kJ day^{-1}, and were thus able to maintain body condition (e.g. body mass, fat and muscle score) and circulating triacylglyceride at levels comparable to those at 21°C. However, when producing eggs. c1dse at

Introduction

Reproduction in seasonally breeding animals is generally timed such that the period of offspring care coincides with seasonal peaks in food availability and quality (Perrins, 1970). However, the seasonal recrudescence of the reproductive axis and early offspring development (i.e. egg production in oviparous animals and gestation in mammals) often occurs well in advance of the period of offspring care. Therefore, these energetically demanding processes (Vézina and Williams, 2002; Zenuto et al., 2002; Korine et al., 2004) often occur prior to the seasonal peak in food availability, at a time when environmental conditions may be sub-optimal. During this time, breeding females must ensure that they can find enough energy and nutrients to produce their offspring while still meeting their own energetic requirements (Perrins, 1970; Scott, 1973). Thus, breeding females will face a trade-off between allocation of resources to offspring their own energetic needs (Bernardo, 1996), and this trade-off will be most acute when reproduction coincides with periods of increased energy demand due to poor environmental conditions, such as exposure to inclement weather, extreme low temperatures or low food availability, which can be common during the early stages of breeding (e.g. during egg production) (Perrins, 1970). Little is known about the physiological mechanisms underlying temperature-induced variation in egg production.

Differential allocation of energy-rich lipids during avian egg production provides a model for studying the physiological basis of a temperature-dependent trade-off between current reproduction and maternal survival, as mediated through female body condition. The estrogen-dependent increase in circulating levels of the egg yolk lipid precursor, yolk-targeted very low density lipoprotein (VLDLy), represents a dramatic shift in lipid metabolism as the primary function of plasma VLDL particles changes from general lipid transport to

maternal tissues (e.g. muscle, adipose), to selective lipid transport to developing egg yolks (Neilson and Simpson, 1973; Chan et al., 1976; Walzem, 1996; Walzem et al., 1999; Williams, 1998). Lipid is the primary energy source for the growing avian embryo (Walzem, 1996). Thus, generic VLDL fuels maternal maintenance activities, including thermoregulation and digestion, while the structurally distinct VLDLy predominates in egg-laying domestic fowl (Hermier et al., 1989; Walzem et al., 1994; Walzem, 1996). The near-total shift in hepatic synthesis towards this relatively non-maternally metabolizable form of VLDL during egg production may compromise the condition of laying females during periods of high energetic demand. Consequently, modulation of the trade-off between reproductive output and maternal survival may be achieved by altering maternal VLDL–VLDLy metabolism, e.g. modulation of plasma concentrations of generic and yolk-targeted VLDL, VLDL particle diameter distribution, efficiency of generic VLDL and VLDLy utilization for maternal energetic needs (cf. the energy requirements of their developing offspring), or efficiency of switching from the exclusive synthesis of non-laying, generic VLDL to an increased synthesis of VLDLy (Lin and Chan, 1981; Griffin et al., 1982; Lin et al., 1986; Nimpf et al., 1988; Walzem, 1996; Williams and Christians, 1997).

The goals of this study were to (1) increase total maternal energy requirements during egg-laying in zebra finches by exposing laying females to low ambient temperatures, (2) determine the effects of exposure to low ambient temperature on maternal body condition and reproductive output (e.g. egg mass, clutch size, egg

not change throughout the laying sequence (Williams and Ternan, 1999). Therefore, measuring food intake per pair was a good indicator of female food intake as the proportion of seeds consumed by both sexes remained unchanged throughout the experimental protocol. Pairs were also provided with 6 g of an egg-food supplement (20.3% protein: 6.6% lipid) (see Williams, 1996) daily between pairing and clutch completion, which was completely consumed within 24 h. Water, grit and cuttlefish bone (calcium) were also provided *m.*

Data on laying interval, egg mass and clutch size were obtained by checking the nest boxes daily between 09.00 h and 11.00 h. All new eggs were weighed (±0.001 g) and numbered

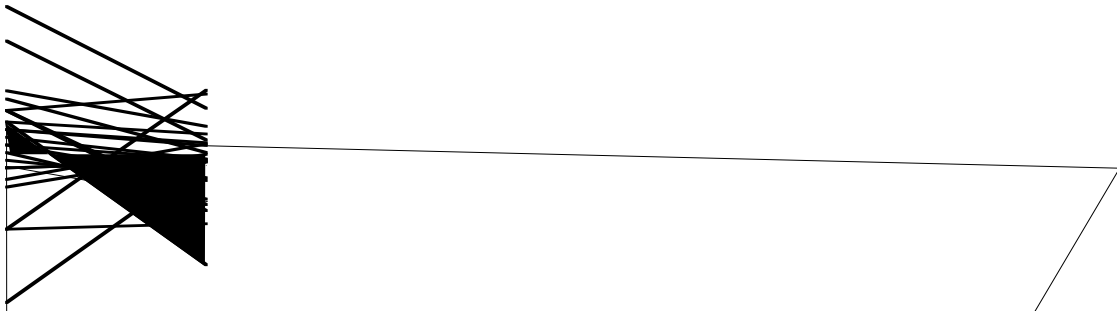
median particle diameter and the range (i.e. width, in nm) of each distribution were also determined.

E m

The second egg of each clutch was subjected to protein and lipid fractional content analysis following published methods (Balzer and Williams, 1998). Briefly, eggs were collected within 6 h of being laid, boiled for 3 min, and frozen (at –20°C) until further analysis. Frozen eggs were thawed and separated into shell, albumen and yolk, which were dried to constant mass in a 50°C drying oven, and then weighed to the nearest 0.0001 g (dry mass). Lipid was removed from the dry yolks by soxhlet extraction for 8 h with petroleum ether as the solvent (Dobush et al., 1985). Lipid-free yolks were then weighed to the nearest 0.0001 g (lean dry mass). Lipid content of the yolks was determined by subtracting lean dry yolk mass from dry yolk mass. Dry albumen mass and lean dry yolk mass were assumed to be approximately 88% protein (Burley and Vadehra, 1989). The yolk lipid, yolk protein and albumen protein content of each egg were calculated as the percentage of each component relative to the fresh mass of the egg without the component of interest to control for part-whole correlations [e.g. %yolk lipid=[yolk lipid mass/(fresh egg mass–yolk lipid mass]×100; all masses in g (Christians, 1999)].

D

All statistical analyses were performed using SAS (SAS Institute, 1999). All data were tested for normality of distribution (Shapiro–Wilk test) (Zar, 1996). All non-normal



water content, dry albumen and yolk mass, yolk lipid content (Fig. 3E) and albumen protein content were all independent of ambient temperature (>0.2 in all cases). However, females laid eggs with 5% more yolk protein, an average of an additional 4.3 mg protein/yolk, at 7°C than at 21°C ($F_{1,23.1}$=4.79, <0.05; Fig. 3F).

Variation in mean egg mass, clutch size, laying interval and laying rate was not related to variation in circulating triacylglyceride levels, VLDL particle diameter distribution range, modal and median VLDL particle diameter or the proportion of VLDL particles within the sVLDLy range, regardless of ambient temperature during egg production (>0.09 in all cases).

Discussion

Zebra finches paired and breeding at 7°C consumed 45% more seed per day, the equivalent of an additional

12.67 kJ energy day^{-1} (assuming 75% digestive efficiency) (Shuman et al., 1989), and laying females were thus able to maintain body condition throughout egg production even at this low ambient temperature. The additional seed consumed represents approximately 25% of the daily energy expenditure (DEE) of non-breeding (53.6±1.0 kJ day^{-1}) and egg-laying

between temperature and both clutch size and laying date, the

(Magrath, 1992); collared flycatcher *F*
(Hargitai et al., 2005)] and with the energetic content of the egg components of great tits and pied flycatchers (Ojanen, 1983). Similarly, egg volumes of great tits laying in heated nest boxes were greater than those of females laying in experimentally cooled nest boxes (Nager and van Noordwijk, 1992). However, most of these studies did not control for laying date, which could potentially confound the relationship between ambient temperature and egg size through correlations with both variables (Magrath, 1992; Lessells et al., 2002). In contrast to these studies, mean egg mass of blue tits was not related to experimentally manipulated nest box temperatures (Yom-Tov and Wright, 1993). As in this study, the masses of the egg components of free-living great tits were not related to ambient temperature when other factors, such as total egg mass, were controlled for. The results from this study and previous studies examining the influence of ambient temperature on reproductive output demonstrate the variety of ways in which females producing eggs in sub-optimal conditions can modulate reproductive output in order to decrease the energetic demands associated with egg production and increase the energy available for fueling maternal self-maintenance and survival, while still producing offspring that can be raised given the current environmental conditions and those predicted for the future. It is not known Tw n sox2mbient tese the eub-op/9s3). A6cing ervedAs in thn,

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Mitchell, M. A. and Carlisle, A. J. (1991). Plasma zinc as an index of vitellogenin production and reproductive status in the domestic fowl. *C m . B m.* . **100A**, 719-724.

Nager, R. G. and van Noordwijk, A. J. (1992). Energetic limitation in the