two main shortcomings: (1) they compared specialized nectarivorous non-passerines with generalized frugivorous/nectarivorous passerines and (2) they were restricted to American (New World) species (Franke et al., 1998; Jackson et al., 1998). It has been shown that specialized passerines display a near perfect (>97%) sucrose assimilation efficiency and as a

result, prefer sucrose solutions (Downs and Perrin, 1996; Lotz and Nicolson, 1996; Franke et al., 1998; Jackson et al., 1998; Brown et al., 2010). Thus the most important distinction is between generalized and specialized nectarivores (Johnson and Nicolson, 2008). The low sucrose concentrations in fruits dispersed by birds could be maintained by birds lacking sucrase because although they are taxonomically restricted, they are geographically widespread (Martinez del Rio et al., 1992).

1.5.7 Sugar concentration

Few studies have considered the role of sugar concentration on fruit selection in birds (Lotz and Schondube, 2006). Birds show a shift from hexose preference at low concentrations to sucrose preference or no preference at high concentrations (Lotz and Schondube, 2006). This pattern has been confirmed to be present in both hummingbirds and passerines (Schondube and Martinez del Rio, 2003). It has been proposed that sucrose solutions increase gastric emptying leading to enhanced energy delivery rate (Jackson et al., 1998; Schondube and Martinez del Rio, 2003). Furthermore, hexoses cause osmotic diarrhoea which dehydrates the gut (Lotz and Schondube, 2006), which explains their lack of preference at high concentrations. However, Napier et al. (2013) suggested that sucrase activity drives the concentration-dependent sugar preference patterns in birds. They showed that specialized nectarivores (which have high sucrase activity) tend to select hexose only in the most dilute solutions while occasional nectarivores (which have low sucrase activity) select hexose up to higher concentrations (Napier et al., 2013). Because sucrose hydrolysis may be a rate-limiting step at low sugar concentrations (Lotz and Schondube, 2006), this explains hexose preference at low concentrations (Fleming et al., 2004).

1.5.8 Secondary compounds

Secondary compounds are compounds with no functional role in plant development but may have secondary roles such as in defence (Beckman, 2013; Whitehead and Bowers, 2013). Fruit is laden with a number of secondary compounds (Barnea et al., 1993; Izhaki, 2002*b*; Izhaki et al., 2002; Cipollini and Levey, 1997*a, b, c*; Cipollini, 2000; Schaefer et al., 2003*b*). Unlike in leaves where consumption is uniformly detrimental, the role of secondary compounds in fruits is complex because fruit consumption can either be neutral, beneficial or detrimental depending on what the frugivore does with seeds after fruit consumption (i.e. whether they destroy or disperse seeds, Cipollini and Levey 1997*a*; Levey et al., 2006; 2007). Therefore it has been suggested that the primary function of secondary compounds is to reduce fruit consumption by organisms that eat fruits without dispersing the seeds or disperse seeds to unsuitable habitats (i.e. directed defence hypothesis, Cipollini and Levey, 1997*a*; Levey et al., 2006).

Secondary compounds have been found to be deterrent to a number of frugivores (Cipollini and Stiles, 1993; Cipollini and Levey, 1997*b, c*; Levey and Cipollini, 1998; Witmer, 2001; Tsahar et al., 2002). Secondary compounds such as tannins interfere with protein digestion which results in body mass loss in birds fed fruit-only diets (Izhaki and Safriel, 1989; Witmer, 1998; Witmer, 2001). It has been suggested that the ingestion of tannins creates an acidic load in the gut of birds which require acid buffering by the breakdown of amino acids to create bicarbonate (Guglielmo et al., 1996; Witmer, 2001). This increases nitrogen and energy requirements by 90% and 10–14% respectively (Guglielmo et al., 1996). Because of such substantial energetic costs associated with detoxification, behavioural adaptations have been suggested as the best way to prevent the negative effects of secondary compounds (but see Struempf et al., 1999). For example, it has been suggested that frugivorous birds forage on a number of fruit types during a foraging bout to prevent the

accumulation of a single secondary compound (Izhaki and Safriel, 1989; Mack, 1990; Levey and Martinez del Rio, 2001; Stanley and Lill, 2001). This also benefits plants by ensuring that seeds are dispersed widely (Cipollini, 2000; Izhaki, 2002*b*). Studies on secondary compounds are likely to contribute greatly to the understanding of fruit-frugivore interactions and dispersal systems as a whole as they did in the studies concerning plant-herbivore interactions, especially if studied at the intraspecific level which represents the scale at which natural selection operates (Izhaki, 2002*b*; Whitehead and Bowers, 2013).

1.5.9 Ethanol content/fruit ripeness

Ethanol is a natural by-product of the fermentation process of fruit sugars mainly by microorganisms, but also by the fruit itself (Dudley, 2004). Ethanol production in fruit occurred concurrently with the shift in dispersal mechanisms in angiosperms from small wind-dispersed seeds to larger, fleshy animal-dispersed fruits from the Cretaceous to the Palaeocene (Benner et al., 2002). This suggests a long historical association between frugivores and ethanol. Because ethanol is widespread in fruits, frugivores consume significant amounts of it (Levey, 2004; Sanchez et al., 2004). Generally, the ingestion of high ethanol diet exerts negative impacts. Although there are few published reports, countless cases of animals becoming inebriated and dying as a result of consuming significant amounts of alcohol under natural conditions have been reported (e.g. Fitzgerald et al., 1990; Milton, 2004; Kinde et al., 2012). However, the effects of ethanol are not completely deleterious. Ethanol represents a nutritional reward because its caloric value is nearly double that of a carbohydrate (Janzen, 1977; Dudley, 2000). Furthermore, alcohol is an appetitive stimulant in humans (Caton et al., 2004; Yeomans, 2004) and the same can be true in birds for alcohol at low concentrations (Sanchez et al., 2004). Due to the abovementioned benefits of ethanol, it has been suggested that frugivores should show a preference for fruits with higher (i.e. overripe) ethanol content (Dudley, 2002). Field and laboratory studies conducted so far seem

to be at odds with this hypothesis (Borowicz, 1988; Valburg, 1992; Cipollini and Stiles, 1993). A further inquiry in this line of research is a promising avenue for future research because it could reveal the complexities involved.

1.6 Frugivore traits affecting fruit selection

1.6.1 Gut structure

Because the mutualistic relationship between frugivores and plants is well established (Howe and Smallwood, 1982), conventional ecological wisdom predicts that frugivores should exhibit distinct gut morphological adaptations to frugivory. However, studies on morphological gut adaptations in frugivorous birds in general have produced inconsistent results which preclude any generalizations regarding morphological adaptations to frugivory or different types of fruits (Walsberg, 1975; Herrera, 1984; Jordano, 1987*b*; Karasov and Levey, 1990). As a consequence, it has been suggested that functional (i.e. relating to fruit processing) rather than morphological adaptations are probably more efficient and important in frugivores (Jordano 1987*b*; Levey and Duke, 1992).

Because sugars in fruits are simple and easily assimilated, fast passage rates have been considered to be a characteristic digestive traits of frugivores specialising on sugary fruits (Karasov and Levey, 1990). Furthermore, passive uptake of sugars through solvent drag (Levey and Cipollini, 1996) and fast intestinal glucose transport (Karasov and Levey, 1990) are thought to be features evolved to ensure high digestive efficiency of sugars despite fast passage rates of sugary fruits (Witmer, 1998; Witmer and van Soest, 1998). On the other hand, since lipids have to be emulsified before being digested and assimilated, frugivores specializing on lipid-rich fruits have been predicted to exhibit slow passage rates (Fuentes, 1994). Studies have indeed found that when birds are switched from fruits to insects or seeds (which are high in lipids), they predictably reduce their gut passage rates (Levey and

Karasov, 1992; 1994; Afik and Karasov, 1995). Therefore frugivores adjust passage rates to match the time required for the food of choice to be digested and assimilated (Levey and Karasov, 1992; 1994; Afik and Karasov, 1995; Witmer, 1998; Witmer and van Soest, 1998). Furthermore, the distribution of amino-peptidase-N activity in the gastro-intestinal tract of frugivores specializing on different types of fruits is different (Witmer and Martinez del Rio, 2001). In the cedar waxwing (*Bombycilla cedrorum*), its activity is highest in the distal part of intestine which suggests that protein digestion occurs in the lower part of the intestine to allow the recovery of precious protein from excreta (Witmer and Martinez del Rio, 2001). On the other hand, the distribution of the amino-peptidase-N activity in the thrushes (*Turdus* spp.) is higher in the proximal part of the intestine suggesting that protein digestion occurs in the duodenum where lipid digestion and emulsification occurs (Witmer and Martinez del Rio, 2001). Therefore waxwings and thrushes display contrasting digestive strategies to specialising on sugary and fatty fruits, respectively (Witmer and van Soest, 1998; Witmer and Martinez del Rio, 2001).

1.6.2 Ability to modulate digestive parameters

In response to seasonal changes in food availability and/or nutritional requirements, many birds switch to alternative food sources, significantly changing the nutrient composition of the diet (Levey and Karasov, 1989; 1992). For example, frugivorous birds increase the intake of lipid-rich fruits during pre-migratory fattening (Bairlein, 2002). In order for switching to be adaptive, the activities of digestive enzymes and transporters associated with the new food type must show corresponding increases which is in accordance with the adaptive modulation hypothesis (Karasov and Diamond, 1988; Levey and Karasov, 1992). Therefore the ability to modulate digestive enzymes to correspond to substrate level in diet can allow or constrain diet switching (Levey and Karasov, 1989; 1992; Ciminari et al., 2001; Levey and Martinez del Rio, 2001). The time scale required for the modulation of the

digestive machinery is also important. For example, when American robins (*Turdus migratorius*) and European starlings (*Sturnus vulgaris*) were switched from a fruit to an insect diet, digestive efficiency of the insect diet was low for the first two days following the switch and increased thereafter (Levey and Karasov, 1989). The lag in response suggests that both species incurred costs immediately after the switch which suggests that the switch should be gradual in order to be effective (Levey and Karasov, 1989). Therefore the most important factors to diet switching are what digestive traits are being modulated, how much are they being modulated and how much time is required for the modulation of the whole digestive machinery to take place (Levey and Martinez del Rio, 2001).

The preponderance of results from modulation of intestinal enzyme activity has shown that passerine birds fail to modulate intestinal carbohydrase activity to match the carbohydrate content in diet which is in stark contrast to amino-peptidases which closely match substrate levels (Martinez del Rio et al., 1995; Sabat et al., 1998; Caviedes-Vidal et al., 2000; Ciminari et al., 2001; Witmer and Martinez del Rio, 2001). Gut retention time, on the other hand, increases when birds are switched from a fruit diet to an insect diet (Karasov and Levey, 1990; Levey and Karasov, 1992; Afik and Karasov, 1995; but see Levey and Karasov, 1994). It is enigmatic why digestive enzymes and transporters in birds associated with carbohydrate digestion appear static while in mammals they show high plasticity (but see Sabat et al., 1999). Afik et al. (1997) proposed that frugivorous birds keep the digestive enzymes associated with protein digestion constant despite increases in carbohydrate content in the diet because protein is a limited resource to them and thus more important. Alternatively, Sabat et al. (1998) proposed that the discrepancy could be due to time scale required for the modulation of the whole digestive machinery to occur. A more convincing explanation is that the predominantly passive uptake of sugars through solvent drag observed in birds (e.g. Levey and Cipollini, 1996) allows automatic adjustment of sugar uptake to

match substrate level and since it is energetically inexpensive, it precludes modulation of nutrient transporters (Levey and Martinez del Rio, 2001). The disadvantage of this approach is that it is unselective which can result in the absorption of toxins (Levey and Cipollini, 1996; Levey and Martinez del Rio, 2001).

1.6.3 Degree of frugivory

Because no bird is completely frugivorous (Izhaki and Safriel, 1989; Jordano, 2000), birds are distributed along a continuum from highly frugivorous to less frugivorous. As a corollary, birds could be expected to exhibit different levels of adaptation to fruits which could be expected to affect their fruit selection patterns. McKey (1975) divided frugivorous birds into specialists and generalists. Specialist frugivores were considered to be those that possess gut adaptations to fruits such as short guts allowing passage of seeds in an intact manner along the gastrointestinal tract (McKey, 1975; Snow, 1981). Generalists, on the other hand, were those species that lacked such gut adaptations (McKey, 1975; Snow, 1981). The specialist-generalist dichotomy has however received a lot of criticism and is considered to be an inaccurate classification as it provided no clear consensus on what constitutes a specialist or a generalist which is understandable as the term tries to fit into one group species with diverse histories and biology (Wheelwright and Orians, 1982; Wheelwright, 1983; Moermond and Denslow, 1985).

In a study of four species of passerines with different levels of frugivory, passage rate was the only parameter that correlated with the degree of frugivory (Karasov and Levey, 1990). That highly frugivorous have fast passage rates has been found in a number of studies and suggest fast passage rates are an important adaptation to strong frugivory (Herrera, 1984; Jordano, 1987*b*; Levey and Karasov, 1992; 1994; Afik and Karasov, 1995). As a result of their fast passage rates, highly frugivorous species select carbohydrate-rich fruits as these

contain easily assimilated sugars while on the other hand, less frugivorous species (omnivores) select lipid-rich fruits because their slow passage rates are suitable for lipid digestion which requires a huge amount of time because of the many steps involved (Levey and Karasov, 1989; 1992; 1994; Fuentes, 1994; Afik and Karasov, 1995; Witmer and van Soest, 1998). Furthermore, highly frugivorous species have high sugar digestive efficiencies and high intestinal glucose uptake (Karasov and Levey, 1990; Levey and Karasov, 1994). Lastly, highly frugivorous birds have incredibly low nitrogen requirements and thus maintain body mass on fruit-only diets whereas in omnivores, a high proportion of insects in the diet is required to achieve the same (Levey and Karasov, 1989; Witmer, 1998; Pryor et al., 2001; Witmer, 2001). As a result, facultative frugivores display abilities to digest both fruits and insects (Herrera, 1984; Jordano, 1987*b*; Levey and Karasov, 1989; Place and Stiles, 1992) and feed to maximize energy gain rather than digestive efficiency (Worthington, 1989; Brown and Downs, 2003).

1.6.4 Tasting ability

For frugivores, the ability to determine subtle differences in fruit by taste is importance for maximizing energy input since fruit is considered to be a nutrient-dilute resource, offering a limited amount of nutrients (Witmer, 1998). Fast passage rates, absence of mastication in the buccal cavity and lack of mixing of saliva with food are some of the factors that are thought to render frugivorous birds to display low taste acuity (Klasing, 1998). However, studies have shown that frugivorous birds display tremendous tasting abilities. For example, Levey (1987*b*) showed that blue-grey tanagers (*Thraupis episcopus*) can detect differences in concentrations of 2% for sugars. In another study, Bosque and Calchi (2003) showed that for proteins, blue-grey tanagers can detect dietary differences as low as 0.09% fresh weight. However, Levey (1987*b*) proposed that interspecific differences in tasting ability could be related to their fruit-handling techniques. For example, he

attributed the finding of sugar-tasting abilities in tanagers (Thraupidae) to the fact that they crush fruits in their bills, making pulp juices come into contact with sensory organs, which is in direct contrast to Manakins (Pipridae) (Levey, 1987*b*). Nevertheless, such fine-scale discriminating abilities reported in the above studies suggest that taste sensitivity in birds could play a bigger role than currently appreciated.

1.6.5 Olfactory abilities

The importance of olfaction in birds has been one of the most controversial issues in ecology for several decades (Roper, 2003; Steiger et al., 2008). It was suggested that birds have comparatively small olfactory bulbs and thus olfaction is negligible compared to other vertebrate taxa (Duncan, 1960; Clark et al., 1993; Rajchard, 2008). An opposing view was that birds have well-developed olfactory bulbs which are used for a variety of purposes such as food acquisition, predator detection and orientation (Steiger et al., 2008; LeClaire et al., 2009). In support of the latter view, Steiger et al., (2008), who tested for the presence of olfactory receptor genes on nine avian species from seven orders, found that all had functional genes for olfactory ability. Therefore, the use of olfaction in birds might be more prevalent than previously thought (Roper, 2003). Despite the huge research effort on olfactory abilities in birds, there is a lack of studies that have tested the olfactory abilities of frugivores which is of particular interest since fruit is a spatially variable resource especially in tropical ecosystems where efficient foraging requires tremendous travel (Jordano 2000; Milton 2004).

1.7. Problem statement

Fruit selection is the first step in fruit removal and thus important for the seed dispersal process. Fruit selection in frugivorous birds is affected by a number of factors which are both intrinsic and extrinsic to fruits (see the Introduction). While some studies have

tried to relate fruit selection patterns to the nutritional value of fruits (Izhaki, 1992; Bosque and Calchi, 2003; Schaefer et al., 2003*b*), others have related it to the energy content of fruits (Sorensen, 1984; Johnson et al., 1985; McPherson, 1987; Lepczyk et al., 2000). However, results have been inconsistent (Izhaki and Safriel, 1989; Martinez del Rio and Restrepo, 1993) with a huge variation in fruit selection patterns between species and within individuals of the same species (Johnson et al., 1985; McPherson, 1987; Willson, 1994; Levey and Martinez del Rio, 2001). The huge inter- and intra-specific variation in fruit selection patterns precludes any generalizations on fruit selection notwithstanding the research effort addressing the issue (Levey and Martinez del Rio, 2001). One factor that could be attributable to this pattern is that most studies on fruit selection in frugivorous birds have studied fruit attributes or physiological adaptations of frugivores to fruits in isolation (Martinez del Rio and Restrepo, 1993; Levey and Martinez del Rio, 2001). Since fruit profitability depends on both pre- and post-ingestional factors (Martin, 1985*b*; Bozinovic and Martinez del Rio, 1996), studying these factors in isolation deprives us of fresh insights into the fruit-frugivore interaction. Therefore by considering simultaneously the pre- and post-ingestional factors affecting fruit selection, this study aims to provide new insights into the fruit-frugivore interaction.

1.8 Aims and objectives

The overall aim of this study is to obtain a better understanding of factors affecting fruit selection in frugivorous birds and how these eventually impact on the seed dispersal process. Studies of this kind are rare in the southern African region but are important because similar to other tropical and subtropical regions, the fruit-frugivore interaction is an important driver of ecosystem processes (Jordano, 2000; Herrera, 2002) and also because the large-scale destruction and fragmentation of forests taking place is threatening the integrity of these fragile ecosystems. Therefore it is hoped that this study will provide information that will be

useful in the formulation of conservation strategies for the region. The objectives of this study were to:

1. Determine the effects of sugar type and concentration on digestive abilities in birds;
2. Determine the deterrent effects of tannins in fruits on fruit selection in birds;
3. Determine the effect of ethanol concentration on fruit selection in birds; and
4. Determine how tasting and olfactory abilities affect fruit selection patterns in a frugivorous bird.

It was hypothesized that: (1) Sugar type and concentration affects digestion in birds which affects fruit selection patterns; (2) Tannins have no effect on fruit selection at low concentrations but act as deterrents at high concentrations; (3) Ethanol act as a foraging cue and thus fruits with high ethanol concentrations should be selected for by frugivorous birds and (4) Frugivorous birds have tasting and olfactory abilities allowing them to make foraging decisions.

1.9 Study species

Three frugivorous birds were selected for this study and these were: Cape white-eyes (*Zosterops virens*, Fig. 1a), red-winged starlings (*Onychognathus morio*, Fig. 1b), and speckled mousebirds (*Colius striatus*, Fig. 1c). Mousebirds are non-passerines (Order Coliiformes) belonging to the family Coliidae whereas starlings and white-eyes are passerines (Order Passeriformes) belonging to families Sturnidae and Zosteropidae, respectively. These families belong to different and unrelated clades in the passerine phylogenetic tree (Sibley and Ahlquist, 1990). Several factors justified the choice of these species. Firstly, these species are some of the most numerous frugivorous birds in South Africa especially along the eastern coastline (Hockey et al., 2005), and thus their fruit

selection patterns could be expected to have a major impact on their environment through their seed dispersing activities. Secondly, these species are on a continuum in body mass, ranging from small (Cape white-eye, body mass = 10.52 ± 0.31 g), through medium (speckled mousebird, body mass = 50.14 ± 3.12 g) to large (red-winged starling, body mass = 118.61 ± 6.54 g). Therefore studying these birds simultaneously allows generalising the results to a much broader assemblage of frugivorous birds. Thirdly, these species, although are broadly categorized as frugivores, have varied diets consuming fruits, nectar, insects and leaves (Downs et al., 2000; Brown and Downs, 2003; Brown et al., 2009; Brown et al., 2012). Because these food items are consumed in different proportions, findings from this study are expected to provide an indirect indication of how diet affects bird response to particular properties of the diet. Red-winged starlings are adaptable feeders with a predominant fruit diet but consume substantial amounts of insects and nectar (Brown et al., 2012). Mousebirds have an omnivorous diet including fruits, insects, nectar and leaves; however, these birds can become completely folivorous during periods of fruit scarcity (Downs et al., 2000). White-eyes are probably one of the most generalist feeders, consuming almost equal proportions of fruits, arthropods and nectar (Brown and Downs, 2003). Because traits of natural fruits vary and covary intractably, artificial fruits were used throughout this study because they allow the researcher to vary only the fruit characteristic of choice, eliminating the confounding effects of covariance among fruit characters (Levey and Grajal, 1991; Lepczyk et al., 2000). However, a problem with experimental studies is that their relevance to the field conditions is questionable. This makes extrapolating from laboratory results risky. Nevertheless, experimental studies are important as a first step in unveiling the intricacies of any system by revealing how factors relate to each other under specific conditions.

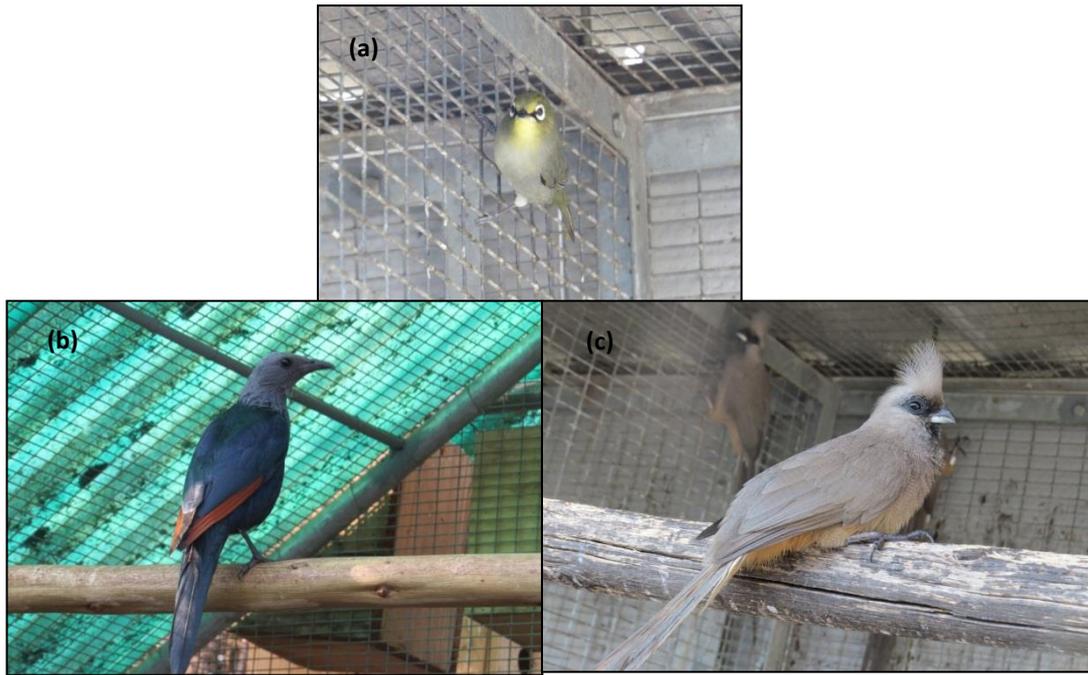


Fig. 1. Frugivorous birds used in this study: (a) Cape white-eyes (*Zosterops virens*), (b) red-winged starlings (*Onychognathus morio*) and (c) speckled mousebirds (*Colius striatus*).

1.10 Thesis structure

The main body of this thesis is organized as manuscripts prepared for publication in peer-reviewed journal articles. The first chapter (Chapter 1) of this thesis is the Introduction which provides the literature review of the concepts covered in this study. The next four chapters (Chapter 2, 3, 4 & 5) are experimental chapters with each one covering a specific objective. Therefore each of these chapters is arranged typically of a manuscript submitted to peer-reviewed journal articles starting with the abstract and ending with tables and figures. Each chapter is formatted according to the journal it is intended to be submitted to. Because of this thesis format, a certain level of repetition especially in the methods section was unavoidable. However, this is deemed to be of little concern as this format allows the reader to read each chapter separately without losing the overall context of the thesis. Chapter 2 investigates the effect of sugar type and concentration on fruit selection. Chapter 3 investigates the deterrent effects of tannins in fruits and how this affects fruit selection by

birds. Chapter 4 is focused on testing how ethanol concentration affects fruit selection. Chapter 5 investigates whether the ability to taste and smell in birds affects their fruit selection patterns. The final chapter (Chapter 6) discusses the main findings of the study and their implications and suggests possible avenues for future research.

References

- Afik, D., Darken, B.W., Karasov, W.H., 1997. Is diet shifting facilitated by modulation of intestinal nutrient uptake? Test of an adaptational hypothesis in yellow-rumped warblers. *Physiological Zoology* 70, 213 – 221.
- Afik, D., Karasov, W.H., 1995. The trade-offs between digestive efficiency and in warblers and their ecological implications. *Ecology* 76, 2247 – 2257.
- Alcantara, J.M., Rey, P.J., 2003. Conflicting selection pressures on seed size: evolutionary ecology of fruit size in a bird-dispersed tree, *Olea europaea*. *Journal of Evolutionary Biology* 16, 1168 – 1176.
- Alpers, D.H., 1987. Digestion and absorption of carbohydrates and proteins. In: Johnson, R., (Ed). *Physiology of the gastrointestinal tract*. Volume 2. Raven, New York.
- Armesto, J.J., Rozzi, R., 1989. Seed dispersal syndromes in the rain forest of Chile: evidence for the importance of biotic dispersal in a temperate rain forest. *Journal of Biogeography* 16, 219 – 226.
- Armesto, J.J., Rozzi, R., Miranda, P., Sabag, C., 1987. Plant-frugivore interactions in South American temperate forests. *Revista Chilena de Historia Natural* 60, 321 – 336.
- Augspurger, C.K., 1983. Seed dispersal of the tropical tree, *Platypodium Elegans*, and the escape of its seedlings from fungal pathogens. *Journal of Ecology* 71, 759 – 771.

- Augsburger, C.K., 1984. Seedling survival of tropical tree species: interactions of dispersal distance, light-gaps, and pathogens. *Ecology* 65, 1705 – 1712.
- Avery, M.L., Goocher, K.J., Cone, M.A., 1993. Handling efficiency and berry size preferences of cedar waxwings. *Wilson Bulletin* 105, 604 – 611.
- Bairlein, F., 2002. How to get fat: nutritional mechanisms of seasonal fat accumulation in migratory songbirds. *Naturwissenschaften* 89, 1 – 10.
- Baker, H.G., Baker, I., 1983. Floral nectar sugar constituents in relation to pollinator type. In: Jones, C.E., Little, R.J., (Eds). *Handbook of experimental pollination ecology*. Scientific and Academic Editions, New York.
- Barnea, A., Harborne, J.B., Pannell, C., 1993. What parts of fleshy fruits contain secondary compounds and why? *Biochemical Systematics and Ecology* 21, 421 – 429.
- Bascompte, J., Jordano, P., 2006. The structure of plant-animal mutualistic networks. In: Pascual, M., Dunne, J., (eds). *Ecological networks*. Oxford University Press, Oxford.
- Beckman, N.G., 2013. The distribution of fruit and seed toxicity during development for eleven tropical trees and vines in central Panama. *PLoS ONE* 8, 1 – 19.
- Benner, S. A., Caraco, M. D., Thomson, J. M., Gaucher E. A., 2002. Planetary biology-paleontological, geological, and molecular histories of life. *Science* 296, 864–868.
- Binggeli, P., 1996. A taxonomic, biogeographical and ecological overview of invasive woody plants. *Journal of Vegetation Science* 7, 121 – 124.
- Blendinger, P.G., Loiselle, B.A., Blake, J.G., 2008. Crop size, plant aggregation, and microhabitat type affect fruit removal by birds from individual melastome plants in the Upper Amazon. *Oecologia* 158, 273 – 283.

- Borowicz, V.A., 1988. Do frugivores avoid decaying fruits? An experimental test with *Cornus amomum* fruits. *Oikos* 53, 74 – 78.
- Borowicz, V.A., Stephenson, A.G., 1985. Fruit composition and patterns of fruit dispersal of two *Cornus* spp. *Oecologia* 67, 435 – 441.
- Bosque, C., Calchi, R., 2003. Food choice by blue-grey tanagers in relation to protein content. *Comparative Biochemistry and Physiology A* 135, 321 – 327.
- Bozinovic, F., Martinez del Rio, C., 1996. Animals eat what they should not: why do they reject our foraging models? *Revista Chilena de Historia Natural* 69, 15 – 20.
- Brown, K.J., Downs, C.T., 2003. Digestive efficiency of a generalist avian feeder, the Cape white-eye (*Zosterops pallidus*). *Comparative Biochemistry and Physiology A* 134, 739 – 748.
- Brown, M., Downs, C.T., Johnson, S.D., 2010. Concentration-dependent sugar preferences of the malachite sunbird (*Nectarinia famosa*). *Auk* 127, 151 – 155.
- Brown, M., Downs, C.T., Johnson, S.D., 2009. Pollination of the red hot poker *Kniphofia caulescens* by short-billed opportunistic avian nectarivores.
- Brown, M., Johnson, S.D., C.T. Downs. 2012. African red-winged starlings prefer hexose solutions, but do not like them too sweet. *Journal of Ornithology* 153, 265 – 272.
- Caton, S.J., Ball, M., Hetherington, M.M., 2004. Dose-dependent effects of alcohol on appetite and food intake. *Physiology and Behaviour* 81, 51 – 58.
- Caviedes-Vidal, E., Afik, D., Martinez del Rio, C., Karasov, W.H., 2000. Dietary modulation of intestinal enzymes of the house sparrow (*Passer domesticus*): testing an adaptive hypothesis. *Comparative Biochemistry and Physiology A* 125, 11 – 24.

- Cazetta, E., Schaefer, H.M., Galetti, M., 2009. Why are fruits colourful? The relative importance of achromatic and chromatic contrasts for detection by birds. *Evolutionary Ecology* 23, 233 – 244.
- Ciminari, M.E., Afik, D., Karasov, W.H., Caviedes-Vidal, E., 2001. Is diet-shifting facilitated by modulation of pancreatic enzymes? Test of an adaptational hypothesis in yellow-rumped warblers. *Auk* 118, 1101 – 1107.
- Cipollini, M.L., 2000. Secondary metabolites of vertebrate-dispersed fruits: evidence for adaptive functions. *Revista Chilena de Historia Natural* 73, 421 – 440.
- Cipollini, M.L., Levey, D.J., 1997a. Antifungal activity of *Solanum* fruit glycoalkaloids: implications for frugivory and seed dispersal. *Ecology* 78, 799 – 809.
- Cipollini, M.L., Levey, D.J., 1997b. Secondary metabolites of fleshy vertebrate-dispersed fruits: adaptive hypotheses and implications for seed dispersal. *American Naturalist* 150, 346 – 372.
- Cipollini, M.L., Levey, D.J., 1997c. Why are some fruits toxic? Glycoalkaloids in *Solanum* and fruit choice by vertebrates. *Ecology* 78, 782 – 798.
- Cipollini, M.L., Stiles, E.W., 1993. Fruit rot, antifungal defense and palatability of fleshy fruits for frugivorous birds. *Ecology* 74, 751 – 762.
- Clark, A., Clark, D.B., 1984. Spacing dynamics of a tropical rainforest tree: evaluating the Janzen-Connell model. *American Naturalist* 124, 769 – 788.
- Clark, L., Avilova, K.V., Bean, N.J., 1993. Odour thresholds in passerines. *Comparative Biochemistry and Physiology A* 104, 305 – 312.
- Corlett, R.T., 1996. Characteristics of vertebrate-dispersed fruits in Hong Kong. *Journal of Tropical Ecology* 12, 819 – 833.
- Courtney, S.P., Sallabanks, R., 1992. It takes guts to handle fruits. *Oikos* 65, 163 – 166.

- Downs, C.T., Perrin, M.R., 1996. Sugar preferences of some southern African nectarivorous birds. *Ibis* 138, 455 – 459.
- Downs, C.T., Wirminghaus, J.O., M.J. Lawes. 2000. Anatomical and nutritional adaptations of the speckled mousebird *Colius striatus*. *Auk* 117: 791 – 794.
- Dudley, R., 2000. Evolutionary origins of human alcoholism in primate frugivory. *The Quarterly Review of Biology* 75, 3 – 15.
- Dudley, R., 2002. Fermenting fruit and the historical ecology of ethanol ingestion: is alcoholism in modern humans an evolutionary hangover? *Addiction* 97, 381 – 388.
- Dudley, R., 2004. Ethanol, fruit ripening, and the historical origin of human alcoholism in primate frugivory. *Integrated Comparative Biology* 44, 315 – 323.
- Duncan, C.J., 1960. Preference tests and the sense of taste in the feral pigeon (*Columbia livia* var *Gmelin*). *Animal Behaviour* 111, 55 – 61.
- Ellis, J.C., 2005. Marine birds on land: a review of plant biomass, species richness, and community composition in seabird colonies. *Plant Ecology* 181, 227 – 241.
- Fitzgerald, S.D., Sullivan, J.M., Everson, R.J., 1990. Suspected ethanol toxicosis in two wild cedar waxwings. *Avian Diseases* 34, 488 – 490.
- Fleming, P.A., Bakken, B.H., Lotz, C.N., Nicolson, S.W., 2004. Concentration and temperature effects on sugar intake and preferences in a sunbird and hummingbird. *Functional Ecology* 18, 222 – 232.
- Fleming, T.H., Kress, W.J., 2011. A brief history of fruits and frugivores. *Acta Oecologica* 37, 521 – 530.
- Fragoso, J.M.V., Silviu, K.M.,Correa, J.A., 2003. Long-distance seed dispersal by tapirs increases seed survival and aggregates tropical trees. *Ecology* 84, 1998 – 2006.

- Franke, E., Jackson, S., Nicolson, S., 1998. Nectar sugar preferences and absorption in a generalist avian frugivore, the Cape white-eye *Zosterops pallidus*. *Ibis* 140, 501 – 506.
- Fuentes, M., 1994. Diets of fruit-eating birds; what are the causes of interspecific differences? *Oecologia* 97, 134 – 142.
- Galetti, M., Keuroghlian, A., Hanada, L., Morato, M.I., 2001. Frugivory and seed dispersal by the lowland tapir (*Tapirus terrestris*) in Southeastern Brazil. *Biotropica* 33, 723 – 726.
- Garcia, D., Zamora, R., Amico, G.C., 2010. Ecosystems: conservation guidelines from real-world landscapes. *Conservation Biology* 24, 1070 – 1079.
- Guglielmo, C.G., Karasov, W.H., Jakubas, W.J., 1996. Nutritional costs of a plant secondary metabolite explain selective foraging by ruffed grouse. *Ecology* 77, 1103 – 1115.
- Herrera, C.M., 1984. Adaptation to frugivory in Mediterranean avian seed dispersers. *Ecology* 65, 609 – 617.
- Herrera, C.M., 1985a. Determinants of plant-animal coevolution: the case of mutualistic dispersal of seeds by vertebrates. *Oikos* 44, 132 – 141.
- Herrera, C.M., 1985b. Habitat-consumer interactions in frugivorous birds. In: Cody, M.L., (Ed.). *Habitat selection in birds*. Academic Press, New York.
- Herrera, C.M., 1986. Vertebrate-dispersed plants: why they do not behave the way they should. In: Estrada, A., Fleming, T.H., (eds). *Frugivores and seed dispersal*. Dr W. Junk Publishers, Dordrecht, Netherlands.
- Herrera, C.M., 1987. Vertebrate-dispersed plants of the Iberian Peninsula: a study of fruit characteristics. *Ecological Monographs* 57, 305 – 331.

- Herrera, C.M., 1998. Long-term dynamics of Mediterranean frugivorous birds and fleshy fruits: a 12-year study. *Ecological Monographs* 68, 511 – 538.
- Herrera, C.M., 2002. Seed dispersal by vertebrates. In: Herrera, C.M., Pellmyr, O., (eds). *Plant-animal interactions: an evolutionary approach*. Blackwell Science.
- Herrera, C.M., Jordano, P., Lopez-Soria, L., Amat, J.A., 1994. Recruitment of a mast-fruited bird-dispersed tree: bridging frugivore activity and seedling establishment. *Ecological Monographs* 64, 315 – 344.
- Hockey, P.A.R., Dean, W.R.J., Ryan, P.G., 2005. Robert's birds of southern Africa. 7th Edition. The Trustees of the John Voelcker Bird Book Fund, Cape Town.
- Holbrook, K.M., Smith, S.B., 2000. Seed dispersal and movement patterns of two species of *Ceratogymna* hornbills in a West African tropical lowland forest. *Oecologia* 125, 249 – 257.
- Holbrook, K.M., Smith, T.B., Hardesty, B.D., 2002. Implications of long-distance movement of frugivorous forest hornbills. *Ecography* 25, 745 – 749.
- Honkavaara, J., Siitari, H., Viitala, J., 2004. Fruit colour preferences of redwings (*Turdus iliacus*): experiments with hand-raised juveniles and wild-caught adults. *Ethology* 110, 445 – 457.
- Howe, H.F., 1977. Bird activity and seed dispersal of a tropical wet forest tree. *Ecology* 58, 539 – 550.
- Howe, H.F., 1984a. Constraints on the evolution of mutualisms. *American Naturalist* 123, 764 – 777.
- Howe, H.F., 1984b. Implications of seed dispersal by animals for tropical reserve management. *Biological Conservation* 30, 261 – 281.

- Howe, H.F., DeSteven, D., 1979. Fruit production, migrant bird visitation and seed dispersal of *Guarea glabra* in Panama. *Oecologia* 39, 185 – 196.
- Howe, H.F., Estabrook, G.F., 1977. On intraspecific competition for avian dispersers in tropical trees. *American Naturalist* 111, 817 – 832.
- Howe, H.F., Smallwood, J., 1982. Ecology of seed dispersal. *Annual Reviews of Ecology and Systematics* 13, 201 – 228.
- Howe, H.F., Vande Kerckhove, G.A., 1981. Removal of wild nutmeg (*Virola surinamensis*) crops by birds. *Ecology* 62, 1093 – 1106.
- Izhaki, I., 1992. A comparative analysis of the nutritional quality of mixed and exclusive fruit diets for yellow-vented bulbuls. *Condor* 94, 912 – 923.
- Izhaki, I., 2002a. The role of fruit traits in determining fruit removal in East Mediterranean Ecosystems. In: Levey, D.J., Silva, W.R., Galetti, M., 2002. Seed dispersal and frugivory: ecology, evolution and conservation. CAB International, UK.
- Izhaki, I., 2002b. Emodin – a secondary metabolite with multiple ecological functions in higher plants. *New Phytologist* 155, 205 – 217.
- Izhaki, I., Safriel, U.N., 1989. Why are there so few exclusively frugivorous birds? Experiments on fruit digestibility. *Oikos* 54, 23 – 32.
- Izhaki, I., Tsahar, E., Paluy, I., Friedman, J., 2002. Within population variation and interrelationships between morphology, nutritional content and secondary compounds of *Rhamnus alaternus* fruits. *New Phytologist* 156, 217 – 223.
- Jackson, S., Nicolson, S.W., Lotz, C.N., 1998. Sugar preferences and “side bias” in Cape sugarbirds and lesser double-collared sunbirds. *Auk* 115, 156 – 165.

- Janzen, D.H., 1977. Why fruits rot, seeds mold, and meat spoils. *American Naturalist* 111, 691 – 713.
- Johnson, R.A., Willson, M.F., Thompson, J.N., Bertin, R.I., 1985. Nutritional values of wild fruits and consumption by migrant frugivorous birds. *Ecology* 66, 819 – 827.
- Johnson, S.D., Nicolson, S.W., 2008. Evolutionary associations between nectar properties and specificity in bird pollination systems. *Biology Letters* 4, 49 – 52.
- Jordano, P., 1987a. Patterns of mutualistic interactions in pollination and seed dispersal: connectance, dependence asymmetries and coevolution. *American Naturalist* 129, 657 – 677.
- Jordano, P., 1987b. Frugivory, external morphology and digestive system in Mediterranean sylviid warblers *Sylvia spp.* *Ibis* 129, 175 – 189.
- Jordano, P., 1995a. Frugivore-mediated selection on fruit and seed size: bird and St. Lucie's cherry, *Prunus mahaleb*. *Ecology* 76, 2627 – 2639.
- Jordano, P., 1995b. Angiosperm fleshy fruits and seed dispersers: a comparative analysis of adaptation and constraints in plant-animal interactions. *American Naturalist* 145, 163 – 191.
- Jordano, P., 2000. Fruits and frugivory. In: Fenner, M., (ed). *Seeds: the ecology and regeneration in plant communities*. Second Edition. CABI Publications, Wallingford, UK.
- Jordano, P., Bascompte, J., Olesen, J.M., 2003. Invariant properties in coevolutionary networks of plant-animal interactions. *Ecology Letters* 6, 69 – 81.
- Karasov, W.H., Diamond, J.M., 1988. The interplay between physiology and ecology in digestion. *Bioscience* 38, 602 – 611.

- Karasov, W.H., Levey, D.J., 1990. Digestive system trade-offs and adaptations of frugivorous passerine birds. *Physiological Zoology* 63, 1248 – 1270.
- Karasov, W.H., Martinez del Rio, C., 2007. *Physiological ecology: how animals process energy, nutrients and toxins*. Princeton University Press, Princeton.
- Kinde, H., Foate, E., Beeler, E., Uzal, F., Moore, J., Poppenga, R., 2012. Strong circumstantial evidence for ethanol toxicosis in cedar waxwings (*Bombycilla cedrorum*). *Journal of Ornithology* 153, 995 – 998.
- Kitamura, S., 2011. Seed dispersal by hornbills (Bucerotidae) in tropical forests. *Acta Oecologia* 37, 531 – 541.
- Klasing, K.C., 1998. *Comparative avian nutrition*. Oxford University Press, Oxford, New York.
- Knight, R.S., Siegfried, W.R., 1983. Inter-relationships between type, size and colour of fruits and dispersal in southern African trees. *Oecologia* 56, 405 – 412.
- Laska, M.S., Stiles, E.W., 1994. Effect of fruit crop size on intensity of fruit removal in *Viburnum prunifolium* (Caprifoliaceae). *Oikos* 69, 199 – 202.
- LeClaire, S., Mulard, H., Wagner, R.H., Hatch, S.A., Danchin, E., 2009. Can Kittiwakes smell? Experimental evidence in a Larid species. *Ibis* 151, 584 – 587.
- Lenz, J., Fiedler, W., Caprano, T., Friedrichs, W., Gaese, B.H., Wikelski, M., Bohning-Gaese, K., 2011. Seed-dispersal distributions by trumpeter hornbills in fragmented landscapes. *Proceedings of the Royal Society B* 278, 2257 – 2264.

- Lepczyk, C.A., Murray, K.G., Winnett-Murray, K., Bartell, P., Geyer, E., Work, T., 2000. Seasonal fruit preferences for sugar and lipids by American robins. *Auk* 117, 709 – 717.
- Levey, D.J., 1987a. Seed size and fruit-handling techniques of avian frugivores. *American Naturalist* 129, 471 – 485.
- Levey, D.J., 1987b. Sugar-tasting ability and fruit selection in tropical fruit-eating birds. *Auk* 104, 173 – 179.
- Levey, D.J., 2004. The evolutionary ecology of ethanol production and alcoholism. *Integrated Comparative Biology* 44, 284 – 289.
- Levey, D.J., Benkman, C.W., 1999. Fruit-disperser interactions: timely insights from a long-term perspective. *Trends in Ecology and Evolution* 14, 41 – 43.
- Levey, D.J., Cipollini, M.L., 1996. Is most glucose absorbed passively in northern bobwhite? *Comparative Biochemistry and Physiology A* 113, 225 – 231.
- Levey, D.J., Cipollini, M.L., 1998. A glycoalkaloid in ripe fruits deters consumption by cedar waxwings. *Auk* 115, 359 – 367.
- Levey, D.J., Duke, G.E., 1992. How do frugivores process fruits? Gastrointestinal transit and glucose absorption in cedar waxwings (*Bombycilla cedrorum*). *Auk* 109, 722 – 730.
- Levey, D.J., Grajal, A., 1991. Evolutionary implications of fruit-processing limitations in cedar waxwings. *American Naturalist* 138, 171 – 189.
- Levey, D.J., Karasov, W.H., 1989. Digestive responses of temperate birds switched to fruit or insect diets. *Auk* 106, 675 – 686.

- Levey, D.J., Karasov, W.H., 1992. Digestive modulation in a seasonal frugivore, the American robin (*Turdus migratorius*). *American Journal of Physiology* 262, 711 – 718.
- Levey, D.J., Karasov, W.H., 1994. Gut passage of insects by European starlings and comparison with other species. *Auk* 111, 478 – 481.
- Levey, D.J., Martinez del Rio, C., 2001. It takes guts (and more) to eat fruits: lessons from avian nutritional ecology. *Auk* 118, 819 – 831.
- Levey, D.J., Tewksbury, J.J., Cipollini, M.L., Carlo, T.A., 2006. A field test of the directed deterrence hypothesis in two species of wild chilli. *Oecologia* 150, 61 – 68.
- Levey, D.J., Tewksbury, J.J., Izhaki, I., Tsahar, E., Haak, D.C., 2007. Evolutionary ecology of secondary compounds in ripe fruits: case studies with capsaicin and emodin. In: Dennis, A.J., (ed). *Seed dispersal: theory and its application in a changing world*. CABI Publications, Wallingford, UK.
- Levin, S.A., Muller-Landau, H.C., Nathan, R., Chave, J., 2003. The ecology and evolution of seed dispersal: a theoretical perspective. *Annual Review of Ecology, Evolution and Systematics* 34, 575 – 604.
- Link, A., Di Fiore, A., 2006. Seed dispersal by spider monkeys and its importance in the maintenance of Neotropical rainforest diversity. *Journal of Tropical Ecology* 22, 234 – 246.
- Lotz, C.N., Schondube, J.E., 2006. Sugar preferences in nectar- and fruit-eating birds: behavioural patterns and physiological causes. *Biotropica* 38, 3 – 15.
- Mack, A.L., 1990. Is frugivory limited by secondary compounds in fruits? *Oikos* 57, 135 – 138.

- Mack, A.L., 1993. The size of vertebrate-dispersed fruits: a Neotropical-paleotropical comparison. *American Naturalist* 142, 840 – 856.
- Magnusson, B., Magnusson, S., Fridrikkson, S., 2009. Developments in plant colonisation and succession on Surtsey during 1999 – 2008. *Surtsey Research* 12, 57 – 76.
- Martin, T.E., 1985a. Selection of second-growth woodlands by frugivorous migrating birds in Panama: an effect of fruit size and plant density? *Journal of Tropical Ecology* 1, 157 – 170.
- Martin, T.E., 1985b. Resource selection by tropical frugivorous birds: integrating multiple interactions. *Oecologia* 66, 563 - 573.
- Martinez del Rio, C., Baker, H.G., Baker, I., 1992. Ecological and evolutionary implications of digestive processes: bird preferences and the sugar constituents of floral nectar and fruit pulp. *Experientia* 48, 544 – 551.
- Martinez del Rio, C., Brugger, K.E., Rios, J.L., Vergara, M.E., Witmer, M., 1995. An experimental and comparative study of dietary modulation of intestinal enzymes in European starlings (*Sturnus vulgaris*). *Physiological Zoology* 68, 490 – 511.
- Martinez del Rio, C., Karasov, W.H., 1990. Digestive strategies of nectar- and fruit-eating birds and the sugar composition of plant rewards. *American Naturalist* 136, 618 – 637.
- Martinez del Rio, C., Karasov, W.H., Levey, D.J., 1989. Physiological bases and ecological consequences of sugar preferences in cedar waxwings. *Auk* 106, 64 – 71.
- Martinez del Rio, C., Restrepo, C., 1993. Ecological and behavioural consequences of digestion in frugivorous birds. *Vegetatio* 107/108, 205 – 216.

- Martinez del Rio, C., Stevens, B.R., 1989. Physiological constraint on feeding behavior: intestinal membrane disaccharidases of the starling. *Science* 243, 794 – 796.
- Martinez del Rio, C., Stevens, B.R., Daneke, D.E., Andreadis, P.T., 1988. Physiological correlates of preference and aversion for sugars in three species of birds. *Physiological Zoology* 61, 222 – 229.
- Mazer, S.J., Wheelwright, N.T., 1993. Fruit size and shape: allometry at different taxonomic levels in bird-dispersed plants. *Evolutionary Ecology* 7, 556 – 575.
- McCarty, J.P., Levey, D.J., Greenberg, C.H., Sargent, S., 2002. Spatial and temporal variation in fruit use by wildlife in a forested landscape. *Forest Ecology and Management* 164, 277 – 291.
- McKey, D., 1975. The ecology of coevolved seed dispersal systems. In: Gilbert, L.E., Raven, P.H., (Eds). *Coevolution of animals and plants*. University of Texas Press, Austin, Texas.
- McPherson, J.M., 1987. A field study of winter fruit preferences of cedar waxwings. *Condor* 89, 293 – 306.
- Milton, K., 2004. Ferment in the family tree: does a frugivorous dietary heritage influence contemporary patterns of human ethanol use? *Integrated Comparative Biology* 44, 304 – 314.
- Moermond, T.C., Denslow, J.S., 1985. Neotropical avian frugivores: patterns of behavior, morphology and nutrition, with consequences for fruit selection. *Ornithological Monographs* 36, 865 – 897.
- Murray, K.G., 1987. Selection for optimum fruit crop size in bird-dispersed plants. *American Naturalist* 129, 18 – 31.

- Murray, K.G., Winnett-Murray, K., Cromie, E.A., Minor, M., Meyers, E., 1993. The influence of seed packaging and fruit colour on feeding preferences of American robins. *Vegetatio* 107/108, 217 – 226.
- Nakanishi, H., 1996. Fruit colour and fruit size of bird-disseminated plants in Japan. *Vegetatio* 123, 207 – 218.
- Napier, K.R., McWhorter, T.J., Nicolson, S.W., Fleming, P.A., 2013. Sugar preferences of avian nectarivores are correlated with intestinal sucrose activity. *Physiological and Biochemical Zoology* 86, 499 – 514.
- Nathan, R., Muller-Landau, H.C., 2000. Spatial patterns of seed dispersal, their determinants and consequences for recruitment. *Trends in Ecology and Evolution* 15, 278 – 285.
- Ortiz-Pilido, R., Rico-Gray, V., 2000. The effect of spatio-temporal variation in understanding the fruit crop-size hypothesis. *Oikos* 91, 523 – 527.
- Pierce, B.J., McWilliams, S.R., 2005. Seasonal changes in composition of lipid stores in migratory birds: causes and consequences. *Condor* 107, 269 – 279.
- Place, A.R., Stiles, E.W., 1992. Living off the wax of the land: bayberries and yellow-rumped warblers. *Auk* 109, 334 – 345.
- Pryor, G.S., Levey, D.J., Dierenfeld, E.S., 2001. Protein requirements of a specialized frugivore, Pesquet's parrot (*Psittichus fulgidus*). *Auk* 118, 1080 – 1088.
- Puckey, H.L., Lill, A., O'Dowd, J., 1996. Fruit colour choices of captive silvereyes (*Zosterops lateralis*). *Condor* 98, 780 – 790.
- Rajchard, J., 2008. Exogenous chemical substances in bird perception: a review. *Veterinarni Medicina* 53, 412 – 419.

- Rey, P.J., Alcantara, J.M., 2000. Recruitment dynamics of a fleshy-fruited plant (*Olea europaea*): connecting patterns of seed dispersal to seedling establishment. *Journal of Ecology* 88, 6212 – 633.
- Rey, P.J., Guitierrez, J.E., Alcantara, J., Valera, F., 1997. Fruit size in wild olives: implications for avian seed dispersal. *Functional Ecology* 11, 611 – 618.
- Ridley, H. N., 1930. *The dispersal of plants throughout the world*. L. Reeve & Co., Ashford, UK.
- Robinson, G.R., Handel, S.N., 1993. Forest restoration on a closed landfill: rapid addition of new species by bird dispersal. *Conservation Biology* 7, 271 – 278.
- Roper, T.J. 2003. Olfactory discrimination in yellow-backed chattering lorries *Lorius garrulus flavopalliatu*s: first demonstration of olfaction in Psittaciformes. *Ibis* 145, 689 – 691.
- Sabat, P., Lagos, J.A., Bozinovic, F., 1999. Test of the adaptive modulation hypothesis in rodents: dietary flexibility and enzyme plasticity. *Comparative Biochemistry and Physiology A* 123, 83 – 87.
- Sabat, P., Novoa, F., Bozinovic, F., Martinez del Rio, C., 1998. Dietary flexibility and intestinal plasticity in birds: a field and laboratory study. *Physiological Zoology* 71, 226 – 236.
- Sallabanks, R., 1993. Hierarchical mechanisms of fruit selection by an avian frugivore. *Ecology* 74, 1326 – 1336.
- Sanchez, F., Korine, C., Pinshow, B., Dudley, R., 2004. The possible roles of ethanol in the relationship between plants and frugivores: first experiments with Egyptian fruit bats. *Integrated Comparative Biology* 44, 290 – 294.

- Schaefer, H.M., Levey, D.J., Schaefer, V., Avery, M.L., 2006. The role of chromatic and achromatic signals for fruit detection by birds. *Behavioural Ecology* 10, 784 – 789.
- Schaefer, H.M., Schmidt, V., Bairlein, F., 2003. Discrimination abilities for nutrients: which difference matters for choosy birds and why? *Animal Behaviour* 65, 531 – 541.
- Schondube, J.E., Martinez del Rio, C., 2003. Concentration-dependent sugar preferences in nectar-feeding birds: mechanisms and consequences. *Functional Ecology* 17, 445 – 453.
- Schmidt, V., Schaefer, H.M., Winkler, H., 2004. Conspicuousness, not colour as a foraging cue in plant-animal signalling. *Oikos* 106, 551 – 557.
- Schupp, E.W., 1992. The Janzen-Connell model for tropical tree diversity: population implications and the importance of spatial scale. *American Naturalist* 140, 526 – 530.
- Sekercioglu, C.H., 2006. Increasing awareness of avian ecological function. *Trends in Ecology and Evolution* 21, 464 – 471.
- Schaefer, H.M., Schmidt, V., Bairlein, F., 2003*a*. Discrimination abilities for nutrients: which difference matters for choosy birds and why? *Animal Behaviour* 65, 531 – 541.
- Schaefer, H.M., Schmidt, V., Winkler, H., 2003*b*. Testing the defence trade-off hypothesis: how contents of nutrients and secondary compounds affect fruit removal. *Oikos* 102, 318 – 328.
- Sibley, C.G., Ahlquist, J.E., 1990. *Phylogeny and classification of birds: a study in molecular evolution*. Yale University Press, New Haven, Connecticut.
- Silva, J.M.C., Tabarelli, M., 2000. Tree species impoverishment and the future flora of the Atlantic forest of northeastern Brazil. *Nature* 404, 72 – 74.
- Snow, D.W., 1971. Evolutionary aspects of fruit eating by birds. *Ibis* 113, 194 – 202.

- Snow, D.W., 1981. Tropical frugivorous birds and their food plants: a world survey. *Biotropica* 13, 1 – 14.
- Sorensen, A.E., 1984. Nutrition, energy and passage time: experiments with fruit preference in European blackbirds (*Turdus merula*). *Journal of Animal Ecology* 53, 545 – 557.
- Stanley, M.C., Lill, A., 2001. Response of silver-eyes (*Zosterops lateralis*) to dietary tannins: the paradox of secondary metabolites in fruits. *Australian Journal of Zoology* 49, 633 – 640.
- Stanley, M.C., Lill, A., 2002a. Avian fruit consumption and seed dispersal in a temperate Australian woodland. *Austral Ecology* 27, 137 – 148.
- Stanley, M.C., Lill, A., 2002b. Does seed packaging influence fruit consumption and seed passage in an avian frugivore? *Condor* 104, 136 – 145.
- Steiger, S.S., Fidler, A.E., Valcu, M., Kampenars, B. 2008. Avian olfactory receptor gene repertoires: evidence for a well-developed sense of smell in birds? *Proceedings of the Royal Society B* 275, 2309 – 2317.
- Strauss, S.Y., Irwin, R.E., 2004. Ecological and evolutionary consequences of multispecies plant-animal interactions. *Annual Reviews of Ecology, Evolution and Systematics* 35, 435 – 466.
- Struempf, H.M., Schondube, J.E., Martinez del Rio, C., 1999. The cyanogenic glycoside amygdalin does not deter consumption of ripe fruits by cedar waxwings. *Auk* 116, 749 – 758.
- Tiffney, B.H., 2004. Vertebrate dispersal of seed plants through time. *Annual Review of Ecology, Evolution and Systematics* 35, 1 – 29.

- Tsahar, E., Friedman, J., Izhaki, I., 2002. Impact on fruit removal and seed predation of a secondary metabolite, emodin in *Rhamnus alaternus* fruit pulp. *Oikos* 99, 290 – 299.
- Valburg, L.K., 1992. Eating infested fruits: interactions in a plant-disperser-pest triad. *Oikos* 65, 25 – 28.
- Vazquez, D.P., 2005. Degree distribution in plant-animal mutualistic networks: forbidden links or random interactions? *Oikos* 108, 421 – 426.
- Vazquez, D.P., Aizen, M.A., 2004. Asymmetric specialization: a pervasive feature of plant-pollinator interactions. *Ecology* 85, 1251 – 1257.
- Vazquez, D.P., Chacoff, N.P., Cagnolo, L., 2009. Evaluating multiple determinants of the structure of plant-animal mutualistic networks. *Ecology* 90, 2039 – 2046.
- Voyobyevev, M., Osorio, D., Bennett, A.T.D., 1998. Tetrachromacy, oil droplets and bird plumage colours. *Journal of Comparative Physiology A* 183, 621 – 633.
- Walsberg, G.E., 1975. Digestive adaptations of *Phainopepla nitens* associated with the eating of mistletoe berries. *Condor* 77, 169 – 175.
- Waser, N.M., Chittka, L., Price, M.V., Williams, N.M., Ollerton, J., 1996. Generalisation in pollination systems, and why it matters. *Ecology* 77, 1043 – 1060.
- Wheelwright, N.T., 1983. Fruits and the ecology of the resplendent quetzal. *Auk* 100, 286 – 301.
- Wheelwright, N.T., 1985. Fruit-size, gape-width and the diets of fruit-eating birds. *Ecology* 66, 805 – 818.
- Wheelwright, N.T., Janson, C.H., 1985. Colours of fruit displays of bird-dispersed plants in two tropical forests. *American Naturalist* 126, 777 – 799.

- Wheelwright, N.T., Orians, G.H., 1982. Seed dispersal by animals: contrasts with pollen dispersal, problems of terminology and constraints on coevolution. *American Naturalist* 119, 402 – 413.
- Whitney, K.D., Fogiel, M.K., Lamperti, A.M., Holbrook, K.M., Stauffer, D.J., Hardesty, B.D., Smith, T.B., 1998. Seed dispersal by *Ceratogymna* hornbills in Dja Reserve, Cameroon. *Journal of Tropical Ecology* 14, 351 – 371.
- Willson, M.F., 1994. Fruit choices by captive American robins. *Condor* 96, 494 – 502.
- Willson, M.F., De Santo, T.L., Sabag, C., Armesto, J.J., 1994. Avian communities of fragmented south-temperate rainforests in Chile. *Conservation Biology* 8, 508 – 520.
- Willson, M.F., Melampy, M.N., 1983. The effect of bicoloured fruit displays on fruit removal by avian frugivores. *Oikos* 41, 27 – 31.
- Willson, M.F., Whelan, C.J., 1990. The evolution of fruit colour in fleshy-fruited plants. *American Naturalist* 136, 790 – 809.
- Witmer, M.C., 1998. Ecological and evolutionary implications of energy and protein requirements of avian frugivores eating sugary diets. *Physiological Zoology* 71, 599 – 610.
- Witmer, M.C., 2001. Nutritional interactions and fruit removal: cedar waxwing consumption of *Viburnum opulus* fruits in spring. *Ecology* 82, 3120 – 3130.
- Witmer, M.C., Martinez del Rio, C., 2001. The membrane-bound intestinal enzymes of waxwings and thrushes: adaptive and functional implications of patterns of enzyme activity. *Physiological and Biochemical Zoology* 74, 584 – 593.
- Witmer, M.C., van Soest, P.J., 1998. Contrasting digestive strategies of fruit eating birds. *Functional Ecology* 12, 728 – 741.

- Worthington, A.R., 1989. Adaptations for avian frugivory: assimilation efficiency and gut transit time of *Manacus vitellinus* and *Pipra mentalis*. *Oecologia* 80, 381 – 389.
- Wunderle, J.M., 1997. The role of animal seed dispersal in accelerating native forest regeneration on degraded tropical lands. *Forest Ecology and Management* 99, 223 – 235.
- Yeomans, M.R., 2004. Effects of alcohol on food and energy intake in human subjects: evidence for passive and active over-consumption of energy. *British Journal of Nutrition* 92, 31 – 34.
- Zamora, R., 2000. Functional equivalence in plant-animal interactions: ecological and evolutionary consequences. *Oikos* 88, 442 – 447.

CHAPTER 2

Digestive efficiencies and digesta transit times of Cape White-eyes (*Zosterops virens*), Red-winged Starlings (*Onychognathus morio*) and Speckled Mousebirds (*Colius striatus*) on equicaloric glucose and sucrose artificial fruit diets

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Abstract

Transit times and digestive efficiencies of three species of birds, the Cape White-eyes (*Zosterops virens*), Red-winged Starlings (*Onychognathus morio*) and Speckled Mousebirds (*Colius striatus*) were investigated on equicaloric glucose and sucrose artificial fruit diets. Three concentrations were used: low (6.6%), medium (12.4%) and high (22%). Digesta transit times of birds increased with an increase in concentration for all diets but were generally higher on glucose diets. Intake rates, on the other hand, decreased with an increase in sugar concentration. All birds failed to maintain a constant assimilated energy intake on glucose diets but Mousebirds and White-eyes maintained it on sucrose diets. Apparent assimilation efficiencies of glucose diets for all species were comparable and typical of those

found in other frugivorous birds. However, assimilation efficiencies for sucrose diets differed widely with Red-winged Starlings displaying very low assimilation efficiencies and as a consequence; they lost significant body mass on all sucrose diets. The lack of significant sucrase activity was attributed to this finding. These results show the importance of digestive physiology in explaining fruit selection patterns in frugivorous birds.

Keywords: Assimilation efficiency; Digesta transit time; digestive efficiency; Frugivore

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1. Introduction

Sucrose, fructose and glucose are the three primary sugars in fruit pulp and nectar of plants and their composition varies widely among plant species (Martinez del Rio et al., 1992; Schondube and Martinez del Rio, 2003; Johnson and Nicolson, 2008). Because pollinator and disperser preference is assumed to exert selection pressures on nectar and fruit pulp composition, the variation in sugar composition in plant rewards could be expected to be reflected in the pollinator and disperser assemblages (Martinez del Rio et al., 1992). For nectar, Baker and Baker (1983) postulated a hummingbird-passerine dichotomy with hummingbird-pollinated flowers having sucrose-rich nectar while those of passerine-pollinated plants contained hexose-rich nectar (Baker and Baker 1983; Baker et al., 1998). Based on this dichotomy, hummingbirds were expected to prefer sucrose-dominant nectar while passerines were expected to prefer hexose-dominant nectar (Baker and Baker, 1983). Indeed laboratory studies showed support for the notion with hummingbirds preferring sucrose solutions (Martinez del Rio, 1990; Martinez del Rio et al., 1992) and passerines preferring hexose solutions over sucrose (Martinez del Rio et al., 1988, Martinez del Rio and

Stevens, 1989; Martinez del Rio et al., 1992; Brugger et al., 1993). However, these studies were confounded by that they compared specialized nectarivorous non-passerines with generalized frugivorous/nectarivorous passerines (Franke et al., 1998; Jackson et al., 1998; Brown et al., 2010*a*). Furthermore, they were conducted on birds from a restricted geographical range (Franke et al., 1998) and assumed that the pattern applies globally. Studies on southern African and Australian specialised nectarivorous passerines have shown that they too digest sucrose as efficiently as hummingbirds and as a result, they prefer sucrose over hexose solutions (Downs and Perrin, 1996; Lotz and Nicolson, 1996; Downs, 1997*a*; Nicolson and Fleming, 2003*a*; Fleming et al., 2004, 2008; Brown et al., 2010*a*). A more recent study by Johnson and Nicolson (2008) showed that nectar of flowers pollinated by specialized nectarivores is characterized by high concentration (15–25%), low volume (10–30 μ l) and high sucrose content (40–60 % of total sugar). On the other hand, nectar of flowers pollinated by generalized nectarivores is dilute (8–12%), copious (40–100 μ l) and sucrose-deficient (0–5%). As a result, they refuted the hummingbird-passerine dichotomy and suggested that a more useful distinction is between generalized and specialized bird-pollination systems (Johnson and Nicolson, 2008).

Conventional ecological wisdom predicts that the selection of a particular diet by an organism should be based on its ability to digest it (Martinez del Rio, 1990; Lotz and Schondube, 2006). While there is constancy in digestive abilities of birds for hexose sugars, the ability to digest sucrose differ markedly, with significant ecological consequences (Martinez del Rio et al., 1988; Schondube and Martinez del Rio, 2003; Lotz and Schondube, 2006). A number of birds lack sucrase, the enzyme responsible for breaking down sucrose to glucose and fructose (Martinez del Rio et al., 1988, 1989; Martinez del Rio and Stevens, 1989; Martinez del Rio and Karasov, 1990; Karasov and Levey, 1990; Martinez del Rio, 1990; Malcarney et al., 1994; Gatica et al., 2006; Bizaare et al., 2012; Brown et al., 2012). As

a result, these birds show an aversion for sucrose as it causes osmotic diarrhoea (Martinez del Rio et al., 1988; Brown et al., 2012). It has been confirmed that the lack of sucrase is restricted to a single lineage, the Sturnidae-Muscicapoidea (Lotz and Schondube, 2006; Gatica et al., 2006; Bizaare et al., 2012; Brown et al., 2012). However, even species with significant sucrase activity prefer hexose solutions as they do not digest sucrose efficiently enough to maintain energy balance on it (Martinez del Rio et al., 1989; Martinez del Rio, 1990). This has been attributed to their fast passage rates which does not allow for sufficient exposure time of food to the digestive enzymes (Martinez del Rio et al., 1992). Therefore the preference for sucrose and lack thereof in birds is not only a function of sucrase activity but depends on the interplay between retention time, sucrose hydrolysis, and glucose and fructose uptake (Martinez del Rio, 1990).

Sugar type and concentration are important determinants of sugar preferences in birds because they affect digestive efficiency and rate of food processing (Schondube and Martinez del Rio, 2003). In the wild, sugar type and concentration in nectar and fruit show considerable variation even at small temporal and spatial scales (Levey, 1987). Therefore sugar selection in birds likely depends on both composition and concentration which suggests that in order elucidate factors affecting sugar preference in birds, these factors should be examined simultaneously (Nicolson and Fleming, 2003*a*). Indeed, studies that have examined these attributes simultaneously have that sugar selection in birds depends on concentration. Specialist nectarivores generally prefer hexose at low concentration, no preference at intermediate levels and sucrose preference at high concentrations (Schondube and Martinez del Rio, 2003; Fleming et al., 2004, 2008; Lotz and Schondube, 2006; Brown et al., 2010*a*). Generalized nectarivores, on the other hand, prefer hexose solutions at low concentrations and show no preference or hexose preference at high concentrations (Brown et al., 2010*b*; Odendaal et al., 2010). Napier et al. (2013) suggested that sucrase activity drives the

concentration-dependent sugar preference patterns in birds. They showed that specialized nectarivores (which have high sucrase activity) tend to select hexose only in the most dilute solutions while occasional nectarivores (which have low sucrase activity) select hexose up to higher concentrations (Napier et al., 2013). The general hexose preference at low concentrations has been attributed to short transit times at this concentration causing sucrose hydrolysis to limit energy delivery rate (Napier et al., 2013).

The effect of sugar type and concentration on sugar preferences in frugivorous birds has not received the same attention it had for nectarivorous birds (Wilson and Downs, 2011). Fruits are broadly categorized into two groups based on their carbohydrate and lipid content (Izhaki, 1992). Energy-dilute fruits are characterized by high water and carbohydrate content and low fibre and lipid content whereas nutrient-dense fruits have high lipid content, variable protein content and low water and carbohydrate content (McKey, 1975; Moermond and Denslow, 1985; Downs, 2008). Studies of fruit characteristics from diverse ecosystems suggest that the majority of fruits are nutrient-dilute (Herrera, 1987; Witmer, 1998). Compounding the dilution of nutrients in fruits is the high seed mass (up to 50% of total dry fruit mass, Moermond and Denslow, 1985) which takes up gut space that could be occupied by fruit pulp (Levey and Grajal, 1991; Murray et al., 1993; Stanley and Lill, 2002). This suggests that in order for frugivores to maintain energy balance on fruits, they should consume a large number of fruits and process them rapidly or select only nutrient-rich fruits (Worthington, 1989; Levey and Karasov, 1989). However, since the fruiting phenology of fruiting trees is highly seasonal and thus fruit shortages frequently occur, being selective cannot fully solve the dilemma faced by frugivores (Worthington, 1989). Therefore the ability to process large amounts of low quality fruits rapidly can allow avian frugivores to maintain energy balance during periods of low fruit abundance (Worthington, 1989).

Digestive efficiency is a measure of how well organisms extract nutrients from food (Levey and Karasov, 1992) and determines whether energetic demands are met (Brown and Downs, 2003; Downs, 2008). The reaction-rate model suggests a trade-off between the rate at which food is processed in the gut (passage rate) and the extent to which food is digested (digestive efficiency) (Levey and Karasov, 1992; Afik and Karasov, 1995; Downs, 2008). Frugivorous birds have high energetic demands, small gut volumes and fast passage rates (Levey and Karasov, 1989; Karasov and Levey, 1990). As a result, they are considered to be poor at assimilating energy (Karasov and Levey, 1990). However, compelling evidence has accumulated to suggest that frugivores do not have inherent fast passage rates but they modulate retention time according to the time required for the complete digestion and assimilation of food (Levey and Karasov, 1992; Afik and Karasov, 1995; Witmer, 1998, 1999; Witmer and van Soest, 1998). Furthermore, digestive efficiencies of sugars by frugivores have been found to be as high as those of hummingbirds when fed sugars of high concentration (Worthington, 1989; Witmer, 1998, 1999; Witmer and van Soest, 1998). The low digestive efficiencies reported in other studies could therefore be attributed to the low concentration of sugar used resulting in low digestive efficiency (Witmer, 1999).

The aim of this study was to determine whether sugar type and concentration affects fruit selection in birds. Three frugivorous bird species (Cape White-eye, *Zosterops virens*, Red-winged Starling, *Onychognathus morio* and Speckled Mousebird *Colius striatus*) were used for this study. These species are indigenous and locally abundant especially along the eastern coast of KwaZulu-Natal (Hockey et al., 2005). In this region, about 233 plant species are known to be dispersed by birds (Pooley, 1993). Although fruiting is seasonal, some plants (57-63) fruit in winter, suggesting that fruits are available throughout the year (Pooley, 1993). Because the foraging behaviour of frugivores have consequences for the process of seed dispersal, examining sugar preferences in frugivores may play a role in understanding the link

between the distribution of frugivores and their food plants by determining precisely which fruits are being selected (Ally, 2010). Equicaloric sucrose and glucose artificial fruit diets of varying concentrations were used in this study. Due to a lack of sugar composition of South African fruits, the artificial fruit diets used were based on those developed by Witmer (1998) for North American birds. These diets were chosen because they (1) they allowed us to compare our findings to those of other studies and (2) they controlled for the effect of seeds and secondary compounds, factors that affect digestion (Bairlein, 1996; Cipollini and Levey, 1997). We hypothesized that sugar type and concentration would affect digestion in these species. We thus predicted that (1) birds would have slower digesta transit times as sugar concentration increased; (2) birds would reduce intake rates as sugar concentration increased to maintain a constant energy flux and (3) birds would show high apparent assimilation efficiency regardless of sugar type (Martinez del Rio and Karasov, 1990; Lotz and Nicolson, 1996; Witmer, 1999).

2. Materials and Methods

2.1 Study species and maintenance

Cape White-eyes ($n = 9$), Red-winged Starlings ($n = 6$) and Speckled Mousebirds ($n = 6$), kept at the Animal House of the University of KwaZulu-Natal (UKZN), Pietermaritzburg ($29^{\circ}37'0''S$; $30^{\circ}23'0''E$, 660 m above sea level), were used. These birds were housed in large outside species-groups holding cages ($1 \times 2.12 \times 2.66$ m) where they were exposed to natural photoperiod, humidity and temperature. All birds used in this study were captured in the vicinity of Pietermaritzburg and had been in captivity for more than a year and thus were fully acclimated to captivity and food preference trials. The maintenance diet these birds were fed on consisted of chopped-up and grated fruit mix including bananas, apples, pears, paw-

paws, oranges and guavas together with an avian supplementary diet (AviPlus Softbill Maintenance Crumble; Aviproducts, Waterfall, KZN). On occasions, Mealworms (*Tenebrio molitor* larvae) were provided. The skin from paw-paws, bananas and oranges was removed and the flesh was cut into small (approximately 1 × 1 × 1 cm) cubes. The other fruits were grated. Water was provided *ad libitum*. Birds were fed every morning between 07:00 and 08:00.

2.2 Experimental trials

Five days prior to the start of the experimental trials, birds were transferred to individual cages in a controlled environment room (12h: 12h L: D photoperiod; 25± 0.1°C, temperature) so that they acclimatize to the experimental conditions. During this acclimation period, they were fed on the same schedule as they were in outside aviaries. On the day preceding each experimental trial, all the food was removed from cages at 18:00 to ensure that birds were post-absorptive. On experimental days, body mass of each bird (g) was measured before and after the experiment. Body masses were rounded off to 0.5g. Three equicaloric glucose and sucrose concentrations were used to investigate the digestive efficiencies and transit times of birds: low (6.6%), medium (12.4%) and high (22%) (Witmer, 1998). Diets were prepared by mixing all ingredients (Table 1) into 1 litre of boiling water, heating the mixture in a microwave oven (for approximately 8 min) until boiling, and then pouring it into a flat tray to cool. While waiting for the mixture to solidify, it was stirred continuously by hand to ensure that ingredients distributed equally. Due to the short life span of the artificial fruit diets, they were prepared on the afternoon preceding the experimental trials and kept in a refrigerator overnight.

2.3 Transit times and digestive efficiencies

Artificial fruits were cut into small pieces (1×1 cm) and administered to birds. Each individual bird was exposed one trial for each diet type. Because the sugar type and concentration trials were run separately, each bird was exposed to a total of 6 trials. The feeders containing artificial fruits were weighed hourly to determine the amount of food eaten. Controls of each experimental diet were used to determine the evaporative water loss from uneaten food. All food items were rounded off to 0.01g. Each experimental diet was dyed using 5ml of Robertson's red food colourant (Libstar Manufacturing Solutions, Chloorkop, South Africa). Digesta transit times were then calculated as the time between birds first ate and the time when dye first appeared in the excreta. Each experimental trial was run for 12 h (06:00-18:00). Successive experimental trials were separated by 48 h to ensure that birds recovered fully from the previous trial. Excreta of the birds were collected by placing plastic sheets under the cages. At the end of each trial (18:00), the excreta from each bird were collected, oven-dried at 60°C before being milled, weighed and bombed using the micro bomb calorimetry for gross energy content (Animal Science Laboratory, UKZN). Controls of each diet were treated similarly to determine gross energy.

For each bird, daily food intake (DFI, calculated as wet mass) was determined for the respective diets. DFI was determined by subtracting the mass of the remaining food from the amount given and accounting for desiccation. This measure was then converted to gross energy intake (GEI) by accounting for the water content of each diet: this was achieved by multiplying the dry mass intake by the energy content of each diet. Daily excreta energy loss (EE) was calculated as the mass of dry excreta produced multiplied by its dry mass energy value. Daily energy assimilated (DEA) was calculated as GEI minus EE. Daily apparent assimilation efficiency (AE) was calculated as DEA divided by GEI. Because AE is a proportion, it was first squareroot transformed and multiplied by 100 to be expressed as a

percentage. For comparison, the data were converted to mass specific values by dividing by the initial body mass in cases where there were no significant differences between initial and final body masses or by the mean of the initial and final body masses in cases where significant differences were found.

2.4 Statistical analyses

As each bird was used more than once, digesta transit times, GEI, EE, DEA and AAE were compared with a generalised linear model (GLM) repeated measures ANOVA (RM ANOVA) and Tukey's *post-hoc* tests. Tukey's *post-hoc* tests were run both between and within each sugar type. Pairwise *t*-tests were used to determine whether there were significant differences in body mass before and after each experimental trial. All statistical analyses were conducted on STATISTICA (Statsoft 7, Tulsa, USA). All values are expressed as mean± S.E, except where specified.

3. Results

3.1 Digesta transit times

For Cape white-eyes, there were significant differences in digesta transit times between glucose and sucrose artificial fruit diets at different concentrations (RMANOVA, $F_{(2, 16)} = 4.366$; $P = 0.030$). Glucose diets had significantly lower digesta transit times than sucrose diets. Digesta transit times increased significantly with an increase in concentration for both glucose and sucrose diets (Table 2). The greatest variability in digesta transit times for the glucose diets were found at the lowest concentration whereas for sucrose diets, it was found at the highest concentration. The lowest transit times were found on the glucose 6.6%

diet while the highest were found on the sucrose 22% diet (Table 2). Similarly for the Red-winged Starlings, significant differences in digesta transit times were found between glucose and sucrose diets (RMANOVA, $F_{(2, 10)} = 6.075$; $P = 0.019$), with sucrose diets having lower digesta transit times. The lowest transit times was found on the sucrose 6.6% diet whereas the highest transit time was on the glucose 22% diet (Table 2). Coincidentally, the greatest variability in transit times was found at these concentrations. For sucrose diets, transit times increased with an increase in concentration whereas this was not the case for glucose diets as the lowest transit times were found at the glucose 12.4% diet (Table 2). For Speckled Mousebirds, no significant differences were found in digesta transit times between glucose and sucrose diets at different concentrations (RMANOVA, $F_{(2, 10)} = 3.072$; $P = 0.091$). However, digesta transit times for sucrose diets were on average higher than those for glucose diets. Similarly to the Cape White-eyes, digesta transit times increased with an increase in concentration for both glucose and sucrose diets in the speckled mousebirds (Table 2). The lowest transit times were observed on the glucose 6.6% diet and the highest were observed on the sucrose 22% diet. The greatest variability in transit times was observed at the highest concentration for both glucose and sucrose diets (Table 2).

3.2 Food intake and assimilation

There were significant differences in food intake for the Cape white-eyes on glucose and sucrose diets at different concentrations (RMANOVA, $F_{(2, 16)} = 5.640$; $P = 0.004$), with the sucrose diets consumed significantly more than glucose diets. The low concentration diets were consumed in significantly greater amounts than the medium and high concentration diets (Tukey's *post-hoc* tests, $P < 0.05$; Fig. 1). There were also significant differences between glucose and sucrose diets in terms of GEI (RMANOVA, $F_{(2, 16)} = 18.731$; $P = 0.001$).

Similarly to intake, GEI was higher on the sucrose diets than on the glucose diets. For glucose diets, GEI differed significantly, being highest on the glucose 6.6% diet and lowest on the glucose 12.4% diet (Tukey's *post-hoc* tests, $P < 0.05$; Fig. 2a). For sucrose diets, there were no significant differences in GEI at different concentrations (Tukey's *post-hoc* tests, $P > 0.05$). There were significant differences in EE between glucose and sucrose diets at different concentrations (RMANOVA, $F_{(2, 16)} = 4.170$; $P = 0.035$), with a higher EE on the sucrose than on the glucose diets. For glucose diets, EE did not differ significantly with concentration (Tukey's *post-hoc* tests, $P > 0.05$; Fig. 2b). For sucrose diets, EE on the sucrose 22% diet was significantly lower than for other diets (Tukey's *post-hoc* tests, $P < 0.05$; Fig. 2b). As a consequence of GEI and EE, DEA differed significantly between glucose and sucrose diets at different concentrations (RMANOVA, $F_{(2, 16)} = 22.928$; $P = 0.001$). For glucose diets, DEA differed significantly, being highest on the glucose 6.6% diet and lowest on the glucose 12.4% diet (Tukey's *post-hoc* tests, $P < 0.05$; Fig. 3a). For sucrose diets, DEA did not differ significantly (Tukey's *post-hoc* tests, $P > 0.05$). AE was not significant between glucose and sucrose diets at different concentrations (RMANOVA, $F_{(2, 16)} = 0.478$; $P = 0.625$; Fig. 3b) and was not affected by sugar concentration.

Red-winged Starlings ingested significantly different amounts of glucose and sucrose diets at different concentrations (RMANOVA, $F_{(2, 10)} = 7.754$; $P = 0.001$). Glucose diets were consumed in greater amounts than sucrose diets (Fig. 4). For all treatments, the low and medium concentration diets were consumed at the same rate (Tukey's *post-hoc* tests, $P > 0.05$) but were consumed significantly more than the high concentration diets (Tukey's *post-hoc* tests, $P < 0.05$). GEI differed significantly between treatments (RMANOVA, $F_{(2, 10)} = 21.345$; $P = 0.001$). There were significant differences in GEI for glucose diets with the glucose 6.6% diet having the highest GEI (Tukey's *post-hoc* tests, $P < 0.05$; Fig. 5a). GEI was not different for sucrose diets (Tukey's *post-hoc* tests, $P > 0.05$). EE differed significantly between glucose

and sucrose diets at different concentrations (RMANOVA, $F_{(2, 10)} = 17.484$; $P = 0.001$), being higher for sucrose than glucose diets. EE increased with an increase in concentration for sucrose diets while it declined with concentration for glucose diets (Fig. 5b). DEA differed significantly between glucose and sucrose diets (RMANOVA, $F_{(2, 10)} = 35.237$; $P = 0.001$), being higher on glucose diets. For glucose diets, DEA was significantly high for glucose 6.6% diet than for other diets. For sucrose diets, DEA was significantly lower for the sucrose 22% diet (Tukey's *post-hoc* tests, $P < 0.05$; Fig. 6a). AE differed significantly between diets at different concentrations (RMANOVA, $F_{(2, 10)} = 56.363$; $P = 0.001$), being higher for glucose than sucrose diets. AE was high on glucose diets and was not affected by sugar concentration but was low and declined with concentration on sucrose diets (Fig. 6b).

Speckled mousebirds consumed significantly different amounts of glucose and sucrose diets at different concentrations (RMANOVA, $F_{(2, 10)} = 7.754$; $P = 0.001$), with sucrose diets consumed in greater amounts. Low concentration diets were consumed in significantly greater amounts than the medium and high concentration diets for all treatments (Tukey's *post-hoc* tests, $P < 0.05$; Fig. 7). There were also significant differences between glucose and sucrose diets in terms of GEI (RMANOVA, $F_{(2, 10)} = 21.345$; $P = 0.001$), with a higher GEI on sucrose diets. For glucose diets, GEI differed significantly being highest on the glucose 6.6% diet and lowest on the glucose 12.4% diet (Tukey's *post-hoc* tests, $P < 0.05$; Fig. 8a). For sucrose diets, GEI was maintained constantly (Tukey's *post-hoc* tests, $P > 0.05$). EE differed significantly between glucose and sucrose diets at different concentrations (RMANOVA, $F_{(2, 10)} = 17.484$; $P = 0.001$; Fig. 8b), with a higher EE on the sucrose than on the glucose diets. EE declined with an increase in concentration for all treatments. DEA differed significantly between glucose and sucrose diets as a consequence of GEI and EE (RMANOVA, $F_{(2, 10)} = 35.237$; $P = 0.001$; Fig. 9a). Again, DEA was maintained constantly for sucrose diets (Tukey's *post-hoc* tests, $P > 0.05$) while it differed significantly for glucose diets (Tukey's

post-hoc tests, $P < 0.05$). AE differed significantly between glucose and sucrose treatments at different concentrations (RMANOVA, $F_{(2, 10)} = 56.363$; $P = 0.001$; Fig. 9b), being higher for glucose than for sucrose diets. For all treatments, AE was influenced by significantly sugar concentration, being highest on the high concentration diets (Fig. 9b).

3.3 Body mass

There was a significant decrease in body mass in the Cape White-eyes on the glucose 6.6% diets (Pairwise *t*-test, $t = 4.318$; $df = 8$; $P = 0.003$; Table 3). In all other diets, no significant differences between initial and final body masses were observed (Table 3). For Red-winged Starlings, a significant increase in body mass was observed on the glucose 12.4% diet (Pairwise *t*-test, $t = -5.391$, $df = 5$; $P = 0.003$; Table 3). In other glucose diets however, no significant differences were found in body mass. For all sucrose diets, Red-winged Starlings showed significant decreases in body mass (Pairwise *t*-test, $t = 5.578$, $df = 5$, $P = 0.003$; $t = 5.357$, $df = 5$, $P = 0.003$; $t = 7.769$, $df = 5$, $P = 0.001$ for the sucrose 6.6%, 12.4% and 22% diets, respectively; Table 3). For Speckled Mousebirds, there was a marginal decrease in body mass on the glucose 22% diet (Pairwise *t*-test, $t = 2.614$, $df = 5$, $P = 0.047$). On other glucose diets, no significant differences in body mass were observed (Table 3). For sucrose diets, there was a significant increase in body mass on the sucrose 6.6% and 12.4% diets (Pairwise *t*-test, $t = -2.673$, $df = 5$, $P = 0.044$; $t = -9.052$, $df = 5$, $P = 0.001$, respectively). On the sucrose 22% diet, there were no significant differences in body mass (Table 3).

4. Discussion

Understanding the nutritional characteristics of fruits, especially the nutritional and energy rewards they provide to frugivores is key towards understanding the relationship

between physiology and behaviour in frugivores (Levey and Martinez del Rio, 2001; Wellmann and Downs, 2009). Frugivorous birds have morphologically simple guts, feed on a chemically simple food and have a feeding behaviour free of constraints associated with external morphology, making their foraging behaviour more directly linked with digestion than other groups (Levey and Martinez del Rio, 2001). As a result, they offer unparalleled opportunities for understanding the link between digestive physiology and behaviour and modelling digestive function. This study was conducted to determine digesta transit times and digestive efficiencies of frugivorous birds fed on equicaloric glucose and sucrose diets of varying concentrations.

Cape White-eyes and Speckled Mousebirds increased food intake as the sugar concentration decreased. This inverse relationship between food intake and nutrient levels, termed the “intake response” (Castle and Wunder, 1995), has been found in a number of nectarivorous and frugivorous birds (Downs, 1997*a, b*; Lopez-Calleja et al., 1997; Levey and Martinez del Rio, 1999; Downs, 2000; McWhorter and Martinez del Rio, 2000; Martinez del Rio et al., 2001; Nicolson and Fleming, 2003*b*; Wellmann and Downs, 2009; Brown et al., 2010*a*; Wilson and Downs, 2011). The intake response has often been attributed to compensatory feeding, which posits that birds respond to nutrient dilution by increasing intake to maintain a constant flow of assimilated energy (McWhorter and Lopez-Calleja, 2000; McWhorter and Martinez del Rio, 2000). Indeed, Cape White-eyes and Speckled Mousebirds managed to maintain a constant assimilated energy intake on sucrose diets. On glucose diets however, these species failed to do so possibly due to the overall low food intake on glucose diets. Red-winged Starlings, on the other hand, did not maintain a constant assimilated energy intake on all diets. The usefulness of tailoring transit times to the nutrient content of food in the field is that birds can obtain the same amount of energy when feeding on fruits that vary greatly in nutrient content (Worthington, 1989; Witmer, 1998). The

shortcoming of this strategy however, is that it increases the handling costs as more time is spent searching and processing these fruits (Witmer, 1998; Wilson and Downs, 2011). The high intake rates on dilute diets found in this study provide an important and often ignored lesson: a high food intake on a particular diet for diets with different concentrations of sugars may not always represent preference for that diet but may be underlain by the energetic requirements of the species consuming them (Wellman and Downs, 2009; Wilson and Downs, 2011).

In this study, digesta transit times increased significantly with an increase in sugar concentration on glucose and sucrose diets for Speckled Mousebirds and Cape White-eyes. For Red-winged Starlings, digesta transit times increased with concentration only on the glucose diets whereas on the sucrose diets, digesta transit times were lowest on the medium concentration diet. The low transit times at this sucrose concentration may be attributed to the fact that Starlings ingested more food at this concentration than on other concentrations, which could have forced food to be processed quicker in the gut. Therefore in general, our results show that digesta transit times increased with an increase in sugar concentration in the diet. Similar results have been found in other previous studies (Downs, 1997a; Witmer, 1998; Wellmann and Downs, 2009; Wilson and Downs, 2011). It has been argued that as transit times increase, nutrient absorption also increases due of the trade-off between the rate of food processing and the thoroughness of digestion (Afik and Karasov, 1995; Downs, 2008). Therefore digesta transit times may determine how well nutrients are absorbed in the gut (Witmer, 1998). In this study, the lowest digesta transit times were observed on the low glucose diet and the highest were observed on the high sucrose diet for the Cape White-eyes and Red-winged Starlings. These results corroborated those obtained by Wellmann and Downs (2009) in the same species and by Wilson and Downs (2011) in Knysna Turacos (*Tauraco corythaix*). However, results on digesta transit times following the methodology

used in this study should be interpreted with caution because they were obtained from fasted birds and thus are unlikely to be applicable under field conditions because birds (frugivores in particular) generally keep their guts full (Levey and Martinez del Rio, 1999).

Digesta transit times in Mousebirds and White-eyes showed more variability at the low glucose and high sucrose diets. For starlings, the greatest variability was found on the high concentration diets, similarly to Wellmann and Downs (2009). This suggested that at these concentrations, transit times depended more on how much birds ate. Previous studies have often attributed variation in retention time to changes in sugar concentration (Witmer, 1998; Levey and Martinez del Rio, 1999; Wellmann and Downs, 2009; Wilson and Downs, 2011). However, ingestion rates may also explain variation in retention time since these attributes are highly correlated in birds (Levey and Grajal, 1991; Levey and Martinez del Rio, 1999). Therefore attributing variation in retention time to variation in sugar concentration is unnecessarily restrictive as ingestion rates may explain variation more at the whole-animal level (Levey and Martinez del Rio, 1999). Appropriate methods that disentangle the effects of each of the above parameters are thus needed to get a better understanding of the interaction between food intake and nutrient levels in food.

Speckled Mousebirds and Cape White-eye's daily energy intake was consistently high on sucrose than on glucose diets and birds maintained constant daily energy assimilation for sucrose diets but not for glucose diets. Surprisingly, both species had higher assimilation efficiency for glucose than for sucrose diets, although this was not significant for Cape White-eyes. This pattern may be explained by the higher food intake rates on the sucrose diets. Thus Mousebirds and White-eyes fed on the sucrose diets so much that they obtained an overall high energy intake than on sucrose diets despite having a high assimilation efficiency on the glucose diets. Results of body mass changes seem to support this point. For example, both species had instances where they lost mass on the glucose diets but they never

lost mass on the sucrose diets. White-eyes lost body mass at the low glucose diet whereas Mousebirds lost body mass on the high glucose diet. Therefore White-eyes and Mousebirds struggled with energy balance on glucose diets. The high assimilation efficiency for glucose over sucrose in occasional nectar visitors (frugivores and granivores) has also been observed in other studies and may explain their preference for hexose-dominant nectar and fruits (Odendaal et al., 2010; Napier et al., 2013). Red-winged Starlings, contrastingly, gained body mass on all glucose diets and significantly lost mass on all sucrose diets. The loss of body mass at all sucrose concentrations for is not surprising as research has shown that members of the Sturnidae-Muscicapoidea lineage from diverse regions lack sucrase, the enzyme responsible for cleaving sucrose into monosaccharides glucose and fructose (Martinez del Rio et al., 1988; Karasov and Levey., 1990; Gatica et al., 2006). Indeed, this species lacks sucrase activity (Bizaare et al., 2012) and fails to assimilate sucrose solutions (Brown et al., 2012). Mousebirds and White-eyes, on the other hand, display significant sucrase activity (Bizaare et al., 2012). The lack sucrase in Starlings demonstrates how a single physiological constraint can have dire ecological consequences (Malcarney et al., 1994).

Apparent assimilation efficiencies displayed by White-eyes in this study were higher for glucose (85.51-94.26%) than for sucrose (80.59-88.57). Wellmann and Downs (2009) similarly obtained high digestive efficiencies for glucose than for sucrose in the same species but the values they obtained were relatively lower (79.3-85.60% and 69.0-78.4%, respectively). The assimilation efficiencies for Mousebirds (64.03-92.51% and 64.09-91.01% for glucose and sucrose, respectively) showed high variability but were comparable to those obtained by Brown et al. (2010*b*) when these birds were given sucrose and hexose solutions (range: 80.23-94.45%). These values are lower than those found in specialized avian nectarivores which are $\geq 97\%$ (Lotz and Nicolson, 1996; Downs, 1997*a*; Franke et al., 1998) but are comparable to those found in other frugivorous birds (Worthington 1989; Witmer,

1998, 1999). The low assimilation values observed in this study and other similar studies using fruits point to the role fibre in fruits plays in reducing the assimilation efficiency compared with nectar.

For Red-winged Starlings, assimilation efficiencies for glucose were comparable to those found in White-eyes and Mousebirds (65.88-87.17%) but for sucrose they were very low (25.59-57.67%). The assimilation of Starlings on sucrose diets differed to those found by Brown et al. (2012) on sucrose solutions where they displayed 0% assimilation. The assimilation of Starlings on sucrose diets obtained in this study were unexpected as we expected them to be similar to those found by Brown et al. (2012) because this species lacks sucrose activity (Bizaare et al., 2012). The assimilation efficiencies observed in this study suggest that Starlings were able to digest large amounts of sucrose. The discrepancy between these findings may be because in the Brown et al. (2012) study, the sucrose diets were in liquid form thus the sugars were completely available to birds (Karasov, 1990) whereas in this study, diets were diluted by an indigestible substance (agar). The mechanism that allowed starlings to digest sucrose is unclear but could be due to a mutualistic relationship between this species and intestinal microorganisms such as the Ciliate (*Polyplaston multivesiculatum*) which is known to hydrolyse larger molecules into simple sugars (Akkada et al., 1963). Further studies on assimilation efficiencies of sucrose diets in asaccharotic birds are required to verify this claim.

The assimilation efficiencies of sugars were not affected by sugar concentration in the Cape White-eyes and Red-winged Starlings except on sucrose diets in the latter. In the speckled Mousebirds, however, sugar concentration significantly affected assimilation efficiencies with high efficiencies on the high concentration diets. In theory, an increase in sugar content in the diet should result in increased luminal concentrations of hexoses which in turn should result in an increased passive component of sugar uptake (Levey and Martinez

del Rio, 1999). Indeed, Napier et al. (2008) showed that the relative contribution of paracellular (passive) glucose uptake in the African White-bellied Sunbirds (*Cinnyris talatala*) and New Holland Honeyeaters (*Phylidonyris nivaehollandiae*) increased with sugar concentration. Thus the high sugar assimilation efficiencies observed at high sugar concentrations in the Speckled Mousebirds may be due to sugar uptake being undertaken by two pathways working together, with the passive pathway playing a supportive role. However, a problem with this explanation is that the discrepancy in the assimilation efficiencies observed may also be an artefact of retention time. Because retention time within the gut may limit the extent of nutrient digestion and absorption that takes place (in this study, retention time was lower for low concentration diets), the low digestive efficiencies observed for lower concentration diets may be due to that absorption efficiencies were compromised at high intake rates (Witmer, 1999). Measurements of faecal sugar solute concentrations on diets differing in sugar concentration may resolve the issue of whether high assimilation efficiencies at high concentrations are due to high absorption rates or high sugar content (Witmer, 1998). This demonstrates the dangers of comparing digestive efficiencies on diets with different nutrient concentrations (Witmer, 1999).

5. Conclusion

The three species used in this study increased digesta transit times with an increase in sugar concentration in the diet, probably to accommodate a high nutrient density. Intake rates, on the other hand, increased with a decrease in sugar concentration, suggesting that birds fed to maintain a constant energy intake. Cape White-eyes and Speckled Mousebirds maintained a constant assimilated energy intake on sucrose diets but not on glucose diets. Red-winged Starlings did not maintain a constant assimilated energy intake at all, especially on sucrose diets due to a lack of significant sucrase activity. The assimilation efficiencies displayed by all species were typical of those found in other frugivorous birds except those displayed by

Red-winged Starlings on sucrose diets where they were very low. Overall, this study shows how physiological limitations can have dire consequences for fruit selection in frugivorous birds.

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References

- Afik, D., Karasov, W.H., 1995. The trade-offs between digestion rate and efficiency in warblers and their ecological implications. *Ecology* 76, 2247–2257.
- Akkada, A.R.A., Eadie, J.M., Howard, B.H., 1963. The biochemistry of rumen protozoa. *Biochemistry Journal* 89, 269–272.
- Ally, E., 2010. Avian fruit selection and sugar preferences. Unpublished MSc thesis, University of KwaZulu-Natal, Pietermaritzburg.
- Bairlein, F., 1996. Fruit eating in birds and its nutritional consequences. *Comparative Biochemistry and Physiology A* 113, 215–224.
- Baker, H.G., Baker, I., 1983. Floral nectar sugar constituents in relation to pollinator type. In: Jones, C.E., Little, R.J., (eds). *Handbook of experimental pollination ecology*, pp. 131–171.
- Baker, H.G., Baker, I., Hodges, S.A., 1998. Sugar composition of nectars and fruits consumed by birds and bats in the tropics and subtropics. *Biotropica* 30, 559–586.

- Bizaare, L., Coetzer, T.H.T., Downs, C.T., 2012. Disaccharidase presence and activities in a range of southern African frugivores. *Ostrich* 83, 165–168.
- Brown, K.J., Downs, C.T., 2003. Digestive efficiency of a generalist avian feeder, the Cape white-eye (*Zosterops pallidus*). *Comparative Biochemistry and Physiology A* 134, 739–748.
- Brown, M., Downs, C.T., Johnson, S.D., 2010a. Concentration-dependent sugar preferences of the malachite sunbird (*Nectarinia famosa*). *Auk* 127, 151–155.
- Brown, M., Downs, C.T., Johnson, S.D., 2010b. Sugar preferences of a generalist nonpasserine flower visitor, the African speckled mousebird (*Colius striatus*). *Auk* 127, 781–786.
- Brown, M., Downs, C.T., Johnson, S.D., 2012. African red-winged starlings prefer hexose solutions, but do not like them too sweet. *Journal of Ornithology* 153, 265–272.
- Brugger, K.E., Nol, P., Phillips, C.I., 1993. Sucrose repellency to European starlings: will high-sucrose cultivars deter bird damage to fruit? *Ecological Applications* 2, 256–261.
- Castle, K.T., Wunder, B.A., 1995. Limits to food intake and fiber utilization in the prairie vole *Microtus ochrogaster*: effects of food quality and energy need. *Comparative Biochemistry and Physiology A* 164, 609–617.
- Cipollini, M.L., Levey, D.J., 1997. Why are some fruits toxic? Glycoalkaloids in *Solanum* and fruit choice by vertebrates. *Ecology* 78, 782–798.
- Downs, C.T., 1997a. Sugar digestion efficiencies of Gurney's sugarbirds, malachite sunbirds and black sunbirds. *Physiological Zoology* 70, 93–99.

- Downs, C.T., 1997b. Sugar preference and apparent sugar assimilation in the red lory. *Australian Journal of Zoology* 45, 613–619.
- Downs, C.T., 2000. Ingestion patterns and daily energy intake on a sugary diet: the red lorry *Eos bornea* and the malachite sunbird *Nectarinia famosa*. *Ibis* 142, 359–364.
- Downs, C.T., 2008. Aspects of diet choice and digestion in the dark-capped bulbul *Pycnototus barbatus*. *Ostrich* 79, 73–78.
- Downs, C.T., Perrin, M.R., 1996. Sugar preferences of some southern African nectarivorous birds. *Ibis* 138, 455–459.
- Fleming, P.A., Bakken, B.H., Lotz, C.N., Nicolson, S.W., 2004. Concentration and temperature effects on sugar intake and preferences in a sunbird and a hummingbird. *Functional Ecology* 18, 223–232.
- Fleming, P.A., Xie, S., Napier, K., McWhorter, T.J., Nicolson, S.W., 2008. Nectar concentration affects sugar preferences in two Australian honeyeaters and a lorikeet. *Functional Ecology* 22, 599–605.
- Franke, E., Jackson, S., Nicolson, S., 1998. Nectar sugar preferences and absorption in a generalist African nectarivore, the Cape white-eye *Zosterops pallidus*. *Ibis* 140, 501–506.
- Gatica, C.D.L., Gonzalez, S.P., Vazquez, R.A., Sabat, P., 2006. On the relationship between sugar digestion and diet preference in two Chilean avian species belonging to the Muscicapoidea superfamily. *Revista Chilena de Historia Natural* 79, 287–294.
- Herrera, C.M., 1987. Vertebrate-dispersed plants of the Iberian Peninsula: a study of fruit characteristics. *Ecological Monographs* 57, 305–331.

- Hockey, P.A.R., Dean, W.R.J., Ryan, P.G., 2005. Robert's birds of southern Africa. 7th Edition. The Trustees of the John Voelcker Bird Book Fund, Cape Town.
- Izhaki, I., 1992. A comparative analysis of the nutritional quality of mixed and exclusive fruit diets for yellow-vented bulbuls. *Condor* 94, 912–923.
- Jackson, S., Nicolson, S.W., Lotz, C.N., 1998. Sugar preferences and “side bias” in Cape sugarbirds and lesser double-collared sunbirds. *Auk* 115, 156–165.
- Johnson, S.D., Nicolson, S.W., 2008. Evolutionary associations between nectar properties and specificity in bird pollination systems. *Biology Letters* 4, 49–52.
- Karasov, W.H., 1990. Digestion in birds: chemical and physiological determinants and ecological implications. In: Morrison, M.L., Ralpa, C.J., Verner, J., Jehl, J.J.R., (Eds), *Avian Foraging Theory, Methodology and Applications: Studies in Avian Biology*. Cooper Ornithological Society Publishing, pp. 391–415.
- Karasov, W.H., Levey, D.J., 1990. Digestive system trade-offs and adaptations of frugivorous passerine birds. *Physiological Zoology* 63, 1248–1270.
- Levey, D.J., 1987. Sugar-tasting ability and fruit selection in tropical fruit-eating birds. *Auk* 104, 173–179.
- Levey, D.J., Grajal, A., 1991. Evolutionary implications of fruit-processing limitations in cedar waxwings. *American Naturalist* 138, 171–189.
- Levey, D.J., Karasov, W.H., 1989. Digestive responses of temperate birds switched to fruit or insect diets. *Auk* 106, 675–686.
- Levey, D.J., Karasov, W.H., 1992. Digestive modulation in a seasonal frugivore, the American robin (*Turdus migratorius*). *American Journal of Physiology* 262, 711–718.

- Levey, D.J., Martinez del Rio, C., 1999. Test, rejection and reformulation of a chemical-reactor based model of gut function in a fruit-eating bird. *Physiological and Biochemical Zoology* 72, 369–383.
- Levey, D.J., Martinez del rio, C., 2001. It takes guts (and more) to eat fruits: lessons from avian nutritional ecology. *Auk* 118, 819–831.
- Lopez-Calleja, M.V., Bozinovic, F., Martinez del Rio, C., 1997. Effects of sugar concentration on hummingbird feeding and energy use. *Comparative Biochemistry and Physiology A* 118, 1291–1299.
- Lotz, C.N., Nicolson, S.W., 1996. Sugar preferences of a nectarivorous passerine bird, the lesser double-collared sunbird (*Nectarinia chalybea*). *Functional Ecology* 10, 360–365.
- Lotz, C.N., Schondube, J.E., 2006. Sugar preferences in nectar- and fruit-eating birds: behavioural patterns and physiological causes. *Biotropica* 38, 3–15.
- Malcarney, H.L., Martinez del Rio, C., Apanius, V., 1994. Sucrose intolerance in birds: simple non-lethal diagnostic methods and consequences for assimilation of complex carbohydrates. *Auk* 111, 170–177.
- Martinez del Rio, C., 1990. Sugar preferences in hummingbirds: the influence of subtle chemical differences on food choice. *Condor* 92, 1022–1030.
- Martinez del Rio, C., Baker, H.G., Baker, I., 1992. Ecological and evolutionary implications of digestive processes: bird preferences and the sugar constituents of floral nectar and fruit pulp. *Experientia* 48, 544–551.
- Martinez del Rio, C., Karasov, W.H., 1990. Digestion strategies in nectar- and fruit-eating birds and the sugar composition of plant rewards. *American Naturalist* 136, 618–637.

- Martinez del Rio, C., Karasov, W.H., Levey, D.J., 1989. Physiological basis and ecological consequences of sugar preference in cedar waxwings. *Auk* 106, 64–71.
- Martinez del Rio, C., Schondube, J.E., McWhorter, T.J., Herrera, L.G., 2001. Intake responses in nectar feeding birds: digestive and metabolic causes, osmoregulatory consequences, and coevolutionary effects. *American Zoology* 41, 902–915.
- Martinez del Rio, C., Stevens, B.R., 1989. Physiological constraint on feeding behaviour: intestinal membrane disaccharidases of the starling. *Science* 243, 794–796.
- Martinez del Rio, C., Stevens, B.R., Daneke, D.E., Andreadis, P.T., 1988. Physiological correlates of preference and aversion for sugars in three species of birds. *Physiological Ecology* 3, 222–229.
- McKey, D., 1975. The ecology of coevolved seed dispersal systems. In: Gilbert, L.E., Raven, P.H., (Eds). *Coevolution of Animals and Plants*. University of Texas Press, Austin, pp. 159–191.
- McWhorter, T.J., Lopez-Calleja, M.V., 2000. The integration of diet, physiology, and ecology of nectar-eating birds. *Revista Chilena de Historia Natural* 73, 451–460.
- McWhorter, T.J., Martinez del Rio, C., 2000. Does gut function limit hummingbird food intake? *Physiological and Biochemical Zoology* 73, 313–324.
- Moermond, T.C., Denslow, J.S., 1985. Neotropical avian frugivores: patterns of behavior, morphology, and nutrition, with consequences for fruit selection. *Ornithological Monographs* 36, 865–897.
- Murray, K.G., Winnett-Murray, K., Cromie, E.A., Minor, M., Meyers, E., 1993. The influence of seed packaging on feeding preferences of American robins. *Vegetatio* 107/108, 217–226.

- Napier, K.R., McWhorter, T.J., Nicolson, S.W., Fleming, P.A., 2013. Sugar preferences of avian nectarivores are correlated with intestinal sucrase activity. *Physiological and Biochemical Zoology* 86, 499–514.
- Napier, K.R., Purchase, C., McWhorter, T.J., Nicolson, S.W., Fleming, P.A., 2008. The sweet life: diet sugar concentration influences paracellular glucose absorption. *Biology Letters* 4, 530 – 533.
- Nicolson, S.W., Fleming, P.A., 2003a. Energy balance in the whitebellied sunbird *Nectarinia talatala*: constraints on compensatory feeding, and consumption of supplementary water. *Functional Ecology* 17, 3–9.
- Nicolson, S.W., Fleming, P.A., 2003b. Nectar as food for birds: the physiological consequences of drinking dilute sugar solutions. *Plant Systematics and Evolution* 238, 139–153.
- Odendaal, T.C., Brown, M., Downs, C.T., Johnson, S.D., 2010. Sugar preferences and digestive efficiency of the village weaver: a generalist avian pollinator of African plants. *Journal of Experimental Biology* 213, 2531–2535.
- Pooley, E., 1993. The complete field guide to trees of Natal Zululand and Transkei. Natal Flora Publications Trust, Durban.
- Schondube, J.E., Martinez del Rio, C., 2003. Concentration-dependent sugar preferences in nectar-feeding birds: mechanisms and consequences. *Functional Ecology* 17, 445–453.
- Stanley, M.C., Lill, A., 2002. Importance of seed ingestion to an avian frugivore: an experimental approach to fruit choice based on seed load. *Auk* 119, 175–184.

- Wellmann, A.E., Downs, C.T., 2009. Sugar preferences and digestion by Cape white-eyes, *Zosterops virens*, fed artificial fruit diets. *African Zoology* 44, 106–116.
- Wilson, A-L, Downs, C.T., 2011. Digestive efficiency of Knysna and purple-crested turacos fed varying concentrations of equicaloric and equimolar artificial fruit. *Journal of Experimental Biology* 214, 607–612.
- Witmer, M.C., 1998. Ecological and evolutionary implications of energy and protein requirements of avian frugivores eating sugary fruits. *Physiological Zoology* 71, 599–610.
- Witmer, M.C., 1999. Do avian frugivores absorb fruit sugars inefficiently? How dietary nutrient concentration can affect coefficients of digestive efficiency. *Journal of Avian Biology* 30, 159–164.
- Witmer, M.C., van Soest, P.J., 1998. Contrasting digestive strategies of fruit-eating birds. *Functional Ecology* 12, 728–741.
- Worthington, A.H., 1989. Adaptations for avian frugivory: assimilation efficiency and gut transit time of *Manacus vitellinus* and *Pipra mentalis*. *Oecologia* 80, 381–389.

Figure legends

Fig. 1. Amount of food eaten by the Cape White-eyes on equicaloric glucose and sucrose artificial fruit diets of varying concentrations ($n = 9$).

Fig. 2. Gross energy intake (a) and excreta energy loss (b) of Cape White-eyes fed equicaloric glucose and sucrose diets of varying concentrations ($n = 9$). All values are expressed as mean \pm S.E.

Fig. 3. Daily energy assimilation (a) and apparent assimilation efficiencies (b) of Cape White-eyes fed equicaloric glucose and sucrose diets of varying concentrations ($n = 9$). All values are expressed as mean \pm S.E.

Fig. 4. Amount of food eaten by Red-winged Starlings on equicaloric glucose and sucrose artificial fruit diets of varying concentrations ($n = 6$). All values are expressed as mean \pm S.E.

Fig. 5. Gross energy intake (a) and excreta energy loss (b) of Red-winged Starlings fed equicaloric glucose and sucrose diets of varying concentrations ($n = 6$). All values are expressed as mean \pm S.E.

Fig. 6. Daily energy assimilation (a) and apparent assimilation efficiencies (b) of Red-winged Starlings fed equicaloric glucose and sucrose diets of varying concentrations ($n = 6$). All values are expressed as mean \pm S.E.

Fig. 7. Amount of food eaten by Speckled Mousebirds on equicaloric glucose and sucrose artificial fruit diets of varying concentrations ($n = 6$). All values are expressed as mean \pm S.E.

Fig. 8. Gross energy intake (a) and excreta energy loss (b) of Speckled Mousebirds fed equicaloric glucose and sucrose diets of varying concentrations ($n = 6$). All values are expressed as mean \pm S.E.

Fig. 9. Daily energy assimilation (a) and apparent assimilation efficiencies (b) of Speckled Mousebirds fed equicaloric glucose and sucrose diets of varying concentrations ($n = 6$). All values are expressed as mean \pm S.E.

Table 1. Composition of the artificial fruit diets used in this study.

Ingredients	Diet composition (g)		
	Low	Medium	High
D-glucose	75	150	300
Equicaloric sucrose	71.2	142.4	284.8
Water (ml)	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. Digesta transit times (min) of Cape White-eyes (*Zosterops virens*, $n = 9$), Red-winged Starlings (*Onychognathus morio*, $n = 6$) and Speckled Mousebirds (*Colius striatus*, $n = 6$) when fed equicaloric glucose and sucrose artificial fruit diets of varying concentrations. Values with the same letter are not significantly different at the 5% level. Significant differences were based on Tukey's *post-hoc* test.

Species	Sugar	Concentration		
		Low	Medium	High
<i>C. striatus</i>	Glucose	25.4 ± 2.7 ^a	34.0 ± 4.2 ^a	57.6 ± 4.5 ^a
	Sucrose	24.6 ± 3.1 ^a	47.0 ± 2.6 ^a	76.0 ± 3.4 ^a
<i>O. morio</i>	Glucose	65.2 ± 1.4 ^a	62.6 ± 2.1 ^a	76.3 ± 2.1 ^b
	Sucrose	36.7 ± 3.3 ^c	40.0 ± 1.3 ^c	42.8 ± 1.6 ^c
<i>Z. virens</i>	Glucose	22.1 ± 1.0 ^a	24.6 ± 0.3 ^b	28.9 ± 0.6 ^c
	Sucrose	25.7 ± 0.3 ^b	28.9 ± 0.4 ^c	30.2 ± 0.7 ^c

Table 3. Changes in body mass in the Cape White-eyes, Red-winged Starlings and Speckled Mousebirds when fed equicaloric glucose and sucrose artificial fruit diets of varying concentrations. Negative values in the mean difference columns indicate a gain in body mass. Significant differences in body masses are highlighted in bold. GL, GM and GH indicate glucose low (6.6%), medium (12.4%) and high (22%), respectively. Similarly, SL, SM and SH indicate sucrose low (6.6%), medium (12.4%) and high (22%), respectively.

Species	Treatment	Mean difference	<i>t</i>	df	<i>P</i>
<i>C. striatus</i>	GL	1.4 ± 0.86	1.655	5	0.159
	GM	-0.2 ± 0.34	-0.631	5	0.556
	GH	2.1 ± 0.80	2.614	5	0.047
	SL	-2.4 ± 0.89	-2.673	5	0.044
	SM	-2.8 ± 0.31	-9.052	5	0.001
	SH	-1.1 ± 0.45	-2.515	5	0.053
<i>O. morio</i>	GL	-3.8 ± 0.65	-5.391	5	0.003
	GM	-1.4 ± 1.22	-1.135	5	0.308
	GH	-1.1 ± 0.67	-1.610	5	0.168
	SL	3.9 ± 0.69	5.578	5	0.003
	SM	4.3 ± 0.80	5.357	5	0.003
	SH	8.2 ± 1.06	7.769	5	0.001
<i>Z. virens</i>	GL	0.3 ± 0.07	4.318	8	0.003
	GM	-0.1 ± 0.14	-0.529	8	0.611
	GH	0.0 ± 0.18	0.025	8	0.981
	SL	0.2 ± 0.09	2.114	8	0.068
	SM	-0.2 ± 0.18	-1.061	8	0.319
	SH	-0.2 ± 0.19	-0.859	8	0.415

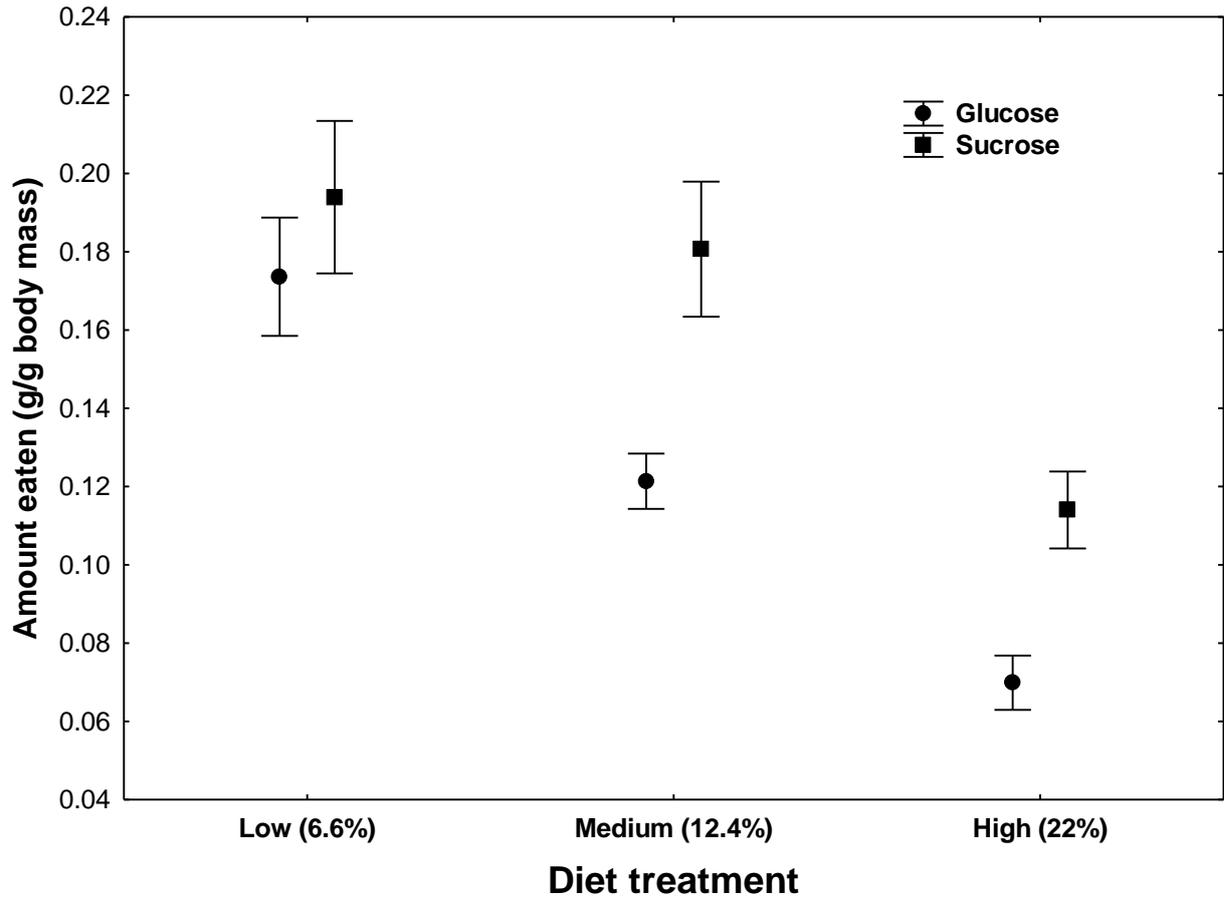


Fig. 1. Amount of food eaten by the Cape White-eyes on equicaloric glucose and sucrose artificial fruit diets of varying concentrations ($n = 9$). All values are expressed as mean \pm S.E.

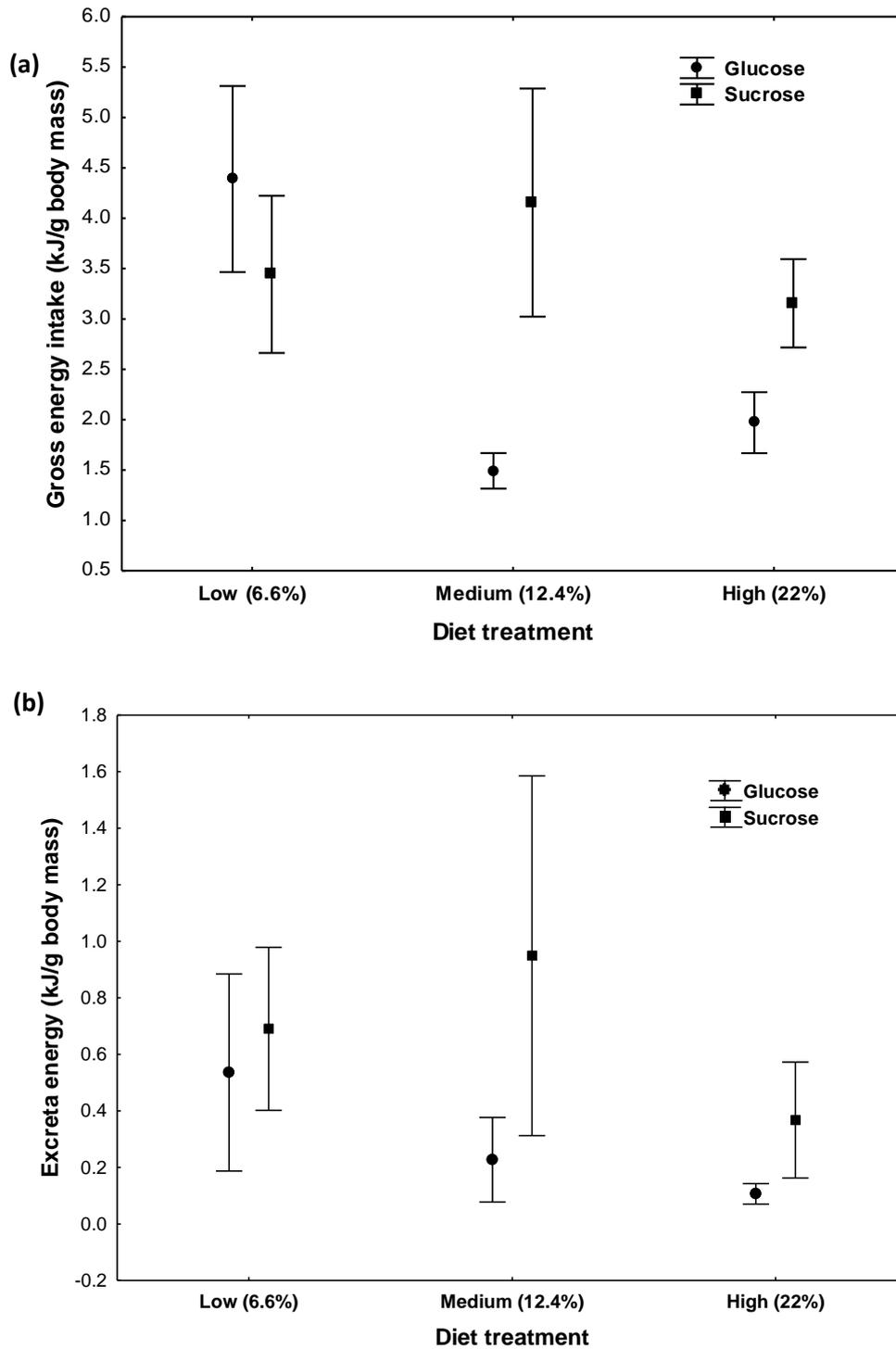


Fig. 2. Gross energy intake (a) and excreta energy loss (b) of Cape White-eyes fed equicaloric glucose and sucrose diets of varying concentrations ($n = 9$). All values are expressed as mean \pm S.E.

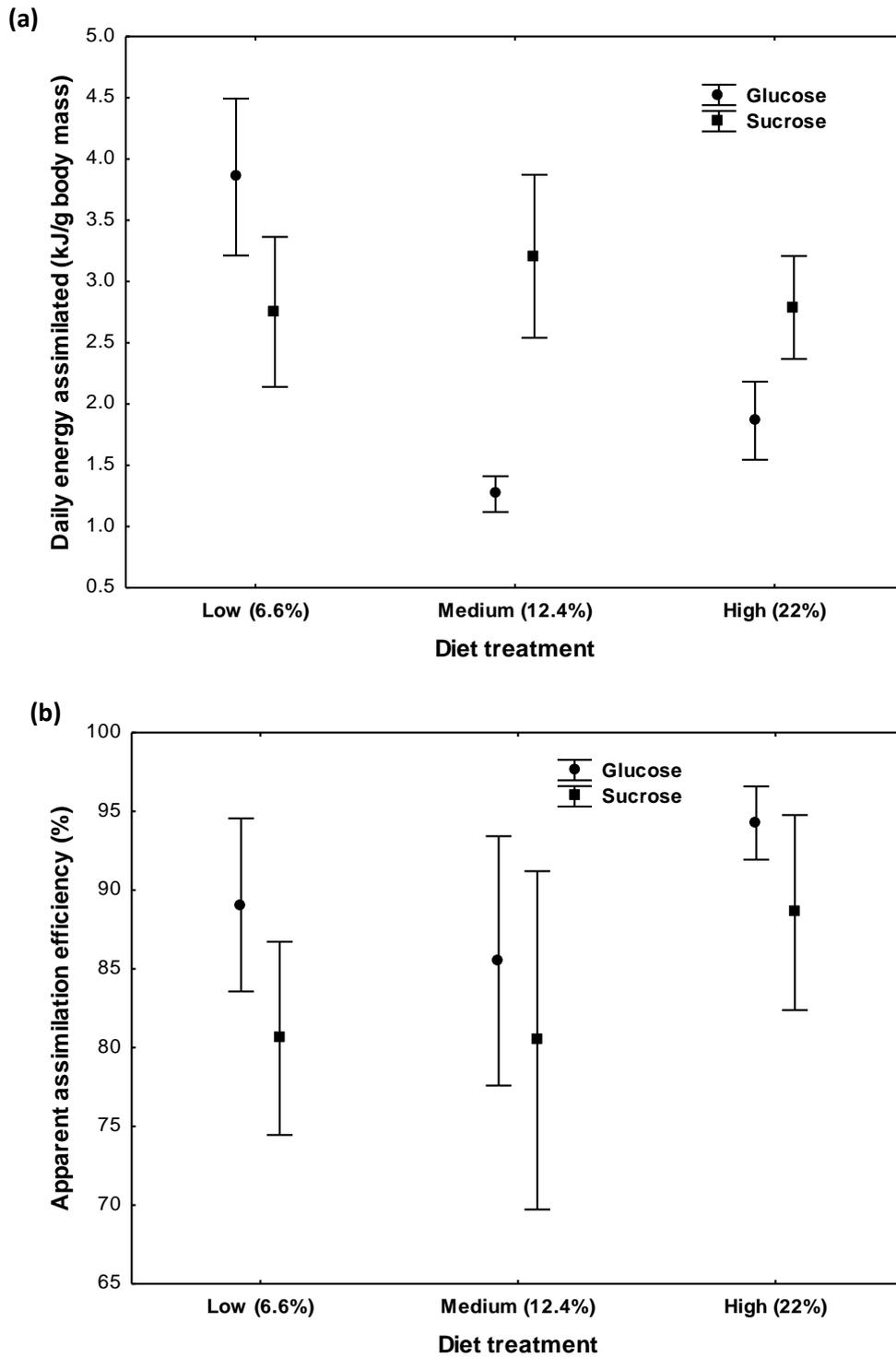


Fig. 3. Daily energy assimilation (a) and apparent assimilation efficiencies (b) of Cape White-eyes fed equicaloric glucose and sucrose diets of varying concentrations ($n = 9$). All values are expressed as mean \pm S.E.

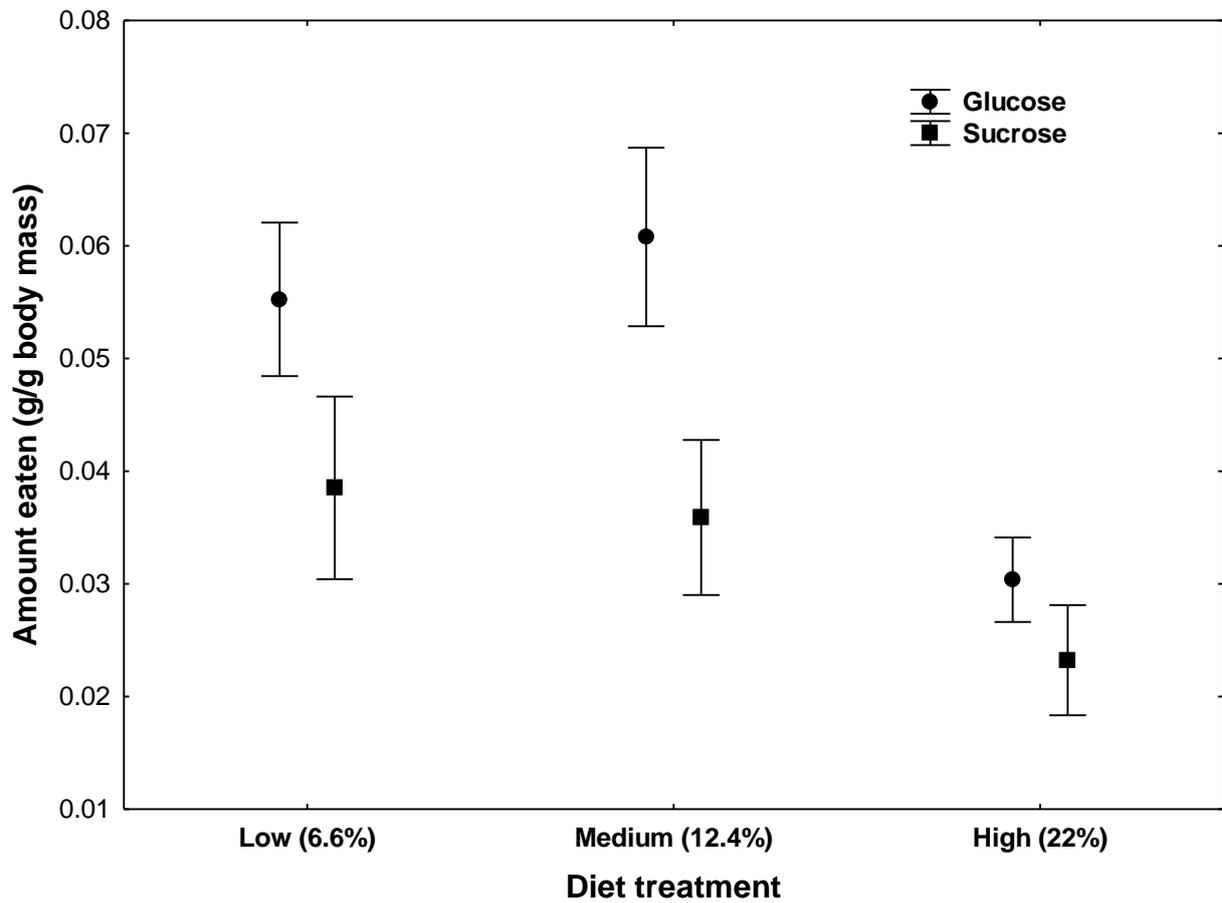


Fig. 4. Amount of food eaten by Red-winged Starlings on equicaloric glucose and sucrose artificial fruit diets of varying concentrations ($n = 6$). All values are expressed as mean \pm S.E.

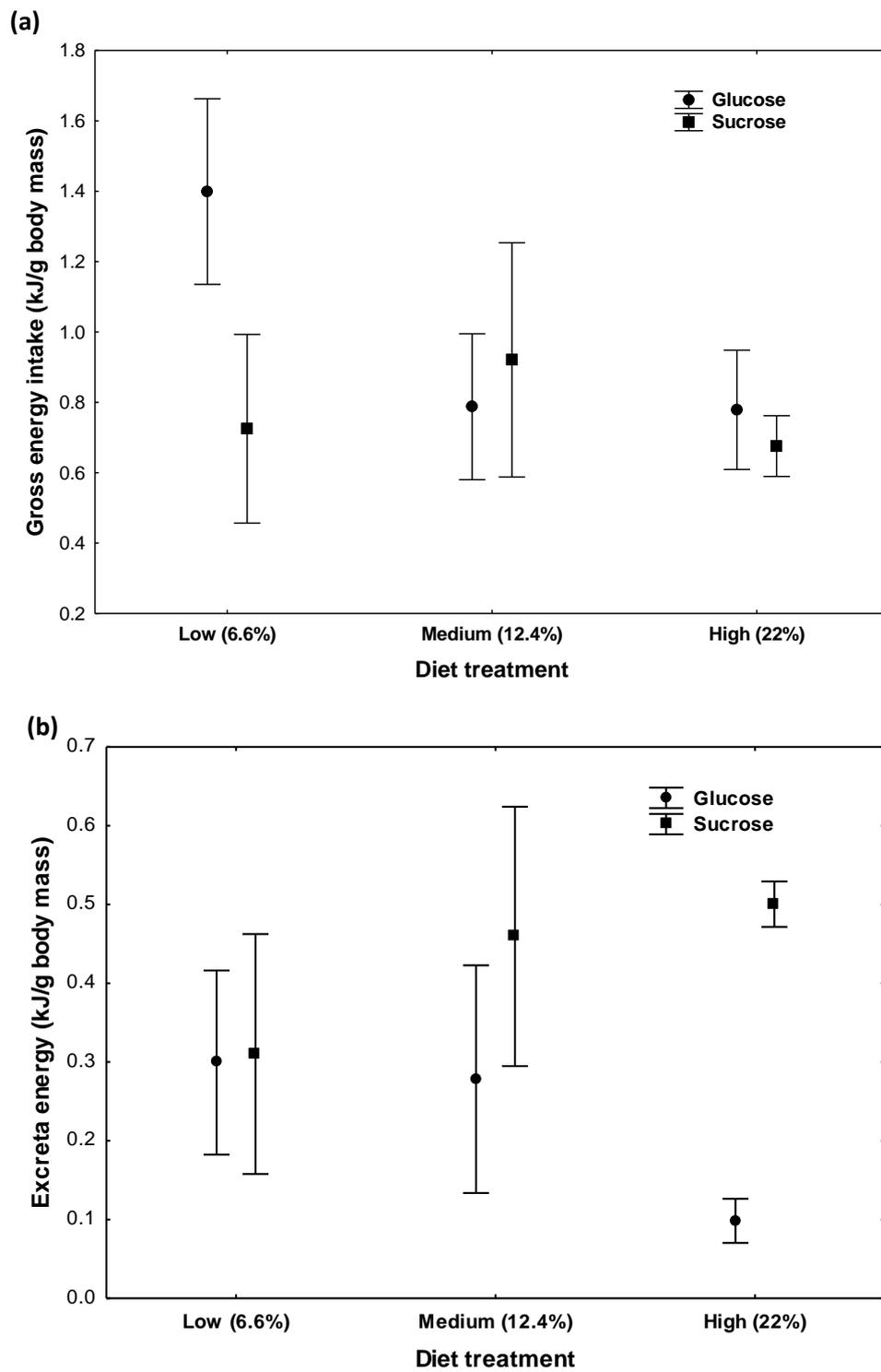


Fig. 5. Gross energy intake (a) and excreta energy loss (b) of Red-winged Starlings fed equicaloric glucose and sucrose diets of varying concentrations ($n = 6$). All values are expressed as mean \pm S.E.

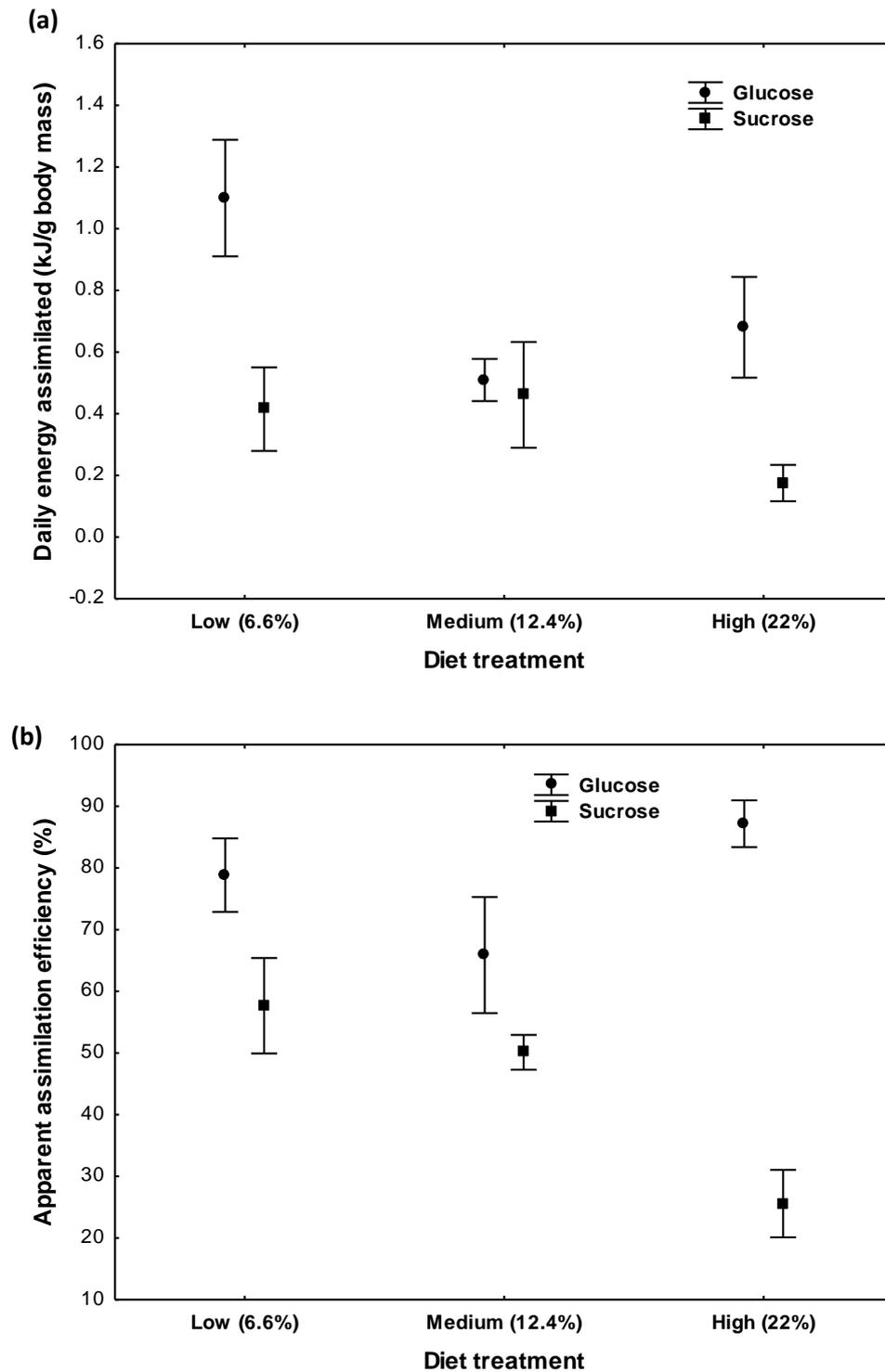


Fig. 6. Daily energy assimilation (a) and apparent assimilation efficiencies (b) of Red-winged Starlings fed equicaloric glucose and sucrose diets of varying concentrations ($n = 6$). All values are expressed as mean \pm S.E.

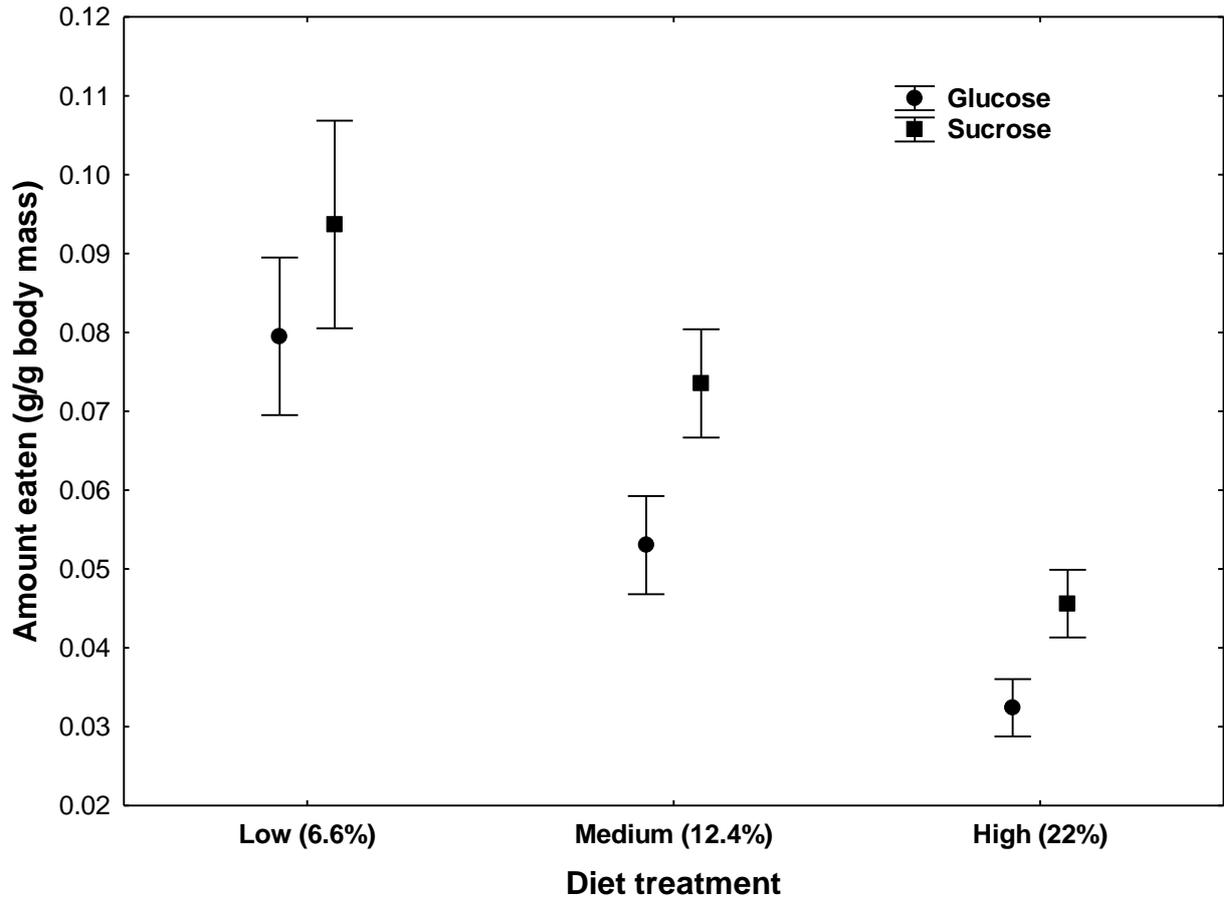


Fig. 7. Amount of food eaten by Speckled Mousebirds on equicaloric glucose and sucrose artificial fruit diets of varying concentrations ($n = 6$). All values are expressed as mean \pm S.E.

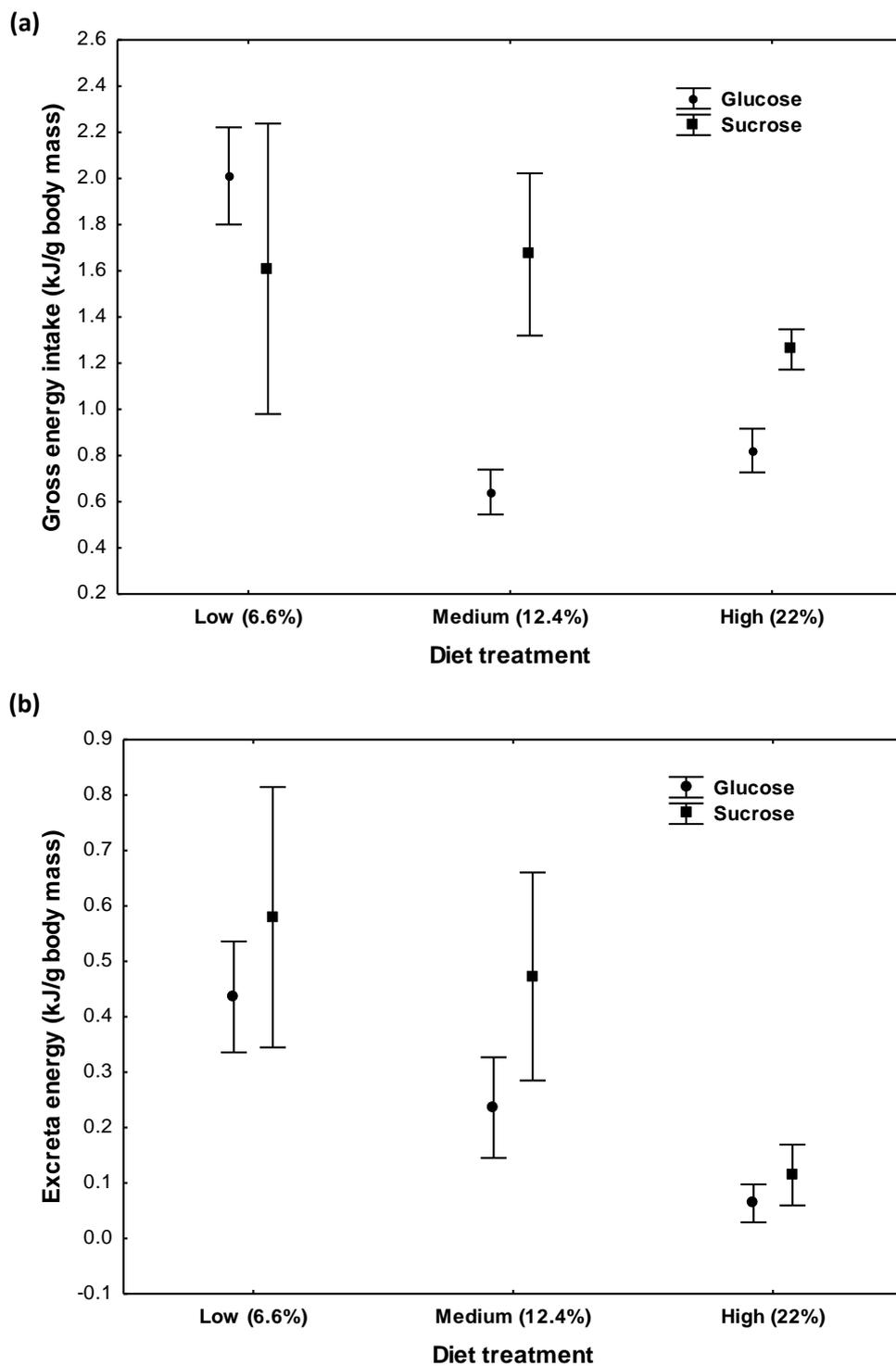


Fig. 8. Gross energy intake (a) and excreta energy loss (b) of Speckled Mousebirds fed equicaloric glucose and sucrose diets of varying concentrations ($n = 6$). All values are expressed as mean \pm S.E.

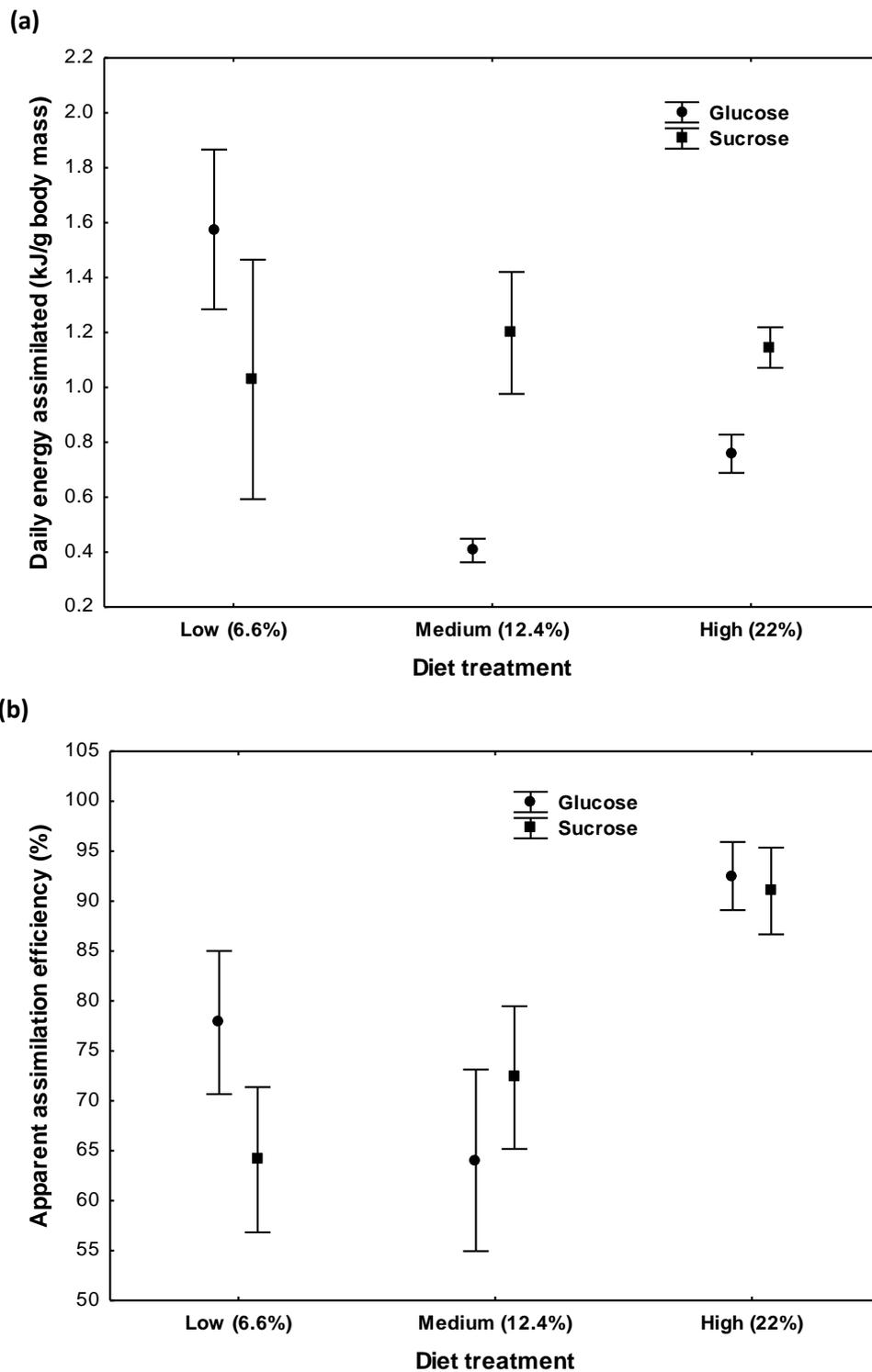


Fig. 9. Daily energy assimilation (a) and apparent assimilation efficiencies (b) of Speckled Mousebirds fed equicaloric glucose and sucrose diets of varying concentrations ($n = 6$). All values are expressed as mean \pm S.E.

CHAPTER 3

Effects of tannins on fruit selection in three southern African frugivorous birds

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ABSTRACT

Tannins are common secondary compounds in plant material and are known for their ability to bind to protein which reduces nitrogen availability in the diet. In fruits, these compounds are responsible for their astringency which is thought to result in reduced food intake. In this study, the repellent effects of tannins were examined in three species of frugivorous birds: red-winged starlings *Onychognathus morio*, speckled mousebirds *Colius striatus* and Cape white-eyes *Zosterops virens*. Birds were fed artificial fruit diets containing varying levels of tannins in paired choice tests with the amount of food eaten by birds used to determine preference. Red-winged starlings preferred the control diet, were indifferent to the medium tannin diet and were deterred by the high tannin diet whereas speckled mousebirds and Cape white-eyes were not deterred at all concentrations. The discrepancy in the results was attributed to differences in taste sensitivity, tolerance levels and detoxification mechanisms of secondary compounds between species. Because fruit selection and ultimately fruit removal rates affect plant community composition, the disparity in the results suggests that

frugivorous birds do not contribute equally to plant community dynamics. However, plant secondary compounds in fruits are diverse and their effects are similarly diverse and there is potential that different groups of secondary compounds generate disparate effects. Similar studies on other types of secondary compounds may thus contribute towards a broader understanding of the role of secondary compounds in mediating fruit-frugivore interactions.

Keywords: birds, seed dispersal, frugivores, secondary compounds, tannins

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1. Introduction

A central theme that has dominated research on plant-frugivore interactions is how the reciprocal selection pressures between frugivores and plants have shaped the morphological, behavioural and chemical traits of each group (Herrera, 1982*a*; Jordano, 1987; 1995). Because plants depend on frugivores for the dissemination of their seeds, conventional ecological wisdom predicts that frugivores exert selection pressures on fruit traits, especially those promoting effective dispersal (Howe and Smallwood, 1982; Herrera 2002). Earlier studies on fruit-frugivore interactions advocated the notion that fruit selection by frugivores exerts selection pressures on fruit traits (Herrera, 1987; Gautier-Hion et al., 1985; Debussche and Isenmann, 1989; Willson et al., 1989). However, findings from these studies cannot be considered as evidence that frugivores influence fruit traits as they did not formulate alternative, phylogenetically informed null models (Herrera, 1992). Indeed, studies utilising rigorous phylogenetic null models have found little correspondence between fruit traits and frugivore selection patterns (Herrera, 1992; Jordano, 1995), suggesting that frugivores exert very weak selective pressures on fruit traits (Howe and Smallwood, 1982; Herrera, 1982*a*; Jordano, 1987). The integration of digestive physiology as a central ingredient in studies on

fruit-frugivore interactions has been seen as a promising approach in deciphering the frugivore influence on fruit traits as it has produced the highest degree of correspondence between digestive abilities and food preference in birds (e.g. Martinez del Rio et al., 1988; 1989; Martinez del Rio and Restrepo, 1993). However, these studies suffer from that (1) the patterns of fruit selection in frugivores may reflect morphological and physiological adaptations to independently evolving fruits and that (2) frugivores may be feeding only on fruits to which they are pre-adapted (Cipollini and Levey, 1997a); thus the selection patterns observed can be fully explained by ecological fitting processes.

Despite the huge research effort, there is still no consensus on selection forces driving plant-frugivore interactions, probably because most studies have focused only on vertebrate frugivores, ignoring the microbial ones (Cipollini and Stiles, 1993; Cipollini and Levey, 1997b; Levey et al., 2007). Fruits are self-advertising structures which may attract a plethora of organisms of which some may be fruit-damaging agents (Herrera, 1982a; Cipollini and Stiles, 1993). Therefore the consideration of fruit-damaging agents is important as they may have far-reaching effects on plant fitness since their impacts may balance out or even override those of seed dispersers (Jordano, 1987b; Cipollini and Stiles, 1993). Tewksbury (2002) suggested that microbial fruit consumers are more likely to exert stronger selection pressures on nutritional, morphological and chemical traits of fruits because they are ever-present, consume fruits and do not disperse seeds. Viewed in this context, plants face a paradox on how to remain attractive to frugivores while being deterrent to fruit-damaging agents (Levey et al., 2006; 2007; Tewksbury et al., 2008). Secondary compounds have been proposed to be the mediators of this conflict (Cipollini and Levey, 1997b; Wahaj et al., 1998; Cazetta et al., 2008) and can change this plant-animal interaction from predation to mutualism and vice versa within an ecological timescale (Samuni-Blank et al., 2012; 2013).

Plant secondary compounds are compounds with no primary metabolic function in plant development (Whitehead and Bowers, 2013). Several studies have shown that fruit pulp is laden with a number of secondary compounds (Barnea et al., 1993; Tewksbury et al., 2006, 2008; Ndhlala et al., 2008). There are two schools of thought regarding the evolution of secondary compounds in fruit (Tsahar et al., 2002; Whitehead and Bowers, 2013). The adaptive approach holds that secondary compounds in fruits have several adaptive roles such as prevention of pre-dispersal predation, reducing the length of foraging bouts, defending fruits against predators and deterring ineffective seed dispersers (Cipollini and Levey, 1997*a*; *b*; Cipollini, 2000). Empirical tests of these hypotheses has been slow but most studies conducted so far seem to support the notion of a trade-off between traits that attract dispersers and those that deter potential damaging agents (Cipollini and Stiles, 1993; Cipollini and Levey, 1997*b*; Tsahar et al., 2002; Cazetta et al., 2008; Tewksbury et al., 2008). The non-adaptive approach posits that secondary compounds in fruits occurred as a happenstance of the general defence mechanism in leaves and other plant tissues (Ehrlen and Eriksson, 1993; Eriksson and Ehrlen, 1998). A number of studies on secondary compounds in floral nectar are in support of this view (reviewed in Adler, 2000). However, that fruit chemistry is neither constrained nor controlled by leaf chemistry (Cipollini et al., 2004), that secondary compounds decline with the fruit ripening process (Cipollini and Stiles, 1993; Levey et al., 2007) and that some secondary compounds are exclusive to ripe fruit (Cipollini, 2000; Whitehead and Bowers, 2013) suggest an adaptive function of secondary compounds in fruits.

Tannins are ubiquitous secondary compounds in fruits and are known for binding with proteins which is already deficient in fruit pulp (Izhaki, 1993), resulting in body mass loss in birds fed fruit-only diets (Izhaki and Safriel, 1989; Izhaki, 1992; Witmer, 1998; Witmer, 2001). The ingestion of tannins creates an acidic load in the gut which requires acid buffering

by the breakdown of amino acids to create bicarbonates (Guglielmo et al., 1996; Witmer, 2001). This leads to increases in nitrogen and energy requirements by 90% and 10–14%, respectively (Guglielmo et al., 1996). Furthermore, tannins attack the gut lining of vertebrates causing considerable damage (Gilardi et al., 1999; Mbatha et al., 2002). Due to the aforementioned negative consequences associated with tannin ingestion, their presence in fruits is expected to result in reduced fruit attractiveness which should result in reduced food intake (Herrera, 1982*b*).

Condensed tannins are ideal compounds for the study of the effects of secondary compounds on frugivores because they are ubiquitous in fruits, have antimicrobial and antifungal properties (Cipollini and Stiles, 1993) and have deterrence effects (Schaefer et al., 2003). There is a paucity of data on the amount of condensed tannins in wild fruits in Africa. Ndhkala et al. (2007) found that the tannin content (wet mass) of three fruits in Zimbabwe (*viz.* *Flacourtia indica*, *Opuntia megacantha* and *Sclerocarya birrea*) ranged from 1.4–6%. In another study on 16 wild fruits from northern Nigeria, the wet mass tannin content ranged from 0.9–7.4% (Umaru et al., 2007).

This study was conducted to determine the deterrent effects of secondary compounds in frugivorous birds. Three frugivorous bird species native to southern Africa were used for this study: red-winged starlings, *Onychognathus morio*, speckled mousebirds, *Colius striatus* and Cape white-eyes *Zosterops virens*. These three frugivores have mixed diets consuming varying proportions of insects, fruits, leaves and nectar (Downs et al., 2000; Brown and Downs, 2003; Brown et al., 2012). Because the proportions of these food types in the diet differ with species, the tannin content in their diet was expected to be different, which we expected to influence their tannin tolerance. Because speckled mousebirds become exclusively folivorous during periods of fruit scarcity (Downs et al., 2000) and because leaves generally have higher tannin content than other plant tissues (Barnea et al 1993),

mousebirds were expected to have high tannin tolerance. Red-winged starlings were expected to have low tannin tolerance because although they are considered frugivores, their diet includes a substantial amount of insects and nectar (Brown et al., 2012) which probably have low tannin content. White-eyes were expected to display intermediate levels of secondary compound tolerance as they consume a mixed diet including fruits, nectar and insects (Brown and Downs, 2003). It was predicted that tannins would have no effect at low concentrations but at higher concentrations would act as deterrents although the levels at which they would become deterrents would differ among species.

2. Materials and Methods

2.1 Study area and animal maintenance

The study was conducted at the Animal House of the University of KwaZulu-Natal (UKZN), Pietermaritzburg (29°37'0"S; 30°23'0"E, 660 m above sea level). Red-winged starlings ($n = 5$), speckled mousebirds ($n = 5$) and Cape white-eyes ($n = 6$) captive at the Animal House were used. All the birds used were captured in the vicinity of Pietermaritzburg. These birds were kept in species groups in large holding cages (1 × 2.12 × 2.66 m) and were exposed to natural photoperiod, humidity and temperature. All birds used were adults and had been in captivity for more than 12 months prior to the study and were fully acclimated to captivity and food preference trials. Birds were fed a maintenance diet consisting of chopped-up fruit mix including bananas, apples, pears, pawpaw and oranges. An avian supplement diet (AviPlus Softbill Maintenance Crumble; Aviproducts, Waterfall, KZN) was administered together with fruits. Feeding took place every morning between 07h00 and 08h00. Five days prior to the start of the experiment, birds were transferred to

individual cages in a controlled environment room (12L: 12D photoperiod; temperature $25 \pm 0.1^{\circ}\text{C}$) and were fed on the same schedule as they were in outside aviaries.

2.2 Experimental approach

Three artificial fruit diets of varying tannin concentrations were used in this study. The first diet had no tannin added to it (0%), thus serving as the control. The 2.5% tannin diet represented the medium tannin concentration and the 5% tannin diet represented the high tannin concentration. The tannin used in this study was the commercialized condensed tannin (Wattle Bark Industry, South Africa) of wattle extract composition. This tannin was used because it was the only available commercialized form in the country.

Artificial fruit diets used in this study were based on those designed by Witmer (1998) for North American frugivores. Diets were prepared by mixing all ingredients (Table 1) into 1 litre of boiling water. The mixture was then heated in a microwave oven (for approximately 8 min) until boiling, and then poured into a flat tray to cool, with continuous stirring by hand to ensure that ingredients distributed equally. After the mixture had cooled but before it had solidified, 5 ml of a Robertson's red food colourant (Libstar Manufacturing Solutions, Chloorkop, South Africa) was poured to improve the appearance of the diets and also to mask the colour differences in diets induced by differing tannin levels. Diets were prepared on the afternoon preceding the experimental trials and kept in a refrigerator overnight due to the short lifespan of the artificial diets.

2.3 Experimental trials

During the experimental trials, food was cut into 1×1 cm cubes and put in metal containers to be presented to birds. Diets were administered to birds in a pairwise manner to compare the amount of food eaten between them. Throughout the experiments arranged in a randomized order, pairs were arranged in the following way: 0% vs 2.5%, 0% vs 5% and 2.5% vs 5%. Containers were placed equidistant from the bird's perches inside the cage and from the sides of the cage to ensure that birds travelled the same distance to acquire food from each dish. Right-left position of food was randomized hourly to minimize the effect of side bias (Jackson et al., 1998). Water was provided *ad libitum* throughout all experimental trials. Each trial lasted for 6 h starting from 06h00 to 12h00. At each hour interval, all containers were removed and weighed to quantify diet consumption and returned to birds thereafter. All food item measurements were rounded-off to the nearest 0.01 g. To allow correction for evaporative water loss, containers of each diet were placed in an empty cage and weighed on the same schedule as the other containers. Total food consumption for each diet was calculated by taking the difference between the initial and final mass of the food and subtracting the amount of mass lost through evaporation.

2.4 Statistical analyses

As each bird was used more than once, a General Linear Model repeated measures ANOVA (RMANOVA) was used to determine the overall effect of tannin concentration on food consumption between diets. The preference values of each diet (calculated as the amount of each diet type consumed divided by the total amount of food consumed that day) were compared to 0.5 (no preference) using one-sample *t*-tests (Brown et al., 2010). Since the preference values were proportions, they were subjected to arcsine square root transformation

before being subjected to one-sample *t*-tests (Brown et al., 2010) and subsequent analyses were based on these transformed values. All statistical analyses were conducted using STATISTICA 7.0 (Statsoft Inc., Tulsa, Oklahoma, USA).

3. Results

Tannin level significantly affected food intake in the red-winged starlings (RMANOVA; $F_{(2, 18)} = 36.71$, $P < 0.05$; Fig. 1a.). Starlings significantly preferred the 0% diet, were indifferent (did not prefer or were not deterred by) to the 2.5% diet and were deterred by the 5% tannin diet (one-sample *t*-tests: $t = 38.89$, $P < 0.05$; $t = 1.42$, $P = 0.19$; $t = -7.26$, $P < 0.05$, respectively). On the other hand, tannin level had no significant effect on food intake in the Cape white-eyes (RMANOVA: $F_{(2, 22)} = 9.20$, $P = 0.41$; Fig. 1b.). Cape white-eyes were not deterred by any of the diets (one-sample *t*-tests: $t = 7.70$, $P = 0.0005$, $t = 9.35$, $P = 0.005$; $t = 6.72$, $P = 0.0005$ for 0%, 2.5% and 5% tannin diets, respectively). Similarly, no significant effect was observed for the speckled mousebirds (RMANOVA; $F_{(2, 18)} = 3.65$, $P = 0.50$, Fig. 1c.). Mousebirds significantly preferred the 0% and 2.5% tannin diets (one-sample *t*-tests: $t = 6.34$, $P < 0.05$ and $t = 6.51$, $P < 0.05$, respectively) but were indifferent to the 5% tannin diet (one-sample *t*-test: $t = 1.80$, $P = 0.11$).

4. Discussion

The three bird species used in this study responded differently to the presence of secondary compounds in the diet. Red-winged starlings preferred the control diet, were indifferent to the medium tannin concentration and were deterred by the high tannin concentration in the diet. These results correspond to a number of previous studies which have found birds to be deterred by high secondary compound concentrations (Cipollini and

Stiles, 1993; Levey and Cipollini, 1998; Struempf et al., 1999; Tsahar et al., 2002). Contrastingly, speckled mousebirds and Cape white-eyes were not deterred by tannins at all concentrations, although mousebirds were indifferent to the high tannin concentration. Similarly, a glycoalkaloid amygdalin did not deter Cedar waxwings, *Bombycilla cedrorum*, even at higher concentrations with birds showing no preference for amygdalin-free fruits (Struempf et al., 1999). In another study, emodin (a secondary compound in the plant family Solanaceae) increased the assimilation efficiency in yellow-vented bulbuls, *Pycnonotus xanthopygos* (Tsahar et al., 2003). Combined, these results put into contention the generalization that all secondary compounds deter fruit consumption by frugivores and cautions against extrapolating the toxicity data for laboratory rats and humans to frugivorous birds (Struempf et al., 1999; Levey and Martinez del Rio, 2001). Bairlein (1996) proposed that secondary compounds may have no effect on frugivores and may even stimulate food intake, although the mechanism involved remains unknown. When garden warblers, *Sylvia borin*, were fed a homogenate mixture of black elderberries *Sambucus nigra* and secondary compounds, they increased food intake by 50% which resulted in body mass gain (Bairlein, 2002). Furthermore, in pair-wise choice tests warblers preferred the diet supplemented with the secondary compound than a pure fruit diet (Bairlein, 2002). This implies a potential beneficial role of secondary compounds to frugivores under certain circumstances provided that they possess mechanisms that allow them to counteract their toxic effects. Frugivorous birds seem to be better equipped to deal with secondary compounds (Bairlein, 1996) as they have larger livers in relation to body size and liver size increases with the degree of frugivory, an indirect indication of preadaptation to a diet high in toxic substances (Herrera, 1984). However, liver masses of seed-eating birds are some of the smallest in birds although seeds generally contain higher secondary compound concentrations than fruits (Stanley and Lill, 2001). Therefore the tolerance of secondary compounds by frugivorous birds may be

attributed to physiological and behavioural, rather than morphological adaptations (Stanley and Lill, 2001).

The tolerance of high secondary compound levels by mousebirds and white-eyes raises an interesting question: how do they cope with their toxic effects once ingested? It could be possible that they detoxify tannins and can thus subsist on a tannin diet. However, this is unlikely as birds that detoxify secondary compounds through physiological mechanisms suffer from a serious energy deficiency because the nutrients in fruits are unable to offset the high energetic costs of detoxification (Guglielmo et al., 1996; Levey and Cipollini, 1998; Witmer, 2001). Because detoxification leads to a negative energy balance, birds would be expected to avoid food with high secondary compound concentrations and instead seek alternative food sources. This strategy is well documented in mammalian herbivores (Provenza et al., 1990). Conversely, they can continue feeding on fruits with high secondary compound concentrations but use alternative ways to detoxify them. Geophagy (ingestion of soil) has been observed in a number of avian species and is considered to be an efficient strategy for the adsorption of secondary compounds (Gilardi et al., 1999). Indeed, mousebirds have been observed engaged in geophagy in the wild (Downs, 2006) and this could be the primary mechanism by which they detoxify secondary compounds. However, since this study was conducted under captive conditions where birds were confined to cages, they could not engage in it. Furthermore, the outside aviaries in which birds spend most of their time is not made of loose soil but concrete which precludes the use of geophagy. Thus geophagy could be expected to be useful only under natural conditions and could not be used to explain the results obtained in this study. An alternative explanation for the tolerance of high tannin concentrations by mousebirds is that their folivorous feeding habits (Downs et al., 2000) could have predisposed them to higher tannin concentrations as secondary compounds are generally more concentrated in the leaves than fruits (Barnea et al., 1993; Cipollini et al.,

2004). The mechanism behind the tolerance of high concentrations of secondary compounds by Cape white-eyes is obscure but tolerance of high tannin concentrations (5%) has been observed in the closely-related Australian silvereyes, *Zosterops lateralis* (Stanley and Lill, 2001; Saxon et al., 2010), although the latter are deterred at higher concentrations (Saxon et al., 2010). Furthermore, the generalist feeding behaviour of Cape white-eyes (Downs and Brown, 2003) may alleviate the effects of tannins by preventing the accumulation of one type of secondary compound (Izhaki and Safriel, 1989; Levey and Martinez del Rio, 2001). There is therefore a possibility that the ability to handle high secondary compounds concentrations in the diet by white-eyes has a dietary as well as a phylogenetic component. Studies incorporating phylogenetically informed analyses are needed to separate the contributions of the two mechanisms.

Tannins are widespread in wild fruits and astringency is the best indication of their presence (Schaefer et al., 2003; Ndhlala et al., 2008). Herrera (1982b) proposed that secondary compounds that result in astringency are likely to be detrimental to plants (in the context of seed dispersal) by deterring legitimate dispersers. The results of this study were at odds with this suggestion as only red-winged starlings were deterred by the presence of tannins in the diet. It could be possible that the disparity of the results obtained in this study was because the tannin levels used were below the concentration at which they could render the food generally deterrent. However, this is unlikely because the concentrations used correspond to the concentrations found in ripe fruits in the wild. An alternative explanation is that because of their ability to detect fine-scaled differences in diet by taste (Zungu et al., *in prep.*); red-winged starlings were able to detect the presence of tannins while speckled mousebirds and Cape white-eyes could not. It could also be possible that red-winged starlings have a lower tannin tolerance which resulted in aversion. In a study by Matson et al. (2004) that tested the taste sensitivity of cockatiels, *Nymphicus hollandicus*, to four types of

secondary compounds, birds were found to be least sensitive to condensed tannins. This implies that although condensed tannins may cause astringency, their effect is not strong enough to render fruits completely deterrent. Therefore the lack of deterrence exhibited in mousebirds and white-eyes may be due to their inability to detect the presence of tannins. However, this argument remains tentative since the taste sensitivity of mousebirds and white-eyes have not been tested. The sole use of food consumption is clearly not enough as a measure of the effects of secondary compounds on animal physiology and behaviour (Witmer, 2001).

In conclusion, this study has shown that frugivores respond differently to the presence of secondary compounds in the diet. Red-winged starlings displayed low tolerance for secondary compounds while mousebirds and Cape white-eyes showed higher tolerance. The differences in tolerance of secondary compounds by the three frugivores under study were attributed to diet, detoxification mechanisms as well as differences in taste sensitivities to secondary compounds. Plant secondary compounds play a pivotal role in mediating fruit persistence patterns and palatability to consumers through their antimicrobial effects (Witmer, 2001) which affect fruit removal rates (Schaefer et al., 2003). Because fruit removal rates determine the rates at which seeds are dispersed and thus have fitness consequences for plants (Moermond and Denslow 1985), plants whose fruits are quickly removed will eventually dominate the community. Therefore fruit removal rates affect plant community composition (Schaefer et al., 2003). The disparity in the results suggests that frugivorous birds affect plant community dynamics differently. Plant secondary compounds in fruits are diverse and their effects are similarly diverse and there is potential that different groups of secondary compounds generate disparate effects (Levey and Martinez del Rio 2001). Similar studies on other types of secondary compounds may contribute towards a broader understanding of the role of secondary compounds in mediating fruit-frugivore interactions.

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References

- Adler, L.S., 2000. The ecological significance of toxic nectar. *Oikos* 91, 409–420.
- Bairlein, F., 1996. Fruit-eating in birds and its nutritional consequences. *Comp. Biochem. Physiol. A* 113, 215–224.
- Bairlein, F., 2002. How to get fat: nutritional mechanisms of seasonal fat accumulation in migratory songbirds. *Naturwissenschaften* 89, 1–10.
- Barnea, A., Harborne, J.B., Pannell, C., 1993. What parts of fleshy fruits contain secondary compounds toxic to birds and why? *Biochem. Syst. Ecol.* 21, 421–429.
- Brown, K.J., Downs, C.T., 2003. Digestive efficiency of a generalist avian feeder, the Cape white-eye (*Zosterops pallidus*). *Comp. Biochem. Physiol. A* 134, 739–748.
- Brown, M., Downs, C.T., Johnson, S.D., 2012. African red-winged starlings prefer hexose sugar solutions, but do not like them too sweet. *J. Ornith.* 153, 265–272.
- Brown, M., Johnson, S.D., Downs, C.T., 2010. Sugar preferences and digestive efficiency in an opportunistic avian nectarivore, the dark-capped bulbul (*Pycnonotus tricolor*). *J. Ornith.* 151, 637–643.

- Cazetta, E., Schaefer, H.M., Galetti, M., 2008. Does attraction to frugivores or defence against pathogens shape fruit pulp composition? *Oecologia* 155, 277–286.
- Cipollini, M.L., 2000. Secondary metabolites of vertebrate-dispersed fruits: evidence for adaptive functions. *Revista Chilena de Historia Natural* 73, 421–440.
- Cipollini, M.L., Levey, D.J., 1997a. Secondary compounds of fleshy vertebrate-dispersed fruits: Adaptive hypotheses and implications for seed dispersal. *Am. Nat.* 150, 346–372.
- Cipollini, M.L., Levey, D.J., 1997b. Why are some fruits toxic? Glycoalkaloids in *Solanum* and fruit choice by vertebrates. *Ecology* 78, 782–798.
- Cipollini, M.L., Paulk, E., Mink, K., Vaughn, K., Fischer, T., 2004. Defence trade-offs in fleshy fruits: Effects of resource variation on growth, reproduction and fruit secondary chemistry in *Solanum carolinense*. *J. Chem. Ecol.* 30, 1–17.
- Cipollini, M.L., Stiles, E.W., 1993. Fruit rot, antifungal defence and palatability of fleshy fruits for frugivorous birds. *Ecology* 74, 751–762.
- Debussche, M., Isenmann, P., 1989. Fleshy fruit characters and the choices of bird and mammal seed dispersers in a Mediterranean region. *Oikos* 56, 327–338.
- Downs, C.T., Wirminghaus, J.O., Lawes, M.J., 2000. Anatomical and nutritional adaptations of the speckled mousebird (*Colius striatus*). *Auk* 117, 791–794.
- Downs, C.T., 2006. Geophagy in the African olive pigeon (*Columba arquatrix*). *Ostrich* 77, 40–44.
- Ehrlen, J., Eriksson, O., 1993. Toxicity in fleshy fruits: A non-adaptive trait? *Oikos* 66, 107–113.

- Eriksson, O., Ehrlen, J., 1998. Secondary metabolites in fleshy fruits: Are adaptive explanations needed? *Am. Nat.* 152, 905–907.
- Gautier-Hion, A., Duplantier, J.M., Quris, R., Feer, F., Sourd, C., Decoux, J.P., Dubost, G., Emmons, L., Erard, C., Hecketsweiler, P., Mougazi, A., Roussillon, C., Thiollay, J.M., 1985. Fruit characters as a basis of fruit choice and seed dispersal in a tropical forest vertebrate community. *Oecologia* 65, 324–337.
- Gilardi, J.D., Duffey, S.S., Munn, C.A., Tell, L.A., 1999. Biochemical functions of geophagy in parrots: Detoxification of dietary toxins and cytoprotective effects. *J. Chem. Ecol.* 25, 897–922.
- Guglielmo, C.G., Karasov, W.H., Jakubas, W.J., 1996. Nutritional costs of a plant secondary metabolite explain selective foraging by ruffed grouse. *Ecology* 77, 1103–1115.
- Herrera, C.M., 1982*a*. Seasonal variation in the quality of fruits and diffuse coevolution between plants and avian dispersers. *Ecology* 63, 773–785.
- Herrera, C.M., 1982*b*. Defence of ripe fruit from pests: its significance in relation to plant-disperser interactions. *Am. Nat.* 120, 218–241.
- Herrera, C.M., 1984. Adaptation to frugivory of Mediterranean avian dispersers. *Ecology* 65, 609–617.
- Herrera, C.M., 1987. Vertebrate-dispersed plants of the Iberian Peninsula: a study of fruit characteristics. *Ecol. Monogr.* 54, 305–331.
- Herrera, C.M., 1992. Interspecific variation in fruit shape: allometry, phylogeny and adaptation to dispersal agents. *Ecology* 73, 1832–1841.

- Herrera, C.M., 2002. Seed dispersal by vertebrates. In: Herrera, C.M., Pellmyr, O., (eds). Plant-animal interactions: an evolutionary approach. Blackwell Science, London.
- Howe, H.F., Smallwood, J., 1982. Ecology of seed dispersal. *Ann. Rev. Ecol. Syst.* 13, 201–228.
- Izhaki, I., 1993. Influence of nonprotein nitrogen on estimation of protein from total nitrogen in fleshy fruits. *J. Chem. Ecol.* 19, 2605–2615.
- Izhaki, I., Safriel, U.N., 1989. Why there are so few exclusively frugivorous birds? Experiments on fruit digestibility. *Oikos* 54, 23–32.
- Izhaki, I., 1992. A comparative analysis of the nutritional quality of mixed and exclusive fruit diets for yellow-vented bulbuls. *Condor* 94, 912–923.
- Jackson, S., Nicolson, W.S., Lotz, C.N., 1998. Sugar preferences and ‘side bias’ in Cape sugarbirds and lesser-collared sunbirds. *Auk* 115, 156–165.
- Jordano, P., 1987. Patterns of mutualistic interactions in pollination and seed dispersal: connectance, dependence asymmetries, and coevolution. *Am. Nat.* 129, 657–677.
- Jordano, P., 1995. Angiosperm fleshy fruits and seed dispersers: a comparative analysis of adaptation and constraints in plant-animal interactions. *Am. Nat.* 145, 163–191.
- Levey, D.J., 1987. Sugar-tasting ability and fruit selection in tropical fruit-eating birds. *Auk* 104, 173–179.
- Levey, D.J., Cipollini, M.L., 1998. A glycoalkaloid in ripe fruit deters consumption by cedar waxwings. *Auk* 115, 359–367.
- Levey, D.J., Martinez del Rio, C., 2001. It takes guts (and more) to eat fruits: lessons from avian nutritional ecology. *Auk* 118, 819–831.

- Levey, D.J., Tewksbury, J.J., Cipollini, M.L., Carlo, T.A., 2006. A field test of the directed-deterrence hypothesis in two species of wild chili. *Oecologia* 150, 61–68.
- Levey, D.J., Tewksbury, J.J., Izhaki, I., Tsahar, E., Haak, D.C., 2007. Evolutionary ecology of secondary compounds in ripe fruit: case studies with capsaicin and emodin. In: Dennis, A.J., Schupp, E.W., Green, R.J., Westcott, D.A., (eds). *Seed dispersal: theory and application in a changing world*. First Edition. Cambridge University Press, Cambridge.
- Martinez del Rio, C., Karasov, W.H., Levey, D.J., 1989. Physiological basis and ecological consequences of sugar preferences in cedar waxwings. *Auk* 106, 64–71.
- Martinez del Rio, C., Restrepo, C., 1993. Ecological and behavioural consequences of digestion in frugivorous animals. *Vegetatio* 107/108, 205–216.
- Martinez del Rio, C., Stevens, B.R., Daneke, D.E., Andreadis, P.T., 1988. Physiological correlates of preference and aversion for sugars in three species of birds. *Physiol. Zool.* 61, 222–229.
- Mbatha, K.R., Downs, C.T., Nsahlai, I.V., 2002. The effects of graded levels of dietary tannin on the epithelial tissue of the gastro-intestinal tract and liver and kidney masses of boer goats. *Anim. Sci.* 74, 579–586.
- Moermond, T.C., Denslow, S.J., 1985. Neotropical avian frugivores: patterns of morphology, behaviour, and nutrition with consequences for fruit selection. *Ornith. Monogr.* 36, 865–897.
- Ndhlala, A.R., Kasiyamhuru, A., Chitindingu, K., Benhura, M.A., Muchuweti, M., 2007. Phenolic composition of *Flacourtia indica*, *Opuntia megacantha* and *Sclerocarya birrea*. *Food Chem.* 103, 82–87.

- Ndhlala, A.R., Muchuweti, M., Chitindingu, K., Benhura, M.A., 2008. Phenolic content and profiles of selected wild fruits of Zimbabwe: *Ximenia caffra*, *Artobotrys brachypelatus* and *Syzigium cordatum*. *Int. J. Food Sci. Tech.* 43, 1333–1337.
- Provenza, F.D., Burritt, E.A., Clausen, T.P., Bryant, J.P., Reichardt, P.B., 1990. Conditioned flavour aversion: A mechanism for goats to avoid tannins in blackbrush. *Am. Nat.* 136, 810–828.
- Samuni-Blank, M., Izhaki, I., Dearing, M.D., Gerchman, Y., Trabelcy, B., Lotan, A., Karasov, W.H., Arad, Z., 2012. Intraspecific directed deterrence by the mustard oil bomb in a desert plant. *Curr. Biol.* 22, 1–3.
- Samuni-Blank, M., Dearing, M.D., Karasov, W.H., Gerchman, Y., Kohl, K.D., Lymberakis, P., Kurnath, P., Arad, Z., 2013. Physiological and behavioural effects of fruit toxins on seed-predating versus seed-dispersing congeneric rodents. *J. Exp. Biol.* 216, 3667–3673.
- Saxon, V.P., Mulder, I., Creasy, G.L., Paterson, A.M., Ross, J.G., Trought, M.C.T., 2010. Comparative behavioural responses of silvereyes (*Zosterops lateralis*) and European blackbirds (*Turdus merula*) to secondary metabolites in grapes. *Austr. Ecol.* 36, 233–239.
- Schaefer, H.M., Schmidt, V., Winkler, H., 2003. Testing the defence trade-off hypothesis: How contents of nutrients and secondary compounds affect fruit removal. *Oikos* 102, 318–328.
- Stanley, M.C., Lill, A., 2001. Response of silvereyes (*Zosterops lateralis*) to dietary tannins: the paradox of secondary metabolites in ripe fruit. *Austr. J. Zool.* 49, 633–640.

- Struempf, H.M., Schondube, J.E., Martinez del Rio, C., 1999. The cyanogenic glycoside amygdalin does not deter consumption of ripe fruit by cedar waxwing. *Auk* 116, 749–758.
- Tewksbury, J.J., 2002. Fruits, frugivores, and the evolutionary arms race. *New Phyt.* 156, 137–139.
- Tewksbury, J.J., Levey, D.J., Huizinga, M., Haak, D.C., Traveset, A., 2006. Costs and benefits of capsaicin-mediated control of gut retention in dispersers of wild chillies. *Ecology* 89, 107–117.
- Tewksbury, J.J., Manchego, C., Haak, D.C., Levey, D.J. 2008. Where did the chilli get its spice? Biogeography of capsaicinoid production in ancestral wild chili species. *J. Chem. Ecol.* 32, 547–564.
- Tsahar, E., Friedman, J., Izhaki, I., 2002. Impact on fruit removal and seed predation of a secondary compound emodin in *Rhamnus alaternus* fruit pulp. *Oikos* 99, 290–299.
- Tsahar, E., Friedman, J., Izhaki, I., 2003. Secondary metabolite emodin increases food assimilation efficiency of yellow-vented bulbuls (*Pycnonotus xanthopygos*). *Auk* 120, 411–417.
- Umaru, H.A., Adamu, R., Dahiru, D., Nadro, M.S., 2007. Level of anti-nutritional factors in some wild edible fruits of Northern Nigeria. *Afr. J. Biotech.* 6, 1935–1938.
- Wahaj, S.A., Levey, D.J., Sanders, A.K., Cipollini, M.L., 1998. Control of gut retention time by secondary metabolites in ripe *Solanum* fruits. *Ecology* 79, 2309–2319.
- Whitehead, S.R., Bowers, M.D. 2013. Evidence for the adaptive significance of secondary compounds in vertebrate-dispersed fruits. *Am. Nat.* 182, 563–577.

- Willson, M.F., Irvine, A.K., Walsh, N.G., 1989. Vertebrate dispersal syndromes in some New Zealand plant communities, with geographic comparisons. *Biotropica* 21, 133–147.
- Witmer, M.C., 1998. Ecological and evolutionary implications of energy and protein requirements of avian frugivores eating sugary fruits. *Physiol. Zool.* 71, 599–610.
- Witmer, M.C., 2001. Nutritional interactions and fruit removal: Cedar waxwing consumption of *Viburnum opulus* fruits in spring. *Ecology* 82, 3120–3130.

Figure legends

Fig. 1. Preference values as a function of diet for the red-winged starlings (**a**), Cape white-eyes (**b**) and speckled mousebirds (**c**). The preference values were calculated as the amount of food consumed for each diet divided by the total food consumed for the day. For all graphs, the values presented are back-transformed means of the square-root transformed preference values and 95% confidence intervals obtained from square-root transformed proportional data. If 0.5 (no preference value) is outside of the 95% confidence interval, the mean preference value is considered significant.

Table 1 The composition of artificial diets of varying tannin levels offered to red-winged starlings, Cape white-eyes and speckled mousebirds.

Ingredient	Diet		
	Control	Medium	High
Water (ml)	1000	1000	1000
Glucose (g)	150	150	150
Wheat bran (g)	50	50	50
Agar (g)	10	10	10
Sodium Chloride (g)	0.75	0.75	0.75
DiCalcium Phosphate (g)	0.80	0.80	0.80
Vitamin Supplement (g)	0.75	0.75	0.75
Condensed tannin (%)	0	2.5	5

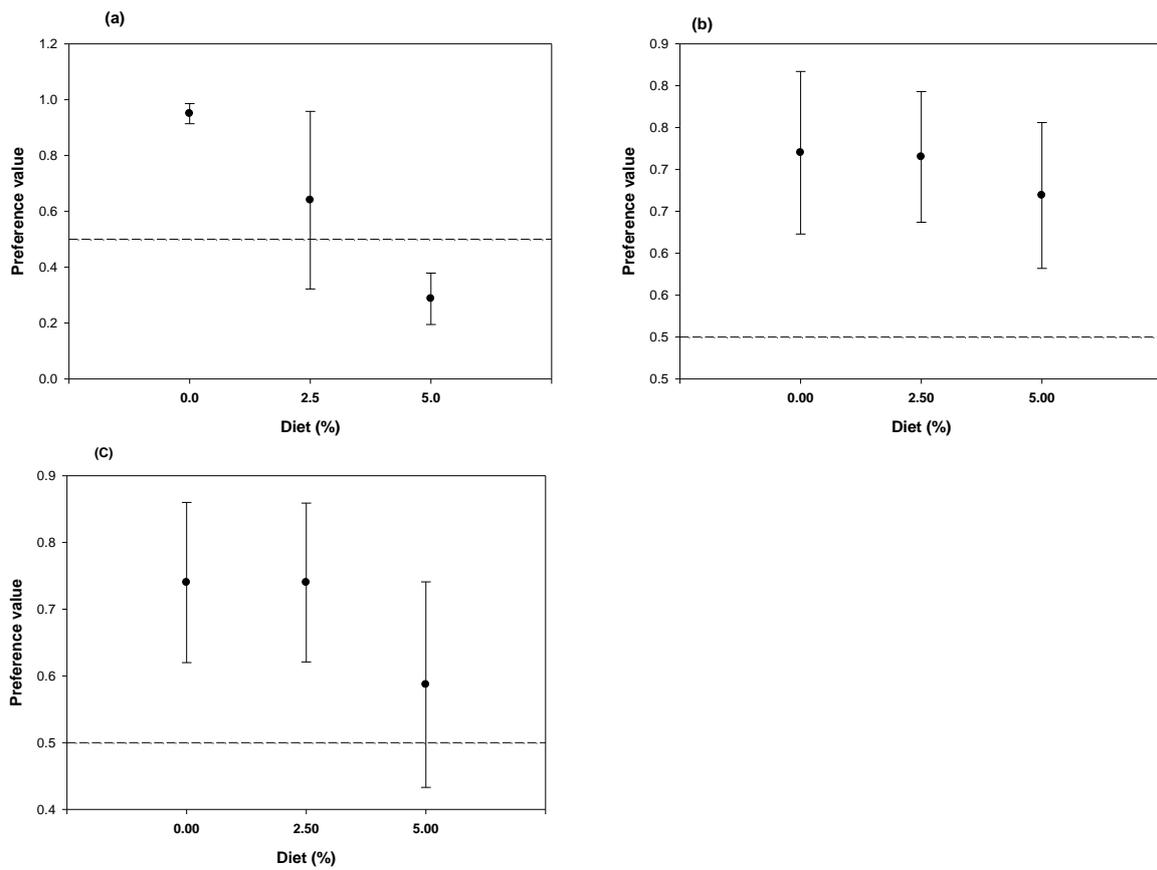


Fig. 1. Preference values as a function of diet for the red-winged starlings (a), Cape white-eyes (b) and speckled mousebirds (c). The preference values were calculated as the amount of food consumed for each diet divided by the total food consumed for the day. For all graphs, the values presented are back-transformed means of the square-root transformed preference values and 95% confidence intervals obtained from square-root transformed proportional data. If 0.5 (no preference value) is outside of the 95% confidence interval, the mean preference value is considered significant.

CHAPTER 4

The role of ethanol in fruit selection by frugivorous birds

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ABSTRACT

Ethanol is a natural by-product of the fermentation process of fruit sugars. Its production started with the advent of fleshy fruits which suggests a long historical association between ethanol and frugivores. Consequently, it has been suggested that because frugivores could use its odour to locate fruiting plants, they should select fruit with high ethanol concentrations. The aim of this study was to test this hypothesis by determining whether frugivorous birds show a preference for fruit laden with alcohol at levels equivalent to those of over-ripe fruits. Three frugivorous bird species were used for this study: the Cape white-eye, *Zosterops virens*, red-winged starling, *Onychognathus morio* and speckled mousebird, *Colius striatus*. Birds were provided with two artificial fruit diets in pairwise choice tests: an experimental diet containing 1% ethanol and an artificial fruit diet with no ethanol. For all species, no significant differences were observed in the amount of fruit consumed between the two food types. Because the concentration of ethanol used in the study represented the ethanol level of over-ripe fruit, we assert that birds are unlikely to prefer over-ripe fruits compared to ripe fruits due to the negative impacts associated with ingesting ethanol at high concentrations. However, it could be possible that the lack of preference observed in this study was because the ethanol concentration used was below the level at which it could be detected. Future

studies using a wide range of ethanol concentrations may help to elucidate how ethanol concentration affects fruit selection in birds. Overall, this study suggests that at high concentrations, ethanol in fruits acts as a deterrent rather than an attractant.

Keywords: artificial fruit; birds; ethanol, frugivore; preference

INTRODUCTION

The search for factors that shape and constrain fruit selection patterns in birds has been at the vanguard of research on fruit-frugivore interactions as these (factors) serve as a mechanistic bridge linking frugivore ecology with plant recruitment and ultimately plant community dynamics. Most studies on plant-frugivore interactions have been viewed from the context of vertebrate frugivores and fruits (Tewksbury 2002; Levey et al. 2007). The lack of tightly coevolved relationships between vertebrate frugivores and fruits (Herrera 1985; Jordano 1995) is a clear testament of the limitations of this unnecessarily restrictive view since fruits are self-advertising structures which attract a diverse array of consumers (Levey et al. 2007). Therefore for a full understanding of fruit-frugivore interactions, studies should be integrative in nature and encompass the whole range of organisms that interact with fruits, including mutualists (i.e. seed dispersers) and antagonists (i.e. fruit-damaging agents) (Herrera 1982; Tewksbury 2002; Levey et al. 2007). Specifically, information on the role of microbes on fruit-frugivore interactions is meagre but their role could be important because the interactions between any of the two players in the fruit-microbe-frugivore triad affect the other player (Borowicz 1988; Buchholz and Levey 1990; Levey 2004). Therefore incorporating the role of microbes in fruit-frugivore interactions could be a crucial step towards elucidating factors responsible for variation in seed dispersal success among plants (Borowicz 1988).

Sugars in fruit pulp are an important energy source to frugivores but serve as the primary template for the fermentation process resulting in the production of ethanol (Mazeh et al. 2008). The production of ethanol in fruits is attributed to the interspecific competition between frugivores and microbes for fruit sugars (Janzen 1977; Levey 2004). Because frugivorous vertebrates can extract energy from fruit sugars instantly upon settling on fruits, they have a competitive advantage in the competition with microbes because for microbes this can take weeks (Dominy 2004; Levey 2004). Since this high-stake game is tilted towards vertebrate frugivores, microbes have to devise ways of gaining an upper hand. One way in which they have been thought to do so is through producing ethanol to make fruits objectionable to vertebrate frugivores (Janzen 1977; Levey 2004). Providing indirect support to this suggestion is the evidence from the fossil record showing that the advent of ethanol production in fruit occurred simultaneously with the shift in seed dispersal mechanisms in angiosperms from wind-dispersed to animal-dispersed seed in the Cretaceous (Benner et al. 2002).

Ethanol concentration in fruits increases with the fruit ripening process and is positively correlated to fruit sugars (Dudley 2004; Levey 2004; Sanchez et al. 2004). Because of the ubiquity of ethanol in fruits, frugivores consume significant amounts of it (Orbach et al. 2010). Generally, ethanol consumption is detrimental to the fitness of organisms as it increases their susceptibility to predation (Janzen 1977) by interfering with motor skills, resulting in a significant loss of coordination (Sanchez et al. 2010). Mounting anecdotal and published accounts of inebriation after the consumption of over-ripe fruits has been observed in a number of vertebrates (Dominy 2004). In extreme cases, death has been reported (Fitzgerald et al. 1990; Kinde et al. 2012). This suggests that at high concentrations, ethanol can reach toxic levels (Sanchez et al. 2010). In vertebrates, ethanol is oxidized in a reaction catalysed by the enzyme alcohol dehydrogenase (ADH) using NAD⁺ as a cofactor which

results in the formation of an intermediate compound acetaldehyde, which becomes catalysed by aldehyde dehydrogenase (ALDH) to produce acetate which enters the Krebs cycle (Kricka & Clark 1979). However, at high concentrations, the enzymatic action is not efficient enough to metabolize ethanol and given its ability to dissolve in solution, it enters the surrounding tissue and affects many important functions (Lieber 1997).

Despite the negative impacts of ethanol, it is however not completely deleterious. Alcohol has been shown to be an appetitive stimulant in humans (Caton et al. 2004; Yeomans, 2004) and the same could be the case in birds for ethanol at low concentrations (Sanchez et al. 2004). In addition, ethanol represents a nutritional reward since its caloric value is nearly double that of carbohydrates (Dudley 2000). This implies that energy metabolism associated with its metabolism is substantial (Janzen 1977; Dudley 2000). Due to the scarcity and the highly heterogeneous nature of fruit distribution especially in tropical ecosystems (Herrera 2002; Milton 2004), the positive correlation between sugars and ethanol and the long historical association between frugivores and ethanol (Hernandez-Tobias et al. 2011), it has been suggested that over long evolutionary times, frugivores associate ethanol with nutritional rewards in fruits (Dudley 2000). Consequently, its plume could serve as a long-distance cue facilitating the localization of highly productive fruit patches (Dudley 2000; 2002) resulting in frugivores should show a preference for fruits with high (i.e. overripe) ethanol content (Dudley 2002). Field (Valberg 1992) and laboratory studies (Borowicz 1988; Cipollini & Stiles 1993), however, suggest that generally, frugivores reject overripe fruit. However, these studies were conducted on temperate frugivores which are generally exposed to lower ethanol concentrations (Levey 2004; Sanchez et al. 2004). It therefore remains to be ascertained whether the same results would be obtained from tropical frugivores since environmental conditions in the tropics are more conducive for the fermentation of fruit sugars (Levey 2004). In addition, a significant preference for fruits with

low ethanol content in preference trials does not preclude consumption of significant amounts of ethanol (Levey 2004).

The aim of this study was to test the hypothesis that frugivores prefer fruits with high ethanol concentrations due to its potential use as a foraging cue. Specifically, we asked: do birds show a preference for fruit with alcohol, such as suggested by Dudley (2000; 2002) at levels equivalent to those in over-ripe fruit? Three frugivorous bird species native to the southern African region were used: Cape white-eyes, *Zosterops virens*, red-winged starlings, *Onychognathus morio* and speckled mousebirds, *Colius striatus*. Although ethanol concentration in fruit is poorly known, studies in temperate and Mediterranean regions suggest that it ranges from 0.04 to 0.72% (Eriksson and Nummi 1982; Dudley 2002; Dominy 2004; Sanchez et al. 2004). For tropical regions, virtually no study has been conducted on ethanol concentration in fruit. Due to faster decomposition rates, high sugar content in fruit and warmer temperatures (conditions there are conducive for microbial proliferation), fruits from tropical regions are expected have higher ethanol content than fruits from other regions (Levey 2004). Consequently, the objective of this study was to compare the amount of food eaten by the three frugivorous avian species between 1% ethanol and control artificial fruit diets. It was predicted that birds would prefer the artificial diet with ethanol as it serves as an important foraging cue which assists in locating fruiting plants (Dudley 2000, 2002).

METHODS

The study was conducted at the Animal House of the University of KwaZulu-Natal (UKZN), Pietermaritzburg (29°37'0"S; 30°23'0"E, 660 m above sea level). Red-winged starlings, *Onychognathus morio* ($n = 10$), speckled mousebirds, *Colius striatus* ($n = 6$) and Cape white-eyes, *Zosterops virens* ($n = 10$) were used. These birds were housed in large outside species-

groups holding cages ($1 \times 2.12 \times 2.66$ m) and were exposed to natural photoperiod, humidity and temperature. All birds used in this study were captured in the vicinity of Pietermaritzburg and had been in captivity for more than a year and thus were fully acclimated to captivity and food preference trials. Birds were fed a maintenance diet consisting of a chopped-up and grated fruit mix including bananas, apples, pears, pawpaw, oranges and guavas together with an avian supplementary diet (AviPlus Softbill Maintenance Crumble; Aviproducts, Waterfall, KZN). Feeding took place every morning between 07h00 and 08h00. Five days prior to the start of the experimental trials, birds were transferred to individual cages in a controlled environment room with temperatures kept constant at room temperature ($25 \pm 0.1^\circ\text{C}$) so that they acclimatize to the experimental conditions and were fed on the same schedule as they were in outside aviaries.

Experimental design

Trials used in this study were based on artificial diets adopted from Witmer (1998). In this study, an ethanol concentration of 1% was used to represent overripe fruits. Trials consisted of a pair-wise choice between a control diet and a 1% ethanol diet, made by adding 10 ml of 98% ethanol to the control diet (Table 1). The mixture was then heated in a microwave oven (for approximately 8 min) until boiling, and then poured into a flat tray to cool, with continuous stirring by hand to ensure that ingredients distributed equally. After the mixture had cooled but before it had solidified, 5 ml of a Robertson's red food colourant (Libstar Manufacturing Solutions, Chloorkop, South Africa) was poured to improve the appearance of the diets. Due to the short life span of the artificial fruit diets, they were prepared on the afternoon preceding the experimental trials and kept in a refrigerator overnight.

Experimental trials

During the experimental trials, food was cut into 1×1 cm cubes and put in metal containers to be presented to birds. One control and one treatment container was assigned to each cage, randomly assigning containers with diet type. Containers were placed equidistant from the bird's perches inside the cage and from the sides of the cage to ensure that birds travelled the same distance to acquire food from either dish. Right-left position of food was randomized hourly to minimize the effect of side bias. Water was provided *ad libitum* throughout all trials. Each trial lasted for a period of six hours from 06h00 to 12h00. Birds were weighed before and after each experimental trial. At each hour interval, all containers were removed and weighed to quantify diet consumption and returned to birds thereafter. To allow correction for evaporative water loss, control and treatment diets were placed in an empty cage and weighed on the same schedule as the other containers. All food items measurements were rounded-off to the nearest 0.01 g. Total food consumption for each trial was calculated by taking the difference between the initial and final mass of the food and subtracting the amount of mass lost through evaporation.

Statistical analysis

An independent samples *t*-test was used to determine whether there were significant differences in the amount of food eaten between the experimental and control diets. Prior to analyses, the amount of food eaten was divided by the initial body mass of each bird and thus expressed as a mass-specific value. Values are expressed as mean \pm S.E. except where specified. All the statistical analyses were conducted using SPSS[®] 16.0 (SPSS Inc., Delaware, Chicago, USA).

RESULTS

There were no significant differences in food consumption between the two food types in the speckled mousebirds ($t = -.413$, $df = 10$, $P = 0.688$; Fig. 1a.). Similarly, no significant differences were observed in consumption between diets in the Cape white-eye ($t = -0.630$, $df = 18$, $P = 0.536$; Fig. 1b.). The results from the red-winged starlings also showed a similar pattern where no significant differences in food consumption between the two diets were observed ($t = -0.419$, $df = 18$, $P = 0.680$; Fig. 1c.). Despite these non-significant differences, the control diet was consumed more than the ethanol diet for all species.

DISCUSSION

Compared with leaves, immature fruits and flowers, fruits are the most heterogeneous food resources in space and time in tropical rainforests (Milton 2004). Therefore efficient foraging requires tremendous travel. Because ethanol concentration increases with the fruit ripening process, it has been suggested that frugivores could use its odour as a long-distance foraging cue, allowing the localisation of fruiting patches (Dudley 2000). A corollary to this hypothesis is that frugivorous birds should select fruit on the basis of ethanol content as they associate it with a nutritional reward. Specifically, they should select fruits with high ethanol concentrations (Dudley 2000; 2002).

The three frugivorous species used in this study showed no preference between a 1% ethanol and a control diet. Although the differences were not significant, the control diet was consumed more than the ethanol diet (Fig. 1.). This suggests that the frugivores used in this study do not show preference for high ethanol concentration in fruit since the ethanol concentration used in this study is similar to ethanol concentration in over-ripe fruits (Eriksson & Nummi 1982; Levey 2004). These results corroborated results of other studies in

birds (Borowicz 1988; Cipollini and Stiles 1993) and primates (Milton 2004) which have found them to avoid overripe fruits in the wild. Therefore the results of the present study in conjunction with available literature brings Dudley's hypothesis (Dudley 2000) into contention and suggest that ethanol at high concentrations may act as a deterrent rather than an attractant (Milton 2004; Sanchez et al. 2004; 2006) whereas at concentrations equivalent to fresh ripe fruits it has no effect on fruit consumption (Sanchez et al. 2004; 2006; Mazeh et al. 2008). Therefore ethanol does not serve as a food-locating cue (Mazeh et al. 2008; Sanchez et al. 2008).

These results are similar to those found in yellow-vented bulbuls, *Pycnonotus goiavierin* (Mazeh et al. 2008). Bulbuls showed no preference for diets containing ethanol within naturally-occurring ranges (up to 1%), but showed aversion to diets containing higher amounts (3%) which resulted in a 36% reduction in food intake (Mazeh et al. 2008). Likewise, Egyptian fruit bats, *Rousettus aegyptiacus* are deterred by ethanol at concentrations greater than 1% (Sanchez et al. 2004; 2008). It has been demonstrated that at high concentrations (3%), ethanol disrupts important body functions in Egyptian fruit bats leading to a reduction in flying speed and disturbed echolocation which has serious fitness consequences (Sanchez et al. 2010). Therefore the reduction in food intake at high ethanol concentrations could be seen an adaptive strategy to prevent intoxicification which can result in death (Fitzgerald et al. 1990; Kinde et al. 2012).

Ethanol is a toxic compound encountered by frugivores but its negative impacts can be alleviated by the presence of nutrients that complement it. In Egyptian fruit bats, Sanchez et al. (2008) found that the amount of ethanol-containing fruit consumed depended on the sugar content and composition of fruits consumed. The authors (Sanchez et al. 2008) found that when ethanol was ingested together with fructose, ethanol metabolism was faster than when ingested with sucrose or glucose. In the current study, the artificial fruits administered

to birds contained glucose as the only sugar. Therefore the lack of preference for the ethanol-containing diet may be attributed to the fact that glucose is not complimentary to ethanol ingestion. However, it could be possible that ethanol metabolism in birds and mammals follow different pathways, which could dispute this claim. Unfortunately, studies on ethanol breakdown in birds have only looked at the rate of breakdown, not the mechanism involved (Eriksson & Nummi 1982). Therefore studies investigating ethanol metabolism and how different sugars in fruit affect it are still needed if we are to get a better understanding on the effects of ethanol on fruit selection in birds.

Eriksson & Nummi (1982) postulated that ethanol metabolism is related to the amount of ethanol in the diet of an organism. This suggests that species that have a diet high in ethanol are more likely to display high ADH activity because their diet could lead to ethanol accumulation if they were incapable of metabolising it rapidly. In that study, they found that waxwings, *Bombycilla garrulus*, which are primarily fruit eaters and thus accustomed to ripe fruit had high ethanol metabolism (900 mg/kg/h) compared to European starlings (*Sturnus vulgaris*; 270 mg/kg/h) and greenfinches (*Chloris chloris*; 130 mg/kg/h) which consume a mixed diet and a seed diet, respectively (Eriksson & Nummi 1982). All the species used in the current study although are broadly categorized as frugivores; have varied diets consuming other foodstuffs in significant proportions (Downs et al. 2000; Brown & Downs 2003; Brown et al. 2012). Red-winged starlings are extremely adaptable feeders with a predominantly frugivorous diet but include substantial amounts of insects and nectar (Brown et al. 2012). Mousebirds have an omnivorous diet including fruits, insects, nectar and leaves and show a tremendous ability to shift from a predominantly frugivorous to a folivorous diet when fruit availability is low (Downs et al. 2000). White-eyes are probably the most generalist feeders eating almost equal proportions of fruits, arthropods and nectar (Brown and Downs 2003). Because of the highly varied nature of the diets of these species and the fact that ethanol

metabolism was not measured in the present study, results obtained cannot be used to validate the findings by Eriksson & Nummi (1982). A possible follow-up to this preliminary study is to conduct a similar study using species that differ markedly in ethanol content in the diet (i.e. a frugivore, nectarivore and a granivore) with the rates of ethanol metabolism/ADH activity measured concurrently to determine whether the same could be observed.

Conclusions

This study provided corroborating evidence that when given a choice, frugivorous birds are unlikely to select fruit containing high ethanol concentrations probably as a result of its deleterious effects when ingested at high concentrations. This has serious ramifications for the process of seed-dispersal as it suggests that fruit production in plants should correspond with high disperser abundance as un-removed fruit rot and contribute negatively towards the seed dispersal process (Janzen 1977; Levey 2004). It could be possible, however; that the lack of preference for high ethanol concentration observed in this study could be because the ethanol concentration used was below the level at which birds could detect it. In future studies, it is recommended that ethanol concentrations should encompass a wide range of concentrations. Furthermore, alcohol metabolism should be measured concurrently with the feeding trials which would add more insight on how physiology affects ethanol tolerance in birds which has remained largely ignored in the past. Overall, the preliminary results provided in this study suggest that ethanol does not serve as a foraging cue but rather as a deterrent.

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References

- Benner, S.A. Caraco, M. D. Thomson, J.M. & Gaucher, E.A.** 2002. Planetary biology-paleontological, geological, and molecular histories of life. *Science*, **296**, 864–868.
- Borowicz, V.A.** 1988. Do vertebrates reject decaying fruit? An experimental test with *Cornus amomum* fruits. *Oikos*, **53**, 74–78.
- Brown, K.J. & Downs, C.T.** 2003. Digestive efficiencies of a generalist avian feeder, the Cape white-eye (*Zosterops pallidus*). *Comparative Biochemistry and Physiology A*, **134**, 739–748.
- Brown, M., Johnson, S.D. & Downs, C.T.** 2012. African Red-winged Starlings prefer hexose solutions, but do not like them too sweet. *Journal of Ornithology*, **153**, 265–272.
- Buchholz, R. & Levey, D.J.** 1990. The evolutionary triad of microbes, fruit and seed dispersers: an experiment in fruit choice by cedar waxwing, *Bombycilla cedrorum*. *Oikos*, **59**, 200–204.
- Cipollini, M.L. & Stiles, E.W.** 1993. Fruit rot, antifungal defence, and palatability of fleshy fruits for frugivorous birds. *Ecology*, **74**, 751–762.
- Dominy, N.J.** 2004. Fruit, fingers and fermentation: The sensory cues available for foraging primates. *Integrated Compositional Biology*, **44**, 295–303.

- Downs, C.T., Wirminghaus, J.O. & Lawes, M.J.** 2000. Anatomical and nutritional adaptations of the speckled mousebird *Colius striatus*. *Auk*, **117**, 791–794.
- Dudley, R.** 2000. Evolutionary origins of Human alcoholism in primate frugivory. *Quarterly Reviews of Biology*, **75**, 3–15.
- Dudley, R.** 2002. Fermenting fruit and the historical ecology of ethanol ingestion: Is alcoholism in modern Humans an evolutionary hangover? *Addictions*, **97**, 381–388.
- Eriksson, K. & Nummi, H.** 1982. Alcohol accumulation from ingested berries and alcohol metabolism in passerine birds. *Ornis Fennica*, **60**, 2–9.
- Fitzgerald, S.D., Sullivan, J.M. & Everson, R.J.** 1990. Suspected ethanol toxicosis in two wild Cedar Waxwings. *Avian Diseases*, **34**, 488–490.
- Hernandez-Tobias, A., Julian-Sanchez, A., Pina, E. & Riveros-Rosas, H.** 2011. Natural alcohol exposure: is ethanol the main substrate for alcohol dehydrogenases in animals? *Chemico-Biological Interactions*, **191**, 14–25.
- Herrera, C.M.** 1982. Defence of ripe fruit from pests: its significance in relation to plant-disperser interactions. *American Naturalist*, **120**, 218–241.
- Herrera, C.M.** 1985. Determinants of plant-animal coevolution: the case of mutualistic dispersal of seeds by vertebrates. *Oikos*, **44**, 132–141.
- Herrera, C.M.** 2002. Seed dispersal by vertebrates. In: Herrera, C.M. & Pellmyr, O. (eds). *Plant-animal interactions: an evolutionary approach*. Blackwell Science.
- Janzen, D.H.** 1977. Why fruit rot, seeds mold, and meat spoils. *American Naturalist*, **111**, 691–713.

- Jordano, P.** 1995. Angiosperm fleshy fruits and seed dispersers: a comparative analysis of adaptation and constraints in plant-animal interactions. *American Naturalist*, **145**, 163–191.
- Kinde, H., Foate, E., Beeler, E. , Uzal, F., Moore, J. & Poppenga, R.** 2012. Strong circumstantial evidence for ethanol toxicosis in cedar waxwings (*Bombycilla cedrorum*). *Journal of Ornithology*, **153**, 995–998.
- Kricka, L.J. & Clark, P.M.S.** 1979. Biochemistry of alcohol and alcoholism. John Wiley and Sons, New York.
- Levey, D.J.** 2004. The evolutionary ecology of ethanol production and alcoholism. *Integrated Compositional Biology*, **44**, 284–289.
- Levey, D.J. Tewksbury, J.J. Izhaki, I. Tsahar, E. & Haak, D.C.** 2007. Evolutionary ecology of secondary compounds in ripe fruit: case studies with capsaicin and emodin. In: Dennis, A.J. Schupp, E.W. Green, R.J. & Westcott, D.A. (eds). Seed dispersal: theory and application in a changing world. First Edition. Cambridge University Press, Cambridge.
- Lieber, C.S.** 1997. Ethanol metabolism, cirrhosis and alcoholism. *Clinical Chemistry Acta*, **257**, 59–84.
- Mazeh, S., Korine, C., Pinshow, B. & Dudley, R.** 2008. The influence of ethanol on feeding in the frugivorous yellow-vented bulbul (*Pycnonotus xanthopigos*). *Behavioural Processes*, **77**, 369–375.
- Milton, K.** 2004. Ferment in the family tree: does a frugivorous dietary heritage influence contemporary patterns of Human ethanol use? *Integrated Comparative Biology*, **44**, 304–314.

- Orbach, D.N., Veselka, N., Dzal, Y., Lazure, L. & Fenton, M.B.** 2010. Drinking and flying: does alcohol consumption affect flight and echolocation performance in Phyllostomid bats? *PloS ONE*, **5**, 1–7.
- Sanchez, F., Korine, C., Pinshow, B. & Dudley, R.** 2004. The possible roles of ethanol in the relationship between plants and frugivores: First experiments with Egyptian fruit bats. *Integrated Compositional Biology*, **44**, 290–294.
- Sanchez, F., Korine, C., Steeghs, M., Laarhoven, L-J., Cristescu, S.M., Harren, F.J.M., Dudley, R. & Pinshow, B.** 2006. Ethanol and methanol as possible odour cues for Egyptian fruit bats. *Journal of Chemical Ecology*, **32**, 1289–1300.
- Sanchez, F., Kotler, B.P., Korine, C. & Pinshow, B.** 2008. Sugars are complementary resources to ethanol in foods consumed by the Egyptian fruit bats. *Journal of Experimental Biology*, **211**, 1475–1481.
- Sanchez, F., Melcon, M., Korine, C. & Pinshow, B.** 2010. Ethanol ingestion affects flight performance and echolocation in Egyptian fruit bats. *Behavioural Processes*, **84**, 555 – 558.
- Tewksbury, J.J.** 2002. Fruits, frugivores, and the evolutionary arms race. *New Phytologist*, **156**, 137–139.
- Valberg, L.K.** 1992. Feeding preferences of common bush tanagers for insect-infested fruits: avoidance or attraction? *Oikos*, **65**, 29–33.
- Yeomans, M.R.** 2004. Effects of alcohol on food and energy intake in human subjects: evidence for passive and active over-consumption of energy. *Britain Journal of Nutrition*, **92**, 31–34.

Figure legends

Fig. 1. The amount of food consumed between the two diets by speckled mousebird, *Colius striatus* (a), Cape white-eye, *Zosterops virens* (b) and the red-winged starling, *Onychognathus morio* (c). Black dots represent the mean and vertical bars indicate the standard error.

Table 1. The composition of artificial diets offered to speckled mousebirds, Cape white-eyes and red-winged starlings.

Ingredient	<u>Diet</u>	
	Control	Experimental
Water (ml)	1000	1000
Glucose (g)	150	150
Wheat bran (g)	50	50
Agar (g)	10	10
Sodium Chloride (g)	0.75	0.75
DiCalcium Phosphate (g)	0.80	0.80
Vitamin Supplement (g)	0.75	0.75
Ethanol (%)	0	1

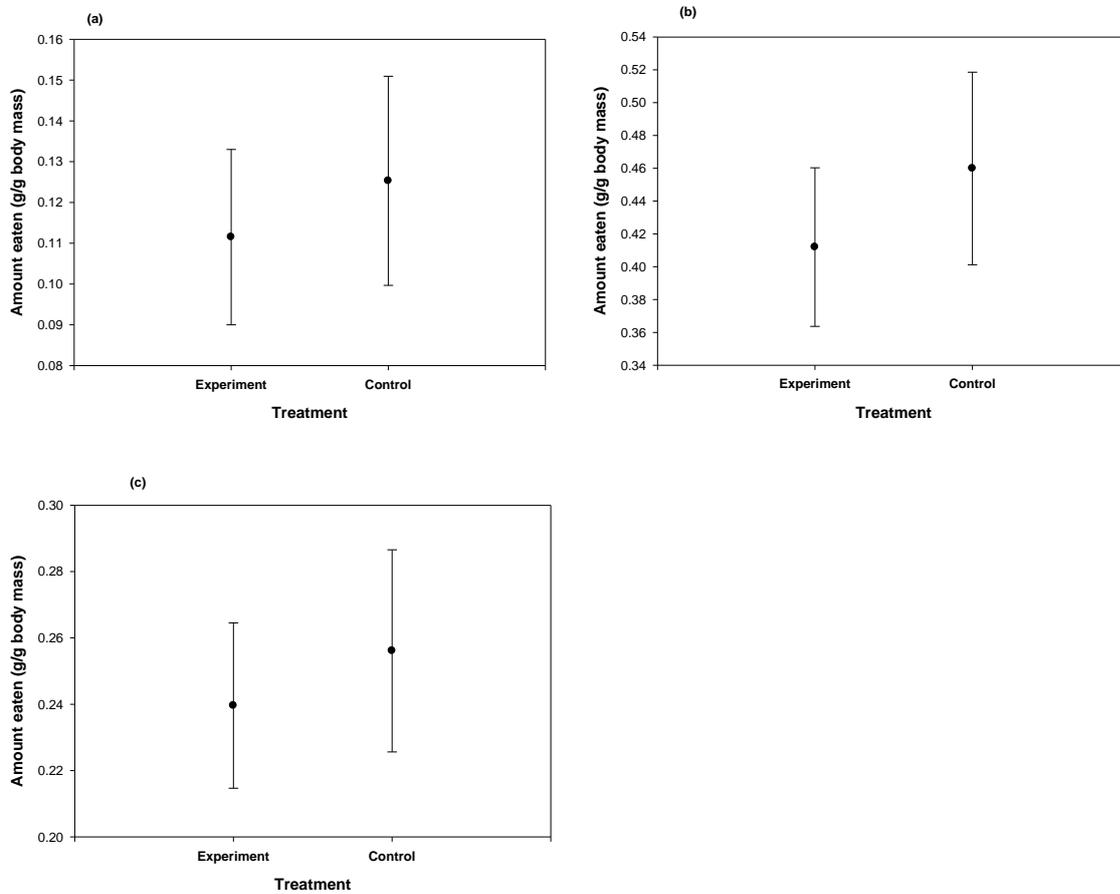


Fig. 1. The amount of food consumed between the two diets by speckled mousebird, *Colius striatus* (a), Cape white-eye, *Zosterops virens* (b) and the red-winged starling, *Onychognathus morio* (c). Black dots represent the mean and vertical bars indicate the standard error.

CHAPTER 5

The use of taste and smell in making foraging decisions in frugivorous birds: an experimental test using Red-winged Starlings *Onychognathus morio*

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Abstract

Birds' reliance on sight in making foraging decisions is well established. However, their reliance on taste and olfactory abilities especially in frugivores has received limited attention. Consequently, this study was conducted to determine the use of taste and olfaction by Red-winged Starlings *Onychognathus morio* in making foraging decisions. To determine whether they use taste in fruit selection, they were concurrently offered a control artificial fruit diet with another artificial fruit diet flavoured with different concentrations of ethanol and various fruit essences. To test whether they were able to use olfaction to locate food, they were provided with two choices: artificial fruit suspended over either banana and orange fruit essences and ethanol of varying concentrations or a control artificial fruit (without essence). It was hypothesized that (1) Red-winged Starlings have tasting ability which helps them in selecting fruits to feed on and that (2) Red-winged Starlings use olfaction to locate food.

Results were consistent with the first hypothesis but inconsistent with the second one. Thus, Red-winged Starlings use taste when selecting fruits to feed on but do not use olfaction to locate fruit sources. The latter was unexpected because birds with olfactory bulb sizes similar to theirs exhibit advanced olfactory abilities. The lack of significant olfactory abilities in Red-winged Starlings was attributed to the fact that this study was conducted outside of this species' breeding season when olfactory abilities were likely to be lowest and also to that olfactory abilities may not have been important at the scale investigated in this study. Overall, the results suggest that in addition to the sense of sight, the sense of taste plays an important role for birds in making optimal foraging decisions.

Keywords: artificial fruit; foraging; frugivore; scent; starlings; taste

Introduction

The role of olfaction in birds has been one of the most hotly debated and controversial issues in ecology (Petit et al. 2002; Balthazart and Taziaux 2008). Previously, olfaction was thought to be unimportant in birds (Balthazart and Taziaux 2008) and as a result, progress in avian olfactory research remained slow (Stager 1967). A turning point was brought about by a study by Bang and Cobb (1968) which compared the olfactory bulbs sizes (using olfactory ratio, calculated as the ratio of the diameter of the olfactory bulb to the diameter of the cortex) of 108 species of birds. The research uncovered huge variability in olfactory bulb sizes which suggested that its size could be beneficial to particular species or lifestyles (Castro et al. 2010). Furthermore, earlier studies were found to have significant flaws in experimental designs and were not conducted over extended periods of time (Stager 1967; Wenzel 2007), making their interpretations dubious (Balthazart and Taziaux 2008). Subsequently, a flurry of research has been conducted and points to the complexity of avian olfactory structures even for species with small olfactory bulbs (Balthazart and Taziaux 2008;

Gsell et al. 2012). The current view is that the gross anatomical and morphological olfactory structure of birds does not differ much from that of mammals and reptiles (Balthazart and Taziaux 2008). Therefore birds exhibit a whole range of olfactory abilities enjoyed by other taxa (Balthazart and Taziaux 2008) and have been found to use them for purposes such as kin recognition (Gsell et al. 2012), predator avoidance (Amo et al. 2008), mate choice (Bonnadonna et al. 2007), locating nesting material (Petit et al. 2002; De Groof et al. 2010), nest location (Caspers and Krause 2011), homing (DeBose and Nevitt 2008), navigation (DeBose and Nevitt 2008; Castro et al. 2010), food acquisition (Cunningham et al. 2009) and the prevention of ingesting toxic insect prey (Avery and Nelms 1990).

Among birds known to have well-developed olfactory abilities, primary candidates include Seabirds (Procellariiforms) (Vehyerden and Jouventin 1994; Nevitt 2000), the Kakapo Parrot (*Strigops habroptilus*) (Hagelin 2004), the Brown Kiwi (*Apteryx australis*) (Cunningham et al. 2009), the Homing Pigeon (*Columba livia domestica*) (DeBose and Nevitt 2008) and New World Vultures (Cathartidae) (Houston 1986). These species are characterized by either being nocturnal or foraging over vast stretches of the landscape (Healy and Guilford 1990; Nevitt 2000). This suggests that olfaction serves as the only efficient mechanism to locate food sources in these birds as commonly-used visual cues are limited at night or over large foraging distances (Mazeh et al. 2008). Thus, olfactory abilities for detecting food sources in birds with large olfactory bulbs are probably similar to those of other vertebrate taxa and challenge the long-held view that birds depend mainly on sight in making foraging decisions (Avery and Nelms 1990).

Fine-scale adjustment in diet selection is important for the maximization of energy gain in animals and forms the basis for the optimal foraging theory (OFT) (Schaefer et al. 2003). However, one shortcoming of the OFT which has limited its applicability in fruit-frugivore interactions is that it ignores the complexities associated with digestion and

nutritional requirements of species, focusing only on energy gain as a measure of animal performance (Bozinovic and Martinez del Rio 1996; Schaefer et al. 2003). An alternative view, the nutrient regulation hypothesis (Simpson and Raubenheimer 2001) takes into account the multi-dimensional nature of nutrient acquisition and states of foragers (Schaefer et al. 2003). This view posits that an optimal forager is the one which forages according to its energetic and nutritional needs (Simpson and Raubenheimer 2001). When viewed in this context, the ability to detect nutrient deficiencies in diet rather than in detecting diet differences *per se* is probably more important in making optimal foraging decisions as it allows birds to adapt to progressive changes in nutrient requirements through the seasonal cycle (i.e. during moult, birds require certain types of amino acids to build up keratin, Murphy and King 1987). This allows birds to regulate intake to match their energetic and nutritional requirements which ensures a sufficient supply of energy and nutrients, a prerequisite for maintaining a balanced diet (Murphy and King 1987). This is probably more important for frugivorous birds since protein is limiting in most fruit types (Bosque and Calchi 2003; Witmer 1998; 2001), resulting in body mass loss when consumed below maintenance levels (Izhaki and Safriel 1989; Witmer 2001).

The ability to detect differences in nutrient and energy concentration in diet by taste is however a prerequisite for both the OFT and nutrient regulation models (Schaefer et al. 2003). Previous studies have shown that some frugivorous birds display remarkable abilities to detect subtle differences in diet composition by taste. For example, Levey (1987) showed that Blue-grey Tanagers *Thraupis episcopus* can detect differences in concentrations of 2% for sugars. In another study, Bosque and Calchi (2003) demonstrated that for proteins, Tanagers can detect dietary differences as low as 0.09% fresh weight. Similarly, four species of Tanagers were able to detect differences in diet of 1% for carbohydrates and 2% for lipids

(Schaefer et al. 2003). Such fine-scale discrimination abilities suggest that taste sensitivity in birds could play a bigger role than currently appreciated.

Ethanol is a natural by-product of the fermentation process of fruit sugars by yeasts (Mazeh et al. 2008). The advent of ethanol production in fruits occurred simultaneously with the shift in dispersal patterns in plants from wind-dispersed to animal-dispersed seed in the Cretaceous (Benner et al. 2002). This suggests a long-term historical association between frugivores and ethanol (Dudley 2000; 2002). Ethanol concentration increases with the fruit ripening process and its concentration is positively correlated to fruit sugars (Dudley 2004; Levey 2004; Sanchez et al. 2004). Because of the ubiquity of ethanol in fruits and the highly heterogeneous nature of fruit distribution especially in tropical ecosystems, it could be plausible that over evolutionary times, frugivores associate it with a nutritional reward (Dudley 2000; 2002). A corollary to this hypothesis is that species that use olfaction may be particularly adept at using its odour in locating fruiting areas (Dudley 2000; Levey 2004; Milton 2004; Sanchez et al. 2006). There is a dearth of knowledge on the use of ethanol as a foraging cue in birds. In studies on birds conducted to date, ethanol was found not to be used as a foraging cue (Mazeh et al. 2008; Chapter 5, this thesis).

Red-winged Starlings *Onychognathus morio* are extremely adaptable feeders with a predominantly frugivorous diet but consume considerable amounts of insects and nectar (Brown et al. 2012). Although their sugar-tasting abilities have been demonstrated (Brown et al. 2012), their discriminatory abilities for other dietary properties are unknown. Furthermore, nothing is known about their olfactory abilities although their small olfactory bulbs (Bang and Cobb 1968) suggest that they could be having poor olfactory abilities. This study was conducted against this backdrop and had two aims. The first aim was to test the ability of Red-winged Starlings to make foraging decisions based on taste. For this part of the study, Starlings were simultaneously provided with two diets: an artificial fruit diet with nothing

added to it and an artificial fruit diet mixed with different fruit essences and ethanol of various concentrations. It was hypothesized that Red-winged Starlings have tasting ability allowing them to make foraging decisions as has been found in other studies on birds (Levey 1987; Bosque and Calchi 2003). The second aim of the study was to assess the ability of the Red-winged Starlings to use olfaction to discriminate between diets. To do this, Starlings were concurrently offered two artificial fruit diets in small dishes which were placed on large plastic dishes: one diet had pure water poured onto the plastic dish and in the other one various fruit essences and ethanol of different concentrations were poured. It was hypothesized that Red-winged Starlings use olfaction to locate food, as has been found in other species with olfactory bulbs similar to theirs (Petit et al. 2002; Mennerat et al. 2005).

Materials and Methods

Study area and animal maintenance

For this study, 8 female Red-winged Starlings kept at the Animal House (29°37'0''S; 30°23'0''E, 660 m above sea level), University of KwaZulu-Natal (UKZN), were used. These birds were kept in a large outside group aviary (4 × 3 × 2m). They were fed everyday between 07h00 and 08h00 on a maintenance diet of chopped and mixed fruits including pawpaws, bananas, apples, pears and oranges, supplemented with AviPlus Softbill Mynah Pellets (Aviproducts, Waterfall, Durban, South Africa). Occasionally, birds were provided with mealworms (*Tenebrio* spp.). Water was provided *ad libitum*. All birds were captured in the vicinity of Pietermaritzburg and had been in captivity for more than a year and thus are fully acclimated to captivity and food preference trials. Five days prior to the start of the experimental trials, birds were transferred to individual cages in a controlled environment room (12L: 12D photoperiod; temperature 25 ± 0.1°C) to acclimate to experimental

conditions. During the acclimation period, they were fed on the same schedule as they were in outside aviaries.

Experimental protocol

Trials used in this study were based on artificial diets designed by Witmer (1998) for North American birds. The artificial fruit diets were prepared by mixing together the ingredients (Table 1) and cooking them in a microwave oven for about 8 minutes. With continuous stirring by hand to ensure that ingredients disseminated evenly, the food was then allowed to cool down. Before it solidified, 5 ml of the Robertson's red food colorant (Libstar Manufacturing Solutions, Chloorkop, South Africa) was poured to the mixture to improve the appearance of the food. Because of the short lifespan of artificial fruits, food was prepared the evening prior to the day of the experiment and kept in a refrigerator overnight. Before the start of the experiment, food was cut into small squares (1×1 cm) in preparation to be consumed by birds. Two trials were run for each experiment and trials lasted 150 min starting from 06h00 in the morning.

Taste trials

In these trials, four diet treatments were used and were offered simultaneously with the control diet. These diets were: a 1% ethanol diet made by adding 10 ml of ethanol to the control diet; a 3% ethanol diet made by adding 30 ml of ethanol to the control diet; a banana-scented diet made by adding 60 ml of banana essence to the control diet and an orange-scented diet made by adding 60 ml of orange essence to the control diet. Two diet treatments

put in metal dishes were administered to each bird: the first containing a control artificial fruit diet and the other contained one of the four treatment diets. These diets represented ‘non-scented’ and ‘scented’ diets respectively. Diets were placed equal distances from bird perches and the side of the cages to ensure that birds travelled a similar distance to feed on each diet. To minimize side bias, birds were rotated in the cages daily. Each trial lasted 150 min after which the remaining food was weighed. Bird body mass was measured before and after each experimental trial. For each diet type, a corresponding control dish placed in the experimental room was weighed on a similar schedule as the experimental diets to control for evaporative water loss. At the end of the experimental trials, birds were given the normal maintenance diet. Total food consumption for each diet was calculated by taking the difference between the initial and final mass of the food and subtracting the amount of mass lost through evaporation. Although not all food removed from the dishes was consumed (for example, food that spilled to the floor was recorded as eaten whereas it was not), this was recorded as such as this indicated a preference for the selected food item.

Scent trials

Artificial fruit diets in these trials were prepared and placed in metal dishes which were put into large plastic dishes. Four solutions, which were poured into the large plastic dishes, were used to elicit response in birds. Fruit-scented solutions (banana and orange) were prepared by mixing 50 ml of each fruit essence with 60 ml of water. The other scented solutions were a pure ethanol solution whereby 60 ml of pure ethanol was poured into the large plastic dish and a dilute (5%) ethanol solution made by adding 5 ml of ethanol to 95 ml of water. In these trials, ethanol was therefore also treated as an “essence”. These represented ‘scented’ treatments. In ‘non-scented’ treatments, control diets were placed inside the large plastic

dishes in which 60 ml of water was poured. During the experimental trials, each bird was given a choice between two diets: one 'scented' and one 'non-scented' diet. The rest of the methodology for the scent trials followed that of taste trials.

Assessment of volatiles in essence scents

An analysis of the volatiles present in each fruit essences was done to determine whether diet preference could be explained by volatile composition of the essences. The analyses of the volatiles present in the headspace of each of the essences were sampled by means of the dynamic headspace extraction methods (see Shuttleworth and Johnson (2009) for details of the method). Briefly, odour samples of the essences were collected from the mouth of the vial for a period of 30 seconds and then analysed by coupled Gas Chromatography-Mass Spectrometry (GC-MS). To identify the compounds present in each essence, the Varian Workstation software with the NIST05 mass spectral library was used (Shuttleworth and Johnson 2009). Because the aim of this study was to compare the volatile composition of the two essences, the retention times of authentic standards and published Kovats indices were not used to verify the identity of compounds in cases where there was uncertainty because the level of details provided by this coarse analysis was sufficient for this study (Shuttleworth, *pers. comm*).

Statistical analyses

As each individual bird was used more than once, a Generalized Linear Model (GLM) Repeated Measures of Analysis of Variance (RMANOVA) was used to compare the amount of food eaten between diets. The amount of food eaten by each bird for the duration of the

trials was divided by the bird's initial body mass and expressed as a mass-specific value. Following significant differences, a Tukey's *Post hoc* test was run to find where the differences were. Data are expressed as means \pm standard error except where specified. All the statistical analyses were conducted using STATISTICA 7.0 (Statsoft Inc., Tulsa, USA).

Results

Taste

Significant differences were found in the amount of food consumed between scented and non-scented diets by Red-winged Starlings (GLM RMANOVA: $F_{(3, 21)} = 6.461$, $p = 0.003$; Fig. 1). Significant differences were observed between orange diets whereby the scented diet was consumed significantly more than the non-scented diet (Tukey's *Post hoc* test, $P < 0.05$). Among scented diets, the banana-scented diet was consumed significantly less than the orange-scented and 1% ethanol diets (Tukey's *Post hoc* test, $P < 0.05$); however, its consumption was not different to that of the 3% ethanol diet (Tukey's *Post hoc* test, $P > 0.05$). No significant differences in consumption were found between the orange-scented, 1% and 3% ethanol diets (Tukey's *Post hoc* test, $P > 0.05$). Among non-scented diets, the banana- and orange-scented diets were consumed significantly less than the 1% and 3% ethanol diets (Tukey's *Post hoc* test, $P < 0.05$).

Scent

There were no significant differences in the amount of food consumed between scented and non-scented diets by Red-winged Starlings (GLM RMANOVA; $df = 7$, $F_{(1,7)} = 1.140$, $p =$

0.321; Fig. 2). However, non-scented diets (0.083 ± 0.022) were consumed more than the scented diets (0.075 ± 0.020). No significant differences were observed among scented and non-scented diets (Tukey's *Post hoc* test, $P < 0.05$). Scent volatile analyses showed that banana and orange essences contained different chemical volatiles (Table 2).

Discussion

Fruits represent a nutrient-dilute food resource as they are typically high in water and carbohydrates but low in lipids and proteins (Herrera 1987; Corlett 1996; Witmer 1998). Therefore to maintain a balanced diet in frugivorous birds, the ability to detect subtle differences in diet composition is indispensable. This is especially the case when animals face changing nutritional requirements which require active changes in food preferences. Therefore by detecting differences in diet composition, frugivores can forage in a way that ensures the acquisition of nutrients/nutrient ratios based on their requirements in the body. In this study, Red-winged Starlings consumed significantly different amounts of scented and non-scented diets in taste trials but not in scent trials. This suggests that they use tasting but not olfactory abilities in making foraging decisions. The latter is surprising as it deviates substantially from results obtained from species with bulb sizes similar to theirs.

Tasting ability

In the past, the sense of taste in birds was considered to be poor because of the relative small number of olfactory bulbs in birds (Duncan 1960). Furthermore, fast passage rates, the absence of mastication in the buccal cavity and lack of mixing of saliva with food are some of the factors that were thought to render birds to have limited tasting ability (Klasing 1998).

However, studies on nectarivorous (Hainsworth and Wolf 1976; Martinez del Rio et al. 1989; Lotz and Nicolson 1996; Jackson et al. 1998; Lane 1998) and frugivorous birds (Levey 1987; Bosque and Calchi 2003; Schaefer et al. 2003) have shown that birds possess fine-scale discrimination abilities. Combined, these results suggest that the tasting abilities of birds do not differ much from those of other taxa.

In this study, significant differences were found in consumption between the scented and non-scented diet types suggesting that Red-winged Starlings are able to adjust dietary preferences based on fine-scale differences in diet. Fruit rinsing and fruit palpating behaviours observed for this species, as well as the rejection of fruits after palpating, provide further support for their ability to use taste (Jordaan and Downs 2012). These results corroborated a number of previous studies on birds (Duncan, 1960; Sorensen 1983; Levey 1987; Murphy and King, 1987; Bosque and Calchi, 2003; Schaefer et al. 2003; 2008). Among the scented diets, the orange-scented diet was consumed significantly more than the banana-scented diet. This preference pattern was in line with observations that when starlings were offered the maintenance diet on non-experimental days, they always consumed the orange fruit more than banana fruit (M.M. Zungu, *pers. obs.*). These results imply that Red-winged Starlings consistently preferred orange over banana fruits. Although this is not to infer that the same could be the case when these fruits are encountered in a 'field' situation, it serves to highlight that these birds showed consistent preference. Because the banana fruit provides almost twice (89 Kcal) the amount of energy provided by the orange (47 Kcal) fruit per unit mass (USDA 2013), the preference for orange over banana fruits may not be based on energy content (e.g. Johnson et al. 1985; Lepczyk et al. 2000) but could be related to the high availability of nutrients that are found in negligible amounts in banana (e.g. vitamins and minerals). This lends support to the proposition that the overall balance of nutrients rather than the energy content of the diet is more important in frugivorous birds (Levey and

Martinez del Rio 2001; Schaefer et al. 2003). The low consumption of the 3% ethanol diet compared to the 1% ethanol diet could be due to that at high concentrations; ethanol becomes a deterrent whereas at 1% it does not affect food intake. The reduction in food intake at high ethanol concentrations has been found in a wide array of frugivores suggesting that ethanol is a deterrent rather than an attractant (Sanchez et al. 2006; 2008; Mazeh et al. 2008; Orbach et al. 2010).

Flavours in food can initiate high food intake but birds soon learn that no energetic benefits are associated with them and thus reduce intake (Forbes and Shariatmadari 1994). This is because birds can associate sensory traits of food with its metabolic effects (i.e. post-ingestive feedback, Schaefer et al. 2008). Therefore continuous sampling of food in birds is important especially when they are feeding on diets where the hierarchy of profitability changes continually as this would allow them to switch diets accordingly (Forbes and Shariatmadari 1994). However, birds need to be exposed to food for a relatively long period of time for post-ingestive feedback to take effect (Bosque and Calchi 2003). For example in European Starlings *Sturnus vulgaris*, post-ingestive feedback was only observed after two 3 hour feeding trials (Avery et al. 1995). The experimental trials in this study lasted only for 150 min which suggests that the preference patterns observed are not based on post-ingestive feedback. The implication of this is that had the experiments been longer, post-ingestive feedback could have masked taste preferences. This argues caution in experimental design with regards to the duration of preference experiments as it can greatly affect the interpretation of the results.

Olfactory ability

In this study, no significant differences in the amount of food eaten between scented and non-scented diets were observed. This suggests that Red-winged Starlings do not use olfaction to locate food. Previous studies postulated that diurnal species should depend more on vision for survival (Avery and Nelms 1990) unlike nocturnal species which require a better-developed olfactory system as visual cues are limited in darkness (Healy and Guilford 1990; Mazei et al. 2008). As a corollary, nocturnal species could be expected to have larger olfactory bulbs compared to their diurnal counterparts (Healy and Guilford 1990). Furthermore, among diurnal species, only species that forage over large oceanic or terrestrial distances could be expected to exhibit efficient olfactory capabilities (Houston 1986; Clark et al. 1993; Nevitt 1999; 2000; DeBose and Nevitt 2008; Mazei et al. 2008). Since Red-winged Starlings do not satisfy any of the above criteria, without sufficient scrutiny, the results obtained from this study would have led us to similar conclusions, reinforcing the supposition that olfactory bulb sizes determine olfactory abilities in birds. However, we believe it is premature to accept this notion and below we provide evidence to substantiate our conclusion.

In passerines, olfaction has long been ignored and is not well understood because they possess small olfactory bulbs and as such, olfaction was thought to be unimportant to them (Clark 1991; Mennerat et al. 2005; De Groof et al. 2010; Mennerat et al. 2005; Mennerat 2008; Caspers and Krause 2011). However, recent studies have shown that even species with small olfactory bulbs show advanced olfactory abilities (Petit et al. 2002; Roper 2003; Gwinner and Begger 2005; Caspers and Krause 2011). Furthermore, a study by Steiger et al. (2008) showed that even in species with small olfactory bulbs, the number of olfactory receptor genes is as high as in species with large olfactory bulbs. Therefore a large olfactory bulb is not a prerequisite for high sensitivity to odour (Castro et al. 2010; Beckstrom 2013)

but may allow species to discriminate easily between different types of odours (Buitron and Neuchterlein 1985). This suggests that species with small olfactory bulbs are not necessarily insensitive to odour but could be sensitive only to those odours that are critical for their survival.

Studies have shown that in the European Starling, the level of olfactory ability varies in relation to the breeding season (Clark et al. 1993; Gwinner and Berger 2005). These birds have high levels of olfaction during the breeding season and low levels outside of it (De Groof et al. 2010). The levels of olfaction are reported to increase in the breeding owing to the need to find herbs to build nests. This was found to be more pronounced in male parents as they are more involved in searching for nest-building materials than females (Gwinner and Berger 2005; 2008). The same could be the case with the closely-related Red-winged Starlings. The breeding season of the red-winged starlings in southern Africa ranges from October to April (Kopij 2009). The current study was conducted in July, using only females. This means that it was conducted outside the breeding season of this species which raises the possibility that it was conducted when their olfactory abilities were lowest. Another consideration is that olfaction may only be used for large-scale foraging, i.e. to smell a fermenting tree from far while flying. At large scales, birds have been shown to use olfaction to locate areas of high resource productivity and at smaller scales; they use it to pinpoint the exact location of the food source (Nevitt 2000; DeBose and Nevitt 2008). Our experiments were conducted on a small scale and perhaps using olfaction at this scale is not necessary for frugivores as they can use tasting ability in choosing fruits to feed on (Levey 1987).

The scent volatile composition in banana and orange fruit essences was significantly different (Table 2) but Red-winged Starlings showed no significant preference between diets scented with them. It is difficult explain why this was the case because there is a general lack of knowledge regarding bird response to different scent volatiles. This could stem from the

fact that classical descriptions of bird pollination syndromes have always classified bird-pollinated flowers as scentless (Knudsen et al. 2004) and also because the olfactory abilities of birds have always been enormously undermined (Balthazart and Taziaux 2008). This has generated limited research on bird response to floral scent volatile composition which is in stark contrast to their insect counterparts (Raguso 2004). Red-winged Starlings, despite being classified as occasional nectarivores, are important pollinators of a number of species especially *Aloe* and red-hot poker (*Kniphofia* spp.) (Brown et al. 2009). Thus their response to particular scent volatiles could elucidate the role they play in the pollination of plants depending on them. It is anticipated that further research on bird-pollination systems is more likely to yield promising insights in this line of research. The explanation for the higher consumption of the artificial diet scented by a dilute ethanol compared to the one scented by the pure ethanol solution is similar to the one already discussed above (see the Discussion on Tasting ability).

In conclusion, this study added to the limited knowledge on how tasting and olfactory abilities affect fruit selection in frugivorous birds. Results showed that Red-winged Starlings are able to use taste but not smell in making foraging decisions. This lack could be attributed to the possibility that this study was conducted when their olfactory abilities were lowest. Furthermore, there is a possibility that to them olfaction may not be important for foraging at the scale examined in this study. It is recommended that future studies should be conducted over the long-term (considering all seasons) to limit the confounding effect of season. Because in some birds the level of olfactory ability differs with sex (Mennerat et al. 2005), future studies should ensure that both sexes should be used so that results obtained could be extrapolated to the whole population.

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References

- Amo L, Tomas G.G, Sanz JJ (2008) Predator odour recognition and avoidance in a songbird. *Functional Ecology* 22: 289 – 293
- Avery ML, Nelms CO (1990) Food avoidance by red-winged blackbirds conditioned with a pyrazine odour. *Auk* 107: 544 – 549
- Avery ML, Decker DG, Humphrey JS, Hayes AA, Laukert CC (1995) Color, size and location of artificial fruits affect sucrose avoidance by cedar waxwings and European starlings. *Auk* 112: 436 – 444
- Balthazart J, Taziaux M (2008) The underestimated role of olfaction in avian reproduction? *Behavioural Brain Research* 200: 248- 259
- Bang BG, Cobb S (1968) The size of the olfactory bulb in 108 species of birds. *Auk* 85: 55 – 61
- Beckstrom J (2013) Olfactory discrimination in a captive turkey vulture *Cathartes aura*. Unpublished Honours Thesis, Wheaton College, Norton, Massachusetts
- Bonnadonna F, Miguel E, Grosbois V, Jouventin P, Bessiere J-M (2007) Individual odour recognition in birds: an endogenous olfactory signature on petrel's feathers? *Journal of Chemical Ecology* 33: 1819 – 1829
- Bosque C, Calchi R (2003) Food choice by blue-grey tanagers in relation to protein content. *Comparative Biochemistry and Physiology A* 135: 321 – 327

- Bozinovic F, Martinez del Rio C (1996) Animals eat what they should not: why do they reject our foraging models? *Revista Chilena de Historia Natural* 69: 15 – 20
- Brown M, Downs CT, Johnson SD (2009) Pollination of the red hot poker *Kniphofia caulescens* by short-billed opportunistic nectarivores. *South African Journal of Botany* 75: 707 – 712
- Buitron D, Neuchterlein GL (1985) Experiments on olfactory detection of food caches by black-billed magpies. *Condor* 87: 92 – 95
- Caspers BA, Krause E (2011) Odour-based natal nest recognition in the zebra finch *Taeniopygia guttata*, a colony-breeding songbird. *Biology Letters* 7: 184 – 186
- Castro I, Cunningham SJ, Gsell AC, Jaffe K, Cabrera A, Liendo C (2010) Olfaction in birds: a closer look at the kiwi (Apterygidae). *Journal of Avian Biology* 41: 213 – 218
- Clark L (1991) Odour detection thresholds in tree swallows and cedar waxwings. *Auk* 108: 177 – 180
- Clark L, Avilova KV, Bean NJ (1993) Odour thresholds in passerines. *Comparative Biochemistry and Physiology A* 104: 305 – 312
- Corlett RT (1996) Characteristics of vertebrate-dispersed fruits in Hong Kong. *Journal of Tropical Ecology* 12: 819 – 833
- Cunningham CJ, Castro I, Potter MA (2009) The relative importance of olfaction and remote touch in prey detection by North Island brown kiwis. *Animal Behaviour* 78: 899 – 905.
- De Groof G, Gwinner H, Steiger S, Kampenaers B, van der Linden A (2010) Neural correlates of behavioural olfactory sensitivity changes seasonally in European starlings. *PLoS ONE* 5: 1 – 7.

- DeBose JL, Nevitt GA (2008) The use of odours at different spatial scales: comparing birds with fish. *Journal of Chemical Ecology* 34: 867 – 881
- Dudley R (2000) Evolutionary ecology of human alcoholism in primate frugivory. *Quarterly Review of Biology* 75: 3 – 15
- Dudley R (2002) Fermenting fruit and the historical ecology of ethanol ingestion: is alcoholism in modern humans an evolutionary hangover? *Addiction* 97: 381 – 388
- Dudley R (2004) Ethanol, fruit ripening and the historical origin of human alcoholism in primate frugivory. *Integrated and Comparative Biology* 44: 315 – 323
- Duncan CJ (1960) Preference tests and the sense of taste in the feral pigeon (*Columbia livia* var *Gmelin*). *Animal Behaviour* 111: 55 – 61
- Forbes JM, Shariatmadari F (1994) Diet selection for protein by poultry. *World's Poultry Science Journal* 50: 7 – 24
- Gsell AC, Hagelin JC, Brunton DH (2012) Olfactory sensitivity in kea and kaka. *Emu* 112: 60 – 66
- Gwinner H, Berger S (2005) European starlings: nestling condition, parasites and green nest material during the breeding season. *Journal of Ornithology* 146: 365 – 371
- Gwinner H, Berger S (2008) Starling males select green nest material by olfaction using experience-independent and experience-dependent cues. *Animal Behaviour* 75: 971 – 976
- Hagelin JC (2004) Observations on the olfactory ability of the kakapo *Strigops habroptilus*, the critically-endangered New Zealand parrot. *Ibis* 146: 161 – 164
- Hagelin JC, Jones IL (2007) Bird odours and other chemical substances: a defence mechanism or overlooked mode of intraspecific communication? *Auk* 124: 741 – 761

- Hainsworth FR, Wolf LL (1976) Nectar characteristics and food selection by hummingbirds. *Oecologia* 25: 101 – 113
- Healy S, Guilford T (1990) Olfactory-bulb size and nocturnality in birds. *Evolution* 44: 339 – 346
- Herrera CM (1982) Defence of ripe fruits from pests: its significance in relation to plant-disperser interactions. *American Naturalist* 120: 218 – 241
- Herrera CM (1987) Vertebrate-dispersed plants of the Iberian Peninsula: a study of fruit characteristics. *Ecological Monographs* 57: 305 – 331
- Houston DC (1986) Scavenging efficiency of turkey vultures in tropical forests. *Condor* 88: 318 – 323
- Hutchinson LV, Wenzel BM (1980) Olfactory guidance in foraging by Procellariiforms. *Condor* 82: 314 – 319
- Izhaki I, Safriel UN (1989) Why are there so few exclusively frugivorous birds? Experiments on fruit digestibility. *Oikos* 54: 23 – 32
- Jackson S, Nicolson SW, Lotz CN (1998) Sugar preferences and “side bias” in Cape sugarbirds and double-collared sunbirds. *Auk* 115: 156 – 165
- Johnson RA, Willson MF, Thompson JN, Bertin RI (1985) Nutritional values of wild fruits and consumption by migrant frugivorous birds. *Ecology* 66: 819 – 827
- Knudsen JT, Tollsten L, Bergstrom G, Raguso RA (2004) Trends in floral scent chemistry in pollination syndromes: floral scent composition in hummingbird-pollinated taxa. *Botanical Journal of the Linnean Society* 146: 191 – 199

- Kopij G (2009) Segregation in sympatrically nesting red-winged starlings *Onychognathus morio* and European starling *Sturnus vulgaris*. Polish Journal of Ecology 57: 589 – 595
- Lane SJ (1998) Preferences and apparent digestibilities of sugars by fruit damaging birds in Japan. Annals of Applied Biology 130: 361 – 370
- Lepczyk CA, Murray KG, Winnett-Murray K, Bartell P, Geyer E, Work T (2000) Seasonal fruit preferences for sugar and lipids by American robins. Auk 117: 709 – 717
- Levey DJ (1987) Sugar-tasting ability and fruit selection in tropical fruit-eating birds. Auk 104: 173 – 179
- Levey DJ (2004) The evolutionary ecology of ethanol production and alcoholism. Integrated and Comparative Biology 44: 284 – 289
- Levey DJ, Martinez del Rio C (2001) It takes guts (and more) to eat fruits: lessons from avian nutritional ecology. Auk 118: 819 – 831
- Lotz CN, Nicolson SW (1996) Sugar preferences of a nectarivorous bird, the lesser double-collared sunbird *Nectarinia chalybea*. Functional Ecology 10: 360 – 365
- Lotz CN, Schondube JE (2006) Sugar preferences in nectar- and fruit-eating birds: behavioural patterns and physiological causes. Biotropica 38: 3 – 15
- Martinez del Rio C, Levey DJ, Karasov WH (1989) Physiological basis and ecological consequences of sugar preferences in cedar waxwings. Auk 106: 64 – 71
- Mazeh S, Korine C, Pinshow B, Dudley R (2008) The influence of ethanol on feeding in the frugivorous yellow-vented bulbul *Pycnonotus xanthopygos*. Behavioural Processes 77: 369 – 375

- Mennerat A, Bonadonna F, Perret P, Lambrechts MM (2005) Olfactory conditioning experiments in a food-searching passerine bird in semi-natural conditions. *Behavioural Processes* 70: 264 – 270
- Mennerat A (2008) Blue Tits *Cyanistes caeruleus* respond to an experimental change in the aromatic plant odour composition of their nest. *Behavioural Processes* 79: 189 – 191
- Milton K (2004) Ferment in the family tree: does a frugivorous dietary heritage influence contemporary patterns of human ethanol use? *Integrated and Comparative Biology* 44: 304 – 314
- Moermond TC, Denslow JS (1985) Neotropical avian frugivores: patterns of behavior, morphology and nutrition, with consequences for fruit selection. *Ornithological Monographs* 36: 865 – 897
- Murphy ME, King JR (1987) Dietary discrimination by moulting White-crowned Sparrows given diets differing only in sulphur amino acid concentration. *Physiological Zoology* 60: 279 – 289
- Nevitt G (1999) Olfactory foraging in Antarctic Seabirds: a species-specific attraction to krill odours. *Marine Ecology Progress Series* 177: 235 – 241
- Nevitt GA (2000) Olfactory foraging by Antarctic Procellariiform seabirds: Life at high Reynolds numbers. *Biological Bulletin* 198: 245 – 253
- Orbach DN, Veselka N, Dzal Y, Lazure L, Fenton MB (2010) Drinking and flying: does alcohol consumption affect the flight and echolocation performance of Phyllostomid bats? *PloS ONE* 5: 1 – 7
- Petit C, Hossaert-McKey M, Perret P, Blondel J, Lambrechts MM (2002) Blue tits use selected plants and olfaction to maintain an aromatic environment for nestlings. *Ecology Letters* 5: 585 – 589

- Raguso RA (2004) Wake up and smell the roses: the ecology and evolution of floral scent. *Annual Reviews of Ecology, Evolution and Systematics* 39: 549 – 569
- Roper TJ (2003) Olfactory discrimination in yellow-backed chattering lorries *Lorius garrulus flavopalliatu*s: first demonstration of olfaction in Psittaciformes. *Ibis* 145: 689 – 691
- Roth TC, Cox JG, Lima SL (2008) Can foraging birds assess predation risk by scent? *Animal Behaviour* 76: 2021 – 2027
- Sager KE 1967. Avian olfaction. *American Zoologist* 7: 415 – 419
- Sanchez F, Korine C, Pinshow B, Dudley R (2004) The possible roles of ethanol in the relationship between plants and frugivores: first experiments with the Egyptian fruit bats. *Integrated and Comparative Biology* 44: 290 – 294
- Sanchez F, Korine C, Steeghs M, Laarhoven L-J, Cristescu SM, Harren FJM, Dudley R, Pinshow B (2006) Ethanol and methanol as possible odour cues for Egyptian fruit bats *Rousettus aegyptiacus*. *Journal of Chemical Ecology* 32: 1289 – 1300
- Schaefer HM, Schmidt V, Bairlein F (2003) Discrimination abilities for nutrients: which differences matter for choosy birds and why? *Animal Behaviour* 65: 531 – 541
- Schaefer HM, Spitzer K, Bairlein F (2008) Long-term effects of previous experience determine nutrient discrimination abilities in birds. *Frontiers in Zoology* 5: 4
- Shuttleworth A, Johnson SD (2009) The importance of scent and nectar filters in a specialized wasp-pollination system. *Functional Ecology* 2009: 931 – 940
- Simpson SJ, Raubenheimer D (2001) The hungry locust. *Advances in the Study of Behaviour* 29: 1 – 44
- Sorensen AE (1983) Taste aversion and frugivore preference. *Oecologia* 56: 117 – 120

- Steiger SS, Fidler AE, Valcu M, Kampenars B (2008) Avian olfactory receptor gene repertoires: evidence for a well-developed sense of smell in birds? *Proceedings of the Royal Society B* 275: 2309 – 2317
- USDA (2013) United States Department of Agriculture, Agricultural Research Service. USDA National Nutrient Database for Standard Reference: Release 26. <http://www.ars.usda.gov/ba/bhnrc/ndl>. Accessed 22/05/2014.
- Verheyden C, Jouventin P (1994) Olfactory behaviour of foraging Procellariiforms. *Auk* 111: 285 – 291
- Witmer M.C (1998) Ecological and evolutionary implications of energy and protein requirements of avian frugivores eating sugary diets. *Physiological Zoology* 71: 599 – 610
- Witmer MC (2001) Nutritional interactions and fruit removal: Cedar waxwing consumption of *Viburnum opulus* fruits in spring. *Ecology* 11: 3120 – 3130

Figure legends

Fig. 1. The amount of food consumed by the Red-winged Starlings in all diets for the for the taste trials ($n = 8$).

Fig. 2. The amount of food consumed by the Red-winged Starlings in all diets for the for the scent trials ($n = 8$).

Table 1. Ingredients of the artificial fruit diet used throughout during the experimental trials.

Ingredient	Amount
Distilled water (ml)	1000
D-Glucose (g)	150
Wheat bran (g)	50
Agar (g)	10
Sodium Chloride (g)	0.75
DiCalcium Phosphate (g)	0.80
Avian Vitamin supplements (g)	0.75

Table 2. The volatiles composition of the banana and orange essences, respectively.

Compound	Percent
Banana essence	
Ethanol	0.6
Ethyl Acetate	7.6
Ethyl butyrate	5.1
Isoamyl acetate	48.9
Butyl butyrate	13.9
Isoamyl butanoate	11.2
Isoamyl isovalerate	0.4
Linalool	0.1
Propylene Glycol	4.9
Beta-Citral	0.2
Benzyl acetate	1.0
Benzyl propanoate	0.0
Benzyl alcohol	0.1
1,2-Diacetate glycerol OR Triacetin	5.3
1,2,3-Propanetriol, 1-acetate	0.3
Unknown, possibly an isomer of above compound	0.1
Orange essence	
Ethanol	1.4
Alpha-Pinene	4.0
Monoterpene: Either Beta-Thujene or beta-Terpinene	1.2
Sabinene	0.8
Limonene	86.3
Beta-Citral	1.2
Alpha-Terpineol	3.5
Alpha-Citral	1.6

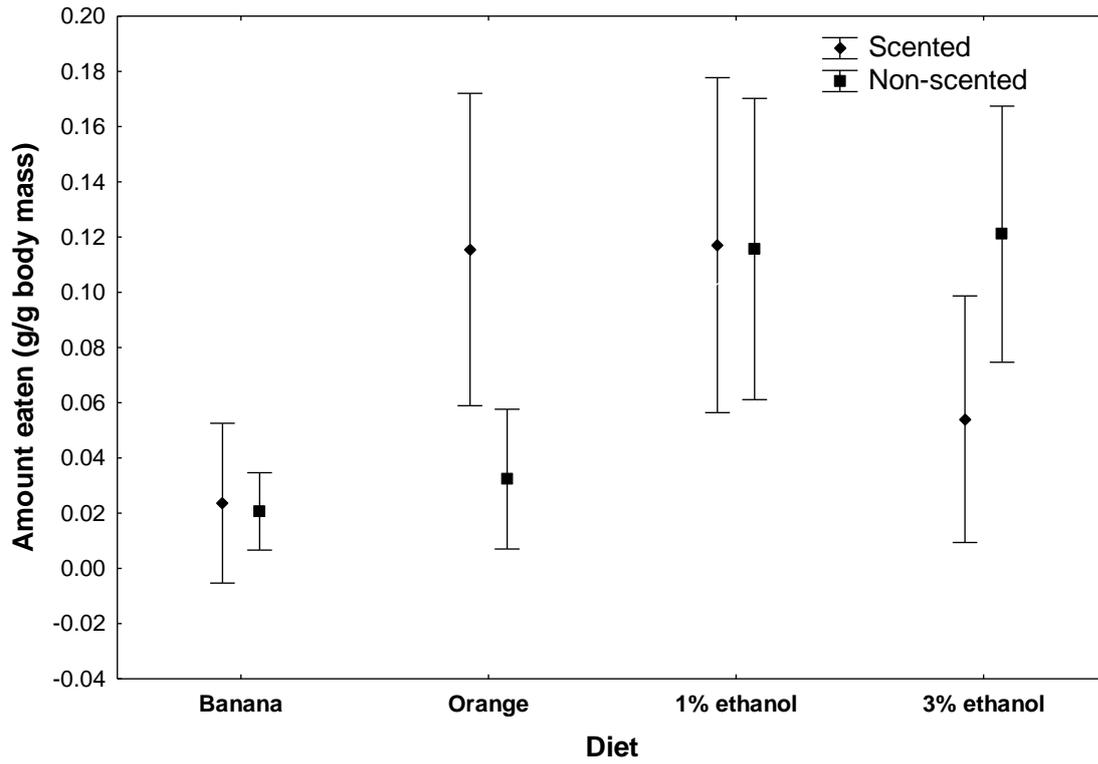


Fig. 1. The amount of food consumed by the Red-winged Starlings in all diets for the for the taste trials ($n = 8$).

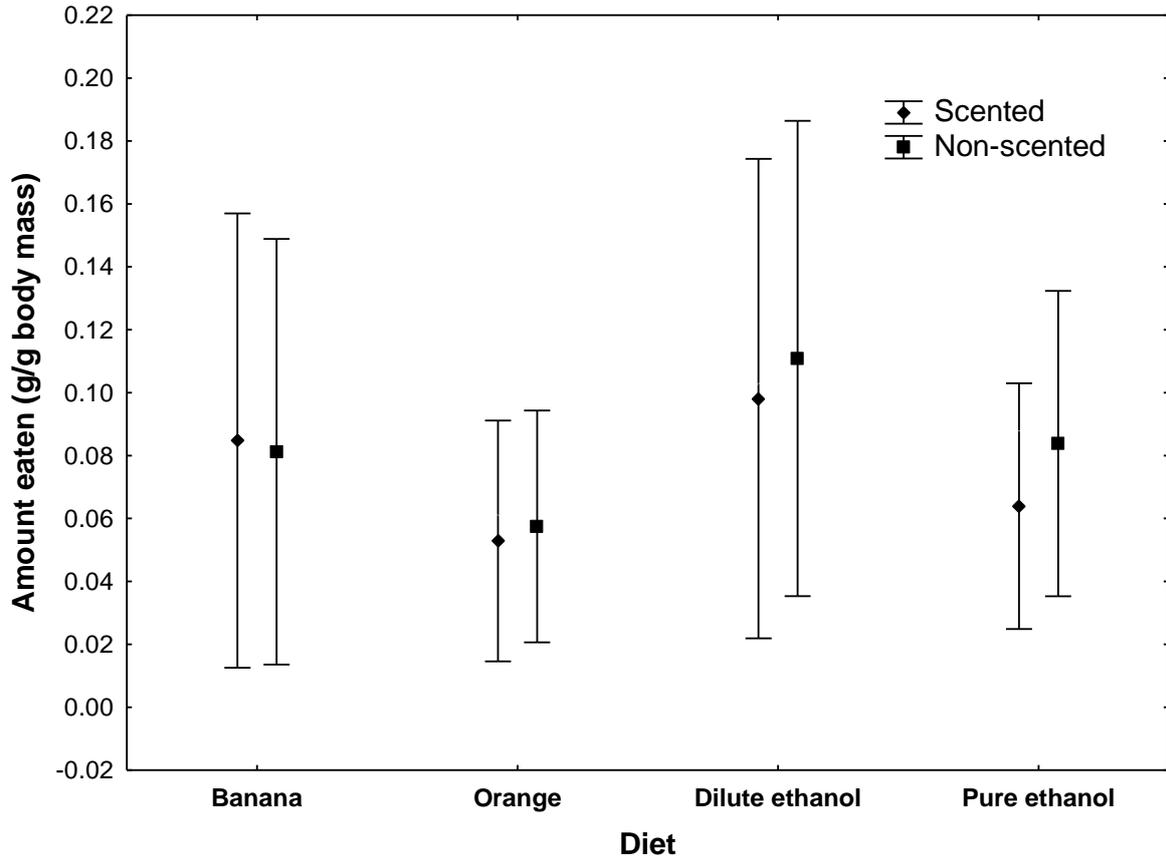


Fig. 2. The amount of food consumed by the Red-winged Starlings in all diets for the for the scent trials ($n = 8$).

CHAPTER 6

Conclusions

Introduction

Although the relationship between frugivores and fruits has been recognised for a very long time, the evolutionary implications for the participants started getting recognition much later (Herrera, 1984). Because of the mutualistic nature of this relationship, each group could be expected to have evolved ways to enhance this relationship (Ally, 2010). As a result, it has been suggested that fruit traits (e.g. colour, size, nutritional composition, seed size, etc.) have evolved with disperser behaviour, ecology and physiology (Herrera, 2002). However, due to the diffuse nature of this interaction, a number of assumptions did not hold up and were eventually rejected (Howe, 1984; Jordano, 1995). Consequently, interest in uncovering the selective pressures driving this mutualistic interaction started to dwindle as researchers started to focus elsewhere (Howe, 1993). The problem with many previous studies is that they focused on narrow a narrow view (e.g. either on fruits or frugivores singularly): they lacked an all-encompassing framework (Levey and Benkman, 1999).

The aim of this study was to investigate the relationship between several fruit traits and frugivore fruit selection patterns. Consequently, the objectives of this study were as follows: (1) to determine how of sugar type and concentration affect digestive abilities in birds, (2) to determine the deterrent effects of tannins in fruits on fruit selection by birds, (3) to determine the effect of ethanol concentration on fruit selection in birds and (4) to determine how tasting and olfactory abilities affect fruit selection patterns in a frugivorous bird.

The effect of sugar type and concentration

Because both sugar type and concentration are implicated in affecting sugar preference patterns in birds (Lotz and Schondube, 2006), a study was conducted to determine the effects of sugar type and concentration on digestive efficiencies of frugivores by feeding them equicaloric sucrose and glucose artificial fruit diets of varying concentrations (Chapter 2). Digesta transit times of birds increased with an increase in concentration for all diets. Because of a trade-off between how quick the food is processed and how much digestion takes place (Afik and Karasov, 1995; Downs, 2008), long digesta transit times increase overall nutrient intake due to prolonged period of exposure of digesta to the digestive enzymes. Therefore digesta transit times may determine how well nutrients are assimilated in the gut (Witmer, 1998). Intake rates, on the other hand, decreased with an increase in sugar concentration. This has been found in a number of nectarivorous and frugivorous birds (reviewed by Martinez del Rio et al., 2001). This suggested that birds fed to maintain a constant intake of assimilated energy. Indeed, Cape white-eyes (*Zosterops virens*) and speckled mousebirds (*Colius striatus*) managed to maintain a constant assimilated energy intake on sucrose diets. Red-winged starlings, on the other hand, did not.

Apparent assimilation efficiencies displayed by Cape white-eyes and speckled mousebirds in this study were higher on glucose than for sucrose diets, a finding similar to other studies generalist nectarivores (Odendaal et al., 2010; Napier et al., 2013). However, the assimilation efficiencies observed in these species were lower than those found in specialized avian nectarivores (Lotz and Nicolson, 1996; Downs, 1997a; Franke et al., 1998) but were similar to those found in other frugivorous birds (Worthington, 1989; Witmer, 1998; 1999). This suggests that fibre reduce assimilation efficiencies in fruits. For red-winged starlings (*Onychognathus morio*), assimilation efficiencies on glucose diets were comparable

to those found in other frugivores but on sucrose diets they were very low. This could be attributed to the lack of sucrase activity in this species (Bizaare et al., 2012).

The assimilation efficiencies of sugars were affected by sugar concentration in the speckled mousebirds but not in the Cape white-eyes and red-winged starlings except on sucrose diets in the latter. In mousebirds, digestive efficiencies were high on high concentration diets. This can be explained in two albeit contrasting ways. The first explanation could be that an increase in sugar content in the diet resulted in increased luminal concentrations of hexoses which in turn increased the passive component of sugar uptake (Levey and Martinez del Rio, 1999). The alternative explanation could be that the digestive efficiencies were higher on high concentration diets because the retention times were also high. Therefore the low digestive efficiencies observed for lower concentration diets may be due to that absorption efficiencies were compromised at high intake rates (Witmer, 1999). This demonstrates the problem of comparing digestive efficiencies on diets with different nutrient concentrations (Witmer, 1999). Measurements of faecal sugar solute concentrations on diets differing in sugar concentration may resolve the issue of whether high assimilation efficiencies at high concentrations are due to high absorption rates or were an artefact of the high sugar content (Witmer, 1998).

The effect of ethanol content

Ethanol is ubiquitous in fruits and its content correlates positively with the sugar content (Dominy, 2004; Levey, 2004, Sanchez et al., 2004). Therefore efficient foraging requires tremendous travel between fruiting trees due to the transient and patchy distribution of fruit food resource especially in tropical regions (Milton, 2004; Dudley, 2000). We tested the hypothesis that the odour of ethanol could represent a valuable foraging cue, allowing frugivores to locate fruiting plants (Dudley, 2000; 2002). This was tested by providing

simultaneously two artificial diets to three species of frugivorous birds: one diet represented the control while the other represented overripe fruits.

Results showed no significant differences in the amount of food eaten between the control and the ethanol-laden diets. These results were at odds with Dudley's hypothesis (Dudley, 2000; 2002) and corroborated a number of previous field and laboratory studies (Sanchez et al., 2006; 2008; 2010) showing that when given a choice, frugivores are unlikely to select overripe fruits. Therefore frugivores do not use ethanol as a foraging cue (Sanchez et al., 2004; 2006; Mazeh et al., 2008). Instead, ethanol at low concentrations does not affect the foraging behaviour of frugivores but at high concentrations, reduces food intake (Sanchez et al., 2004; 2006; Mazeh et al., 2008). Therefore the odour of ethanol should be seen as a deterrent rather than an attractant (Sanchez et al., 2004; 2006; Mazeh et al., 2008). However, this study was based on foraging decisions made at a small scale; they could only apply at this scale. Therefore it is unwarranted to dispute the possibility that ethanol may be used as a long-distance foraging cue, allowing frugivores to locate fruiting plants rather than as a short distance cue allowing them to assess fruit quality (Sanchez et al., 2006).

The effects of secondary compounds

The ingestion of tannins creates an acidic load in the gut which requires acid buffering by the breakdown of amino acids to create bicarbonates (Witmer, 2001), leading to increases in nitrogen and energy requirements (Guglielmo et al., 1996). In addition, tannins attack the gut lining of vertebrates causing considerable damage to the absorptive structures (Mbatha et al., 2002). Lastly, tannins are thought to be responsible for the astringency of fruits (Herrera 1982; Ndhlala et al., 2008). Because of this, their presence in fruit should be associated with reduced intake rates. A study was conducted (Chapter 3) to test this hypothesis by

determining the deterrence effects of tannins using three species of avian frugivores in choice tests with artificial fruit diets of varying tannin levels.

The results showed that birds respond differently to the presence of secondary compounds in the diet. Red-winged starlings preferred the control diet, were indifferent to the medium tannin diet and were deterred by the high tannin diet. Speckled mousebirds and Cape white-eyes were not deterred by tannins at all concentrations used. These results are at odds with the generalization that all secondary compounds are deterrents and argue against making sweeping generalisations regarding the effects of secondary compounds on fruit selection in birds based on the toxicity data from laboratory rats and humans (Struempf et al., 1999; Levey and Martinez del Rio, 2001). They further support the suggestion that frugivores are better adapted to deal with toxic substances than other vertebrates (Eriksson and Nummi, 1982; Herrera, 1984; Bairlein, 1996). The tolerance of high tannin levels by mousebirds could be due to their folivorous feeding habits (Downs et al., 2000) which could have predisposed them to higher tannin concentrations. Furthermore, wild speckled mousebirds have been seen engaged in geophagy which could be their primary detoxification mechanism (Downs, 2006). The mechanism behind the tolerance of high tannin levels by Cape white-eyes is unclear but similar tolerance of high secondary compounds has been reported in the closely-related Australian silvereyes (*Zosterops lateralis*) (Saxon et al., 2010). Furthermore, the generalist feeding behaviour of Cape white-eyes (Downs and Brown, 2003) may alleviate the negative effects of toxins in food because it prevents the accumulation of one type of secondary compound (Levey and Martinez del Rio, 2001). Therefore it could be asserted that the response of frugivores to secondary compounds has a dietary as well as a phylogenetic component. The latter suggests that studies on the interaction between frugivores and secondary compounds should be analysed in a phylogenetic context to separate the effects of the two mechanisms.

Plant secondary compounds in fruits are diverse and their effects on the fruit-frugivore interaction are similarly diverse (Levey et al., 2007). Because of such diverse roles, finding general answers regarding the role of secondary compounds in fruit-frugivore interactions is a challenging venture (Levey and Martinez del Rio, 2001). There is therefore a need to determine whether a particular group of secondary compounds generate specific effects on frugivores (Levey and Martinez del Rio, 2001). Such an undertaking may allow the research community to assign particular adaptive functions to particular groups of secondary compounds. Ultimately, this may resolve the long-standing debate on the role of frugivores in shaping fruit traits (Levey and Martinez del Rio, 2001). For example, because secondary compounds directly affect fruit consumption, they are believed to exert a stronger selective pressure on fruit characteristics unlike the role of pulp nutrients which has created a diffuse interaction between fruits and frugivores (Levey and Cipollini, 1998).

The effect of olfactory and tasting abilities

Motivated by the lack of knowledge of olfactory abilities of frugivorous birds and by the need to expand knowledge of tasting abilities of frugivorous birds, a study was conducted to examine the olfactory and tasting abilities of red-winged starlings, respectively by offering them simultaneously: (1) two artificial fruit diets in small dishes which were placed on large plastic dishes: one diet had water poured onto the plastic dish and the other was scented with various fruit essences and ethanol of different concentrations and (2) a control artificial fruit diet and a control diet mixed with banana and orange fruit essences and ethanol of varying concentration (Chapter 5).

In the scent trials, no significant differences in the amount of food eaten between scented and non-scented diets were observed. This suggested that starlings displayed no olfactory abilities. This was unexpected because bird species with olfactory bulb sizes similar

to those theirs display advanced olfactory abilities (Gwinner and Begger, 2005; Roper, 2003). Furthermore, even in species with small olfactory bulbs, the number of olfactory receptor genes is as high as in species with large olfactory bulbs which suggests that they should display similar olfactory abilities (Steiger et al., 2008). The lack of olfactory abilities in red-winged starlings could be attributed to the possibility that this study was conducted when their olfactory abilities were lowest as it was conducted outside of its breeding season, as is the case in the closely-related European starlings (*Sturnus vulgaris*) (Clark et al., 1993; Gwinner and Berger, 2005). Furthermore, red-winged starlings could be having adequate olfactory abilities but using them for purposes other than foraging. For example, European starlings have been shown to use their olfactory abilities for navigation (Wallraff et al., 1995) and locating aromatic nesting materials (Gwinner and Berger, 2008). Lastly, the experiments of this study were conducted on a small scale and perhaps starlings do not use olfaction at this scale but use it as a long-distance foraging cue allowing the localization of fruiting plants because they can use taste at a smaller scale to assess fruit quality (Levey, 1987).

In taste trials, significant differences were found in the amount of food consumed between the scented and non-scented diets. The orange-scented diet was consumed significantly more than the banana scented diet. This was in line with the feeding patterns of red-winged starlings when offered the maintenance diet whereby they always consumed the orange fruit more than banana fruit. The preference for orange over banana fruits was attributed to the balanced representation of different nutrients in the orange than on the banana fruits (USDA, 2013). It was also found that the 1% ethanol diet was consumed more than the 3% ethanol diet, although the results were non-significant. This was attributed to the deterrence effects of ethanol at high concentrations (Sanchez et al., 2006; 2008; Mazeh et al., 2008).

Plants differ widely in fruit composition and this affects fruit removal rates and ultimately, the rate at which seeds are dispersed (Levey, 1987). Rapid removal of fruit is important from the plant perspective because ripe fruits rot quickly which may render them unattractive to legitimate seed dispersers (Herrera, 1982; Levey, 2004). In view of the discrimination abilities of frugivores for diets differing in composition shown in this study and elsewhere (Levey, 1987; Bosque and Calchi, 2003; Schaefer et al., 2003), plants which offer more resources than their conspecifics are more likely to receive more visits from mutualists than those that do not. Therefore tasting abilities of frugivorous birds could be seen as mediating the competitive interactions between plants for seed dispersers.

Conclusions

This study was all about acquiring a deeper understanding of fruit selection patterns in frugivorous birds. In this study, it was shown how dietary properties (e.g. sugar type and concentration, secondary compound content, ethanol content and subtle different in dietary composition) act in concert with physiological attributes (e.g. digestive efficiencies, detoxification abilities, ethanol metabolism and olfactory and tasting abilities) of frugivorous birds to affect fruit selection patterns. Thus physiological attributes of frugivores have a significant impact on fruit selection. Therefore by relating physiological attributes of frugivores to fruit attributes, a deeper understanding of fruit-frugivore interactions can be acquired. One major advantage of the experimental approach used in his study was that it controlled for covariance among fruit characters and also removed the confounding effects of as seed size and secondary compound composition, factors that affect fruit selection. Therefore the results obtained in this study are not influenced by these factors as field studies. However, the shortcoming of this approach is that its applicability to the field is questionable. The most worrying factor was the one of scale: all the experiments conducted in this

dissertation were conducted on a small scale and thus it is risky to extrapolate these results to larger scales. Overall, the findings of this study can be summarised as follows:

- (1) Sugar type and concentration affect digestive efficiencies in frugivorous birds which in turn influence their fruit selection patterns;
- (2) Diet affects the response of birds to tannin level in the diet: species with diets high in chemically defended food are better able to deal with tannins than those that feed on other substances;
- (3) Ethanol does not serve as a foraging cue in frugivorous birds: it acts as a deterrent rather than an attractant; and
- (4) The sense of taste in frugivorous birds plays an important role for making optimal foraging decisions.

References

- Afik, D., Karasov, W.H., 1995. The trade-offs between digestive efficiency and in warblers and their ecological implications. *Ecology* 76, 2247 – 2257.
- Ally, E., 2010. Avian fruit selection and sugar preferences. Unpublished MSc thesis, University of KwaZulu-Natal, Pietermaritzburg.
- Bairlein, F., 1996. Fruit-eating in birds and its nutritional consequences. *Comparative Biochemistry and Physiology A* 113, 215–224.
- Bizaare, L., Coetzer, T.H.T., Downs, C.T., 2012. Disaccharidase presence and activities in a range of southern African frugivores. *Ostrich* 83, 165–168.

- Bosque, C., Calchi, R., 2003. Food choice by blue-grey tanagers in relation to protein content. *Comparative Biochemistry and Physiology A* 135, 321 – 327.
- Brown, K.J., Downs, C.T., 2003. Digestive efficiency of a generalist avian feeder, the Cape white-eye (*Zosterops pallidus*). *Comparative Biochemistry and Physiology A* 134, 739–748.
- Clark, L., Avilova, K.V., Bean, N.J., 1993. Odour thresholds in passerines. *Comparative Biochemistry and Physiology A* 104, 305 – 312
- Dominy, N.J., 2004. Fruit, fingers and fermentation: The sensory cues available for foraging primates. *Integrated Compositional Biology* 44, 295–303.
- Downs, C.T., 2006. Geophagy in the African olive pigeon (*Columba arquatrix*). *Ostrich* 77, 40–44.
- Downs, C.T., 2008. Aspects of diet choice and digestion in the dark-capped bulbul *Pycnototus barbatus*. *Ostrich* 79, 73–78.
- Downs, C.T., Wirminghaus, J.O., Lawes, M.J., 2000. Anatomical and nutritional adaptations of the speckled mousebird (*Colius striatus*). *Auk* 117, 791–794.
- Dudley, R., 2000. Evolutionary origins of Human alcoholism in primate frugivory. *Quarterly Reviews of Biology* 75, 3–15.
- Dudley, R., 2002. Fermenting fruit and the historical ecology of ethanol ingestion: Is alcoholism in modern Humans an evolutionary hangover? *Addictions* 97, 381–388.
- Eriksson, K., Nummi, H., 1982. Alcohol accumulation from ingested berries and alcohol metabolism in passerine birds. *Ornis Fennica* 60, 2–9.

- Guglielmo, C.G., Karasov, W.H., Jakubas, W.J., 1996. Nutritional costs of a plant secondary metabolite explain selective foraging by ruffed grouse. *Ecology* 77, 1103–1115.
- Gwinner, H., Berger, S., 2005. European Starlings: nestling condition, parasites and green nest material during the breeding season. *Journal of Ornithology* 146, 365 – 371
- Gwinner, H., Berger, S., 2008. Starling males select green nest material by olfaction using experience-independent and experience-dependent cues. *Animal Behaviour* 75, 971 – 976
- Herrera, C.M., 1982. Defence of ripe fruit from pests: its significance in relation to plant-disperser interactions. *American Naturalist* 120, 218–241.
- Herrera, C.M., 1984. Adaptation to frugivory of Mediterranean avian dispersers. *Ecology* 65, 609–617.
- Herrera, C.M., 2002. Seed dispersal by vertebrates. In: Herrera, C.M., Pellmyr, O., (eds). *Plant-animal interactions: an evolutionary approach*. Blackwell Science, London.
- Howe, H.F., 1984. Constraints on the evolution of mutualisms. *American Naturalist* 123, 764 – 777.
- Howe, H.F., 1993. Specialized and generalized dispersal systems: where does ‘the paradigm’ stand? *Vegetation* 107/108, 3–13.
- Jordano, P., 1995. Angiosperm fleshy fruits and seed dispersers: a comparative analysis of adaptation and constraints in plant-animal interactions. *American Naturalist* 145, 163 – 191.
- Levey, D.J., 1987. Sugar-tasting ability and fruit selection in tropical fruit-eating birds. *Auk* 104, 173 – 179.

- Levey, D.J., 2004. The evolutionary ecology of ethanol production and alcoholism. *Integrated and Comparative Biology* 44, 284 – 289.
- Levey, D.J., Benkman, C.W., 1999. Fruit-disperser interactions: timely insights from a long-term perspective. *Trends in Ecology and Evolution* 14, 41 – 43.
- Levey, D.J., Cipollini, M.L., 1998. A glycoalkaloid in ripe fruit deters consumption by cedar waxwings. *Auk* 115, 359–367.
- Levey, D.J., Martinez del Rio, C., 1999. Test, rejection and reformulation of a chemical-reactor based model of gut function in a fruit-eating bird. *Physiological and Biochemical Zoology* 72, 369–383.
- Levey, D.J., Martinez del Rio, C., 2001. It takes guts (and more) to eat fruits: lessons from avian nutritional ecology. *Auk* 118, 819–831.
- Lotz, C.N., Schondube, J.E., 2006. Sugar preferences in nectar- and fruit-eating birds: behavioural patterns and physiological causes. *Biotropica* 38, 3 – 15.
- Martinez del Rio, C., Schondube, J.E., McWhorter, T.J., Herrera, L.G., 2001. Intake responses in nectar feeding birds: digestive and metabolic causes, osmoregulatory consequences, and coevolutionary effects. *American Zoology* 41, 902–915.
- Mazeh, S., Korine, C., Pinshow, B., Dudley, R., 2008. The influence of ethanol on feeding in the frugivorous yellow-vented bulbul (*Pycnonotus xanthopigos*). *Behavioural Processes* 77, 369–375.
- Mbatha, K.R., Downs, C.T., Nsahlai, I.V., 2002. The effects of graded levels of dietary tannin on the epithelial tissue of the gastro-intestinal tract and liver and kidney masses of boer goats. *Animal Science* 74, 579–586.

- Milton, K. 2004. Ferment in the family tree: does a frugivorous dietary heritage influence contemporary patterns of human ethanol use? *Integrated Comparative Biology* 44, 304 – 314.
- Napier, K.R., McWhorter, T.J., Nicolson, S.W., Fleming, P.A., 2013. Sugar preferences of avian nectarivores are correlated with intestinal sucrase activity. *Physiological and Biochemical Zoology* 86, 499–514.
- Ndhlala, A.R., Muchuweti, M., Chitindingu, K., Benhura, M.A., 2008. Phenolic content and profiles of selected wild fruits of Zimbabwe: *Ximenia caffra*, *Artobotrys brachypelatus* and *Syzigium cordatum*. *International Journal of Food Science and Technology* 43, 1333–1337.
- Odendaal, T.C., Brown, M., Downs, C.T., Johnson, S.D., 2010. Sugar preferences and digestive efficiency of the village weaver: a generalist avian pollinator of African plants. *Journal of Experimental Biology* 213, 2531–2535.
- Roper, T.J., 2003. Olfactory discrimination in yellow-backed chattering lorries *Lorius garrulus flavopalliatu*s: first demonstration of olfaction in Psittaciformes. *Ibis* 145, 689 – 691.
- Sanchez, F., Korine, C., Pinshow, B., Dudley, R., 2004. The possible roles of ethanol in the relationship between plants and frugivores: First experiments with Egyptian fruit bats. *Integrated Compositional Biology* 44, 290–294.
- Sanchez, F., Korine, C., Steeghs, M., Laarhoven, L-J., Cristescu, S.M., Harren, F.J.M., Dudley, R., Pinshow, B., 2006. Ethanol and methanol as possible odour cues for Egyptian fruit bats. *Journal of Chemical Ecology* 32, 1289–1300.

- Sanchez, F., Kotler, B.P., Korine, C., Pinshow, B., 2008. Sugars are complementary resources to ethanol in foods consumed by the Egyptian fruit bats. *Journal of Experimental Biology* 211, 1475–1481.
- Sanchez, F., Melcon, M., Korine, C., Pinshow, B., 2010. Ethanol ingestion affects flight performance and echolocation in Egyptian fruit bats. *Behavioural Processes* 84, 555 – 558.
- Saxon, V.P., Mulder, I., Creasy, G.L., Paterson, A.M., Ross, J.G., Trought, M.C.T., 2010. Comparative behavioural responses of silvereyes (*Zosterops lateralis*) and European blackbirds (*Turdus merula*) to secondary metabolites in grapes. *Austral Ecology* 36, 233–239.
- Schaefer, H.M., Schmidt, V., Winkler, H., 2003. Testing the defence trade-off hypothesis: How contents of nutrients and secondary compounds affect fruit removal. *Oikos* 102, 318–328.
- Steiger, S.S., Fidler, A.E., Valcu, M., Kampenars, B., 2008. Avian olfactory receptor gene repertoires: evidence for a well-developed sense of smell in birds? *Proceedings of the Royal Society B* 275, 2309 – 2317.
- Struempf, H.M., Schondube, J.E., Martinez del Rio, C., 1999. The cyanogenic glycoside amygdalin does not deter consumption of ripe fruit by cedar waxwing. *Auk* 116, 749–758.
- USDA., 2013. United States Department of Agriculture, Agricultural Research Service. USDA National Nutrient Database for Standard Reference: Release 26. <http://www.ars.usda.gov/ba/bhnrc/ndl>. Accessed 22/05/2014.

- Wallraff, H.G., Kiepenheuer, J., Neumann, M.F., Streng, A., 1995. Homing experiments with starlings deprived of the sense of smell. *Condor* 97, 20-26.
- Witmer, M.C., 1998. Ecological and evolutionary implications of energy and protein requirements of avian frugivores eating sugary fruits. *Physiological Zoology* 71, 599–610.
- Witmer, M.C., 1999. Do avian frugivores absorb fruit sugars inefficiently? How dietary nutrient concentration can affect coefficients of digestive efficiency. *Journal of Avian Biology* 30, 159–164.
- Witmer, M.C., 2001. Nutritional interactions and fruit removal: Cedar waxwing consumption of *Viburnum opulus* fruits in spring. *Ecology* 82, 3120–3130.
- Worthington, A.H., 1989. Adaptations for avian frugivory: assimilation efficiency and gut transit time of *Manacus vitellinus* and *Pipra mentalis*. *Oecologia* 80, 381–389.