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The influence of temperature on egg composition in the grey partridge *Perdix perdix*

M. Cucco [1], B. Guasco, R. Ottonelli, V. Balbo and G. Malacarne

Università del Piemonte Orientale, Dipartimento di Scienze dell'Ambiente e della Vita, Via Bellini 25, 15100 Alessandria, Italy

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Variations in egg size and composition can have important consequences for the quality of offspring. We investigated the effect of temperature, in the 5 days preceding laying, on yolk, albumen, shell and total egg mass. Moreover, we examined the influence of temperature on three important egg components, i.e. the yolk carotenoids and the albumen lysozyme and avidin. In our experimental condition food was provided ad libitum, hence we were able to remove the possible indirect effects of temperature due to a concomitant effect of temperature on food availability. Temperature on the day immediately before laying (day –1) was positively related to total egg mass and albumen mass, and negatively related to shell mass. Yolk mass was positively related to temperature in the day farthest from laying (day –5). These findings are in line with the predicted timing of the maturation of egg follicle, with yolk deposition preceding albumen and shell formation. Carotenoids and lysozyme concentration did not vary in relation to temperature, while avidin concentration was positively related to the temperature on day –1. Avidin is an albumen antibacterial substance, that could play a beneficial role during the embryonic development phases. Our results suggest that ambient temperature is an environmental factor that should be considered together with genetic and maternal effects in studies of egg quality and reproductive success.

KEY WORDS: avidin, egg components, lysozyme, temperature, yolk and albumen.

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[1] Corresponding author: Marco Cucco, Università del Piemonte Orientale, DISAV, Via Bellini 25, 15100 Alessandria, Italy (Tel. +39.0131 360276, Fax +39.0131 360243, E-mail: cucco@unipmn.it).

INTRODUCTION

Phenotypic plasticity of reproductive traits can be viewed as a mechanism to synchronize the events involved in reproduction and to maximize lifetime breeding success (Perrins 1991, Kawecki & Stearns 1993). Two major environmental factors playing a role in this optimization strategy are food abundance and weather conditions (Eeva et al. 2002). In homeotherms living in temperate or boreal climates, temperature plays a key role in reproductive events. Temperature variability (Pendlebury et al. 2004) impacts reproductive functions both indirectly (e.g. by affecting foraging and thermoregulation) or more directly (e.g. egg production, incubation and brood-rearing). An extensive literature concerning oviparous animals, and in particularly birds, documents the positive influence of high *vs* low temperatures for the timing of deposition, clutch size, egg size and hatchability (Magrath 1992, Robertson 1995, Brown & Brown 1999). In a few studies, however, a reversed pattern is observed: for example in the snow goose *Anser caerulescens* cold temperatures induce the laying of bigger eggs (Williams & Cooch 1996), a possible adaptation to the extreme climate, and in the hen *Gallus gallus*, Japanese quail *Coturnix coturnix japonica* and pheasant *Phasianus colchicus* heat stress depresses egg production and egg weight (Sahin et al. 2002, Mashaly et al. 2004, Nowaczewski & Kontecka 2005).

On the nutritional side, many studies have explored the influence of maternal food availability and composition on clutch size and on the energetic composition of eggs (e.g. Royle et al. 1999, McGraw 2006). For example, experiments with diets differing in fat and protein composition show that the nutritional quality of a diet can influence egg size and composition (fat, proteins, water), that may be fundamental to a chick's growth and survival (Nager 2006).

Recently, interest in the effects of maternal investment in the eggs has focused also on other aspects of egg quality, i.e. substances deposited in the yolk (antioxidants, in particular vitamin E and carotenoids, hormones and proteins involved in the acquired immunity as immunoglobulins) and in the albumen (water and substances involved in innate immunity) (Saino et al. 2002, Royle et al. 2003, Cucco et al. 2006b). This is an important aspect of egg quality, since all these substances are now acknowledged to play a major role in the prospects of chick survival, in some cases independently of egg size. Since all these substances impose a metabolic cost or are costly to acquire, an optimal adaptive allocation strategy has been hypothesised (Schwabl 1993, Blount et al. 2000).

Until now few studies have explored the influence of climatic conditions on the deposition of these important substances. Saino et al. (2004) have shown that lysozyme and lutein, the main carotenoid in the barn swallow *Hirundo rustica*, co-vary with temperature during the days preceding the laying of individual eggs. However, since aerial insects, the food of barn swallow, co-vary with temperature (Cucco & Malacarne 1996), it is difficult to disentangle the two environmental effects (Saino et al. 2004).

In this study we examined how the environmental conditions (temperatures in the days preceding laying) can influence egg mass and composition.

Food availability was kept constant, so that we could specifically explore the effect of temperature regardless of the indirect effects of temperature on food abundance. We choose to study the grey partridge *Perdix perdix*, a species in which females can lay more that twenty eggs, thus making a huge investment in this reproductive trait. Egg quality was assessed measuring yolk, albumen, shell and total egg mass, and the concentration of three important egg components, i.e. the yolk carotenoids and the albumen lysozyme and avidin.

MATERIALS AND METHODS

The study was conducted on grey partridges reared in 2002, 2003, 2004 and 2005 at a game breeding farm in S. Giuliano Nuovo, Alessandria, NW Italy (Cucco et al. 2006a, 2007). In total 32 breeding pairs in 2002, 32 in 2003, 16 in 2004, and another 25 in 2005, were housed in individual outdoor reproduction cages (4 m long × 1 m wide × 0.5 m high). Throughout the year, the birds were maintained in natural light and temperature conditions. Each pair had food and water available ad libitum.

When females started laying, all pens were inspected daily to collect eggs (in the afternoon, all within 1 to 5 hr after laying). Using a non-toxic marker pen, we marked each egg with the female's code, the position in the laying order and date of laying. Just after collection, we weighted the eggs with an electronic balance (± 0.01 g accuracy). From April to June, the hens laid a mean of 33.1 eggs, with a total of 3476 eggs in the 4 years. In this study, we analyzed only the first 20 eggs laid by each female in order to restrict the analysis to the range occurring in natural conditions. The total number was 1910 eggs (565, 593, 302, and 450 eggs in 2002, 2003, 2004 and 2005 respectively).

Most of the eggs were incubated for two side studies concerning chicks (Cucco et al. 2006b, 2008), while 344 eggs were brought to the laboratory for chemical analysis (N = 100, 100, 80 and 66 eggs, laid by 20, 20, 16 and 25 randomly chosen different females in 2002, 2003, 2004 and 2005, respectively). Eggs utilized in composition analyses derived from different positions in the laying sequence (specifically the 5th, 7th, 10th, 13th and 16th eggs).

In the laboratory, the eggs were carefully separated into their constituent parts (shell, albumen and yolk, all weighed to the nearest ± 0.01 g). The yolk was then homogenized and stored at – 20 °C until analysis, while the albumen was frozen without centrifugation. The yolk was chemically analysed to assess total carotenoid concentration, while the albumen was used to assess lysozyme and avidin content.

The concentration of total egg yolk carotenoids was measured spectrophotometrically. Egg yolk samples were mixed with hexane, acetone, toluene and ethanol (10/7/7/6), and centrifuged at 10000 rpm for 10 min. In the resulting supernatant, we determined the absorbance of the carotenoids peak at 450 nm using a Beckman Du-640 spectrophotometer. Carotenoid concentrations (µg/ml) were calibrated according to standard curves of ß-carotene (Sigma).

Lysozyme activity was measured by the method of Osserman & Lawlor (1966): an agar gel with a dried strain of *Micrococcus lysodeikticus* (M-3770; Sigma), which is particularly sensitive to lysozyme activity, was inoculated with 25 µl of albumen. Standard dilutions of crystalline hen egg-white lysozyme (L-6876, Sigma) (25, 100, 500 and 1000 µg/ml) were run with each group of test samples. The plates were incubated in a laboratory incubator at 25 °C for 18 hr, during which bacterial growth was inhibited in the area of the gel surrounding the albumen inoculation site. The diameters of the cleared zones are proportional to the log of the lysozyme concentration. This area was measured using an ad hoc ruler, and converted on a semilogarithmic plot into hen egg lysozyme equivalents (HEL equivalents, expressed in µg/ml) according to the standard curve. Each egg albumen was assayed twice, and for each sample we measured lysozyme activity in triplicate. Intra-assay coefficient of variation was 1.53%, while inter-assay coefficient of variation was 1.84%.

The biotin-binding capacity (active avidin) of samples from egg-whites was measured using biotinylated insulin and biotinylated alkaline phosphatase (AP, Sigma), according to the method reported in Groman et al. (1990). The 96-well plate was coated with biotinylated insulin (10 µg/ml) in sodium carbonate buffer (50 mM, pH 9.6) at 37 °C for 2 hr, followed by washing 3 times with PBS-Tween and blocking with 1% BSA in PBS (PBS-BSA). Egg-white samples were diluted 1:6 with 0.5% BSA in PBS, vortexed briefly and incubated for 2 hr at 25 °C. Duplicate samples were allowed to bind to biotinylated insulin at 37 °C for 1 hr at 100 µl volume, followed by washing 5 times with PBS-Tween. Biotin saturated samples (biotin 17 mg/l, Sigma) and BSA were used as negative controls and the assay was standardised with chicken avidin (Sigma), diluted to known concentrations. Biotinylated AP was used to probe the bound biotin-binding proteins diluted 1:3000 in PBS-BSA (1 hr, 37 °C). Para-nitrophenyl phosphate (1 mg/ml, Sigma) was used as a signal molecule and absorbencies were measured at 405 nm with a Bio-Rad 450 Microplate-reader. Each egg albumen was assayed twice. Intra-assay coefficient of variation was 9.51%, while inter-assay coefficient of variation was 10.7%, as assessed from a subsample of 12 eggs that were assayed twice.

Meteorological data. Daily mean temperatures were collected by the weather station of Lobbi owned by the Regione Piemonte. This weather station is the closest to the study area (5 km away, across flat countryside). The mean temperature values in the laying period ranged from 7.9 to 28.0 °C, with an average of 18.6 ± 4.7 SD. We associated the temperatures from the 5 days preceding the laying of each egg to the mass and components, since this time span probably included the period of rapid yolk development and albumen allocation (Lipar et al. 1999, Young & Badyaev 2004).

Statistics. We utilized linear mixed models to examine the effect of temperature on egg characteristics. In the models, the egg mass, the yolk, albumen and shell weight, and the concentration of total carotenoids, avidin and lysozyme were the dependent variables. The year was considered as an independent factor, while female identity was inserted as a random effect. Egg position in the laying order and temperatures in the days preceding laying were inserted as covariates. We did not entered laying date in the models because laying date and position in the laying order were highly correlated (in our captivity condition there was good breeding synchronization. Hence, all breeders started to lay within a few days of each other, and all breeders ended the breeding season at a similar date).

Initially, we entered temperatures at all 5 days before laying simultaneously (from day –1 to –5). However, temperature values were highly collinear. Hence, we used a stepwise backward procedure of selection of independent variables by eliminating, at each step, the day on which temperatures had the smallest predictive power. The stepwise backward procedure led to a final model containing only the variables reaching the 0.05 level of significance.

All stepwise models were evaluated using the corrected Akaike information criterion (AICc), and each time we checked whether the final stepwise selected model was also the model with the smallest AICc value. The model with the smallest AICc was considered as the most parsimonious model, assuming that models that differed by more than 2 in AIC were different (Burnham & Anderson 2002). Statistics were computed by SYSTAT 12 (Wilkinson 2007).

RESULTS

Table 1 shows the results of the mixed models used to analyze egg mass in relation to year, position in the laying order, and temperature. In the complete model, there was a year by year difference, while the position in the laying order was only marginally related to egg mass. Temperature of the day preceding laying was significantly related to egg mass, with a positive relation with temperature of day –1. The stepwise backward selection procedure confirmed this result, with year, laying order and temperature of day –1 retained in the final model (Table 1). The mean egg mass was 14.01 g ± 1.04 SD. The variation related to temperature of day –1 explained 0.6% of variance, and the regression line between temperature and mass predicted a variation of 0.26 g (1.88% of mean egg mass) in the range of observed temperatures.

The albumen, yolk, and shell mass did not differ between years (Table 2). Egg position in the laying order was positively related to albumen mass, and negatively to yolk and shell mass. In the complete model, the temperature of the day immediately before laying (day –1) was positively related to albumen mass, and negatively to shell mass, while temperature of day –5 was positively related to yolk mass. The analyses run with a stepwise backward procedure led to final models that confirmed the relationships between temperature of day –1 and both albumen (Fig. 1) and shell mass (Fig. 2), whereas the temperature of day –5 was not retained in the stepwise model. The mean albumen mass was 6.09 g ± 0.85 SD, and the mean shell mass was 1.92 g ± 0.25 SD. The variation related to temperature of day –1 explained respectively 4.5% and 25.5% of variance in albumen and shell mass. The regression line between temperature and albumen predicted a variation of 0.92 g (15.1% of mean albumen mass; Fig. 1) in the range of observed temperatures, while the

Table 1.

Multivariate analysis of egg mass (N = 1910) with year as a factor, and laying order and mean daily temperature in the days preceding laying of the focal egg as covariates.

Variable	Complete model			Stepwise model	
	F	*P*	Coefficient (SE)	F	*P*
Year	14.06	**0.001**	—	13.50	0.001
Laying order	3.17	0.075	– 0.005 (0.003)	4.38	0.037
T day 1	5.26	**0.022**	0.020 (0.009)	5.41	0.020
T day 2	1.79	0.180	– 0.015 (0.011)	—	—
T day 3	0.44	0.507	– 0.007 (0.010)	—	—
T day 4	0.04	0.836	– 0.002 (0.010)	—	—
T day 5	0.21	0.650	0.004 (0.008)	—	—
AICc	*3502.7*			*3494.3*	

In the models, female identity was inserted as a random factor.

Table 2.

Multivariate analysis of albumen mass (N = 186), yolk mass (N = 185), and shell mass (N= 200), with year as a factor, and laying order and mean daily temperature in the days preceding laying of the focal egg as covariates.

Variable	Complete model			Stepwise model	
	F	*P*	Coefficient (SE)	F	*P*
Albumen mass					
Year	1.98	0.162	—	—	—
Laying order	9.37	**0.003**	0.053 (0.017)	10.71	0.001
T day 1	4.19	**0.043**	0.069 (0.038)	4.67	0.032
T day 2	0.08	0.785	– 0.013 (0.046)	—	—
T day 3	0.46	0.500	– 0.027 (0.040)	—	—
T day 4	0.69	0.408	0.033 (0.039)	—	—
T day 5	1.66	0.200	– 0.045 (0.030)	—	—
AICc	*446.6*			*433.4*	
Yolk mass					
Year	1.90	0.170	—	—	—
Laying order	5.98	**0.016**	– 0.033 (0.014)	6.79	0.010
T day 1	0.91	0.342	– 0.029 (0.03)	—	—
T day 2	1.02	0.314	0.037 (0.037)	—	—
T day 3	0.06	0.810	0.008 (0.032)	—	—
T day 4	2.16	0.144	– 0.046 (0.031)	—	—
T day 5	4.24	**0.041**	0.049 (0.024)	—	—
AICc	*360.68*			*338.7*	
Shell mass					
Year	1.50	0.223	—	—	—
Laying order	24.77	**0.001**	– 0.019 (0.004)	34.23	0.001
T day 1	16.56	**0.001**	– 0.035 (0.009)	26.90	0.001
T day 2	0.27	0.603	0.006 (0.011)	—	—
T day 3	0.08	0.773	0.003 (0.011)	—	—
T day 4	0.63	0.428	– 0.008 (0.010)	—	—
T day 5	0.03	0.864	0.001 (0.008)	—	—
AICc	*– 41.4*			*– 71.1*	

In the models, female identity was inserted as a random factor.

regression line between temperature and shell predicted a variation of 0.53 g (27.5% of mean shell mass; Fig. 2).

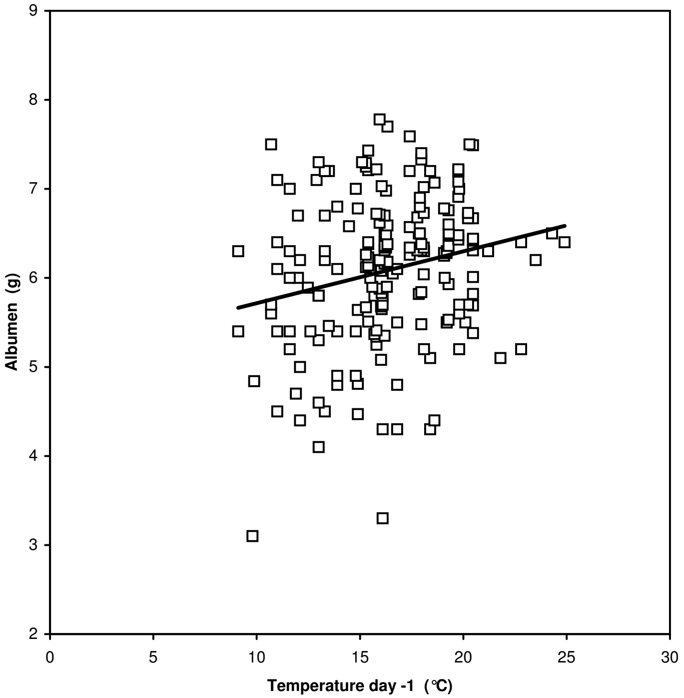

Fig. 1. — Relationship between egg albumen mass and mean daily temperature on the day preceding laying (day –1).

The lysozyme and avidin concentration differed between years (Table 3). Egg position in the laying order was negatively related to lysozyme concentration, but was unrelated to carotenoid and avidin concentrations. In the complete models, temperature of the day immediately before laying (day –1) was positively related to avidin concentration (Fig. 3), while lysozyme and carotenoid concentration were independent of temperature (Table 3). The analyses run with a stepwise backward procedure led to final models that confirmed the results obtained with the complete models. The variation related to temperature of day –1 explained 10.0% of variance, and the regression line between temperature and avidin predicted a variation of 0.41 mg/L (75.1% of mean avidin concentration) in the range of observed temperatures.

DISCUSSION

In this study we found a significant relationship between temperatures in the days before laying and some characteristics of the eggs laid. In our experimental condition the food was provided ad libitum and we were, thus,

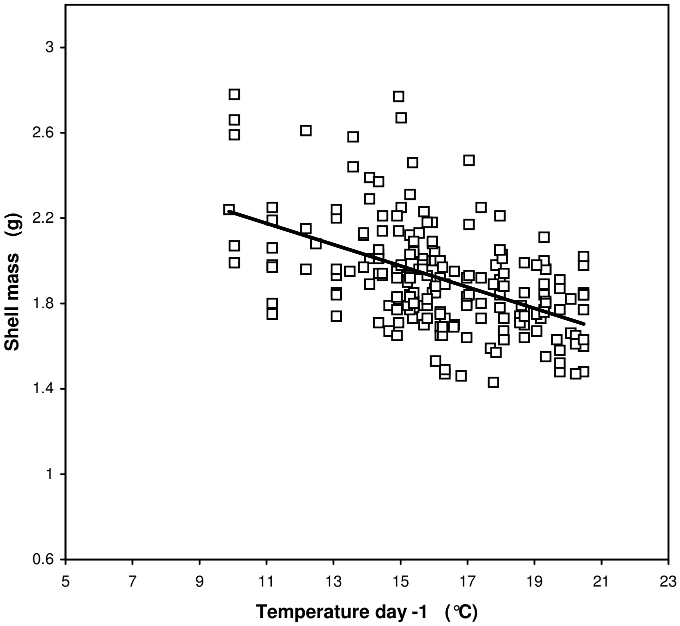

Fig. 2. — Relationship between egg shell mass and mean daily temperature on the day preceding laying (day –1).

able to remove the possible indirect effects of temperature due to a concomitant effect of temperature on food availability.

Temperature can be important in determining the amount of time and energy that can be allocated to the different stages of the life cycle. For birds, this has been demonstrated for foraging activity (ACQUARONE et al. 1998, 2001), egg production (WARD 1996, STEVENSON & BRYANT 2000), incubation (BRYAN & BRYANT 1999) and brood-rearing (SPENCER & BRYANT 2002). Experimental manipulation of temperatures has demonstrated the effects of mean temperature on the timing of laying (MEIJER et al. 1999) and egg mass (NAGER & VAN NOORDWIJK 1992). This implies that temperature can alter thermoregulation costs, leading to a reallocation of energy resources available for reproduction. In this way, mean temperature could directly influence fitness via the quality of eggs (WILLIAMS 1994, PERRINS 1996, BOTH et al. 1999, CHRISTIANS 2002).

In the present study, we found a positive relationship between air temperature at day –1 and egg mass. A similar effect of temperature on egg mass has been reported for the barn swallow, blackbird *Turdus merula*, great tit *Parus major* and pied flycatcher *Ficedula hypoleuca* (OJANEN 1983, MAGRATH 1992, SAINO et al. 2004, HARGITAI et al. 2005, WARD & BRYANT 2006). In other cases, egg mass variation was not related to temperature (LESSELLS et al. 2002), while a negative relationship was reported in galliformes (indicating a possible heat stress effect from high temperatures: SAHIN et al. 2002, NOWACZEWSKI & KONTECKA 2005), and in the snow goose (WILLIAMS & COOCH 1996).

Table 3.

Multivariate analysis of carotenoids (N = 100), lysozyme (N = 130), and avidin (N = 146) concentrations with year as a factor. and laying order and mean daily temperature in the days preceding laying of the focal egg as covariates.

Variable	Complete model			Stepwise model	
	F	*P*	Coefficient (SE)	F	P
Carotenoids					
Year	—	—	—	—	—
Laying order	2.07	0.155	0.105 (0.073)	—	—
T day 1	2.37	0.128	– 0.229 (0.149)	—	—
T day 2	0.03	0.858	0.032 (0.179)	—	—
T day 3	0.12	0.727	– 0.063 (0.178)	—	—
T day 4	0.26	0.614	0.091 (0.179)	—	—
T day 5	0.01	0.953	– 0.008 (0.142)	—	—
AICc	*432.6*			*425.1*	
Lysozyme					
Year	11.56	**0.001**		12.08	0.001
Laying order	3.91	**0.050**	– 0.008 (0.004)	5.431	0.022
T day 1	0.34	0.563	– 0.006 (0.010)	—	—
T day 2	1.14	0.289	0.013 (0.012)	—	—
T day 3	0.50	0.484	– 0.009 (0.013)	—	—
T day 4	1.02	0.315	– 0.012 (0.011)	—	—
T day 5	0.08	0.784	– 0.002 (0.009)	—	—
AICc	*– 56.9*			*– 84.9*	
Avidin					
Year	11.82	**0.001**	– 0.096 (0.028)	11.97	0.001
Laying order	2.11	0.149	– 0.007 (0.005)	—	—
T day 1	4.16	**0.044**	0.026 (0.013)	4.85	0.030
T day 2	1.32	0.253	– 0.020 (0.017)	—	—
T day 3	0.01	0.961	– 0.001 (0.016)	—	—
T day 4	1.55	0.216	0.020 (0.016)	—	—
T day 5	0.05	0.831	0.003 (0.012)	—	—
AICc	*49.5*			*14.6*	

In the models, female identity was inserted as a random factor.

The percentage variation of egg mass explained by temperature in our model study was small. This result confirms the general pattern observed in birds,

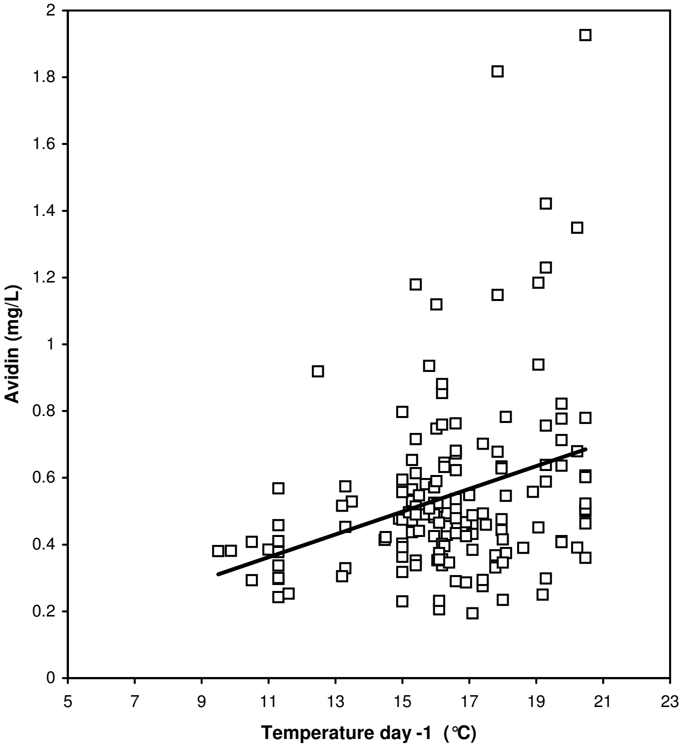

Fig. 3. — Relationship between avidin concentration and mean daily temperature on the day preceding laying (day –1).

where egg size is a characteristic with a small environmental variation and a high repeatability (Christians 2002). In our study, the environmental conditions were standardized, excluding the possible effect of food abundance, territory quality, predators presence, etc. It is conceivable that foraging costs are higher for wild birds than captive birds provided with food ad libitum, thus birds in the wild may frequently experience relatively poor conditions, and temperature variation may be more likely to affect female conditions and egg characteristics variations.

There are several views on which egg component determines egg size. Williams (2001) suggested that egg size is mainly affected by yolk size, while others proposed that variation in albumen content is the main factor contributing to differences in egg size (Parson 1976, Ruiz et al. 2000). To a lesser extent, egg shell, also, can contribute to egg mass variation (Mänd & Tilgar 2003, Roberts 2004). The yolk and albumen fractions have distinctive roles during development. The yolk is the main source of lipids and is implicated in mass gain, whereas the albumen is a key source of water and proteins (Carey et al. 1980, Sotherland & Rahn 1987). An increase in yolk and albumen could be beneficial to embryonic development and offspring survival (Williams 1994, Braun & Fehlhaber 1996, Ferrari et al. 2006, Bonisoli-Alquati et al. 2007).

In our study, yolk mass was positively related to temperature at day –5, while albumen mass was positively related to temperature at day –1, and shell mass was negatively related to temperature at day –1. Interestingly, the period when the temperature influenced egg components was the day immediately before laying for the albumen and the shell, and the day far from laying for the yolk: these are the times when these elements are predicted to be completed in the oviduct (RICKLEFS 1974, BOARD 1982, CAREY 1996, CHRISTIANS & WILLIAMS 2001). A positive effect of temperature on egg components was reported for the great tit and the pied flycatcher (OJANEN 1983), while no effect was found in the great tit by LESSELLS et al. (2002).

In the grey partridge, we found a negative relationship between shell mass and temperature at day –1. This result could be related to two different reasons, (a) a possible trade-off between the observed increase of albumen and the concomitant decrease of shell, or (b) a possible detrimental effect of temperature on shell deposition (heat stress: SAUVEUR & PICARD 1987, BALNAVE & MUHEEREZA 1997). The shell protects the embryo against mechanical stress, water loss and infection and is the main source of calcium for the growing embryo (BOARD 1982). Hence, a reduction in shell mass could be detrimental for offspring development (CAREY 1996, TILGAR et al. 2005, NAGER 2006).

In our study we also found variations of yolk, albumen and shell mass, and lysozyme concentration in relation to laying order. This fact could suggest the presence of some differential resource allocation between first and late laid eggs. However, in our study conditions it was not possible to disentangle the effect of date and laying order because these two variables were highly correlated.

In our study, carotenoid and lysozyme concentrations were not related to temperature, while we found a positive relationship between avidin and temperature on the day immediately before laying. The last day of egg formation is important for albumen production. Albumen is thought to provide protein reserves for the growing embryo (FINKLER et al. 1998, NAGER 2006), and it is important for hatching success and offspring performance (BONISOLI-ALQUATI et al. 2007). The albumen is rich in water and proteins, but albumen also contains antimicrobial proteins that protect the embryo from infections that may pass through the eggshell. Among these proteins, we analyzed lysozyme and avidin. Lysozyme concentration did not vary in relation to temperature. There is only one study concerning the effect of pre-laying temperature on lysozyme in birds (SAINO et al. 2004), where a positive influence of temperature in the barn swallow was reported. More studies are needed to explain the difference between the two studied species, and it would be interesting to compare birds differing both phylogenetically and with different life-history traits.

To the best of our knowledge, no other studies have analyzed the influence of temperature on avidin. Avidin has a very strong affinity for biotin, thus its role is important for innate immunity (BOARD & FULLER 1974, ELO et al. 1980, KORPELA et al. 1983). Indeed, biotin is an essential nutrient for bacteria to grow and avidin, by strongly binding it, will make biotin unavailable to microorganisms. The positive effect of temperature on avidin, in this way, could play a favourable role for offspring fitness prospects.

In this study, carotenoids concentration was unrelated to temperature. The result is similar to that reported by SZIGETI et al. (2007) in the blue tit *Parus caer-*

uleus, where yolk colour was used as an index of carotenoids concentration. On the other hand, a positive relationship between carotenoids and temperature was found in the barn swallow eggs (Saino et al. 2004), and, in the collared flycatcher, Hargitai et al. (2006) found that in a year with high temperatures there were more carotenoids in the eggs. In all these studies the positive effects of temperature could be due to an indirect effect of weather on food abundance. Moreover, weather condition, and air temperature in particular, can vary considerably, thus mothers that lay eggs may deliver more carotenoids to their eggs in order to buffer the negative effects of environmental stress on the developing embryo.

In conclusion, studies examining correlations between ambient temperatures during egg formation and egg size or components, yield mixed results, including positive and negative relationships, as well as no effect. Even within a species, there may be significantly positive and significantly negative relationships (Christians 2002). Given the lack of consistent results in the observational studies reported up to now, more experimental studies on the effects of temperature on egg production are necessary. Further attention should be dedicated to key substances contained in the yolk and the albumen, that have been little considered until now, in particular molecules with antibacterial activity, like lysozyme and avidin.

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