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Salt intake and regulation in two passerine nectar drinkers: whitebellied sunbirds and New Holland honeyeaters

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Abstract

Avian nectarivores face the dilemma of having to conserve salts while consuming large volumes of a dilute, electrolyte-deficient diet. This study evaluates the responses to salt solutions and the regulation of salt intake in whitebellied sunbirds (*Cinnyris talatala*) and New Holland honeyeaters (*Phylidonyris novaehollandiae*). Birds were first offered a choice of four sucrose diets, containing no salt or 25, 50 or 75 mM NaCl. The experiment was repeated using five sucrose concentrations (0.075 to 0.63 M) as the base solution. Both species ingested similar amounts of all diets when fed the concentrated base solutions. However, when birds had to increase their intake to obtain enough energy on the dilute sucrose diets, there was a general avoidance of the higher salt concentrations. Through this diet switching, birds maintained constant intakes of both sucrose and sodium; the latter may contribute to absorption of their sugar diets. A second, no-choice experiment was designed to elucidate the renal concentrating abilities of these two nectarivores, by feeding them

0.63 M sucrose containing 5-200 mM NaCl over a 4 h trial. In both species, cloacal fluid osmolalities increased with diet NaCl concentration, but honeyeaters tended to retain ingested Na^+, while sunbirds excreted it. Comparison of Na^+ and K^+ concentrations in ureteral urine and cloacal fluid showed that K^+, but not Na^+, was reabsorbed in the lower intestine of both species. The kidneys of sunbirds and honeyeaters, like those of hummingbirds, are well suited to diluting urine; however they also appear to concentrate urine efficiently when necessary.

Key words: salt balance; nectarivores; renal function; osmoregulation

Introduction

Avian nectarivores consume a physiologically challenging diet, high in preformed water and variable in predominant sugar type and concentration (Baker and Baker 1982; Johnson and Nicolson 2008; Nicolson and Fleming 2003b). Nectar ion composition is also highly variable, although there are few data available. Hiebert and Calder (1983) found higher mean K^+ (24.7 mM) than Na^+ (3.4 mM) concentrations in the nectar of 19 hummingbird-pollinated plant species. The nectar of some bird-pollinated plants in South Africa, such as *Aloe* and *Erica* species, is generally low in both K^+ (4.2-4.9 mM) and Na^+ (3.3-3.5 mM) while in *Protea* species K^+ averages 17.3 mM and Na^+ averages 18.0 mM (Nicolson and Thornburg 2007). Nectar ion levels are too low to be a good dietary source of ions, but insect feeding supplements the electrolyte intake of avian nectarivores. In wild-caught hummingbirds and honeyeaters, K^+ and Na^+ concentrations measured in excreted fluids were much higher than would be expected from a nectar diet alone (Calder and Hiebert 1983; Goldstein and Bradshaw 1998a) and could be attributed to the insect portion of the diet (Brice 1992; Stiles 1995).

The prioritisation of sugar over water intake is characteristic of specialist avian nectarivores (for review see Köhler et al. 2012). The ability to adjust volumetric intake to maintain a steady energy intake is extremely important for these birds in view of the wide range of nectar concentrations and associated water loads (Fleming et al. 2004a; Nicolson and Fleming 2003a). Electrolyte balance is potentially a major problem for birds that process several times their body mass in water each day

(Beuchat et al. 1990; Martínez del Rio et al. 2001; McWhorter and Martínez del Rio 1999; Nicolson and Fleming 2003a). Eight species of hummingbird feeding on dilute nectars were shown to eliminate excess water in chronic diuresis: these hummingbirds conserved solutes by reducing their urine osmolality to a fifth of plasma levels (Calder and Hiebert 1983). Ion concentrations in the fluid excreted by sunbirds can also be remarkably low. When Lotz and Nicolson (1999) fed southern double-collared sunbirds (*Cinnyris chalybeus*) 0.4 M sucrose with no electrolytes, the birds excreted only 0.4 mM K^+ and 1.6 mM Na^+ in their cloacal fluid. Similarly, whitebellied sunbirds (*C. talatala*) feeding on artificial diets under laboratory conditions can dramatically dilute their cloacal fluid to only 6.2±2.6 SD mOsmol/kg H_2O when feeding on a 0.25 M sucrose diet, thereby minimising their electrolyte losses on this diet (Fleming and Nicolson 2003). However, this same study demonstrated that the osmolality of cloacal fluid and total osmotic excretion (i.e. electrolyte loss) actually increased when sunbirds were challenged on more dilute (0.07 and 0.1 M sucrose) diets devoid of electrolytes. The fact that the addition of small amounts of NaCl to extremely dilute diets enables both sunbirds and honeyeaters to increase food intake (Purchase et al. 2010) suggests that salt loss on dilute diets is a serious problem for these birds. Together these data suggest a significant interplay between sodium concentration and the ability of sunbirds and honeyeaters to deal with dilute nectar diets. We therefore predicted that these birds should be acutely sensitive to electrolyte concentrations in their diets.

Very few studies have examined dietary salt preferences in birds. In 1976, Broom observed hummingbirds (ten species) at artificial feeders in the wild. He recorded feeding bout times at feeders containing 0.27 M sucrose but differing in salt concentration. At 70 mM NaCl and below, hummingbirds showed no preference for one feeder over another; however they avoided diets containing 125 mM NaCl and above (Broom 1976). Another study demonstrated that NaCl and KCl preference of cockatiels (*Nymphicus hollandicus*) varies widely between individuals (Matson et al. 2001). Bob-white quails *Colinus virginianus* demonstrate the ability to distinguish between diets of different salt content: diets with NaCl included were evidently the most palatable, followed by those with $CaCl_2$ and then KCl (Hamrum 1953). As far

back as 1909, the lethal dose of table salt (NaCl) was tested in chickens (*Gallus gallus domesticus*), with little interest in the birds' actual preference (Mitchell et al. 1926).

Limits to salt loading are important when investigating renal abilities and the ability of birds to cope in extreme environments. The urine concentrating ability of birds has been reasonably well documented (Ambrose and Bradshaw 1988; Beuchat 1996; Goldstein et al. 1990; Skadhauge and Bradshaw 1974). However, because water conservation is a major challenge to most terrestrial vertebrates, including the majority of bird species, ion regulation experiments have usually involved birds subjected to dehydration (Goldstein and Bradshaw 1998b; Skadhauge and Bradshaw 1974), where no water is made available to trial birds. The urine concentration ability of birds that have access to sufficient water, in the face of electrolyte loading, has received little attention. Lotz & Martinez del Rio (2004) examined electrolyte excretion in rufous hummingbirds (*Selasphorus rufus*) and found that these nectarivores were unable to excrete all the salt ingested when NaCl concentration in their diets exceeded 35 mM. The authors argued that this reflected adaptation to generally dilute diets, low in electrolytes, and this is supported by studies of the kidney morphology of hummingbirds and honeyeaters (Casotti et al. 1998; Casotti and Richardson 1992, 1993; Casotti et al. 1993), which suggest limited capacity to deal with high electrolyte loads in these two lineages of nectar-feeding birds. When renal function of red wattlebirds was tested in response to varying fluid intake, Goldstein and Bradshaw (1998b) found that rates of urine flow differed twofold between the most dilute and most concentrated diets, while water fluxes differed sevenfold. This implies that the intestinal tract plays an integral role in the processing of fluid and electrolyte loads, involving either water shunting through the gut or substantial postrenal ion reabsorption by the lower intestine.

The present study examines how African whitebellied sunbirds and Australian New Holland honeyeaters (*Phylidonyris novaehollandiae*) maintain electrolyte balance when they have to adjust their diet intake (and therefore water and electrolyte loading) in order to maintain energy intake. Firstly, we measured the effects of sugar concentration on the relative intake of four simultaneously-offered salt concentration (0 – 75 mM NaCl) diets (termed the '*choice*' experiment). These diet preferences were examined over successive trials where the energy value of the diets was adjusted

4

to alter the total food intake required to maintain energy balance, testing the hypothesis that birds would be more likely to avoid salt solutions on more dilute diets. In the second experiment, we measured electrolyte handling (renal and intestinal) on a series of diets where the birds were required to ingest increasing concentrations of NaCl in order to maintain energy balance (termed the '*no-choice*' experiment). This experiment tested the hypothesis of Lotz & Martinez del Rio (2004) that improved diluting ability compromises the concentrating ability of nectar-feeding birds. Together these experiments examine the interplay between electrolyte balance and the regulation of energy intake.

Methods

Bird capture and maintenance

Eight whitebellied sunbirds (body mass 8.8 ± 1.2 SD g) and eight New Holland honeyeaters (body mass 20.4 ± 1.5 g) were captured by mist netting in Jan Celliers Park in Pretoria and on Murdoch University campus in Perth, respectively. Both are common species, ensuring ease of capture and little impact upon local populations. Birds were housed in individual cages (sunbirds: 45 x 45 x 32 cm; honeyeaters: 46 x 56 x 45 cm) at 20 ± 1 °C with an automatic photophase (sunbirds: 0700 to 1900; honeyeaters: 0600 to 1800). Both species were fed a maintenance diet *ad libitum* from inverted stoppered syringes hanging from the cage sides. Sunbirds received 20% (w/w) sucrose (0.63 mol·L^{-1}) and 5% Ensure$^®$ (Abbott Laboratories, Johannesburg, South Africa); honeyeaters received 20% (w/w) sucrose and 15% Wombaroo$^®$ powder (Wombaroo Food Products, Adelaide, Australia). These maintenance diets contain low concentrations of Na$^+$: 3.1 and 2.5 mM respectively.

Choice experiment

Birds were offered four sucrose-based diets at a time containing A: 0, B: 25, C: 50 and D: 75 mM NaCl. The experiment was repeated using five different sucrose concentrations (0.075, 0.1, 0.15, 0.315 and 0.63 M) as the base solution. Trials were carried out over 6 h (commencing 0.5 h after lights on), with the positions of feeders rotated every 1.5 h in order to eliminate side bias. Each bird was randomly assigned to a sucrose concentration and each experimental diet was given over two consecutive

days (acclimation and test day), with at least one recovery day between trials, when the maintenance diet was given *ad libitum* (the maintenance diet was also offered for the remaining 6 h of photophase on acclimation and test days). Test syringes were weighed at the beginning and end of each trial, as were paraffin collection jars that were placed under each syringe to collect any spillage. The amount of each diet consumed was calculated by mass difference [(before mass – after trial mass) – spillage]. The salt intake (mmol) over 6 h was calculated by adding the products of volume consumed (V; in litres) and salt concentration (mM) for each diet in the trial:

Salt intake (mmol) = [diet B V x 25] + [diet C V x 50] + [diet D V x 75)]

No-choice experiment

In the second experiment, ion intake and excretion were recorded for birds fed diets of varying NaCl concentration. Sunbirds and honeyeaters were fed, in random order, 0.63 M sucrose containing the following concentrations of NaCl: 5, 9.9, 19.8, 29.7, 39.7, 59.5, 79.3, 100 and 200 mM. These concentrations were used to enable direct comparison with the earlier study of rufous hummingbirds (Lotz and Martínez del Rio 2004). Birds were fed the experimental diet from 0700 until 1100. Feeders were weighed hourly to measure intake, which was corrected to individual body mass. Cloacal fluid was collected under liquid paraffin, in trays that were removed and replaced hourly, for determination of its osmolality and Na^+ and K^+ concentrations. Plasma osmolality was not measured because we consider the birds, especially the sunbirds, to be too small for repeated blood sampling. At the end of every experimental session (1100) a ureteral urine sample was collected from each bird for comparison of Na^+ and K^+ levels with the cloacal fluid samples. All samples of cloacal fluid and ureteral urine were frozen at -20 °C until analysis. The Na^+ and K^+ concentrations were measured by flame photometry (Model 420, Sherwood Scientific Ltd., Cambridge, UK) and osmolality of cloacal fluid was measured with a vapour pressure osmometer (Vapro 5520, Wescor Inc., Utah, USA). At no stage did any bird have to be removed from the trial; both species were able to cope on all diets without any visible ill effects.

Statistical analysis

For the choice experiment, MANOVA was carried out to test whether there was a significant effect of species and sucrose concentration upon the arcsine square root transformed proportions of each diet consumed. MANOVA indicated significant differences in diet preferences between species ($F_{4,67}$= 2.92, P=0.027) and with sucrose concentration ($F_{16,205}$=3.85, P<0.01), and therefore each diet was analysed for each species separately by one way t-test comparing the transformed proportion data with the arsine square root of 0.25 (i.e. equal consumption of all four diets). A Bonferroni adjustment corrected for the multiple tests within each sucrose concentration.

Data from the no-choice experiment were analysed by repeated-measures ANOVA with consumption on each diet (nine NaCl concentrations) and for each hour included as the repeated dependent measures. *Post hoc* comparisons were carried out by Tukey HSD test. Generalised linear mixed model analyses were used (with individual bird ID included as a random factor to take into account repeated measures on individuals) to detect an association between sodium ingestion, retention, cloacal fluid osmolality and dietary NaCl intake. Even when individual body mass was taken into account, there were significant differences between the species in their ingestion rates and therefore salt ingestion rates ($F_{1,336}$= 170.70, P<0.001), so each species was analysed separately. All data are presented as means $\pm$ 1 SD.

Results

Choice experiment

Sunbirds consumed equal amounts of each of the four simultaneously-offered salt solutions on the most concentrated 0.63 M sucrose diets (Fig. 1A). However, there was increasing avoidance of the high salt concentrations as the sucrose concentration decreased. On the 0.315 M sucrose diets, sunbirds showed significant avoidance of the 75 mM salt solution (P < 0.05). On the 0.15 M sucrose diets, both the 75 mM and 50 mM salt solutions were avoided (P < 0.01 and 0.05 respectively) and significantly more of the no-salt solution was consumed (P < 0.05). A further decrease to

Figure 1. Proportions (mean+1 SD) of four simultaneously-offered diets (varying in NaCl concentration) consumed by whitebellied sunbirds (A) and New Holland honeyeaters (B) varied according to the concentration of sucrose in the base solution. Significant change from an equal proportion of each diet (i.e. 0.25, shown by the horizontal lines) is indicated by asterisks, where (* $p <$ 0.05, ** $p < 0.01$ and *** $p <$

0.001).
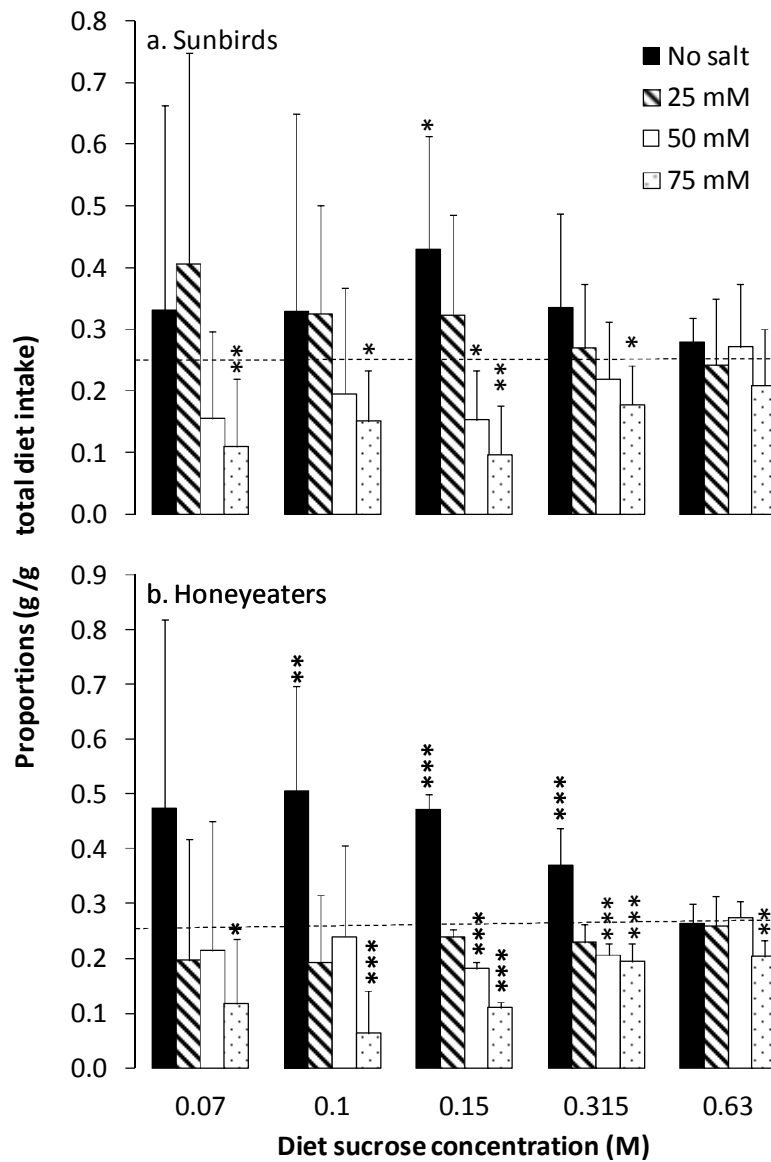
extremely dilute sucrose diets (0.1 and 0.07 M sucrose) resulted in significant avoidance of the 75 mM salt solution ($P < 0.05$ and 0.01, respectively).

A similar pattern was observed for the honeyeaters. On the 0.63 M sucrose diets, honeyeaters showed significant avoidance of the 75 mM salt solution ($P < 0.01$) (Fig. 1B). On 0.315 and 0.15 M sucrose diets, honeyeaters avoided both the 75 and 50 mM salt solutions ($P < 0.001$) and showed significant preference for the no-salt solution ($P < 0.001$). On the 0.1 M sucrose diets, honeyeaters significantly avoided the 75 mM salt solution ($P < 0.001$) and preferred the no-salt solution ($P < 0.01$), while on the 0.07 M sucrose diet, only an avoidance of the 75 mM salt solution was significant ($P < 0.05$).

With the exception of honeyeaters on the most concentrated (0.63 M) sucrose diets, the selective feeding shown by both sunbirds and honeyeaters resulted in their maintaining a steady NaCl intake (Fig. 2). Sunbirds maintained an intake of 0.408 ± 0.216 mmol NaCl over 6 h (no effect of diet sucrose concentration: $F_{4,28}=1.99$, $P=0.124$). Honeyeaters maintained an intake of 1.06 ± 0.46 mmol NaCl over 6 h on the 0.07 to 0.315 M sucrose diets, but only 0.54 ± 0.05 mmol NaCl on the 0.63 M sucrose diets ($F_{4,28}=3.38$, $P=0.022$).

No-choice experiment
Both species demonstrated significantly greater food intake (g diet/h) in the first hour compared with subsequent hours (sunbirds: $F_{3,21}= 12.56$, $P<0.001$; honeyeaters: $F_{3,21}= 102.71$, $P<0.001$). Although there was a significant effect of NaCl concentration on consumption for sunbirds (Fig. 3a; $F_{8,56}= 5.64$, $P<0.001$), there were no trends apparent in the data, and for honeyeaters, this effect was not statistically significant (Fig. 3b; $F_{8,56}= 1.84$, $P=0.088$). The mass of cloacal fluid collected was approximately half the mass of diet ingested (Fig. 3) for both honeyeaters and sunbirds (honeyeaters: $F_{1,8}= 1927.79$, $P<0.001$; sunbirds: $F_{1,8}= 2173.41$, $P<0.001$).

The osmolality of sunbird and honeyeater excreta increased linearly with dietary NaCl intake (sunbirds: $R^2 = 0.969$, $F_{1,64}=175.90$, $P<0.001$; honeyeaters: $R^2 = 0.978$, $F_{1,64}=183.69$, $P<0.001$) (Fig. 4). On the most concentrated NaCl diet, cloacal fluid

Figure 2. NaCl intake over 6 h (mmol NaCl, mean±1 SD) of whitebellied sunbirds (A) and New Holland honeyeaters (B) varied according to the concentration of sucrose in the base solution. Columns with the same letters were not significantly different (post hoc analyses).
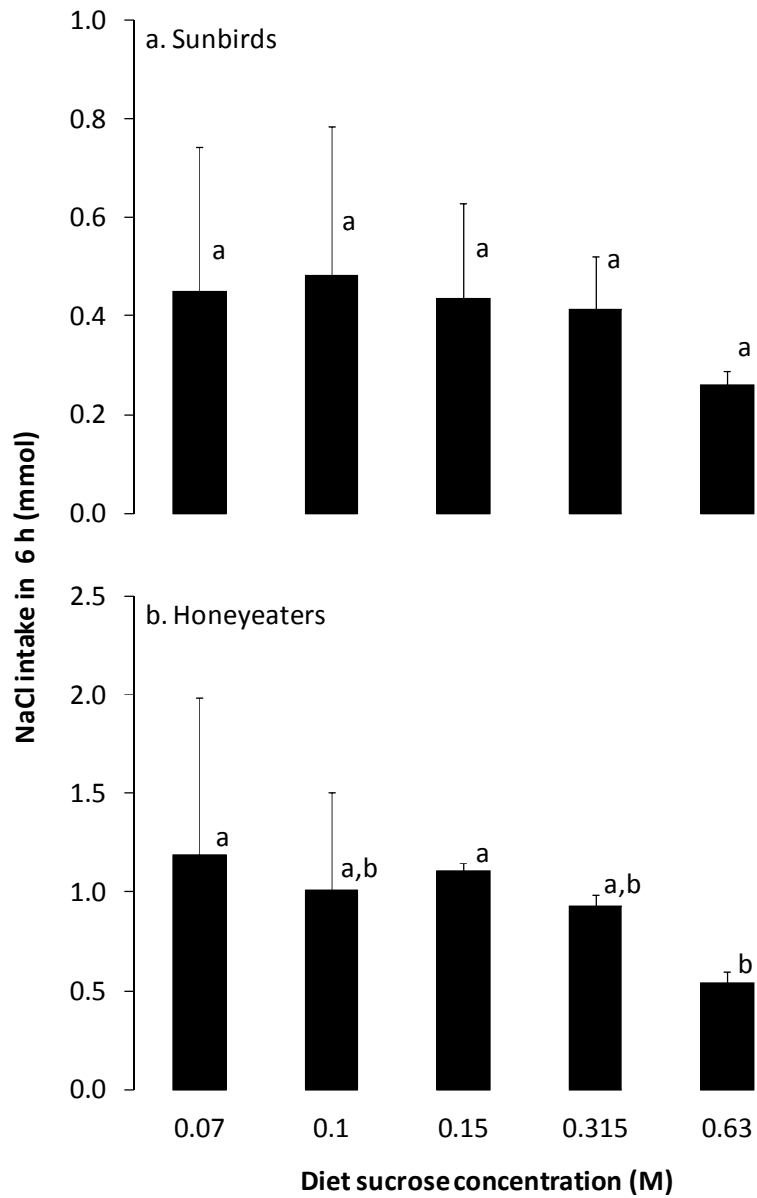
Figure 3. Mass of diet consumed and cloacal fluid excreted (g) during the 4 h no-choice trial of whitebellied sunbirds (A) and New Holland honeyeaters (B) across all nine NaCl concentrations in 0.63 M sucrose. The mass of cloacal fluid excreted is about half the mass of the food ingested. Values are means +1 SD.

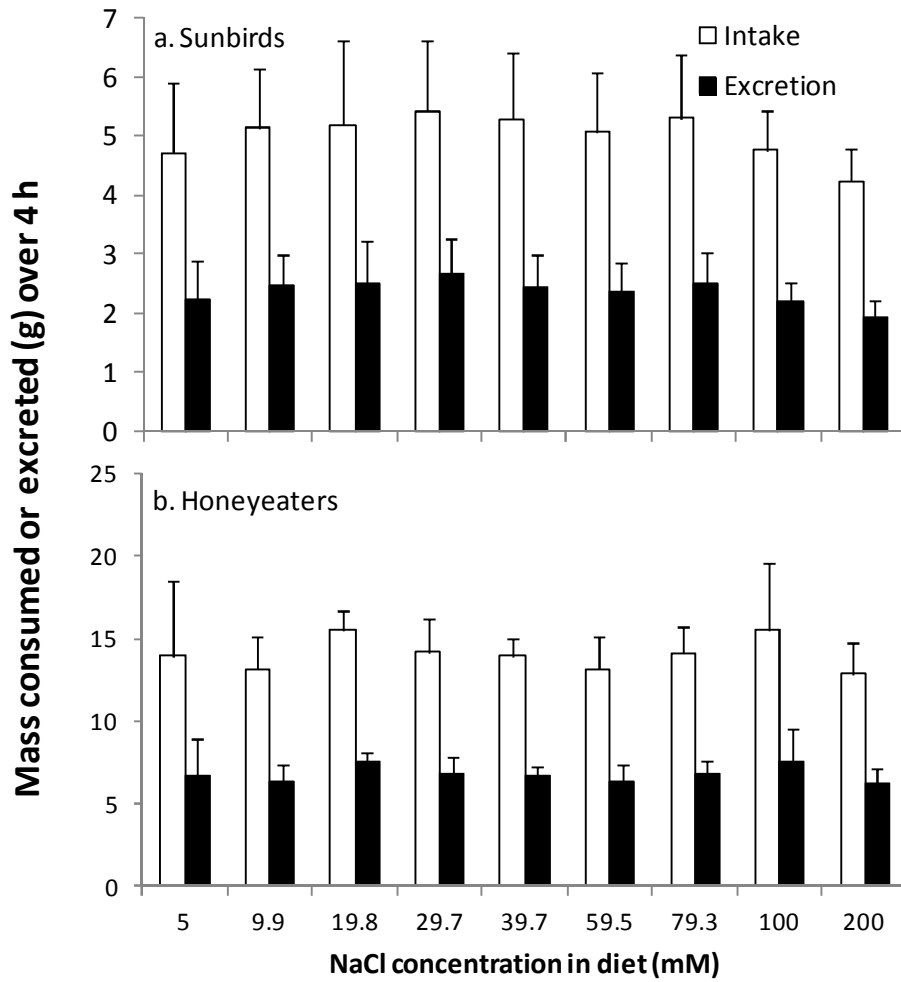**Figure 4.** Osmolality of cloacal fluid (mOsmol/kg H_2O) over the last hour as a function of Na^+ intake (mmol over 4h) of whitebellied sunbirds (A) and New Holland honeyeaters (B) consuming nine diets of the same sucrose concentration (0.63 M), but varying in NaCl concentration.
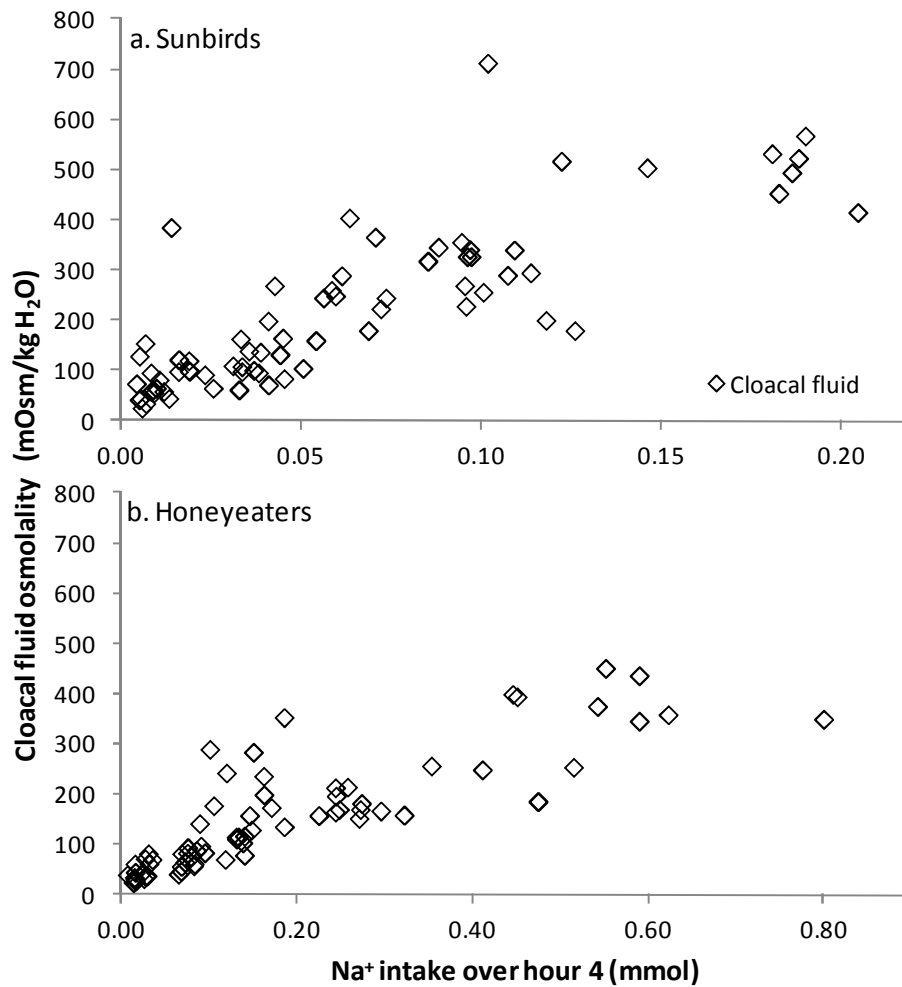
Table 1. Minimum and maximum osmolality values (mOsmol/kg H_2O; mean ± SD) of cloacal fluid in 4 avian nectarivores.

* estimated from figure

Species	Osmolality (mOsm.kg^{-1})				reference
	min	conditions	max	conditions	
Rufous hummingbird (*Selasphorus rufus*)	16	Feeders and flowers	383	Resource competition stress	Calder & Hiebert 1983
Rufous hummingbird (*Selasphorus rufus*)	60*	0.63 M sucrose, 5 mM NaCl	560*	0.63 M sucrose, 200 mM NaCl	Lotz & Martínez del Rio 2004
Whitebellied sunbird (*Cinnyris talatala*)	7.1 ± 4.7	0.25 M sucrose	460.9 ± 253.3	2.5 M sucrose	Fleming & Nicolson 2003
Whitebellied sunbird (*Cinnyris talatala*)	62.6 ± 28.2	0.63 M sucrose, 5 mM NaCl	498.6 ± 36.7	0.63 M sucrose, 200 mM NaCl	This study
Palestine sunbird (*Cinnyris oseus*)	47 ± 20	0.29 M sucrose, 3 mg/day NaCl & KCl	754 ± 233	1.46 M sucrose, 30 mg/day NaCl & KCl	Roxburgh & Pinshow 2002
New Holland honeyeater (*Phylidonyris novaehollandiae*)	85.4 ± 70.3	0.63 M sucrose, 5 mM NaCl	367.5 ± 26.3	0.63 M sucrose, 200 mM NaCl	This study

osmolalities of sunbirds averaged 498.6 ± 36.7 mOsmol/kg H_2O and those of honeyeaters averaged 367.5 ± 26.3 mOsmol/kg H_2O (Table 1).

In both species, there was a significant correlation between Na^+ intake and Na^+ excretion rates. In whitebellied sunbirds, NaCl excretion closely matched NaCl intake (although there was nevertheless a statistically significant difference between intake and excretion rates; RM-ANOVA with intake and excretion on each of the nine diets as the repeated dependent measures: $F_{1,56}=15.92$, $P=0.005$). Honeyeaters excreted far less NaCl than they consumed ($F_{1,56}=284.10$, $P<0.001$). The relationship between Na^+ intake and Na^+ excretion is shown graphically for hour 4 (Fig. 5), where excretion in both ureteral urine and cloacal fluid is shown. Sodium excretion in cloacal fluid (sunbirds: $F_{1,67}=234.37$, $P<0.001$; honeyeaters: $F_{1,64}=272.16$, $P<0.001$) and ureteral urine (sunbirds: $F_{1,68}=75.80$, $P=0.005$; honeyeaters: $F_{1,67}=101.13$, $P<0.001$) in hour 4 were correlated with sodium intake (Fig. 5). There was no statistically significant difference in Na^+ concentration between cloacal fluid and ureteral urine for either species (sunbirds: $F_{1,71}=2.08$, $P=0.154$; honeyeaters: $F_{1,71}=1.64$, $P=0.204$). Maximum cloacal fluid Na^+ concentrations (recorded when birds were fed the 200 mM NaCl diet) reached 335 ± 19.37 mM Na^+ in sunbirds and 252 ± 38.96 mM Na^+ in honeyeaters. Both sunbirds and honeyeaters showed a linear relationship between Na^+ retention during the 4 h trial and increasing NaCl in the diet (Fig. 6). Honeyeaters showed a significant increase in sodium retention with increased dietary load ($F_{1,65}=1949.38$, $P<0.001$); the pattern was less marked for sunbirds, albeit still statistically significant ($F_{1,64}=14.26$, $P<0.001$).

Ureteral urine had a significantly higher K^+ concentration than cloacal fluid in both species (RM-ANOVA; sunbirds: $F_{1,71}=39.40$, $P<0.001$; honeyeaters: $F_{1,71}=67.87$, $P<0.001$) (Fig. 7). For sunbirds, excretion of K^+ in ureteral urine was correlated with sodium intake (sunbirds: $F_{1,65}=31.25$, $P<0.001$), but honeyeaters did not show an increase in cloacal fluid K^+ excretion with NaCl load ($F_{1,65}=1.23$, $P=0.272$) (Fig. 7).

Figure 5. Sodium (Na$^+$) excretion in cloacal fluid (◊) and ureteral urine (▲) over the last hour as a function of Na$^+$ intake (mmol) of whitebellied sunbirds (A) and New Holland honeyeaters (B) consuming nine diets over 4 h of the same sucrose concentration (0.63 M), but varying in NaCl concentration.
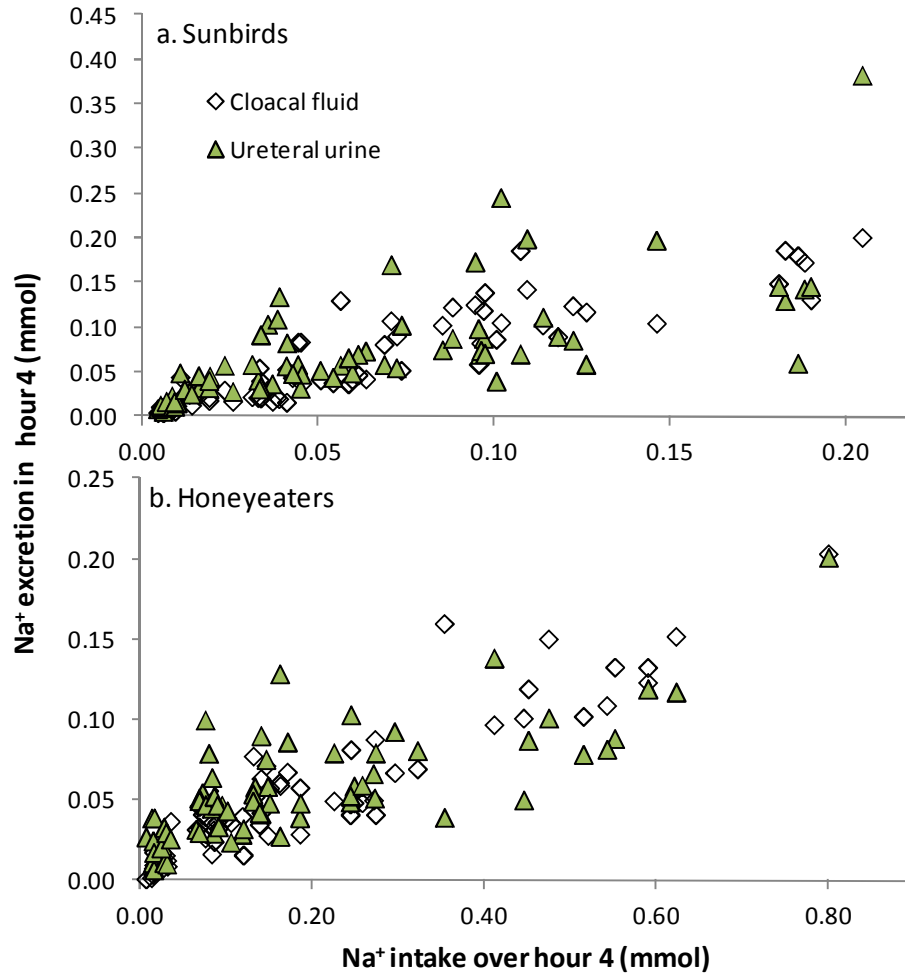
Figure 6. Retention rates of sodium compared with NaCl consumption (mmol over 4 h) of whitebellied sunbirds (A) and New Holland honeyeaters (B) consuming nine diets of the same sucrose concentration (0.63 M), but varying in NaCl concentration.
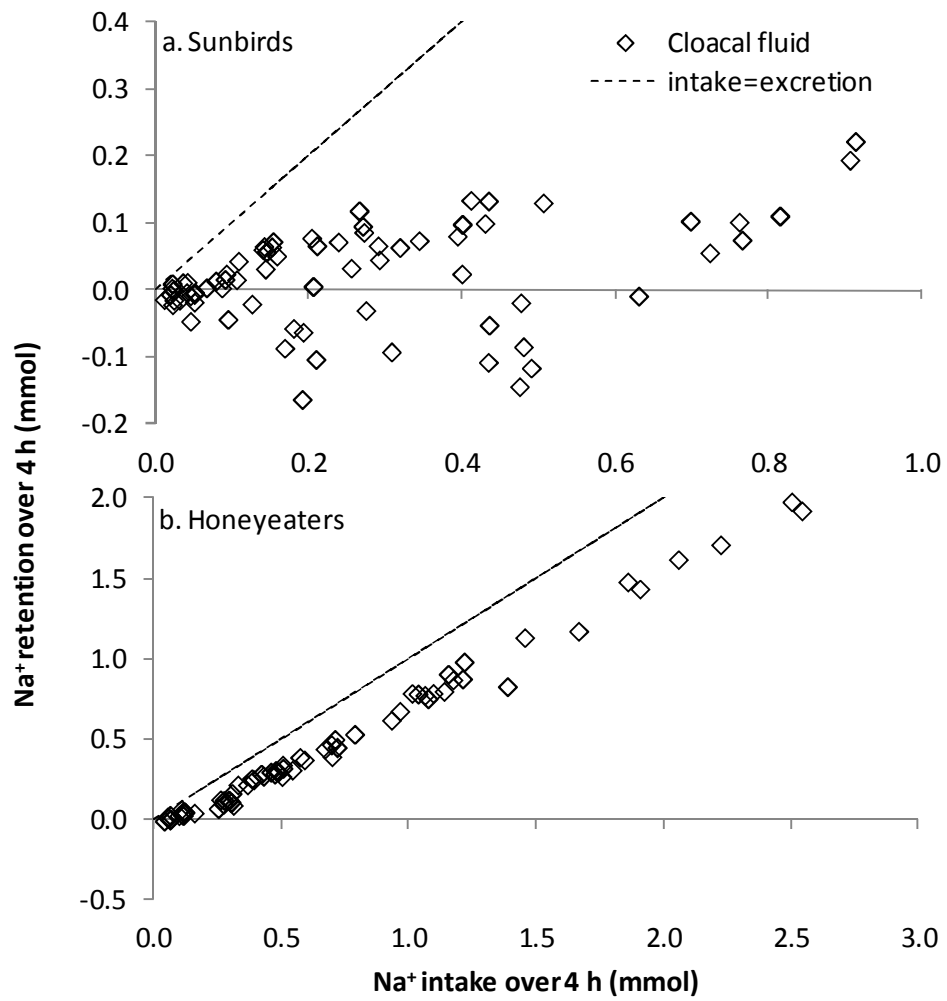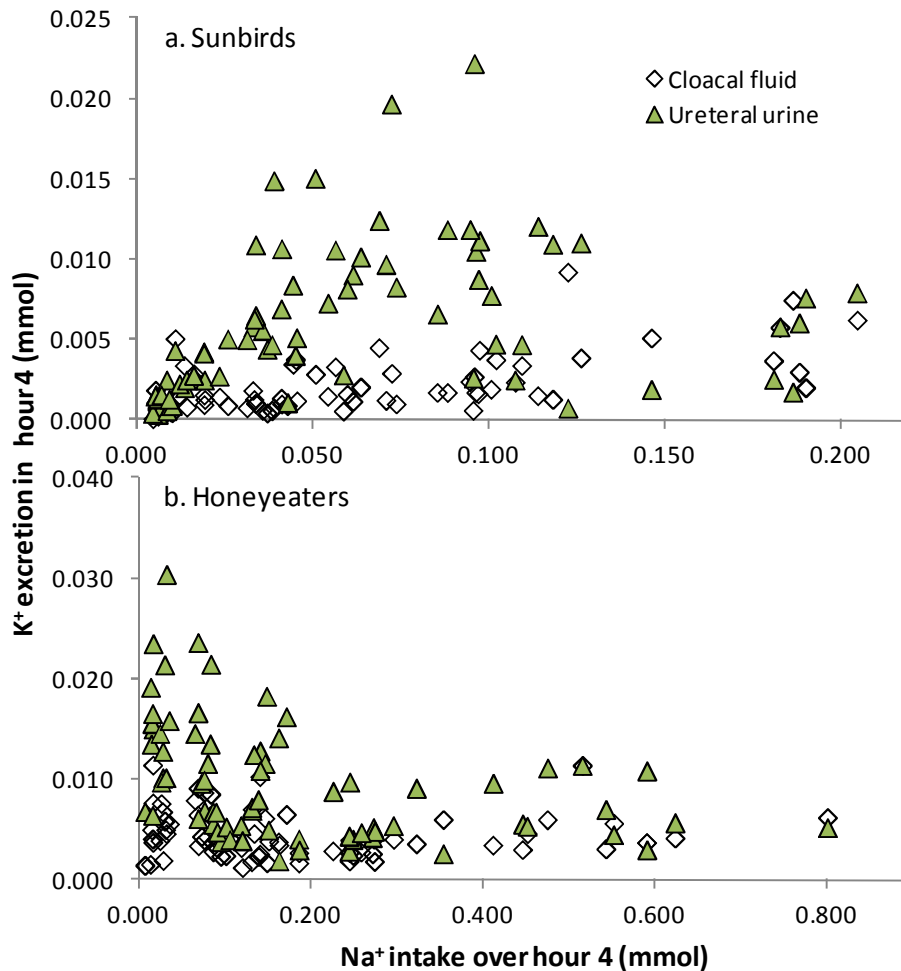
Figure 7. Potassium (K^+) excretion in cloacal fluid (◊) and ureteral urine (▲) over the last hour as a function of Na^+ intake (mmol) of whitebellied sunbirds (A) and New Holland honeyeaters (B) consuming nine diets over 4 h of the same sucrose concentration (0.63 M), but varying in NaCl concentration. Note different y-axis scale compared with Figure 6.

Discussion

Avian nectarivores maintain a constant, high energy intake despite markedly variable diet concentration and composition. Consequently they may have to switch between water excretion and conservation, as well as dealing with either electrolyte deficiency or loading. In the present study, we took advantage of this compensatory feeding to examine how whitebellied sunbirds and New Holland honeyeaters deal with electrolytes through two experiments. Firstly, we examined diet selection where the birds had a choice between diets that differed in salt concentration, and secondly, we examined salt excretion where the birds did not have choice in their diet. We discuss

16

the findings and implications of these two experiments in terms of our understanding of nectarivore osmoregulation.

Choice experiment

The first set of experiments investigated diet preference when salt was added to sucrose diets. An important finding was that whitebellied sunbirds and New Holland honeyeaters did not avoid all salt in their diets. As a consequence of selective feeding – switching to a low salt concentration when they increased consumption on more dilute diets – these nectarivores maintained a steady salt intake, consuming a total of about 0.41 (sunbirds) and 1.1 mmol NaCl (honeyeaters) during the 6 h trials. This salt may play an important role in glucose absorption and/or osmoregulation in these birds, as discussed below.

While obtaining energy from a dilute and electrolyte-deficient diet, nectarivores are required to ingest and excrete enormous volumes of preformed water, so that electrolyte conservation is vital. Previous research has shown that hummingbirds (Lotz and Martínez del Rio 2004) and sunbirds (Fleming and Nicolson 2003) are able to recover almost all solutes from the excreta. However, on extremely dilute sucrose diets devoid of electrolytes, hummingbirds go into torpor, whilst honeyeaters and sunbirds suffer decreased plasma sodium levels and are unable to maintain energy balance (Fleming et al. 2004b; Goldstein and Bradshaw 1998a; Lotz and Martínez del Rio 2004). We have found that whitebellied sunbirds and New Holland honeyeaters are limited in their intake of extremely dilute diets by increasing losses of sodium, confirmed by a significant decrease in plasma sodium levels in the absence of dietary sodium (Purchase et al. 2010). Excessive sodium excretion (natriuresis) and subsequent hyponatremia affect the digestive capacity of nectarivores. Through Na^+/K^+ pumps on the basolateral membrane of intestinal cells, a sodium concentration gradient is established that causes Na^+ ions to enter the cells passively across the apical membrane, accompanied by glucose. The sodium-linked glucose transporter SGLT-1 transports one glucose molecule along with two Na^+ ions from the intestinal lumen to the cytosol (Scheepers et al. 2004). The presence of sodium in the diet therefore aids the uptake of glucose. The addition of even small amounts of sodium (5-10 mM) to very dilute sucrose diets enables whitebellied sunbirds and New Holland honeyeaters to increase intake of such diets (Purchase et al. 2010). Diet

switching and modulation of sodium intake, as demonstrated in the present study, allows the birds to maintain sodium intake levels sufficient to assist with sugar digestion/absorption, without wasting energy processing more salt than is required.

Reduction of the sucrose concentration forces the birds to increase volumetric food intake to maintain constant energy intake, thus increasing water intake. However, except on the most concentrated diet of 0.63 M sucrose, the sodium intake of whitebellied sunbirds and New Holland honeyeaters in the choice experiment was unaffected by preformed water intake (i.e. sodium intake remained constant over these diets through diet selection, despite a 4.5-fold increase in water intake between 0.315 and 0.07 M sucrose diets). Although we recognise that sodium plays an important role in the water balance of every animal, this result suggests that the significance of sodium for homeostasis in these animals is not directly or solely linked to water balance.

We were interested in whether the regulated sodium intake recorded in the choice experiment reflected estimated sodium intake of these birds under wild conditions. A rough calculation of sodium intake from natural nectars for whitebellied sunbirds (these birds consume $0 \cdot 313 \pm 0 \cdot 038$ g sugar per g body mass daily under laboratory conditions and in the field consume, on average, nectars of 20% w/w sucrose and 3.4 mM sodium, Nicolson and Fleming 2003a; Nicolson and Thornburg 2007) shows that these birds would naturally consume around 0.0206 mmol of sodium in 6 h. This is about one twentieth of their sodium intake during feeding trials in the present study. If the sodium preferences apparent in these laboratory experiments can be taken as an indication of the ideal sodium requirements of the birds in the wild, then it is clear that these nectarivores could not meet their sodium requirements from nectar alone. We assume similar values for the New Holland honeyeaters, although we know little about the electrolyte concentrations of Australian nectars. Therefore, if we can assume that their voluntary salt intake in the laboratory reflects sodium requirements, it is likely that arthropods, in addition to being an important source of protein (Paton 1982; Stiles 1995), are also a source of electrolytes for avian nectarivores.

No-choice experiment

The second set of experiments investigated electrolyte handling by whitebellied sunbirds and New Holland honeyeaters when these birds were fed diets with added salt to test the salt loading point. Both species demonstrated the capacity to concentrate their excreta and to modify urine in the lower intestine by recovering potassium on these K^+-free diets. The smaller sunbirds showed greater excreta concentrating ability than the honeyeaters and a better ability to excrete excess dietary sodium. Similarly, when southern double-collared sunbirds were fed 15 mM each of K^+ and Na^+ in 0.4 M sucrose, they maintained cation balance by producing cloacal fluid with concentrations of each ion around 17 mM (Lotz 1999).

Both whitebellied sunbirds and New Holland honeyeaters surpassed the urine concentrating abilities reported for hummingbirds. Rufous hummingbirds become salt loaded when feeding on 0.63 M sucrose diets with 35 mM NaCl added (Lotz and Martínez del Rio 2004), where their sodium intake would be around 0.0859 mmol over the 3 h trial (calculated from their estimated intake rate of 5.77 ml/day and assuming that they feed for 12 h in the day). Consequently, hummingbirds cannot maintain energy balance on these solutions (Lotz and Martínez del Rio 2004). Similarly, Rooke *et al.* (1983) found that frugivorous silvereyes (*Zosterops lateralis*) feeding in vineyards during the dry season, where grapes and brackish water were their only water sources, were dehydrated and probably salt loaded. By contrast with rufous hummingbirds and silvereyes, whitebellied sunbirds and New Holland honeyeaters were far more tolerant of salt added to their diet, ingesting reasonable quantities of 200 mM NaCl, similar to the salt tolerance of arid-adapted granivores, such as zebra finches and scrubwrens, both of which can tolerate salty solutions of up to 800 mM NaCl and show a maximum salt intake of 3.6 mmol in 24 h when drinking 300 mM NaCl solutions (Ambrose and Bradshaw 1988; Skadhauge and Bradshaw 1974).

Previous suggestions that, even under conditions of water deficiency, nectarivores cannot produce urine of higher osmolality than plasma ($\approx$ 350 mOsmol/kg H_2O) may be accurate for some hummingbirds (Beuchat et al. 1990; Lotz and Martínez del Rio 2004). However, under extreme conditions, sunbirds certainly are capable of producing relatively concentrated urine (sunbirds: 499 mOsmol/kg H_2O; present

study), while honeyeaters excrete somewhat less concentrated excreta (368 mOsmol/kg H_2O; present study). Some avian nectarivores can therefore produce copious quantities of dilute excreta, but can also concentrate excreta when necessary (Table 1).

While the kidney morphology of both hummingbirds and honeyeaters suggests that these birds are adapted to produce dilute rather than concentrated urine, post-renal modification also plays a role in osmoregulation in birds. Concentration or dilution of excreta can occur in the gastrointestinal tract, with lower intestinal modification of urine described for a variety of bird species, although the focus has been on reabsorption of sodium (Goldstein and Skadhauge 2000). In the present study, postrenal modification was shown for potassium, with more K^+ present in ureteral urine than the excreted cloacal fluid. Conservation of K^+ is important when there is no dietary source (such as on these experimental diets) and has been demonstrated previously for sunbirds on salt-free sucrose diets (Fleming and Nicolson 2003; Lotz and Nicolson 1999). However, the finding that K^+ was reabsorbed, but not Na^+, is an artefact of our experimental design using diets that included Na^+ but not K^+.

Dietary sodium, which is naturally deficient in nectars, clearly plays a significant role in the maintenance of energy balance in nectarivorous birds, and therefore we suspect alternative salt sources may be important for these birds to supplement their nectar diet. This will be especially important under wet or cold conditions, where nectar has been diluted by rain or dew, insects are in short supply, and the birds are required to increase intake to maintain energy balance due to reduced ambient temperatures. There is a dearth of information on nectar ion levels and the extent of arthropod foraging amongst nectarivorous birds. Information on both is required before we can interpret the ecological consequences of varying tolerance to dietary sodium by nectarivorous birds. We also have some way yet to go in terms of understanding the mechanisms of action or role of sodium in the diet of nectarivorous birds; this is compounded by the high level of variation in field water and ion balance shown in these birds (Goldstein and Bradshaw 1998a). Measurements of the Na^+ and K^+ concentrations in excreta of sunbirds in the field would give us a better understanding of the ecological relevance of these data and enable comparison with previous field research on honeyeaters (Goldstein and Bradshaw 1998a).

Acknowledgements

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