Article (refereed) - postprint

Caro, Samuel P.; Schaper, Sonja V.; Dawson, Alistair; Sharp, Peter J.; Gienapp, Phillip; Visser, Marcel E. 2013. **Is microevolution the only emergency exit in a warming world?: temperature influences egg laying but not its underlying mechanisms in great tits.**

NOTICE: this is the author's version of a work that was accepted for publication in *General and Comparative Endocrinology*. Changes resulting from the publishing process, such as peer review, editing, corrections, structural formatting, and other quality control mechanisms may not be reflected in this document. Changes may have been made to this work since it was submitted for publication. A definitive version was subsequently published in *General and Comparative Endocrinology* (2013), 190. 164-169. 10.1016/j.ygcen.2013.02.025

www.elsevier.com/

Prepared for General and Comparative Endocrinology

Is microevolution the only emergency exit in a warming world? Temperature influences egg laying but not its underlying mechanisms in great tits

Samuel P. Caro[1,†,*], Sonja V. Schaper[1,†], Alistair Dawson[2], Peter J. Sharp[3], Phillip Gienapp[1], Marcel E. Visser[1]

[1]Netherlands Institute of Ecology (NIOO-KNAW), P.O. Box 50, 6700PB Wageningen, The Netherlands
[2]Centre for Ecology & Hydrology, Bush Estate, Penicuik, Edinburgh, Midlothian, EH26 0QB, Scotland, UK
[3]Roslin Institute, University of Edinburgh, Easter Bush, Midlothian, EH25 9RG, Scotland, UK

* author for correspondence. E-mail: s.caro@nioo.knaw.nl, Tel: +31(0)317.47.34.51
† these authors equally contributed to the study

<u>Keywords</u>: adaptation, heritability, climate change, phenology, Parus major, Cyanistes caeruleus, endocrinology, seasonal timing

Abstract

Many bird species have advanced their seasonal timing in response to global warming, but we still know little about the causal effect of temperature. We carried out experiments in climate-controlled aviaries to investigate how temperature affects luteinizing hormone, prolactin, gonadal development, timing of egg laying and onset of moult in male and female great tits. We used both natural and artificial temperature patterns to identify the temperature characteristics that matter for birds. Our results show that temperature has a direct, causal effect on onset of egg-laying, and in particular, that it is the pattern of increase rather than the absolute temperature that birds use. Surprisingly, the pre-breeding increases in plasma LH, prolactin and in gonadal size are not affected by increasing temperature, nor do they correlate with the onset of laying. This suggests that the decision to start breeding and its regulatory mechanisms are fine-tuned by different factors. We also found similarities between siblings in the timing of both the onset of reproduction and associated changes in plasma LH, prolactin and gonadal development. In conclusion, while temperature affects the timing of egg laying, the neuroendocrine system does not seem to be regulated by moderate temperature changes. This lack of responsiveness may restrain the advance in the timing of breeding in response to climate change. But as there is heritable genetic variation on which natural selection can act, microevolution can take place, and may represent the only way to adapt to a warming world.

Introduction

Matching the timing of breeding with the local peak of food abundance is of critical importance for many animals such as insectivorous birds that rely on external food sources for feeding their nestlings [1,28,66]. Any deviation from this favorable period has dramatic consequences in terms of energy expenditure, reproductive success and for the survival of both the parents and the offspring [55,58,68]. Because the time at which the local food peak occurs varies from year to year, and because the decision to start breeding is taken weeks in advance of the period of maximal food demand from the offspring, birds must use predictive environmental cues to try to match the annual optimal breeding period. These cues include photoperiod, temperature, vegetation development and/or social interactions (e.g. opposite-sex behaviors have been shown to enhance gonadal development and/or advance laying in a variety of species [2,29,35,75]). While photoperiod plays an important role in seasonal timing [17,22], it cannot account for the year to year variation in optimal timing. As a consequence, in most geographic zones of the world, birds have to use additional cues that allow fine-tuning the best moment to start breeding within the broad time-window opened by the stimulatory effect of increasing photoperiod on the reproductive system. In the temperate zone ambient temperature is the environmental variable that generally best correlates with this variation in seasonal timing: many observational field studies have shown robust correlations between mean population timing of reproduction and ambient temperature [15,18].

In recent years, the observation that the world's climate is changing at an unprecedented rate has induced a clear upsurge of interest in the influence of temperature on ecological mechanisms. One of the best-documented impacts of global warming on living organisms is on phenology (i.e. seasonal timing). Clear phenological shifts have been observed in all taxonomic groups in terrestrial, aquatic and marine environments over time [14,38,43,54]. However, the rate at which different species change their phenology is highly variable, causing phenological mismatches in food chains [42,62,64]. These mismatches can influence population viability and can lead to natural selection on the mechanisms underlying timing, especially on the intensity with which ambient temperature affects timing. Establishing the causal effect of temperature on phenology is thus critical if we want to predict the maximum rate of temperature increase organisms can cope with, or adapt to in the coming decades [26]. At present, this causal effect of temperature on seasonal timing and the underlying physiological basis of temperature integration in birds and mammals is still poorly understood.

The first experiments investigating the effect of temperature on birds' reproduction date back to 1937 [53] and since then many more studies have addressed this question [16,19,20,30,39,49,50,69,70,72-74]. Very few studies have looked at the effect of temperature on timing of reproduction directly, by obtaining laying dates under controlled conditions [34,44]. Most experiments used temporal patterns of hormone concentration or gonadal growth, generally in males [6], as proxies for timing of reproduction. No clear pattern emerges from these studies: there is variation both among species and between populations of the same species on how temperatures affect the timing of reproductive development. There are moreover potential problems with using proxies for timing of breeding as their relationship with laying dates is not always straightforward (see below) [46].

Temperature can affect seasonal timing of reproduction in two ways. Particularly low or high ambient temperatures can lead to an unfavorable energy balance and thus no energy can be allocated to breeding. In these cases temperature may constrain reproduction [40,52,60]. Alternatively, temperature may be a source of information. In that case, the effect of temperature on timing is that temperature acts as a cue predicting future environmental conditions, i.e. the optimal period for breeding to take place, and by using this predictive value of temperature, animals increase their fitness [75]. It should be noted that this predictive character of temperature may sometimes only be indirect, via a third variable such as vegetation phenology or food abundance. If temperature acts as a constraint or as an indirect predictive cue, birds would not need to directly sense and interpret temperature information to adapt to a changing climate. In addition, we would not only need to incorporate the relationship between temperature and timing in the models predicting the future consequences of global warming, but we would also need to take the effects of temperature on the third variable into account. As a consequence, one critical question for understanding and predicting the consequences of climate change is whether temperature has a direct signaling effect on seasonal timing of reproduction in birds.

Temperature has a direct effect on timing of breeding in great tits

Demonstrating a direct effect of ambient temperature on timing requires experiments under controlled conditions in the laboratory [48]. In 1999 we started a research program that aims at deciphering the possible causal relationships between temperature and breeding phenology in great tits (*Parus major*). This research program makes use of 36 climate-controlled aviaries, in which single pairs of great tits are housed. During the first 6 years, birds were exposed to a slowly increasing photoperiod and to temperature patterns mimicking the actual temperature variation of a year when great tits bred respectively very early (1998, the "warm" treatment) and very late (1986, the "cold" treatment) in the wild [65] (Fig. 1A). In this set-up, the two temperature profiles to which birds were exposed varied in many different aspects, but the average temperature difference between the treatments was only 4°C. Although there was large between-year variation in the effect of temperature on the onset of laying in aviaries, on average birds exposed to the warm treatment did start laying earlier than birds exposed to the cold treatment. Temperatures over a 3-week period prior to mean egg–laying date were the best predictor of variation in timing of breeding [65]. As birds were fed *ad libitum* and were maintained under artificial conditions that limit the array of environmental cues available, these results demonstrated that temperature does have a direct, causal effect on the onset of breeding in great tits. Furthermore, for a number of individual females, we had laying dates both from the aviaries and from the wild (either from the year(s) before they were brought into captivity or the year(s) after they were released at the end of the experiment). Overall those females laid later in captivity than in the wild, but there was a strong correlation between the laying dates recorded in the two kinds of environments: females that laid early in captivity also laid early in the wild [65]. This result helped validate investigations of reproduction of wild birds in captivity.

An increasing temperature is the critical cue

At the time when the direct influence of ambient temperature on timing of breeding in great tits was demonstrated, the precise characteristics of the seasonal temperature profiles modulating a bird's decision to lay was still unknown. Most observational and experimental studies on the effect of temperature on reproduction use the mean value of temperature in their analyses. Correlational studies conducted in the wild generally calculate the average temperature or "warmth sums" of daily minimal, mean or maximal temperatures over fixed periods of time, and relate these temperature values with the phenotypic traits of interest [3,4,12,32,57]. Experimental studies in captivity generally use temperatures that do not vary in time and are therefore set to remain constant around an average value [e.g. 30,50,72]. As a consequence, we first experimentally tested whether it is the mean absolute value of ambient temperature that Dutch great tits use to time their breeding period. Pairs of birds were exposed to two temperature treatments that increased stepwise by 0.65°C per week throughout the spring but constantly differed by 4°C between the two groups (the same overall temperature difference that allowed the demonstration of a direct effect of temperature in the earlier experiment) (Fig. 1B). In this experiment, the laying dates did not differ between the two groups, suggesting that the average temperature value is not the critical temperature characteristic for the onset of egg laying in great tits. However, the birds terminated laying and started molting earlier when exposed to warm temperatures. A similar early shutting down of the reproductive machinery under warm temperatures was shown in European starlings (*Sturnus vulgaris*) [16] and other populations of great tits [50]. Although the evolutionary significance of such an effect remains to be discovered, it suggests that in a warmer climate, birds' breeding seasons might become shorter, resulting in fewer offspring produced [25].

In addition to the absolute temperature not playing a role, the daily amplitude of temperature variation also does not seem to be used as a cue for breeding. In temperate zones, the difference between daily minimum and maximum temperatures increases progressively during spring. Sensing this variation in daily amplitude could thus be a relevant time-cue for seasonally breeding birds. We exposed great tits to temperatures that presented either a high or a low daily fluctuation around two different mean temperatures (Fig. 1C). The temperature profiles remained the same throughout spring, i.e. no seasonal variation in temperature. Such temperature treatments did not influence when birds initiated and terminated laying, suggesting that the daily variation in temperature is not relevant for predicting the annual optimal breeding window [47].

The relevant temperature information that female great tits use to time their breeding period seems to reside in the pattern of increase in temperature. From the different experiments in which great tits were exposed to artificial patterns of temperature variation, it appeared that females started laying earlier when they were exposed to a temperature increase at specific times during their pre-breeding period. A sharp temperature increase around 15 and 45 days prior to laying (black and blue lines on Fig. 1D) did significantly advance the laying of the first egg compared to no increase at all (dashed-red and -grey on Fig. 1D). A moderate and progressive increase one month before laying (red and light-blue lines on Fig. 1E) was more influential than increases occurring earlier in the pre-breeding season (orange and dark-blue lines on Fig. 1E). How the temperature rise influenced the onset of laying was also highly dependent on the genetic background of the females (see below). The importance of a temperature increase relatively close to breeding resembles findings by Meijer et al [34], who observed that pairs of starlings started laying as soon as one week after a 5°C temperature increase at different dates in April.

No effect of temperature on pre-breeding physiology

Reproduction is preceded by a cascade of neuro-endocrine reactions triggered by the stimulatory effects of various environmental cues that forecast the optimal timing for breeding. When the environment changes, one could thus predict that it is not so much the trait value itself (i.e. onset and termination of breeding) that natural selection acts on, but rather the neuro-endocrine response mechanisms underlying the phenotype [63]. The effect of temperature that we observe on timing of reproduction would thus be mediated through its influence on the hypothalamo-pituitary-gonadal (HPG) axis, and the selective pressures of a warming climate would operate on its components.

Demonstrating an effect of ambient temperature on the activity of the HPG axis in great tits has proven difficult. In most of the experiments described earlier, monthly measurements of luteinizing hormone (LH), prolactin (PRL) and/or gonadal size (male testis and female follicle volumes) were performed. Overall, no convincing effect of temperature was found on any of these physiological mechanisms preceding breeding, even in the cases where temperature had been shown to influence the onset of laying [47] or the basal metabolic rates [11]. The only temperature effects that were found on the HPG axis were on the mechanisms associated with termination of breeding: in some experiments, warm temperatures (especially when held constant) were shown to accelerate testis regression and LH level decrease [47,67] in a similar fashion as previously described in other studies [16,49,50].

Thus, although ambient temperature does influence the onset of breeding, it does not seem to reliably impact the underlying physiological mechanisms. This suggests that the decision of when to start breeding and the neuro-endocrine pathways that lead to breeding are modulated by different environmental cues. The temperature information that is perceived and integrated by the central nervous system would influence the laying decision without affecting the HPG axis. In contrast, the seasonal recrudescence of the HPG axis activity would be regulated by other environmental cues, such as photoperiod and maintained in a close-to-ready state until the decision of when to start laying is taken and the final maturation of the female follicles takes place. This hypothesis was supported by studies in blue tits that have shown a disconnection between the spring activation of the reproductive system and the actual breeding period [8,9]. Alternatively, temperature might affect components of the HPG axis that were not measured here. It has for example recently been hypothesized that the physiological regulation of reproduction could lie at the level of the gonads, particularly at the level of the female ovary [7,71], where receptors for GnRH and GnIH have been identified [56]. The temperature influence on timing of breeding might thus be mediated via a pathway that directly connects the temperature perception and integration at the brain level to the female ovary. This hypothesis and more generally, the pathways linking temperature to seasonal timing, still need to be tested [10]. However, in great tits temperature does not seem to affect gonadal development in males and females, and gonadal growth is hardly related to timing of breeding (see below). How the environment controls the HPG axis has mostly been studied in relation to photoperiod, and we urgently need to better understand how other, non-photic cues such as temperature influence the pre-breeding physiology [e.g. 51,75,76].

No link between pre-breeding physiology and timing of breeding

Given that ambient temperature was found to modulate the onset of egg-laying, but not its underlying mechanisms (gonadal size, hormonal concentrations, etc.), one could argue that these mechanisms do not reliably predict the onset of reproduction. Most physiological studies on avian reproduction do not measure timing of breeding directly but rather measure the underlying mechanisms, generally in males [5,6], assuming that they mirror the successive breeding stages [46]. Few attempts have been made to validate these physiological proxies against the actual breeding dates [24,37,45,63]. Using the data we collected in great tits breeding in captivity, we have tried to validate some of these proxies, and found very few reliable correlations with the onset of laying [46]. Figure 2 illustrates the relationship between follicle size and laying dates from 28 individual females that were used in an experiment testing for the effect of different absolute temperature values on the onset of breeding (see Fig. 1B). Individual laying dates did not correlate with the overall size of the largest follicles (i.e. intercept on Fig. 2B), nor with the rate of follicle development (i.e. slope on Fig. 2C), meaning that females that have large or fast growing follicles are not necessarily the first to initiate laying. On very few occasions, the different physiological measurements were found to correlate with each other or with laying dates at one particular sampling period, but overall no robust trend was described, meaning that laying dates can hardly be predicted based on these commonly used physiological proxies [but see 37,46]. Recording laying dates in the wild and in captivity thus seems to be necessary in studies investigating the physiological orchestration of breeding.

Genetic effects on timing of breeding and the HPG axis

Both in the wild and in captivity, the observed variation in timing of breeding in response to variation in temperature is largely due to individual phenotypic plasticity: the same individual starts breeding at different times under different climatic conditions [41]. In response to global warming, birds have often been shown to use their phenotypic plasticity to adjust to the new environmental conditions [12,31]. However, there are also consistent differences among individual birds, some individuals always breed earlier than others and/or advance breeding more when spring is warmer [36]. These individual differences reflect genetic variation in the energetic costs associated with laying [13,40] or in the way they respond to environmental cues [36]. Such genetic variation is a necessary substrate for natural selection to operate and micro-evolution to occur, in response to a changing environment [61]. To estimate the rate of micro-evolution under climate change, we need to understand to what extent individuals consistently differ in their timing of reproduction and where the genetic variation lies within the neuro-endocrine cascade that mediates timing [33,63]. The importance of micro-evolutionary responses of physiological mechanisms is starting to be acknowledged, and recent years have seen an upsurge of interest for studies addressing that question [21,27,33,59] .

Most of the experiments that we performed to test for an effect of temperature on seasonal timing of reproduction in great tits used animals of known genetic background, including siblings, that were allocated across the different treatments. By comparing the pre-breeding physiology and laying dates between birds exposed to different temperatures, but with *ad* *libitum* food (to exclude potential genetic variation in the energetic costs of laying), we could determine if birds differed genetically in cue sensitivity or in the way these cues are transduced in physiological pathways. We found clear differences between families of birds (each year we collected whole broods of chicks in the wild from early- or late-laying maternal lines, and we compared those siblings in captivity, see [47,67] for details) in the onset and termination of breeding, as well as in the underlying physiological mechanisms. For example, females that have mothers that lay early in the wild laid early themselves in the aviaries, and some families of males had larger testis volumes than others. Overall, and even if the results were not always consistent across successive experiments and traits, we found some genetic effects in all the physiological measures that we performed: LH, PRL, testis and follicle volumes [47,67, unpub. data]. Interestingly, some of these effects were influenced by the temperature treatments to which the birds were exposed. For instance, the effect of a temperature increase on the advancement of laying was mostly visible in females originating from genetically early families, demonstrating genetic differences in how birds perceive or respond to temperature variation [47].

Is microevolution the only emergency exit in a warming world?

In conclusion, temperature has a direct effect on timing of breeding [34,65], which in itself is an important step towards assessing the implication of climate change on seasonal timing. In addition, we showed that the most important temperature characteristic that great tits use to time their reproduction is its pattern of increase [47]. Originally we thought that this effect of temperature on timing would be mediated via the HPG axis, meaning that a temperature increase in spring would elevate the concentrations of the gonadotropins in the blood and stimulate the growth of the gonads. Temperature does however not seem to influence these commonly studied mechanisms [16,39,47,67], and we found no convincing evidence for a relationship between the development of the HPG axis and the onset of laying [46]. This suggests that the effector pathways by which temperature acts on timing by-pass some major components of the reproductive system, and that timing of breeding and its underlying mechanisms are regulated by different environmental cues. It implies that, under global warming, great tits could use temperature information to adequately advance their laying period, but that this advancement of phenology might at some point become constrained by the lack of responsiveness of the HPG axis to an increasing temperature.

However, we found some genetic variation both in the onset and termination of laying, and in the underlying mechanisms, sometimes in interaction with temperature [47,67, unpub. data]. This suggests that some families of birds will be able to respond more quickly than others to the increasing temperature, which will lead to difference in fitness between families [36]. Such heritable genetic variation in cue sensitivity (this could be temperature or photoperiod) will allow natural selection to modulate a bird's response to the information coming from its environment. In the case of birds breeding too late in an advancing season, this should lead to an increase in their sensitivity to these cues, i.e. they would start breeding under shorter photoperiods or cooler temperatures. And if phenotypic plasticity becomes insufficient to face the changes, which has already been observed in some populations [31], the only way birds might be able to restore the synchrony between their timing of breeding and the changing optimal period for rearing chicks, would be via microevolution. But microevolution is generally a slow process and the pivotal question now is whether adaptation will be fast enough [23,61].

Acknowledgements

We thank Laura Reparaz for comments made on an earlier version of this manuscript. We are grateful to Floor Petit, Marylou Aaldering and Janneke Venhorst for taking good care of the birds, and Ab and Gilles Wijlhuizen for technical support with the aviaries. P.J.S. thanks the Roslin Institute, Edinburgh University, for providing access to laboratory facilities. M.E.V. was supported by a NWO-VICI grant, S.P.C. by a NWO-VENI grant. Experiments were carried out in accordance with Dutch law under licenses CTO.0501, CTE.9903, 0201, 0809 and 0907 of the Animal Experimentation Committee of the Royal Dutch Academy of Sciences (DEC-KNAW).

References

[1] J.R. Baker, The evolution of breeding seasons, in: G.R. de Beer (Ed.), Evolution, Essays on Aspects of Ev. Biology Presented to Prof. E.S. Goodrich on His 70th Birthday. Oxford University Press, London, New York, 1938, pp.

[2] G.E. Bentley, J.C. Wingfield, M.L. Morton, G.F. Ball, Stimulatory effects on the reproductive axis in female songbirds by conspecific and heterospecific male song, Horm. Behav. 37 (2000) 179-189.

[3] P. Bourgault, D. Thomas, P. Perret, J. Blondel, Spring vegetation phenology is a robust predictor of breeding date across broad landscapes: a multi-site approach using the Corsican blue tit (Cyanistes caeruleus), Oecologia 162 (2010) 885-892.

[4] J.E. Brommer, K. Rattiste, A.J. Wilson, Exploring plasticity in the wild: laying date-temperature reaction norms in the common gull Larus canus, Proc. R. Soc. B.-Biol. Sci 275 (2008) 687-693.

[5] W.J. Burger, A review of experimental investigations on seasonal reproduction in birds, Wilson Bull. 61 (1949) 211-230.

[6] S.P. Caro, Avian ecologists and physiologists have different sexual preferences, Gen. Comp. Endocrinol. 176 (2012) 1-8.

[7] S.P. Caro, A. Charmantier, M.M. Lambrechts, J. Blondel, J. Balthazart, T.D. Williams, Local adaptation of timing of reproduction: females are in the driver's seat, Func. Ecol. 23 (2009) 172-179.

[8] S.P. Caro, M.M. Lambrechts, J. Balthazart, Early seasonal development of brain song control nuclei in male blue tits, Neurosci. Let. 386 (2005) 139-144.

[9] S.P. Caro, M.M. Lambrechts, O. Chastel, P.J. Sharp, D.W. Thomas, J. Balthazart, Simultaneous pituitary-gonadal recrudescence in two Corsican populations of male blue tits with asynchronous breeding dates, Horm. Behav. 50 (2006) 347-360.

[10] S.P. Caro, S.V. Schaper, R.A. Hut, G.F. Ball, M.E. Visser, The case of the missing mechanism: how does temperature influence seasonal timing in endotherms, PLoS Biol. in press (2013).

[11] S.P. Caro, M.E. Visser, Temperature-induced elevation of basal metabolic rate does not affect testis growth in great tits, J. Exp. Biol. 212 (2009) 1994-1998.

[12] A. Charmantier, R.H. McCleery, L.R. Cole, C. Perrins, L.E.B. Kruuk, B.C. Sheldon, Adaptive phenotypic plasticity in response to climate change in a wild bird population, Science 320 (2008) 800-803.

[13] O. Chastel, A. Lacroix, M. Kersten, Pre-breeding energy requirements: thyroid hormone, metabolism and the timing of reproduction in house sparrows *Passer domesticus*, J. Avian Biol. 34 (2003) 298-306.

[14] E.E. Cleland, I. Chuine, A. Menzel, H.A. Mooney, M.W. Schwartz, Shifting plant phenology in response to global change, TREE 22 (2007) 357-365.

[15] H.Q.P. Crick, C. Dudley, D.E. Glue, D.L. Thomson, UK birds are laying eggs earlier, Nature 388 (1997) 526-526.

[16] A. Dawson, The effect of temperature on photoperiodically regulated gonadal maturation, regression and moult in starlings - potential consequences of climate change, Func. Ecol. 19 (2005) 995-1000.

[17] A. Dawson, V.M. King, G.E. Bentley, G.F. Ball, Photoperiodic control of seasonality in birds, J. Biol. Rhythms 16 (2001) 365-380.

[18] P. Dunn, Breeding dates and reproductive performance, Adv. Ecol. Res. 35 (2004) 69-87.

[19] M.E. El Halawani, J.L. Silsby, E.J. Behnke, S.C. Fehrer, Effect of ambient temperature on serum prolactin and luteinizing hormone levels during the reproductive life cycle of the female turkey (Meleagris gallopavo), Biol. Reprod. 30 (1984) 809-815.

[20] W.L. Engels, C.E. Jenner, The effect of temperature on testicular recrudescence in Juncos at different photoperiods, Biological Bulletin 110 (1956) 129-137.

[21] M.R. Evans, M.L. Roberts, K.L. Buchanan, A.R. Goldsmith, Heritability of corticosterone response and changes in life history traits during selection in the zebra finch, J. Evol. Biol. 19 (2006) 343-352.

[22] D.S. Farner, Time measurement in vertebrate photoperiodism, Am. Nat. 98 (1964) 375-386.

[23] P. Gienapp, M. Lof, T.E. Reed, J.M. McNamara, S. Verhulst, M.E. Visser, Predicting demographically-sustainable rates of adaptation: can great tit breeding time keep pace with climate change?, Philos. Trans. R. Soc. B-Biol. Sci. in press (2013).

[24] B. Helm, Geographically distinct reproductive schedules in a changing world: Costly implications in captive Stonechats, Integr. Comp. Biol. 49 (2009) 563-579.

[25] A. Husby, L.E.B. Kruuk, M.E. Visser, Decline in the frequency and benefits of multiple brooding in great tits as a consequence of a changing environment, Proc. R. Soc. B.-Biol. Sci 276 (2009) 1845-1854.

[26] IPCC, Summary for policymakers, in: S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis (Eds.), Climate change 2007: the physical science basis. Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, 2007, pp. 1-18.

[27] B. Kempenaers, A. Peters, K. Foerster, Sources of individual variation in plasma testosterone levels, Philos. Trans. R. Soc. B-Biol. Sci. 363 (2008) 1711-1723.

[28] D. Lack, Ecological adaptations for breeding in birds. Methuen, London, 1968.

[29] D.S. Lehrman, The reproductive behavior of ring doves, Scientific American 211 (1964) 48-54.

[30] D.L. Maney, T.P. Hahn, S.J. Schoech, P.J. Sharp, M.L. Morton, J.C. Wingfield, Effects of ambient temperature on photo-induced prolactin secretion in three subspecies of white-crowned sparrow, *Zonotrichia leucophrys*, Gen. Comp. Endocrinol. 113 (1999) 445-456.

[31] E. Matthysen, F. Adriaensen, A.A. Dhondt, Multiple responses to increasing spring temperatures in the breeding cycle of blue and great tits (*Cyanistes caeruleus, Parus major*), Glob. Change Biol. 17 (2011) 1-16.

[32] R.H. McCleery, C.M. Perrins, ... temperature and egg-laying trends, Nature 391 (1998) 30-31.

[33] J.W. McGlothlin, D.J. Whittaker, S.E. Schrock, N.M. Gerlach, J.M. Jawor, E.A. Snajdr, E.D. Ketterson, Natural selection on testosterone production in a wild songbird population, Am. Nat. 175 (2010) 687-701.

[34] T. Meijer, U. Nienaber, U. Langer, F. Trillmich, Temperature and timing of egg-laying of European starlings, Condor 101 (1999) 124-132.

[35] M.L. Morton, M.E. Pereyra, L.F. Baptista, Photoperiodically induced ovarian growth in the white-crowned sparrow (*Zonotrichia leucophrys gambelii*) and its augmentation by song, Comp. Biochem. Physiol. A-Physiol. 80 (1985) 93-97.

[36] D.H. Nussey, E. Postma, P. Gienapp, M.E. Visser, Selection on heritable phenotypic plasticity in a wild bird population, Science 310 (2005) 304-306.

[37] J.Q. Ouyang, P.J. Sharp, A. Dawson, M. Quetting, M. Hau, Hormone levels predict individual differences in reproductive success in a passerine bird, Proc. R. Soc. B.-Biol. Sci 278 (2011) 2537-2545.

[38] C. Parmesan, G. Yohe, A globally coherent fingerprint of climate change impacts across natural systems, Nature 421 (2003) 37-42.

[39] N. Perfito, S.L. Meddle, A.D. Tramontin, P.J. Sharp, J.C. Wingfield, Seasonal gonadal recrudescence in song sparrows: Response to temperature cues, Gen. Comp. Endocrinol. 143 (2005) 121-128.

[40] C. Perrins, The timing of birds' breeding season, Ibis 112 (1970) 242-255.

[41] M. Pigliucci, Phenotypic plasticity: beyond nature and nurture: syntheses in ecology and evolution. Johns Hopkins University Press, Baltimore, 2001.

[42] E. Post, M.C. Forchhammer, Climate change reduces reproductive success of an Arctic herbivore through trophic mismatch, Philos. Trans. R. Soc. B-Biol. Sci. 363 (2008) 2369-2375.

[43] T.L. Root, J.T. Price, K.R. Hall, S.H. Schneider, C. Rosenzweig, J.A. Pounds, Fingerprints of global warming on wild animals and plants, Nature 421 (2003) 57-60.

[44] K.G. Salvante, F. Vezina, T.D. Williams, Evidence for within-individual energy reallocation in cold-challenged, egg-producing birds, J. Exp. Biol. 213 (2010) 1991-2000.

[45] K.G. Salvante, R.L. Walzem, T.D. Williams, What comes first, the zebra finch or the egg: temperature-dependent reproductive, physiological and behavioural plasticity in egg-laying zebra finches, J. Exp. Biol. 210 (2007) 1325-1334.

[46] S.V. Schaper, A. Dawson, P.J. Sharp, S.P. Caro, M.E. Visser, Individual variation in avian reproductive physiology does not reliably predict variation in laying date, Gen. Comp. Endocrinol. 179 (2012) 53-62.

[47] S.V. Schaper, A. Dawson, P.J. Sharp, P. Gienapp, S.P. Caro, M.E. Visser, Increasing temperature, not mean temperature, is a cue for avian timing of reproduction, Am. Nat. 179 (2011) E55-E69.

[48] F. Seebacher, C.E. Franklin, Determining environmental causes of biological effects: the need for a mechanistic physiological dimension in conservation biology, Philos. Trans. R. Soc. B-Biol. Sci. 367 (2012) 1607-1614.

[49] B. Silverin, P.A. Viebke, Low temperatures affect the photoperiodically induced LH and testicular cycles differently in closely related species of tits (*Parus spp.*), Horm. Behav. 28 (1994) 199-206.

[50] B. Silverin, J.C. Wingfield, K.A. Stokkan, R. Massa, A. Jarvinen, N.A. Andersson, M.M. Lambrechts, A. Sorace, D. Blomqvist, Ambient temperature effects on photo induced gonadal cycles and hormonal secretion patterns in Great Tits from three different breeding latitudes, Horm. Behav. 54 (2008) 60-68.

[51] T.W. Small, P.J. Sharp, P. Deviche, Environmental regulation of the reproductive system in a flexibly breeding Sonoran Desert bird, the Rufous-winged Sparrow, Aimophila carpalis, Horm. Behav. 51 (2007) 483-495.

[52] I.R. Stevenson, D.M. Bryant, Avian phenology - Climate change and constraints on breeding, Nature 406 (2000) 366-367.

[53] H. Suomalainen, The effect of temperature on the sexual activity of non-migratory birds, stimulated by artificial lighting, Ornis Fennica 14 (1937) 108-112.

[54] S.J. Thackeray, T.H. Sparks, M. Frederiksen, S. Burthe, P.J. Bacon, J.R. Bell, M.S. Botham, T.M. Brereton, P.W. Bright, L. Carvalho, T.I.M. Clutton-Brock, A. Dawson, M. Edwards, J.M. Elliott, R. Harrington, D. Johns, I.D. Jones, J.T. Jones, D.I. Leech, D.B. Roy, W.A. Scott, M. Smith, R.J. Smithers, I.J. Winfield, S. Wanless, Trophic level asynchrony in rates of phenological change for marine, freshwater and terrestrial environments, Glob. Change Biol. 16 (2010) 3304-3313.

[55] D.W. Thomas, J. Blondel, P. Perret, M.M. Lambrechts, J.R. Speakman, Energetic and fitness costs of mismatching resource supply and demand in seasonally breeding birds, Science 291 (2001) 2598-2600.

[56] T. Ubuka, N.L. McGuire, R.M. Calisi, N. Perfito, G.E. Bentley, The control of reproductive physiology and behavior by gonadotropin-inhibitory hormone, Integr. Comp. Biol. 48 (2008) 560-569.

[57] J.H. van Balen, A comparative study of the breeding ecology of the gret tits *Parus major* in different habitats, Ardea 61 (1973) 1-93.

[58] A.J. van Noordwijk, R.H. McCleery, C.M. Perrins, Selection for the timing of great tit breeding in relation to caterpillar growth and temperature, J. Anim. Ecol. 64 (1995) 451-458.

[59] K. van Oers, K.L. Buchanan, T.E. Thomas, P.J. Drent, Correlated response to selection of testosterone levels and immunocompetence in lines selected for avian personality, Anim. Beh. 81 (2011) 1055-1061.

[60] F. Vézina, T.D. Williams, Metabolic costs of egg production in the European starling (Sturnus vulgaris), Physiol. Biochem. Zool. 75 (2002) 377-385.

[61] M.E. Visser, Keeping up with a warming world; assessing the rate of adaptation to climate change, Proc. R. Soc. B.-Biol. Sci 275 (2008) 649-679.

[62] M.E. Visser, C. Both, Shifts in phenology due to global climate change: the need for a yardstick, Proc. R. Soc. B.-Biol. Sci 272 (2005) 2561-2569.

[63] M.E. Visser, S.P. Caro, K. van Oers, S.V. Schaper, B. Helm, Phenology, seasonal timing and circannual rhythms: towards a unified framework, Philos. Trans. R. Soc. B-Biol. Sci. 365 (2010) 3113-3127.

[64] M.E. Visser, L.J.M. Holleman, Warmer springs disrupt the synchrony of oak and winter moth phenology, Proc. R. Soc. B.-Biol. Sci 268 (2001) 289-294.

[65] M.E. Visser, L.J.M. Holleman, S.P. Caro, Temperature has a causal effect on avian timing of reproduction, Proc. R. Soc. B.-Biol. Sci 276 (2009) 2323-2331.

[66] M.E. Visser, L.J.M. Holleman, P. Gienapp, Shifts in caterpillar biomass phenology due to climate change and its impact on the breeding biology of an insectivorous bird, Oecologia 147 (2006) 164-172.

[67] M.E. Visser, S.V. Schaper, L.J.M. Holleman, A. Dawson, P. Sharp, P. Gienapp, S.P. Caro, Genetic variation in cue sensitivity involved in avian timing of reproduction, Func. Ecol. 25 (2011) 868-877.

[68] M.E. Visser, A.J. van Noordwijk, J.M. Tinbergen, C.M. Lessells, Warmer springs lead to mistimed reproduction in great tits (*Parus major*), Proc. R. Soc. B.-Biol. Sci 265 (1998) 1867-1870.

[69] M. Wada, Low temperature and short days together induce thyroid activation and suppression of LH-release in Japanese quail, Gen. Comp. Endocrinol. 90 (1993) 355-363.

[70] M. Wada, F. Hatanaka, H. Tsuyoshi, Y. Sonoda, Temperature modulation of photoperiodically induced LH secretion and its termination in Japanese quail (*Coturnix coturnix japonica*), Gen. Comp. Endocrinol. 80 (1990) 465-472.

[71] T.D. Williams, Hormones, life-history, and phenotypic variation: Opportunities in evolutionary avian endocrinology, Gen. Comp. Endocrinol. 176 (2012) 1145-1154.

[72] J.C. Wingfield, T.P. Hahn, D.L. Maney, S.J. Schoech, M. Wada, M.L. Morton, Effects of temperature on photoperiodically induced reproductive development, circulating plasma luteinizing hormone and thyroid hormones, body mass, fat deposition and molt in mountain white-crowned sparrows, *Zonotrichia leucophrys oriantha*, Gen. Comp. Endocrinol. 131 (2003) 143-158.

[73] J.C. Wingfield, T.P. Hahn, M. Wada, L.B. Astheimer, S. Schoech, Interrelationship of day length and temperature on the control of gonadal development, body mass, and fat score in white-crowned sparrows, *Zonotrichia leucophrys gambelii*, Gen. Comp. Endocrinol. 101 (1996) 242-255.

[74] J.C. Wingfield, T.P. Hahn, M. Wada, S.J. Schoech, Effects of day length and temperature on gonadal development, body mass, and fat depots in white-crowned sparrows, *Zonotrichia leucophrys pugetensis*, Gen. Comp. Endocrinol. 107 (1997) 44-62.

[75] J.C. Wingfield, G.J. Kenagy, Natural regulation of reproductive cycles, in: P.K.T. Pawg, M.P. Schreibman (Eds.), Vertebrate endocrinology: fundamentals and biomedical implications. Academic Press Inc., New York, 1991, pp. 181-241.

[76] J.C. Wingfield, K. Sullivan, J. Jaxion-Harm, S.L. Meddle, The presence of water influences reproductive function in the song sparrow (Melospiza melodia morphna), Gen. Comp. Endocrinol. (2012).

Figure captions

Figure 1: Temperature profiles used during experiments conducted in climate-controlled aviaries. **A**: Temperature fluctuations mimicking actual patterns of a "warm" (red line) and a "cold" spring (blue line) during which wild great tits respectively bred exceptionally early (1998) and late (1986). On average the two treatments differed by 4°C. **B-E**: Artificial patterns of temperature variation. **B**: Stepwise increase of temperature throughout spring (increase of 0.65°C per weak) with a constant 4°C difference between the two treatments. **C**: Variation in the daily amplitude of temperature. Birds were exposed to one of four temperature treatments, each composed of a high or low mean temperature (14 *vs.* 8°C) with either a high or low day-night amplitude (6 *vs.* 2°C). Note that the x-axis only ranges over 24h, i.e. there was no seasonal variation. **D**: Variation in the onset and termination of a cold period. Birds were kept at 15°C from December onwards. One group remained at 15°C for the whole spring (dashed red), while the other three groups were temporarily exposed to a lower temperature (7°C) in February (black), March (light blue) or April (dashed grey). This latter group remained at 7°C until laying. **E**: Variation in the onset and the rate of increase of temperature. Four groups of birds were exposed to two consecutive temperature increases. A first increase occurred either in early (orange and red), or late (light and dark blue) February. A second increase occurred either in early (red and light blue) or late (orange and dark blue) March. Temperatures increased at different rates in February, but not in March. A day-night fluctuation of 2°C was superimposed to each of the four temperature profiles. Adapted from [46,47,65,67].

Figure 2: Relationship between female ovarian follicle size and onset of laying in captive great tits. **A**: Individual ovarian follicle growth profiles of 28 females. Each line represents the regression obtained based on the laparotomy data collected on four different occasions (once per month). **B**. Relationship between the size of the follicles and the onset of laying. The y-axis represents the elevation (calculated at the middle point of each regression line) of the regression lines in panel A, each point represent one individual female. Females that have large follicles (high elevations) do not necessarily lay earlier (i.e. no correlation between the variables). **C**. Relationship between the rate of follicle growth and the onset of laying. The y-axis represents the slopes of the regression lines in panel A, each point represent one individual female. Females that have fast growing follicles (steep slopes) do not necessarily lay earlier (i.e. no correlation between the variables).

Figure1

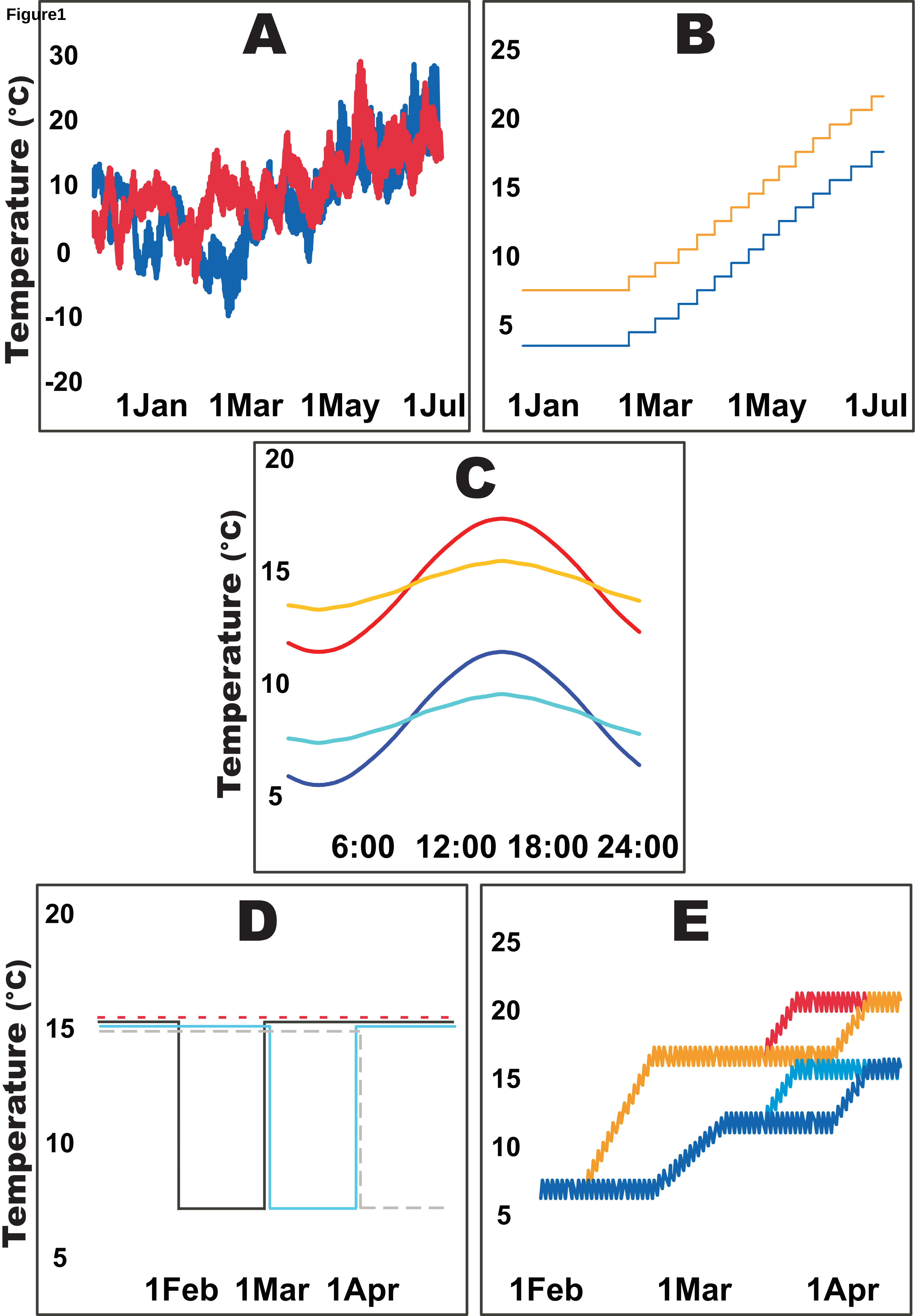
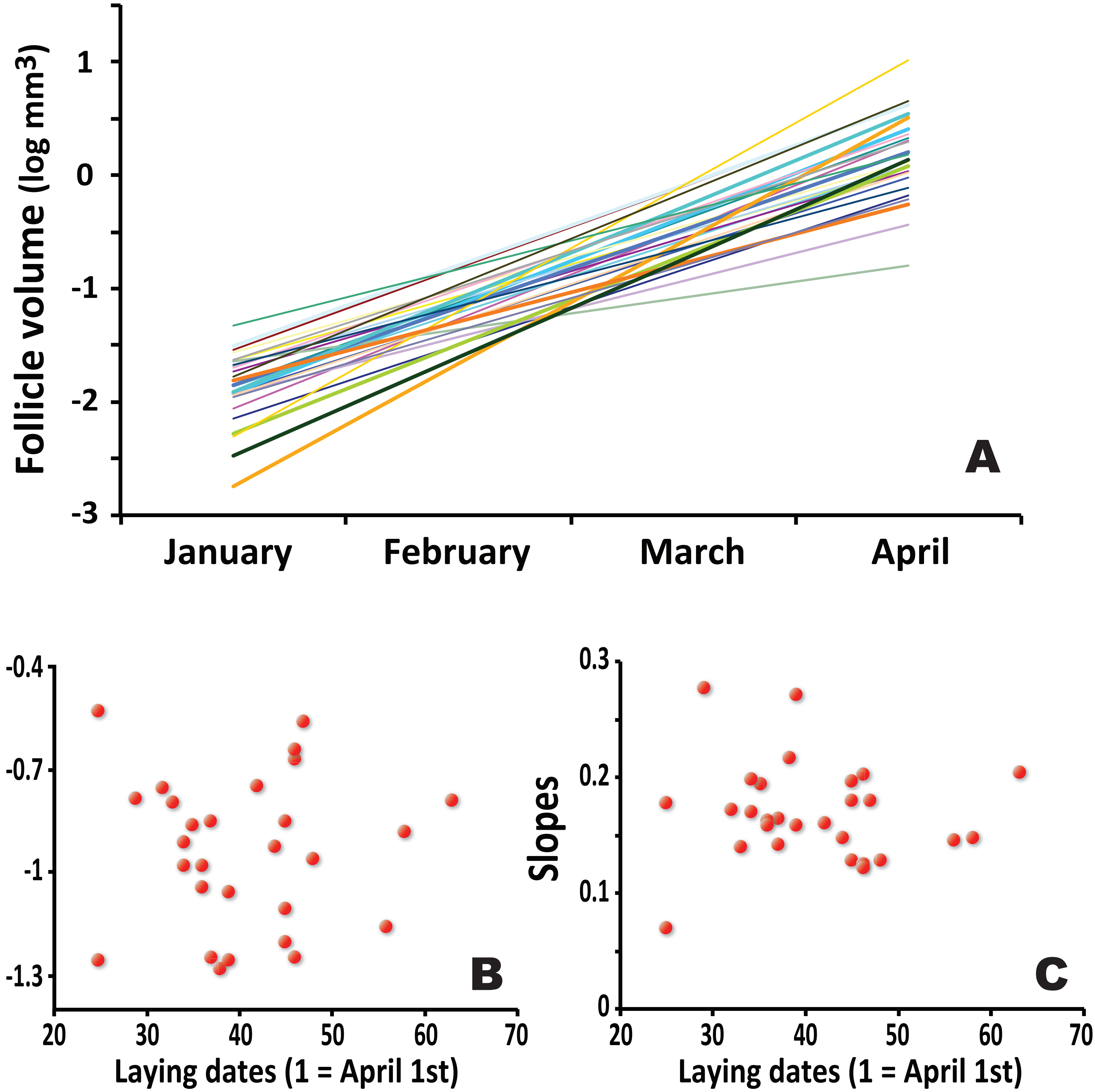

A
Temperature (°C)
30
20
10
0
-10
-20
1Jan
1Mar
1May
1Jul
B
25
20
15
10
5
1Jan
1Mar
1May
1Jul
C
Temperature (°C)
20
15
10
5
6:00
12:00
18:00
24:00
D
Temperature (°C)
20
15
10
5
1Feb
1Mar
1Apr
E
25
20
15
10
5
1Feb
1Mar
1Apr

Figure2.eps


Follicle volume (log mm3)
1
0
-1
-2
-3
January
February
March
April
A
-0.4
-0.7
-1
-1.3
Elevations
20
30
40
50
60
70
Laying dates (1 = April 1st)
B
0.3
0.2
0.1
0
Slopes
20
30
40
50
60
70
Laying dates (1 = April 1st)
C