

METHODS & TECHNIQUES

A simple technique to manipulate foraging costs in seed-eating birds

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Accepted 6 January 2011

SUMMARY

Food availability is a key factor in ecology and evolution, but available techniques to manipulate the effort to acquire food in vertebrates are technically challenging and/or labour intensive. We present a simple technique to increase foraging costs in seed-eating birds that can be applied with little effort and at low monetary cost for prolonged periods (years) to solitary or group-housed animals. The essence of the technique is that food is offered in a container above ground level, with holes in the sides from which the food can be taken, forcing birds into energetically demanding hovering flight to forage. As a control treatment we offered a similar container but with perches mounted beneath the holes, allowing birds to eat without extra flights. Increasing foraging costs in this way induced zebra finches to double the time spent foraging, and to decrease their basal metabolic rate, in agreement with results obtained using more laborious techniques to increase foraging costs. The technique was not too severe because mortality was low during a winter with sub-zero temperatures. As foraging costs under natural conditions are generally higher than those under standard laboratory conditions, we suggest that measuring behaviour and physiology when animals have to work for food may better reflect their natural state.

Key words: net intake rate, energetics, time budget.

INTRODUCTION

Food availability is a key variable in behaviour and ecology, in that animal physiology, behaviour, survival and reproduction are usually modified by food availability. The effects of food availability have often been studied by limiting access to food through caloric restriction; caloric restriction also generally extends life span (Weindruch and Walford, 1988; Barger et al., 2003). Although such manipulations can be effective when one is interested in altering energy consumption *per se*, they differ from natural reductions in food availability in important ways. Firstly, for free-living animals, reduced food availability generally implies that more effort is required per unit of food that is obtained. Such an increase in foraging costs may result in a decrease, no change or an increase in the total amount of energy consumed (reviewed by Wiersma and Verhulst, 2005) and it probably always results in reallocation of the energy budget (Wiersma et al., 2005; Schubert et al., 2009). This differs markedly from caloric restriction, where the total amount of energy consumed is always reduced, and there is no profitable opportunity to allocate extra energy to foraging. Secondly, the pattern of food intake over the day is also likely to differ markedly between free-living animals facing low food availability, which may forage throughout the day, and captive animals subjected to caloric restriction, which usually receive one meal per day. Thus, techniques to manipulate food availability through a change in the foraging costs per reward are likely to provide a setting that is more informative with respect to the effects of variation in food availability in free-living animals.

Several techniques have been developed to manipulate the foraging costs per reward. Rodents can be trained to run for food in running wheels, and the experimenter can set the number of revolutions required per reward (e.g. Perrigo and Bronson, 1983; Schubert et al., 2009). Similarly, birds can be trained to fly between

perches for their food, and the number of return flights required per reward can be set at different levels (e.g. Deerenberg et al., 1998; Wiersma et al., 2005). These techniques can be effective, but require a large investment of time and money to develop and operate. Another disadvantage is that, at least with current systems, the animals need to be housed individually. Application of this technique is therefore restricted to limited numbers of animals for limited time periods. Diluting food in a medium has also been applied to increase the foraging costs per reward. For seed-eating birds, seed and chaff can be mixed in different ratios, where a higher proportion of chaff decreases intake rate (Lemon, 1993; Wiersma and Verhulst, 2005). However, recovering the chaff, and keeping the chaff:seed ratio within acceptable limits, is a major effort, as birds continuously increase this chaff:seed ratio by eating seeds and dropping chaff in the food trays. Furthermore, the foraging effort induced by this method is modest when expressed in terms of energy, as it involves relatively little locomotion. Advantages of this technique are that it is easy to manipulate, by changing the chaff:seed ratio, and that it can be applied to groups of birds. Nectarivorous species can be trained to forage in flight from artificial flowers containing sugar of different dilutions (e.g. Markman et al., 2002), which is an elegant technique but such species are not common laboratory animals. Because all these techniques are either very laborious or can be applied to a very limited species range, we suggest that there is a need for reliable techniques to increase the foraging costs per reward that can be applied at low cost for prolonged periods.

Here, we introduce a technique to manipulate the foraging costs per reward in seed-eating birds that can be applied for prolonged periods with little effort. In brief, food (seeds) is offered in a container suspended from the ceiling, and birds are forced to fly and hover to collect the seeds. As finches remove the chaff before consuming a seed, and they do this only when perched, they fly

back and forth from a distant perch to the food container for each seed that is consumed. Because flight is energetically relatively expensive (Masman and Klaassen, 1987; Hambly et al., 2004), this technique substantially increases the foraging costs per food item obtained. The situation for control (low foraging cost) birds is identical except for the presence of a perch beneath each foraging hole. We present data using zebra finches, *Taeniopygia guttata*, to illustrate that on the one hand the birds clearly adjust their behaviour and physiology to the increased foraging costs, while on the other hand the birds acquire sufficient food to survive even prolonged periods with sub-zero temperatures, and hence this technique manipulates foraging costs within a scientifically interesting range.

MATERIALS AND METHODS

Manipulating foraging costs

Food was offered in transparent Plexiglas containers (L×W×H: 120×10×60 cm) suspended from the roof of the aviary, with holes low on the sides to allow access to seeds (Fig. 1). The holes were fitted with horizontal PVC tubes (outer tubes in Fig. 1), with an i.d. of 2.7 cm and an o.d. of 3.2 cm. The tubes have a 120 deg opening on the bottom, so that food flows to the inside but not out of the tubes. By rotating the outer tubes, the level of food in the tube can be controlled, and this affects how easily the birds can collect the seeds. Turning the opening too far upward increases the 'spilling rate', increasing the frequency of container refills needed. Turning the opening straight down results in low food levels in the tube, so the birds are unable to reach the seeds. The outer tubes were equipped with small inner tubes (o.d. 1.6 cm) to prevent birds from

fixing their heads in the outer tubes to collect seeds without having to fly. These inner tubes were 2.8 cm shorter than the outer tubes in which they were fitted, to leave the birds 1.4 cm on each side for foraging. For the low foraging cost treatment, we fitted PVC perches beneath the holes (diameter 1.0 cm), on which the birds could sit while foraging; the presence of these perches is the only difference between the two treatments. The absence of perches in the high foraging cost treatment forces the bird to fly to the foraging hole and hover briefly to collect a seed, after which it returns to a perch 80 cm from the food container to consume the seed. The mean foraging trip duration (from perch to food container and back) of experienced birds was 1.7 s (s.d.=0.44, N=45 successful foraging attempts). The foraging costs can be modified up or down through alteration of the distance between food container and perch, and the vertical distance can also be modified (perches and holes were at a similar height in our set-up).

To train birds to collect their food in the high foraging cost condition, we gradually shortened the perches underneath the feeding holes. The perches extended 5 cm in the low foraging cost condition; for 60% of the foraging holes we shortened the perches to 3.5 cm at the start of training and subsequently shortened the perches 0.5 cm each day. The other 40% of the perches were removed entirely at the start of training, because at some intermediate perch length some birds do not succeed in obtaining food and these birds can then forage from the tubes without a perch instead. As the perches became shorter, the birds gradually lost their grip, and were forced to forage half sitting and half flying, gradually shifting to flying. Eventually all perches were removed, forcing all birds to

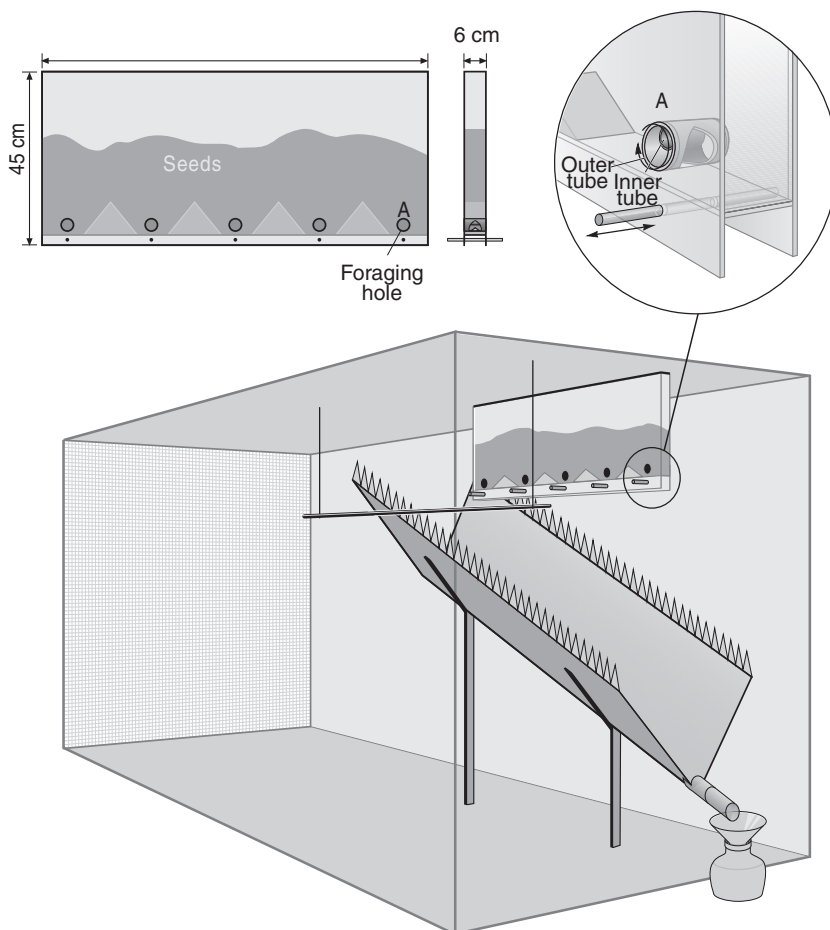


Fig. 1. Experimental setup. Bottom: overview of the aviary with the food container suspended from the roof, with the system to catch any dropped seeds below. Top left: seed container viewed from the side. Top right: detail of tube A from which food is obtained and the perch underneath, which was removed in the high foraging cost treatment.

fly and hover to obtain food. To ensure animal welfare, we housed birds in groups of 10 during the training phase, which is a small enough group size that birds can be monitored individually, using unique combinations of colour rings to identify individuals. In our experience so far, >95% of all birds we tested ($n>300$) learned to forage in the high foraging cost condition within a week.

Any seeds that are spilled can potentially be eaten on the aviary floor, and to prevent this we fitted a wooden trough under the food container to catch spilled seeds. The seeds collect in a tube mounted under the trough, with a 1.5 cm slit along its length, which was wide enough for the seeds but too narrow for the birds (Fig. 1). The tube was fitted at an angle, exiting through the aviary wall and hence gravity moved the seeds out of the aviary. A plastic sheet with a serrated edge was mounted on the top edge of the trough to prevent the birds using it as a perch.

We offered a mixture of tropical seeds in the food containers; thus, it was possible that the seed selection the birds made could differ between treatments. We therefore separated the dropped seeds from the chaff using a ventilator and recycled the seeds to the aviaries, to ensure that the foraging cost manipulation did not affect diet (more detailed information available from the authors on request).

Housing

The experiment was carried out in outdoor aviaries (L×H×W: 310×210×150 cm) covered with opaque roofing and with wire mesh on the front side, and visually but not acoustically isolated from neighbouring aviaries by wooden partitions. There were no artificial lights and hence birds were exposed to the local day–night cycle. We used 6–15 month old Zebra finches (*T. guttata*, Vieillot 1817) hatched in our laboratory and housed in eight single-sex groups (four of each sex) of 32–37 birds. Drinking water, tropical seed mixture, grit and cuttlebone were provided *ad libitum*, of which the tropical seed was offered as described in the previous section (four aviaries with low foraging costs and four aviaries with high foraging costs, divided equally between the sexes). To ensure a balanced diet, 1.25 g bird⁻¹ week⁻¹ of fortified canary food (Bogena, Hedel, The Netherlands) was supplied, spread over three portions per week. To prevent monopolization of the food by individual zebra finches this food was distributed over a 1 m long shelf.

Experiments were carried out under licence 5150 of the Animal Experimentation committee of the University of Groningen.

Behavioural observations

Behaviour during treatment was scored for individual birds for a period of 10 min. Observations were carried out from 16 September to 12 December 2008 either between 09:00 and 12:30 h or between 14:00 and 16:30 h. The time and date of observations were balanced between treatments. Birds could be individually recognized using a combination of colour rings and spots on the breast feathers made with a black marker (the spots were visible for up to 2 weeks). The number of foraging flights and flights for other purposes (drinking, social behaviour) were scored using a database program (MS Access) that automatically stored the exact time of all data entries. Time spent foraging was calculated as the sum of time spent handling seeds plus time spent flying to and from the food container. Other behaviours were scored as well, but will be reported elsewhere as part of a larger study.

Energy expenditure

Overnight energy expenditure was measured in 39 females before and during treatment using an open flow respirometer situated in a dark climatized chamber. We did not measure male basal metabolic

rate (BMR). Individual birds were put in 21 boxes between 1.5 and 0 h before sunset, and measurements ended between 08:00 and 09:00 h the next morning. Eight birds were measured simultaneously, with a reference measurement after every four bird measurements, yielding one measurement per 10 min. The temperature was set at 36°C, within the thermoneutral zone for zebra finches (Calder, 1964). BMR (in W) was calculated using both oxygen consumption and carbon dioxide production, and the lowest 60 min running mean was taken to be the BMR. For technical details, see Verhulst et al. (Verhulst et al., 2005), where the same procedure was followed using the same equipment.

Analysis

Data were analysed using JMP 7.0. Aviary was entered as random effect in the models but explained a non-significant part of the variance in all cases and was therefore omitted from the models we report. The proportion of time spent foraging was arcsine square-root transformed prior to analysis.

RESULTS

Time spent foraging

In total 136 observations of 10 min duration were carried out, almost equally distributed over aviaries and treatments (48% low foraging cost treatment; 50% females, equally balanced across treatments). Birds on the high foraging cost treatment spent 18.2±2.6% (mean ± s.e.m.) of their time foraging, more than twice as much as control birds (8.5±2.6%) (Fig. 2; $F_{1,134}=8.9$, $P=0.003$). When comparing total flight activity, including foraging flights and flights for other purposes, we found that birds on the high foraging cost treatment performed significantly more flights per 10 min than control birds (11.3±1.8 vs 5.7±1.9; $F_{1,134}=5.4$, $P=0.02$, tested using ln-transformed values).

BMR

We measured BMR in 39 females before the high foraging cost treatment started, and 425–471 days after the start of treatment on 9 December 2007. Pre-treatment BMR did not differ between birds that were assigned to the two treatment groups ($F_{1,37}=0.20$, $P=0.7$). In a multiple regression model, BMR during treatment was positively

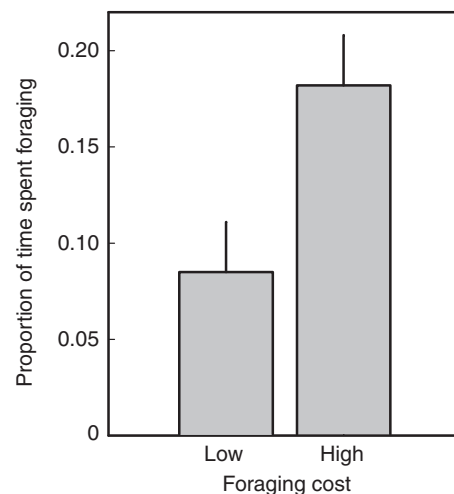


Fig. 2. The high foraging cost treatment significantly increased the proportion of time that birds spent foraging. Data shown are means (+s.e.) of 10 min observations on 136 birds evenly distributed over sexes and treatments.

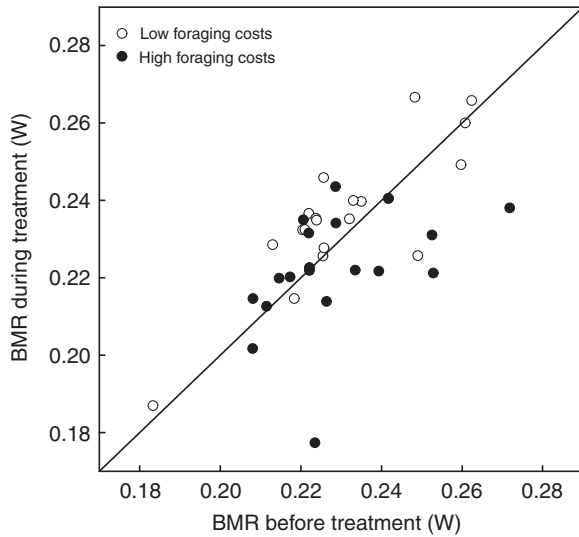


Fig. 3. Foraging costs and basal metabolic rate (BMR) of female zebra finches. Data show BMR of birds on the low and high foraging cost treatment, plotted against their BMR before the treatment started. Pre-treatment BMR and BMR during treatment were significantly correlated, but females exposed to higher foraging costs had a significantly lower BMR.

correlated with pre-treatment BMR despite the long period between measurements (Fig. 3; $F_{1,35}=26.0$, $P<0.0001$), and the mass difference between the first and the second measurement ($F_{1,35}=19.7$, $P<0.0001$). Controlling for these factors, birds on the high foraging cost treatment had a significantly lower BMR than controls ($F_{1,35}=11.8$, $P<0.002$).

Winter survival

Temperatures during winter regularly dropped below zero (Fig. 4), but survival of low and high foraging cost groups during the first winter (9 December 2007 to 10 April 2008) was nevertheless high and did not differ between treatments (Fig. 4; low foraging cost, 8/129 died; high foraging cost, 7/126 died: $\chi^2=0.048$, d.f.=1, $P=0.8$).

DISCUSSION

Food availability is a key variable in animal ecology and evolution. Lower food availability in the wild usually results in an increase in foraging costs per unit of food obtained. We have developed a new

technique to manipulate foraging costs in captive seed-eating birds. Two technical advantages of the technique we introduce are that (i) the costs in terms of time and money are low, and (ii) the reliability of the method is high, because of the lack of mechanical or electric components. With these advantages, it is feasible to apply our technique to large numbers of birds over prolonged periods. Our data show that the increase in foraging costs results in a doubling in foraging effort and a reduction in BMR. This is encouraging, because similar results were obtained using more labour intensive techniques to increase foraging costs in zebra finches and other species of birds and mammals (for a review, see Wiersma and Verhulst, 2005). Moreover, the birds were able to gather sufficient energy to survive even a cold winter, and hence foraging costs were manipulated within a suitable range. Ethical considerations are of growing importance when it comes to the licensing of experiments. It is worth mentioning, therefore, that the level of suffering resulting from this treatment was estimated to be minor by the Dutch legal inspectors of animal experiments. The fact that there was no significant difference in winter survival (Fig. 4) supports this view.

We studied only two levels of foraging costs but changing the distance between perches and food containers would allow further modification. It remains to be investigated to what extent our technique can be applied to species other than seed-eating birds, but in principle other kinds of food can be offered in similar food containers, as long as the food can be packed in small discrete units comparable to the seeds that we used. Hence, there is the potential that our technique can be widely applied, but this requires further study.

Captive animals used in physiological and/or behavioural studies can usually acquire their food at negligible energy cost, which contrasts markedly with the conditions that most free-living animals find themselves in. It can therefore be argued that animals that have to work for their food provide more relevant data for understanding variation in animal physiology and behaviour under natural conditions. Furthermore, long-term effects of experimental interventions may be more conspicuous in a more natural environment where animals are slightly challenged. Such interactions have repeatedly been reported in studies using *Drosophila* (e.g. Kraaijeveld and Godfray, 1997), and recently also in seed-eating birds using short-term food deprivation (Krause et al., 2009). The technique we introduce provides the means by which to explore such interactions in seed-eating birds.

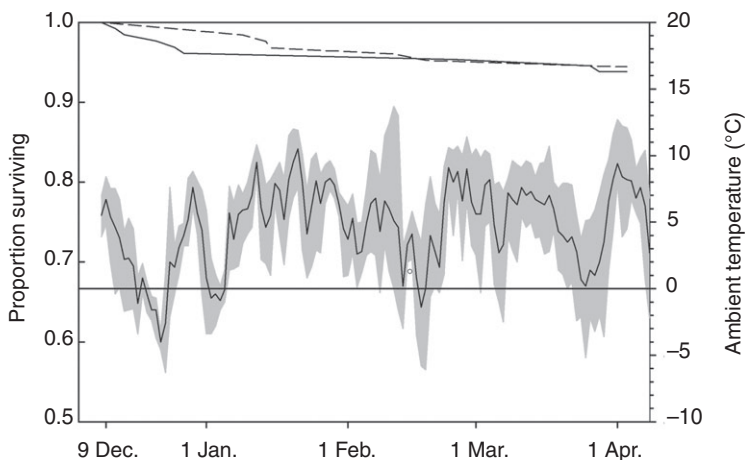


Fig. 4. Survival of birds with low and high foraging costs during winter (dashed and continuous lines, respectively, at the top of the graph; $n=126$ –129 birds per group, sexes pooled). Survival was high, independent of treatment. Lower line shows mean daily ambient temperature with the temperature range indicated by the shaded area.

ACKNOWLEDGEMENTS

Maartje Kouwen, Femke Tamminga and Martijn Salomons assisted in building and developing the design. Dick Visser prepared Fig. 1. Detailed comments of a reviewer improved the manuscript. This work was supported by an NWO Vici-grant to S.V.

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