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Parental energy and fitness costs in birds

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CHAPTER 6

HARD WORK IMPINGES ON FITNESS: AN EXPERIMENTAL STUDY WITH ZEBRA FINCHES

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ABSTRACT

The experiment tested the effect of manipulated levels of diurnal activity on later reproductive decisions of Zebra Finches *Taeniopygia guttata*. Using restricted access to food, on the condition of a minimum amount of flight activity, a low and a high workload were imposed on the birds. The level of daily activity was increased, while body mass, and, concurrently, food intake, were reduced during the higher workload. After completion of the workload schedules, restricted access to the food was relaxed, and the birds were allowed to breed. The experimentally imposed workloads affected the subsequent interval till laying. Following a period with a high workload, the birds delayed the onset of laying with, on average, 6 days. Clutch size and brood size of the reproductive event did not vary with the previous work level. The interval till laying was not related to body mass at the end of the workload episode, neither in males, nor in females. An ethological mechanism and two physiological mechanisms, through which the effect of previous work level on later reproduction may have been attained, are suggested and discussed. We demonstrate that daily activity *per se*, thus independently of parental care, delays later reproduction, and thereby identify a causal mechanism for reduction in parental residual fitness.

INTRODUCTION

In many species of birds and mammals, reproduction is the phase in the year with the highest rate of energy turnover. This is especially true for altricial birds with an energy-intensive foraging method (Wijnandts 1984; Masman *et al* 1988a; review in: Weathers & Sullivan 1989). The extra energy above maintenance level devoted to reproduction, the parental effort, has been postulated to reduce the expected future reproduction of the parent (Fisher 1930; Williams 1966; Drent & Daan 1980; Daan *et al.* 1990a). This reduction in fitness is the cost of reproduction or parental investment (Trivers 1974). A cost of reproduction has been indicated for instance in Willow Tits (*Parus montanus*) by a reduced local survival rate of breeding males compared with non-breeding males (Ekman & Askenmo 1986). Experimental support for costs of reproduction has been provided by a number of studies, in which parental effort was manipulated by an experimental change in clutch size or brood size (reviews in: Dijkstra *et al* 1990; Stearns 1992).

Several types of costs are potentially involved. The first type of costs is of temporary, recoverable nature, and no longer affects fitness once the individual has started another reproductive attempt. These costs arrive through risk factors (Calow 1979; Partridge 1987), or somatic changes (Nur 1984; Slagsvold 1984; Tinbergen 1987; Martins & Wright 1993), which may impinge on fitness through reduced fecundity at the next reproductive event. Non-recoverable fitness costs may arise from permanently reduced fertility in later years (Gustafsson & Pärt 1990), or from reduced life span (Lemon 1991; 1993).

As an indicator of parental effort, parental daily energy expenditure (DEE) may be used. The evidence that parental effort is effectively manipulated in brood manipulation experiments, is commonly evaluated by food provisioning rate (review in: Lessells 1993), which often is closely related to DEE (Bryant 1988). Several recent studies reported on direct estimates of DEE as a function of brood size manipulation, either by constructing a time-energy budget for the parent (Dijkstra *et al* 1990; Green & Ydenberg 1994), or on the basis of the doubly labelled water method (Williams 1987; Deerenberg *et al* 1995; Moreno *et al* 1995; Verhulst 1995; Chapter 5). There is thus experimental evidence for effects of brood size manipulation both on parental energy expenditure and on subsequent survival and/or fecundity. The suggestion that DEE is instrumental in causing these fitness costs (Reyer 1984; Ekman & Askenmo 1986; Dijkstra *et al* 1990; Bryant & Tatner 1991; Lemon 1991; Deerenberg *et al* 1995; Chapter 2; Chapter 5) predicts that also other manipulations of DEE should have consequences for residual fitness.

We therefore designed a laboratory experiment to examine the effect of effort on later reproduction in Zebra Finches *Taeniopygia guttata*. We aimed at manipulating DEE through diurnal activity of individual birds, independently of parental work, to disentangle the intrinsic effects of parental care and the effects of energy expenditure on later reproduction. The principal rationale was to modify work rate of the zebra finches by restricting access to food. To obtain access to food, the birds had to perform a certain amount of activity. The required amount of activity was variable, while time-limited access to food was fixed. By varying the amount of activity per reward, we aimed at manipulating daily activity. Following such manipulations, birds were paired and their reproductive activities monitored.

METHODS

Male and female zebra finches, originating from domesticated stock, were kept in climate rooms at 25 °C, and relative humidity 60%, in a LD cycle of 14:10 (lights on at 0800 h). The birds were held individually in cages of 40·80·40 cm (h·w·d) with two perches 56 cm apart, equipped with drinking water *ad libitum*, and an automatic feeder. The food was a mixture of tropical and herb seeds (ratio 1:1), provided *ad libitum*. The birds could reach the food in the feeder by pushing the front door. Every opening of the door was recorded as a feeding peck. An infra-red light beam situated just in front of one of the perches, enabled us to record the activity of flying between the two perches. Activity and feeding pecks were recorded continuously, in intervals of two min (DataQuest System III, Data Sciences). The door in front of the feeder could be locked and unlocked under control of a central processing unit (Series III System, GE).

Training procedure

For one week, the door of each feeder was left open, leaving no obstruction to reach the food. Then the door of the feeder was closed, but not locked, and the birds had to push the door to feed. Most birds learned this in one to four days. In the next phase of the training procedure, the birds had to fly a fixed number (X) of times to unlock the feeder, which was then programmed to remain unlocked for 10 sec. When this feeding interval had elapsed, the door was locked and the bird had to fly again X times before the feeder would be unlocked. The value of X was gradually increased in about weekly steps (X = 5, 10, 20, 30 and 40) providing the bird was performing well on the previous schedule. The performance of the bird was evaluated from the daily amount of seeds that it removed from the feeder (assessed by daily weighing of the remaining food), and from the body mass and general appearance of the bird.

When a bird had successfully completed the series of increasing schedules, it was allowed an episode on a schedule with X=10 that lasted at least one month. This was introduced to have an episode of relaxed effort as a starting point for the experiment, and to reset the timing of the experiment.

The experiment

In the experiment, we used successful breeding pairs, which had produced a brood and had raised the young to independence. We imposed two different workloads on the birds: a 'low' workload with X=20, and a 'high' workload with X=40. In a pilot experiment, we had found that activity could be modified to about 4500 flights per day on the low workload, and about 6500 flights per day on the high workload. Spontaneous activity was about 2000 flights per day. Both members of a pair were exposed to the same workloads simultaneously, but in separate cages.

Starting from a schedule of X=10, the required schedule again was built stepwise with steps of 10 (schedule 10 to 20, or 10 to 20, 30, 40). Each step took three to four days. The final, experimental, low or high workload lasted four weeks. During the

Table 1. Flights, food and mass in episode 1 and 3 with the same workloads. Means and standard deviation of the variables are given for the group on the low workload (n=8) and on the high workload (n=9). Test results from paired t-tests are included.

	episode 1	episode 3	T	P
LOW workload				
activity	6379 ± 1962	7673 ± 3362	-1.20	>.10
food (g)	3.75 ± 0.40	4.11 ± 0.57	-3.72	<.01
mass (g)	13.54 ± 1.61	13.60 ± 1.70	-0.67	>.10
HIGH workload				
activity	7325 ± 3188	7907 ± 2479	-1.36	>.10
food (g)	3.37 ± 0.36	3.47 ± 0.44	-0.86	>.10
mass (g)	13.70 ± 1.23	13.67 ± 0.96	0.14	>.10

episode of work, the food supply was weighed each day before noon. Each bird was weighed once or twice weekly.

At the end of a workload episode of four weeks, the birds were weighed, and each pair was reunited in a cage with suitable breeding opportunities: a nest box, nesting material, a water bath, and unrestricted access to food. As long as the pair had not yet started to lay, we weighed them once or twice weekly. From daily checks of the nest box, we determined laying dates of the first egg, and clutch size. A few days after hatching of the eggs, we removed the brood and separated male and female again to expose them to a new episode of work.

The full experiment comprised three four-week episodes of work, each episode followed by breeding. One group (eight pairs) was first given an episode on the low workload, then an episode on the high workload, and another episode, again on the low workload. The other group (nine pairs) was given the high workload in the first episode, the low workload in the second episode, and again the high workload in the third episode.

Data analysis

The differences in activity, food intake, and body mass of the bird between the low and high workloads, and between the corresponding first and third episodes on the same workload, were tested intra individually using paired t-tests. The differences between episodes one and three were analyzed for the group on the low or on the high workload separately. We estimated the relations between the variables measured during work or reproduction with multiple linear regression models, with the two experimental workloads included as a factor (low = 1, high = 2). The contribution of a variable to the model was examined with an F-ratio test on the difference in deviance (= sum of squares) between models with and without the variable in question (and its interactions). Significance was accepted at $P \leq .05$ (two-tailed).

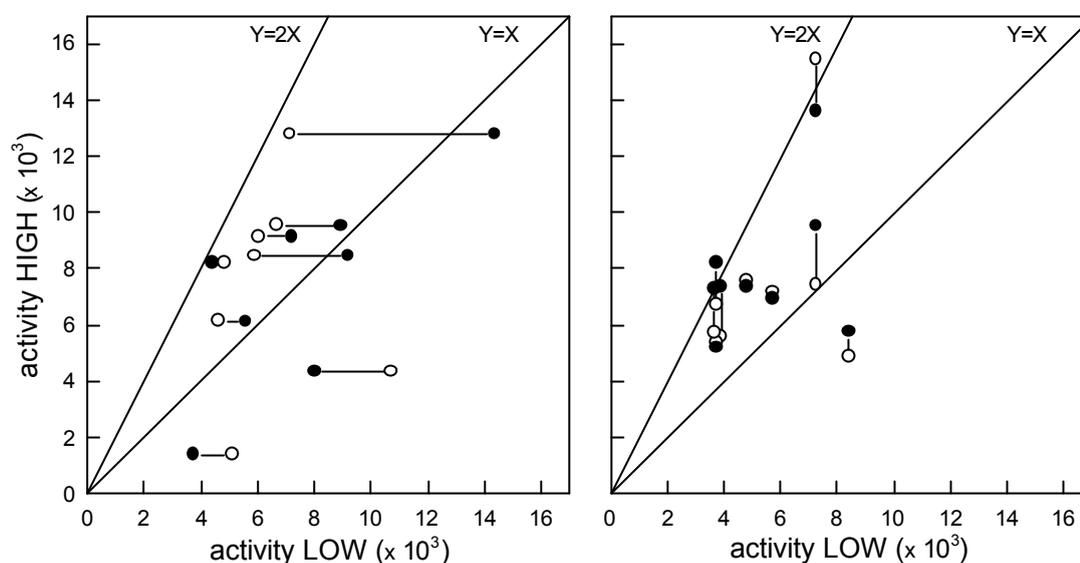


Figure 1. Individual mean daily flight activity on the high and the low workload schedule, averaged over a workload episode of four weeks. Activity in the first episode is represented by the open symbols, in the third episode by filled symbols. The line ($Y=X$) represents equal activity under both workloads. The data points of three birds below the $Y=X$ line indicate a disproportional low activity rates (in relation to food intake) on the high workload.

RESULTS

Working for food

Comparison of episode one and three Prior to analysis of the results of the two workloads, we needed to compare the results from the first and the third episode, when the birds were exposed to the same workload. Only if performance of the birds did not differ between these episodes, we could disregard the episode effect, pool the data, and concentrate on effects imposed by the workloads only. We measured the variables 'activity' (= number of flights between the two perches per day), 'food' (= amount of food removed from the feeder, g per day), and 'mass' (= mean body mass during work, g).

Mean values for activity, food intake and body mass in both episodes are listed in Table 1. Neither activity, nor body mass differed significantly between the two episodes, though individual variation was considerable. The same applied to food intake on the high workload. Only food intake on the low workload was higher in the third episode.

Activity We averaged data from episode one and three to compare activity on the high workload with activity on the low workload. Most zebra finches increased their activity on the high workload in comparison to the low workload ($t_{13}=-5.72$, $P<.001$), though not twofold, as we had expected (Figure 1). Three birds showed opposite results, due to

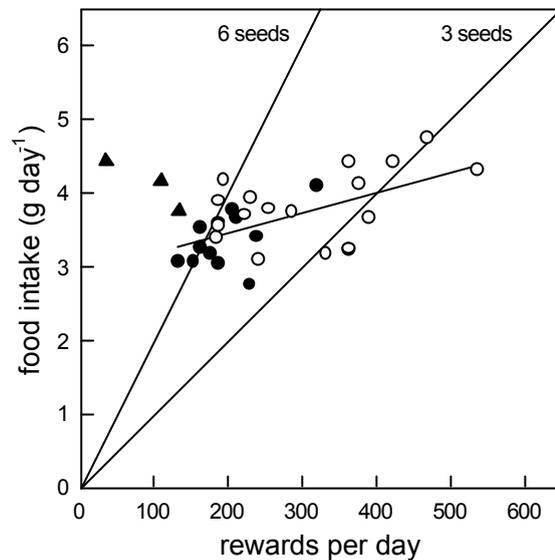


Figure 2. Mean daily food intake in relation to maximum number of rewards earned, given the daily flight activity and the current workload, and assuming 100% efficiency (see text). The area between the lines gives the possible food intake, based on the observed range of number of seeds taken per feeding bout. Open symbols represent birds on the low workload, filled symbols birds on the high workload. Triangles indicate three birds with a disproportional high food intake (in relation to flight activity) on the high workload (see also Figures 1 and 4). The regression line is described by the equation: $\text{Food} = 2.7 \cdot 10^{-3} \text{ rewards}$, $r^2 = 0.34$, $n = 31$, $P < .001$.

relatively low levels of activity on the high workload, while food intake was (above) normal (4.11 g, $sd = 0.34$, $n = 3$), and were thus excluded from further analysis. Mean daily activity was 3675 ($sd = 2045$, $n = 3$) without a workload, 6158 ($sd = 2167$, $n = 17$) on the low workload, and 8393 ($sd = 2545$, $n = 14$) on the high workload ($t_{13} = -5.72$, $P < .001$). This amounted to a duration of activity of about $0.6 \text{ h} \cdot \text{day}^{-1}$, $1.0 \text{ h} \cdot \text{day}^{-1}$, and $1.4 \text{ h} \cdot \text{day}^{-1}$ respectively, based on the maximum number of flights per two-minute interval observed, which converted to a flight speed of 0.6 sec per flight (= 56cm).

The increase in activity on the high workload when compared to the low workload, was smaller than expected and may be ascribed tentatively to the birds performing more efficiently on the high workload. Higher efficiency might have been achieved for example by a reduced surplus of activity over the activity required for feeding, by a larger food intake per feeding interval, or by a combination of both. We visualized efficiency by comparing recorded food intake with maximum number of rewards. The maximum number of rewards was calculated from the observed activity, assuming no more than 20 (low workload), or 40 (high workload) flights in between feeding bouts (= 100% efficiency). We had observed a range of three to six pecks per feeding interval of 10 sec, and, assuming that each peck resulted in one seed being ingested, we calculated the possible range in maximum food intake in relation to activity (Figure 2). Birds with high levels of activity were increasingly less efficient.

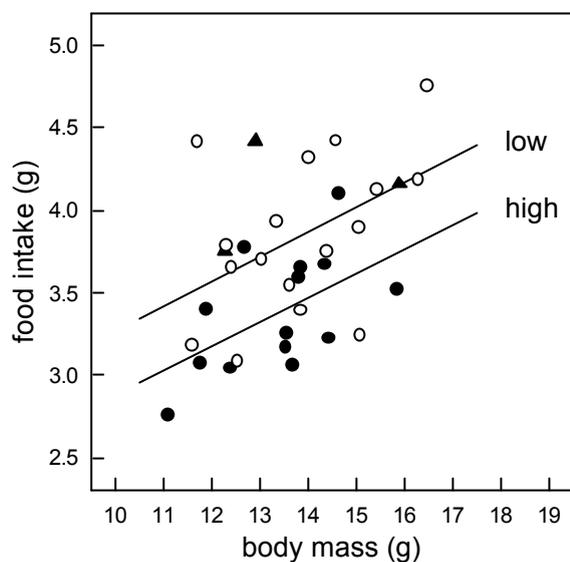


Figure 3. Mean daily food intake as a function of average body mass per workload schedule. The regression lines for the two workloads are drawn: Food = 2.17 + 0.15 mass - 0.4 workload (low=1, high=2), $r^2=0.42$, $n=31$, $t_{\text{mass}}=2.97$, $P<.01$, $t_{\text{workload}}=-2.84$, $P<.01$. Open symbols represent birds on the low workload, filled symbols birds on the high workload. The triangles indicate the three birds with a disproportional high food intake (in relation to flight activity) on the high workload (see also Figure 1).

Body mass and food intake Contrary to *a priori* expectations, food intake on the high workload ($3.38 \text{ g}\cdot\text{day}^{-1}$, $\text{sd}=0.36$, $n=14$) was less than on the low workload ($3.84 \text{ g}\cdot\text{day}^{-1}$, $\text{sd}=0.48$, $n=17$; $t_{13}=4.64$, $P<.001$). Body mass of zebra finches on the two workloads differed significantly, with a lower mass on the high workload (Low: 13.85 g , $\text{sd}=1.50$, $n=17$; High: 13.38 g , $\text{sd}=1.29$, $n=14$; $t_{13}=3.75$, $P<.01$). The observed reduction in body mass was anticipated, and complied with the data on food intake. Food intake was positively associated with body mass (Food, $\text{g}\cdot\text{day}^{-1} = 1.26 + 0.17 \text{ mass, g}$, $r^2=0.25$, $P<.01$, $n=31$; Figure 3). In a multiple linear regression model, 52% of the variation in food intake could be explained by body mass and activity of the bird, and by the imposed workload (Table 2).

Later reproduction

To analyze timing and clutch size of reproductive events following each episode of work, again we first compared the data from episode one and three, and then tested differences between the two workloads. The first comparison also served to test for non-recoverable costs of reproduction. Paired sample sizes were too small to analyze differences between episode one and three separately for the two workloads (low: 3 pairs, high: 5 pairs), therefore the data of both workloads were pooled. Neither clutch size (mean difference = 0.5 egg, $t_7=1.32$, ns), nor brood size at hatching (mean difference = 0.25 young, $t=0.39$, ns) differed significantly between the first and third

Table 2. Multiple linear regression model on the observed daily food intake of zebra finches working for food on two different workloads. All first order interaction terms between the independent variables were dropped from the model.

	(increase in) deviance	(increase in) df	P	coefficient	se
null model	7.03	30	<.001		
final model	3.34	27	<.001		
constant		1		2.059	0.694
workload	1.82	1	<.001	-0.533	0.144
mass (g)	1.09	1	<.01	0.139	0.047
activity ($\cdot 10^{-3}$)	0.73	1	<.02	0.068	0.028

episode. This implied that we could not show any non-recoverable effect of work on fecundity. The interval till laying of the first egg seemed smaller in the third episode of the experiment (mean difference = 5.9 days, $t_7=2.09$, $n=8$, $P=.08$), but the difference was not significant, and therefore we averaged the data over the two episodes.

Mean clutch size, initial brood size and interval till laying following an episode of work on the low and the high workload, are listed in Table 3. One pair failed to produce a clutch following the low workload. A within-pair comparison of these reproductive parameters yielded no significant differences between the workloads in clutch size and initial brood size of the subsequent reproductive event (Table 3). However, the interval till laying did differ between the workloads (Table 3, Figure 4). Most pairs showed a prolonged interval to the start of reproduction after an episode of work on the high workload, with an extra delay of 5.9 days on average.

Potential causes of time cost other than work

We showed before that not only activity, but also body mass at the low and the high workload differed significantly, and that there were considerable individual differences. Therefore, we tested whether activity and body mass were associated with subsequent reproductive decisions. For both sexes separately, deviations of the interval from the mean per workload (δ interval), were related to both activity and body mass in the preceding episode of work. Males showed a weak correlation of δ interval with previous activity (δ interval = $-5.78 + 0.82 \cdot 10^{-3}$ activity, $r^2=0.27$, $n=15$, $P<.05$). However, one male, with a work level of 12,790 flights and the longest subsequent interval (28 days), determined the significance of the relationship. There was no relation of δ interval with previous activity for females (linear regression, $r^2=0.07$, $n=17$, ns).

To breed after an episode of work, the birds may be expected to first have to restore body condition. Because body mass was lower while working on the high workload than on the low workload, time needed to restore mass might have been

Table 3. Interval till breeding, clutch size and brood size following work on the low or the high workload. One pair failed to produce a clutch after an episode on the low workload, leaving 8 pairs of which to compare the data. Means and standard deviation of the variables are given; test results from paired t-tests (episode 1 and 3 combined) are included.

workload:	LOW	HIGH	T_7	P
Laying interval (days)	8.6±1.9	14.5±6.1	3.45	=.01
Clutch size	3.4±1.0	2.9±0.9	1.51	>.10
Initial brood size	2.4±1.5	2.1±1.2	0.51	>.10

proportionally longer after completion of the high workload. There are two aspects of the body mass data which argue against such a role for restoring body mass following a workload episode. First, in a multiple regression model, controlling for individual and workload, the interval till laying was not related to body mass at the end of an episode of work (Male mass: coefficient=6.76, $F_{1,7}=3.10$, ns; Female mass: coefficient=-1.45, $F_{1,7}=0.06$, ns). Second, males did not seem to gain weight gradually before the onset of laying ($\delta\text{mass} = 0.21 + 0.01 \text{ days}$, $r^2=0.02$, $n=65$, ns), though females did ($\delta\text{mass} = 0.70 + 0.05 \text{ days}$, $r = 0.21$, $n=65$, $P<.001$; Figure 5). The increase in female body mass over the last four days prior to laying (mean mass 14.85 g, $sd=1.80$, versus 14.03 g, $sd=1.76$, earlier, $t_8=-6.67$, $P<.001$) could be attributed tentatively to development of eggs (Houston, Donnan & Jones 1995b). There was no change in body mass of females before those last four days prior to laying ($\delta\text{mass} = 0.16 + 0.01 \text{ days}$, $r^2=0.01$, $n=46$, ns).

DISCUSSION

Hard work delays subsequent reproduction

The central idea of this experiment was to study the effects of manipulated levels of activity on later reproduction. We successfully created two levels of daily flight activity, and we thereby imposed two levels of work on the experimental birds. Most pairs readily started a clutch after an episode of work (25 out of 27 trials). Previous work on a higher level delayed the onset of reproduction with on average 6 days (range 1 to 16), representing a recoverable fitness cost of hard work. The experiment yielded no evidence for non-recoverable costs of hard work in terms of reduced fertility: clutch sizes did not differ between consecutive episodes, or between workloads.

Potential causes: ethological aspects

We designed the experiment to test the effect of daily activity *per se* on later reproduction. The setup of the experiment does not allow to identify how the effect came about. As a result of the design of the experiment that was based on reward ratios, there may have been an ethological cost factor, the partial reinforcement extinction effect (PREE). PREE is a well-known effect in animal learning, and it is based on the

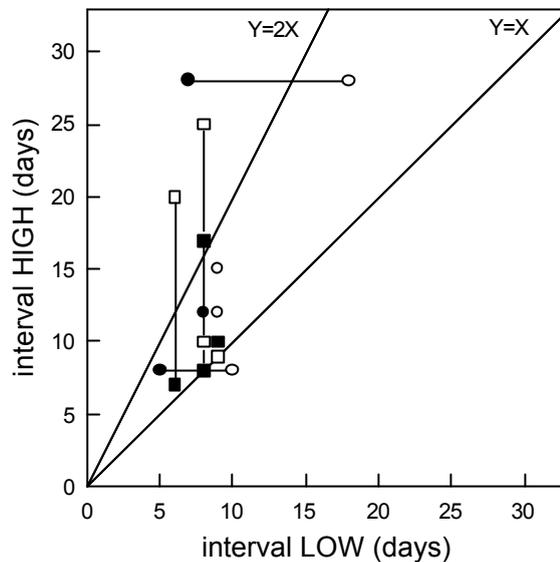


Figure 4. Average interval till laying of individual pairs on the high versus the low workload. The line ($Y=X$) represents equal intervals after completion of the workload schedules. The open symbols represent data from the first episode, the filled symbols represent the third episode.

incomplete information model concerning the food supply (McNamara & Houston 1980), changing from 'good' ($p\%$ rewards) to 'bad' (no rewards). It predicts that if the probability of a reward is low, it takes longer (= more trials) to convince the animal that the source has run out (no or fewer rewards). In general terms, a higher similarity between the prior and posterior situations makes discrimination more difficult, and thus time-consuming (Stephens & Krebs 1986).

In our experiment, however, we deal with a situation in which the food supply changes from 'bad' (two levels) to 'good'. Essentially, the probability of a reward (= feeder unlocked) on the high workload, is half the probability on the low workload. The similarity between the experimentally manipulated situation ('bad') and the unrestricted situation after completion of the workload episode ('good'), is higher for the low workload than for the high workload. Therefore, after completion of the low workload, it is predicted that it should take longer for the birds to realize that the food availability has changed, and that the new conditions allow breeding. Thus, the argument of a PREE predicts a result opposite of the observed effect. Moreover, though McNamara & Houston (1980) did not tabulate the probability of a change at high similarities, which we need to estimate the number of trials that is required to detect the change, this number may be well within the range of trials attained in one day (100-500).

Potential causes: physiological aspects

What indications do we have from this experiment for physiological pathways through which the detrimental effects of DEE on fitness may have come about? First, we should

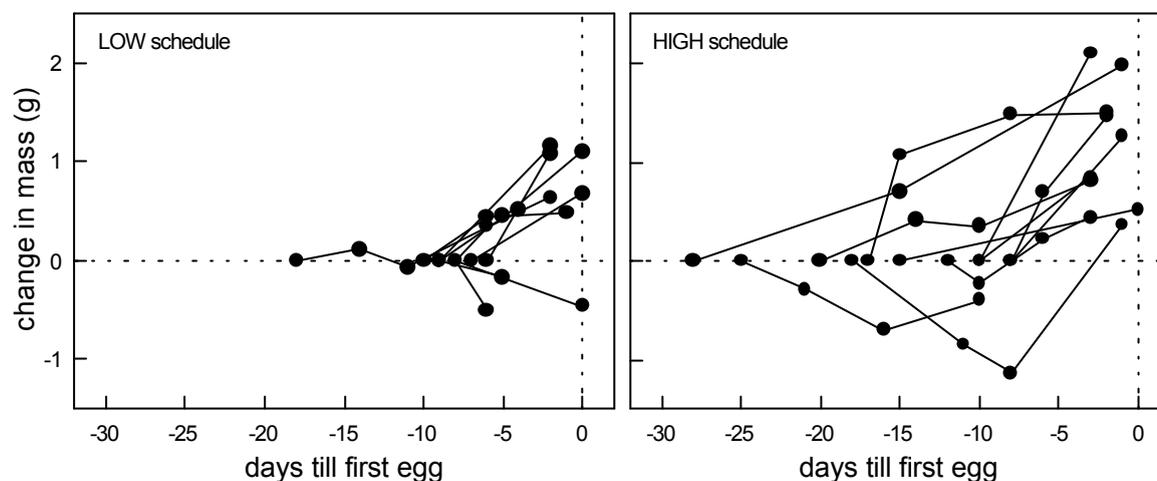


Figure 5. Observed change in female body mass during the interval till laying, relative to the individual's mass at the end of the preceding episode of work. Repeated measures (max. 4) of the same bird are connected by lines.

consider potential responses of the birds to the imposed levels of work. Responses have been categorized as primary, secondary and tertiary effects, and include release of hormones, depletion of nutrient reserves and impaired reproduction and/or increased vulnerability to disease respectively (Koehn & Bayne 1989). We did not measure anything that may be categorized as primary effects. The observed reduction in body mass on the high workload comes under the category of secondary effects, and may be a symptom of reduced allocation of resources to maintenance. In a cascade of effects, a bird that reduces its body mass during hard work may need more time to restore its condition afterwards. The extra time may then be reflected in a delayed start of reproduction following episodes of work, which is an effect of the tertiary category. However, there was no evidence in this experiment that the birds gained weight gradually until their body condition allowed reproduction.

Another pathway may have been through a primary effect of an increased release of free radicals (Sibly & Calow 1989), inducing secondary effects consisting of somatic, cellular or molecular damage. The observed time cost effect of the tertiary category may be explained in this case by an extended recovery phase, when energy will be allocated preferably to expensive (Sibly & Calow 1989) repair processes. During recovery, other activities with a high demand of energy, such as reproduction, may be put on hold temporarily. An additional argument for this hypothesis of a trade-off between allocation of energy to repair and reproduction following hard work, is provided by the observation of a time lag before increase in body mass in females (developing eggs) after completion of the workload episodes.

Work and costs of reproduction

The effects of imposed levels of work on later reproduction agree with results from brood size manipulation experiments, in zebra finches (Chapter 5), as well as in Great Tits (*Parus major*)

(Slagsvold 1984; Tinbergen 1987; Smith, Kalländer & Nilsson 1987; Tinbergen & Van Balen 1988). In all of these experiments, the timing, but not the size of the next clutch was affected. The results from brood size manipulation experiments are open to other explanations, however, such as interference by the experimental brood through duration of parental (post fledging) care, food depletion, or condition of the parents. In the experiment we report on here, opportunities for breeding were equally favourable after work on both the low and the high workload. For the argument of duration of parental care, the situation was thus comparable to the work of Slagsvold (1984), who removed the nestlings before fledging. The food depletion argument is not applicable to our experiment, because food was continuously available *ad libitum*. Working for food (this experiment) and parental work (Chapter 5) affected parental body condition of zebra finches negatively, and in neither experiment a direct association could be shown between body mass during work and subsequent costs of reproduction. We therefore conclude that the prior experimental situation, thus the level of work, delayed the subsequent timing of laying.

The fitness consequences of this delay are expressed in a smaller total amount of broods that can be raised in one season. Towards the end of the reproductive season, for instance, it may not be opportune to start yet another clutch, which would reduce parental survival rates (Bryant 1979; Ekman & Askenmo 1987; Tinbergen 1987).

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