Energy and water balance in the lesser double-collared sunbird, Nectarinia chalybea

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Dedication

I dedicate this PhD thesis to:

my parents for many years of good advice and unfailing support,

my brother and sister for their good influence in my life,

my friends for enriching my life,

the sunbirds for their perfection, and their Designer.

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Abstract

Nectarivores feed on aqueous sugar solutions, and their water balance is tightly linked to their energy balance. When nectar is dilute and energy demands are high, consumption of a large excess of preformed water is inevitable. Physiological implications of nectarivory for the lesser double-collared sunbird, Nectarinia chalybea, have been investigated here. Sunbirds consumed 2.7 times their body mass (8 g) per day when feeding on 0.4 M sucrose at an ambient temperature of 10 °C, and excreted 87 % of the water. When feeding on 1.2 M sucrose at 30 °C, sunbirds drank only 0.5 times their body mass of water daily. In view of the sometimes high flux of water, combined with the low electrolyte concentrations of nectar, sunbirds must be efficient at conserving ions to maintain electrolyte balance. When 15 mM each of KCl and NaCl were included in a diet of 0.4 M sucrose, sunbirds precisely maintained electrolyte balance by increasing cation excretion from 2 to 17 mM.

Considering the high preformed water content of dilute nectar, sunbirds may expend large amounts of energy warming their food to body temperature, which was measured as 42 °C irrespective of diet. Modelling revealed that sunbirds feeding on dilute (0.4 M) sucrose at 18 °C would use the same proportion of their daily energy intake (4 %) to warm their food as high-latitude aquatic endotherms feeding on fish or invertebrates just above freezing point. The evaporative water loss (and therefore evaporative heat loss) of N. chalybea increased as dietary sucrose concentration decreased (more than two-fold between 1.2 and 0.2 M). To maintain a constant body temperature despite differences in evaporative heat

loss and food warming costs, the metabolic rates of sunbirds were higher when feeding on relatively dilute nectar.

General introduction

Many animals, including insects, mammals and birds, rely on floral nectar as their primary energy source. Nectar energy occurs in the easily assimilable form of sugar, dissolved in water. Sucrose, glucose and/or fructose are the predominant sugars of floral nectar (Percival 1961, Baker and Baker 1982, van Wyk and Nicolson 1995). All three of these sugars are assimilated with 97 to 100 % efficiency by nectarivorous birds, including sunbirds, sugarbirds, honeyeaters, hummingbirds and a nectarivorous parrot (Collins and Morellini 1979, Collins et al. 1980, Karasov et al. 1986, Hainsworth 1988, Lotz and Nicolson 1996, Downs 1997a and b, Lopez-Calleja et al. 1997, Jackson et al. 1998a). In contrast, the pentose monosaccharide xylose is a major nectar sugar of some species of Proteaceae (van Wyk and Nicolson 1995), but neither sunbirds nor sugarbirds, both of which often feed on Proteaceae nectar, are able to assimilate xylose (Lotz and Nicolson 1996, Jackson et al. 1998a).

In addition to nectar, all nectarivorous bird species also consume arthropods (Austin 1963, Scott 1979). The exact contribution of this supplementary arthropod feeding to the energy balance of nectarivorous birds is unclear, but generally appears to be small or negligible. For example, hummingbirds are able to subsist for at least ten days solely on pure sucrose solution in the laboratory, but cannot survive for more than a day when offered only fruit flies (Drosophila melanogaster) and water (Brice 1992). In addition, captive sunbirds, honeyeaters and hummingbirds can maintain body mass for months at a time on sucrose solution containing nutritional supplements such as electrolytes and amino acids (this study, Beuchat et al. 1979, Collins 1981). Arthropod feeding provides negligible energetic rewards for at least some honeyeater and hummingbird species (Recher and Abbott 1970, Ford and Paton 1975, Gass and Montgomerie 1981). For example, the honeyeater Philidonyris novaehollandiae expends as much energy hawking insects as it gains from ingesting them (Ford and Paton 1975). Territorial behaviour of certain sunbird and hummingbird species around nectar sources (Wolf 1969, Stiles and Wolf 1970, Stiles 1971, Wolf 1975, Wolf et al. 1975) further highlights the importance of this food.

Although arthropod feeding is apparently unimportant for the energy balance of many nectarivorous bird species, it may be necessary for nitrogen balance, as the nectar of bird-pollinated flowers contains only trace quantities of amino acids (Baker and Baker 1982, Martinez del Rio 1994). Laboratory data reveal that pollen can also meet the nitrogen requirements of nectarivorous birds, including the lesser double-collared sunbird, Nectarinia chalybea (van Tets and Nicolson, submitted), but it is unknown whether these birds actually ingest pollen in the field. Arthropod- and possible pollen-feeding may also provide a range of micro-nutrients, including electrolytes (see below), that are scarce in nectar (Baker and Baker 1982).

The water balance of nectarivorous animals is inextricably linked to their energy balance, because nectar sugars come dissolved in water. Nectar-feeding birds usually consume a substantial excess of preformed water which they need to lose through excretion or evaporation. Calder (1979) predicted the "threshold" nectar concentrations for nectarivorous birds, at which the preformed and metabolic water gains would exactly balance the evaporative water loss, so that birds would neither drink nor excrete water. Based on Calder's predictive equation, nectarivorous birds at an ambient temperature of

30 °C should consume preformed water in excess of evaporative water loss unless the nectar concentration exceeds 1.5 M sucrose (= 42 % w/w). In reality, the nectar of bird-pollinated flowers seldom reaches this concentration, averaging only 0.8 M sucrose (= 23 %, Pyke and Waser 1981). Calder's "threshold" nectar concentration increases exponentially as ambient temperature decreases below 30 °C. It is thus not surprising that the water influx of wild sunbirds, honeyeaters and hummingbirds (Powers and Nagy 1988, Williams 1993, Weathers et al. 1996, Goldstein and Bradshaw 1998b) far exceed allometric predictions (see chapter 1).

Bat-pollinated flowers produce similarly dilute nectar to bird-pollinated ones (Pyke and Waser 1981, Baker and Baker 1982), and two nectar-feeding bat species have high water influx (Carpenter 1969, Helverson and Reyer 1984) comparable to those of nectarivorous birds. Some insects excrete copious, dilute fluid because of their nectar diet (Bertsch 1984, Nicolson 1990), whereas others gain just enough preformed water to maintain water balance (Willmer 1988), or require additional free water (Nicolson 1998).

The electrolyte concentrations in the nectar of insect- and bird-pollinated flowers are generally low (Hiebert and Calder 1983, Nicolson and Worswick 1990). The few studies that have considered the electrolyte balance of nectarivorous insects (Arms et al. 1974, Barrows 1974, Nicolson and Louw 1982, Nicolson 1990, Nicolson and Worswick 1990) indicate that these animals may have difficulty in maintaining cationic balance, particularly when water influx is high (Nicolson and Louw 1982, Nicolson 1990). Data on electrolyte balance of nectarivorous birds are even scarcer than for insects. The copious excretory fluid of hummingbirds, although dilute, may sometimes contain cationic concentrations higher than those in the nectar of their food plants (Calder and Hiebert 1983, see chapter 2), so that these birds may depend on supplementary arthropod feeding to maintain electrolyte balance, as the water volumes ingested by hummingbirds are lower than the volumes excreted. The honeyeater Anthochaera carunculata evidently has difficulty in maintaining ionic balance when fed calorifically dilute nectar containing low cationic concentrations (Goldstein and Bradshaw 1998a).

The ingestion of large volumes of preformed water by endothermic nectarivores may have interesting thermoregulatory implications, which have not yet been explored. It is possible that at low ambient temperatures a substantial amount of energy is needed to warm the large nectar volumes to body temperature. In addition, the evaporative water loss (and therefore the evaporative heat loss) of two honeyeater species is known to increase as the ingested nectar becomes more dilute (Collins 1981).

Nine families from three orders of birds have at least some representative species which are nectarivorous (Table 0.1, Austin 1963, Scott 1979). Many other species not shown in Table 0.1 feed opportunistically on nectar, such as troupials (Icteridae), orioles (Oriolidae), drongos (Dicruridae), leaf-birds (Irenidae), bulbuls (Pycnonotidae) and chats (Turdidae, Austin 1963, Calder 1979, Scott 1979, Maclean 1985, Burd 1995).

In terms of the general biology of the widespread and speciose sunbirds, Skead (1967) provides detailed information on all the southern African species. A book aimed at providing a comprehensive account of what is known about all sunbird species world-wide is currently under preparation by R. A. Cheke.

Table 0.1: Distribution of avian nectarivory (from Austin 1963, Scott 1979).

Family	Distribution	Species	Nectarivorous species
Psittaciformes			
Parrots (Psittacidae)	all continents	339	few
Trochiliformes			
Hummingbirds (Trochilidae)	N and S America	331	all
Passeriformes			
False sunbirds (Philepittidae)	Madagascar	4	2
Flowerpeckers (Dicaeidae)	Asia, Australasia	51	few
Sunbirds (Nectariniidae)	Africa, Asia, Australasia	104	all
Sugarbirds (Promeropidae)	Africa	2	2
White-eyes (Zosteropidae)	Africa, Asia, Australasia	80	many
Honeyeaters (Meliphagidae)	Australasia	160	most
Honeycreepers (Drepanididae)	Hawaii	22	some

Energy balance and especially water balance of sunbirds are poorly known. Time-energy budgets have been quantified for male malachite sunbirds, Nectarinia famosa, territorial around Aloe graminicola flowers (Wolf 1975), golden-winged sunbirds, N. reichenowi, defending Leonotus nepetifolia stands (Gill and Wolf 1975),.olive sunbirds, N. olivacea, defending L. leonurus (Frost and Frost 1980) and orange-breasted sunbirds, N. violacea, feeding on Mimetes hirtus and Erica perspicua flowers (Collins 1983). Wolf et al. (1975) compared the relationship between foraging efficiency and time and energy budgeting of sunbirds and hummingbirds, using laboratory data of resting and flying metabolic rates and body temperatures for the bronze sunbird, N. kilimensis, combined with the field data for N. famosa from Wolf (1975). Resting metabolic rates and body temperatures of 14 other sunbird species, including the lesser double-collared sunbird, N. chalybea, have been measured at different ambient temperatures in the laboratory (Prinzinger et al. 1989, Prinzinger et al. 1992, Leon and Nicolson 1997). The body temperatures of five sunbird species have been measured at their sites of capture high up in East African mountains (Cheke 1971). Finally, the metabolic rate and water flux of incubating female orange-breasted sunbirds, N. violacea, have been measured using doubly-labelled water during winter breeding (Williams 1993).

The energy balance of hummingbirds and honeyeaters has received a relatively large amount of attention, and the water balance of these birds has also received some attention (see below). Hummingbirds are the New World counterparts of sunbirds. However, hummingbirds are in a separate order (Table 0.1), are smaller than sunbirds (hummingbirds typically weigh 4 – 6 g, whereas sunbirds typically weigh 7 – 15 g), and hummingbirds normally hover while feeding, whereas sunbirds usually perch while feeding. Australasian honeyeaters, like sunbirds, are in the order Passeriformes, and also perch while feeding, but only the smallest honeyeaters resemble sunbirds in size.

The lesser double-collared sunbird, Nectarinia chalybea (8 g), is endemic to South Africa, but forms part of a species complex which is widely-distributed over the African continent. It is represented by four subspecies, which occupy many different habitats ranging from semi-desert to temperate forest, and which feed from the nectar of a wide range of flower species. The subspecies N. chalybea chalybea is abundant in the winter rainfall fynbos biome (see Cowling 1992) of the south-western Cape, South Africa, where it particularly favours dune thicket, riparian woodland and suburban gardens (Skead 1967, Maclean 1985, Harrison et al. 1997).

Outline and contributions of this PhD thesis

In this PhD thesis, energy and water balance are examined from an integrated perspective in captive N. chalybea. All sunbirds were captured in the southwestern Cape, South Africa, and were of the subspecies chalybea. Individual sunbirds were kept for no longer than eight months before being released at their sites of capture. Throughout the study, sunbirds were fed solutions of sucrose to simulate nectar. Sucrose is a common nectar sugar of ornithophilous flowers in South Africa, is favoured by N. chalybea as much as other common nectar sugars (glucose and fructose), and is assimilated with 100 % efficiency (Barnes et al. 1995, Lotz and Nicolson 1996, Jackson et al. 1998a, Nicolson and van Wyk 1998).

In **chapter 1**, nectar intake and excretion were measured every hour in sunbirds kept at 20 °C and fed different nectar concentrations (0.4, 0.8 or 1.2 M sucrose). Similar studies have been done on honeyeaters (Collins and Morellini 1979, Collins 1981), but the review of Beuchat et al. (1990) highlighted the paucity of water balance data for hummingbirds, even though the energy balance of these birds had received considerable attention. Since then, Lopez-Calleja et al. (1997) have measured hourly nectar intake but not excretion in Sephanoides sephanoides hummingbirds fed different nectar concentrations. The experiment of **chapter 1** provides water and energy balance data for sunbirds for comparison with honeyeaters and hummingbirds, and it also provides a solid foundation for the experiments of chapters 2 to 5.

In **chapter 2**, the ability of N. chalybea to conserve or excrete electrolytes while feeding on 0.4 M sucrose, which produces a high water flux (twice its body mass daily, chapter 1), was investigated. The data of this chapter clearly show how N. chalybea is able to maintain electrolyte balance from the nectar of its food plants. The study of Calder and Hiebert (1983) on hummingbirds, although pioneering, did not reveal much about electrolyte balance. The work of Goldstein and Bradshaw (1998a) on Anthochaera carunculata honeyeaters fed artificial nectar of different calorific and ionic concentrations apparently provides the first data on electrolyte balance in this bird family. Ionic concentrations of the excreted fluid of wild hummingbirds (Calder and Hiebert 1983) and honeyeaters (Goldstein and Bradshaw 1998b) have been measured, but these data await laboratory studies like that of **chapter 2** on N. chalybea for meaningful interpretation.

In **chapter 3**, nectar intake and excretion of N. chalybea kept at different ambient temperatures (10, 20 or 30 °C) were measured every hour. Various studies have examined the effect of ambient temperature on the energetics of nectarivores, including hummingbirds (e.g. Beuchat et al. 1979), honeyeaters (e.g. Collins et al. 1980b), Hawaiian honeycreepers (MacMillen 1974) and sunbirds (Prinzinger et al. 1989, Prinzinger et al 1992, Leon and Nicolson 1997). However, only the theoretical analysis of Calder (1979), which focused on hummingbirds but also included other nectarivorous birds, has considered the effect of ambient temperature on water and energy balance from an integrated perspective. **Chapter 3** provides data which complements Calder's theoretical analysis.

The cost of warming nectar of different concentrations and temperatures to body temperature in endotherms is modelled in **chapter 4**. Wilson and Culik (1991) proposed a more general model for warming other foodstuffs (e.g. fruit and fish) to body temperature, and this model is developed further in **chapter 4** so that the cost of warming various other foodstuffs can be compared with the cost of warming nectar. Apart from the recent studies of Wilson and Culik (1991), Croll and McLaren (1993), Wiersma (1995), Hawkins et al. (1997) and de Leeuw et al. (1998), which have all been on aquatic birds feeding on fish or invertebrates, studies of thermoregulation and specific dynamic action (SDA) have ignored the cost of food warming, which is thus a confounding variable. **Chapter 4** addresses not only the question of the potentially high costs associated with warming the large preformed water excesses contained in nectar, but also highlights the importance of considering food warming costs in studies of thermoregulation and SDA in endotherms, irrespective of their diet.

In chapter 1 it was found that the difference between water gain and excreted water, which should represent evaporative water loss (EWL), was considerably higher in sunbirds fed relatively dilute nectar. In two honeyeater species, directly-measured EWL was also higher when birds were fed dilute nectar (Collins 1981), but this has not been observed in hummingbirds. In fact, no other studies have examined the effect of diet on EWL in birds. **Chapter 5** describes experiments in which both night- and day-time EWL of N. chalybea were monitored continuously while birds had access to food, either 0.2 or 1.2 M sucrose.

This confirmed the dietary difference in EWL predicted in chapter 1. Further experiments were performed to ascertain how rapidly the dietary change in EWL occurred, and calculations were done to determine whether the change was cutaneous or respiratory in origin.

The thermoregulatory consequences of dietary changes in EWL, together with the cost of warming food, were also examined in **chapter 5**. Body temperatures and metabolic rates were measured in birds fed 0.2 or 1.2 M sucrose. Body temperature was unaffected by diet, but metabolic rate was higher in sunbirds fed the more dilute solution. These thermoregulatory consequences of ingesting large preformed water excesses because of a nectar diet have not been examined before.

Chapter 1

Energy and water balance of Nectarinia chalybea fed different nectar concentrations

Summary

The water balance of nectarivores is tightly linked to their energy balance. When nectar is dilute, consumption of a large water excess is inevitable. Energy and water balance were investigated in lesser double-collared sunbirds, Nectarinia chalybea (8 g), kept at 20 °C and fed different nectar concentrations (0.4, 0.8 or 1.2 M sucrose). The mass of sucrose ingested, body mass, day-time mass gain and night-time mass loss were the same irrespective of diet, the birds compensating energetically for changes in sucrose concentration by drinking greater volumes of the more dilute solutions. Sunbirds consumed between 0.5 and 1.8 times their body mass in preformed water per day, depending on sucrose concentration, and excreted around 75 % of the water. The difference between water gain (preformed and metabolic water) and excreted water is assumed to equal evaporative water loss, and was similar on 1.2 and 0.8 M sucrose, but was higher on a diet of 0.4 M sucrose. The osmolalities and K^+ and Na^+ concentrations of the excreted fluid were extremely low, so that the excretory fluid of sunbirds resembles that of hummingbirds and freshwater vertebrates rather than that of typical terrestrial vertebrates. Nectarinia chalybea is able to maintain energy and water balance over a range

of nectar concentrations by adjusting the volume of solution consumed and by excreting copious, dilute fluid.

Introduction

The water balance of nectarivores is tightly linked to their energy balance, because they are forced to consume the preformed water component of nectar to obtain their energy needs (Beuchat et al. 1990). Calder (1979) predicted threshold nectar concentrations for nectar-feeding birds, which would compensate for evaporative water losses at different ambient temperatures, and found that the threshold concentration ranged from 9.3 M sucrose at 10 °C to 0.8 M at 37 °C. Ornithophilous plants assumed to be pollinated by hummingbirds, honeyeaters and sunbirds produce nectar of only about 0.75 M sucrose on average (23 % w/w; Pyke and Waser 1981), which means that the volume of preformed and metabolic water consumed by nectarivorous birds will be in excess of their evaporative water loss even when ambient temperatures are as high as 37 °C. At lower ambient temperatures, the water excess would increase greatly, presumably far more than necessary to dissolve and excrete metabolic waste.

The water influx and excretion rates measured in nectarivorous birds are indeed high, sometimes more than twice the values predicted from allometry (Williams et al. 1993). Daily water influx (preformed water consumed and metabolic water produced) has been measured in free-living nectarivorous hummingbirds, sunbirds and honeyeaters using labelled water, and ranges from 0.9 to 2.4 times body mass (Weathers and Stiles 1989; Williams 1993; Weathers et al. 1996, Goldstein and Bradshaw 1998b). Similarly, preformed water intake in laboratory-kept hummingbirds and honeyeaters ranges from 0.6 to 3.3 times body mass, and at the highest intake rates 90 % of the water gain is excreted

(Collins 1981; Beuchat et al. 1990). Considering the exceptionally large volumes of water excreted by nectarivorous birds, excessive loss of electrolytes is also a potential problem.

The lesser double-collared sunbird, Nectarinia chalybea (8 g), is endemic to South Africa, where it occupies a variety of habitats from semi-desert to temperate forest and is known to feed from nectar of a wide range of plant species (Skead 1967, Harrison 1997). The aim of the present chapter is to compare energy and water balance in N. chalybea feeding on different nectar concentrations, to see how closely this species fits Calder's model.

Materials and methods

Seven female and six male Nectarinia chalybea were kept in individual cages (52 x 52 x 52 cm), in a constant environment room set on a 12L:12D photoperiod (light from 07:30 to 19:30), at 20 °C and 65 % r.h., and maintained on a diet of 12.5 g sucrose and 2.5 g Complan® (Boots Pharmaceuticals, Isando, South Africa) per 100 ml of water, which gives a concentration of 0.37 M sucrose excluding Complan®. Complan® is characterised in full in Appendix 1.

During experiments, birds were transferred to plastic cylindrical cages, 50 cm high and 36 cm in diameter, with nylon gauze tops. The cages rested on chicken wire stands above trays containing liquid paraffin, for collection of excreted fluid without evaporation. Experimental feeders were modified 25 ml glass pipettes which did not leak. Birds were fed either 0.4, 0.8 or 1.2 M sucrose (equal to 13, 25 and 36 % w/w). These concentrations were chosen because they cover the range of nectar concentrations of ornithophilous flowers, and for direct comparison with a previous study of water balance in honeyeaters (Collins 1981). Birds were acclimated to their experimental diets for two days. Preliminary experiments showed that from the second day onwards after a dietary switch, sunbirds would drink highly constant 24 h volumes on consecutive days, so that even a single day of acclimation would have been adequate. On the third day, from 07:30 (lights on) to 19:30 (lights off), their intake of sucrose solution was monitored (to the nearest 0.1 ml) every hour, and the excreted fluid produced was collected every hour and immediately frozen for later analysis. During the dark period, the birds did not drink, but excreted small volumes of fluid, which was collected at 07:30. Birds were weighed at 07:30 every day, and also at 19:30 on the experimental (third) day. During experiments, sunbirds lost between 1 and 3 % of their 07:30 body mass every 24 h, irrespective of dietary sucrose concentration (see results), possibly because of a lack of protein in the experimental diet.

All 13 birds were fed all three sucrose concentrations, so a total of 13 * 3 = 39 trials were carried out. Four trials were conducted simultaneously. The order in which an individual bird received the three sucrose concentrations was randomised, but individual birds were not included in consecutive experiments, so that they had at least ten days on Complan® - supplemented food between trials.

The volume of preformed water consumed was calculated using the equation

$$gH_2O\ (\text{ml solution})^{-1} = (-0.21644 * M) + 1.0007,$$

where M = mol l^{-1} (derived from Weast 1985). The hourly excreted fluid samples were later thawed and weighed. To determine the volume of water excreted, excreted fluid was assumed to be pure water, because volumes of some samples were measured to an accuracy of 0.1 ml and found to be equivalent to masses measured to the nearest 0.1 g. The osmolalities of the pooled excreted fluid samples were measured using a Wescor 5500 vapour pressure osmometer, and the K^+ and Na^+ concentrations were measured by flame photometry (Instrumentation Laboratory Model IL 243).

Independent sample t-tests were used to test for differences in body mass, sucrose solution intake and excreted fluid volume between males and females. To test for differences in the various components of energy, water and electrolyte balance among the three sucrose concentrations and among the twelve hours of the light period, ANOVA's with repeated measures were performed, followed by Tukey multiple range tests. A paired sample t-test was used for morning and evening body mass data pooled for the three sucrose concentrations. All statistics were performed using STATISTICA (Statsoft, Tulsa), following the methodology of Zar (1996). Data are reported as the mean ± SE.

Results

Comparisons between sexes

Body mass at both 07:30 and 19:30 on the experimental day was significantly higher in males than in females, on all three diets ($P < 0.05$). Body mass at 07:30 pooled for all three diets averaged 8.17 ± 0.12 g in males and 7.27 ± 0.10 g in females; body mass at 19:30 was 8.53 ± 0.13 g in males and 7.58 ± 0.11 g in females. However, sucrose solution intake and excreted fluid volume were the same in both sexes, on all three diets ($P >$ 0.13).

Sucrose concentration

Body masses at 07:30 and at 19:30 did not differ significantly among diets (Table 1.1). The mean mass at 19:30 on the experimental day, 8.13 ± 0.12 g, was significantly higher than the mean mass at 07:30, 7.73 ± 0.11 g ($P < 0.00001$). Day-time mass gain and night-time mass loss were the same irrespective of diet. The birds lost 1 - 3 % of their 07:30 body mass every 24 h. The total mass loss over the entire experiment (days 1 to 4, 72 h) did not differ among diets, and was 3.8 % of initial body mass.

Table 1.1: Body mass, day-time mass gain, night-time mass loss and mass loss over the entire experimental period (g, mean ± SE, n = 13), of birds fed 0.4, 0.8 or 1.2 M sucrose. Results of ANOVA's, testing for differences among sucrose concentrations, are also shown.

Measurement	0.4 M	0.8 M	1.2 M	F	P
Body mass, 07:30, day 1	8.02 ± 0.21	7.99 ± 0.19	7.96 ± 0.16	0.10	0.901
Body mass, 07:30, day 2	7.94 ± 0.21	7.92 ± 0.19	7.91 ± 0.17	0.02	0.977
Body mass, 07:30, exp. day	7.72 ± 0.23	7.63 ± 0.20	7.83 ± 0.21	1.44	0.264
Body mass, 19:30, exp. day	8.15 ± 0.21	8.10 ± 0.21	8.15 ± 0.20	0.12	0.890
Body mass, 07:30, day 4	7.74 ± 0.20	7.72 ± 0.18	7.70 ± 0.18	0.08	0.925
Day-time mass gain, exp. day	0.28 ± 0.01	0.29 ± 0.03	0.32 ± 0.04	0.70	0.511
Night-time mass loss	0.42 ± 0.02	0.43 ± 0.02	0.47 ± 0.03	2.57	0.099
Mass loss, day 1 to day 4	0.31 ± 0.04	0.27 ± 0.05	0.32 ± 0.22	0.28	0.759

The volume of sucrose solution consumed decreased with increasing sucrose concentration (Fig. 1.1, Appendix 2.1), such that the mass of sucrose ingested over the entire light period did not differ significantly between sucrose concentrations (2.12 ± 0.06 g for 0.4 M, 2.12 ± 0.06 g for 0.8 M and 2.16 ± 0.05 g for 1.2 M; $P = 0.736$).

The effects of dietary sucrose concentration on the different components of the 24 h water balance of N. chalybea are shown in Fig. 1.2 and Appendix 2.2. Preformed water intake and excretory water loss were measured directly. The 24 h metabolic water production was calculated from the measured mass of sucrose consumed by N. chalybea during the day, assuming that 1 g of sugar yields 0.60 g of metabolic water (Collins 1981).

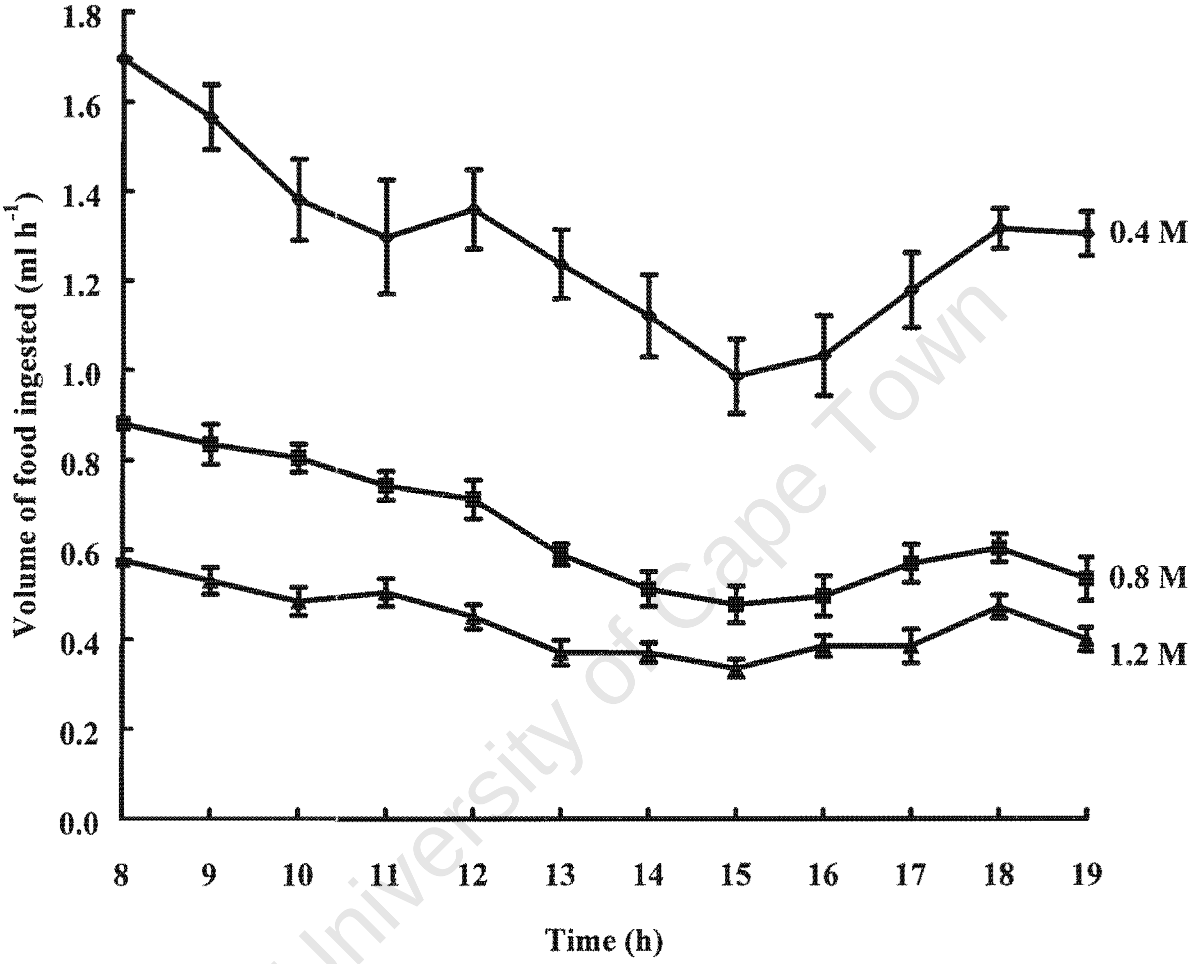

Fig. 1.1: Hourly volumes (ml h^{-1} ; mean ± SE, n = 13) of sucrose solution ingested by <u>N. chalybea</u> fed 0.4, 0.8 or 1.2 M sucrose. The times indicated on the x-axis are the mid-points of each hourly interval in which food intake was measured.

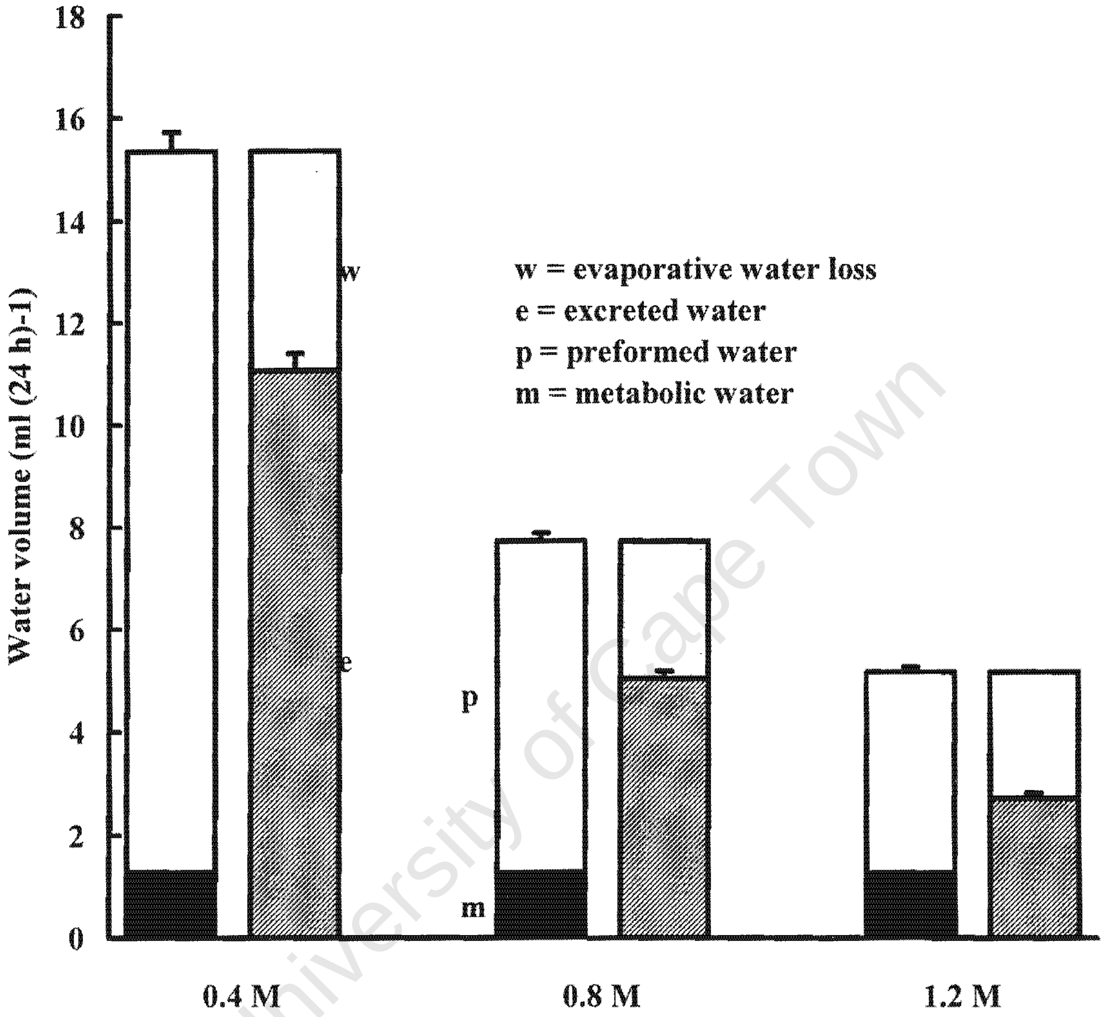

Fig. 1.2: Components of water gain and loss (mean ± SE, n = 13) in N. chalybea fed 0.4, 0.8 or 1.2 M sucrose. For explanation see text.

The birds did not drink at all during the dark period, and therefore all preformed water intake was during the 12-hour light period. Similarly, only 0.5 - 1.9 % of total excretory water loss occurred at night (0.06 ± 0.02 ml on 0.4 M, 0.04 ± 0.01 ml on 0.8 M and 0.05 ± 0.02 ml on 1.2 M sucrose). The birds gained 1.9 times their body mass per day in preformed and metabolic water when fed 0.4 M sucrose, but only 0.6 times their body mass when fed 1.2 M. The volume of water excreted was always lower than the volume of preformed and metabolic water, the difference presumably representing the 24 hour evaporative water loss (Fig. 1.2, Appendix 2.2). Twenty-four hour evaporative water loss predicted in this way did not differ significantly between birds fed 0.8 M and 1.2 M sucrose, but was 1.6 times higher when they were fed 0.4 M sucrose ($P < 0.05$).

Diurnal patterns

The volume of sucrose solution ingested (and therefore mass of sucrose and volume of preformed water) varied through the day (Fig. 1.1, Appendix 2.1). The diurnal patterns were similar for all three sucrose concentrations. The volume ingested was highest in the first hour of light, and decreased to a low around 15:00. There was a smaller secondary peak towards the end of the light period. Intake in the first three to five hours of light was significantly higher than intake between 14:30 and 15:30 (by 72 to 85 %, depending on sucrose concentration), and also 22 to 46 % higher than the secondary peak after 15:30 ($P < 0.05$). For 1.2 M sucrose, the secondary peak around 18:00 was significantly higher (by 41 %) than the low around 15:00, and although the same trend was apparent for 0.8 and 0.4 M sucrose, it was not significant.

The hourly volume of excreted water was always lower than, but varied in a similar pattern to, the preformed water intake (Fig. 1.3, Appendix 2.2). However, the difference between preformed and excreted water volumes showed a significant peak ($P < 0.05$) in the first hour of light, because of a high preformed water intake and low excreted water volume during that hour. The difference between preformed and excreted water was also low in the last hour of light, so it did not appear that the birds were conserving water in anticipation of the nocturnal fast.

Excreted fluid electrolytes

The osmolality of the excreted fluid increased significantly from 61 mmol kg^{-1} when birds were fed 0.4 M sucrose to 74 mmol kg^{-1} when they were fed 1.2 M sucrose (Table 1.2, $P < 0.00001$). Concentrations of K^+ and Na^+ in the excreted fluid also increased significantly with dietary sucrose concentration (Table 1.2, $P < 0.00001$). The K^+ concentration was higher than the Na^+ concentration, but only significantly so in birds fed 1.2 M sucrose ($P < 0.05$). The concentrations of K^+ and Na^+ did not exceed 7 and 2 mM respectively in the excreted fluid of individual birds.

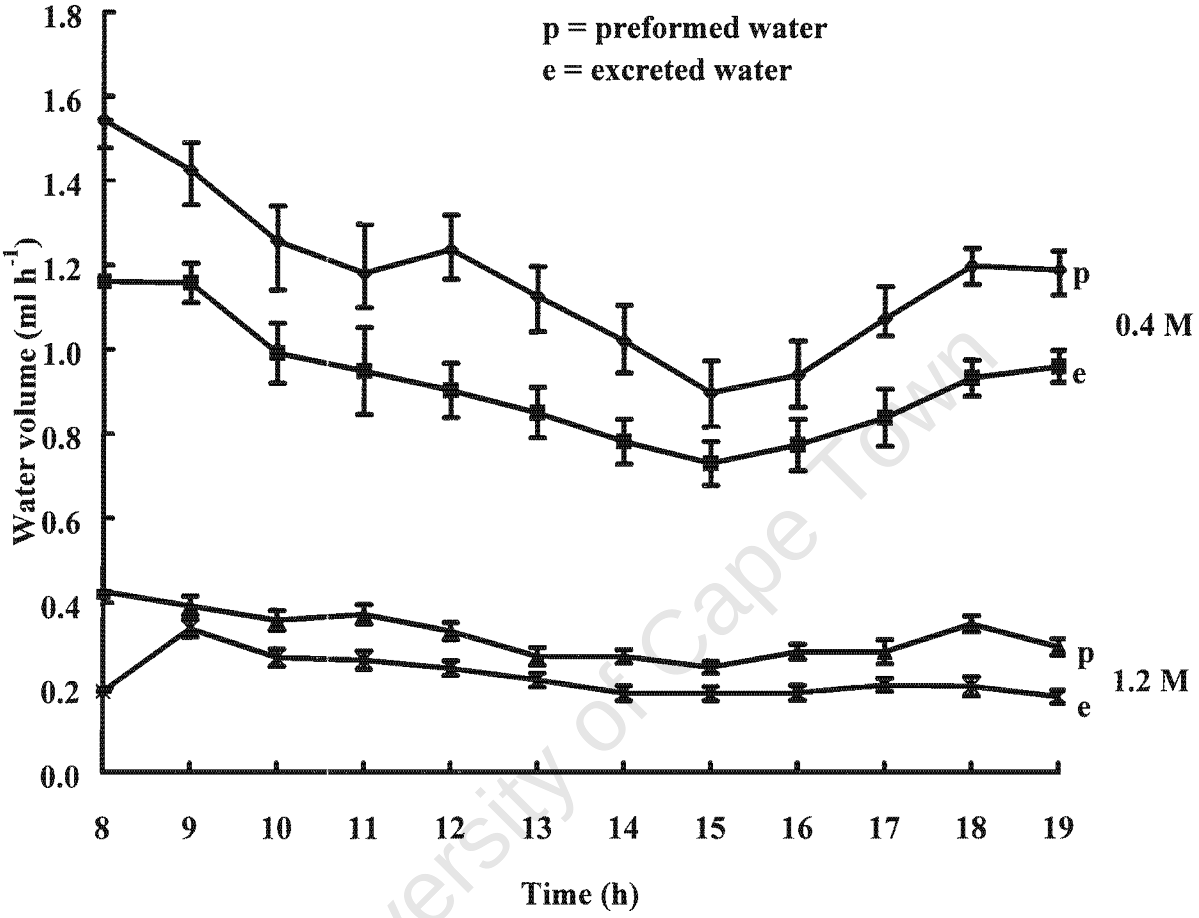

Fig. 1.3: Hourly volumes (ml h^{-1}; mean ± SE, n = 13) of preformed and excreted water in N. chalybea fed 0.4 or 1.2 M sucrose. Data for 0.8 M sucrose are omitted for the sake of clarity. The times indicated on the x-axis are the mid-points of each hour of the day in which food intake and excreted fluid were measured.

Table 1.2: Osmolalities (mmol kg^{-1}) and cation concentrations (mM) of total excreted fluid produced during the light period (pooled hourly collections, mean ± SE), in birds fed 0.4, 0.8 or 1.2 M sucrose.

	0.4 M	0.8 M	1.2 M
Osmolality (mmol kg^{-1})	60.6 ± 1.1	65.8 ± 1.2	73.9 ± 1.8
K^+ (mM)	1.5 ± 0.3	2.7 ± 0.3	6.4 ± 0.9
Na^+ (mM)	0.6 ± 0.5	1.0 ± 1.5	1.7 ± 2.3

Discussion

Energy balance

The mass of sucrose ingested was independent of dietary sucrose concentration, and was similar to that predicted from the metabolic requirements of N. chalybea (Leon and Nicolson 1997). In this species, sucrose is assimilated with 100 % efficiency, according to HPLC measurement of sugars in the excreted fluid (Lotz and Nicolson 1996). Body mass, day-time mass gain, night-time mass loss, and loss of body mass over the 72 hour experiment were the same for all three sucrose concentrations. Therefore, the birds maintain energy balance, compensating for changes in sucrose concentration by adjusting the volumes of solution consumed. Regulation of energy intake over a range of nectar concentrations, as found in N. chalybea, is apparently widespread in nectarivorous birds

Table 1.3: **Nectarivorous bird species that compensate energetically for differences in dietary sugar concentration by adjusting the volume of food ingested, and the ranges in sugar concentration over which they compensate.**

Species	Order	Range of sugar[a] conc.	Duration of test	Reference/s
Nectarinia chalybea (sunbird)	Passeriformes	0.4, 0.8 and 1.2 M	24 hours	present study
N. amethystina (sunbird)	Passeriformes	0.25 and 0.73 M	1.5 hours	Downs 1997
N. famosa (sunbird)	Passeriformes	0.25 and 0.73 M	1.5 hours	Downs 1997
N. afra (sunbird)	Passeriformes	0.25, 0.5 and 1.0 M	24 hours	Lloyd 1991
Promerops gurneyi (sugarbird)	Passeriformes	0.25 and 0.73 M	1.5 hours	Downs 1997
Lichmera indistincta (honeyeater)	Passeriformes	0.4, 0.8, 1.2 and 1.6 M	24 hours	Collins et al. 1980a; Collins 1981
Acanthorhynchus superciliosis (honeyeater)	Passeriformes	0.4, 0.8 and 1.2 M	24 hours	Collins 1981
Anthochaera carunculata (honeyeater)	Passeriformes	0.25, 1.0 and 1.75	> 24 hours	Goldstein and Bradshaw 1998
Trichoglossus haematodus (rainbow lorikeet)	Psittaciformes	0.4 and 1.2 M glucose	48 hours	Karasov and Cork 1996
Sephanoides sephanoides (hummingbird)	Trochiliformes	0.25, 0.5 and 0.75 M	24 hours	Lopez-Calleja et al. 1997

[a] sucrose unless otherwise stated

(Table 1.3). Lloyd (1991) classified Nectarinia afra as a food energy maximizer (Hixon 1982, Schoener 1983) which is limited by a food processing constraint. Similarly, Downs (1997) suggested that two species of sunbird and Gurney's sugarbird (Promerops gurneyi) are limited by digestion processes, as transit times were found to be longer in birds fed 0.73 M than in those fed 0.25 M sucrose. However, neither their data nor the present study indicate that the birds have reached, or are close to reaching, a limit on the amount of energy they can ingest and use. It is more likely that, as metabolic rate changes with activity and ambient temperature, the birds will adjust their intake to balance expenditure. The birds are most simply classified as time minimizers (Hixon 1982, Schoener 1983) which consume just enough energy to meet their 24 h expenditure, and do not exceed this minimum amount, as Lopez-Calleja et al. (1997) suggested recently for the Chilean hummingbird Sephanoides sephanoides.

Although the honeyeater Meliphaga virescens drinks greater volumes of 0.4 than 0.8 M sucrose, it cannot compensate completely and does not maintain body mass on the more dilute solution (Collins and Morellini 1979). In the honeyeater Acanthorhynchus superciliosis, energy intake surprisingly increases slightly as sucrose concentration decreases from 0.82 to 0.37 to 0.20 M sucrose. However, the birds are unable to maintain mass on 0.20 M sucrose, probably because of greater costs involved in processing such dilute food (Collins and Clow 1978, see also chapters 4 and 5 of this thesis). It appears that these species, when fed the most dilute solutions, have either reached, or are close to reaching, a limit on the amount of food they are able to ingest. The data of appendix 3 suggests that N. chalybea cannot compensate energetically below a sucrose concentration somewhere between 0.13 and 0.20 M at 20 °C. At or below this lower limit, these birds probably switch from being time minimisers to energy maximisers, because they now need to feed at their maximal possible rate to meet their 24 h energy requirements.

Food intake in N. chalybea peaked in the first hour of light, decreased gradually to a low around 15:00, and then increased to a secondary peak around 18:00, an hour before dark. This pattern was similar on all three diet concentrations. This is the feeding pattern most typical of small birds, especially passerines, both in captivity and in the wild (Aschoff 1966, Bednekoff and Houston 1994).

A high early morning feeding rate, caused by uncertainty about food supply, has been predicted using an optimisation model (Bednekoff and Houston 1994). Similarly, the models of McNamara et al. (1994) predict a declining feeding rate throughout the day when feeding is not interrupted by bad weather, but predict a secondary afternoon peak when feeding is interrupted.

Unlike N. chalybea, many nectarivorous birds show a pattern of steadily decreasing feeding rate throughout the day, with no secondary afternoon peak (Collins and Clow 1978, Collins and Morellini 1979, Wheeler 1980). The hummingbird Sephanoides sephanoides shows a steadily decreasing feeding rate when fed 0.75 or 0.50 M sucrose, but shows an afternoon peak when fed 0.25 M sucrose (Lopez-Calleja et al. 1997). Similarly, the honeyeater Acanthorhynchus superciliosis shows an obvious decline in feeding rate throughout the day when fed 0.82 or 0.37 M sucrose, but shows an afternoon peak when fed 0.20 M sucrose, and cannot maintain energy balance at this low sucrose concentration (Collins and Clow 1978). These birds are probably increasing their feeding rates towards the end of the day in an attempt to compensate energetically for the low nectar concentrations. Bednekoff and Houston (1994) predict in their model that digestive constraints will cause feeding rates to be spread more evenly through the day. It can be predicted that N. chalybea (and other nectarivores), when fed more dilute sucrose than in this study, or when facing greater energetic demands (for example, lower ambient temperatures, see chapter 3), will display a more steady feeding rate through the day.

Water balance

When the birds compensate energetically for changes in sucrose concentration, their 24 h water gain ranges from 1.9 times body mass, in birds fed 0.4 M sucrose, to 0.7 times body mass in those fed 1.2 M sucrose (Fig. 1.2). The daily water influx of an 8 g bird predicted from allometry (46 species, body mass range 4.5 g to 88.25 kg, $r^2 = 0.89$) is only 0.8 times body mass (Williams et al. 1993). This value is often greatly exceeded in nectarivorous birds. Williams (1993) measured a 24 h water influx of 1.6 times body mass in incubating female N. violacea (9.5 g) using the doubly-labelled water method. Water influx in the honeyeater Acanthorhynchus superciliosus (10 g) has been measured as 2.3 times body mass per day (Goldstein and Bradshaw 1998b), and in wild hummingbirds (4.5 to 9.7 g) as between 0.9 and 2.4 times body mass (Weathers and Stiles 1989). Similarly, water influx in the frugivorous white-eye Zosterops lateralis (9.9 g) varies between 0.6 and 2.2 times body mass, depending on habitat (Rooke et al. 1983). Thermoregulatory implications of the high water influx of nectarivorous birds are considered in chapters 4 and 5.

A large volume of literature addressing the question of why bird-pollinated flowers produce such dilute nectar (which leads to high water flux in nectarivorous birds), has accumulated, although the answer remains unclear. Two major approaches have been used to address the question: 1) determining the preferences of birds for different nectar concentrations, and 2) measuring or modelling the "optimal" nectar concentration, at which energy uptake is maximised, and foraging time is minimised. The first approach fails to shed light on the question, because hummingbirds prefer nectar exceeding 1.6 M (= 45 % w/w) sucrose, twice the concentration of that actually produced on average by hummingbird-pollinated flowers (Stiles 1976, Pyke and Waser 1981, Tamm and Gass 1986). Roberts (1996) suggested that this paradox may be an experimental artefact, from offering birds unrealistically large volumes of nectar during preference experiments. However, when he offered hummingbirds realistically low nectar volumes, the paradox remained, because birds preferred 2.5 M = 65 % sucrose over more dilute solutions. The second approach, which depends on the fact that nectar viscosity, and therefore uptake rate, decreases with decreasing nectar concentration, has yielded contradictory results. In honeyeaters and hummingbirds offered different concentrations but at equal (small) volumes, optimal concentrations during individual visits to flowers lie well above those actually produced by flowers (Tamm and Gass 1986, Mitchell and Paton 1990). However, when the energy reward is held constant by lowering the volume of nectar offered as its concentration is increased, the measured optimum for honeyeaters decreases to 0.6 M = 20 % sucrose, which is indeed similar to the average concentration occurring in honeyeater-pollinated flowers (Pyke and Waser 1981, Mitchell and Paton 1990). Confounding this, Gass and Roberts (1992) showed that as the overhead time (time not actually spent licking), increases, so the optimal nectar concentration for nectarivorous birds increases.

Even when the handling time of flowers is only 0.5 seconds, the modelled optimal nectar concentration exceeds 1.4 M = 40 %. If the handling time is longer, or if the time spent moving between individual flowers and between flower patches is included, the optima shift upwards even further. These results indicate that nectar concentration of ornithophilous flowers did not evolve to maximise the rate of energy gain. Thus, the paradox of why bird-pollinated flowers produce such dilute nectar remains unanswered.

It is clear that the nectar concentrations commonly occurring in the field yield high water influxes, and presumably large water excesses, for nectarivorous birds. Chapters 3, 4 and 5 reveal further challenges, resulting indirectly from high water influx, imposed on nectarivorous birds when feeding on the more dilute nectars they encounter.

Daily excretory water loss was 1.4 times body mass in N. chalybea fed 0.4 M sucrose, 28 times higher than the value predicted from allometry (5 species, body mass range 13 - 149 g, $r^2 = 0.89$; Calder and Braun 1983). The difference between water gain (preformed and metabolic water) and excreted water, which should represent evaporative water loss (EWL), was the same in birds fed 0.8 and 1.2 M sucrose, but was higher in those fed 0.4 M sucrose (Fig. 1.2, see chapter 5).

Nectarinia chalybea (family Nectariniidae) has the same body mass (8 g) as the Australian honeyeater Lichmera indistincta (family Meliphagidae), and Table 1.4 compares these two species' 24-hour water budgets. Data for L. indistincta were obtained from a study by Collins (1981), where birds were kept at 20 °C and fed 0.4, 0.8 or 1.2 M sucrose, as in the present study on N. chalybea. Metabolic water production, preformed water intake, excretory water loss and EWL are slightly higher in L. indistincta than in N. chalybea, but vary similarly with dietary sucrose concentration. In L. indistincta, EWL is similar in birds fed 0.8 and 1.2 M sucrose, but significantly higher in those fed 0.4 M sucrose (Collins 1981) and the same pattern is evident in the calculated values for N. chalybea. Clearly, these two small nectarivorous birds from different families have similar strategies for maintaining water balance. An 8 g hummingbird, on the other hand, may be expected to have a higher water flux, given the same food concentrations, because it hovers while feeding, unlike sunbirds and honeyeaters, which perch while feeding (see General Introduction).

Active bumblebees (Bombus lucorum) feeding on 1.8 M (= 50 %) sucrose must excrete 0.62 times their body mass in 24 h (Bertsch 1984). This is comparable to the value of 0.63 times body mass in N. chalybea feeding on 0.8 M sucrose, and to the values calculated for honeyeaters (Collins 1981) and hummingbirds (Calder and Hiebert 1983) feeding on nectar of between 0.6 and 1.3 M sucrose. The nectarivorous desert bat Leptonycteris sanborni (22 g) excretes 0.53 times its body mass daily when fed 0.6 M sucrose (Carpenter 1969). The water balance of bumblebees feeding on concentrated nectar is comparable to that of nectarivorous birds and bats feeding on substantially more dilute nectar, because their metabolic water production : evaporative water loss ratios are higher (Bertsch 1984). This fits data on nectar concentrations of flowers pollinated by these groups, with bumblebee-pollinated plants producing substantially more concentrated nectar than those pollinated by

Table 1.4: Total 24 h water budgets of Nectarinia chalybea and a honeyeater of the same body mass, Lichmera indistincta (from Collins 1981). All units are ml.

	Preformed	Metabolic[a]	Excreted		Evaporative[b]
			Day	Night	(Day + night)
N. chalybea					
0.4 M	14.07	1.27	11.02	0.06	4.59
0.8 M	6.44	1.27	5.00	0.04	3.00
1.2 M	3.89	1.30	2.68	0.05	2.76
L. indistincta					
0.4 M	16.83	1.57	12.84	-	4.61
0.8 M	10.01	1.50	7.48	-	3.66
1.2 M	4.76	1.73	2.53	-	3.88

[a] refer to "results" for calculation of metabolic water production in N. chalybea. Metabolic water production in the honeyeater was calculated from measured metabolic rates.

[b] refer to "results" for calculation of evaporative water loss in N. chalybea. Evaporative water loss in the honeyeater was measured directly.

birds and bats (Pyke and Waser 1981).

The difference between preformed and excreted water in N. chalybea was high in the first hour of light, and constant for the rest of the day. The honeyeaters Lichmera indistincta and Acanthorhynchus superciliosis show a similar pattern, but with a distinct increase in the last hour of light, in apparent anticipation of the nocturnal fast (Collins 1981). The early morning peak in apparent water retention in N. chalybea results partly from a high preformed water intake and partly from a low water excretion during that hour, both of which probably occur in response to the water deficit incurred during the nocturnal fast.

Excreted fluid osmolalities of sunbirds were extremely low, ranging from 61 to 76 mmol kg^{-1}. These values are only 18 and 23 %, respectively, of typical avian plasma concentration (332 mmol kg^{-1}, Skadhauge 1981) and excreted fluid : plasma ratios of sunbirds resemble those of wild hummingbirds (Calder and Hiebert 1983), as well as the urine : plasma ratios of freshwater fish and amphibians (Dantzler 1989). Terrestrial vertebrates typically have urine : plasma ratios of around 100 % when drinking water is available ad. lib. (Goldstein and Braun 1986, Dantzler 1989). Nicolson and Louw (1982) measured a urine : plasma ratio of 29 % in carpenter bees (Xylocopa capitata) feeding on 2.3 M (= 60 %) Virgilia divaricata nectar (mainly sucrose). Once again, insects feeding on concentrated nectar resemble birds feeding on substantially more dilute nectar.

The concentrations of K^+ and Na^+ in the excreted fluid of N. chalybea were also exceptionally low. Nectar containing the same cation concentrations as the excreted fluid would more than replace excreted cations, because nectar volumes consumed are greater than volumes excreted. Thus, nectar equivalent to 1.2 M sucrose would need less than 7 mM K^+ and 2 mM Na^+. Cation concentrations in nectars used by N. chalybea vary widely, but most contain adequate electrolytes. This is discussed in detail in chapter 2.

The sum of the K^+ and Na^+ concentrations, multiplied by two to account for assumed monovalent anions, $2 * K^+ + Na^+$, accounts for only 7 to 22 % of the total osmolality of the excreted fluid, depending of the dietary sucrose concentration. At least some of the difference may be accounted for by undigested sugar in the excretory fluid. Indeed, Lotz and Nicolson (1996) measured 0.62 % sucrose equivalents in the excretory fluid of N. chalybea fed 0.632 M (= 20 %) sucrose (which translates to a 97 % assimilation efficiency for sucrose), and this would account for 19.6 mM, or 27 to 32 % of the total osmolality of the excreted fluid of the sunbirds in the present study. Apart from undigested sucrose, nitrogenous waste probably also contributed significantly to the total osmolality of the excreted fluid. Current nitrogen balance work on the orange-tufted sunbird (N. osea) in Israel (B. Pinshow and L. Roxburgh), should help to quantify the contribution of nitrogenous waste.

Chapter 2

The ability of Nectarinia chalybea to conserve or excrete electrolytes while feeding on dilute nectar

Summary

Favoured food plants of Nectarinia chalybea produce nectar with K^+ and Na^+ concentrations ranging between 2 and 19 mM of each cation. Sunbirds will need to precisely regulate the amounts of cations they excrete to maintain electrolyte balance. When feeding on energetically dilute nectar and excreting large preformed water excesses, sunbirds will need to conserve electrolytes, especially when the nectar contains cationic concentrations at the lower extreme of those produced in food plants (2 mM). To determine the ability of N. chalybea to conserve or excrete electrolytes while feeding on dilute nectar, sunbirds were fed pure 0.4 M sucrose without electrolytes for 1 h, then 0.4 M sucrose with 15 mM each of KCl and NaCl for 2 h, and finally pure 0.4 M sucrose again for 2 h. The birds excreted only 0.4 mM K^+ and 1.6 mM Na^+ when fed electrolyte-free sucrose, and should thus be able to maintain electrolyte balance even on nectars with the lowest cation concentrations occurring in the field. When fed 15 mM of each electrolyte, sunbirds exactly maintained cation balance by excreting 17 mM of each ion.

Introduction

The sunbird Nectarinia chalybea is abundant in the fynbos biome of the south-western Cape, South Africa (Harrison et al. 1997), where favourite food plants include various species of Protea, Leucospermum and Erica (Skead 1967, Harrison et al. 1997). The nectar of seven species of Protea contain K^+ and Na^+ concentrations averaging 19.0 ± 3.8 and 17.8 ± 1.5 mM respectively (S. W. Nicolson, unpublished data), similar to that of five Leucospermum species (Nicolson and Worswick 1990). The nectar of five Erica species contains lower cation concentrations: 4.9 ± 1.5 and 3.5 ± 0.2 mM (S. W. Nicolson, unpublished data). In the fynbos biome and other mesic habitats in which N. chalybea occurs, this and other sunbird species are known to congregate around stands of Leonotus leonurus (Frost and Frost 1980, Harrison et al. 1997, C. N. Lotz, personal observation), the nectar of which contains K^+ and Na^+ concentrations of 2.4 and 2.3 mM respectively (S. W. Nicolson, unpublished data).

Nectarinia chalybea also occurs in semi-desert, where the nectar of Aloe species is an important food source (Harrison et al. 1997). The K^+ and Na^+ concentrations in the nectar of nine Aloe species average 8.6 ± 4.5 and 3.5 ± 0.6 mM (S. W. Nicolson, unpublished data).

Nectarivores have to excrete large preformed water excesses (see general introduction and chapter 1), and are uniquely challenged in their ability to maintain electrolyte balance. Carpenter bees, which excrete copious, dilute fluid, have difficulty in maintaining electrolyte balance from the low cationic concentrations present in their nectar (Nicolson 1990). It is unclear whether hummingbirds can maintain electrolyte balance from their nectar, although the ionic concentrations in their excretory fluid are correlated with those in the nectar they have been feeding on (Calder and Hiebert 1983).

When feeding on dilute nectar (equivalent to 0.4 M sucrose), N. chalybea has to excrete a preformed water excess of almost twice its body mass daily (chapter 1). Considering the low cationic concentrations of some of its favourite nectars, N. chalybea should have an ability to conserve electrolytes. At the same time, it must be able to regulate its electrolyte balance precisely, considering the variation in the cation concentrations in the nectar of its favourite food plants. The aim of the present experiment is to examine the ability of N. chalybea to conserve or excrete electrolytes while feeding on dilute nectar. Changes in cation excretion were monitored when 15 mM each of KCl and NaCl were included in a diet of 0.4 M sucrose.

Materials and methods

Two male and three female N. chalybea were used during these experiments. Prior to each experiment, birds were fed an acclimation diet of 12.5 g sucrose and 3.1 g Ensure® (Abbott Laboratories) per 100 ml of water, giving a concentration of 0.37 M sucrose excluding Ensure®. Ensure® is characterized in appendix 1. Complan®, used during the experiment of chapter 1, was replaced with Ensure® during the present and all further experiments because the former could no longer be purchased. As in the experiments of chapter 1, the constant environment room was set at 20 °C, 65 % r.h. and a 12L:12D photoperiod (with lights-on at 07:30). The same cages and feeders used for chapter 2 were again used. Birds were transferred individually to the constant environment room at 14:30 the day prior to experimentation and fed 0.4 M sucrose. At 09:30 the following day (two hours after lights-on), measurements were started. Birds remained on pure 0.4 M sucrose until 10:30. Birds were then fed 0.4 M sucrose containing 15 mM KCl and 15 mM NaCl for two hours, until 12:30. From 12:30 until 14:30, birds were again fed pure sucrose without electrolytes. This 5 h measurement period was started at 09:30 to ensure that birds' water and electrolyte flux had reached typical day-time levels, after 2 h of feeding since lights-on.

Only one bird was experimented upon at a time. Every 5 minutes during the 5 h measurement period, sucrose volumes consumed were recorded, and the trays underneath the cages containing liquid paraffin were exchanged so that excretory fluid could be collected outside the constant environment room to minimize disturbance to the bird. The constant environment room was only entered for a few seconds during each 5 minute period. Excretory fluid was frozen, and was later thawed, weighed, and its osmolality, K^+ and Na^+ concentrations measured, using the same methodology as in chapter 1.

The amounts (in µmoles) of ions excreted were calculated by multiplying the concentrations (in mM) by the volumes (in ml) of water excreted.

Repeated measures ANOVA's were performed to test for differences in the hourly volumes of preformed water consumed, of water excreted, and of the difference between preformed and excreted water, between the initial 1 h of the experiment (with no electrolytes in the diet), the following 2 h (with electrolytes in the diet), and the final 2 h (with no electrolytes in the diet). These tests were performed using STATISTICA (Statsoft, Tulsa), following the methodology of Zar (1996). Data are reported as the mean ± SE.

Results

The hourly volume of preformed water consumed was significantly higher in the 2 h period during which electrolytes were included in the diet, 1.87 ± 0.17 ml h^{-1}, than in the 1 and 2 h periods during which birds were fed pure sucrose solution without electrolytes, 1.42 ± 0.17 and 1.58 ± 0.16 ml h^{-1} ($P = 0.035$, see Fig. 2.1 and Appendix 2.3). However, the hourly volumes excreted were not significantly different during the initial 1 h period (no electrolytes), the middle 2 h period (electrolytes included), and the final 2 h period (no electrolytes): 1.17 ± 0.11, 1.32 ± 0.09 and 1.17 ± 0.12 ml h^{-1} ($P = 0.279$, Fig. 2.1, Appendix 2.3). During the first ten minutes after electrolytes were included in the diet, there was a distinct low in the volumes of fluid consumed and excreted, but this was compensated for by a peak immediately thereafter (Fig. 2.1, Appendix 2.3). The difference between the hourly volumes of preformed and excreted water was significantly higher in the middle 2 h of the experiment (electrolytes included), 0.55 ± 0.15 ml, than in the first 1 h of the experiment (no electrolytes), 0.25 ± 0.24 ml ($P = 0.017$), but was not significantly different from that in the final 2 h of the experiment, 0.41 ± 0.18 ($P > 0.179$). This must have been due to differences in evaporative water loss and/or short-term retention of water.

During the initial 1 h period in which sunbirds were fed pure 0.4 M sucrose solution without electrolytes, the osmolality of the excreted fluid averaged 59.30 ± 0.38 mmol kg^{-1} (Fig. 2.2, Appendix 2.3), the same as that measured in birds fed 0.4 M sucrose in the experiments of chapter 1. When electrolytes were included in the diet, the osmolality immediately started to increase, within the first 5 minutes, until it reached a stable, maximal level of 96.02 ± 0.72 mmol kg^{-1} 1 h later. This level was maintained for a

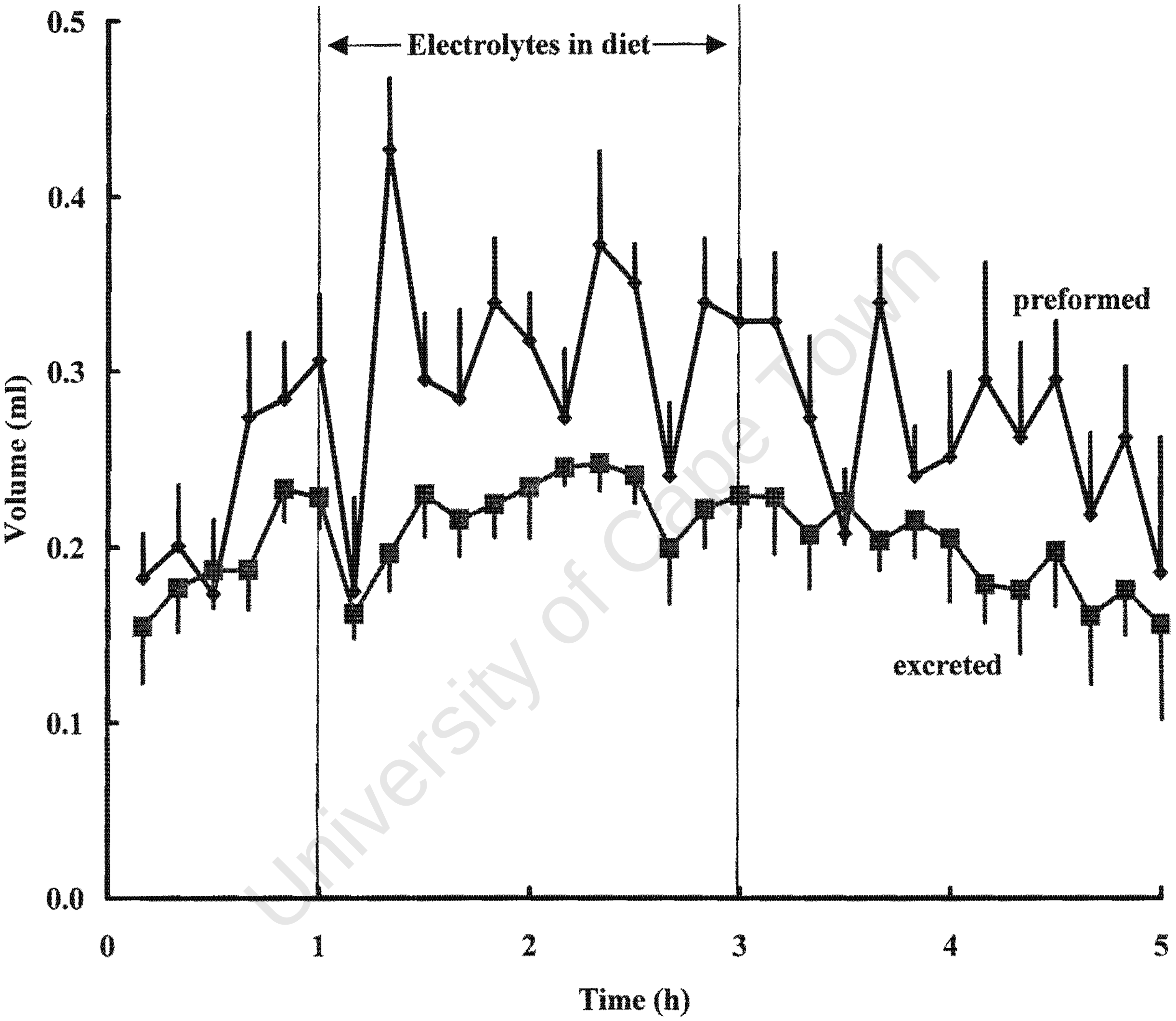

Fig. 2.1: Volumes of preformed and excreted water during the 5 h experiment. For clarity, every two consecutive 5 min intervals were summed, so that the water volumes for each 10 minute interval are shown on the graph. Points are mean ± SE, n = 5.

further 1 h, until electrolytes were again excluded from the diet. After 2 h with no electrolytes, the osmolality returned to initial levels.

The concentrations of K^+ and Na^+ excreted in the first 1 h of the experiment, before electrolytes were included in the diet, were exceptionally low, 0.40 ± 0.03 mM in the case of K^+ and 1.55 ± 0.13 mM in the case of Na^+ (Fig. 2.3, Appendix 2.3). When 15 mM KCl and 15 mM NaCl were included in the diet, the concentration of Na^+ in the excreted fluid started to increase immediately (within the first 5 minutes), and reached a maximal level of about 20 mM after 1 h. In contrast to this, the K^+ concentration only started to increase about 30 minutes after electrolytes had been included in the diet, and took 5 minutes longer to reach maximal levels of 20 mM. After electrolytes were again excluded from the diet, the excreted concentrations of both cations started to decrease immediately (within the first 5 minutes), and reached initial levels by the end of the 5 h experiment.

The sum of the K^+ and Na^+ concentrations, multiplied by two to account for assumed monovalent anions, 2 * (K^+ and Na^+), was initially 3.9 mM before electrolytes were included in the diet, but increased by 68.7 to 72.6 mM after electrolytes were included in the diet. The osmolality of the excreted fluid, on the other hand, only increased by 36.7 mmol kg^{-1} after electrolytes were included in the diet, from 59.3 to 96.0 mmol kg^{-1}. Thus the "solute gap" was considerably smaller when electrolytes were present in the diet (refer to Fig. 2.2 and Appendix 2.3).

In the initial 1 h of the experiment, when the five birds were not ingesting electrolytes, they excreted an average of 0.42 ± 0.06 μmoles of K^+ and 1.72 ± 0.33 μmoles of Na^+ (Table 2.1). In the following 2 h period, birds ingested 51.30 μmoles of KCl and the same amount of NaCl. During this 2 h period, plus the following 2 h period in which electrolytes were again excluded from the diet, birds excreted 43.09 ± 4.11 μmoles of K^+ and 51.84 ± 2.73 μmoles of Na^+. By the end of the experiment the concentrations of both ions had reached minimal levels, and yet 8.21 ± 3.07 μmoles of K^+ had been retained. In contrast, the sunbirds had excreted marginally (0.54 ± 3.39 μmoles) more Na^+ than they consumed after this 4 h period. However, individual birds had retained as much as 11.73 μmoles, or had lost as much as 8.07 μmoles of Na^+ after the 4 h.

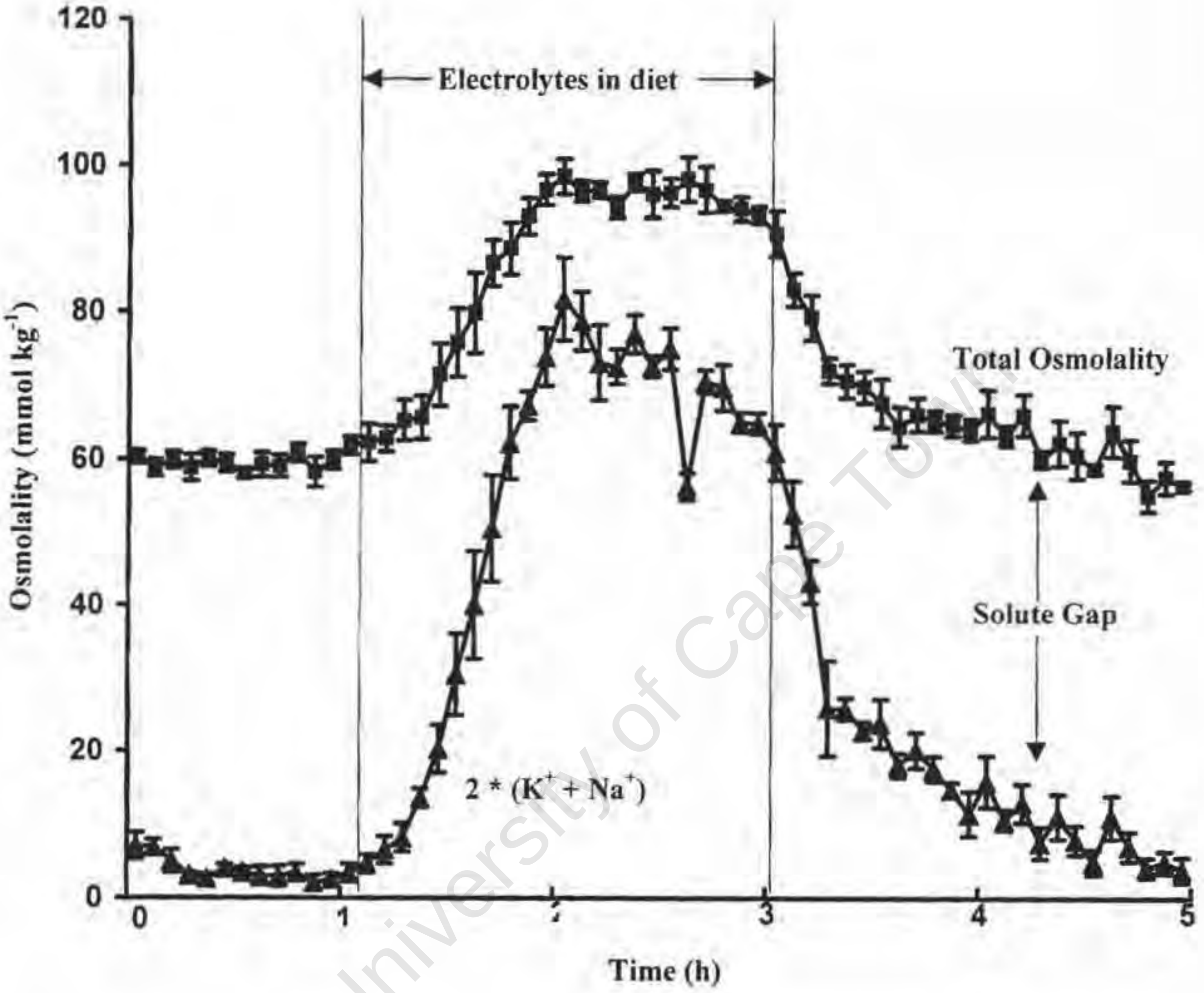

Fig. 2.2: Osmolality (mmol kg^{-1}) of excreted fluid of <u>N. chalybea</u>, measured every 5 minutes during the 5 h experiment, and the sum of the K$^+$ + Na$^+$ concentrations, multiplied by 2 to account for assumed monovalent anions (mM). The "solute gap" is indicated. See text for details. Points are mean ± SE, n = 5.

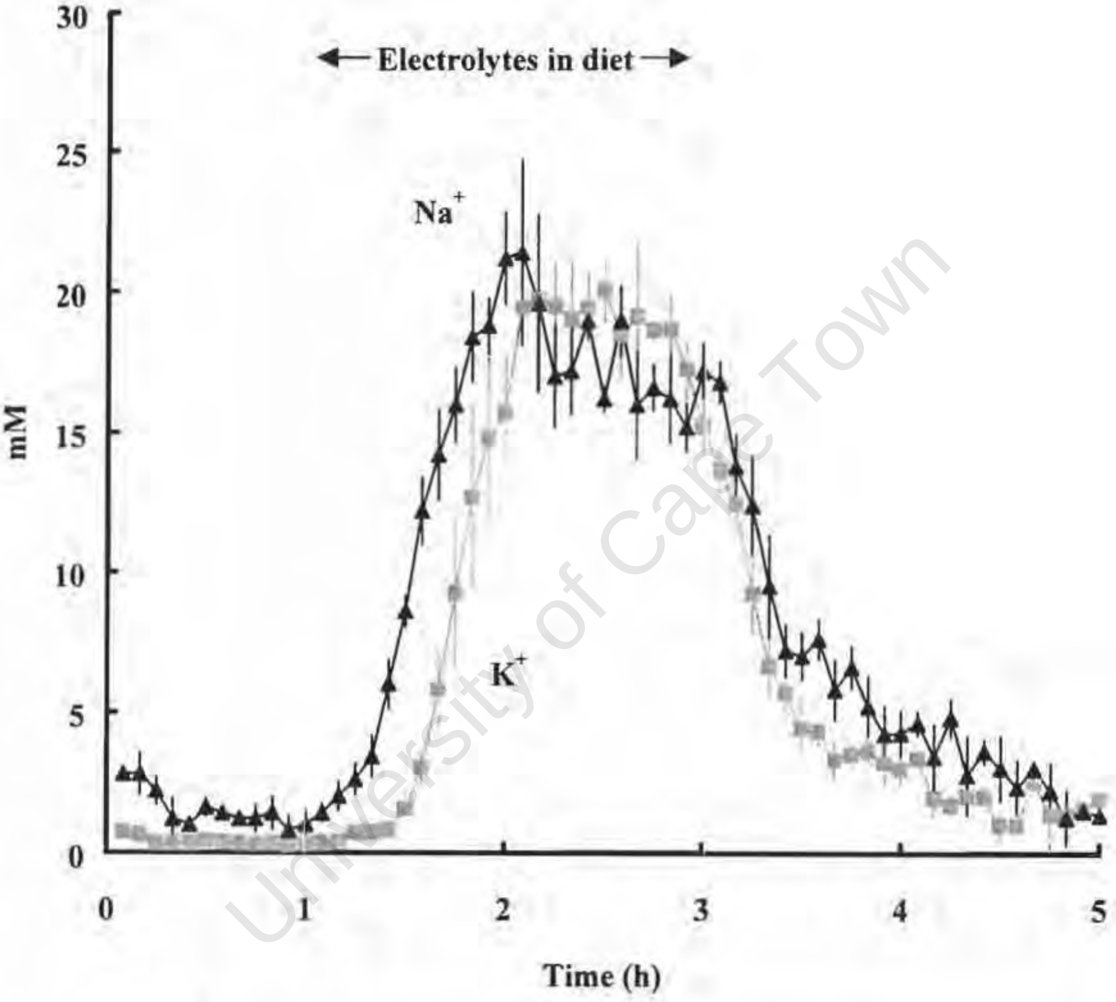

Fig. 2.3: K^+ and Na^+ concentrations (mM) of excreted fluid of N. chalybea, measured every 5 minutes during the 5 h experiment. Points are mean ± SE, n = 5.

Table 2.1: The amounts (μmoles) of K^+ and Na^+ consumed, excreted and retained during different stages of the 5 h experiment.

			K^+		
Bird no.	**Int h1[a]**	**Exc, h1[a]**	**Int h2-5**	**Exc, h2-3**	**Int - Exc, h2-5**
1	0	0.34	41.25	35.02	6.23
2	0	0.57	60.00	40.10	19.9
3	0	0.25	60.75	58.68	2.07
4	0	0.48	51.00	43.06	7.94
5	0	0.48	43.50	38.57	4.93
Ave ± SE	0	0.42 ± 0.06	51.30 ± 4.04	43.09 ± 4.11	8.21 ± 3.07
			Na^+		
Bird no.	**Int, h1**	**Exc, h1**	**Int h2-5**	**Exc, h2-3**	**Int - Exc, h2-5**
1	0	1.25	41.25	44.15	-2.90
2	0	2.58	60.00	48.27	11.73
3	0	0.80	60.75	59.84	0.91
4	0	2.27	51.00	55.39	-4.39
5	0	1.69	43.50	51.57	-8.07
Ave ± SE	0	1.72 ± 0.33	51.30 ± 4.04	51.84 ± 2.73	-0.54 ± 3.39

[a] Int = Intake, Exc = Excretion, h = hour

Discussion

Nectarinia chalybea excreted exceptionally low concentrations of K^+ and Na^+ when feeding on pure sucrose: 0.40 ± 0.03 and 1.55 ± 0.13 mM respectively. Similarly low concentrations were measured in the experiments of chapter 1 after 48 h of feeding on pure 0.4 M sucrose: 1.5 mM K^+ and 0.6 mM Na^+. The ionic concentrations measured during the experiments of chapter 1 increased as dietary sucrose concentration increased from 0.4 to 0.8 to 1.2 M sucrose, but only reached 6.4 mM K^+ and 1.7 mM Na^+ even when birds were fed the most concentrated diet (Table 1.2 of chapter 1). When sunbirds were fed pure 0.4 or 1.2 M sucrose at 10, 20 or 30 °C during the experiments of chapter 3, the excreted fluid K^+ and Na^+ concentrations did not exceed 3.3 and 1.3 mM respectively (Table 3.3). These values probably represent the minimal ionic concentrations that sunbirds are able to excrete while feeding on particular sucrose concentrations, assuming that after 48 h of feeding on electrolyte-free food the birds are maximally conserving electrolytes.

Nectarinia chalybea will easily gain adequate K^+ and Na^+ from nectar containing at least these minimal ionic concentrations they are able to excrete, because preformed water volumes consumed exceed water volumes excreted (Fig. 2.1, chapter 1). In Fig. 2.4, these minimal K^+ and Na^+ concentrations that sunbirds can excrete, together with the K^+ and Na^+ concentrations measured in the nectars of 25 flower species from 6 different genera (Nicolson 1990, Nicolson and Worswick 1990, S. W. Nicolson, unpublished data), all favoured food plants of N. chalybea (Skead 1967, Harrison et al. 1997), are plotted as a function of sucrose concentration. All of these flower species, except for Erica glandulosa, have nectar with K^+ and Na^+ concentrations that match or exceed the minimal concentrations that N. chalybea can excrete, thus providing adequate amounts of these ions for electrolyte balance. Erica glandulosa produces nectar equivalent to 1.2 M sucrose, with 2.3 mM K^+ (S. W. Nicolson, unpublished data), only a third of the K^+ concentration excreted by sunbirds fed pure 1.2 M sucrose during the experiment of chapter 1. However, E. glandulosa normally occurs in association with a host of other plant species in the fynbos biome, especially with other Erica species, and with species of Proteaceae. Four species of Erica excluding E. glandulosa produce nectar with K^+ and Na^+ concentrations averaging 5.5 ± 1.8 and 3.5 ± 0.3 mM, and seven species of Protea produce nectar with ionic concentrations averaging 19.0 ± 3.8 and 17.8 ± 1.5 mM (Fig. 2.4, S. W. Nicolson, unpublished data). Sunbirds feeding from a range of flower species (typical of N. chalybea, C. N. Lotz, personal observation), occurring together in the species-rich fynbos would probably need to excrete excess electrolytes rather than to conserve them, even without supplementary insect feeding.

Calder and Hiebert (1983) captured four hummingbird species and collected excretory fluid "for 24 h" while they were fed pure 0.6 M sucrose. The K^+ and Na^+ concentrations measured in their excretory fluid are shown in Table 2.2. Unfortunately, excretory fluid produced immediately after capture was not separated from that produced later, so the values reported probably lie in between the values for wild hummingbirds feeding on natural nectar (which are substantially higher, Table 2.2), and the minimal values that would be reached after acclimation to pure sucrose. It is nevertheless clear that hummingbirds would be able to gain adequate K^+ from a seasonally-dominant flower species of the study site, Delphinum nelsoni, because its nectar contains 39 mM of this cation (Hiebert and Calder 1983). On the other hand, it is unclear whether hummingbirds

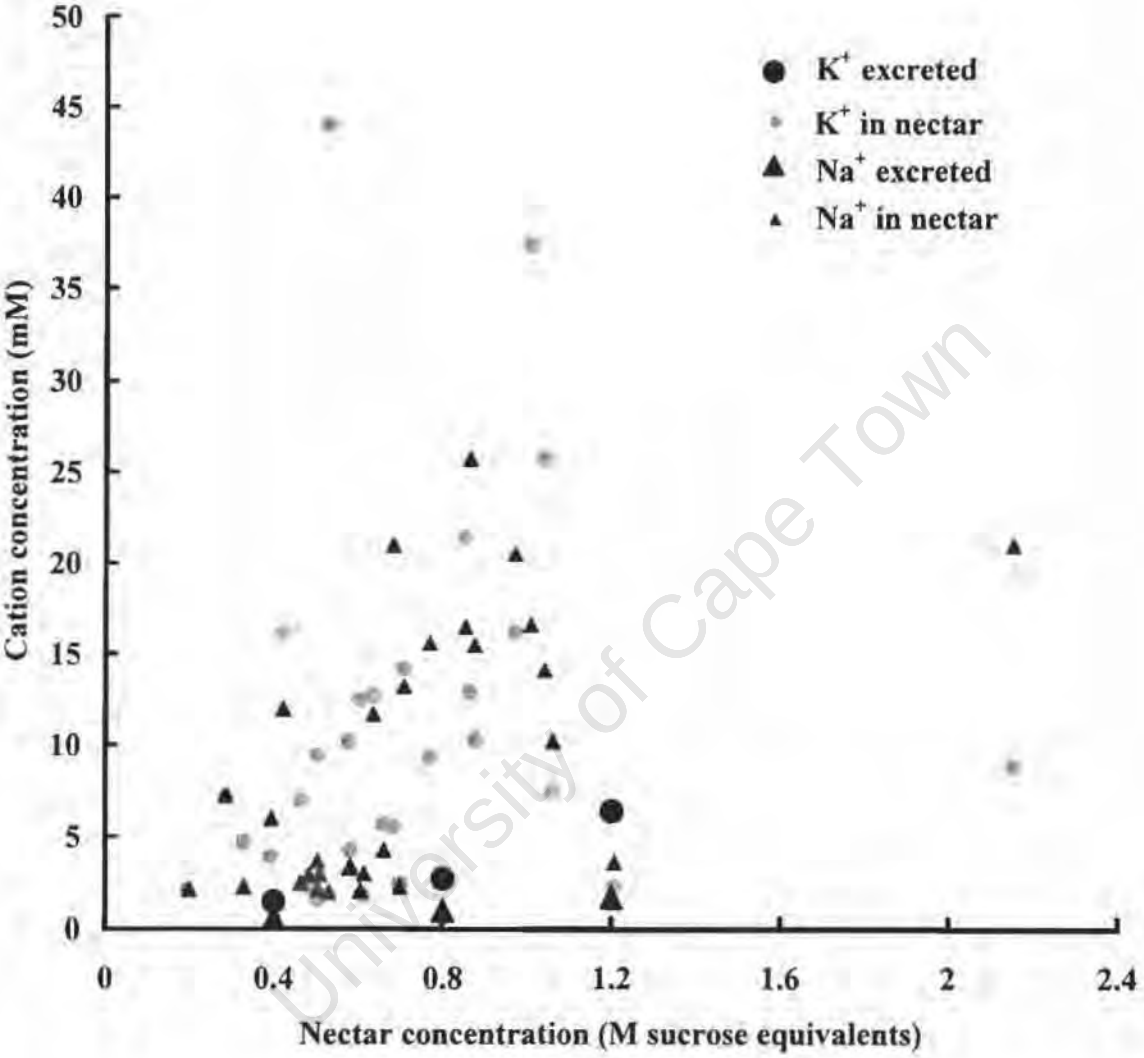

Fig. 2.4: Minimal K^+ and Na^+ concentrations excreted by N. chalybea kept at 20 °C and fed 0.4, 0.8 or 1.2 M nectar (in sucrose equivalents, from chapter 1), and K^+ and Na^+ concentrations (mM) in nectar of food plants of N. chalybea, plotted as a function of nectar concentration (in sucrose equivalents).

would gain adequate Na^+ from the nectar of this flower species, because its Na^+ concentration is only 2.1 mM. The predominant flower species of the study site later on in the year, Ipomopsis aggregata, produces nectar containing only 3.6 mM of K^+ and 2.0 mM of Na^+ (Hiebert and Calder 1983), and it is unclear whether hummingbirds would be able to maintain either K^+ or Na^+ balance from its nectar. The ionic concentrations of 16 other hummingbird-pollinated flower species average 23.4 ± 5.7 mM K^+ and 3.9 ± 1.2 mM Na^+ (Hiebert and Calder 1983).

Table 2.2: Concentrations (mM) of K^+ and Na^+ measured in the excretory fluid or ureteral urine of captive or wild nectarivorous animals feeding on different diets. The table lists all values that could be found in the literature.

Species	Treatment	Excretory fluid		Ureteral urine		Reference
		K^+	Na^+	K^+	Na^+	
Sunbird:						
N. chalybea	0.4 M	0.4 - 1.5	0.6 – 1.6	-	-	Present chapter, chapter 1
N. chalybea	0.8 M	2.7	1.0	-	-	Chapter 1
N. chalybea	1.2 M	6.4	1.7	-	-	Chapter 1
N. chalybea	0.4 M; 15 mM KCl, NaCl	16 - 20	16 – 20	-	-	Present chapter
Hummingbirds:						
4 species	0.6 M	3.6 - 7.7	4.5 – 8.7	-	-	Calder and Hiebert (1983)
Selasphorus platycercus	0.6 M, 4 mM KCl	5.5	1.5	-	-	Calder and Hiebert (1983)
S. platycercus	0.6 M, 4 mM NaCl	5.0	5.5	-	-	Calder and Hiebert (1983)
S. platycercus	0.6 M, 38 mM KCl	115.0	8.4	-	-	Calder and Hiebert (1983)
S. platycercus	0.6 M, 38 mM NaCl	29.4	100.2	-	-	Calder and Hiebert (1983)
4 species	Wild	17.9	5.2	-	-	Calder and Hiebert (1983)

Table 2.2 (cntd)

Species	Treatment	Excretory fluid		Ureteral urine		Reference
		K^+	Na^+	K^+	Na^+	
Hummingbirds (cntd):						
2 species	wild, suburban	13.1	16.9	-	-	Calder and Hiebert (1983)
Honeyeaters:						
Anthochaera carunculata	0.25 mmol kg^{-1}, 4/4 mM K^+/Na^+	-	-	9.2	20.9	Goldstein and Bradshaw (1998a)
A. carunculata	1.00 mmol kg^{-1}, 12/15 mM K^+/Na^+	-	-	20.8	46.3	Goldstein and Bradshaw (1998a)
A. carunculata	1.75 mmol kg^{-1}, 23/30 mM K^+/Na^+	-	-	28.0	39.9	Goldstein and Bradshaw (1998a)
A. carunculata	Wild	38	103	-	-	Goldstein and Bradshaw (1998b)
Phylidonyris novaehollandaiae	Wild	53	29	-	-	Goldstein and Bradshaw (1998b)
Manorina flavigula (insectiv.)	Wild	68	48	-	-	Goldstein and Bradshaw (1998b)
Carpenter bee:						
Xylocopa capitata	Wild	9.9	11.2	-	-	Nicolson and Louw (1982)

Anthochaera carunculata honeyeaters maintain the same plasma K^+ and Na^+ concentrations irrespective of whether they are fed 1.00 or 1.75 mmol kg^{-1} artificial nectar containing 12/15 mM and 23/30 mM of K^+/Na^+ respectively. However, when fed 0.25 mmol kg^{-1} nectar containing K^+/Na^+ concentrations of 4/4 mM, birds no longer maintain the same plasma cation concentrations (Goldstein and Bradshaw 1998b). Data on the cation concentrations in the nectar of flowers pollinated by these honeyeaters will shed more light on whether they are able to maintain electrolyte balance from nectar.

Nectarivorous insects are faced with the same challenge of maintaining ionic balance as nectarivorous birds, because of their shared diet which is low in electrolytes, but which yields high water influx and excretion rates (see chapter 1). Carpenter bees, Xylocopa capitata, excrete fluid of 9.9 mM K^+ and 11.2 mM Na^+ while foraging on Virgilia divaricata flowers (Table 2.2). These flowers secrete nectar of only 1.4 mM K^+ and 3.8 mM Na^+ (Nicolson and Louw 1982). This means that bees would have to excrete only 14 % (1.4 / 9.9 mM) of the volume of water they consumed if they were to maintain K^+ balance exclusively from nectar (34 % for Na^+ balance). However, during flight, metabolic water production exceeds evaporative water loss in these bees (Nicolson and Louw 1982), so that excretory water loss would actually be greater than preformed water intake (assuming that preformed + metabolic water gain = evaporative + excretory water loss). Unless carpenter bees drastically reduced their ratio of metabolic water production : evaporative water loss when not flying, they would be unable to maintain ionic balance from their nectar. Although female carpenter bees supplement their diet with cation-rich pollen, male bees feed solely on nectar (Nicolson 1990), and would thus seem unable to maintain ionic balance. Nicolson (1990) showed that male carpenter bees have remarkably low Na^+ contents in comparison to other insects, and suggested that bees' total Na^+ content may decline with age, even though the concentration of this ion in their haemolymph is maintained. There is similar evidence that butterflies (Arms et al. 1974) and bees (Barrows 1974, Nicolson and Worswick 1990) may have difficulty in maintaining Na^+ balance from their nectar.

During the present experiment, when 15 mM KCl and 15 mM NaCl were included in the diet of sunbirds, the concentrations of these ions in the excreted fluid increased until reaching stable levels of between 16 and 20 mM. The sunbirds consumed 1.42 ml h^{-1} of preformed water and excreted 1.23 ml h^{-1}, averaged over the 5 h experiment. To maintain electrolyte balance while continuously feeding on 0.4 M sucrose with 15 mM of each cation, these birds would therefore have to excrete fluid with 1.42 / 1.23 = 1.15 times the ionic concentrations of the solution they consume, which translates to a value of 17.3 mM, indistinguishable from that actually measured. This means that sunbirds feeding on 0.4 M sucrose containing KCl and NaCl were exactly maintaining cation balance.

The concentrations of K^+ and Na^+ excreted by wild sunbirds should give an indication of diet, because it is clear from the present experiment that the amounts of these ions excreted closely resemble those consumed. In particular, the contribution of arthropods to the diet of these sunbirds can be estimated by measuring the concentrations of ions in the excreted fluid of freshly-caught birds. A diet consisting purely of arthropods is likely to yield a Na^+ : H_2O flux ratio somewhere around 0.032 mM ml^{-1}, as measured in the insectivorous honeyeater Manorina flavigula (Goldstein and Bradshaw 1998b). The exact value will depend on the particular Na^+: H_2O ratios of the arthropods that the birds are feeding on. These Na^+: H_2O ratios can be obtained by analyzing captured insects, or estimated from literature values for different insects, such as those published by Nicolson

(1990). Knowledge of the nectar Na^+ concentrations of the food plants of sunbirds in a particular area, combined with measurement of the Na^+ concentrations of fluid excreted by freshly-caught sunbirds, will allow calculation of the percentage of water contributed by each food source. Knowledge of the average water contents of the nectar and of arthropods would then allow calculation of the percentage in terms of mass that each food contributes to the diet. The water content of the nectar in a particular area can easily be measured using a hand-held refractometer (Nicolson and Worswick 1990). The water content of most arthropods can be assumed to be around 77 % (Peters 1983), but literature values of the water contents of particular insect orders can be used to yield more accurate results, if the insect orders that the birds are feeding on can be narrowed down.

Apart from having to estimate the ionic concentrations of insect versus nectar food, this proposed method of evaluating the contribution of insects to the diet of sunbirds makes a number of assumptions. It assumes that birds do not drink. This assumption is probably seldom violated in N. chalybea, because these birds apparently consume excess preformed water even when they are feeding on concentrated (1.2 M sucrose) nectar at an ambient temperature as high as 30 °C (see chapter 3). Moreover, sunbirds are rarely observed drinking, except in particularly hot weather (C. N. Lotz, personal observation). A second assumption of this method is that the ratio of excreted water: evaporative water loss is the same in the field as it is in the laboratory. If this assumption is violated, then the same influx of water and ions could result in different patterns of excretory fluid composition. It is indeed plausible that the rate of evaporative water loss will be higher in wild birds, because they are more active, than in captive ones (see chapter 3), reducing the ratio of excreted water: evaporative water loss. This would yield higher K^+ and Na^+ concentrations in the excretory fluid of wild sunbirds than of laboratory ones, at a particular nectar concentration, because smaller volumes of more concentrated fluid would be excreted. Knowledge of the evaporative water loss measured in birds performing different activities in the laboratory, combined with time-energy budget data (Wolf 1975, Gill and Wolf 1975, Frost and Frost 1980), will allow correction to be made for violation of this assumption. This method of evaluating the diet of birds can indeed usefully be combined with time-energy budget studies. A third assumption of this method is that there is no temporal variation in the ionic composition of the excreted fluid. This assumption can easily be tested and corrected for by taking more than one sample, at different times. A fourth assumption of this method is that the composition of the excretory fluid does not change with capture and/or handling of the birds. This can easily be tested by capturing birds in the laboratory, analyzing their excretory fluid, and seeing whether it differs from the excretory fluid collected from birds as during the present experiment.

Accurate and unbiased quantification of the proportion of arthropods in the diet of nectarivores, although important information, has proved difficult using classical methodology such as time-energy budgets (Pyke 1980, Paton 1982). It is also possible to accurately measure the number of arthropods consumed using stomach dissection (as done on the sugarbird Promerops cafer by Mostert et al. 1980), but this involves killing birds, and moreover it cannot quantify nectar intake (or the relative role of insects and nectar in the diet). Therefore, this proposed new method may be useful, especially when it has been tested more. It is technically easy, and can be usefully combined with time-activity budget studies.

At Cape Point Nature Reserve, N. chalybea and another sunbird species, N. famosa, congregate around a large monospecific stand of Leonotus leonurus during the flowering season (C. N. Lotz, personal observation). This flower species contains nectar with a sugar concentration of 0.7 M (in sucrose equivalents), and K^+ and Na^+ concentrations of 2.4 and 2.3 mM (S. W. Nicolson, unpublished data). Whereas N. famosa appears to be territorial around the flowering stand, N. chalybea probably only moves through it while foraging over a much wider area (C. N. Lotz, personal observation, Skead 1967, Harrison 1997), and feeding additionally from other fynbos flower species. Measurement of the K^+ and Na^+ concentrations in the excretory fluid of both sunbird species may give a more quantitative understanding of the relative foraging site fidelities of these two species, and/or of the extent of arthropod feeding. It takes 1 h for the cation concentrations in the excreted fluid of N. chalybea to decrease from 17 mM to minimal levels after feeding on sucrose solution containing 15 mM KCl and 15 mM NaCl (Fig. 2.3), and therefore sunbirds should excrete fluid containing about 2.5 mM of each cation as long as they have spent at least 1 h foraging solely from L. leonurus nectar, even if they had previously been excreting 17 mM (possibly from Protea feeding). Both sunbird species are easily mist-netted at this site (C. N. Lotz, personal observation).

Cationic concentrations excreted by captive Selasphorus rufus hummingbirds fed 4 mM KCl or 4 mM NaCl were indistinguishable from baseline levels when the diet contained no electrolytes (around 5 mM, Table 2.2), and this would allow birds to maintain ionic balance. However, when 38 mM KCl or 38 mM NaCl were included in the diet, these hummingbirds excreted 2.6 and 3.0 times the concentrations of K^+ and Na^+, respectively, that they consumed (Table 2.2). These birds must have been excreting far too much K^+ and Na^+ to maintain ionic balance, because hummingbirds excrete between 80 and 90 %

of the preformed water they consume (Beuchat et al. 1990). Similarly, although the K^+ concentrations measured in the excretory fluid of freshly-caught S. platycercus and S. rufus were significantly correlated with the average K^+ concentrations of the plants flowering in the area, the excretory fluid concentrations were 8.2 and 13.2 times higher than the nectar concentrations (Calder and Hiebert 1983). Further study is needed to resolve the paradox of why hummingbirds apparently excrete concentrations of electrolytes far too high to maintain ionic balance, before measurement of the K^+ and Na^+ concentrations in the excretory fluid of wild hummingbirds can be used to indicate diet. Without further study, the K^+ and Na^+ concentrations measured in the excretory fluid of wild hummingbirds and honeyeaters (Table 2.2) are difficult to interpret. This is in contrast to the results of the present experiment on the sunbird N. chalybea, in which birds exactly balanced their excretion of cations while feeding on 0.4 M sucrose containing 15 mM each of KCl and NaCl by excreting 17 mM of each cation. However, even for N. chalybea, it would be informative to repeat the present experiment using different dietary electrolyte concentrations.

During the electrolyte-loading experiment on N. chalybea, differences between K^+ and Na^+ excretion were detected. The concentration of Na^+ in the excreted fluid already started to increase during the first 5 minutes after 15 mM each of KCl and NaCl were included in the diet, whereas there was a delay of about half an hour before the concentration of K^+ started to increase (Fig. 2.3, Appendix 2.3). By the end of the 5 h experiment, none of the Na^+ that the birds had consumed was retained. In fact, marginally more of this ion had been excreted than retained, on average, by the end of the experiment, and in one individual bird the deficit was as much as 8.1 μmoles (Table 2.1). In contrast, because of the initial delay in the excretion of K^+, sunbirds had retained 8.2

μmoles of this cation, on average, by the end of the 5 h experiment. The lowest amount of K^+ retained by any individual bird was 2.1 μmoles. Because the concentrations of both ions had reached the minimal levels attainable by N. chalybea (see earlier) by the end of the 5 h experiment, it is unlikely that the birds needed to excrete further K^+, and thus the retention of 8.2 μmoles of this ion probably compensated for a deficit incurred while birds had been fed pure sucrose solution without electrolytes, since 14:30 the previous day. The pre-experimental (acclimation) food was a solution of 0.4 M sucrose containing 5.3 mM K^+, and 4.9 mM Na^+, so the deficits of both of these ions was probably incurred since the start of the experiment.

It will be interesting in the future to examine the mechanisms of ionic excretion in N. chalybea. The more immediate increase in excretion of Na^+ than of K^+ after electrolytes were included in the diet of sunbirds could involve differences in the mechanisms of excretion of these two ions. This can be tested by feeding birds first a low concentration of each cation (for example 4 mM of each), so that they are definitely not deficient of either cation, and then feeding them higher concentrations (for example 15 mM), to see whether birds still excrete Na^+ more rapidly than K^+.

The urine of birds, unlike that of mammals, enters the cloaca and can move retrograde into the intestine before being excreted, allowing post-renal modification (Ohmart et al. 1970, Goldstein and Braun 1986). In Anthochaera carunculata honeyeaters fed diets with different energetic, K^+ and Na^+ concentrations, the measured amounts of K^+ and Na^+ in the ureteral urine always considerably exceeded those ingested (Table 2.2), and yet the birds apparently maintained ionic balance because the plasma cation concentrations were conserved, except when feeding on the most dilute diet (Goldstein and Bradshaw 1998a).

Although the cation concentrations of the excreted fluid were not measured (only those of the ureteral urine were), it can be inferred that post-renal re-absorption must have occurred to maintain cation balance. It will be interesting to measure both ureteral urine and excretory fluid of N. chalybea fed either pure sucrose solution or sucrose solution containing electrolytes, to determine the contribution of post-renal modification of urine to the maintenance of electrolyte balance.

The "solute gap" (which is the difference between the total osmolality, and 2 * (K^+ + Na^+)), was higher during the period when electrolytes were included in the diet (Fig. 2.2, Appendix 2.3). This means that the concentrations of other contributors to total osmolality, apart from K^+ and Na^+, may have decreased after electrolytes were included in the diet. An alternative, non-exclusive hypothesis is that cations may have been bound to uric acid microspheres, thereby remaining unmeasured and resulting in an underestimate of the K^+ + Na^+ concentrations excreted (Dantzler 1989). This area requires further research. At present, the laboratory of B. Pinshow in Israel is studying nitrogen balance in the sunbird N. osea, and their work may shed light on the contribution of nitrogenous waste to the solute gap, in birds fed different diets. For comparison, the increase in osmolality of the excreted fluid of Selasphorus platycercus hummingbirds was only marginally greater than the increase in (K^+ + Na^+) * 2, after 38 mM KCl or 38 mM NaCl were included in their diet (Calder and Hiebert 1983).

It can be concluded that N. chalybea is able to tightly regulate its excretion of K^+ and Na^+ ions, so that it can maintain electrolyte balance over the range of nectar sugar and cation concentrations that is likely to encounter in the field. When feeding on dilute nectar containing low cation concentrations, this sunbird is highly efficient at conserving electrolytes.

Chapter 3

Changes in energy and water balance of Nectarinia chalybea with ambient temperature

Summary

The lesser double-collared sunbird, Nectarinia chalybea, has a widespread distribution in South Africa, and inhabits semi-desert to temperate forest. It occurs where average 24 h winter temperatures are as low as 10 °C, and also where average summer temperatures approach 30 °C. Sunbirds were kept at 10, 20 or 30 °C, and fed 0.4 or 1.2 M sucrose, to see how they would maintain energy and water balance in the field. As expected, the amount of energy ingested increased with decreasing temperature. In terms of body mass, birds always maintained energy balance. The diurnal feeding patterns of sunbirds varied with temperature, decreasing through the day at 30 and 20 °C, but being more constant through the day at 10 °C. To maintain energy balance, sunbirds consumed between 0.6 and 2.9 times their body mass in preformed water daily. The difference between water gain (preformed and metabolic water) and excreted water, which should represent evaporative water loss, increased with ambient temperature in birds fed 1.2 M sucrose, but decreased with temperature in those fed 0.4 M sucrose.

Introduction

Calder (1979) predicted the threshold nectar concentrations for nectarivorous birds, at which the preformed and metabolic water gains would exactly balance the evaporative water loss, so that birds would neither drink nor excrete water. The threshold nectar concentration increases exponentially with decreasing ambient temperature (T_a, Fig. 3.1). Based on Calder's prediction, nectarivorous birds should consume preformed water in excess of evaporative water loss even when the T_a is as high as 30 °C, unless the average concentration of the nectar they are feeding on exceeds 1.5 M sucrose (= 42 % w/w). In fact, nectar produced by ornithophilous flowers averages only 0.8 M (= 23 %, Pyke and Waser 1981). When the T_a is as low as 10 °C, the water excess consumed by nectar-feeding birds should be enormous.

The sunbird Nectarinia chalybea (8 g) is endemic to South Africa, where it is widespread (Fig. 3.2), occupying a wide variety of habitats from semi-desert to temperate forest (Harrison et al. 1997). In many of the regions where this sunbird occurs, the average 24 h T_a in mid-winter may be as low as 10 °C, whereas in mid-summer T_a frequently averages 25 to 30 °C (Harrison et al. 1997). The species breeds in winter in the south-western Cape (Harrison et al. 1997), when low T_a, relatively short light period, high energetic demands of breeding, frequent bad weather, and nectar which may be diluted by winter rainfall, combine so that sunbirds may be under considerable energetic stress.

The aim of the present chapter is to investigate how N. chalybea maintains energy and water balance over the range of ambient temperatures it encounters in the field.

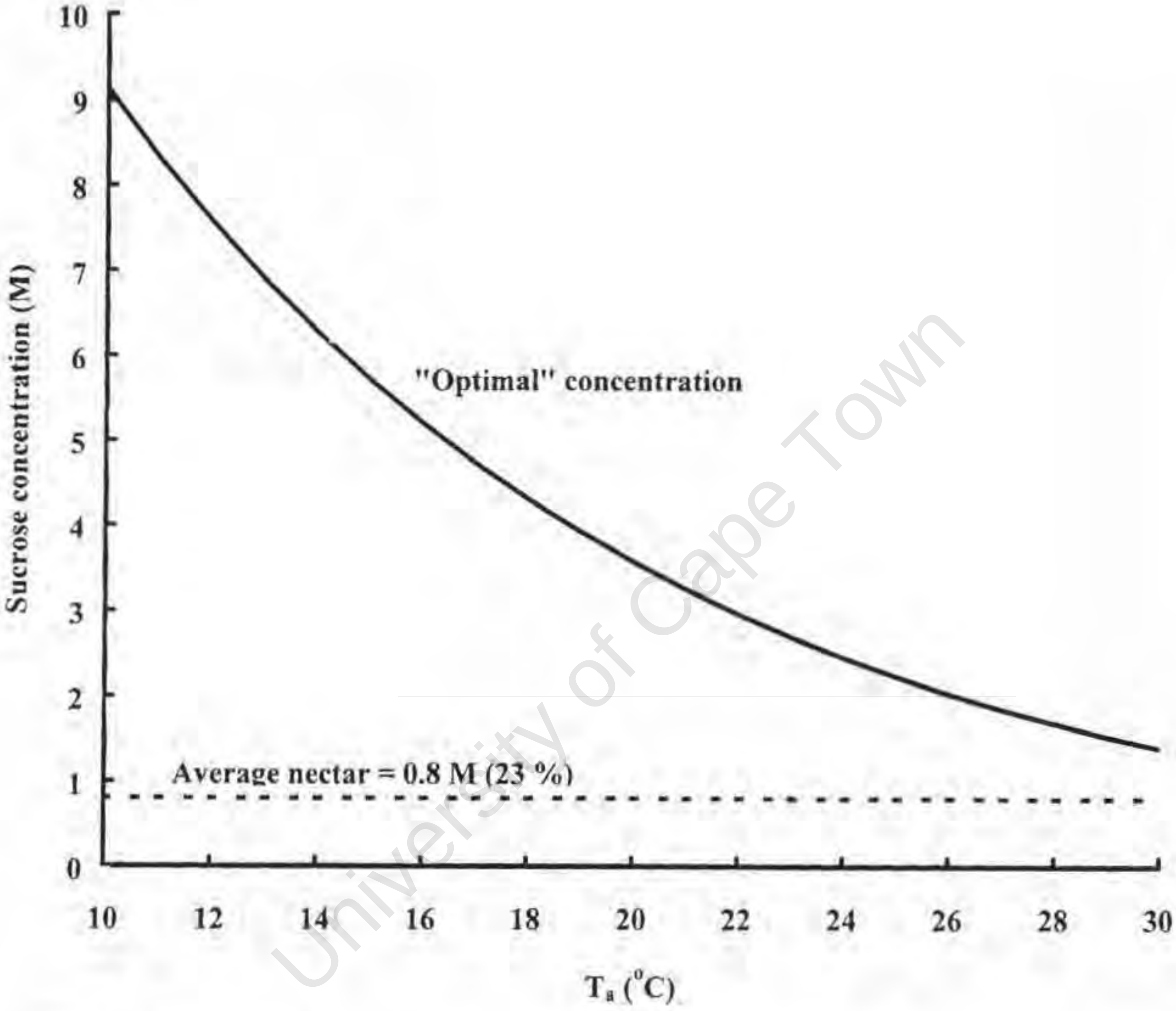

Fig. 3.1: Threshold nectar concentrations for nectarivorous birds, at which birds neither have to drink nor excrete water, over a range of T_a's. Plotted from the equation $M_{threshold} = 23.3 * e^{-0.092 * Ta}$ ($R^2 = 0.972$) in Calder (1979). The average nectar concentration of ornithophilous flowers (Pyke and Waser 1981), and the saturation concentrations of sucrose over the T_a range (Calder 1979), are also shown.

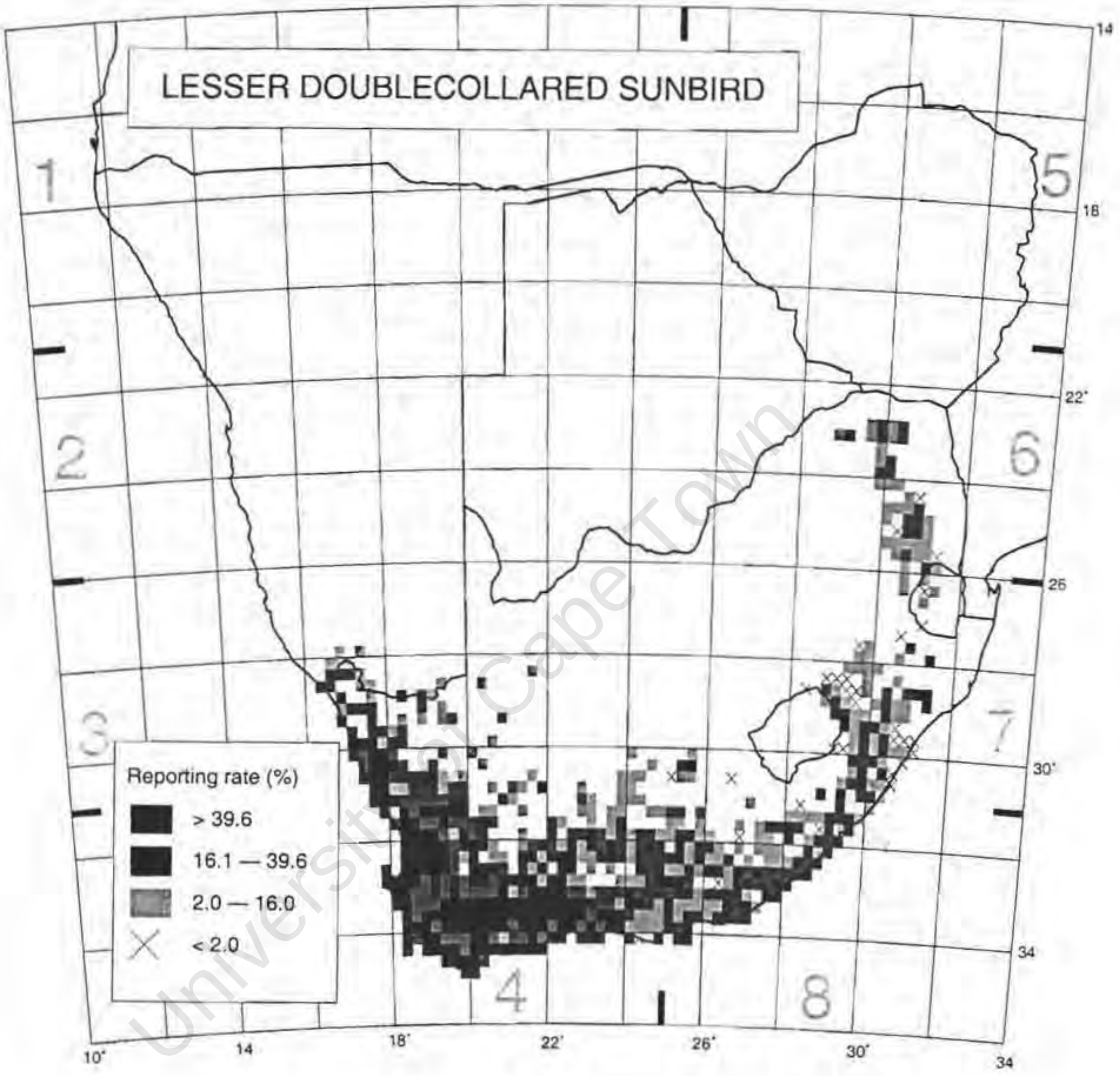

Fig. 3.2: Distribution map of N. chalybea. Reproduced with kind permission from Harrison et al. (1997).

Materials and methods

Four female and four male N. chalybea were kept in a laboratory partially lit by natural light and air-conditioned at 20 °C. They were kept in the same cages as in chapter 1 and fed the same acclimation diet as in chapter 2 (containing Ensure®). During experiments, birds were transferred to the experimental cages in the constant environment room as in chapter 1, but they were kept at 10, 20 or 30 °C and fed 0.4 or 1.2 M sucrose. These T_a's and nectar concentrations span the ranges they are likely to encounter in the field. Birds were acclimated to their experimental T_a's and diets for one day. One day of acclimation was deemed adequate, because during a preliminary experiment, birds consumed indistinguishable volumes of food from the second to the fifth day after a change in diet, with only the volume consumed on the first day being slightly different. On the second day, the intake of sucrose solution was monitored every hour, and the excreted fluid produced was collected every hour, as in the experiments of chapter 1. Birds were weighed at 07:30 and 19:30 on the experimental day, and at 07:30 on the following day. Birds lost 1 to 2 % of their 07:30 body mass in 24 h. As discussed in chapter 1, this does not imply that the birds were inadequately acclimated to their experimental conditions, but rather that they lacked Ensure® and therefore protein in their diet during the experiments. Birds had at least five days on Ensure®-supplemented food between trials.

The volumes of preformed water, and the volumes, osmolalities and K^+ and Na^+ concentrations of the excreted fluid were determined as in chapter 1. Metabolic water production was calculated as in chapter 1. The difference between water gain (preformed and metabolic water) and excreted water was assumed to equal evaporative water loss (EWL). The r.h. of the constant environment room was maintained at 65 %, so that the absolute humidity (Q_{va}) ranged from 6.1 g m^{-3} at 10 °C to 19.7 g m^{-3} at 30 °C. The possible effects of this confounding variable (Q_{va}) on the calculated EWL values are discussed below.

The experimental protocol (Table 3.1) was designed so that the data could be analysed using the computer program Genstat 5 (Release 3.1, Lawes Agricultural Trust, 1994). Males and females were numbered from one to four. Each female was randomly paired with a male. Two of these male-female pairs were fed 0.4 M sucrose, and two were fed 1.2 M sucrose. Four trials were conducted simultaneously using one 0.4 M pair and one 1.2 M pair. Each pair was tested at all three ambient temperatures, but the order of being kept at the different temperatures was randomised.

Table 3.1: Experimental protocol

Exp. no.	**1**	**2**	**3**	**4**	**5**	**6**
T_a	**10 °C**	**20 °C**	**30 °C**	**10 °C**	**20 °C**	**30 °C**
Male 1	0.4 M		0.4 M		0.4 M	
Female 3	0.4 M		0.4 M		0.4 M	
Male 2	1.2 M		1.2 M		1.2 M	
Female 1	1.2 M		1.2 M		1.2 M	
Male 3		1.2 M		1.2 M		1.2 M
Female 2		1.2 M		1.2 M		1.2 M
Male 4		0.4 M		0.4 M		0.4 M
Female 4		0.4 M		0.4 M		0.4 M

To test for differences in the intake of sucrose among the twelve hours of the light period, repeated measures ANOVA's were performed, followed by Tukey multiple range tests, as in chapter 1. Data are reported as the mean ± SE.

Results

Energy balance

At each T_a, the mass of sucrose ingested did not differ significantly on the two diets ($P = 0.202$). The mass of sucrose ingested decreased with increasing T_a ($P < 0.001$). The relationship between 24 h energy intake and T_a is described by the equation

g sucrose per 24 hr = $-0.052\ T_a + 3.56$ (Fig. 3.3, see also Appendix 2.4).

Leon and Nicolson (1997) measured metabolic rates in N. chalybea kept at T_a's from 7 to 35 °C (35 °C being at or just below the lower critical temperature of N. chalybea), and when their hourly night-time and day-time metabolic rates are added together, multiplied by 12 to give 24 h values, converted from ml O_2 expended to g sucrose required, and multiplied by 1.03 to account for the fact that N. chalybea only assimilates sucrose with 97 % efficiency (Lotz and Nicolson 1996), an equation is yielded which can be compared to the equation obtained above using the data from the present experiment,

g sucrose per 24 hr = $-0.055\ T_a + 3.14$

The slopes of the two equations are almost identical, but the masses of sucrose ingested in the present study were on average 28 % higher than those calculated from Leon and Nicolson (1997).

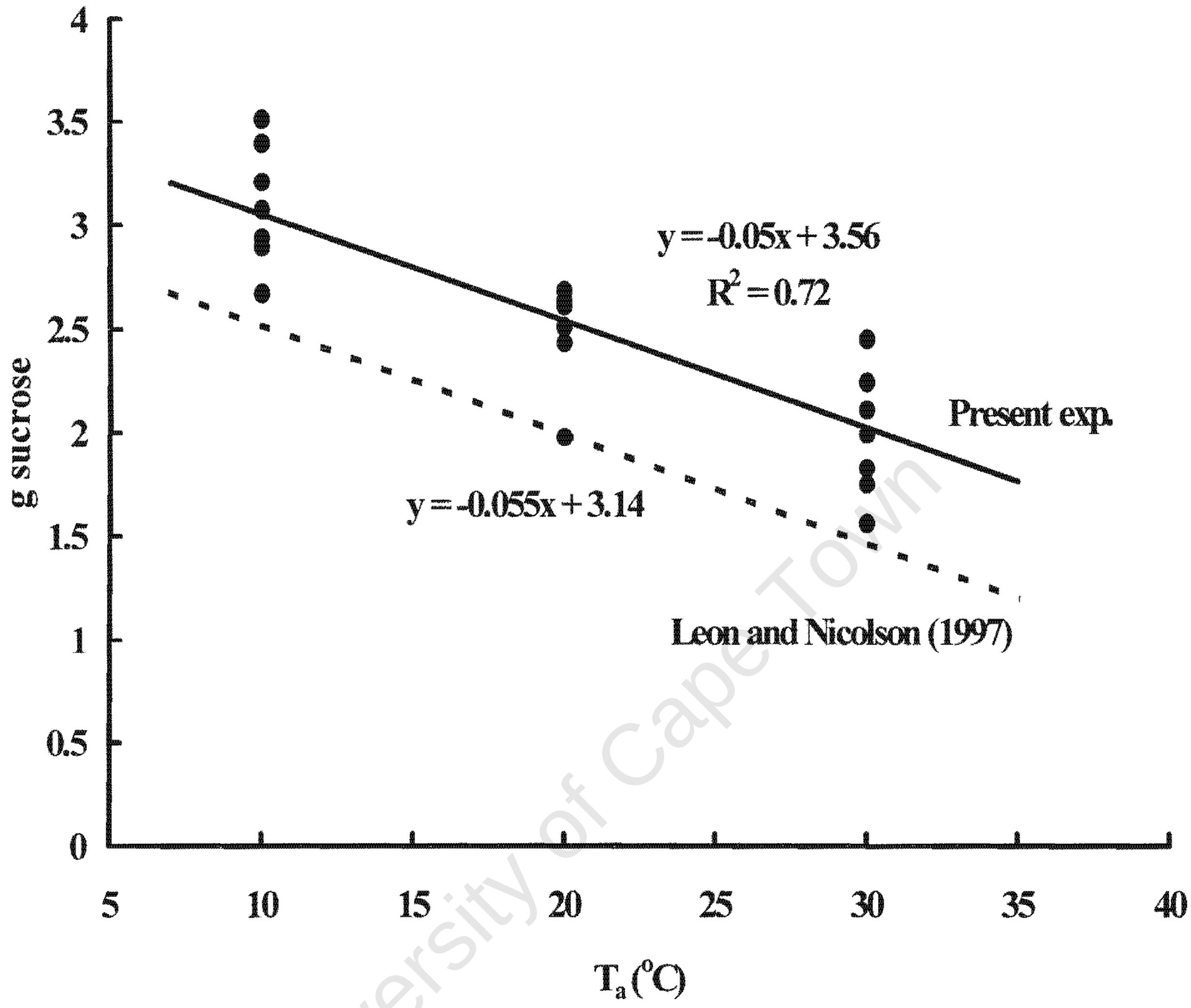

Fig. 3.3: The daily mass of sucrose ingested by N. chalybea plotted as a function of T_a. Birds consumed the same mass of sucrose whether fed 0.4 or 1.2 M sucrose, and values for each individual bird are shown on the graph (n = 8). The slope representing the daily mass of sucrose that would have to be ingested to fuel the 24 h (night-time + day-time) metabolic rates of N. chalybea measured by Leon and Nicolson (1997) over a range in T_a from 7 to 35 °C are also plotted.

Although the body masses of birds fed 0.4 M sucrose were on average 5 % higher than those fed 1.2 M sucrose, this was not significant ($P = 0.657$, Table 3.2). It should be noted that different individual birds were fed the two different diets. Birds kept at 30 °C maintained significantly but only slightly (2 %) higher masses than those kept at 20 or 10 °C ($P = 0.004$). The same individual birds were kept at all three T_a's, for each diet. The day-time mass gain and night-time mass loss were unaffected by diet ($P > 0.8$), but decreased significantly with T_a ($P < 0.001$). The birds lost 1 to 2 % of their 07:30 body mass in 24 hours, the mass loss being the same irrespective of dietary sucrose concentration and T_a ($P > 0.696$).

Diurnal patterns

The diurnal feeding patterns of sunbirds varied with T_a but not with diet (Fig. 3.4, Appendix 2.5). At 10 °C, there were no significant differences among the volumes of sucrose solution consumed each hour, whether birds were fed 0.4 or 1.2 M sucrose ($P > 0.064$). Birds at 20 °C showed a peak in the volumes consumed during the second hour of light, significantly greater than the volumes consumed in the late afternoon ($P < 0.05$). There was no significant secondary afternoon peak, unlike in chapter 1. Birds at 30 °C showed a similar pattern to those at 20 °C, except that the volumes consumed in the first hour of light were significantly lower than those consumed in the second hour of light.

Table 3.2: Body mass, day-time mass gain, night-time mass loss and 24 h mass loss (g, mean ± SE, n = 4) of N. chalybea fed 0.4 or 1.2 M sucrose and kept at 10, 20 or 30 °C.

		0.4 M	
	10 °C	**20 °C**	**30 °C**
Body mass, 07:30, exp. Day	8.11 ± 0.57	8.18 ± 0.60	8.39 ± 0.58
Body mass, 19:30, exp. Day	8.59 ± 0.58	8.52 ± 0.56	8.65 ± 0.56
Body mass, 07:30, next day	8.00 ± 0.57	8.00 ± 0.58	8.23 ± 0.55
Day-time mass gain, exp. Day	0.48 ± 0.03	0.34 ± 0.05	0.26 ± 0.03
Night-time mass loss	0.60 ± 0.05	0.52 ± 0.03	0.42 ± 0.03
Mass loss, 07:30 - 07:30	0.12 ± 0.05	0.18 ± 0.04	0.42 ± 0.03
		1.2 M	
	10 °C	**20 °C**	**30 °C**
Body mass, 07:30, exp. Day	7.71 ± 0.52	7.79 ± 0.50	7.98 ± 0.48
Body mass, 19:30, exp. Day	8.20 ± 0.60	8.14 ± 0.52	8.27 ± 0.55
Body mass, 07:30, next day	7.65 ± 0.56	7.67 ± 0.52	7.84 ± 0.57
Day-time mass gain, exp. Day	0.50 ± 0.09	0.35 ± 0.03	0.28 ± 0.09
Night-time mass loss	0.55 ± 0.04	0.47 ± 0.04	0.43 ± 0.02
Mass loss, 07:30 - 07:30	0.06 ± 0.05	0.12 ± 0.04	0.15 ± 0.10

Water balance

The effects of T_a and dietary sucrose concentration on the different components of the 24 hr water balance of N. chalybea are shown in Fig. 3.5 and Appendix 2.4. Preformed water intake and excretory water loss were measured directly. Metabolic water production was calculated as in chapter 1. The birds gained 2.8 times their body mass per day in preformed and metabolic water when fed 0.4 M sucrose at 10 °C, but only 0.6 times their body mass when fed 1.2 M at 30 °C. The volume of water excreted was always lower than the volume of preformed and metabolic water, the difference presumably representing the 24 h evaporative water loss (EWL, Fig. 3.5, Appendix 2.4). The EWL calculated in this way was higher in birds fed 0.4 M sucrose than in those fed 1.2 M sucrose ($P < 0.018$). In birds fed 0.4 M sucrose, EWL increased significantly with decreasing T_a ($P < 0.05$). In birds fed 1.2 M sucrose, the opposite trend appeared to occur, but it was not significant ($P > 0.175$).

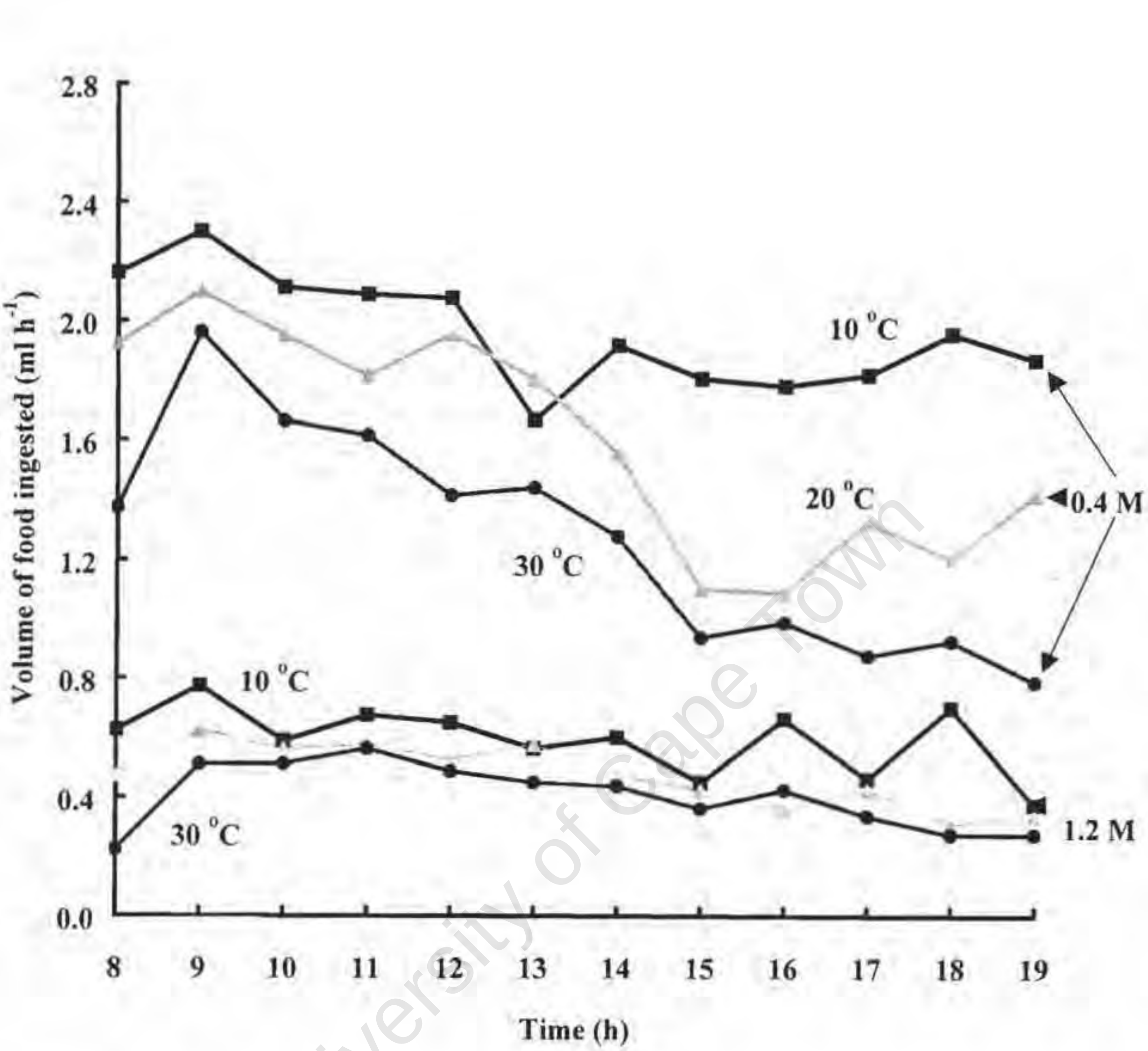

Fig. 3.4: Hourly volumes (ml h^{-1}, mean, n = 4) of sucrose solution consumed by <u>N. chalybea</u> fed 0.4 or 1.2 M sucrose at T_a's of 10, 20 or 30 °C. Standard errors are omitted for the sake of clarity. The times indicated on the x-axis are the mid-points of each hour of the day in which food intake was measured.

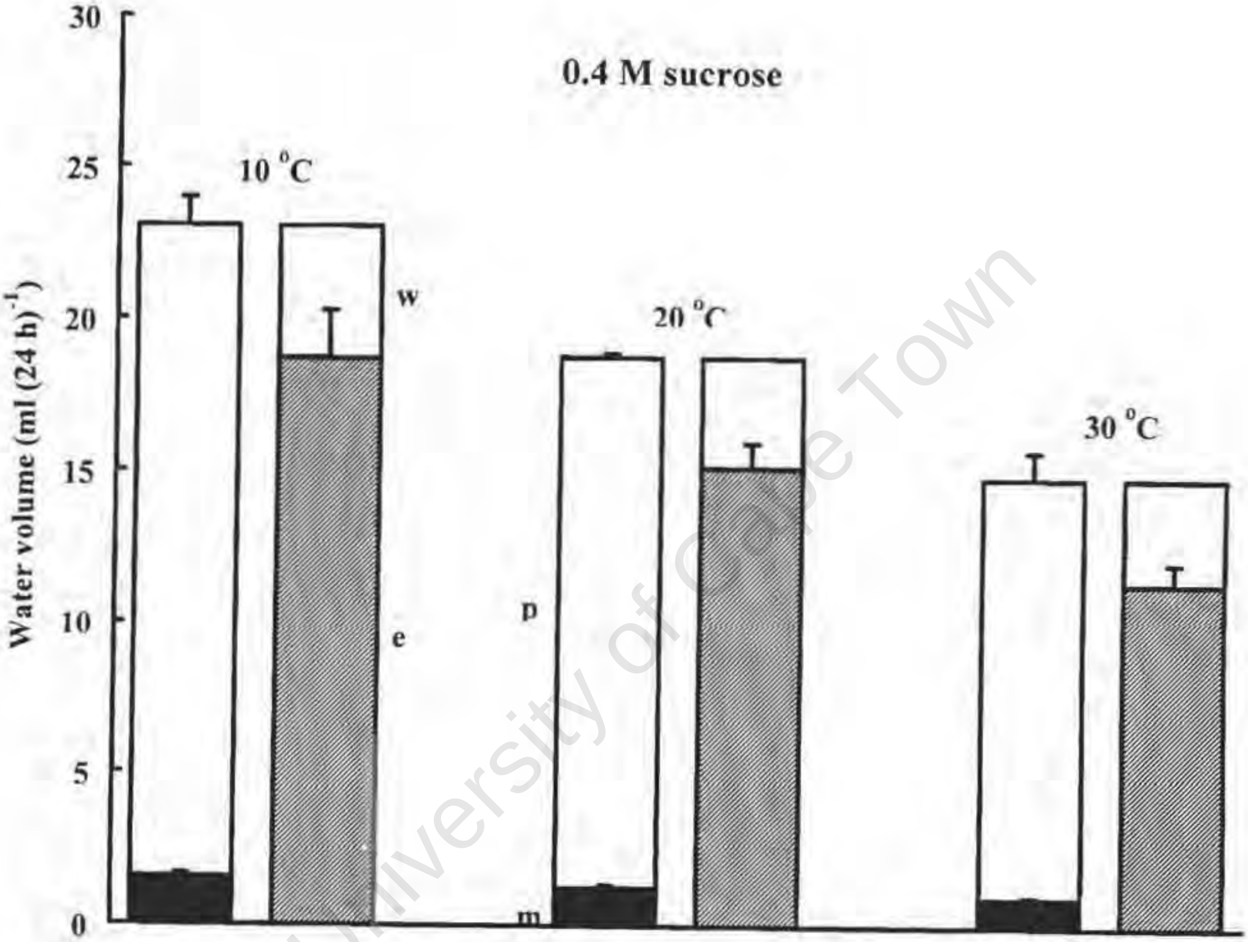

Fig. 3.5a: Components of water gain and loss (mean ± SE, n = 4) in <u>N. chalybea</u> kept at T_a's of 10, 20 or 30 °C, and fed 0.4 M sucrose. Refer to Fig. 3.5b for legend. For explanation see text.

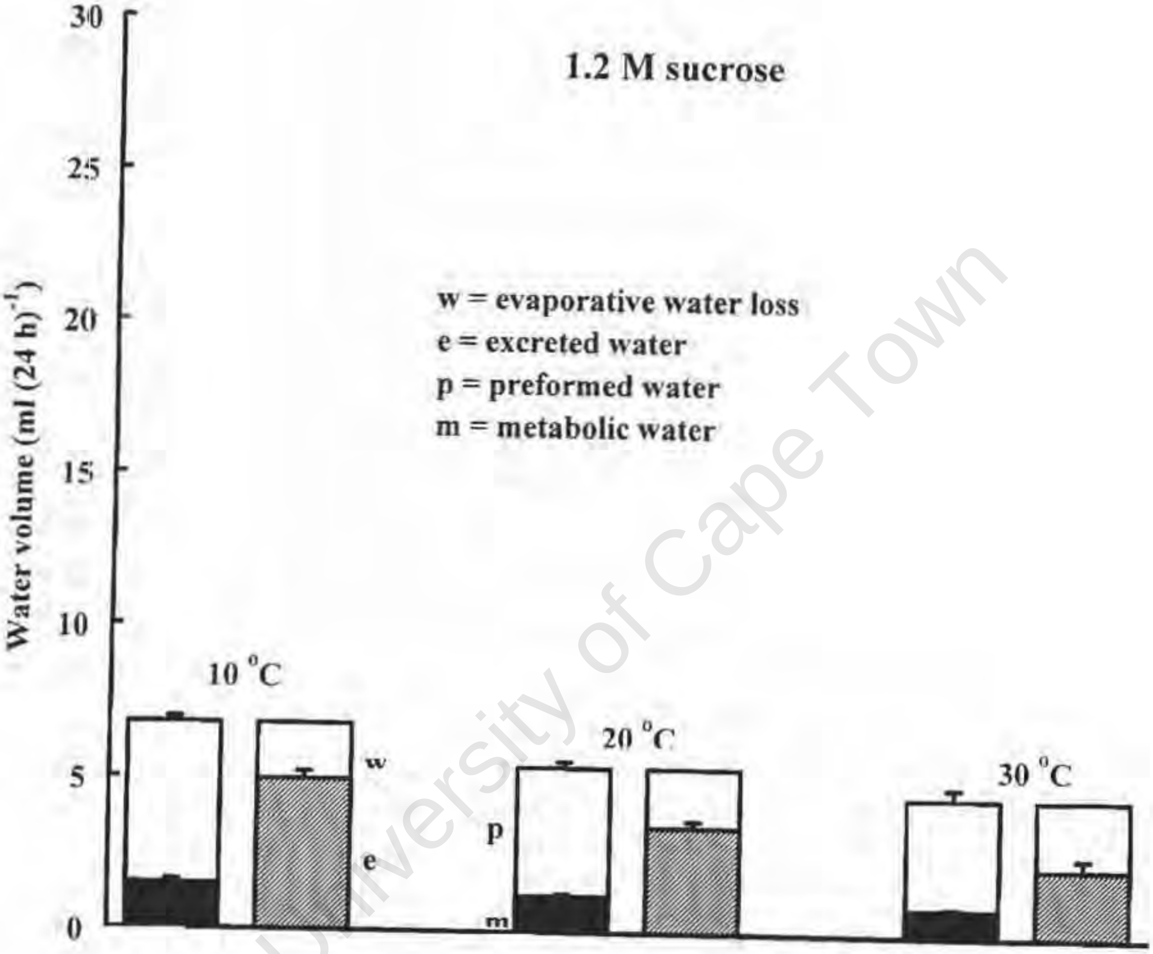

Fig. 3.5b: Components of water gain and loss (mean ± SE, n = 4) in N. chalybea kept at T_a's of 10, 20 or 30 °C, and fed 1.2 M sucrose. For explanation see text.

Excreted fluid electrolytes

The osmolalities of the excreted fluid were always exceptionally low, never exceeding 78 mmol kg^{-1} (Table 3.3). Excreted fluid osmolalities were significantly lower in birds fed 0.4 M sucrose than in those fed 1.2 M sucrose ($P = 0.036$). In birds fed the dilute solution, osmolality was unaffected by T_a, but it increased significantly with T_a in birds fed the concentrated solution ($P = 0.002$).

Table 3.3: Osmolalities (mmol kg^{-1}) and cation concentrations (mM) of total excreted fluid produced during the light period (pooled hourly collections, mean ± SE, n = 4), in birds fed 0.4 or 1.2 M sucrose and kept at 10, 20 or 30 °C.

		0.4 M	
	10 °C	20 °C	30 °C
Osmolality (mmol kg^{-1})	46.6 ± 2.3	45.3 ± 1.9	48.1 ± 1.5
K$^+$ (mM)	0.4 ± 0.1	0.5 ± 0.0	0.6 ± 0.2
Na$^+$ (mM)	0.1 ± 0.1	0	0.9 ± 0.9
		1.2 M	
	10 °C	**20 °C**	**30 °C**
Osmolality (mmol kg^{-1})	60.1 ± 4.1	64.8 ± 2.2	77.8 ± 8.1
K$^+$ (mM)	0.9 ± 0.3	1.3 ± 0.4	3.3 ± 1.8
Na$^+$ (mM)	0.3 ± 0.1	0.4 ± 0.2	1.0 ± 0.4

The concentrations of K$^+$ and Na$^+$ in the excretory fluid were exceptionally low (Table 3.3), not exceeding 9 and 2 mM respectively in individual birds. Concentrations of these ions were slightly higher in birds fed 1.2 M sucrose and at higher T_a ($P < 0.058$).

Discussion

Energy balance

As T_a increased towards the thermoneutral zone, the mass of sucrose ingested decreased. The slope of the curve of energy intake with T_a was almost identical to that calculated from metabolic rates measured in N. chalybea by Leon and Nicolson (1997), but the daily intake of energy was consistently 28 % higher (Fig. 3.3). The energy intake calculated from the metabolic rate measured in sunbirds fed 1.2 M sucrose at 20 °C during the experiments of chapter 5, was also 28 % lower than the corresponding value (20 °C, 1.2 M sucrose) measured from sucrose intake during the present experiment, being very similar to Leon and Nicolson's value. Evidently, the birds in the present food intake experiments had higher metabolic rates, perhaps because they were kept in larger cages. Indeed, sunbirds appeared far more active during the present experiments than during the metabolic rate measurements. Sunbirds made frequent flights around their cages during the present experiments, unlike in the metabolic rate measurements, during which they seldom if ever flew. Powers (1991) explained higher than expected measured metabolic rates in hummingbirds because birds were kept in unusually large metabolic chambers, so that they flew frequently within the chambers.

Birds kept at 30 °C maintained marginally (2 %) but significantly higher body masses than those kept at 20 or 10 °C (see below). The night-time mass loss decreased with increasing T_a, which is expected because of the decreasing energy requirements. The day-time mass gain also decreased with increasing T_a, to compensate for the differences in night-time loss. In hummingbirds, there is evidence that the daily energy ingestion rate is dictated by the extent of decrease in stored energy overnight (Hainsworth 1978, Hainsworth and Wolf 1983). The mass loss of N. chalybea over 24 h (1 - 2 % of initial mass) was the same irrespective of T_a.

The field metabolic rate (FMR) predicted for an 8.1 g nectarivorous bird is 46 kJ per day (equivalent to 2.6 g sucrose), based on an allometric equation calculated from five species ranging in body mass from 4 to 17 g (MacMillen and Carpenter 1977), or 54 kJ per day (equivalent to 3.1 g sucrose), based on a more recent equation calculated from nine nectarivorous bird species ranging in body mass from 3.7 to 17.3 g (Weathers et al. 1996). Similarly, the FMR predicted by an allometric equation calculated from 62 bird species (all diets), is 41 kJ = 2.3 g (Williams et al. 1993). The daily amounts of energy ingested by N. chalybea in the present study are 3.1 g = 54 kJ at 10 °C, 2.5 g = 44 kJ at 20 °C, and 2.0 g = 35 kJ at 30 °C. These values are similar to the expected FMR values, which also means that the daily water influx in these laboratory-kept sunbirds should approximate the values found in wild birds feeding on nectar equivalent to 0.4 or 1.2 M sucrose. Field metabolic rates estimated from time-energy budgets in orange-breasted sunbirds (N. violacea, 9.5 g, Collins 1983), olive sunbirds (N. olivacea, 12 g, Frost and Frost 1980), malachite sunbirds (N. famosa, 13.5 g, Wolf 1975) and golden-winged sunbirds (N. reichenowi, 15 g, Gill and Wolf 1975), are 53, 57, 71 and 54 kJ $(24\ h)^{-1}$, respectively.

The basal metabolic rate (BMR) of N. chalybea is 13 kJ per 24 hours (Leon and Nicolson 1997). The daily energy intakes of N. chalybea during the present study were thus 2.7 times BMR at 30 °C, and 4.2 times BMR at 10 °C. Drent and Daan (1980) suggested that the maximal sustainable energy expenditure rate in birds should be 4 times BMR, indicating that the metabolic rate of N. chalybea at 10 °C is at its upper limit. However, Weathers and Sullivan (1989) suggested that the limit is closer to 5 times BMR, and Williams (1993) measured an average FMR (66 kJ) that was 6.5 times BMR in incubating female orange-breasted sunbirds (N. violacea, 9.5 g, n = 10), which breed in winter.

The diurnal feeding patterns observed in N. chalybea varied with T_a but not with diet. The feeding patterns of birds at 20 °C were similar to those described in chapter 1 (when birds were also kept at 20 °C, but fed 0.4, 0.8 or 1.2 M sucrose), showing a significant decline in feeding rate through the day. However, during the present experiments, feeding rate peaked in the second and not in the first hour of light, and the secondary peak in the late afternoon was not significant. The reasons for these slight differences in the diurnal feeding pattern are unclear, although it can be noted that different birds were used in the two sets of experiments, and that the experiments were also conducted during different months (August and September for those in chapter 1 and in the present chapter, respectively).

When birds were at 30 °C, the decrease in feeding rate through the day was more marked than when they were at 20 °C (irrespective of dietary sucrose concentration). However, food intake in the first hour of light was significantly and substantially lower than in the second hour of light. Body masses at 07:30 were 2 % higher than those in birds at 20 or 10 °C, and perhaps the birds consumed less food in the first hour of light to regulate their morning body mass.

Sunbirds at 30 or 20 °C showed the feeding pattern typical of small birds (Aschoff 1966), and the pattern predicted by an optimisation model (Bednekoff and Houston 1994, see chapter 1), with a declining feeding rate through the day. However, birds at 10 °C showed the pattern expected in the presence of a digestive constraint: a constant feeding rate through the day (Bednekoff and Houston 1994). It is likely that sunbirds are constrained by the rate at which they can absorb energy, rather than the rate at which they can process preformed water, because birds fed 0.4 M sucrose always consumed more preformed water than those fed 1.2 M sucrose, at each T_a, whereas birds at 10 °C always ingested more energy than those at higher T_a. Moreover, sunbirds feeding on 0.09 M sucrose at 20 °C are able to consume 2.5 times more water than they do when feeding on 0.4 M sucrose at 10 °C (appendix 3). Sunbirds feeding on 0.09 M sucrose show a constant feeding rate through the day at 20 °C, and are probably constrained by the volume of water (and not by the amount of energy), that they are able to process, as they fail to ingest as much energy as birds fed higher sucrose concentrations at this T_a (appendix 3).

Nectarivorous birds including N. chalybea breed in winter in the south-western Cape, South Africa (Harrison et al. 1997), when nectar is abundant but may be diluted by winter rainfall. Daily (24 h) T_a often averages 10 °C (Harrison et al. 1997). During breeding, avian FMR typically increases substantially over non-breeding levels (Bryant 1991), and the FMR's of N. chalybea during winter breeding probably substantially exceed the daily metabolic rate calculated from the energy ingestion rate of sunbirds at 10 °C during the present experiment. Incubating female orange-breasted sunbirds (N. violacea) have an exceptionally high FMR of 6.5 times BMR during winter breeding (Williams 1993). Moreover, the time available for feeding during winter breeding will be considerably lower than during the present experiment: the mid-winter light period in the western Cape is only 10 h, bad weather will sometimes disrupt feeding, and time will have to be allocated to provisioning fledglings with food. If sunbirds at 10 °C during the present experiment fed at a constant rate through the day because they were constrained by the rate at which they could absorb energy, the constraint would be expected to become far more intense during winter breeding.

One way in which the constraint could potentially be alleviated during winter breeding would be if gut acclimation occurred. It is plausible that the density in the gastro-intestinal tract of at least one of the following could increase during winter: 1) sucrase enzymes, 2) glucose transporters, and/or 3) fructose transporters. This type of acclimation of the gastro-intestinal tract has been documented in various bird species, commonly occurring as a response to a change in diet (see Sabat 1998).

Without major gut acclimation, N. chalybea would probably have to employ marked nocturnal hypothermia during winter breeding to maintain energy balance, and it is highly likely that this does indeed occur. In the laboratory, only mild nocturnal hypothermia (about 4 °C below diurnal body temperature, T_b) occurs in N. chalybea and other sunbird species when they have free access to food over light periods similar to those of the present study, and over a T_a range of 7 to 35 °C (Prinzinger et al. 1989, Leon and Nicolson 1997). In addition, the magnitude of the hypothermia only increases marginally with decreasing T_a, the reduction in T_b always being close to 4 °C below diurnal T_b. However, when deprived of food for the last three hours of a thirteen hour light period, at 10 °C, the nocturnal T_b's of four captive N. chalybea averaged 13 °C below diurnal T_b's (unpublished data of J.R.B. Lighton and B. Leon, cited in Leon and Nicolson 1997). Similarly, T_b measured at night in several East African sunbird species at high altitudes was 5 to 18 °C below day-time T_b (Cheke 1971). Williams (1993) measured egg temperatures below incubating female N. violacea sunbirds in the south-western Cape during winter. One particular bird continuously maintained egg temperature above 34 °C on two consecutive nights, but on the third night, following stormy weather the previous day, the egg temperature dropped to 29 °C for more than 3 h.

Some Australian honeyeaters also breed in winter (Jackson 1998), during which time they probably experience similar energetic stresses to N. chalybea. Captive Meliphaga virescens honeyeaters (25 g), which breed in winter in certain parts of their range (Frith 1988), reduced T_b at night by 1 to 3 °C when they were kept at T_a's ranging between 10 and 20 °C, and on an 11.5 h light period, (Collins and Briffa 1984). When deprived of food for the last two hours of the day, night-time hypothermia was 9 to 11 °C below day-time T_b.

Apart from potentially using gut acclimation and probably using marked nocturnal hypothermia during winter breeding, N. chalybea may still fail to maintain energy balance, and may lose body mass, when breeding, especially during bad weather. Ten female N. violacea lost an average of 2.6 % of their body mass daily while incubating eggs (Williams 1993). All except one of these ten birds lost mass daily, the maximum value for any individual bird being 7.7 % of initial body mass over 24 h.

Water balance

The 24 h water gain of N. chalybea ranged from 0.6 times body mass in birds fed 1.2 M sucrose at 30 °C, to 2.9 times body mass in those fed 0.4 M sucrose at 10 °C. The value predicted from allometry is 0.8 times body mass (Williams et al. 1993). Birds excreted 60 to 79 % of their preformed water intake, the percentage increasing with the volume of preformed water consumed. The rate of water excretion during any hour of the day was never less than 0.07 ml h^{-1}, even when birds were fed 1.2 M sucrose at 30 °C. This is consistent with the prediction of Calder (1979), that nectarivorous birds at 30 °C will exactly balance their evaporative water loss with preformed and metabolic water (so that they don't excrete any water) only when they encounter an average nectar concentration exceeding 1.5 M sucrose.

In sunbirds fed 1.2 M sucrose, the calculated evaporative water loss (EWL) did not vary significantly with T_a. However, in birds fed 0.4 M sucrose, EWL actually decreased significantly as T_a increased from 10 to 30 °C. However, the changes in EWL with T_a have to be viewed with caution because they are confounded by differences in absolute humidity (Q_{va}), which increased from 6.1 to 19.7 g m^{-3} between 10 and 30 °C during the present experiment. All other things being equal, EWL is known to decrease with increasing Q_{va} in birds (Webster and King 1987, Powers 1992), because the driving force for evaporation is directly proportional to the absolute humidity gradient between the skin and the surrounding air ($\Delta p_v = Q_{vs} - Q_{va}$, where Q_{vs} is the absolute humidity below the skin, Webster et al. 1985). This means that the decrease in EWL with increasing T_a in <u>N. chalybea</u> can potentially be explained by the change in Q_{va}. Dividing by Δp_v would remove the effect of this confounding variable, but calculation of this term requires accurate knowledge of skin temperature (for calculation of Q_{vs}), at each T_a. Skin temperature has not been measured in <u>N. chalybea</u>, and its relation to T_a is poorly understood in birds (Webster et al. 1985).

The EWL of the hummingbird Calypte anna has been measured over a range of T_a's and absolute humidities, and is described by the equation:

$$\text{EWL} = 2.314 - 0.490 * Q_{va} + 0.427 * T_a,$$

where EWL is in mg g^{-1} h^{-1} and Q_{va} is in g m^{-3} (Powers 1992). Substituting the absolute humidities and T_a's of the present study into this equation yields EWL values of 3.6 and 5.5 mg g^{-1} h^{-1} at 10 and 30 °C. This means that the EWL of hummingbirds would increase substantially with T_a despite the associated decrease in Q_{va} of the present experimental conditions. In contrast, the EWL of sunbirds feeding on 0.4 M sucrose increased with decreasing T_a.

If the EWL of N. chalybea feeding on dilute nectar does indeed increase with decreasing ambient temperature at a particular Q_{va}, then it is possible that N. chalybea actively regulates its EWL as a mechanism for maintaining water balance, increasing its EWL to compensate for an increase in water influx. If EWL was controlled in order to maintain water balance, this could explain why it changes in a different fashion than predicted by passive biophysical considerations. This possibility is discussed further in chapter 5. Based on the calculated values of EWL, sunbirds fed 1.2 M sucrose at 10 °C will lose 4 kJ, and those at 30 °C 6 kJ, of heat daily through evaporative heat loss. On the other hand, birds fed 0.4 M sucrose will lose 11 and 8 kJ at 10 and 30 °C. This means that sunbirds will have to increase their metabolic rate and/or decrease their T_b to a greater degree with decreasing T_a when they are feeding on relatively dilute nectar. This will exacerbate their energetic stress during winter breeding, especially when feeding on nectar which has been diluted by winter rainfall or high relative humidities.

Chapter 4

The cost of warming nectar to body temperature

Summary

Food ingested by endothermic animals must be warmed (or cooled) to body temperature. When feeding on dilute nectar, nectarivorous endotherms may use large amounts of energy to warm the preformed water consumed. The costs of warming nectar of different temperatures and concentrations are modelled. For comparison, a more general model from the literature for warming other foodstuffs to body temperature is improved. The percentage of the energy ingested that is used for nectar warming (% H') increases linearly with decreasing nectar temperature, and exponentially with decreasing nectar concentration. Endotherms feeding on nectar equivalent to 0.4 M sucrose at 18 °C will have the same % H' value (4 %) as those feeding on fruit at the same temperature, or as high-latitude aquatic endotherms feeding on fish or invertebrates just above freezing point. The % H' value of endotherms feeding on 0.2 M sucrose at 10 ° C will be 11 %. Studies of specific dynamic action (SDA) and thermoregulation have seldom considered the cost of warming food to body temperature. Future studies will have to disentangle the costs of warming food, thermoregulation (excluding food warming), SDA and activity, especially for endotherms feeding on certain food types such as dilute nectar.

Introduction

Food ingested by endothermic animals must be warmed (or cooled) to body temperature. The amount of energy required to warm the food will depend on the food's temperature, its specific heat capacity, and the amount ingested. The amount ingested will depend in turn on the animal's energetic needs, the food's calorific concentration, and the efficiency with which the food is assimilated by the animal (Wilson and Culik 1991).

Birds and mammals living near the poles that ingest non-endothermic food (e.g. fish or invertebrates) may expend a large amount of energy on food warming. The Adelie penguin (Pygoscelis adeliae) uses 2.5 % of its daily energy expenditure warming its food (krill), from 0 °C to body temperature (Wilson and Culik 1991). Based on calorimetric calculations, the Brunnich's guillemot (Uria lomvia) uses 4.3 % of its daily energy cost warming its food (fish) from 2 °C to body temperature (Hawkins et al. 1997), and the great crested grebe (Podiceps cristatus) uses 6 % on warming food (also fish), from an initial temperature of 3 °C during the Dutch winter (Wiersma et al. 1994). High-latitude endotherms that ingest frozen food will also have to overcome the cost of the latent heat of fusion of ice, which is 0.34 kJ g^{-1} (Withers et al. 1979).

Meat, including fish and krill, has a relatively high calorific concentration, around 6.0 kJ per g of fresh mass (Peters 1983). This may compensate for the low temperatures at which aquatic endotherms ingest it, and result in these animals spending only small percentages of their daily energy costs warming food. Relatively more energy may be needed to warm food with lower calorific concentrations even when it is at higher temperatures. In particular, nectar can have an exceptionally low calorific concentration. One of the lowest concentrations measured in endotherm-pollinated flowers is 1.1 kJ per g of solution (0.21 M sucrose, or 7 % w/w, Pyke and Waser 1981, Nicolson 1993). Typically, nectar concentrations of flowers pollinated by birds (sunbirds, honeyeaters and hummingbirds) range between 1.9 kJ g^{-1} (0.4 M, 13 %), and 5.3 kJ g^{-1} (1.2 M, 36 %), averaging 3.4 kJ g^{-1} (0.75 M, 23 %, Baker 1975, Pyke and Waser 1981). Mussel-feeding tufted ducks (Aythya fuligula) are analogous to nectarivores, ingesting up to three times their body mass per day of calorifically-dilute food (which includes the mussel shells), and in winter use 13 - 20 % of their daily energy intake warming their food to body temperature (de Leeuw et al. 1998).

It is well-documented that the metabolic rate of a wide range of animal taxa increases directly after a meal. Most authors (e.g. Costa and Kooyman 1984, MacArthur and Campbell 1994, Janes and Chappell 1995) have assumed that this increase results from specific dynamic action (SDA), the energy costs of digesting, absorbing and storing or processing food. Specific dynamic action can also include a facultative component which is poorly understood but which is thought to be an adaptive mechanism for dissipating unneeded energy as heat (Trayhurn and James 1981, Hawkins et al. 1997). Few authors (Wilson and Culik 1991, Hawkins et al. 1997) have accounted for the simple cost of warming the meal to body temperature. Similarly, few studies (Wiersma et al. 1994, de Leeuw et al. 1998) on the effect of ambient temperature on thermoregulation in endotherms have considered food warming costs.

In this chapter, the cost of warming nectar to body temperature is modelled. A more general model for the cost of warming food, proposed by Wilson and Culik (1991), is then modified, and used to calculate the cost of warming fruit, grain / seeds, insect food and meat / fish to body temperature, over a range of food temperatures, for comparison with the cost of warming nectar.

The model

If a nectarivorous animal ingests a total of X kJ of energy per day, then the total amount (in g) of sucrose (or of an equal-parts mixture of glucose and fructose), that it ingests, will be,

$$\text{g sucrose ingested} = \frac{X}{17.57} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

because 1 g sugar $\equiv$ 17.57 kJ

The cost of warming the water and sugar components of nectar to body temperature will now be considered separately.

Energy required to warm the preformed water

Using equation (1), the volume (ml) of nectar required by the animal per day can be calculated,

$$\text{ml nectar needed} = \frac{\text{sucrose mass ingested}}{\text{nectar concentration}}$$

$$= \frac{X * 1000}{17.57 * C * 342.3}$$

$$= \frac{0.166 * X}{C}$$

where C is equal to the concentration of sucrose in mol l^{-1} and 342.3 is the molar mass of sucrose. The volume of preformed water per volume of sucrose solution, is

$$\text{ml preformed water per ml solution} = \frac{1000.7 - 216.44 * C}{1000}$$

(derived from Weast 1985). Multiplying this by the volume of nectar ingested by the animal per day yields the volume (ml) of preformed water the animal will consume per day to meet its energetic requirements,

$$\text{ml preformed water consumed per day} = X * (-0.036 + \frac{0.166}{C})$$

This preformed water has to be heated by an amount equal to the difference between the animal's body temperature and the temperature of the nectar, (T_b - T_n). The specific heat capacity of water is 4.17 J ml^{-1} ^{o}C^{-1}. Therefore, the amount of energy (kJ) required to warm the daily preformed water intake will be,

$$\text{Energy for warming preformed water} = \frac{4.17 * (T_b - T_n) * X * \left(-0.036 + \frac{0.166}{C}\right)}{1000} \qquad \ldots\ldots\ldots(2)$$

Energy required to warm the sucrose (solid)

Nectarivorous birds generally compensate energetically for changes in sucrose concentration by drinking greater volumes when the nectar is relatively dilute (see chapter 1). Therefore, the absolute amount of energy required to warm the sugar component of nectar will be independent of concentration, but that required to warm the preformed water component will be tightly linked to concentration.

In addition, the amount of energy required to warm the sugar component of nectar will be trivial in comparison to that needed to warm the preformed water component. The specific heat capacity of sucrose (Perry and Green 1984), is only 1.25 J g^{-1} ^{o}C^{-1}, 30 % that of water. This means that in animals feeding on 0.2 and 1.2 M sucrose, respectively, only 2 and 19 % of the total cost of warming the food will be accounted for by the energy required to warm the actual sucrose, the rest required to warm the preformed water.

Equation (1) gave the mass (g) of sucrose ingested. The amount of energy (kJ) required to warm the mass of sucrose ingested to body temperature will be,

$$\text{Energy for warming sugar} = \frac{1.25 * (T_b - T_n) * X}{17.57 * 100}$$

$$= \frac{0.071144 * (T_b - T_n) * X}{1000} \quad \ldots\ldots\ldots\ldots\ldots(3)$$

Energy required to warm the nectar

By adding equations (2) and (3) (the amounts of energy required to warm the preformed water and sugar components of the nectar, respectively), the total energy (kJ) required to warm the nectar is yielded,

$$\text{energy for warming nectar} = \frac{(T_b - T_n) * X * (-0.079 + \frac{0.692}{C})}{10}$$

The proportion of the energy in ingested food that is left after obligatory specific dynamic action,

SDA' = 1 – (proportion of ingested energy used for specific dynamic action)

and the proportion of the energy in ingested food that is assimilated by the animal,

AE = proportion of ingested energy assimilated need to be taken into account to yield the energetic requirement (Y kJ) of the animal for energy balance, for storage (either positive or negative), and for reproduction. This is done by simply dividing the equation by SDA' and AE,

$$\text{energy for warming nectar} = \frac{(T_b - T_n) * Y * \left(-0.079 + \frac{0.692}{C}\right)}{SDA' * AE * 10}$$

It is useful to express this as a percentage of the total daily energy requirement (excluding food warming), Y kJ, because it then becomes independent of metabolic rate, and therefore of body mass and ambient temperature, except that ambient temperature will generally determine nectar temperature, T_n.

The percentage of the 24 h energy intake (excluding that expended in food warming) required to warm the daily nectar intake to body temperature, will be,

$$\% H = \frac{(T_b - T_n) * \left(-0.079 + \frac{0.692}{C}\right)}{10 * SDA' * AE} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

Wilson and Culik (1991) derived a more general equation for the relative cost of heating ingested food, for endotherms,

$$\% H = \frac{417 * (T_b - T_{food})}{ACV * SDA' * AE},$$

where ACV is the calorific concentration of the ingested food, in kJ kg^{-1}. When ACV is in kJ g^{-1}, the equation becomes:

$$\% H = \frac{0.417 * (T_b - T_{food})}{ACV * SDA' * AE},$$

This equation of Wilson and Culik (1991) assumes that the specific heat capacity of any food ingested by an endotherm is the same as that of water, 4.17 J g^{-1} $^{o}C^{-1}$. In reality, specific heat capacities of particular foodstuffs (see Table 4.1, values derived from Peters 1983, Perry and Green 1984) can be much lower. For example, the specific heat capacity of grain is only 40 % that of water, and this means that the equation will yield values for granivorous birds that are 2.5 times too high. Foodstuffs like meat, fruit and dilute nectar, which have relatively high water contents, will have specific heat capacities closer to that of water, and so the equation will yield more realistic results. The specific heat capacity of meat, fish and insects is around 77 % that of water, so values would be 1.3 times too high. The specific heat capacity of fruit is more variable, but is typically similar to that of meat, fish and insects. Nectar of 0.2 and 1.2 M sucrose would have specific heat capacities 95 and 76 % that of water, respectively, resulting in values 1.1 and 1.3 times too high. The equation of Wilson and Culik (1991) can be made more accurate simply by multiplying it by the particular foodstuff's specific heat, as a proportion of that of water (= s), so that the equation becomes,

$$\% H = \frac{s * 417 * (T_b - T_{food})}{ACV * SDA' * AE} \quad \text{......................(5)}$$

Equation (4) is still more useful for nectar than this modified equation of Wilson and Culik (1991), because it is easier to work in mol l^{-1} (= C) than in kJ g^{-1} (= ACV) when dealing with liquid food.

Equations (4) and (5) still have to be taken through an additional step. If an endotherm uses % H of its energy requirements to warm the food it needs to meet its metabolic requirements excluding food warming costs, then it will actually have to ingest an additional % H of energy, and will have to expend % H of this additional energy gained to warm it to body temperature. The amount of energy (kJ) ingested will then be,

$$\begin{aligned} X' &= X + (X * H) + (X * H * H) + (X * H * H * H) + \ldots \\ &= X + (X * H) + (X * H^2) + (X * H^3) + \ldots \\ &= X * (1 + H + H^2 + H^3 + \ldots) \\ &= X + X * \frac{H}{1 - H}, \end{aligned}$$

where X is the amount of energy ingested, excluding that needed for food warming, and H is % H expressed as a proportion (i.e. % H / 100).

The total percentage of the energy expenditure used to warm the food will then become,

$$\% H' = \frac{100 * \% H}{100 - \% H} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (6).$$

When Wilson and Culik (1991) derived their equation, they assumed that food ingestion rates did not vary with differences in food warming costs. They assumed that the amount of energy ingested, X, was equal to 924.5 * (body mass)$^{0.70}$ kJ day $^{-1}$, from Peters (1983). Although this relationship is empirical and generally holds true, from the present analysis it is clear that it will vary, at least to some degree, with the actual cost of warming food.

The error resulting from using % H instead of % H', increases as the value of % H' increases (Fig. 4.1). When % H is 5, 20 and 50, % H' will be 5, 25 and 100 %.

The assimilation efficiencies of hummingbirds, a nectarivorous parrot, honeyeaters, sugarbirds and sunbirds for sucrose have been measured as between 97 and 100 % (Collins et al. 1980, Collins and Morellini 1979, Lotz and Nicolson 1996, Downs 1997a,b, Lopez-Calleja et al. 1997, Jackson et al. 1998a), and the AE term of equation (4) can therefore be assumed to equal 1 (effectively ignored).

The obligatory specific dynamic action (SDA) for carbohydrate is generally 6 % of the energy content of the food (Peters 1983), so the SDA' term in equation (4) is assumed to be 1 - 0.06 = 0.94.

The day-time body temperature (T_b) of the sunbird Nectarinia chalybea is 42.2 °C, irrespective of whether it is fed 0.2 or 1.2 M sucrose (see chapter 5).

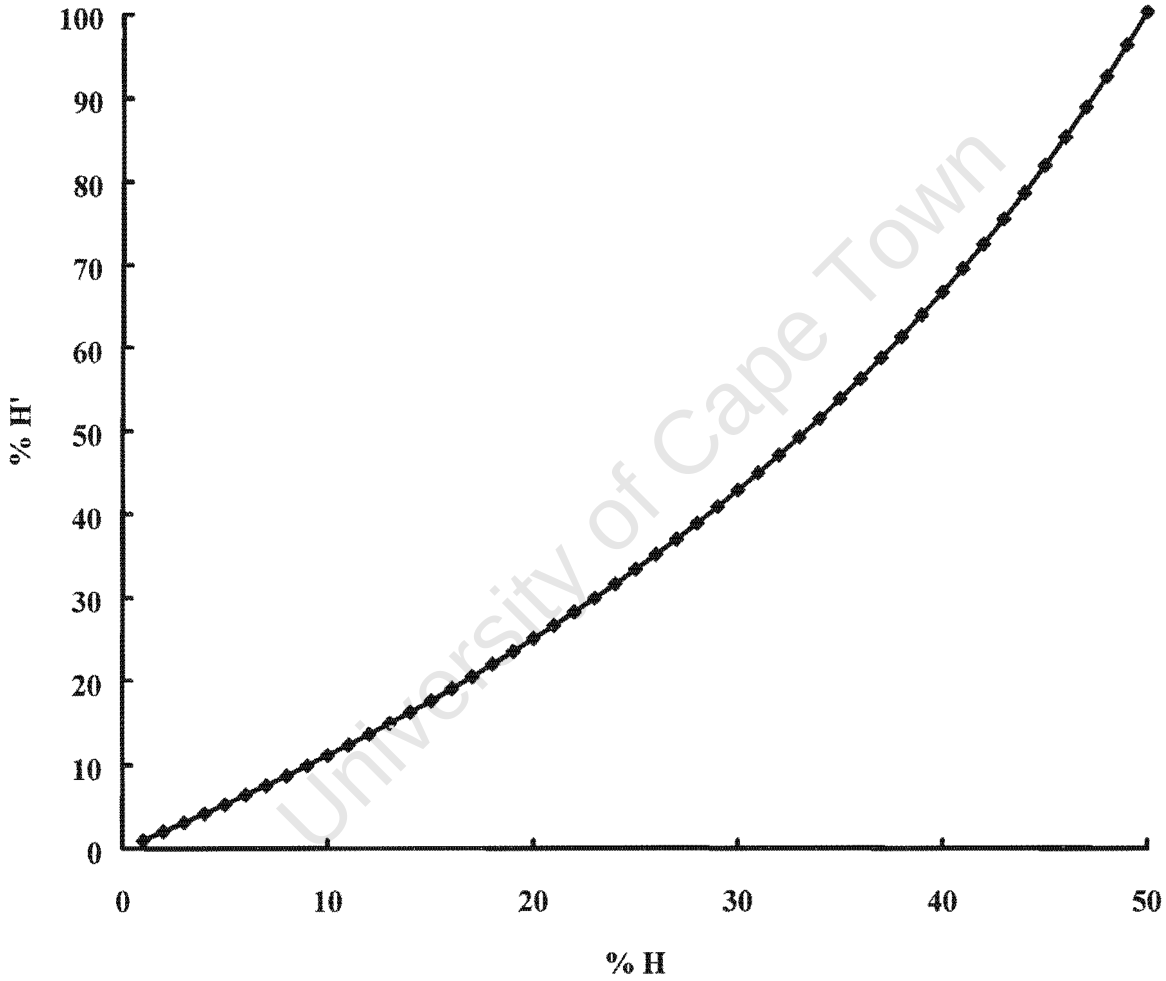

Fig. 4.1: Comparison between % H and % H'. Values were plotted using equation (6) (see text).

Equation (4) thus becomes,

$$\% H = \frac{(42.2 - T_n) * \left(-0.079 + \frac{0.692}{C}\right)}{10 * 0.94}$$

leaving only two variables, the nectar temperature, T_n (which will generally be determined by the ambient temperature), and the nectar concentration, C. This equation must be substituted into equation (6) to calculate % H'.

Results and discussion

Values of % H' are plotted in Fig. 4.2 over a range of nectar concentrations from 0.05 to 2.00 M sucrose and at two possible nectar temperatures, 10 and 20 °C. The percentage of the daily energy expenditure used to warm the nectar (% H') increases exponentially with decreasing nectar concentration. Therefore, although the cost of warming the nectar is low over a wide range of nectar concentrations, it rapidly becomes important below a particular concentration. When $T_n = 20$ °C, % H' increases from 1, to 4, to 17 % in birds feeding on 1.2, 0.4 and 0.1 M sucrose.

Values of % H' are plotted in Fig. 4.3 over a range of possible nectar temperatures between 1 and 42 °C (= T_b), and at two possible nectar concentrations, 0.4 and 1.2 M sucrose. At a given nectar concentration, % H' decreases linearly as T_n increases.

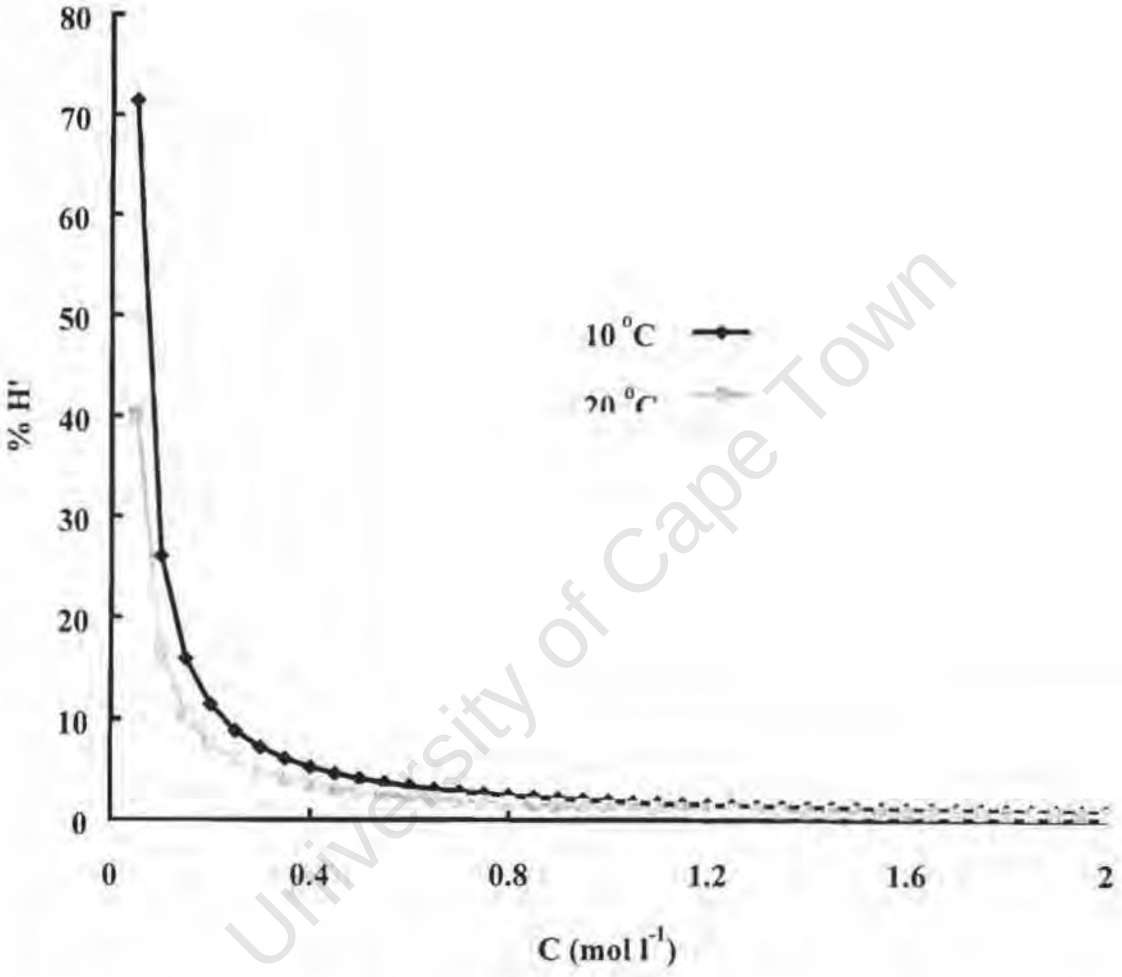

Fig. 4.2: The percentage of the energy intake used by endotherms to warm nectar to body temperature (% H'), plotted for two nectar temperatures (10 and 30 °C), over a range of nectar concentrations (C, g l^{-1}) from 0.05 to 2.0 M sucrose.

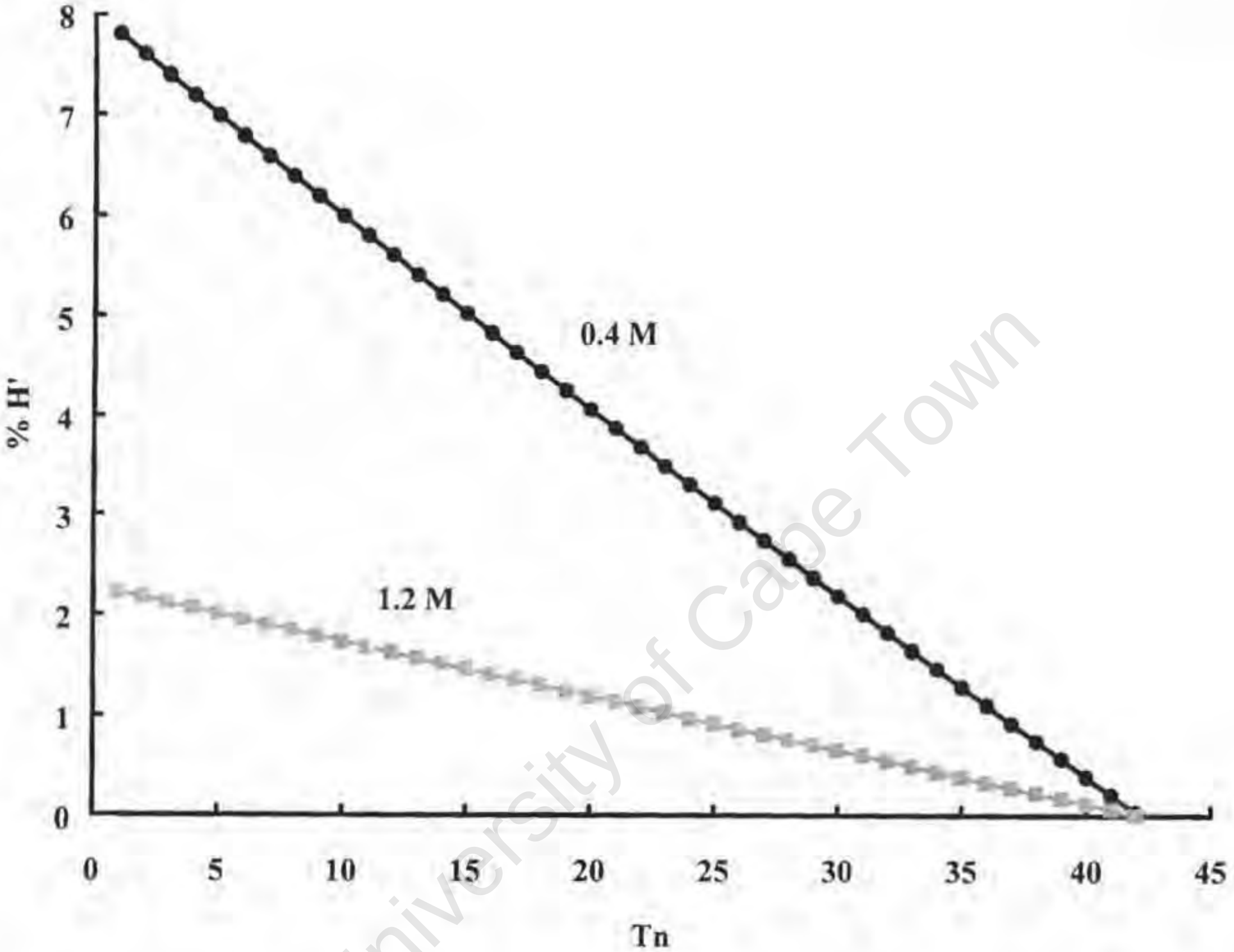

Fig. 4.3: The percentage of the energy intake used by endotherms to warm nectar to body temperature (% H'), plotted for two nectar concentrations (0.4 and 1.2 M sucrose), over a range of nectar temperatures (T_n, °C) from 1 to 42 °C.

Wilson and Culik (1991) plotted values of % H (not % H') for herbivorous and piscivorous birds at different food temperatures, using values of SDA', AE and ACV derived from Peters (1983). However, their plotted values are erroneous, being three times too high. Re-calculation of % H using their equation (unmodified) and the data they used from Peters (1983), yields values which are only 36 % of their plotted values, and this does not change when AE and ACF values are obtained from Karasov (1990) instead of deriving them from Peters (1983). Hawkins et al. (1997) independently calculated a % H value for eating fish at 2 °C, which is also only 30 % of the equivalent value on the graph of Wilson and Culik (1991). Furthermore, when their equation is modified to account for the difference in specific heat capacity between water and nectar of different concentrations, it yields % H values for nectar which are the same as those produced by equation (4), as modelled in the present study. It is therefore not surprising that Wilson and Culik failed to explain why the % H values they actually measured (2.5 %) in krill-eating Adelie penguins were substantially lower than the values they estimated calorimetrically (13 %).

In the present study, % H' values for birds feeding on different foodstuffs over a range of temperatures were calculated using equation (6) above, and the modified equation of Wilson and Culik (1991), equation (5), and these values are plotted in Fig. 4.4. The plotted values for nectar of different concentrations were calculated using equations (6) and (4). A T_b of 42 °C was assumed throughout. Avian body temperatures range between 39 and 44 °C (Peters 1983), and the maximal possible error in % H values resulting from an incorrect T_b assumption will be 6 % (assuming that $T_b = 39$ °C). The

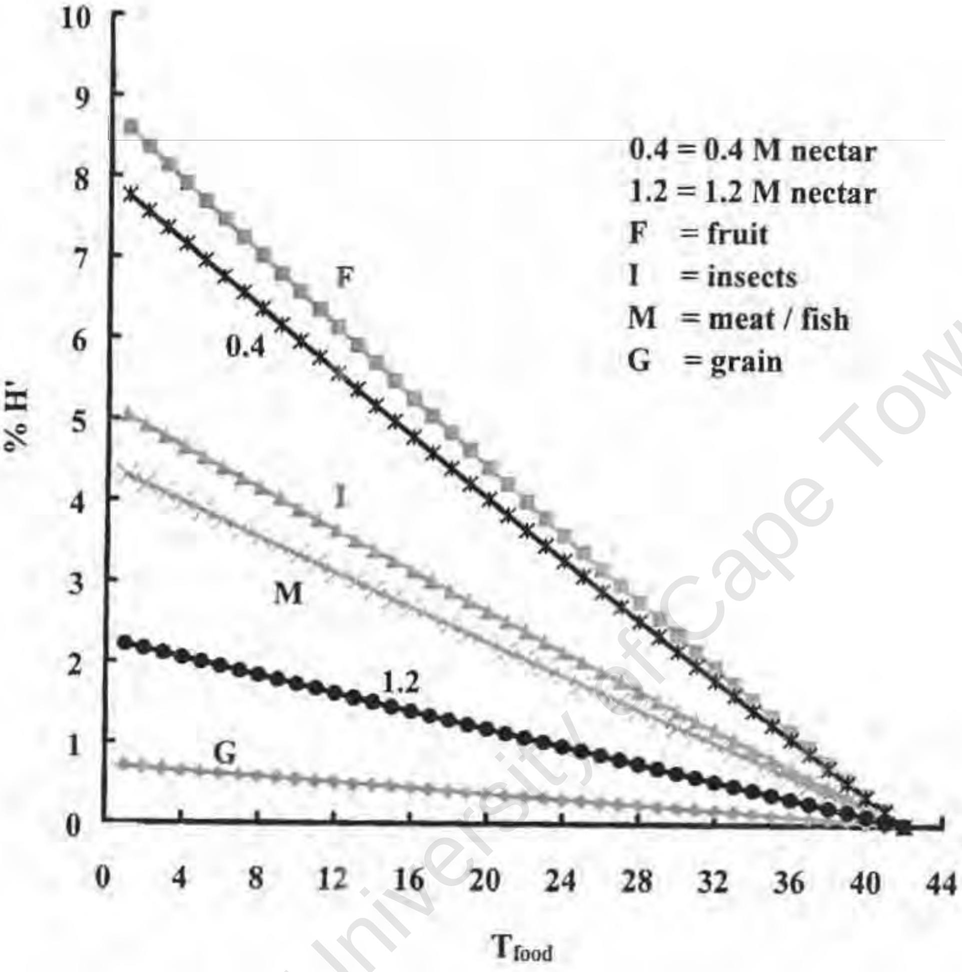

Fig. 4.4: The costs of warming a range of different foodstuffs to body temperature (% H'), over a range of food temperatures (T_{food}, °C) from 1 to 42 °C.

SDA', AE and ACV values were obtained from Peters (1983) and Karasov (1990), and are shown in Table 4.1.

The % H' values for dilute (0.4 M sucrose) nectar, and for fruit, are comparably high (Fig. 4.4), but for different reasons. The high % H values of 0.4 M nectar are due to this food's outstandingly low calorific concentration, ACV (see Table 4.1). However, the values for the dilute nectar would be even higher if its AE was not exceptionally high, 100 %. Typical fruit, on the other hand, generally has only a moderately low ACV (twice as high as that of 0.4 M nectar), and its high % H' values can be largely explained by its unusually (see Table 4.1) low AE, 51 % (Karasov 1990). The specific heat capacities of both foods are similarly high (Table 4.1). Fruit, however, is particularly variable in terms of water content, specific heat capacity, ACV and assimilation efficiency. For example, Peters (1983) reports an AE of only 31 % for berries, which would lead to substantially higher % H' values.

As nectar becomes more dilute than that commonly occurring in nature, so its ACV decreases and its % H' increases exponentially. Nectar of 0.1 M sucrose has a % H' which is twice that of typical fruit. Nectar of 0.2 M sucrose, which does occur in some bird- and bat-pollinated flowers (Pyke and Waser 1981, Nicolson 1993), will yield a % H' value of 7.6 and 11 % when at 20 and 10 ^{o}C respectively.

Table 4.1: % H' values and their determinants, for different foods.

	Protein	Fat	Carbo.	Water	SHC[b]	ACV[c]	SDA'[d]	AE[e]	% H', 2 °C	% H', 20 °C
	%	%	%	%	% SHC_{water} / 100	KJ g^{1}	% / 100	% / 100	%	%
0.1 M nectar[a]	0	0	3.4	96.6	0.98	0.53	0.940	1.00	41.4	19.3
0.2 M nectar	0	0	6.5	93.5	0.95	1.01	0.940	1.00	16.9	8.7
0.4 M nectar	0	0	12.4	87.6	0.91	1.92	0.940	1.00	7.6	4.1
0.75 M nectar	0	0	22.2	77.8	0.84	3.43	0.940	1.00	3.6	2.0
1.2 M nectar	0	0	34.3	65.7	0.76	5.30	0.940	1.00	2.2	1.2
1.6 M nectar	0	0	44.7	55.3	0.69	6.92	0.940	1.00	1.5	0.8
Fruit	1.2	4.0	12.8	82.1	0.88	4.18	0.901	0.51	12.9	6.9
Grain	10.9	2.4	73.2	13.5	0.40	16.75	0.763	0.76	1.7	0.4
Meat / fish	19.2	3.3	0.3	77.1	0.85	6.01	0.728	0.81	4.0	2.2
Insects	17.7	3.4	2.3	76.6	0.84	6.02	0.748	0.67	4.6	2.6

[a] sucrose equivalents [b] SHC = Specific heat capacity, [c] ACV = Absolute calorific value, [d] SDA = Specific dynamic action, [e] AE = assimilation efficiency (see text for details).

Nectar with a concentration at the upper extreme of that commonly produced in bird-pollinated flowers in nature, 1.2 M sucrose, has a low % H' value, lower than all the other foodstuffs considered except for grain (Table 4.1 and Fig. 4.4). Its ACV is similar to that of meat (including fish), and of insects. However, its specific heat capacity is substantially lower, and its assimilation efficiency substantially higher, than these foods, and so overall its % H' values are lower. Grain has the highest ACV value, the lowest specific heat capacity (seeds contain very little water), and therefore the lowest % H' of any of the foods considered, except for 1.6 M sucrose.

The % H' of meat / fish at 2 °C is equivalent to that of 0.4 M sucrose at 18 °C, or of fruit at 20 °C, so the cost of warming dilute nectar or fruit at moderate temperature is comparable to the cost of warming fish or krill in high-latitude piscivorous endotherms such as penguins, seals, whales and grebes. In addition, quite a number of marine birds and mammals have an ability to reduce body temperature during foraging dives (Wilson and Gremillet 1996, Handrich et al. 1997), which would reduce their % H' values further. In contrast to this, the day-time body temperature of the sunbird N. chalybea is maintained at 42 °C irrespective of whether it is fed 0.2 or 1.2 M sucrose (see chapter 5).

The cost of warming nectar will be reduced if flowers maintain nectar temperature above ambient temperature. Few data exist on nectar temperatures, but it is known that some hummingbird-pollinated flowers track the sun, which leads to increased nectar temperature (Kevan 1975, Corbet and Willmer 1981). In addition, it is known that a few insect-pollinated flowers maintain flower temperatures up to 20 °C above ambient temperature (Lamarck 1778, Meeuse 1978, Knutson 1979). Warming nectar would not only reduce the cost of % H', but would also allow more rapid nectar uptake because of reduced viscosity, as discussed by Heyneman (1983).

It is well-established that the metabolic rate in a wide range of different animals increases directly after a meal. Some of this increase results from the obligatory SDA, which is the energy expended during digestion, absorption and processing or storage of the ingested food (Trayhurn and James 1981, Janes and Chappell 1995). However, in endotherms, at least some of it may also result from the cost of warming food to body temperature. Indeed, Wilson and Culik (1991) found that SDA was not measurable in Adelie penguins fed krill warmed to their body temperature, and that metabolic rate increased only when birds were fed freezing krill. The same difference in metabolic rate was observed when penguins were fed water either at body temperature or freezing. Most authors have ignored the cost of warming food when measuring SDA, and so their calculation of obligatory SDA is confounded. On the other hand, studies of SDA in ectotherms, for example that of Hailey (1998) on tortoises, and that of Wang et al. (1995) on toads, are not confounded by food warming costs.

Knowledge of the temperature of the ingested food, its specific heat capacity, calorimetric concentration and the efficiency with which it is assimilated allows calculation of the cost of warming it. If the food's temperature is measured, the other components can usually be estimated from known literature values. Hawkins et al. (1997) did such calculations on Brunnich's guillemot feeding on fish, and found that

30 % of the increase in metabolic rate after ingestion could be accounted for by food warming.

It is possible that heat produced from obligatory SDA contributes to food warming, so that if the heat produced from SDA is greater than or equal to that required to warm the food, then additional heat may not have to be produced. It is known that "waste" heat produced during foraging activity in verdins (Auriparus flaviceps) substitutes for that which is otherwise produced solely for thermoregulation, as ambient temperature decreases (Webster and Weathers 1990). Hawkins et al. (1997) ignored this possibility when concluding that 30 % of the post-meal increase in metabolic rate was caused by food warming, and that the other 70 % by SDA, in fish-fed guillemots. In fact, 100 % of the increase could have been due to SDA, 30 % of which may then have been channelled into food warming. In the light of the above discussion, the most rigorous method of measuring SDA would be to heat food to the animal's body temperature, as Wilson and Culik (1991) did for Adelie penguins.

At an ambient temperature of 20 °C, the non-resting, day-time MR of N. chalybea is 14.6 % higher when feeding on 0.2 than when feeding on 1.2 M sucrose, 9.96 and 8.69 ml O_2 g^{-1} h^{-1} respectively (see chapter 5). The night-time MR is 5.04 ml O_2 g^{-1} h^{-1}, irrespective of diet. In birds fed the dilute solution, 16.2 % of the increase in day- over night-time MR can be accounted for by food warming, whereas only 2.7 % of the increase can be accounted for by food warming in birds fed the concentrated solution. This represents a difference of 16.2 – 2.7 = 13.5 %, which could almost account for the dietary difference in day-time MR of 14.6 %. However, the energy cost of evaporation also needs to be considered, and in fact is a much larger expense than that of food warming, especially in sunbirds fed dilute sucrose: 57 and 30 % of day-time metabolic rate in birds kept at 20 °C, and fed 0.2 and 1.2 M sucrose, respectively (see chapter 5). This difference in evaporative heat loss between birds fed 0.2 and 1.2 M sucrose should lead to a further 21.7 % difference in MR between the two diets, in addition to the 13.5 % difference accounted for by food warming costs. However, the dietary difference in day-time MR is actually only 14.6 %, and this can possibly be explained by a higher insulation in birds fed the relatively dilute food, as discussed in chapter 5.

Leon and Nicolson (1997) measured the day- and night-time MR of N. chalybea fed 0.3 M sucrose, and kept under a range of ambient temperatures from 7 to 35 °C. The percentages of the day-time values accounted for by food warming (% H') are now calculated, and added to the actual night-time values, to yield the elevation in RMR that would occur solely from food warming, in the absence of SDA and / or activity. These values are plotted in Fig. 4.5, together with the actual night- and day-time MR's. It is clear from Fig. 4.5 that only a fraction of the measured increase in day- over night-time MR can be accounted for by food warming, which means theoretically that no additional energy needs to be expended to warm the food, because the heat produced from activity and SDA can be used to warm the food. However, it may be more difficult for aquatic endotherms to redirect activity-related heat production to food warming, because the thermal conductance of water is 25 times greater than that of air (Withers 1992).

Numerous studies have quantified field metabolic rate (FMR) in nectarivorous birds, using either time-energy budgets or labelled water (e.g. Wolf 1975, Gill and Wolf

1975, Frost and Frost 1980, Collins 1983, Weathers and Stiles 1989, Williams 1996, Weathers et al. 1996, Goldstein and Bradshaw 1998b). Knowledge of the magnitude of the increase in metabolic rate caused by food warming would improve the accuracy of the time-energy budget studies, and would improve interpretation of the labelled water data. In addition, the accuracy of validations of time-budget data by labelled water data (e.g. Weathers et al. 1996), would be improved if food warming costs were taken into account. For example, the labelled water study of Williams (1993) on the sunbird N. violacea breeding during the Cape winter, revealed that sunbirds were feeding on 0.6 M nectar (sucrose equivalents, assuming that the birds obtained all their energy from nectar, and calculating nectar concentration from FMR and water flux). At the low winter temperatures, the cost of warming such dilute nectar to body temperature cannot be negligible.

In conclusion, the energy required to warm food to body temperature may be substantial, especially for endotherms consuming certain food types such as dilute nectar. To disentangle the costs of warming food, thermoregulation (excluding food warming), SDA and activity, future studies will have to ensure that these variables are not confounded.

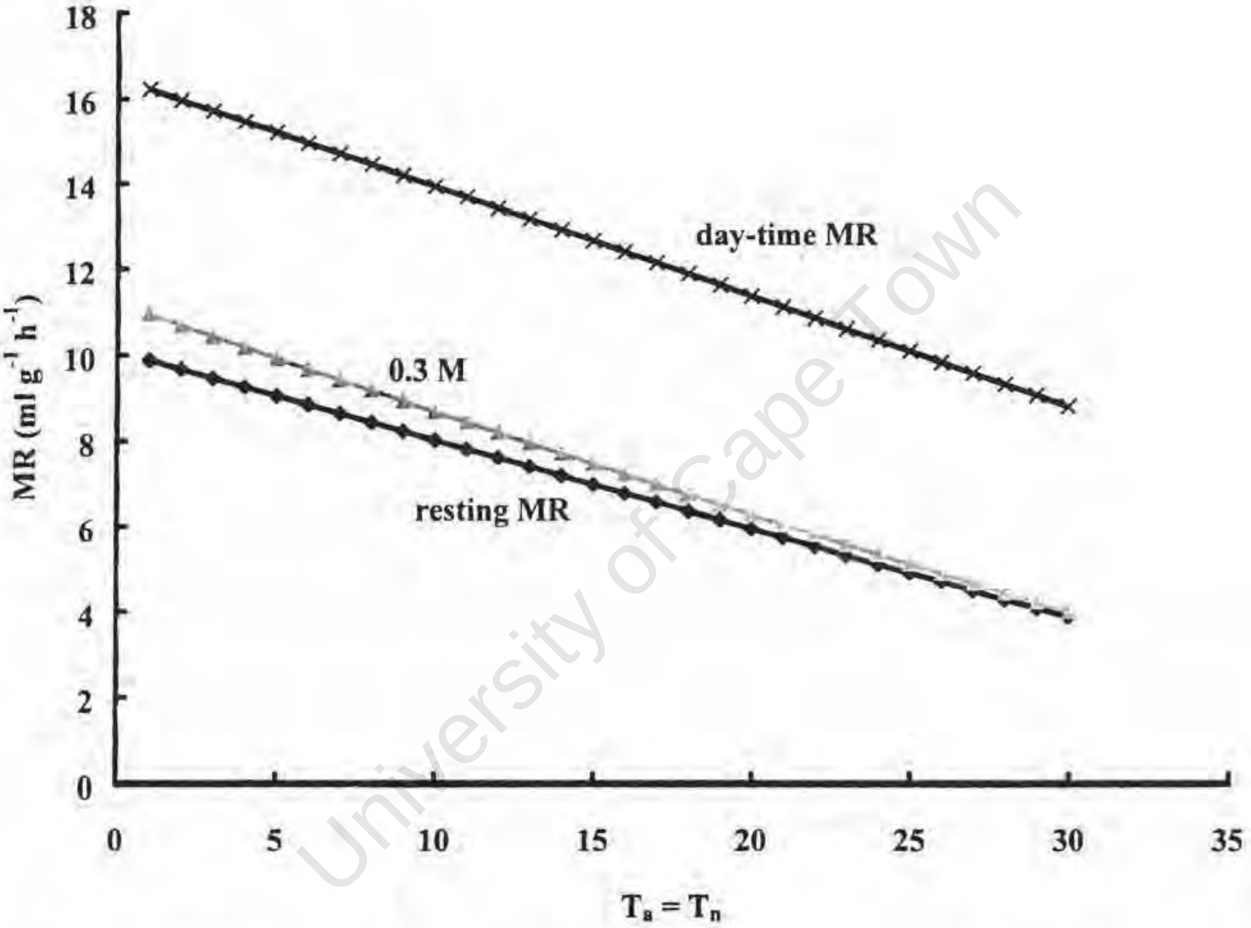

Fig. 4.5: elevation of resting (night-time) metabolic rate that would occur from food (0.3 M sucrose) warming alone, compared with measured day-time metabolic rate in N. chalybea. Resting and day-time metabolic rates are from Leon and Nicolson (1997). The elevation in resting metabolic rate was calculated using % H' values (see text for details).

Chapter 5

Evaporative water loss, metabolic rate and body temperature of Nectarinia chalybea fed 0.2 or 1.2 M sucrose

Summary

In the sunbird Nectarinia chalybea, the difference between water gain (preformed and metabolic water) and excreted water, which should represent evaporative water loss (EWL), is higher when birds are fed relatively dilute nectar (chapters 1 and 3). If EWL and therefore evaporative heat loss is higher in sunbirds fed more dilute nectar, then they will have to increase their metabolic rate (MR) and/or decrease their dry thermal conductance if they are to maintain the same body temperature (T_b). During the present experiments, EWL, MR and T_b were measured directly in N. chalybea kept at an ambient temperature of 20 °C and fed either 0.2 or 1.2 M sucrose. The day-time EWL was indeed 2.15 times higher in birds fed the dilute solution. This change occurred immediately after a switch in diet, and from calculations of respiratory EWL it was apparently cutaneous in origin. The T_b of N. chalybea was 42 °C irrespective of diet. To maintain this T_b, the MR of sunbirds was higher, and the dry thermal conductance lower, when fed the dilute solution. These responses, like the change in EWL, were effected immediately.

Introduction

A large number of studies have measured total evaporative water loss (EWL) in birds. A reduction in EWL occurs in a range of bird species when kept on low water rations ("dehydrated"). For example, in the sparrow Spizella pallida (11.0 g), EWL at $T_a = 25$ °C is 4.6 mg g^{-1} h^{-1} when it has access to water ad lib., but only 2.1 mg g^{-1} h^{-1} when it is dehydrated (Dawson et al. 1979). The house finch Carpodacus mexicanus (17.9 g) can reduce its EWL from 6.1 to 5.7 mg g^{-1} h^{-1} between normally-hydrated and dehydrated states ($T_a = 26$ °C, MacMillen and Hinds 1998). In the budgerigar (30 g), kept at $T_a = 40$ °C, EWL is 5.0 mg g^{-1} h^{-1} when hydrated, and 4.3 mg g^{-1} h^{-1} when dehydrated (Greenwald et al. 1967).

In some studies, either cutaneous or respiratory EWL has been measured, or both components have been measured so that their relative contributions to total EWL are known. A reduction in total EWL with water deprivation can occur through either a reduction in cutaneous or in respiratory EWL. In the zebra finch, Poephila guttata (12 g), cutaneous EWL (measured with an electrolytic moisture analyser), is reduced markedly when birds are water-deprived for six weeks (Menon et al. 1989). In this species kept at $T_a = 25$ °C, total EWL is normally 8.8 mg g^{-1} h^{-1}, but falls to 5.5 after dehydration. At T_a = 40 °C, the difference is more marked, 22 and 8 mg g^{-1} h^{-1}, respectively (Macmillen 1990). The ostrich (Struthio camelus) reduces its respiratory EWL (measured with a mask) when dehydrated (Crawford and Schmidt-Nielsen 1967). In the emu (Dromaius novaehollandiae) at $T_a = 45$ °C, total EWL is reduced from 2.4 to 1.9 mg g^{-1} h^{-1} when dehydrated, and calculation of respiratory EWL from expired air temperature and minute volume reveals that this change in total EWL is accounted for by a change in cutaneous

EWL (Maloney and Dawson 1998). In pigeons (Columba livea) kept at $T_a = 45$ °C in two-compartment chambers for separating the head and the rest of the body, which allows measurement of both components of total EWL, cutaneous EWL decreases from 20 to 6 mg g^{-1} h^{-1} when dehydrated, but respiratory EWL increases such that there is actually no difference in total EWL between normally-hydrated and dehydrated birds (Arad et al. 1987).

Clearly, the state of hydration (whether normal or dehydrated) affects EWL in birds. In addition, birds from mesic areas have higher EWL rates than those from arid areas (Williams 1996), presumably because the former generally have easier access to drinking water. It is likely that if the availability of drinking water influences EWL, then the preformed water content of food also affects EWL. Birds that feed on nectar should have higher rates of EWL than those that consume other foods. Moreover, nectarivorous birds feeding on dilute nectar may have higher EWL rates than those feeding on more concentrated nectar.

The effect of diet on EWL has rarely been examined in birds. However, in honeyeaters, it is known that the day-time EWL increases with decreasing nectar concentration. In Lichmera indistincta (8 g) kept at $T_a = 20$ °C, the EWL is 30.3 mg g^{-1} h^{-1} when the birds are fed 0.4 M, but only 24.7 mg g^{-1} h^{-1} when fed 1.2 M sucrose. Similarly, in Acanthorhynchus superciliosis (10 g), EWL is 34.0 and 28.1 mg g^{-1} h^{-1}, respectively, in birds fed 0.4 and 1.2 M sucrose (Collins 1981). In chapter 1, it was established that in the sunbird Nectarinia chalybea, the difference between water gain (preformed and metabolic water), and excreted water, which should represent EWL, was higher in birds fed 0.4 M sucrose than in those fed 0.8 or 1.2 M sucrose.

If EWL is higher in birds fed more dilute nectar at a particular T_a, then interesting thermoregulatory considerations result. Because birds would lose more heat (through evaporative heat loss) when feeding on dilute food, at least one of the following would have to occur to maintain heat balance on the dilute diet:

1) body temperature could decrease,
2) metabolic rate could increase,
3) dry thermal conductance (the reciprocal of insulation) could decrease.

The cost of warming nectar to body temperature was considered in chapter 4, and was found to increase dramatically at low nectar concentrations. This cost should exacerbate 1) to 3) above.

In the present chapter, the effect of nectar concentration on EWL, body temperature, metabolic rate and thermal conductance is examined in N. chalybea.

Materials and Methods

All experiments were performed in a constant environment room set at $T_a = 20$ °C, and on a 12L : 12D photoperiod, with lights-on at 07:30, as in the experiments described in previous chapters. The Ensure® acclimation diet was used, as in chapters 2 and 3 (Ensure® is characterised in Appendix 1)

The body temperature (T_b), metabolic rate (MR), and EWL were measured separately, on different birds. Birds were fed either 0.2 or 1.2 M sucrose. These two concentrations were chosen because it is known that N. chalybea easily survives on both diets (C. N. Lotz, unpublished data), but they nevertheless represent extremes in concentration, so that dietary changes in T_b, MR or EWL should be easily detected.

T_b

The T_b's of three male and three female N. chalybea each fed, in turn, 0.2 and 1.2 M sucrose for 24 h, were measured over a 10 min period by inserting a copper-constantan thermocouple 1 - 2 cm into the cloaca. During the measurement period, the birds were restrained in cloth jackets, and they were allowed to feed ad lib. on their particular experimental diet. Birds always fed a few times during the 10 min period. The thermocouple was connected to a Fluke thermocouple thermometer, and calibrated against a mercury thermometer calibrated by the South African Bureau of Standards. Birds were handled with surgical gloves, kept at the same T_a, to minimise conductive heat transfer. After insertion of the thermocouple, T_b readings were taken every minute for ten minutes. Further methodological considerations concerning the measurement of T_b are discussed below.

During measurement of MR and EWL, birds were placed singly in a 3 l volume chamber (Fig. 5.1). The chamber was a modified glass funnel, 27 cm in diameter, inverted and fastened to a tray containing a layer of liquid paraffin 2 cm deep. The bird sat directly on a chicken wire stand 5 cm above the liquid paraffin. The shape of the chamber prevented excreta from coming into contact with its walls, which would lead to erroneously high EWL measurements. The spout of the funnel was cut off, and a 50 ml syringe feeder was attached in its place. The feeder extended far enough into the chamber so that the bird could feed without changing its position on the chicken wire stand. An inlet and an outlet for air onto which tubing could be snugly attached, were made near the top and bottom, respectively, of the chamber.

Before reaching the chamber, air passed through copper tubing (115 cm long, 3 mm diameter) in a water bath to buffer its temperature beyond the capabilities of the constant environment room (± 0.3 °C without the water bath). Air then passed through soda lime and then through silica gel before entering the chamber. After the chamber, the air was led through a tube containing the sensor of a Vaisala relative humidity meter, then through soda lime and silica gel again, before being led through the oxygen sensor and into an air pump which "pulled" the air through the system. After the air pump, air was passed into a 50 ml burette which allowed accurate measurement of flow rate by timing the speed with which a soap bubble introduced into the system travelled the length of the burette. The whole system was linked together using silicone rubber tubing. Data were recorded on a computer using the interface program "Data Capture System".

The Vaisala humidity meter was carefully calibrated over saturated solutions of four different salts, LiCl, 12.4 % r.h.; $MgCl_2$, 33 %; NaCl, 75.5 %, and K_2SO_4, 97.2 % Winston and Bates 1960). The calibration was checked at least every two weeks.

Although the system was set up for the measurement of both MR and EWL, the former could only be measured accurately at low flow rates due to limitations of the oxygen meter, whereas the latter could only be measured at high flow rates when the r.h. was well below saturation. The MR and EWL were therefore measured separately. During the MR measurements the flow rate was set at 180 ml min^{-1}, and during the EWL measurements it

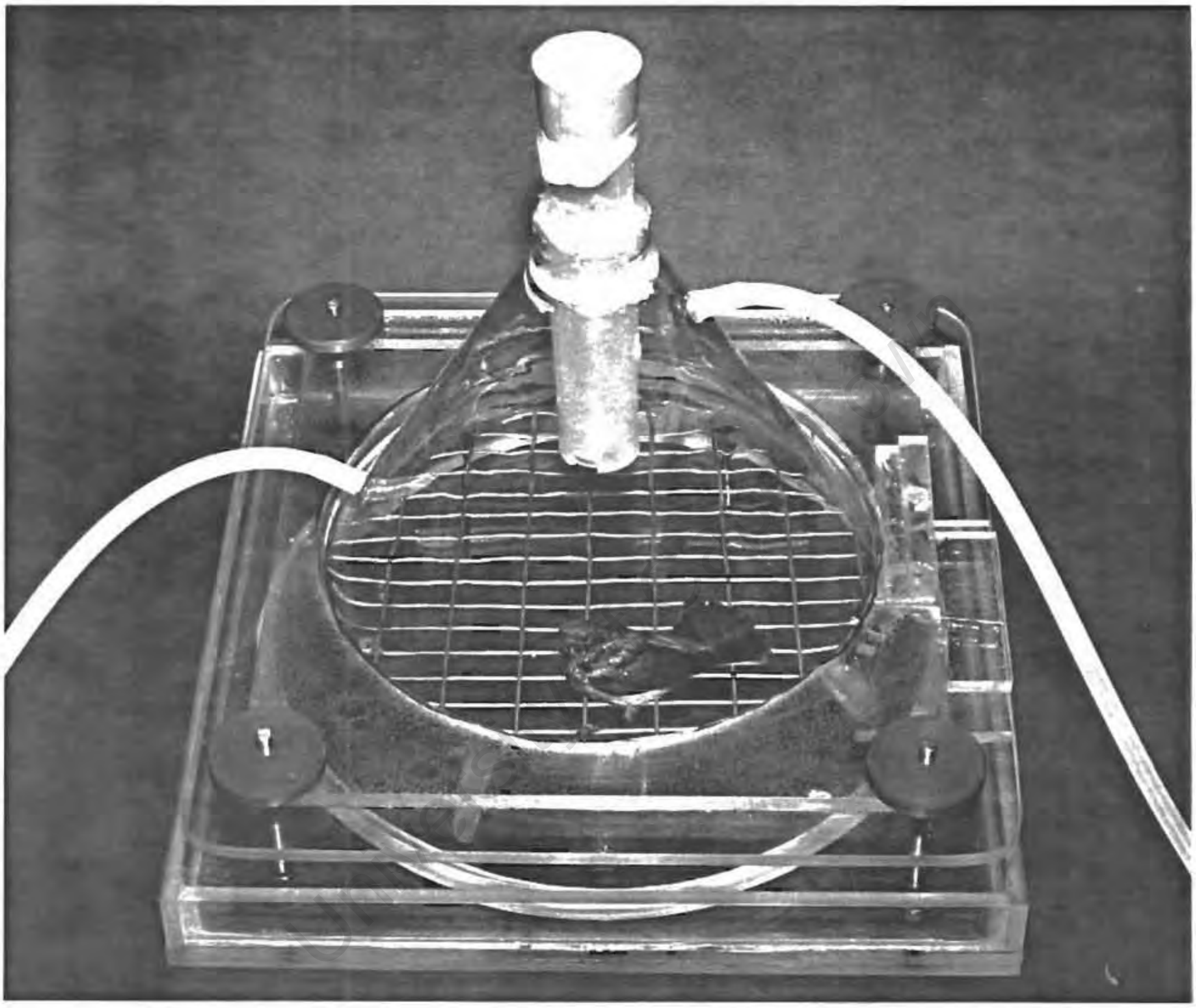

Fig. 5.1: A photograph of the 3 l chamber used for measurement of MR and EWL

was set at 540 ml min^{-1}. The day-time chamber r.h. during the MR experiments, once stabilised, averaged around 100 and 70 % while birds were fed 0.2 and 1.2 M sucrose, respectively. The day-time chamber r.h. during the EWL experiments was 70 and 36 % while birds were fed 0.2 and 1.2 M sucrose, respectively. The night-time r.h. averaged 12 and 15 %. The MR and EWL were only calculated from readings obtained once the r.h. in the chamber had stabilised (see below).

MR

For the MR experiments, a total of two male and two female N. chalybea were fed 0.2 and 1.2 M sucrose, in turn. One male and one female received 0.2 M sucrose during their first trial, the other two birds receiving 1.2 M sucrose first. After a full 24 h of acclimation to their experimental diets, birds were weighed and placed in the chamber at 16:30 and allowed to settle down before recording was started at 18:30. The MR was then monitored for one full night, and for part of the following day. It was considered unnecessary to monitor MR for a full day because it was already known that the MR of N. chalybea does not vary substantially through the day (Leon and Nicolson 1997). At 12:30, during the day-time measurement of MR, the diet was switched to see how quickly, if at all, MR would change with a dietary change. Experiments were terminated 2.5 h later, at 15:00, before birds were returned to their acclimation cages and diet. Individual birds were fed the acclimation diet for a minimum of three full days between trials.

The washout time expected for the system to reach 99 % equilibrium, given a volume of 3 l and a flow rate of 180 ml min^{-1}, was 77 min (Lasiewski et al. 1966). The actual night- and day-time oxygen analyzer readings stabilized slightly faster than expected, after 60 min. In addition, during the last 30 minutes of the dark period, there was a marked increase in the average MR, in apparent anticipation of lights-on, and this was more pronounced in some individuals than in others. Average night-time oxygen consumption was thus calculated from readings obtained between 21:00 and 07:00. The day-time oxygen consumption was calculated from all the readings obtained between 10:30 (after three hours of light), and 12:30, at which time the diet was switched. The MR after the dietary switch was calculated for the last hour of the experiment (from 14:00 to 15:00).

Oxygen consumption was calculated, in ml O_2 h^{-1}, as

$$(\text{flow rate} * 60) * (F_iO_2 - F_eO_2) / (1 - F_iO_2),$$

where flow rate is in ml air min^{-1}, and F_iO_2 and F_eO_2 are the fractional concentrations of oxygen in the incurrent and excurrent air, respectively (Bucher and Chappell 1997). Mass-specific oxygen consumption, in ml O_2 g^{-1} h^{-1} was also calculated, by dividing by body mass. Volumes were converted to STP.

EWL

During the measurement of EWL, the same protocol as for the MR experiments was followed, except that the r.h. was monitored for one full night, followed by one full day and another full night. It was deemed important to measure EWL for an entire day because it was unknown how much EWL would vary through the day, as it has not been measured in sunbirds before, and in fact day-time EWL while normally feeding has rarely been measured in birds.

The night-time chamber r.h. decreased gradually before reaching stable levels between 03:30 and 07:30 (light-on, see Fig. 5.5). This may have been due to retention of water vapour within plumage (J. B. Williams, pers comm.), and/or to the inside of the chamber and system tubing, because the expected washout time (the time for the system to reach 99 % equilibrium), given a system volume of 3 l and a flow rate of 540 ml min^{-1}, is only 26 min (Lasiewski et al. 1966). The night-time EWL (mass-specific, area-specific and total night-time), was calculated (see below) only from these r.h. values between 03:30 and 07:30. Also, the day-time chamber r.h. did not stabilise until 11:30 when birds were fed 0.2 M sucrose (earlier when fed 1.2 M sucrose), and EWL was calculated from r.h. values between 11:30 and 19:30.

The mass-specific EWL, in mg g^{-1} h^{-1}, was calculated as

$$(\text{flow rate} * 60 / \text{body mass}) * (\text{r.h.} / 100) * 0.0173,$$

where flow rate is in ml min^{-1}, body mass is in g, and 0.0173 is the mass, in mg, of water in 1 ml of saturated aqueous vapour at 20.0 °C (Weast 1985). The r.h. of the air flowing into the chamber was assumed to be 0 %. The r.h. of the chamber without a bird was consistently measured, prior to each experiment, as less than 1 %, which would lead to negligible errors in the calculation of EWL. This calculation is modelled after that of Bernstein et al. (1977).

The method of calculating EWL from r.h. measured with a humidity meter was carefully validated. Twice, a small bowl containing a known mass of water was placed in the chamber instead of a bird, for 24 h, to simulate typical EWL rates of N. chalybea.

Positioned after the chamber, was the Vaisala humidity sensor, followed by a weighed tube of drierite. At the end of the experiment, the drierite tube, as well as the bowl, were re-weighed to give separate measures of the mass of water evaporated, and compared with the water volumes calculated using the measured r.h. The error was less than 5 %.

The surface area-specific EWL, in mg cm^{-2} h^{-1}, was calculated from the mass-specific values by multiplying by the body mass and dividing by the surface area. The surface area was estimated from the body mass using the formula of Walsberg and King (1978),

$$\log S_{ext} = 0.667 * \log(\text{body mass}) + \log 8.11,$$

where S_{ext} is the external surface area of the plumage. The S_{ext} was used instead of S_{skin}, because it excludes surfaces that are not directly exposed to the environment, such as the under-surface of the folded wing (Walsberg and King 1978).

The total night-time and day-time EWL, in ml 12 h^{-1}, were calculated as the respective mass-specific EWL * 1000 * 12 h / body mass. The two were added to yield the total daily EWL, in ml 24 h^{-1}.

After the finding that diet had a large effect on EWL, further experiments were performed to determine how rapidly EWL changes with a switch in diet. The chamber was modified so that the feeder could be switched rapidly during experimental runs. Preliminary tests showed that switching of feeders did not influence chamber r.h., and therefore would not influence EWL readings during experiments. One female and one male N. chalybea, in turn, was placed in the chamber at 16:30 and fed 1.2 M sucrose, and EWL was monitored for a full night and until 11:00 the following day, at which time the diet was changed to 0.2 M sucrose. Monitoring continued for the rest of the day, for the following night, and for another full day, whilst 0.2 M sucrose was still available. This was repeated on another male and another female bird, except that the initial diet was 0.2 M sucrose.

Evaporative heat loss (H_e, J g^{-1} h^{-1}) was calculated from EWL assuming a latent heat of vaporisation of 2.428 J (mg H_2O)$^{-1}$, and night- and day-time respiratory quotients of 0.775 and 1.2, respectively (see chapter 1). Metabolic heat production (H_m, J g^{-1} h^{-1}) was also calculated assuming these respiratory quotients, for determination of the ratio of $H_e : H_m$.

Thermal conductance

The dry thermal conductance (DTC), in ml O_2 (g h °C)$^{-1}$, was calculated as

$$DTC = (MR - H_e) / (T_b - T_a),$$

where MR and H_e are in ml O_2 g^{-1} h^{-1}. Similarly, wet thermal conductance (WTC) was calculated as

$$WTC = MR / (T_b - T_a) \text{ (McNab 1980).}$$

Paired sample t-tests were performed using STATISTICA (Statsoft, Tulsa), following the methodology of Zar (1996), to test for dietary differences in body mass, T_b, MR and EWL. Values are reported as the mean ± SE. Statistics were not used when calculations involved more than one of the measured variables of EWL, MR and T_b (i.e. $H_e : H_m$ ratios and thermal conductance) because these variables were obtained during separate experiments on different birds.

Results

Body mass

The body masses at 16:30, after 24 h of acclimation to the experimental diet, and just prior to EWL measurement, did not differ significantly ($P = 0.732$) between birds fed 0.2 M sucrose (7.89 ± 0.24 g) and those fed 1.2 M sucrose (7.97 ± 0.31 g). However, during the MR measurements. the body mass of birds fed 0.2 M sucrose (8.63 ± 0.32 g), was significantly lower than that of birds fed 1.2 M sucrose (9.27 ± 0.33 g, $P = 0.0382$).

Body temperature (T_b)

Body temperatures measured every minute remained constant over the ten minute measuring period (Fig. 5.2). Body temperatures averaged over the ten minutes for each bird were the same irrespective of diet (Table 5.1, $P = 0.367$).

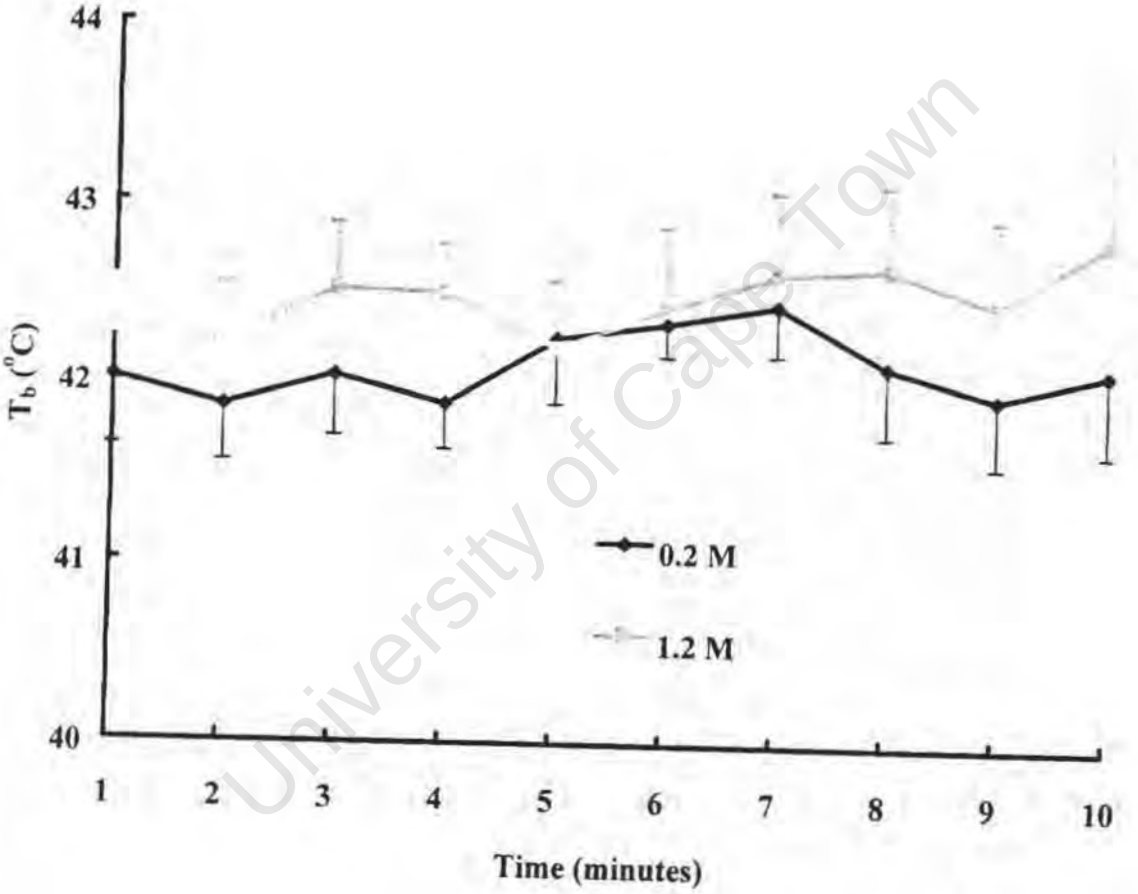

Fig. 5.2: Body temperature (T_b, °C, mean ± SE n = 6) of N. chalybea fed 0.2 or 1.2 M sucrose, measured every minute for a ten minute period.

Table 5.1: Body temperature (T_b), metabolic rate (MR), evaporative water loss (EWL), percentage of metabolic heat production (H_m) dissipated by evaporation (H_e), dry thermal conductance (DTC) and wet thermal conductance (WTC) in *N. chalybea* fed 0.2 or 1.2 M sucrose, at $T_a = 20\ ^{\circ}C$. Values represent the mean ± SE, n = 4, except that n = 6 in the case of T_b.

		Night, 0.2 M	Night, 1.2 M	Day, 0.2 M	Day, 1.2 M
T_b	°C	-	-	42.04 ± 0.21	42.43 ± 0.61
MR	**$ml\ O_2\ h^{-1}$**	42.64 ± 2.79	45.17 ± 2.41	84.09 ± 3.14	78.93 ± 3.94
MR	**$ml\ O_2\ g^{-1}\ h^{-1}$**	5.06 ± 0.41	5.01 ± 0.41	9.96 ± 0.34	8.69 ± 0.25
EWL	**$mg\ g^{-1}\ h^{-1}$**	10.68 ± 1.31	8.43 ± 0.87	49.44 ± 2.84	23.04 ± 0.72
EWL	**$mg\ cm^{-2}\ h^{-1}$**	2.61 ± 0.30	2.08 ± 0.23	12.18 ± 0.81	5.65 ± 0.14
H_e:H_m	%	25.78	20.35	57.07	29.93
DTC	**$mlO_2\ (g\ h\ ^{\circ}C)^{-1}$**	-	-	0.20	0.29
WTC	**$mlO_2\ (g\ h\ ^{\circ}C)^{-1}$**	-	-	0.45	0.40

Metabolic rate (MR)

Because birds fed 0.2 M sucrose maintained slightly lower body masses than those fed 1.2 M sucrose (see above), the night-time MR in ml $O_2\ h^{-1}$ (Fig. 5.3) was lower in birds fed the dilute solution. However, average mass-specific MR during the night (ml $O_2\ g^{-1}\ h^{-1}$, Fig. 5.4) was the same irrespective of diet (see Table 5.1 for values). Average day-time MR in ml h^{-1} and in ml $g^{-1}\ h^{-1}$ was significantly higher in birds fed 0.2 M sucrose than in those fed 1.2 M sucrose ($P < 0.025$), but the differences in the mass-specific values were of greater magnitude, again because of the dietary differences in body mass.

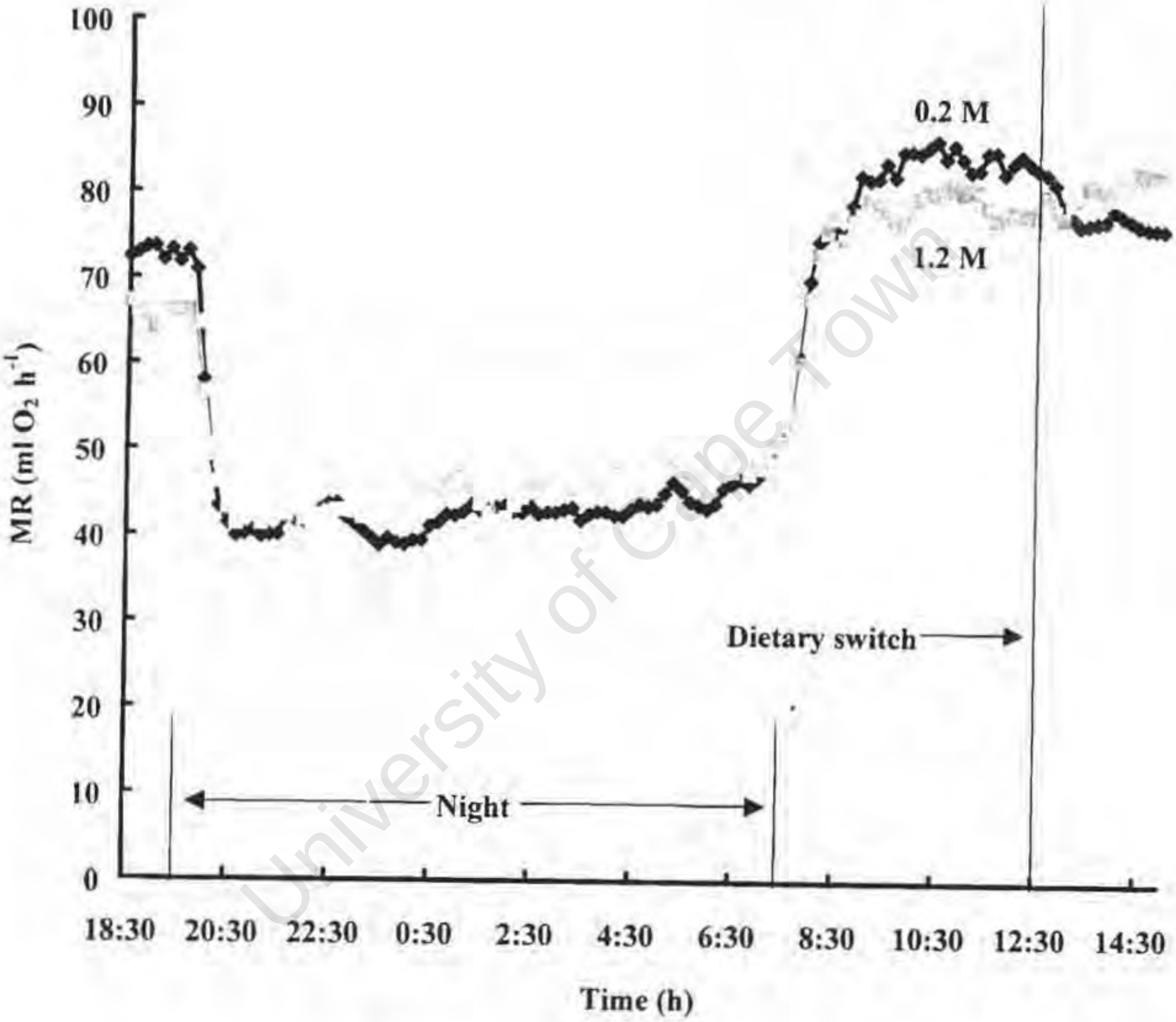

Fig. 5.3: Metabolic rate (MR, ml O_2 h^{-1}, mean n = 4) of N. chalybea fed 0.2 or 1.2 M sucrose. Standard errors are omitted for the sake of clarity.

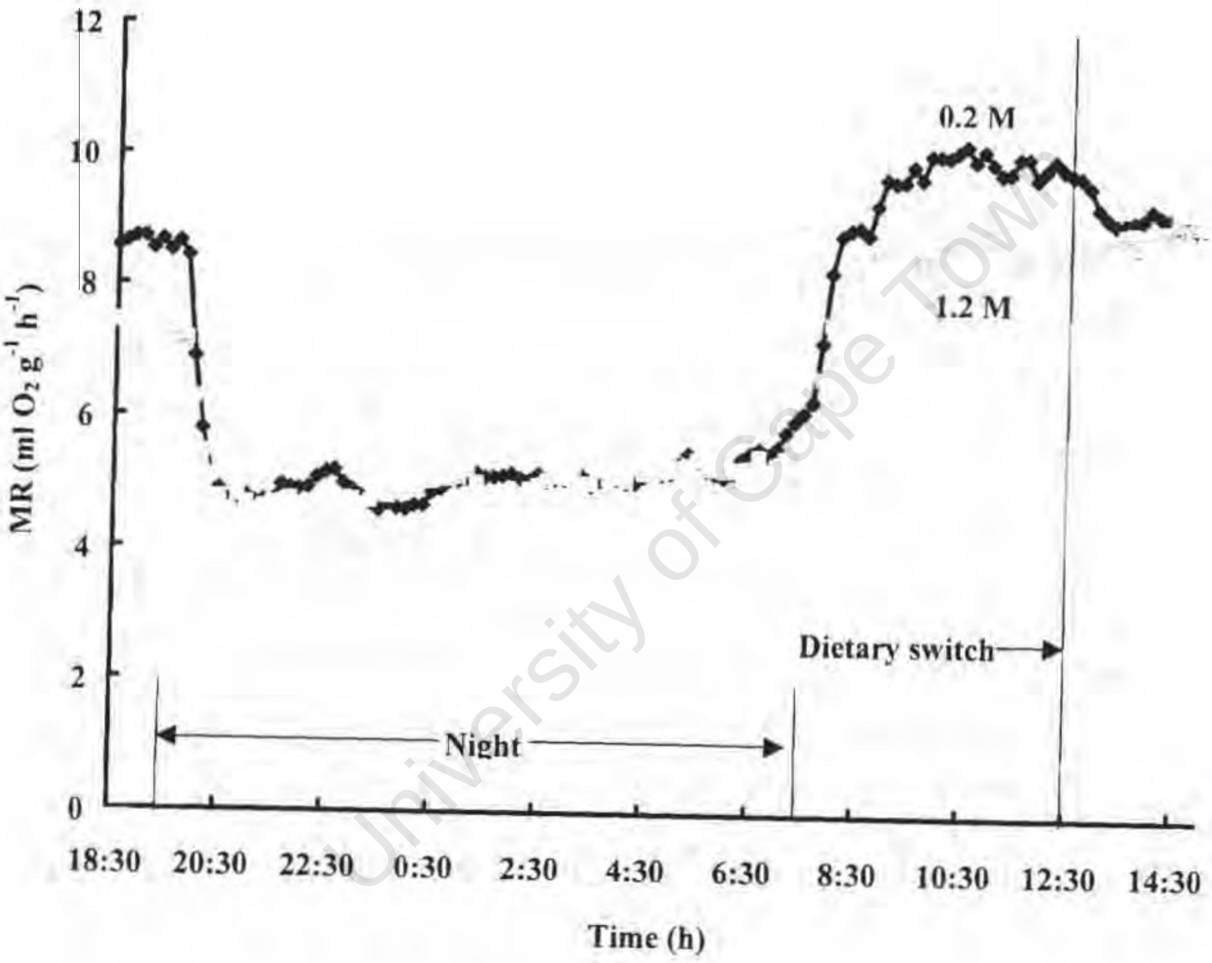

Fig. 5.4: Metabolic rate (MR, ml O_2 g^{-1} h^{-1}, mean, n = 4) of N. chalybea fed 0.2 or 1.2 M sucrose. Standard errors are omitted for the sake of clarity.

When the diet was switched from 1.2 to 0.2 M sucrose at 12:30 (see Fig. 5.3), the MR immediately started to increase, and vice versa, so that 1.5 hours after the dietary change, the metabolic rates of birds fed the different solutions were once again significantly different from one another, at the $P < 0.025$ level. In other words, the MR changed immediately after the dietary switch.

Evaporative Water Loss (EWL)

The day-time EWL values (in mg g^{-1} h^{-1}) were 2.15 times higher in birds fed 0.2 than in those fed 1.2 M sucrose ($P < 0.0025$, Fig. 5.5, Table 5.1). The night-time EWL was higher in birds fed 0.2 than in those fed 1.2 M sucrose, but the difference was not significant ($P = 0.361$, Fig. 5.5, Table 5.1). The 24 h EWL values were 5.70 ± 0.30 ml in birds fed 0.2, and 3.81 ± 0.81 ml in those fed 1.2 M sucrose.

In the EWL experiments during which the diet was switched from 1.2 to 0.2 M sucrose at 11:00 (see Fig. 5.6a), the EWL was initially low (16.63 mg g^{-1} h^{-1}), similar to that maintained throughout the day in birds fed 1.2 M sucrose continuously during previous experiments (16.63 mg g^{-1} h^{-1}). When the diet was switched to 0.2 M sucrose at 11:00, the EWL immediately started to increase, at the same rate as at the start of the light period in birds which had been acclimated to 0.2 M sucrose (during previous experiments). In one bird, the EWL increased by a factor of 3.1 within an hour, and in the other bird, it increased by a factor of 2.7 within two hours after the change in diet, reaching levels comparable to those found in birds fed 0.2 M sucrose continuously during previous

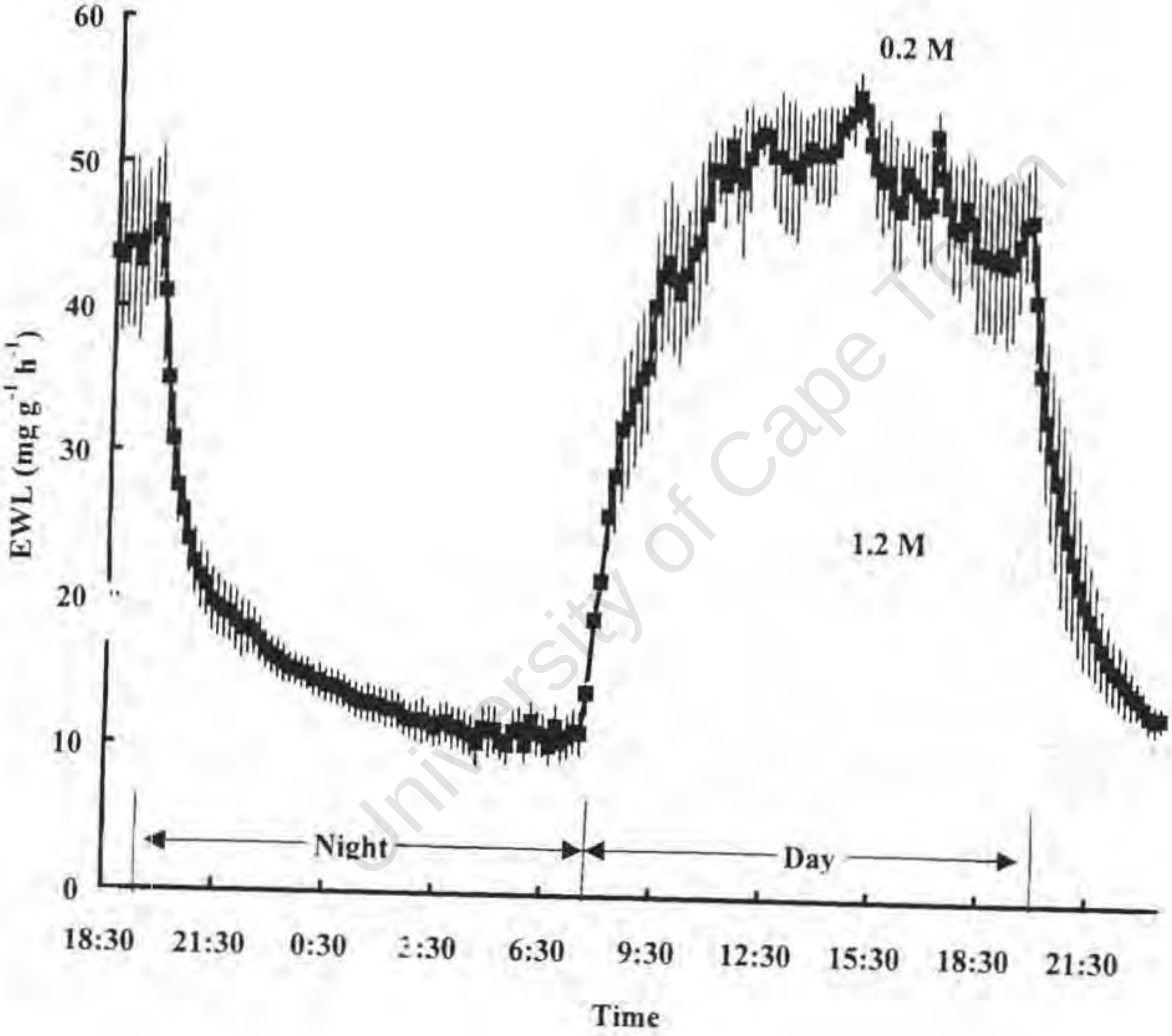

Fig. 5.5: Evaporative water loss (EWL, mg g^{-1} h^{-1}, mean ± SE, n = 4) of *N. chalybea* fed 0.2 or 1.2 M sucrose.

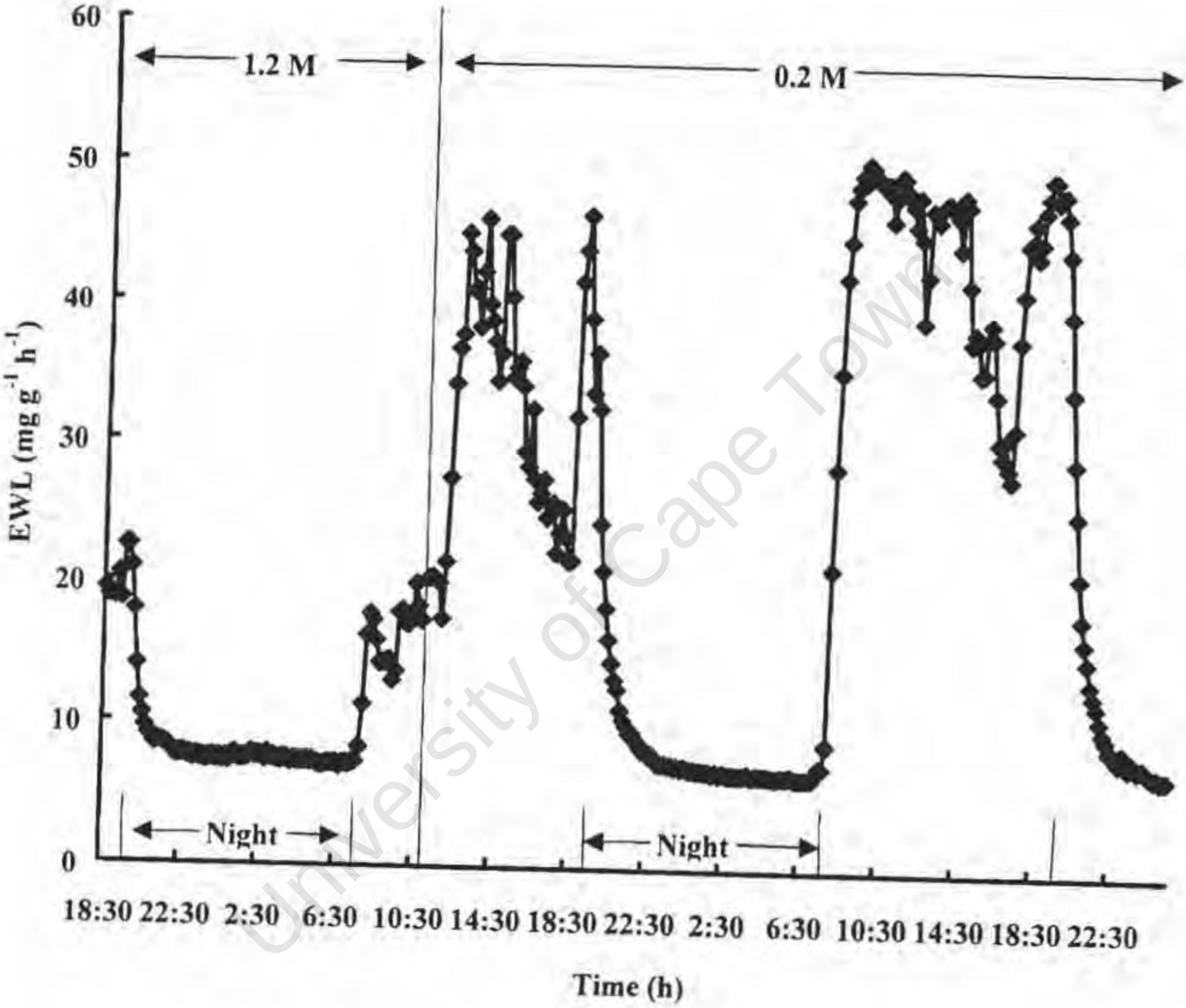

Fig. 5.6a (bird no. 1): Evaporative water loss (EWL, mg g^{-1} h^{-1}) of an individual <u>N. chalybea</u> initially fed 1.2 M sucrose, and then fed 0.2 M sucrose.

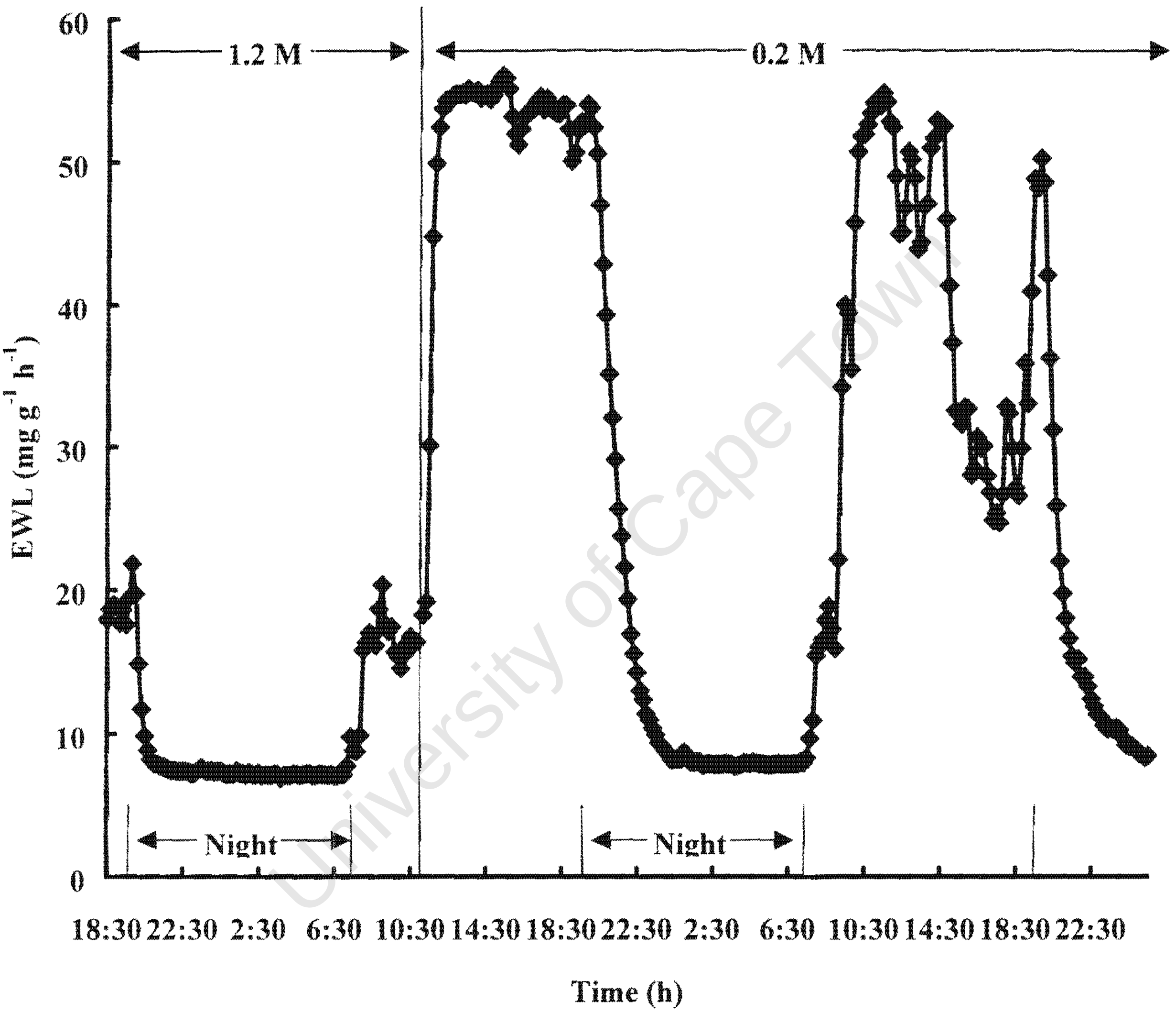

Fig. 5.6a (bird no. 2): Evaporative water loss (EWL, mg g^{-1} h^{-1}) of an individual <u>N. chalybea</u> initially fed 1.2 M sucrose, and then fed 0.2 M sucrose.

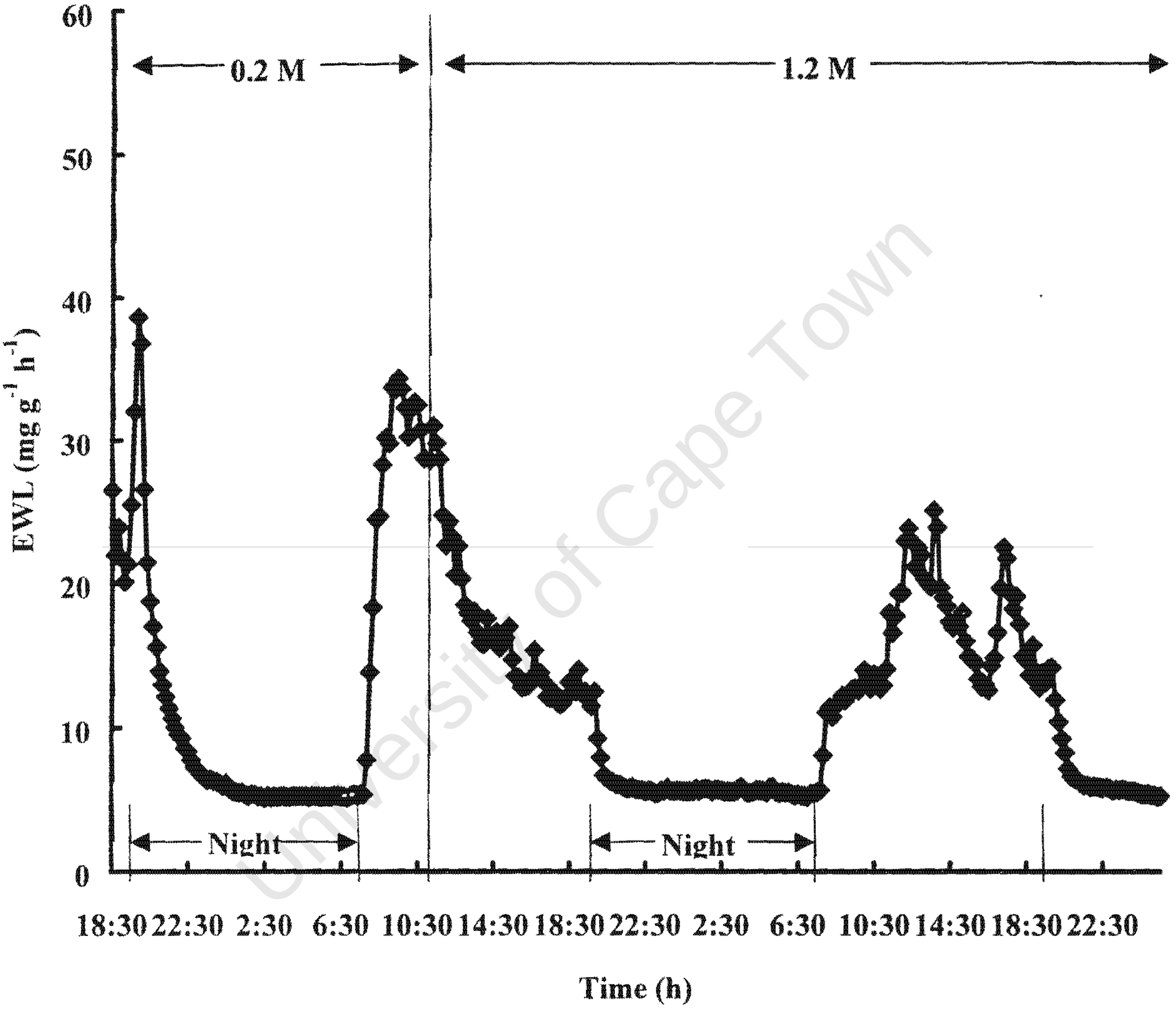

Fig. 5.6b (bird no. 1): Evaporative water loss (EWL, mg g^{-1} h^{-1}) of an individual N. chalybea initially fed 0.2 M sucrose, and then fed 1.2 M sucrose.

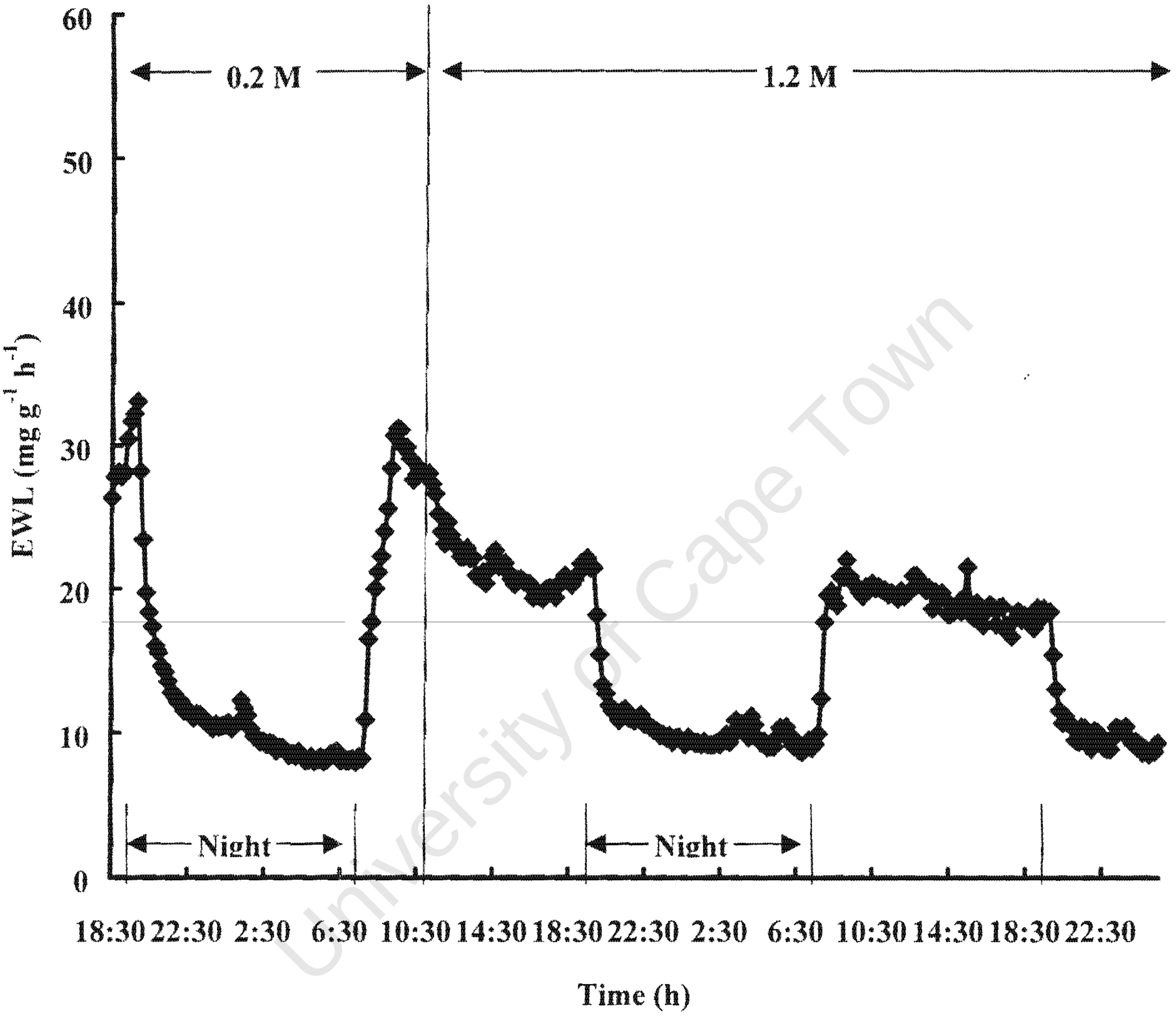

Fig. 5.6b (bird no. 2): Evaporative water loss (EWL, mg g^{-1} h^{-1}) of an individual <u>N. chalybea</u> initially fed 0.2 M sucrose, and then fed 1.2 M sucrose.

experiments. On the second day of the experiment, while the birds were still being fed 0.2 M sucrose, the birds maintained the same high EWL rates expected in birds fed 0.2 M sucrose. In the two birds that were initially fed 0.2 M sucrose (Fig. 5.6b), the EWL started off at high levels, and after the 11:00 diet change, it immediately started to decrease to the low levels normally occurring in birds fed 1.2 M sucrose. During the second day, the low levels were maintained.

The EWL of individual birds was far more constant at night than it was during the day, both in birds fed 0.2 and in those fed 1.2 M sucrose (Figs. 5.5a and 5.5b).

Thermal conductance

Thermal conductance (the inverse of insulation) is a measure of the ease with which heat is transferred between the body and its environment. The day-time dry thermal conductance, which excludes H_e (see "methods" for its calculation) and is therefore technically a more precise term than wet thermal conductance (WTC), was higher in birds fed 0.2 M than in those fed 1.2 M sucrose (see Table 5.1). The WTC showed the opposite trend, because it includes H_e which was 2.15 times higher in birds fed the dilute solution than in those fed the concentrated one.

$H_e : H_m$ ratios

The percentage of the metabolic heat production dissipated by evaporation is given by the ratio of $H_e : H_m$. The night-time $H_e : H_m$ ratio was slightly (1.27 times) higher in birds fed 0.2 than in those fed 1.2 M sucrose (Table 5.1). This is because the night-time H_e (which is directly proportional to EWL, see earlier), was slightly but insignificantly higher in birds fed the dilute solution, even though the MR was unaffected by diet. The day-time H_e : H_m ratio was 1.91 times higher in birds fed the dilute diet. This is because the day-time H_e was 2.15 times higher in birds fed 0.2 M than in those fed 1.2 M sucrose, but the day-time H_m was only 1.15 times higher.

Discussion

Body temperature (T_b)

The T_b of N. chalybea kept at 20 °C averaged 42.2 °C, irrespective of diet (0.2 or 1.2 M sucrose). The lowest T_b measured in any individual bird was 41.3, and the highest was 43.7 °C. Leon and Nicolson (1997) measured an average day-time T_b of only 39.8 °C in the same species, also kept at 20 °C, and fed 0.3 M sucrose. These authors inserted the thermocouple "about 1 cm" into the cloaca, and recorded T_b within 30 s. In the present study, the thermocouple was inserted until it could no longer easily be pushed further, 1 - 2 cm, and T_b was measured immediately and then every minute for ten minutes. During the measurement periods, T_b remained constant (Fig 5.2). During preliminary experiments, the thermocouple was only inserted about 1 cm into the cloaca, and T_b measurements comparable to those of Leon and Nicolson (1997) were obtained. During these preliminary measurements, the T_b would suddenly increase during the voiding of excretory fluid, and then decrease to initial levels within a minute or two. This did not occur when the thermocouple was inserted further.

The day-time T_b's of other sunbirds are similar to those measured in N. chalybea. Prinzinger et al. (1989) measured an average day-time T_b of 41.6 °C in 13 sunbird species kept at 20 °C, by inserting a thermocouple "at least 1.0 cm" into the cloaca, and taking readings within 30 s of insertion. This T_b is 0.6 °C lower than that measured in N. chalybea during the present study. In three Afro-montane sunbird species, maintained in cages at their sites of capture for less than 24 h, day-time T_b's ranged between 41 and 43 °C (measured by insertion of a thermistor into the cloaca, T_a = 8 to 20 °C, Cheke 1971).

Body temperatures have also been measured in other nectarivorous birds. In the hummingbird Sephanoides sephanoides, day-time T_b's of between 40 and 45 °C were measured by inserting a thermocouple "about 1 cm" into the cloaca, over a T_a range of 10 - 25 °C (Prinzinger et al. 1992). The average T_b of the hummingbird Calypte anna at T_a = 20 °C, measured by insertion of a thermocouple into the pectoralis muscle, was 42.0 $\pm$ 2.0 °C. The T_b's of sunbirds and hummingbirds are thus in the same range. However, there is evidence that honeyeaters have slightly lower T_b's. Collins et al. (1980b) measured an average day-time T_b of 39 °C in two honeyeater species, Lichmera indistincta and Meliphaga virescens, maintained at 20 °C. These measurements are probably reliable because a thermistor was inserted 2 cm into the cloaca, and sometimes T_b was also monitored continuously with a thermocouple implanted.

Metabolic rate (MR)

The metabolic rate measured at night in N. chalybea was the same irrespective of diet, 5.04 ± 0.27 ml O_2 g^{-1} h^{-1}. The metabolic rate increased by 98 % during the day, to 9.96 ± 0.20 ml O_2 g^{-1} h^{-1} when the birds were fed 0.2 M, but by only 76 %, to 8.85 ± 0.28 ml O_2 g^{-1} h^{-1}, when they were fed 1.2 M sucrose. Leon and Nicolson (1997) measured a night-time metabolic rate of 5.96 ml O_2 g^{-1} h^{-1} in N. chalybea kept at 20 °C, which was elevated by 91 % to 11.38 ml O_2 g^{-1} h^{-1} during the day, during which time they consumed 0.3 M sucrose. In the honeyeater Lichmera indistincta, which has the same body mass as N. chalybea (8 g), the MR increased by 99 % from 5.16 ml O_2 g^{-1} h^{-1} at night, to 10.27 ml O_2 g^{-1} h^{-1} during the day, irrespective of whether the birds were fed 0.4, 0.8 or 1.2 M sucrose (Collins 1981). In hummingbirds weighing around 8 g, the elevation of day- over night-time MR is also approximately 100 % (Kruger et al. 1982). The expected increase in day-over night-time MR in passerine birds is only 49.4 % (Aschoff and Pohl 1970). From the above discussion, it is apparent that nectarivores in general seem to have values that are twice as high.

The difference of 1.11 ml O_2 g^{-1} h^{-1} in the day-time MR between birds fed 0.2 and those fed 1.2 M sucrose is equivalent to the change which occurs during the day in N. chalybea over a 4.4 °C decrease in T_a below the thermoneutral zone (Leon and Nicolson 1997). The dietary difference in absolute (not mass-specific) MR was even higher (see Table 5.1), because birds fed 0.2 M sucrose maintained a lower body mass than those fed 1.2 M sucrose. The MR started to change immediately after a dietary switch (Figs. 5.3 and 5.4). Clearly, feeding on the dilute nectar involves a cost (see below), which can be compared with that associated with a decrease in T_a.

Evaporative water loss (EWL)

The night-time EWL was higher in N. chalybea fed 0.2 than in those fed 1.2 M sucrose (Table 5.1), but the difference was not significant. Values of EWL from the literature should be comparable to these night-time values, as they are largely based on measurements done on birds resting in the dark (Dawson 1982, Williams 1996). There are ample data in the literature showing that EWL is considerably higher in birds that are active or feeding than in those that are resting (for example, Tucker 1968, Taylor et al. 1971, Collins 1981, Hudson and Bernstein 1981). The night-time values of 10.68 and 8.43 mg g h^{-1}, measured in N. chalybea fed 0.2 and 1.2 M sucrose respectively, are slightly higher than predicted by the equations of Williams (1996) for mesic birds, of which N. chalybea is an example (7.53 mg g h^{-1}), and especially for arid and mesic birds pooled (6.42 mg g h^{-1}). The data of Williams (1996) are from birds kept at 25 °C, but are still comparable to those of the present study at 20 °C because ambient temperature only has an important effect on EWL when it exceeds thermoneutral temperatures (Calder and King 1974).

It is possible that the high night-time EWL values measured in N. chalybea are typical of nectarivorous birds, which consume food with a high preformed water content. The EWL, in ml 24 h^{-1}, of nine hummingbird species ranging in body mass from 3.2 to 19.1 g (from Williams 1996) are plotted in Fig. 5.7, and the night-time values measured in N. chalybea are also shown for comparison. The value for the sunbird feeding on 0.2 M sucrose closely resembles that expected for an 8 g hummingbird. In addition, the 8 g honeyeater Lichmera indistincta has a night-time EWL of 12.5 mg g^{-1} h^{-1}, 1.7 times higher than that predicted for a mesic bird (Williams 1996).

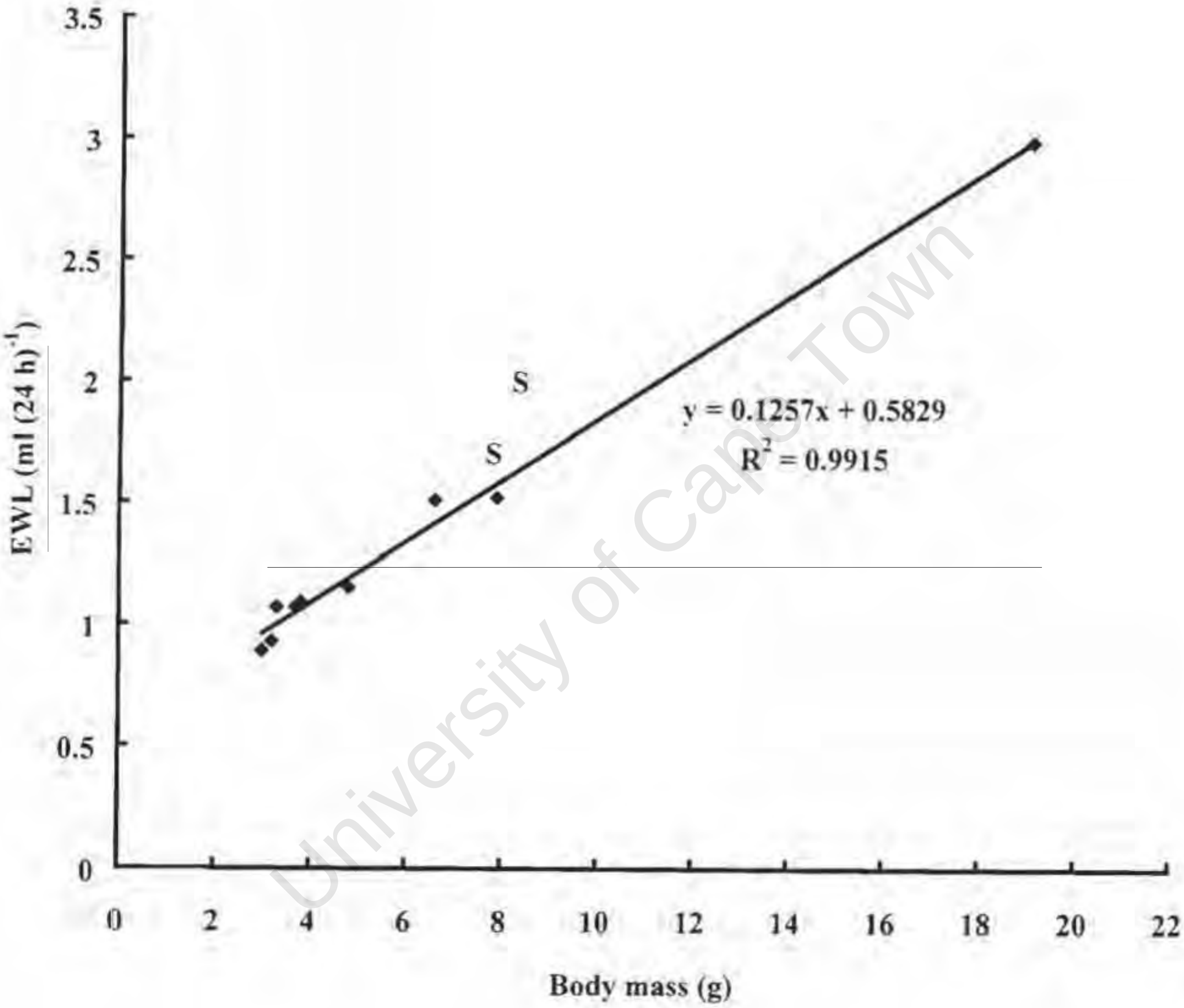

Fig. 5.7: Resting evaporative water loss (EWL, ml 24 h^{-1}) of nine hummingbird species as a function of body mass (data from Williams 1996), with the night-time values of N. chalybea fed either 0.2 or 1.2 M sucrose indicated.

fed 0.2 and 1.2 M sucrose is qualitatively similar to that occurring between normally-hydrated and dehydrated individuals of various bird species (post-absorptive and resting in the dark), but the actual EWL values involved are substantially higher in the actively-feeding sunbirds (see Table 5.2). The EWL values measured during the day in sunbirds fed 0.2 and 1.2 M sucrose respectively, averaged 4.6 and 2.7 times those measured at night. The honeyeaters Lichmera indistincta (8 g) and Acanthorhynchus superciliosis (10 g) have comparable day-time EWL values to N. chalybea, which are affected similarly by diet (whether fed 0.4, 0.8 or 1.2 M sucrose, see Table 5.3). Few if any other studies have measured day-time EWL in normally-feeding birds.

Nectarinia chalybea loses 5.69 ml of water by evaporation per 24 h (4.68 ml during the day and 1.01 ml during the night) when fed 0.2 M sucrose, and 3.01 ml 24 h^{-1} (2.20 ml during the day and 0.81 ml during the night), when fed 1.2 M sucrose. Although the absolute 24 h volume of water lost evaporatively is much larger (almost double) in birds fed the dilute solution, the percentage of the total 24 h water gain (preformed + metabolic water, from chapters 1 and 3), lost evaporatively is considerably smaller (13 % of 46 ml in birds fed 0.2 M, and 55 % of 5.5 ml in those fed 1.2 M sucrose). This means that EWL is a less important component of water balance in N. chalybea fed dilute sucrose than in those fed more concentrated sucrose, and excretion accounts for most of the increase in water turnover on the dilute diet. Therefore, if the increase in EWL on the dilute nectar is an active mechanism for getting rid of excess water, rather than an unavoidable, passive process resulting from a high water influx (see below), it is not a very effective mechanism, and excretion is far more important.

Table 5.2: Changes in resting EWL (mg g^{-1} h^{-1}) with state of hydration in various bird species.

Species	BM	T_a	Hydrated	Dehydrated	Reference
	g	°C	mg g^{-1} h^{-1}	mg g^{-1} h^{-1}	
Spizella pallida (sparrow)	11.0	25	4.6	2.1	Dawson et al. (1979)
S. passerina	11.8	25	2.8	2.5	Dawson et al. (1979)
Carpodacus mexicanus (house finch)	17.9	26	5.0	4.5	MacMillen and Hinds (1998)
Poephila guttata (zebra finch)	11.8	25	8.8	5.5	MacMillen (1990)
P. guttata	11.8	40	22	8	MacMillen (1990)
Melopsittacus undulatus (budgerigar)	30	40	5	4.3	Greenwald et al. (1967)
Columba livia (pigeon)	207	30	6	7	Arad et al. (1987)
C. livea	207	45	17	15	Arad et al. (1987)
Dromaius novaehollandiae (emu)	42400	45	2.4	1.8	Maloney and Dawson (1998)

Table 5.3: Changes in night- and day-time EWL (mg g^{-1} h^{-1}) with sucrose concentration in N. chalybea and in two honeyeater species, Lichmera indistincta and Acanthorhynchus superciliosis

Species	BM	0.2 M	0.4 M	0.8 M	1.2 M	Reference
	g	mg g^{-1} h^{-1}				
		Night				
N. chalybea	7.9	10.7	-	-	8.4	Present study
L. indistincta	8.0	-	12.5	12.5	12.5	Collins (1981)
A. superciliosis	10.0	-	10.0	10.0	10.0	Collins (1981)
		Day				
N. chalybea	7.9	49.4	-	-	23.0	Present study
L. indistincta	8.0	-	30.3	29.0	24.7	Collins (1981)
L. indistincta	8.0	-	-	42.5	-	Collins (1980b)
A. superciliosis	10.0	-	34.0	31.0	28.1	Collins (1981)

The mechanism of EWL change

From available data, it is possible to determine whether the higher EWL in birds fed 0.2 M sucrose than in those fed 1.2 M sucrose results from an increase in cutaneous or respiratory EWL. The EWL resulting directly from breathing,

$$EWL_b = \text{mg water vapour expired - mg water vapour inspired}........(1)$$

Knowledge of the temperature and relative humidity of the expired and inspired air allows calculation of the water content per unit volume expired/inspired, as

$$\text{mg water (ml air)}^{-1} = (\text{r.h.} / 100) * 0.0052 * e^{0.059 * T},$$

where T is the temperature of the expired/inspired air (derived from Weast 1985). This can then be multiplied by the volume of air expired/inspired to yield the mg water expired/inspired for equation (1).

Schmidt-Nielsen et al. (1970) found that the temperature of the air expired by birds (T_{ex}) lies between the T_b and the ambient temperature (T_a), and is described by the equation $T_{ex} = 23.0 + 0.43 * T_a$. Using this equation, the T_{ex} of N. chalybea would be 31.6 °C. Assuming that air is expired saturated, N. chalybea would then expire 0.0321 mg water (ml air)$^{-1}$, irrespective of diet.

The temperature of the inspired air was 20 °C, and its relative humidity averaged 69.9 and 32.5 % respectively when sunbirds were fed 0.2 and 1.2 M sucrose. Therefore, sunbirds inspire 0.0121 and 0.0056 mg water (ml air)$^{-1}$, depending on their diet.

The water contents per ml of air expired and inspired have now been calculated. The volumes of air respired still need to be calculated to determine the mass of water lost as a direct result of breathing. The volumes (at STP) of air expired and inspired are the same. The volume of respired air,

$$= \text{ml } O_2 \text{ g}^{-1} \text{ h}^{-1} \text{ used} * \frac{100}{\% \ O_2 \text{ extraction efficiency}} * \frac{100}{\% \ O_2 \text{ in air respired}}$$

The MR's (in ml O_2 g^{-1} h^{-1}) and chamber oxygen percentages (which are lower than the oxygen percentage in normal air because the birds remove some oxygen), were measured for N. chalybea fed the two diets during the present experiments. The oxygen extraction rate has also been measured in N. chalybea kept at 20 °C (at night), as 15.10 ± 0.02 % (n

= 5, unpublished data, B. Leon). The volume (at STP) of air inspired can therefore be calculated for the sunbirds using the equation above. This must be converted to air (from standard) temperature for calculation of the masses of water respired. The resulting volumes of air respired during the day are higher in birds fed 0.2 M sucrose than in those fed 1.2 M sucrose, because the former have higher metabolic rates. In birds fed 0.2 M sucrose, the volume (at air temperature) expired is 370 mg g^{-1} h^{-1}, and that inspired is 356 mg g^{-1} h^{-1}. In birds fed 1.2 M sucrose, the values are 322 and 309 mg g^{-1} h^{-1}.

The day-time respiratory water loss calculated in this way is lower in birds fed 0.2 M sucrose, 7.6 mg g^{-1} h^{-1}, than in those fed 1.2 M sucrose, 8.6 mg g^{-1} h^{-1}. Clearly, the 2.15 times higher day-time EWL in birds fed 0.2 M sucrose than in those fed 1.2 M sucrose cannot be accounted for by breathing. The percentage of the water loss that results directly from breathing is 15 % of the total EWL in birds fed 0.2 M sucrose, compared with the 37 % in birds fed 1.2 M sucrose (refer to Table 5.4).

Table 5.4: Respiratory (calculated) and total (measured) EWL (mg g^{-1} h^{-1}) in <u>N. chalybea</u> fed 0.2 or 1.2 M sucrose, during the day and at night.

	Day, 0.2 M	Day, 1.2 M	Night, 0.2 M	Night, 1.2 M
Respiratory EWL	7.6	8.6	5.8	5.9
Total EWL	49.4	23.0	10.7	8.4
% respiratory	15.4	37.4	54.2	70.2

In contrast to the dietary difference in total EWL, the differences between night- and day-time values can be explained partly by differences in respiratory EWL. The day-time values are 1.3 and 1.5 times higher than the night-time values, depending on diet (see Table 5.4).

The above analysis of respiratory EWL assumes that the oxygen extraction rate is the same, 15.10 %, irrespective of diet. This assumption would be violated during panting, when the volume of air breathed increases (due to increased respiratory frequency and/or tidal volume), and the oxygen extraction rate decreases (Dawson 1982). However, panting would not be expected to occur in N. chalybea under the present experimental conditions, because it is normally a response to the need to dissipate a heat load resulting from high ambient temperatures or vigorous activity (Dawson 1982). Consistent with this, open-mouthed panting was never observed during the present experiments, or during any previous experiments during which N. chalybea were kept at 20 or 10 °C, although it was observed frequently during the experiments in which sunbirds were maintained at 30 °C (chapter 3). Also, a lack of open-mouthed panting would preclude EWL from occurring directly off the moist respiratory surfaces.

It is therefore proposed that the entire increase in EWL between different diets may be cutaneous. This can be tested directly by measuring respiratory and cutaneous EWL (cEWL) separately. There are various possible methods of doing this. Whole body plethysmography, where pressure sensors are used to measure tidal volumes and respiratory frequency, combined with respirometry, would be an effective method (Maloney and Dawson 1998). Some authors (for example Bernstein 1971, Webster and King 1987, Wolf and Walsberg 1996) have used two-compartment metabolism chambers to separate the bird's head from the rest of its body. However, although this method allows measurement of EWL in resting birds, it will be difficult to use it for normally-feeding birds. This is because it requires that the opening between the two compartments be just large enough for the bird's neck, to prevent leakage, and so the birds are restrained. Bartholomew and Lighton (1986) measured the metabolic rate of feeding hummingbirds by attaching a flow-through mask to a feeder, so that the hummingbirds had to respire within the mask to feed. A mask could be attached to the feeder in the present study, so that respiratory (while feeding) and total (continuously) EWL are both measured in N. chalybea. The respiratory EWL could then be subtracted from the total EWL to calculate cEWL. Some authors (like Webster and Bernstein 1987, on mourning doves) have measured cEWL using the ventilated capsule method, in which small capsules through which air is flowed are attached to different parts of the bird's skin, and the water contents of the incurrent and excurrent air are measured. This method could be used on N. chalybea while they are in their metabolism chamber, so that total EWL is simultaneously measured to allow calculation of respiratory EWL. However, it may be difficult to obtain accurate results using ventilated capsules on birds that are as small as sunbirds, and the method also involves restraint of the bird. Menon et al. (1989) measured cEWL in zebra finches using an electrolytic moisture analyser. Other authors (like Marder and Ben-Asher 1983, in various bird species) have calculated cEWL by measuring all the components of equation (2), below. However, these methods do not allow simultaneous measurement of respiratory or total EWL, and once again cannot be done on normally-feeding birds.

If the entire increase in EWL in sunbirds fed dilute sucrose is cutaneous, then cEWL increases by 12.14 - 5.67 = 6.47 mg cm^{-2} h^{-1} (see Table 5.1). This value can be compared with the increase in cEWL which occurs with increasing T_a in resting columbiform birds, which are unusual amongst birds because they can dissipate virtually their entire heat loads using cEWL and not respiratory EWL at high T_a (Wolf and Walsberg 1996). In the pigeon (Columba livia), cEWL increases by 5.9 mg cm^{-2} h^{-1}, from 1.6 to 7.5 mg cm^{-2} h^{-1}, as T_a increases from 20 to 40 °C. At 52 °C, cEWL reaches 20.9 mg cm^{-2} h^{-1}. Similar trends occur in collared and palm doves (Marder and Ben-Asher 1983). In most other bird species, cEWL increases only marginally, if at all, over wide T_a changes. For example, in the quail, cEWL is 2.0 and 2.1 mg cm^{-2} h^{-1} at T_a = 20 and 40 °C, respectively (Marder and Ben-Asher 1983), and in the verdin (Auriparus flaviceps), it is 0.75 and 2.5 mg cm^{-2} h^{-1} at 20 and 50 °C (Wolf and Walsberg 1996). Apart from changes with T_a, a change in cEWL between resting and activity which is comparable to that occurring with the dietary change in N. chalybea, has been documented in the white-necked raven (Corvus cryptoleucus), in which cEWL increases by 4.76 mg cm^{-2} h^{-1}, from 2.08 to 6.84 mg cm^{-2} h^{-1}, between resting and flying, at a constant T_a of 22 °C (Hudson and Bernstein 1981).

The exact mechanisms by which EWL changes in N. chalybea need further investigation. The rate of cEWL depends on 1) the surface area available for evaporation (SA), 2) the absolute humidity differential between the skin and the surrounding air ($\Delta\rho_v$), and 3) the resistance of the skin and boundary layer to water vapour diffusion (r_v),

$$cEWL = SA * \Delta\rho_v / r_v \text{ ....(2)}$$

(modified from Webster et al. 1985). The different components of this equation are discussed below in relation to possible mechanisms by which N. chalybea increases cEWL when the diet is switched from 1.2 to 0.2 M sucrose. In addition, methods for testing each possible mechanism are discussed.

The surface area available for EWL can be altered through postural changes by the bird. The postures of sunbirds fed 0.2 or 1.2 M sucrose could be carefully observed, and differences quantified.

With respect to $\Delta\rho_v$, the chamber absolute humidity in birds fed 0.2 M sucrose (12.10 g m^{-3}) is 2.15 times higher than that in birds fed 1.2 M sucrose (5.63 g m^{-3}). Therefore, all other things being equal, the driving force for cEWL in birds fed 0.2 M sucrose should actually be lower than in those fed 1.2 M sucrose. If the chamber air humidity around birds fed 0.2 M sucrose was as low as that around those fed 1.2 M sucrose, it is likely that the difference in EWL between diets would be even greater than measured in the present experiments.

The absolute humidity of the air above an evaporating surface, as well as that of the evaporating surface itself, depends on surface temperature (Campbell 1977), and therefore a change in skin temperature (T_{skin}) should yield a change in cEWL. Webster et al. (1985) found that in pigeons, T_{skin} and cEWL are both strongly correlated with T_a, and estimated that 60 % of the increase in cEWL between 0 and 30 °C T_a can be accounted for by the change in T_{skin}, the remainder being accounted for by changes in r_v. An increase in T_{skin} could be effected rapidly, possibly through peripheral vasodilation (see below), and could potentially at least partly explain the rapid increase in EWL observed in N. chalybea after the switch in diet from 1.2 to 0.2 M sucrose. It would be easy to test this T_{skin} hypothesis by measuring T_{skin} in birds fed either 0.2 or 1.2 M sucrose, using a thermocouple attached with surgical tape, or implanted subcutaneously, ideally on different parts of the bird's body.

The absolute humidity of the air directly above the skin is also affected by the rate at which the water vapour produced is removed from the evaporating surface. The chamber flow rate during the present experiments was the same irrespective of diet (540 ml min^{-1}), so this could not have affected the rate of removal of water vapour from the skin. However, the activity of the bird would also influence the amount of air passing over the skin and therefore the rate of removal of water vapour. If birds were more active when fed 0.2 M sucrose, then the humid boundary layer above their skin would be disrupted more, $\Delta\rho_v$ would increase, and cEWL would increase. Birds fed 0.2 M sucrose may be more active than those fed 1.2 M, because their metabolic rates are 1.14 times higher on average, perhaps because birds have to feed more frequently when fed dilute nectar. However, the higher MR in birds fed the dilute diet may not result from increased activity, but from shivering thermogenesis to compensate for the cost of feeding on dilute nectar. It is necessary to measure activity directly in birds fed the two diets, possibly using the methods of Beuchat et al. (1979), who measured perching and flying times in captive hummingbirds. Unfortunately, even if a dietary difference in activity was found, it would be difficult to establish whether it was the cause of the EWL change, because of potential confounding factors such as changes in T_{skin} or r_V. It would be better to test the effect of removal of water vapour from near the skin on cEWL by altering wind speed in the chamber, while keeping all other variables (such as overall chamber humidity) constant.

Plumage impedes the movement of water vapour away from the skin, thereby decreasing the gradient for cEWL. Webster et al. (1985) estimated that the presence of feathers accounts for between 5 and 20 % of the total resistance to water loss in pigeons, depending on T_a (from 10 to 40 °C). Diet-dependent plumage adjustments in N. chalybea, such as fluffing of the feathers, could at least partially account for the observed differences in EWL. Plumage adjustments could occur quickly and therefore contribute to the rapid change in EWL after a switch in diet. Fluffing of the feathers is often observed in N. chalybea, but it is unknown whether or not it is less frequent in birds fed the dilute solution. Changes in plumage disposition need to be quantified for sunbirds fed either 0.2 or 1.2 M sucrose.

The other component of equation (2) is the skin's resistance to water vapour diffusion, r_v. In some birds, r_v, and therefore cEWL, can be altered through changes in the arrangement of lipids in the skin. In zebra finches dehydrated for six weeks, alterations in the arrangement of lipids in both the stratum corneum and in the viable epidermis effect a 50 % reduction in cEWL (Menon et al. 1989). After five days of rehydration, cEWL reaches levels intermediate between dehydrated and baseline levels. The turnover time of stratum corneum exceeds five days, and therefore one would also expect the full increase in cEWL with rehydration to exceed five days. However, changes in the arrangement of lipids in the viable epidermis may occur faster, and it is not impossible (although unlikely), that the rapid change in EWL (within an hour) after a dietary switch in N. chalybea is at least partly caused by a change in the arrangement of skin lipids.

In columbiform birds, which substantially increase their cEWL with T_a (as discussed earlier), r_v measured directly using a diffusion porometer decreases dramatically with T_a. For example, in the pigeon (Columba livea), r_v decreases from 99.8 sec cm^{-1} at 20 °C, to 9.4 sec cm^{-1} at 52 °C (Marder and Ben-Asher 1983, Webster et al. 1985). These temperature-dependent changes in r_v in pigeons are effected partly through changes in extracellular lipid distribution and skin vascularisation, but these changes are not immediate (Peltonen et al. 1997). Peripheral vasodilation also occurs in pigeons as T_a increases, and this can occur immediately (Webster et al. 1985). This, like increased skin vascularisation, effectively allows some of the water in liquid phase to bypass some of the skin, and in addition would increase T_{skin}, and therefore $\Delta\rho_v$ (see above, Webster et al. 1985).

The diffusional resistance of the non-living stratum corneum (which is dry, compact and keratinized) greatly exceeds that of the aqueous, viable epidermis (by 1000 times in humans). Hydration and swelling of the cells of the stratum corneum, possibly through increased peripheral blood flow, may decrease their resistance to water loss (Scheuplin and Blank 1971, Webster et al. 1985). It is plausible that the stratum corneum of birds feeding on 0.2 M sucrose may become hydrated. This could either be an inevitable, passive result of a large water load, or it could be a controlled mechanism for getting rid of excess water. However, complete in vitro hydration of human stratum corneum takes three full days (Scheuplin and Blank 1971), suggesting that this mechanism cannot account for the rapid change in EWL observed in N. chalybea with a switch in diet.

To study the possible mechanisms of altering r_v, and therefore cEWL, with a change in diet, it would be useful to use transmission electron microscopy (Menon et al. 1989). Skin sections of birds fed 0.2 and 1.2 M sucrose could be compared to detect differences in the arrangement of lipids, peripheral blood flow, and the degree of hydration of the stratum corneum.

Dry thermal conductance (DTC)

The calculated day-time DTC was 0.20 and 0.29 ml O_2 (g h °C)$^{-1}$, respectively, in birds fed 0.2 and 1.2 M sucrose. This means that birds fed the dilute solution are better insulated against non-evaporative heat loss than those fed the concentrated solution. Dry thermal conductance (= 1 / insulation) can be decreased through various mechanisms which fall into two basic types: 1) a decrease in the surface area available for radiation, conduction and/or convection, and 2) changes in the nature of the heat exchange surface (i.e. the skin and plumage), which decrease the tendency for radiation (emmisivity), conductivity and/or convection (McNab 1980). These mechanisms of decreasing DTC contradict some, but not all, of the possible mechanisms of altering cEWL discussed above. Changes in the skin surface area exposed to the environment (SA in equation (2) above), skin temperature, activity of the bird, plumage disposition (which affect $\Delta\rho_v$) and peripheral vasodilation (which affects r_v) are potential mechanisms of increasing cEWL on the dilute food, but because they would also lead to an increase in thermal conductance, they are actually unlikely to be of major importance for cEWL. On the other hand, changes in the arrangement of skin lipids, or hydration of the cells of the stratum corneum (both of which affect r_v), could alter cEWL without a change in DTC. It is proposed that the dietary change in cEWL is effected by one of these mechanisms which does not alter DTC, and that DTC is then altered, as a thermoregulatory response, through another mechanism which is unimportant for cEWL. In the emu (Dromaius novaehollandiae), cEWL decreases when dehydrated, and unlike in N. chalybea this is associated with a decrease in DTC (Maloney and Dawson 1998).

The relatively high EWL of birds fed the dilute solution could either be a passive physical process, which cannot be avoided when water influx is high, or it could be an active mechanism for excreting excess water. Changes in the arrangement of skin lipids could easily be an active process, whereas hydration of the cells of the stratum corneum could easily be a passive process.

It is well-documented that insulation can increase as a response to decreasing T_a in birds (e.g. Drent and Stonehouse 1971, Withers and Williams 1990), but dietary differences in insulation such as seem to occur in N. chalybea are apparently unreported.

Drent and Stonehouse (1971) tabulate, for 83 bird species ranging in body mass from 3.0 g to 5 kg, "minimal" DTC, i.e. that occurring at or below the lower critical temperature. Their data can be summarised in the equation

$$\mathrm{DTC} = 3.216 * M^{-0.1497}\ (R^2 = 0.51),$$

where DTC is in kcal $(m^2\ h\ ^{\circ}C)^{-1}$, and M is body mass in g. Assuming an RQ of 0.775 and that log (surface area) = 0.667 * log (M) + log (8.11) (Walsberg and King 1978, see methods), the predicted DTC for a 7.93 g bird would be 0.20 ml O_2 $(g\ h\ ^{\circ}C)^{-1}$, which is identical to that calculated for N. chalybea fed 0.2 M sucrose.

The DTC at the lower critical temperature is normally assumed to be minimal, but it can actually continue to decrease below the lower critical temperature (Drent and Stonehouse 1971). Although the DTC's reported here for N. chalybea occur at $T_a = 20\ ^{\circ}C$, which is well below this species' lower critical temperature of about 33 °C (Leon and Nicolson 1997), the lowest (possibly minimal) DTC is dependent on diet.

Wet thermal conductance (WTC)

The day-time WTC is 0.45 ml O_2 (g h °C)$^{-1}$ in birds fed 0.2 M sucrose, and 0.40 in those fed 1.2 M. Leon and Nicolson (1997) obtained a day-time value of 0.58 ml O_2 (g h °C)$^{-1}$ in N. chalybea at 20 °C (in birds fed 0.3 M sucrose). Part of this discrepancy can be accounted for by differences in the measured T_b's; recalculation according to their body temperature (39.8 °C) yields values of 0.50 and 0.45 ml O_2 (g h °C)$^{-1}$. The remainder has to be accounted for by differences in the metabolic rates measured in the two studies. Calculation of day-time WTC, $T_a = 20$ °C, from the metabolic rate (9.04 ml O_2 g^{-1} h^{-1}) and T_b (41.6 °C), averaged for thirteen sunbird species ranging in body mass from 4.7 to 15.2 g (excluding N. chalybea, Prinzinger et al. 1989), yields a value of 0.42 ml O_2 (g h °C)$^{-1}$, very similar to that obtained in N. chalybea during the present study.

The predicted active-phase, "minimal" (i.e. measured at the lower critical temperature), WTC for a 7.93 g passerine bird is 0.33 ml O_2 (g h °C)$^{-1}$ (Aschoff 1981). The difference between this predicted value and the values calculated for N. chalybea and other sunbirds could easily result because sunbirds have higher-than-expected day-time metabolic rates. Day-time MR in birds is expected to be 49.4 % higher than night-time MR (Aschoff and Pohl 1970), but in N. chalybea it is 96.6 and 75.6 % higher when fed 0.2 and 1.2 M sucrose, respectively, similar to the value measured by Leon and Nicolson (1997), and the values measured in the thirteen other sunbird species by Prinzinger et al. (1989). If the day-time WTC is re-calculated in N. chalybea assuming that the day-time MR is 6.3 ml

O_2 g^{-1} h^{-1}, 49.4 % higher than the measured night-time MR, as predicted by Aschoff and Pohl (1970), then it is reduced to 0.28 ml O_2 (g h °C)$^{-1}$.

$H_e : H_m$ ratios

The night-time ratio of evaporative heat loss to metabolic heat production, $H_e : H_m$ is 25.78 and 20.35 %, respectively, in sunbirds fed 0.2 and 1.2 M sucrose. In birds fed 0.2 M sucrose, the day-time ratio more than doubles, to 57.07 %, whereas in those fed 1.2 M sucrose, it only increases to 29.93 %. Therefore, N. chalybea dissipates far more of its metabolic heat production via evaporation, during the day, when fed 0.2 than when fed 1.2 M sucrose.

The predicted $H_e : H_m$ ratio for birds at $T_a = 20$ °C (body-mass independent) is only 13.43 % (from equation 56, Calder and King 1974). Therefore, even at night and when fed the concentrated diet, N. chalybea dissipates more of its heat production via evaporation than expected. The lowest ratio measured in N. chalybea (20.35 %, night-time, 1.2 M sucrose), is equivalent to that expected for birds at $T_a = 26.89$ °C, and the highest ratio (57.07 %, day-time, 0.2 M sucrose), is expected at $T_a = 40.93$ °C. The predicted ratio when $T_a = T_b = 42.2$ °C, is 63.15 %, marginally (1.11 times) higher than the actual ratio measured at 20 °C in N. chalybea fed the dilute diet.

In the honeyeater Lichmera indistincta (8 g), the night- and day-time $H_e : H_m$ ratios are similar to those of N. chalybea. The night-time ratio is 29.47 %. The day-time ratio is 31.49 % in honeyeaters fed 1.2 M sucrose, and 38.06 in those fed 0.4 M sucrose (Collins 1981). On the other hand, in the hummingbird Calypte anna resting in the dark without food, the $H_e : H_m$ ratio is lower than predicted by Calder and King (1974), over a T_a range from 20 to 40 °C, being 5 to 10 at 20 °C, depending on ambient humidity (Powers 1992).

The discrepancy between the predicted $H_e : H_m$ ratio for birds at 20 °C, and those actually measured in sunbirds, honeyeaters and hummingbirds, can be explained if H_e and H_m are considered separately. As discussed earlier, all these nectarivores have higher-than expected EWL (and therefore H_e) values. On the other hand, the H_m's of sunbirds are in the expected range for birds (Prinzinger et al. 1989), and those of honeyeaters (including L. indistincta), are lower than predicted (Aschoff and Pohl 1970, Collins et al. 1980), and therefore the high $H_e : H_m$ ratios are not surprising. However, the hummingbird Calypte anna, although having a high H_e, also has an H_m that is twice as high as predicted from body mass, explaining its low $H_e : H_m$ ratio.

The relatively high H_e values of nectarivorous birds (sunbirds, honeyeaters and sunbirds), mean that these birds lose more heat through evaporation than other birds, and need to compensate somehow if they are to maintain the same body temperature as other small birds. The body temperatures of sunbirds and hummingbirds are around 42 °C, amongst the highest for any birds, whereas the body temperatures of honeyeaters seem to be lower, around 39 °C (see earlier). As mentioned above, the metabolic heat production (H_m) of hummingbirds is higher than expected from body mass. In sunbirds, on the other hand, body temperature as well as H_m values are in the expected range, and it can therefore be predicted that they have a more efficient insulation (= 1/DTC), than other small birds. This area warrants further research.

The MR of N. chalybea is 12.54 % higher in sunbirds fed 0.2 than in those fed 1.2 M sucrose. It is possible to calculate whether this difference in MR is accounted for by the thermoregulatory cost of feeding on dilute versus concentrated nectar. Assume that the MR of birds fed 0.2 M sucrose is the same as that of birds fed 1.2 M sucrose, 8.85 ml O_2 g^{-1} h^{-1}. From the EWL measured in birds fed the two diets, the day-time H_e : H_m ratios would then be 64.22 and 29.93 % in birds fed 0.2 and 1.2 M sucrose, respectively. This translates to a difference in cost of 64.22 - 29.93 = 34.29 %. In addition to this, it costs more to warm the greater volumes of preformed water consumed when feeding on 0.2 than when feeding on 1.2 M sucrose (see chapter 4). Birds feeding on 0.2 and 1.2 M sucrose should use 7.59 and 1.05 %, respectively, of their metabolic requirements, the difference being 6.54 %. It should therefore cost sunbirds 34.29 + 6.54 = 40.83 % more energy to feed on the dilute diet. However, because the dry thermal conductance (= 1 / insulation) is lower in birds fed the dilute diet (see earlier), it actually only costs the birds 12.54 % more energy when feeding on the dilute diet (from the MR measurements).

In conclusion, the EWL and therefore evaporative heat loss is considerably higher in N. chalybea fed 0.2 M sucrose than in those fed 1.2 M sucrose. Despite this, the birds maintain the same body temperature on both diets, by increasing their metabolic rate and decreasing their dry thermal conductance on the dilute solution. The mechanisms of altering evaporative water loss and dry thermal conductance are probably cutaneous in origin, but need further investigation.

Synthesis

Consistent with the prediction of Calder (1979) for nectarivorous birds in general, the lesser double-collared sunbird, Nectarinia chalybea, consumed excess preformed water, even when ambient temperatures were high (30 °C) and when the diet was concentrated nectar (1.2 M sucrose). When kept at an ambient temperature of 10 °C while fed dilute (0.4 M) sucrose, sunbirds consumed 2.7 times their body mass (8 g) of preformed water daily, and excreted 87 % of the water. These high water influx and excretion rates resemble those of honeyeaters and hummingbirds, but far exceed the rates predicted from allometry.

Such high water influx and excretion rates present unique challenges for the maintenance of homeostasis in birds that feed on nectar. The maintenance of electrolyte balance in the face of high water excretion rates, combined with the sometimes low ion concentrations in nectar, is one such challenge. However, N. chalybea was found to be highly efficient at conserving cations when provided with calorifically dilute nectar lacking ions. Indeed, sunbirds precisely maintained electrolyte balance by increasing both K^+ and Na^+ excretion from 2 to 17 mM when 15 mM each of KCl and NaCl were included in their diet of 0.4 M sucrose.

Another challenge resulting from the high water influx typical of nectarivorous birds, especially when feeding on relatively dilute nectar, involves the maintenance of a constant body temperature. The day-time body temperature of N. chalybea was measured as 42 °C irrespective of dietary sucrose concentration. To maintain this high body temperature, the food must be warmed to the same temperature. The modelling in chapter 4 revealed that sunbirds or other endothermic nectarivores feeding on dilute (0.4 M) sucrose at 18 °C would use the same proportion (4 %) of their daily energy intake to warm their food as high-latitude aquatic endotherms feeding on fish or invertebrates just above freezing point. Endothermic nectarivores feeding on 0.2 M sucrose at 10 °C would use 11 % of their daily energy intake to warm their food.

The day-time evaporative water loss (and therefore evaporative heat loss) of N. chalybea more than doubled when dietary sucrose concentration was changed from 1.2 to 0.2 M. This provides a further challenge to sunbirds in their ability to thermoregulate, especially since the latent heat of vaporisation of water is high (2.5 kJ g^{-1}). A similar dietary effect on EWL has been observed in honeyeaters (Collins 1981), but the influence of diet on EWL has otherwise not been examined in birds.

To compensate for both food warming costs and evaporative heat loss, and thus maintain a constant body temperature of 42 °C, both the day-time metabolic rates and the day-time insulation of sunbirds were found to be higher when fed relatively dilute nectar. Such increases in metabolic heat production and/or insulation are well-documented in birds and mammals as thermoregulatory responses to decreasing ambient temperature, but have previously not been observed as thermoregulatory responses to a changing diet.

The night-time evaporative heat loss of N. chalybea was found to resemble that of other nectarivorous birds (honeyeaters and hummingbirds), but was seemingly higher than predicted allometrically, probably because of the watery diet. The implications of the higher-than-expected evaporative heat loss of nectar-feeding birds are that the body temperature of these birds has to be lower than that of other birds, and/or the metabolic heat production has to be higher, and/or that insulation has to be higher. There is preliminary evidence that the body temperature of honeyeaters is a few degrees lower than expected for small birds, and that the metabolic heat production of hummingbirds is higher than expected. Since the body temperature of sunbirds, including N. chalybea, is high (around 42 °C), and the metabolic rates are not higher than expected allometrically, it is hypothesised that the thermal insulation of sunbirds is particularly effective when compared to other small birds. This area warrants further research.

Clearly, nectarivorous birds are faced with unique thermoregulatory challenges because of their diet, and these problems increase as the nectar they are feeding on becomes more dilute. It is energetically more expensive for N. chalybea to feed on relatively dilute nectar: their metabolic rate has been shown to be higher when fed dilute food. This should have implications for the evolution of the nectar concentrations produced by ornithophilous flowers. It can be predicted that there will be natural selection on plants to produce relatively concentrated nectar, which is less energetically costly to their pollinators. However, the actual nectar concentrations found in ornithophilous flowers are commonly as low as 0.4 M sucrose (= 13 % w/w), and can be much lower. These nectar concentrations are low enough to add a substantial energy cost to the energy budgets of nectarivorous birds, and it is unclear why such dilute nectar is so common in the field.

Some sunbird and honeyeater species breed in winter, so that the high energy cost of breeding exacerbates the high energy cost of thermoregulating in cold weather. Moreover, nectar may be diluted by winter rainfall. Thus, these winter-breeding nectarivorous birds are particularly challenged to maintain energy balance, and may undergo nocturnal hypothermia and lose body mass while breeding (see chapter 3).

Although the sunbird N. chalybea has major energetic disadvantages when feeding on dilute nectar, it does not seem to have a problem maintaining water balance. This species has an impressive ability to excrete excess water. When feeding on nectar which is far more dilute than that commonly occurring in the field (0.09 M sucrose), it is able to process a water flux of more than 7 times its body mass (8 g) daily (see Appendix 3). Apart from excretion, it is plausible that this sunbird also uses evaporative water loss as a controlled mechanism of getting rid of excess water. However, when fed 0.2 M sucrose, only 13 % of the total of 46 ml of water consumed per day is lost through evaporation, so that excretory water loss is still by far the more important route of water loss.

In conclusion, a diet of nectar has profound physiological consequences for the sunbird N. chalybea and other nectarivorous birds. These birds are able to maintain water and electrolyte balance when feeding on dilute nectar, but suffer numerous energetic disadvantages in the process.

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Appendices

Appendix 1: characterization of Complan® and Ensure®. Amounts are per 100 g dry powder

	Complan®	Ensure®
Protein	20 g	15.8 g
Fat	13.8 g	15.8 g
Carbohydrate	56.7 g	61.8 g
Vitamin A	2682 i.u.	1130 i.u.
Vitamin D	151 i.u.	90 i.u.
Vitamin E	8.6 i.u.	10.2 i.u
Vitamin K		18 µg
Vitamin C	72 mg	68 mg
Vitamin B1	1.44 mg	0.68 mg
Vitamin B2	1.76 mg	0.81 mg
Vitamin B6	1.51 mg	0.94 mg
Vitamin B12	4.51 µg	2.73 µg
Nicotinic Acid		9.03 mg
Biotin	49.1 µg	135.4 µg
Folic Acid	192 µg	181 µg
Pantothenic Acid	4.7 mg	4.5 mg
Choline	80.4 mg	135.4 mg
Calcium	720 mg	225 mg
Phosphorus	587 mg	225 mg
Magnesium	76 mg	90 mg

Appendix 1 (cntd)

	Complan®	Ensure®
Potassium		666 mg
Sodium	293 mg	360 mg
Chloride		560 mg
Iodine	185 µg	34 µg
Iron	7.1 mg	4.1 mg
Copper	0.69 mg	0.47 mg
Zinc	7.1 mg	5.1 mg
Manganese	0.9 mg	1.2 mg
Selenium		16.2 µg
Chromium		22.3 µg
Molybdenum		34 µg
Ash		2.5 g
Water		4.25 g

Appendix 2.1: Hourly and total daily volumes (ml h^{-1}; mean ± SE, n = 13) of sucrose solution ingested by N. chalybea fed 0.4, 0.8 or 1.2 M sucrose, during the experiments of chapter 1.

Time	0.4 M	0.8 M	1.2 M
8	1.70 ± 0.07	0.88 ± 0.04	0.58 ± 0.03
9	1.57 ± 0.09	0.83 ± 0.03	0.53 ± 0.03
10	1.38 ± 0.13	0.80 ± 0.03	0.48 ± 0.03
11	1.30 ± 0.09	0.74 ± 0.04	0.50 ± 0.03
12	1.36 ± 0.08	0.71 ± 0.02	0.45 ± 0.02
13	1.24 ± 0.09	0.59 ± 0.04	0.37 ± 0.02
14	1.12 ± 0.08	0.51 ± 0.04	0.37 ± 0.02
15	0.99 ± 0.09	0.48 ± 0.04	0.33 ± 0.02
16	1.03 ± 0.08	0.50 ± 0.04	0.38 ± 0.04
17	1.18 ± 0.05	0.57 ± 0.03	0.38 ± 0.03
18	1.32 ± 0.05	0.60 ± 0.05	0.47 ± 0.03
19	1.30 ± 0.07	0.53 ± 0.03	0.40 ± 0.03
Total	15.46 ± 0.40	7.75 ± 0.20	5.26 ± 0.13

Appendix 2.2: Hourly volumes (ml h^{-1}; mean ± SE, n = 13) of preformed and excreted water in N. chalybea fed 0.4, 0.8 or 1.2 M sucrose, during the experiments of chapter 1.

Time	Preformed Water			Excreted Water		
	0.4 M	**0.8 M**	**1.2 M**	**0.4 M**	**0.8 M**	**1.2 M**
8	1.54 ± 0.07	0.73 ± 0.04	0.43 ± 0.02	1.16 ± 0.05	0.45 ± 0.02	0.19 ± 0.02
9	1.42 ± 0.08	0.69 ± 0.03	0.39 ± 0.02	1.16 ± 0.07	0.62 ± 0.02	0.34 ± 0.02
10	1.26 ± 0.12	0.67 ± 0.03	0.36 ± 0.02	0.99 ± 0.10	0.56 ± 0.02	0.27 ± 0.02
11	1.18 ± 0.08	0.62 ± 0.04	0.37 ± 0.02	0.95 ± 0.06	0.49 ± 0.02	0.26 ± 0.02
12	1.24 ± 0.07	0.59 ± 0.02	0.33 ± 0.02	0.90 ± 0.06	0.47 ± 0.03	0.25 ± 0.02
13	1.12 ± 0.08	0.49 ± 0.03	0.27 ± 0.02	0.85 ± 0.05	0.39 ± 0.03	0.22 ± 0.02
14	1.02 ± 0.08	0.42 ± 0.03	0.27 ± 0.01	0.78 ± 0.05	0.34 ± 0.03	0.19 ± 0.02
15	0.90 ± 0.08	0.40 ± 0.04	0.25 ± 0.02	0.73 ± 0.06	0.30 ± 0.03	0.19 ± 0.02
16	0.94 ± 0.08	0.41 ± 0.04	0.28 ± 0.03	0.77 ± 0.07	0.33 ± 0.03	0.19 ± 0.02
17	1.07 ± 0.04	0.47 ± 0.03	0.28 ± 0.02	0.84 ± 0.04	0.33 ± 0.03	0.21 ± 0.02
18	1.20 ± 0.04	0.50 ± 0.04	0.35 ± 0.02	0.93 ± 0.04	0.35 ± 0.03	0.20 ± 0.02
19	1.19 ± 0.06	0.44 ± 0.03	0.30 ± 0.03	0.96 ± 0.06	0.37 ± 0.03	0.18 ± 0.01
Total	14.07 ± 0.37	6.44 ± 0.17	3.89 ± 0.09	11.02 ± 0.34	5.00 ± 0.15	2.68 ± 0.10

Appendix 2.3: Volumes (ml) of preformed and excreted water, and osmolality (mmol kg^{-1}), K^+ and Na^+ concentrations (mM) of excreted fluid, measured every 5 minutes during the 5 h experiment of chapter 2(mean ± SE).

Time	Preformed water	Excreted water	Osmolality	K+	Na+
09:35	0.09 ± 0.03	0.08 ± 0.02	60.25 ± 0.85	0.76 ± 0.27	2.80 ± 0.73
09:40	0.09 ± 0.01	0.08 ± 0.02	58.80 ± 1.16	0.66 ± 0.12	2.80 ± 0.49
09:45	0.09 ± 0.03	0.09 ± 0.02	59.80 ± 1.11	0.34 ± 0.07	2.20 ± 0.73
09:50	0.11 ± 0.03	0.09 ± 0.01	58.80 ± 1.80	0.34 ± 0.04	1.20 ± 0.20
09:55	0.06 ± 0.02	0.09 ± 0.02	60.00 ± 1.05	0.34 ± 0.02	1.00 ± 0.32
10:00	0.11 ± 0.03	0.10 ± 0.01	59.40 ± 1.21	0.44 ± 0.09	1.60 ± 0.24
10:05	0.16 ± 0.02	0.09 ± 0.01	58.00 ± 0.55	0.38 ± 0.06	1.40 ± 0.24
10:10	0.12 ± 0.04	0.09 ± 0.01	59.20 ± 1.66	0.34 ± 0.13	1.20 ± 0.49
10:15	0.11 ± 0.01	0.12 ± 0.01	59.00 ± 1.52	0.30 ± 0.10	1.20 ± 0.58
10:20	0.13 ± 0.03	0.12 ± 0.01	60.80 ± 1.20	0.34 ± 0.09	1.40 ± 0.51
10:25	0.08 ± 0.03	0.12 ± 0.01	58.20 ± 2.08	0.26 ± 0.07	0.80 ± 0.49
10:30	0.17 ± 0.02	0.11 ± 0.01	59.80 ± 1.24	0.30 ± 0.07	1.00 ± 0.32
10:35	0.08 ± 0.03	0.08 ± 0.01	61.80 ± 1.24	0.34 ± 0.08	1.40 ± 0.51
10:40	0.06 ± 0.02	0.09 ± 0.01	62.20 ± 2.52	0.38 ± 0.09	2.00 ± 0.55
10:45	0.19 ± 0.03	0.09 ± 0.02	62.75 ± 1.77	0.70 ± 0.28	2.60 ± 0.75
10:50	0.16 ± 0.02	0.11 ± 0.01	65.20 ± 2.75	0.72 ± 0.12	3.40 ± 0.87
10:55	0.14 ± 0.03	0.12 ± 0.01	65.60 ± 2.96	0.82 ± 0.19	6.00 ± 0.87
11:00	0.11 ± 0.02	0.11 ± 0.01	71.40 ± 4.24	1.54 ± 0.56	8.60 ± 1.21
11:05	0.15 ± 0.03	0.11 ± 0.01	75.80 ± 4.62	3.02 ± 1.36	12.20 ± 1.59
11:10	0.09 ± 0.03	0.11 ± 0.01	79.80 ± 5.52	5.80 ± 2.64	14.20 ± 1.32
11:15	0.11 ± 0.04	0.10 ± 0.01	86.60 ± 3.14	9.24 ± 3.17	16.00 ± 1.61
11:20	0.17 ± 0.03	0.12 ± 0.01	88.60 ± 3.54	12.68 ± 3.10	18.40 ± 0.98
11:25	0.10 ± 0.02	0.11 ± 0.01	93.00 ± 2.51	14.80 ± 2.04	18.80 ± 1.62
11:30	0.16 ± 0.03	0.13 ± 0.02	96.60 ± 2.11	15.70 ± 1.33	21.20 ± 3.29
11:35	0.13 ± 0.03	0.12 ± 0.01	98.40 ± 2.38	19.46 ± 0.39	21.40 ± 3.14
11:40	0.10 ± 0.03	0.12 ± 0.01	96.40 ± 1.44	19.74 ± 1.54	19.60 ± 1.81
11:45	0.16 ± 0.03	0.12 ± 0.01	96.60 ± 1.08	19.52 ± 1.94	17.00 ± 1.55
11:50	0.16 ± 0.02	0.12 ± 0.01	94.20 ± 1.46	19.04 ± 1.25	17.20 ± 0.58
11:55	0.15 ± 0.02	0.12 ± 0.01	97.60 ± 1.17	19.48 ± 1.14	19.00 ± 0.45
12:00	0.15 ± 0.03	0.12 ± 0.01	96.00 ± 3.16	20.08 ± 1.58	16.20 ± 1.24
12:05	0.14 ± 0.03	0.10 ± 0.02	96.20 ± 1.93	18.48 ± 2.66	19.00 ± 1.92
12:10	0.06 ± 0.02	0.10 ± 0.03	98.00 ± 3.03	19.15 ± 0.29	16.00 ± 0.82
12:15	0.14 ± 0.02	0.12 ± 0.02	96.60 ± 3.14	18.66 ± 1.32	16.60 ± 1.57
12:20	0.15 ± 0.02	0.10 ± 0.01	94.40 ± 0.75	18.68 ± 1.04	16.20 ± 0.86
12:25	0.11 ± 0.03	0.12 ± 0.01	94.00 ± 1.64	17.28 ± 0.92	15.20 ± 0.97
12:30	0.16 ± 0.02	0.11 ± 0.01	93.20 ± 1.16	15.22 ± 0.84	17.20 ± 0.73
12:35	0.16 ± 0.02	0.11 ± 0.01	90.60 ± 3.12	13.66 ± 0.99	16.80 ± 1.16
12:40	0.11 ± 0.03	0.12 ± 0.02	83.00 ± 2.30	12.46 ± 1.35	13.80 ± 1.74
12:45	0.07 ± 0.02	0.10 ± 0.01	79.20 ± 3.06	9.22 ± 1.11	12.40 ± 1.83
12:50	0.16 ± 0.02	0.11 ± 0.02	72.00 ± 1.75	6.65 ± 0.92	9.50 ± 0.93

Appendix 2.3 (cntd)

Time	Preformed water	Excreted water	Osmolality	K+	Na+
12:55	0.08 ± 0.03	0.11 ± 0.01	70.60 ± 2.29	5.68 ± 1.09	7.20 ± 0.86
13:00	0.09 ± 0.01	0.11 ± 0.01	69.80 ± 2.18	4.42 ± 0.48	7.00 ± 0.71
13:05	0.13 ± 0.02	0.11 ± 0.01	67.60 ± 3.30	4.30 ± 0.66	7.60 ± 1.08
13:10	0.16 ± 0.03	0.10 ± 0.01	64.40 ± 2.68	3.26 ± 0.51	5.80 ± 0.73
13:15	0.12 ± 0.03	0.12 ± 0.01	66.00 ± 2.24	3.48 ± 0.39	6.60 ± 1.08
13:20	0.08 ± 0.03	0.10 ± 0.02	65.20 ± 1.46	3.62 ± 0.74	5.20 ± 1.02
13:25	0.14 ± 0.04	0.11 ± 0.01	64.60 ± 1.50	3.20 ± 0.60	4.20 ± 0.80
13:30	0.07 ± 0.02	0.10 ± 0.03	64.00 ± 1.46	2.95 ± 0.14	4.25 ± 0.43
13:35	0.12 ± 0.04	0.08 ± 0.02	66.20 ± 3.20	3.34 ± 0.65	4.60 ± 1.17
13:40	0.13 ± 0.03	0.10 ± 0.01	63.20 ± 1.39	1.94 ± 0.40	3.40 ± 0.68
13:45	0.08 ± 0.04	0.09 ± 0.02	66.00 ± 2.81	1.70 ± 0.28	4.80 ± 1.39
13:50	0.14 ± 0.04	0.08 ± 0.02	60.00 ± 1.15	2.00 ± 0.35	2.75 ± 0.43
13:55	0.07 ± 0.02	0.10 ± 0.01	62.20 ± 3.07	1.98 ± 0.54	3.60 ± 1.17
14:00	0.17 ± 0.02	0.09 ± 0.02	60.50 ± 3.15	0.98 ± 0.17	3.00 ± 0.97
14:05	0.10 ± 0.02	0.07 ± 0.03	58.67 ± 0.68	0.97 ± 0.05	2.33 ± 0.26
14:10	0.08 ± 0.03	0.09 ± 0.02	63.80 ± 3.43	2.52 ± 1.27	3.00 ± 1.00
14:15	0.11 ± 0.03	0.09 ± 0.01	59.80 ± 2.85	1.34 ± 0.24	2.20 ± 0.97
14:20	0.11 ± 0.04	0.08 ± 0.02	55.00 ± 2.13	1.35 ± 0.30	1.25 ± 0.22
14:25	0.07 ± 0.03	0.09 ± 0.03	57.50 ± 2.24	1.58 ± 0.28	1.50 ± 0.26
14:30	0.08 ± 0.04	0.07 ± 0.03	56.33 ± 0.52	1.90 ± 0.41	1.33 ± 0.26
Total	7.09 ± 0.65	6.15 ± 0.45			

Appendix 2.4: The daily mass of sucrose ingested, and components of water gain and loss (mean ± SE) in N. chalybea fed 0.4 or 1.2 M sucrose and kept at 10, 20 or 30 °C, during the experiments of chapter 3.

		0.4 M	
	10 °C	20 °C	30 °C
Sucrose mass ingested	3.22 ± 0.14	2.63 ± 0.02	2.09 ± 0.13
Preformed water	21.49 ± 0.95	17.56 ± 0.11	13.94 ± 0.88
Metabolic water	1.56 ± 0.11	1.24 ± 0.09	0.94 ± 0.06
Excreted water	18.71 ± 1.58	15.17 ± 0.83	11.41 ± 0.70
Evaporative water loss	4.34 ± 0.59	3.63 ± 0.71	3.47 ± 0.38

		1.2 M	
	10 °C	20 °C	30 °C
Sucrose mass ingested	2.93 ± 0.11	2.33 ± 0.12	2.00 ± 0.21
Preformed water	5.3 ± 0.2	4.22 ± 0.22	3.62 ± 0.37
Metabolic water	1.49 ± 0.11	1.19 ± 0.08	0.90 ± 0.06
Excreted water	4.96 ± 0.26	3.48 ± 0.23	2.26 ± 0.36
Evaporative water loss	1.83 ± 0.02	1.93 ± 0.07	2.26 ± 0.32

Appendix 2.5: Hourly volumes (ml h^{-1}, mean ± SE, n = 4) of sucrose solution consumed by N. chalybea fed 0.4, 0.8 or 1.2 M sucrose at T_a's of 10, 20 or 30 °C, during the experiments of chapter 3.

		0.4 M	
Time	10 °C	20 °C	30 °C
8	2.16 ± 0.26	1.93 ± 0.12	0.19 ± 0.07
9	2.30 ± 0.09	2.10 ± 0.13	0.27 ± 0.04
10	2.11 ± 0.08	1.95 ± 0.11	0.23 ± 0.02
11	2.09 ± 0.25	1.81 ± 0.07	0.22 ± 0.04
12	2.08 ± 0.16	1.95 ± 0.11	0.19 ± 0.03
13	1.66 ± 0.12	1.80 ± 0.14	0.20 ± 0.06
14	1.91 ± 0.06	1.55 ± 0.15	0.17 ± 0.06
15	1.80 ± 0.17	1.10 ± 0.12	0.13 ± 0.04
16	1.78 ± 0.12	1.09 ± 0.14	0.14 ± 0.03
17	1.81 ± 0.16	1.33 ± 0.17	0.12 ± 0.05
18	1.95 ± 0.13	1.20 ± 0.15	0.13 ± 0.05
19	1.86 ± 0.11	1.41 ± 0.13	0.11 ± 0.05
Total	23.51 ± 1.04	19.21 ± 0.12	2.09 ± 0.26

		1.2 M	
Time	10 °C	20 °C	30 °C
8	0.26 ± 0.03	0.20 ± 0.02	0.09 ± 0.03
9	0.32 ± 0.02	0.26 ± 0.02	0.21 ± 0.02
10	0.24 ± 0.02	0.23 ± 0.02	0.21 ± 0.03
11	0.28 ± 0.02	0.24 ± 0.03	0.23 ± 0.04
12	0.27 ± 0.02	0.22 ± 0.02	0.20 ± 0.02
13	0.23 ± 0.03	0.24 ± 0.04	0.18 ± 0.02
14	0.25 ± 0.01	0.20 ± 0.03	0.18 ± 0.02
15	0.18 ± 0.05	0.17 ± 0.04	0.15 ± 0.03
16	0.27 ± 0.03	0.15 ± 0.03	0.17 ± 0.01
17	0.19 ± 0.04	0.17 ± 0.03	0.14 ± 0.01
18	0.29 ± 0.06	0.13 ± 0.01	0.11 ± 0.02
19	0.15 ± 0.04	0.14 ± 0.01	0.11 ± 0.02
Total	2.93 ± 0.11	2.33 ± 0.12	2.00 ± 0.21

Appendix 3: The maximal water flux of Nectarinia chalybea

To establish the maximal water flux of Nectarinia chalybea, and the minimal nectar concentration below which sunbirds are unable to ingest the same mass of sucrose and therefore to compensate energetically, was established by keeping birds at 20 °C, feeding them 0.09, 0.13, 0.2 or 0.4 M sucrose, and measuring hourly intake of sucrose solution after 1 full day of acclimation. The mass of sucrose ingested was the same when birds were fed 0.4 or 0.2 M sucrose, but was lower in birds fed 0.13 M sucrose, and even lower in those fed 0.09 M sucrose (Fig. X.1). Consistent with this, the volume of preformed water consumed levelled off when sunbirds were fed increasingly dilute nectar, indicating that birds were approaching the maximal water volume they were able to process, when fed the most dilute nectar (Fig. X.2). The mean preformed water intake of birds fed the most dilute solution was 58.82 ± 2.79 ml.

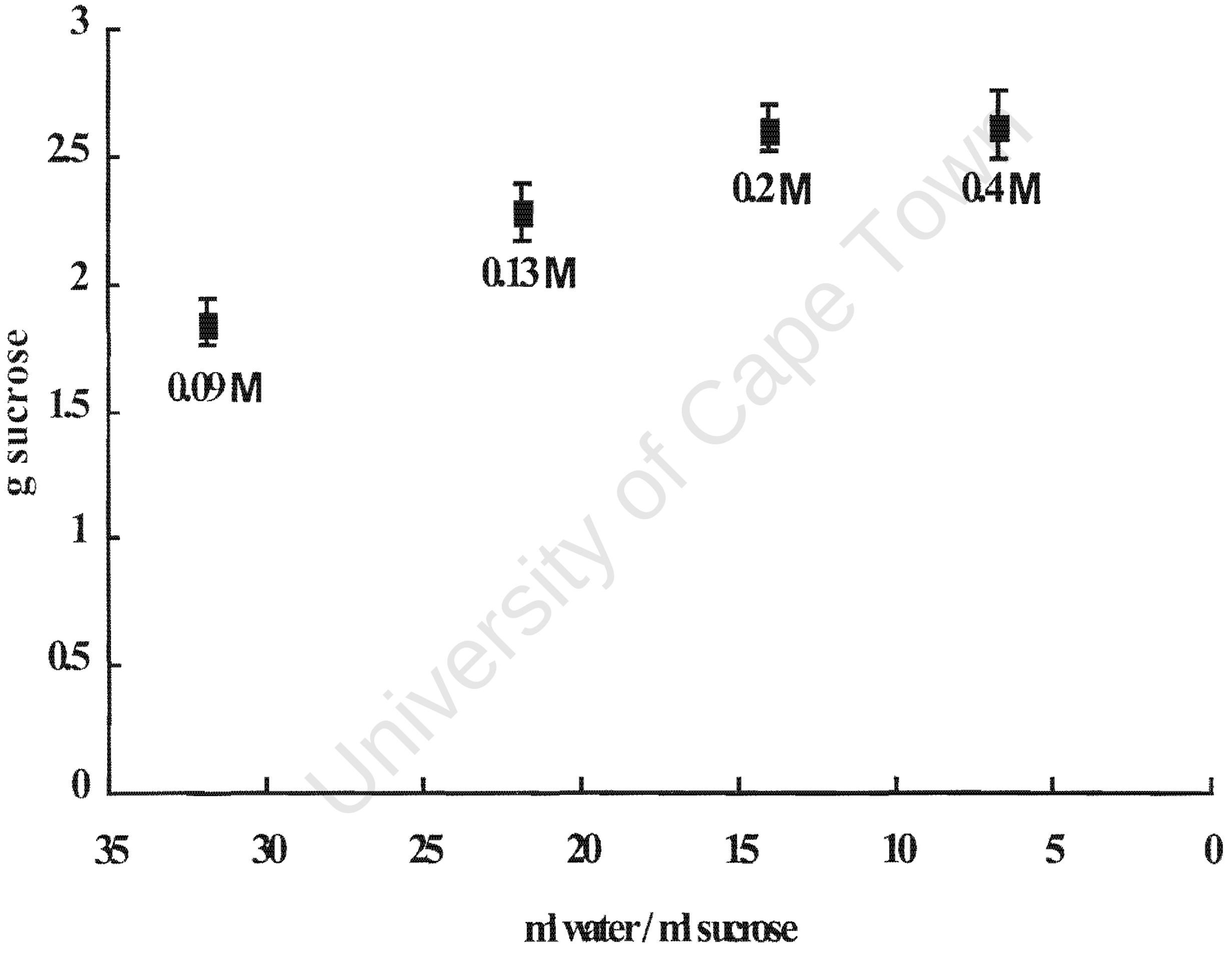

Figure X.1: The mass of sucrose ingested (g, mean ± SE) by N. chalybea fed 0.09, 0.13, 0.2 or 0.4 M sucrose.

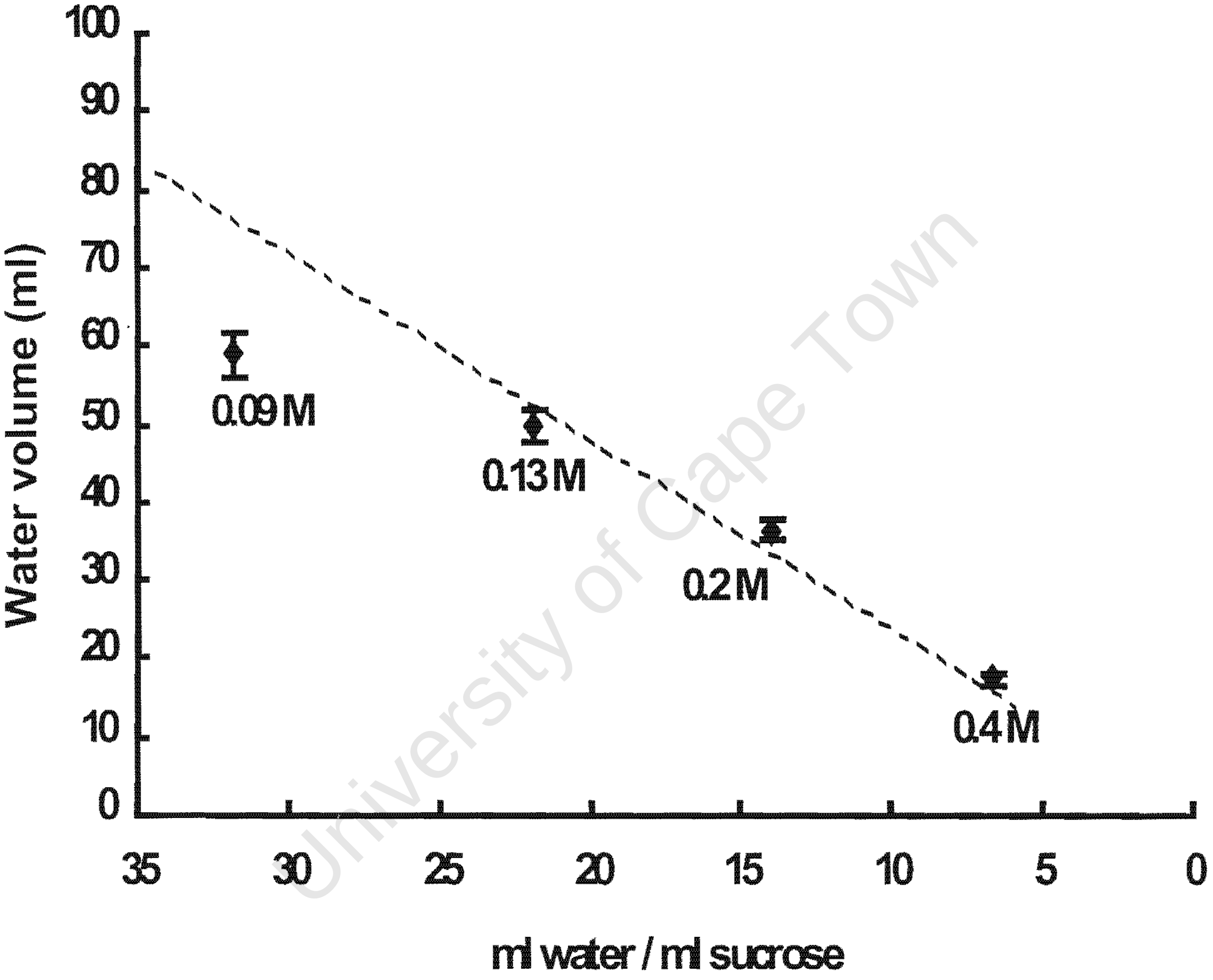

Fig. X.2: The volume of preformed water consumed by N. chalybea fed 0.09, 0.13, 0.2 or 0.4 M sucrose.