

CONCLUSION

The importance of studying animal responses to energy challenges

The regulation of food intake allows an animal to balance its energy intake and expenditure, which is crucial for survival and fitness. When the energy content of the food source is low or energy requirements are high, animals must increase their food intake. Exposure to low food quality, fasting periods and low temperature under laboratory conditions provides an understanding of how animals cope in terms of foraging energetics with adverse environmental conditions. All three energy challenges in my study are ecologically relevant. Firstly, nectarivorous birds encounter food plants providing low nectar concentrations, or nectar may be diluted by rain. Secondly, the time available for foraging may be restricted by storms and heavy rain, or chases by predators and competitors may prevent feeding. Thirdly, the birds also experience sudden drops in environmental temperature during a storm, or are exposed to longer-term periods of cold during winter.

Studies in the field of ecological physiology provide insight into animal physiology and behaviour, including energy availability and utilization, thermoregulation, body size effects on physiological traits and behavioural adjustments (Bennett 1987a; Karasov and Martínez del Río 2007). Data deriving from these studies can be used to predict the physiological responses of animals exposed to changing environmental conditions. Since the physiology of an animal determines the range of environmental conditions under which it can persist, this knowledge may serve conservation purposes. It can help biologists to anticipate future problems, find explanations for population declines, develop countermeasures and monitor the success of management strategies (Wikelski and Cooke 2006).

The response of hummingbirds to energetically challenging conditions, such as low ambient temperature and fasting periods, has been extensively investigated (e.g.

Beuchat et al. 1979; Tooze and Gass 1985; Carpenter and Hixon 1988; Gass et al. 1999; McWhorter and Martínez del Rio 2000; Fernández et al. 2002). Similar challenges in sunbirds (Lotz 1999; Nicolson et al. 2005) and honeyeaters (Collins and Briffa 1984) have received less attention, but it is just as important to investigate the effect of energy stress on these two families of avian nectarivores. My study therefore provides an important contribution, as it focuses on the behavioural and physiological responses of the main families of passerine nectarivores to variations in food quality and availability and low ambient temperature.

Suitability of avian nectarivores for my study

Avian nectarivores are diurnal and can easily be observed while foraging. For this reason, the feeding behaviour and inter-individual interactions of wild avian nectarivores have been investigated in various field studies (e.g. Gill and Wolf 1975 and 1977; Vaughton 1990; Lott 1991; Mendonça and Dos Anjos 2006). Nectarivorous birds are also ideal subjects for investigating the compensatory feeding response and physiological constraints to this response under energetically challenging conditions. Firstly, their diet consists mainly of nectar, which is a relatively simple food and easily assimilated. The energy intake rates of nectarivorous birds can be readily quantified, since nectar consists mainly of sugar and water. Secondly, the energy balance of the birds is linked to their water balance. Birds compensate for low nectar concentrations and increased energy demands by ingesting larger amounts of the watery diet, which may pose osmoregulatory challenges (Lotz and Nicolson 1999; Fleming and Nicolson 2003). Thirdly, avian nectarivores are characterized by high mass-specific metabolic rates and energetic lifestyles. The birds have to feed frequently to meet their high energy requirements. As shown for hummingbirds, the metabolism is fuelled by recently ingested sugars, rather than stored fat (Carleton et al. 2006; Welch and Suarez 2007). These features make the birds susceptible to low temperatures and periods of food scarcity.

The study species, namely amethyst (*Chalcomitra amethystina*) and whitebellied (*Cinnyris talatala*) sunbirds and brown honeyeaters (*Lichmera indistincta*), were chosen

for this study as they occur in similar habitats, but belong to two different families of avian nectarivores. The body size of the brown honeyeater is comparable to that of the two sunbird species. The brown honeyeater is one of the smallest honeyeater species and mainly nectarivorous, while larger honeyeaters also ingest fruits and insects as major portions of their diet (Pyke 1980). Since the species are ecologically alike, they are expected to experience similar energy challenges in their natural environment. If sunbirds and honeyeaters differ in their response to energy challenges, phylogenetic effects are likely, since other ecological and physiological aspects are comparable (Liknes and Swanson 1996).

Brown honeyeaters and whitebellied sunbirds accustom easily to captivity, and have consequently been useful research subjects (e.g. Collins et al. 1980; Collins and Briffa 1984; Nicolson et al. 2005; Leseigneur et al. 2007). Whitebellied sunbirds, in particular, learn to feed from artificial feeders within a few hours of capture and settle down quickly in individual cages. They can also be kept together in an aviary, without major competitive and aggressive behaviour (pers. observation). Amethyst sunbirds do not habituate to captivity so easily: individual birds take several days to learn to feed from artificial feeders, and the birds are generally more alert even after long periods in captivity. When both species were kept in a group in an aviary, I observed adult male amethyst sunbirds chasing each other and whitebellied sunbirds of both sexes, with the subordinate birds being excluded from feeding, even though plenty of feeding stations were provided.

The response of sunbirds and honeyeaters to energy challenges

In response to a decrease in nectar sugar concentration, whitebellied sunbirds increased their food intake to maintain a constant energy intake. This supports the compensatory feeding hypothesis, which states that nectarivorous birds adjust their food intake and defend a constant sugar intake over a wide range of nectar concentrations (Martínez del Rio et al. 2001). On a very dilute diet (2.5% w/w), however, sunbirds were not able to increase their food intake sufficiently and had a lower daily sugar intake than on more

concentrated diets, indicating the influence of physiological constraints. When switched from a very dilute to a more concentrated diet, the birds increased their food intake instantly in an attempt to compensate for the previous low energy intake. In contrast, the response was much slower following a decrease in nectar sugar concentration: birds increased their food intake within 10 min after the diet was changed. The ecological significance of this may be that when wild birds encounter low nectar sugar concentrations, they may first search for flowers that provide nectar with higher energy content before increasing their intake of dilute nectar. How fast nectarivorous birds respond to a change in dietary sugar concentration has not been determined before, except for one preliminary study in which a single rufous hummingbird (*Selasphorus rufus*) responded to an increase in concentration faster than to a decrease (Gass 1978).

When whitebellied sunbirds and brown honeyeaters were exposed to a fasting period, food intake and energy accumulation were accelerated following the fast. However, compensation for the loss in foraging time was incomplete, as the birds had a lower daily sugar intake and weighed less in the evening than on a day with uninterrupted feeding. During acute exposure to low ambient temperature, which increases energy requirements for thermoregulation, all three nectarivore species increased their food and thus energy intake. Despite this increased food consumption, however, the birds lost more body mass in the cold than at the highest temperature.

It is therefore concluded that the intake response of whitebellied and amethyst sunbirds and brown honeyeaters is shaped by both compensatory feeding and physiological constraint. As discussed in the individual chapters, limitations to nectar ingestion (Bednekoff and Houston 1994; Mbatha et al. 2002), digestion (McWhorter and Martínez del Rio 2000; McWhorter et al. 2006) and osmoregulatory processes (Fleming and Nicolson 2003; Hartman Bakken and Sabat 2006) may result in energy deficits. To overcome digestive constraints, avian nectarivores may show physiological adjustments that lead to a higher rate of digestion. Brown honeyeaters in my study were able to increase their food intake after only a few days of exposure to high feeding rates, which suggests that physiological adjustments had taken place. These may consist of

upregulation of digestive processes or an increase in intestine size, and thus increased amounts of nutrient transporters and digestive enzymes (McWilliams and Karasov 2001; Karasov and McWilliams 2005). These physiological changes occur in a wide variety of animals and may occur rapidly (Starck 1999).

In terms of food ingestion, my study showed that the rate at which nectar can be licked may constrain ingestion at high nectar concentrations. As diet concentration increased, lick durations of sunbirds increased, and licking frequency and food consumption per lick decreased. High nectar concentrations impede the ingestion of the solution, thus reducing energy intake rates. Consequently, birds and other nectarivorous animals face a trade-off between feeding on concentrated nectar, which provides more energy per unit volume than dilute nectar, and feeding on nectar that is dilute enough for optimal ingestion (Nicolson 2007). The effect of nectar concentration on the licking behaviour of avian nectarivores was investigated in early studies (e.g. Hainsworth 1973; Collins et al. 1980) but improved techniques allow for more accurate results in the present study. Future studies should include independent manipulation of the concentration and viscosity of sugar solutions to investigate which fluid characteristic affects the licking behaviour of the birds.

When looking at the feeding patterns of sunbirds, it was apparent throughout this study that nectar intake rate is mainly adjusted by increasing feeding frequency and not meal size. However, sunbirds do not seem to be constrained by the amount of food that they can ingest and process at once, as starved individuals that were previously exposed to a very dilute diet or deprived of food were able to ingest larger meals. So why do sunbirds not adjust their food intake rate by changing meal size? A large meal results in an increase in body mass, which is associated with higher energetic costs for flight (DeBenedictis et al. 1978). The adjustment of nectar intake via feeding frequency rather than meal size may therefore be interpreted as optimal feeding behaviour based on energetic costs and benefits. The hovering capacity of ruby-throated hummingbirds (*Archilochus colubris*) was reduced when body mass was increased (Chai and Dudley 1999; Chai et al. 1999). A reduction in body mass, on the other hand, saves energy as

shown in European starlings (*Sturnus vulgaris*) (Bautista et al. 1998). It must be noted however, that the metabolic costs of short flights of sunbirds are relatively inexpensive compared to those of starlings (Hambly et al. 2004), and the hovering flight of hummingbirds. Alternatively, the increase in feeding frequency may also represent the natural foraging behaviour of sunbirds. The birds visit many different flowers to meet their energy requirements, as the volume of nectar per flower is limited. Consequently, sunbirds ingest frequent small meals instead of few large meals.

Comparing the responses of sunbirds and honeyeaters to energy challenges, it is concluded that they showed convergence in their behavioural and physiological responses. Both sunbirds and honeyeaters compensated for a period of food deprivation, low food quality and increased energy requirements by increasing their food intake. However, species of both lineages of nectarivorous birds appear to face constraints to food intake and processing, since their compensatory feeding response was incomplete. Although sunbirds and honeyeaters are unrelated and geographically isolated (except for the far northeast of Australia, New Guinea and nearby islands), the adaptation to a similar nectar-feeding lifestyle and comparable environmental conditions probably has resulted in similar behaviour and physiology. In my study, major phylogenetic effects on the responses to energy challenges were not apparent.

Individual variation

The feeding patterns of sunbirds were recorded on a novel short-term scale using a photodetection system. The detailed measurements highlighted unexpectedly large variation in feeding behaviour between individual birds. Meal sizes, feeding frequencies and licking behaviour differed greatly between birds, but the behaviour of individual birds was very consistent. For instance, if one bird took many short meals, while a second bird took few long meals, they would demonstrate the same characteristic feeding pattern on different days and under various experimental conditions. However, the data are insufficient to statistically demonstrate this repeatability since birds were not exposed to

the same experimental conditions several times to avoid training effects and physiological adjustments.

Between-individual differences have already been observed on a coarse time scale, when the energy intake of whitebellied sunbirds was measured in an earlier study (Nicolson and Fleming 2003). Jackson et al. (1998) found variations in sugar type preferences between individual southern double-collared sunbirds (*Cinnyris chalybeus*) and Cape sugarbirds (*Promerops cafer*). The responses of rufous hummingbirds (*Selasphorus rufus*) and European starlings exposed to energetically challenging conditions differed between individuals (Tooze and Gass 1985; Bautista et al. 1998). Individual differences may conceal trends in the behavioural response of nectarivorous birds to experimental conditions. Earlier studies on avian nectarivores sometimes tested only one individual (Carpenter and Hixon 1988; Roberts 1995) or very few (e.g. Wolf and Hainsworth 1977; Hainsworth et al. 1981; Tamm and Gass 1986; Roberts 1996). Researchers should increase their sample size in order to be able to draw conclusions that are representative of the entire species.

However, the investigation of between-individual differences has the potential to contribute to physiological and ecological studies. In earlier ecophysiological studies, the uniqueness of an individual often received limited attention, with researchers rather attempting to describe the physiological and behavioural response of the average animal of an experimental group (Bennett 1987b). If researchers find repeatable individual traits of animals that are in good physical condition, and experimental errors have been ruled out, the causes and consequences of individual variation can be analyzed (Bennett 1987b). What are the physiological and morphological factors that underlie individual differences? Is the performance of an animal correlated with differential survivorship under natural conditions and what are the evolutionary consequences?

The integration of physiology and behaviour

The exposure of avian nectarivores to energetically challenging conditions was short-term in my study. Since the results indicate physiological limitations, it remains a question how sunbirds and honeyeaters cope with longer-term unfavourable environmental conditions. The birds are likely to adjust their behaviour, such as reducing their flight activity to save energy. Behavioural adjustments probably occurred, especially when birds maintained their body mass despite an insufficient energy intake. During cold exposure, for instance, sunbirds and honeyeaters were observed to be less active and ptiloerected their feathers to conserve body heat. Birds may also have reduced their body temperature to reduce the amount of energy needed to maintain body heat (McKechnie and Lovegrove 2002).

This emphasizes that physiological and behavioural responses are linked and can not be investigated in isolation. In addition to an increased food intake, animals may conserve energy by reducing their activity and metabolic rate when they need to maintain energy balance under energetically stressful conditions. Behavioural adjustments have been observed in earlier studies in which birds were exposed to energetically challenging conditions (e.g. Dall and Witter 1998; Fernández et al. 2002). If the perches are connected to electronic balances interfaced to a computer, and the bird can not rest anywhere else, activity can be calculated from missing and unstable body mass recordings. In Chapter 2, the flights between the two perches in the cage were used as a measure of flight activity. The daily number of flights did not differ when birds were fed different nectar concentrations. On the dilute diet treatment however, sunbirds were found to reduce the number of flights in the afternoon, which indicates activity adjustments to conserve energy.

Directions for future research

Several new questions and directions for future experiments have arisen during the course of my research. Firstly, a more detailed study on the activity and time budget of the birds may enhance our understanding of behavioural adjustments during energy challenges.

Under laboratory conditions, the activity of birds can easily be quantified by connecting all available perches to electronic balances and recording body mass. Alternatively, the activity of a caged bird can be analyzed using video recordings or motion detectors. Future studies could quantify flight activity, along with other activities such as hopping, jumping from perch to perch and grooming. From these records, researchers can calculate what proportions of the day the bird is active or resting. The recording of the time spent feeding, using a photodetection system or motion detectors next to the feeder, completes the time budget data.

These time budget data can then be used to calculate the energy requirements of the birds. The basal metabolic rate can be determined by measuring the oxygen consumption of resting birds in a metabolic chamber. The energetic costs for flights can be calculated from the body mass, time spent flying and the unit flight cost for the particular species (Wolf et al. 1975; Collins and Morellini 1979). This would result in a complete daily energy budget, which could then be compared between control and energetically challenging conditions. Such calculations of energy budgets have been done for hummingbirds exposed to different ambient temperatures and diet qualities (López-Calleja and Bozinovic 2003). In a future experiment, one could also show whether energetic costs for flight increase with increasing body mass in sunbirds, and whether the size of a large meal affects flight costs. If so, this would explain why sunbirds in the present study adjusted their food intake via feeding frequency rather than meal size.

In addition to activity measurements and energy budgets, the body temperature of avian nectarivores during energy challenges should be recorded. This was not incorporated in my study, as regular measurements with a thermocouple involve catching of the birds and this would interrupt their normal feeding patterns. Alternatively, iButtons could be implanted and body temperatures monitored using telemetry. Various bird species reduce their body temperature (leading to either shallow hypothermia or torpor), which in turn leads to a reduction in metabolic rate (McKechnie and Lovegrove 2002). For example, southern double-collared sunbirds and malachite sunbirds (*Nectarinia famosa*) reduce their body temperature with decreasing ambient temperature (Leon and

Nicolson 1997; Downs and Brown 2002). Body temperature measurements in future experiments involving energy challenges would enable us to detect possible facultative hypothermic responses, and would complete the investigations of physiological and behavioural responses of avian nectarivores to energy challenges.

A different aspect, that could not be resolved in the present study, is the physiological constraint to food intake. Body mass losses during individual energy challenges indicate that the birds were not able to increase their food intake sufficiently to maintain body mass. Which factors are limiting? Sucrose hydrolysis rates may constrain food intake in avian nectarivores (McWhorter and Martínez del Rio 2000). If all experiments were to be repeated on hexose diets and the birds maintained their body mass, then sucrose hydrolysis is likely to be the constraining factor. However, repetition of parts of the experiment on hexose diets also revealed limitations to food intake (Chapters 4 and 5). This limitation on hexose diets is in agreement with other studies, and suggests constraints to hexose absorption rates (Levey and Martínez del Rio 1999; Fleming et al. 2004). Furthermore, birds may be challenged by the handling of large amounts of ingested fluid (Fleming and Nicolson 2003) and by electrolyte and metabolite losses in the excreta (Lotz and Nicolson 1999; Lotz and Martínez del Rio 2004). In future experiments, the nectar diets could be supplemented with electrolytes to investigate whether this allows the birds to maintain energy balance during energetically stressful conditions.

Lastly, the knowledge on feeding behaviour and energy balance of avian nectarivores gained from experiments on captive birds should be confirmed by studies on free-living individuals. Captivity is associated with reduced activity levels compared to wild individuals and affects the basal metabolic rate of birds (Warkentin and West 1990; Nudds and Bryant 2001). The present study showed that housing conditions affect the activity of avian nectarivores and their energy requirements. When kept in isolation in a Perspex cage, individual whitebellied sunbirds were less active than when they were able to see or hear the other birds (A. Köhler, pers. observation). Consequently, their sugar intake was lower in the Perspex cage (Chapter 1: $220\text{--}240 \text{ mg}\cdot 24 \text{ h}^{-1}\cdot \text{g body mass}^{-1}$ at

20°C on 0.32–1.35 M sucrose diets) than when birds were kept in separate cages standing next to each other (Chapter 4: 280–300 mg•24 h⁻¹•g body mass⁻¹ at 15 and 25°C on 0.25–1 M sucrose diets). Furthermore, it is possible that the pure sucrose diet (with added Ensure[®]), which was available *ad libitum* during captivity, leads to upregulation of intestinal sucrase activity, increased food intake and body mass gain. Since the sunbirds were kept together in a large outdoor aviary between experiments, with exposure to natural vegetation and weather conditions, they were in good physical condition and did not gain mass during captivity. They were kept in captivity for up to one year, after which they were released and new individuals were captured. Honeyeaters were captured and kept in the laboratory for six weeks prior to experiments, and were kept for a total of three months. Their diet consisted of honeyeater and lorikeet nectar (Wombaroo[®]) and additional sucrose, and body mass did not change after capture. I am therefore confident that the responses of the sunbirds and honeyeaters used in my study can be extrapolated from captive to wild individuals. However, captivity and the presence of a researcher induce stress in the birds and may affect physiological and behavioural results.

Wild birds can be caught in mist-nets and colour coded rings attached to their legs for recognition during later observations. Alternatively, radio-transmitters can be implanted, so that released birds can be tracked. Many sunbird and honeyeater species are resident, at least seasonally, and can easily be observed foraging on flowers, especially when territorial (Gill and Wolf 1975 and 1977; Frost and Frost 1981). The feeding behaviour, activity and roosting of a single rufous hummingbird was recorded by observing the wild bird (Carpenter and Hixon 1988). In addition, body temperature can be recorded with implanted iButtons. Field metabolic rate and water turnover can be quantified using the doubly labelled water method, as has been done in free-living female orange-breasted sunbirds (*Anthobaphes violacea*) during the incubation period (Williams 1993). Field studies are time-consuming and often difficult to implement, but they are necessary to confirm the responses of avian nectarivores to energy challenges under natural conditions.

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APPENDIX

The model of sucrose hydrolysis

The chemical reactor model of digestive capacity was developed by McWhorter and Martínez del Rio (2000) and was extended by Martínez del Rio et al. (2001). In this approach, it is assumed that the intestine of avian nectarivores functions as a plug-flow chemical reactor (Penry and Jumars 1987).

The assumptions of the model

- 1) Digesta flows unidirectionally (Jumars and Martínez del Rio 1999)
- 2) Rate of sucrose hydrolysis in the gut ($-r_s$) follows simple Michaelis-Menten kinetics

Therefore

$$-r_s = S_{\max} \cdot C_s \cdot (K_m + C_s)^{-1} \quad (1)$$

S_{\max}	Rate of hydrolysis along the gut [$\mu\text{mol} \cdot \text{min}^{-1} \cdot \mu\text{l}^{-1}$]
K_m	Michaelis constant of sucrase [$\mu\text{mol} \cdot \mu\text{l}^{-1}$]
C_s	Sucrose concentration [$\mu\text{mol} \cdot \mu\text{l}^{-1}$] down the intestine or over time (Jumars and Martínez del Rio 1999)

The integration of Equation (1) yields the throughput time (τ) that is required to reduce the initial sucrose concentration (C_{s0}) to a given final sucrose concentration (C_{sf}) which is based on the assimilation efficiency:

$$\tau = S_{\max}^{-1} \cdot [K_m \cdot \ln(C_{s0} \cdot C_{sf}^{-1}) + (C_{s0} - C_{sf})] \quad (2)$$

If the volume of gut contents (G) and the throughput time (τ) are known, intake rate (v_0) can be estimated for plug-flow reactors:

$$v_0 = G \cdot \tau^{-1} \quad (3)$$

v_0	Intake rate [$\mu\text{l} \cdot \text{min}^{-1}$]
G	Volume of gut contents [μl]
τ	Throughput time [min]

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Photographs of the experimental equipment



A. Köhler

Fig. 1. The experimental equipment used in Chapters 1–3. The cage was constructed from Perspex with one-way mirrors on two sides to minimize disturbance of the bird. An infrared photodetection system was mounted inside the cage on either side of the feeding aperture to record feeding events. The feeder was mounted on an electronic balance and the two perches were also connected to electronic balances. A tray with liquid paraffin was placed on the balance beneath the feeder to collect dripping fluid. The photodetectors and all balances were interfaced to a computer and feeding events, food intake and body mass of the bird were recorded continuously, using custom-designed software (L. Verburgt).



Fig. 2. The Perspex cages used for the fasting experiment in brown honeyeaters (Chapter 3). One side of each cage consisted of a one-way mirror allowing for observation of the bird with minimal disturbance. The only perch available in the cage was suspended from a balance which was placed on top of the cage. Body mass was recorded manually when the bird was resting on the perch. The feeder was attached outside the cage, allowing for weighing of the feeder without disturbance of the bird.

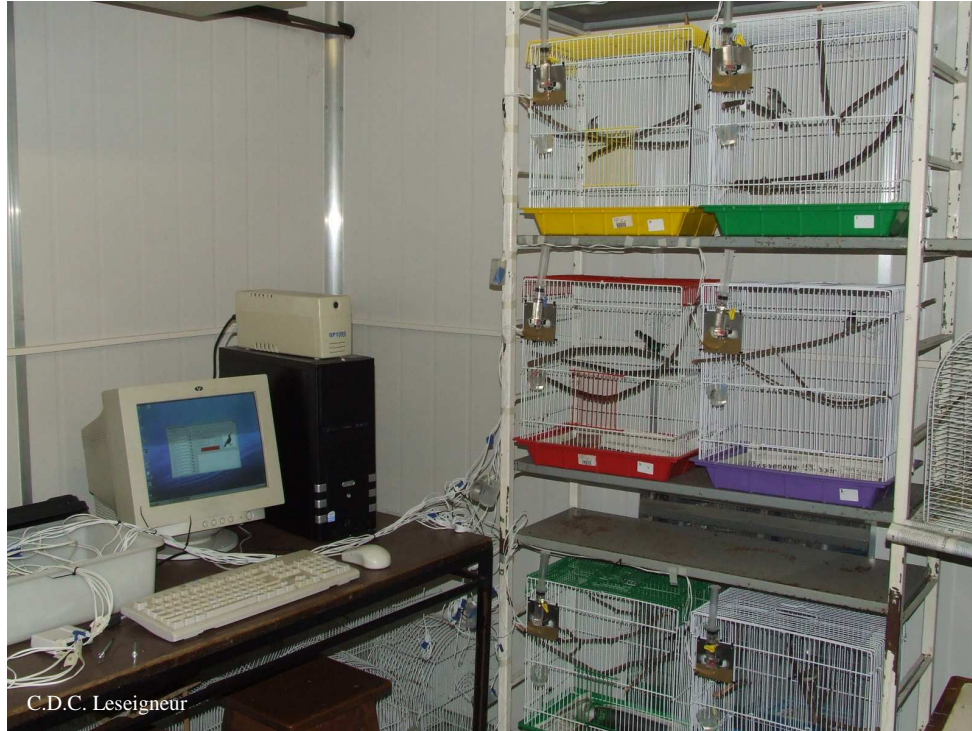


Fig. 3. The experimental equipment used in Chapter 4. Feeding behaviour of nine sunbirds was recorded simultaneously by photodetection systems interfaced to a computer. Custom-designed software (L. Verburgt) was used.

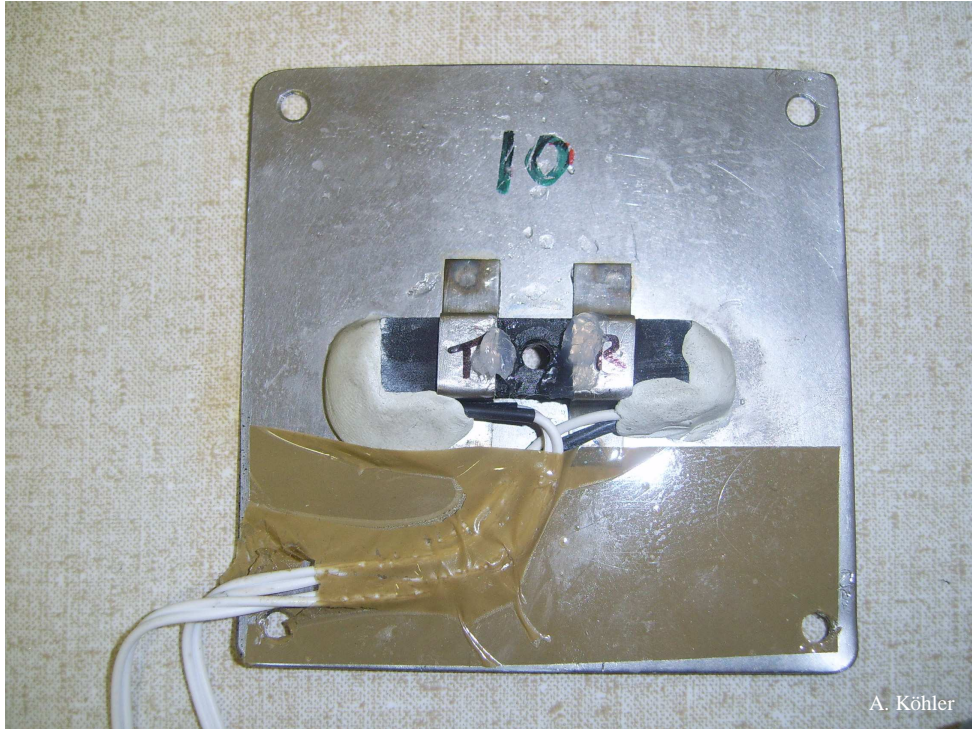


Fig. 4. The photodetection system used in Chapter 4. Transmitter (T) and receiver (R) were mounted on either side of the 3 mm feeding aperture, between a metal plate and the feeder (not shown). Photodetectors were surrounded by a plastic cover to shield them from light and protect them from contamination. The light beam was interrupted by the insertion of the bill into the hole for feeding, recorded as a feeding event.

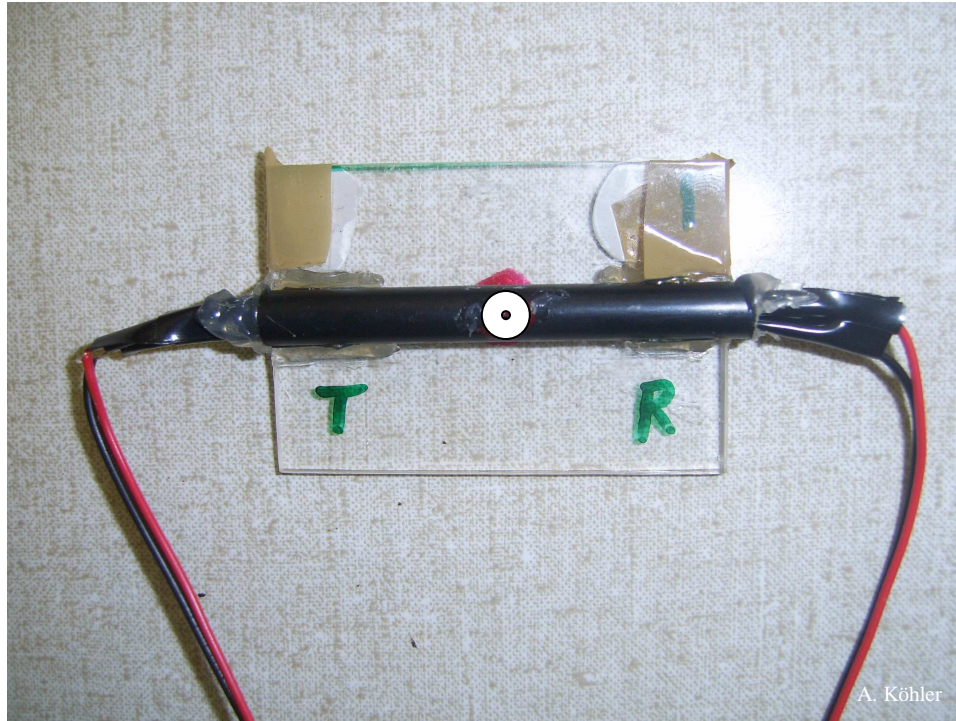


Fig. 5. The feeding aperture used to record licking behaviour of sunbirds (Chapter 6). A black pipe containing photodetectors was mounted between the feeder (not shown here) and a 3 mm thick Perspex plate. The birds extended their tongues through a 1.2 mm hole in the Perspex and the pipe, and this movement interrupted the light beam of the infrared light source. The photodetection system was interfaced to a computer, and tongue licks were recorded continuously.

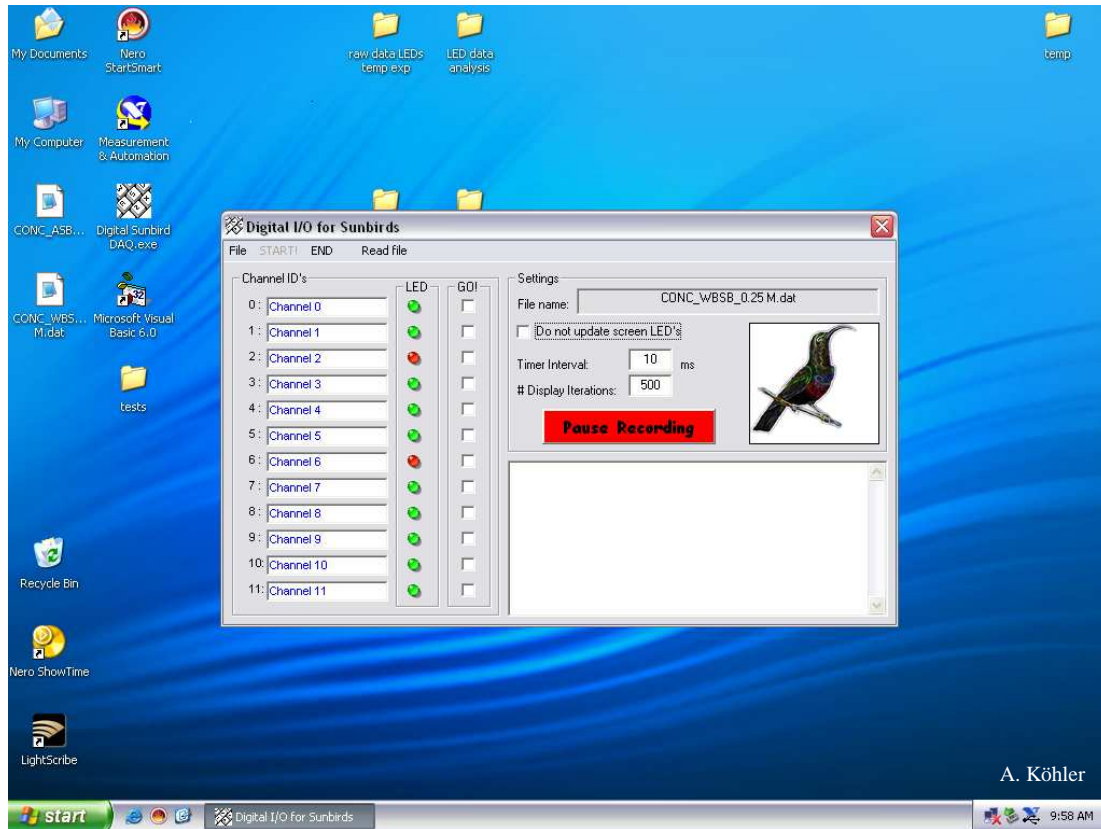


Fig. 6. Image of the custom-designed software developed by Luke Verburgt, which was used to record licking behaviour of up to 12 birds simultaneously (Chapter 6). Licking events of individual birds were indicated by colour changes next to the channel. The recording could be started separately for each bird. The time interval for recordings could be set manually; data were recorded every 1 ms. Recorded data could be displayed on the screen.