GOBIERNO DE ESPAÑA
MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD

UNIÓN EUROPEA
Fondo Social Europeo
El FSE invierte en tu futuro

Incubation behaviour of Great Tits *Parus major* in response to ambient temperature in three contrasting Mediterranean habitats
David Diez Méndez
2021

Incubation behaviour of Great Tits *Parus major* in response to ambient temperature in three contrasting Mediterranean habitats

Doctoral Thesis by
David Diez Méndez

Supervisors
Emilio Barba Campos
Juan José Sanz Cid

Doctoral Program in Biodiversity and Evolutionary Biology (RD 99/2011) **Valencia, March 2021**

CSIC
Consejo Superior de Investigaciones Científicas

mncn
museonacionaldecienciasnaturales

Universitat de València

VNIVERSITAT
D VALÈNCIA

Incubation behaviour of Great Tits *Parus major* in response to ambient temperature in three contrasting Mediterranean habitats

Doctoral Thesis by

David Diez Méndez

Supervisors:
Emilio Barba Campos
Juan José Sanz Cid

Doctoral Program in Biodiversity and Evolutionary Biology (RD 99/2011)
March 2021, Valencia

Recommended citation:
Diez-Méndez, D. (2021). Incubation behaviour of Great Tits *Parus major* in response to ambient temperature in three contrasting Mediterranean habitats. PhD Thesis. Universitat de València, Valencia, Spain.

Cover image is an original creation of Nicolás Fernández (www. ilustracionesnaturaleza.com), who remains its intellectual ownership. Section photographs were taken during the fieldwork that led into this PhD thesis. Any form of reproduction, distribution, public communication or transformation of the cover image or section photographs without prior authorization of the authors is forbidden.

Summary: Great Tit female, Pina. Author: David Diez Méndez.
General Introduction: Great Tit nest, Sagunto. Author: Jéssica Jiménez Peñuela.
Aims and Objectives: Female Great Tit incubating, Sagunto. Author: David Diez Méndez.
Chapter 1: Great Tit hatchlings, Sagunto. Author: Sara Guillamón Prats.
Chapter 2: Female Great Tit incubating, Sagunto. Author: Jéssica Jiménez Peñuela.
Chapter 3: Great Tit hatchlings, Sagunto. Author: Sara Guillamón Prats.
Conclusions: Great Tit male, Pina. Author: David Diez Méndez.
Appendix 1: Great Tit male, Pina. Author: David Diez Méndez.
Appendix 2: The village of Pina from Monte de Santa Bárbara. Author: David Diez Méndez.
Appendix 3: A nestbox in Pina. Author: David Diez Méndez.
Resumen: The village of Pina (de Montalgrao). Author: David Diez Méndez.
References: David checking a nestbox, Pina. Author: Sara Guillamón Prats.

D. Emilio Barba Campos, Profesor Titular de Ecología de la Facultad de Ciencias Biológicas de la Universitat de València, y
D. Juan José Sanz Cid, Investigador Científico del Museo Nacional de Ciencias Naturales del Consejo Superior de Investigaciones científicas,

CERTIFICAN que D. David Diez Méndez ha realizado bajo nuestra dirección, y con el mayor aprovechamiento, el trabajo de investigación recogido en esta memoria, y que lleva por título “Incubation behaviour of Great Tits *Parus major* in response to ambient temperature in three contrasting Mediterranean habitats”, para optar al grado de Doctor en Ciencias Biológicas.

Y para que así conste, en cumplimiento de la legislación vigente, expedimos el presente certificado en Valencia, a 3 de marzo de 2021.

Firmado: Emilio Barba Campos

Firmado: Juan José Sanz Cid

A mis padres y hermana
A Ana y Olivia

Acknowledgements / Agradecimientos

Los agradecimientos al finalizar una tesis no sólo abarcan aquellos hechos y personas que te han acompañado a través de estos largos años, sino que siempre hay un bagaje que incluye a muchas más personas y abarca casi toda tu vida anterior.

Lo primero de todo, el agradecimiento a mis directores, Emilio y Juanjo, por elegirme para llevar a cabo este proyecto. Ha costado, pero he llegado a la meta. Para siempre quedará el recuerdo de 2011, hace ya 10 años, cuando me presenté en el despacho de Emilio preguntando por la posibilidad de que me dirigiera el trabajo de máster. Una semana más tarde estaba ayudando a colocar cajas-nido en Sagunto. Tampoco olvidaré la impresión que me causó la primera visita al Museo en 2015 y el sonido de mis botas contra esos suelos quejicosos.

En Sagunto, y después en Pina, he pasado muchas primaveras de mi vida. Tantas que es casi mejor no contarlas, pero que me han servido para aprender esto que llamamos "trabajo de campo". Cada nueva temporada nuevos estudiantes que nos ayudaban. Aunque muchas veces prefiriera la soledad, gracias a todos por la compañía en esas largas y calurosas horas de campo. Entre todas las personas no puedo dejar de mencionar a Elena y a Samuel. Con Elena empecé a recorrer los campos de naranjos en lo que, aún sin saberlo, sería el principio de muchos años de revisión de cajas y anillamientos. ¡Y qué decir de Samuel y los incontables días de campo que pasamos juntos!, y esas idas y venidas primero en el Terrano y luego en el Dacia. Tampoco me puedo olvidar de Jose y su ayuda durante mis semanas de cuasi-aislamiento en Pina. Siempre con energía a primera hora de la mañana y dispuesto a hacer campo. Aprovecho también para recordar a Dani, mi casero de Pina, un buen hombre al que le gusta presumir de sus embutidos de jabalí, y con razón.

Como no podía ser de otra forma, al acabar el máster empezó un periodo de emigración. Primero un par de meses a Oxford, en el legendario, para fans de los carboneros, bosque de Wytham. Semanas intensas que se llevaron mejor gracias a la compañía de Pepe, tanto en el campo como comprando en Tesco, y de Nico. *I cannot forget Nico's confussion face when he was not allowed to step into a pub showing his football team t-shirt.* Meses después le siguió un año en Alemania aunque, cosas de la vida, incluía campo en Granada. Por aquel tiempo en Seewiesen había una pequeña invasión española que, obviamente, trabajaba con carboneros (Irene, Víctor, Javi, Ana y Marta). Un grupete majo. Me perdonarán el resto, pero tengo que destacar a Javi y Marta, o más bien a sus padres, que tuvieron a bien, aún años después, acogerme en sus casas en mis visitas al Museo. Tampoco me olvido de Leti y Liviu que me acompañaron durante la segunda parte de mi estancia en la *guesthouse* de Seewiesen. *Liviu has been an awesome friend during these years and I hope we keep visiting each other in the days to come wherever we are.*

Dejé Alemania para empezar el doctorado, aunque los viajes siguieron, esta vez en forma de estancias. Fueron cortas (Carolina del Norte, Ciudad del Cabo y el Kalahari) pero difíciles de olvidar. *Thank you, Caren and Susie for accepting me in your labs for a few months, and specially to Susie and an awesome driving trip to the Kalahari, flat tire included.* Y como donde quiera que uno vaya hay españoles, ¡incluso en el desierto!, no me puedo olvidar de Juanma, compañero de piso inesperado, y de Diana.

Pero no todas las personas son nuevas, muchas han estado ahí desde el principio y su apoyo directo o indirecto han hecho más fáciles estos años. Tan al principio como el instituto (Vecín qué haríamos si esas cadenas de correos infinitas); la universidad (malditos priapúlidos); o los años valencianos (paellas, rutas, y noches en Chiva). Ahora más que nunca con una pandemia de por medio, es difícil veros a todos (y a vuestras nuevas criaturillas), pero al menos tenemos las videollamadas.

Durante todos estos años no han sido pocos los viajes a České Budějovice para ver a Ana, empezando por aquel verano de 2013. Nunca llegué a imaginar que acabaría viviendo y trabajando aquí. De nuevo otro grupo de españoles variadito (Carmen y Héctor de la Roda, los andaluces, los madrileños y un francés) asiduo al Singer y a la música en directo nos acompañaron durante esos años. Gracias también a Astrid por acogerme en su laboratorio durante mis visitas y hacerme un hueco donde sentarme con el portátil. Ahora, después de mudarme a esta parte del mundo hace ya casi dos años, he tenido mucha suerte por haber sido contratado en un laboratorio tan interesante como el de *Multitrophic interactions. Thank you Katka for trusting me, a random guy with no PhD yet, that just knocked on your door asking for a job, děkuji!*

No puedo acabar los agradecimientos sin mencionar a mis padres y mi hermana. Mis padres siempre me han apoyado incondicionalmente, esfuerzos económicos mediante, para que pudiera llegar donde estoy hoy. Seguramente no entiendan del todo lo que hago, pero nunca han puesto en duda lo que quería hacer, aunque suponga vivir a más de 2000 km de distancia y vernos una o dos veces al año. Gracias por todo. Qué decir de Marimar, durante nuestros años jóvenes tuvimos nuestros más y menos, pero en los últimos años hasta nos llevamos bien y nos videollamamos. Gracias también por estar ahí. También me gustaría acordarme de las personas que desde hace poco ya no están. Wolfgang me solía preguntar por cómo iba mi tesis, se hubiera alegrado de saber que ya he llegado hasta aquí. Y mi abuela. Me encontraba arrastrando un carro de la compra en Kathu, algo así como el culo del mundo (lo siento por sus habitantes), cuando mi madre me llamó para darme la noticia. Creo que nunca entendió porque me iba tanto y tan lejos. Pues era por esto, por esta tesis y la investigación en general, espero que haya merecido, y merezca, la pena estar tan alejados de la familia.

Por último, Ana. Qué puedo decir de Ana. Algo más de nueve años juntos y siempre apoyándome, constantemente a mi lado. Ella sabe mejor que nadie lo que ha costado llegar hasta aquí. Ha habido momentos muy felices, pero también otros tantos duros. Meses separados, extenuantes temporadas de campo, y mucho más que ha tenido que aguantar, y aguantarme. Gracias por todo, pero sobre todo por estar ahí. Hemos renunciado a muchas cosas, y pospuesto otras tantas, por esta tesis, pero como ya he dicho antes, espero que haya merecido la pena. Además, ahora ya no es tiempo de seguir cancelando planes, siempre que la pandemia lo permita, porque con Olivia ya no hay excusas, está creciendo tan rápido que no podemos permitirnos perder un solo segundo más. A falta de otras colaboraciones entre la parasitología y la ornitología, esta sí que será nuestra "tesis" común.

Y no puedo más que despedirme mientras escucho a través de la ventana a un par de carboneros. Es un día soleado de invierno, de esos que ya huelen a primavera.

České Budějovice, a 3 de marzo de 2021

TABLE OF CONTENTS

Summary

Avian incubation is a complex behaviour that, in female-only incubator species, entails key trade-offs between egg warming periods and time off the nest for female self-maintenance. The dynamics between on- and off-bouts are thought to be mainly influenced by ambient temperature, because changes in egg cooling rates would influence how females allocate their time during the incubation period. Incubating females need to keep an adequate and narrow thermal environment for the egg, with small deviations causing long-term effects on survival and reproductive success.

Females would need to adjust their bout duration to ambient temperatures. Both on- and off-bouts are expected to lengthen when temperatures increase because incubation constraints are eased. Females usually lengthen on-bouts at a higher rate, thus increasing incubation effort. The opposite response, i.e., increasing self-maintenance time with increasing ambient temperature, has been also reported in both different species and different populations of the same species. While these opposite behavioural responses might be the result of different breeding strategies adapted to habitat conditions, it might also be that they arise as artefacts of limited datasets, different methodological approaches, or the timescale at which incubation behaviour is measured.

Determining the onset of incubation also implies certain complexities derived from trying to delimit a progressive behaviour that is gradually settled during the egg-laying period. Despite classic studies, performed forty years ago in Great Tits *Parus major* describing both diurnal and nocturnal incubation behaviours and their respective periods of partial and full incubation, little is known about how the onset of incubation relates to ambient temperature and its effects on hatching asynchrony. Ambient temperature might have a major role in the onset of incubation if incubating females use it as a cue to synchronize their hatchlings maximum growth period with the expected prey peak. Prey development, mainly caterpillars for Great Tits, accelerates with increasing temperatures. Once females start laying eggs, they could only keep track of faster prey development by an earlier onset of incubation. But an earlier onset also implies that incubation happens before the clutch is complete, which could cause hatching asynchrony.

Hatching asynchrony could still occur even if incubation is delayed after clutch completion. Thermal gradients within the clutch during incubation might be a potential factor behind this residual hatching asynchrony. Females keep eggs under their brood patch warmer than peripheral ones, because they are not able to cover the whole clutch. If females do not distribute the heat properly by repositioning eggs within the nest-cup it could cause a differential embryo development, potentially resulting in hatching asynchrony.

In this thesis project I have chosen a commonly studied species, the Great Tit, and collected high-quality incubation data during three consecutive years in three different breeding populations. The main aim of the project was a better understanding of the dynamics of incubation behaviour, its onset, and the consequences on hatching asynchrony in relation to ambient temperature. During an additional fourth breeding season, I also investigated whether differential egg repositioning within the clutch, a rarely studied behaviour, had a role in hatching asynchrony. Incubation behaviour was recorded using temperature data loggers placed in the nest-cup and egg repositioning was calculated from photographed clutches during the incubation period. First, I delimited the different incubation behaviours (diurnal and nocturnal, and partial and full incubation), quantified them, and assessed how ambient temperature affected their onset relative to the egg-laying sequence. I also assessed the association between the onset and duration of these incubation behaviours and the extent of hatching asynchrony. Secondly, I tried to comprehend incubation rhythms (i.e., the relation between on- and off- bouts during diurnal full incubation), their number and duration, in relation to ambient temperature. I aimed to investigate whether divergent patterns of nest attentiveness were a result of local adaptations or the consequence of incomplete datasets and different analysed timescales (hourly, daily, overall incubation period). Finally, by using an experimental approach, I deterred clutches from being partially incubated and assessed the effect on hatching asynchrony. I also investigated if residual hatching asynchrony could be endorsed to differential egg repositioning within the clutch by taking twice-a-day photographs of marked eggs during the diurnal full incubation period. The duration of the diurnal full incubation was analysed under the effect of different factors related to each objective.

I found that both ambient temperature and clutch size affected the onset of incubation behaviour (**Chapter 1**). Increasing ambient temperature during the egg-laying period advanced diurnal partial incubation relative to the laying sequence, but larger clutches delayed the onset of both nocturnal full incubation and diurnal partial incubation. Only diurnal incubation affected hatching asynchrony despite nocturnal periods being longer. Both partial incubation and full incubation occurring before clutch completion increased hatching asynchrony.

In **Chapter 2** I showed that incubating females allocated time into self-maintenance at higher ambient temperatures, i.e., reducing nest attentiveness when constraints alleviate, as a generalized response among populations. Females maximised the duration of incubation bouts based on local temperatures and not absolute values as suggested in previous studies. This behaviour translated into different nest attentiveness patterns depending on the timescale, even showing contrary incubating behaviours.

Going beyond correlational studies, in **Chapter 3** clutches where partial incubation was prevented showed longer incubation periods and reduced hatching asynchrony. However, egg repositioning within the clutch did not seem to be the cause behind the observed residual hatching asynchrony.

Ambient temperature is a key variable for incubation behaviour, both for its onset and rhythms. It affects the onset of incubation asymmetrically, advancing only diurnal partial incubation. Female incubation rhythms differ among populations because they maximise on-bout duration at different local ambient temperatures. Ambient temperature, both during the egg-laying and full incubation period, is indirectly associated with the duration of the full incubation period and the extent of hatching asynchrony.

General Introduction

1. The importance of incubation in the avian breeding cycle

Incubation, "the process by which the heat necessary for embryonic development is transferred to an egg after it has been laid" (Beer 1964), is a key period in the avian breeding cycle that later shapes fitness and survival of both parents and nestlings (Berntsen & Bech 2016, Nord & Nilsson 2016, Ricklefs *et al.* 2017, Ospina *et al.* 2018; reviewed in Durant *et al.* 2013).

The study of avian incubation behaviour has gained importance in the last decades after a strong focus on the chick rearing period (reviewed in Monaghan & Nager 1997). Lack's seminal studies (1947, 1948) linked clutch size evolution to the number of hatchlings that parents are able to rise, spotlighting the brood-rearing period in detriment to the egg-laying or incubation period. Later studies also diminished the energy costs of incubation behaviour and proposed that basal metabolism could cope with heat requirements of embryo development, in contrast to the energetically demanding nestling period (King 1973, Walsberg & King 1978). Forty years after Lack's studies, new findings that associated clutch size and foraging constraints with duration of the incubation period and hatching success (reviewed in Nord & Williams 2015) started to unveil the tight link between incubation behaviour and breeding performance. Williams (1996) highlighted evidence of the energy expenditure in incubating individuals being equal to that from the rearing period, or even exceeding it for small species (see also Nord & Williams 2015), especially when they incubate out of their thermoneutral zone, which is common in temperate habitats (Williams 1996, Tinbergen & Williams 2002). Optimization of energy expenditure, time allocation between incubation and self-maintenance, and how it translates into breeding success, are key factors to understand incubation behaviour.

2. Incubation strategies and post-hatching development

The heterogeneity of avian incubating strategies may be classified in two main groups: Species in which both members of the pair incubate and species where only one of the parents does it (Skutch 1957). Bi-parental incubation (Williams 1996) happens in approximately 50 % of avian families, from which 80 % are non-passerines. Female-only incubation (gyneparental incubation, Williams 1996) is found in 37 % of avian families, but predominantly in passerines (62 %). In contrast, male-only incubation (androparental incubation, Williams 1996) only happens in 6 % of the families, mainly restricted to paleognaths (tinamous, kiwis, cassowaries, emus, ostriches and rheas) and the Emperor Penguin (*Aptenodytes forsteri*). Other strategies such as collaborative incubation between members of a group (> 2 members incubate, e.g., Southern Pied-Babblers *Turdoides bicolor*, bush tits *Psaltriparus minimus*), brood parasitism (cuckoos, cowbirds, indigobirds and whydahs), or birds that do not incubate their eggs with their bodies but bury them instead (megapodes) are found in a minority of species (Deeming 2002a, Marasco & Spencer 2015).

To some extent independent from incubation strategies, avian species show a continuum in developmental patterns from altriciality to precociality (reviewed in Starck & Ricklefs 1998). In one end, altricial species hatch with closed eyes, no down and little motor activity; incapable of departing from the nest and in need of parental feeding (e.g., passerines). In the other end of the spectrum, precocial species hatch with open eyes, feathered, independent from their parents and able to fly from the first day (e.g., megapodes). Intermediate stages show precocial hatchlings that do not immediately depart from the nest and need brooding or food showing (e.g., some galliforms), semi-precocial species that hatch with down but can only leave the nest after few days from hatching and need stronger parental care, and semi-altricial birds (e.g., raptors, herons) that show a more reduced mobility but with open eyes and still covered with down (Starck & Ricklefs 1998).

For precocial birds it is advantageous to hatch together or within a short lapse of time, synchronous hatching, since they leave the nest shortly after. In altricial species, the need for parental feeding, and a protracted developmental period in the nest, allows a wider range of hatching variation (Stoleson & Beissinger 1995).

3. Incubation behaviour basics in songbirds

Passerines, commonly known as perching birds or songbirds, show asynchronous hatching, altricial development, and female-only incubation as the most common strategy. Intermittent daytime incubation behaviour (Deeming 2002a) is the solution carried out by females, when they are the only incubators, to deal with energy expenditure since they have to allocate time between clutch incubation (on-bouts) and self-maintenance activities off the nest (off-bouts) (White & Kinney 1974, Haftorn 1981). Additionally, males may help by feeding their partner during the incubation period (assisted gyneparental incubation, Nord & Williams 2015) in the nest or the surroundings (Bambini *et al.* 2019), alleviating to some extent energy constraints (Matysioková & Remeš 2014).

Incubating females do not just sit on the eggs and provide heat. Embryos need a narrow range of temperatures for optimal development (37–40.5 ^{o}C, Drent 1975, Durant *et al.* 2013). Females consistently keeping incubation temperature a few degrees below the optimum (e.g., 36.5–35.0 ^{o}C) might already reduce hatching success because of negative effects on embryo development (Hepp *et al.* 2006, Olson *et al.* 2006, Nord & Nilsson 2011). If females neglect their clutch at ambient temperatures between the physiological zero temperature (i.e., 24–27 ^{o}C, hereafter PZT, Drent 1975) and optimal incubation values, it might also result in abnormal development of embryo tissues, asynchronous growth, and embryo mortality

(Drent 1975, Webb 1987). In species with intermittent incubation, females cannot avoid recurring temperature drops in the nest during their off-bouts, but they can limit off-bout duration. Otherwise if eggs reach temperatures lower than the PZT, embryos might pause their development and could eventually die (Drent 1975, Haftorn 1988).

Besides providing an adequate fine-tuned range of temperatures, incubating females need to actively move their eggs to accomplish their hatching (Drent 1975). Egg movements in the nest have been described either as egg turning or repositioning. Females turn their eggs around their short axis laying on the long one, from one up to 12 times per hour, by pushing them with their beaks with a trembling motion (Deeming 2002b, 2009); along with certain unquantified repositioning, i.e., shifting positions (Drent 1975, Deeming 2002b). Early observations of birds repositioning their eggs were proposed as a necessity for redistribution of the heat provided by the incubating parent (Drent 1975 and references therein). The subjacent idea was that clutches experience thermal gradients from the centre, directly under the female brood patch, to the periphery (Huggins 1941), because females are not able to completely cover the clutch with their bodies (Caldwell & Cornwell 1975, Löhrl 1986). Within-clutch thermal gradients remained however unexplored (until recently, see Boulton & Cassey 2012, Hope *et al.* 2018), and research focused on egg turning solely. Eggs still needed to be turned in artificial incubators, where there is no temperature gradient, for them to hatch (Drent 1975, Deeming 2002b). Indeed poultry science focused on optimizing hatchability, and the role of egg turning was a key factor on this matter (Deeming 1991). Lack of turning, especially in the first third of the incubation period, affects a variety of aspects of embryonic development including membrane growth, fluid transport, embryo growth and hatchability (Deeming 1991, 2002b, 2009).

I resumed the idea of egg repositioning as a consequence of thermal gradients in **Chapter 3**. Moreover, I quantified the relative distances of individual eggs to the centre of the clutch and, by following an experimental approach, investigated the potential link between this behaviour and the extent of hatching asynchrony (see below).

4. Hatching asynchrony and the onset of incubation

The proximate cause of hatching asynchrony is the warming of eggs before the clutch is complete, which triggers embryo development of early-laid eggs, advancing their growth stage at the time last eggs are laid, thereby hatching earlier (Stoleson & Beissinger 1995). The earlier a female starts incubation in relation to clutch completion, the higher the hatching asynchrony (Stoleson & Beissinger 1995). However some degree of hatching asynchrony can be observed even in the absence of early incubation (Clark & Wilson 1981, Magrath 1990). A potential explanation might be egg differential composition (Williams & Groothuis 2015), which would cause faster embryonic development in last-laid eggs to buffer detrimental laying order effects (Hadfield *et al.* 2013), even if incubation before clutch completion does not take place. Alternatively, the occurrence of clutch thermal gradients could drive incubating females to promote certain eggs over the rest of the clutch by positioning them more frequently under the brood patch, hence developing faster (Poláček *et al.* 2017a, see **Chapter 3**).

Hatching asynchrony, as already mentioned, creates a nestling hierarchy by size that could lead to an early death of late-hatched chicks, although not necessarily. For example the whole brood may be favoured if hatching asynchrony reduce sibling competition via avoiding scramble competition (sibling rivalry hypothesis, Hahn 1981, Gilby *et al.* 2011); or by spreading the peak of food demand so parents can better cope with feeding (peak load reduction hypothesis, Hussell 1972), although this is likely to happen only under a moderate degree of asynchrony (Gilby *et al.* 2011). Hatching asynchrony usually ends with the death of the smallest last-hatched chick, outcompeted by the largest early-hatched ones. Lack (1947) proposed the brood reduction hypothesis trying to explain this phenomenon: parents would lay an optimistic clutch and later brood size would be adjusted depending on parental abilities or prey availability. Brood reduction would be especially intense in scenarios of food scarcity (Lack 1947, Ricklefs 1965). These hypotheses share the view of hatching asynchrony as an adaptive mechanism (reviewed in Stoleson & Beissinger 1995) that gives certain advantages to the whole or part of the brood.

Hatching asynchrony has been however shown to be prejudicial for the whole brood even in years of food abundance (Szöllosi *et al.* 2007). Females would try to avoid negative effects of hatching asynchrony by further investing in late-laid eggs by increasing their yolk content or size (Rosivall *et al.* 2005, Morales *et al.* 2006, Hadfield *et al.* 2013). This suggests that hatching asynchrony might be an undesired outcome, at least under certain circumstances. In this line of though, the egg viability hypothesis proposed by Veiga (1992, based on Arnold *et al.* 1987) would explain hatching asynchrony as a by-product from the adaptive role of incubation onset. Hatching asynchrony would be the consequence of females incubating the clutch during the egg-laying period to prevent embryos from dying in neglected clutches. As previously highlighted, embryo development may start as soon as ambient temperatures reach 24–27^{o}C (PZT), a source for abnormal growth. Egg-laying females under these circumstances would need to start incubation to avoid hatchability reduction, which has been reported in eggs after three days of exposure to ambient temperature (Veiga 1992, Stoleson & Beissinger 1999). Furthermore, the combination of mild ambient temperatures and humidity could promote bacterial growth and trans-shell microbial infection in unattended eggs during the egg-laying period, reducing egg viability (Cook *et al.* 2003, Beissinger *et al.* 2005, Ruiz-De-Castañeda *et al.* 2011, 2012). Incubation during the egg-laying period would be therefore a protective mechanism that, incidentally, causes hatching asynchrony.

From an ecological perspective, the timing of the breeding cycle in insectivorous passerines may further explain the observed extent of hatching asynchrony. Mainly studied in Great Tits *Parus major*, the starting point of incubation behaviour might be affected by ambient temperatures during the egg-laying period, not because of potential hatchability loss when ambient temperature surpasses PZT, but because females would try to synchronize hatching dates and caterpillar peak (Perrins 1991, Noordwijk *et al.* 1995). Caterpillars are the main food source for Great Tit hatchlings and, as ectotherms, their seasonal peak depends on ambient temperatures (Noordwijk *et al.* 1995, Buse *et al.* 1999, Visser *et al.* 2006). If females need to synchronize their offspring maximum food demands with the peak of caterpillars, they would need to keep track of ambient temperature fluctuations not only to start laying eggs but afterwards, during the egg-laying period, to achieve the temporal match. At increasing temperatures, females would start incubating earlier in the laying sequence to hatch earlier, causing higher hatching asynchrony (Cresswell & McCleery 2003). On the contrary, a delayed peak of caterpillars would be expected under cold spells, implying that females would have to delay their onset of incubation beyond clutch completion, indirectly reducing hatching asynchrony (Naef-Daenzer *et al.* 2004, García-Navas & Sanz 2011). I used this hypothesis as the baseline to investigate how ambient temperature during the egg-laying period was associated with the onset of incubation, and how the onset of incubation affected both the duration of the incubation period and the extent of hatching asynchrony (see **Chapter 1**).

5. The onset of diurnal and nocturnal incubation behaviour

Passerine females incubate both during daylight and at night. Throughout the nocturnal time females do not carry any other activity, continuously incubating without leaving the nest, displaying a single on-bout. This period, inaccurately called 'inactive time', comprises not only the night *per se* but from the last time a female enters the nest to overnight until it leaves the nest to forage for the first time in the early morning. Female's active day would then encompass from the first off-bout in the morning to the last on-bout in the evening, during which intermittent incubation is displayed (i.e., alternating on- and off-bouts). The proportion of active (or inactive) time that a female spends incubating (sum of on-bout duration) is defined as nest attentiveness (Skutch 1962). Nest attentiveness could be measured hourly, daily or for the overall incubation period; moreover, it could also refer to both active and inactive hours, resulting in approx. 24h-periods.

The incubation behaviour is progressive, increasing as hatching date gets closer, but it might be possible to decompose it in different stages. Haftorn (1981) described how females in a Great tit population started to incubate shortly in the evening after the first egg was laid and, as days went by, the nocturnal incubation bout extended to last the whole night. In the meantime, females started to display incubation bouts in the afternoon few days after egg-laying started, gradually increasing in number and expanding into the morning, until they achieved a more or less regular daytime intermittent incubation. This behavioural sequence may be however different from other passerine species in which diurnal incubation precedes the nocturnal phase (e.g., White-Crowned Sparrow *Zonotrichia leucophrys* Zerban & Morton 1983, Dusky Flycatcher *Empidonax oberholseri* Morton & Pereyra 1985, Red-Winged Blackbird *Agelaius phoeniceus* Clotfelter & Yasukawa 1999, Mountain Bluebird *Sialia currucoides* Johnson *et al.* 2013, but see Wang & Beissinger 2009); but each diurnal and nocturnal stage usually starts as an irregular and low nest attentiveness period, which progressively becomes a stable stage of incubation.

The occurrence of an early irregular and a later stable period of incubation was early noticed in passerines, and researchers gave them different names (reviewed in Wang & Beissinger 2011). Wang and Beissinger (2011) defined the widespread denominations and narrowed down partial incubation to denote the irregular incubation period, in contrast to full incubation that would refer to the stable period. Although named, establishing a cut-off point between partial and full behaviours would not be that straightforward. Nocturnal incubation may be easier to delimit as females stabilize their behaviour when they incubate for the whole night (100 % nest attentiveness during inactive time) (see Wang & Beissinger 2009, 2011 for an alternative definition of nocturnal full incubation). Diurnal incubation shows however a blurred line between partial and full periods since nest attentiveness values during the so called full incubation could vary from 50 to 100 %, with average values of 75 % (Deeming 2002a).

The delimitation of the diurnal full incubation onset based on fieldwork observations relies on the finding of an incubating female in the nest or uncovered warm eggs for the first time (e.g., Nilsson 1993, Álvarez & Barba 2014a). It is worthy to notice that Great and Blue Tits *Cyanistes caeruleus*, the two most studied passerine species in Europe, usually cover their eggs with nest material during the egg-laying period until they start incubation (Loukola *et al.* 2020), facilitating incubation detection. Fieldwork is usually performed in the early morning, so it is unlikely to detect diurnal partial incubation (which seems to occur mostly in the afternoon), and the detection of an incubating female implies that diurnal incubation already extends into the morning. A more technical detection of full incubation could be based on nest attentiveness. Following Haftorn (1981), Cresswell and McCleery (2003) proposed a minimum 50 % of nest attentiveness during the active day as the breaking point between diurnal partial and full incubation. The first day a female reaches half of her active time incubating would be the onset of full incubation. Moreover, when females reach this value, they have already expanded their diurnal bouts into the morning (Simmonds *et al.* 2017). Alternatively, Wang and Beissinger (2009, 2011), proposed a more complex methodology, by calculating the upper and lower 90 % interval of nest attentiveness from clutch completion to hatching date, and applying it retrospectively to the whole period to delimit full incubation days. Other slightly different solutions, from estimating the cut-off point as a minimum random duration of nest attentiveness (Ardia *et al.* 2006, Bambini *et al.* 2019) to the reverse counting from the hatching date (Cooper & Voss 2013), have also been adopted in previous studies.

To avoid the problematic issue of discriminating full from partial incubation, many studies have used the 'apparent incubation period' (e.g., Matthysen *et al.* 2011, Aldredge *et al.* 2014, Aldredge 2017), considering the day of clutch completion as the beginning of incubation and the hatching of the first egg as its end. This approach totally ignore the onset of incubation, but it has been useful to explore how environmental factors such as ambient temperature could shorten or lengthen the 'apparent incubation period', with consequences for the extent of hatching asynchrony or the synchronization between hatchlings and maximum prey abundance (e.g., Cresswell & McCleery 2003). In **Chapter 1** I delimited partial and full incubation in daylight and nocturnal periods by measuring nest attentiveness, later investigating the association of the onset of the diurnal and nocturnal incubation behaviours with the duration of the full incubation period and the extent of hatching asynchrony.

6. Consequences of advancing the incubation onset into the egg-laying phase

Delimiting partial from full incubation based on the clutch completion day is to some extent problematic, but the day the last egg is laid in a clutch draws a clear line before which any incubation behaviour may have caused differential embryo development, resulting in hatching asynchrony (Magrath 1990, Stoleson & Beissinger 1995). The difficulties appear when researchers try to tell apart the effects from nocturnal and diurnal periods, or from partial and full incubation happening before the clutch is completed.

Nocturnal incubation behaviour can exceed PZT and reach incubation temperature (Haftorn 1978, 1979, 1981, Lord *et al.* 2011, Vedder 2012, Podlas & Richner 2013a, but see Pendlebury & Bryant 2005), that is, it has the capacity to cause embryo development. Furthermore, nocturnally heated experimental nests undergoing incubation have revealed that nocturnal incubation is an energetically demanding period for the female (Bryan & Bryant 1999, Vedder 2012, Bleu *et al.* 2017). Alleviating nocturnal energy constraints would reduce egg-laying gaps (Yom-Tov & Wright 1993) and favour an earlier onset of diurnal incubation (Vedder 2012, Bleu *et al.* 2017). To assess nocturnal incubation effects on hatching asynchrony, or in other incubation related traits, faces the problem of pulling together nocturnal incubation and early diurnal incubation (Lord *et al.* 2011). In **Chapter 1** I approached this problem and investigated whether nocturnal incubation affected hatching asynchrony to some extent and/or the duration of full incubation period.

Unlike nocturnal behaviour, diurnal incubation has been systematically studied. As previously mentioned, the onset of diurnal incubation before clutch completion would shorten the period of full incubation, (Nilsson 1993, Ardia & Clotfelter 2007, Mainwaring *et al.* 2012a), enhancing hatching success (Veiga 1992, Stoleson & Beissinger 1999) and increasing the extent of hatching asynchrony (Veiga 1992, Ardia & Clotfelter 2007, Lord *et al.* 2011, Johnson *et al.* 2013, Aldredge 2017). The contrary (i.e., longer incubation periods, lower hatching success and hatching asynchrony) would be true when diurnal incubation starts after the clutch is complete (Naef-Daenzer *et al.* 2004, García-Navas & Sanz 2011, Kluen *et al.* 2011, but see Monrós *et al.* 1998). Which effects can be attributed to diurnal partial or full incubation before clutch completion are still under debate, and some hypothesis point out to early full incubation (i.e. diurnal full incubation displayed before the clutch is complete) as the only cause of shorter incubation periods and higher hatching asynchrony (Wang & Beissinger 2009, Podlas & Richner 2013a). In **Chapter 1** I tried to reach clear conclusions about the association between both partial and full diurnal incubation before clutch completion and hatching asynchrony; and in **Chapter 3** I tried to discriminate both behavioural effects by experimentally avoiding partial incubation.

7. Incubation rhythms

If approaching the onset of incubation encounters difficulties by the scarce previous research and the lack of clear concepts, exploring incubation rhythms during the full incubation period might be overwhelming given the numerous studies and the reported different patterns of nest attentiveness.

Incubation data has been extensively collected using a variety methodologies (see Smith *et al.* 2015), which could be grouped into two categories depending on the type of data recorded. Researchers collecting egg or nest-cup temperature have used iButtons (e.g., Amininasab *et al.* 2016, Bueno-Enciso *et al.* 2017) or thermocouples attached to data loggers (e.g., Ardia *et al.* 2006, Ardia & Clotfelter 2007, Cooper & Voss 2013), that allow to extrapolate female behaviour from off-bouts by identifying drops and rebounds of temperature (Cooper & Mills 2005). Researchers recording female movements have used pit-tagged birds (e.g., Álvarez & Barba 2014a, Bambini *et al.* 2019) or video cameras (e.g., Kovařík *et al.* 2009, Amininasab *et al.* 2017b, Capp *et al.* 2018) that would directly detect females entering and leaving the nest. Both methods have advantages and flaws that should be individually evaluated depending on the aims of the project, although the detection of bouts highly correlate between them (e.g., Bueno-Enciso *et al.* 2017, Bambini *et al.* 2019).

Most studied passerine species could be classified into two broad categories depending on the nest structure, which could be critical in shaping incubation responses to environmental factors: hole-nesters and birds that build nests with a dome, and open-cup and ground nesters. Building the nest in a tree-hole, nestbox, or building a dome, provides a buffer against overheating from direct sunlight (Tieleman *et al.* 2008, Griffith *et al.* 2016). Open-cup or ground nesters, especially in hot environments, not only have to allocate time between warming the eggs and self-maintenance but they would also need to display egg-cooling behaviours (e.g., shading the eggs, Walsberg & Voss-Roberts 1983) during the hours of maximum sun incidence. Unattended eggs could overpass their upper thermal limit (40.5–44 ºC, Drent 1975, Williams 1996), reaching temperatures over 50 ºC and potentially causing embryo death (Nord & Williams 2015). In comparison, hole-nesting passerines are an ideal model to test incubation behavioural responses to ambient temperatures, since females would just allocate time between incubation and self-maintenance activities, unless ambient temperature overpass 40.5 ºC (see Conway & Martin 2000a).

Incubation is a temporal dynamic behaviour. Females increase daily nest attentiveness throughout the incubation period, likely a compensating response to the increasing loss of heat from the developing embryo circulatory system (Cooper & Voss 2013). Females would also increase daily nest attentiveness later in the season because continuous nocturnal incubation becomes shorter (daylight also shortens), thus females would need to invest more time incubating during the daylight hours (Ardia *et al.* 2009, Bueno-Enciso *et al.* 2017). The last temporal variable that affects nest attentiveness patterns is the time of the day. The effects of hour are not straightforward due to a strong interaction with ambient temperature. Females would need to forage in the early morning to replenish the energy budget after a demanding night incubating, showing low values of nest attentiveness. However, if ambient temperature is low in the morning, females would need to protect their clutch, increasing nest attentiveness values (MacDonald *et al.* 2014, Nord & Cooper 2020). In **Chapter 2** I assessed the potential differences of interpretation derived from measuring nest attentiveness over different timescales (hourly, daily and full incubation period) given the different type of variables in play, together with the effect of ambient temperature.

The role of ambient temperature in the avian breeding period is of major importance, from before egg production (Stevenson & Bryant 2000, Cresswell & McCleery 2003), throughout nest construction (Deeming *et al.* 2012, Mainwaring *et al.* 2012b, 2014) and egg-laying (Monrós *et al.* 1998, Naef-Daenzer *et al.* 2004, García-Navas & Sanz 2011, Kluen *et al.* 2011) to the post-fledging period (Rodríguez *et al.* 2016a, Andreasson *et al.* 2018). Conway and Martin (2000a) showed that increasing ambient temperature decrease egg cooling rates, alleviating female incubation constraints, allowing them to lengthen both on- and off-bouts. Bout lengthening entails an increase in nest attentiveness because on-bouts increase at a higher rate than off-bouts (Conway & Martin 2000a, Cooper & Voss 2013). This response implies that nest attentiveness is lower at low temperatures, and bouts last fewer minutes and are more numerous, although it is expected a minimum threshold below which it is not possible to keep shortening them (Conway & Martin 2000a). Nevertheless, the effects of increasing temperature on nest attentiveness are not homogeneous across species or populations, and many studies have found opposing patterns: decreasing nest attentiveness and shortening bout duration at increasing temperatures (e.g., Camfield & Martin 2009, Kovařík *et al.* 2009, Walters *et al.* 2016). The different outcomes could be due to different breeding strategies in different habitats, or data artefacts from incomplete and partial datasets, which are common in incubation studies. I approached this problem in **Chapter 2** trying to find a generalized response to ambient temperature during the incubation period in three Great Tit populations breeding under different environmental conditions (see **Chapter 1** or **2** for habitat description, and Figure 1).

We cannot undervalue other factors, intertwined or not with ambient temperature, that could affect nest attentiveness (reviewed in Marasco and Spencer 2015). Rain (Basso & Richner 2015, Coe *et al.* 2015, Schöll *et al.* 2019), nest predation (Conway & Martin 2000b, Basso & Richner 2015), personality (Cole & Quinn 2014), blood parasites (Marzal *et al.* 2005), prey availability (Nilsson & Smith 1988, Sanz 1996, Londoño *et al.* 2008) or male feeding (Matysioková & Remeš 2014, Bambini *et al.* 2019) could alter females incubation responses, but in **Chapter 2** I focused on two variables closely related to incubation behaviour: clutch size and nest size. For example, nest properties are adapted to habitat conditions (Mainwaring *et al.* 2012b, 2014) and species building nests with higher insulator capacity have shown lower values of nest attentiveness (Deeming & Gray 2016) likely owing to eased constraints after a reduction in egg cooling rates. Females laying larger clutches may also find advantages in higher clutch thermal inertia, (i.e., lower egg cooling rates), cooling down and warming-up slower compared to smaller clutches (Reid *et al.* 2000a, Cooper *et al.* 2005).

8. The thesis project

Carrying out research in passerine incubation behaviour produce contradictory feelings. On one hand it seems that everything is already said. On the other, uncertainties appear with every concept: the onset of incubation, the effects of ambient temperature, proximate and ultimate causes of hatching asynchrony, etc. Even the methodological approaches to collect and analyse incubation data might be behind some of the described patterns (or their absence). Therefore, most questions are still open. This project aims to go back to the very seminal works about incubation behaviour in the last forty years, and build from there with the help of generous Great Tits (Figure 2) who tolerated my meddling during four breeding seasons. Thanks to accurate methods collecting high-quality incubation data and the will of the co-authors in revisiting basic concepts, this project has tried to delimit and quantify incubation behaviour and to find general effects of ambient temperature across populations.

Figure 1. Photographs showing the three breeding populations where the thesis project took place. a) Quintos de Mora National Wildlife Reserve (Quintos), b) Sagunto orange-tree plantations and, c) Monte de Santa Bárbara de Pina (Pina). Authors: Juan José Sanz (a) and David Diez Méndez (b, c).

The Great Tit is a hole-nesting female-only incubator passerine. The species has been widely studied in Europe, owing to its readiness to breed in wooden or concrete nestboxes provided by humans (Lack 1947, Kluijver 1950, Haftorn 1981). Nests are easily recognized by the use of moss (Álvarez & Barba 2011, Álvarez *et al.* 2013, Wesołowski & Wierzcholska 2018) and the variable clutch size allows to explore breeding trade-offs (Belda *et al.* 1998, de Heij *et al.* 2006). Females lay speckled eggs in a daily basis (Sanz & García-Navas 2009), although laying gaps occur (Nilsson & Svensson 1993b). Among passerines, hole-nesting species tend to hatch their eggs more asynchronously than open-cup nesters (Clark & Wilson 1981) and to lay larger clutches, thereby Great Tits are an adequate species for researching incubation behaviour and hatching asynchrony. Incubation behaviour was described in detail by Haftorn (1981) almost forty years ago in Norway (see **Chapter 1** for further details on the species). Previous incursions into the topic using data from Mediterranean habitats in the Iberian Peninsula (Álvarez & Barba 2014a, Bueno-Enciso *et al.* 2017) were invaluable studies that led to the present project.

Figure 2. Photographs of a) a Great Tit male in Sagunto and b) a 15-days old Great Tit nestling in Pina. Authors: Jéssica Jiménez Peñuela (a) and David Diez Méndez (b).

To sum up previously scatter mentions to the specific work carried out in this project, in **Chapter 1** I addressed the association between ambient temperature during the egg-laying period and the onset of incubation behaviours, by splitting them in four different periods: diurnal and nocturnal partial and full incubation. In **Chapter 2** I used three different timescales to assess how ambient temperature during the full incubation period could affect the number and duration of on- and off-bouts. The effect of partial incubation in hatching asynchrony was assessed in **Chapter 1** and later in **Chapter 3**, where we experimentally impeded females from partially incubating their clutches, and analysed the association between the residual hatching asynchrony and the role of egg repositioning within the nest-cup. The duration of the full incubation period was addressed in **Chapter 1** and **Chapter 2** by investigating how the partial stages of incubation and the intensity of incubation respectively affected this period. Additionally, in **Chapter 3** I also assessed how the experimental avoidance of partial incubation affected the duration of the full incubation period.

This project would have not existed without the funding granted by former Spanish Ministry of Economy and Competitiveness (MINECO) (CGL2013-48001-C2-1-P and CGL2016-79568-C3-1-P) thanks to the European funds "Fondo Social Europeo" (FSE). To work on this project, I was supported by an FPI fellowship (BES-2014-069191, MINECO and FSE) and two short-term research stays in North Carolina Museum of Natural Sciences (Raleigh, NC, USA) (grant EEBB-I-16-11668, MINECO), supervised by Dr Caren Cooper; and in the FitzPatrick Institute of African Ornithology (Cape Town, South Africa) (grant EEBB-I-18-12903, MINECO), supervised by Dr Susan J. Cunningham, who greatly contributed to achieve my objectives.

Aim and Objectives

The main aim of the project was to achieve a better understanding of incubation behaviour in a passerine species, the Great Tit *Parus major*, where females are the only-incubator. I revisited basic incubation concepts by collecting high-quality data of incubating females breeding under different environmental pressures, and tried to identify generalized behavioural responses to both biotic and abiotic factors. Specific objectives were:

1. To describe and quantify the onset of incubation behaviour. To assess how ambient temperature was associated with the onset of each incubation period (i.e., diurnal partial and full incubation, and nocturnal partial and full incubation) and the duration of partial incubation behaviours. To investigate whether these associations showed the same patterns among breeding populations. Moreover, to assess the role of breeding variables such as the egg-laying date, clutch size and year on the different onsets of incubation and the duration of the partial behaviours.

2. To assess how incubation rhythms, once diurnal full incubation is established, were associated with increasing ambient temperatures. To investigate if previously described divergent female responses, either increasing incubation effort or self-maintenance time with increasing temperatures, were population-dependent or they could be generalized. To analyse how nest attentiveness patterns could change depending on the timescale of analysis (hourly, daily and full incubation period).

3. To investigate the role of the different incubation periods before diurnal full incubation on the duration of this period. To analyse the potential role of incubation effort (i.e., higher nest attentiveness) on the duration of diurnal full incubation. To assess, following an experimental approach, how deterring clutches from being incubated during the egg-laying period affected the duration of the full incubation period.

4. To assess the effect of diurnal and nocturnal incubation behaviours before clutch completion on the extent of hatching asynchrony. To investigate how hatching asynchrony decreased after experimentally deterring clutches from being partially incubated. To quantify within-clutch egg repositioning throughout the incubation period and assess their role on hatching asynchrony.

Chapter 1

Chapter 1

Impacts of ambient temperature and clutch size on incubation behaviour onset in a female-only incubator songbird

Original paper published in:
Diez-Méndez D, Sanz JJ & Barba E. (2021) Ambient temperature and clutch size are the main drivers of the incubation behaviour onset of a female-only incubator songbird. *Ibis, doi: 10.1111/ibi.12937.*

Data are available from the Dryad Digital Repository:
https://doi.org/10.5061/dryad.pnvx0k6kz

ABSTRACT

Ambient temperature is assumed to be the major cue used by passerines to synchronize their laying and hatching dates to the expected peak of prey availability. While laying eggs, females are still able to fine-tune their hatching date following increasing or decreasing patterns of ambient temperature, mostly via changes in incubation onset. The onset of incubation behaviour in relation to the laying sequence could have later consequences for the duration of the incubation period and the extent of hatching asynchrony. Clutch size is also known to affect incubation patterns and might therefore condition potential responses to changing temperatures. In this study we assessed the association of ambient temperature and clutch size with the onset of four different incubation behaviours: partial and full nocturnal incubation, and partial and full diurnal incubation. We also evaluated how the onset and duration of each incubation behaviour might predict the duration of diurnal full incubation and the extent of hatching asynchrony. To achieve our aims, we monitored incubation behaviour using temperature data loggers during the egg-laying period in three Mediterranean Great Tit *Parus major* populations in three consecutive years. Our results showed that increasing temperatures were related to an advance of diurnal partial incubation, but not to its duration, nor to the onset of full incubation behaviour. We did not find any effect of ambient temperatures on nocturnal incubation. However, females lengthened nocturnal partial incubation and delayed the onset of nocturnal full and diurnal partial incubation, when laying larger clutches. Longer diurnal incubation before clutch completion was associated with greater hatching asynchrony. Moreover, longer diurnal partial incubation shortened the duration of the full incubation period. In conclusion, increasing ambient temperatures during the egg-laying period advanced diurnal partial incubation, indirectly shortening the full incubation period and increasing hatching asynchrony.

Keywords: Great Tit, *Parus major*, hatching asynchrony, nest attentiveness, nocturnal incubation, partial incubation.

INTRODUCTION

Birds are able to track environmental cues to synchronize the peak of food demand by their nestlings with maximum prey abundance (Perrins 1991, Cresswell & McCleery 2003, Simmonds *et al.* 2017). For passerines that feed caterpillars to their nestlings, early spring temperature is a potential cue for future prey availability (Noordwijk *et al.* 1995, Visser *et al.* 1998, Matthysen *et al.* 2011), and females might use it to advance or delay their egg-laying dates accordingly (Cresswell & McCleery 2003, Both & Visser 2005, Matthysen *et al.* 2011). Because ambient temperature still varies once egg laying has started, females may need to track these fluctuations to readjust egg-hatching dates to the changing, expected caterpillar peak (Cresswell & McCleery 2003, Both & Visser 2005, García-Navas & Sanz 2011, Matthysen *et al.* 2011).

Females advance hatching dates at increasing temperatures (Visser *et al.* 1998, Cresswell & McCleery 2003, Both & Visser 2005, Matthysen *et al.* 2011) or delay them during cold spells (Monrós *et al.* 1998, Naef-Daenzer *et al.* 2004, García-Navas & Sanz 2011, Kluen *et al.* 2011, Gądalski *et al.* 2018). The onset of incubation behaviour is the major determinant of reproductive timing once egg laying has started (Tomás 2015), and may open a temporal window of up to 16 days to readjust hatching dates within a population (García-Navas & Sanz 2011, Simmonds *et al.* 2017).

Earlier incubation onset within the laying sequence shortens the incubation period (Nilsson & Svensson 1993a, Ardia & Clotfelter 2007), but increases hatching asynchrony (Nilsson & Svensson 1993a, Ardia *et al.* 2006, Johnson *et al.* 2013), which could end in brood reduction and unbalanced offspring growth (Slagsvold *et al.* 1995, Szöllosi *et al.* 2007, Stenning 2008). Conversely, delaying incubation onset beyond clutch completion may lengthen the incubation period and reduce hatching asynchrony. This may lead to heavier fledglings, but has the drawback of reducing hatching success (Naef-Daenzer *et al.* 2004, García-Navas & Sanz 2011, Kluen *et al.* 2011).

The variability in the onset of incubation caused by ambient temperature may be narrowed by the effects of clutch size. Both laying eggs and incubation are energetically costly behaviours (Williams 2005, Nord & Williams 2015) and it may be difficult for females to incubate while laying eggs. For example, egg-laying females are more able to advance incubation onset when energy constraints are alleviated via supplementary food during the egg-laying period (Nilsson 1993), and may lay larger clutches when they are not constrained by an earlier prey peak so that there is no need to advance incubation behaviour into the egg-laying period (Cresswell & McCleery 2003). Nevertheless, several studies have shown that either the onset of incubation is not affected by clutch size (Nilsson & Svensson 1993a, Ardia & Clotfelter 2007), or that females may start incubation earlier relative to clutch completion

(Aldredge *et al.* 2014, Álvarez & Barba 2014a), or earlier in the laying sequence (Wang & Beissinger 2009), when clutches are larger.

Incubation onset, as addressed in previous studies, can be equated to the onset of diurnal full incubation (Wang & Beissinger 2009, reviewed in Wang & Beissinger 2011), defined as the stable period of incubation during the daylight (Haftorn 1981, Simmonds *et al.* 2017). But some diurnal incubation may start earlier if females display what is known as partial incubation, a developing and irregular period of incubation (Haftorn 1981, Simmonds *et al.* 2017). To date, the consequences of diurnal partial incubation are contested. While several studies highlight its importance in shortening the incubation period (Ricklefs 1993, Stoleson & Beissinger 1995, Wang & Beissinger 2011) and increasing hatching asynchrony (Veiga 1992, Lord *et al.* 2011, Johnson *et al.* 2013), others attribute such effects to the early onset of diurnal full incubation (Wang & Beissinger 2009, Podlas & Richner 2013a). Moreover, nocturnal incubation is rarely considered (but see Wang & Beissinger 2009, Lord *et al.* 2011). Passerines incubate continuously during the night, in contrast to intermittent diurnal incubation (Deeming 2002a), but females also go through a period of irregular partial incubation, that lasts from a few minutes to several hours per night (Haftorn 1981, Pendlebury & Bryant 2005). Both nocturnal, partial and full incubation behaviours might also have an effect on the duration of the incubation period, hatching asynchrony, and hatching success (Wang & Beissinger 2009), but their exact role is unknown.

In this study we explored the early incubation behaviour of Great Tits *Parus major* in three different populations, focusing first on the onset and duration of the partial incubation behaviours, and then on the onset of nocturnal and diurnal full incubation. We assessed the association of ambient temperature with the different incubating periods and whether this was consistent among populations. We also investigated the size of the clutch as a correlate of onset of incubation in every population. We then explored the association of the different incubation behaviours with the duration of the incubation period (i.e., the duration of diurnal full incubation) and the extent of hatching asynchrony.

We expected that 1) increasing temperatures would be associated with an advance of the incubation behaviour into the egg-laying period, and shorter partial incubation (i.e., speeding up the onset of full incubation behaviour); 2) larger clutches would be associated with delayed onset of incubation behaviour; 3) longer incubation behaviour during the egg-laying period would be associated with increased hatching asynchrony; 4) longer incubation before reaching diurnal full incubation would be associated with a shorter incubation period; and 5) increasing ambient temperatures would have similar associations with the onset of incubation behaviour across breeding populations.

METHODS

Study species

The Great Tit is a secondary hole-nesting passerine species that easily accepts nestboxes. Females lay clutches of 4–13 eggs (Álvarez & Barba 2014b); the usual pattern is laying one egg per day although they may skip a day, producing gaps in the laying sequence (Nilsson & Svensson 1993b, Monrós *et al.* 1998). The incubation period lasts 13 days on average (Álvarez & Barba 2014b) and females are the only incubator. Clutches hatch in a range of 0–3 days (Álvarez & Barba 2014b). Females usually start nocturnal incubation shortly after laying the first egg, and diurnal incubation somewhat later in the laying sequence (see Haftorn 1981 for a detailed description). Full diurnal incubation behaviour is considered to start around the day the clutch is complete, although this behaviour is highly variable (Cresswell & McCleery 2003, Álvarez & Barba 2014b).

Study areas

We collected data on first clutches during three breeding seasons (2015–2017) in three Spanish populations, where wooden nestboxes were available for the birds to breed as part of long-term studies on the species. The study area of Pina (40.02° N, 0.63° W, 1200 m asl) is a mixed forest composed by Portuguese Oaks *Quercus faginea* scattered within Maritime Pines *Pinus pinaster* woodlands. The population is located at Monte de Santa Bárbara de Pina in Eastern Spain and includes 200 nestboxes. Blue Tits *Cyanistes caeruleus* and Coal Tits *Periparus ater* also breed into the nestboxes (Alambiaga *et al.* 2020). The study area of Quintos (39.73° N, 3.98° W, 900 m asl) is part of the Quintos de Mora National Wildlife Reserve in Central Spain. Forest patches here are dominated by Pyrenean Oaks *Quercus pyrenaica* and, to a lesser extent, by Portuguese Oaks (e.g., Barrientos *et al.* 2015). Blue Tits, Eurasian Nuthatches *Sitta europaea* and Rock Sparrows *Petronia petronia* also breed in the 200 nestboxes available in the area. The study area of Sagunto (39.70° N, 0.25° W, 30 m asl) is within a homogeneous extensive orange *Citrus aurantium* plantation in Eastern Spain, where orange trees are uniformly distributed (e.g., Rodríguez *et al.* 2016b). From approximately 500 available nestboxes, a subarea of 200 boxes was selected for the present study. Apart from the Great Tit, a few House Sparrows *Passer domesticus* and Tree Sparrows *P. montanus* also occupy some nestboxes.

General field methods

Nestboxes in each study area were inspected weekly from early spring, looking for signs of nest building. We increased the frequency of the visits during the nest building period up to daily after we found lining material. This allowed us to detect the first laid egg at each nest (i.e., laying date, 1st of April = 1) in most cases (see below). Daily visits during the laying period allowed us to detect incubation behaviour (presence of uncovered and warm eggs, or direct observation of an incubating female). This was an approximation to the onset date of diurnal full incubation behaviour (which was determined more precisely later) and was useful for estimating hatching dates during fieldwork. Eleven days after the detection of incubation we started visiting the nests twice a day to measure hatching asynchrony. We weighed the hatchlings at every visit (electronic balance; 0.1 g), until the last egg hatched, or new hatchlings were absent for two consecutive visits. We used the weights from the visit at which the last egg was found to have hatched and defined hatching asynchrony as the ratio of the lightest and heaviest hatchlings (following Ardia *et al.* 2006), so lower ratios indicate higher asynchrony. Breeding parameters for each study area by year are provided in Appendix 1, Table S1.

Temperature data collection

We assessed incubation behaviour from recorded temperatures in the nest-cup at each nest. When we detected eggs in a nest, the nest was carefully removed from the nestbox to place an inverted U-shape mesh of galvanized steel covered by a cardboard platform. In the space between the mesh and the nestbox floor (15 mm height) we placed a HOBO UX100-014M Single Channel Thermocouple data logger (Onset Computer Corporation, USA) fitted to collect data every 10 s. We connected a Type T 6 ft Beaded Thermocouple probe (30 wire gauge, accuracy ± 1.0°C; Onset Computer Corporation, USA) to the data logger and placed the nest back in the nestbox, over the cardboard (Figs. 1 & S1). The raw probe was anchored to the centre of the clutch, being surrounded by the eggs. The raw probe was still highly mobile and females could bury or displace it from the centre of the nest-cup, reducing chances of collecting high quality data. In order to increase data collection efficiency, in 2017 the raw probe was implanted in a plastic craft egg (Factory Direct Craft Supply, USA) filled with wire-pulling lubricant (Clear Glide, Ideal industries, USA), as used successfully in previous studies (Ardia *et al.* 2006, 2010, Cooper & Voss 2013). The plastic eggs (15.9 x 11.5 mm) resembled speckled Great Tit eggs, though slightly smaller (e.g., eggs in Sagunto population averaged 18.1 x 13.3 mm; Encabo *et al.* 2001) (see Fig. S2).

We set thermocouples in 29, 62, and 95 nests in Pina, Quintos and Sagunto respectively. Three in Pina (10.3 %) and six in Quintos (9.7 %) failed before incubation started due to female desertion, and an additional nest was preyed upon in Pina. In Sagunto, 21 nests failed before clutch completion (22.1 %) due to female desertion (16) and outsider human manipulation (5). From the 25 deserted nests among the three populations, breeding females stopped laying eggs after datalogger placement in eleven of them (6 % of nests with dataloggers). From the resulting successful nests, we discarded those with low quality temperature data caused by the displacement of the probe from the centre of the clutch, or by being buried during the recording period, after a preliminary visual inspection of temperature patterns (see Fig. S3 for a comparison). In total, we had 77 nests available for fine-scale incubation analyses (16, 22 and 39 nests in Pina, Quintos and Sagunto, respectively). From these nests, six were not used in analyses of duration of the incubation period because females deserted few days before hatching (i.e., incubation day 11 onwards), and four additional nests were not used in analyses of hatching asynchrony because we could not weigh hatchlings on focal days. For the 77 nests, we set thermocouples on the day of the first egg day in 52 nests (67.5 %). In the remaining 25, thermocouples were set during the second egg-laying day in 11 nests, the third day in eight nests, the fourth in five nests, and the fifth in one nest.

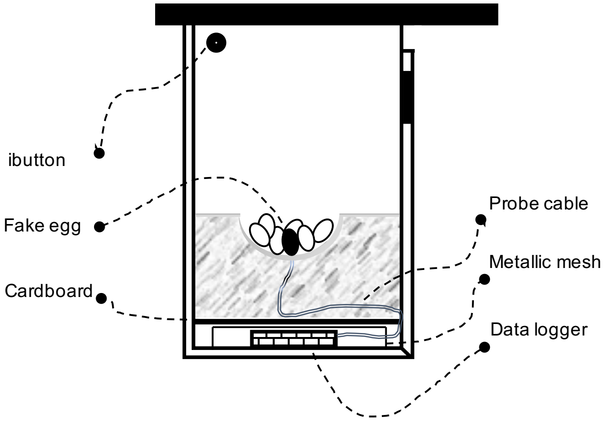


Figure 1. Diagram of a lateral view of a nestbox showing the experimental setup to measure both nest-cup temperature and ambient temperature.

To measure ambient temperature at the nest, we placed Thermochron iButton data loggers (accuracy ± 0.5°C, Model DS1922L-F5, Maxim Integrated, USA) in the upper part of one of the inner walls of the nestbox, fixed with brown adhesive tape, recording local ambient temperature every 520 s. When an iButton was lost (i.e., a member of the breeding pair detached it from the inner wall), we used temperature data from the closest nestbox, choosing the dates of interest. During 2015, temperature in Quintos was only provided by two empty nestboxes installed for that purpose in the study area. Ambient temperature for each study area by year and incubation behaviour is provided in Table S2.

Incubation behaviour

We used the software combination of Rhythm and Raven (Cooper & Mills 2005) to analyse recorded temperatures in the nest-cup and discriminate between on-bouts (i.e., periods of time that a female is incubating the clutch in the nest) and off-bouts (i.e., periods of time that a female is foraging or carrying self-maintenance activities out of the nest). Our criteria to delimit off-bouts in Rhythm were a minimum decrease in egg temperature of 2.0 °C, a minimum initial cooling slope of 0.2 °C/min, and a minimum off-bout duration of 2 minutes. The output of Rhythm was visually inspected in Raven to verify the timing and duration of incubation off- and on-bouts.

The cut-off point between diurnal partial and full incubation was set as the first day that incubation behaviour lasted at least 50 % of the active day; that is, the first day a female incubated at least half of the time between the first off-bout in the early morning and the last on-bout in the evening (following Cresswell & McCleery 2003, Simmonds *et al.* 2017). For nocturnal incubation, the cut-off point was considered when females incubated steadily the whole night (100 % of the inactive day) for the first time (Haftorn 1981) (Fig. 2). The incubation period, or more accurately the full incubation period, was defined as the elapsed days from onset of full incubation to the day preceding hatching date, measured in days.

We reported the onset of incubating periods as both the number of elapsed days since the first egg was laid (laying date = egg day 1) and the number of days since clutch completion (clutch completion day = 0), following recommendations in Wang and Beissinger (2011). However, we only used the former for statistical analyses, as this variable better allowed us to account for when the females start to incubate in the laying sequence, and to account for the effect of clutch size by adding the latter variable into the models. The duration of nocturnal and diurnal partial incubation was reported as the total sum of minutes and the number of days of partial incubation (Table 1). For statistical models we only used partial incubation duration in minutes as a more accurate variable.

Statistical analyses

We conducted statistical analyses using R software 3.6.1 (R Core Team 2019). We built several linear models (LMs) to assess the association between mean ambient temperature and the onset and duration of diurnal partial incubation, the duration of nocturnal partial incubation, and the onset of both diurnal and nocturnal full incubation (see Fig. 3 for further model details). Laying date and clutch size were added to models as covariates and study population and year as categorical effects. For the model for onset of diurnal partial incubation, we also added mean ambient temperature (from laying date to the day prior to the onset of diurnal partial incubation) as a covariate. For the models for duration of nocturnal and diurnal partial incubation, we added mean ambient temperature during the partial incubation period and the egg day on which partial incubation began as covariates. For the models for onset of full diurnal and nocturnal incubation, we added the mean

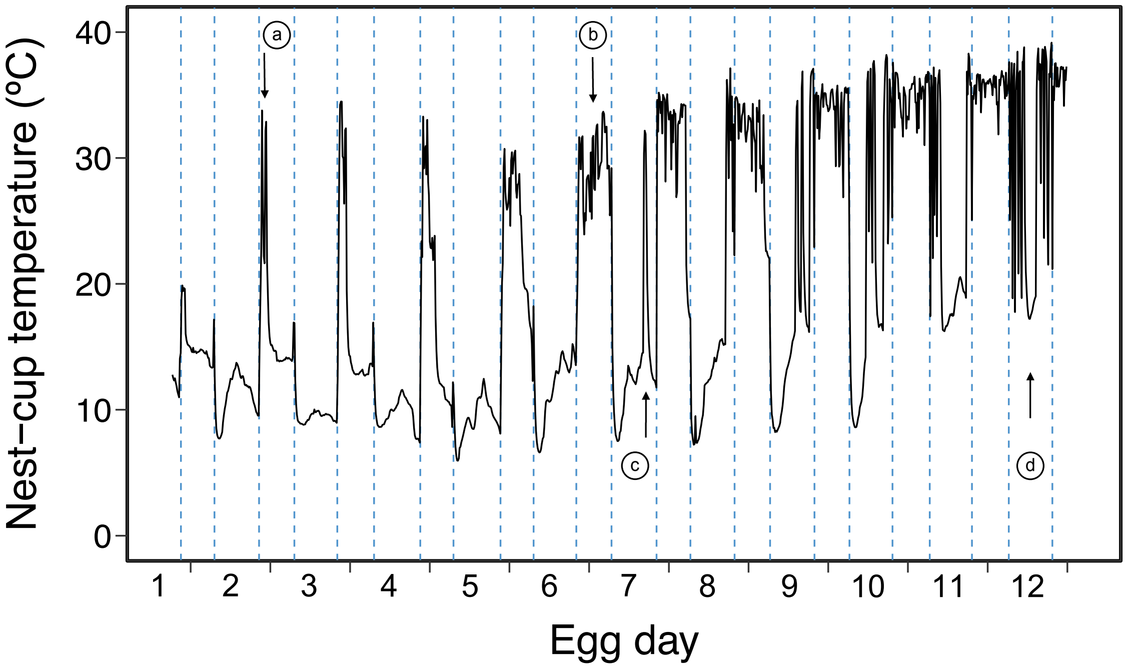


Figure 2. Raw data showing the variation in nest-cup temperature since the laying date (egg day 1) until the onset of diurnal full incubation. a) onset of nocturnal partial incubation, b) onset of nocturnal full incubation, c) onset of diurnal partial incubation, and d) onset of diurnal full incubation. Shadowed areas highlight nocturnal inactive time.

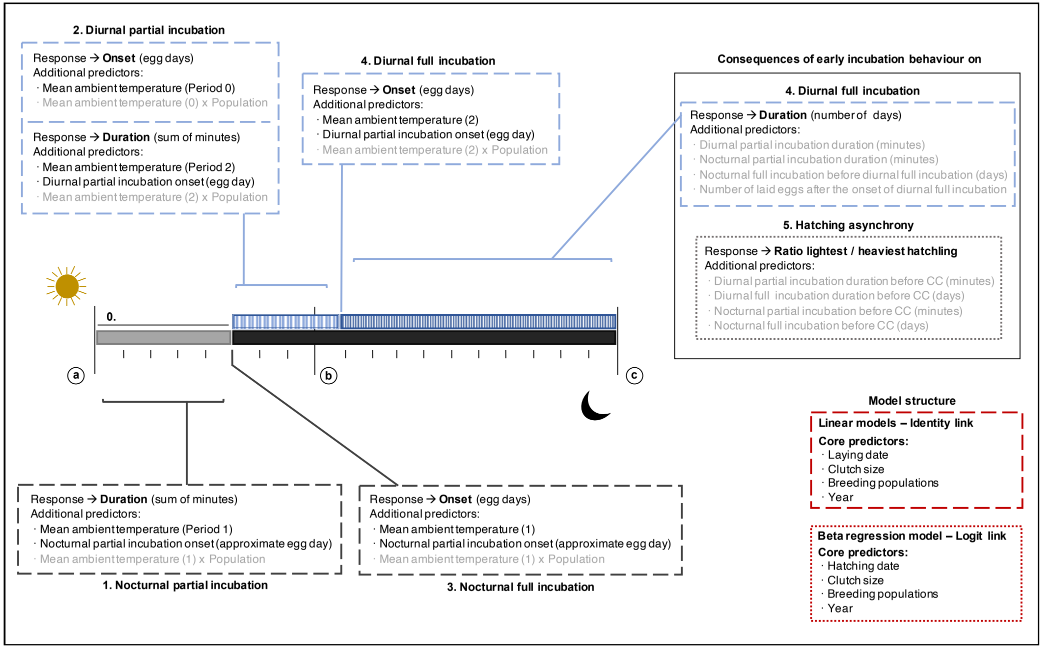


Figure 3. Schematic representation of the different incubation behaviour periods displayed by an incubating female and the variables used in the statistical models. Variables in grey were subject to model selection. a) refers to laying date, b) to clutch completion day (CC), and c) to hatching date. Horizontal striped bars show diurnal partial (2) and full (4) incubation and filled bars represent nocturnal partial (1) and full (3) incubation.

temperature value for the preceding partial incubation period, and the egg day each partial incubation started as covariates. We only provided descriptive statistics for the onset of nocturnal partial incubation, because most of the females started to incubate the day they laid the first egg, and we lack of temperature data before the laying date to model any association between ambient temperature and the onset of this behaviour. In the case of nests where the data logger was set after egg day 1, we considered the onset of nocturnal partial incubation to happen on the egg day when data collection started, as the better approximation for data analysis, although we are aware that this could have resulted in under-estimation of nocturnal partial incubation periods. To test our hypothesis about the effect of ambient temperature across populations, we built two candidate models for each dependent variable, one of them including the interaction between the breeding population and ambient temperature and the other one without such interaction. We selected the most informative model based on Akaike's Information Criterion (AIC) following Burnham and Anderson (2002) via the *MuMIn* package (Bartón 2015). If both models were equally informative (ΔAICc < 2.0) we selected the most parsimonious one, with no interactions (see Table S3 for model selection results).

Following the same rationale, to explore the consequences of the different incubating behaviours for the duration of the full incubation period and the extent of hatching asynchrony, we built a set of LMs and beta regression models respectively. Beta regression models allowed us to use ratios from hatching asynchrony data as a dependent variable by applying a logit link (*betareg* package, Cribari-Neto & Zeileis 2010). We added laying date, clutch size, population and year as predictors in LMs for the duration of the full incubation period and hatching date, clutch size, population and year as predictors in beta regression models for hatching asynchrony. Both LMs and beta regression models contained different combinations of the incubation behaviours potentially displayed by females until the onset of the full incubation period (i.e., diurnal partial incubation and nocturnal partial and full incubation) or until clutch completion respectively (i.e., diurnal partial and full incubation and nocturnal partial and full incubation). We compared candidate models and selected the most informative one based on AIC values (Table S4).

We analysed final selected models, reported their R^2 values, and considered as significant *P*-values lower than 0.05. Numerical predictors were centred, and residuals of the response variable visually inspected to check normality and homogeneity of the variance. Because final LMs showed residual data points with high leverage values or outliers, we reran the models applying an iterated re-weighted least squares methodology, that weighs down the effect of outliers and recalculate estimates and standard errors, reducing the influence of these data

Table 1. Descriptive statistics of the nocturnal and diurnal partial incubation behaviour. Egg day refers to the number of days since the first egg was laid at each nest (laying date = egg day 1) and clutch completion refers to the number of days since the clutch was completed (clutch completion day = 0). *n* denotes the number of nests.

	Pina			Quintos			Sagunto		
	n	**mean ± sd**	**range**	***n***	**mean ± sd**	**range**	***n***	**mean ± sd**	**range**
Nocturnal partial incubation									
Duration (approximate)									
Number of days	16	5.2 ± 2.51	1 – 10	22	5.4 ± 1.59	3 – 9	38	4.7 ± 1.80	0 – 8
Sum of minutes	16	1364 ± 681	374-3084	22	1258 ± 369	758 – 2140	38	1077 ± 506	0 – 2006
Nocturnal full incubation									
Onset									
Egg day	16	7.0 ± 2.03	4 – 11	22	7.7 ± 1.55	5 – 10	38	7.0 ± 1.46	3 – 10
Clutch completion	16	0.8 ± 1.75	(-2) – 5	22	-0.8 ± 1.85	(-3) – 1	38	-0.8 ± 1.09	(-6) – 1
Diurnal partial incubation									
Onset									
Egg day	15	7.7 ± 2.19	5 – 12	20	7.7 ± 1.78	4 – 11	38	6.7 ± 1.40	4 – 9
Clutch completion	15	0.5 ± 1.92	(-2) – 5	20	-1.8 ± 1.51	(-4) – 2	38	-2.1 ± 1.25	(-5) – 0
Duration									
Number of days	15	3.5 ± 1.13	2 – 5	21	2.1 ± 1.00	0 – 4	39	2.3 ± 1.08	0 – 5
Sum of minutes	14	696 ± 214	207 – 1035	21	331 ± 215	0 – 933	39	366 ± 182	0 – 710
Diurnal full incubation									
Onset									
Egg day	16	11.4 ± 2.50	7 – 16	22	9.9 ± 1.58	7 – 12	39	9.0 ± 1.06	7 – 11
Clutch completion	16	4.2 ± 2.54	0 – 9	22	0.4 ± 1.37	(-2) – 4	39	0.3 ± 0.86	(-1) – 2

points on the model estimates (*Robustbase* package, Maechler *et al.* 2015). We reported mean ± sd unless otherwise stated.

RESULTS

Nocturnal incubation

Nocturnal partial incubation was a ubiquitous behaviour in our breeding populations, lasting 1203 ± 522 min (for population level summaries see Table 1). Females breeding in Pina and Quintos displayed partial incubation from the evening when egg laying commenced in all of the 16 nests where the thermocouple was set from the first day. The other 22 females breeding in these two populations were engaged in nocturnal partial incubation when data collection started, except one female from Quintos that started on egg day 5. In contrast, only 15 of 36 females from Sagunto (in nests where we collected data since egg day 1) started nocturnal partial incubation that first evening. Except for one female that immediately displayed nocturnal full incubation, females breeding in Sagunto started nocturnal partial behaviour 1.91 ± 0.98 days after laying the first egg (range = 1 – 4, $n = 35$).

We did not find any effect of ambient temperature on the duration of partial nocturnal incubation (estimate ± se = -7.1 ± 80.60, $t = -0.088$, $P = 0.930$), but the larger the clutch the longer nocturnal partial incubation lasted (estimate ± se = 164.6. ± 65.99, $t = 2.494$, $P = 0.015$, Table 2, Fig. 4a). Our model also showed that the duration of nocturnal partial incubation was similar among the three studied populations (Table 2), although we had reduced power to detect such differences because we did not collect complete nocturnal partial incubation data in 23 out of 77 nests.

The onset of nocturnal full incubation was not related to ambient temperatures (estimate ± se = -0.11 ± 0.186, $t = -0.611$, $P = 0.543$), but the larger the clutch the later it started (estimate ± se = 1.01 ± 0.154, $t = 6.520$, $P < 0.001$, Table 2, Fig. 4b). Females showed a great variability in nocturnal full incubation onset relative to the laying sequence, from the egg day 3 to day 11, or as early as six days before clutch completion and up to five days after it (Table 1). We discarded one nest from Sagunto where nocturnal full incubation was not achieved during the recording of incubation behaviour (see Fig. S4). The starting day of nocturnal full incubation was similar at the three breeding populations (Table 2), but differed among years (reference year 2015; 2016 estimate ± se = 1.04 ± 0.296, $t = 3.505$, $P = 0.001$; 2017 estimate ± se = 0.65 ± 0.321, $t = 2.039$, $P = 0.045$).

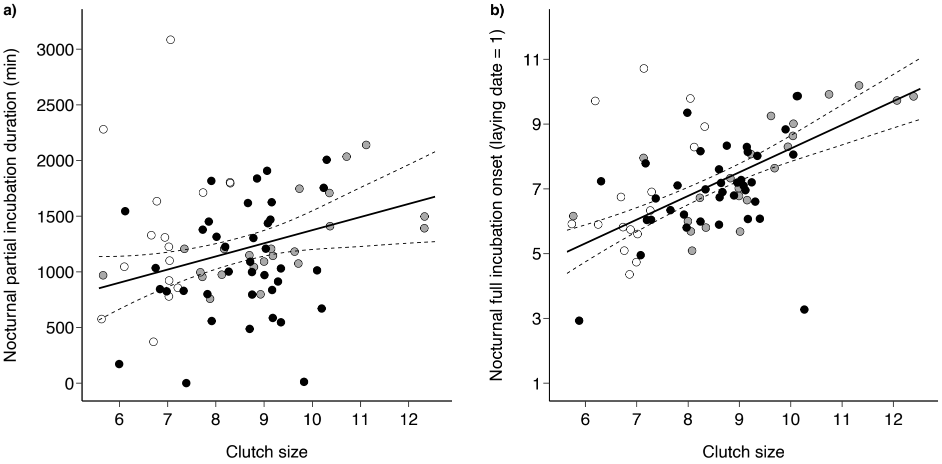


Figure 4. Variation of a) the duration of partial incubation (sum of minutes) and b) the onset of the nocturnal full incubation in relation to the clutch size. Dashed lines denote 95 % confidence intervals obtained with the package *effects* (Fox 2003). Circles represent raw data from the three populations: empty circles show nests from Pina, grey circles from Quintos and black ones from Sagunto.

Diurnal incubation

Diurnal partial incubation was also displayed by almost all females, except by one in Quintos and one in Sagunto, which directly started diurnal full incubation (i.e., ≥ 50 % of the active day incubating). The earliest a female started diurnal partial incubation was after laying the fourth egg, or five days before clutch completion. Females displayed 427 ± 259 min of diurnal partial incubation (for population level summaries see Table 1). We discarded one nest from Pina and another from Quintos because females partially incubated for twice as long time as the maximum recorded (10 and 8 days compared to 5 and 4 days respectively) and showed erratic behaviour, not progressing as expected or even interrupting partial incubation (0 % of nest attentiveness at some point during the period) (see Figs. S5 & S6). Diurnal partial incubation always started after partial nocturnal incubation behaviour had already begun (Figs. S4-S6).

Females displayed diurnal partial incubation earlier in the laying sequence when temperatures were higher (estimate ± se = -0.8 ± 0.25, t = -3.233, P = 0.002, Table 3, Fig. 5a). However, larger clutches delayed its onset (estimate ± se = 0.8 ± 0.20, t = 3.671, P < 0.001, Table 3, Fig. 5b). Diurnal partial incubation onset differed among years (reference year 2015; 2016estimate ± se = 1.0 ± 0.39, t = 2.577, P = 0.012; 2017 estimate ± se = -0.1 ± 0.40, t =

-0.356, *P* = 0.723), but we did not find differences among breeding populations (Table 3). Females allocated fewer minutes to diurnal partial incubation as it started later in the laying sequence (estimate ± se = -69.4 ± 29.96, *t* = -2.317, *P* = 0.024), but we did not find a clear effect of ambient temperature on the duration of diurnal partial incubation (estimate ± se = -13.6 ± 33.84, *t* = -0.402, *P* = 0.689, Table 3). When comparing populations, females from Pina showed a longer diurnal partial incubation period than those from the other sites (Quintos estimate ± se = -421.3 ± 103.06, *t* = -4.088, *P* < 0.001; Sagunto estimate ± se = -450.1 ± 127.30, *t* = -3.536, *P* = 0.001, Table 3).

Table 2. Results of the linear models analysing the effects of laying date, clutch size, breeding population, mean ambient temperature and year on the nocturnal partial incubation duration (sum of minutes) and the onset of nocturnal full incubation (days since the laying date). The categorical variables year and population were assessed in comparison to the reference year 2015 and the reference population Pina. Significant effects are highlighted in bold.

	Estimate	**se**	***t***	***P***
Nocturnal partial incubation duration				
R^2 = 0.17				
Intercept	1197.0	255.33	4.688	
Laying date	-28.3	100.15	-0.283	0.778
Clutch size	**164.6**	**65.99**	**2.494**	**0.015**
Quintos	-274.5	236.06	-1.163	0.249
Sagunto	-326.1	320.28	-1.018	0.312
Mean temperature	-7.1	80.60	-0.088	0.930
Year 2016	**308.4**	**127.76**	**2.414**	**0.019**
Year 2017	**328.9**	**139.39**	**2.360**	**0.021**
Nocturnal partial incubation onset	-63.8	53.48	-1.193	0.237
Nocturnal full incubation onset				
R^2 = 0.54				
Intercept	6.97	0.582	11.968	
Laying date	-0.11	0.230	-0.497	0.621
Clutch size	**1.01**	**0.154**	**6.520**	**< 0.001**
Quintos	-0.61	0.539	-1.132	0.262
Sagunto	-0.64	0.733	-0.878	0.383
Mean temperature	-0.11	0.186	-0.611	0.543
Year 2016	**1.04**	**0.296**	**3.505**	**0.001**
Year 2017	**0.65**	**0.321**	**2.039**	**0.045**
Nocturnal partial incubation onset	**0.41**	**0.122**	**3.330**	**0.001**

Females started to display full incubation behaviour as early as two days before clutch completion, and as late as nine days after. Relative to the laying sequence, no female started diurnal full incubation before the seventh egg day (Table 1). The night before diurnal full incubation started, all females, except one in Sagunto, already displayed nocturnal full incubation. The later a female began to partially incubate the clutch during the day, the later diurnal full incubation started (estimate ± se = 1.06 ± 0.145, $t = 7.328$, $P < 0.001$, Table 3). We did not find a clear association of ambient temperature with the onset of diurnal full incubation at any breeding population (estimate ± se = -0.2 ± 0.16, $t = -1.446$, $P = 0.153$) (Table 3). Full incubation onset was similar among years but differed among populations: females from Pina started later in the laying sequence than females elsewhere (Quintos estimate ± se = -1.9 ± 0.50, $t = -3.785$, $P < 0.001$; Sagunto estimate ± se = -2.0 ± 0.61, $t = -3.327$, $P = 0.001$, Table 3).

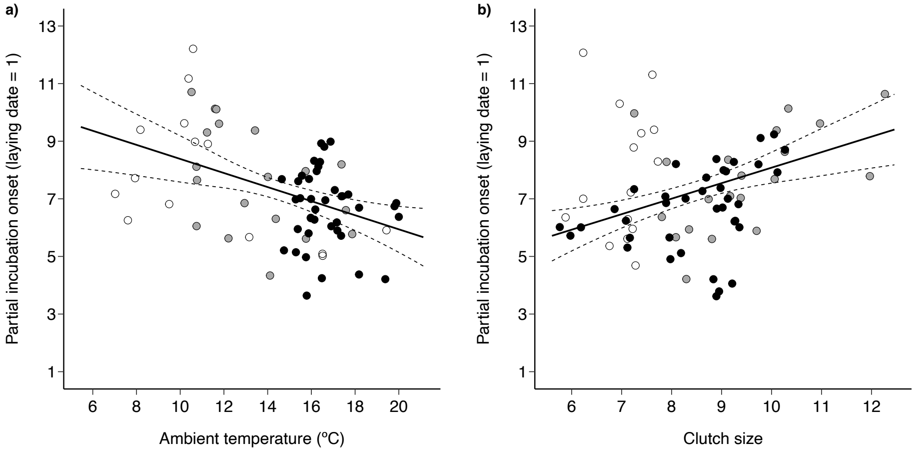


Figure 5. Variation of the onset of partial incubation in relation to a) mean ambient temperature since the laying date and b) clutch size. Dashed lines denote 95 % confidence intervals obtained with the package *effects*. Circles represent raw data from the three populations: empty circles show nests from Pina, grey circles from Quintos and the black ones from Sagunto.

Effects of early incubation behaviour

The duration of the full incubation period was 12.3 ± 1.02 days (for population level summaries see Table 1). Among the different incubation periods subject to model selection, the final model only contained diurnal partial incubation (Table S2): the longer was diurnal partial incubation, the shorter was the period of diurnal full incubation (estimate ± se = -0.4

± 0.13, t = -3.289, P = 0.002, Table 4). Similarly, the longer females partially incubated a clutch before laying all the eggs, the higher was the hatching asynchrony (i.e., a negative effect on the ratio between the lightest and the heaviest hatchling) (estimate ± se = -0.3 ± 0.08, t = -3.232, P = 0.001, Table 4). Also, the longer diurnal full incubation was exhibited before clutch completion, the higher was hatching asynchrony (estimate ± se = -0.2 ± 0.007, t = -2.606, P = 0.009, Table 4). It should be noted, however, that diurnal full incubation before clutch completion only happened in 11 out of 77 nests, while diurnal partial incubation prior to clutch completion occurred in 61 nests (Figs. S4-S6)

DISCUSSION

Ambient temperature during the egg-laying period was associated only with advance of the onset of diurnal partial incubation, and the effect was similar in the three breeding populations. We did not find any independent association of ambient temperature with the onset of diurnal full incubation, but the diurnal partial incubation advance pulled it forward. Clutch size showed an important association with early incubation behaviour: larger clutches were associated with delayed onset of diurnal partial incubation, lengthened nocturnal partial incubation, and delayed onset of nocturnal full incubation. Only the diurnal stages of incubation were directly associated with duration of the full incubation period and the extent of hatching asynchrony. However, both ambient temperature and clutch size, through their associations with the duration of diurnal partial incubation in opposite directions, indirectly predicted full incubation duration and hatching asynchrony.

Nocturnal incubation behaviour

Nocturnal incubation has been proposed to be a protective response for embryos against cold temperatures (Haftorn 1978) while warmer temperatures would delay its onset (Wang & Beissinger 2009). However, incubating female songbirds do not display nocturnal incubation during the egg-laying period when ambient temperatures are close to freezing (Zerban & Morton 1983, Morton & Pereyra 1985, Clotfelter & Yasukawa 1999), or at least not regularly (Johnson *et al.* 2013). Moreover, if nocturnal partial incubation is displayed, it does not last until the coldest temperatures occurring close to the sunrise (this study, see also Haftorn 1981, Pendlebury & Bryant 2005, Lord *et al.* 2011). An alternative explanation for the early onset of nocturnal incubation maybe be protection against warm nights to preserve egg hatchability (Lord *et al.* 2011, see also the egg viability hypothesis; Veiga 1992, Stoleson & Beissinger 1999). However, our data also disagrees with the latter hypothesis since females from the warmest population (Sagunto) started nocturnal incubation on average two days later than the others. We lack suitable data to propose a strong alternative hypothesis, although body size, hormone levels or prey availability may play an important role in early stages of incubation (see below). Moreover, pre-laying abiotic factors such as ambient temperature (Simmonds *et al.* 2017) or photoperiod (Lambrechts *et al.* 1997) that affect laying date may also impact on the onset of nocturnal partial incubation.

Clutch size is unlikely to affect the onset of nocturnal partial incubation but was the only variable that was associated with nocturnal behaviour (see also Haftorn 1981, Vedder 2012). That only smaller clutches were associated with advanced onset of nocturnal full incubation (but see Wang & Beissinger 2009), might suggest that breeding females cannot cope with both laying eggs and full nocturnal incubation, or at least not early in the laying sequence. In this scenario, it might be expected that females with better body condition could increase nocturnal nest attentiveness faster and reach nocturnal full incubation earlier, although a previous study found that better body condition correlated with longer partial nocturnal incubation periods in precocial Wood Ducks *Aix sponsa* (Grimaudo *et al.* 2020).

Diurnal incubation behaviour

Incubation behaviour (equated to our definition of full incubation) starts earlier in the laying sequence at increasing ambient temperatures (Naef-Daenzer *et al.* 2004, Kluen *et al.* 2011) or earlier relative to clutch completion (Ardia *et al.* 2006, García-Navas & Sanz 2011, but see Wang & Beissinger 2009). Our results showed that increasing temperatures since the laying date were associated with an advance of the onset of diurnal partial incubation, but not of diurnal full incubation. Since the main driver for advancing the onset of full incubation was an earlier onset of partial incubation, an apparent advance of diurnal full incubation might still be expected even when partial incubation is not measured. We cannot discount, however, the possibility that temperatures themselves could directly advance the onset of diurnal full incubation in other populations or species.

We found that breeding females showed a potential time window of up to nine days (from egg day 4 to 12) to start diurnal partial incubation. Unfortunately, we lack of data on caterpillar phenology to explore whether a better synchronization was achieved by changes in hatching dates, although previous studies in Sagunto (Monrós *et al.* 1998) and Quintos (García-Navas & Sanz 2011) suggest this. Besides the synchronization hypothesis, females could have advanced diurnal incubation into the laying sequence to protect eggs against bacteria (Cook *et al.* 2003, Beissinger *et al.* 2005, Ruiz-De-Castañeda *et al.* 2012) or to maintain hatchability of early-laid eggs (Veiga 1992, Stoleson & Beissinger 1999). In our studied populations, females modified the onset of diurnal partial incubation over the whole range of mean temperature values between 6 and 20 ^{o}C (see Figure 3), and not only at high temperatures (e.g., >26 ^{o}C, Ardia *et al.* 2006). Furthermore, most females heated the clutch after the first day by displaying nocturnal partial incubation, with diurnal partial incubation happening several days later, which questions the role of diurnal partial incubation in maintaining egg viability.

Table 3. Results of the linear models analysing the effects of laying date, clutch size, mean ambient temperature, breeding population and year on the onset (days since the laying date) and duration (in minutes) of diurnal partial incubation and the onset of diurnal full incubation (days since the laying date). The categorical variables year and population were assessed in comparison to the reference year 2015 and the reference population Pina. Significant effects are highlighted in bold.

	Estimate	**se**	***t***	***P***
Diurnal partial incubation onset				
$R^2 = 0.40$				
Intercept	6.9	0.81	8.597	
Laying date	0.2	0.32	0.512	0.610
Clutch size	**0.8**	**0.20**	**3.671**	**< 0.001**
Quintos	-0.3	0.77	-0.361	0.719
Sagunto	0.1	1.00	0.090	0.929
Mean temperature	**-0.8**	**0.25**	**-3.233**	**0.002**
Year 2016	**1.0**	**0.39**	**2.577**	**0.012**
Year 2017	-0.1	0.40	-0.356	0.723
Diurnal partial incubation duration				
$R^2 = 0.38$				
Intercept	796.6	106.96	7.447	
Laying date	-60.1	43.78	-1.373	0.175
Clutch size	-27.1	32.22	-0.842	0.403
Quintos	**-421.3**	**103.06**	**-4.088**	**< 0.001**
Sagunto	**-450.1**	**127.30**	**-3.536**	**0.001**
Mean temperature	-13.6	33.84	-0.402	0.689
Year 2016	-12.7	59.71	-0.213	0.832
Year 2017	-21.2	59.73	-0.355	0.724
Diurnal partial incubation onset	**-69.4**	**29.96**	**-2.317**	**0.024**
Diurnal full incubation onset				
$R^2 = 0.67$				
Intercept	11.1	0.51	21.701	
Laying date	-0.4	0.21	-1.666	0.101
Clutch size	0.1	0.16	0.465	0.644
Quintos	**-1.9**	**0.50**	**-3.785**	**<0.001**
Sagunto	**-2.0**	**0.61**	**-3.327**	**0.001**
Mean temperature	-0.2	0.16	-1.446	0.153
Year 2016	0.3	0.29	1.070	0.288
Year 2017	0.3	0.29	1.062	0.292
Diurnal partial incubation onset	**1.06**	**0.145**	**7.328**	**< 0.001**

Table 4. Results of the linear and beta regression models analysing the effects of laying date, clutch size, breeding population, year and diurnal partial incubation on the duration of diurnal full incubation period and the extent of hatching asynchrony (ratio between the smallest and the heaviest hatchling) respectively. The hatching asynchrony model was built using hatching date instead of the laying date, and the addition diurnal partial and full incubation duration before clutch completion. The categorical variables year and population were assessed in comparison to the reference year 2015 and the reference population Pina. Significant effects are highlighted in bold.

	Estimate	**se**	***t*** [a]	***P***
Diurnal full incubation duration				
$R^2 = 0.19$				
Intercept	12.8	0.47	27.213	
Laying date	-0.1	0.18	-0.529	0.599
Clutch size	0.1	0.13	0.721	0.474
Quintos	-0.8	0.47	-1.733	0.088
Sagunto	-0.9	0.51	-1.794	0.077
Year 2016	0.2	0.26	0.929	0.357
Year 2017	-0.1	0.26	-0.366	0.720
Diurnal partial incubation duration	**-0.4**	**0.13**	**-3.289**	**0.002**
Hatching asynchrony				
$R^2 = 0.42$				
Intercept	0.5	0.31	1.568	
Hatching date	-0.2	0.13	-1.534	0.125
Clutch size	-0.1	0.09	-0.860	0.390
Quintos	-0.5	0.31	-1.590	0.112
Sagunto	**0.1**	**0.02**	**0.113**	**0.040**
Year 2016	0.2	0.17	1.187	0.235
Year 2017	0.1	0.18	0.822	0.411
Diurnal partial incubation duration	**-0.3**	**0.08**	**-3.232**	**0.001**
Diurnal full incubation duration	**-0.2**	**0.07**	**-2.606**	**0.009**

[a] For the hatching asynchrony model we provided *z-values*.

None of the breeding females displayed diurnal incubation before the fourth egg day, or nocturnal full incubation before the third egg day. We have mentioned that the onset of nocturnal full incubation was delayed in larger clutches, and we observed the same in the diurnal partial incubation onset. The association with clutch size may be explained to some extent by the close relationship between both diurnal and nocturnal behaviours. Exploratory analysis showed that the onset of both behaviours is positively correlated ($r = 0.64$, $P < 0.001$); that is, females initiated nocturnal full incubation on average the same evening after they began to display diurnal partial incubation (0.1 ± 1.67 days, range = (-5) – 7, $n = 71$). Although larger clutch sizes delayed both diurnal partial and nocturnal full incubation behaviour, we did not find a delaying effect of clutch size on the onset of diurnal full incubation. This lack of effect is in line with most previous studies (Nilsson & Svensson 1993a, Matthysen *et al.* 2011), including those where the onset of incubation was measured relative to the clutch completion date, which did not find an effect of clutch size on the onset of incubation (Ardia *et al.* 2006, García-Navas & Sanz 2011).

The fact that only two of 77 females skipped diurnal partial incubation strongly suggests that it may be a necessary precursor to full, stable incubation behaviour. As for nocturnal partial incubation, female body condition may be a key determinant of diurnal partial incubation. For example, experimental approaches have shown that females provided with extra food during the laying period started to incubate earlier (Nilsson 1993, Nilsson & Svensson 1993a, Eikenaar *et al.* 2003, Gilby *et al.* 2013). Physiological factors may contribute as well to limit incubation behaviour: hormones such as prolactin increase gradually while laying eggs (reviewed in Vleck 2002, Marasco & Spencer 2015), and the brood patch develops throughout the laying period (e.g., Haftorn 1981, Massaro *et al.* 2006, Barrionuevo & Frere 2016). Our results also showed that the later in the laying sequence the diurnal partial incubation started, the shorter it was, suggesting that females are able to speed up incubation behaviour but later in the laying sequence.

Consequences of early incubation behaviour

Diurnal incubation behaviour during the egg laying period is known to shorten the (full) incubation period (Stenning 1996, Ardia *et al.* 2006, García-Navas & Sanz 2011, Lord *et al.* 2011, Vedder 2012, Aldredge 2017, Diez-Méndez *et al.* 2020, but see Podlas & Richner 2013a). There is also some evidence that nocturnal behaviour might contribute to shortening that period (Lord *et al.* 2011). Our results showed that, among the considered incubation stages, shortening the full incubation period could be only attributed to a longer diurnal partial incubation. Starting full incubation earlier than clutch completion was not associated with the duration of full incubation. We have previously discussed that females advanced diurnal partial incubation at increasing temperatures, and the earlier into the laying sequence they started, the longer it lasted. In this scenario, females would indirectly shorten the full incubation period at increasing temperatures, which contributes to advanced hatching dates and hatching success (Nord & Nilsson 2012, Macdonald *et al.* 2013, Diez-Méndez *et al.* 2020) but also entails higher hatching asynchrony (see below).

Although we did not detect a direct association of clutch size with full incubation duration, larger clutches would indirectly cause the opposite effect from increasing ambient temperature: lengthening the full incubation period by delaying, and then shortening, diurnal partial incubation. The role of clutch size in the duration of diurnal full incubation period is still controversial. In line with our findings, females may have difficulties incubating larger clutches, resulting in eggs reaching lower temperatures during full incubation, and thus lengthening the period (Nord & Nilsson 2012). On the contrary, larger clutches show higher thermal inertia, cooling down more slowly when females leave the nest (Boulton & Cassey 2012) and facilitating higher intensity of incubation and shortening the full incubation period (Cooper *et al.* 2005). Positive and negative effects of clutch size may cancel each other resulting in the lack of effect found in this study.

Incubation behaviour before the clutch is fully laid causes hatching asynchrony (Nilsson & Svensson 1993a, Ardia *et al.* 2006, Johnson *et al.* 2013, but see Wang & Beissinger 2009, Podlas & Richner 2013a). We found that this effect was mainly due to females displaying diurnal partial incubation before completing the clutch. Diurnal full incubation before clutch completion also contributed to hatching asynchrony, but females displayed this behaviour in only 11 of 77 nests. We did not find any association between nocturnal incubation behaviour and hatching asynchrony, even though nocturnal partial incubation lasted twice to three times longer than diurnal partial incubation and mostly started immediately after the first egg was laid. Both partial and full nocturnal incubation could exceed physiological zero temperature (26–28 ^{o}C, (Drent 1975)) and reach incubation temperatures (36–38 ^{o}C, Haftorn 1978, 1979, 1981, Lord *et al.* 2011, Vedder 2012, Podlas & Richner 2013a, Simmonds *et al.* 2017; but see Pendlebury & Bryant 2005), potentially affecting embryo development. However, we did not detect any clear effect of these nocturnal periods on hatching asynchrony.

Breeding females only reacted to increasing temperatures by advancing diurnal partial incubation into the laying period, which indirectly increased the degree of hatching asynchrony (see also Naef-Daenzer *et al.* 2004, Ardia *et al.* 2010, Johnson *et al.* 2013). Larger clutches, however, seemed to deter earlier onsets of incubation. In a scenario of uneven warmer springs, breeding females may follow different strategies in different populations. Assessing how phenological changes in clutch size, incubation behaviour and hatching spreads occur in each population may help to understand avian responses to climate change.

Chapter 2

Chapter 2

Deconstructing incubation behaviour in response to ambient temperature over different timescales

Original manuscript:

Diez-Méndez D, Cooper C, Sanz JJ, Verdejo J & Barba E. Deconstructing incubation behaviour in response to ambient temperature over different timescales. Submitted.

Data are available from the Dryad Digital Repository:
https://doi.org/10.5061/dryad.5qfttdz4h

ABSTRACT

Avian embryos need a stable thermal environment to develop optimally, while incubating females need to allocate time to self-maintenance off the nest. In species with female-only incubation, eggs are exposed to ambient temperatures that usually cool them down during female absences. The lower the ambient temperature the sooner females should return to re-warm the eggs. When incubation constraints ease at increasing ambient temperatures, females respond by increasing either incubation effort or self-maintenance time. These responses are population-dependent even within the same species; but it is uncertain whether they are caused by local environmental conditions or they are an artefact from limited datasets, different methodological approaches or the timescale over which incubation behaviour is measured. In this study, we collected incubation data from three Mediterranean Great Tit *Parus major* populations during three consecutive years. We measured the duration of each off- and on-bout event, used these variables to compute nest attentiveness at three different timescales (full incubation, daily, and hourly periods) and assessed the impact of ambient temperature on bout duration and nest attentiveness. We found that females maximized on-bout duration at different local temperatures, ranging from 10 to 20 ºC; but lengthened off-bouts linearly across a range of 0–38 ºC in all three populations. These local differences translated into opposite linear nest attentiveness patterns at the full incubation scale: Females increased either incubation effort, longest on-bouts between 15–20 ºC, or self-maintenance time, longest on-bouts at 10 ºC. It was at daily and hourly periods when we detected non-linear nest attentiveness patterns, as expected from on-bout duration, peaking at different local ambient temperatures. Females first increased incubation effort up to a certain temperature value and then increased self-maintenance time at the highest ambient temperatures. Further research is needed to understand which factors are behind the turning points from one behaviour to the other.

Keywords: Incubation bouts, incubation duration, nest attentiveness, songbird, *Parus major*.

INTRODUCTION

Incubation is an energetically demanding period of avian reproduction, especially for species with small body sizes, in which energy expenditure could reach, and exceed, chick rearing values (Williams 1996, Nord & Williams 2015). Embryos need an optimal thermal environment (36–40.5 ºC) to develop adequately (Drent 1975, Nord & Nilsson 2011, DuRant *et al.* 2013), and even small but persistent deviations of ~2–3 ºC might cause short- (Hepp *et al.* 2006, Nord & Nilsson 2011, Ospina *et al.* 2018) and long-term effects on nestling condition (Berntsen & Bech 2016; reviewed in DuRant *et al.* 2013). Daytime intermittent incubation behaviour is a way of dealing with high-energy expenditure when only one member of the pair incubates, by optimally allocating time between incubation bouts (on-bouts) and self-maintenance time off of the nest (off-bouts). In temperate habitats, intermittent incubation causes inevitable cooling periods during off-bouts, thereby females should adjust their duration to prevent egg temperature from dropping below 24–26 ºC, the physiological zero temperature (PZT), when embryo development is suspended (Drent 1975, Haftorn 1988).

Incubation constraints alleviate with increasing temperatures: female's energy stores deplete slowly and unattended eggs approach PZT at a slower rate, allowing females to lengthen on-bouts (Reid *et al.* 1999, Ardia *et al.* 2009) and off-bouts respectively (Conway & Martin 2000a). Since on-bouts usually lengthen more than off-bouts, females increase their overall time spent incubating (i.e., nest attentiveness) (Conway & Martin 2000a, Cooper & Voss 2013). The increase in nest attentiveness might also be achieved by just lengthening on-bouts (Morton & Pereyra 1985, Kovařík *et al.* 2009, MacDonald *et al.* 2014, Amininasab *et al.* 2016), or shortening off-bouts (Ardia *et al.* 2009, Schöll *et al.* 2019) at increasing temperatures. When ambient temperature reaches PZT, both on- and off-bouts would reach their maximum duration and the positive relationship with ambient temperature would cease, resulting in an overall non-linear effect (Conway & Martin 2000a). Increasing incubation effort when constraints alleviate provides advantages by shortening the incubation period (Hepp *et al.* 2006, Nord & Nilsson 2011, Coe *et al.* 2015), which is associated with a reduction of predation risk (Clark & Wilson 1981, Conway & Martin 2000b), and an increase in hatching success (Nord & Nilsson 2011, Macdonald *et al.* 2013, Diez-Méndez *et al.* 2020).

Despite the reproductive benefits of increasing incubation effort, females might prioritize self-maintenance (e.g., preening, foraging or avoidance of nest predators) at increasing ambient temperatures, by having longer (Walters *et al.* 2016, Capp *et al.* 2018), or more frequent off-bouts (MacDonald *et al.* 2014). Either investing in incubation or self-maintenance time is not species-specific but population dependent, since both behavioural responses have been reported within the same species (e.g., Great Tits *Parus major*: Bueno-

Enciso *et al.* 2017, Schöll *et al.* 2019; Blue Tits *Cyanistes caeruleus*: Bueno-Enciso *et al.* 2017, Bambini *et al.* 2019). Reported increases in self-maintenance behaviour might be related to exceptional environmental conditions (near freezing temperatures in alpine habitats, MacDonald *et al.* 2014) or harsh habitats (arid zones, Capp *et al.* 2018).

Apart from the potential effects of ambient temperature, bout duration has been shown to vary with time of day (Conway & Martin 2000a) and vary throughout the incubation period (Cooper & Voss 2013). Energetic needs push to shorter bouts early and late in the day, reaching a maximum by midday (Conway & Martin 2000a). However the opposite relationship, with minimum bout duration around midday, has been also reported (Camfield & Martin 2009, Kovařík *et al.* 2009), and some studies have even found a linear decrease of nest attentiveness throughout the day (Walters *et al.* 2016, Bambini *et al.* 2019). On the other hand, embryo development entails increasing egg-cooling rates, causing a linear decrease of bout duration along the incubation period (Cooper & Voss 2013). Given the potential interaction between ambient temperature and temporal variables, the actual pattern of bout duration would depend on the timescale considered. Thus, if nest attentiveness is measured in an hourly basis, it would be under the non-linear influence of time of the day (Weathers & Sullivan 1989, Kovařík *et al.* 2009, MacDonald *et al.* 2014) and temperature (Conway & Martin 2000a), and the linear effect of incubation day (Reid *et al.* 1999, Stein *et al.* 2010) (Figure 1a). On the other hand, if it is measured on a daily basis, only the non-linear effect of temperature (Bambini *et al.* 2019; but see Simmonds *et al.* 2017, Schöll *et al.* 2019 for a linear effect) and the linear effect of day of incubation would affect it (Figure 1b). Finally, if it is measured considering the full incubation period, only the linear effects of ambient temperature would prevail (Figure 1c).

Given the temporal dynamism of incubation behaviour, when and for how long incubation behaviour is measured could severely affect nest attentiveness assessments. Most studies to date have recorded incubation behaviour for only a few days (Álvarez & Barba 2014a, Amininasab *et al.* 2016, Schöll *et al.* 2019), just one day (Kovařík *et al.* 2009, Amininasab *et al.* 2017a, Bueno-Enciso *et al.* 2017), or even a few hours per day (Weathers & Sullivan 1989, Hatchwell *et al.* 1999, Capp *et al.* 2018). This limitation may have driven previous studies to under-detect effects on nest attentiveness (e.g., Álvarez & Barba 2014a, Amininasab *et al.* 2016). This opens the question of whether the different responses to ambient temperature among populations of the same species (i.e., investing in incubation or self-maintenance with increasing temperatures) are, at least partially, a consequence of the limited datasets and different timescales of the measurements, or they are truly two different responses to contrasting local temperatures.

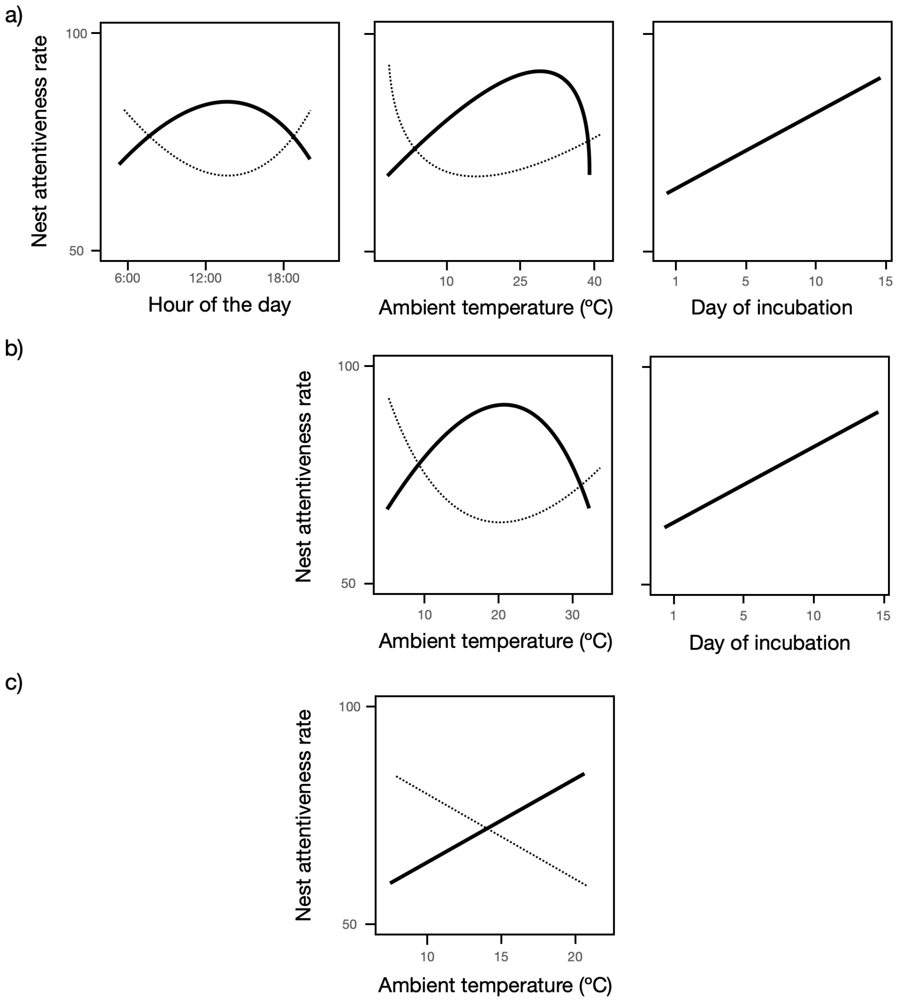


Figure 1. Schematic representation of how nest attentiveness patterns would vary with hour of the day, ambient temperature and incubation day over different timescales. a) represents nest attentiveness rate measured in an hour basis, b) nest attentiveness rate measured daily and, c) nest attentiveness rate considering the full incubation period, Solid lines represent the expected main effect, and dash lines show alternative effects also described in the literature.

Other important breeding variables such as clutch size and nest volume, along with calendar date, could also affect nest attentiveness, but in a similar way across timescales. For example, eggs cool slower in large clutches (Boulton & Cassey 2012, Cooper & Voss 2013) and in better-insulated nests (Hilton *et al.* 2004, reviewed in Deeming & Mainwaring 2015). Thus, females incubating larger clutches, and/or in bigger nests (as a proxy for nest insulation capacity, Bueno-Enciso *et al.* 2017), might be able to re-allocate time to increase incubation effort, since they decrease their energy consumption. However, to date, incubating larger clutches (Cooper & Voss 2013) and building better insulated nests (Grubbauer & Hoi 1996, Deeming & Gray 2016) have only been reported to lengthen off-bouts (i.e., increasing self-maintenance time). Finally, females increase incubation effort later in the breeding season, independently of the effect of ambient temperature (Ardia *et al.* 2006, Bueno-Enciso *et al.* 2017).

In this study, we investigated whether behavioural responses to ambient temperature could be generalized across populations and timescales by using a unified methodology and coherent variable effects. For this, we collected incubation data during three years in three different breeding populations of a monogamous, female only-incubator passerine, the Great Tit, for which a variety of incubation behaviours has been reported (Álvarez & Barba 2014a, Bueno-Enciso *et al.* 2017, Schöll *et al.* 2019). We explored (1) how on- and off-bout duration was associated with ambient temperature, time of day, and day of incubation; and (2) the influence of the timescale (full incubation, daily, and hourly periods) on the interpretation of nest attentiveness. We also investigated (3) how ambient temperature was associated with duration of the active day, and (4) how nest attentiveness values could predict the duration of the full incubation period. Finally, we assessed (5) the effect of relevant breeding parameters (clutch size, nest volume, calendar date) on active day duration, and hourly, daily and full incubation nest attentiveness.

METHODS

Study areas and general field procedures

Breeding data of Great Tits were collected as part of long-term studies in three Mediterranean study areas in Spain: Sagunto, Quintos and Pina. Sagunto study area (39.70° N, 0.25° W, 30 m asl) is part of an extensive orange-tree *Citrus aurantium* intensive monoculture (e.g., Rodríguez *et al.* 2016b). Quintos (39.73° N, 3.98° W, 900 m asl) is found at the Quintos de Mora National Wildlife Reserve and has forest patches that are dominated by Pyrenean Oaks *Quercus pyrenaica*, and secondarily by Portuguese Oaks *Quercus faginea* (e.g., Barrientos *et al.* 2015). Pina (40.02° N, 0.63° W, 1200 m asl) is a mountain area containing a mixed forest dominated by Maritime Pines *Pinus pinaster* with Portuguese Oaks scattered within (Alambiaga *et al.* 2020). Only first clutches produced during three consecutive years (2015–2017) were included in this study. During these three years, Quintos registered the lowest mean ambient temperature during the incubation period of each breeding female (13.3 ± 1.31 ºC, range = 10.4–15.5 ºC), followed by Pina (14.9 ± 2.05 ºC, range = 10.1–17.1 ºC) and Sagunto (17.4 ± 1.29 ºC, range = 15.2–19.7 ºC). Nestboxes present in the study areas were made of pinewood, with a squared bottom panel surface of approx. 149.5 cm^2. All the nestboxes present in Quintos and Pina, 200 per site, were used for the present study, while only part of a larger area in Sagunto, containing about 200 nestboxes, was used here.

We checked all the nestboxes weekly starting in mid-March. We increased the frequency of the visits to those nestboxes where nest building started, until we detected the first laid egg. At the day when the fifth egg was expected to be laid (assuming the laying of one egg per day) in a particular nest, we started daily visits to detect the start of full incubation (i.e., the observation of an incubating female during the daytime or warm eggs in the absence of the female). This observed onset was only used to estimate hatching dates, and a more accurate onset was estimated later based on nest attentiveness (see below). We visited the nest 4–5 days after incubation was detected to confirm clutch sizes. Eleven days from the initiation of incubation, we visited the nest twice per day until hatching, to accurately accurately quantify hatching date and confirm the end of the incubation period. While assessing the hatching date, we measured the height of the front wall of the nest with a calliper (to the nearest 0.1 cm), and then multiplied it by the area of the bottom panel of the nestbox as a surrogate of nest volume (e.g., Bueno-Enciso *et al.* 2017).

Incubation temperature collection

The incubation behaviour of Great Tits was assessed from estimated nest-cup temperatures recorded in each nest during the incubation period. We collected incubation data from the laying date until the hatching date, except for Quintos and Sagunto in 2015, when we were only able to collect incubation data up to six days after the onset of incubation. We used thermocouple data loggers (HOBO UX100-014M Single Channel, Onset Computer Corporation, USA) connected to a Type T beaded Thermocouple probe (30 wire gauge, accuracy ±1.0°C; Onset Computer Corporation, USA) that recorded temperature every 10 s. The probe was fixed in the centre of the clutch. We used a raw probe during the first two breeding seasons (2015-2016). During 2017, the probe was inserted into a plastic crafted egg (Factory Direct Craft Supply, USA) filled with wire-pulling lubricant (Clear Glide, Ideal industries, USA) similar in shape and colour to Great Tit eggs but somewhat smaller (15.9 x 11.5 mm vs. 18.1 x 13.3 mm; average egg size from Sagunto; Encabo *et al.* 2001). The shift in methodology was caused by the low rate of high-quality incubation data obtained from the raw probes (i.e., number of nests available for incubation analysis/number of nests where thermocouples were placed). Probes could be easily pushed aside the clutch, to the nest-cup periphery, or buried by the incubating females. The latter fake-egg methodology has been proven to be more successful (Ardia *et al.* 2006, 2010, Cooper & Voss 2013). The change in methodology did not affect collected incubation data because we focused in detecting temperature changes, drops during off-bouts and rebounds when females return to the nest, instead of absolute temperature values. Moreover, we did not detect any negative effect on clutch size caused by the use of fake eggs (see Appendix 2 Table S1). Additionally, we fixed a Thermochron iButton data logger (accuracy ±0.5°C, Model DS1922L-F5, Maxim Integrated) to the upper inner area of a nestbox wall to collect local ambient temperature every 520 s. When an iButton data logger was removed by a breeding bird, we used the ambient temperature data from the closest nestbox.

Incubation behaviour data

We used the software Rhythm (Cooper & Mills 2005) to delimit off-bouts, the criteria being to have a minimum duration of 2 minutes and a minimum drop in temperature of 2.0 ^{o}C. We visualized Rhythm output in Raven software (Cornell Laboratory of Ornithology) and modified it if we detected any misidentification or off-bouts that did not meet the criteria.

We collected temperature data during the incubation period in 74 nests from Sagunto, 56 from Quintos and 25 from Pina. Some nests were abandoned (six in Sagunto, two in Quintos and two in Pina), preyed upon (two in Sagunto, and one in each Quintos and Pina), or deserted due to human activities nearby (one in Sagunto). Another nine nests, three from each population, were discarded from the analyses because we did not measure nest height. We also excluded nests that showed low-quality data, in which we could not discriminate off- and on-bouts properly because probes were simply registering ambient temperature after being buried or displaced by the females as previously mentioned. Overall, 69 nests were available for statistical analyses: 36, 19, and 14 from Sagunto, Quintos and Pina respectively.

Following Cresswell and McCleery (2003) and Simmonds *et al.* (2017), we established the onset of full incubation the day that nest attentiveness reached 50 % of the active day for the first time. The full incubation period was considered to last between the day full incubation started (incubation day 1) and the day before hatching date. Sunrise and sunset times for each breeding population were obtained from www.timeanddate.com. Nest attentiveness is a composite variable that can be defined as the proportion of active time (i.e., the lapse between the first off-bout in the morning and the last on-bout in the evening) that a female spends incubating (Skutch 1962). For analytical reasons nest attentiveness units were adjusted to model requirements at each analysed timescale (see below). For calendar dates, we considered 1st of April as day 1. Descriptive statistics of main breeding and incubation parameters at the population level are provided in Table 1.

Table 1. Descriptive statistics of breeding and incubation parameters at population level. Median values are provided in brackets in the mean ± sd column.

	Pina		Quintos		Sagunto	
	mean ± sd	**range**	**mean ± sd**	**range**	**mean ± sd**	**range**
Breeding parameters [a]						
Clutch size	6.9 ± 0.73	5–8	9.1 ± 1.56	6–12	8.4 ± 1.05	6–10
Full incubation onset (date)	39.4 ± 7.06	26–47	23.6 ± 5.33	17–35	17.1 ± 4.74	6–30
Partial incubation length (min)	704 ± 280	297–1333	333 ± 226	0–933	382 ± 180	0–710
Nest volume (cm3)	754 ± 168	588–1244	710 ± 254	375–1241	730 ± 91	568–1027
Incubation parameters						
Duration off-bouts (min) [b]	(10.6) 12.9 ± 10.9	0.9–117	(8.9) 10.6 ± 8.17	0.6–113	(9.7) 11.2 ± 8.11	1.2–119
Duration on-bouts (min) [c]	(30.0) 34.7 ± 22.7	0.5–266	(29.3) 34.1 ± 22.0	0.5–268	(27.4) 31.7 ± 19.7	1.1–226
Daily off-bout number [d]	(15) 15.9 ± 5.91	3–32	(17) 17.3 ± 5.46	3–36	(18) 17.5 ± 4.55	2–32
Daily active day (min) [e]	787 ± 43.4	678–880	753 ± 35.3	663–829	736 ± 37.4	575–847
Daylight (min) [e]	863 ± 15.6	821–888	828 ± 14.6	798–864	811 ± 12.8	773–841
First off-bout (min after sunrise) [e]	(16) 17 ± 13.5	(-39.4)–59.2	(18) 22 ± 19.8	(-22.2)–103	(10) 13 ± 18.7	(-64)–109
Last on-bout (min to sunset) [e]	(54) 60 ± 31.5	151– (-2)	(52) 53 ± 29.1	148– (-11)	(60) 62 ± 29.6	207—10)

[a] Pina $n = 14$, Quintos $n = 19$, Sagunto $n = 36$

[b] Pina $n = 2696$, Quintos $n = 3360$, Sagunto $n = 5651$.

[d] Pina $n = 2571$, Quintos $n = 3175$, Sagunto $n = 5349$.

[d] Pina $n = 170$, Quintos $n = 194$, Sagunto $n = 323$.

[e] Pina $n = 171$, Quintos $n = 193$, Sagunto $n = 324$

Statistical analyses

We conducted statistical analyses using R software 3.6.1 (R Core Team 2019). We established three timescale levels (see Bambini et al. 2019 for a similar structuration) to evaluate nest attentiveness in relation to ambient temperature and breeding parameters: (1) hourly, (2) daily and (3) full incubation period. For clarity, we grouped the rest of the incubation variables of interest in each level depending on their measured timescale: duration of the full incubation period in level 3, and duration of the active day and number of off-bouts in level 2. Additionally, on- and off-bout duration as single-event variables were grouped within the hourly level. To test whether the response of each variable to ambient temperature was population dependent we built a set of two models for each independent variable, containing the interaction between ambient temperature and breeding population or not, and applied a model selection approach. We used Akaike's Information Criterion (AIC, following Burnham and Anderson (2002) via the MuMIn package (Bartón 2015) to find the model that best explained our data (ΔAICc > 2.0, see Tables S2-S4 for model selection results).

For the first level, analysing on- and off- bout duration, we selected clutch size, incubation day, hour of the day (quadratic term via "poly-" function), ambient temperature (quadratic term via "poly-" function), nest volume (cm^3), calendar date, breeding population, and year as explanatory variables, being the last two categorical variables. Hour variable was the exact time at which the event started, and ambient temperature refers to when the off-bout event started. The "poly-" function calculates orthogonal polynomials and avoids correlations between linear and quadratic terms. We built linear mixed-effects models (LMMs) for on- and off-bout duration (in minutes and log transformed because of right skewness in model residuals) and added nest identity as a random factor. Data exploration showed a non-independent temporal structure of residuals (following Zuur *et al.* 2009 pp. 146–147), and we therefore added a temporal autocorrelation structure with an autoregressive-moving average to deal with it (Pinheiro & Bates 2000, package nlme). Off-bouts longer than 60 min have been considered to be outliers in previous studies, even though they account up to 10 % of the recesses (Bueno-Enciso *et al.* 2017, Bambini *et al.* 2019). We decided to include off-bouts of up to 120 min (~98.5 % off-bouts recorded in this study) and on-bouts up to 360 min, three times the longest off-bout (see results).

Similar LMMs were built for hourly nest attentiveness. For these models, ambient temperature was considered as mean value per hour (quadratic term using "poly-" function). Hour of the day was restricted from 06:00 to 21:00 (quadratic term via "poly-" function) when most female activity happened (except for extremely early off-bouts, see below). We also detected temporal autocorrelation and models were built accordingly using the nlme package as described before. When adding a variance structure into a model, the response variable needs to be continuous. We therefore needed to consider hourly nest attentiveness as a continuous variable, defining it as the summed on-bout duration (minutes) within an hour, and cubed it to reduce strong left skewness in the residuals.

For the second level of analysis, that covered daily periods, we assessed the effect of clutch size, incubation day (1 = incubation onset day), daily mean ambient temperature (quadratic term of the mean temperature measured from sunrise to sunset, using the "poly-" function), date, nest volume, breeding population, and year on daily nest attentiveness. We built LMMs and added nest identity as a random factor. Data exploration again showed a non-independent temporal structure of residuals and models were built accordingly. We needed to define daily nest attentiveness as the sum of daily on-bout duration (hours). Since duration of the active day may affect nest attentiveness hours by definition, we added daily active day duration (hours) as an explanatory variable to control for the duration of the on-bouts. Lastly, daily nest attentiveness was cubed in order to reduce left skewness in model residuals.

At the same daily level, we built LMMs for the daily number of off-bouts and the daily duration of the active day. Model structure, random and explanatory variables were the same as described for daily nest attentiveness. For the active day model, we used daylight duration (hours) instead of date as it better correlates with active day duration. We discarded 9 data points (out of 695) when active day was more than 120 min longer than the daylight period, which originated from extreme early first off-bouts in the morning (i.e., several hours prior to sunrise).

For the third level of analysis, that covered the full incubation period, nest attentiveness was best considered as the summed total hours of on-bouts vs. summed total hours of off-bouts, to fit as a response variable in generalized linear models (GLMs, lme4 package) with a logit link (binomial family). Nest volume, clutch size, calendar date when full incubation started, mean ambient temperature for the period at each nest, breeding population, and year were the explanatory variables. Mean temperature was calculated from sunset before incubation day 1 to sunset of the day before hatching.

To investigate the duration of the full incubation period (in days) we created linear models (LMs) using an identity link (Gaussian family). Clutch size, date when full incubation started, nest volume, breeding population and year were selected as explanatory variables. To assess the relative contribution of incubation behaviour to the full incubation period, we added the nest attentiveness rate for the overall period, and considered two additional behaviours: (1) partial incubation behaviour (i.e. sum of minutes of diurnal incubation before the onset of full incubation behaviour) because it has been previously reported that the longer the partial incubation the shorter the full incubation period (Ricklefs 1993, Wang & Beissinger 2011, Diez-Méndez *et al.* 2021) and; (2) the number of days that full incubation started before clutch completion, because if females keep laying eggs may lengthen the full incubation period (Diez-Méndez *et al.* 2020). Since nest attentiveness correlates with ambient temperature (see results), the latter was not included in these models. In this case, the different combinations of incubation behaviours were subject to model selection, choosing the model that best explained our data.

Because we found residuals showing high leverage values or outliers, we corrected the final GLM from nest attentiveness and LM from full incubation period duration using the *Robustbase* package (*lmrob* function, Maechler *et al.* 2015) and recalculating model estimates and standard errors in order to weigh down their influence.

We analysed final selected models, reported their R^2 values, and considered p-values lower than 0.05 as significant. Numerical predictor variables were scaled by subtracting the mean and dividing by the standard deviation. We visually inspected residuals of the response variable to check for normality and homogeneity of the variance. As previously mentioned, we found left skewness in daily nest attentiveness model residuals and right skewness in off- and on-bout duration model residuals. These variables were cubed or log transformed respectively. We have reported mean ± sd unless otherwise stated.

RESULTS

Bout duration

On-bouts lasted on average about three times more than off-bouts. A median off-bout lasted 9.6 minutes (11.4 ± 8.88, range = 0.6-118.8, n = 11707) compared to a median on-bout of 28.3 minutes (33.1 ± 21.13, range = 0.5-268.4, n = 11095) (see Table 1 for population level values).

Females lengthened off-bouts linearly with increasing ambient temperatures throughout the full range of recorded temperatures (0–38 ºC), although it happened at a slower pace in Sagunto than in Pina and Quintos (Table 2, Fig. 2a). Hour of the day caused a positive quadratic effect on off-bout duration: females shortened off-bouts until reaching a minimum duration in the afternoon (approx. between 13:00h and 17:00h) followed by a later lengthening (Table 2, Fig. S1). Females shortened off-bouts as incubation progressed, i.e., as hatching date was getting closer (Table 2). Lastly, females that had built larger nests had longer off-bouts (Table 2).

Table 2. Results of linear mixed-effect models analysing the effects of the incubation day, hour of the day, clutch size, hourly mean ambient temperature, breeding population, year, nest volume and date on hourly nest attentiveness and the duration of on- and off-bouts. Nest identity was added as a random factor. The categorical variables year and population were assessed in comparison to the reference year 2015 and the population Pina. Significant results are highlighted in bold.

	Estimate	**se**	***t***	***P***
Hourly nest attentiveness				
($R^2_{\text{fixed factors}} = 0.33$, $R^2_{\text{model}} = 0.37$)				
Intercept	12.35	0.748	16.51	
Incubation day	**0.92**	**0.145**	**6.36**	**<0.001**
Hour	**65.43**	**7.259**	**9.01**	**<0.001**
Hour^2	**338.12**	**8.500**	**39.78**	**<0.001**
Clutch size	0.01	0.224	0.05	0.961
Temperature	**-35.09**	**14.515**	**-2.42**	**0.016**
Temperature^2	**-83.43**	**11.191**	**-7.45**	**<0.001**
Quintos	-0.93	0.783	-1.19	0.238
Sagunto	**-1.87**	**0.901**	**-2.08**	**0.042**
Year 2016	0.75	0.442	1.70	0.094
Year 2017	0.58	0.479	1.22	0.228
Date	**-1.41**	**0.399**	**-3.55**	**<0.001**
Nest volume	**-0.42**	**0.186**	**-2.26**	**0.028**
Temperature x Quintos	**-84.99**	**17.691**	**-4.80**	**<0.001**
Temperature^2 x Quintos	**63.12**	**15.165**	**4.16**	**<0.001**
Temperature x Sagunto	-6.10	18.191	-0.34	0.737
Temperature^2 x Sagunto	**53.53**	**17.316**	**3.09**	**0.002**
Off-bout duration				
($R^2_{\text{fixed factors}} = 0.07$, $R^2_{\text{model}} = 0.16$)				
Intercept	2.29	0.110	20.87	
Incubation day	**-0.15**	**0.020**	**-7.84**	**<0.001**
Hour	**-2.84**	**0.833**	**-3.41**	**0.001**
Hour^2	**2.86**	**0.834**	**3.42**	**0.001**
Clutch size	0.02	0.032	0.48	0.634
Temperature	**19.61**	**1.579**	**12.42**	**<0.001**
Temperature^2	2.19	1.213	1.80	0.072
Quintos	-0.13	0.115	-1.16	0.251
Sagunto	-0.20	0.131	-1.51	0.137
Year 2016	0.11	0.063	1.78	0.080
Year 2017	0.11	0.068	1.66	0.102
Date	0.00	0.058	0.04	0.966
Nest volume	**0.08**	**0.027**	**2.79**	**0.005**
Temperature x Quintos	-0.34	1.875	-0.18	0.858
Temperature^2 x Quintos	-0.57	1.631	-0.35	0.728
Temperature x Sagunto	**-5.42**	**1.936**	**-2.80**	**0.005**
Temperature^2 x Sagunto	1.60	1.880	0.85	0.393

Table 2. Continued.

	Estimate	**se**	***t***	***P***
On-bout duration				
($R^2_{\text{fixed factors}} = 0.13$, $R^2_{\text{model}} = 0.22$)				
Intercept	3.51	0.108	32.62	
Incubation day	-0.04	0.020	-1.81	0.070
Hour	**-4.40**	**0.801**	**-5.49**	**<0.001**
Hour^2	**-5.08**	**0.846**	**-6.00**	**<0.001**
Clutch size	0.03	0.031	1.07	0.290
Temperature	**-6.89**	**1.596**	**-4.31**	**<0.001**
Temperature^2	**-5.39**	**1.227**	**-4.39**	**<0.001**
Quintos	**-0.36**	**0.113**	**-3.17**	**0.002**
Sagunto	**-0.47**	**0.129**	**-3.67**	**0.001**
Year 2016	**0.25**	**0.062**	**4.02**	**<0.001**
Year 2017	0.09	0.067	1.39	0.169
Date	**-0.15**	**0.057**	**-2.57**	**0.010**
Nest volume	-0.02	0.027	-0.79	0.428
Temperature x Quintos	**-7.20**	**1.943**	**-3.71**	**<0.001**
Temperature^2 x Quintos	-0.20	1.700	-0.12	0.904
Temperature x Sagunto	**6.87**	**2.062**	**3.33**	**0.001**
Temperture^2 x Sagunto	-0.38	1.981	-0.19	0.848

Females in Quintos shortened on-bouts at temperatures higher than 10 ºC (Table 2, Fig. 2b). Females in Pina and Sagunto displayed a clearer negative quadratic pattern, increasing on-bout duration up to approx. 15 ºC and 20 ºC respectively, followed by a later shortening (Table 2, Fig. 2b). Contrary to the effect found in off-bouts, females lengthened on-bouts reaching a maximum around midday and then shortened them again (Figure S1). Off-bouts shortened as the season progressed (Table 2). Among populations, females in Pina had longer on-bouts compared to females in Sagunto and Quintos (Table 2).

Daily bout numbers

The number of daily off-bouts, and consequently on-bouts, increased at increasing daily temperatures (Table 3). Females also increased bout number with incubation day (i.e., as the hatching date was getting closer), and later in the season (Table 3). Among populations, females in Pina had fewer off-bouts per day than females in Quintos and Sagunto (see Table 1 for population level descriptive statistics and Table 3).

Daily active day duration

Females left the nest for the first time in the morning 16.4 ± 18.24 min after sunrise (range = 64 min before to 109 min after sunrise, *n* = 688 days) and entered the nest to overnight 59.0 ± 30.20 min before sunset (range = from 207 before to 11 min after), being active on

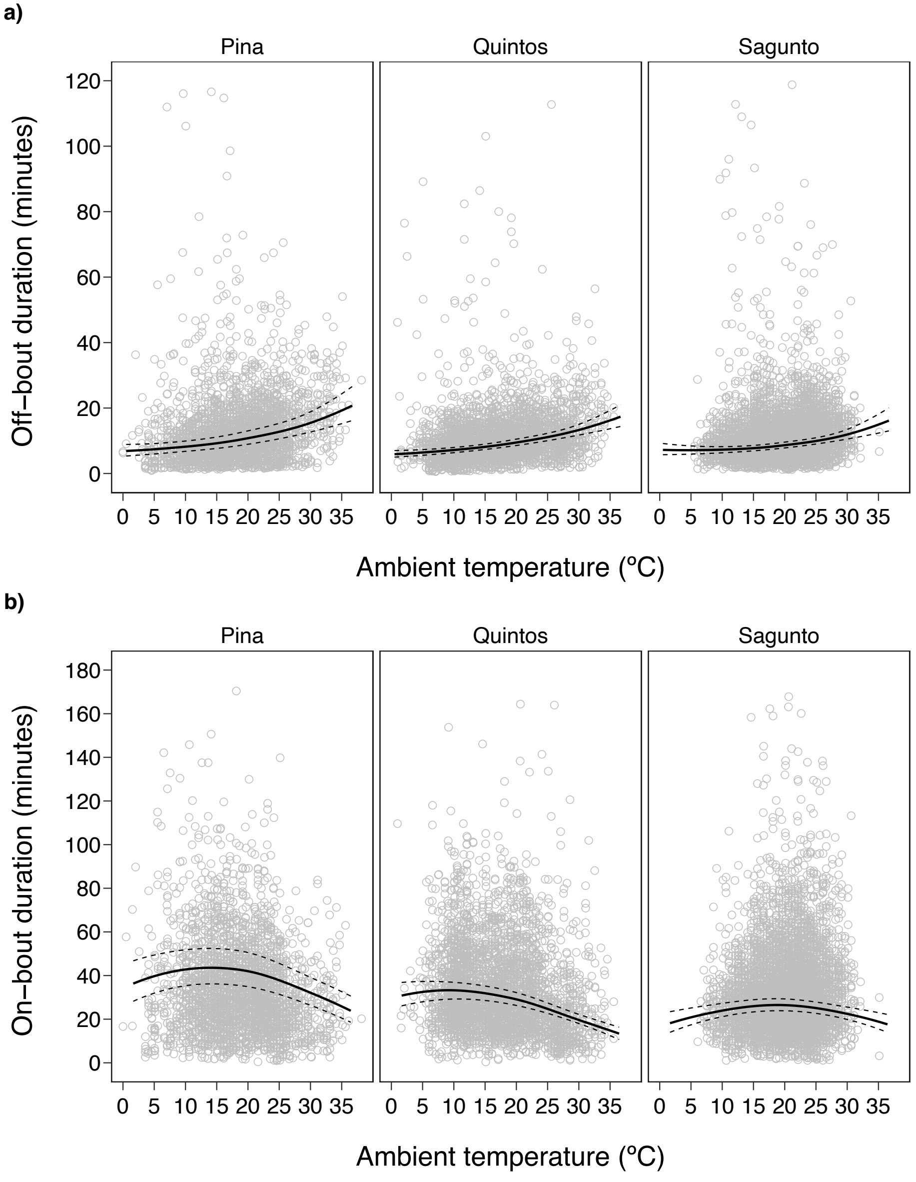


Figure 2. Variation of a) off-bout duration and b) on-bout duration in relation to ambient temperature. Dashed lines denote 95 % confidence intervals. Circles represent jittered raw data. For a better visualization, raw data of on-bouts longer than 180 min have been omitted.

average 753 ± 43.6 min per day or 12.55 hours (range = 574.8–879.8, *n* = 688 days) (see Table 1 for population level descriptive statistics).

We did not find a clear effect of ambient temperature on active day duration in Pina or Quintos (Table 3), but females breeding in Sagunto shortened active days during the coldest and warmest days of their incubation period (Table 3, Figure S2). Females shortened active days with incubation day, but lengthened them as the season progressed (i.e., as daylight duration increased) (Table 3).

Table 3. Results of linear mixed-effect models analysing the effects of incubation day, clutch size, daily mean ambient temperature, breeding population, year, nest volume and date on daily attentiveness, daily active day duration and daily number of off-bouts. Nest identity was added as a random factor. In the nest attentiveness model, we added the length of the active day as a fixed factor. In the active day model, we substituted date by daylight duration. The categorical variables year and population were assessed in comparison to the reference year 2015 and the population Pina. Significant results are highlighted in bold.

	Estimate	**se**	***t***	***P***
Daily nest attentiveness ($R^2_{\text{fixed factors}}$ = 0.32, R^2_{model} = 0.32)				
Intercept	-9.66	1.950	-4.95	
Active day	**1.36**	**0.139**	**9.84**	**<0.001**
Incubation day	**1.22**	**0.214**	**5.69**	**<0.001**
Clutch size	0.08	0.256	0.30	0.769
Temperature	**-12.85**	**5.534**	**-2.32**	**0.021**
Temperature^2	**-8.73**	**3.606**	**-2.42**	**0.016**
Quintos	-0.23	0.911	-0.25	0.804
Sagunto	-1.05	1.061	-0.99	0.325
Year 2016	0.22	0.542	0.41	0.685
Year 2017	-0.21	0.591	-0.35	0.729
Date	**-1.21**	**0.472**	**-2.56**	**0.011**
Nest Volume	-0.41	0.212	-1.94	0.057
Temperature x Quintos	**-24.17**	**7.471**	**-3.24**	**0.001**
Temperature^2 x Quintos	**12.85**	**5.803**	**2.22**	**0.027**
Temperature x Sagunto	1.91	8.368	0.23	0.819
Temperature^2 x Sagunto	-9.27	7.456	-1.24	0.214

Table 3. Continued.

	Estimate	**se**	***t***	***P***
Daily active day ($R^2_{\text{fixed factors}} = 0.34$, $R^2_{\text{model}} = 0.46$)				
Intercept	12.56	0.184	68.13	
Daylight	**0.52**	**0.099**	**5.25**	**<0.001**
Incubation day	**-0.14**	**0.042**	**-3.29**	**0.001**
Clutch size	0.04	0.056	0.75	0.458
Temperature	0.68	1.225	0.56	0.579
Temperature^2	-0.07	0.841	-0.08	0.937
Quintos	0.16	0.196	0.80	0.428
Sagunto	0.07	0.226	0.32	0.746
Year 2016	-0.20	0.1144985	-1.76	0.084
Year 2017	-0.15	0.1239146	-1.23	0.223
Nest Volume	-0.01	0.0457482	-0.18	0.860
Temperature x Quintos	0.43	1.6471994	0.26	0.794
Temperature^2 x Quintos	1.66	1.4056666	1.18	0.238
Temperature x Sagunto	**5.65**	**1.939316**	**2.91**	**0.004**
Temperature^2 x Sagunto	**-5.36**	**1.7705564**	**-3.03**	**0.003**
Daily number off-bouts ($R^2_{\text{fixed factors}} = 0.21$, $R^2_{\text{model}} = = 0.42$)				
Intercept	14.07	1.903	7.39	
Incubation day	**1.00**	**0.407**	**2.46**	**0.014**
Clutch size	-0.39	0.563	-0.70	0.488
Temperature	**32.17**	**9.047**	**3.56**	**<0.001**
Temperature^2	-4.59	5.890	-0.78	0.436
Quintos	**6.50**	**1.988**	**3.27**	**0.002**
Sagunto	**6.24**	**2.289**	**2.73**	**0.008**
Year 2016	**-3.36**	**1.133**	**-2.96**	**0.004**
Year 2017	-0.97	1.233	-0.78	0.436
Date	**2.08**	**1.008**	**2.06**	**0.040**
Nest Volume	0.05	0.467	0.12	0.908
Temperature x Quintos	7.07	12.164	0.58	0.561
Temperature^2 x Quintos	11.73	9.461	1.24	0.216
Temperature x Sagunto	3.13	13.622	0.23	0.818
Temperature^2 x Sagunto	-17.40	12.075	-1.44	0.150

Hourly nest attentiveness

Effects of ambient temperatures on hourly nest attentiveness differed among populations: Females in Sagunto and Quintos decreased nest attentiveness at increasing temperatures while females in Pina firstly showed an increase, peaking around 15 ºC, to decrease thereafter

(Table 2, Figure 3c). Hourly nest attentiveness followed a positive quadratic pattern, reaching the highest values early in the morning and late in the afternoon (Table 2, Figure 4). Hourly nest attentiveness increased with the day of incubation but decreased as the season progressed (Table 2). Females that built larger nests also displayed lower nest attentiveness (Table 2).

Daily nest attentiveness

Females in Quintos reduced daily nest attentiveness with increasing daily ambient temperatures, from a range of 8-26 ºC , (Table 3, Figure 3b). On the other hand, females showed relatively constant nest attentiveness values in days with ambient temperatures from 4ºC to approx. 20 ºC in Pina and from 12ºC to approx. 25 ºC in Sagunto, and a later decrease at higher temperatures, following a negative quadratic effect (Table 3, Figure 3b). Daily nest attentiveness increased with incubation day (i.e., the closer to hatching date the higher the nest attentiveness) and decreased as the season progressed (Table 3).

Full incubation period nest attentiveness

Females in Quintos incubated 73.6 ± 4.55 % of their active time during the incubation period (range = 66.2-83.6 %, *n* = 19 nests). Females in Pina incubated during 65.7 ± 8.25 % of their time (range 48.5-78.4 %, *n* = 14 nests) and females in Sagunto 69.8 ± 6.47 % (range = 55.1-82.6 %, *n* = 36).

Nest attentiveness differed between populations and because of the temperature (Table 4), but we will focus on the interaction between these two variables. The effect of ambient temperature on nest attentiveness differed among populations: while in Pina and Sagunto females increased nest attentiveness with increasing temperatures, females in Quintos reduced it (Table 4, Figure 3a). Females showed lower values of nest attentiveness when they started incubating later in the season (Table 4). Females that built larger nests also had lower nest attentiveness (Table 4).

Duration of the full incubation period

The incubation period was longer in Pina (12.6 ± 1.45 days, range = 10-15, *n* = 14 nests) than in Quintos (12.2 ± 0.86 days, range 10-14, *n* = 19 nests) and Sagunto (12.2 ± 0.95 days, range = 11-15, *n* = 36 nests) (Table 4). Females that displayed higher nest attentiveness and/or longer partial incubation (i.e., more minutes of partial incubation before full incubation started) shortened their full incubation period (Table 4).

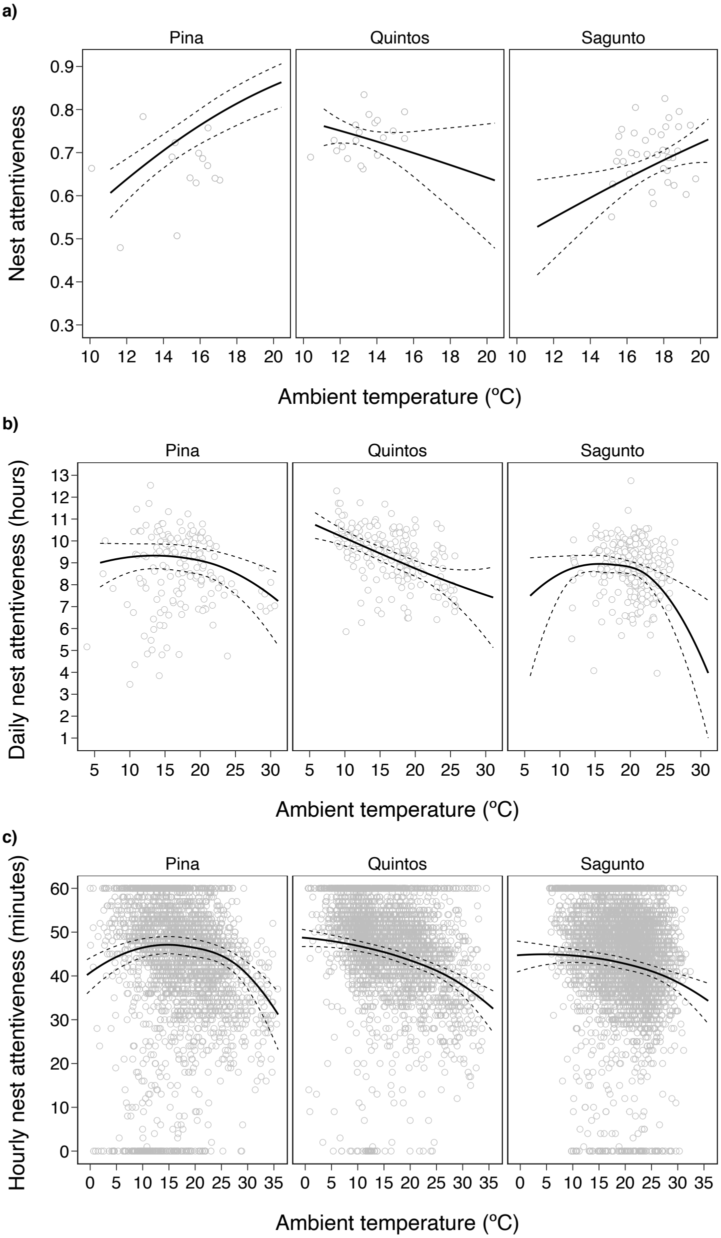


Figure 3. Modelled variation of a) nest attentiveness rate during the full incubation period, b) daily nest attentiveness and c) hourly nest attentiveness in relation to mean ambient temperatures. Dashed lines denote 95 % confidence intervals. Circles represent jittered raw data.

DISCUSSION

Incubation behavioural responses to increasing ambient temperatures differed across populations and timescales (hourly, daily and full incubation period). For nest attentiveness, each population showed either female investment in incubation effort or in self-maintenance at the full incubation scale; however, for daily and hourly nest attentiveness, both responses occurred within each population. The transition between them was associated with local ambient temperatures. Maximum on-bout duration occurred at different temperatures depending on the population, not at a fixed range as suggested in previous studies, while off-bout duration increased linearly with increasing temperatures. This combination of the longest on-bouts at population-dependent temperatures together with off-bout linear increments caused the different observed patterns of nest attentiveness across timescales. Analysed breeding factors (i.e., incubation day, date, nest volume and clutch size) showed a clear association with incubation behaviour across timescales: females increased nest attentiveness time as incubation progressed, but decreased it later in the season. Females that built larger nests displayed shorter on-bouts and therefore lower levels of nest attentiveness, but we did not find any clear association between clutch size and incubation behaviour. Lastly, higher overall nest attentiveness values (i.e., higher incubation effort) were associated with shorter full incubation periods.

Table 4. Results of a generalized linear model and the linear model analysing the effect of clutch size, full incubation onset date, mean ambient temperature, breeding population, nest volume and year on the overall nest attentiveness and the duration of the full incubation period respectively; For the latter we dropped ambient temperature and added nest attentiveness rate and partial incubation duration. The categorical variables year and population were assessed in comparison to the reference year 2015 and the population Pina. Significant results are highlighted in bold.

	Estimate	**se**	***t***	***P***
Overall nest attentiveness				
$R^2 = 0.27$				
Intercept	0.95	0.129	7.37	
Clutch size	0.00	0.034	0.06	0.952
Incubation onset date	**-0.24**	**0.059**	**-3.99**	**<0.001**
Temperature	**0.34**	**0.074**	**4.64**	**<0.001**
Quintos	-0.29	0.192	-1.54	0.125
Sagunto	**-0.56**	**0.160**	**-3.49**	**<0.001**
Year 2016	**0.25**	**0.072**	**3.44**	**0.001**
Year 2017	**0.31**	**0.094**	**3.29**	**0.001**
Nest volume	**-0.07**	**0.028**	**-2.45**	**0.014**
Temperature x Quintos	**-0.50**	**0.148**	**-3.36**	**0.001**
Temperature x Sagunto	-0.14	0.114	-1.24	0.215

Table 4. Continued.

	Estimate	se	*t*	*P*
Full incubation period duration				
$R^2 = 0.47$				
Intercept	13.29	0.547	24.30	
Clutch Size	0.05	0.117	0.47	0.641
Incubation onset date	-0.31	0.197	-1.56	0.124
Quintos	**-1.31**	**0.539**	**-2.44**	**0.018**
Sagunto	**-1.64**	**0.591**	**-2.77**	**0.008**
Year 2016	**0.58**	**0.267**	**2.17**	**0.035**
Year 2017	0.04	0.283	0.13	0.900
Nest volume	-0.17	0.105	-1.64	0.107
Nest attentiveness	**-0.39**	**0.119**	**-3.25**	**0.002**
Partial incubation duration	**-0.37**	**0.129**	**-2.86**	**0.006**

Temporal patterns of nest attentiveness

As incubation progresses, females may shorten off-bouts (Walters *et al.* 2016, Schöll *et al.* 2019) or both on- and off-bouts (Camfield & Martin 2009, Cooper & Voss 2013) to meet embryo energetic needs (Cooper & Voss 2013). It usually translates into higher daily nest attentiveness (Bueno-Enciso *et al.* 2017, Simmonds *et al.* 2017, Bambini *et al.* 2019) but many studies have failed to detect any effect at all (e.g., Conway & Martin 2000a, Álvarez & Barba 2014a, Capp *et al.* 2018) even after observing shorter bouts (Camfield & Martin 2009, MacDonald *et al.* 2014). Our results showing an increase in daily and hourly nest attentiveness agree with the premise of increasing egg-cooling rates as hatching date approaches, and it is achieved solely by shortening off-bouts. Although higher egg-cooling rates would cause faster female energy depletion and hence shorter on-bouts (Reid *et al.* 1999, Cooper & Voss 2013), females in our populations seemed to be able to cope with increasing energy expenditure, keeping a constant on-bout duration. Higher foraging efficiency facilitated by increasing preferred prey availability (Perrins 1991, Cresswell & McCleery 2003, Simmonds *et al.* 2017) during the incubation period (Shutt *et al.* 2020) could explain our results, despite shorter off-bouts. Alternatively, females could keep on-bout duration without any compensating mechanism, worsening their body condition as incubation progresses to meet embryo needs. Alongside shorter off-bouts, we found that females shortened their daily active day as incubation progressed, thus increasing their nocturnal continuous incubation time (e.g., Reid *et al.* 1999), that contributes to a net increase in incubation effort over 24h.

Females have also been reported to increase diurnal nest attentiveness as the breeding season progresses (Ardia *et al.* 2009), which would compensate for shorter nocturnal incubation periods as daylight lengthens throughout the spring (Bueno-Enciso *et al.* 2017). We found however that females shortened on-bouts, decreasing nest attentiveness along the season (see also Camfield & Martin 2009). It should be noted that this effect appeared even though our analyses were restricted to first clutches, with a range for the onset of incubation of 7-21 days depending on year and population, so the variation in daylength was relatively small. We lack adequate data to explain this decrease in incubation activity, but we should look for factors that follow within-season fast changes besides ambient temperature. As mentioned above, prey availability increases throughout the incubation period, usually increasing nest attentiveness (Rastogi *et al.* 2006, Londoño *et al.* 2008) and then unlikely explaining on-bout shortenings. Perceived nest predation risk is a factor that could decrease nest attentiveness (e.g., Ghalambor & Martin 2002, Kovařík & Pavel 2011), and might favour early breeding in Great tits (Götmark 2002). However, increased predation risk hardly could explain our results because it usually entails a reduction in bout numbers (Basso & Richner 2015), and we found the opposite effect of more numerous bouts.

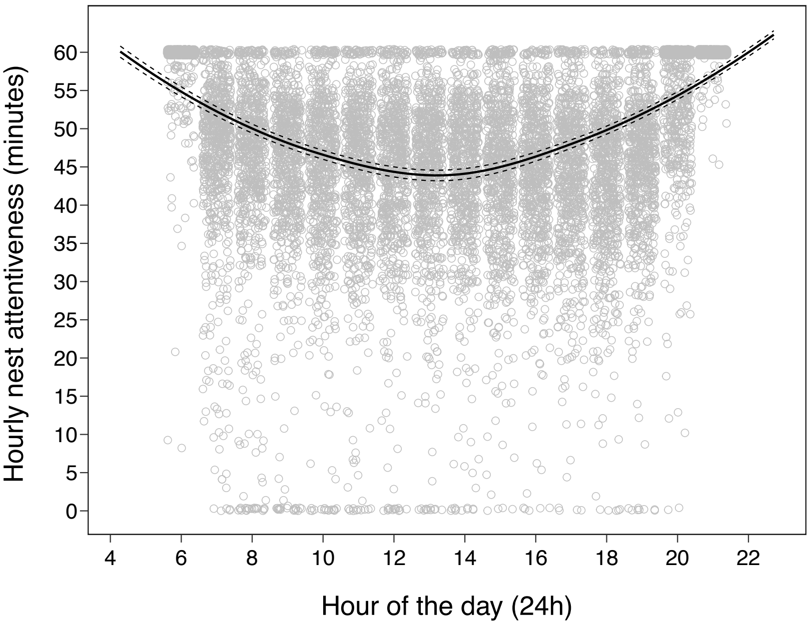


Figure 4. Modelled variation of hourly nest attentiveness in response to hour of the day. Dashed lines denote 95 % confidence intervals. Circles represent jittered raw data.

Breeding parameters and nest attentiveness

We did not detect any effect of clutch size on nest attentiveness at any timescale, agreeing with that previously found in several studies (e.g., Amininasab *et al.* 2017a, Capp *et al.* 2018, Bambini *et al.* 2019, Schöll *et al.* 2019, but see Cooper & Voss 2013). On one hand, incubating additional eggs is expected to be costly (Haftorn & Reinertsen 1985, de Heij *et al.* 2007, Nord & Nilsson 2012 but see Nord & Williams 2015) especially if females incubate below their thermoneutral zone (Nord & Williams 2015). On the other hand, larger clutches reduce egg-cooling rates (Reid *et al.* 2000a, Boulton & Cassey 2012), alleviating female incubation costs. The net balance between costs and benefits would depend on the exact number of eggs and the interaction with local ambient temperature at each nest. It is likely that the costs of incubating larger clutches are balanced out by the benefit of reduced cooling rates (see Cooper *et al.* 2005), except for experimentally modified clutches (Nord & Nilsson 2012), where the intricate relationship between clutch size, ambient temperature and female body condition would be disrupted, which would explain why this effect is rarely detected.

Previous research in one of our focal populations found that females building larger (supposedly, better insulated) nests displayed higher nest attentiveness (Quintos, Bueno-Enciso *et al.* 2017). By using complete datasets, that is at 24h of incubation data spanning the whole incubation period, we found a decreasing nest attentiveness pattern with increasing nest volume across timescales, a consequence of longer off-bouts associated with larger nests. Because better insulated nests would slow down egg cooling, females would be able to invest in self-maintenance time, lengthening off-bouts. Interspecific comparisons among passerines have also shown that larger nests lead to lower nest attentiveness (Deeming & Gray 2016). If females that build better insulated nests reduce their incubation effort, it could have positive carry-over effects in their nestlings (Reid *et al.* 2000b, Pérez *et al.* 2008) or in their own survival prospects (Reid *et al.* 2000a, de Heij *et al.* 2006). However, several studies have reported weak associations between nest size and breeding performance (e.g., Tomás *et al.* 2013, Álvarez *et al.* 2013, Lambrechts *et al.* 2016), suggesting that potential energy savings are capitalized by females, probably ending the incubation period in better physical condition.

Behavioural responses to ambient temperature

Conway and Martin (2000a) proposed twenty years ago that bout duration would follow a non-linear relationship with ambient temperature, reaching maximum values at temperatures between the PZT and the upper thermal limit (approx. 40.5 ºC Drent 1975), when females are no longer constrained . We only found a non-linear relationship regarding on-bouts, while females lengthened off-bouts with increasing temperatures throughout the full range of recorded ambient temperatures (0–38 ºC). The duration of on-bouts reached its maximum at different ambient temperatures depending on the population (see Figure 2), but females shortened them during the warmest periods in each population. This pattern would explain why we also found more numerous bouts with increasing temperatures, also in contradiction to Conway and Martin (2000a). We cannot discard that other passerine species, under different local ambient temperatures, would follow the patterns described in Conway and Martin (2000a), but our findings clearly pointed to site-specific temperature thresholds, rather than fixed ranges, to explain the turning point from increasing to decreasing incubation effort. Interestingly, the longest on-bouts happened at the most frequent temperatures in each population (10-12 ºC for Quintos, 15-17 ºC for Pina, and 20-22 ºC for Sagunto). At low temperatures it is expected that females need to leave the nest for feeding more frequently or for longer (i.e., lower nest attentiveness). At higher temperatures, on-bout shortening, and declining nest attentiveness, would be associated to reduced egg-cooling rates. As constraints alleviate females would allocate longer time to self-maintenance, not necessarily foraging, out of the nest.

Each timescale gives a different perspective of nest attentiveness variation. While overall values of nest attentiveness may be useful when assessing the duration of the incubation period (see below), daily and hourly nest attentiveness give a more accurate picture of females' responses to ambient temperature. The discussion about females investing time in incubation or self-maintenance could be reformulated into finding the turning point when one behavioural response transitions into the other, and the factors behind it. Females in Quintos maximized on-bout duration at low temperatures, which resulted in a negative relationship between ambient temperature and nest attentiveness at every timescale. In contrast, females in Pina and Quintos maximized on-bout duration at intermediate temperatures, which resulted in changing incubation patterns at daily and hourly periods, but a positive relationship between ambient temperature and nest attentiveness at the full incubation scale (see Figure 3). We recommend that future incubation studies report at least, independently of their object of study, both on- and off-bout duration, number of daily bouts, and hourly or daily nest attentiveness; and ambient temperature, incubation day and calendar date as main factors shaping these incubation variables.

How females deal with energy expenditure to reach similar on-bout duration at different temperatures, and the factors behind the different nest attentiveness shapes, are some questions raised by our results. Given the factors we measured, clutch size differed among populations (table 1) but we did not detect any association between clutch size and bout duration or nest attentiveness. It could also be expected that larger nests facilitate the observed higher nest attentiveness at lower temperatures in Quintos, but nest volume was similar among populations (see table 1). Among the non-measured factors, differential male help in feeding during incubation could boost female nest attentiveness (Matysioková & Remeš 2014), especially at lower temperatures when male feeding assistance might occur at higher rates (Bambini *et al.* 2019), which could explain nest attentiveness patterns in Quintos. As mentioned before, perceived predation might reduce nest attentiveness (Ghalambor & Martin 2002, Martin 2002, Kovařík & Pavel 2011, but see Fontaine & Martin 2006), by lengthening bouts and decreasing their daily number (Basso & Richner 2015). If perceived predation was higher in Pina, it would explain observed incubation patterns in this population. Differential predator communities associated with a type of habitat could also affect incubation behaviour (Thompson 2007) depending on the time of the day (DeGregorio *et al.* 2015) and thus at different temperatures. Finally, prey availability is a habitat dependent variable that could shape nest attentiveness patterns (e.g., Rastogi *et al.* 2006, Londoño *et al.* 2008). While Quintos provides large amounts of caterpillars, typical in oak forests, Pina is a pine forest that usually holds lower prey abundance (e.g., Van Balen 1973, Mänd *et al.* 2005).

Finally, we found that higher values of nest attentiveness at the full incubation level were associated with shorter incubation periods, as previously reported (Nord & Nilsson 2012 but see Hatchwell *et al.* 1999, Amininasab *et al.* 2017a). Our analyses did not include ambient temperature because of the correlation with nest attentiveness at the full incubation scale, but Simmonds *et al.* (2017) did not find any association between ambient temperature and the incubation period, although it would be expected to find a negative relationship between them. In a scenario where breeding females keep track of temperature fluctuations to synchronize their hatchlings with the peak of their preferred prey, i.e., caterpillars for Great tits, increasing temperatures would push females to increase incubation effort to shorten the incubation period, following caterpillar development (Perrins 1991, Noordwijk *et al.* 1995, Cresswell & McCleery 2003, Matthysen *et al.* 2011). But females in Quintos do just the opposite, reducing nest attentiveness at increasing temperatures, which could affect trophic synchronization. Further analyses are needed to ascertain all the details of the fitness consequences of this response to ambient temperature.

Chapter 3

Chapter 3

The role of partial incubation and egg repositioning within the clutch in hatching asynchrony and subsequent effects on breeding success

Original paper published in:
Diez-Méndez D, Rodríguez S, Álvarez E & Barba E. (2020) The role of partial incubation and egg repositioning within the clutch in hatching asynchrony and subsequent effects on breeding success. *Ibis*, 162:63–74.

Data are available from the Dryad Digital Repository:
https://doi.org/10.5061/dryad.gc7qf50

ABSTRACT

The main mechanism to achieve hatching asynchrony (HA) for incubating birds is to start heating the eggs before clutch completion. This might be achieved through partial incubation and/or early incubation. Even in the absence of incubation behaviour during the laying phase, clutches still experience a certain degree of asynchrony. Recent studies have shown that eggs located in the centre of the nest receive more heat than peripheral ones during incubation. Since eggs receiving more heat would develop faster, we hypothesised that HA should be shorter in nests where eggs were moved homogeneously along the centre-periphery space during incubation compared to those nests where eggs repeatedly remained in the same locations, either centrally or peripherally. We explored the relative roles of egg repositioning and partial incubation in determining HA in wild birds by (1) removing eggs from 20 Great Tit *Parus major* nests on day of laying and replacing them with fake eggs to avoid partial incubation, and returning them when full incubation began; (2) monitoring twice a day the position of each individually marked egg relative to the clutch centre during incubation, and estimating the coefficient of variation of the distances ($CV_{distance}$); (3) determining HA in each nest. Preventing partial incubation reduced HA by 51 % days in experimental nests. It also caused negative effects for the incubating females (lengthening the full incubation period) and positive effects for the brood (increasing fledging success). However, our hypothesis about the role of egg repositioning on HA was not supported: all the females moved the eggs with remarkable consistency, generally attaining a $CV_{distance}$ around 33 %, and it was not related to the HA experienced. We therefore conclude that partial incubation is an important factor regulating HA, and females compensate for the potential effects of differential heating by moving the eggs homogeneously within the clutch.

Keywords: Egg turning, egg recognition, Great Tits, incubation period, thermal gradients.

INTRODUCTION

In avian species laying more than one egg per clutch, eggs do not hatch exactly at the same time. The degree of hatching asynchrony (the time span between the hatching of the first and the last egg, 'HA' hereafter) varies both between and within species. To set a limit to categorise the hatching pattern, when hatchings happen within 24 hours is usually classified as 'synchronous', and when they take longer as 'asynchronous' (Magrath 1990, Ricklefs 1993, Stoleson & Beissinger 1999). Asynchronous hatching creates a size hierarchy among nestlings which could be either beneficial (Gilby *et al.* 2011), partially beneficial (Slagsvold *et al.* 1995, Mainwaring *et al.* 2012a) or detrimental (Stenning 1996, Szöllosi *et al.* 2007) for the brood, depending on the circumstances. Accordingly, many hypotheses have been proposed to explain the occurrence of HA (Stenning 1996) and its degree, spanning from its adaptive value (e.g. 'the brood reduction hypothesis', Lack 1947, Ricklefs 1965, Amundsen & Slagsvold 1998), to being the inevitable result of other constraints affecting the incubation onset, e.g. 'the egg viability hypothesis' (Veiga 1992, Stoleson & Beissinger 1995), or 'the nest-failure hypothesis' (Clark & Wilson 1981).

Independently of whether it has adaptive value, the main mechanism to achieve asynchronous hatching is to begin to heat the eggs before clutch completion. Two non-mutually exclusive behaviours could promote HA: (1) partial incubation, and (2) full incubation before clutch completion (early incubation hereafter). If we focus on gyneparental species, where only females incubate, partial incubation occurs when the female displays short incubation on-bouts during the egg-laying phase, but the eggs do not always reach full incubation temperature (36-39 ^{o}C) during these bouts (Wang & Beissinger 2009, 2011, Podlas & Richner 2013a). On the other hand, early incubation occurs when the female advances the beginning of full incubation into the laying phase (Wang & Beissinger 2011) and keeps a steady incubation rhythm until hatching (Cooper & Voss 2013). Either partial or early incubation may cause an increase in HA (e.g. Veiga 1992, Veiga & Vinuela 1993, Lord *et al.* 2011a, Johnson *et al.* 2013; but see Wang & Beissinger 2009, Podlas & Richner 2013a). Moreover, partial incubation could reduce the length of the incubation period (Ricklefs 1993, Stoleson & Beissinger 1995, Wang & Beissinger 2011, Mainwaring *et al.* 2012a).

Both partial and early incubation take place during the egg-laying phase. However when females delay the beginning of the incubation until the clutch is complete, it is still possible to observe some degree of HA (Clark & Wilson 1981, Magrath 1990). A potential explanation is that eggs, even if they are incubated for the same length of time, receive different thermal inputs from the incubating adult depending on their position within the nest. Although the existence of thermal gradients from the centre to the periphery of the clutch has been known for a long time (Drent 1975 and references therein), this fact and its potential effects have been largely overlooked. Recent studies have confirmed the presence of these thermal gradients (Boulton & Cassey 2012, Sálek & Zárybnická 2015, Hope *et al.* 2018), with central eggs (i.e., directly positioned under the brood patch) being warmer than peripheral ones (Boulton & Cassey 2012, Hope *et al.* 2018). These central eggs would therefore receive more heat, and thus develop faster (Reid *et al.* 2000b, Hepp *et al.* 2006, Nord & Nilsson 2011), than those placed in the periphery, potentially causing HA in clutches otherwise expected to be synchronous.

It has also been known for a long time that eggs are moved by the incubating bird from one side of the nest to the other (e.g., Drent 1975). Early studies pointed out that egg displacements within the nest were necessary for the females to keep eggs equally warm (Caldwell & Cornwell 1975, Löhrl 1986), since they usually are not able to cover the whole clutch with their brood patches (Bortolotti & Wiebe 1993, Niizuma *et al.* 2005). However, this behaviour has not attracted much attention from researchers, and has been usually associated with egg turning. For example, Deeming (2002b) defined egg turning as 'the process in which the egg is moved around within the nest changing both its location and, through rotation, its orientation'. However, these should be considered as two different movements, with probably different functions. While egg turning is necessary for normal embryo development (e.g. Tullett & Deeming 1987, Deeming 1989, 1991), egg repositioning would be needed to keep the eggs equally warm throughout the incubation period (Boulton & Cassey 2012). Linking egg repositioning with thermal gradients within the nest means that, if the position of each egg is not equally distributed in the centre-periphery space throughout the incubation period, i.e. spending more time directly under the brood patch than out of its reach or *vice versa*, this might create differences in embryo development leading to alterations in the expected HA (Eichholz & Towery 2010, Poláček *et al.* 2017a).

Our aim here was to elucidate the relative roles of egg repositioning and partial incubation in determining HA, choosing a Mediterranean population of Great Tits *Parus major* breeding in nestboxes as a model species. Female Great Tits perform partial incubation (Lord *et al.* 2011, Podlas & Richner 2013a), and full incubation has been described before clutch completion (Haftorn 1989, Álvarez & Barba 2014b). Our main research questions addressed (1) how much partial incubation affects HA, and (2) whether the degree of HA was affected by the different distances of each egg from the centre of the clutch throughout the incubation period, once partial incubation was controlled for as a potential source of HA. We expected the degree of HA to be lower (1) in nests where partial incubation was avoided, and (2) in those where eggs were moved homogeneously along the centre-periphery space, compared to those nests where eggs repeatedly stayed in the same locations, either centrally or peripherally. We also expected longer incubation periods when partial incubation was avoided. Other breeding parameters, such as hatching success and fledging success, could also be affected if preventing partial incubation effectively shortens HA and extends incubation periods, so these potential effects were also investigated.

METHODS

Study area and general field procedures

Data used for this experiment were collected during 2014 from a wild Great Tit population breeding within an extensive orange *Citrus aurantium* plantation near Sagunto (Valencia, eastern Spain; 39°42'N, 0°15'W, 30 m asl). The study site is a homogeneous habitat, where orange trees are uniformly distributed (Andreu & Barba 2006). Wooden nestboxes, hanging from branches between 0.5 and 1.0 m above ground (see for details), were put in place by the end of February for the birds to use as breeding sites. Each nestbox was inspected weekly until a nest under construction was detected. Then, the frequency of the visits was readjusted so as to allow detection of first egg date and clutch size. The start of full incubation was determined by daily inspections after the fifth egg was laid. The detection of warm eggs in the absence of the female or the direct observation of an incubating female was used to establish the incubation onset. We checked the nests to record the laying of any additional egg three days after the incubation onset. We resumed daily nest inspections from incubation day 12 (day 1 = onset of incubation) to determine the exact hatching date. These daily visits continued until the last egg hatched, or absence of new hatchlings during two consecutive days, to determine the degree of HA accurately (e.g. Greño *et al.* 2008). We calculated HA as the days elapsed between the hatching of the first and the last nestling, being 0 if all the eggs had hatched the first day. Dates were defined numerically with 1 equalling the 1st of April. Females were captured with traps at the nest when feeding 10-12 days-old nestlings, and their sex and age were recorded based on the presence of brood patch and plumage coloration (Svensson 1992). All 15-day old nestlings were weighed (electronic balance; ± 0.1 g).

Selection of nests and procedures in experimental and control nests

Among the available nests during the first weeks of the breeding season, we randomly selected 20 nests during their construction phase. These experimental nests followed a specific schedule during the nest construction, laying and incubation periods. First, we visited these nests every 3-4 days when the nest was initiated, and daily when they were in the last phases of nest construction. Once the first egg was detected, we continued with the daily visits, and substituted every new laid egg for a fake one. Both the fake and the original eggs were identically marked with a colour code corresponding to its position in the laying order (see Appendix 3 Fig. S1 and Table S1). Real eggs were placed in an additional nestbox, close to the one where the original experimental nest was, containing an artificial nest built with moss, wool and hair. This additional nestbox had a blocked entrance hole, preventing access to other breeding birds but allowing air flow. The day when the onset of full incubation was detected, we placed the real eggs back in their nest. From the day after incubation onset, and until hatching was detected, we visited the experimental nests twice per day (i.e., early in the morning and around midday) and took a photograph each time, where the position and code of every egg in the nest cup could be identified. A paper scale was placed close to the eggs to allow measurement of distances between the eggs and the centre of the nest.

To explore the implications of preventing partial incubation in experimental nests, we randomly chose another subset of 20 nests from the rest of the breeding population, with similar laying dates to the experimental ones (estimate ± se = -1.39 ± 1.43, t = -9.78, P = 0.336), and a similar clutch size (estimate ± se = -1.14 ± 0.11, z = -1.26, P = 0.207). We acknowledge that our 'control' nests are not strictly controls because of the lack of egg coding and different checking patterns during the incubation period. However, we think that the parameters we aimed to compare (effects of partial incubation) have not been compromised by the methodological differences.

From the 20 experimental nests, one was abandoned and another was preyed upon during the laying phase, and two more were abandoned during incubation. We therefore used data from 16 nests (mean laying date ± sd = 9.31 ± 4.42, range 1–16; mean clutch size ± sd = 9.17 ± 1.19, range 7–11), to compare them to nests which experienced partial incubation (i.e., controls). We discarded two more nests from the egg repositioning analysis because of problems with the confident visualization of the codes throughout the incubation phase. In five out of 16 experimental nests (and four out of the 14 used for egg repositioning analysis), one additional egg was laid after full incubation began, so these nests were categorised as nests with early incubation.

From 20 control nests, three were abandoned during the incubation period, so 17 nests were used for statistical analyses (mean laying date ± sd = 10.71 ± 3.75, range 4–17; mean clutch size ± sd = 9.00 ± 1.37, range 6–12). In five of these remaining 17 control nests, one additional egg was laid after incubation started (i.e., nests with early incubation).

Distance analyses from photographs

We used the image processing software ImageJ (Schneider *et al.* 2012) to calculate the relative position of each egg in relation to the centre of the clutch. As we took two photographs per day, from the day after full incubation began until the hatching of the first egg, we produced between 20 and 30 photographs per nest, depending on the incubation duration. Each photograph was scaled and a minimum ellipse that contained the clutch was built to establish the centre of the nest, i.e. the focal point from where to measure the distances. Afterwards, the silhouette of each egg was created to calculate its approximate centre. Finally, we extracted the ‘x’ and ‘y’ coordinates of the clutch focal point and the centre of the eggs and calculated the distances using the ‘distance formula’ derived from the Pythagorean Theorem.

$$\text{Distance} = \sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2}$$

Once we knew the raw distances, as a standardised measure of variation, we used the coefficient of variation of the distances ($CV_{distance}$ = sd / mean x 100) to calculate the variation of the distance of each egg to the nest centre throughout the incubation period. The logic behind the use of $CV_{distance}$ lies in the interest of knowing whether an egg changes its position or remains in the same spot. If the $CV_{distance}$ is low, that would mean that the focal egg was moved little, no matter whether it was in the centre or in the periphery. On the contrary, if an egg shows a high $CV_{distance}$, it would mean that its position was often changed relative to the nest centre. Translating this into our objective, experimental nests with eggs of, on average, low $CV_{distance}$ (i.e., little exchange between centre and periphery) may show higher HA than nests with high $CV_{distance}$.

Statistical analysis

First we analysed whether the experimental nests were subjected to a reduced HA compared to control nests. Since the dependent variable was HA (number of days), we used a GLM (generalized linear model) with log link (Poisson family), with group (experimental/control), clutch size, incubation period (days), female age (first breeding year or older) and the presence of early incubation (yes/no) as explanatory variables. Thereafter, we analysed whether the $CV_{distance}$ of each egg within an experimental nest had an effect on the HA, among other potential explanatory variables such as clutch size, incubation period and early incubation. We used a GLMM (generalized linear mixed model) model with log link (Poisson family) fitted by Laplace Approximation (Bates *et al.* 2015), including ‘nest’ as a random effect. No interactions were built due to low sample sizes (*n* = 14 nests).

To compare experimental nests without partial incubation to control ones, we chose several variables potentially affected by partial incubation. We analysed the potential effects on incubation period using a LM with identity link (Gaussian family) and group (experimental/control), clutch size, early incubation and female age as explanatory variables. Effects on hatching success (i.e., the proportion of hatched eggs relative to the clutch size) were analysed using a GLM with a complimentary log-log link (binomial family) and group, clutch size, incubation period, early incubation and female age as explanatory variables. We chose the complimentary log-log link over the classical logit link because it works best with binomial data that shows skewed distributions (e.g., Dobson 2002).

We also analysed the influence of preventing partial incubation on fledglings. First, we checked for potential effects on fledging success (i.e., number of fledglings in relation to the total number of hatchlings) using a GLM with a complimentary-log-log link, and group, clutch size, female age and incubation period as explanatory variables. Secondly, we analysed the weight of chicks at fledging using a LMM (linear mixed model) with identity link, nest as the random factor, and group, female age, fledgling number and incubation period as explanatory variables.

We conducted statistical analyses using the R software, version 3.5.1 (R Core Team 2019). To select each final model, the following procedure was followed: we set up a number of models combining the explanatory variables to the different dependent variables described above, selecting and analysing the most informative model based on the Akaike information criterion (AIC), following (Burnham & Anderson 2002) and using the MuMIn package (Bartón 2015). When more than one model was in the best model set (ΔAICc < 2.0), the most parsimonious model was selected (Burnham & Anderson 2002). For model selection results see Appendix 3 (Tables S2-S7). For the selected models, we presented the adjusted-R^2 in LMs, the D^2 as the amount of deviance accounted for in GLMs (Guisan & Zimmermann 2000), and the R^2, following (Nakagawa & Schielzeth 2013), in GLMMs. All numeric predictors were scaled and only biologically-meaningful two-way interactions were considered, except for analyses with low sample sizes (see above) where no interactions were examined. All models were built using lme4 package (Bates *et al.* 2015). We corrected final GLMs using Robustbase package and LMs using MASS package (Venables & Ripley 2003) when we found influential points. Using this correction, we tried to balance their influence in the model output recalculating estimates and standard errors.

RESULTS

Experimentally preventing partial incubation reduced hatching asynchrony by 0.84 days (51 %), from 1.65 ± 0.70 days in control nests to 0.81 ± 0.54 days in experimental ones (D^2 = 45 %, estimate = 0.46, se = 0.207, z = 0.21, P = 0.026). For experimental nests, HA spanned from 0 (four nests) to 2 days (one nest), and in 11 nests HA was 1 day. For control nests, HA spanned from 0 (one nest) to 3 days (one nest), being 1 day in 5 and 2 days in 10 nests.

The coefficient of variation of the distances was not significant in explaining variation in HA (Table 1). Average values of $CV_{distance}$ did not differ between nests containing clutches with different degrees of HA ($CV_{distance}$ = 33.22, 33.33 and 33.98 % for nests with HA = 0, 1 and 2 respectively; Fig. 1), and the range of $CV_{distance}$ values for individual eggs within the clutch was moderate (23.89–43.25 % for HA = 0; 16.26–46.38 % for HA = 1; and 27.91–40.37 % for HA =2). However, HA increased with clutch size and decreased as the incubation period lengthened (Table 1). Contrary to expected, early incubation (i.e., the onset of full incubation before clutch completion) was not selected as a relevant factor explaining HA. It should be noted, however, that sample size was very low for this analysis, since only four out of the 14 nests included in the analysis showed early incubation.

Table 1. Estimates, standard errors, z-values and p-values from the best informative generalized linear mixed model explaining the variables influencing the degree of hatching asynchrony in experimental nests. Nest is the random factor. R^2explained by fixed factors = 0.29; R^2 explained by both fixed and random factors = 0.32. Significant terms are highlighted in bold.

	Estimate	**se**	***z***	***P***
Intercept	-0.21	0.604	-0.35	
$CV_{distance}$	0.00	0.018	-0.10	0.922
Clutch size	**0.45**	**0.147**	**3.08**	**0.002**
Incubation period	**-0.41**	**0.139**	**-2.95**	**0.003**

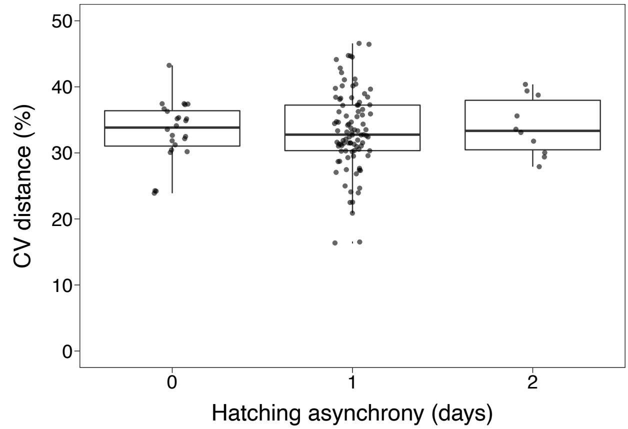


Figure 1. Box and whisker plots of coefficients of variation of the egg distances to the centre of the clutch throughout the incubation period in relation to the degree of hatching asynchrony in experimental nests. Black horizontal lines represent the median, boxes represent 50 % of the values, upper and lower whiskers represent values greater than 75th and lesser than 25th percentile. Solid circles represent jittered raw data from individual egg $CV_{distance}$ values.

The prevention of partial incubation caused an increase in the full incubation period in experimental nests compared to controls (Table 2) or, in other words, partial incubation reduced the full incubation period (experimental nests 14.06 ± 1.00 days, range 13–16;

control nests 12.24 ± 1.30 days, range 11–15). The incubation period also increased when females laid an egg after beginning full incubation (Table 2). On average, incubation in experimental nests was 1.82 days longer than in control ones. As shown above, HA was 0.84 days shorter in experimental nests. Therefore, the reduction in the hatching period in experimental nests did not compensate for the lengthening of the incubation period, resulting in experimental nests showing a longer incubation + hatching phase (0.98 days longer on average).

Table 2. Estimates, standard errors, t-values and p-values from the best informative linear model explaining the variables influencing the length of the incubation period. $R^2 = 0.58$. Significant terms are highlighted in bold.

	Estimate	**se**	***t***	***P***
Intercept	13.57	0.216	62.72	
Group	**-1.14**	**0.206**	**-5.56**	**<0.001**
Female age	0.39	0.206	1.87	0.075
Early incubation	**-0.76**	**0.216**	**-3.50**	**0.002**

Table 3. Estimates, standard errors, z-values and p-values from the best informative generalized linear model explaining the variables influencing hatching success. $D^2 = 0.82$. Significant terms are highlighted in bold.

	Estimate	**se**	***z***	***P***
Intercept	3.13	0.473	6.62	
Group	-0.02	0.364	-0.06	0.953
Incubation period	**-0.85**	**0.362**	**-2.35**	**0.019**
Clutch size	**1.20**	**0.348**	**3.46**	**0.001**
Female age	0.51	0.338	1.50	0.134
Group x Incubation period	**0.79**	**0.395**	**1.99**	**0.047**

Hatching success was positively affected by clutch size and decreased with the duration of the full incubation period (Table 3). Additionally, there was a weak interaction (Table 3; Fig. 2) between nest group and the duration of the incubation period. This interaction showed a trend for a lower hatching success in experimental nests with long incubation periods.

Preventing partial incubation in experimental nests did not affect the weight of chicks at fledging (R^2 explained by fixed factors = 0 %; R^2 explained by both fixed and random factors = 50 %; estimate = -0.04, se = 0.293, $t = -0.15$, $P = 0.881$). However, fledging success was higher in experimental nests (i.e., without partial incubation; Table 4). We also found that clutch size negatively affected fledging success, and older females (i.e., those which were at least in their second breeding season) achieved higher fledging success than first-years (Table 4).

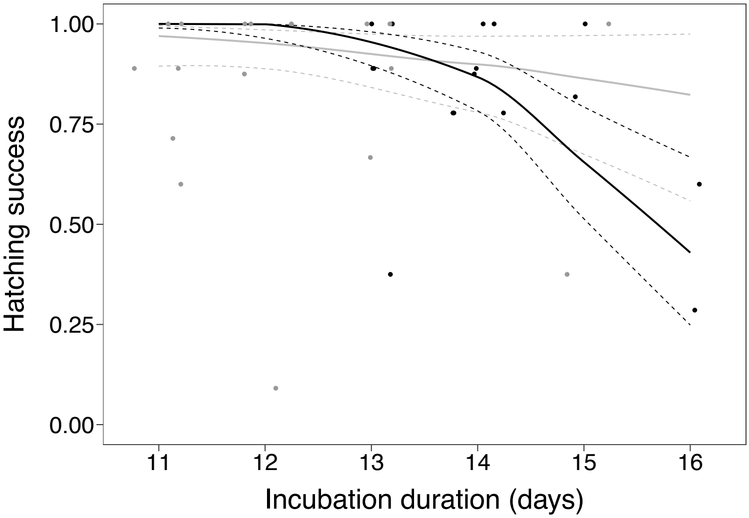


Figure 2. Variation of hatching success depending on incubation duration in experimental (black line) and controls nests (grey line). Dashed lines denote 95 % confidence intervals. Black and grey circles represent jittered raw data from experimental and control nest respectively.

DISCUSSION

The avoidance of partial incubation in the experimental nests caused a reduction of 51 % days in HA compared to controls, a lengthening of the full incubation period and a higher fledging success. Additionally, fledging success was also positively influenced by female age and negatively by clutch size. Hatching success was not clearly affected by the experimental procedure, but it was negatively affected by long incubation periods and positively by clutch size. Finally, egg repositioning did not influence the degree of HA in experimental nests, but HA increased with clutch size and decreased as the incubation period lengthened.

Consequences of preventing partial incubation

The effect of early incubation on HA is well stablished in the literature (Stoleson & Beissinger 1997, Wiebe *et al.* 1998, Wang & Beissinger 2009, Gilby *et al.* 2011), while the occurrence and consequences of partial incubation has received less attention (Wang & Beissinger 2011).

Moreover, in species where both partial and early incubation occur, the lack of discrimination of the onset and duration of both behaviours has further complicated the assessment of their separate effects on HA (Wang & Beissinger 2011).

Podlas and Richner (2013b), simulating partial incubation in a laboratory-based experiment with Great Tits, failed to show an effect of partial incubation on HA, suggesting that early incubation was the major factor responsible for the observed HA. Contrasting with this conclusion, we found a shortening of HA (i.e., 51 % days) when partial incubation was experimentally suppressed in field conditions, strongly suggesting that partial incubation is an important factor affecting HA. On the other hand, we did not find a significant effect of early incubation on HA (but see Stenning 2008a) for a experimentally induced early incubation), although our sample size for this analysis was low and we should be cautious about reaching a firm conclusion.

Table 4. Estimates, standard errors, z-values and p-values from the best informative generalized linear mixed model explaining the variables influencing fledging success. $D^2 = 0.68$. Significant terms are highlighted in bold.

	Estimate	**se**	***z***	***P***
Intercept	1.88	0.277	6.80	
Group	**-0.57**	**0.255**	**-2.24**	**0.025**
Clutch size	**-0.87**	**0.268**	**-3.25**	**0.001**
Female age	**0.88**	**0.263**	**-3.34**	**0.001**

Podlas and Richner (2013b) did not find any effect of partial incubation on the incubation period. Our results in the field again disagree with those obtained in the laboratory, showing that preventing partial incubation, and thus probably also avoiding embryo development during the egg-laying phase, caused the expected effect of lengthening the full incubation period of experimental nests compared to controls. This result supports the idea that, if partial incubation effectively increases egg temperature over 26-28^{o}C (i.e., Physiological Zero Temperature, Webb 1987), first-laid eggs would have started embryo development before full incubation onset occurred, and therefore the period of time to the first hatching would be reduced (Haftorn 1981, Lord *et al.* 2011, Wang & Beissinger 2011).

Another important consequence of preventing partial incubation in our population was that the period of time between the start of full incubation and the hatching of the last egg (i.e., the incubation + hatching period) became longer. Long incubation periods may increase energy expenditure by females (Nord & Williams 2015) and predation risk to the clutch (Clark & Wilson 1981, Conway & Martin 2000b). We cannot totally rule out the possibility that our experimental procedure, photographing the clutch twice a day, could have contributed to lengthening of the incubation period. However, we believe that the potential effect would be negligible, since females in this population leave the nest an average of 23 times per day during the incubation period (Álvarez & Barba 2014b). We had to force the female to leave in 68 % of our visits, so we think that one or two forced departures from the nestbox per day could be integrated into the female on- and off-bout pattern.

The prevention of partial incubation did not affect hatching success, but hatching success was lower in nests with long incubation periods and in those with small clutch sizes. These associations may be mediated by differences in female body condition, as they affected both control and experimental nests. Females that exhibit poor body condition lay fewer eggs and incubate at lower temperatures (Hepp *et al.* 2006), and/or display more recesses during incubation (Bueno-Enciso *et al.* 2017), thereby lengthening incubation and resulting in lower hatching success (Nord & Nilsson 2011, Macdonald *et al.* 2013).

Fledging success (proportion of hatched nestlings which eventually fledge) was higher in smaller clutches, in nests attended by second-year breeding females, and in nests where partial incubation was prevented. The first two relationships are commonplace: smaller clutches are easier to raise, and second-year females show higher breeding outputs than first-years (Saether 1990, Andreu & Barba 2006). However, we highlight the effect that preventing partial incubation, and thereby reducing HA, had on fledgling success. HA creates a size hierarchy among the nestlings which could be argued to be generally detrimental for the whole brood (Szöllosi *et al.* 2007, Stenning 2008) or, at best, to have no effects if ambient conditions are favourable (Veiga & Vinuela 1993, Podlas & Richner 2013b). Positive effects, such as a decreasing sibling rivalry (Gilby *et al.* 2011) and long-term sex-specific advantages (Mainwaring *et al.* 2012a), have been described, but so far with very specific situations: under moderate degrees of HA and under conditions of *ad libitum* food availability, respectively. HA usually ends with brood reduction, benefiting only early hatched nestlings (Magrath 1990, Amundsen & Slagsvold 1998). Our results show that shortening HA, as a result of preventing partial incubation, reduced nestling mortality, thereby potentially increasing parental fitness.

Egg displacement and hatching asynchrony

The occurrence of HA, even in the absence of partial and early incubation, was noticed decades ago (Magrath 1990, Amundsen & Slagsvold 1998), and our results showed hatching spread of eggs of up to 2 days in nests where partial incubation was avoided and early incubation did not occur. We tested here the hypothesis that the different positions of the eggs within the clutch, with more peripheral eggs taking longer to hatch than more central ones, could explain the additional HA once partial incubation was controlled for and early incubation was taken into account. This hypothesis was not supported by our results, since the overall variation in egg distances from the centre of the nest was similar in experimental nests containing clutches exhibiting variable degrees of HA. The observed pattern of egg repositioning might be accomplished by the females simply reacting to thermal gradients within the clutch (Boulton & Cassey 2012, Sálek & Zárybnická 2015), moving cooler peripheral eggs to the centre of the clutch, and warmer central eggs outwards. By this simple rule of thumb, females could maintain homogeneity in heat transfer. Although eggs within a clutch can show differences up to 30 % of the $CV_{distance}$, our results suggest that this variation between individual eggs should be below the threshold to produce detectable HA (which, considering the precision of the present study, would have meant differences of more than one day).

In contrast with our results, there are two previous studies that have shown non-homogeneous egg repositioning within nests (Eichholz & Towery 2010, Poláček *et al.* 2017a). These studies considered the possibility that females could recognize individual eggs and move them differentially. It is known in songbirds that eggs within a clutch could differ in size (Krist 2011) and colour (Morales *et al.* 2006, Walters & Getty 2010, Krištofík *et al.* 2013), and these differences may be indicators of potential body mass growth (Rosivall *et al.* 2005, Poláček *et al.* 2017b). Eichholz and Towery (2010) showed that smaller last-laid eggs were found more frequently placed in central positions within the clutch of precocial waterfowl with synchronous hatching. Placing these last laid-eggs in central positions within nests accelerates their embryonic development, being able to catch up earlier laid eggs and thus reduce (or even avoid) HA. In the second study, (Poláček *et al.* 2017a) found darker eggs in central positions more frequently than expected by chance. They hypothesised that darker eggs (i.e., those in the middle positions of the laying sequence) were recognised and favoured, but only under adverse breeding conditions, when females would try to avoid complete brood failure. Unfortunately, they did not provide HA or nestling mortality data to support this hypothesis. To the best of our knowledge, there is no evidence that Great Tits are able to discriminate between their own eggs, although differences in pigmentation patterns exist (Gosler *et al.* 2000, Stoddard *et al.* 2012). Further studies are needed to disentangle the possibility of individual egg recognition in passerines and its potential link to egg repositioning and HA.

It should also be noted that females laying bigger clutches displayed a higher degree of HA. Incubating big clutches may involve greater difficulties for the incubating adults to cover all eggs with the brood patch compared with smaller clutches (Bortolotti & Wiebe 1993, Niizuma *et al.* 2005), resulting in colder peripheral eggs in larger, than in smaller, clutches (Hope *et al.* 2018). Despite between-clutch similarities in egg repositioning, a higher degree of HA in bigger clutches might indicate that females struggled to move the eggs within the clutch. Potential small differences in egg repositioning that we could have been missed, together with the lower temperatures that the eggs reach in peripheral positions in big clutches, might have led to the observed differences in HA.

The possibility also exists that part of the remaining HA might be a side-effect of our experimental manipulation. Depending on the laying sequence, eggs differ not only in external characteristics, but also in their internal composition (Hadfield *et al.* 2013, Williams & Groothuis 2015, Noguera *et al.* 2016). Last-laid eggs could show higher embryo development rates (Viñuela 1997, Griffith & Gilby 2013, Hadfield *et al.* 2013), supposedly to compensate for their position in the laying sequence, thus reducing HA. If this were true for our population, we would have created an 'artificial' HA by triggering the development of all the embryos at the same time. Alternatively, experimental eggs were kept under ambient temperatures until the incubation onset, and this 'storage time' could have also affected embryo development rates, lengthening the time the embryos needed to hatch (Fasenko 2007, Stenning 2008).

Finally, we are aware that two daily photographs surely do not show all the displacements experienced by the eggs, or even the frequency of this repositioning, but it is a first step in recording this overlooked behaviour. It would be of major interest to study egg displacement and egg turning movements separately. These two behaviours could follow opposite paths through incubation. Since cooling rates increase throughout this period (Boulton & Cassey 2012, Cooper & Voss 2013), eggs in the periphery of the clutch would undergo faster cooling as incubation proceeds, and therefore displacements to the centre of the clutch would need to be more frequent. On the contrary, egg turning has been described to be more important in the early phase of incubation (Deeming 2009, Clatterbuck *et al.* 2017). These and other considerations should be taken into account in future research, as well as developing technologies to record egg repositioning within avian nests.

Conclusions

1. This thesis project, focused on incubation behaviour of Great Tits *Parus major* in three Mediterranean breeding populations, shows that ambient temperature is a key variable to understand female breeding behaviour.

2. Increasing ambient temperature during the egg-laying period advances the onset, and lengthens, diurnal partial incubation, pulling forward the onset of diurnal full incubation in a similar way among the three breeding populations. On the other hand, nocturnal incubation is not associated with ambient temperature, but it could have been affected by temperature values before egg-laying started.

3. Larger clutches lengthen nocturnal partial incubation and delay the onset of the nocturnal full period. Moreover, larger clutches also delay the onset of diurnal partial incubation, that could explain the correlation between the onset of diurnal partial incubation and nocturnal full incubation. Clutch size effects would point to a high energy expenditure scenario that constraints females from fully incubating the clutch during the egg-laying period, pushing back the onset of diurnal partial incubation and counteracting the increasing temperature effect.

4. During diurnal full incubation, females maximize on-bout duration at different temperatures depending on the population, instead of showing a generalized incubating response to a common range of temperatures. Opposite observed patterns of nest attentiveness for the overall incubation period, either increasing incubation effort or self-attentiveness time, seem to occur because females maximize on-bout duration at either 10 ºC or 15-20 ºC in different populations. The quadratic effect of ambient temperature on hourly and daily nest attentiveness is more informative and accurate, regarding on- and off-bout patterns, than linear nest attentiveness for the overall incubation period.

5. Females increase incubation effort with increasing local temperatures up to a certain threshold, thereafter self-maintenance time increases in every population. The cause behind local turning points in incubation behaviour when females increase self-maintenance time over incubation needs to be further investigated.

6. Longer diurnal partial incubation shortens the diurnal full incubation period, an effect that could be indirectly caused by higher ambient temperatures during the egg laying period. The opposite, an indirect lengthening of the diurnal full incubation period because of a later onset of diurnal partial incubation, could be caused by larger clutches. The effect of diurnal partial incubation on the full incubation period is consistent in observational and experimental approaches: in the absence of diurnal partial incubation, the diurnal full incubation period is longer. Not only an earlier onset of incubation but a

higher incubation effort (i.e., higher nest attentiveness) also shortens the diurnal full incubation period. On the other hand, neither the duration of partial nor the onset of full nocturnal incubation is associated to the duration of the diurnal full incubation period.

7. Diurnal partial incubation is a key period for subsequent hatchling survival because of its role in hatching asynchrony. In comparison, diurnal full incubation before clutch completion showed a smaller effect. Nocturnal incubation was not associated to hatching asynchrony, despite nocturnal partial incubation lasts at least the double than the diurnal partial period and the nocturnal full incubation starts days before the diurnal full period. Experimental avoidance of incubation before the onset of diurnal full incubation also shows higher hatching asynchrony, in accordance with observational studies.

8. Egg repositioning within the clutch is similar among nests with different values of hatching asynchrony. After experimentally deterring clutches from being incubated during the egg laying period, the residual hatching asynchrony is not associated with egg repositioning.

Appendix 1

Table S1. Descriptive statistics of the breeding parameters given by year and population. *n* denotes the number of nests.

		Pina			Quintos			Sagunto		
		n	**mean ± sd**	**range**	***n***	**mean ± sd**	**range**	***n***	**mean ± sd**	**range**
Laying date [a]	2015	4	35.2 ± 2.06	33 – 38	6	13.3 ± 5.05	6 – 21	14	11.1 ± 4.87	3 – 23
	2016	6	33.3 ± 5.20	23 – 36	8	17.3 ± 6.88	7 – 26	14	6.4 ± 4.52	(-2) – 13
	2017	6	20.2 ± 5.74	15 – 30	8	11.9 ± 5.03	3 – 18	11	10.0 ± 3.07	5 – 16
Clutch size [b]	2015	4	7.0 ± 0.00	7 – 7	6	8.7 ± 0.82	8 – 10	14	8.0 ± 1.36	6 – 10
	2016	6	7.0 ± 0.89	6 – 8	8	9.1 ± 2.03	6 – 12	14	8.9 ± 0.86	7 – 10
	2017	6	7.0 ± 0.63	6 – 8	8	9.8 ± 1.28	8 – 12	11	8.5 ± 0.93	6 – 9
Incubation period [c]	2015	4	13.2 ± 1.50	12 – 15	5	11.8 ± 1.30	10 – 13	13	11.8 ± 0.69	11 – 13
	2016	5	13.0 ± 1.22	12 – 15	8	12.5 ± 0.76	12 – 14	12	12.8 ± 1.14	12 – 15
	2017	5	11.2 ± 0.84	10 –12	8	12.2 ± 0.46	12 – 13	11	12.0 ± 0.78	11 – 13
Hatching asynchrony [d]	2015	4	0.50 ± 0.100	0.44 – 0.65	5	0.50 ± 0.109	0.41 – 0.68	13	0.52 ± 1.143	0.26 – 0.79
	2016	5	0.63 ± 0.154	0.50 – 0.81	7	0.63 ± 0.131	0.43 – 0.83	9	0.50 ± 0.096	0.32 – 0.65
	2017	5	0.72 ± 0.180	0.46 – 0.92	8	0.40 ± 0.141	0.25 – 0.61	11	0.52 ± 0.196	0.30 – 0.99

[a] Laying dates are numerically defined by the 1st April = 1.

[b] Clutch size equals to the number of eggs laid by a breeding female.

[c] Incubation period refers to the time lapse in days between the onset of diurnal full incubation (i.e. first day that nest attentiveness lasts ≥ 50% of the active day) to the day preceding hatching date (i.e. the day the first egg hatches).

[d] Hatching asynchrony refers to the ratio between lightest and the heaviest hatchling.

Table S2. Descriptive statistics of mean ambient temperature (°C) during different incubation behaviours. *n* denotes the number of nests which we could assign local ambient temperature data to, from their own datalogger or from the closest nestbox.

	Pina			**Quintos**			**Sagunto**		
	n	mean ± sd	range	*n*	mean ± sd	range	*n*	mean ± sd	range
From laying date to diurnal partial incubation									
2015	4	17.1 ± 1.57	16.0 – 19.4	6	11.8 ± 1.43	10.7 – 14.1	14	16.2 ± 1.37	14.6 – 19.8
2016	6	10.4 ± 0.59	9.5 – 11.3	8	12.5 ± 1.38	10.5 – 14.6	14	17.2 ± 1.60	15.6 – 20.0
2017	6	8.4 ± 2.41	6.5 – 13.2	8	16.0 ± 2.15	11.6 – 17.9	11	16.9 ± 0.90	15.8 – 18.2
During diurnal partial incubation									
2015	4	18.8 ± 1.62	17.5 – 21.2	6	13.7 ± 1.24	11.7 – 15.3	14	17.5 ± 1.63	14.3 – 20.8
2016	6	13.4 ± 1.43	11.2 – 14.8	8	13.4 ± 2.38	11.0 – 18.1	14	17.7 ± 1.38	15.6 – 19.8
2017	6	12.5 ± 2.05	9.01 – 15.2	8	16.1 ± 1.80	13.7 – 18.1	11	17.7 ± 1.48	14.4 – 19.8
During nocturnal partial incubation									
2015	4	17.3 ± 1.00	16.2 – 18.6	6	11.7 ± 1.38	10.7 – 14.2	13	16.1 ± 1.47	13.8 – 19.8
2016	6	10.5 ± 0.62	9.5 – 11.3	8	12.6 ± 1.48	10.5 – 15.1	14	17.3 ± 1.41	15.8 – 19.6
2017	6	8.6 ± 1.37	6.7 – 10.3	8	16.1 ± 1.34	13.7 – 17.4	11	17.2 ± 0.82	16.3 – 19.2

Table S3. Model selection results from linear models analysing the influence of laying date, clutch size, ambient temperature, breeding population and year on the duration of nocturnal partial incubation, diurnal partial incubation, the onset of nocturnal full incubation and diurnal partial and full incubation. We added the day that diurnal (DPI) or nocturnal (NPI) partial period started as an explanatory variable to both the duration of the partial behaviour itself or the subsequent full incubation behaviour.

Candidate models	AICc[a]	ΔAICc[b]	AICw
Nocturnal partial incubation duration			
Laying date + Clutch size + Year + Population + Temperature + NPI onset	**1144.7**	**0.00**	**0.83**
Laying date + Clutch size + Year + Population*Temperature + NPI onset	1147.9	3.20	0.17
Nocturnal full incubation onset			
Laying date + Clutch size + Year + Population + Temperature + NPI onset	**266.0**	**0.00**	**0.83**
Laying date + Clutch size + Year + Population *Temperature + NPI onset	269.2	3.22	0.17
Diurnal partial incubation onset			
Laying date + Clutch size + Year + Population + Temperature	**265.4**	**0.00**	**0.93**
Laying date + Clutch size + Year + Population*Temperature	270.6	5.25	0.07
Diurnal partial incubation duration			
Laying date + Clutch size + Year + Population +Temperature + DPI onset	**968.3**	**0.00**	**0.91**
Laying date + Clutch size + Year + Population*Temperature + DPI onset	972.9	4.62	0.09
Diurnal partial incubation onset			
Laying date + Clutch size + Year + Population*Temperature + DPI onset	**237.1**	**0.00**	**0.69**
Laying date + Clutch size + Year + Population + Temperature + DPI onset	238.6	1.59	0.31

[a] The *AICc* measures the relative quality of a candidate model, adjusted for sample size.

[b] *ΔAICc* is defined as the *AICc* differences between two proposed models. When *ΔAICc* > 2.0, the difference between the two competing models is substantial (Burnham & Anderson 2002). When *ΔAICc* < 2.0, the most parsimonious model is selected (final models are indicated in bold).

‘*’Denotes an interaction between two variables.

Table S4. Model selection results from linear and beta regression models on the full incubation period (days) and the hatching rate respectively. Common explanatory variables were laying date, clutch size, year and breeding population. We added the duration of the following variables before the onset of diurnal full incubation: diurnal partial incubation (DPI) and nocturnal partial incubation (NPI) in minutes, and the nocturnal full incubation (NFI) in days. We also added the number of days diurnal full incubation lasted before clutch completion (DFI). We added the same first three previous variables, but including their duration until clutch completion, when assessing the effect on hatching asynchrony ratio, and substituting laying date for hatching date.

Candidate models	AICc	ΔAICc	AICw[a]
Duration of the diurnal full incubation period			
Laying date + Clutch size + Year + Population + DPI	**205.2**	**0.00**	**0.47**
Laying date + Clutch size + Year + Population + DPI + NPI	207.7	2.58	0.13
Laying date + Clutch size + Year + Population + DPI + DFI	207.8	2.60	0.13
Laying date + Clutch size + Year + Population + DPI + NFI	207.9	2.72	0.12
Hatching asynchrony ratio			
Hatching date + Clutch size + Population + Year + DPI + DFI	**-72.0**	**0.00**	**0.42**
Hatching date + Clutch size + Population + Year + NPI + NFI + DFI	-69.4	2.62	0.11
Hatching date + Clutch size + Population + Year + DPI + NPI + DFI	-69.4	2.62	0.11
Hatching date + Clutch size + Population + Year + DFI	-68.5	3.51	0.07
Hatching date + Clutch size + Population + Year + DPI	-68.3	3.67	0.07

[a] *AICw* measures the relative weight that the model has in relation to the rest of proposed models. Only models with AICw ≥ 0.05 are shown.

Figure S1. Photograph showing the experimental setup from a frontal view after removing the nestbox front door. Author: David Diez Méndez.

Figure S2. Photographs showing the experimental setup from above the nest. In a) and b) white arrows reveal the raw probe while in c) and d) they point to the fake egg. Since we were interested in temperature changes (and not in raw temperature) caused by off- and on-bouts, the probes, either raw or inserted in fake eggs, were accurate detecting when females left (drops of temperature) or entered the nest (rebounds). There is an evident different in thermal inertia between methodologies (raw probes have lower thermal inertia, showing sharper variations of temperature) but both methodologies accurately detected temperature changes. Author: David Diez Méndez.

Figure S3. Comparison between recorded nest-cup temperature during the egg laying period within a 24-hour time frame. a) High quality temperature data that shows nocturnal incubation, and diurnal partial incubation starting in the afternoon; and b) low-quality data resulting from a displaced probe. In the latter, nest-cup temperature matches ambient temperature, deterring any analysis of incubation behaviour. We discarded low-quality data after visual inspection of temperature patterns. Blue lines are raw nest-cup temperatures and grey lines raw ambient temperatures.

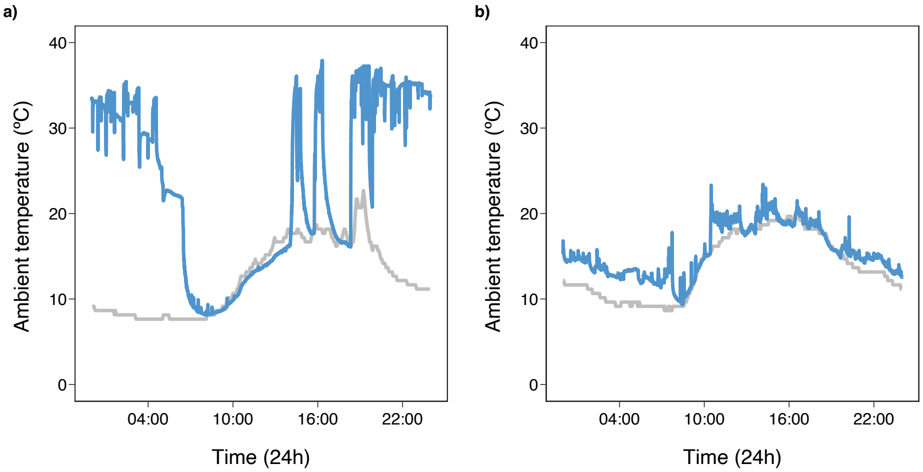
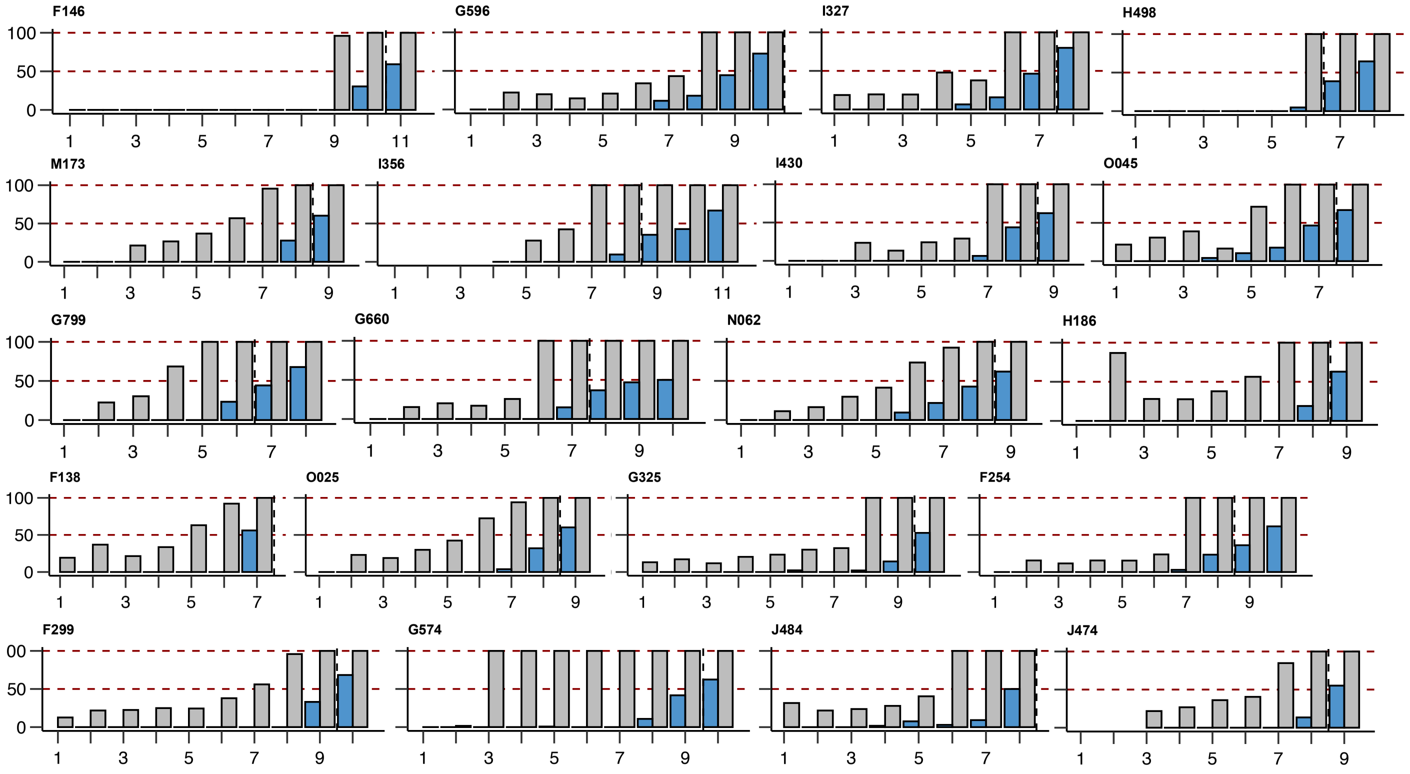


Figure S4. Bar graphs showing the increase of diurnal and nocturnal nest attentiveness until the onset of diurnal full incubation behaviour in Sagunto. Vertical axes show nest attentiveness rate and horizontal axes refer to egg days (1 = Laying date). Grey bars show nocturnal incubation and blue bars diurnal incubation. Nocturnal full incubation is accomplished when nest attentiveness reaches 100% for the first time. Diurnal full incubation is accomplished when females reach 50% of diurnal nest attentiveness for the first time. Vertical dashed lines show the day the clutch was completed. Alphanumerical codes identify each nestbox.



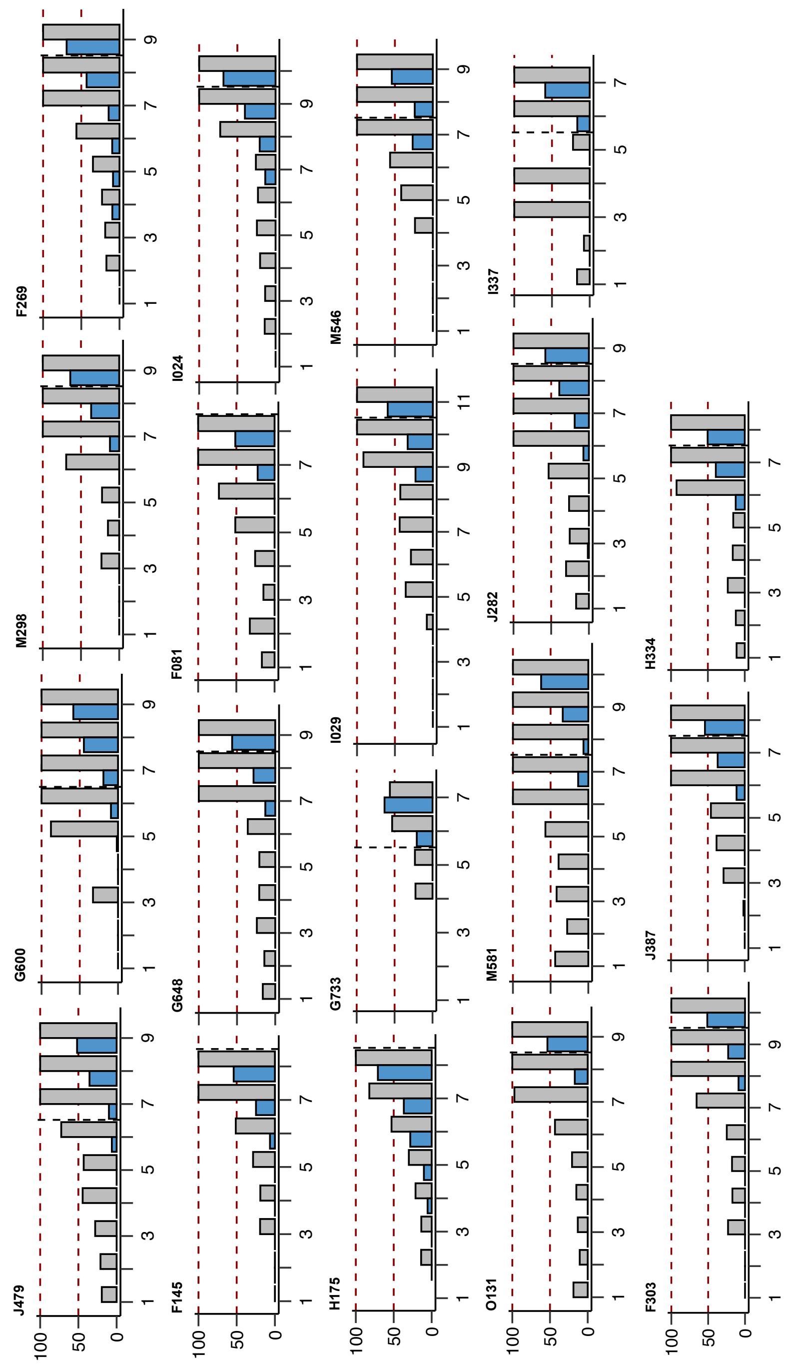


Figure S4. Continued

Figure S5. Bar graphs showing the increase of diurnal and nocturnal nest attentiveness until the onset of diurnal full incubation behaviour in Quintos. Vertical axes show nest attentiveness rate and horizontal axes refer to egg days (1 = Laying date). Grey bars show nocturnal incubation and blue bars diurnal incubation. Nocturnal full incubation is accomplished when nest attentiveness reaches 100% for the first time. Diurnal full incubation is accomplished when females reach 50% of diurnal nest attentiveness for the first time. Vertical dashed lines show the day the clutch was completed. Alphanumerical codes identify each nestbox. Asterisk highlights a nest that was removed from diurnal partial incubation statistics because the female displayed an unusual and lasting behaviour.

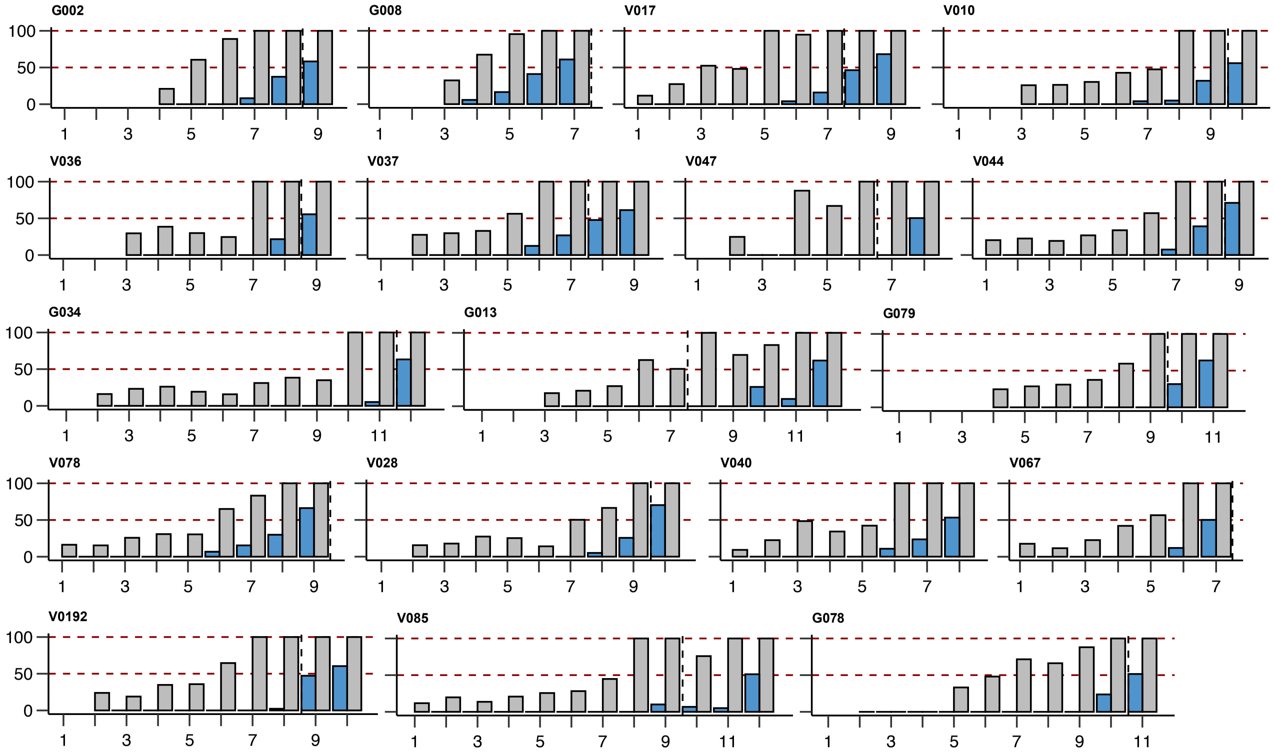


Figure S5. Continued

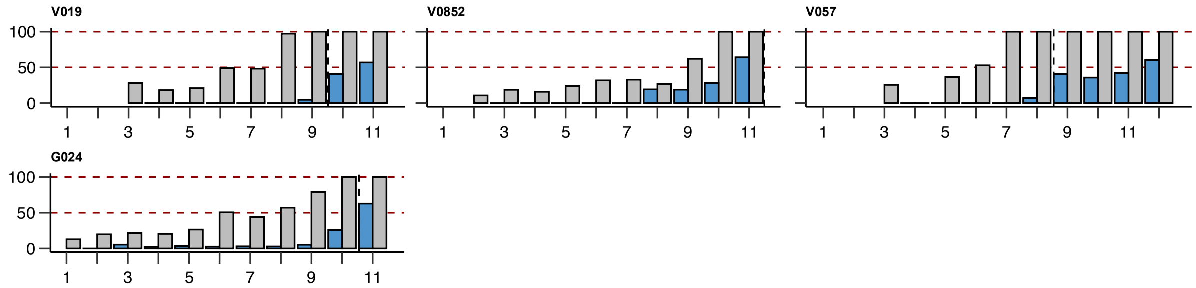
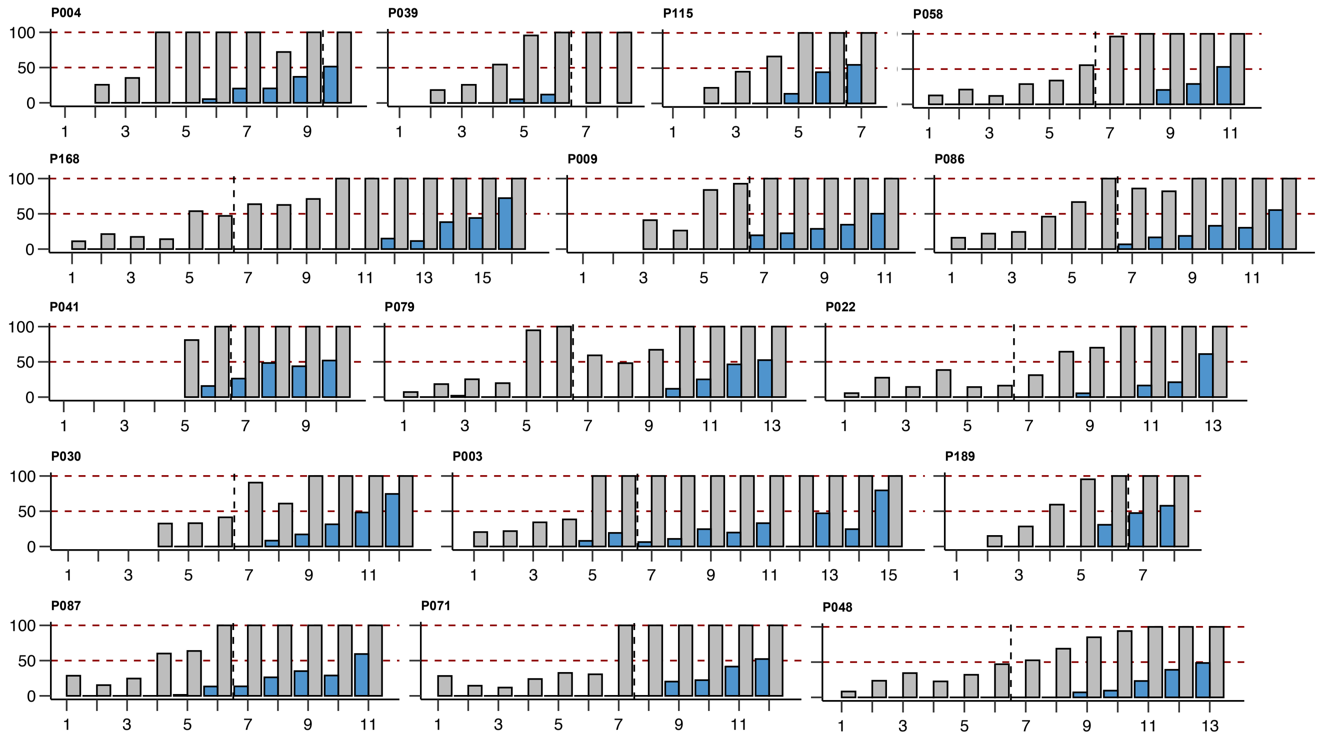


Figure S6. Bar graphs showing the increase of diurnal and nocturnal nest attentiveness until the onset of diurnal full incubation behaviour in Pina. Vertical axes show nest attentiveness rate and horizontal axes refer to egg days (1 = Laying date). Grey bars show nocturnal incubation and blue bars diurnal incubation. Nocturnal full incubation is accomplished when nest attentiveness reaches 100% for the first time. Diurnal full incubation is accomplished when females reach 50% of diurnal nest attentiveness for the first time. Vertical dashed lines show the day the clutch was completed. Alphanumerical codes identify each nestbox. Asterisk highlights a nest that was removed from diurnal partial incubation statistics because the female displayed an unusual and lasting behaviour.



Appendix 2

Table S1. Descriptive statistics of clutch size in the three breeding populations during three years. We used raw probes in years 2015-2016. In 2017 the probes were inserted in a fake egg.

	Pina			**Quintos**			**Sagunto**		
	n	**mean ± sd**	**range**	***n***	**mean ± sd**	**range**	***n***	**mean ± sd**	**range**
2015	5	6.6 ± 0.89	5-7	5	8.4 ± 0.55	8-9	13	8.2 ± 1.28	6-10
2016	5	7.2 ± 0.84	6-8	8	9.1 ± 2.03	6-12	12	8.8 ± 0.87	10-12
2017	4	7.0 ± 0.00	7-7	6	9.7 ± 1.37	8-12	11	8.5 ± 0.94	9-11

Table S2. Model selection results from linear mixed models analysing the influence of incubation day, hour of the day, clutch size, ambient temperature (T), population, year and nest volume (NV) in nest attentiveness, on- and off-bout duration. The interaction between temperature and population was subject to model selection. Nest identity was added as a random variable. Selected models are highlighted in bold.

Candidate models	AICc	ΔAICc	AICw
Hourly nest attentiveness			
Inc. day + Hour^2 + Clutch size + T^2*Population+ Year + NV	**67565.85**	**0.00**	**1.00**
Inc. day + Hour^2 + Clutch size + T^2 + Population+ Year + NV	67641.50	75.65	0.00
Off-bout duration			
Inc. day + Hour^2 + Clutch size + T^2*Population+ Year + NV	**20340.39**	**0.00**	**1.00**
Inc. day + Hour^2 + Clutch size + T^2 + Population+ Year + NV	20355.20	14.81	0.00
On-bout duration			
Inc. day + Hour^2 + Clutch size + T^2*Population+ Year + NV	**97228.47**	**0.00**	**1.00**
Inc. day + Hour^2 + Clutch size + T^2 + Population+ Year + NV	97297.66	69.19	0.00

[a] The *AICc* measures the relative quality of a candidate model, adjusted for sample size.

[b] *ΔAICc* is defined as the *AICc* differences between two proposed models. When *ΔAICc* > 2.0, the difference between the two competing models is substantial (Burnham & Anderson 2002)

'*' Denotes an interaction between two variables.

Table S3. Model selection results from linear mixed models analysing the influence of the incubation day, clutch size, ambient temperature (T), population, year, date and nest volume (NV) on daily nest attentiveness, number of daily off-bouts and active day length. For nest attentiveness models we added active day as explanatory variable. For active day models, we substituted date for daily daylight duration. The interaction between temperature and population was subject to model selection. Nest identity was added as a random variable. Selected models are highlighted in bold.

Candidate models	AICc	ΔAICc	AICw
Daily nest attentiveness			
Active day + Inc. day + Clutch size + T^2*Population + Year + Date + NV	**2964.32**	**0.00**	**1.00**
Active day + Inc. day + Clutch size + T^2 + Population + Year + Date +NV	2999.62	35.30	0.00
Daily number of off-bouts			
Inc. day + Clutch size + T^2*Population + Year + Date + NV	**3657.16**	**0.00**	**1.00**
Inc. day + Clutch size + T^2 + Population + Year + Date + NV	3681.00	23.85	0.00
Daily active day duration			
Daylight length + Inc. day + Clutch size + T^2*Population + Year + NV	**1148.22**	**0.00**	**1.00**
Daylight length + Inc. day + Clutch size + T^2 + Population + Year + NV	1194.162	45.94	0.00

Table S4. Model selection results assessing the effect of clutch size, incubation onset date, year, breeding population and nest volume (NV) on nest attentiveness (generalized linear models) and duration of the full incubation period (linear models). For nest attentiveness models we added mean ambient temperature of the full incubation period. The interaction between temperature and population was subject to model selection. For incubation duration models we added duration of partial incubation, nest attentiveness rate for the full incubation period, and number of days that a female incubated before clutch completion (CC). These last variables were under model selection. Selected models are highlighted in bold.

Candidate models	AICc[a]	ΔAICc[b]	AICw
Full incubation nest attentiveness			
Clutch size + Inc. onset + Year + Population*Temperature + NV	**481.36**	**0.00**	**0.98**
Clutch size + Inc. onset + Year + Population + Temperature + NV	488.89	7.53	0.02
Full incubation duration			
Clutch size + Inc. onset + Year + Population + NV + Attentiveness + Partial inc.	**192.09**	**0.00**	**0.67**
Clutch size + Inc. onset + Year + Population+ NV + Partial inc.	195.26	3.17	0.14
Clutch size + Inc. onset + Year + Population+ NV + Attentiveness + Partial inc. + CC	196.74	4.65	0.07
Clutch size + Inc. onset + Year + Population+ NV + Attentiveness	197.04	4.94	0.06
Clutch size + Inc. onset + Year + Population+ NV + Partial inc. + CC	198.10	6.00	0.03
Clutch size + Inc. onset + Year + Population+ NV + Attentiveness + CC	198.58	6.49	0.03
Clutch size + Inc. onset + Year + Population+ NV	200.84	8.75	0.01
Clutch size + Inc. onset + Year + Population+ NV + CC	203.46	11.37	0.00

Figure S1. Modelled variation of individual off-bouts (black lines) and on-bouts (grey lines) in relation to hour of the day. Dashed lines denote 95 % confidence intervals.

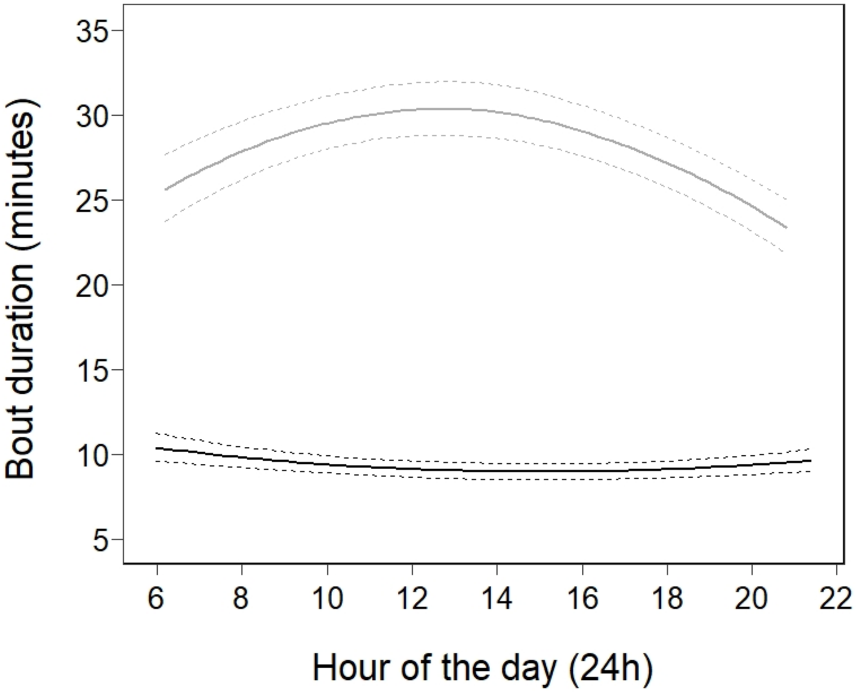
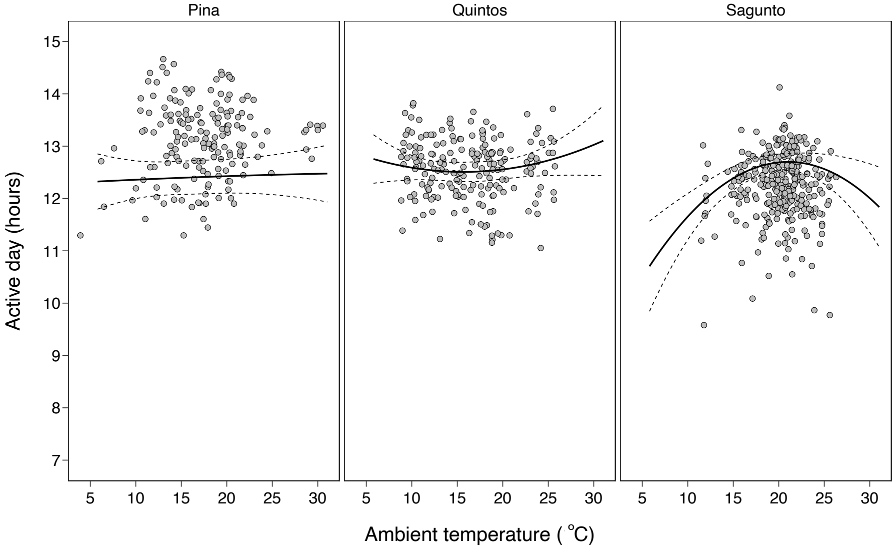


Figure S2. Modelled variation of active day duration (sum of daily hours) in relation to daily mean ambient temperatures. Dashed lines denote 95% confidence intervals. Circles represent jittered raw data.



Appendix 3

Figure S1. Photography of the experimental set up showing a nest with the eggs coded based on their laying order. This is nest ‘O463’ with a clutch size of 9 eggs. The photograph was taken on the 25th April during the first check of the day. Author: Samuel Rodríguez.

Table S1. Colour codes assigned to the experimental eggs in relation to their laying order within the clutch.

Laying order	Colour
1st	Blue
2nd	Black
3rd	Orange
4th	Green
5th	Blue-Blue
6th	Black-Black
7th	Orange-Orange
8th	Green-Green
9th	Blue-Orange
10th	Blue-Green
11th	Orange-Green
12th	Black-Orange

Table S2. Model selection results from Poisson GLMs testing the influence of group, incubation period, early incubation, clutch size and female age on hatching asynchrony.

Candidate models	**AICc** [a]	**ΔAICc** [b]	**AIC_W** [c]
Group + Female age	**63.5**	**0.00**	**0.34**
Group + Inc. period + Female age	64.7	1.24	0.18
Group + Female age + Early incubation	65.6	2.13	0.12
Group + Clutch size + Female age	65.9	2.40	0.10
Group + Inc. period*Female age	66.8	3.35	0.06
Group*Inc. period + Female age	67.4	3.91	0.05

[a] The *AICc* measures the relative quality of a candidate model, adjusted for sample size.

[b] *ΔAICc* is defined as the *AICc* differences between two proposed models. When ΔAICc > 2.0, the difference between the two competing models is substantial (Burnham & Anderson 2002). When *ΔAICc* < 2.0, the most parsimonious model is selected (final models are indicated in bold).

[c] *AIC_W* measures the relative weight that the model has in relation to the rest of proposed models. Only models with *$AIC_W \geq 0.05$ are shown.*

''* Denotes an interaction between two variables*.*

Table S3. Model selection results from Poisson GLMMs testing the influence of group, incubation period, early incubation, clutch size and the coefficient of variation of the eggs' distance ($CV_{distance}$) on hatching asynchrony of experimental nests. Nest is the random variable.

Candidate models	AICc	ΔAICc	AIC_W
$CV_{distance}$ + Clutch size + Inc. period	**257.9**	**0.00**	**0.57**
$CV_{distance}$ + Clutch size + Inc. period + Early incubation	259.3	1.44	0.28
$CV_{distance}$ + Inc. period + Early incubation	262.8	4.94	0.05

Table S4. Model selection results from LMs testing the influence of group, incubation period, early incubation, clutch size and female age on the length of the incubation period.

Candidate models	AICc	ΔAICc	AIC_W
Group + Female age + Early incubation	**78.9**	**0.00**	**0.80**
Group + Clutch size + Female age + Early incubation	81.8	2.93	0.19

Table S5. Model selection results from Binomial GLMs testing the influence of group, incubation period, early incubation, clutch size and female age on hatching success.

Candidate models	AICc	ΔAICc	AIC_W
Group*Inc. period + Clutch size + Female age	**69.2**	**0.00**	**0.79**
Group + Clutch size + Inc. period + Female age	72.5	3.25	0.16

Table S6. Model selection results from LMMs testing the influence of group, incubation period, female age and number of fledglings on the weight of chicks at fledgling. Nest is the random factor.

Candidate models	AICc	ΔAICc	AIC_W
Group + Female age	**457.9**	**0.00**	**0.35**
Group + Fledgling no + Female age	458.4	0.44	0.28
Group + Female age + Inc. period	460.1	2.17	0.12
Group*Inc. period + Female age	461.3	3.36	0.07
Group + Fledgling no + Female age + Inc. period	461.7	3.74	0.05
Group*Inc. period + Fledgling no + Female age	461.8	3.81	0.05
Group + Female age + Inc. period	461.9	3.99	0.05

Table S7. Model selection results from Binomial GLMs testing the influence of group, clutch size, incubation period, and female age on fledging success.

Candidate models	AICc	ΔAICc	AIC_W
Group + Clutch size + Female age	**82.7**	**0.00**	**0.51**
Group + Inc. period*Female age + Clutch size	83.7	1.01	0.31
Group + Clutch size + Inc. period + Female age	85.1	2.42	0.15

Resumen

INTRODUCCIÓN

La incubación es el proceso por el cual el calor necesario para el desarrollo embrionario es transferido al huevo después de su puesta (Beer 1964). El periodo de incubación es clave en el ciclo reproductor de las aves e influye en la posterior supervivencia y éxito reproductor tanto de los progenitores como de los polluelos (revisado en DuRant *et al.* 2013).

La incubación puede parecer un proceso simple, pero requiere de un estrecho rango de temperaturas (37-38 ºC) para el desarrollo de los embriones (Drent 1975), movimientos de rotación (Deeming 2002b, 2009) y posiblemente el desplazamiento de los huevos dentro del nido durante el periodo de incubación (Drent 1975). Si las temperaturas de incubación se mantienen de forma prolongada por debajo de 36,5-35,0 ºC, el desarrollo de los huevos y el éxito de eclosión podría verse afectado negativamente (Hepp *et al.* 2006, Olson *et al.* 2006, Nord & Nilsson 2011). Sin embargo, las hembras que incuban solas (especies gineparentales, ver más abajo) no pueden evitar caídas periódicas en la temperatura de los huevos cuando se alejan del nido para buscar comida. En estos casos, las hembras deberían impedir que los huevos alcancen temperaturas por debajo de 24-27 ºC, la temperatura del cero fisiológico (PZT en sus siglas en inglés, Drent 1975). Las hembras también deben evitar tiempos prolongados entre la PZT y la temperatura de incubación, ya que puede provocar malformaciones en el desarrollo de los tejidos del embrión (Drent 1975, Webb 1987). La rotación de los huevos es otro comportamiento imprescindible por parte de la hembra para conseguir que la puesta eclosione. La falta de rotación, sobre todo en el primer tercio del periodo de incubación, puede provocar problemas en el desarrollo de las membranas, transporte de fluidos y crecimiento embrionario en general (Deeming 1991, 2002b, 2009). Por último, el cambio de posición o desplazamiento de los huevos dentro del cuenco del nido podría deberse a la imposibilidad de que las hembras cubran toda la puesta con su cuerpo (Caldwell & Cornwell 1975, Löhrl 1986). Si el reposicionamiento de los huevos no se produce, se crearían diferencias térmicas entre los huevos que se encuentran directamente bajo la placa incubadora de la hembra y los huevos periféricos de la puesta (Boulton & Cassey 2012, Hope *et al.* 2018). Esto obligaría a las hembras a un intercambio de posiciones homogéneo entre el centro y la periferia para evitar diferencias en la velocidad de desarrollo de los embriones.

Aunque las estrategias de incubación son diversas y dependientes de la especie, este proyecto doctoral pondrá el foco en la estrategia mayoritaria observada en paseriformes o aves cantoras: especies donde la hembra es la única que incuba (62% de las especies de paseriformes, Williams 1996). Las puestas en los paseriformes eclosionan normalmente de forma asíncrona y los polluelos recién nacidos tienen los ojos cerrados, carecen de plumón y presentan una capacidad de movimiento muy restringida, necesitando del cuidado parental hasta que abandonan el nido (Starck & Ricklefs 1998). Como se ha mencionado en el párrafo anterior, el hecho de que la hembra sea la única que incuba conlleva ciertas limitaciones asociadas al mantenimiento de la correcta temperatura de la puesta. La hembra deberá repartir su tiempo entre los recesos, es decir tiempo para su propio mantenimiento alejada del nido (búsqueda de comida, acicalamiento y evitación de la depredación), y sesiones de incubación en el nido, tratando mantener un equilibrio adecuado (Conway & Martin 2000a). El ritmo de incubación, es decir, la combinación de las sesiones y los recesos, es la variable de mayor importancia para describir el comportamiento de una hembra incubadora, lo que se ha llamado atención al nido (Skutch 1962).

La duración de las sesiones y recesos es en buena medida dependiente de la temperatura ambiental. Si las temperaturas suben, la tasa de enfriamiento de los huevos se reduce, facilitando que las hembras puedan alargar el tiempo fuera del nido, pero también las sesiones de incubación, porque disminuye el gasto energético (Conway & Martin 2000a, Cooper & Voss 2013). Las sesiones suelen alargarse más, por lo que un incremento de temperaturas normalmente conlleva un incremento de la atención al nido. En cambio, en respuesta al aumento de la temperatura ambiente durante la incubación, las hembras también pueden disminuir la atención al nido, dedicando más tiempo a actividades fuera del mismo (p. ej. Walters *et al.* 2016). Ambos comportamientos, el incremento de la atención al nido, o del tiempo fuera del nido, se han descrito tanto en poblaciones de distintas especies de aves cantoras como en distintas poblaciones de la misma especie. Las posibles causas que llevan a uno u otro tipo en el comportamiento durante la incubación no son claras.

Junto con la atención al nido, el comienzo mismo de la incubación puede ayudar a entender el comportamiento de las hembras durante este periodo y su efecto posterior en la eclosión de los huevos. Empezando por el final, la causa próxima de que los huevos eclosionen de manera no sincrónica es que las hembras empiecen a incubar antes de que finalice la puesta, haciendo que los primeros huevos de la puesta comiencen a desarrollarse antes de que los últimos se hayan puesto (Stoleson & Beissinger 1995). La falta de sincronía en la eclosión ha sido tratada históricamente como un mecanismo adaptativo, sobre todo en escenarios de escasez de alimento (Lack 1947, Ricklefs 1965), para reducir la competencia entre hermanos (Hahn 1981, Gilby *et al.* 2011) o disminuir la carga de alimentación de los padres (Hussell 1972). Hipótesis alternativas la presentan como un subproducto de la necesidad de comenzar la incubación antes de completar la puesta, por ejemplo para amortiguar temperaturas ambientales que pueden provocar un desarrollo anormal de los embriones (Arnold *et al.* 1987, Veiga 1992) o para disminuir la proliferación de bacterias, que reducen la viabilidad de los huevos. El adelanto del comienzo de la incubación también se puede ver como un intento de las hembras para sincronizar el periodo de máxima demanda de comida de sus polluelos con la máxima disponibilidad de presas (Perrins 1991, Noordwijk *et al.* 1995). Esto podría ocurrir en escenarios donde la temperatura ambiente aumenta una vez la puesta ha comenzado, y las hembras necesitan reajustar la fecha de puesta esperada ante un crecimiento más rápido de sus principales presas, como podrían ser las orugas.

El comienzo de la incubación sin embargo no es tan fácil de medir porque es gradual, empezando por lo que se denomina incubación parcial y acabando en incubación completa. Ambos pudiendo iniciarse antes del fin de la puesta. Además, se la incubación se compone de un periodo diurno y uno nocturno que difieren en el tipo de incubación: intermitente y continua respectivamente (Deeming 2002a). El comienzo gradual dificulta establecer límites entre los tipos de incubación y analizar correctamente el papel que desempeña cada uno de ellos en la duración de la eclosión.

OBJETIVOS

La finalidad de esta tesis doctoral fue mejorar nuestro entendimiento del comportamiento de incubación en paseriformes en los que sólo la hembra incuba. Durante la elaboración de la tesis revisité conceptos básicos sobre incubación gracias a la recolección de datos de alta resolución en tres poblaciones reproductoras de Carbonero común *Parus major*. El objetivo general ha sido intentar identificar tanto respuestas generalizadas como locales a cambios en la temperatura ambiente y variables reproductoras asociadas. Los objetivos concretos fueron:

1. Describir y cuantificar el comienzo de la incubación. Analizar cómo la temperatura ambiente se relacionó con el inicio de cada periodo de incubación (diurna parcial y completa, y nocturna parcial y completa) y la duración de los periodos parciales. Investigar si estas relaciones mostraron el mismo patrón entre las poblaciones reproductores. Evaluar además el papel que variables reproductoras como la fecha de puesta, el tamaño de puesta y el año pueden tener sobre los diferentes comienzos de la incubación y la duración de las incubaciones parciales.

2. Evaluar cómo el comportamiento de incubación, una vez establecida la incubación completa diurna, se relaciona con el incremento de la temperatura ambiente. Investigar si las respuestas de las hembras al incremento de temperatura, aumentando la atención al nido o dedicando más tiempo a su propio mantenimiento fuera del nido, fueron dependientes de la población o podrían generalizarse. Evaluar además cómo los patrones de atención al nido cambiaron dependiendo de la escala temporal de interés (p. ej. atención al nido horaria, diaria o abarcando todo el periodo de incubación).

3. Investigar el papel de los diferentes periodos de incubación antes de la incubación completa diurna en la propia duración de este periodo. Analizar el posible papel del esfuerzo de incubación, es decir la atención al nido, en la duración de este periodo de

incubación diurna completa. Evaluar, mediante una aproximación experimental, cómo evitar la incubación gracias durante el periodo de puesta de los huevos afectó a la duración de la incubación diurna completa.

4. Evaluar el efecto de la incubación diurna y nocturna antes del comienzo de la puesta en la duración de la asincronía de eclosión. Investigar como la asincronía se redujo después de impedir experimentalmente que la puesta fuera incubada de forma parcial. Además, cuantificar el intercambio de posiciones de los huevos llevado a cabo por la hembra en el cuenco del nido y evaluar su papel en la asincronía de eclosión.

CAPÍTULO 1

Los efectos de la temperatura ambiente y el tamaño de puesta en el comienzo del comportamiento de incubación de un ave cantora donde la hembra es la única que incuba.

Introducción

Las aves son capaces de seguir los cambios ambientales para sincronizar el pico de demanda de comida de sus polluelos con la máxima abundancia de presas (Perrins 1991, Cresswell & McCleery 2003). Para las aves cantoras que alimentan a sus polluelos con orugas, la temperatura ambiente durante la primavera es una señal para la futura disponibilidad de presas (Noordwijk *et al.* 1995) y las hembras la pueden usar para decidir cuándo comenzar la puesta. Sin embargo, una vez que comienzan a poner huevos, las hembras necesitarían mantener en seguimiento los cambios de temperatura para reajustar sus fechas de eclosión con el esperado pico de orugas (García-Navas & Sanz 2011). Con el aumento de temperaturas, las hembras adelantarían sus fechas de eclosión en previsión de un crecimiento más rápido de las orugas (Visser *et al.* 1998), por el contrario intentarían retrasar la eclosión de la puesta en caso de un descenso de las temperaturas (García-Navas & Sanz 2011).

Adelantar o retrasar el comienzo de la incubación es el principal mecanismo mediante el cual las hembras pueden ajustar las fechas de eclosión en respuesta a los cambios en la temperatura ambiente (Tomás 2015). Adelantar el comienzo de la incubación acorta el periodo de incubación (Nilsson & Svensson 1993a), pero también favorece la eclosión asíncrona (Ardia *et al.* 2006, Johnson *et al.* 2013). En cambio, retrasar el comienzo de la incubación reduce el éxito de eclosión de los huevos (Naef-Daenzer *et al.* 2004). Por otra parte, puestas más grandes dificultarían el adelanto del comienzo de la incubación, porque poner huevos e incubar a la vez es muy costoso energéticamente (Nord & Williams 2015).

El comienzo de la incubación es algo esquivo en la literatura. Solemos hablar del comienzo de la incubación como el comienzo de la incubación diurna completa (Wang & Beissinger 2011). Aunque la incubación puede empezar antes en forma de incubación diurna parcial, un periodo irregular de desarrollo de la incubación (Haftorn 1981). Los efectos de la incubación parcial están en disputa, habiendo estudios que muestran como una incubación parcial más larga aumenta el grado de eclosión asíncrona y acorta el periodo de incubación (Stoleson & Beissinger 1995), mientras que otros achacan estos mismos efectos al comienzo de la incubación completa antes de que finalice la puesta, lo que se llama incubación temprana (Podlas & Richner 2013a). Pero no sólo por el día, la incubación también se produce por la noche. La incubación nocturna es continua, en contraposición a la intermitente diurna (Deeming 2002a), y tiene sus propios periodos de incubación parcial y completa. La incubación nocturna también podría tener efectos en la eclosión y el periodo de incubación, pero su papel exacto es desconocido.

En este estudio, exploramos el comportamiento incubador de hembras de carbonero común en tres poblaciones, centrándonos en el comienzo y duración de la incubación parcial y en el comienzo de la incubación completa. Evaluamos la relación entre la temperatura ambiente y el comienzo de la incubación, junto con variables reproductoras como el tamaño de puesta, y si la relación es generalizable entre las poblaciones. Por último, investigamos la duración del periodo de incubación diurna completa, comúnmente denominado como periodo de incubación, en relación al comienzo de los distintos periodos de incubación previos.

Métodos

Recogimos datos de incubación durante tres años en tres poblaciones reproductoras del ámbito mediterráneo donde los carboneros usan cajas-nido para criar. Las poblaciones se encuentran en la misma latitud, pero diferente altitud, y muestran diferente composición de hábitat. Mediante el uso de registradores de datos de temperatura en el nido extrapolamos el comportamiento de incubación de las hembras. La técnica consiste en introducir un termopar, incrustado en un huevo falso relleno de gel, entre los huevos de la puesta. La temperatura en el nido la medimos cada 10 segundos, a la máxima resolución posible. De esta forma se registran descensos y subidas de temperatura, que equivalen a salidas por recesos y entradas por sesiones de incubación de la hembra. Al mismo tiempo registramos la temperatura ambiente de cada caja-nido gracias al uso de registradores de temperatura iButton sujetos a una de las paredes internas de cada una de las cajas-nido. Obtuvimos 77 nidos en total con datos de buena calidad sobre incubación. Además del registro de temperaturas, tuvimos en cuenta el tamaño de puesta, la duración del periodo de incubación y la sincronía en la eclosión.

Establecimos que la incubación diurna completa comienza el primer día que la hembra supera el 50% de la atención al nido durante su día activo (ver también Cresswell & McCleery 2003), es decir, el primer día que la suma de la duración de todas las sesiones de incubación llega o supera a la mitad del tiempo entre el primer receso del día y la última vez que la hembra entra al nido para pasar la noche. La incubación nocturna comenzaría cuando la hembra alcanza el 100% de la atención al nido durante la parte inactiva del día. La duración de la incubación parcial diurna y nocturna se calculó como la suma del número de minutos de las sesiones de incubación. El análisis de las temperaturas del nido extrapolándolas a comportamientos de incubación se llevó a cabo gracias al software Raven y Rhythm (Cooper & Mills 2005).

Creamos varios modelos lineales para analizar la relación entre la temperatura ambiente durante la puesta y el comienzo de la incubación diurna parcial y completa, la duración de la incubación parcial tanto diurna como nocturna, y el comienzo de la incubación nocturna. El comienzo de la incubación parcial nocturna no fue modelizado porque la mayoría de las hembras comenzaron el mismo día del inicio de la puesta. Como variables predictoras añadimos parámetros reproductores que pudieran afectar a la incubación: el tamaño de puesta, la fecha de puesta, la población y el año. Para evaluar si el efecto de la temperatura es generalizable en las tres poblaciones estudiadas, creamos dos modelos estadísticos para cada periodo de incubación analizado, uno incluyendo la interacción entre la temperatura ambiente y la población y otro que carecía de ella. Además, creamos un grupo de modelos lineales para evaluar cómo la duración de las incubaciones parciales nocturna y diurna, y el comienzo de la incubación nocturna completa, afectan a la duración del periodo de incubación completa diurna. Para evaluar el efecto de los distintos comportamientos de incubación en el grado de eclosión asíncrona creamos modelos de regresión tipo beta, y añadimos como variables a seleccionar la duración de los periodos de incubación que suceden antes de que la puesta finalice.

Resultados

El incremento de la temperatura ambiente durante la puesta sólo estuvo relacionado con el avance de la incubación parcial diurna, siendo el efecto similar en las tres poblaciones (Tabla 3, Figura 5a). En cambio, cuanto mayor fue la puesta, más se alargó la incubación parcial nocturnal (Tabla 2, Figura 4a) y más se retrasó el comienzo de la incubación completa nocturna (Tabla 2, Figura 4b). Sólo los periodos diurnos de incubación estuvieron asociados con la duración del periodo de incubación completa y con la eclosión asíncrona. Cuanto más larga fue la incubación parcial más corto fue el periodo de incubación (Tabla 4). En la misma línea, cuanto más largas fueron la incubación parcial y la incubación completa antes de finalizar la puesta, mayor grado de asincronía en la eclosión se observó (Tabla 4).

Discusión

La incubación nocturna parece estar relacionada a la condición física de la hembra, ya que no encontramos una relación clara entre la temperatura ambiente y su comienzo o duración, pero sí con el tamaño de puesta. Es posible que las hembras no puedan lidiar a la vez con la incubación nocturna completa y la puesta de huevos. A pesar de que la incubación nocturna tuvo una duración mayor que la diurna, antes de que la hembra finalizase la puesta, no detectamos ninguna relación con la duración del periodo de incubación o en la duración de la eclosión. Sin embargo, las temperaturas de incubación durante las fases nocturnas suelen superar el PZT, teniendo la capacidad de iniciar el desarrollo embrionario (Lord *et al.* 2011, Vedder 2012).

El hecho de que el comienzo de la incubación parcial diurna esté relacionado con la temperatura ambiente pero no así el comienzo de la incubación completa es muy interesante. La incubación completa sólo comenzó antes cuando la parcial se adelantó, es decir, los estudios anteriores que sí encontraron un efecto de la incubación completa (Naef-Daenzer *et al.* 2004, Kluen *et al.* 2011) podrían haber detectado un efecto arrastre, pero al no medir la incubación parcial no se puede asegurar.

La respuesta de la incubación parcial diurna ante el incremento de temperaturas es clave para los subsiguientes procesos reproductivos. La mayor duración de la incubación parcial acorta el periodo de incubación (Nord & Nilsson 2012, Macdonald *et al.* 2013) pero alarga la eclosión. El primero favorecería la sincronización con el pico de presas, pero la eclosión asíncrona suele tener efectos negativos en el crecimiento de los polluelos nacidos más tarde (Amundsen & Slagsvold 1998, Szöllosi *et al.* 2007). Aunque carecemos de datos sobre la abundancia de presas en este estudio, nuestros resultados indican que las hembras sí regulan el comienzo de la incubación, afectando a la duración del periodo, en respuesta a la temperatura ambiente.

CAPÍTULO 2

Deconstruyendo el comportamiento de incubación en respuesta a temperaturas ambientales en diferentes escalas temporales

Introducción

Durante la incubación completa diurna, las hembras de carbonero común tienen que distribuir su tiempo entre las sesiones de incubación y los recesos fuera del nido. Esta incubación intermitente causa caídas regulares de temperatura en la puesta, aunque que la hembra debe evitar que sean por debajo de 24-27ºC, el cero fisiológico bajo el cual el crecimiento se suspendería (Drent 1975, Haftorn 1988). Por lo tanto, la hembra debe adaptar su comportamiento a la temperatura ambiental y regular la duración de las sesiones y los recesos.

Se espera que las hembras aumenten la duración de recesos y sesiones cuando las temperaturas aumentan (Conway & Martin 2000a), aumentando en el proceso su atención al nido. Un mayor esfuerzo de incubación cuando las limitaciones se relajan, es decir cuando la temperaturas aumentan, acortaría el periodo de incubación, asociado a una reducción del riego de depredación (Conway & Martin 2000b) y un incremento del éxito de eclosión (Nord & Nilsson 2011). En cambio, se han estudiado poblaciones donde las hembras aumentan el tiempo dedicado a su propio mantenimiento, en lugar de aumentar la atención al nido, con el aumento de temperaturas (Walters *et al.* 2016). Esta respuesta en el comportamiento de incubación ocurre tanto en poblaciones de distintas especies como en poblaciones de una misma especie. Incrementar el tiempo de mantenimiento podría ser una estrategia alternativa bajo ciertas condiciones ambientales (MacDonald *et al.* 2014, Capp *et al.* 2018), pero la disparidad de resultados también podría deberse a problemas derivados de grupos de datos limitados a unos pocos días u horas dentro del periodo de incubación (Bueno-Enciso *et al.* 2017), o relacionados con las distintas escalas de tiempo en las que se analizan estos datos.

Además de la temperatura, hay otras variables que afectan al comportamiento durante la incubación porque afectan a las tasas de enfriamiento de los huevos. Por ejemplo, en puestas más grandes los huevos se enfrían más despacio (Boulton & Cassey 2012). Lo mismo pasa con un mayor volumen del nido, que aislaría mejor a la puesta, permitiendo que la atención al nido se incremente (Bueno-Enciso *et al.* 2017). En ambos casos la reducción en las tasas de enfriamiento fue aprovechada por las hembras para aumentar el tiempo dedicado a su propio mantenimiento fuera del nido en lugar de para aumentar el esfuerzo de incubación. Además, variables temporales como el día de incubación o la fecha del año también causarían cambios en la atención al nido. Cuánto más cerca de la fecha de eclosión se encuentra una hembra, mayor esfuerzo de incubación necesita hacer (Cooper & Voss 2013), y el mismo efecto tendría incubar más tarde en la temporada reproductora (Ardia *et al.* 2006).

En este trabajo, evaluamos cómo la temperatura afecta a la duración de los recesos y las sesiones de incubación, y cómo la atención al nido derivada de éstos varía según la escala de tiempo en la que se analice (atención al nido cada hora, diaria o para todo el periodo de incubación). Buscamos saber si las respuestas a la temperatura ambiental son generalizables entre poblaciones de carbonero común con distinta temperatura ambiente media. Además, evaluamos la relación entre el tamaño de puesta, el volumen del nido, el día de incubación y el día del año con la atención al nido. Por último, investigamos el papel de la atención al nido en la duración del periodo de incubación y la duración del día activo de la hembra.

Métodos

La metodología para la recolección de datos en el campo es similar a la utilizada en el capítulo 1. Medimos el comportamiento de incubación de las hembras en 69 nidos durante tres años en tres poblaciones reproductoras de la península ibérica. Mediante el uso de termopares acoplados a registradores de datos fuimos capaces de detectar caídas y rebrotes de temperatura en el nido que equivalen a recesos y sesiones de incubación de la hembra. El extremo del termopar que es sensible a la temperatura se colocó en el centro de la puesta y se mantuvo durante todo el periodo de incubación midiendo la temperatura del nido cada 10 segundos. La temperatura ambiente se registró en cada caja-nido mediante un registrador de datos tipo iButton.

Igualmente, establecimos el comienzo de la incubación como el primer día que la atención al nido alcanzó el 50 % del tiempo activo de la hembra (Cresswell & McCleery 2003, Simmonds *et al.* 2017). Es decir, la suma de minutos de todas las sesiones de incubación fue al menos la mitad del tiempo transcurrido desde que la hembra deja el nido por primera vez por la mañana, hasta que vuelve al nido de forma definitiva por la tarde para pasar la noche. Por lo tanto, el periodo de incubación abarcó desde el primer día que la atención al nido alcanzó el 50 % hasta el día antes de la eclosión del primer huevo.

Analizamos la duración de las sesiones y recesos en relación a la temperatura ambiental y diferentes variables reproductivas. Creamos modelos lineales mixtos y, como la duración de cada receso o sesión de incubación está relacionada con la duración del anterior y afectará al siguiente, nuestros modelos incluyeron un ajuste a la autocorrelación temporal que presentan los datos.

La atención al nido fue analizada desde tres escalas temporales distintas: horaria, diaria y abarcando el periodo de incubación completo. Para las dos primeras escalas temporales usamos modelos lineales mixtos y, para la última, modelos lineales generalizados.

La duración del día activo y la duración del periodo de incubación fueron analizados con modelos lineales mixtos y modelos lineales respectivamente. No incluimos la temperatura ambiental en relación a la duración del periodo de incubación porque incluimos la atención al nido, y las dos variables están correlacionadas.

Para cada variable dependiente elegida, construimos dos modelos distintos, uno con la interacción entre la temperatura ambiental y la población y otro sin dicha interacción, para poder discernir si nuestros resultados son generalizables a las tres poblaciones objetivo o no.

Resultados

Las respuestas a un incremento de temperatura fueron diferentes dependiendo de la población y la escala temporal medida. Dependiendo de la población, las hembras mostraron o un incremento del esfuerzo de incubación, o un incremento del tiempo dedicado al propio mantenimiento, en la escala temporal que abarca todo el periodo de incubación (Tabla 4, Figura 3). En cambio, para la atención al nido en la escala horaria o diaria, ambas respuestas ocurrieron en cada población (Figura 3). La transición entre un comportamiento y el otro estuvo relacionada con la temperatura ambiente local.

La duración máxima de las sesiones de incubación se registró a diferentes temperaturas dependiendo de la población (Tabla 2, Figura 2b). Los recesos aumentaron linealmente con el incremento de temperaturas en todas las poblaciones (Tabla 2, Figura 2a). La combinación entre sesiones y recesos alcanzando máximos a distintas temperaturas explica los distintos patrones de atención al nido observados en cada escala temporal y entre poblaciones (Figura 3).

Los otros parámetros reproductivos medidos mostraron un mismo efecto en las distintas escalas temporales: las hembras aumentaron la atención al nido según la incubación sucedió más tarde en la temporada reproductora (Tablas 2-4). Las hembras que construyeron nidos más grandes tuvieron sesiones de incubación más cortas, disminuyendo la atención al nido (Tablas 2 y 4). No encontramos ningún efecto del tamaño de puesta en la incubación. Por último, observamos que un mayor esfuerzo de incubación acortó el periodo de incubación completa diurna (Tabla 4).

Discusión

La dicotomía entre invertir tiempo en comportamientos de incubación o en el mantenimiento propio no apareció entre poblaciones, pero sí dentro de ellas. En cada una de las poblaciones estudiadas se pudieron observar ambos comportamientos. Las hembras respondieron a la temperatura ambiente local, y no acorde a un rango de temperaturas generalizado, como se había sugerido hasta el momento en estudios previos con aves cantoras (Conway & Martin 2000a). Las combinaciones de recesos y sesiones según la temperatura local causarían los distintos patrones que se observan en las poblaciones. Las escalas temporales diaria y horaria dan información suficiente sobre la atención al nido y cómo cambia según la temperatura ambiente, mientras que la atención al nido cómo un único valor describiendo todo el periodo de incubación puede llevar a confusión, concluyendo que las hembras desarrollan patrones de incubación contrarios. Aun así, puede ser útil usar un valor único de atención al nido para estimar el efecto general que el esfuerzo de incubación pueda tener sobre la duración del propio periodo de incubación (ver más abajo).

Las hembras disminuyeron su atención al nido y aumentaron el tiempo empleado en su propio mantenimiento si los nidos eran más voluminosos y conforme avanza la estación reproductora, indicando que las hembras en general no incrementan su esfuerzo de incubación cuando las limitaciones se lo permiten, sino que ese tiempo lo usan en beneficio propio. La abundancia de presas (Londoño *et al.* 2008), el riesgo de depredación (Ghalambor & Martin 2002), o la ayuda del macho alimentando a la hembra durante la incubación (Matysioková & Remeš 2014) pueden ser factores detrás de algunos de los patrones locales observados que no pueden ser explicados únicamente por la temperatura ambiente, y que sería interesante considerar en futuros estudios.

El acortamiento del periodo de incubación estuvo relacionado con un incremento del esfuerzo de incubación. Aunque parezca un resultado lógico, en Quintos esto implicaría que se acorta el periodo de incubación diurna completa cuando las temperaturas descienden. Sin embargo, en un marco ecológico, este comportamiento podría dar lugar a un desequilibrio trófico. Teóricamente un mayor esfuerzo de incubación se asociaría a un incremento de temperaturas, para poder mantener una hipotética sincronización con el pico de abundancia de presas (Perrins 1991, Noordwijk *et al.* 1995) mediante un acortamiento del periodo como vimos en el capítulo 1.

CAPÍTULO 3

El papel de la incubación parcial y el reposicionamiento de los huevos en la eclosión asíncrona y los subsiguientes efectos en el éxito reproductor

Introducción

Las aves pueden mostrar patrones de eclosión síncronos, cuando todos los huevos eclosionan en menos de 24 horas, o asíncronos, cuando eclosionan con más de 24 horas de diferencia (Stoleson & Beissinger 1995). En las especies asíncronas, muchas de ellas paseriformes, se crea una jerarquía entre los polluelos que puede tener consecuencias tanto positivas (Gilby *et al.* 2011) como negativas para la pollada (Szöllosi *et al.* 2007). Diversas teorías han intentado explicar este fenómeno pero, independientemente de si la eclosión asíncrona es adaptativa o no (ver Stoleson & Beissinger 1995), el principal mecanismo estudiado que la provoca es la hembra incubando los huevos antes de que la puesta se complete. Como hemos visto en el capítulo 1, la incubación durante la puesta de los huevos puede ser parcial, una fase inicial e irregular de la incubación que es escasamente tenida en cuenta (Podlas & Richner 2013b), o completa, cuando el ritmo de incubación es constante (Wang & Beissinger 2011). Si la incubación completa comienza antes de que la puesta finalice, se denomina incubación temprana (Wang & Beissinger 2011). Tanto la incubación parcial como temprana causan eclosiones asíncronas (Veiga 1992, Lord *et al.* 2011, Johnson *et al.* 2013) y adicionalmente causan un acortamiento del periodo de incubación (Ricklefs 1993, Stoleson & Beissinger 1995). Sin embargo, la eclosión asíncrona de los huevos puede suceder incluso cuando ambos tipos de incubación están ausentes (Clark & Wilson 1981, Magrath 1990), por lo que debe haber algún o algunos mecanismos adicionales que causen la asincronía.

Uno de ellos puede ser la presencia de gradientes térmicos en el nido (Drent 1975). La hembra incuba a mayor temperatura los huevos que se encuentran directamente debajo de su placa incubadora que los que se encuentran en posiciones más periféricas (Boulton & Cassey 2012, Hope *et al.* 2018), lo que causaría un desarrollo más rápido en los huevos centrales comparados con los periféricos (Nord & Nilsson 2011). La observación de hembras que cambian la posición de sus huevos dentro de la puesta (Caldwell & Cornwell 1975), podría indicar que, o bien las hembras tratan homogeneizar la distribución de calor en la puesta, o tratan de beneficiar a algunos huevos de la puesta llevándolos más frecuentemente al centro.

El experimento se desarrolló en una población de carbonero común, una especie que se sabe que tiene tanto incubación parcial (Podlas & Richner 2013a) como temprana (Haftorn 1981, Álvarez & Barba 2014a). Las principales preguntas fueron: 1) cuánto afecta la incubación parcial a la sincronía de eclosión, y 2) conocer si el movimiento de los huevos dentro del nido afecta a la eclosión asíncrona una vez se ha eliminado la incubación parcial y se ha cuantificado la incubación temprana. Nuestra hipótesis es que la eclosión asíncrona será menor en nidos donde no ha habido incubación parcial o donde los huevos se han reposicionado homogéneamente. Esperamos además un alargamiento del periodo de incubación en los nidos sin incubación parcial.

Métodos

Durante la temporada reproductora de 2014 seleccionamos entre las primeras puestas 20 parejas de nidos, con fechas de puesta y tamaños de puesta similares. Un nido de cada pareja se asignó al grupo experimental, sirviendo el otro de control. Los nidos experimentales fueron visitados diariamente durante las últimas fases de construcción. Cuando el primer huevo apareció en cada uno de los nidos, se marcó y reemplazó por uno falso con la misma marca. El huevo original se conservó en una caja-nido cercana a la experimental. Con los siguientes huevos de cada nido se realizó el mismo procedimiento, sustituyéndolos el día de puesta. Cuando se detectó el comienzo de la incubación, la puesta fue devuelta al nido original. Al día siguiente se realizaron dos fotografías, a primera hora de la mañana y a mediodía, de forma perpendicular a la puesta y mostrando claramente todos los huevos y sus marcas, junto con una regla de papel. Se siguieron sacando dos fotografías al día durante todo el periodo de incubación hasta que los huevos eclosionaron. Los nidos control se visitaron con menor frecuencia durante el periodo de incubación debido fueron utilizados únicamente para comparar parámetros reproductivos con los nidos experimentales pero no el movimiento de los huevos. Quince días después de la eclosión de los huevos, los polluelos fueron anillados y pesados tanto en los nidos experimentales como en los nidos control.

Los movimientos de reposicionamiento de los huevos fueron medidos como el coeficiente de variación de la distancia desde el centro de cada huevo al punto focal de la puesta. Para medir las distancias en las fotografías usamos el programa de procesamiento de imágenes ImageJ. El coeficiente de variación nos permite saber si un huevo cambia de posición entre la periferia y el centro (valores altos) o se mantiene en una posición relativamente estable (valores bajos) entre dos visitas al nido.

Llevamos a cabo análisis estadísticos entre los nidos control y experimentales para comparar la sincronía y éxito de eclosión y el porcentaje de polluelos que logran volar del nido (modelos lineales generalizados), la duración del periodo de incubación (modelos lineales) y el peso de los polluelos quince días después de nacer (modelos mixtos lineales). Para investigar el efecto del reposicionamiento de los huevos dentro de las puestas y su efecto en la eclosión, usamos modelos lineales mixtos generalizados.

Resultados

Impedir la incubación parcial de la puesta redujo la asincronía en la eclosión 0.84 días de media, un 51 % del total, pero no encontramos ningún efecto del coeficiente de variación de las distancias de los huevos en la eclosión (Tabla 1). La incubación temprana, es decir la incubación completa antes de la que la puesta finalizara, tampoco tuvo ningún efecto en la duración de la eclosión. La eclosión asíncrona se vio reforzada con el tamaño de puesta, pero disminuyó en incubaciones largas (Tabla 1). Impedir la incubación parcial alargó el periodo de incubación 1.82 días de media, lo que hizo que los nidos experimentales tuvieran un periodo con huevos en el nido 0.98 días más largo. El éxito de eclosión fue menor en nidos con la incubación más larga (Figura 2), pero mayor en nidos con puestas más grandes (Tabla 3). Evitar la incubación parcial, es decir, una mayor sincronía en la eclosión, no tuvo efectos en el peso de los polluelos, pero sí que aumentó el porcentaje de polluelos que sobrevivieron hasta volar del nido (Tabla 4).

Discusión

La incubación temprana ha sido considerada como el principal mecanismo que provoca la eclosión asincrónica (Stoleson & Beissinger 1997, Wang & Beissinger 2009) y el acortamiento del periodo de incubación (Podlas & Richner 2013a), descartando un posible efecto de la incubación parcial. Nuestros resultados muestran que, en realidad, la incubación parcial es la principal causante de una eclosión asíncrona de la puesta y que en su ausencia el periodo de incubación se alarga. Siendo esto último perjudicial para la tasa de eclosión de los huevos. En cambio, evitar la incubación parcial y, por tanto, reducir la asincronía de eclosión, causó un incremento en el porcentaje de polluelos que sobrevivieron hasta abandonar el nido. Reducir la jerarquía por tamaño con eclosiones más sincrónicas evitó efectos negativos comúnmente descritos (Szöllosi *et al.* 2007, Stenning 2008), como la reducción de pollada (Amundsen & Slagsvold 1998).

Nuestra hipótesis sobre el papel del reposicionamiento de los huevos en la duración de la eclosión no se vio refrendada por los datos. Es probable que las hembras reaccionen simplemente a los gradientes térmicos moviendo homogéneamente los huevos desde la periferia al centro del cuenco del nido, debajo de su placa incubadora. El hecho de que las hembras reposicionaran sólo algunos huevos de la puesta, y otros no, querría decir que pueden identificarlos por medio de alguna característica distintiva (Eichholz & Towery 2010, Poláček *et al.* 2017a), y hasta el momento no tenemos constancia de que esto pueda suceder en los carboneros. Por otro lado no podemos obviar que un mayor tamaño de puesta incrementó la eclosión asíncrona, lo que podría indicar que realmente las hembras tienen dificultades para cubrir la puesta con su cuerpo (Bortolotti & Wiebe 1993, Niizuma *et al.* 2005), resultando en huevos periféricos más fríos en puestas grandes, reduciendo la temperatura media de toda la puesta (Hope *et al.* 2018), a pesar de que el reposicionamiento dentro del cuenco fuera relativamente homogéneo.

CONCLUSIONES

1. Este proyecto de tesis, centrado en el comportamiento de incubación del carbonero común *Parus major* en tres poblaciones reproductoras mediterráneas, muestra que la temperatura ambiente es un factor clave para entender el comportamiento reproductor de las hembras.
2. El incremento de la temperatura ambiente desde que comienza la puesta de los huevos adelanta el comienzo, y alarga, la incubación parcial diurna, arrastrando a la incubación completa diurna a comenzar antes siguiendo un patrón similar entre las tres poblaciones reproductoras. Por otro lado, la incubación nocturna no está relacionada con la temperatura ambiente durante el periodo de puesta, pero podría haberse visto afectada por la temperatura antes de que comenzara la puesta.
3. Puestas más grandes alargan la incubación parcial nocturna y retrasan el comienzo de la incubación completa nocturna. Además, puestas más grandes también retrasan el comienzo de la incubación parcial diurna, explicando así la correlación que existe entre el comienzo de la incubación parcial diurna y la incubación completa nocturna. El efecto del tamaño de puesta señala a un alto gasto energético que limita a las hembras incubar

totalmente durante la puesta, retrasando el comienzo de la incubación parcial diurna, y contrarrestando el efecto del incremento de la temperatura.

4. Durante la incubación completa diurna, las hembras maximizan la duración de las sesiones de incubación dependiendo de las temperaturas locales de cada población, en lugar de mostrar un comportamiento de incubación generalizado en un rango común de temperaturas. Los patrones contrarios que se observan en la atención al nido medida para todo el periodo de incubación, es decir, aumentar el tiempo para el mantenimiento propio o el tiempo de incubación, se explican porque las hembras maximizan la duración de las sesiones de incubación a 10ºC o 15-20ºC en diferentes poblaciones. El efecto cuadrático de la temperatura ambiental en la atención del nido horaria o diaria provee mayor información y es más preciso, teniendo en cuenta los patrones de sesiones y recesos, que el efecto lineal de la atención al nido para todo el periodo de incubación.
5. Las hembras aumentan el esfuerzo de incubación con el incremento de temperaturas hasta cierto punto de inflexión, a partir del cual el tiempo para el mantenimiento propio aumenta en cada población. La causa detrás de la existencia de puntos de inflexión locales a partir de los cuales las hembras cambian su comportamiento de incubación e incrementan el mantenimiento propio necesita ser investigada en mayor detalle.
6. Cuando la incubación parcial diurna es más larga, el periodo de incubación completa diurna es más corto, efecto que puede ser indirectamente provocado por un incremento de la temperatura ambiente durante el periodo de incubación parcial diurna. Lo contrario, un alargamiento indirecto del periodo de incubación completa diurna debido a un acortamiento de la incubación parcial diurna sería causado por puestas más grandes. El efecto de la incubación parcial diurna sobre el periodo de incubación completa es congruente tanto en aproximaciones observacionales como experimentales: en ausencia de incubación parcial diurna, el periodo de incubación es más largo. No solo un comienzo más temprano pero también un mayor esfuerzo de incubación, una mayor atención al nido, acortó el periodo de incubación completa diurna. Por otro lado, la incubación nocturna, ya sea parcial o completa, no está relacionada con la duración del periodo de incubación.
7. La incubación parcial diurna es un periodo clave para la posterior supervivencia de los polluelos por su papel en la eclosión asíncrona, en comparación con la incubación completa diurna antes de completar la puesta, que mostró un efecto menor. La incubación nocturna, parcial o completa, no se relacionó con la asincronía en la eclosión a pesar de que la incubación parcial nocturna duró al menos el doble que la parcial diurna y de que el periodo de incubación completa nocturna comenzó días antes que la completa diurna. Evitar experimentalmente la incubación antes del comienzo de la incubación completa diurna también muestra que la asincronía en la eclosión aumenta, confirmando los resultados observacionales.

8. El reposicionamiento de los huevos dentro de la puesta es similar en nidos con diferente grado de eclosión asíncrona. Después de impedir que los huevos sean incubados durante el periodo de puesta, la eclosión asíncrona residual no tuvo relación con el reposicionamiento de los huevos.

References

Alambiaga, I., Álvarez, E., Diez-Méndez, D., Verdejo, J. & Barba, E. 2020. "The tale of the three little tits": Different nest building solutions under the same environmental pressures. *Avian Biol. Res.* **13**: 40–56.

Aldredge, R.A. 2017. Incubation onset maintains survival of most embryos and growth and survival of late-hatched young. *Anim. Behav.* **134**: 35–43.

Aldredge, R.A., Boughton, R.K., Rensel, M.A., Schoech, S.J. & Bowman, R. 2014. Hatching asynchrony that maintains egg viability also reduces brood reduction in a subtropical bird. *Oecologia* **174**: 77–85.

Álvarez, E. & Barba, E. 2011. Nest characteristics and reproductive performance in Great Tits *Parus major*. *Ardeola* **58**: 125–136.

Álvarez, E. & Barba, E. 2014a. Within and between population variations of incubation rhythm of Great Tits *Parus major*. *Behaviour* **151**: 1827–1845.

Álvarez, E. & Barba, E. 2014b. Incubation and hatching periods in a Mediterranean Great Tit *Parus major* population. *Bird Study* **61**: 152–161.

Álvarez, E., Belda, E.J., Verdejo, J. & Barba, E. 2013. Variation in Great Tit nest mass and composition and its breeding consequences: A comparative study in four Mediterranean habitats. *Avian Biol. Res.* **6**: 39–46.

Amininasab, S.M., Birker, M., Kingma, S.A., Hildenbrandt, H. & Komdeur, J. 2017a. The effect of male incubation feeding on female nest attendance and reproductive performance in a socially monogamous bird. *J. Ornithol.* **158**: 687–696.

Amininasab, S.M., Hammers, M., Vedder, O., Komdeur, J. & Korsten, P. 2017b. No effect of partner age and lifespan on female age-specific reproductive performance in blue tits. *J. Avian Biol.* **48**: 544–551.

Amininasab, S.M., Kingma, S.A., Birker, M., Hildenbrandt, H. & Komdeur, J. 2016. The effect of ambient temperature, habitat quality and individual age on incubation behaviour and incubation feeding in a socially monogamous songbird. *Behav. Ecol. Sociobiol.* **70**: 1591–1600.

Amundsen, T. & Slagsvold, T. 1998. Hatching asynchrony in Great Tits: A bet-hedging strategy? *Ecology* **79**: 295–304.

Andreasson, F., Nord, A. & Nilsson, J.-Å. 2018. Experimentally increased nest temperature affects body temperature, growth and apparent survival in Blue Tit nestlings. *J. Avian Biol.* **49**: jav-01620.

Andreu, J. & Barba, E. 2006. Breeding dispersal of Great Tits *Parus major* in a homogeneous habitat: effects of sex, age, and mating status. *Ardea* **94**: 45–58.

Ardia, D.R. & Clotfelter, E.D. 2007. Individual quality and age affect responses to an energetic constraint in a cavity-nesting bird. *Behav. Ecol.* **18**: 259–266.

Ardia, D.R., Cooper, C.B. & Dhondt, A. 2006. Warm temperatures lead to early onset of incubation, shorter incubation periods and greater hatching asynchrony in Tree Swallows *Tachycineta bicolor* at the extremes of their range. *J. Avian Biol.* **37**: 137–142.

Ardia, D.R., Pérez, J.H., Chad, E.K., Voss, M.A. & Clotfelter, E.D. 2009. Temperature and life history: Experimental heating leads female tree swallows to modulate egg temperature and incubation behaviour. *J. Anim. Ecol.* **78**: 4–13.

Ardia, D.R., Pérez, J.H. & Clotfelter, E.D. 2010. Experimental cooling during incubation leads to reduced innate immunity and body condition in nestling tree swallows. *Proc. R. Soc. B Biol. Sci.* **277**: 1881–1888.

Arnold, T.W., Rohwer, F.C. & Armstrong, T. 1987. Egg viability, nest predation, and the adaptive significance of clutch size in Prairie Ducks. *Am. Nat.* **130**: 643–653.

Bambini, G., Schlicht, E. & Kempenaers, B. 2019. Patterns of female nest attendance and male feeding throughout the incubation period in Blue Tits *Cyanistes caeruleus*. *Ibis* **161**: 50–65.

Barrientos, R., Bueno-Enciso, J., Serrano-Davies, E. & Sanz, J.J. 2015. Facultative interspecific brood parasitism in tits: a last resort to coping with nest-hole shortage. *Behav. Ecol. Sociobiol.* **69**: 1603–1615.

Barrionuevo, M. & Frere, E. 2016. Delayed start of full incubation is related to a delayed brood patch development in Magellanic Penguins *Spheniscus magellanicus*. *Bird Study* **63**: 353–358.

Bartón, K. 2015. *MuMIn: multi-model inference*. R package.

Basso, A. & Richner, H. 2015. Predator-specific effects on incubation behaviour and offspring growth in Great Tits. *PLoS ONE* **10:** e0121088.

Bates, D., Maechler, M., Bolker, B.M. & Walker, S. 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **67**: 1–48.

Beer, C. 1964. Incubation. In: *A new dictionary of birds* (D. L. Thomson, ed), pp. 396–398. Nelson, London.

Beissinger, S.R., Cook, M.I. & Arendt, W.J. 2005. The shelf life of bird eggs: testing egg viability using a tropical climate gradient. *Ecology* **86**: 2164–2175.

Belda, E.J., Barba, E., Gil-Delgado, J.A., Iglesias, D.J., López, G.M. & Monrós, J.S. 1998. Laying date and clutch size of Great Tits (*Parus major*) in the Mediterranean region: a comparison of four habitat types. *J. Ornithol.* **139**: 269–276.

Berntsen, H.H. & Bech, C. 2016. Incubation temperature influences survival in a small passerine bird. *J. Avian Biol.* **47**: 141–145.

Bleu, J., Agostini, S. & Biard, C. 2017. Nest-box temperature affects clutch size, incubation initiation, and nestling health in Great Tits. *Behav. Ecol.* **28**: 793–802.

Bortolotti, G.R. & Wiebe, K.L. 1993. Incubation behaviour and hatching patterns in the American Kestrel *Falco sparverius*. *Ornis Scand.* **24**: 41–47.

Both, C. & Visser, M.E. 2005. The effect of climate change on the correlation between avian life-history traits. *Glob. Change Biol.* **11**: 1606–1613.

Boulton, R.L. & Cassey, P. 2012. How avian incubation behaviour influences egg surface temperatures: Relationships with egg position, development and clutch size. *J. Avian Biol.* **43**: 289–296.

Bryan, S.M. & Bryant, D.M. 1999. Heating nest-boxes reveals an energetic constraint on incubation behaviour in Great Tits, *Parus major*. *Proc. R. Soc. B Biol. Sci.* **266**: 157.

Bueno-Enciso, J., Barrientos, R. & Sanz, J.J. 2017. Incubation behaviour of Blue *Cyanistes caeruleus* and Great Tits *Parus major* in a Mediterranean habitat. *Acta Ornithol.* **52**: 21–34.

Burnham, K.P. & Anderson, D.R. 2002. *Model selection and multimodel inference: A practical information-theoretic approach*, Second edition. Springer-Verlag, New York.

Buse, A., Dury, S.J., Woodburn, R.J.W., Perrins, C.M. & Good, J.E.G. 1999. Effects of elevated temperature on multi-species interactions: the case of Pedunculate Oak, Winter Moth and Tits. *Funct. Ecol.* **13**: 74–82.

Caldwell, P.J. & Cornwell, G.W. 1975. Incubation behavior and temperatures of the Mallard Duck. *The Auk* **92**: 706–731.

Camfield, A. & Martin, K. 2009. The influence of ambient temperature on horned lark incubation behaviour in an alpine environment. *Behaviour* **146**: 1615–1633.

Capp, E., Liebl, A.L., Cones, A.G. & Russell, A.F. 2018. Advancing breeding phenology does not affect incubation schedules in Chestnut-Crowned Babblers: Opposing effects of temperature and wind. *Ecol. Evol.* **8**: 696–705.

Clark, A.B. & Wilson, D.S. 1981. Avian breeding adaptations: Hatching asynchrony, brood reduction, and nest failure. *Q. Rev. Biol.* **56**: 253–277.

Clatterbuck, C.A., Young, L.C., VanderWerf, E.A., Naiman, A.D., Bower, G.C. & Shaffer, S.A. 2017. Data loggers in artificial eggs reveal that egg-turning behavior varies on multiple ecological scales in seabirds. *The Auk* **134**: 432–442.

Clotfelter, E.D. & Yasukawa, K. 1999. The function of early onset of nocturnal incubation in Red-Winged Blackbirds. *The Auk* **116**: 417–426.

Coe, B.H., Beck, M.L., Chin, S.Y., Jachowski, C.M.B. & Hopkins, W.A. 2015. Local variation in weather conditions influences incubation behavior and temperature in a passerine bird. *J. Avian Biol.* **46**: 385–394.

Cole, E.F. & Quinn, J.L. 2014. Shy birds play it safe: personality in captivity predicts risk responsiveness during reproduction in the wild. *Biol. Lett.* **10**: 20140178.

Conway, C.J. & Martin, T.E. 2000a. Effects of ambient temperature on avian incubation behavior. *Behav. Ecol.* **11**: 178–188.

Conway, C.J. & Martin, T.E. 2000b. Evolution of passerine incubation behavior: influence of food, temperature, and nest predation. *Evolution* **54**: 670–685.

Cook, M.I., Beissinger, S.R., Toranzos, G.A., Rodriguez, R.A. & Arendt, W.J. 2003. Trans-shell infection by pathogenic micro-organisms reduces the shelf life of non-incubated bird's eggs: a constraint on the onset of incubation? *Proc. R. Soc. B Biol. Sci.* **270**: 2233–2240.

Cooper, C.B., Hochachka, W.M., Greg, B. & Dhondt, A.A. 2005. Seasonal and latitudinal trends in clutch size: thermal constraints during laying and incubation. *Ecology* **86**: 2018–2031.

Cooper, C.B. & Mills, H. 2005. New software for quantifying incubation behavior from time-series recordings. *J. Field Ornithol.* **76**: 352–356.

Cooper, C.B. & Voss, M.A. 2013. Avian incubation patterns reflect temporal changes in developing clutches. *PLoS ONE* **8**: e65521.

Cresswell, W. & McCleery, R.H. 2003. How Great Tits maintain synchronization of their hatch date with food supply in response to long-term variability in temperature. *J. Anim. Ecol.* **72**: 356–366.

Cribari-Neto, F. & Zeileis, A. 2010. Beta Regression in R. *J. Stat. Softw.* **34**: 1–24.

de Heij, M.E., van den Hout, P.J. & Tinbergen, J.M. 2006. Fitness cost of incubation in Great Tits (*Parus major*) is related to clutch size. *Proc. R. Soc. B Biol. Sci.* **273**: 2353–2361.

de Heij, M.E., van der Graaf, A.J., Hafner, D. & Tinbergen, J.M. 2007. Metabolic rate of nocturnal incubation in female Great tits, *Parus major*, in relation to clutch size measured in a natural environment. *J. Exp. Biol.* **210**: 2006–2012.

Deeming, D.C. 1989. Failure to turn eggs during incubation: Development of the area vasculosa and embryonic growth. *J. Morphol.* **201**: 179–186.

Deeming, D.C. 1991. Reasons for the dichotomy in egg turning in birds and reptiles. In: *Egg incubation: its effects on embryonic development in birds and reptiles* (D. C. Deeming & M. W. J. Ferguson, eds), pp. 306–323. Cambridge University Press, Cambridge, England.

Deeming, D.C. 2002a. Behaviour patterns during incubation. In: *Avian incubation: Behaviour, environment and evolution* (D. C. Deeming, ed), pp. 63–87. Oxford University Press, Oxford.

Deeming, D.C. 2002b. Patterns and significance of egg turning. In: *Avian Incubation: Behaviour, Environment and Evolution* (D. C. Deeming, ed), pp. 161–178. Oxford University Press, Oxford, England.

Deeming, D.C. 2009. The role of egg turning during incubation. *Avian Biol. Res.* **2**: 67–71.

Deeming, D.C. & Gray, L.A. 2016. Incubation attentiveness and nest insulatory values correlate in songbirds. *Avian Biol. Res.* **9**: 32–36.

Deeming, D.C. & Mainwaring, M.C. 2015. Functional properties of nests. In: *Nests, Eggs, and Incubation* (D. C. Deeming & S. J. Reynolds, eds), pp. 29–49. Oxford University Press.

Deeming, D.C., Mainwaring, M.C., Hartley, I.R. & Reynolds, S.J. 2012. Local temperature and not latitude determines the design of Blue Tit and Great Tit nests. *Avian Biol. Res.* **5**: 203–208.

DeGregorio, B.A., Sperry, J.H., Ward, M.P. & Weatherhead, P.J. 2015. Wait until dark? Daily activity patterns and nest predation by snakes. *Ethology* **121**: 1225–1234.

Diez-Méndez, D., Rodríguez, S., Álvarez, E. & Barba, E. 2020. The role of partial incubation and egg repositioning within the clutch in hatching asynchrony and subsequent effects on breeding success. *Ibis* **162**: 63–74.

Diez-Méndez, D., Sanz, J.J. & Barba, E. 2021. Impacts of ambient temperature and clutch size on incubation behaviour onset in a female-only incubator songbird. *Ibis*, doi: 10.1111/ibi.12937.

Dobson, A.J. 2002. *An introduction to generalized linear models*, 2nd ed. Chapman & Hall/CRC, Boca Raton.

Drent, R. 1975. Incubation. In: *Avian Biology* (D. Farmer & J. King, eds), pp. 333–420. Academic Press, New York.

DuRant, S.E., Hopkins, W.A., Hepp, G.R. & Walters, J.R. 2013. Ecological, evolutionary, and conservation implications of incubation temperature-dependent phenotypes in birds. *Biol. Rev.* **88**: 499–509.

Eichholz, M.W. & Towery, B.N. 2010. Potential influence of egg location on synchrony of hatching of precocial birds. *The Condor* **112**: 696–700.

Eikenaar, C., Berg, M.L. & Komdeur, J. 2003. Experimental evidence for the influence of food availability on incubation attendance and hatching asynchrony in the Australian Reed Warbler *Acrocephalus australis*. *J. Avian Biol.* **34**: 419–427.

Encabo, S.I., Monrós, J.S. & Barba, E. 2001. Egg size variation in a Mediterranean Great Tit *Parus major* population. *Ardeola* **48**: 63–70.

Fasenko, G.M. 2007. Egg storage and the embryo. *Poult. Sci.* **86**: 1020–1024.

Fontaine, J.J. & Martin, T.E. 2006. Parent birds assess nest predation risk and adjust their reproductive strategies. *Ecol. Lett.* **9**: 428–434.

Fox, J. 2003. Effect displays in R for generalised linear models. *J. Stat. Softw.* **8**: 1–27.

Gądalski, M., Bańbura, M., Kaliński, A., Markowski, M., Skwarska, J., Wawrzyniak, J., Zieliński, P. & Bańbura, J. 2018. Hatching delays in Great Tits and Blue Tits in response to an extreme cold spell: a long-term study. *Int. J. Biometeorol.* **62**: 1437–1445.

García-Navas, V. & Sanz, J.J. 2011. Short-term alterations in songbird breeding schedule lead to better synchronization with food availability. *The Auk* **128**: 146–155.

Ghalambor, C.K. & Martin, T.E. 2002. Comparative manipulation of predation risk in incubating birds reveals variability in the plasticity of responses. *Behav. Ecol.* **13**: 101–108.

Gilby, A.J., Mainwaring, M.C. & Griffith, S.C. 2011. The adaptive benefit of hatching asynchrony in wild Zebra Finches. *Anim. Behav.* **82**: 479–484.

Gilby, A.J., Mainwaring, M.C. & Griffith, S.C. 2013. Incubation behaviour and hatching synchrony differ in wild and captive populations of the Zebra Finch. *Anim. Behav.* **85**: 1329–1334.

Gosler, A.G., Barnett, P.R. & Reynolds, S.J. 2000. Inheritance and variation in eggshell patterning in the Great Tit *Parus major*. *Proc. R. Soc. B Biol. Sci.* **267**: 2469–2473.

Götmark, F. 2002. Predation by sparrowhawks favours early breeding and small broods in great tits. *Oecologia* **130**: 25–32.

Greño, J.L., Belda, E.J. & Barba, E. 2008. Influence of temperatures during the nestling period on post-fledging survival of Great Tit *Parus major* in a Mediterranean habitat. *J. Avian Biol.* **39**: 41–49.

Griffith, S.C. & Gilby, A.J. 2013. Egg development time in the Zebra Finch *Taeniopygia guttata* varies with laying order and clutch size. *Ibis* **155**: 725–733.

Griffith, S.C., Mainwaring, M.C., Sorato, E. & Beckmann, C. 2016. High atmospheric temperatures and 'ambient incubation' drive embryonic development and lead to earlier hatching in a passerine bird. *R. Soc. Open Sci.* **3**: 150371.

Grimaudo, A.T., Hope, S.F., DuRant, S.E., Kennamer, R.A., Hallagan, J.J. & Hopkins, W.A. 2020. Ambient temperature and female body condition are related to night incubation behavior in Wood Ducks (*Aix sponsa*). *J. Avian Biol.*: e02379.

Grubbauer, P. & Hoi, H. 1996. Female Penduline Tits (*Remiz pendulinus*) choosing high quality nests benefit by decreased incubation effort and increased hatching success. *Écoscience* **3**: 274–279.

Guisan, A. & Zimmermann, N.E. 2000. Predictive habitat distribution models in ecology. *Ecol. Model.* **135**: 147–186.

Hadfield, J.D., Heap, E.A., Bayer, F., Mittell, E.A. & Crouch, N.M.A. 2013. Intraclutch differences in egg characteristics mitigate the consequences of age-related hierarchies in a wild passerine. *Evolution* **67**: 2688–2700.

Haftorn, S. 1978. Egg-laying and regulation of egg temperature during incubation in the Goldcrest *Regulus regulus*. *Ornis Scand.* **9**: 2–21.

Haftorn, S. 1979. Incubation and regulation of egg temperature in the Willow Tit *Parus montanus*. *Ornis Scand.* **10**: 220–234.

Haftorn, S. 1981. Incubation during the egg-laying period in relation to clutch-size and other aspects of reproduction in the Great Tit *Parus major*. *Ornis Scand.* **12**: 169–185.

Haftorn, S. 1988. Incubating female passerines do not let the egg temperature fall below the 'physiological zero temperature' during their absences from the nest. *Ornis Scand.* **19**: 97–110.

Haftorn, S. 1989. Seasonal and diurnal body weight variations in titmice, based on analyses of individual birds. *Wilson Bull.* **101**: 217–235.

Haftorn, S. & Reinertsen, R.E. 1985. The effect of temperature and clutch size on the energetic cost of incubation in a free-living Blue Tit (*Parus caeruleus*). *The Auk* **102**: 470–478.

Hahn, D.C. 1981. Asynchronous hatching in the Laughing Gull: Cutting losses and reducing rivalry. *Anim. Behav.* **29**: 421–427.

Hatchwell, B.J., Fowlie, M.K., Ross, D.J. & Russell, A.F. 1999. Incubation behavior of Long-Tailed Tits: Why do males provision incubating females? *The Condor* **101**: 681–686.

Hepp, G.R., Kennamer, R.A. & Johnson, M.H. 2006. Maternal effects in Wood Ducks: Incubation temperature influences incubation period and neonate phenotype. *Funct. Ecol.* **20**: 307–314.

Hilton, G.M., Hansell, M.H., Ruxton, G.D., Reid, J.M. & Monaghan, P. 2004. Using artificial nests to test importance of nesting material and nest shelter for incubation energetics. *The Auk* **121**: 777–787.

Hope, S.F., DuRant, S., Hallagan, J.J., Beck, M., Kennamer, R.A. & Hopkins, W.A.

2018. Free-moving artificial eggs containing temperature loggers reveal remarkable within-clutch variance in incubation temperature. *J. Avian Biol.*: e01685.

Huggins, R.A. 1941. Egg temperatures of wild birds under natural conditions. *Ecology* **22**: 148.

Hussell, D.J.T. 1972. Factors affecting clutch size in Arctic passerines. *Ecol. Monogr.* **42**: 317–364.

Johnson, L.S., Napolillo, F.M., Kozlovsky, D.Y., Hebert, R.M. & Allen, A. 2013. Variation in incubation effort during egg laying in the Mountain Bluebirds and its association with hatching asynchrony. *J. Field Ornithol.* **84**: 242–254.

King, J.R. 1973. Energetics of reproduction in birds. In: *Breeding biology of birds* (D. Farner, ed), pp. 78–107. National Academy of Science, Washington DC.

Kluen, E., de Heij, M.E. & Brommer, J.E. 2011. Adjusting the timing of hatching to changing environmental conditions has fitness costs in blue tits. *Behav. Ecol. Sociobiol.* **65**: 2091–2103.

Kluijver, H.N. 1950. Daily routines of the Great Tit, *Parus m. major* L. *Ardea* **38**: 99–135.

Kovařík, P. & Pavel, V. 2011. Does threat to the nest affect incubation rhythm in a small passerine? *Ethology* **117**: 181–187.

Kovařík, P., Pavel, V. & Chutný, B. 2009. Incubation behaviour of the Meadow Pipit (*Anthus pratensis*) in an alpine ecosystem of Central Europe. *J. Ornithol.* **150**: 549–556.

Krist, M. 2011. Egg size and offspring quality: A meta-analysis in birds. *Biol. Rev.* **86**: 692–716.

Krištofík, J., Darolová, A., Griggio, M., Majtán, J., Okuliarová, M., Zeman, M., Zídková, L. & Hoi, H. 2013. Does egg colouration signal female and egg quality in Reed Warbler (*Acrocephalus scirpaceus*)? *Ethol. Ecol. Evol.* **25**: 129–143.

Lack, D. 1947. The significance of clutch-size. *Ibis* **89**: 302–352.

Lack, D. 1948. The significance of clutch-size. Part III.-Some Interspecific Comparisons. *Ibis* **90**: 25–45.

Lambrechts, M.M., Blondel, J., Maistre, M. & Perret, P. 1997. A single response mechanism is responsible for evolutionary adaptive variation in a bird's laying date. *Proc. Natl. Acad. Sci.* **94**: 5153–5155.

Lambrechts, M.M., Marrot, P., Fargevieille, A., Giovannini, P., Lucas, A., Demeyrier, V., Midamegbe, A., Perret, P., Grégoire, A., Charmantier, A. & Doutrelant, C. 2016. Nest size is not closely related to breeding success in Blue Tits: A long-term nest-box study in a Mediterranean oak habitat. *The Auk* **133**: 198–204.

Löhrl, H. 1986. Experimente zur Bruthöhlenwahl der Kohlmeise (Parus major). *J. Ornithol.* **127**: 51–59.

Londoño, G.A., Levey, D.J. & Robinson, S.K. 2008. Effects of temperature and food on incubation behaviour of the northern mockingbird, *Mimus polyglottos*. *Anim. Behav.* **76**: 669–677.

Lord, A.M., Mccleery, R.H. & Cresswell, W. 2011. Incubation prior to clutch completion accelerates embryonic development and so hatch date for eggs laid earlier in a clutch in the Great Tit *Parus major*. *J. Avian Biol.* **42**: 187–191.

Loukola, O.J., Adamik, P., Adriaensen, F., Barba, E., Doligez, B., Flensted-Jensen, E., Eeva, T., Kivelä, S.M., Laaksonen, T., Morosinotto, C., Mänd, R., Niemelä, P.T., Remeš, V., Samplonius, J.M., Sebastiano, M., Senar, J.C., Slagsvold, T., Sorace, A., Tschirren, B., Török, J. & Forsman, J.T. 2020. The roles of temperature, nest predators and information parasites for geographical variation in egg covering behaviour of tits (Paridae). *J. Biogeogr.* **47**: 1482–1493.

Macdonald, E.C., Camfield, A.F., Jankowski, J.E. & Martin, K. 2013. Extended incubation recesses by alpine-breeding Horned Larks: A strategy for dealing with inclement weather? *J. Field Ornithol.* **84**: 58–68.

MacDonald, E.C., Camfield, A.F., Jankowski, J.E. & Martin, K. 2014. An alpine-breeding songbird can adjust dawn incubation rhythms to annual thermal regimes. *The Auk* **131**: 495–506.

Maechler, M., Rousseeuw, P., Croux, C., Todorov, V., Ruckstuhl, A., Salibian-barrera, M., Verbeke, T. & Koller, M. 2015. *robustbase: Basic Robust Statistics.*

Magrath, R.D. 1990. Hatching asynchrony in altricial birds. *Biol. Rev.* **65**: 587–622.

Mainwaring, M.C., Blount, J.D. & Hartley, I.R. 2012a. Hatching asynchrony can have long-term consequences for offspring fitness in Zebra Finches under captive conditions. *Biol. J. Linn. Soc.* **106**: 430–438.

Mainwaring, M.C., Deeming, D.C., Jones, C.I. & Hartley, I.R. 2014. Adaptive latitudinal variation in Common Blackbird *Turdus merula* nest characteristics. *Ecol. Evol.* **4**: 851–861.

Mainwaring, M.C., Hartley, I.R., Bearhop, S., Brulez, K., du Feu, C.R., Murphy, G., Plummer, K.E., Webber, S.L., James Reynolds, S. & Deeming, D.C. 2012b. Latitudinal variation in Blue Tit and Great Tit nest characteristics indicates environmental adjustment. *J. Biogeogr.* **39**: 1669–1677.

Mänd, R., Tilgar, V., Lõhmus, A. & Leivits, A. 2005. Providing nest boxes for hole-nesting birds - Does habitat matter? *Biodivers. Conserv.* **14**: 1823–1840.

Marasco, V. & Spencer, K.A. 2015. Improvements in our understanding of behaviour during incubation. In: *Nests, Eggs, and Incubation* (D. C. Deeming & S. J. Reynolds, eds), pp. 142–151. Oxford University Press.

Martin, T.E. 2002. A new view of avian life-history evolution tested on an incubation paradox. *Proc. R. Soc. B Biol. Sci.* **269**: 309–316.

Marzal, A., de Lope, F., Navarro, C. & Møller, A.P. 2005. Malarial parasites decrease reproductive success: an experimental study in a passerine bird. *Oecologia* **142**: 541–545.

Massaro, M., Davis, L.S. & Davidson, R.S. 2006. Plasticity of brood patch development and its influence on incubation periods in the Yellow-Eyed Penguin *Megadyptes antipodes*: an experimental approach. *J. Avian Biol.* **37**: 497–506.

Matthysen, E., Adriaensen, F. & Dhondt, A.A. 2011. Multiple responses to increasing spring temperatures in the breeding cycle of Blue and Great Tits (*Cyanistes caeruleus*, *Parus major*). *Glob. Change Biol.* **17**: 1–16.

Matysioková, B. & Remeš, V. 2014. The importance of having a partner: male help releases females from time limitation during incubation in birds. *Front. Zool.* **11**: 24.

Monaghan, P. & Nager, R.G. 1997. Why don't birds lay more eggs? *Trends Ecol. Evol.* **12**: 270–274.

Monrós, J.S., Belda, E.J. & Barba, E. 1998. Delays of the hatching dates in Great Tits *Parus major*: Effects on breeding performance. *Ardea* **86**: 213–220.

Morales, J., Sanz, J.J. & Moreno, J. 2006. Egg colour reflects the amount of yolk maternal antibodies and fledging success in a songbird. *Biol. Lett.* **2**: 334–336.

Morton, M.L. & Pereyra, M.E. 1985. The regulation of egg temperatures and attentiveness patterns in the Dusky Flycatcher (*Empidonax oberholseri*). *Auk* **102**: 25–37.

Naef-Daenzer, L., Nager, R.G., Keller, L.F. & Naef-Daenzer, B. 2004. Are hatching delays a cost or a benefir for Great Tit *Parus major* parents? *Ardea* **92**: 229–238.

Nakagawa, S. & Schielzeth, H. 2013. A general and simple method for obtaining R2 from generalized linear mixed-effects models. *Methods Ecol. Evol.* **4**: 133–142.

Niizuma, Y., Takagi, M., Senda, M., Chochi, M. & Watanuki, Y. 2005. Incubation capacity limits maximum clutch size in Black-Tailed Gulls *Larus crassirostris*. *J. Avian Biol.* **36**: 421–427.

Nilsson, J.-Å. 1993. Energetic constraints on hatching asynchrony. *Am. Nat.* **141**: 158–166.

Nilsson, J.-Å. & Smith, H.G. 1988. Incubation feeding as a male tactic for early hatching. *Anim. Behav.* **36**: 641–647.

Nilsson, J.-Å. & Svensson, E. 1993a. Energy constraints and ultimate decisions during egg-laying in the Blue Tit. *Ecology* **74**: 244–251.

Nilsson, J.-Å. & Svensson, J.A. 1993b. The frequency and timing of laying gaps. *Ornis Scand.* **24**: 122–126.

Noguera, J.C., Metcalfe, N.B., Reichert, S. & Monaghan, P. 2016. Embryonic and postnatal telomere length decrease with ovulation order within clutches. *Sci. Rep.* **6**: 25915.

Noordwijk, A.J.V., McCleery, R.H. & Perrins, C.M. 1995. Selection for the timing of Great Tit breeding in relation to caterpillar growth and temperature. *J. Anim. Ecol.* **64**: 451-458.

Nord, A. & Cooper, C.B. 2020. Night conditions affect morning incubation behaviour differently across a latitudinal gradient. *Ibis* **162**: 827–835.

Nord, A. & Nilsson, J.-Å. 2011. Incubation temperature affects growth and energy metabolism in Blue Tit nestlings. *Am. Nat.* **178**: 639–651.

Nord, A. & Nilsson, J.-Å. 2012. Context-dependent costs of incubation in the Pied Flycatcher. *Anim. Behav.* **84**: 427–436.

Nord, A. & Nilsson, J.-Å.. 2016. Long-term consequences of high incubation temperature in a wild bird population. *Biol. Lett.* **12**: 6–9.

Nord, A. & Williams, J.B. 2015. The energetic costs of incubation. In: *Nests, Eggs, and Incubation: new ideas about avian reproduction* (D. C. Deeming & S. J. Reynolds, eds), pp. 152–170. Oxford University Press, Oxford.

Olson, C.R., Vleck, C.M. & Vleck, D. 2006. Periodic cooling of bird eggs reduces embryonic growth efficiency. *Physiol. Biochem. Zool.* **79**: 927–936.

Ospina, E.A., Merrill, L. & Benson, T.J. 2018. Incubation temperature impacts nestling growth and survival in an open-cup nesting passerine. *Ecol. Evol.* **8**: 3270–3279.

Pendlebury, C.J. & Bryant, D.M. 2005. Night-time behaviour of egg-laying tits. *Ibis* **147**: 342–345.

Pérez, J.H., Ardia, D.R., Chad, E.K. & Clotfelter, E.D. 2008. Experimental heating reveals nest temperature affects nestling condition in Tree Swallows (*Tachycineta bicolor*). *Biol. Lett.* **4**: 468–71.

Perrins, C.M. 1991. Tits and their caterpillar food supply. *Ibis* **133**: 49–54.

Pinheiro, J.C. & Bates, D.M. 2000. *Mixed effects models in S and S-plus*. Springer-Verlag, New York.

Podlas, K.A. & Richner, H. 2013a. Partial incubation and its function in Great Tits (*Parus major*) - an experimental test. *Behav. Ecol.* **24**: 643–649.

Podlas, K.A. & Richner, H. 2013b. The adaptive function of hatching asynchrony: an experimental study in Great Tits. *Anim. Behav.* **86**: 567–576.

Poláček, M., Bartíková, M. & Hoi, H. 2017a. Intraclutch eggshell colour variation in birds: are females able to identify their eggs individually? *PeerJ* **5**: e3707.

Poláček, M., Griggio, M., Mikšík, I., Bartíková, M., Eckenfellner, M. & Hoi, H.

2017b. Eggshell coloration and its importance in postmating sexual selection. *Ecol. Evol.* **7**: 941–949.

R Core Team. 2019. *R: A language and environment for statistical computing.* R Foundation for Statistical Computing, Viena, Austria.

Rastogi, A.D., Zanette, L. & Clinchy, M. 2006. Food availability affects diurnal nest predation and adult antipredator behaviour in Song Sparrows, *Melospiza melodia. Anim. Behav.* **72**: 933–940.

Reid, J.M., Monaghan, P. & Ruxton, G.D. 1999. The effect of clutch cooling rate on starling, *Sturnus vulgaris*, incubation strategy. *Anim. Behav.* **58**: 1161–1167.

Reid, J.M., Monaghan, P. & Ruxton, G.D. 2000a. The consequences of clutch size for incubation conditions and hatching success in starlings. *Funct. Ecol.* **14**: 560–565.

Reid, J.M., Monaghan, P. & Ruxton, G.D. 2000b. Resource allocation between reproductive phases: the importance of thermal conditions in determining the cost of incubation. *Proc. R. Soc. B Biol. Sci.* **267**: 37–41.

Ricklefs, R.E. 1965. Brood reduction in the Curve-Billed Thrasher. *The Condor* **67**: 505–510.

Ricklefs, R.E. 1993. Sibling competition, hatching asynchrony, incubation period, and lifespan in altricial birds. In: *Current Ornithology* (D. M. Power, ed), pp. 199–276. Springer US, Boston, MA.

Ricklefs, R.E., Austin, S.H. & Robinson, W.D. 2017. The adaptive significance of variation in avian incubation periods. *The Auk* **134**: 542–550.

Rodríguez, S., Diez-Méndez, D. & Barba, E. 2016a. Negative effects of high temperatures during development on immediate post-fledging survival in Great Tits *Parus major*. *Acta Ornithol.* **51**: 235–244.

Rodríguez, S., van Noordwijk, A.J., Álvarez, E. & Barba, E. 2016b. A recipe for postfledging survival in Great Tits *Parus major*: Be large and be early (but not too much). *Ecol. Evol.* **6**: 4458–4467.

Rosivall, B., Szöllosi, E. & Török, J. 2005. Maternal compensation for hatching asynchrony in the Collared Flycatcher *Ficedula albicollis*. *J. Avian Biol.* **36**: 531–537.

Ruiz-De-Castañeda, R., Vela, A.I., González-Braojos, S., Briones, V. & Moreno, J. 2011. Drying eggs to inhibit bacteria: Incubation during laying in a cavity nesting passerine. *Behav. Processes* **88**: 142–148.

Ruiz-De-Castañeda, R., Vela, A.I., Lobato, E., Briones, V. & Moreno, J. 2012. Early onset of incubation and eggshell bacterial loads in a temperate-zone cavity-nesting passerine. *The Condor* **114**: 203–211.

Saether, B.-E. 1990. Age-specific variation in reproductive performance of birds. In: *Current Ornithology* (D. M. Power, ed), pp. 251–283. Plenum Press, New York.

Sálek, M.E. & Zárybnická, M. 2015. Different temperature and cooling patterns at the blunt and sharp egg poles reflect the arrangement of eggs in an avian clutch. *PLoS ONE* **10**: e0117728.

Sanz, J.J. 1996. Effect of food availability on incubation period in the Pied Flycatcher (*Ficedula hypoleuca*). *The Auk* **113**: 249–253.

Sanz, J.J. & García-Navas, V. 2009. Eggshell pigmentation pattern in relation to breeding performance of Blue Tits *Cyanistes caeruleus*. *J. Anim. Ecol.* **78**: 31–41.

Schneider, C.A., Rasband, W.S. & Eliceiri, K.W. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* **9**: 671–675.

Schöll, E.M., Aparisi, M.P. & Hille, S.M. 2019. Diurnal patterns of ambient temperature but not precipitation influence incubation behavior in Great Tits. *J. Ornithol.* **161**: 529–538.

Shutt, J.D., Nicholls, J.A., Trivedi, U.H., Burgess, M.D., Stone, G.N., Hadfield, J.D. & Phillimore, A.B. 2020. Gradients in richness and turnover of a forest passerine's diet prior to breeding: A mixed model approach applied to faecal metabarcoding data. *Mol. Ecol.* **29**: 1199–1213.

Simmonds, E.G., Sheldon, B.C., Coulson, T. & Cole, E.F. 2017. Incubation behavior adjustments, driven by ambient temperature variation, improve synchrony between hatch dates and caterpillar peak in a wild bird population. *Ecol. Evol.* **7**: 9415–9425.

Skutch, A.F. 1957. The incubation patterns of birds. *Ibis* **99**: 69–93.

Skutch, A.F. 1962. The constancy of incubation. *Wilson Bull.* **74**: 38.

Slagsvold, T., Amundsen, T. & Dale, S. 1995. Costs and benefits of hatching asynchrony in Blue Tits *Parus caeruleus*. *J. Anim. Ecol.* **64**: 563–578.

Smith, J.A., Cooper, C.B. & Reynolds, S.J. 2015. Advances in techniques to study incubation. In: *Nests, Eggs, and Incubation* (D. C. Deeming & S. J. Reynolds, eds), pp. 179–195. Oxford University Press.

Starck, J.M. & Ricklefs, R.E. 1998. Patterns of development: The altricial-precocial spectrum. In: *Avian growth and development. Evolution within the altricial-precocial spectrum* (J. M. Starck & R. E. Ricklefs, eds), pp. 2–30. Oxford University Press, New York.

Stein, L.R., Oh, K.P. & Badyaev, A.V. 2010. Fitness consequences of male provisioning of incubating females in a desert passerine bird. *J. Ornithol.* **151**: 227–233.

Stenning, M.J. 1996. Hatching asynchrony, brood reduction and other rapidly reproducing hypotheses. *Trends Ecol. Evol.* **11**: 243–246.

Stenning, M.J. 2008. Hatching asynchrony and brood reduction in Blue Tits *Cyanistes caeruleus* may be a plastic response to local oak bud burst and caterpillar emergence. *Acta Ornithol.* **43**: 97–106.

Stevenson, I.R. & Bryant, D.M. 2000. Climate change and constraints on breeding. *Nature* **406**: 366–7.

Stoddard, M.C., Fayet, A.L., Kilner, R.M. & Hinde, C.A. 2012. Egg speckling patterns do not advertise offspring quality or influence male provisioning in great tits. *PLoS ONE* **7**: e40211.

Stoleson, S.H. & Beissinger, S.R. 1995. Hatching asynchrony and the onset of incubation in birds, revisited. When is the critical period? In: *Current Ornithology* (D. M. Power, ed), pp. 191–270. Plenum Press, New York.

Stoleson, S.H. & Beissinger, S.R. 1997. Hatching asynchrony, brood reduction, and food limitation in a Neotropical parrot. *Ecol. Monogr.* **67**: 131–154.

Stoleson, S.H. & Beissinger, S.R. 1999. Egg viability as a constraint on hatching asynchrony at high ambient temperatures. *J. Anim. Ecol.* **68**: 951–962.

Svensson, L. 1992. *Identification guide to European passerines*. British trust for Ornithology.

Szöllosi, E., Rosivall, B. & Török, J. 2007. Is hatching asynchrony beneficial for the brood? *Behav. Ecol.* **18**: 420–426.

Thompson, F.R. 2007. Factors affecting nest predation on forest songbirds in North America. *Ibis* **149**: 98–109.

Tieleman, B.I., Van Noordwijk, H.J. & Williams, J.B. 2008. Nest site selection in a hot desert: Trade-off between microclimate and predation risk? *The Condor* **110**: 116–124.

Tinbergen, J.M. & Williams, J.B. 2002. Energetics of incubation. In: *Avian incubation: Behaviour, environment and evolution* (D. C. Deeming, ed), pp. 299–313. Oxford University Press, Oxford.

Tomás, G. 2015. Hatching date vs laying date: what should we look at to study avian optimal timing of reproduction? *J. Avian Biol.* **46**: 107–112.

Tomás, G., Merino, S., Martínez-de la Puente, J., Moreno, J., Morales, J. & Rivero-de Aguilar, J. 2013. Nest size and aromatic plants in the nest as sexually selected female traits in blue tits. *Behav. Ecol.* **24**: 926–934.

Tullett, S.G. & Deeming, D.C. 1987. Failure to turn eggs during incubation: effects on embryo weight, development of the chorioallantois and absorption of albumen. *Br. Poult. Sci.* **28**: 239–249.

Van Balen, J. 1973. A comparative study of the breeding ecology of the Great Tit *Parus major* in different habitats. *Ardea* **61**: 1–93.

Vedder, O. 2012. Individual birds advance offspring hatching in response to increased temperature after the start of laying. *Oecologia* **170**: 619–628.

Veiga, J.P. 1992. Hatching asynchrony in the House Sparrow: a test of the egg-viability hypothesis. *Am. Nat.* **139**: 669–675.

Veiga, J.P. & Vinuela, J. 1993. Hatching asynchrony and hatching success in the House Sparrow: Evidence for the egg viability hypothesis. *Ornis Scand.* **24**: 237.

Venables, W.N. & Ripley, B.D. 2003. *Modern applied statistics with S*, Fourth edt. Springer, New York.

Viñuela, J. 1997. Laying order affects incubation duration in the Black Kite (*Milvus migrans*): Counteracting hatching asynchrony? *The Auk* **114**: 192–199.

Visser, M.E., Holleman, L.J.M. & Gienapp, P. 2006. Shifts in caterpillar biomass phenology due to climate change and its impact on the breeding biology of an insectivorous bird. *Oecologia* **147**: 164–172.

Visser, M.E., van Noordwijk, A.J., Tinbergen, J.M. & Lessells, C.M. 1998. Warmer springs lead to mistimed reproduction in Great Tits (*Parus major*). *Proc. R. Soc. Lond. Ser. B-Biol. Sci.* **265**: 1867–1870.

Vleck, C. 2002. Hormonal control of incubation behaviour. In: *Avian incubation: Behaviour, environment and evolution* (D. C. Deeming, ed), pp. 54–62. Oxford University Press, Oxford.

Walsberg, G.E. & King, J.R. 1978. The energetics consequences of incubation for two passerine species. *The Auk* **95**: 644–655.

Walsberg, G.E. & Voss-Roberts, K.A. 1983. Incubation in desert-nesting doves: mechanisms for egg cooling. *Physiol. Zool.* **56**: 88–93.

Walters, L.A. & Getty, T. 2010. Are brighter eggs better? Egg color and parental investment by House Wrens. *J. Field Ornithol.* **81**: 155–166.

Walters, L.A., Webber, J.A., Jones, B.A. & Volker, C.L. 2016. Taking a break: The relationship between ambient temperature and nest attendance patterns of incubating Carolina Chickadees (*Poecile carolinensis*). *Wilson J. Ornithol.* **128**: 719–726.

Wang, J.M. & Beissinger, S.R. 2009. Variation in the onset of incubation and its influence on avian hatching success and asynchrony. *Anim. Behav.* **78**: 601–613.

Wang, J.M. & Beissinger, S.R. 2011. Partial incubation in birds: Its occurrence, function, and quantification. *The Auk* **128**: 454–466.

Weathers, W.W. & Sullivan, K.A. 1989. Nest attentiveness and egg temperature in the Yellow-Eyed Junco. *The Condor* **91**: 628–633.

Webb, D.R. 1987. Thermal tolerance of avian embryos: A review. *The Condor* **89**: 874–898.

Wesołowski, T. & Wierzcholska, S. 2018. Tits as bryologists: patterns of bryophyte use in nests of three species cohabiting a primeval forest. *J. Ornithol.* **159**: 733–745.

White, F.N. & Kinney, J.L. 1974. Avian incubation: Interactions among behavior, nest, and eggs result in regulation of egg temperatue. *Science* **186**: 107–115.

Wiebe, K.L., Wiehn, J. & Korpimäki, E. 1998. The onset of incubation in birds: can females control hatching patterns? *Anim. Behav.* **55**: 1043–52.

Williams, J.B. 1996. Energetics of avian incubation. In: *Avian Energetics and Nutritional Ecology* (C. Carey, ed), pp. 375–415. Springer, Boston, MA.

Williams, T.D. 2005. Mechanisms underlying the costs of egg production. *BioScience* **55**: 39–48.

Williams, T.D. & Groothuis, T.G.G. 2015. Egg quality, embryonic development, and post-hatching phenotype: an integrated perspective. In: *Nests, Eggs, and Incubation: new ideas about avian reproduction* (D. C. Deeming & S. J. Reynolds, eds), pp. 113–126. Oxford University Press, Oxford.

Yom-Tov, Y. & Wright, J. 1993. Effect of heating nest boxes on egg laying in the Blue Tit (*Parus caeruleus*). *The Auk* **110**: 95–99.

Zerban, E. & Morton, M.L. 1983. The rhythm of incubation from egg laying to hatching in mountain White-Crowned Sparrows. *Ornis Scand.* **14**: 188–197.

Zuur, A.F., Ieno, E.N., Walker, N., Saveliev, A.A. & Smith, G.M. 2009. *Mixed effects models and extensions in ecology with* R. Springer, New York, NY.