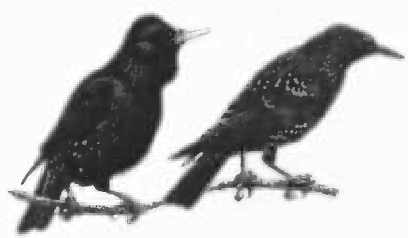
Effects of workload on the energy budget of the European starling
(Sturnus vulgaris)

Zoological Laboratory of the University of Groningen

Martijn Salomons, August 2001



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Introduction

Under natural conditions animals have to work to gain food and (in altricial animals) feed nestlings. Time and energy is used for foraging to provide the daily amount of energy needed to survive. Not only will the energy demand vary between different periods in the life of an animal, for example during the reproductive phase or during migration, the amount of energy and time needed to find a certain amount of food will also be highly variable within and between individuals.

Individuals faced with a change in food availability may use different compensatory strategies. If an animal for example is faced with a reduction in food supply it may show an increase in foraging activity to maintain the same intake rate, or a decrease in overall activity (including foraging activity) to reduce energy demand (Houston & McNamara 1993). Also changes in foraging method may occur, as shown in starlings (Kacelnik 1984) and other animals (Caldow & Furness 1991; Bautista et al. 2001). Next to these behavioural changes animals can make certain physiological adaptations. A reduction of body mass for example can result in lower foraging costs through its effect on travel costs (Freed 1981; Norberg 1990). Also an animal can increase digestion efficiency, or control daily energy expenditure by changing variables such as overnight metabolism (Ricklefs et al. 1996; Deerenberg et al. 1998), energy allocated to the immune system (Sheldon & Verhulst 1996; Norris & Evans 2000) or maintenance processes, such as growth, and behaviour, for example preening.

In this experiment starlings (*Sturnus vulgaris)* were made to work in a laboratory set-up (by flying between perches in an aviary) to obtain their daily supply of food, thus creating a model for food acquisition in the wild. Food distribution was based on a possibility factor, creating a situation in which the average amount of foraging effort needed to obtain a certain amount of food is constant, but the effort is variable for separate food items. This is in contrast to earlier studies by Bautista et al. (1998b), using a similar set-up, where the amount of flights per food item was fixed.

As the amount of work needed to obtain a certain amount of food is also variable in free-living animals we expected this schedule to be better than a fixed-ratio schedule. Moreover, because of the finding by Fotheringham (1998) that starlings were more willing to work for food, especially at a high workload, in variable-ratio schedules, such a schedule was preferred over a fixed-ratio schedule.

By sequentially assigning the starlings to three different levels of workload, adaptations to changes in workload, or foraging success, could be closely investigated. The goal of this study was to see how the daily energy budget is affected by workload and how body condition changes, and to find out how possible adaptations in the energy budget are achieved. Furthermore by answering these questions a contribution can be made to a better understanding of the role of parental effort in the overall study of costs of reproduction.

To answer these questions we focussed on the effect of changes in food availability on:

1. Daily activity
2. Body condition
3. Intake rate and digestion efficiency
4. Energy expenditure during non-foraging periods
5. Immune function

Daily activity

The daily routine of each starling was closely monitored to see what adaptations in the level of activity, if any, were made following a change in food availability. Do starlings increase their foraging activity, and thus increase the daily amount of energy allocated to foraging, in order to maintain the same level of net energy income, or perhaps keep foraging activity at the same level but decrease overall activity to keep energy demand as low as possible?

Also the timing of activity is of interest. In the wild, starlings show a bimodal foraging routine with activity peaking at the beginning and the end of the day (Aschoff 1966). A study by Bautista et al (1998a) (using a fixed-ratio schedule) however failed to show this phenomenon in starlings working for food in a laboratory set-up.

Body mass

Body mass was continuously recorded to find out if it was different between the three treatments. Judging by the result of other studies (Freed 1981; Jones 1994) it is most likely that a body mass change is not simply the result of homeostatic mechanisms, but that it can be seen as one of the strategies of an individual to cope with changes in its environment. An increase in body mass (through fat deposition) acts as an insurance against (unpredictable) negative changes in the environment such as low temperatures and low food availability (Meijer et al. 1994). However a high body mass also has negative effects as it causes an increase in maintenance costs, and, as mentioned earlier, travel costs (Daan et al. 1990; Deerenberg et al. 1998). Finally, being heavy means less manoeuvrability and therefore an increased predation risk (Gosler et al. 1995).

Intake rate and digestion efficiency

Since intake rate in this set-up is closely related to foraging activity the same question of increase or decrease in activity also holds for intake rate. A difference in digestion efficiency between treatments is also to be expected. Because starlings with high workload face low food availability, they might increase digestion efficiency to obtain more energy from a specific amount of food. However, the opposite effect of treatment can also be expected since there may be a bottleneck in the amount of time available for digestion (Kersten & Visser 1996), or the composition of the digestive tract may change to lower body mass and maintenance costs (Kersten & Piersma 1987).

Energy expenditure during non-foraging periods

An increase in daily energy expenditure has to be compensated in some way. One way is to increase energy intake, but since this leads to an even higher energy-expenditure it might be more favourable to decrease energy expenditure where possible. This can be achieved by allocating less energy to behavioural elements like singing and preening but also to processes concerning (feather-)growth, thermoregulation and maintenance (Daan et al. 1990). Compensation during non-foraging periods is to be expected since earlier studies do show a decrease in basal metabolic rates and mass specific BMR as a result of increased workload (Ricklefs et al. 1996; Deerenberg et al. 1998; Bautista et al. 1998b) and resting metabolic rates (Nudds & Bryant 2001).

Immune function

Recent studies have emphasised that the immune system should also be taken into account when investigating costs of (parental) effort. They suggest a trade-off between energy or other resources allocated to the immune system on the one hand and other physiological functions on the other (Sheldon & Verhulst 1996; Norris & Evans 2000; Lochmiller & Deerenberg 2000; Ilmonen et al. 2000).

To see whether differences in workload between treatments in our study affect the immune function, the starlings were challenged with an injection with phytohaemagglutinin (PHA). PHA is a plant lectin injected intradermally into the wing web (the patagium). PHA has a strong mitogenic effect on T-lymphocytes. After injection it causes a swelling of the wing web through a local inflammatory response and lymphocyte-induced accumulation of phagocytic cells at the site of the injection. The magnitude of this swelling is routinely used as an index of the efficiency of cell-mediated immunity in poultry but it is used in an ecological context as well (Smits et al. 1999; Johnsen & Zuk 1999; Hõrak et al. 2000). For example in red jungle fowl (*Gallus gallus*) females parasitized with an intestinal nematode showed less swelling of the wing web after PHA-injection compared to females that were not parasitized (Johnsen & Zuk 1999). Also Hõrak et al. (2000) found a lower cutaneous response to PHA inoculation in great tit (*Parus major*) chicks that grew poorly compared to well growing chicks.

Total white blood cell count is used as a second measure of the immune system. Because birds tend to respond to an infection, with pathogenic agents, with an increase in the number of white blood cells, it offers general information about infection status and can thus function as a screening test (Gustafsson et al. 1994).

Materials and methods

Subjects and apparatus

Eight wild-caught male starlings were individually housed in flight cages in the Zoological Laboratory of the University of Groningen. These birds had been used previously in the same flight cages in a similar experiment about two years ago and were, therefore, expected to be familiar with the experimental set-up. Nevertheless, all birds were in the cages 5 weeks before the actual experiment began to get accustomed to the cages and their feeding scheme. Throughout the entire experiment the starlings were visually isolated, but were able to hear each other. Room temperature was controlled and was on average 20°C.

The flight cages were 5.45 meters in length, 68 centimetres in width and 80 centimetres in height (Figure 1). In each cage there were three perches, one small perch the starling had to use to reach to the feeder ('feeding perch') and two larger perches. One perch was placed 45 cm from the end of the flight cage and the second was placed at the other end. The distance between the two foraging perches was exactly 5 meters.

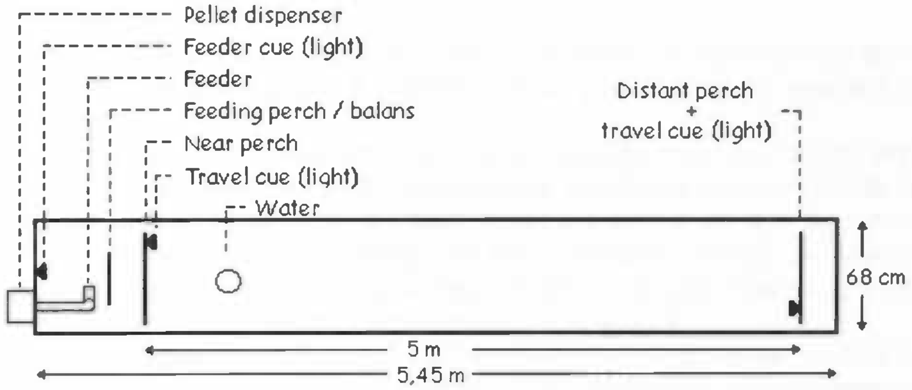


Figure 1 Schematic representation of a flight cage viewed from above. Starlings had to fly back and forth between the two feeding perches a number of times in order to receive a reward. This reward, a food pellet, was automatically distributed by a pellet dispenser.

The two perches were equipped with microswitches that registered starlings landing on them. The data from these switches was send to a Programmable Logic Controller (PLC) that was connected to a computer to store the data. Depending on the number of flights, a signal was given by the PLC to activate the pellet dispenser. The feeding perch was functioning as a balance and each time the starling would sit on it, its weight was measured (at a 0.5-second interval) and stored on a second computer. As an extra stimulus to guide the starling through the 'flight-reward schedule', a small red light would switch on near the perch the starling had to go to next. In addition a small green light near the food tray would switch on every time a food pellet was given.

The food used in this experiment consisted of trout-pellets (0.02 g) (see Table 1 for specifications). These pellets were distributed automatically by a pellet dispenser. This pellet dispenser was controlled by a PLC that received data from the two perches, and was activated, with a certain probability, when the starling landed on the perch closest to the feeding perch.

Fresh water was always available in a drinking bottle at the bottom of the cage and two to three times per week two mealworms were offered as an additional source of nutrients. Bathing water was also offered regularly.

The daylight period lasted 14 hours and the night 10 hours. At the beginning and end of the daylight period, the lighting in the flight cages was automatically turned off, except for a small light bulb that remained on for five more minutes to create a smooth transition from day to night and vice versa.

Table 1 Specifications of the food pellets used in this experiment

Name:	Trouvit Europa paling
Ingredients:	Fish-meal, Fish-oil, Wheat, Blood-meal, Vitamins, Minerals
Composition:	Rough albumen 44,0 % Rough ashes 9,4 % Rough fat 30,0 % NFE 10,8 % Rough cellulose 0,6 %
Gross energy: Digestible energy:	23,9 MJ/Kg (5,710 Kcal/Kg) 21,3 MJ/Kg
Additions:	Vitamin A 23.000 IE/Kg Vitamin D_3 2.250 IE/Kg Vitamin E 225 mg/Kg Antioxidant
Manufacturer:	Trouw France s.a.

Experimental design

By using a chance factor in the distribution of the food pellets, a flight-reward schedule could be designed in which the starling got a reward (1 food pellet) after an average instead of a fixed number of flights.

Two experimental groups of four starlings each were formed and assigned to a LOW workload, in which the starlings had to fly, on average, two times back and forth between the two perches to get a food reward, or a HIGH workload where an average number of 5.5 return flights was needed. In the middle of the transition period between these two treatments, all birds had to fly on average 4 times to get a reward. This treatment was called TRANSITION treatment and lasted for approximately two weeks.

To be able to investigate the effects of workload within an individual, half of the starlings were first assigned to the easy treatment and then their workload was increased before the second experimental period. The other half received the opposite treatment starting with a high workload.

The experiment lasted for four months (December until April). During the entire experiment daily flight activity, intake rate and change in body mass was recorded. For practical reasons the start of the light period for the starlings was at midnight.

When starlings were first faced with a particular treatment, or their treatment was changed, they first needed some time to get accustomed to it. Therefore each treatment lasted for several weeks until a more or less 'steady-state' was reached in which body mass was stable (Figure 2).

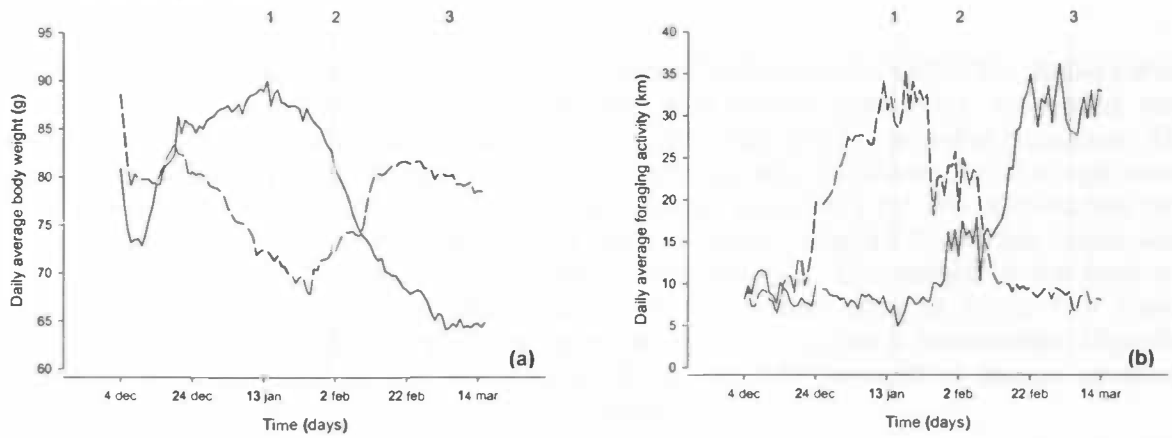


Figure 2 These figures show the timing of the energy budget measurements (1, 2 and 3) in relation to the average body mass change (a) and the daily foraging activity (b) during the experimental period for birds first assigned to a low workload (solid line) or a high workload (dashed line).

Body parameters

As mentioned above body mass (± 0.1 g) was measured the entire time that the starlings were in the flight cages. These measurements were checked on a regular basis by weighing the starlings on a scale and by checking the balances used in the flight cages with known weights. Other body parameters measured were tarsus length, wing length and the thickness of the pectoral muscle. Tarsus and wing length were measured at the beginning and the end of the experiment. The pectoral muscle was measured every time the starling was caught during the experiment. Tarsus length was measured as the length between the notch behind the intertarsal joint and the bend of the foot (± 0.1 mm) (Svensson 1976). Wing length was determined by measuring the flattened maximum cord (± 0.5 mm) (Svensson 1976). The thickness of the pectoral muscle was determined using a 'muscle-meter' (Figure 3). It is used by simply placing the device on the breast of a bird, with the cavity in the front plate directly above the crest of the sternum, and gently lowering the frontplate until it rests on the skin. The difference between the top of the back plate and the front plate equals the distance between sternum crest and muscle surface, and can be measured with callipers (± 0.1 mm). The leaner the pectoral muscle, the bigger this distance is.

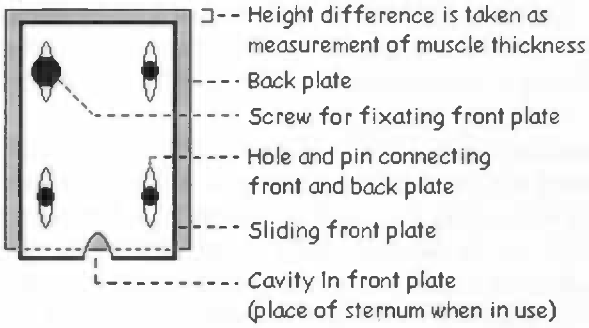


Figure 3 Schematic representation of a muscle-meter. It is used by placing the device on the breast and lowering the frontplate. The difference between the top of the back plate and the front plate is used as measurement of the thickness of the pectoral muscle. A high value means a lean muscle.

Blood parameters

All birds were bled once before, during and after each treatment to see whether workload had an effect on the haematological health state of the starlings and for future analysis and comparison of plasma levels of glucose, urea, uric acid and/or cholesterol. Each time a sample of about 0.2 ml was taken from the vena brachialis, using capillaries of 70 µl. Then each blood sample was centrifuged at 12000 rpm for 10 minutes. After measuring the total plasma volume and the percentage of red (haematocrit) and white (buffy coat) blood cells, the plasma was collected and stored at -20°C. Blood smears were also made, for future microscopical analysis of the blood.

Energy budget measurements

All measurements regarding the energy budget lasted for two weeks within the stable period. To get an accurate measurement of intake rate and faeces production, the cages were cleaned thoroughly and a known amount of food (60 g) was put in the pellet dispenser. This was done at the end of the daylight period, as we did not want to disturb the starlings during the active phase. Daily intake rate was then determined accurately for two consecutive days by weighing the food in the system after 24 and 48 hours. After 48 hours the cages were cleaned again and all the faeces was collected and weighed. Dry weight of the food and faeces were determined after storage in an oven for several days at 70°C. The energy contents of the food and faeces samples were determined in a bomb calorimeter. Digestion efficiency was calculated as the energy content of the total amount of faeces produced divided by the total energy intake. Using the formula:

$$\frac{E_{tot} - E_{faec}}{E_{tot}} \times 100\%$$

Basal metabolic rate (BMR) was measured in each bird in each treatment. To do so the birds were placed in darkness in a respirometer. This was done for all the birds at the same time and during the night. BMR was calculated as the minimum value of a 30-min running mean of oxygen consumption. The energy equivalent of oxygen consumption was assumed to be 19.9 kJ/L O_2. We calculated mass specific BMR using the average of measurements of body mass of the individual starlings before and after they had been in the respirometer.

A theoretical value of energy expenditure during flight was taken from a study by Hambly et al. (in prep.) in the same experimental set-up at the Zoological Laboratory of the University of Groningen, and was 20.5W.

Estimates of maintenance energy demand in starlings were calculated from the data of another study on starlings at the Zoological Laboratory (unpublished data) and were 1.95*BMR. The same study also provided an estimate of the energetic equivalent of body mass change in starlings, and was 18.0 kJ/g. This value corresponded with the value of 19.2 kJ/g found by Masman in the Kestrel (*Falco tinnunculus)* (Masman et al. 1986).

Immune function

Responsiveness of the cell-mediated immune system was tested twice in each individual starling. During both the easy and the hard treatment, 0.2 mg of PHA in 0.04 ml of phosphate buffered saline (PBS) was injected intradermally into the wing web of one of the wings. At the same time the other wing was injected with 0.04 ml of PBS only, to control for a possible swelling caused by the injection itself.

This method is routinely used in many avian studies (Smits et al. 1999). We decided to use PHA in our experiment because we wanted to test the response of the immune system two times in the same individual. We decided the PHA-test would be the best option because until now there is no evidence found of long term effects of PHA (Hõrak et al. 2000). Nevertheless each wing was injected with PHA only once during the experiment in order to further minimise the chance of interference of the second test by the first. Swelling of the wing web was measured right before and 24, 48, 96 and 216 hours after injection, using a spessimeter (Mitutoyo, No. 2046F-60) with an accuracy of 0.01 mm. Each time four measurements were made and the average of these measurements was used in the calculation of the immune response to the injection with PHA. The PHA response was measured by subtracting the average amount of swelling in the wing web injected with PBS from the average amount of swelling in the wing web injected with PHA.

Statistics

The effect of workload on the different parameters was tested using a 'repeated measures, linear model', in this model both within and between subjects characteristics are used. To control for possible effects of the order of treatment, order was put in the model as a 'between subjects factor'. Analysis of differences between the two experimental groups was done using an 'independent samples t-test'. For analyses the statistical program SPSS (version 10.0) and Mlwin (version 1.1) were used. Graphs were made in Sigmaplot (version 2000).

Results

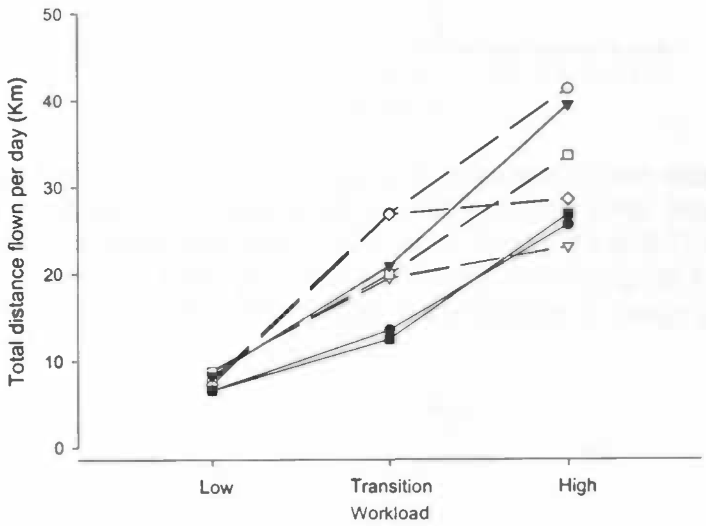
In the first week of the experiment two of the eight starlings that we used showed undesirable behaviour. One was flying continuously but refused to eat the pellets and the second showed no activity at all. These birds were taken out of the flight cages and replaced by other starlings. These as well as the other six starlings quickly got used to the set-up and their task, as became clear from individual observations.

Unfortunately during the experiment one of the starlings became ill, and in the end died. We think the data provided by this individual starling was unreliable and therefore decided not to use it in our analysis. As a result the group that was first assigned to the easy treatment ultimately consisted of only three starlings.

The effect of treatment on activity

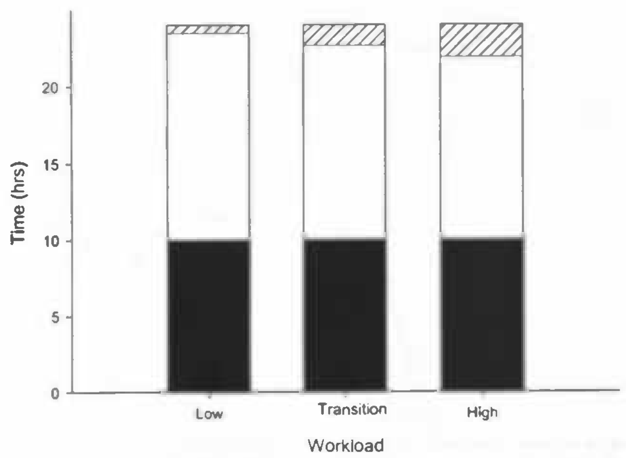
When starlings were faced with a higher workload, they increased their foraging activity. The starlings flew, on average, 7.7 km per day at low workload, 20.0 km per day at transition workload and 31.2 km per day at high workload (Figure 4).

Figure 4 The relation between workload and foraging activity, shown as the total distance flown per day. (Closed symbols connected by solid lines represent individual starlings first assigned to low workload.) Differences are significant between all workloads (low/transition $p<.005$, transition/high $p<.001$ and low/high $p<.001$)



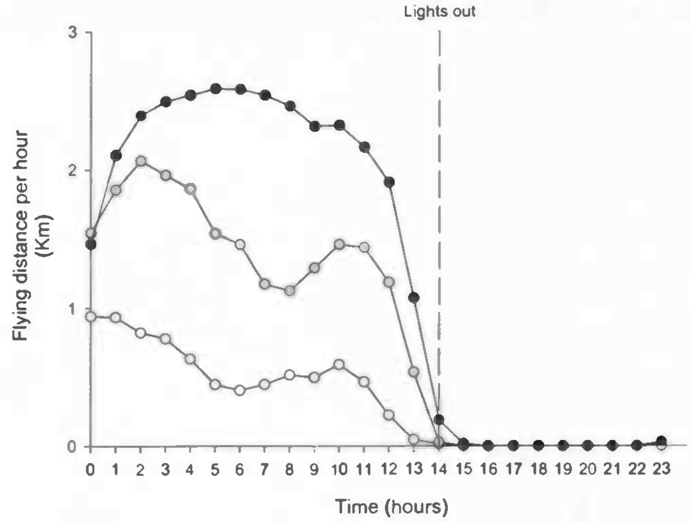
Larger flight distances implicate that a starling needs to allocate more of its time to foraging. Therefore the daily activity of starlings also differed between the different workloads as is shown in figure 5. Although total foraging time was increased, time spent foraging during high workload was still only a small fraction of the total daylight period (3.7%, 9.6% and 15.0% for low, transition and high workload resp.).

Figure 5 Daily schedule of starlings at three different workloads. Dark areas represent nighttime, white areas total nonforaging time during the day and dashed areas total foraging time.



Also there was a change in the timing of activity as is shown in figure 6. At low and transition workload the starlings showed peaks in activity in the morning and evening, but at high workload activity was lowest in the first two hours and remained more or less constant higher activity level during the rest of the day.

Figure 6 Workload and distribution of foraging activity in the distance flown per hour. For low workload (white dots), Transition workload (grey dots) and high workload (black dots).



This becomes even more clear when hourly activity is expressed as a fraction of the total daily activity (Figure 7). At transition workload there was a difference between birds that previously had to work at a low workload and birds that had to work hard (Figure 7a and 7b). The two peaks in activity during the day were far more pronounced in birds first assigned to low workload. Birds that came from the high treatment still showed a distribution of foraging activity fairly similar to the high treatment.

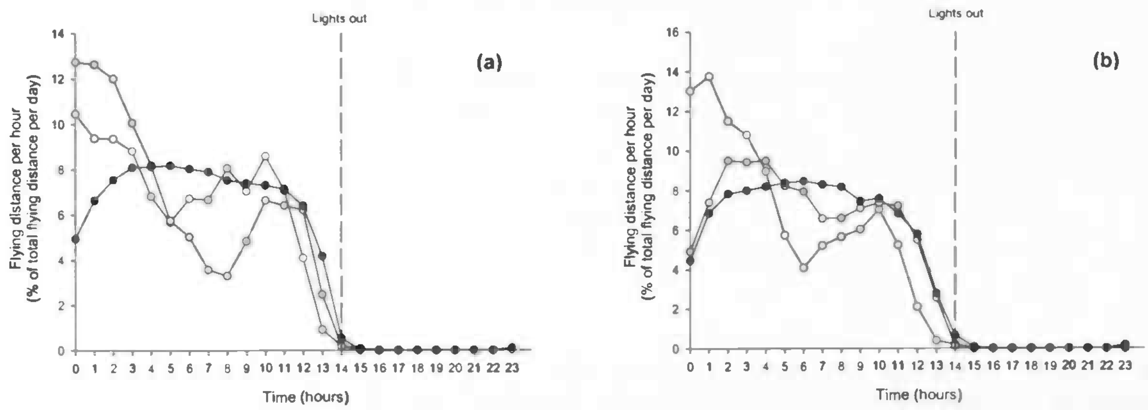
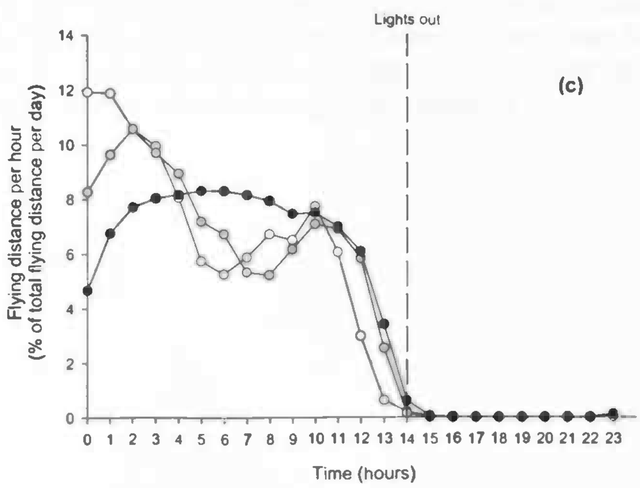


Figure 7 Workload and distribution of foraging activity. For low workload (white dots), transition workload (grey dots) and high workload (black dots). At transition workload, a much higher part of the foraging activity is found in the early morning in birds first assigned to low workload (a) compared to birds first assigned to high workload (b). Striking is the disappearance of the two activity peaks in the high treatment. Figure (c) shows combined data for both experimental groups.



The effect of treatment on body mass and condition

As is shown in figure 8, body mass was negatively correlated with workload. At high workload the body mass of some starlings was very low (some even dropped below 65 g, which is very low compared to their body mass of around 85-90 g when fed ad libitum) indicating that this level of workload was (close to) their maximum capacity. Order of treatment also had an effect on body mass. The starlings that were first assigned to the low workload maintained a higher body mass at this level than starlings that first had to cope with the high workload (Table 2).

Figure 8 The effect of workload on body mass, measured at the start of the day, for individual starlings. (Closed symbols connected by solid lines represent individual starlings first assigned to low workload). The difference in body mass between treatments was significant (all $p<.01$). Order of treatment also had an effect ($p<.05$). Body mass of each starling before the experiment began (Ad lib) is shown in the graph as a reference point.

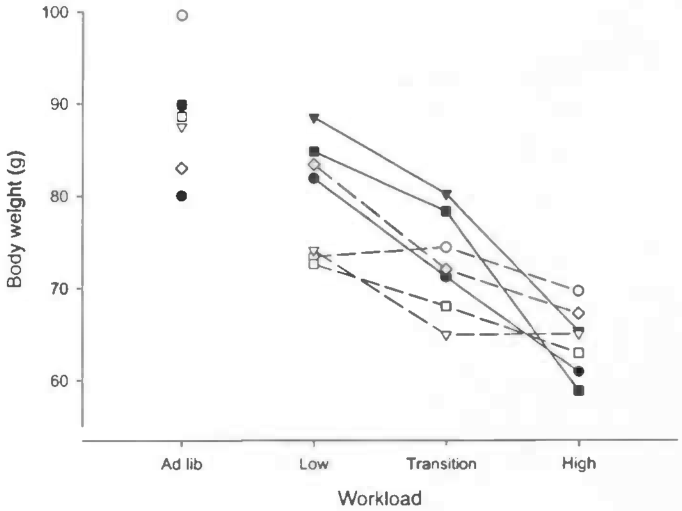


Table 2 Order of treatment and body mass at three workload levels.

Workload	Body mass Low 1st	Body mass High 1st	t_5	P (2-tailed)
Low	85.07 (± 1.91)	75.88 (± 2.53)	-2.71	.042
Transition	76.57 (± 2.74)	69.83 (± 2.11)	-1.99	.103
High	61.67 (± 1.92)	66.18 (± 1.44)	1.93	.112

Not only was body mass lower at high workload, also the dynamics of daily weight changes varied between different workloads (Figure 9). Especially at transition workload this difference was greatly influenced by order of treatment. Birds shifting from a low workload to transition workload showed a different pattern of body mass changes over the day than birds first assigned to the high workload (Table 3). They had smaller changes in body mass during the day and night, but, more important, their overall weight change was negative.

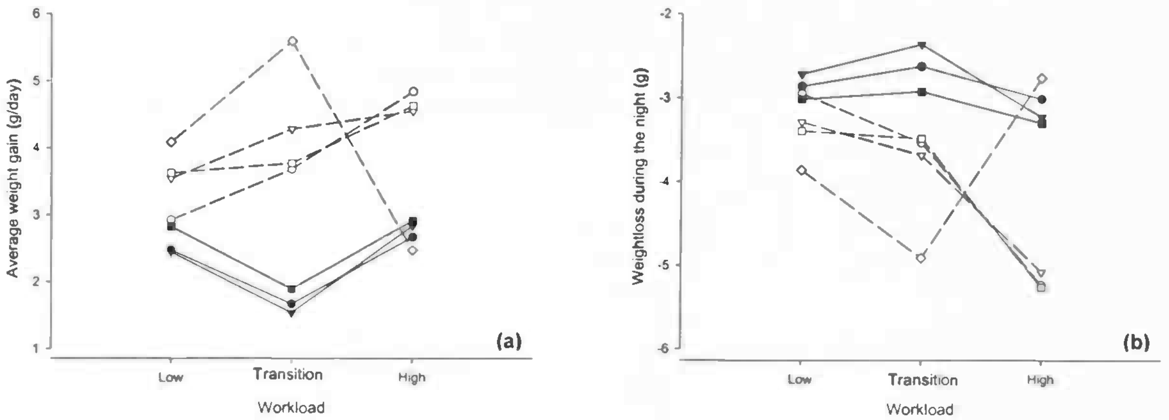


Figure 9 The effect of workload on body mass changes during the daylight period (a) and the night (b) and total 24-h weight changes (c). (Closed symbols connected by solid lines represent individual starlings first assigned to low workload). When analyzing the effect of workload on weight change during the day and the night separately they were only significant between low and transition workload ($p<.005$ and $p<.05$ resp.). The effect of workload on total weight change in 24 hours was different between all treatments (all p-values <.005). Order of treatment also had an effect on weight changes during the day, the night and total 24-hrs. ($p<.05$, $p<.01$ and $p<.005$ resp.)

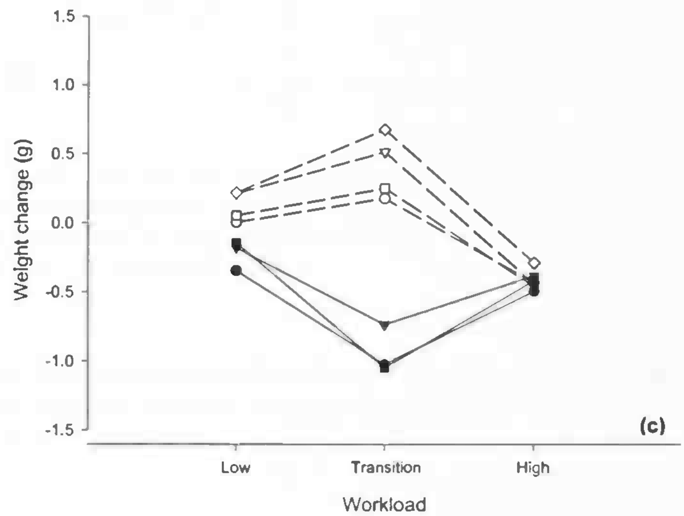


Table 3 Order of treatment and weight change during the daylight period (14-h) (a), the night (10-h) (b) and total 24-h. (c).

	Workload	Body mass Low 1st	Body mass High 1st	t_5	P (2-tailed)
(a)	Low	2.58 (± .12)	3.54 (± .24)	3.20	.024
	Transition	1.70 (± .10)	4.33 (± .44)	4.98	.004
	High	2.80 (± .07)	4.12 (± .55)	2.00	.102
(b)	Low	-2.87 (± .09)	-3.38 (± .19)	-2.18	.082
	Transition	-2.64 (± .16)	-3.91 (± .34)	-3.00	.030
	High	-3.18 (± .09)	-4.59 (± .61)	-1.94	.111
(c)	Low	-.22 (± .06)	-.12 (± .05)	4.19	.009
	Transition	-.94 (± .10)	.40 (± .12)	8.34	.000
	High	-.43 (± .03)	-.41 (± .04)	.42	.694

The effect of treatment on blood composition

Haematocrit level and the number of white blood cells (buffy coat) also seemed to vary between workloads (Figure 10). The combination of a lower haematocrit-level and a thicker buffy coat in the plasma at higher workload is a possible indicator of lower body condition. However these differences were just not significant.

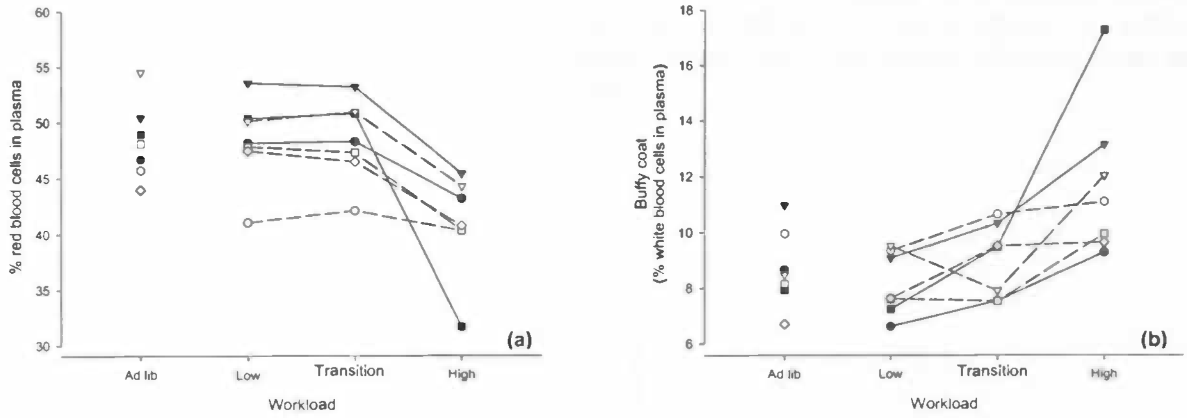
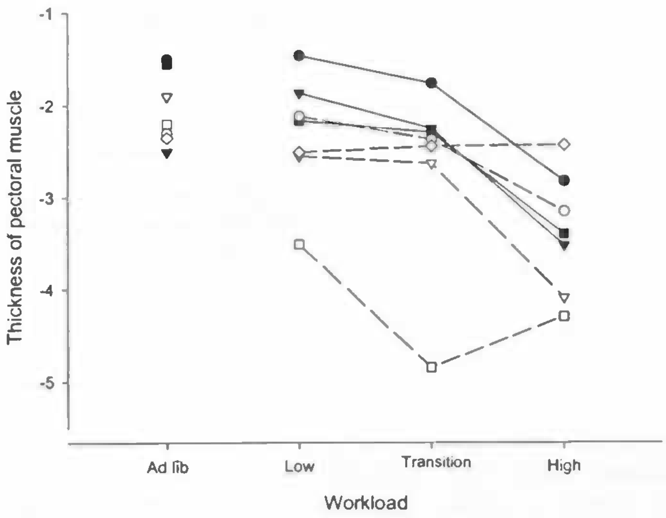


Figure 10 The effect of workload on haematocrit level (a) and the amount of white bloodcells in the plasma (b). (Closed symbols connected by solid lines represent individual starlings first assigned to low workload). Both a decrease in haematocrit levels and an increase in white bloodcells are observed when workload is high but this is not significant ($p=.051$ and $p=.050$ resp.)

The lower body mass of the starlings at the higher workload seemed, at least partly, to be caused by a reduction of the size of the pectoral muscle (Figure 11). Exactly what part of the total weight change can be attributed to this change in size of the pectoral muscle could not be determined in this experiment.

Figure 11 The effect of workload on the thickness of the pectoral muscle. (Closed symbols connected by solid lines represent individual starlings first assigned to low workload.) The difference in size of the pectoral muscle between low and high and between transition and high workload is significant ($p<.005$ and $p<.05$ resp.)



The effect of treatment on intake rate and digestion efficiency

Food consumption was higher in starlings assigned to a high workload compared to starlings that did not have to work that hard (Figure 12). Due to a high variability in food consumption in the transition treatment, food consumption at transition workload did not differ from low and high workload. This variability was especially high between the two experimental groups, these differences were not significant however.

As can be seen in figure 13, digestion efficiency was not affected by workload. Order of treatment (or time) however seemed to have an effect on digestion efficiency, with an increase in efficiency with time spent in the flight cages. But no significant effect of order was found at the separate levels of workload (Table 4).

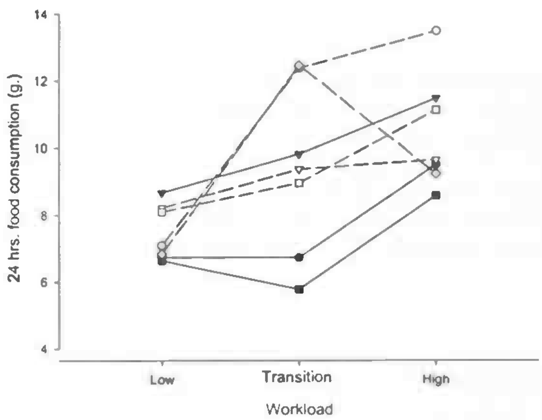


Figure 12 The relation between workload and 24-h food consumption. (Closed symbols connected by solid lines represent individual starlings first assigned to low workload). Increase in food consumption between low and high workload is significant (p<.01).

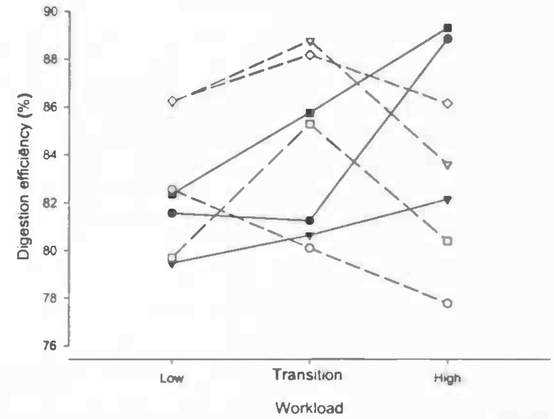


Figure 13 The effect of workload and digestion efficiency. (Closed symbols connected by solid lines represent individual starlings first assigned to low workload). There are no significant differences in digestion efficiency between workloads. Order of treatment however does have an effect

Table 4 Order of treatment and digestion efficiency

Workload	Digestion efficiency Low 1st	Digestion efficiency High 1st	T_5	P (2-tailed)
Low	81.13 (± .87)	83.67 (± 1.58)	1.27	.261
Transition	82.54 (± 1.61)	85.57 (± 1.98)	1.12	.312
High	86.75 (± 2.31)	81.96 (± 1.83)	-1.65	.160

Since there were no differences in digestion efficiency between the three levels of workload, the increase in food consumption at higher workload also meant a higher total metabolizable energy intake (MEI) (Figure 14). MEI during 24 hours was different between all three treatments, except between the transition and the high workload. As was the case in food consumption there was a lot of variation in MEI at the transition workload. When MEI was corrected for body mass it was significantly different between all three levels of workload. Also the effect of treatment order at the transition workload was significant (p<.01), with MEI in birds first assigned to the low workload being lower compared to birds first assigned to the high workload (Figure 15).

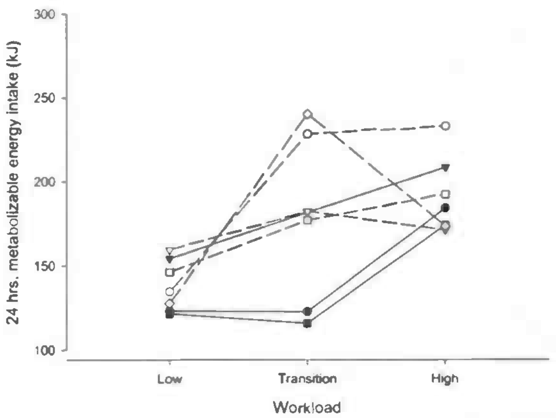


Figure 14 The relation between workload and 24-h metabolizable energy intake. (Closed symbols connected by solid lines represent individual starlings first assigned to low workload.) Increase in metabolizable energy intake is significant from low to transition workload and from low to high workload ($p<.05$ and $p<.01$ resp.)

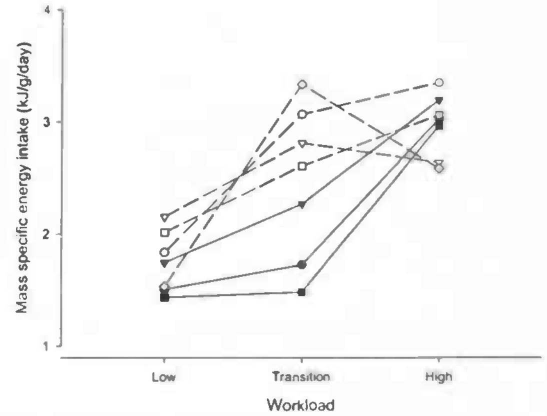


Figure 15 Workload and mass-specific energy intake. (Closed symbols connected by solid lines represent individual starlings first assigned to low workload). Differences are significant between all workloads (low/transition $p<.05$, transition/high $p<.05$ and low/high $p<.001$)

The effect of treatment on the energy budget

The basal metabolic rate of starlings is associated with workload (Figure 16). Starlings assigned to high workload have lower basal metabolic rates compared to starlings that have to work less hard for their food. However BMR is also highly dependent on body mass as is shown in Figure 17a. When BMR is corrected for body mass in our model, the effect of treatment on BMR is not significant. This is also in the fact that mass specific BMR (or metabolic intensity, BMR/g) is not dependent on treatment (Figure 17b.)

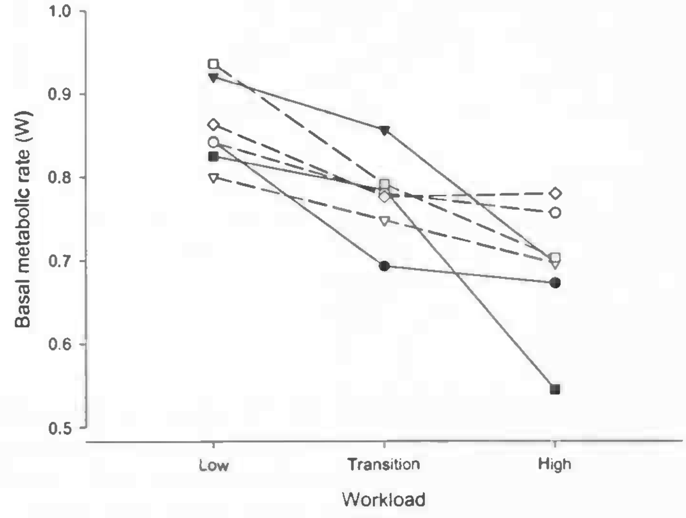


Figure 16 The relation between workload and basal metabolic rate (Closed symbols connected by solid lines represent individual starlings first assigned to low workload). BMR decreases when workload is higher (low/transition $p<.01$, transition/high $p<.05$ and low/high $p<.005$).

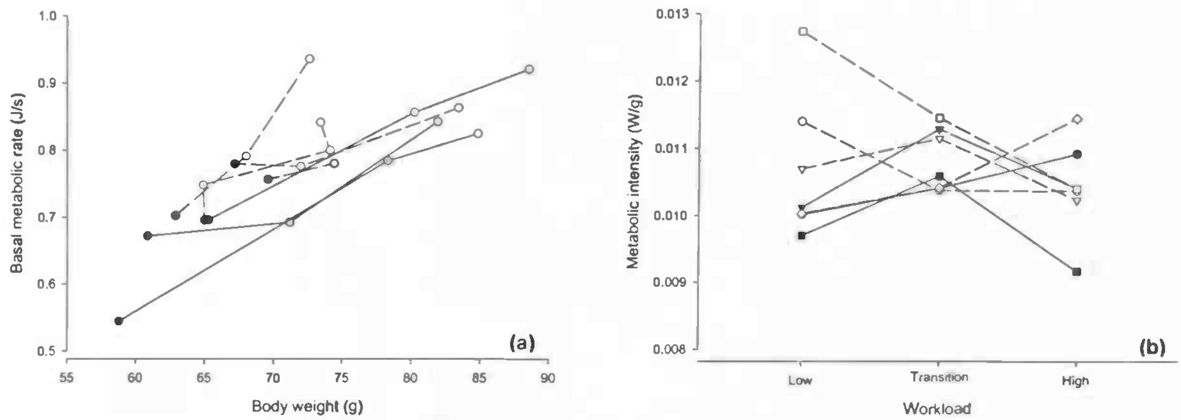
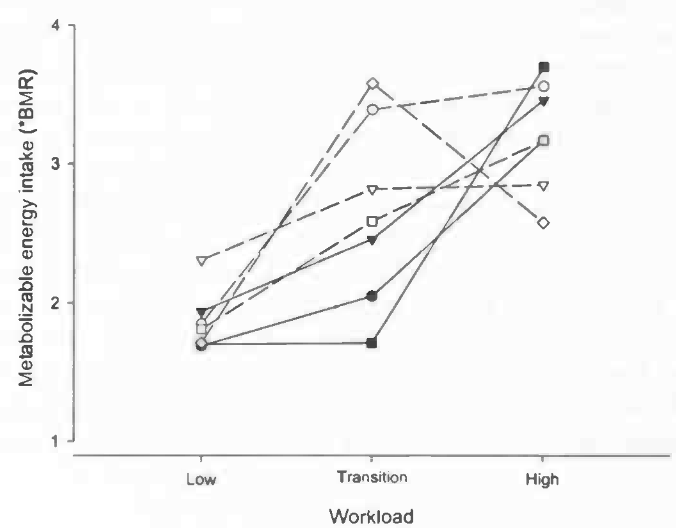


Figure 17 (a) The relation between bodyweight and BMR. This figure shows that starlings with a higher body weight also have higher BMR (repeated measures p<.001). (b) Mass specific BMR, or metabolic intensity is not dependent of workload. (Closed symbols connected by solid lines represent individual starlings first assigned to low workload.)

MEI expressed as a multiple of (individually measured) BMR, is different for all three workload levels. At the low workload MEI was equal to on average 1.9*BMR, at the transition workload 2.7*BMR and at the high workload 3.2*BMR (Figure 18). Order of treatment had an effect at the transition workload, in birds first assigned to the low workload the MEI divided by BMR was lower than in birds first assigned to the high workload (p<.05)

Figure 18 The relation between workload and metabolizable energy intake expressed as a multiple of BMR (Closed symbols connected by solid lines represent individual starlings first assigned to low workload). Metabolizable energy intake at low workload is on average equal to 1.9*BMR, at high workload this has increased to 3.2*BMR. Again variation at the transition workload is very big. Differences are significant between all three levels of workload (low/transition p<.05, transition/high p<.05 and low/high p<.001).



Next to the basal metabolic rate three other parts of the energy budget are energy demanding, namely maintenance, foraging activity and body tissue deposition. In figure 19 the estimates of these variables are shown. They are based on the estimates of 1.95*BMR for maintenance energy and 18kJ/g for mass deposition gain (calculated from unpublished data of a study on starlings at the Zoological Laboratory), and 20.5W for flight costs (Hambly et al. In press.) Energy income was divided into two components, metabolizable energy intake on the one hand and the energy gain from loss of body mass (assumed 18kJ/g) on the other.

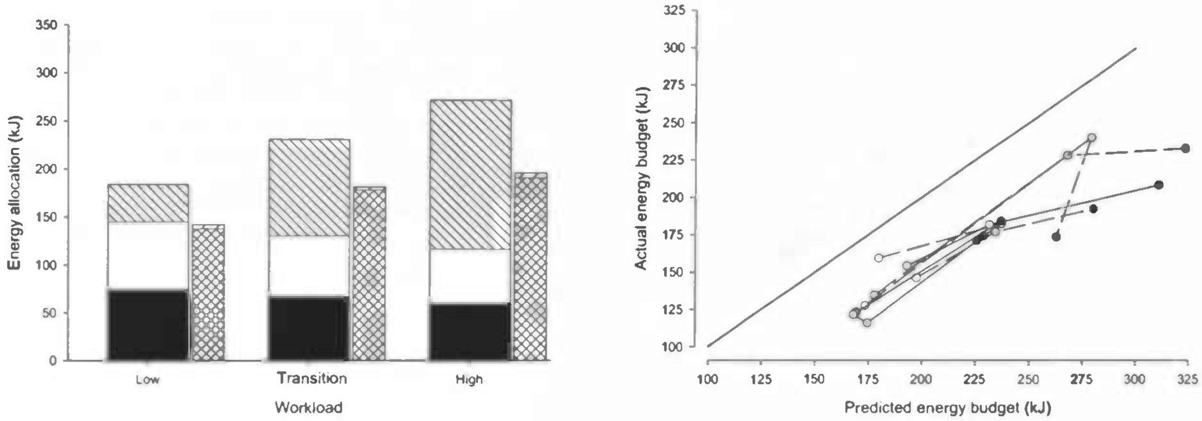
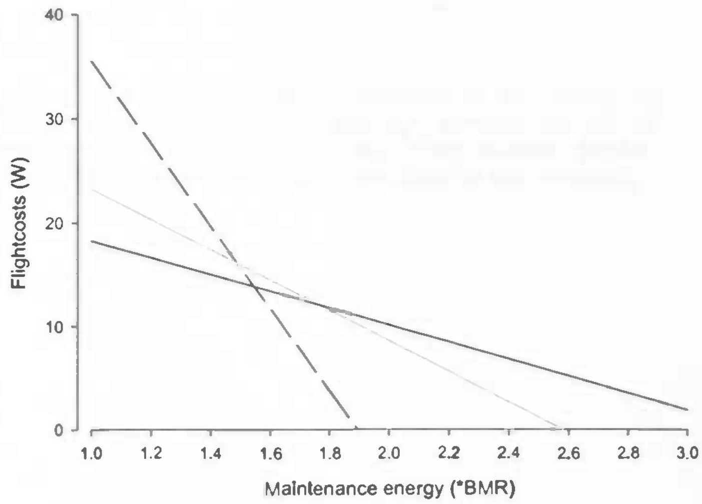


Figure 19 (a) The relation between workload and energy budget. The wide areas represent energy used for BMR (dark), maintenance (based on estimated value of 1.95*BMR) (white) and foraging activity (based on costs of flight of 20.5W) (dashed). The narrow areas represent energy gained through food intake (crossed) and loss of weight (18kJ/g) (dashed). (b) The relation between predicted and actual energy budget. (White dots represent values at low workload, grey dots transition workload and solid dots high workload. Symbols connected by solid lines represent individual starlings first assigned to low workload.) Both graphs show an overestimation of energy expenditure at all three levels of workload.

Using the theoretical values for the variables in our model leads to an overestimation of daily energy use in starlings, for all three levels of workload (Figure 19). The overestimation in our model is most likely caused by an overestimation of either maintenance costs or flight costs or both. BMR and food intake (and its energy content) are accurately measured and weight changes were so small that their effects were negligible. Looking at figure 19a it becomes clear that the prediction of both maintenance costs and flight costs are too high, because at low workload predicted energy use is already above energy intake when flight costs are not taken into account. At the same time, at high workload adding only energy used for flight and BMR leads to an overestimation of energy use.

Figure 20 shows the relation between flight costs and maintenance energy, assuming all other values are accurate. In this figure flight costs are calculated by summarising all other estimates of energy expenditure, and comparing this to the total amount of metabolizable energy intake at different estimates of maintenance energy expenditure. The x-axis starts at 1*BMR since maintenance energy can not be lower than BMR. If assumed that both maintenance energy and flight costs are the same for all workload levels (the point where the three lines in figure 19 cross each other), they should respectively be 1.5*BMR and 14.8W. Figure 21 shows the correlation between predicted and actual energy budget when these values are used in our model.

Figure 20 Relation between maintenance energy and flightcosts based on metabolizable energy intake. This relationship is different for birds assigned to low (dashed line), transition (grey line) and high (solid line) workload.



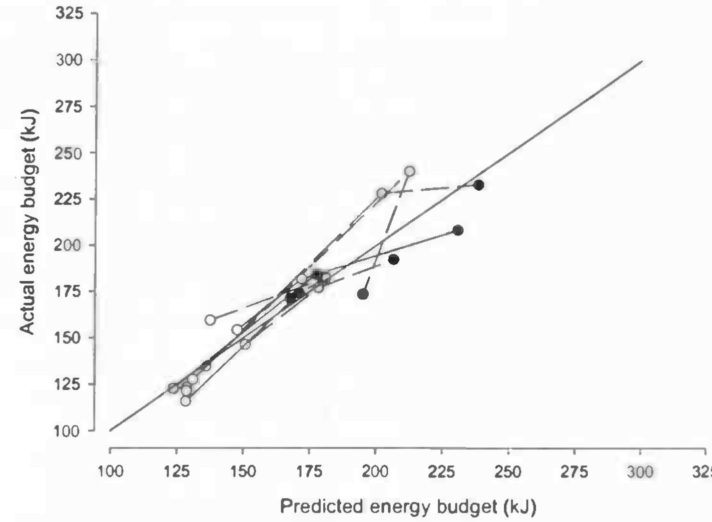


Figure 21 The relation between predicted and actual energy budget. Prediction is based on the values 1.52*BMR for maintenance costs and 14.8W for flight costs (White dots represent values at low workload, grey dots transition workload and solid dots high workload. Symbols connected by solid lines represent individual starlings first assigned to low workload).

The effect of workload on immune function

The increased thickness of the buffy coat in starlings at the high workload shown in figure 9b, suggests that these starlings are in poorer condition and possibly suffer from infections. However there were no differences found in the response to the immune challenge with PHA (Figure 22) All starlings showed thickening of the wing web after they were injected with PHA, in most cases a peak in response was found after 24 h, after which it remained constant or dropped. 216 hours after injection the wing web was only 1 mm thicker than normal. Measurement of wing web thickness showed large variation that could not be explained by either workload or order of treatment.

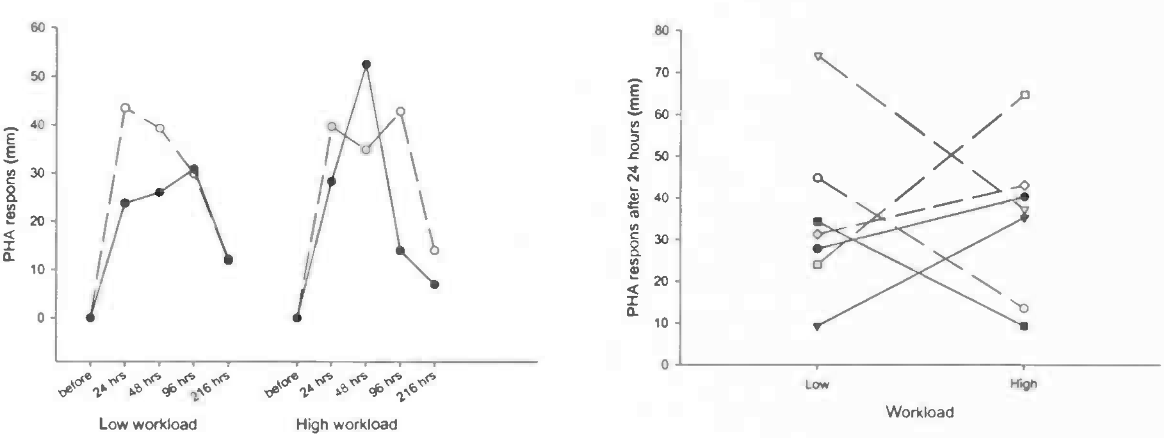


Figure 22 Workload and PHA-responsiveness. The PHA-response is expressed in the change in thickness of the wing web at four different times after injection for low and high workload (a) and 24 hours after injection separately (b). No differences in PHA-responsiveness were found between the two workloads. (Closed symbols connected by solid lines represent starlings first assigned to low workload).

Table 6 Summary of experimental results

Workload	Low	Transition	High
BMR (W)	0,86	0,78	0,69
Body mass (g)	79,81	72,71	64,24
Buffy coat (%)	8,15	8,99	11,77
DEE (kJ/d)	138,09	178,28	190,95
Digestion efficiency (%)	82,58	84,27	84,02
Flying distance (Km/d)	7,73	19,97	31,17
Haematocrit level (%)	48,34	48,44	40,85
Metabolic intensity (W/g)	$1{,}07 * 10^{-2}$	$1{,}08 * 10^{-2}$	$1{,}04 * 10^{-2}$
Pectoral muscle (mm)	-2,31	-2,66	-3,39

Discussion

During our experiment it became clear that in some cases the starlings from our two experimental groups did not react equally to the different levels of workload. Starlings first assigned to high workload had lower body mass at low workload compared to starlings that had to work at this level at the beginning of the experiment. And, perhaps more important, the group of starlings first assigned to low workload seemed to have greater difficulty coping with the following higher workload. The differences between the two groups at transition workload are most likely an artefact caused by the short period the starlings were assigned to the transition workload, and consequently an absence of a steady state at this working level. This explanation might also hold for the differences at low and high workload, as the period of recovery from the first treatment might have been too short. Also the possible detrimental effects on body condition, caused by the long period the starlings had been in the cages, can not be ignored.

Different levels of workload lead to a number of behavioural and physiological changes within an individual starling, these will be discussed below.

The effect of treatment on activity

In contrast to the findings of an earlier study, using a very similar laboratory set-up (Bautista et al. 1998b) the starlings in our experiment were able to increase the daily food intake observed at low workload. These different findings are most likely caused by the earlier mentioned differences in work and food ratio. In our study this ratio was variable instead of fixed, apparently this influences the foraging activity in starlings as was found in the study by Fotheringham et al. (1998). Total foraging time however still only made up a small portion of the total daylength, indicating that time was not a constraint of total foraging effort.

Workload also affected the timing of activity. At low and transition workload the starlings showed peaks in activity in the morning and evening, but at high workload activity was lowest in the first two hours and after an increase remained more or less constant during the rest of the day. At all three levels of workload the starlings reduced their foraging effort two to three hours before the end of the light period, but the timing of this reduction was later at the higher workload.

The effect of treatment on body mass and condition

We found that body mass was reduced when the level of workload became higher. In some cases body mass even was as low as 60 grams. This is extremely low compared to the typical body mass for this species in the wild (± 75 g (Meijer et al. 1994)). Body mass was lowered not only by a reduction of fat deposits, but also by reducing the size of the pectoral muscle. Although no effect of workload on digestion efficiency was found, this can not be seen as direct evidence for a reduction in the size of the digestive tract. It was not possible to measure the digestive tract, or any of the other internal organs, therefore nothing can be said about possible adaptations at that level.

The variation found in changes in body mass overnight and during the day, within individual starlings and between treatments, are most likely not only caused by variation in fat reserves. Because body mass is measured at the end of the working period, birds that stop working at a later time will have more food in their stomach and therefore be heavier than birds that were already fasting for a while. As a result measurements of overnight weight change in these birds will also be higher.

As mentioned earlier a reduction in body mass can be seen as an adaptation to an increase in workload, through a reduction in maintenance and flight costs. Unfortunately in our experiment no comparison of these separate variables could be made. However we did find a lower basal metabolic rate in starlings that were assigned to high workload compared to starlings with easier tasks.

The decrease in haematocrit levels that we found at higher workload, is in contrast to findings by Hõrak et al. (Hõrak et al. 1998) who found just the opposite in Great Tits that had to work harder after enlargement of their clutch (*Parus major*). This decrease in haematocrit levels, combined with an increase in the number of white blood cells found in the plasma of starlings assigned to a high workload, suggests that these starlings are in worse condition than they were at low workload. Elevated leukocyte number is symptomatic of stress syndrome and inflammatory processes, while low values of haematocrit are indicative of bacterial infections and gastrointestinal disorders (Ots et al. 1998). Although it is likely that the starlings did have lower body conditions at high workload, it is also possible that the reduction in size of the pectoral muscle, found in these starlings, lowered the demand for oxygen transport and thereby permitted a lower amount of red blood cells in the plasma. However behavioural observations already suggested in some cases that the birds were less healthy at high workload, with some birds suffering from diarrhea towards the end of the experiment, and one bird even dying.

The effect of treatment on intake rate and digestion efficiency

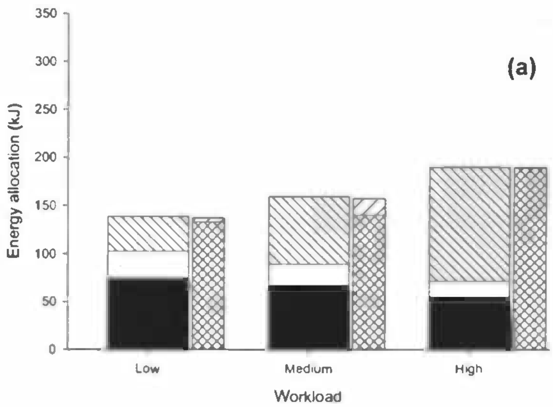
As mentioned above, in our experiment the starlings increased their foraging effort at higher workload. This increase was such that daily intake rate also increased with the level of workload. Since we found no effect of workload on digestion efficiency, increased food intake at higher workload also meant that metabolizable energy intake was higher. This is also in contrast to the findings by Bautista et al. (Bautista et al. 1998b), who found a decrease in total energy expenditure. This difference is most likely also caused by the variable work and food ratio used in our study. The probability of receiving a food pellet after each flight is apparently highly motivating for a starling to keep flying, resulting in a higher foraging activity in our study compared to the study by Bautista.

The effect of treatment on the energy budget

Since the measurements regarding the energy budget were made in a period without considerable changes in body mass, total metabolizable energy intake equals total energy expenditure. Although the starlings apparently were able to respond to the decrease in food availability, it is unlikely that they did so only by increasing foraging effort. It would increase energy demand even more through higher foraging costs. Therefore a combination with other energy saving adaptations could be expected (Deerenberg et al. 1998; Bautista et al. 1998b). One possible adaptation is lowering the amount of energy allocated to body maintenance. In our experiment it was not possible to directly measure maintenance costs at the three different levels of workload, however BMR was measured as an indicator. BMR was lower in starlings assigned to higher workload, reducing energy demand by 10% at transition workload and 20% at high workload. BMR was correlated to body mass, this however does not diminish its role as a possible adaptation to changes in food availability and workload.

From our data it became clear that the value of 20.5W for flight costs suggested by Hambly et al. (in prep.) could not be used for all three levels of workload, because this would lead to an overestimation of energy expenditure, thereby leading to an impossibly low estimate of the amount of energy allocated to maintenance at high workload. Also the use of a model where both maintenance energy and flight costs are assumed to be the same at all levels of workload, results in a prediction of these values that is too low.

Flight costs within individuals can be corrected for body mass, where costs increase with body mass as $(BM_1/BM_2)^{7/6}$ (Norberg 1990). Since the estimated flight costs of 20.5W in the experiment by Hambly were determined at low workload, this would mean that flightcosts at the transition and the high workload in our experiment would respectively be 18.4W and 15.9W. These values are very close to our earlier estimates of flightcosts at a level of 1.38*BMR for maintenance costs. Using these estimates of flightcosts in our model results in maintenance costs varying between 1.3*BMR and 1.4*BMR at the three levels of workload. Figure 23 shows the relation between workload and the energy budget using this final model and compares the estimated energy expenditure to the actual amount of energy intake.



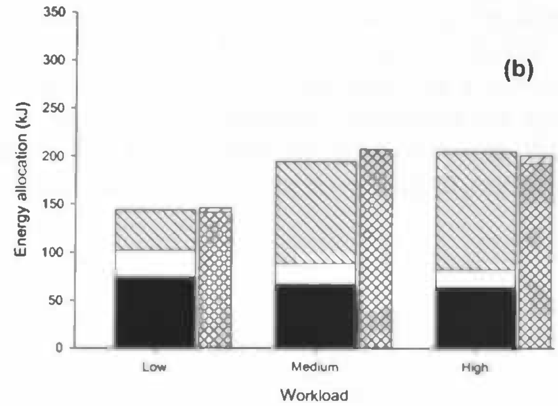
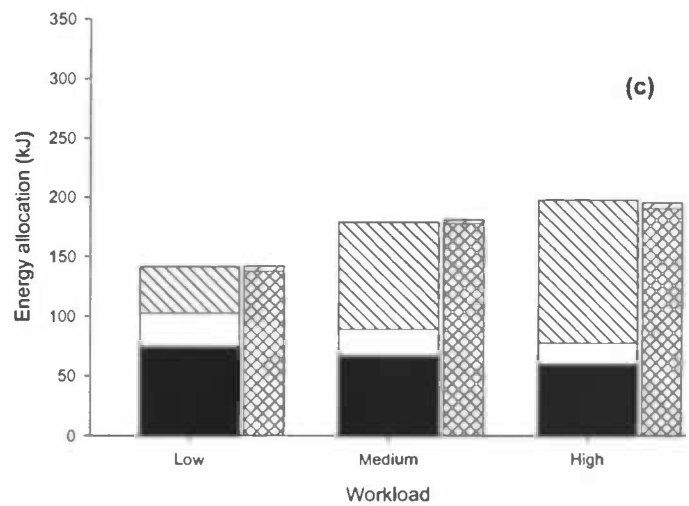
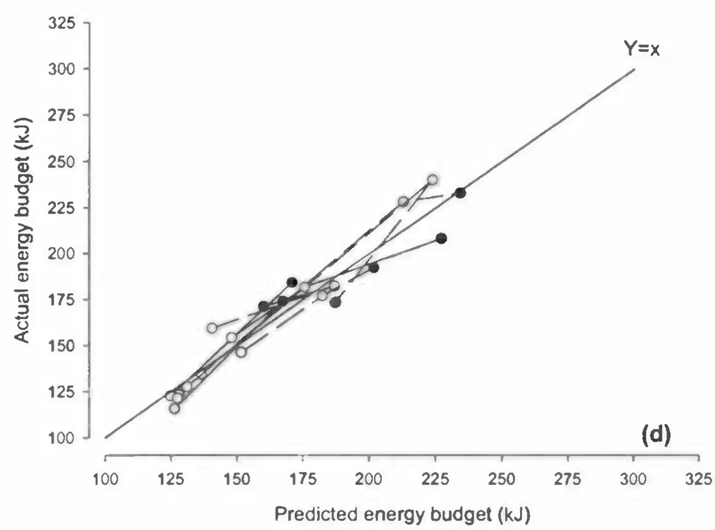


Figure 23 Workload and energy budget of starlings first assigned to low workload (a) and high workload (b) and combined data for the two groups (c). The wide bars represent energy used for BMR (dark), maintenance (calculated as of 1.38, 1.33 and 1.30*BMR for low, transition and high treatment) (white) and foraging activity (flightcosts of 20.5W, 18.4W and 15.9W for low, transition and high treatment) (dashed). The narrow bars represent energy gained through food intake (crossed) and weightloss (18kJ/g) (dashed).
(d) shows the relation between predicted and actual energy budget. The prediction is based on the values 1.38, 1.33 and 1.30*BMR for maintenance costs and 20.5W, 18.4W and 15.9W for flight costs (for low, transition and high treatment respectively) (White dots represent values at low workload, grey dots transition workload and solid dots high workload. Symbols connected by solid lines represent individual starlings first assigned to low workload).





A possible explanation for the problems in accurately predicting energy expenditure using our data might be that some starlings did not always fly to the other side of the flight cage but sometimes walked instead. Since walking is a less costly form of locomotion compared to flying, this would always lead to an overestimation of foraging costs in our experiment.

The effect of treatment on immune function

No effects of workload on the response to an injection with PHA were found. The responses of the starlings were equal to those of starlings in another experiment in outdoor pens where food was available ad libitum (unpubl. data). The fact that we already found an overestimation of energy expenditure in our model without taking into account a possible energy cost of the PHA-challenge, suggests that this cost is negligible. However the lower amount of white blood cells found in starlings at the high workload suggests that these starlings are possibly suffering from infections, and that their immune system therefore is affected by the fact that they have to work that hard.

References

Aschoff,J. 1966. Circadian activity pattern with two peaks. *Ecology* **47**, 656-662.

Bautista,L.M., Kacelnik,A., Tinbergen,J.M. & Wiersma,P. 1998a. Patterns of avian daily foraging when food intake is highly predictable and there is no predation risk. *American Naturalist* **in press**.

Bautista,L.M., Martin-Beatriz, Martinez-Laura & Mayo-Carolina 2001. Risk-sensitive foraging in coal tits. *Behaviour* **138**, 69-83.

Bautista,L.M., Tinbergen,J., Wiersma,P. & Kacelnik,A. 1998b. Optimal foraging and beyond: How starlings cope with changes in food availability. *American Naturalist* **152**, 543-561.

Caldow,R.W.G. & Furness,R.W. 1991. The relationship between kleptoparasitism and plumage polymorphism in the Arctic Skua Stercorarius parasiticus. *Functional Ecology* **5**, 331-339.

Daan,S., Masman,D. & Groenewold,A. 1990. Avian basal metabolic rates: their association with body composition and energy expenditure in nature. *American Journal of Physiology* **259**, R333-R340.

Deerenberg,C., Overkamp,G.J.F., Visser,G.H. & Daan,S. 1998. Compensation in resting metabolism for experimentally increased activity. *Journal of Comparative Physiology B Biochemical Systemic and Environmental Physiology* **168**, 507-512.

Fotheringham,J.R. 1998. Feeding patterns in fixed- versus variable-ratio schedules. In: *Starlings Working for Food in a Closed Economy: Empirical Studies of Feeding Regulation*, pp. 4-35. Oxford, Oxford University, Dept. of Zoology.

Freed,L.A. 1981. Loss of mass in breeding wrens: stress or adaptation? *Ecology* **62**, 1179-1186.

Gosler,A.G., Greenwood,J.J.D. & Perrins,C.M. 1995. Predation risk and the cost of being fat. *Nature* **377**, 621-623.

Gustafsson,L., Nordling,D., Andersson,M.S., Sheldon,B.C. & Qvarnström,A. 1994. Infectious diseases, reproductive effort and the cost of reproduction in birds. *Philosophical Transactions of the Royal Society of London Series B Biological Sciences* **346**, 323-331.

Houston,A.I. & McNamara,J.M. 1993. A theoretical investigation of the fat reserves and mortality levels of small birds in winter. *Ornis Scandinavica* **24**, 205-219.

Hõrak,P., Ots,I. & Murumägi,A. 1998. Haematological health state indices of reproducing great tits: a response to brood size manipulation. *Functional Ecology* **12**, 750-756.

Hõrak,P., Ots,I., Tegelmann,L. & Møller,A.P. 2000. Health impact of phytohaemagglutinin-induced immune challenge on great tit (*Parus major*) nestlings. *Canadian Journal of Zoology* **78**, 905-910.

Ilmonen,P., Taarna,T. & Hasselquist,D. 2000. Experimentally activated immune defence in female pied flycatchers results in reduced breeding success. *Proceedings Of The Royal Society Of London Series B Biological Sciences* **267**, 665-670.

Johnsen,T.S. & Zuk,M. 1999. Parasites and tradeoffs in the immune response of female red jungle fowl. *Oikos* **86**, 487-492.

Jones,I.L. 1994. Mass changes of Least Auklets Aethia pusilla during the breeding season: evidence for programmed loss of mass. *Journal of Animal Ecology* **63**, 71-78.

Kacelnik,A. 1984. Central place foraging in Starlings (Sturnus vulgaris). 1. Patch residence time. *Journal of Animal Ecology* **53,** 283-300.

Kersten,M. & Piersma,T. 1987. High levels of energy expenditure in shorebirds: metabolic adaptations to an energetically expensive way of life. *Ardea* **75,** 175-187.

Kersten,M. & Visser,W. 1996. The rate of food processing in the oystercatcher: Food intake and energy expenditure constrained by a digestive bottleneck. *Functional Ecology* **10,** 440-448.

Lochmiller,R.L. & Deerenberg,C. 2000. Trade-offs in evolutionary immunology: just what is the cost of immunity? *Oikos* **88,** 87-98.

Masman,D., Daan,S. & Dijkstra,C. 1986. Ecological energetics of the kestrel: field estimates of energy intake throughout the year. *Ardea* **74,** 24-39.

Meijer,T., Mohring,F.J. & Trillmich,F. 1994. Annual and daily variation in body mass and fat of Starlings Sturnus vulgaris. *Joumal of Avian Biology* **25,** 98-104.

Norberg,U.M. 1990. *Vertebrate Flight*. berlin: Springer verlag.

Norris,K. & Evans,M.R. 2000. Ecological immunology: life history trade-offs and immune defense in birds. *Behavioral Ecology* **11,** 19-26.

Nudds,R.L. & Bryant,D.M. 2001. Exercise training lowers the resting metabolic rate of zebra finches, *Taeniopygia guttata*. *Functional Ecology* **15,** 458-464.

Ots,I., Murumägi,A. & Hõrak,P. 1998. Haematological health state indices of reproducing great tits: methodology and sources of natural variation. *Functional Ecology* **12,** 700-707.

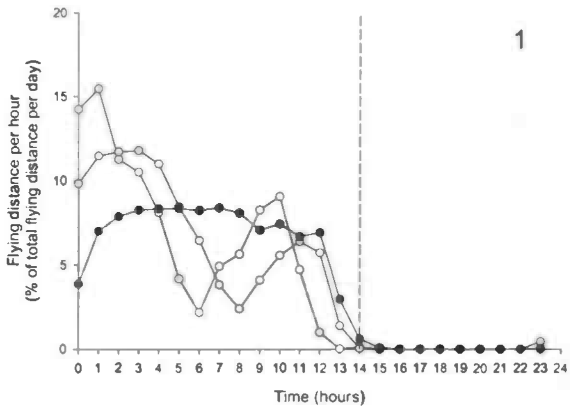
Ricklefs,R.E., Konarzewski,M. & Daan,S. 1996. The relationship between basal metabolic rate and daily energy expenditure in birds and mammals. *American Naturalist* **147,** 1047-1071.

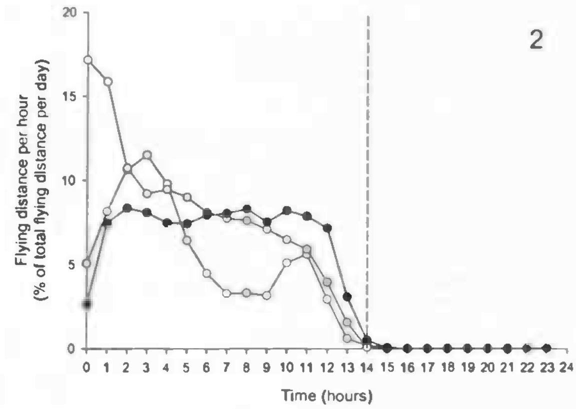
Sheldon,B.C. & Verhulst,S. 1996. Ecological immunology: costly parasite defense and trade-offs in evolutionary ecology. *Trends in Ecology & Evolution* **11,** 317-321.

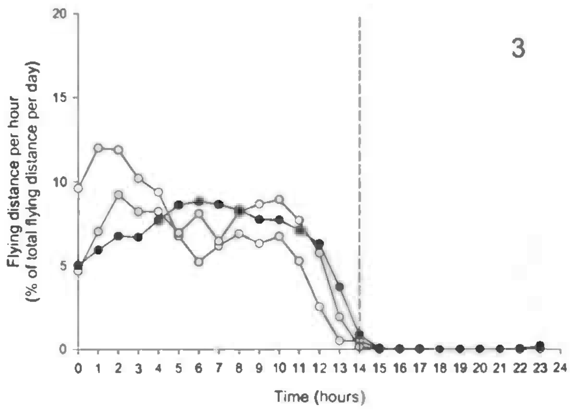
Smits,J.E., Bortolotti,G.R. & Tella,J.L. 1999. Simplifying the phytohaemagglutinin skin-testing technique in studies of avian immunocompetence. *Functional Ecology* **13,** 567-572.

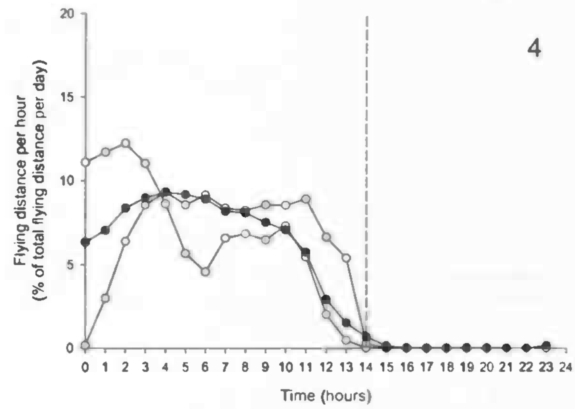
Svensson,S. 1976. Svenska haeckfageltaxeringen - arsrapport 1975. *Var Fagelv.* **35,** 83-89.

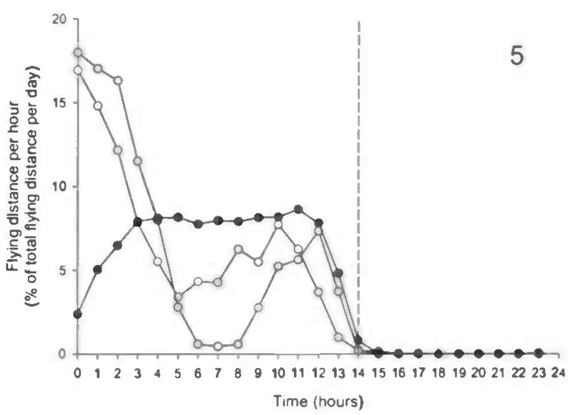
Appendix

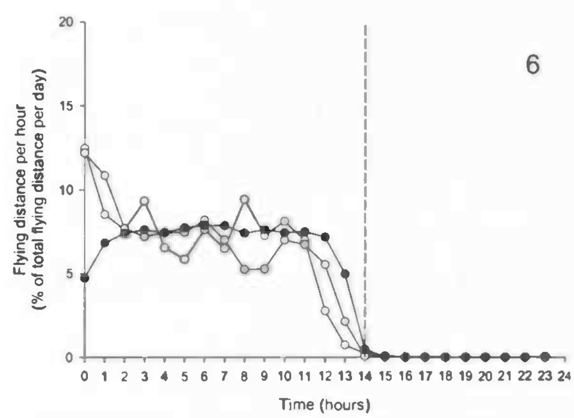


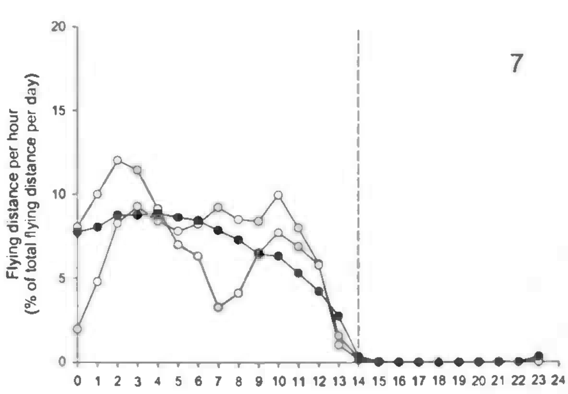




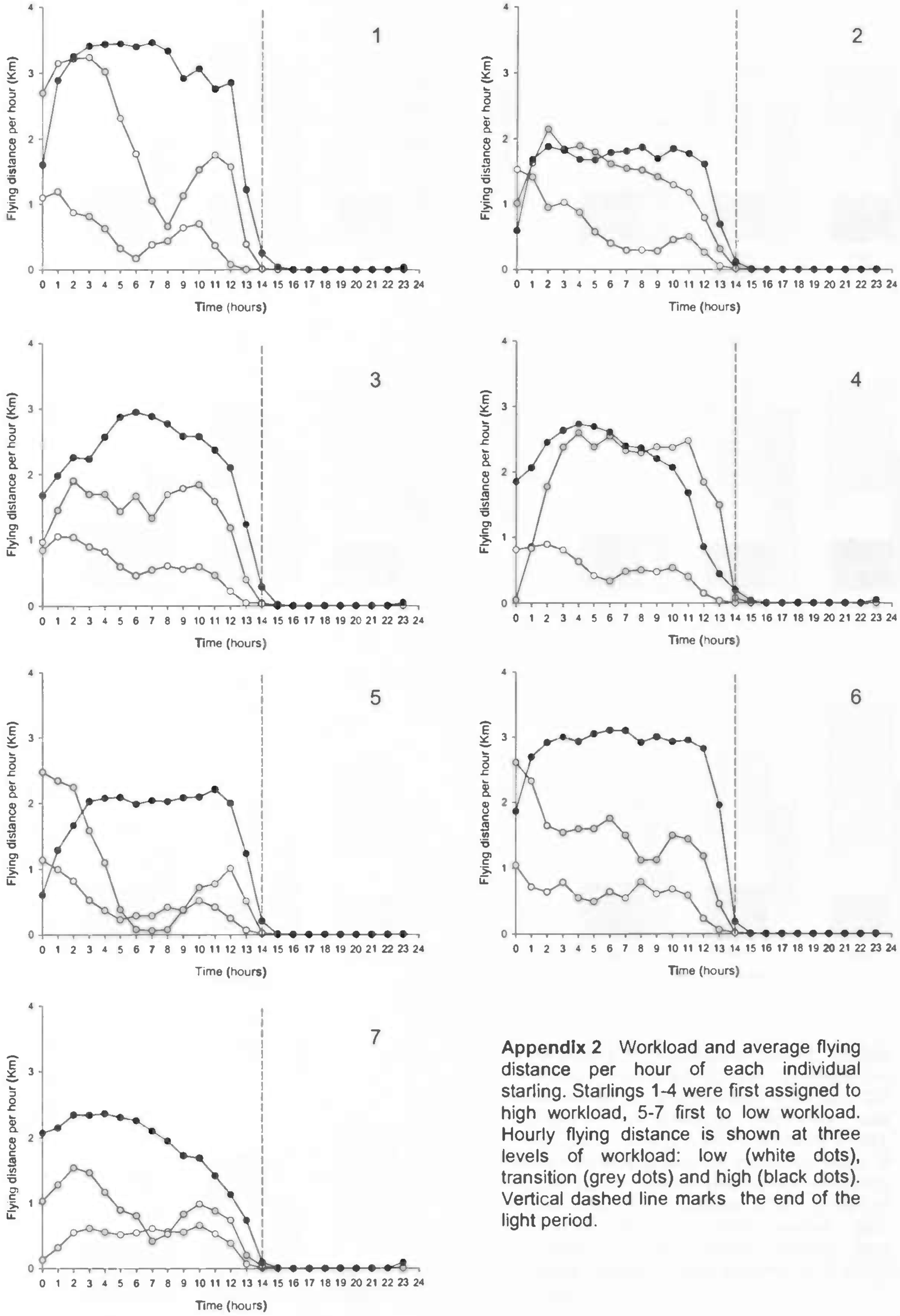




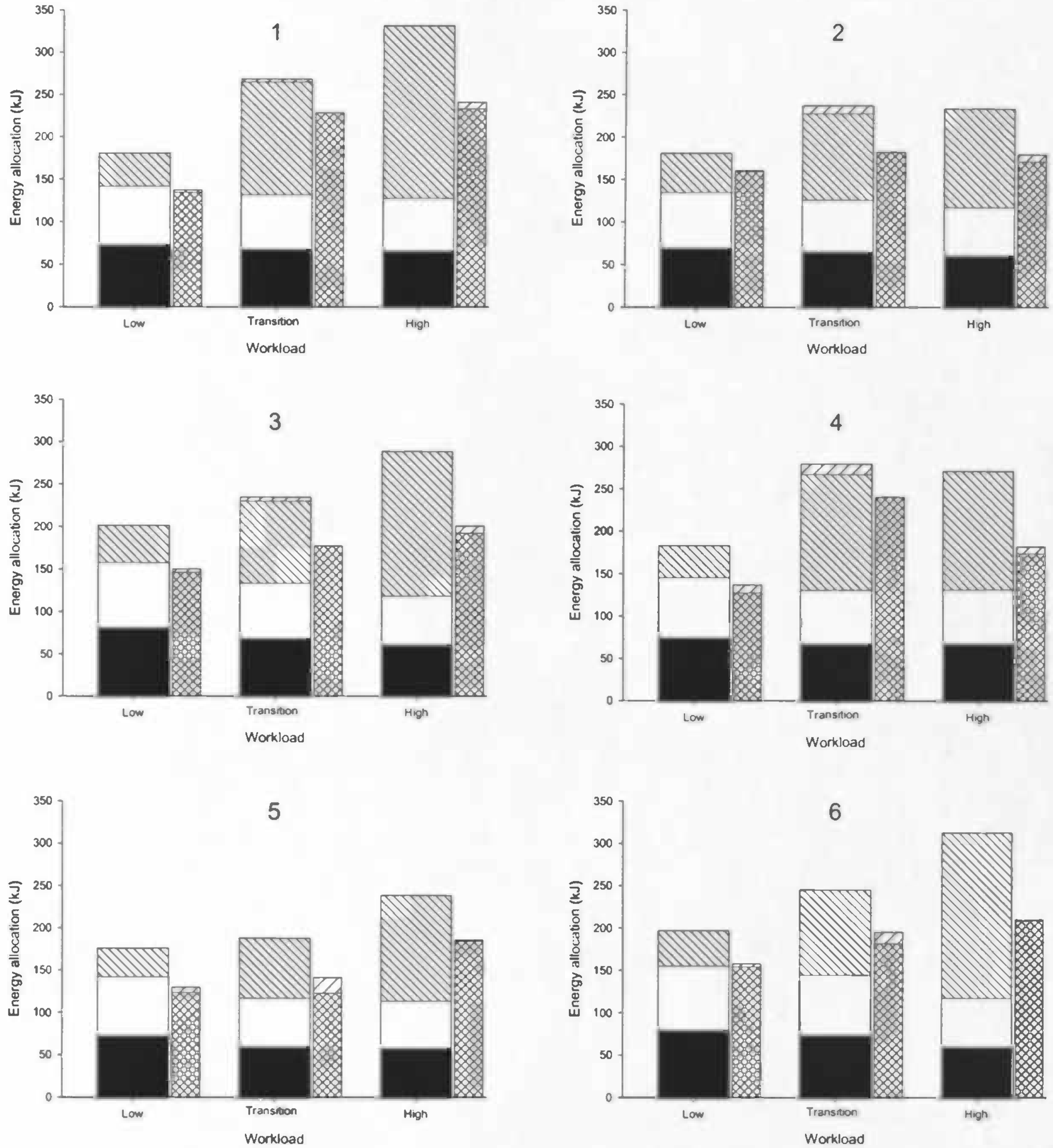


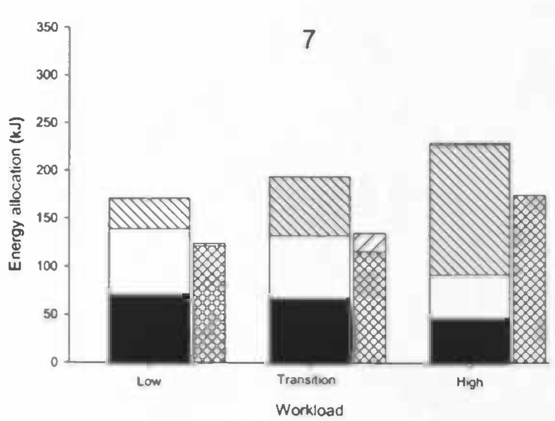


Appendix 1 Workload and average hourly activity of each individual starling, shown as a percentage of total flying distance per day. Starlings 1-4 were first assigned to high workload, 5-7 first to low workload. Hourly activity is shown at three levels of workload: low (white dots), transition (grey dots) and high (black dots). Vertical dashed line marks the end of the light period.



Appendix 2 Workload and average flying distance per hour of each individual starling. Starlings 1-4 were first assigned to high workload, 5-7 first to low workload. Hourly flying distance is shown at three levels of workload: low (white dots), transition (grey dots) and high (black dots). Vertical dashed line marks the end of the light period.





Appendix 3 Workload and energy budget of each individual starling. Starlings 1-4 were first assigned to high workload, 5-7 first to low workload. The wide bars represent energy used for BMR (dark), maintenance (based on theoretical value of 1.95*BMR) (white) and foraging activity (based on the theoretical costs of flight of 20.5W) (dashed). The narrow bars represent energy gained through food intake (crossed) and weightloss (18kJ/g) (dashed).