Avian fruit selection and sugar preferences

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ABSTRACT

It has been suggested that fruit features such as nutrient content, size and colour have co-evolved with dispersal agent behaviour, physiology and morphology. Avian nectarivore feeding ecology is relatively well studied; however, less is known about fruit selection in avian frugivores. Previous work highlights the importance of individual factors that contribute towards fruit preferences, but few studies bring these factors together. Consequently the aim of this dissertation was to attempt this in terms of frugivory by investigating behavioural, physiological and morphological aspects of fruit selection in generalist avian dispersers. This was achieved by manipulating the nutritional content, size and colour of fruits (artificial fruits) under controlled conditions.

The first part of the dissertation addresses physiological aspects of fruit selection in Red-winged Starling *Onychognathus morio*, Speckled Mousebird *Colius striatus* and Dark-Capped Bulbul *Pycnonotus tricolor*. Assimilation efficiency of birds fed glucose and sucrose diet treatments of varying concentration was observed. All study species showed high apparent assimilation efficiency irrespective of artificial fruit sugar concentration and type (with the exception of Red-winged Starlings on an all sucrose diet).

The second part of the dissertation addresses behavioural aspects of fruit selection in the same three species by observing selective preferences between glucose and sucrose fruits of varying concentration and molarity. While Mousebirds displayed no preference for any of the diet treatments, Bulbuls occasionally favoured

glucose diets over sucrose diets and Starlings always favoured glucose diets over sucrose diets.

Another behavioural aspect of fruit selection was addressed in the third part of the dissertation. Colour preferences of Red-winged Starling and Speckled Mousebird were observed. Although study species did not conform to trends by favouring black and red fruits, they did show avoidance of green fruits and (Starlings) presented evidence of learning.

A morphological aspect of fruit selection is presented in the fourth part of the dissertation. Starlings, Bulbuls and Mousebird beak morphology was measured to investigate if this has an effect on fruit size preferences. Starlings with the largest beak dimensions were more selective of fruit size classes than Bulbuls and Mousebirds which displayed the importance of feeding method (thrashing/swallowing/mashing) to compensate for larger fruit sizes.

The final section of this dissertation is a synthesis of the observed behavioural, physiological and morphological aspects of fruit selection in Red-winged Starlings, Dark-capped Bulbuls and Speckled-Mousebirds.

PREFACE

The experimental work described in this dissertation was carried out in the School of Biological and Conservation Sciences, University of KwaZulu-Natal, Pietermaritzburg from January 2007 to December 2008, under the supervision of Professor Colleen T. Downs.

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

………………………….

Ebrahim Ally

December 2010

I certify that the above statement is correct.   …………………………..

Professor Colleen T. Downs

Supervisor

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DECLARATION 1 - PLAGIARISM

I, Ebrahim Ally, declare that

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DECLARATION 2 - PUBLICATIONS

DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this dissertation (include publications in preparation, submitted, *in press* and published and give details of the contributions of each author to the experimental work and writing of each publication)

Publication 1

E Ally and CT Downs. 2010. Will frugivorous birds select hexose and sucrose proportions to maximize assimilation efficiency?

Author contributions:

EA and CTD conceived paper. EA collected and analysed data. EA wrote the paper. CTD contributed valuable comments to the manuscript.

Publication 2

E Ally and CT Downs. 2010. Does sugar type and concentration affect food selection by frugivorous South African birds: A combined methodological approach.

Author contributions:

EA and CTD conceived paper. EA collected and analysed data. EA wrote the paper. CTD contributed valuable comments to the manuscript.

Publication 3

E Ally and CT Downs. 2010. Use of colour in fruit to attract avian dispersers

Author contributions:

EA conceived paper. EA collected and analysed data. EA wrote the paper. CTD contributed valuable comments to the manuscript.

Publication 4

E Ally and CT Downs. 2010. Does size matter? Can beak size be used to predict fruit selection in avian frugivores?

Author contributions:

EA and CTD conceived paper. EA collected and analysed data. EA wrote the paper. CTD contributed valuable comments to the manuscript.

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December 2010

Acknowledgments

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Chapter 1

Introduction

Plant frugivore interactions are said to be mutualistic: plants provide frugivores with digestible nutrients and frugivores provide effective seed dispersal for plants (Peres and van Roosmalen 1996). If fruits have indeed originated as a means of dispersing seeds via the provision of food to dispersal agents (Herrera 1982) then frugivorous birds have also evolved to optimize this relationship. It has consequently been suggested that fruit features such as colour, size and nutrient content of flesh as well as fruiting patterns (crop size, ripening rates and phenology) have co-evolved with dispersal agent behaviour, morphology and physiology (Herrera 1982; Levey and Grajal 1991). Figure 1 summarises the primary factors involved in food selection for a typical avian frugivore.

This study investigates the relationship between various fruit features and the potential effects these have on three southern African avian frugivores (Speckled Mousebird *Colius striatus*, Dark-capped Bulbul *Pycnonotus tricolor* and Red-winged Starling *Onychognathus morio* (Hockey et al. 2005)). Increasing our understanding of the relationship between these avian frugivores and the fruits they consume in addition to contributing towards an established pool of knowledge regarding feeding behaviour and food preferences in birds, will also aid in the development of more effective conservation efforts and predictive models, especially in the face of rapid climate change.

Learning

First and foremost, preferential selection of any food type depends on the subject's ability to learn. Learning allows animals to behave in an optimal manner

when faced with a trade-off. By definition learning is "a relatively permanent change in behaviour as a result of experience" p112 (Dugatkin 2004). Animals with the ability to distinguish between food sources and their attributes are more likely to take advantage of this to improve fitness compared with those without this ability (Dukas and Bernays 2000; Dugatkin 2004). For example food colour and odour were associated with nutritional rewards and significant increases in growth rate for grasshoppers (*Schistocera americana*) (Dukas and Bernays 2000). In the present study experiments were not designed to test learning abilities but rather what birds have already learned and are applying to fruit selection under neutral conditions.

It is worth mentioning that associating fruit colour with nutritional rewards is a second-order response, the first-order association would be the digestive response towards eating fruit X (Dugatkin 2004). If for example consumption of fruit X always results in indigestion, the individual will learn to associate indigestion with a fruit X trait (e.g. colour, odour, size, shape, taste) to prevent ingestion in the first place. The pairing of visual cues with gustatory cues has been questioned with evidence suggesting that rats (*Rattus rattus*) tend to pair gustatory cues with other gustatory cues (e.g. taste and indigestion) and audiovisual cues with other audiovisual cues (e.g. eagle cry and predator presence) as opposed to one with the other (e.g. colour and indigestion) (Garcia and Koelling 1966). Temporally speaking, it is also less likely that cues separated by longer periods of time are associated with each other (Dugatkin 2004).

Feeding behaviour and fruit selection in avian frugivores

To date most studies investigate the nutritional content of fruits without considering sugar composition (Lotz and Schondube 2006). It has, however, been shown that the nutritional value of fruits and fruit preferences are often not correlated

(Izhaki and Safriel 1989). Not to mention that fact that under natural circumstances the intraspecific nutritional composition of fruits varies considerably depending on season, rainfall, age of fruit and stage of ripening (Johnson et al. 1985; Herrera 1995; Izhaki et al. 2002).

Studies have shown that frugivorous birds show preferences for specific compositions and concentrations of sugar (Ciminari et al. 2001; Levey and Martinez del Rio 2001; Lotz and Schondube 2006). Thus far numerous theories have been proposed to describe mechanisms behind sugar preferences in avian frugivores. A question as simple as "do birds prefer hexose dominant fruits, sucrose dominant fruits or neither?" has consequently raised various evolutionary, behavioural, physiological and morphological hypotheses. Context-dependent sugar preference hypotheses include digestive flexibility which potentially constrains feeding ecology in birds (Levey and Grajal 1991; Karasov 1996; Ciminari et al. 2001). The modulation of dietary enzymes is possible but sufficient acclimation time is needed thus making sugar preferences dependent on the spatiotemporally dominant sugar type (Ciminari et al. 2001; Karasov 1996). Downs and Perrin (1996) suggest that post ingestional constraints could determine sugar preferences if energy thresholds are reached by sugar solutions on offer.

Frugivores will exhibit preferential selection of hexose dominant fruits, particularly if they no longer have the ability to digest sucrose as in some passerines (Martinez del Rio and Stevens 1989; Lotz and Schondube 2006). Hexose sugars may be absorbed passively due to their small molecule sizes (Karasov 1996; Levey and Martinez del Rio 2001). Rainbow Lorikeets (*Trichoglossus heamatodus*) absorbed eighty percent of the glucose in an artificial nectar solution passively (Karasov and

Cork 1994) while Cedar Waxwings (*Bombycilla cedrorum*) appear to absorb more than eighty percent of glucose in an artificial fruit diet passively (Karasov 1996).

In the digestive system, carriers are membrane proteins that facilitate the movement of specific molecules through the intestinal membrane (Karasov and Martinez del Rio 2007). Carrier mediated active transport allows glucose molecules for example to be moved against the electrochemical gradient during intestinal absorption (Karasov and Martinez del Rio 2007). This is more costly than passive absorption as transported molecules must be coupled with a solute to overcome the electromagnetic gradient (McWhorter et al. 2006; Karasov and Martinez del Rio 2007). Avian frugivores may show a preference for hexose dominant fruits to take advantage of less costly passive absorption as a result (Levey and Martinez del Rio 2001). Another hypothesis that favours hexose dominant fruits takes into account that fruits in general are digested relatively poorly (summarised in Levey and Martinez de Rio 2001). To compensate, frugivores may have rapid transit rates and so need to process fruits more quickly resulting in a preference for easily digestible hexose sugars (Herrera 1998; Levey and Martinez del Rio 2001).

While consumption of hexose dominant food sources has several benefits (above) digestive physiology of sunbirds and even some passerines (summarised in Fleming et al 2004) result in these species favouring sucrose dominant food sources under certain circumstances (Fleming et al 2004, Brown et al 2008). Lotz and Schondube (2006) highlight the importance of sugar composition preferences at varying concentrations of nectar. While Lesser Double-collared Sunbirds *Nectarinia chalybea* for example, prefer sucrose at higher concentrations, they favour glucose at lower concentrations (Lotz and Schondube 2006). Fleming et al (2004) suggest water balance as a potential determinant of sugar preferences in nectarivores. At equal

energy levels, sucrose nectars have about half the osmolality of hexose nectars making them favourable as this reduces water ballast (Fleming et al. 2008).This may be explained in terms of digestive processes where at higher concentrations when glucose and fructose carriers become saturated, a 1:1 ratio of glucose to fructose would be absorbed more efficiently than a glucose or fructose dominant food source (glucose and fructose have independent intestinal carrier systems) (Martinez del Rio 1990). Thus a sucrose dominant food source which is hydrolysed into equal amounts of fructose and glucose (Martinez del Rio 1990) should also be digested more efficiently than a glucose or fructose dominant source at high concentrations. While most of the above theories are plausible, few have been conclusively verified.

Optimality

Frugivorous birds have been found to digest sugars with varying efficiency ranging from 92% (Cedar Waxwings *Bombycilla cedrorum*) (Martinez del Rio et al. 1989) to as low as 69% (Cape White-eyes *Zosterops virens*) (Wellmann and Downs 2009b). Earlier predictive models suggest that all frugivorous birds will favour hexose sugars over sucrose as the rate of sucrose sugar absorption is limited by hydrolysis (Martinez del Rio and Karasov 1990). The fact that hummingbirds prefer sucrose solutions over 1:1 fructose, glucose solutions of the same energetic value (Martinez del Rio and Karasov 1990) has, however, puzzled researchers and left room for further investigation into digestive processes and sugar selection. Evidence from more recent studies indicate that sugar preferences investigated in a large number of previous studies made use of apparently flawed experimental designs such as the percentage weight and equimolar sugar concentrations (Fleming et al. 2004; Brown et al. 2008). Trends observed in these studies are now viewed with caution leaving room for more equal energy studies.

Lack of agreement between researchers over the usefulness of optimal foraging theory (Pierce and Ollason 1987; Dawkins 1995) has left the field of study floundering with half completed and untested alternative theories (Stephens et al. 2007). The current study makes use of optimal foraging theory to generate and test hypotheses simply because this theory has remained the most robust and comparable technique for interpreting foraging behaviour in birds. We do not rule out alternative theories such as Johnson and Nicolson's nectar property and pollination system (2008) or McNamara and Houston's 'Evo-mecho' approach (2009). On the contrary, this study seeks to place optimal foraging theory inspired findings into context within more recent theoretical developments.

Optimal foraging can be divided into two categories, long-term optimality and short-term optimality. Long-term optimality is "the reproductive success of an animal over its entire life compared to its rivals" (Dawkins 1995) whereas short-term optimality is the apparent ability an animal has to optimize functions in its daily life (e.g. optimal foraging – taking the path of least resistance towards the most rewarding food sources) (Dawkins 1995). To date most studies investigating optimality focus on the easily applied short-term optimality to make inferences about long-term optimality (Dawkins 1995).

Finding the optimal foraging strategy used by an organism has traditionally followed three steps:

1. Choosing a currency,
2. choosing an appropriate cost benefit function and
3. solving for the optimum (Pyke et al. 1977).

Most optimality studies to date have opted to use energy as the currency but some work has been done investigating the importance of molarity (decisions motivated by taste or taste intensity and not energy) as the correct currency (Downs 1997) summarised in (Pyke et al. 1977; Greig-Smith 1985; Werner et al. 2007; Brown et al. 2008; Fleming et al. 2008). Even though both have received criticism, energy has remained the more popular of the two. This study aims to provide insight into the usefulness of both equal energy and equimolar concentration by observing responses of target species towards both experimental designs.

Effects of fruit size and colour on avian dispersers

Before a fruit can be eaten it must be detected. Effective seed dispersal is a consequence of successful advertising by a given fruiting plant species (Knight and Siegfried 1983; Schaefer et al. 2008; Burns et al. 2009). To increase encounter rates between fruits and dispersers plants must increase the conspicuousness of their fruits. Numerous studies have narrowed down the primary methods used to increase fruit conspicuousness to three (Knight and Siegfried 1983; Gautier-Hion et al. 1985; Giles and Lill 1999; Cazetta et al. 2009):

1. Colour variation and contrast against a background,

2. size variation,

3. frequency of fruit availability and occurrence.

Chromatic variation in fruits apart from highlighting their presence also indicates ripeness, aids in disperser learning and association (Herrera 1982; Willson and Whelan 1990; Dugatkin 2004; Burns 2005; Schaefer et al. 2008). Studies to date have found that black and red fruits are generally selected over other colours by birds (Knight and Siegfried 1983; Willson and Whelan 1990; Burns 2005). Black and red

fruits are also the most prevalent in South Africa (Figure 1, Chapter 5) and in general (Fischer and Chapman 1993). It is crucial to highlight the importance of interpreting fruit colours in the correct context, not all colour variations exist to attract avian dispersers (Willson and Whelan 1990; Gamberale-Stille and Tullberg 2001; Endler and Day 2006). In addition to this, avian frugivores are so taxonomically diverse that selective forces driving fruit colour variation are unlikely as a result of frugivory alone (Willson and Whelan 1990; Schmidt and Schaefer 2004; Burns et al. 2009). Nevertheless, a higher prevalence of smaller red and black fruits on the ends of long thin branches is evidence of adaptation that is beneficial to avian dispersal (Thompson and Willson 1979; Knight and Siegfried 1983; Gautier-Hion et al. 1985; Fischer and Chapman 1993).

Fruit size can be indicative of nutritional value. Studies on Mediterranean Buckthorn (*Rhamnus alaternus*) revealed that ripe fruits will differ chemically and morphologically within the same species with larger fruits being richer in carbohydrates (nonstructural), water and phosphorus, medium sized fruits showing a tendency to be richer in lipids, magnesium and calcium and smaller fruits tending to be richer in protein, structural carbohydrates potassium and zinc (Izhaki et al. 2002). Fruit size has, however, been brought into question as a category for fruit selection by avian frugivores. Migratory birds in North America showed no preference for fruits based on fruit size (Johnson et al. 1985).

Seasonal fruit resources are also unlikely to be taken advantage of if morphological adaptation is required to make use of them (Levey and Grajal 1991). Beak morphology is said to be adapted to feeding on the primary resources in an area (Clayton et al. 2005). Feeding behaviour works hand in hand with beak morphology with fruit eating birds being classified as Swallowers/gulpers, squashers/mashers,

thrashers and foot/beak coordinators (Levey 1987; Symes and Downs 2001; Brown and Hopkins 2002) (Chapter 5 for details). If approached from this angle, it can still be argued whether beak morphology for instance has an effect on feeding behaviour and consequently fruit size preferences in avian dispersers.

Objectives

There are many reasons why avian frugivores limit fruit consumption (Levey and Martinez del Rio 2001). To date most studies investigate single factors and their effects on avian frugivore fruit preferences. This thesis investigated fruit preferences from a morphological, behavioural and physiological point of view in order to gain a more comprehensive idea of feeding ecology in avian dispersers. The resulting findings should, in addition to contributing towards the understanding of frugivory in three abundant South African frugivorous species, aid in the formation of new hypotheses for future studies.

The dissertation is presented as chapters in manuscript form for publication in peer reviewed journals. Some overlap is unavoidable. These chapters are:

- Will frugivorous birds select hexose and sucrose proportions to maximize assimilation efficiency?
- Does sugar type and concentration affect food selection by frugivorous South African birds: A combined methodological approach.
- Use of colour in fruit to attract avian dispersers
- Does size matter? Can beak size be used to predict fruit selection in avian frugivores?

The various hypotheses addressed in each are presented in the chapters.

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Figure

Physical → Thorns

Detection probability

U.V. Properties

Protection

Shells

Colour preference*

Contrast with background

Tannins ← Chemical

?

Fruit size

Secondary compounds

Beak morphology

Toxic concentrations

Fruit abundance

Digestive Ability

Energy intake

Sugar preferences

Post ingestional feedback
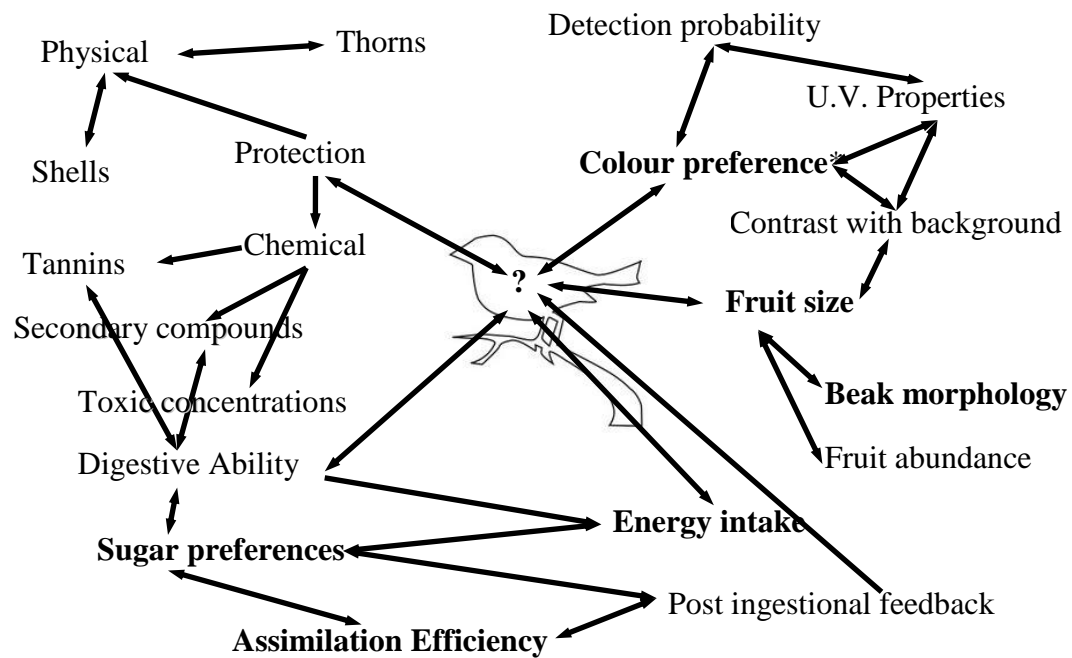
Assimilation Efficiency

Figure 1: Interaction of factors involved in the decision making process of a typical avian frugivore. Factors in **bold** were tested for each study animal in this experiment (* not tested on Dark-capped Bulbuls).

Chapter 2

Will frugivorous birds select hexose and sucrose proportions to maximize assimilation efficiency?

(formatted for *Physiological and Biochemical Zoology*)

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Abstract

Although the sugar preference (hexose vs. sucrose) debate has dominated avian foraging ecology since the conception of the optimal foraging theory, little work has investigated the effects of a mixed proportion hexose and sucrose combined diet. This study investigated intake and digestive responses in avian frugivores (Red-winged Starling *Onychognathus morio*, Speckled Mouse-bird *Colius striatus* and Dark-Capped Bulbul *Pycnonotus tricolor*) towards diets where choices of artificial fruits containing different sugar types and proportions are presented. We also examined the effects of variable concentration on digestive efficiency with a free choice of sugar experimental design. Finally we investigated how much use and applicability equicaloric and equimolar solutions have in determining sugar preferences. Results indicated that all bird species consume proportions of both sugar types under the free sugar choice experimental design. We also observed very high

assimilation efficiencies for study species on all diets where more than one type of sugar was offered (> 95 %). Concentration dependent sugar type preferences were apparent in Starlings which generally had higher gross energy intake and assimilation efficiencies on low equimolar and low equicaloric diets. Mousebird and Bulbul assimilation efficiency was unaffected by all equimolar and equicaloric diet sugar concentrations. Further research into the digestive processes involved in sucrose and glucose absorption as well as transit rates and transit rate flexibility may be able to contribute further towards the mechanisms that result in this phenomenon.

Key words: Red-winged Starling, Speckled Mousebird, Dark-capped Bulbul, Apparent assimilation efficiency, Avian Frugivore, Sugar preference

Introduction

Optimal foraging theory has come a long way since its first conception (MacArthur and Pianka 1966; Stephens et al. 2007). Energy intake maximization in particular has remained a popular theme at the forefront of optimal foraging theory because of its logical reasoning and relatively simple theoretical nature. Finding conclusive support for energy intake maximization has, however, remained an issue as numerous studies have both supported and discredited that it is used by foraging animals e.g. (Pierce and Ollason 1987; López-Calleja et al. 1997), see review (Jumars and Martinez del Rio 1999). A common misconception regarding energy intake maximization is that it requires the forager to base choices on the results of a mathematical decision process under which individuals are expected to consider all costs and benefits associated with a particular food type and its immediate

surroundings (Pierce and Ollason 1987; Stephens et al. 2007). On the contrary, energy intake maximization is merely a tool to understand food selection choices and predict foraging behaviour with a higher degree of accuracy.

We cannot ignore the fact that in its natural environment, an organism will need to make tradeoffs and consider various unique patch characteristics before being considered an optimal forager. Laboratory studies aim to control for the effects of tradeoffs made under natural conditions (e.g. foraging costs) and pinpoint the importance of the effects of sugar type and concentration specifically. This study investigates the nutritional gain achieved by selecting the most appropriate food source and the possibility that this could be related to energy intake maximization.

Frugivorous birds are considered ideal test subjects to investigate energy intake maximisation as they have relatively unconstrained feeding habits, morphologically simple guts and chemically simple food (Jumars and Martinez del Rio 1999; Levey and Martinez del Rio 2001). A combination of relatively rapid food transit rates (Levey and Duke 1992) and generally minimal energy storage (fat < 5% body mass in non-migratory passerines (McWilliams and Karasov 2001)) also mean that frugivorous birds need a relatively constant source of energy (especially evident in smaller birds with small or absent crops (Levey and Duke 1992)) and are more likely to regulate energy intake and optimize feeding (Pyke et al. 1977; Jumars and Martinez del Rio 1999).

We used three southern African avian frugivores (Red-winged Starling *Onychognathus morio*, Speckled Mouse-bird *Colius striatus* and Dark-Capped Bulbul *Pycnonotus tricolour* (Hockey et al. 2005)) to test our hypotheses. Red-winged Starlings eat fruits primarily but also forage on invertebrates and even drink aloe

nectar (Hoffman 1988; Brown et al. 2009). The Sturnidae family lack the ability to digest sucrose (Martinez del Rio and Stevens 1989; Avery et al. 1995). Recent work observing Red-winged Starling responses towards artificial nectar solutions finds that hexose sugars are preferred over sucrose ones indicating this species may lack sucrase (Brown 2009). We investigated if Red-winged Starlings display a similar aversion towards sucrose presented as artificial fruits containing fibre and other nutritional differences compared with artificial nectar used in Brown's (2009) study. Dark-capped Bulbuls also forage on fruits, invertebrates and nectar (Symes and Downs 2001), their widespread presence makes them an important species for seed dispersal. Dark-capped Bulbuls show a preference of hexose sugar solutions irrespective of concentration (Brown et al. 2010a). Speckled Mousebirds feed on fruit, leaves, seeds and nectar (summarised in Hockey et al. 2005). Speckled Mousebirds show concentration-dependent preferences (favouring hexose at low concentrations and sucrose at high concentrations) when presented with sugar solutions (Brown et al. 2010b)

Thus far most work regarding sugar preferences and digestion has been conducted on nectarivores (Lotz and Schondube 2006; Brown et al. 2008;). Studies observing food preferences in nectarivores have mainly focused on selective preferences for differing sugar types at varying concentrations (Fleming et al. 2008; Brown et al. 2010a; b). Pollination theory on sugar preferences in nectarivores suggests that there is a dichotomy in nectarivorous birds: those that are specialist pollinators (specialist nectarivores from here on) and those that are generalist pollinators (generalist nectarivores from here on) (Johnson and Nicolson 2008). Generally for equicaloric solutions, specialist nectarivores prefer disaccharides at high concentrations and hexose sugars at lower concentrations while generalist pollinators

show preference for monosaccharides irrespective of diet concentration (Fleming et al. 2004; Fleming et al. 2008; Johnson and Nicolson 2008; Brown et al. 2010a; b).

Less is known about fruit sugar properties and their digestion in birds compared to nectar properties (Lotz and Schondube 2006). Firstly, fruit composition (unlike nectar) has varying proportions of fat, protein, fibre and secondary compounds over and above the contained sugars (Snow 1981; Johnson et al. 1985; Afik and Karasov 1995; Sabat and Gonzalez 2003). We have limited understanding of the effects these additional elements have on digestive efficiency in frugivorous birds but it is generally accepted that these additional diet components will decrease digestive efficiency relative to nectar diets (Martinez del Rio and Karasov 1990; Levey and Duke 1992). In terms of monosaccharide assimilation and disaccharide digestion, glucose should be assimilated more efficiently as there is no need for the relatively smaller molecules to be hydrolyzed before absorption (Afik and Karasov 1995; Sabat and Gonzalez 2003). Sucrose on the other hand must first be hydrolyzed before being absorbed (Martinez del Rio and Karasov 1990). Adjusting retention time can result in equal extraction efficiencies for both mono- and disaccharide sugars (Afik and Karasov 1995). Retention time however, does not make a difference to sucrose extraction efficiency if insufficient amounts of sucrase are available to break molecules down (Martinez del Rio and Karasov 1990; Afik and Karasov 1995). Sucrase activity in turn is modulated by the amount of sucrose in the frugivores' diet although it has been argued whether variable diets determine digestive strategy (via digestive plasticity for example) or digestive ability limits diet strategy (Afik and Karasov 1995). Concentration also plays a role here, glucose molecules are small enough to be absorbed passively as long as the concentration of monosaccharides in the blood stream are lower compared to those in the intestinal lumen (the larger the

gradient the more efficiently glucose will be absorbed) (Renner et al. 1972; Martinez del Rio and Karasov 1990; McWhorter et al. 2006). Carrier mediated transport is responsible for monosaccharide uptake at lower concentrations (Renner et al. 1972; Martinez del Rio 1990; McWhorter et al. 2006).

It was hypothesised that study animals would show greater control in digestive ability (and consequently higher digestive efficiency) if allowed to choose freely between the amounts of glucose and sucrose foods being ingested as glucose and sucrose utilize different digestive pathways. This study aimed to investigate if offering avian frugivores different sugar types at various concentrations has an effect on digestive ability.

Materials and Methods

Test subjects

Individuals (6-10) of each species were mist netted in KwaZulu-Natal (South Africa) between July 2007 and February 2008 (Waterfall (29°44'56.64"S 30°48'49.68"E), Pietermaritzburg (29°37'31.88"S 30°24'5.22"E), Darvill (29°35'50.92"S 30°26'16.78"E) and Hilton College (29°29'32.12"S 30°18'6.86"E) under permit from Ezemvelo KZN Wildlife. Birds were housed in outdoor aviaries at University of KwaZulu-Natal, Pietermaritzburg (South Africa). Individuals were dewormed (Mediworm Powder, Medpet, Benrose) and given one week to acclimate to full laboratory conditions before experimentation (12:12 photoperiod, temperature controlled at 25°C ± 1°C). During the acclimation period, all birds were housed individually in wire cages (50 x 50 x 50cm) and fed a standard maintenance diet consisting of a mixture of apple, pear, banana, grape, pawpaw, orange (additional

spinach and broccoli for mousebirds) and medium concentration (2350 kJ/l, Table 1) glucose or sucrose artificial fruits (method Witmer 1998, ingredients Table 1). The above ingredients were mixed in a fruit-salad with Mynah pellets and Mynah Softbill crumbs (Avi-Products, Hillcrest). Water was provided *ad libitum* and mealworms (*Tenebrio molitor*) (for Bulbuls and Starlings only) every second day during this acclimation period.

Assimilation efficiency trials

Experiments were carried out with a minimum period of 48 h between trials. Birds were fed a standard maintenance diet between experiments. On experiment days, individually housed birds were fed artificial fruits containing agar (Table 1) (Witmer 1998) in the light phase of the 12:12 cycle. Sugar type and concentration in artificially created fruits presented to birds during trials were manipulated to expose birds to low, medium and high equicaloric and equimolar solutions of glucose and sucrose sugars (Table 2). Individuals were exposed to one day (06h00 to 18h00) on each experimental diet. Individuals were free to choose the amounts of each type of sugar they preferred to eat in trials where both were offered (see Chapter 3 for more details on sugar choices). Birds were weighed prior to each trial (06h00) and at 18h00 for the respective diet trials. Experimental diets were chosen randomly but limited to one on each experimental day given the short shelf-life of agar fruits (2-3 days).

Artificial fruits of medium-sized balls (20 – 25 mm diameter) were placed in equal amounts in separate feeders on either side of the cage, equal distances from a central perch. In trials where only one type of food was on offer (experimental controls), equal amounts of the one type of food were placed in each feeder. Where

both sucrose and glucose artificial fruits were used, they were not mixed (i.e. sucrose in one tray and glucose in the other).

Feeders with artificial fruit were weighed at the beginning and end of trials to determine the total amount eaten from each. Control food trays containing the same amount and type of agar fruit as experimental food trays were placed in the room to calculate and compensate for food evaporative water loss.

At the end of each trial (18h00), excreta from each bird was collected (from the bottom of cages) and oven dried to constant mass at 50 °C before being milled, weighed and bombed (micro bomb calorimetry) for gross energy content (Animal Science Department, UKZN, Pietermaritzburg). Controls of each diet were also dried and bombed to determine gross energy.

The daily gross energy intake (GEI), excreta energy loss (EE), daily energy assimilation (DEA) and percentage daily apparent assimilation efficiency (AAE) were then calculated as per Wellmann and Downs (2009a) and mass corrected using the average body mass of initial and final weights. GEI was calculated by multiplying the equivalent dry mass of food eaten by the energy content of the diet. Where birds were offered sucrose and glucose fruits at the same time, energy content of the diet was calculated by taking the proportion of each type of food eaten and multiplying it by the energy levels obtained from the controls of each respective type of food. Energy levels calculated for each type of food were then added together and used in further calculations.

Excreta energy loss was calculated by multiplying the dried excreta mass by the excreta energy values obtained from the bombed samples. DEA was equal to the daily gross energy intake (GEI) subtracted by the excreta energy loss (EE). Finally,

the percentage daily apparent assimilation efficiency (AAE) was calculated by dividing the DEA by the GEI before multiplying by 100.

Statistical analyses

Significant differences in body mass before and after each experiment were identified using paired t-tests. General linear models (GLM) repeated measures ANOVA (RMANOVA) and post hoc Tukey HSD tests were used to compare experimental diets of medium concentration (i.e. medium concentration -equal energy glucose and sucrose, -matched molar glucose and sucrose, -glucose only and -sucrose only). Finally, RMANOVA and post hoc Tukey tests were used to find significant differences between matched molar experiments and equicaloric experiments as well as observing trends in GEI and % AAE at low, medium and high concentrations. Since % AAE values are effectively proportions, these were arcsine-square root transformed prior to statistical analyses (Brown et al. 2010a). Analyses were done using STATISTICA (Statsoft, V.7, Tulsa, OK, USA).

Results

Body mass

Paired t-tests indicate that Speckled Mousebirds only lost a significant amount of body mass on the medium energy equicaloric diet (Table 3, T-tests). Dark-capped Bulbuls on the other hand lost a significant amount of body mass on the low concentration equimolar diet as well as glucose and sucrose control diets (Table 3). Red-winged Starlings failed to maintain body mass on the low and medium concentration equimolar diets and glucose and sucrose control diets respectively (Table 3).

Equimolar and equicaloric sugar combinations vs. single sugar diets

Gross energy intake (GEI) in Speckled Mousebirds only differ significantly between the equicaloric diet and equimolar diet with the former being significantly higher (Fig. 1a), (RMANOVA, $F_{(3,24)} = 4.70$, $p = 0.01$, post hoc Tukey HSD, $p = 0.01$, $df = 24$). Apparent assimilation efficiency (AAE) on all diets averaged between 97 - 98 % with no significant differences between diet treatments (Fig. 1b) (RMANOVA, $F_{(3,24)} = 0.27$, $p = 0.84$).

Dark-capped Bulbuls on the other hand show significantly lower GEI on the equimolar diet compared to the equicaloric and sucrose diet treatments (Fig. 2a), (RMANOVA, $F_{(3,12)} = 7,0$, $p = 0.006$, post hoc Tukey HSD, $p = 0.011$, $df = 12$ and $p = 0.008$, $df = 12$ respectively). AAE for Dark-capped Bulbuls averaged between 97 - 99 % with no significant differences between diet treatments (Fig. 2b) (RMANOVA, $F_{(3,12)} = 0.97$, $p = 0.44$).

It should be noted that faecal sample collection for Red-winged Starlings may be biased due to the fact that individuals developed osmotic diarrhoea when fed exclusively on sucrose (Fig. 3b). Red-winged Starling GEI was significantly higher when fed the all sucrose diets compared with all other diets (Fig. 3a), (RMANOVA, $F_{(3,24)} = 21.0$, $p < 0.001$, post hoc Tukey HSD, $p < 0.001$, $df = 24$ for sucrose vs. all other diets). AAE for Red-winged Starlings also averaged between 97 - 99 % with no significant difference between diets (RMANOVA, $F_{(3,21)} = 1.50$, $p = 0.24$).

Matched molar vs. equal energy

Matched molar diets (0.42 mol/l, 0.83 mol/l and 1.66 mol/l; sucrose and glucose) and equicaloric diets (1175 kJ, 2350 kJ and 4701 kJ; sucrose and glucose) of varying concentrations were compared in Speckled Mousebirds (Fig. 4). Speckled

Mousebirds show significantly higher GEI of the medium concentration equimolar diet compared with the medium concentration equicaloric diet (RMANOVA, $F_{(2,16)} = 9.2$, $p = 0.002$, post hoc Tukey HSD, $p = 0.014$, $df = 16$), (Fig. 4a). Differences between equi-molar and -caloric diet treatments were not sufficient to affect GEI significantly at low and high concentrations (Fig. 4a). Mean AAE on equimolar and equicaloric diets remained between 97 and 98 % irrespective of diet concentration (RMANOVA, $F_{(2,16)} = 1.33$, $p = 0.29$), (Fig. 4b).

Dark-capped Bulbuls only showed significantly higher GEI when fed a low concentration equicaloric diet compared with a low concentration equimolar diet (RMANOVA, $F_{(2,8)} = 3.9$, $p = 0.065$, post hoc Tukey HSD, $p = 0.027$, $df = 8$) (Fig. 5a). Mean AAE for Dark-capped Bulbuls was between 98 - 100 % and did not differ significantly between equicaloric and equimolar diets (RMANOVA, $F_{(2,6)} = 2.3$, $p = 0.19$), (Fig. 5b).

Red-winged Starlings showed a similar GEI response to Dark-capped Bulbuls, equicaloric diet being significantly higher than the equimolar diet at low concentrations (RMANOVA, $F_{(2,16)} = 3.6$, $p = 0.05$, post hoc Tukey HSD, $p = 0.039$, $df = 16$) but no significant differences at medium and high concentrations (Fig. 6a). Mean AAE for Red-winged Starlings was between 96 - 100 % with the only significant difference being between equicaloric and equimolar diets at low a high concentration (equicaloric being favoured) (RMANOVA, $F_{(2,14)} = 84.89$, $p < 0.001$, post hoc Tukey HSD, $p < 0.001$, $df = 14$), (Fig. 6b).

Effects of concentration

Speckled Mousebirds GEI on equicaloric diets (sucrose and glucose) was significantly higher on the low concentration diet (1175 kJ/l) compared with medium

(2350 kJ/l) and high (4701 kJ/l) concentration diets (RMANOVA, $F_{(2,16)} = 9.2$, $p = 0.002$, post hoc Tukey HSD, $p < 0.001$ for both, $df = 16$), (Fig. 4a). AAE did not differ significantly between low, medium and high concentration diet treatments (RMANOVA, $F_{(2,16)} = 1.33$, $p = 0.29$), (Fig. 4b). In terms of equimolar diets, Speckled Mousebirds GEI was significantly higher when fed the low (0.42 mol/l) match molar diet compared with the high (1.66 mol/l) matched molar diet (RMANOVA, $F_{(2,16)} = 9.2$, $p = 0.002$, post hoc Tukey HSD, $p < 0.001$ $df = 16$), (Fig. 4a) AAE on each diet did not however, differ significantly (RMANOVA, $F_{(2,16)} = 1.33$, $p = 0.29$) ,(Fig. 4b).

Dark-capped Bulbul GEI at varying concentrations showed a similar trend for both equicaloric and equimolar diets (Fig. 5). GEI was significantly higher on low caloric and molar concentration diets compared with respective medium and high concentration diets (RMANOVA, $F_{(2,8)} = 3.9$, $p = 0.065$, post hoc Tukey HSD, $p < 0.01$ for all instances, $df = 8$), (Fig. 5a). AAE did not differ significantly between the diets irrespective of caloric or molar concentration (RMANOVA, $F_{(2,6)} = 2.25$, $p = 0.19$), (Fig. 5b).

Red-winged Starling GEI mirrored Dark-capped Bulbul GEI with low caloric and molar concentration diets intake being significantly higher than medium and high concentrations (RMANOVA, $F_{(2,16)} = 3.6$, $p = 0.05$, post hoc Tukey HSD, $p < 0.001$ for all instances, $df = 16$), (Fig. 6a). AAE for Red-winged Starlings displayed interesting concentration dependent trends (Fig. 6b). In terms of equicaloric diets, the medium energy diet (2350 kJ/l) was assimilated with significantly lower efficiency than low (1175 kJ/l) and high (4701 kJ/l) equicaloric diets (RMANOVA, $F_{(2,14)} = 84.89$, $p < 0.001$, post hoc Tukey HSD, $p = 0.003$ vs. low and $p < 0.001$ vs. high, $df = 14$), (Fig. 6b). For matched molar diets, we observe a significant decrease in AAE with each increase in molar concentration (RMANOVA, $F_{(2,14)} = 84.89$, $p < 0.001$,

post hoc Tukey HSD, $p = 0.005$ for low vs. medium, $p = 0.016$ for medium vs. high, $p < 0.001$ for low vs. high $df = 14$), (Fig. 6b).

Discussion

Sugar combinations

A study on Blackcaps (*Sylvia atricupilla*) and Garden Warblers (*S. borin*) found that these frugivores managed to maintain body mass on an exclusively fruit diet, for birds to increase fat reserves and body mass they had to feed on insects (Jordano 1988). Changes in body mass suggest that Dark-capped Bulbuls and Red-winged Starlings maintain body mass when given a choice to feed on glucose and sucrose fruits as opposed to when offered exclusively glucose or sucrose equicaloric diet treatments. Speckled Mousebirds in contrast, failed to maintain body mass on the free choice diet treatments and maintained body mass when fed exclusively on glucose or sucrose when fed medium concentration equicaloric diet treatments.

Red-winged Starling and Dark-capped Bulbul GEI values indicated that energy intake regulation occurred in these species in general, with the exception of Red-winged Starlings which ate over 60 % more when fed on an all sucrose diet. Despite this, both species consistently showed greater than 95 % AAE (barring Red-winged Starling on the medium concentration all sucrose diet, due to osmotic diarrhea), both species did not maintain body mass on both sucrose and glucose single sugar diet treatments. Brown (2009) found that Red-winged Starlings fed on artificial nectar solutions were unable to digest sucrose at 5% and 25% concentration and avoided feeding on sucrose solutions below 25% concentration. Previous work on other Sturnidae including Purple-headed Glossy Starling (*Lamprotornis pupureiceps*),

Gray Starling (*Sturnus cineraceus*) and European Starling (*S. vulgaris*) indicated that these birds preferred a 1:1 mixture of glucose and fructose over sucrose (from 12% to 15% sugar mass/total volume) (Martinez del Rio and Stevens 1989; Malcarney et al. 1994; Lane 1997). This study indicates that Red-winged Starlings appear to follow similar sucrose aversion trends when fed an artificial fruit diet.

Speckled Mousebirds also assimilated all single sugar and sugar combination diets with over 95 % efficiency. Limited understanding of non-Passerine assimilation efficiencies indicates that this group has retained sucrose assimilation as a primitive ancestral trait (Lotz and Schondube 2006). Speckled Mousebirds lost body mass when GEI was significantly low. Consequently digestive limitations appear less important for this species.

Matched molar verses equicaloric diets

In our experiments equimolar diets differed from equicaloric diets only in energy with sucrose artificial fruits containing twice that of equicaloric sucrose fruits. Thus any differences or trends observed are assumed to be primarily as a result of this energy difference. For Red-winged Starlings and Speckled Mousebirds, a difference only manifested when birds were fed a low concentration diet treatment. Both species showed higher GEI when fed the equicaloric diet compared with the equimolar diet. In terms of AAE, only Red-winged Starlings showed a difference and this was between the high concentration equicaloric and equimolar diet treatments. GEI was not different between the equimolar and equicaloric diets yet AAE figures showed that the starlings were assimilating the equicaloric diet more effectively than the equimolar diet at high concentrations.

Speckled Mousebirds showed no difference between matched molar and equicaloric diet GEI at low and medium concentrations but had higher GEI on the medium equimolar diet compared with the medium concentration equicaloric diet. Once again, we see that AAE was fairly constant for Speckled Mousebirds irrespective of the type of diet being consumed (equimolar or equicaloric artificial fruits), sugars were digested with high efficiency (97-98 % AAE) as predicted for non-Passerines (Witmer 1999; Lotz and Schondube 2006). After modelling avian frugivore guts as chemical reactors, Martinez del Rio and Karasov (1990) predicted near 100 % assimilation efficiency for birds with low foraging costs (similar to foraging condition in our laboratory).

Concentration effects

GEI either increased or remained constant with decreasing concentration on both equicaloric and matched molar diets for Speckled Mousebirds, Dark-capped Bulbuls and Red-winged Starlings. We predicted that birds would consume a larger volume of lower concentration food to meet energetic demands as in other studies (Stephens et al. 2007; Brown et al. 2010a). If consumed energy was assimilated with 100% efficiency for all species, GEI (kJ/g Body mass) would have been consistent irrespective of diet concentration under the energy intake regulation model. GEI, however, increased significantly with decreasing sugar concentration in all species suggesting that the birds are compensating for example, with quicker transit rates (Herrera 1998), a trend noticed in some nectarivores (Karasov et al. 1986).

AAE values of Speckled Mousebird and Dark-capped Bulbul were consistently high suggesting that birds fed more at low concentrations to achieve

intake needs. Red-winged Starling AAE decreased with increasing concentration when fed equimolar diets and displayed lowest AAE for medium concentration equicaloric diets. The decrease in AAE when fed an equimolar diet of increasing concentration is considered as energy intake optimization. Even though Red-winged Starlings are less capable of digesting sucrose compared with glucose (Brown 2009), the sucrose fruits on offer in equimolar experiments had approximately twice the energy per gram. Red-winged Starlings appear to trade assimilation efficiency for higher intake in this scenario, possible evidence that trace amounts of sucrose can be digested. Literature suggests that all Sturnidae are incapable of producing the enzyme sucrase and therefore incapable of digesting sucrose (reviewed in Lotz and Schondube 2006) . Red-winged Starlings in particular were unable to digest sucrose in a liquid form (artificial nectar) (Brown 2009). If this is also true when fed on an a sucrose artificial fruit diet, then it is expected that birds are only able to digest trace elements found in the artificial fruit provided and negligible amount of sucrose, possibly as a result of a mutualistic relationship with intestinal microorganisms (bacterial or protozoan) such as the ciliate *Polyplastron multivesiculatum* which is known to produce simple sugars by the hydrolyses of larger molecules (Akkada et al. 1963).

There are numerous results from this study that do not fit with the hypotheses, model predictions and trends observed in previous studies of this nature. Most anomalies arise from our assimilation efficiency findings. We believe that our findings differ in this respect because of an experimental protocol that allowed study animals a decision making process giving them a choice over how much of each sugar type to consume. Levey and Grajal (1991) suggest that frugivores select fruits to maximise nutrient absorption. If selecting specific proportions of hexose and sucrose

sugars had an effect on digestive efficiency we would have expected the assimilation efficiencies to be high. This trend was observed, giving us evidence that avian frugivores can indeed optimize digestive energy gain by regulating the amount of sucrose and glucose being ingested. Further research into the digestive processes involved in sucrose and glucose absorption as well as transit rates and transit rate flexibility (eg. anti-peristalsis) would help explain the observed trends.

Acknowledgements

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Ingredients (all values in grams)	Energy content					
	Low		Med		High	
	Sucrose	Glucose	Sucrose	Glucose	Sucrose	Glucose
Anhydrous Sucrose/Glucose	**71.2** *(142.5)*	**75.0**	**142.5** *(284.9)*	**150.0**	**284.9** *(569.8)*	**300.0**
Distilled Water	1000	1000	1000	1000	1000	1000
Wheat Bran	50	50	50	50	50	50
Agar	10	10	10	10	10	10
Sodium Chloride	0.75	0.75	0.75	0.75	0.75	0.75
Dicalcium Phosphate	0.8	0.8	0.8	0.8	0.8	0.8
Vitamin supplement*	0.75	0.75	0.75	0.75	0.75	0.75
Energy of Sugar (kJ/l)	1175.3	1175.3	2350.5	2350.5	4701.0	4701.0

*AviPlus (Hillcrest. South Africa)

Table 2: Summary of energy contents, molecular masses and sugar combinations used in assimilation efficiency trails. Shaded cells represent equivalent molecular mass for equicaloric trials and equivalent energy content for equimolar trials.

Sugar	Trial	Energy content (kJ/l)		Molecular mass (mol/l)	
		Sucrose	Glucose	Sucrose	Glucose
Gluc + Sucr	low energy	1175.25	1175.25	**0.21**	**0.42**
Gluc + Sucr	med energy	2350.50	2350.50	**0.42**	**0.83**
Gluc + Sucr	high energy	4701.00	4701.00	**0.83**	**1.66**
Gluc + Sucr	low mol	**2350.70**	1175.25	0.42	0.42
Gluc + Sucr	med mol	**4701.40**	2350.50	0.83	0.83
Gluc + Sucr	high mol	**9402.80**	4701.00	1.66	1.66
Gluc only	med energy	2350.50		0.42	
Sucr only	med energy		2350.50		0.83

Gluc = Glucose, **Sucr** = Sucrose

Table 3: Body mass differences, pre- verses post-experiment, for Speckled Mousebirds (SMB), Dark-capped Bulbul (DCB) and Red-winged Starling (RWS). Paired t-test values in **bold** indicate where a significant amount of body mass was lost and *italics* indicates a significant gain in body mass.

		P	t	n	Percentage change in body mass (pre-experiment range; post-experiment range) Body mass grams
SMB	*low cal*	*0.0074*	*3.439*	*10*	*2.14 (42.5-51.0; 43.9-52.3)*
	low mol	0.153	1.561	10	-0.64 (43.6-53.0; 43.1-51.9)
	med cal	**0.0195**	**2.836**	**10**	**-1.58 (42.0-51.5; 41.7-49.8)**
	med mol	0.0705	2.051	10	-1.48 (42.5-54.0; 43.7-53.8)
	high cal	0.1	1.828	10	1.48 (43.5-51.5; 44.6-50.4)
	high mol	*0.019*	*2.842*	*10*	*2.27 (42.3-52.1; 44.5-52.4)*
	med gluc contr	0.649	0.471	10	0.34 (42.7-53.0; 43.3-51.1)
	med sucr contr	0.75	0.329	10	0.11 (41.8-50.8; 41.4-50.6)
DCB	low cal	0.89	0.1404	5	-0.14 (41.1-45.2; 41.1-45.3)
	low mol	**0.002**	**7.109**	**5**	**-3.13 (42.0-44.2; 40.4-43.6)**
	med cal	0.16	1.707	5	-1.06 (39.4-45.8; 39.3-44.4)
	med mol	0.103	1.993	6	-0.66 (44.2-46.0; 43.8-46.4)
	high cal	0.097	2.034	6	-1.37 (40.7-43.9; 41.1-43.1)
	high mol	0.34	1.053	6	-1.68 (41.6-44.1; 41.1-44.1)
	med gluc contr	**0.023**	**3.228**	**6**	**-1.17 (43.1-45.1; 42.4-44.8)**
	med sucr contr	**0.0012**	**8.227**	**5**	**-2.41 (42.2-44.8; 40.9-43.8)**
RWS	low cal	0.896	0.136	9	0.07 (100.6-121.5; 100.6-124.1)
	low mol	**0.0323**	**2.587**	**9**	**-1.84 (104.5-123.9; 101.2-125.7)**
	med cal	0.49	0.723	9	0.42 (100.5-123.0; 101.1-125.7)
	med mol	**0.006**	**3.724**	**9**	**-1.34 (102.4-128.1; 102.1-124.8)**
	high cal	*0.041*	*2.427*	*9*	*0.94 (102.1-124.1; 105.5-125.4)*
	high mol	0.329	1.039	9	0.46 (102.4-126.6; 102.5-125.3)
	med gluc contr	**0.0018**	**4.6**	**9**	**-0.75 (102.1-127.7; 101.3-127.4)**
	med sucr contr	**<0.0001**	**8.807**	**9**	**-4.44 (101.5-122.2; 95.1-118.7)**

Cal = equicaloric diet, **mol** = equimolar diet, **gluc contr** = experimental control with only glucose sugars, **sucr contr** = experimental control with only sucrose sugars
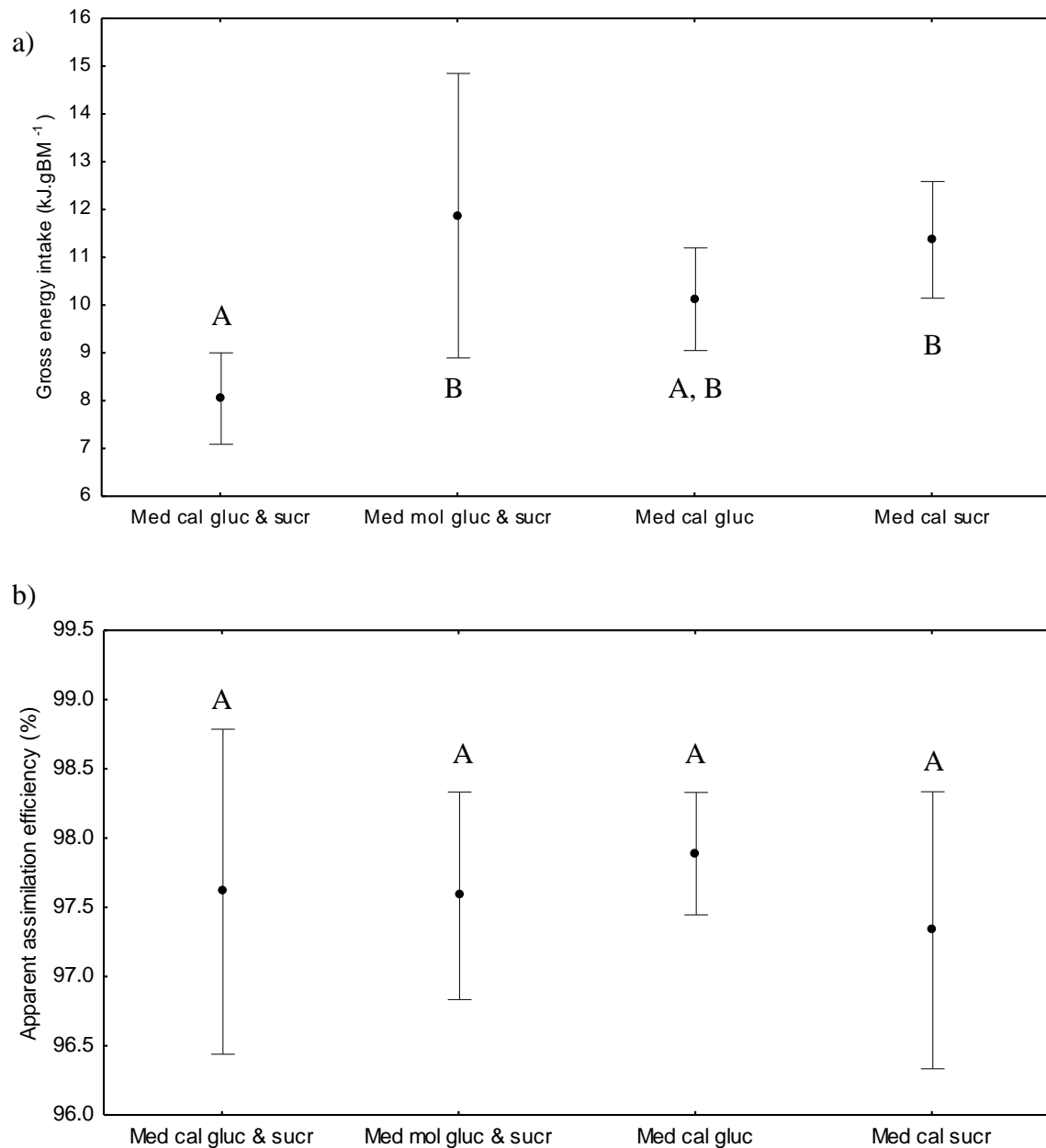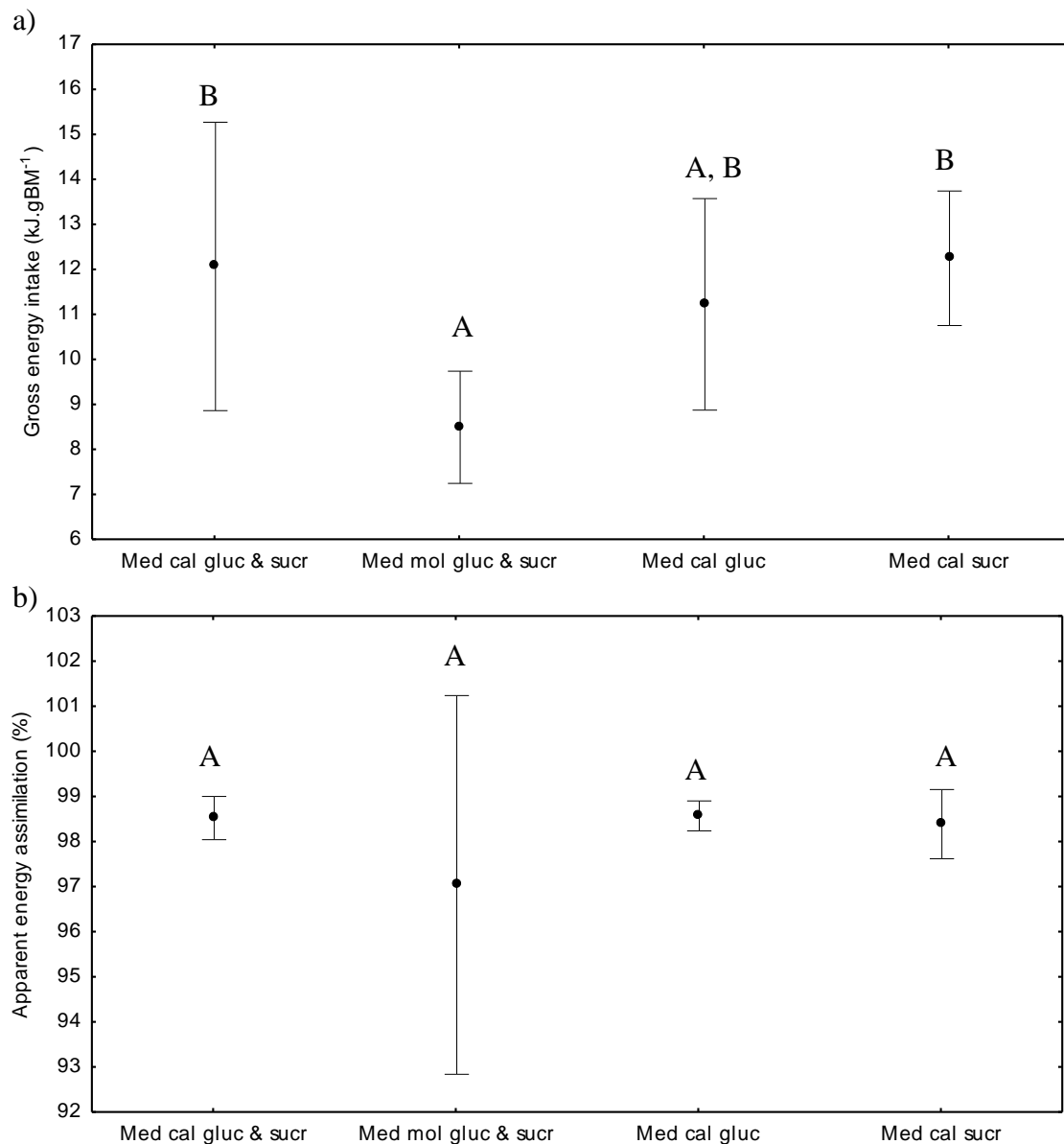
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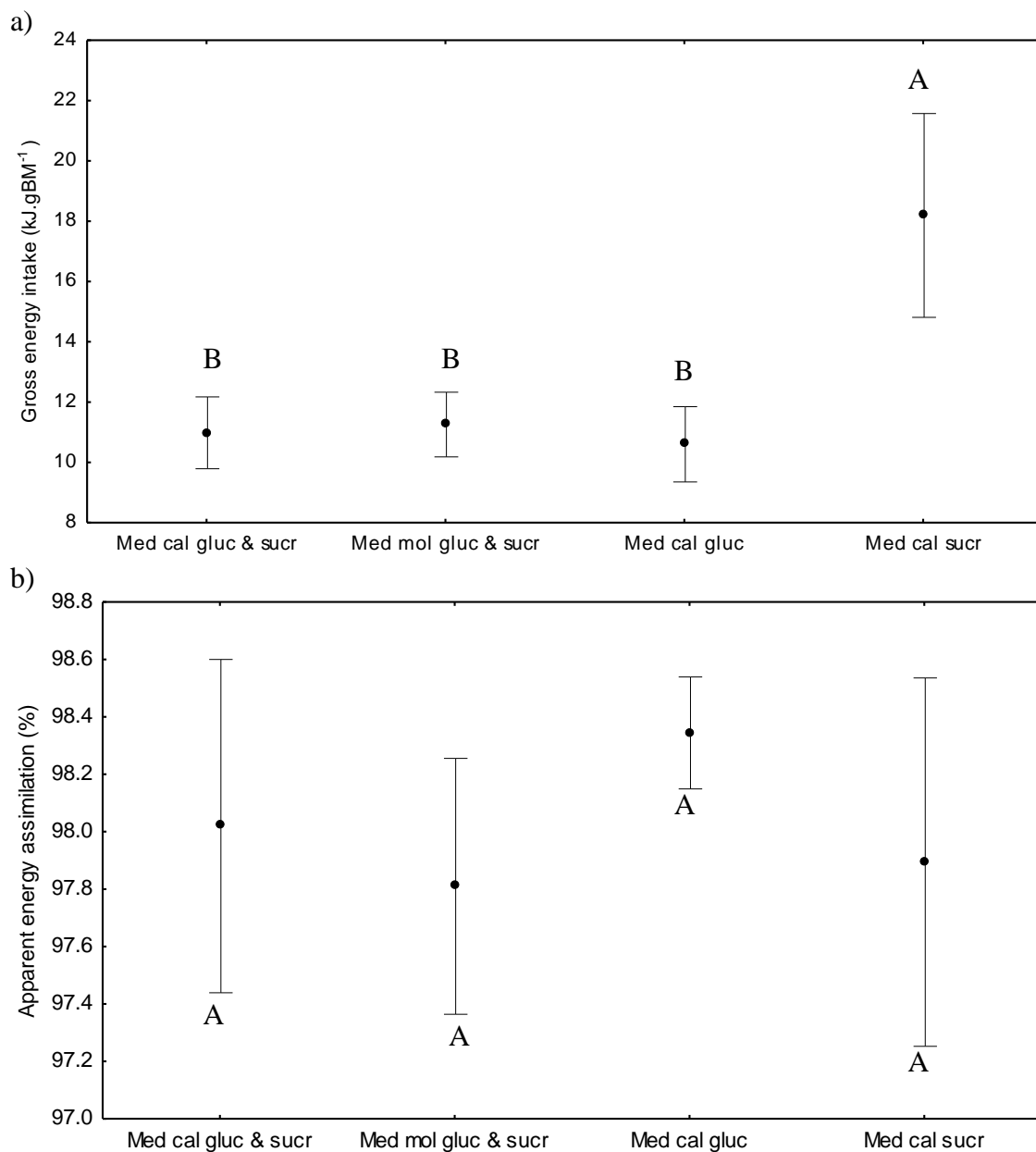
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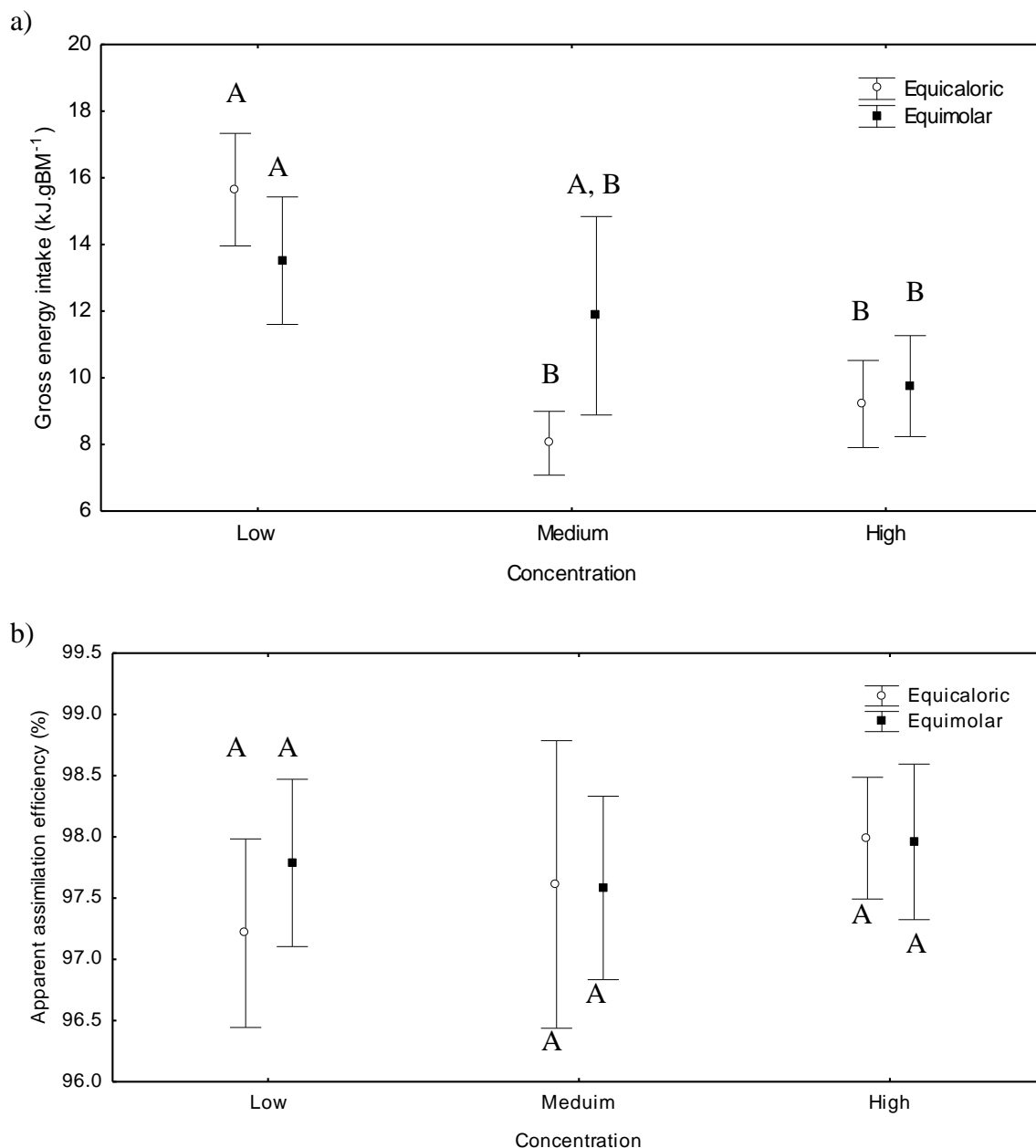
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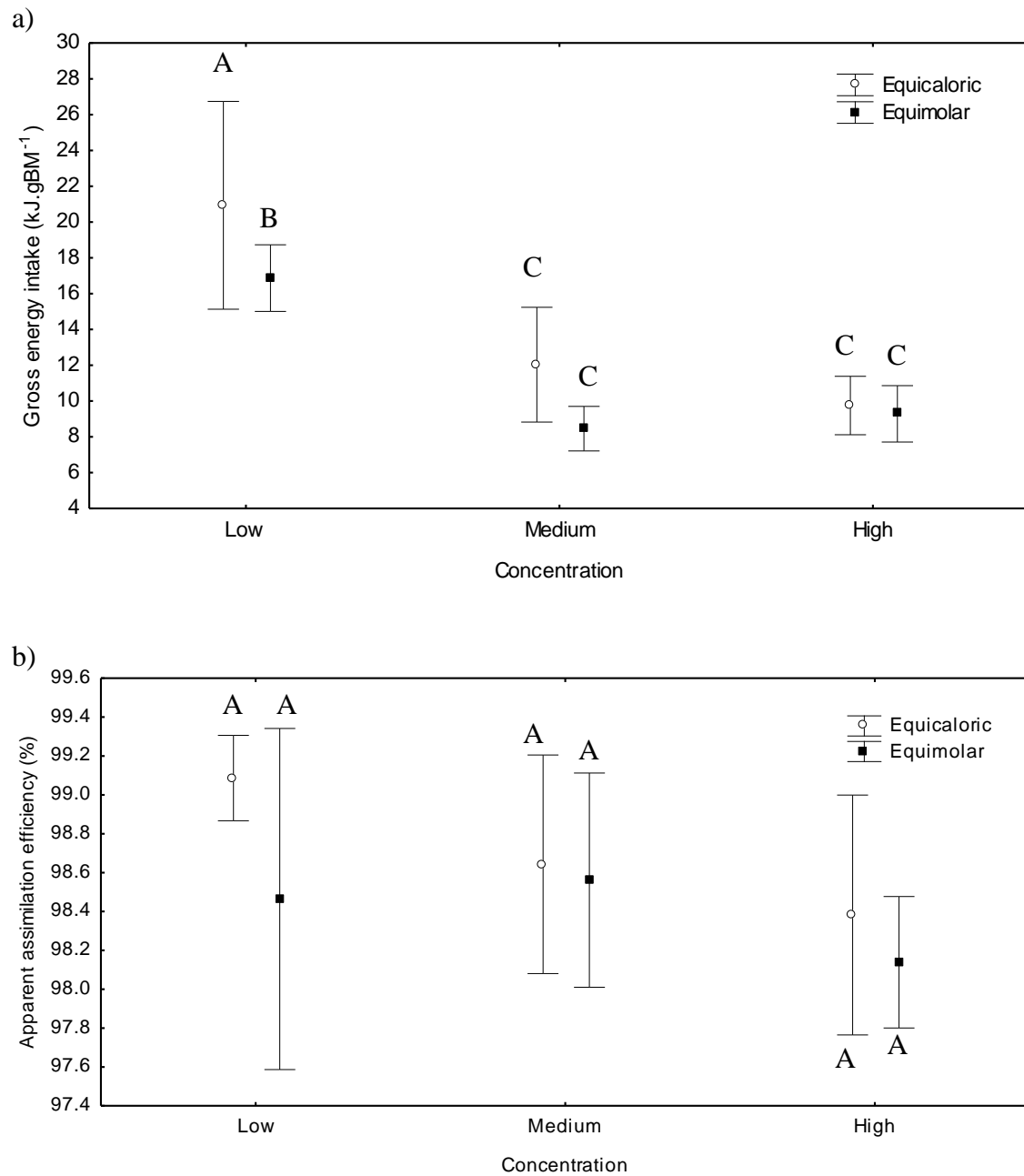
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a)

b)

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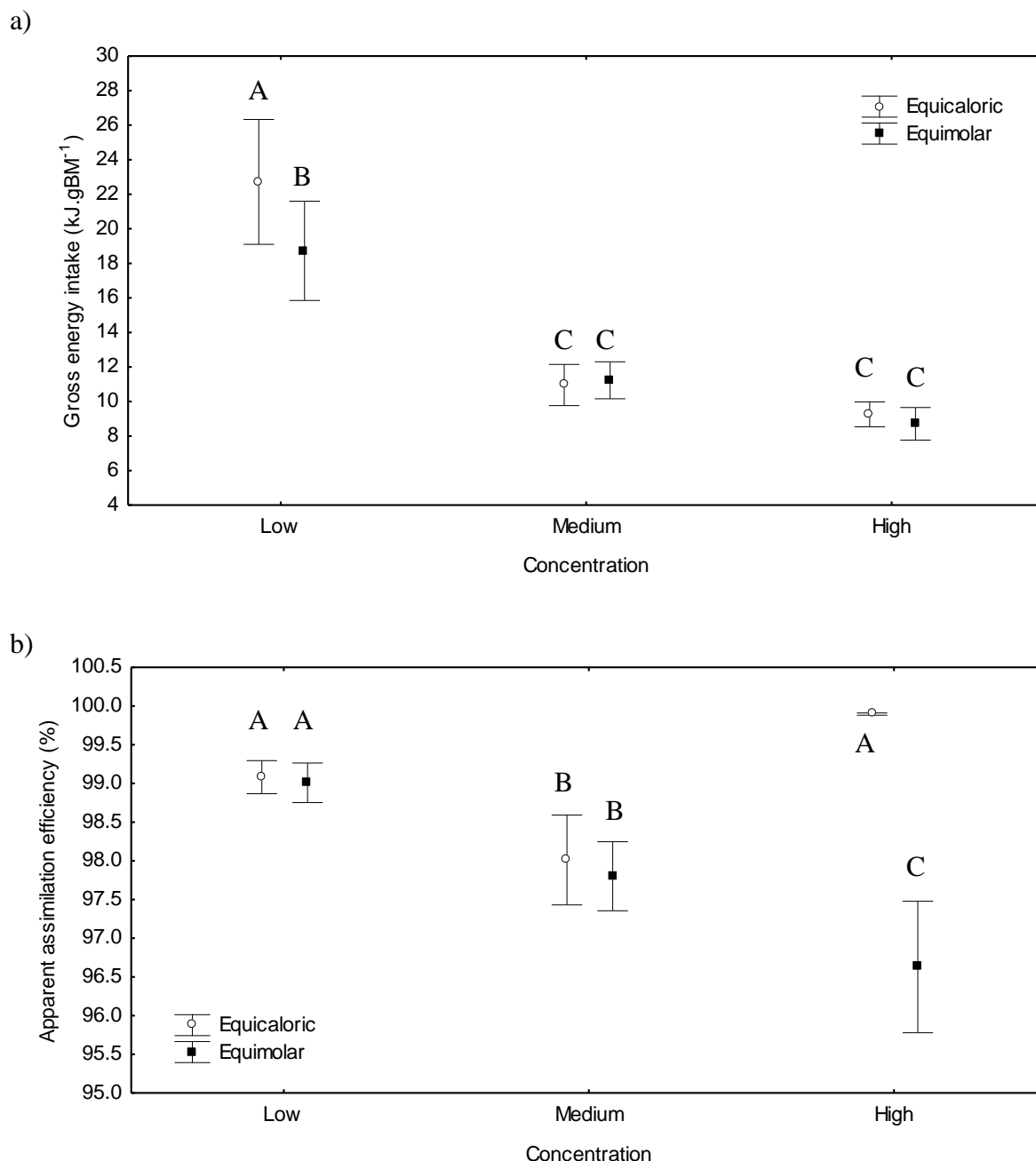
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Chapter 3

Does sugar type and concentration affect food selection by frugivorous South African birds: A combined methodological approach

(formatted for *Functional Ecology*)

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Summary

1. Understanding mechanisms behind foraging behaviour in organisms can greatly aid in conservation efforts. An understanding of foraging ecology in avian frugivores is essential in the effort to understand how organisms respond to changing environments

2. This study investigated if birds show a preference for specific types of sugar in food selection. Three frugivorous species (Red-winged Starling *Onychognathus morio*, Speckled Mouse-bird *Colius striatus* and Dark-Capped Bulbul *Pycnonotus tricolor*) were exposed to diet treatments containing artificial fruits to determine if sucrose or glucose were preferred at varying controlled equicaloric and equimolar sugar concentrations.

3. Each study species presented different results, Speckled Mousebirds consistently showed no sugar preferences on any diet treatments (concentration and sugar type), Dark-capped Bulbuls occasionally favoured glucose over sucrose while Red-winged Starlings always favoured glucose over sucrose.

4. Birds can exhibit differing behavioural preferences for sugar types in fruits compared with nectars. A preference (or lack thereof) for specific sugars can potentially be used to predict foraging behaviour in avian frugivores.

Key words: Artificial fruit, Digestion, Equicaloric vs. equimolar, Glucose, Sucrose, Sugar preferences

Introduction

It has been suggested that frugivorous birds be studied to investigate the hazy link between physiology and behaviour (Levey and Martinez del Rio 2001; Tewksbury 2002). Frugivorous birds have morphologically simple guts, chemically simple foods and relatively unconstrained feeding behaviour (Levey and Martinez del Rio 2001). To date most work investigating possible sugar preferences in organisms has focused on nectarivorous birds (summarized in Fleming et al. 2008; Brown et al. 2010a; b). The results of these studies indicate that specialist avian nectarivores favour hexose sugars at low concentrations and sucrose at higher concentrations when solutions are equicaloric (Fleming et al. 2008; Brown et al. 2010b).

Little work has been done on sugar preferences in fruit eating birds (Lotz and Schondube 2006). Fruits generally differ from nectars as they contain a larger proportion of complex molecules (e.g. lipids, protein and fats), secondary compounds and bulk (e.g. fibre and seeds) (Levey and Martinez del Rio 2001; Downs 2008). The composition of naturally occurring fruits is however, inconsistent as the content of fruits change during fruit expansion and maturation (Klann et al. 1993). Consistency in methodology for testing sugar preferences in avian frugivores was only achieved after Witmer (1998) made use of synthetic fruits containing controlled concentrations of sugar and other constituents (Chapter 2, Table 1). To date, most studies investigating avian sugar preferences have either based findings on results generated from experiments which give birds a choice between sucrose and hexose sugar solutions (glucose and fructose) of equal sugar weight, equal molecular sugar mass or equal energy levels (Greig-Smith 1985; Brown et al. 2008; Fleming et al. 2008; Johnson and Nicolson 2008) (see Chapter 1 *Optimality*). Researchers call for the standardization of methodology to determine sugar preferences in nectarivores

(Fleming *et al.* 2004). Brown *et al.* (2008) also agreed that each methodology (except the flawed % weight model) has merits depending on the question being asked (Brown *et al.* 2008; Johnson and Nicolson 2008). By using a both matched molar (equimolar) solution and equal energy (equicaloric) solution experiments respectively, we wanted to compare results and acquire a full understanding of sugar preferences for various frugivorous avian species.

Three frugivore species (Speckled Mouse-birds, Dark-capped Bulbuls and Red-winged Starlings: *Colius striatus, Pycnonotus tricolor* and *Onychognathus morio* respectively) were chosen for study. Speckled Mousebird feed on fruit, leaves, seeds and nectar (summarised in Hockey *et al.* 2005). When offered nectars containing equicaloric sugar types and concentrations, Speckled Mousebirds showed a preference for hexose sugars at low concentrations and a preference for sucrose at higher concentrations (Brown *et al.* 2010b). Dark-capped Bulbul forage on fruits, invertebrates and nectar (Symes and Downs 2001; Hockey et al. 2005). Brown et al. (2010a) suggest that Dark-capped Bulbuls always favour hexose sugars in nectars of varying sugar concentration. Previous work on Red-winged Starlings has determined that these birds avoid sucrose sugars at low concentrations when fed artificial nectar solutions (Brown 2009). Red-winged Starling responses towards sucrose and hexose sugars in artificial fruits containing fibre and other trace elements have yet to be investigated. Including other members of the Sturnidae family results in general, agree that this family lacks the ability to digest sucrose (Martinez del Rio and Stevens 1989; Avery et al. 1995; summarised in Lotz and Schondube 2006;).

Each of these bird species is indigenous and locally abundant in the KwaZulu Natal region of South Africa (Hockey *et al.* 2005). Their feeding behaviour consequently has a great effect on seed dispersal (Deckers et al. 2008; Howe and

Smallwood 1982). Primary frugivorous species in an area have the greatest effect on the evolutionary path taken by a fruit bearing plant species (Herrera 1998). Thus geographic distributions of study animals should be closely linked to the distribution of fruit bearing vegetation (Herrera 1998; Kissling *et al.* 2007), a relationship which is not well understood in the selected study species. Examination of sugar preferences may contribute towards understanding the link between the distribution of fruit bearing trees and their dispersers by determining which fruit are selected preferentially based on sugar content.

We investigated Speckled Mousebird, Dark-capped Bulbul and Red-winged Starling sugar preferences at varying concentrations on an artificial fruit diet. It was hypothesized that sugar type and concentration affect fruit preferences. It was predicted that birds would display sugar preferences similar to those observed when fed nectar solutions in previous studies (summarised above). Any differences in sugar preferences are therefore expected to be as a result of sugars being presented in the form of artificial fruits and not nectar.

Materials and methods

Study Animals

Six to ten individuals of Speckled Mousebird, Dark-capped Bulbul and Red-winged Starling were mist netted in KwaZulu-Natal between July 2007 and February 2008 (Waterfall (29°44'56.64"S 30°48'49.68"E), Pietermaritzburg (29°37'31.88"S 30°24'5.22"E), Darvill (29°35'50.92"S 30°26'16.78"E) and Hilton College (29°29'32.12"S 30°18'6.86"E) under permit from Ezemvelo KZN Wildlife. Birds were housed in outdoor aviaries at University of KwaZulu-Natal, Pietermaritzburg.

Individuals were dewormed (Mediworm Powder, Medpet (Pty.) Ltd., Benrose, South Africa) and given one week to acclimate to laboratory conditions before experimentation (12:12 photoperiod, temperature controlled at 25°C ±1°C). During the acclimation period, all birds were housed individually in wire cages (50 x 50 x 50 cm) and fed a standard maintenance diet consisting of a mixture of apple, pear, banana, grape, pawpaw, orange (additional spinach and broccoli for mousebirds) and medium concentration glucose or sucrose artificial fruits (Witmer 1998). The above ingredients were mixed in a fruit-salad with Mynah pellets and Mynah Softbill crumbs (Avi-Products (PTY) LTD). Water was provided *ad libitum* and mealworms *Tenebrio molitor* (for Bulbuls and Starlings only) every second day during this acclimation period.

Sugar preference trials

Experiments were carried out with a minimum period of 48 h between trials. Birds were fed a standard maintenance diet in the time between experiments. On experiment days, individually housed birds were fed artificial fruits containing agar (Witmer 1998) in the light phase of the 12:12 cycle (see Chapter 2, Table 1). Sugar type and concentration in artificially made fruits presented to birds during trials was manipulated to expose birds to low, medium and high equicaloric and equimolar solutions of glucose and sucrose sugars (Table 1). Individuals were exposed to one day on each experimental diet. Experimental diets were chosen randomly but limited to one on each experimental day given the short shelf-life of agar fruits (2-3 days).

Artificial fruits were carved into medium sized balls (20 − 25 mm diameter) out of a pre set agar solution. Equal amounts of food were placed into separate food trays and placed on either side of the cage at equal distances from a central perch. In the case of trials where only one type of food was on offer (i.e. control diets), equal

amounts of the one type of food were placed in each tray. Where both sucrose and glucose artificial fruits were used, they were not mixed (i.e. sucrose in one tray and glucose in the other).

Food trays were weighed at the beginning and end of trials as well as every hour on the hour (from 06:00 – 18:00) to determine total amount eaten from each tray and hourly energy intake rate respectively. Evidence suggests that daily ingestion patterns could affect the outcome of experiments if study animals gorge on artificial diets initially (Downs 2000) before distinguishing between different food sources. Observing hourly food intake of each experimental diet allowed diet switching events to be detected. Control food trays containing the same amount and type of agar fruit as experimental food trays were placed in the room to calculate and compensate for food evaporative water loss. Individuals were weighed at 06:00 and 18:00 (see Chapter 2 for detailed body mass analyses), the mean body mass was then used to calculate the total mass specific energy intake and hourly mass specific energy intake from each tray.

Data analyses

All raw data values from sugar preference trials were transformed to account for variation in body mass between test subjects and hourly evaporative water loss from food trays. Only the manipulated data was used for analyses. Preferential selection of one type of sugar over another at various concentrations and energy levels was determined and plotted against time using General Linear Models (GLM) Repeated Measures Analysis of Variance (RMANOVA). Post-hoc Tukey HSD tests determine any significant differences between hourly tray readings. Analyses were done using STATISTICA (Statsoft, V.7, Tulsa, OK, USA).

Results

Equicaloric sugar preference experiments

Speckled Mousebirds showed no significant preferences for glucose or sucrose at low (1175.25 kJ/l), medium (2350.5 kJ/l) and high (4701 kJ/l) energy equicaloric diet treatments (RMANOVA, $F_{(11,99)}$ = 1.64, 1.16, 1.52, p = 0.1, 0.33, 0.88 respectively) (Table 2).

Dark-capped Bulbuls showed no preference for either sugar type at low and high energy equicaloric diet treatments (RMANOVA, $F_{(11,55)}$ = 0.73, 0.44, p = 0.70, 0.93 respectively) (Table 2). Interestingly, Dark-capped Bulbuls showed a significant preference for glucose over sucrose when fed the medium energy equicaloric diet treatment (Fig. 1) (RMANOVA, $F_{(11,44)}$ = 3.66, $p < 0.001$).

Red-winged Starlings on the other hand showed a distinct preference for glucose over sucrose when fed low (Fig. 2), medium (Fig. 3) and high (Fig. 4) energy equicaloric diet treatments (RMANOVA, $F_{(11,88)}$ = 3.60, 2.17, 4.85, $p < 0.001$, = 0.02, <0.001 respectively) (Table 2).

Equimolar sugar preference experiments

Results from these experiments are presented in kilojoules of sugar consumed per gram body mass and mass of artificial fruits eaten (grams) per gram body mass. Similar to equicaloric experiments, Speckled Mousebirds displayed no preferences for sugar types irrespective of molar concentration. There was no significant preference for either sugar type on low (0.42 mol/l), medium (0.83 mol/l) and high (1.66 mol/l) equimolar diet treatments (RMANOVA, $F_{(11,99)}$ = 1.02, 1.24, 0.41, p = 0.43, 0.27,

0.95 respectively for volumetric fruit intake) (RMANOVA, $F_{(11,99)}$ = 1.03, 0.8, 0.72, p = 0.42, 0.64, 0.71 respectively for mass specific energy intake) (Table 2).

Dark-capped Bulbuls showed a preference for glucose over sucrose when offered the high equimolar (1.66 mol/l) diet treatment (Fig. 5). A significant preference for glucose was evident for both mass specific volumetric food intake (RMANOVA, $F_{(11,55)}$ = 3.95, $p < 0.001$) (Fig 5a) and energy intake (RMANOVA, $F_{(11,55)}$ = 4.05, $p < 0.001$) (Fig. 5b) when fed a 1.66 mol/l diet.

Red-winged Starlings always favoured glucose over sucrose when fed low (Fig. 6), medium (Fig. 7) and high (Fig. 8) equimolar diet treatments. Both mass specific volumetric intake (Figs 6a, 7a and 8a) and energy intake (Figs 6b, 7b and 8b) displayed significant results in favour of glucose at low, medium and high molar concentrations (RMANOVA, $F_{(11,88)}$ = 3.31, 9.90, 10.10, $p < 0.001$ for volumetric intake) (RMANOVA, $F_{(11,88)}$ = 3.20, 6.66, 9.5, $p \leq 0.001$ for mass specific energy intake) (Table 2).

Energy intake vs. mass intake

Having analyzed both energy intake and mass intake data for all experiments the following trends were observed: Given the nature of equicaloric experiment diets, mass specific volumetric food intake and energy consumption yielded the same results. Volumetric analyses were consequently left out of the results in favour of energy intake analyses for these experiments. Equimolar diet experiments differ with regards to energy levels present per unit food consumed. Results from mass intake and energy intake differed as a consequence (Table 2). Differences due to variation in energy levels of consumed artificial fruits on the equimolar diet treatments were, however, not pronounced enough to affect results statistically. All significant results

remained significant and non-significant results remained non-significant when comparing energy intake and volumetric food intake analyses (Table 2).

Discussion

Speckled Mousebirds had no preference for sucrose or glucose sugars and appeared to feed equally on both sugar types at all concentrations (equicaloric and equimolar). This is in contrast to a study by Brown *et al.* (2010b) which reports a preferential selection of hexose sugars at low concentrations and sucrose at high concentrations when these were presented as liquid solutions. This highlights the differences when feeding on artificial fruit versus artificial nectar (primarily water and sugar) diet. Fast food transit rates on a liquid diet may result in lower digestive efficiency (Karasov *et al.* 1986) resulting in the selective preferences observed by Brown et al. (2010b). On a fruit diet, digestion appears to be aided by a more controlled process involving separation of fruit constituents, use of the crop and antiperistalsis allowing birds to be less selective about what they eat (Levey and Duke 1992; Symes and Downs 2001).

Dark-capped Bulbuls on the other hand show concentration dependent preferences for glucose fruits over sucrose fruits (on medium equicaloric and high equimolar diets). This was in contrast with Brown et al. (2010a) who found that glucose was always the preferred choice under a range of equicaloric nectar concentrations on offer. Consequently it appears that Dark-capped Bulbuls are less particular about preferential selection of sugar types when feeding on equal energy fruits as opposed to nectars. The fact that birds maintained body mass (see Chapter 2

Table 3) on low, medium and high energy (equicaloric) diets but only showed a preference for glucose on the medium energy diet is unusual.

Dark-capped Bulbuls are classified as facultative frugivores, they feed opportunistically on arthropods and nectar when fruit sources are scarce or nutritionally poor (Hockey et al. 2005; Downs 2008; Brown et al. 2010a). It stands to reason that if offered two fruits, the fruit with greater energetic rewards will be favoured. Results, however, indicated that Dark-capped Bulbuls preferred feeding on high concentration glucose fruits with half the energy content of sucrose fruits in equimolar diets. Since birds maintain body mass (see Chapter 2 Table 2) on the high molar glucose diet we suggest that the preference is due to other factors. If energy levels in two fruits are both sufficiently high enough to maintain body mass, birds will select fruits based on secondary factors such as post ingestional constraints due to energy thresholds being reached (Downs and Perrin 1996), taste preferences (Downs 1997), or relative ease of digestion of glucose compared with sucrose (Levey and Martinez del Rio 2001). At lower concentrations neither glucose nor sucrose are favoured as digestive enzymes and transporter molecules are not limited (Afik and Karasov 1995; Lotz and Schondube 2006; Martinez del Rio and Karasov 1990).

Brown (2009) observed a preference for hexose nectars over sucrose nectars of low concentration for Red-winged Starlings. In the present study Red-winged Starlings always selected glucose fruits over sucrose fruits irrespective of energy levels or molar concentration. It has been suggested that sucrose digestion is an ancestral trait that has been dropped in some passerines (Lotz and Schondube 2006). Previous work on sugar preferences in other Starling species indicate that the Sturnidae family have lost their ability to digest sucrose (Martinez del Rio and Stevens 1989; Malcarney et al. 1994; Avery et al. 1995; summarized in Fleming et al.

2008). Our results also suggest that Red-winged Starlings may lack the ability to digest sucrose.

In general, we found that sugar preference in the three avian frugivore species was dependent on the form of a target food source. Avian frugivores appear to have differing criteria for sugar preferences when it comes to fruit (present study) versus nectar (Brown 2009; Brown et al. 2010a; b). This should be taken into account in future studies and conservation efforts involving resource distribution and foraging behaviour. Underlying physiological limitations, however, may form the primary basis of sugar preferences in avian frugivores. Interestingly, birds either favoured glucose or ate both sucrose and glucose with no preference, sucrose was never selected preferentially over glucose at any energy level or molar concentration.

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gram body mass (mean ± SE). b) Values are energy consumed per gram body mass (mean ± SE).

Figure 6: Sugar preferences observed for Red-winged Starlings fed a low equimolar (0.42 mol/l) glucose and sucrose diet (n = 9). a) Values are mass consumed per gram body mass (mean ± SE). b) Values are energy consumed per gram body mass (mean ± SE).

Figure 7: Sugar preferences observed for Red-winged Starlings fed a medium equimolar (0.83 mol/l) glucose and sucrose diet (n = 9). a) Values are mass consumed per gram body mass (mean ± SE). b) Values are energy consumed per gram body mass (mean ± SE).

Figure 8: Sugar preferences observed for Red-winged Starlings fed a high equimolar (1.66 mol/l) glucose and sucrose diet (n = 9). a) Values are mass consumed per gram body mass (mean ± SE). b) Values are energy consumed per gram body mass (mean ± SE).

Table 1: Summary of energy contents, molecular masses and sugar combinations used in sugar preference trails. Light shaded cells represent equivalent molecular mass for equicaloric trials and equivalent energy content for equimolar trials.

Sugar	Trial	Energy content (kJ/l)		Diet Molarity (mol/l)	
		Sucr	Gluc	Sucr	Gluc
Gluc + Sucr	low energy	1175.25	1175.25	0.21	0.42
Gluc + Sucr	med energy	2350.50	2350.50	0.42	0.83
Gluc + Sucr	high energy	4701.00	4701.00	0.83	1.66
Gluc + Sucr	low mol	2350.70	1175.25	0.42	0.42
Gluc + Sucr	med mol	4701.40	2350.50	0.83	0.83
Gluc + Sucr	high mol	9402.80	4701.00	1.66	1.66
Gluc only	med energy	2350.50		0.42	
Suc only	med energy		2350.50		0.83

Table 2: Summary of RMANOVA results from equicaloric (energy intake) and equimolar (energy intake and volumetric intake) diets of varying concentration. Where **bold** values indicate a significant preference for glucose fruits over sucrose fruits.

| Equicaloric sugar diets	low - 1175 kJ/l			medium -2350 kJ/l			high - 4701 kJ/l		
Energy intake only	F	df	p	F	df	P	F	df	p
Speckled Mousebird	1.64	99	0.10	1.16	99	0.33	1.52	99	0.88
Dark-capped Bulbul	0.73	55	0.70	3.66	44	**< 0.001**	0.44	55	0.93
Red-winged Starling	3.60	88	**< 0.001**	2.17	88	**0.02**	4.85	88	**< 0.001**
Equimolar sugar diets	low - 0.42 mol/l			medium - 0.83 mol/l			high - 1.66 mol/l		
Energy intake	F	df	p	F	df	P	F	df	p
Speckled Mousebird	1.03	99	0.43	0.80	99	0.64	0.72	99	0.71
Dark-capped Bulbul	1.95	44	0.06	1.00	55	0.46	4.05	55	**< 0.001**
Red-winged Starling	3.20	88	**< 0.001**	6.66	88	**< 0.001**	9.50	88	**< 0.001**
Volumetrc intake									
Speckled Mousebird	1.02	99	0.43	1.24	99	0.27	0.41	99	0.95
Dark-capped Bulbul	1.67	44	0.11	1.07	55	0.40	3.95	55	**< 0.001**
Red-winged Starling	3.31	88	**< 0.001**	9.90	88	**< 0.001**	10.10	88	**< 0.001**
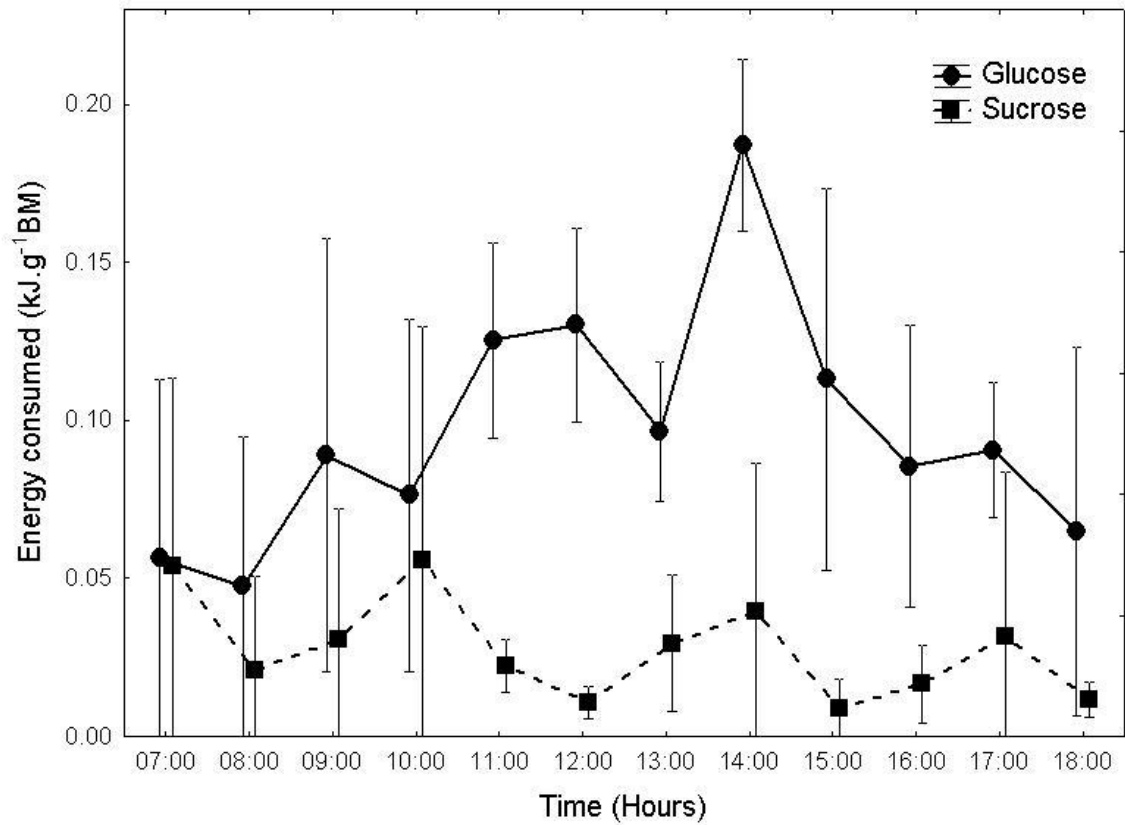
Figure 1: Sugar preferences observed for Dark-capped Bulbuls fed a medium energy (2350.5 kJ/l) glucose and sucrose diet (n = 5). Values are energy consumed per gram body mass (mean ± SE).
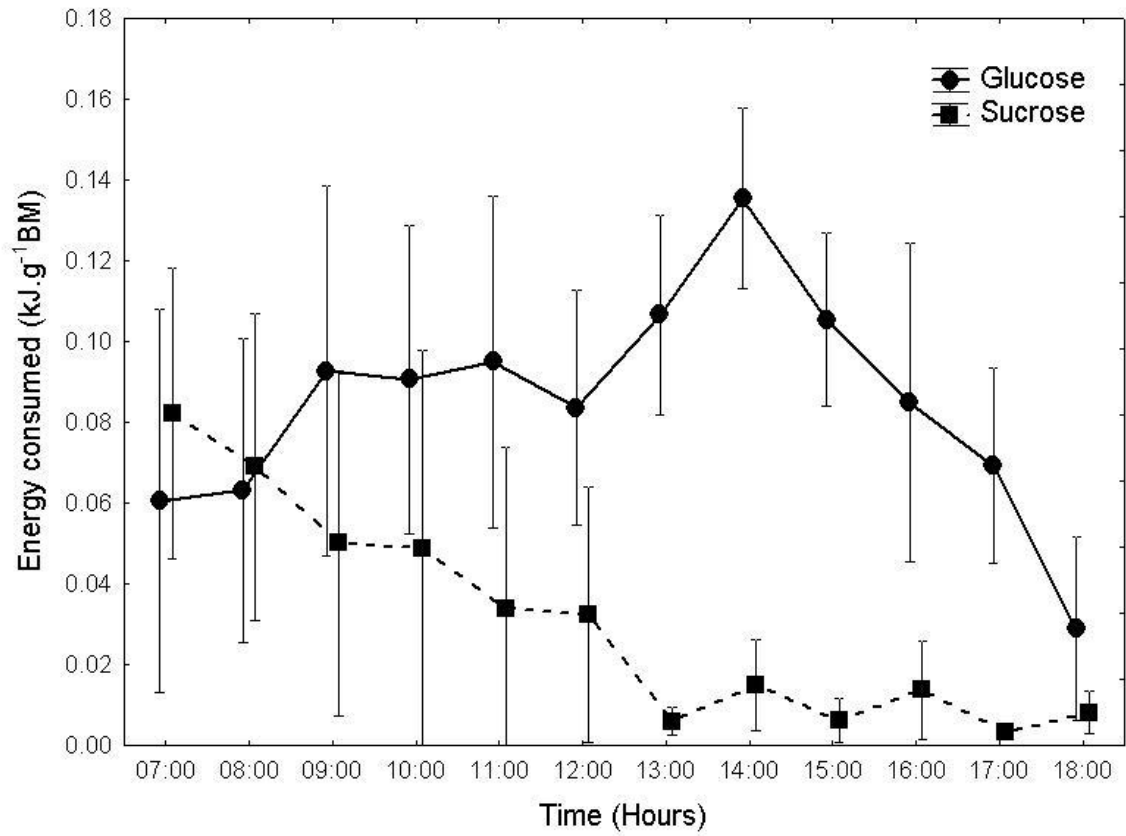
Figure 2: Sugar preferences observed for Red-winged Starlings fed a low energy (1175.25 kJ/l) glucose and sucrose diet (n = 9). Values are energy consumed per gram body mass (mean ± SE).
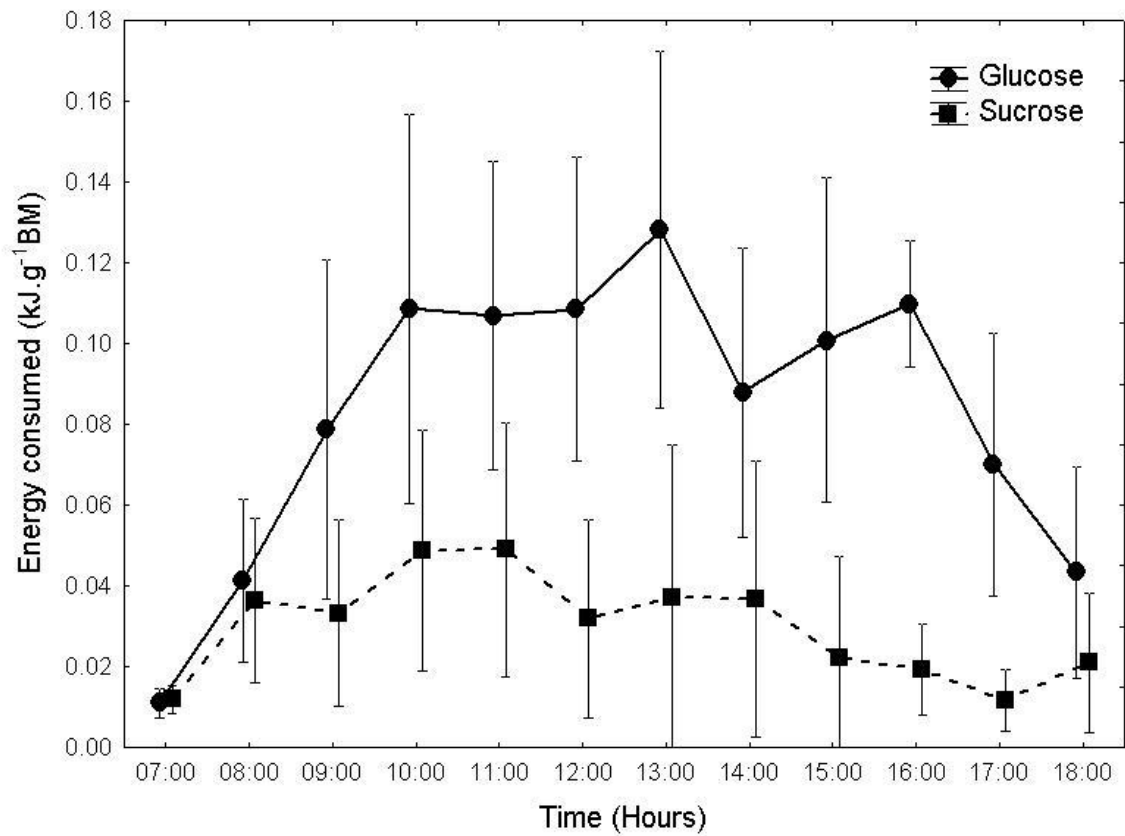
Figure 3: Sugar preferences observed for Red-winged Starlings fed a medium energy (2350.5 kJ/l) glucose and sucrose diet (n = 9). Values are energy consumed per gram body ass (mean ± SE).
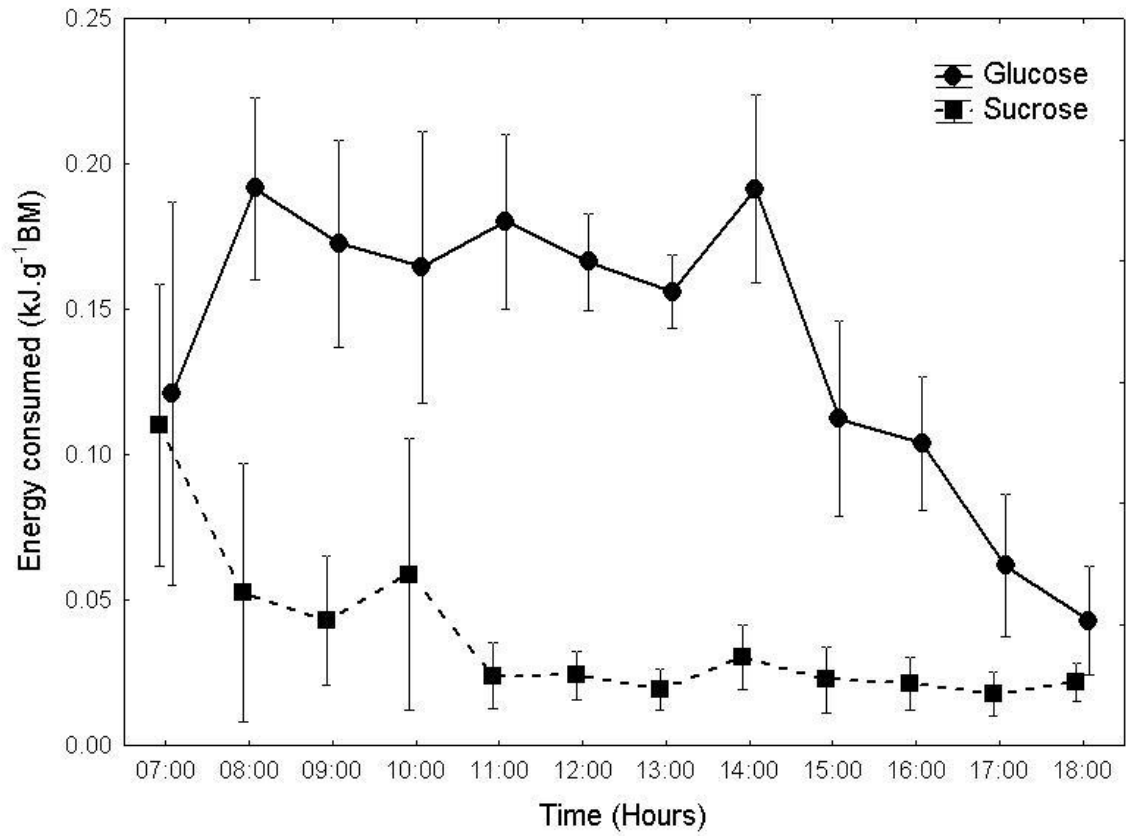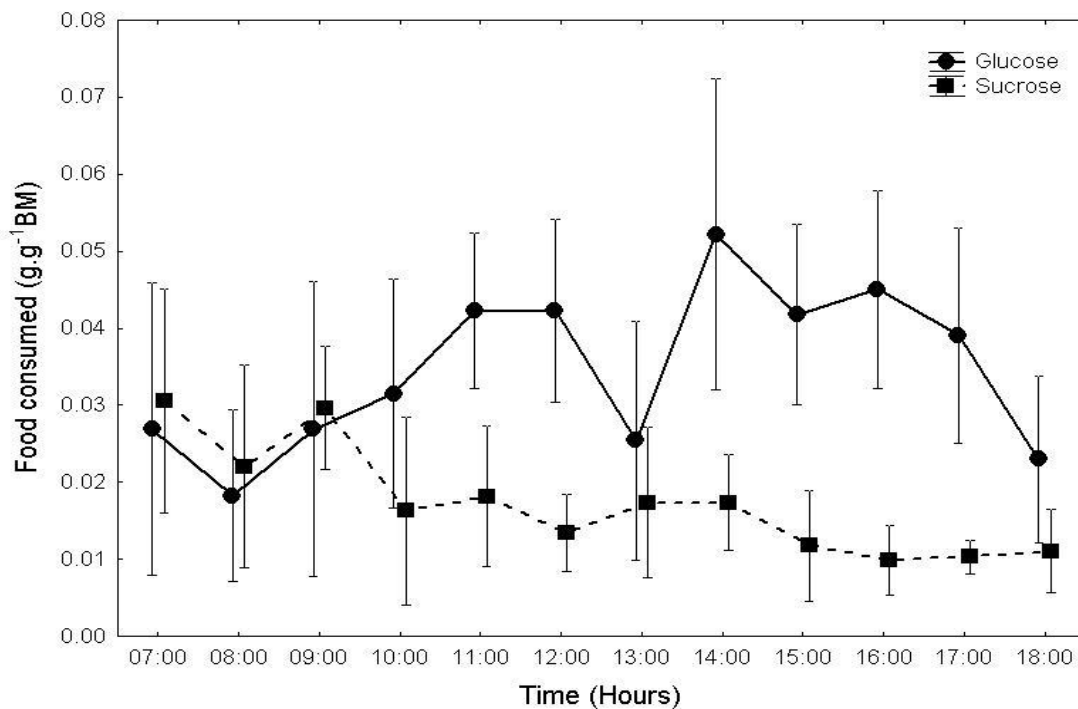
Figure 4: Sugar preferences observed for Red-winged Starlings fed a high energy (4701 kJ/l) glucose and sucrose diet (n = 9). Values are energy consumed per gram body mass (mean ± SE).
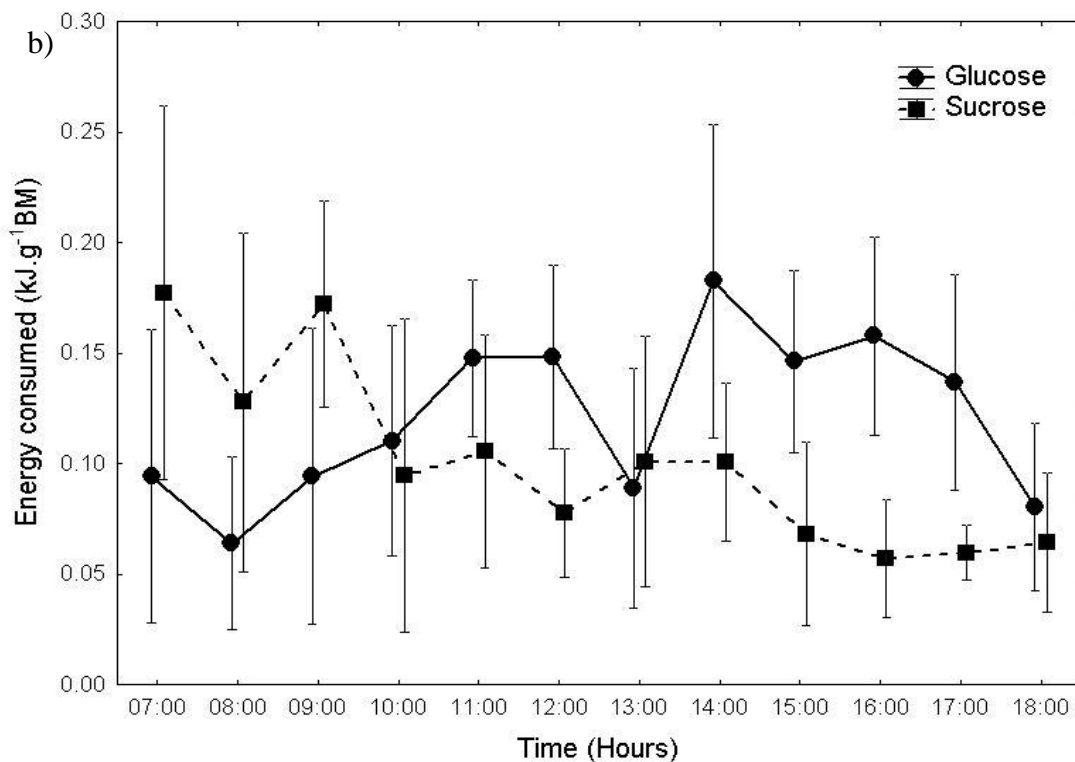
a)

b)

Figure 5: Sugar preferences observed for Dark-capped Bulbuls fed a high equimolar (1.66 mol/l) glucose and sucrose diet (n = 6). a) Values are mass consumed per gram body mass (mean ± SE). b) Values are energy consumed per gram body mass (mean ± SE).
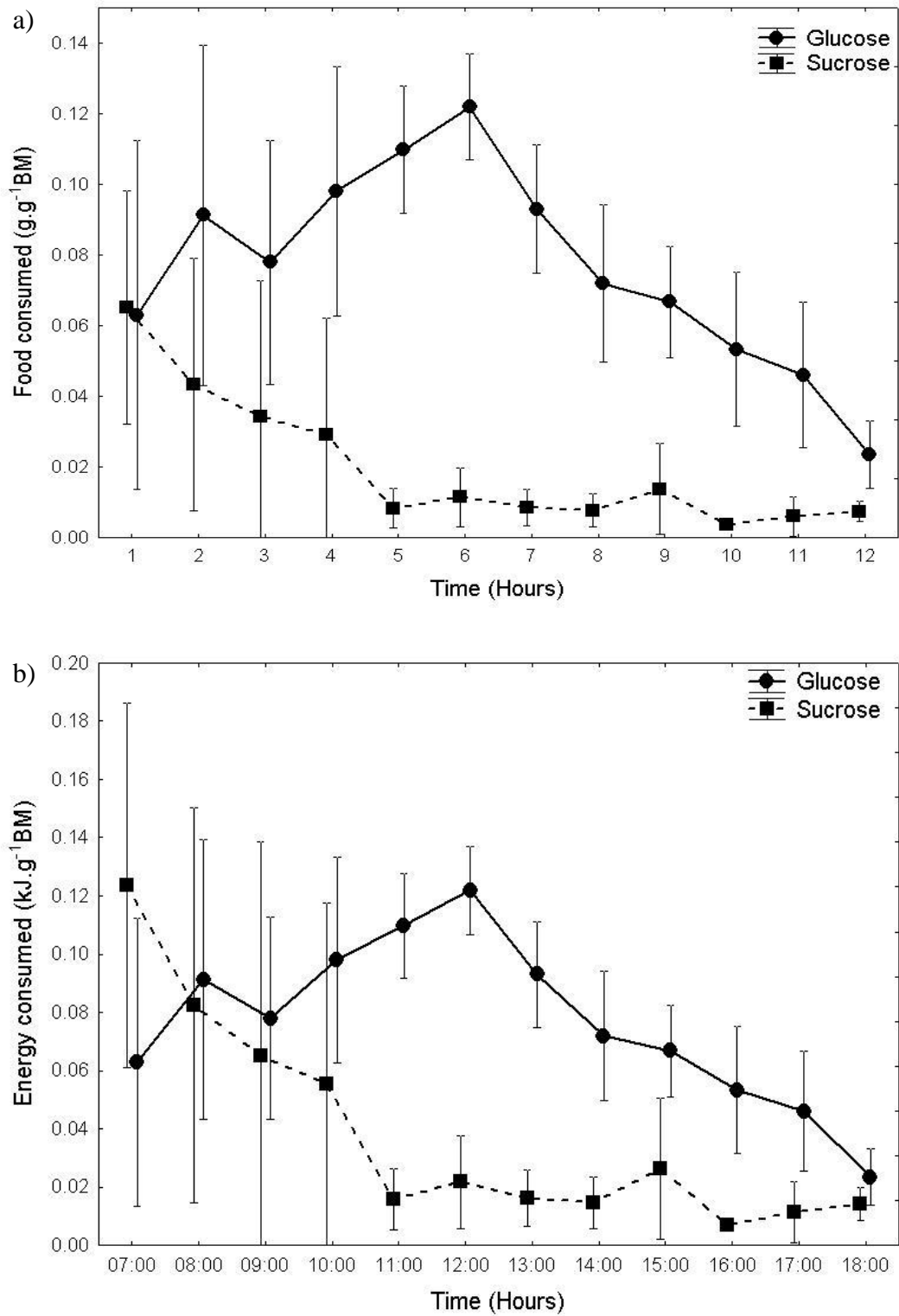
Figure 6: Sugar preferences observed for Red-winged Starlings fed a low equimolar (0.42 mol/l) glucose and sucrose diet (n = 9). a) Values are mass consumed per gram

body mass (mean ± SE). b) Values are energy consumed per gram body mass (mean ± SE).

Figure 7: Sugar preferences observed for Red-winged Starlings fed a medium equimolar (0.83 mol/l) glucose and sucrose diet (n = 9). a) Values are mass consumed per gram body mass (mean ± SE). b) Values are energy consumed per gram body mass (mean ± SE).

Figure 8: Sugar preferences observed for Red-winged Starlings fed a high equimolar

(1.66 mol/l) glucose and sucrose diet (n = 9). a) Values are mass consumed per

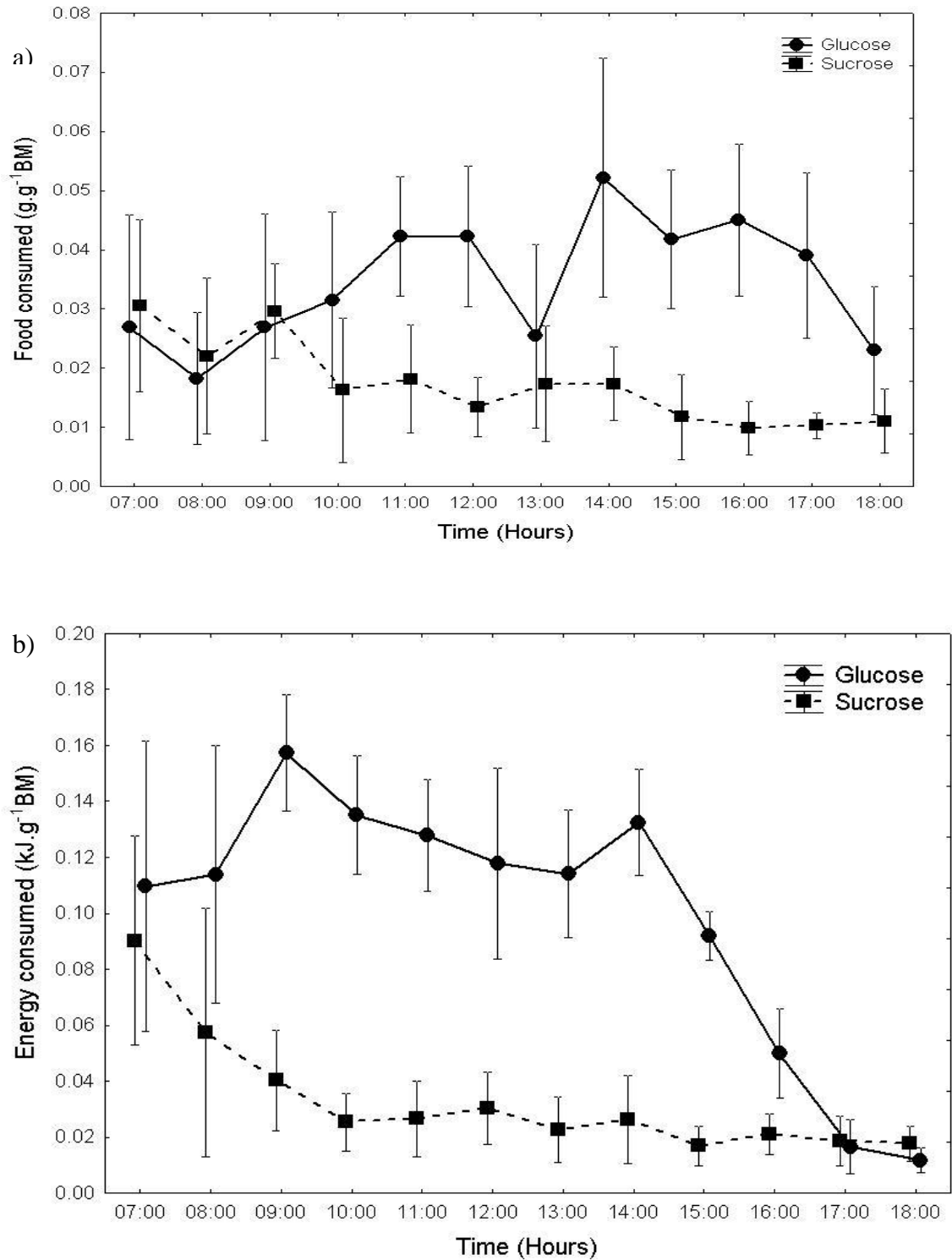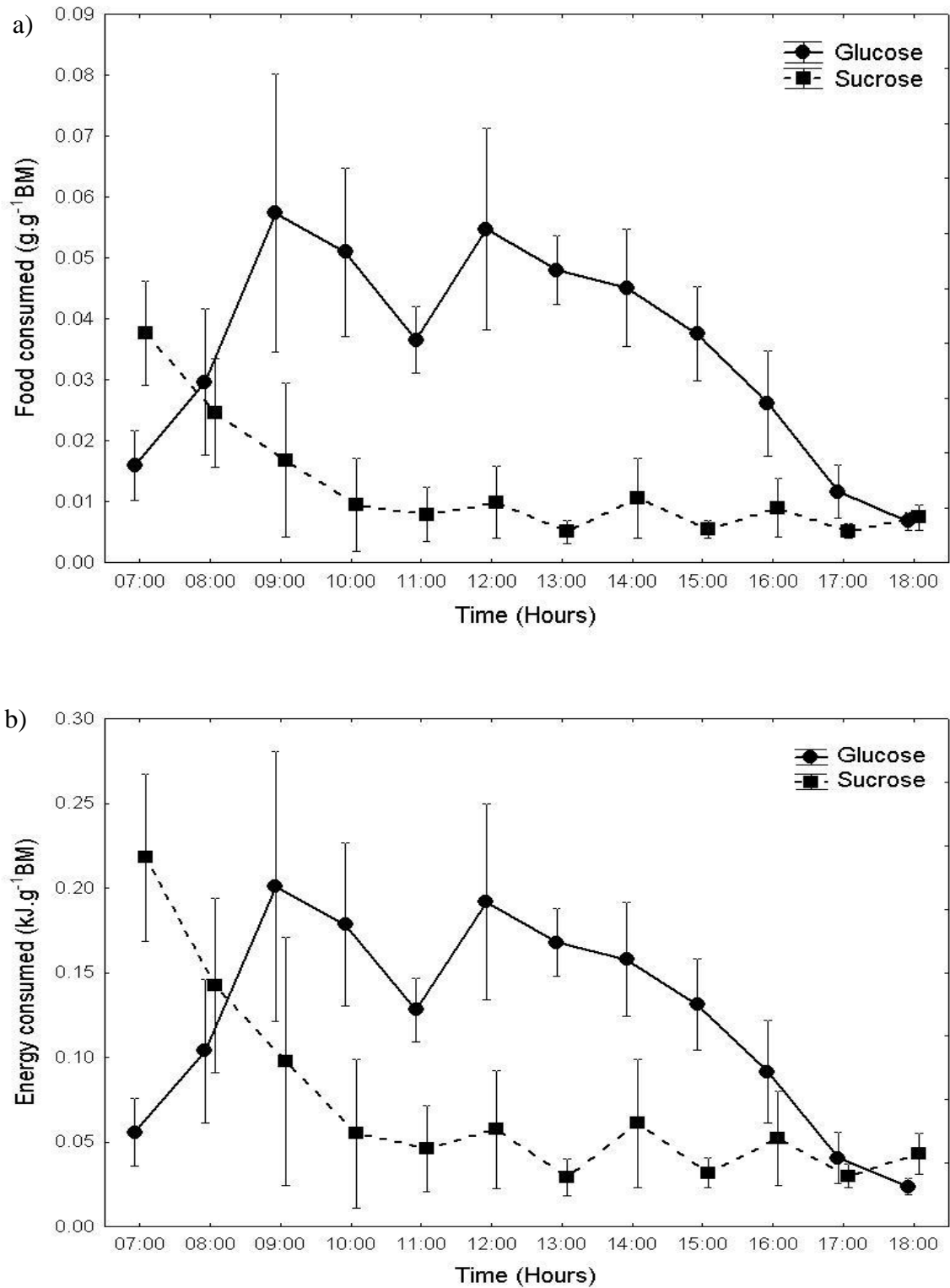grambody mass (mean ± SE). b) Values are energy consumed per gram body mass

(mean ± SE).

Chapter 4

Use of colour in fruit to attract avian dispersers

(formatted for *Oecologia*)

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Abstract

It is widely accepted that red-coloured and black-coloured fruits are targeted over other colours by avian frugivores. We tested if this trend exists in two South African species Red-winged Starlings (*Onychognathus morio*) and Speckled Mouse-bird (*Colius striatus*). We also looked at instinctual versus learned preferences for fruit colours. Individuals were fed a neutral colour diet for ten days before experiments to ensure that birds chose fruits based on instinct as opposed to experience. Colour preference experiments exposed individuals to six colours of nutritionally identical artificial fruits for 3h a day over three consecutive days. Neither species showed a preference for red or black coloured fruits but both showed some signs of learning. We concluded that non-specialist/facultative frugivore species may show lower fruit colour preference than specialist frugivores. Instinctual colour

preferences may play an initial role in fruit selection but individuals will likely alter colour preferences as they learn about target fruits.

Key words: colour preference, frugivores, fruit preference, artificial fruit, learned verses instinctual behaviour

Introduction

First and foremost, preferential fruit selection in birds relies on birds finding said fruits. Fruits must therefore advertise to birds to initiate feeding and consequently seed dispersal (Knight and Siegfried 1983; Schaefer et al. 2008; Burns et al. 2009). One could therefore argue that the more effectively a fruit can advertise with its dispersal agent, the better its chances of reproductive success (Herrera 1998; Cazetta et al. 2009).

Various studies have investigated the channels used by plants to effectively enhance seed dispersal. Most plausible of the mechanisms resulting in more efficient fruit encounter rates by avian frugivores are related to fruit detectability (Knight and Siegfried 1983; Gautier-Hion et al. 1985; Giles and Lill 1999; Cazetta et al. 2009). Detectability is enhanced by variation in fruit colour (Gautier-Hion et al. 1985; Willson and Whelan 1990; Traveset and Willson 1998; Gamberale- Stille and Tullberg 2001; Werner et al. 2007; Burns et al. 2009) and increased fruit contrast/conspicuousness against a background colour (Knight and Siegfried 1983; Willson and Whelan 1990; Giles and Lill 1999; Burns 2005; Cazetta et al. 2009).

Optimal foraging theory suggests that fruit size and frequency play important roles in fruit conspicuousness and therefore selection (MacArthur and Pianka 1966; Pyke et al. 1977; Carlo and Morales 2008). In addition to this, fruit protection and accessibility are also considered fruit selection influencing factors (Gautier-Hion et al. 1985; Fischer and Chapman 1993). Generally, birds preferentially select some fruits over others in the same patch but observed patterns fail to highlight a general explanation as to why fruit selection is so biased (Gautier-Hion et al. 1985; Carlo and Morales 2008; Skelhorn et al. 2008).

Current levels of understanding of fruit selection in avian frugivores suggest that black and red fruits are most often preferred over other colours (Knight and Siegfried 1983; Willson and Whelan 1990; Burns 2005). Higher prevalence of red and black fruits in the wild (e.g. in South Africa Fig. 1) and trends of frugivorous feeding habits tend to support this theory (Fischer and Chapman 1993). In terms of ontogeny, avian frugivore preference for these colours may be learned or instinctual (Willson and Whelan 1990; Gamberale-Stille and Tullberg 2001; Schmidt and Schaefer 2004; Stephens et al. 2007; Werner et al. 2007) (see Chapter 1 *Learning).* Studies observing new born chick behaviour have found that colour preference may be inherited and displayed as instinctual behaviour but findings are inconsistent (Gamberale-Stille and Tullberg 2001; Schmidt and Schaefer 2004; Skelhorn et al. 2008). How much emphasis can be placed on inherited behaviour? Can it account for all frugivore fruit selection decisions or are these instinctual colour preferences coupled with learning and experience to create a more effective and efficient frugivore? If so, how does one test this?

Since no two fruits are identical, each should illicit a different 'best response' from an avian frugivore (i.e. the best response when faced with a low energy fruit that

is high in secondary compounds and well defended should be to leave it and move to the next fruit) (Stephens et al. 2007). The 'best response' is a compromise that is made by an individual to achieve the highest payoff and lowest risk depending on the state of the individual and its environment (Stephens et al. 2007). The 'best response' also depends on whether the forager is well informed or uninformed, a well informed forager will base selective preferences on experience and learned rewards while an uninformed forager is expected to display an averaged or instinctive response (Willson and Whelan 1990; Schmidt and Schaefer 2004; Stephens et al. 2007).

Apart from the obvious conspicuousness, to enhance encounter rates between fruits and their dispersal agents, many studies have tried to find more specific relationships between fruit colouration and feeding habits of seed dispersers (Giles and Lill 1999; Gamberale-Stille and Tullberg 2001; Burns 2005; Werner et al. 2007). After all, if avian frugivores only targeted red and black fruits, surely other colours of bird eaten fruits would not exist (Carlo and Morales 2008). Since other colours do exist and are evidently fed on by birds (Burns 2005), there should be more to the relationship. Fischer and Chapman (1993) suggested that fruits are categorized according to dispersal syndromes in which highly frugivorous, partly frugivorous, generalist and specialist frugivores target different colours although this has been criticized (Burns et al. 2009).

Studies have also tried to correlate fruit colouration with nutritional value and secondary compounds with limited success (Werner et al. 2007, Schaefer et al. 2008). The most reliable trend observed thus far is that fruit colour changes to indicate ripeness (Herrera 1982; Willson and Whelan 1990; Schmidt and Schaefer 2004; Schaefer et al. 2008). If this is indeed the primary function of chromatic variation in fruits, then it appears that it is the plants that are in control, dictating to birds, the most

appropriate time to disperse their seeds. Preferential selection of specific colours of fruits would be negligible in such a scenario as avian frugivores target only fruits that bearing plants 'intended' them to select.

Fruit colour variation may have a number of functions and it is important that this is considered and colour signals interpreted in the correct context (Gamberale-Stille and Tullberg 2001; Endler and Day 2006) without assuming that fruit colour variations exist purely to attract dispersal agents (Willson and Whelan 1990). For instance, Burns et al. (2009) suggest that fruit colours are evolutionarily related to leaf reflectance properties and not solely to disperser selection.

Evolutionarily speaking, a relationship between fruit colour and selective preference by dispersers would be evident if a fruit bearing plants seeds were dispersed primarily by frugivorous birds (Thompson and Willson 1979; Peres and van Roosmalen 1996). It has been argued that avian frugivores are so taxonomically diverse that the resulting selective process would not have an influence on fruit colours and therefore on fruit colour/frugivore selection patterns (Willson and Whelan 1990; Schmidt and Schaefer 2004; Burns et al. 2009). There is however, some evidence supporting the relationship, red and black fleshy fruits commonly targeted by avian frugivores are usually smaller and found on the end of long thin branches, an adaptation that is said to benefit avian dispersal (Thompson and Willson 1979; Knight and Siegfried 1983; Gautier-Hion et al. 1985; Fischer and Chapman 1993).

This study investigated instinctive and learned colour preferences in two frugivorous species (Red-winged Starlings *Onychognathus morio* and Speckled Mouse-birds *Colius striatus* (Hockey et al. 2005)) occurring in KwaZulu-Natal, South Africa. We tested instinctive colour preferences by observing colour selection after a

period of unlearning (in which individuals were fed a diet of unstained artificial fruits). Frugivorous birds have the ability to learn and alter colour preferences and preferences for novel food items in the space of 2-6 days (Turdidae) and in some cases (Sturnidae), a few hours (Willson and Whelan 1990; Avery et al. 1995). We will test the learning ability of frugivorous birds by observing changes in colour preferences of nutritionally identical fruits over a period of time.

We predicted that avian frugivores should show an instinctual preference for black and red coloured fruits and an aversion to green fruits. We also predicted that individuals will learn that fruits of differing colour are nutritionally identical and the preferences for colours will not influence fruit selection by the end of the experiment.

Materials and methods

Study animals

Six-ten adult individuals of each species were mist-netted in KwaZulu-Natal, between July 2007 and February 2008 (Waterfall (29°44'56.64"S 30°48'49.68"E), Pietermaritzburg (29°37'31.88"S 30°24'5.22"E), Darvill (29°35'50.92"S 30°26'16.78"E) and Hilton College (29°29'32.12"S 30°18'6.86"E) under permit from Ezemvelo KZN Wildlife. Bird species were housed in separate outdoor aviaries at University of KwaZulu-Natal, Pietermaritzburg (South Africa). During this time all birds were fed a standard maintenance diet consisting of a mixture of apple, pear, banana, grape, pawpaw and orange (additional spinach and broccoli for mousebirds). Individuals were then moved indoors and given ten days to acclimate to full laboratory conditions before experimentation (12:12 photoperiod, temperature controlled at 25°C ±1°C). During the acclimation period, all birds were housed

individually in wire cages (50 x 50 x 50cm) and fed a standard unstained medium concentration glucose artificial fruit diet *ad libitum* (Witmer 1998). Water was provided *ad libitum* throughout.

Colour preference trials

Colour preference experiments consisted of placing study animals into a situation where they would be able to select fruits of identical nutritional value but different colours. Records of bird eaten fruits occurring in KwaZulu-Natal and their respective colours were obtained from Pooley (1993). Fruits were categorized according to their externally visible primary and secondary colours displayed when ripe and 6 prevalent colours were selected for colour preference experiments. Preferences for specific fruit colours would be inferred from how much of each colour offered was eaten.

Previous work has highlighted the primary methodological flaws in multiple choice feeding preference experiments as a lack of independence of simultaneously offered food choices and variation in food choice proportions caused by uneven consumption (Roa 1992). Recent work has, however noted that certain hypotheses are only able to be addressed by means of multiple choice feeding preference experiments (Larrinaga 2010). Independence of simultaneously offered food samples for example cannot be avoided by testing each food individually as this will not be useful in determining preferences (Larrinaga 2010). Larrinaga (2010) suggests that statistical violations in multiple choice feeding preference experiments can be negated if variation in food proportions over the duration of the experiment are too small to be noticed. This can be achieved by reducing the duration of the experiment and/or increasing the amount of each food type. The statistical power of the experiment can

be increased further by increasing sample size and repeating the experiments (Larrinaga 2010).

To accomplish this, individually housed birds of each species were exposed to three days of colour choice experiments post-acclimation as described above. All stained artificial fruits were made using an agar formula (Witmer and Soest 1998). Agar fruits contained a standardized amount of energy and were made up as a medium concentration (2350 kJ/l) glucose solution (Appendix 1) to which food colouring (Robertsons, Unilever, SA) was applied before setting. Food colouring was mixed with artificial fruits to ensure that the desired final colour was even throughout the final set artificial fruits. Colours were classified according to the PANTONE ® Colour Formula Guide (Swatch 747XR, USA).

Unstained maintenance diets were removed from bird cages at 18:00 (beginning of the dark cycle) the day before experiments. A white food tray containing randomly arranged 30 x 20 x 10 mm weighed cubes of red, orange, yellow, green, purple and black stained artificial fruits (Table 1) was placed into each cage at 06:00 (beginning of the light cycle) on each experiment day. A control tray was also placed into the room to account for evaporative water loss. All trays were removed from the cages at 09:00 (including the control) and each colour of artificial fruit was weighed again to determine the total amount eaten by each individual bird. Due to the short duration of experiments, data for total amount eaten by each bird for each repetition (not mass specific) corrected for evaporative water loss only were analysed. Unstained agar maintenance diet resumed from 09:00 till 18:00 on experiment days. The protocol was repeated over the next two days.

Statistical Analyses

General linear models (GLM) Repeated measures analyses of variation (RMANOVA) were used to analyze differences between amounts of specific colours of fruits eaten, experiment days and a combination of the two. Post hoc Tukey HSD tests were used to identify lower level differences in colour selection and differences between days. All analyses were conducted using Statistica (Version 7, Statsoft, Tulsa, OK, USA).

Results

Percentage prevalence of primary (ripe) fruit colours indicated that red and black fruits accounted for nearly half of all fruiting species consumed by avian frugivores (Fig. 1). Brown fruits were in third representing the fruits of around 17.5% of all target fruiting tree species (Fig. 1). Brown was left out of the experimental design to prevent bias as the unstained maintenance diet fed to birds prior to and during the experiment was brown in appearance. Thus around 80% of fruiting colours were represented in the employed experimental design.

Red-winged Starlings showed no significant preference for any fruit colours based on amount eaten (RMANOVA, $F_{(5, 40)} = 1.50$, $p = 0.21$) nor significant differences between experiment days (RMANOVA, $F_{(2, 16)} = 1.09$, $p = 0.36$). A significant difference was however, observed when colours and days were combined (RMANOVA, $F_{(10, 80)} = 2.61$, $p = 0.009$) (Fig. 2). Post hoc Tukey analyses revealed a significant preference for purple fruits over yellow ($p = 0.031$) and green ones ($p = 0.03$) on day one (Fig. 2). When it came to variation in colour preferences over time, post hoc analyses also showed that preference for purple fruits declined after the first

day and significantly declined by the third day (p = 0.038) (Fig. 2). It is worth noting that green fruits were not selected over the first two days (significantly lower than purple on day one with low variation) (Post hoc Tukey, p = 0.031 and p = 0.033 for green 1 and 2 verses purple 1 respectively). No significant patterns were observed for colour selection in red, orange and black fruits which all displayed a high amount of variation between individuals (Fig. 2).

Speckled Mousebirds displayed significant preferences for fruit colour based on amount eaten (RMANOVA, $F_{(5, 45)}$ = 13.28, p < 0.001) (Fig. 3) but no significance was found when looking at the effect of days (RMANOVA, $F_{(2,18)}$ = 1.86, p = 0.18) nor the combination of colour and days (RMANOVA, $F_{(10,90)}$ = 1.23, p = 0.28). Post hoc analyses on colour preferences showed that Speckled Mousebirds selected orange and yellow fruits significantly over red fruits (RMANOVA, $F_{(5, 45)}$ = 13.28, p < 0.001, post hoc Tukey, p = 0.006 and p = 0.002 respectively), green fruits (p = 0.001 and p < 0.001 respectively) purple fruits (p < 0.001 for both) and black fruits (p < 0.001 for both) (Fig. 3).

Discussion

The widely accepted norm of red and black fruits being favoured over other colours by frugivorous birds (Knight and Siegfried 1983; Willson and Whelan 1990; Willson 1994; Burns 2005) was not supported. When Red-winged Starlings and Speckled Mousebirds were given equal access to six different colours of artificial fruit (of equal nutritional value) neither of the species showed a significant preference for black and/or red fruits. Although Red-winged Starlings are described as frugivores, they opportunistically fed on insects, nectar and even lizards (summarised in Hockey

et al. 2005). Speckled Mousebirds primarily eat fruit but also consume leaves, seeds, nectar and occasionally insects (Downs et al. 2000; Symes and Downs 2001). Perhaps obligate frugivores (diets comprised of over 90% fruits) would display clearer preference for red and black fruits than facultative frugivores (Herrera 1995, 1998).

Green fruits were avoided initially by Red-winged Starlings and although Speckled Mousebirds did eat some green fruits, they were eaten less frequently than preferred colours (orange and yellow). Thus perceptions that avian frugivores tend to avoid green fruits (Willson and Whelan 1990; Avery et al. 1995) were supported.

Another theory that was contested by these results is that of red and black coloured fruits facilitating learning in uninformed birds (Willson and Whelan 1990). If the pre-experimental feeding regime (unstained artificial fruits for 10 days) had effectively zeroed study animal colour preferences, we would have expected Red-winged Starlings and Speckled Mousebirds to show a preference (or at least an initial preference) for red and/or black coloured fruits, this was not the case. It appears that uninformed bird colour selection behaviour was closer to an averaged response predicted by Stephens *et al.* (2007) judging from the high level of variation in colour preference displayed.

Changes in colour preferences over time were analysed to investigate the learning ability of Red-winged Starlings and Speckled Mousebirds (as mentioned - a change in colour preference over time representing a learned feeding response to that specific colour). European Starlings (*Sturnus vulgaris*) have demonstrated an ability to associate fruit colours with nutritional content of fruits over a period of three hours in a previous study (Avery et al. 1995). Our experiments exposing uninformed Red-winged Starlings to nutritionally identical coloured fruits for 3 h a day for three

consecutive days showed that Red-winged Starlings did indeed alter colour preferences over the duration of the experiment. Changes in colour preference over time were, however, limited to just two colours (purple and yellow). Other colours (apart from green) were fed on with a high degree of individual variation. This could be indicative of a learning process which involves uninformed individuals assessing a limited number of fruits at a time all the while building up associations between colours and post ingestional feedback. Allowing a similar experiment to progress for a longer duration may verify this.

Speckled Mousebirds, on the other hand, showed no evidence of learning. Differing groups of birds display different learning rates (Patel et al. 1997). A pattern similar to those displayed by Red-winged Starlings may develop for Speckled Mousebirds over a different time scale, three hours a day for three days was too short a duration to assess Speckled Mousebird learning abilities.

In conclusion, non-specialist/facultative frugivore species may show less fruit colour preference than specialist frugivores. Instinctual colour preferences play an initial role in fruit selection but individuals will likely alter colour preferences as they learn about target fruits via postingestional feedback mechanisms and previous experience. This study has contributed to avian fruit preference knowledge by providing evidence that birds do indeed select fruits based on instinctual and learned behaviour. In short, an instinctual avoidance of green fruits is followed by a duration of averaged response to other fruit colours to facilitate learning. This is by no means an end to the extent of our understanding, further studies on avian eye structure and ultraviolet fruit colour emissions bring this element of fruit selection into the picture suggesting that avian frugivores may be better at identifying target fruits than other non avian dispersers and fruit predators (Willson and Whelan 1990; Jones et al. 2007;

Schaefer et al. 2008; Burns et al. 2009; Cazetta et al. 2009). Unfortunately this is exceedingly difficult to test as the spectrum of light visible to human eyes excludes ultraviolet. Including ultra violet properties of fruits in future experiments may provide further insight into avian frugivore foraging ecology.

Acknowledgements

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Figure 2: Amount of artificial fruits of each colour type eaten by Red-winged Starlings on three experimental days (Mean ± SE) (n = 9 for each experiment).

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Appendix 1: Ingredients used to make 2350 kJ/l artificial fruits (modified from Witmer and Soest 1998).

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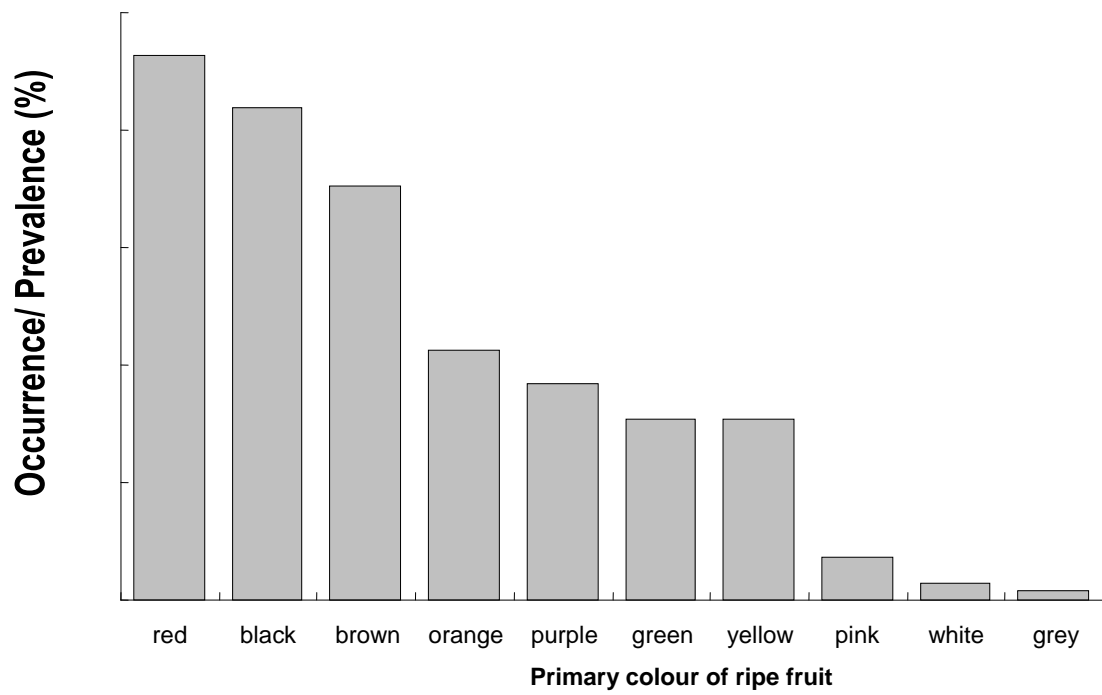| Colour	Parts food colouring (1ml dye per 100ml fruit)	Colour code
Red	7 red	193 U
Orange	1 red, 3 yellow	145 U
Yellow	4 yellow	117 U
Green	1 green	362 U
Purple	1 pink, 1 blue	4985 U
Black	5 red, 5 yellow, 5 green, 5 blue	Process Black C

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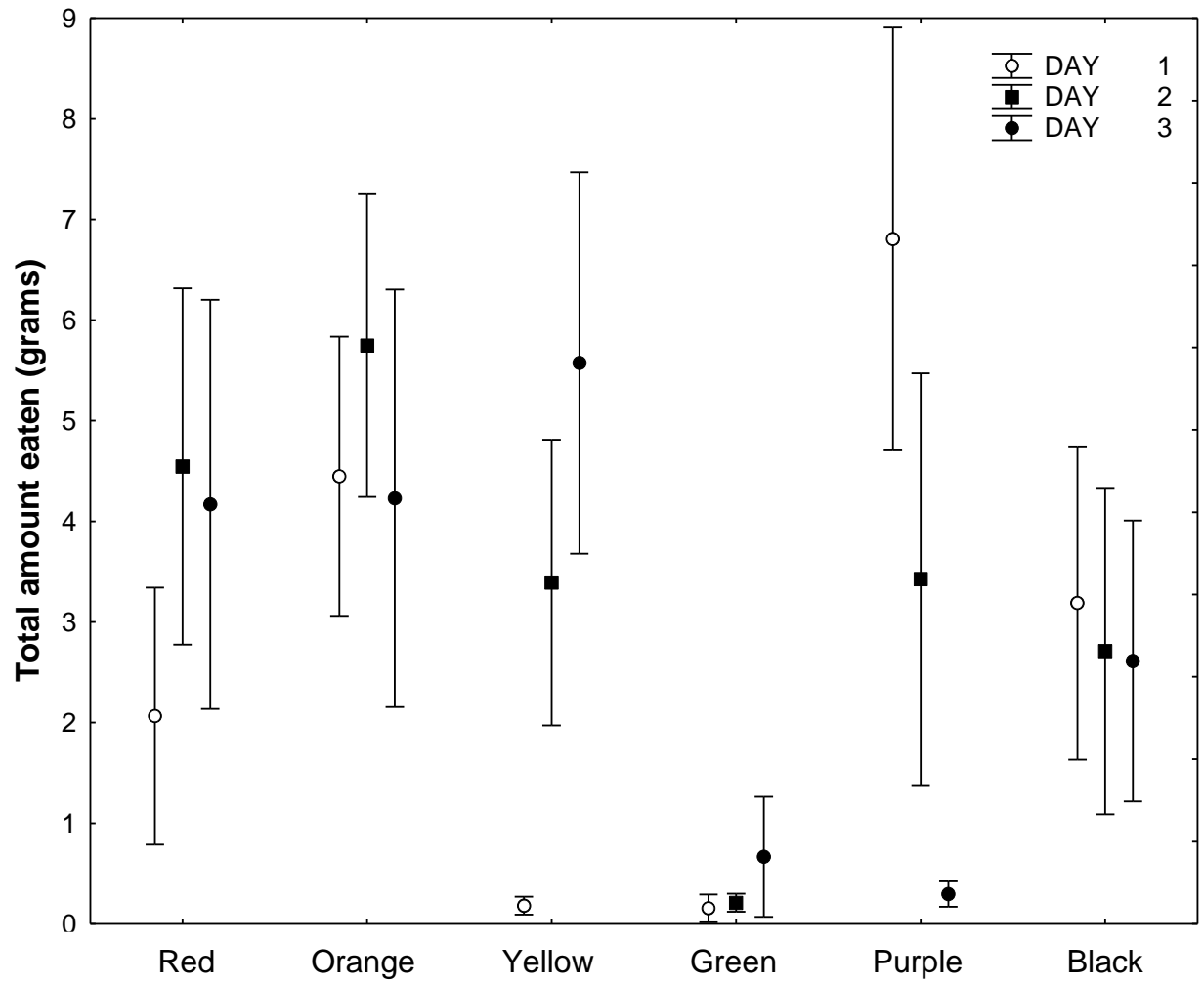
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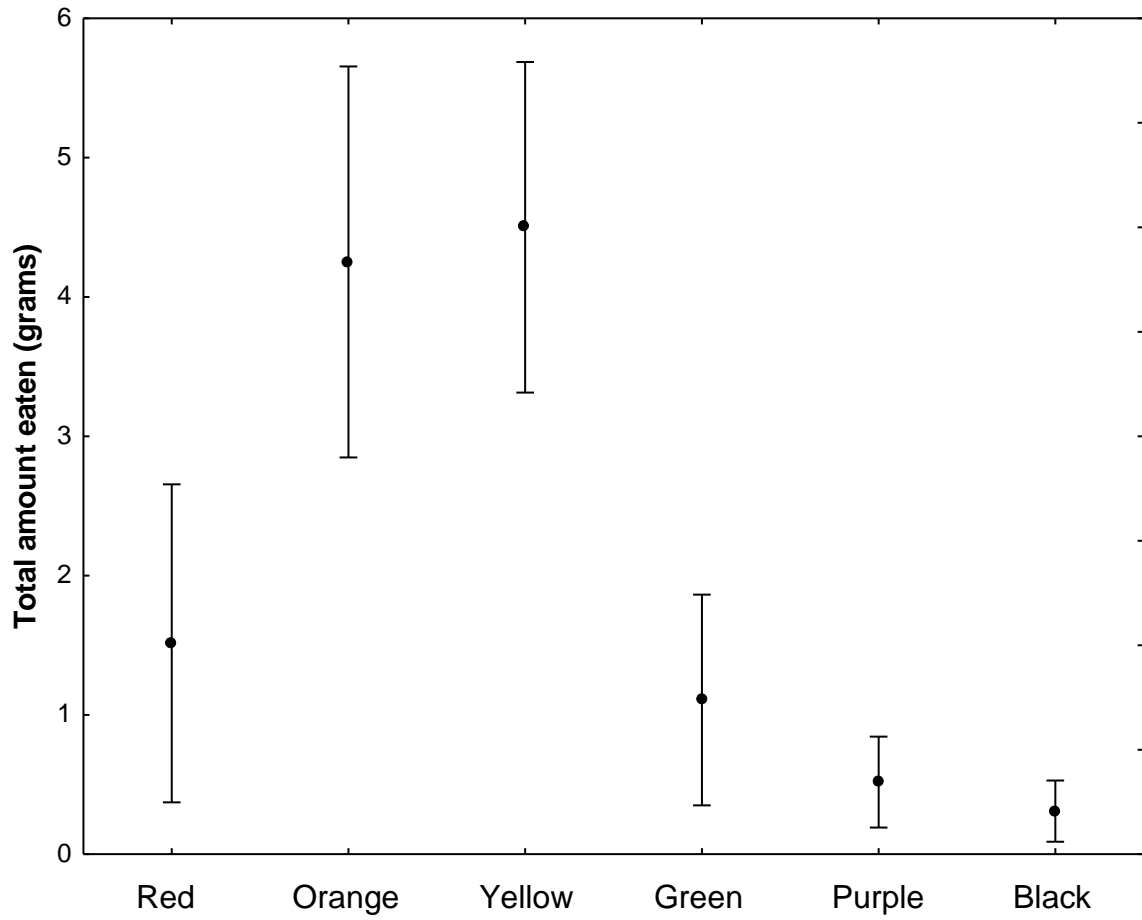
Figure 3: Artificial fruit colour selection in Speckled Mousebirds (mean ± SE) (n = 10 for each experiment).

Appendix 1. Ingredients used to make 2350 kJ/l artificial fruits (modified from Witmer and Soest 1998).

Ingredients (all values in grams)	
Anhydrous Glucose	150
Distilled Water	1000
Wheat Bran	50
Agar	10
Sodium Chloride	0.75
Dicalcium Phosphate	0.8
Vitamin supplement*	0.75

*AviPlus (Hillcrest. South Africa)

Chapter 5

Does size matter? Can beak size be used to predict fruit selection in avian frugivores?

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Abstract

Avian frugivores are responsible for the distribution of a large percentage of seeds from fruiting plants. Our understanding of mechanisms underlying choices made by birds is limited. This study investigated if preferences based on fruit size exist in frugivorous birds as a result of beak morphology. Interactions between three abundant South African frugivores (Red-winged Starlings, *Onychognathus morio*; Speckled Mousebirds, *Colius striatus*; Dark-capped Bulbuls, *Pycnonotus tricolor*) and amount of controlled artificial fruits consumed (divided into three size classes) were observed under laboratory conditions. Beak measurements (maxilla to forehead (culmen length), maxilla tip to gape and maximum width at gape) were also taken to investigate the effects of beak size on fruit size selection. Each study species had unique beak measurements and fruit size preferences. Red-winged Starlings show alternative feeding dependent on the size of fruit on offer. Dark-capped Bulbuls displayed no significant preferences for any fruit size classes, likely a result of their squashing/mashing fruit eating behaviour. Speckled Mousebirds, well known for their foot/beak coordinated feeding behaviour, displayed their true generalist nature by not favouring any size of the artificial fruits on offer. This study clarifies the relationship between beak morphology and food selection in avian frugivores. Beak morphology alone cannot be used to predict fruit selection in avian frugivores. Feeding behaviour also plays a large role in determining what size of fruit birds will select to feed on.

Key words: Beak morphology, fruit size preference, feeding behaviour, artificial fruits

Introduction

Fruit features (colour, size, nutrient content etc.) are thought to have co-evolved with dispersal agent features (Herrera 1982). Brown and Hopkins (2002) thus make the assumption that fruiting plants have restricted sets of seed dispersers. Along with the information that beak morphology is often adapted to successful acquisition of food resources in an area (Clayton *et al.* 2005), a relationship between fruit size and beak morphology of birds that disperse their seeds should be apparent. Yet the theoretical framework supporting the interactions between frugivores and the morphological traits of fruits consumed by them are limited (Tewksbury 2002). Fuentes (1994) found a correlation between fruit size and avian frugivores selective preferences but only went so far as to say that birds prefer fruits that they can swallow whole to maximize energy intake and minimize fruit handling time thus predicting that avian frugivores will prefer fruits that are narrower than their gape widths. A study by Johnson *et al.* (1985) on fruit consumption by migratory birds in North America found that fruit size had no effect on fruit selection. It was also noticed that areas that had few specialist frugivores were rich or poor in fruit abundance depending on the season (Morton 1973). It has consequently been argued that taking advantage of seasonal resources such as fruit would only pay off if switching diet was achieved at relatively low costs by frugivores. If morphological adaptations were required, fewer birds would be able to exploit seasonal fruit (Levey and Grajal 1991).

Individual preferences for specific fruit attributes are important as seeds of a preferred fruiting plant have greater probability of being dispersed than a plant that is not preferred (Herrera 1998). Generally it appears that bird preferences for fruits are

not random but there is a limited understanding of the mechanisms underlying choices made on what to eat and why these choices are made by birds (Levey and Martinez del Rio 2001). Herrera (1982) suggested that if viewed from an evolutionary perspective, dispersers select fruits based on the avoidance of harmful chemicals, maximization of energy intake and achievement of a balanced diet (Herrera 1982). However from a behavioural point of view fruit choice in birds is based on fruit size, fruit structure, fruit availability and bird feeding behaviour (Brown and Hopkins 2002).

All fruit eating birds can be classified into one of four categories for feeding behaviour (Levey 1987; Brown and Hopkins 2002). Swallowers/gulpers pluck fruit and swallow them whole, squashers/mashers crush fruits in their beaks, thrashers break fruit into pieces before swallowing and foot/beak coordinators manipulate fruits with their feet while tearing off pieces with their beaks (Levey 1987; Symes and Downs 2001; Brown and Hopkins 2002). To date most studies addressing beak morphology have involved granivore adaptations towards seed diets (summarised in Herrel *et al.* 2010) these have generally suggested that bill-shape is a product of selective forces based on the primary food source available to target species. Little work has been done on the importance of beak morphology in the feeding behaviour of avian frugivores. A predictive model could well be generated for avian frugivores if beak morphology is adapted towards fruit selection in a similar way granivores are adapted towards certain types of seeds. In addition to this, Levey and Grajal (1991) specify that successful fruit consumption also depends on the correct physiological adaptations by dispersers. A combination of morphological, behavioural and physiological adaptations will likely contribute to the understanding of why so many

bird species rarely or never consume fruit and why avian frugivores limit fruit consumption to such a narrow range (Levey and Martinez del Rio 2001).

In KwaZulu Natal (South Africa) there are 233 species of fruiting plants that are known to be fed on by birds (Pooley 1993). Fruit production is seasonal but staggered for the majority of species. Most species (137-155) fruit in summer (January – March) and the least (57-63) during the winter months (June – August) (data from Pooley 1993). So fruits, although seasonally rich, are available throughout the year. One would expect resident frugivorous species to be supplied throughout the year whilst migratory species would only exploit the boom in fruit numbers during the summer months (Thompson and Willson 1979). Thus morphological adaptations of the beak towards fruit traits are more likely to occur in resident frugivorous species and not migratory ones. It is for this reason that resident avian frugivores were chosen to study.

For this study, morphological adaptations birds have to cope with a fruit diet were observed. Morphological adaptations to a fruit diet do occur in birds; they are however, inconsistent (Levey and Grajal 1991). Morphological adaptations to a fruit diet in birds were accounted for by narrowing down morphological traits to beak dimensions and the link between morphology in frugivores and plant fruit adaptations to fruit size preferences. Unlike other attributes (e.g. gut morphology, digestion, fruit selection behaviour etc.) beak morphology for birds in general is not flexible. Thus any relationship observed between beak morphology and fruit attributes should represent morphological adaptations towards successful consumption of a specific fruit trait. Previous work by Symes and Downs (2001) measured three beak

dimensions to estimate gape size in avian frugivores; maxilla tip to forehead, maxilla tip to gape and maximum width at gape (Fig. 1).

This study investigated if preferences based on fruit size exist in frugivorous birds as a result of beak morphology. Study animals were expected to maximize energy intake by choosing fruits sizes which minimize handling time (Herrera 1982; Symes and Downs 2001). Energy levels in offered fruits of varying size were identical. Larger beak dimensions were expected to lead to preferential selection of larger fruits and smaller beak dimensions to favour feeding on smaller sized fruits.

Materials and Methods

Study Animals

Wild caught Red-winged Starlings, Speckled Mousebirds and Dark-capped Bulbuls (*Onychognathus morio, Colius striatus* and *Pycnonotus tricolor*) were dewormed and acclimated to laboratory conditions (see Chapter 2 for details) before tests. Permits for study animal use were provided by Ezemvelo KZN Wildlife.

Beak Measurements

Three beak dimensions were measured on 37 sexed adult Red-winged Starling, 50 sexed adult Dark-capped Bulbuls and 50 sexed adult Speckled Mousebirds. Birds were randomly selected from the Durban Natural Science Museum (DNSM) collection. Maxilla to forehead (culmen length), maxilla tip to gape and maximum width at gape were measured (Symes and Downs 2001) using Vernier calipers (0.01 mm accuracy) (Fig. 1).

Fruit size preference experiments

These experiments focused on determining what fruit size the three study species were capable of ingesting. Individually housed test subjects were exposed to choice experiments to determine any behavioural feeding adaptations towards artificial fruits of different sizes. Artificial fruits used in experiments were made from a medium energy level (2350 kJ/l) glucose agar solution (Witmer 1998). Artificial fruits were presented to birds as small (5 mm) medium (25 mm) and large (40 mm diameter) sized spherical shaped 'fruits' which were carved out of a pre-set agar solution. Food trays were filled to three quarters with each size class to appear equal to study animals (masses did differ as a result of this). Two food trays containing one size class of fruit each were placed at equal distances from a central perch in a neutral wire cage (50 x 50 x 50 cm). To avoid any further possible negative effects of spatial bias on food selection, food trays were swapped around for half of the test subjects. Water was provided *ad libitum* during experiments.

Trails were conducted in a manner that exposed each test subject to two fruit size classes per experiment. Trails included all possible combinations of fruit size in two way choice tests. Access to food and water was not given to study animals overnight (12 hours) before each trial. Experimental days were spaced with at least one day of standard maintenance diet between to prevent any positional bias from carrying over from previous experiments. Trials were conducted during the light phase of the 12L: 12D photoperiod starting at 06:00. Mass of birds was measured at 06:00 and 18:00.

Food trays were weighed every hour on the hour for the duration of the experiment to observe any possible changes in feeding behaviour during the

experiment. Individuals were exposed to one day on each experimental diet. Preferential selection of one fruit size class over another was determined using paired t-tests for overall amounts (per unit body mass) of each size eaten. General Linear Models (GLM) Repeated Measures Analysis of Variance (RMANOVA) was used to analyze the same data on an hourly basis from food tray readings. Post-hoc Tukey HSD tests determined any significant differences between hourly tray readings. Two way ANOVA was used to determine any significant differences in beak morphology between species groups. Post-hoc Tukey HSD tests determined which beak dimension differed significantly.

Results

Beak measurements

Red-winged Starling, Dark-capped Bulbul and Speckled Mousebird beak sizes differed significantly for each of the three measurements (ANOVA Maxilla tip to forehead, n = 136, f = 2750.20, $p < 0.001$; Maxilla tip to gape, n = 136 f = 3763.90, $p < 0.001$; Maxilla width at gape, n = 136, f = 1520.46, $p < 0.001$). Red-winged Starlings had the largest overall beak dimensions when compared with Dark-capped Bulbuls and Speckled Mousebirds (Post hoc Tukey HSD, $p < 0.001$ for all measurements) (Fig. 2). Dark-capped Bulbul beak dimensions were significantly larger than Speckled Mousebirds for maxilla tip to forehead and maxilla tip to gape measurments (Post hoc Tukey HSD, $p < 0.001$ for both) while maxilla width at gape did not differ significantly between the two species (Post hoc Tukey HSD, $p = 0.14$) (Fig 2).

Fruit size preference experiments

When observing total amounts of fruit eaten by Red-winged Starlings, Dark-capped Bulbuls and Speckled Mousebirds there is no significant preference for any size class (Paired t-test, $p = 0.075\text{-}0.99$, d.f. = 5-9) (Table 1). However, hourly readings of amounts of fruit eaten did show significant preferences. Red-winged Starlings showed significant preferences for small sized artificial fruits (5 mm diameter) over large fruits (40 mm diameter) (RMANOVA; $F_{11,77} = 3.52$; $p < 0.001$; n = 8) (Fig. 3a). When Starlings were given the option between small sized fruits and medium sized fruits (25 mm diameter) a significant preference was observed for medium sized fruits (RMANOVA; $F_{11,88} = 2.13$; $p = 0.02$; n = 9) (Fig. 3b). No significant preferences for either size class were observed in the medium verses large fruit size trial (Table 1). Overall, Red-winged Starlings only ever showed significant preferences for medium and small sized fruits, yet there was no aversion displayed towards large sized fruits (Table 1).

Despite consuming more than twice as much of the medium sized fruits (per unit body mass) compared to large fruits (Table 1), Dark-capped Bulbuls showed no significant preference for medium sized fruits over large sized fruits (RMANOVA; $F_{11,55} = 1.83$; $p = 0.07$; n = 6) (Fig. 4). No significant preferences were observed in large verses small, and small verses medium sized fruit choice trials either (Table 1). Speckled Mousebirds, on average, ate a greater volume of the smaller fruits on offer but this trend was not significant (Table 1).

Discussion

Overall beak dimensions differed between Red-winged Starlings, Dark-capped Bulbuls and Speckled Mousebirds. Red-winged Starlings had the largest overall beak dimensions and were expected to favour large fruits over smaller ones as a consequence. Results showed that Red-winged Starlings actually favoured small fruits over large ones. This may be related to the idea that larger more nutritious fruits are eaten by large specialized frugivores while smaller less nutritious fruits are eaten by a wider range of opportunistic frugivores (Pratt and Stiles 1985). Since the larger fruits had no nutritional advantage over smaller fruits, it is very likely that Red-winged Starlings were favouring smaller ones as these appear easier to manipulate than large fruits. When offered only medium or large fruits, no preference was shown by Red-winged Starlings as both require greater manipulation. Medium-sized fruits were preferred over small-sized fruits but medium sized fruits were not preferred over large ones while small fruits were (Table 1). This can only be explained by the use of multiple feeding behaviours dependent on the size of the target fruit. Most birds are said to only be able to occupy one behavioural feeding group (Levey 1987). In this case Red-winged Starlings are thought to use mashing behaviour for large fruits (pecking out bits from the fruit), thrashing behaviour for medium sized fruit (picked up and broken up into smaller pieces before being eaten) and swallowing behaviour for small fruits. Whilst swallowing small fruit whole is preferred over mashing large fruit and thrashing medium fruits over swallowing small ones, no preference emerged when the option was between squashing large fruits and thrashing medium sized ones. Evidence of these eating methods was noted in this experiment from observed

indentations in leftover artificial fruits. Direct behavioural observations would, however, be required to verify this.

In a previous study, Dark-capped Bulbuls showed no preference for fruit size (Symes and Downs 2001). However, in this prior study, there was inconsistency in nutritional value of the fruit used. The present study showed that Dark-capped Bulbuls have no preference for fruit size with a controlled experimental diet (2350 kJ/l artificial fruits). Studies on jaw bone and muscular structure of *Pycnonotus* species reveal that they manipulate fruits by using a squashing action (Kalyakin and Dzerzhinsky 1996). This feeding action may be the reason Dark-capped Bulbuls are able to feed on fruits of any size indiscriminately. Symes and Downs (2001) note that Bulbuls display a preference for smaller sized grapes over large ones. Due to the nature of Dark-capped Bulbul feeding behaviour (squasher/masher strategy), it can be suggested that fruit choice is more dependent on fruit structure (Brown and Hopkins 2002) and to a lesser extent on fruit size.

Speckled Mousebirds showed no preference for any size class of fruit. For the most part (barring maxilla width at gape versus Dark-capped Bulbuls) Speckled Mousebirds have morphologically distinct beaks compared with Red-winged Starlings and Dark-capped Bulbuls. Lack of a preference for varying fruit sizes is a behaviour that is shared with Dark-capped Bulbuls. In terms of feeding behaviour, Mousebirds fall into the foot/beak coordinator category (Symes and Downs 2001; Hockey et al. 2005; pers. obs.). Speckled Mousebirds are also generalist foragers and accounts of them eating plant matter ranging from flowers and leaves to fruits exist (Hockey *et al.* 2005). It is no surprise that such well adapted generalists show no preference for any fruit size.

The elimination of factors such as inconsistencies in fruit energy levels, colour and fruit structure allowed for an unbiased view of the effects of beak morphology on fruit selection in these three common South African frugivores. Overall, results indicated that beak morphology alone cannot be used to predict fruit selection in avian frugivores. Feeding behaviour also plays a large role in determining what size of fruit birds will select to feed on (Levey 1987; Brown and Hopkins 2002). Thus a combination of beak morphology and feeding method (swallowing; squashing; thrashing; Foot/beak coordination) are needed to get a more accurate understanding of food selection in avian frugivores.

There are also a number of secondary functions associated with beak structure. These include vocalization, sexual display, and parasite removal (Podos 2001; Clayton et al. 2005). Differences in beak structure between sexes of a species are however more likely as a result of sexual selection and not as adaptation towards fruit selection e.g. maxilla tip to forehead measurements in male Speckled Mousebirds are much larger than in females (Fig. 2). Such factors should not be ignored in a study such as this, it is however difficult to account and control for them.

Acknowledgements

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Figure 2: Beak dimensions from the three frugivorous South African bird species. All values are mean ± SE a) males, Red-winged Starling n = 19, Dark-capped Bulbuls n = 24 and Speckled Mousebirds n = 25 b) females, Red-winged Starling n = 18, Dark-capped Bulbuls n = 26 and Speckled Mousebirds n = 25. See Appendix 1 for actual values, SE too small to be seen in Figure 2.

Figure 3: Red-winged Starling fruit size preferences in terms of amount eaten hourly a) Large (40mm) verses small (5mm) sized artificial fruits (mean ± SE; n = 8). b) Medium (25mm) versus small (5mm) sized artificial fruits (mean ± SE; n = 9).

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Appendix 1: Mean ± SE (n) for Red-winged Starling, Dark-capped Bulbul and Speckled Mousebird beak dimensions for males and females.

Table 1: GLM RMANOVA results from fruit size preference experiments. **Bold** values indicate significant preference. Mean and Standard error of total amounts eaten over 12 hours also included (values in grams eaten per gram body mass).

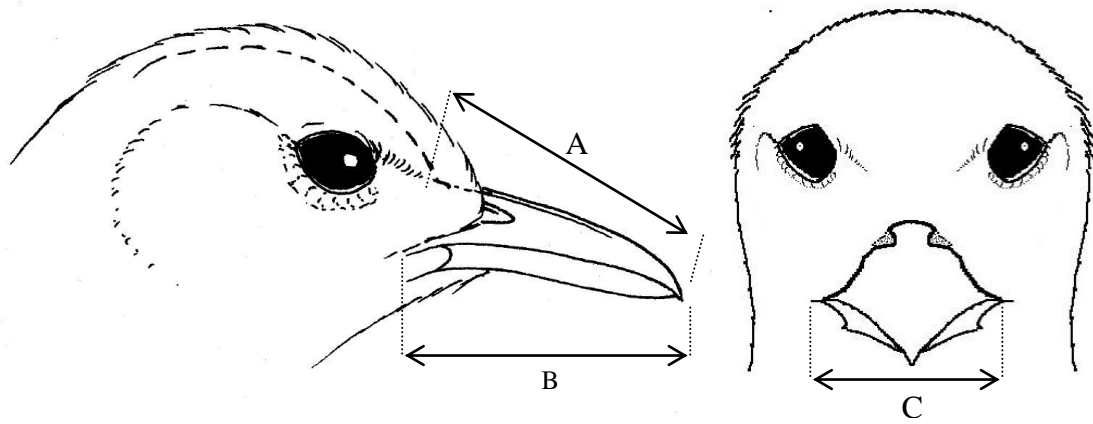| Species	Fruit size trial (mm diameter)	F (df)	p value	Preference	40mm		25mm		5mm	
					Mean	SEM	Mean	SEM	Mean	SEM
Red winged Starlings	**40 v 5**	**3.52 (11,77)**	**<0.001**	**5mm**	0.30	0.06			0.42	0.06
	25 v 40	1.06 (11,88)	0.4	none	0.35	0.06	0.35	0.06		
	5 v 25	**2.13 (11,88)**	**0.02**	**25mm**			0.45	0.07	0.27	0.06
Dark-capped Bulbuls	40 v 5	1.69 (11,55)	0.1	none	0.36	0.09			0.50	0.09
	25 v 40	1.83 (11,55)	0.07	none	0.21	0.07	0.56	0.01		
	5 v 25	0.24 (11,55)	0.99	none			0.53	0.10	0.39	0.11
Speckled Mousebirds	40 v 5	0.58 (11,99)	0.84	none	0.40	0.05			0.43	0.06
	25v 40	0.52 (11,99)	0.89	none	0.33	0.13	0.39	0.05		
	5 v 25	0.44 (11,99)	0.93	none			0.35	0.05	0.58	0.06

Figure 1: Beak measurements used. **A**- Maxilla tip to forehead. **B**- Maxilla tip to gape.

C- Maxilla width at gape.

a)

b)
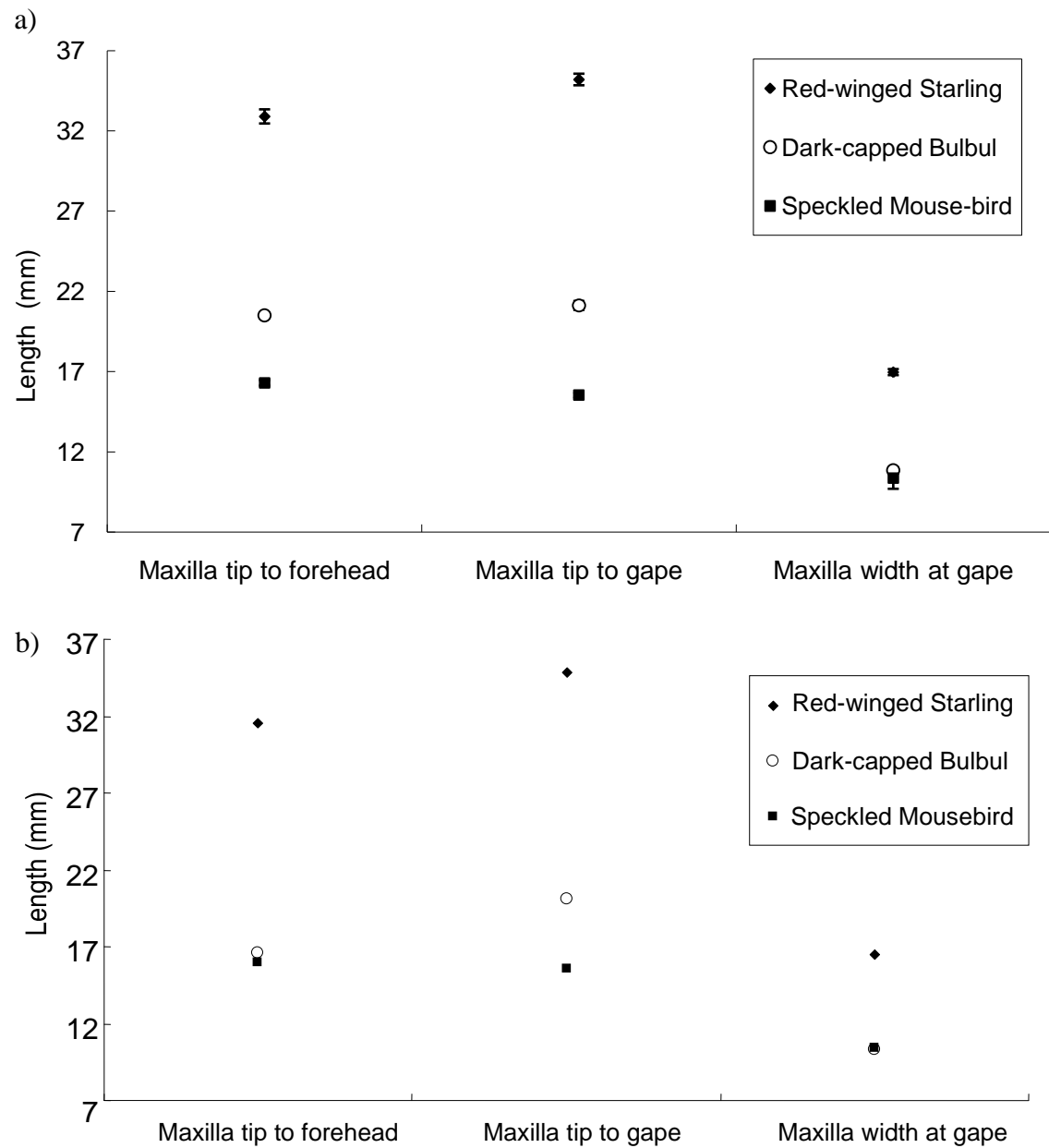

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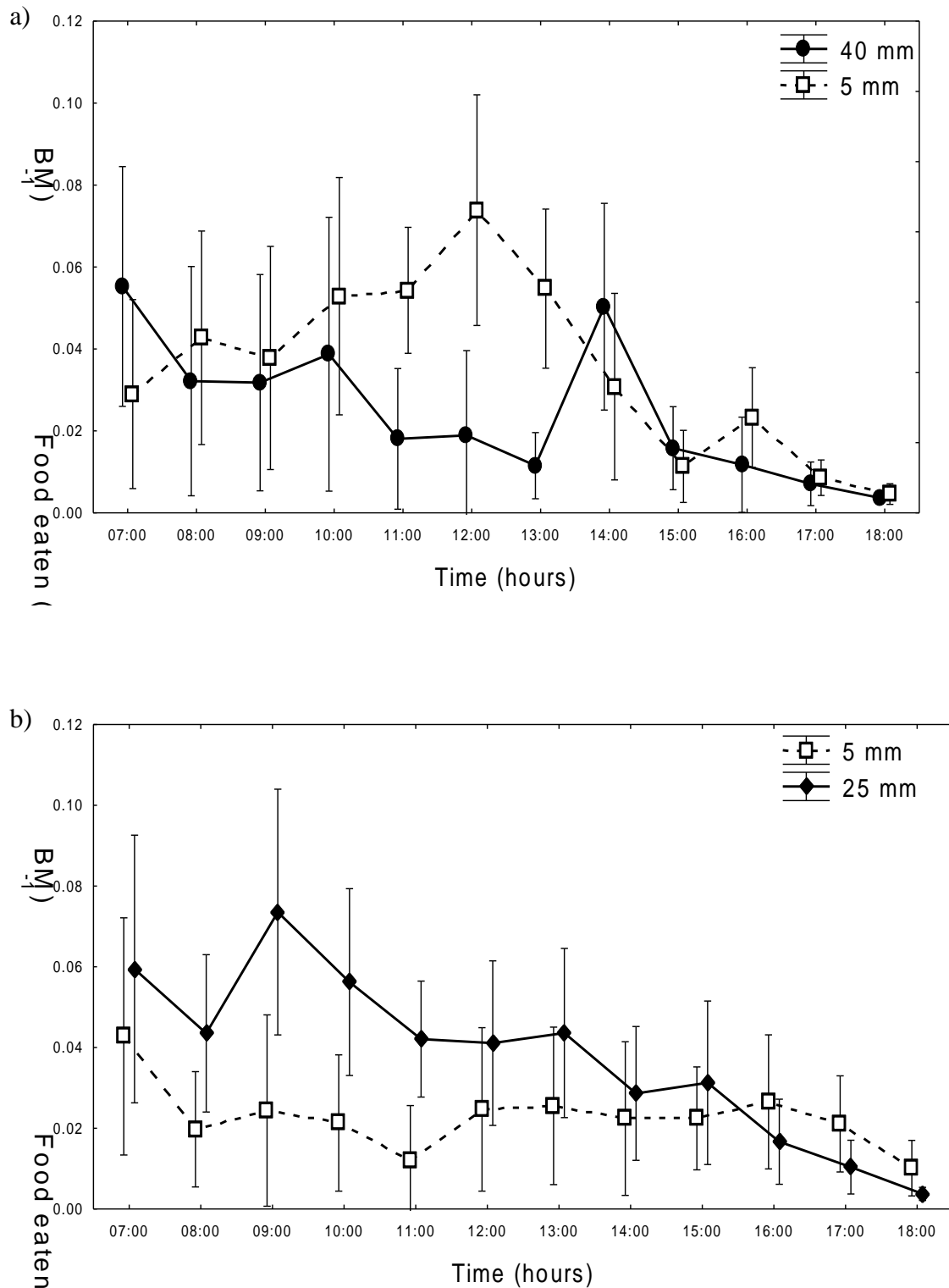
a)

b)

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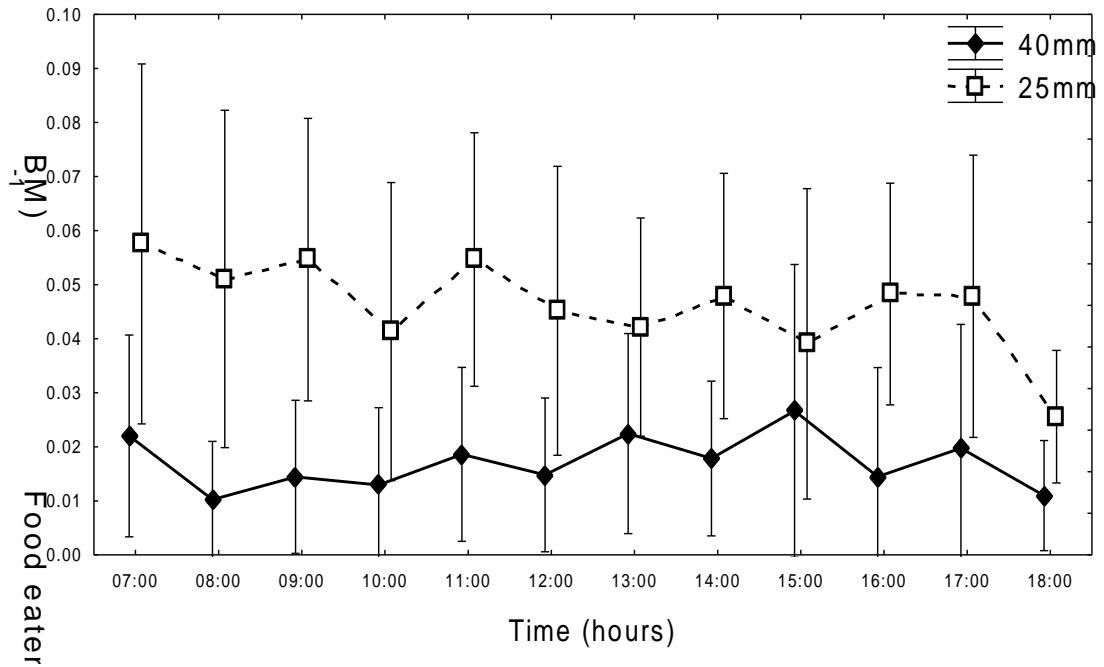
Figure 4: Dark-capped Bulbul fruit size preferences in terms of amount eaten hourly between large (40 mm) and medium (25 mm) sized artificial fruits (mean ± SE; n = 6).

Appendix 1: Beak dimensions (Mean ± SE (n)) for male and female Red-winged Starlings, Dark-capped Bulbuls and Speckled Mousebirds.

		Red-winged Starling	Dark-capped Bulbul	Speckled Mouse-bird
Male	Maxilla tip to forehead	32.90±0.44 (19)	20.51±0.15 (24)	16.30±0.17 (24)
	Maxilla tip to gape	35.20±0.36 (19)	21.15±0.28 (24)	15.54±0.14 (24)
	Maxilla width at gape	16.97±0.19 (19)	10.85±0.10 (24)	10.37±0.67 (24)
Female	Maxilla to forehead	31.53±0.25 (18)	19.60±0.12 (26)	15.95±0.14 (26)
	Maxilla to gape	34.84±0.25 (18)	20.07±0.16 (26)	15.53±0.14 (26)
	Maxilla width at gape	16.46±0.10 (18)	10.31±0.12 (26)	10.36±0.10 (26)

Chapter 6

Conclusions

Fruit features including fruit sugar type, sugar concentration, size and colour were successfully isolated in this study. Morphological, physiological and behavioural aspects of feeding ecology in Speckled Mousebird *Colius striatus*, Dark-capped Bulbul *Pycnonotus tricolor* and Red-winged Starling *Onychognathus morio* (Hockey et al. 2005) were consequently investigated. While each chapter examined a narrow range of responses towards specific stimuli, this synthesis endeavours to combine these findings to construct a more holistic view of avian fruit preferences in a southern African context.

Speckled Mousebirds

Regarded as a generalist non-passerine, various aspects of Speckled Mousebird (*Colius striatus*) feeding ecology have been studied previously (Downs et al. 2000; Symes and Downs 2001; Mlikovski 2002; Lotz and Schondube 2006; Brown et al. 2010a). Energy intake, fruit size, feeding behaviour and bill morphology were observed by Symes and Downs (2001). The experiments were carried out using apples and grapes as diet treatments. Naturally occurring fruits are known to have variable amounts of sugars, nutrients and secondary compounds depending on climatic factors and ripeness of fruits (Klann et al. 1993; Ayaz et al. 1999). The present study controlled for natural variation in fruit composition by using artificially created agar fruits (Witmer 1998).

The current study investigated Speckled Mousebird energy intake (Chapter 2), assimilation efficiency (Chapter 2), sugar preferences (Chapter 3), colour preferences (Chapter 4), size preferences (Chapter 5) and feeding behaviour (Chapter 5) on artificial fruit diets. The results from the above mentioned studies have been summarised in a flow diagram (Fig. 1). This study verifies foot-beak co-ordination as the preferred method of feeding by Speckled Mousebirds (Symes and Downs 2001) (Chapter 5, Fig. 1). Previously when offered apple (pieces), mousebirds chose large sizes over smaller ones and when offered grapes they preferred smaller sizes over larger ones and struggled to penetrate grape skins on whole large fruit (Symes and Downs 2001). In contrast in the current study, Speckled Mousebirds had no preference for fruit size using controlled artificial fruit diets ranging in size from 5 to 40 mm (Chapter 5, Fig. 1). Previous research showed that Speckled Mousebirds prefer hexose sugars at low concentrations and sucrose at higher concentrations when fed equal energy nectar solutions (Brown et al. 2010a). In contrast, a lack of preference for any sugar type irrespective of concentration in fruit was shown by Speckled Mousebirds (Chapter 3, Fig. 1). This indicates that selection criteria for fruits and nectars may be different for Speckled Mousebirds. A species known for its broad dietary tolerances including the ability to maintain body mass on a relatively nutrient poor leaf diet (Downs et al. 2000). Interestingly Mousebirds preferred orange and yellow coloured fruits over green ones (Chapter 3, Fig. 1).

Lastly, information gathered from this study indicated that Speckled Mousebirds were able to maintain body mass on single sugar equicaloric diets but consistently gained body mass on equicaloric diets where multiple sugars were presented (Chapter 2, Fig. 1). They also increased energy intake significantly on low concentration diets all the while maintaining a digestive efficiency of over 98 %

irrespective of diet concentration (equimolar and equicaloric) (Chapter 2, Fig. 1). After modelling guts as chemical reactors which utilize both active and passive transport of sugars into the blood stream, it was predicted that frugivores with low foraging costs should exhibit near 100 % assimilation efficiency (Martinez del Rio and Karasov 1990). The same study however, predicted that hexose fruits should be preferred over sucrose fruits of equal energy (especially at higher concentrations) (Martinez del Rio and Karasov 1990) this was not the case in the present study which showed a lack of preference for both sucrose and glucose fruits.

Dark-capped Bulbuls

Records of Dark-capped Bulbul (*Pycnonotus tricolor*) foraging behaviour acknowledge that this species generally feeds on fruits but will opportunistically feed on insects and nectar (Symes and Downs 2001; Hockey et al. 2005; Downs 2008; Brown et al. 2010b). Energy intake, assimilation efficiency, fruit size preferences, feeding behaviour and bill morphology have already been investigated for this species (Symes and Downs 2001; Downs 2008). Similarly to Speckled Mousebirds, these earlier investigations made use of fruits (and insects) with variable nutritional content and were consequently re-examined under stricter laboratory conditions with controlled diets in the present study. This study investigated Dark-capped Bulbul energy intake (Chapter 2), assimilation efficiency (Chapter 2), sugar preferences (Chapter 3), size preferences (Chapter 5) and feeding behaviour (Chapter 5) on artificial fruit diets, the results of which are summarised in a flow diagram (Fig. 2).

Previous research indicated that Dark-capped Bulbuls regulated energy intake (grams food eaten per gram body mass) irrespective of food type (Symes and Downs

2001). The current study, however, found that when viewing food intake from an energy point of view (i.e. kilojoules ingested per gram body mass) birds increased their energy intake on lower concentration diets (Chapter 2, Fig. 2). In terms of fruit size selection and feeding behaviour of Dark-capped Bulbuls results from the current study verified previous research (Kalyakin and Dzerzhinsky 1996; Symes and Downs 2001) by showing no preferential selection of any fruit size and using a squashing strategy for fruit consumption (Chapter 5, Fig. 2). Previous research found that Dark-capped Bulbuls were able to gain body mass on diet treatments where multiple food options were made available (apple and insects) but only managed to maintain body mass on a single food option diet treatment (only apple) (Downs 2008). In addition, the present study found that Dark-capped Bulbuls lost body mass on single sugar diets (glucose only or sucrose only) but were able to maintain body mass when offered multiple sugar diet treatments (glucose and sucrose) (Chapter 2, Fig. 2).

In terms of sugar preferences, Brown et al. (2010b) found that when offered equicaloric nectar solutions containing hexose and sucrose, Dark-capped Bulbuls always preferred hexose sugars irrespective of concentration. The present study (Chapter 3) found that if offered equicaloric glucose and sucrose fruits, Dark capped Bulbuls only preferred glucose at medium concentrations (2250kJ/l) (Fig. 2). In addition to this, sucrose was usually consumed but never favoured over glucose on both equicaloric and equimolar diets (Chapter 3, Fig. 2). Lastly, this study found high apparent assimilation efficiency of artificial fruit diets (> 99 %) irrespective of sugar type and concentration (Chapter 2, Fig. 2).

Red-winged Starlings

Previous work indicates that Red-winged Starlings feed primarily on fruits but also feed opportunistically on invertebrates and nectar (Hoffman 1988; Brown et al. 2009). Records of European Starlings (*Sturnus vulgaris*) indicate that these birds lack the ability to digest sucrose (Martinez del Rio and Stevens 1989; Avery et al. 1995) and work on other species from the Sturnidae family indicate that the entire family lacks the ability to digest sucrose (Lotz and Schondube 2006). Brown (2009) suggests that Red-winged Starlings also lack the ability to digest sucrose.

This study investigated Red-winged Starling energy intake (Chapter 2), assimilation efficiency (Chapter 2), sugar preferences (Chapter 3), colour preferences (Chapter 4), size preferences (Chapter 5) and feeding behaviour (Chapter 5) on artificial fruit diets. The results from the above mentioned studies have been summarised in a flow diagram (Fig. 3) Red-winged Starlings preferentially select glucose artificial fruits over sucrose artificial fruits irrespective of concentration on equimolar and equicaloric diets (Chapter 3,Fig. 3). Birds lost body mass when offered single sugar diets containing only sucrose but maintained body mass and showed heavy bias towards glucose fruits when offered multiple sugar diets containing both glucose fruits and sucrose fruits of equal energy (Chapters 2 and 3; Fig 3). Diet treatments not limited exclusively to sucrose sugars were assimilated with over 97% efficiency and apparent assimilation efficiency decreased with increasing fruit sugar concentration (Chapter 2, Fig. 3). Mass specific energy intake also increased with decreasing fruit sugar concentrations (Chapter 2, Fig. 3). In terms of colour preferences, Red-winged Starlings avoided green fruits and displayed an average initial response to other colours with evidence of subsequent learning over a 3 h period (Chapter 4, Fig. 3). Beak structure allowed Red-winged Starlings to swallow

small fruits whole, thrash at medium sized fruits and squash large fruits with birds preferring small fruits over large fruits and medium fruit over small fruits (Chapter 5, Fig. 3).

Summary

In conclusion, this dissertation has comprehensively accounted for fruit preferences of Speckled Mousebirds, Dark-capped Bulbuls and Red-winged Starlings. New understanding of morphological, physiological and behavioural aspects of avian frugivore feeding ecology and the direct affects these have on selective preferences for fruit colour, size and nutrient content was successfully gained. Earlier (Chapter 1, pp3) a simple question was posed:

"Do birds prefer hexose dominant fruits, sucrose dominant fruits or neither?"

The results of the present study indicate that this deceptively simple question does not have a simple answer. Each species studied had unique selection criteria for sugars in fruits (not to mention other investigated fruit characteristics). Assimilation efficiencies consistently higher than 95 % on multiple sugar diets indicate that although selection criteria were unique for each species these selection criteria were, nevertheless, optimized. The above findings indicate the complexity of the plant-disperser relationship and how having unique fruit selection criteria makes frugivory feasible when so many organisms depend of fruits for sustenance (Baker & Baker, 1998).

This is an important stepping stone as together with knowledge of the properties of naturally occurring fruits in southern Africa the information can be used to

generate useful predictive models. Knowing and being able to predict changes in avian frugivore distributions based on the fruiting patterns of target fruiting plant species for instance would greatly assist conservation efforts, especially with global warming and rapid climate change threatening our biodiversity. It is essential that future research focuses on the nutritional composition of indigenous fruits in southern Africa as this information may allow us to accurately predict foraging behaviour in avian frugivores under natural conditions. Due to the natural intraspecific variation in fruit composition this may prove difficult (Johnson et al. 1985; Klann et al. 1993; Herrera 1995; Ayaz et al. 1999; Izhaki et al. 2002). Examination of sucrose synthase and acid invertase in naturally occurring fruits could, however, hold the key to obtaining an accurate reflection of fruit sugar properties in a given species (Klann et al. 1993). Amounts of sucrose, fructose and hexose sugars in fruits are dependent on the amounts of sucrose synthase and acid invertase present in fruits during expansion and maturation (Klann et al. 1993; Wu et al. 2005).

To further improve our understanding of avian frugivore feeding ecology, avian disperser responses towards fruit chemical defences (secondary compounds, tannins and toxic concentrations) and physical defences (shells, skin and thorns) should be investigated. Further research on avian frugivore digestive flexibility and enzyme activity will also contribute towards our understanding of fruit selection criteria. If digestive ability has a primarily phylogenetic foundation, comparative studies observing variation within clade groups could account for feeding behaviour and fruit selection on a larger scale (Lotz and Schondube 2006).

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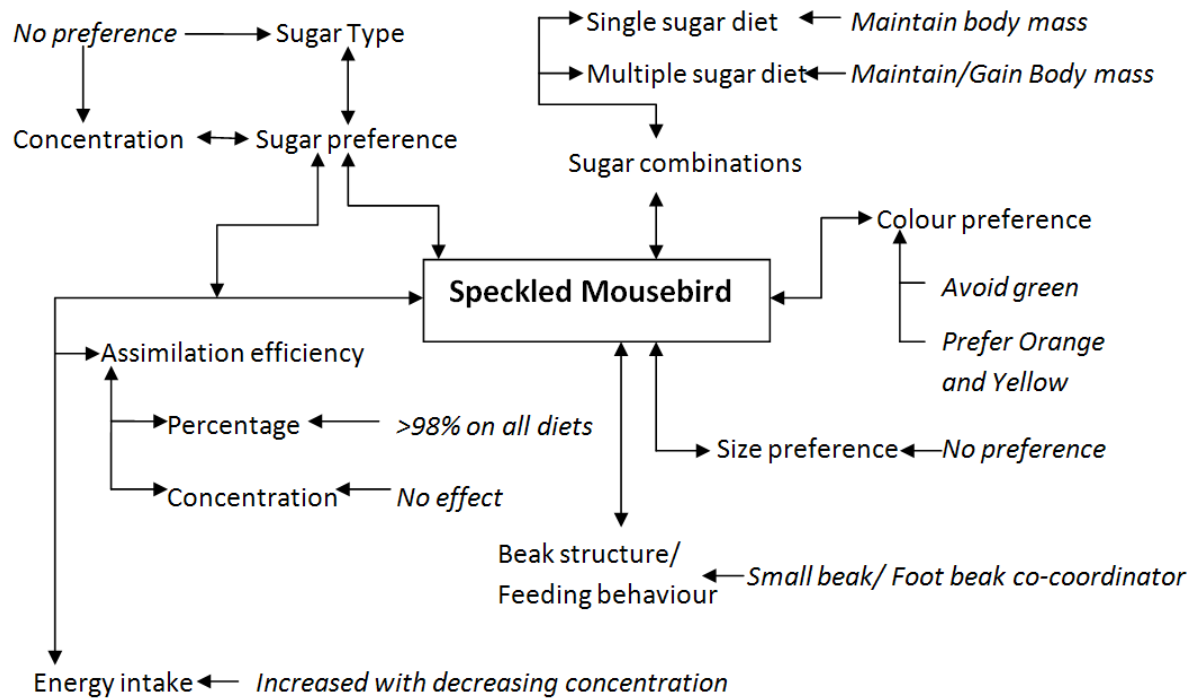
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Figure 1: Flow diagram detailing the factors affecting fruit selection in Speckled Mousebirds and experimental findings gained in this study (in *italics*).
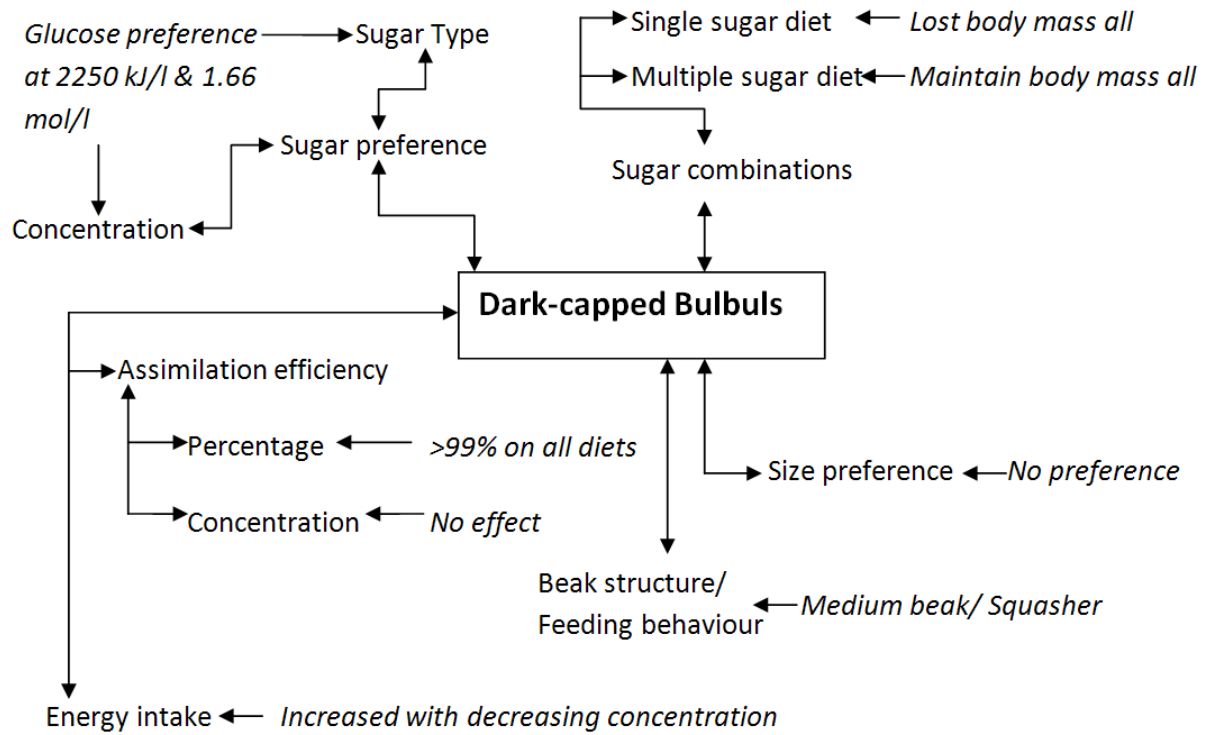
Figure 2: Flow diagram detailing the factors affecting fruit selection in Dark-capped Bulbuls and experimental findings gained in this study (in *italics*).
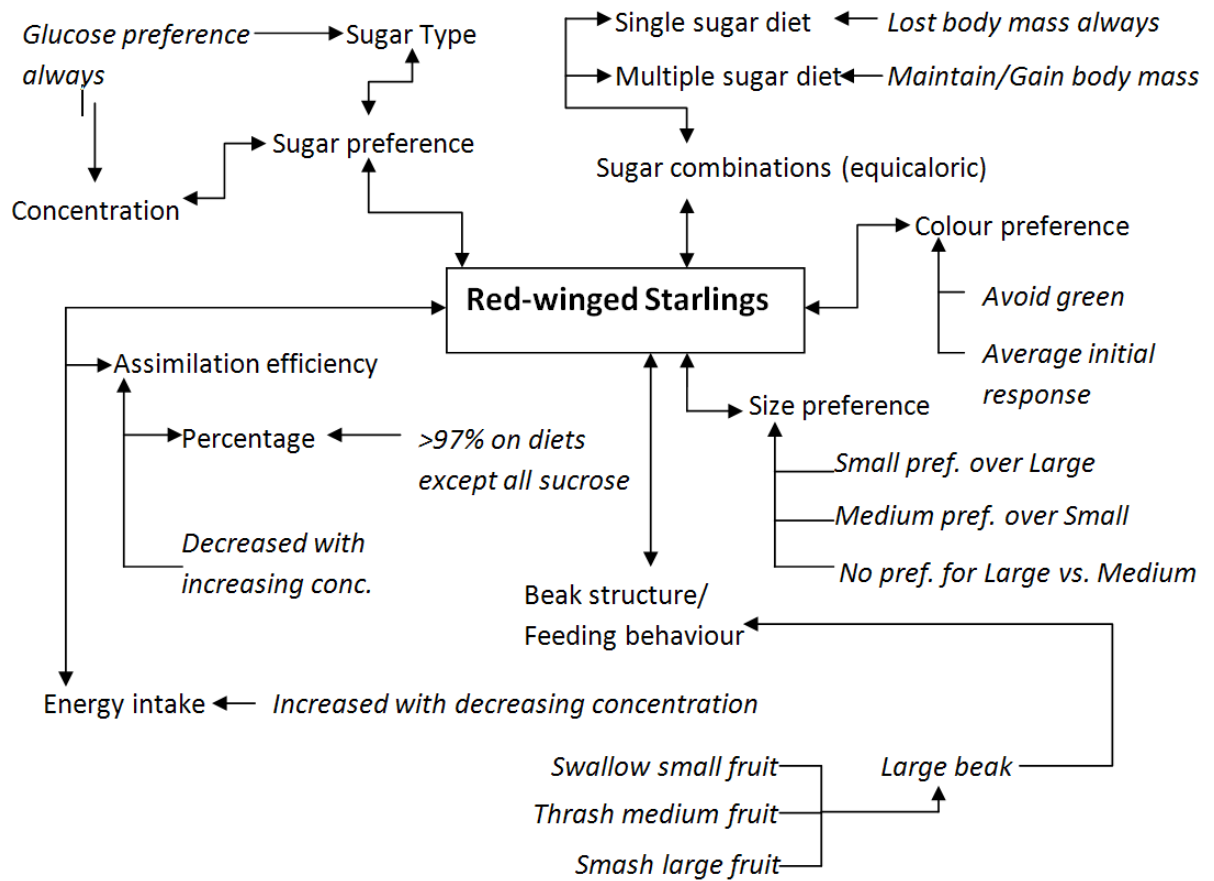
Figure 3: Flow diagram detailing the factors affecting fruit selection in Red-winged Starlings and experimental findings gained in this study (in *italics*).