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Temperature-induced elevation of basal metabolic rate does not affect testis growth in great tits

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SUMMARY

The timing of reproduction varies from year to year in many bird species. To adjust their timing to the prevailing conditions of that year, birds use cues from their environment. However, the relative importance of these cues, such as the initial predictive (e.g. photoperiod) and the supplemental factors (e.g. temperature), on the seasonal sexual development are difficult to distinguish. In particular, the fine-tuning effect of temperature on gonadal growth is not well known. One way temperature may affect timing is *via* its strong effect on energy expenditure as gonadal growth is an energy-demanding process. To study the interaction of photoperiod and temperature on gonadal development, we first exposed 35 individually housed male great tits (*Parus major*) to mid-long days (after 6 weeks of 8 h L:16 h D at 15°C, photoperiod was set to 13 h L:11 h D at 15°C). Two weeks later, for half of the males the temperature was set to 8°C, and for the other half to 22°C. Unilateral laparotomies were performed at weeks 5 (i.e one week before the birds were transferred to mid-long days), 8 and 11 to measure testis size. Two measures of basal metabolic rate (BMR) were performed at the end of the experiment (weeks 11 and 12). Testis size increased significantly during the course of the experiment, but independently of the temperature treatment. BMR was significantly higher in birds exposed to the cold treatment. These results show that temperature-related elevation of BMR did not impair the long-day-induced testis growth in great tits. As a consequence, temperature may not be a crucial cue and/or constraint factor in the fine-tuning of the gonadal recrudescence in male great tits, and testis growth is not a high energy-demanding seasonal process.

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Key words: energy expenditure, respirometry, gonadal cycles, timing of reproduction, seasonal reproduction, phenology, supplemental factors, environmental cues, captive experiments, predictability, day length.

INTRODUCTION

In most organisms, breeding occurs seasonally, and the ability to match the periodicity of appropriate reproductive conditions has major fitness consequences. In anticipation of the most suitable season for breeding, birds generally show tremendous seasonal cycles of recrudescence/regression of their reproductive organs (Balthazart, 1983; Bergtold, 1926; Murton and Westwood, 1977). Even if this seasonal (generally annual) gonadal cycle is considered as an adaptation to save energy expenditure in carrying and maintaining developed reproductive organs all year round (Dawson et al., 2001; Piersma and Lindstrom, 1997), the annual growth of the gonads still requires a substantial increase in energy allocation. This increase may be particularly costly as it has to take place upstream of the period of maximal food abundance, when resources are generally limited (Perrins, 1970; Williams, 2005).

To achieve breeding condition, birds have evolved response mechanisms to a host of environmental stimuli, which function as signals heralding the approach of suitable breeding times (Murton and Westwood, 1977). These proximate factors have been sorted into classes that, combined, orchestrate the life cycle in relation to environmental contingencies (Jacobs and Wingfield, 2000). The seasonal change in length of day is considered as an initial predictive cue because it allows the organisms to make long-term predictions about their habitat. There is an outstanding amount of knowledge on the effect and the mechanisms of photoperiodism, the main initial predictive cue, on bird reproductive cycles (for reviews, see Ball and Balthazart, 2002; Dawson et al., 2001; Farner, 1985; Murton and Westwood, 1977; Sharp, 1996). On the other hand, there are supplementary cues, such as temperature, which enable fine-tuning of responses to initial predictive cues, and also provide short-term predictive information about the environment (Wingfield and Kenagy, 1991). The effect of temperature on the timing of breeding has been well documented (McCleery and Perrins, 1998; Meijer et al., 1999; Nager and van Noordwijk, 1995; O'Connor, 1978; Perrins and McCleery, 1989; Salvante et al., 2007; van Noordwijk et al., 1995; Visser et al., 2003), but the proximate mechanisms that precede the behavioural response to temperature cues are still poorly understood (Dawson, 2007; Visser et al., 2009). The effect of temperature on gonadal growth is one striking example. Perfito and colleagues (Perfito et al., 2004) have found a clear correlation between ambient temperature and gonadal growth in the field in two song sparrow (*Melospiza melodia morphna*) populations breeding at similar latitudes but at different altitudes. However, temperature manipulation in captivity showed a much less convincing influence, with a modest effect on testis volume that was furthermore limited to one population (Perfito et al., 2005). Other studies have shown that temperature influence on gonadal growth may depend on the latitudinal origin of the population/species considered (Silverin et al., 2008; Wingfield et al., 2003; Wingfield et al., 1996; Wingfield et al., 1997) and that temperature may influence gonadal regression rather than its recrudescence (Dawson, 2005; Silverin et al., 2008; Wingfield et al., 2003) (M.E.V.,

A. Dawson, S.P.C. and S. V. Schaper, unpublished data). The effect of temperature on spring gonadal growth and its synergetic effect with the energy needed to achieve this several thousand-fold recrudescence require further investigation. In the context of climate change, such knowledge is highly advocated because an adaptive response to the new environmental characteristics is necessary to maximize fitness components (Visser et al., 2004).

The aim of this study was two-fold; we wanted to (1) decipher the respective roles of photoperiod and temperature on male testis seasonal growth, and (2) see whether the temperature-associated energy requirements may impair testis recrudescence in great tits.

MATERIALS AND METHODS

We performed an experiment in the autumn of 2007, using 35 male great tits, *Parus major* L., from our long-term studied population in the Hoge Veluwe (The Netherlands). These birds were obtained from the field 10 days after hatching and subsequently hand-reared in the laboratory in the spring of 2006. In the spring of 2007, each male was paired to a female and settled in climate-controlled aviaries, one pair per aviary, as part of another study. During that study, birds experienced a photoperiod that mimicked natural changes in day length. After breeding, at the end of their moult cycle in late August (for convenience, we will set this period as week 1), all males were settled together under a short photoperiod (8 h L:16 h D, which included half an hour of illumination by an 8 W light bulb, both in the morning and the evening, to mimic dawn and dusk) at 15°C for 6 weeks (Fig. 1). During all experiments, water and food were provided *ad libitum*. These experiments were carried out under licence from the Animal Experimental Committee of the KNAW (Addendum IX of DEC protocol CTO 05.01).

Treatments

At the beginning of week 7, males were individually housed in climate-controlled aviaries (2 m×2 m×2.25 m), and exposed to mid-long days (13 h L:11 h D, which included half an hour of illumination by an 8 W light bulb, both in the morning and the evening, to mimic dawn and dusk), with temperature kept constant at 15°C (Fig. 1). At the end of week 8, males were exposed to an acute change in temperature. Temperature was set to 8°C (cold treatment) in 17 aviaries and to 22°C (warm treatment) in the remaining 18 aviaries (Fig. 1). In The Netherlands, natural day length reaches 13 h in early April. In the field, the mean daily temperature in April in a cold year is around 6°C, and around 13°C in a warm year. The 14°C difference between the two experimental groups is therefore greater than in natural conditions, which should maximize any possible effect of temperature on testis growth rates. Successive aviaries were alternately assigned as cold or warm.

Testis size measurements

Testis size was measured three times during the experiment: at the end of week 5 (while males were exposed to short days: 8 h L:16 h D, and temperature set at 15°C); at the end of week 8 (mid-long days: 13 h L:11 h D, and temperature kept at 15°C) to assess the influence of mid-long days on early testis development; and at the end of week 11 (mid-long days: 13 h L:11 h D, temperature set at 8 or 22°C) to assess the fine-tuning effect of temperature on the on-going testis recrudescence. Males were unilaterally laparotomized under anaesthesia with isoflurane (Forene, Abbott b.v., Hoofddorp, The Netherlands). Testis length and width were measured to the nearest 0.1 mm, using a scale engraved in the ocular of a binocular. Testis volumes were calculated using the equation: $V=4/3\pi\alpha^2\beta$ where α is half the testis width and β is half the testis length.

Basal metabolic rate measurements

We measured basal metabolic rate (BMR) in terms of oxygen consumption, over four successive nights at weeks 11 and 12, in an open-circuit respirometer. Birds were isolated in six sealed respirometer chambers (0.76 l) and placed in the darkness of a climate cabinet (Sanyo MIR-553, Sanyo E&E Europe BV, Etten-Leur, The Netherlands) at 26°C, i.e. within their thermoneutral zone. Chamber allocation was randomized according to the temperature treatment of the birds used. H_2O and CO_2 were removed from the inlet air (blown into the animal chamber) respectively with Drierite® (6 mesh, Sigma-Aldrich Chemie b.v., Zwijndrecht, The Netherlands) and Ascarite® (5–20 mesh, Fluka, Zwijndrecht, The Netherlands). Air flow rate was set to 250 ml min^{-1} with flowmeters (Brooks Instrument b.v., Ede, The Netherlands) previously calibrated using a soap bubble method (Bubble-O-Meter, LLC, Dublin, OH, USA). Oxygen content of outlet air was measured with an oxygen analyser (Servomex 4100, Servomex BV, Zoetemeer, The Netherlands). Oxygen consumption (ml O_2 min^{-1}) was calculated as the difference in oxygen concentration between air from the respirometer chambers and reference air from an empty chamber. As only one oxygen analyzer was used, measurements alternated between the six experimental plus one reference chamber every 7.5 min. The oxygen consumption was converted to metabolic rate (kJ $24\,h^{-1}$) by assuming an energetic equivalence of 20 kJ l^{-1} O_2. As BMR could only be measured in six birds every night, we decided to sample 24 of the 35 male great tits included in the experiment, to avoid long time gaps between the first and the last measured birds, which could have biased the testis sizes subsequently measured. These 24 males were equally divided between the cold and warm treatments.

After the first set of BMR measurements, temperature was set back to 15°C for one week (week 12). A second set of BMR measurements was then performed on the same 24 birds 5 days later

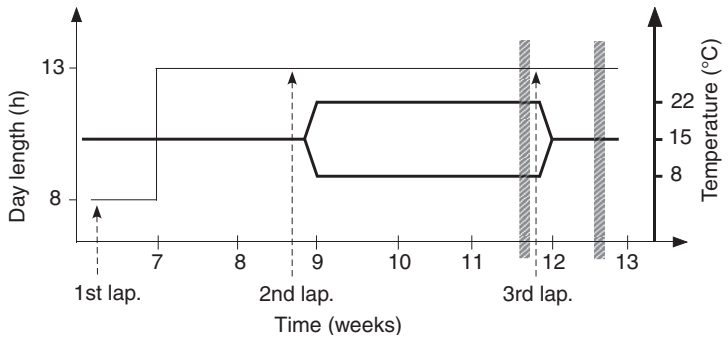

Fig. 1. Schematic representation of the protocol used in the experiment (see details in Materials and methods). Thin line indicates day length. Thick line, temperature profiles. Dashed arrows, laparotomies (lap.). Shaded areas, basal metabolic rate (BMR) measurements.

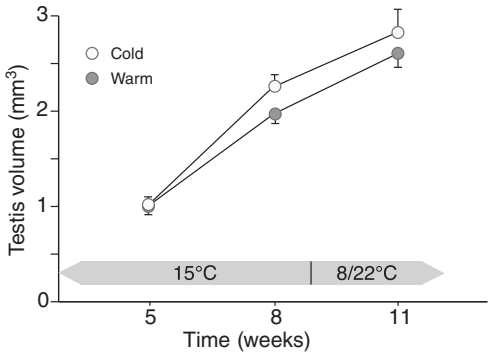

Fig. 2. Changes in testis size of male great tits exposed to 8 h L:16 h D at 15°C (week 5), 13 h L:11 h D at 15°C (week 8), and 13 h L:11 h D at either 8°C (cold) or 22°C (warm; week 11). Error bars are drawn only as +s.e.m. for the cold treatment group and –s.e.m. for the warm treatment group.

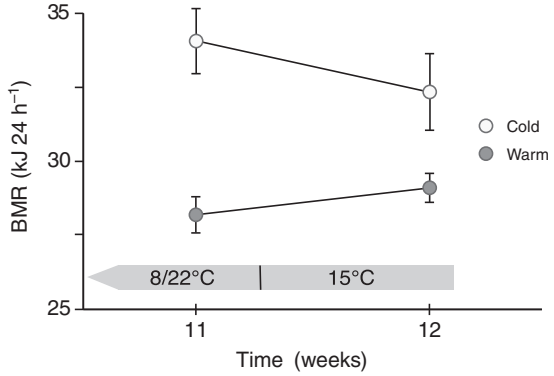

Fig. 3. Changes in BMR induced by variations in ambient temperature. After BMR measurements at week 11, birds were transferred from a cold (8°C) or a warm (22°C) treatment to the same temperature regime (15°C). Five days after the transfer back to 15°C, the difference in BMR is no longer significant (P=0.0535, see text for details). Error bars are ± s.e.m.

to assess the long-lasting effects of the cold and warm treatments on BMR (Fig. 1).

Statistical analysis

The effect of temperature (warm *vs* cold treatments) on testis growth was tested using repeated measures analysis of variance (RM-ANOVA), assessing temperature treatment overall effect (between factor) and temporal differences during the course of the experiment (within factor). Temperature influence on BMR was tested using an analysis of covariance (ANCOVA) with treatment as a factor and body mass as a covariate. Additional analyses, aimed at comparing first and second sets of BMR measurements, were performed using RM-ANOVA (same factors as above). The influence of BMR on testis growth was tested using an ANCOVA, with temperature treatment as a factor and BMR as a covariate. Non-significant interactions were removed from the models and are not presented in the results. Effects were considered significant at $P \leq 0.05$.

RESULTS

Testis volume

Great tit testis grew during the course of the experiment ($F_{2,62}$=106.30, P<0.0001) but did not differ between the temperature treatments ($F_{1,62}$=1.47, P=0.234; Fig. 2). To isolate the overall effect of a mid-long photoperiod on the onset of testis growth from the combined effect of both photoperiod and a temperature change on the subsequent testis growth, we performed two supplemental RM-ANOVA on the first two testis measurements (effect of an increase of photoperiod) and the two last ones (combining effect of photoperiod and temperature change). In both periods, testis grew significantly (first period: $F_{1,33}$=176.29, P<0.0001; second period: $F_{1,32}$=18.50, P<0.0001), but temperature had no effect on testis volume during the second period ($F_{1,32}$=1.98, P=0.169), showing that the additive change of temperature had no significant effect on mid-long day-induced testis growth.

BMR

BMR differed significantly between the groups at the end of the temperature treatments, at week 11 ($F_{1,21}$=16.93, P<0.001), but this significant difference had gone 5 days later, when birds were again held under the same temperature (15°C), at week 12 ($F_{1,21}$=4.19, P=0.054; Fig. 3). Comparing these two sets of BMR measurements using a repeated measures ANOVA (temperature treatments as a factor), the effect of treatment ($F_{1,22}$=12.85, P=0.002) and the interaction between treatment and temporal effects ($F_{1,22}$=9.47, P=0.006) were significant (Fig. 3). There was, however, no significant overall temporal difference between the BMR measurements ($F_{1,22}$=0.82, P=0.376).

Influence of BMR on testis growth

When including both temperature and BMR in the analysis of testis growth, the overall effect of temperature was still not significant ($F_{1,68}$=1.03, P=0.313), and there was no overall influence of the BMR level on testis growth ($F_{1,68}$=0.351, P=0.555).

DISCUSSION

We have demonstrated that, in male great tits, photoperiodic-induced testis growth is not affected by a major modification of ambient temperatures, even when these temperatures induced a significant difference in BMR. As a consequence, temperature, which is one of the most variable components of the environment, does not seem to act as a constraint factor, nor as a fine-tuning cue for male great tit testis recrudescence.

Temperature as a constraint factor

Although males held under cold temperature (8°C) showed a significantly higher mean BMR than males kept under warm treatment (22°C), their testis growth rates did not differ. The cold temperature treatment induced a 20% increase in BMR compared with the warm treatment, which is comparable to the costs induced by follicle growth in starlings (*Sturnus vulgaris*) (Vézina and Williams, 2002) and is twice the increase in BMR after an immune challenge in great tits and blue tits (*Cyanistes caeruleus*) (Ots et al., 2001; Svensson et al., 1998). Furthermore, in the present experiment, birds were fed *ad libitum*, which suggests that the difference in BMR may even have been higher if the food resources had been limited. If testis recrudescence was a high energy-consuming process, and temperature a limiting constraint factor, we would have expected a reduction in the testis growth rate in birds exposed to the cold treatment. As this was not observed, testis growth in great tits may be considered as a low energy-demanding process. A few old studies investigated the relationship between metabolic rate and testis development in birds, but they generally came to the same conclusion, that testis development is not energetically costly (for reviews, see King, 1973; Walsberg, 1983).

However, the exact metabolic costs of testis enlargement and maintenance are notoriously hard to determine (Greenman et al., 2005). As an example, the cost of gonadal growth may be met through reallocation of resources among different physiological systems, rather than in an additive manner (Vézina et al., 2003). In this respect, testis growth rate in our cold temperature treatment group may have been maintained thanks to a reallocation of energy originally dedicated to e.g. general maintenance or immunity. On the other hand, the recrudescence of the testes may itself not be costly, but sperm/steroid production and its consequences, like the immunosuppressive effect of testosterone (Folstad and Karter, 1992), may well be. Because testes were not fully developed at the end of the present experiment (see Fig. 2), the production of sperm and steroids may still have been low at this stage (Silverin et al., 2008) and, therefore, their potential costs would not have been expressed.

Temperature as a cue

While the potential metabolic cost of testis recrudescence has still to be discovered, temperature may also act as a cue predicting the optimal timing for breeding and/or developing the gonads.

There are several lines of evidence that bird egg laying dates are partly decided according to past ambient temperatures, including that in great tits (McCleery and Perrins, 1998; Meijer et al., 1999; Nager and van Noordwijk, 1995; O'Connor, 1978; Perrins and McCleery, 1989; Salvante et al., 2007; Visser et al., 2003; Visser et al., 1998). From an ecological point of view, this makes sense as bird fitness is closely related to their ability to match their reproduction with the annual, short period of arthropod abundance, which is highly dependent upon temperature (Visser and Holleman, 2001; Visser et al., 2006).

On the other hand, the influence of temperature on sexual physiology is still highly debated. Some studies have demonstrated a clear effect of temperature on gonadal, generally testis, growth (Engels and Jenner, 1956; Wingfield et al., 2003), while others produced more inconsistent results (Dawson, 2005; Farner and Wilson, 1957; Perfito et al., 2005; Silverin and Viebke, 1994; Suomalainen, 1937; Wingfield et al., 1996). Among these studies, several support a potential latitudinal variation in the physiological response to temperature cues, with birds from southern latitudes relying more on temperature cues than birds from the north (Silverin et al., 2008; Wingfield et al., 2003; Wingfield et al., 1996; Wingfield et al., 1997). The degree to which organisms use supplemental factors (e.g. temperature) to time their sexual activity would depend on the predictability of their breeding environment. This predictability is a function of varying degrees of constancy (the habitat is predictable because it is always the same) and contingency (the habitat is predictable in the degree of change between seasons) (Colwell, 1974; Wingfield et al., 1992). The ratio between contingency and constancy (Ie: environmental information factor) reflects the relative importance of supplemental factors as a source of predictive information (Wingfield et al., 1992). Populations breeding at low latitudes generally have a higher Ie than populations breeding at high latitude, meaning that these low latitude populations are expected to closely rely on supplemental information to time their breeding period.

In a recent paper, Silverin and colleagues (Silverin et al., 2008) compared the predictability (Pr) and the Ie factors between different populations of great tits breeding at different latitudes. They demonstrated that populations breeding in southern Europe have higher Ie indexes than populations breeding in northern Europe, and these populations were also the most sensitive to temperature

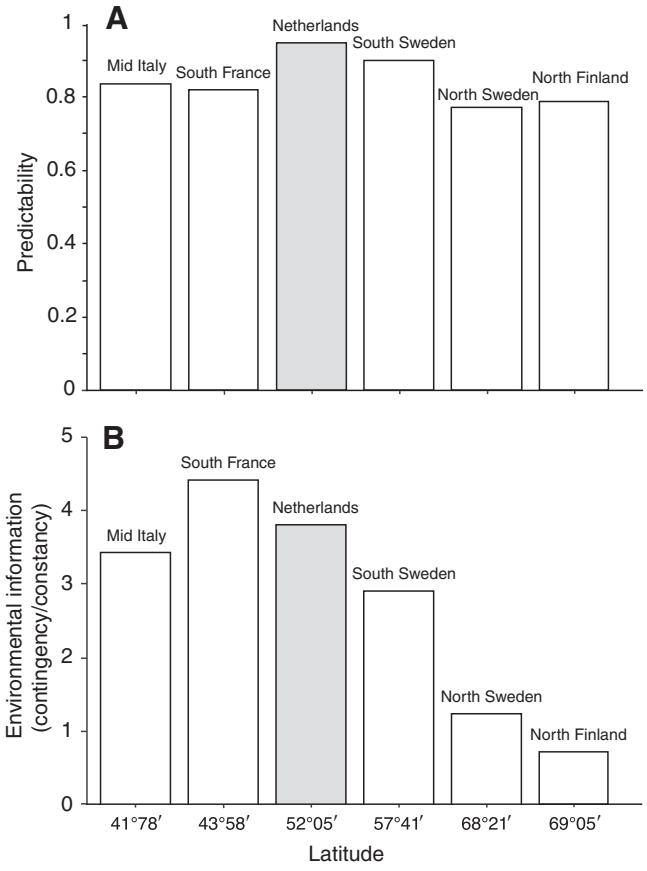

Fig. 4. Predictability (A) and environmental information factor (B) of six populations of great tits breeding at different latitudes. Data in this figure were calculated using laying date data tables published by Silverin and colleagues (Silverin et al., 2008), and laying dates collected over 34 years from the Dutch, Hoge Veluwe, great tit population (grey bars). See supplementary material (Table S1) for methods and Hoge Veluwe data.

treatments in aviaries. Birds from Italy held under warm temperature (20°C) grew gonads sooner than birds held under cold temperature (4°C), while no temperature effect on physiological development was observed in northern populations of the same species (Silverin et al., 2008). The male great tits used in the present study originate from a population that has a Pr of 0.95 and an Ie of 3.8 (Fig. 4; see supplementary material Table S1 for the breeding data of the Dutch population). Both Pr and Ie of our long-term-studied Dutch population are very high, and the Ie is similar to that found in southern Europe (Silverin et al., 2008). The absence of a temperature effect on photoperiodically induced testis development in great tits from the Netherlands is therefore surprising and somewhat contradicts the predictability model.

One must, however, be very cautious when interpreting these models of predictability, as the calculations are based on the annual repartition of egg laying, while the conclusions are generally applied to gonadal development. This may lead to erroneous conclusions given that egg laying and gonadal development are, to some extend, two different processes, induced by different temporal decisions that are not necessarily closely correlated (S.P.C. and M.E.V., manuscript in preparation).

The overall temperature difference between the cold and warm treatments in the study by Silverin and colleagues (Silverin et al., 2008) was 2°C more than in the present study, and in particular the

cold treatments differed by 4°C between studies [4 *vs* 20°C in Silverin et al. (Silverin et al., 2008); 8 *vs* 22°C in the present study]. Although we cannot exclude the possibility that this slight temperature range difference may explain the discrepancies in the results of the two studies, if the Ie factor could reliably predict population sensitivities to temperature in a natural context, we might expect that the somewhat unnatural 14°C temperature difference between the treatments in our study would have been sufficient to induce differential testis growth rates.

The effect of temperature on bird seasonal reproduction clearly needs more investigation. We need to clarify how the sexual physiology is affected by temperature, how temperature cues and constraints respectively affect male and female pre-breeding development, and how the temperature effects on gonadal growth and on laying dates can be linked. As climate change mainly influences the supplementary cues species use to time reproduction, understanding its consequences in terms of population adaptation will depend highly on our knowledge of the mechanisms by which temperature, and its interaction with photoperiod, affects the reproduction of organisms (Visser et al., 2004).

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Table S1. Great tit breeding data over 34 consecutive years at the Hoge Veluwe (The Netherlands)

	J	F	M	A	M	J	J	A	S	O	N	D
No eggs	34	34	34	0	0	3	33	34	34	34	34	34
Eggs	0	0	0	34	34	31	1	0	0	0	0	0

Columns represent months of the year, rows represent the number of years where eggs where present/absent during each particular month. Predictability and environmental information factor (Ie) were calculated using the mathematical model developed by Colwell (Colwell, 1974). We validated our calculations by recalculating the contingency, constancy, predictability and Ie values using the data tables published by Colwell (Colwell, 1974) and Wingfield and colleagues (Wingfield et al., 1992; Wingfield et al., 2003). However, using the same equations with the tables published by Silverin and colleagues (Silverin et al., 2008) gave slightly different results. In order to allow direct comparison of the predictabilities and Ie factors of the different European great tit populations investigated by Silverin and colleagues (Silverin et al., 2008) with the Hoge Veluwe population, Fig. 4 shows the results of our calculations.