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Evidence for within-individual energy reallocation in cold-challenged, egg-producing birds

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SUMMARY

Recent studies have shown that the metabolic cost of avian egg production involves a 16–27% increase in metabolic rate (MR) above non-reproductive basal or resting values (BMR and RMR, respectively). To determine how the metabolic cost of egg production interacted with the costs of other essential processes (such as cold acclimation and active heat production), we measured the MR of non-breeding and egg-producing zebra finches (*Taeniopygia guttata*) while (a) warm-acclimated (to 19–21°C) and measured within their thermoneutral zone (at 35°C), (b) cold-acclimated (to 7°C) and measured at thermoneutrality (at 35°C, i.e. not actively producing heat), and (c) cold-acclimated and measured below thermoneutrality (at 7°C) (i.e. during active heat production). The metabolic cost of egg production was small (24% above BMR) compared with the additive costs of cold acclimation and active heat production (224% above BMR). Exposure to low ambient temperatures was accompanied by an increase in seed consumption (by 72%) and a decrease in locomotor activity (by 72%) compared with warm-acclimated, non-breeding values. By contrast, egg production in heat-producing females was associated with an 11% decrease in MR and a 22% decrease in seed consumption compared with non-breeding thermoregulating values. Our data suggest that while the increase in MR associated with egg production is small in relation to the birds' capacity to increase MR in response to other energetically demanding processes, the addition of egg production to these metabolically costly activities may be enough to necessitate the use of energy-saving strategies, such as internal energy reallocation, to cope with the additional energetic demands.

Key words: egg production, cold acclimation, metabolic rate, thermoregulation, energy reallocation, *Taeniopygia guttata*.

INTRODUCTION

Egg production in birds is a complex process that has measurable energetic costs. Indeed, in passerine birds the physiological changes associated with egg formation (Williams, 1998; Johnson, 2000) have been shown to result in a 16–27% increase in metabolic rate (MR) above that of non-breeding females [house sparrows, *Passer domesticus*, 16% above non-breeding basal metabolic rate (BMR) (Chappell et al., 1999); great tits, *Parus major*, 27% over wintering BMR (Nilsson and Raberg, 2001); European starlings, *Sturnus vulgaris*, 22% over pre-reproductive BMR (Vézina and Williams, 2002; Vézina and Williams, 2003); zebra finches, *Taeniopygia guttata*, 22% above non-breeding resting metabolic rate (RMR) (Vézina and Williams, 2005b)]. This metabolic investment in egg production is comparable with the changes in MR associated with other reproductive activities. For example, in comparison with non-breeding values, incubating zebra finches exhibit a 20% increase in RMR (Vleck, 1981), while the RMR of chick-rearing individuals is 13% higher (Vézina and Williams, 2005b). However, it is unclear whether the changes in MR associated with reproduction are substantial in the context of the birds' ability and capacity to upregulate metabolism, i.e. how the metabolic costs of reproduction (egg production, incubation and chick rearing) interact with those of other, potentially energetically demanding, physiological processes that occur throughout a bird's lifetime.

Reproduction in seasonally breeding birds is generally timed such that the period of chick rearing coincides with favorable environmental conditions [i.e. warmer weather, increased food availability and quality (Williams, 1998)] so that nestlings have ample nutrients and resources for their rapid growth and development. However, the egg-laying period necessarily precedes the period of chick rearing. Consequently, free-living birds are often faced with the energetically demanding task of producing, laying and incubating eggs while being simultaneously exposed to relatively colder temperatures and more unstable weather conditions earlier in the breeding season (Perrins, 1970). This means that early breeding individuals may face conflicting demands due to the simultaneous need for energy to fuel reproduction and cold acclimatization. In cold-acclimated or acclimatized birds, energy expenditure related to life in the cold can be divided into two components. First, there are the costs associated with cold acclimation itself, i.e. changes in thermoregulatory physiology and maintenance of tissues involved in heat production or fueling heat production. In several cases, although this may not be true for large species, this results in a mean increase in BMR up to 50% due to elevated physiological maintenance costs (reviewed in Swanson, 2009). To the best of our knowledge, the highest reported increase in BMR in response to cold challenge was 85% in siskins *Carduelis spinus* (Gelineo, 1964). Second, there is the cost of active heat production, which is apparent when the animals face substantial heat loss that is not fully compensated by other physiological heat sources such as digestion (Biebach, 1984; Meienberger and Dauberschmidt, 1992; Chappell et al., 1997; Bech and Praesteng, 2004) or locomotor activity (Webster and Weathers, 1990; Bruinzeel and Piersma, 1998). In a resting state, the cost of active heat production corresponds to the increase in MR measured at ambient temperatures below thermoneutrality and is proportional to body heat loss (Blaxter,

1989). One way individuals can modulate heat loss in response to varying environmental conditions is to adjust their thermal conductance, i.e. the heat flow between an individual and its surroundings. This is done by (1) raising the feathers to trap and hold air, thereby increasing the thickness of the insulative barrier above the skin, (2) reducing peripheral blood flow; thus, decreasing exposure of warm blood to cold ambient temperatures, and (3) minimizing evaporative heat loss (Johansen and Bech, 1984; Kadoya et al., 1985; Schmidt-Nielsen, 1990; Alexander, 1999; McNab, 2002; Jofré and Caviedes-Vidal, 2003).

The first goal of this study was to determine how the metabolic cost of egg production compares with the investment in other essential and metabolically demanding activities associated with life in the cold, namely cold acclimation and active heat production. A second objective was to determine whether the energetic costs of cold acclimation, active heat production and egg production were additive when these activities occurred simultaneously. Our third goal was to assess the magnitude of inter-individual variation in MR and behavioral modification associated with these energetically demanding activities. We also considered potential differences in wet thermal conductance at 7°C between reproductive stages and thermal treatments to determine whether they could explain part of the variation in the measured energy expenditure associated with cold acclimation, active heat production and egg production. Working in a controlled laboratory environment, we measured and compared the MRs and thermal conductances of female zebra finches in a variety of thermal and reproductive states.

MATERIALS AND METHODS

Animals and husbandry

Zebra finches, *Taeniopygia guttata* Vieillot 1817, with previous breeding experience (i.e. produced at least one previous clutch) were randomly chosen from our breeding colony housed in the Simon Fraser University Animal Care Facility, Burnaby, BC, Canada. All birds were housed in cages (61 cm × 46 cm × 41 cm), exposed to a constant light schedule of 14 h:10 h L:D (lights on at 09:00 h) and ambient temperatures ranging from 19 to 21°C, and provided with a mixed-seed diet (Panicum and white millet, 50:50; approximately 12.0% protein, 4.7% lipid; Just for Birds, Surrey, BC, Canada), water, grit and cuttlefish bone (calcium) *ad libitum*. All experiments and animal husbandry were carried out under a Simon Fraser University Animal Care Committee permit (no. 692B-94) following guidelines of the Canadian Committee on Animal Care.

Acclimation protocol and non-breeding MR measurements

The male and female zebra finches that were chosen for this study were divided into two study groups: a warm-acclimated (19–21°C) group and a cold-acclimated (7°C) group. Females in the warm-acclimated group (N=10; initially housed as N=5 per cage) were weighed (±0.1 g) and randomly assigned to same-sex pairs (N=2 birds per cage) in 'warm' ambient conditions (i.e. temperature 19–21°C) for a minimum of seven days. Males in the warm-acclimated group (N=10) were group housed in same-sex cages (N=5 birds per cage) under the same environmental conditions as the warm-acclimated females. By contrast, birds in the cold-acclimated group (N=12 females and 12 males) were weighed (±0.1 g) and transferred to same-sex group cages (N=6 birds per cage) within a Conviron E15 plant growth chamber (Controlled Environments, Winnipeg, MB, Canada). The temperature within the chamber at the beginning of the acclimation period was 14°C, and was decreased slowly over four weeks (i.e. one week at 14°C, one week at 10°C, then two weeks at 7°C). The acclimation period was based on the time it took for all birds to return to and maintain their pre-acclimation body mass at each temperature. Following the four-week acclimation period, cold-acclimated females were randomly assigned to same-sex pairs (N=2 birds per cage) for a minimum of seven days. All warm- and cold-acclimated, same-sex female pairs were visually but not acoustically isolated from the opposite sex. The MR (see protocol below) of each female was measured twice, once while the females were in same-sex, non-breeding pairs (NBr sample), and again while the females were paired with males and actively laying eggs (LAY sample). MR measurements began on the seventh night following same-sex pairing and continued nightly until all females were measured.

Breeding protocol and laying MR measurements

Following measurement of non-breeding MR, all females were randomly paired with males acclimated to the same thermal conditions. One warm-acclimated female died prior to pairing for breeding. Therefore, all data from this female were excluded from the analyses. Males and females were weighed (±0.1 g) at the time of pairing and housed in cages equipped with an external nest box (15 cm × 14.5 cm × 20 cm). Breeding pairs were provided with 6 g of an egg-food supplement [20.3% protein, 6.6% lipid (see Williams, 1996)] daily between pairing and clutch completion. This food supplement was always completely consumed by the next day. Nest boxes were checked daily between 09:00 h and 11:00 h. The MR (see protocol below) of each egg-laying female was measured during the night following the laying of the second egg. Female zebra finches in our captive breeding colony lay a mean of 6 eggs while housed at 21°C and 5.5 eggs while housed at 7°C (Salvante et al., 2007). Therefore, the MR measurement coincided with albumen and shell formation of the third egg and the rapid yolk development period of the remainder of the clutch. Clutches were considered complete if no new eggs were laid over three days. At this time each pair was returned to same-sex cages.

Locomotor activity and seed consumption

The locomotor activity of all same-sex and breeding pairs was monitored using a micro-switch system connected to a cage perch as described by Williams and Ternan (Williams and Ternan, 1999). This system does not discriminate potential differences in locomotor activity between members of a pair, but previous work by Williams and Ternan (Williams and Ternan, 1999) involving direct observations of activity showed that, at 19–21°C, activity does not differ between sexes throughout the breeding period from non-breeding to the end of egg laying. Therefore, we assumed that, locomotor activity did not differ between members of same-sex and breeding pairs when exposed to either of our acclimation temperatures. Locomotor activity data for one warm-acclimated, non-breeding, same-sex pair and two warm-acclimated breeding pairs were lost due to switch failure.

To obtain a gross estimate of energy intake by cold-acclimated birds, seed consumption was measured by providing all pairs with 30.0 g of the mixed-seed diet daily in open Ziploc™ (Brantford, ON, Canada) containers (946 ml) placed on the cage floor. This method avoided any spillage and allowed for the measurement of daily seed consumption by same-sex and breeding pairs by weighing the remaining seeds in the container after 24 h (±0.1 g). Birds were able to feed *ad libitum* as 30.0 g of seed was always in excess of their daily intake. Williams and Ternan showed that, at 19–21°C, females consistently ate slightly more food (4.5%) than males, regardless of their breeding status, and that this effect did not change across the laying sequence, from non-breeding to the end of egg

laying (Williams and Ternan, 1999). Therefore, we assumed that measuring food intake per pair was a good indicator of female food intake at both of our acclimation temperatures. Seed consumption for cold-acclimated, same-sex and breeding pairs were compared with published data for warm-acclimated zebra finch pairs that were maintained in the same facility, under the same conditions as the warm-acclimated pairs in this study (Vézina et al., 2006b).

Measurement of MR

BMR is defined as the energy consumed by a resting, non-growing, post-absorptive animal during the inactive phase of the circadian cycle at a temperature within the thermoneutral range (Commission for thermal physiology of the International Union of Physiological Sciences, 2001), and can be applied to the MR of the non-breeding females in this study. However, birds were also measured during egg production, and we considered these laying females to be in an 'active physiological state' (Vézina and Williams, 2002; Vézina and Williams, 2005b). To avoid any confusion in terminology, we therefore refer to 'non-breeding MR' (which is in fact BMR) and 'laying MR' in the following text.

All MR measurements were completed using a flow-through respirometry system (Sable Systems International; Las Vegas, NV, USA) as follows. At 21:00 h, three hours prior to the beginning of measurement, the food was removed from the cages of females undergoing metabolic measurement that night. At 23:00 h, warm-acclimated females were taken from their cages, their body masses were measured (±0.1 g), and they were placed randomly in one of four metabolic chambers (1.5 l stainless steel coffee canisters, Great Canadian Superstore, Coquitlam, BC, Canada) for one hour prior to the beginning of measurements. All chambers continuously received approximately 500 ml min^{-1} of dry CO_2-free air (using Drierite™ and ascarite™ as scrubbers; Sigma-Aldrich, Oakville, ON, Canada; mean flow 518.37±0.99 ml min^{-1}; Sierra Instruments 810C-NR-2 mass flow valve, Sierra Instruments, Monterey, CA, USA; Sable Systems MF-8 Airflow Manifold and 2-channel mass flow controller version 1.0) and were maintained at 35°C, which is within the thermoneutral zone for this species (lower critical temperature=33°C) (Marschall and Prinzinger, 1991; Meijer et al., 1996). Our respirometry system consisted of four metabolic chambers placed into a temperature-control cabinet (Sable Systems PTC-1 Peltier effect temperature-controlled portable cabinet), which was programmed to maintain the ambient temperature at 35°C throughout the night using a Peltier controller (Sable Systems PELT-3). Each chamber was connected to a divided air line with a valve multiplexer (Sable Systems TR-TM4), which allowed us to sample air coming from either an open, ambient baseline air line (scrubbed for water and CO_2) or from one metabolic chamber at a time. The air then passed through a mass flow valve (Sierra Instruments) for proper air flow reading [standard temperature and pressure (STP) corrected] and through CO_2 and oxygen analyzers (model CA-1 and FC-1 Sable Systems, respectively; air was scrubbed to remove water before entering the CO_2 analyzer and scrubbed to remove water and CO_2 before entering the O_2 analyzer). Measurements were always started at 00:00 h. The measurement sequence was as follows: baseline air was recorded for 10 min, then the out-flowing air from the first chamber for 33 min and then the second chamber for 33 min, then baseline air again for 10 min, followed by the out-flowing air from the third chamber for 33 min and then the fourth chamber for 33 min, and finally ending with baseline air for 10 min. This sequence was repeated three times throughout the night giving 99 min of recording per chamber spanning 8 h. After measurement the birds were weighed for a second time and released back into their cages (approximately 30 min to one hour before the lights were turned on), and their food was returned. The mean of first and second body masses was used in subsequent analyses. The oxygen consumption ($\dot{V}_{O_2}$) of each bird was calculated using eqn 4a from Withers (Withers, 1977) integrated in the Datacan software program (Sable Systems):

$$\dot{V}_{O_2} = \frac{\dot{V}_E (F_{I_{O_2}} - F'_{E_{O_2}})}{1 - F_{I_{O_2}} + RQ(F_{I_{O_2}} - F'_{E_{O_2}})}, \quad (1)$$

where $\dot{V}_{O_2}$ is the rate of oxygen consumption corrected to STP, $\dot{V}_E$ is the rate of airflow out of the chamber, F_{IO2} is the fractional concentration of O_2 entering the chamber, F'_{EO2} is the fractional concentration of O_2 exiting the chamber after CO_2 has been removed, and RQ is the respiratory quotient, which was fixed at 0.8. A running mean representing 10 minutes of recording was passed through the data for each bird, with the lowest mean taken as non-breeding or laying MR. This value was always found during the second or third rounds of measurement (i.e. during the last 5 h of the night). Preliminary analysis showed that our sequential respirometry protocol did not generate a time effect (*sensu* Hayes et al., 1992) on metabolic variables.

MRs of cold-acclimated females were measured using the same protocol as described above with the following exceptions. Firstly, females were placed randomly in one of two metabolic chambers (1.5 l) for one hour prior to the beginning of measurements. The temperature was maintained at 7°C for the first part of the night, and then increased to and maintained at 35°C for the remainder of the measurement period by placing the metabolic chambers into the pre-programmed temperature-control cabinet. The measurement sequence was as follows: starting at 00:00 h, baseline air was recorded for 2 h and 40 min, then the out-flowing air from the first chamber for 45 min, baseline air again for 15 min, and then the out-flowing air from the second chamber for 45 min, all at 7°C. Following the first set of measurements, the temperature was increased to 35°C, which took approximately 40 min. Once 35°C was maintained, birds were allowed to adjust to the new temperature for approximately 55 min. Baseline air was recorded while the temperature was increased to 35°C and during the adjustment period. The measurement sequence was then repeated (i.e. baseline during the adjustment period, chamber 1 for 45 min, baseline for 15 min, chamber 2 for 45 min) and, finally, baseline air was recorded for the last 15 min. This measurement protocol was designed such that the air recordings from the test chambers occurred during the last 5.5 h of the night, and is therefore comparable with the protocol for warm-acclimated females (described above), wherein air recordings occurred in the last 5 h of the night during the second and third rounds of MR measurement. $\dot{V}_{O_2}$ at each temperature (i.e. 7°C and 35°C) was calculated using the protocol described above.

Measurement of thermal conductance

To determine whether thermal conductance at 7°C changed in response to cold acclimation, egg production or a combination of the two, we randomly chose 44 female and 12 male zebra finches with previous breeding experience (i.e. produced at least one previous clutch) from our breeding colony. The male and female zebra finches were divided into two temperature-acclimation groups: a warm-acclimated (19–21°C) group (N=24 females, N=6 males) and a cold-acclimated (7°C) group (N=20 females, N=6 males). Warm- and cold-acclimation followed the same protocol as described above. Following the acclimation period, the MR of each female was measured only once, either while the females were in same-sex, non-breeding pairs (N=18 warm-acclimated NBr, N=14

Table 1. Comparisons between experimental treatment groups for analysis of metabolic costs

Metabolic cost of:	Comparison between:		*t*	d.f.	*P*-value
Egg production	Warm-acclimated NBr–35	Warm-acclimated LAY–35	–2.90	4.9	*P*<0.04
Cold acclimation	Warm-acclimated NBr–35	Cold-acclimated NBr–35	–5.13	18.0	*P*<0.0001
Heat production	Cold-acclimated NBr–35	Cold-acclimated NBr–7	–9.66	32.9	*P*<0.0001
Heat production while producing eggs	Cold-acclimated LAY–35	Cold-acclimated LAY–7	–8.81	32.9	*P*<0.0001
Cold acclimation and heat production	Warm-acclimated NBr–35	Cold-acclimated NBr–7	–15.31	18.0	*P*<0.0001
Egg production while cold-acclimated, not producing heat	Cold-acclimated NBr–35	Cold-acclimated LAY–35	1.73	35.3	*P*>0.09
Egg production while producing heat	Cold-acclimated NBr–7	Cold-acclimated LAY–7	2.56	35.3	*P*<0.015
Egg production, cold acclimation and heat production	Warm-acclimated NBr–35	Cold-acclimated LAY–7	–16.91	17.0	*P*<0.0001

Group names indicate temperature-acclimation group (warm- or cold-acclimated), reproductive stage (NBr=non-breeding; LAY=egg laying) and ambient temperature at which metabolic rate measurements were taken (35°C or 7°C).

cold-acclimated NBr) or while the females were paired with males and actively laying eggs, following the breeding protocol described above (*N*=6 warm-acclimated LAY, *N*=6 cold-acclimated LAY).

MRs of warm- and cold-acclimated females for measurement of thermal conductance at 7°C were measured using the same protocol as warm-acclimated females described above with the following exceptions. Ambient temperature during MR measurement was maintained at 7°C for the entire night. Additionally, following MR measurement, the body temperature of each female was measured by inserting a copper constantan thermocouple probe (3 mm diameter; attached to a Sable Systems TC-1000 Thermometer NIST traceable and set to fast response) into the female's cloaca within 30 s of removing the female from the metabolic chamber. We calculated wet thermal conductance (C, ml $O_2\,h^{-1}\,°C^{-1}$) of each bird at 7°C using the following equation: $C=\dot{V}_{O2}/(T_b–T_a)$, where $\dot{V}_{O2}$ is the rate of oxygen consumption (ml $O_2\,h^{-1}$), T_b is body temperature (°C) and T_a is ambient temperature (in this case, 7°C) (McNab, 1980; Aschoff, 1981). Our measurements of thermal conductance reflect overall heat loss at 7°C, including heat lost by evaporation of water [i.e. wet thermal conductance (Schleucher and Withers, 2001)]. Evaporative heat loss represents a small component of total heat loss and remains relatively constant below thermoneutrality (Schleucher and Withers, 2001).

Data analysis

The metabolic costs of the various activities (e.g. egg production, cold acclimation, heat production) and combinations of these activities (e.g. egg production, cold acclimation and heat production) were measured by performing the comparisons in Table 1. Because daily energy budgets are dependent on the amount of energy taken in and expended, similar comparisons were made to determine how daily seed consumption and locomotor activity were affected by egg production and thermoregulation (Table 2).

All statistical analyses were performed using either SAS (version 9; SAS Institute, Cary, NC, USA) or JMP (version 7.0.2; SAS Institute). All data were tested for normality [Shapiro-Wilk test (Zar, 1996)], and all non-normal variables were $\log_{10}$-transformed prior to analysis (although some non-transformed values were used for graphical purposes). We compared MRs, locomotor activity and seed consumption (1) within the acclimation groups, between different reproductive stages (e.g. non-breeding *vs* laying) or (2) within and between the warm- or cold-acclimation group, between different reproductive stages measured at different temperatures (e.g. non-breeding MR measured at 35°C *vs* laying MR measured at 7°C). To perform these analyses, we used mixed model, repeated-measures analysis of variance (ANOVA) or analysis of covariance (ANCOVA) (with female body mass as a covariate) with stage (i.e. reproductive stage or 'reproductive stage–measurement temperature') as a fixed, repeated factor and individual female as a random factor [PROC MIXED (SAS Institute)]. Comparisons between acclimation groups (i.e. warm *vs* cold) were examined using *t*-tests or ANCOVA (with female body mass as a covariate). Comparisons of thermal conductance among thermal-reproductive stage groups were performed using ANCOVA with female body mass as a covariate.

In the main study, MRs of all females were measured twice, first as non-breeders and then again during egg laying. However, females in the thermal conductance study were only measured once, either as non-breeders or during egg laying. Therefore, to test whether the decrease in MR observed in cold-acclimated females between the non-breeding and egg-laying periods (see Results below) can be attributed to an order effect, we used an ANCOVA (with female body mass as a covariate) to compare MR measured at 7°C of the cold-acclimated, egg-laying females from the two studies.

All tests were two-tailed, and the overall significance level was $P<0.05$. As one of the main goals of this study was to assess the

Table 2. Comparisons between experimental treatment groups for analysis of locomotor activity and seed consumption

	Comparison between:		*t*	d.f.	*P*-value
Locomotor activity during:					
Egg production	Warm-acclimated NBr	Warm-acclimated LAY	5.84	4.53	*P*<0.003
Cold acclimation and heat production	Warm-acclimated NBr	Cold-acclimated NBr	3.52	12.1	*P*<0.005
Egg production while producing heat	Cold-acclimated NBr	Cold-acclimated LAY	1.75	11.0	*P*>0.1
Egg production, cold acclimation and heat production	Warm-acclimated NBr	Cold-acclimated LAY	4.79	12.0	*P*<0.0005
Seed consumption during:					
Egg production	Warm-acclimated NBr	Warm-acclimated LAY	–0.56	29.1	*P*>0.5
Cold acclimation and heat production	Warm-acclimated NBr	Cold-acclimated NBr	–12.63	35.8	*P*<0.0001
Egg production while producing heat	Cold-acclimated NBr	Cold-acclimated LAY	2.98	11.0	*P*<0.013
Egg production, cold acclimation and heat production	Warm-acclimated NBr	Cold-acclimated LAY	–2.49	13.5	*P*<0.03

Group names indicate temperature-acclimation group (warm- or cold-acclimated) and reproductive stage (NBr=non-breeding; LAY=egg laying).

Table 3. Body mass and metabolic rate of zebra finches in different thermal-reproductive stages

	Warm acclimated		Cold acclimated			
	NBr–35	LAY–35	NBr–35	LAY–35	NBr–7	LAY–7
Female body mass (g)	14.7+0.5 (9) [12.9–16.8]	16.4+0.4 (9) [13.8–18.7]	15.2+0.5 (12) [12.8–18.7]	15.9+0.4 (12) [13.9–17.9]	15.2+0.5 (12) [12.8–18.7]	15.9+0.4 (12) [13.9–17.9]
$\dot{V}_{O_2}$ (ml O_2 h^{-1})	36.11+1.4 (9) [27.20–42.10]	48.39+1.8 (9) [40.64–58.59]	67.80+5.23 (12) [38.70–97.20]	64.06+3.50 (12) [36.39–77.96]	113.60+5.76 (12) [87.50–152.90]	105.85+3.52 (12) [82.18–121.95]
$\dot{V}_{O_2}$ correcting for female body mass (ml O_2 h^{-1})	35.82+1.65 (9)	44.59+1.84 (9)	70.12+3.19 (12)	61.72+3.23 (12)	115.92+3.19 (12)	103.53+3.23 (12)

All values are means ± s.e.m. with sample size in parentheses and minimum and maximum values in brackets, with the exception of rate of oxygen consumption ($\dot{V}_{O_2}$) correcting for female body mass values, which are least-square means ± s.e.m. with sample size in parentheses. Female body mass at each stage was included as a covariate for statistical analysis of metabolic rate. NBr=non-breeding; LAY=egg laying.

extent of individual variation in MR and behavioral energy reallocation to fuel the energy demands associated with thermoregulation and egg production, all data are visually displayed in reaction norm-style graphs with lines joining repeated measures from individuals across thermal-reproductive stages (reviewed in Williams, 2008).

RESULTS

RMR

MRs were positively related to body mass ($F_{1,22.5}$=23.54, P<0.0001). Therefore, female body mass was included as a covariate in all analyses, and the percentage change in MR between comparison groups was calculated using least squares mean MR controlling for body mass at the stages being compared. MR exhibited marked inter-individual variation within the six thermal-reproductive stages (ranging from 1.4-fold variation among warm-acclimated laying females measured at 35°C to 2.5-fold variation among cold-acclimated non-breeding females measured at 35°C; Table 3; Fig. 1) and varied significantly between the six different thermal-reproductive stages ($F_{5,47.9}$=86.53, P<0.0001; Table 1; Fig. 1).

Taken individually, the physiological processes of egg production, cold acclimation and active heat production were all associated with an increase in mass-corrected MR. Egg production and cold acclimation resulted in a 24% and 96% increase in mass-corrected MR, respectively, above that of warm-acclimated, non-breeding females (i.e. BMR) (Tables 1 and 3; Fig. 1). Similarly, active heat production alone induced a 65% and 68% increase in mass-corrected MR over cold-acclimated, non-breeding and cold-acclimated, egg-laying values, respectively (Tables 1 and 3; Fig. 1).

When combined, cold acclimation and active heat production by non-breeding females resulted in a 224% increase in mass-corrected MR above warm-acclimated, non-breeding MR (Tables 1 and 3; Fig. 1). This is comparable with the predicted increase in mass-corrected MR of 212% to 224% (based on laying and non-breeding females, respectively) if the metabolic costs of cold acclimation and heat production were additive [i.e. (cold acclimation + active heat production) / BMR × 100%]. We were not able to detect any significant change in mass-corrected MR associated with egg production in cold-acclimated females that were not actively producing heat (Tables 1 and 3; Fig. 1). Furthermore, egg production during active heat production was actually associated with an 11% decrease in mass-corrected MR compared with cold-acclimated, non-breeding values measured at 7°C (Tables 1 and 3; Fig. 1). As a result, the combination of cold-acclimation, active heat production and egg formation induced a 189% increase in mass-corrected MR above non-breeding, warm-acclimated values (Tables 1 and 3; Fig. 1), well below the predicted 237% to 248% increase (based on laying and non-breeding females, respectively) if the metabolic costs of all three activities were additive.

Locomotor activity and seed consumption

Locomotor activity was not related to female or pair body mass (P>0.05) but did differ between stages ($F_{3,6.2}$=14.32, P<0.005). Egg production was associated with a 64% decrease in locomotor activity compared with warm-acclimated, non-breeding values (Tables 2 and 4; Fig. 2A). A similar 72% decrease in hopping was observed by female pairs that were cold acclimated and actively producing heat (Tables 2 and 4; Fig. 2A). In contrast to the warm-acclimated birds, egg production by females that were actively producing heat was not associated with any change in locomotor activity compared with cold-acclimated, non-breeding values (Tables 2 and 4; Fig. 2A). However, the combination of egg production, cold acclimation and heat production was associated with an 81% decrease in locomotor

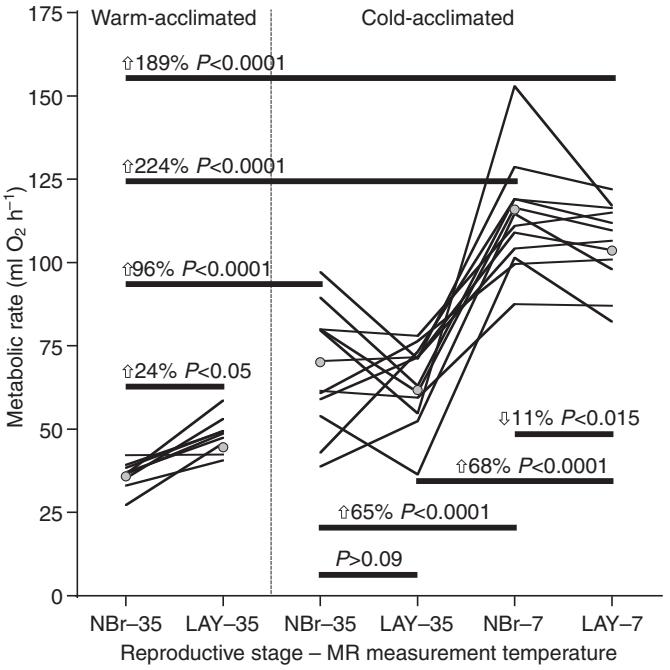

Fig. 1. Metabolic rate (MR, ml O_2 h^{-1}) for warm- and cold-acclimated females as non-breeders and during egg production. MR measured at 7°C represents values for actively heat-producing birds. Thin lines join repeated measures from individual females, and gray circles represent least square means MR controlling for female body mass at each stage. Female body mass at each stage was included as a covariate. Thick lines underneath P-values indicate the experimental groups being compared statistically. NBr, non-breeding; LAY, egg laying.

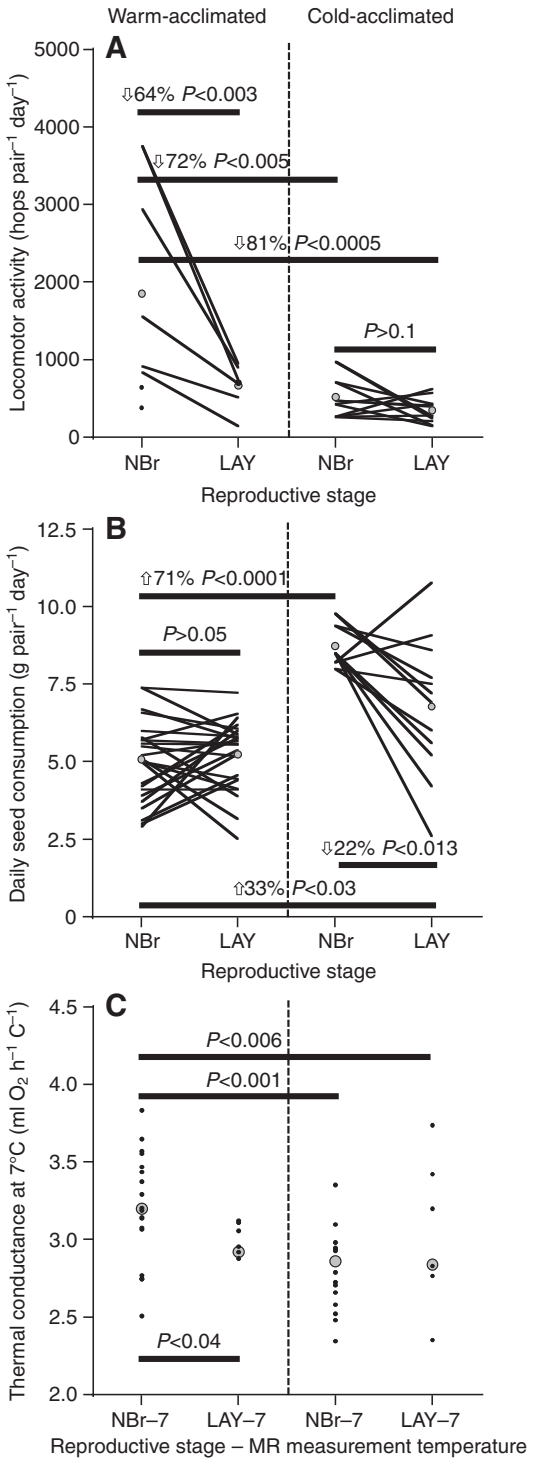

Fig. 2. (A) Locomotor activity (hops pair^{-1} day^{-1}), (B) seed consumption (g pair^{-1} day^{-1}) and (C) wet thermal conductance at 7°C (ml O_2 h^{-1} °C^{-1}) of warm- and cold-acclimated zebra finches as non-breeders and during egg production [warm-acclimated seed consumption data from Vézina et al. (Vézina et al., 2006b)]. For locomotor activity and seed consumption, thin lines join repeated measures from individual females, and gray circles represent stage means. Locomotor activity and seed consumption were $\log_{10}$-transformed for statistical analysis. For thermal conductance at 7°C, gray circles represent least square means controlling for female body mass. Female body mass at each stage was included as a covariate for statistical analysis of thermal conductance. Thick lines underneath *P*-values indicate the experimental groups being compared statistically. NBr, non-breeding; LAY, egg laying.

activity compared with warm-acclimated, non-breeding female pairs (Tables 2 and 4; Fig. 2A). Variation among pairs in locomotor activity decreased from 9.9-fold variation in warm-acclimated, non-breeding female pairs to 3.7-fold and 4.3-fold variation in cold-acclimated, non-breeding, female pairs and cold-acclimated breeding pairs, respectively (Table 4; Fig. 2A).

Seed consumption, our indicator of daily raw energy intake, was not related to female or pair body mass at the various stages (P>0.05) but did differ between stages ($F_{3,18.3}$=64.01, P<0.0001). Gross energy intake of warm-acclimated birds was not affected by egg production, and neither was variation between pairs in energy intake [2.6-fold variation among non-breeding female pairs compared with 2.9-fold variation among breeding pairs; Tables 2 and 4; Fig. 2B; data from Vézina et al. (Vézina et al., 2006b)]. By contrast, cold acclimation and active heat production induced a 71% increase in seed consumption in non-breeding birds (Tables 2 and 4; Fig. 2B), with very little variation (only 1.2-fold) between female pairs. Interestingly, while cold-acclimated, egg-laying birds involved in active heat production exhibited marked variation (over 4-fold) in gross energy intake, mean seed consumption decreased by 22% compared with cold-acclimated, non-breeding values (Tables 2 and 4; Fig. 2B). Despite this decrease in gross energy intake, the combination of egg production, cold acclimation and active heat production still induced a 33% increase in seed intake above warm-acclimated, non-breeding values (Tables 2 and 4; Fig. 2B).

Thermal conductance

Body temperature following MR measurements did not differ among the different thermal-reproductive stages ($F_{3,39}$=1.06, P>0.3) but thermal conductance at 7°C varied among the stages ($F_{3,39}$=6.04, P<0.002) and was positively related to female body mass ($F_{1,39}$=30.46, P<0.0001). Therefore, female body mass was included as a covariate in all analyses. Warm-acclimated, non-breeding females exhibited 9–13% higher thermal conductance at 7°C than warm-acclimated, egg-laying females (t=21.8, d.f.=38, P<0.04), cold-acclimated, non-breeding females (t=3.67, d.f.=38, P<0.001), and cold-acclimated, egg-laying females (t=2.92, d.f.=38, P<0.006) (Table 4; Fig. 2C). By contrast, females from all three of these thermal-reproductive stages did not differ from each other in their thermal conductance at 7°C (all P>0.5; Table 4). Thermal conductance exhibited little variation among females within thermal-reproductive stages (ranging from 1.1-fold variation in warm-acclimated laying females to 1.6-fold variation in cold-acclimated laying females; Table 4; Fig. 2C).

DISCUSSION

In this study we have shown that the metabolic cost of egg production in passerine birds [16–27% above BMR or RMR (Chappell et al., 1999; Nilsson and Raberg, 2001; Vézina and Williams, 2002; Vézina and Williams, 2003; Vézina and Williams, 2005b) (this study)] is relatively small compared with the additive costs of cold acclimation and active heat production (224% above BMR). Birds exposed to low ambient temperatures dealt with this increase in energy expenditure by increasing food intake (seed consumption) by 72% and decreasing locomotor activity by 72% compared with warm-acclimated, non-breeding birds. By contrast, egg production in cold-acclimated, heat-producing females was associated with an 11% decrease in MR and a 22% decrease in seed consumption but with no change in body temperature compared with non-breeding, thermoregulating values. This suggests that while the increase in MR associated with egg production is relatively small in relation to the birds' capacity to increase MR in response to other

Table 4. Locomotor activity, seed consumption and thermal conductance at 7˚C of zebra finches in different thermal-reproductive stages

	Warm acclimated		Cold acclimated	
	NBr	LAY	NBr	LAY
Locomotor activity (hops pair^{-1} day^{-1})	1852+1418 (8) [379–3760]	662+270 (7) [144–945]	520+264 (12) [266–975]	346+158 (12) [145–629]
Seed consumption (g pair^{-1} day^{-1})	5.1+0.2 (23) [2.9–7.4]	5.2+0.2 (23) [2.5–7.2]	8.7+0.2 (12) [8.0–9.8]	6.8+0.7 (12) [2.6–10.8]
Thermal conductance at 7°C (ml O_2 h^{-1} °C^{-1})	3.19+0.09 (18) [2.49–3.83]	3.01+0.05 (6) [2.87–3.12]	2.78+0.07 (14) [2.32–3.34]	3.00+0.19 (6) [2.33–3.64]
Thermal conductance at 7°C correcting for female body mass (ml O_2 h^{-1} °C^{-1})	3.20+0.06 (18)	2.94+0.10 (6)	2.86+0.07 (14)	2.84+0.11 (6)

All values are means ± s.e.m. with sample size in parentheses and minimum and maximum values in brackets, with the exception of thermal conductance correcting for female body mass values, which are least-square means ± s.e.m. with sample size in parentheses. Female body mass at each stage was included as a covariate for statistical analysis of thermal conductance. Locomotor activity and seed consumption were $\log_{10}$-transformed for statistical analysis. NBr=non-breeding; LAY=egg laying.

energetically demanding processes, the addition of the costs of egg production on top of these metabolically costly activities may be enough to necessitate the use of energy-saving strategies, such as internal energy reallocation, to cope with the additional or combined energetic demands.

There is marked variation across avian species in the reported metabolic costs associated with acclimatization or acclimation to cold ambient temperatures. For example, cold-acclimated (5°C) house finches, *Carpodacus mexicanus*, exhibited a 15% increase in BMR above that of warm-acclimated (25˚C) birds (O'Connor et al., 2000), while cold-acclimation (to 8˚C) in great tits (*Parus major*) induced a 21% increase in MR above warm-acclimated (to 22°C) BMR (Caro and Visser, 2009). Similarly, winter-acclimatized American goldfinches (*Carduelis tristis*) demonstrated 23% and 46% higher BMR than spring- and summer-acclimatized birds (Liknes et al., 2002). By contrast, Carleton and del Rio found no measurable metabolic cost of cold-acclimation in house sparrows, *Passer domesticus*, as warm-acclimated (to 22°C) and cold-acclimated (to 5°C) birds had comparable MRs (measured at 5°C and 22°C) (Carleton and del Rio, 2005). However, Arens and Cooper reported a 64% increase in BMR of winter-acclimatized house sparrows compared with summer-acclimatized birds (Arens and Cooper, 2005). In fact, across species the effects of cold acclimation or acclimatization on BMR range from having no effect to increasing BMR by 85% (Gelineo, 1964; Swanson, 2009; McKechnie, 2008), and are affected by differences between seasons (Swanson and Olmstead, 1999) and populations (Broggi et al., 2004). Therefore, the 96% increase in MR (measured at 35°C) associated with cold acclimation in this study was higher than previously reported metabolic costs of cold acclimation in other passerine species. Because of their relatively high body heat conductance, species from warm climates are characterized by a small temperature range of thermoneutrality and relatively high lower critical temperature (Scholander et al., 1950; Schmidt-Nielsen, 1990). Consequently, for a desert species such as the zebra finch, acclimation to 7°C represents a greater cold challenge than for temperate species because of the greater relative difference between the acclimation temperature and the lower limit of thermoneutrality [i.e. 26°C difference in zebra finches *vs* 15–18°C in house sparrows and house finches (Hudson and Kimsey, 1966; Weathers, 1981; Dawson et al., 1985; Marschall and Prinzinger, 1991)].

The metabolic cost of egg production in this study (24% above BMR) is comparable with previous studies on free-living and captive passerine birds, which reported 16–27% increases in MR above values for non-breeding females (Chappell et al., 1999; Nilsson and Raberg, 2001; Vézina and Williams, 2002; Vézina and Williams, 2003, Vézina and Williams, 2005b). However, the metabolic cost of egg production was small when compared with the additive metabolic costs of cold acclimation and active heat production alone (224% above BMR). Interestingly, we could not detect any additional increase in metabolism in association with the egg formation process in cold-acclimated females when MR was measured at thermoneutrality. Indeed, all but four cold-acclimated birds had MRs that were either stable or decreased between the non-breeding and laying stages (Fig. 1). Furthermore, the mean MR of these same cold-acclimated females decreased by 11% when egg production coincided with active heat production. As the body temperature of these females did not differ from that of warm-acclimated, egg-laying females, the 11% decrease was not caused by facultative hypothermia. Furthermore, the decrease in mass-corrected MR during egg production in cold-acclimated females was also not due to the fact that females had previous experience with the respirometry system as non-breeders. Indeed, mass-corrected, egg-laying MR of cold-acclimated females measured at 7°C during the thermal conductance study (102.31±4.75 ml O_2 h^{-1}) did not differ from that of cold-acclimated females from the main study (P>0.7). Furthermore, warm-acclimated, egg-laying females also had previous experience with respirometry as non-breeders and exhibited a 24% increase in mass-corrected MR (Table 3; Fig. 1).

In our study, if the metabolic costs of cold acclimation, active heat production and egg formation were additive, the predicted increase in MR was 3.5 times the BMR. However, female zebra finches faced with this combination of thermal and reproductive challenges only increased MR by 224% above BMR, the equivalent of 2.9 times the BMR. This suggests that the addition of the energetic demands associated with egg production (Chappell et al., 1999; Nilsson and Raberg, 2001; Vézina and Williams, 2002; Vézina and Williams, 2003, Vézina and Williams, 2005b) may result in downregulation of non-reproductive functions and thus energy reallocation within an individual, potentially at the expense of somatic maintenance (Deerenberg et al., 1997; Deerenberg et al., 1998; Wiersma and Verhulst, 2005). Within individuals energy reallocation can take several forms. MR in resting animals reflects the energy consumption of all metabolically active tissues. Because RMR is often positively correlated with the masses of organs such as the liver, pectoral muscle and gut (O'Connor, 1995; Chappell et al., 1999; Vézina et al., 2006a), individuals could modulate MR by changing the size of major metabolically active internal organs (Swanson, 2009; McKechnie, 2008). Metabolic intensity, the energy consumed per unit tissue mass, can also vary reversibly, and birds may change metabolic intensity to reallocate energy between demanding systems. For example, red knots (*Calidris canutus*)

forced to generate larger digestive tracts by diet manipulations responded by decreasing BMR (Piersma et al., 2004), suggesting a reduction in the tissue's metabolic intensity. Similarly, free-living European starlings (*Sturnus vulgaris*) were found to downregulate liver metabolic intensity during egg production and chick rearing (Vézina and Williams, 2005a). In this study, physiological downregulation of non-reproductive function and reallocation of this energy towards thermoregulation and egg production could ultimately lead to undetectable metabolic changes or even decreases in MR in cold-acclimated, egg-laying birds when comparing cold-acclimated, non-breeding and laying MR within-individuals, regardless of the measurement temperature. Future experimental work is needed to address the questions of whether downregulation of non-reproductive functions occurs when egg production, cold acclimation and heat production are combined, which non-reproductive functions are affected, and to what extent are these functions downregulated to meet the combined energy requirements of these three processes.

Respirometry is an indirect way of measuring total heat production [i.e. indirect calorimetry (Blaxter, 1989)]. This means that egg production generates heat, as shown by a 24% increase in MR in warm-acclimated birds, and this heat increment of egg production can probably substitute for part of the thermostatic costs involved at 7°C. It has been shown that the heat increment of feeding, i.e. the increase in MR following consumption of a meal [also referred to as specific dynamic action or diet-induced thermogenesis (Ricklefs, 1974) (reviewed in Jobling, 1983; Aoyagi et al., 1990)] can substitute for thermoregulatory heat production in birds exposed to low ambient temperatures (Biebach, 1984; Meienberger and Dauberschmidt, 1992; Chappell et al., 1997). Previous studies have reported that a large proportion of the increase in MR following ingestion of a meal is due to the assimilation of food, primarily the accelerated rates of protein synthesis (Aoyagi et al., 1990; Brown and Cameron, 1991a; Brown and Cameron, 1991b). Thus, the marked increase in protein synthesis associated with egg development may be the main heat source resulting from egg production. The most likely contributors to the heat increment of egg production are the oviductal production of egg albumen (Yu et al., 1971; Yu and Marquardt, 1973) (reviewed in Williams, 1998) and the physiological activity of the shell gland (reviewed in Williams, 1998), as Vézina et al. found no detectable effects of the hepatic production of the egg yolk precursors (vitellogenin and yolk-targeted very-low density lipoprotein) on RMR in zebra finches (Vézina et al., 2003). While laying females could exploit this metabolic by-product of egg development to compensate for a portion of the costs of active heat production at low temperatures, the maximal amount of heat generated by egg production (corresponding to a $\dot{V}_{O_2}$ of 8.77 ml $O_2\,h^{-1}$; Table 3) only represents 20% of the energy needed for active heat production (i.e. a mean of 43.81 ml $O_2\,h^{-1}$; Table 3). Therefore, in this specific experiment, the compensatory effect of the heat increment of egg production did not fully match the thermoregulatory needs at 7°C. We observed an 11% decrease in MR from the non-breeding to the egg-laying stage in cold-acclimated, heat-producing females. As there was no difference in thermal conductance at 7°C between these two reproductive stages, the observed decline in MR reflects a reduction in total heat production. We suspect this observation may have resulted from the over-compensatory effect of downregulating non-reproductive functions to fuel the added costs of egg formation in the cold environment. Vézina and colleagues found a similar over-compensation effect in zebra finches, wherein some egg-laying females were able to decrease their daily energy expenditure (DEE) compared with the non-breeding state, despite an increase in MR during egg production (Vézina et al., 2006b).

The cold-acclimated females in this study faced obvious increases in energetic costs. One could predict that the combination of these energetically challenging processes would affect egg production if different physiological systems have to compete for energy, and this is exactly what we found. Compared with their own warm-acclimated (to 21°C) reproductive values, female zebra finches did not maintain reproductive output when egg laying coincided with cold acclimation and heat production at 7°C. Cold-acclimated females took approximately 0.5 days longer to initiate egg laying and ultimately laid a mean of 0.4 fewer eggs at a slower rate (0.90 eggs day^{-1} compared with 0.95 eggs day^{-1} while warm acclimated). Furthermore, cold-acclimated females 'skipped' more days in between eggs, when no egg was laid (0.7 skipped days compared with 0.5 skipped days while warm-acclimated) (Salvante et al., 2007). Therefore, exposure to the combined metabolic costs of egg production, cold acclimation and active heat production appeared to compromise the processes involved in egg formation. Consequently, there may be downregulation of multiple physiological systems, reproductive and non-reproductive, to fuel the substantial, combined energetic demands associated with egg production and thermoregulation. Furthermore, the metabolic costs of the downregulated processes involved in egg production by cold-acclimated, heat-producing females may be lower than the MR associated with egg production in more favorable energetic conditions (i.e. 8.77 ml $O_2\,h^{-1}$ in warm-acclimated females). Consequently, this potentially smaller increase in MR associated with egg production in the cold may be masked by the substantial decrease in MR associated with down-regulation of reproductive and non-reproductive functions.

Daily locomotor activity of warm-acclimated zebra finches decreased by 64% from the non-breeding to egg-producing stage, confirming previous findings that warm-acclimated laying zebra finches decrease activity by 61% with no associated change in food intake (Vézina et al., 2006b). Vézina et al. also showed, using the doubly labeled water technique, that DEE in these birds did not change between the non-breeding and egg-producing stages (Vézina et al., 2006b). Taken together, these findings suggest that the 61–64% decrease in locomotor activity of warm-acclimated, egg-producing birds along with the observed decrease in thermal conductance may conserve sufficient energy on a daily basis to meet the energy demands of egg production in favorable thermal conditions. Consequently, warm-acclimated zebra finches do not need to increase energy intake when producing eggs.

Compared with warm-acclimated, non-breeding birds, cold-acclimated, non-breeding zebra finches decreased both daily locomotor activity (by 72%) and wet thermal conductance, suggesting that these birds employ energy-saving behavioral modification as a first strategy to reallocate the energy needed to fuel expensive metabolic processes. Cold-acclimated individuals also increased energy intake by consuming more seed per day (71% as non-breeders and 33% during egg production) than warm-acclimated, non-breeding birds, suggesting that birds increase energy intake to fuel the additional energy requirements of cold acclimation and active heat production. Similarly, cold-acclimated (15°C) hoopoe larks, *Alaemon alaudipes*, were fed 3-times more food than larks maintained at thermoneutrality (36°C), suggesting that these cold-acclimated birds also increased energy intake (Williams and Tieleman, 2000). Therefore, while zebra finches are capable of increasing food intake above levels generally observed in favorable conditions, they only do so in certain

circumstances. In warm environments, behavioral energy reallocation by individuals allows them to minimize large variations in DEE (Vézina et al., 2006b; Williams et al., 2009). Under cold challenge, however, reducing locomotor activity to its minimum is not sufficient, and the birds must also increase their energy intake.

An interesting finding regarding daily food intake in cold-acclimated birds is that females experiencing the combined costs of cold acclimation, active heat production and egg production exhibited a mean daily food intake that was 22% lower than when at the non-breeding stage. This suggests a reduced overall DEE despite the additional energetic demands associated with egg production and could be the result of a metabolic downregulation of reproductive and non-reproductive physiological systems as discussed above. Alternatively, changes in digestive assimilation efficiency in response to increased energy demands could also explain the general decline in seed consumption by cold-acclimated females during egg production. Cold-acclimated (to –9°C), force-exercised house wrens, *Troglodytes aedon*, assimilated 2.3 times more energy per day than warm-acclimated, non-exercised birds by modulating food intake and increasing their energy assimilation quotient [i.e. 1 – (energy excreted / energy ingested)]. This was done by increasing the length of their small intestines by 21%, resulting in a larger small intestine volume and absorptive surface area (Dykstra and Karasov, 1992). A larger gut in cold-acclimated birds is consistent with findings of Williams and Tieleman (Williams and Tieleman, 2000) and Cavieres and Sabat (Cavieres and Sabat, 2008) and would explain part of the increase in MR reported in both groups of cold-acclimated birds above that of warm-acclimated birds. It is possible that our cold-acclimated, egg-laying females may have increased digestive assimilation efficiency above cold-acclimated, non-breeding levels *via* further changes in digestive tract morphology, retention time in the digestive tract or an increase in the rate of nutrient absorption, which could have led to an assimilation of comparable amounts of energy from less seed without necessarily increasing MR as the measurements were taken in the post-absorptive state.

The combination of the energetic requirements of cold acclimation, heat production and egg formation was associated with the most marked variation among birds in daily seed consumption (over 4-fold), with some birds decreasing seed consumption to levels comparable with warm-acclimated, non-breeding females, and others maintaining a high level of gross energy intake that was comparable with their elevated levels as cold-acclimated non-breeders (see Fig. 2B). By contrast, this combination of energetically demanding activities was also associated with the lowest mean value and smallest range (i.e. max.–min.) in locomotor activity between pairs (Table 4). These data suggest that while all of the cold-acclimated, heat-producing, egg-laying females used behaviorally based energy reallocation (*sensu* Williams and Ternan, 1999) to meet their elevated energy demands, these same females varied in the extent to which they altered gross energy intake. This plasticity is consistent with previous findings of individually variable energy management strategies used by females to meet the energetic demands of reproduction (Vézina et al., 2006b), which have been found to be repeatable among individuals between breeding attempts (Williams et al., 2009).

LIST OF ABBREVIATIONS

BMR	basal metabolic rate
C	wet thermal conductance
DEE	daily energy expenditure
LAY	egg laying
MR	metabolic rate
NBr	non-breeding/non-breeder
RMR	resting metabolic rate
STP	standard temperature and pressure
T_a	ambient temperature
T_b	body temperature
$\dot{V}_{O_2}$	rate of oxygen consumption

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