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# Patrones de incubación y eclosión del Carbonero Común *Parus major* en un ambiente mediterráneo: causas y consecuencias

Memoria de Tesis presentada por

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*Dedicada al niño de mis*
*ojos, mi hijo Marcos*
*(alias "OuiOui")*

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# ÍNDICE

# 1. RESUMEN DE LA TESIS

*Objetivos generales*: Los principales objetivos de esta tesis son describir los patrones de incubación y eclosión de una población de carbonero común *Parus major* en un ambiente mediterráneo, profundizar en algunas de las causas que afectan a dichos patrones, centrándonos sobre todo en las características del nido, y describir sus posibles consecuencias en el desempeño reproductor.

*Hipótesis general*: Las características parentales, las del nido, y las condiciones ambientales pueden afectar a la duración de los periodos de incubación y eclosión, teniendo consecuencias en el desempeño reproductor.

Para poder alcanzar los objetivos generales, hemos identificado varios objetivos particulares, agrupados en dos grandes apartados. Por un lado, los artículos del 1 al 4 se han centrado en las características del nido, puesto que es un elemento fundamental para crear un microclima adecuado para los huevos durante la incubación. En la segunda parte de la tesis, que abarca los artículos del 5 al 7, nos hemos centrado en los procesos de incubación y eclosión. A continuación resumimos los principales resultados y conclusiones de cada uno de los artículos.

## Artículo 1

Los nidos de las aves son estructuras especiales construidas con fines reproductivos. El tamaño y la estructura del nido pueden surgir de compromisos evolutivos entre beneficios, como el aislamiento frente a condiciones desfavorables, el mantenimiento de huevos o pollos, o seguridad frente a la depredación, y costes, como el gasto de energía en la construcción del nido y el riesgo de depredación en nidos más visibles. La existencia de estos costes sugiere que probablemente sólo los “buenos” padres deberían poder construir “buenos” nidos. Nuestros objetivos en este capítulo fueron estudiar la posible relación entre algunas características biométricas de los padres y su edad (calidad parental) con algunos indicadores de la calidad del nido, y entre la calidad del nido y algunos parámetros reproductores. Para ello tomamos como modelo una población de carbonero común *Parus major* que cría en cajas-nido en plantaciones de naranjos en Sagunto (Este de España). Por lo comentado arriba, esperamos una relación positiva entre la calidad de los padres y la calidad del nido, y entre la calidad del nido y el éxito reproductor. Visitamos los nidos con la frecuencia necesaria para determinar los parámetros reproductores básicos. Medimos y pesamos todos los nidos durante el periodo de incubación, tomamos las características biométricas de los padres (longitud del tarso y del ala, peso y condición). Utilizamos análisis de componentes principales para reducir el número de variables del nido a tres componentes relevantes. Encontramos relaciones positivas entre los diferentes componentes de la calidad del nido y componentes del desempeño reproductor, pero no encontramos ninguna relación significativa entre calidad de los padres y la del nido. También encontramos una correlación positiva significativa, aunque débil, entre el tamaño de la hembra y la tasa de éxito reproductor. Por tanto, concluimos que la calidad del nido afecta al éxito reproductor, aunque queda abierta la cuestión de si las características de los nidos por sí mismas fueron importantes para el éxito, o fue la calidad parental la variable importante que hay detrás de esta relación.

### Artículo 2

Para las aves, construir un nido implica una serie de costes, pero otorga importantes beneficios, ya que la calidad del nido está a menudo relacionada positivamente con el desempeño reproductor. Nuestros objetivos en este capítulo fueron estudiar las relaciones entre las características del nido y los parámetros reproductores, las posibles variaciones interanuales en dichas relaciones, y explorar las relaciones entre las características parentales y las variables relacionadas con el tamaño del nido y con el desempeño reproductor, puesto que las características parentales también pueden afectar a las características del nido y al éxito reproductor. Para ello, en este trabajo hemos ampliado el estudio del Artículo 1, añadiendo un año más, y considerando además nuevos componentes del éxito reproductor, como la biometría y capacidad inmune de los pollos producidos. Para ello, además de los datos recolectados para el trabajo anterior, tomamos las características biométricas de pollos (longitud del tarso, peso y condición), y su capacidad inmune (prueba de la fitohemaglutinina). En conjunto, 2007 parece que fue un año más favorable que 2006 (mayor tamaño de puesta y pollos en mejor condición). La fecha de puesta fue más temprana en nidos con mayor diámetro de copa, el éxito de eclosión aumentó al aumentar la profundidad relativa del nido en 2007, y el éxito de vuelo aumentó con el tamaño global del nido en 2007. La única relación significativa entre las características de los padres y las del nido fue que las hembras de un año construyeron nidos relativamente más profundos que las de más edad. Las hembras de tarso más largo y en peor condición comenzaron antes la puesta. El éxito de vuelo aumentó al disminuir el tarso de los machos, y la condición de los pollos fue mejor en nidos atendidos por machos mayores de un año. En general, las características del nido estuvieron positivamente relacionadas con el éxito reproductor, aunque las relaciones específicas entre los distintos componentes variaron entre años. Estas relaciones fueron más evidentes en un año "bueno" que en uno "malo" en términos de disponibilidad de recursos. Parece ser que tener un buen nido es sólo importante cuando otros factores limitantes, como la disponibilidad de alimento o la temperatura ambiente, están sobre un umbral, pero puede ser relativamente poco importante en años de mayor estrés.

### Artículo 3

Los nidos de las aves son estructuras cuyas propiedades afectan al desempeño reproductor. Así, la gran mayoría de las especies de aves construyen nidos característicos, seleccionando materiales adecuados para ello. Sin embargo, en hábitats donde la disponibilidad de materiales "ideales" es baja, sería deseable la capacidad para utilizar materiales alternativos, aún con el coste de tener un éxito reproductivo reducido. El carbonero común *Parus major* puede criar en lugares con condiciones ambientales muy diferentes. Sus nidos están compuestos principalmente por musgo, aunque muy pocos estudios han cuantificado la composición del nido. Nuestros objetivos en este capítulo fueron describir el peso y composición del nido del carbonero común en cuatro hábitats mediterráneos diferentes, desde plantaciones homogéneas de naranjos hasta bosques mediterráneos bien conservados, y explorar sus consecuencias en la eficacia biológica. Esperamos que el peso y la composición del nido difieran entre hábitats, y que la cantidad y/o proporción de los materiales clave afecten al desempeño reproductor dentro de los hábitats. Para ello, obtuvimos los parámetros reproductores básicos, recogimos los nidos al volar los pollos, y descompusimos una muestra de ellos para determinar su composición, en cuatro poblaciones al Este de España: Pina (pinos y robles), Mariola (pinos), Font Roja (encinas), y Sagunto (naranjos). Los nidos fueron más pesados en Sagunto y Mariola y más ligeros en Font Roja, y los de Sagunto tuvieron la menor cantidad de musgo. El tamaño de puesta disminuyó cuando el peso del musgo aumentó en los cuatro sitios. En Sagunto, el éxito de eclosión aumentó al aumentar el peso del musgo. En todos los hábitats, la condición de los pollos fue más baja cuando la cantidad de palitos y plumas fue más alta. Concluimos que el peso y composición del nido del carbonero común varía considerablemente entre hábitats, y la cantidad de diferentes materiales podría afectar a diferentes parámetros reproductivos, entre ellos al éxito de eclosión.

### Artículo 4

La composición y características del nido afectan a sus propiedades térmicas, y la temperatura del nido afecta al proceso de incubación. Nuestro principal objetivo en este capítulo fue determinar experimentalmente si la calidad del nido por sí misma afecta al proceso de incubación. Para ello, manipulamos algunos nidos de carbonero común *Parus major* con objeto de aumentar o empeorar su calidad respecto a nidos control, desvinculando así la posible contribución de la calidad de los padres de la de los nidos que atienden. En base a esto, predecimos que, si el proceso de incubación depende de la calidad del nido, (1) la duración media del periodo de incubación será menor en los nidos experimentales de buena calidad y mayor en los de mala calidad respecto a los nidos control, y/o (2) el éxito medio de eclosión será mayor en los nidos experimentales de buena calidad y menor en los de mala calidad respecto a los nidos control. Para ello, cambiamos aleatoriamente los nidos originales por nidos artificiales de “buena” y “mala” calidad al comienzo de la incubación. Las aves añadieron material en casi todos los nidos experimentales, pero no hubo diferencias en la cantidad de material añadido entre los nidos buenos y malos. La proporción de nidos donde voló al menos un pollo no difirió entre los nidos control y buenos, siendo significativamente más baja en los nidos malos. El periodo de incubación no difirió entre los nidos control y los buenos. El éxito de eclosión fue mayor en los nidos buenos que en los control. Nuestros resultados sugieren que la calidad del nido *per se* afecta al proceso de incubación, independientemente de la calidad de los padres. Por lo que conocemos, este es el primer trabajo que muestra esta relación de forma experimental.

### Artículo 5

Cuándo iniciar la incubación en relación a la secuencia de puesta, y cuánto tiempo y energía invertir en esta actividad, son decisiones importantes que deben tomar las aves incubantes, puesto que afectarán a su desempeño reproductor. Las principales consecuencias están relacionadas con el grado de asincronía en la eclosión de los huevos, que a su vez afecta a las tasas de crecimiento y a la supervivencia de cada pollo, y a la longitud total del periodo de pollo, que a su vez afecta a la exposición de la nidada a los depredadores o a las inclemencias climáticas. Nuestro objetivo para este capítulo fue utilizar datos de un estudio a largo plazo (1988-2010) de una población de carbonero común *Parus major* que cría en cajas-nido en plantaciones de naranjos en Sagunto (Este de España) para investigar (1) cuándo comienza la incubación en relación a la secuencia de puesta; (2) la longitud del periodo de incubación; (3) el grado de asincronía; y (4) qué factores próximos afectan a todos esos rasgos. En promedio, la incubación empezó con la puesta del último huevo, aunque hubo diferencias entre años. El periodo de incubación (desde la fecha de inicio de la incubación hasta el día previo a la eclosión del primer huevo) duró 13,3 días. El periodo medio de incubación fue más corto en años donde el tamaño medio de puesta (como una aproximación de la disponibilidad de alimento) fue más grande. La duración del periodo de incubación fue más corto cuando la incubación empezó más tarde en la secuencia de puesta, sugiriendo que la incubación es menos efectiva durante el periodo de puesta. Contrastado con algunos estudios previos, la incubación además fue más corta si el tamaño de puesta fue mayor – quizás las puestas más grandes se mantienen calientes más fácilmente cuando las hembras dejan el nido para buscar alimento. Además, el periodo de incubación fue más corto si los huevos estaban descubiertos antes de comenzar la incubación completa, sugiriendo que las hembras aplican algo de calor durante esos días previos. El periodo medio de eclosión fue 1,7 días, aunque las medias para los años individuales estuvieron entre 1,0 y 2,9 días. Como se esperaba, la eclosión asincrónica fue mayor cuando el periodo de incubación decreció, cuando el tamaño de puesta aumentó, y cuando el inicio de la incubación fue más temprano en relación a la secuencia de puesta.

### Artículo 6

La incubación uniparental significa frecuentemente que los huevos quedan desatendidos durante los periodos en los que el ave incubante está buscando alimento fuera del nido. La determinación de los ritmos de incubación (i.e. la longitud y el patrón temporal de las sesiones de incubación), y los factores que los afectan, son también importantes para comprender los compromisos del ciclo vital. Nuestros objetivos en este capítulo fueron: (1) describir el patrón de incubación en una población de carbonero común *Parus major* que cría en cajas-nido en plantaciones de naranjos en Sagunto (Sur de Europa), y determinar algunos de los factores que afectan al ritmo de incubación dentro de esta población, y (2) revisar la información publicada sobre los patrones de incubación en otras poblaciones europeas de carbonero común, y examinar sus variaciones a lo largo de un gradiente latitudinal. Por primera vez mostramos en una especie de ave que la atención al nido (% de tiempo incubando) es bastante similar (83-88%) entre poblaciones que van desde Noruega a España. La duración media de las sesiones y los recesos de incubación no siguen un patrón latitudinal claro – quizás los recesos sean más largos en las poblaciones más extremas (10-12 min) que en las centrales (7-8 min). Dentro de la población española, la atención aumentó a lo largo del periodo de incubación, debido a recesos más cortos, y las hembras dejaron el nido más temprano por la mañana y se retiraron más tarde por la tarde (prolongando así su día activo) cuando las temperaturas ambientales eran más elevadas. Mostramos por primera vez que la duración media de los recesos era más larga cuando el tamaño del huevo, el tamaño de puesta y el volumen total de los huevos de la puesta disminuyeron en la fase más temprana de la incubación (días 3-6). Finalmente, las hembras de mejor calidad, y/o las emparejadas con machos de mejor calidad, pasaron más tiempo fuera del nido, probablemente para evitar la depredación del nido.

### Artículo 7

En las especies de aves donde sólo un miembro de la pareja incuba los huevos, existe un compromiso entre emplear el tiempo en el cuidado de los huevos, o dejar el nido para buscar alimento o desempeñar otras actividades de mantenimiento. De esta manera, la incubación durante el día activo es intermitente, y el número de sesiones y recesos, y sus longitudes, condicionan las temperaturas de los huevos, y con ello el desarrollo embrionario. Nuestro principal objetivo en este capítulo fue evaluar las decisiones de inversión de la hembra, y sus consecuencias sobre diferentes factores relacionados con la eficacia biológica, incluyendo la condición y el comportamiento parental, longitud del periodo de incubación, y el desempeño reproductor, calentando o enfriando experimentalmente nidos de una población de carbonero común *Parus major* que cría en cajas-nido en plantaciones de naranjos en Sagunto (Este de España) durante el periodo de incubación. Por lo que conocemos, este es el primer estudio en el que ambos tratamientos se aplican simultáneamente en una población de aves. Aunque existen trabajos con resultados contradictorios, inicialmente predecimos que el enfriamiento experimental incrementaría los costes de la incubación, mientras que el calentamiento los reduciría, y las hembras distribuirían esos costes extras (en los nidos enfriados), o la energía ahorrada (en los nidos calentados) entre ellas mismas y/o su progenie. Las hembras cuyos

nidos fueron calentados redujeron el tiempo que pasan incubando, incrementando la duración de sus recesos. Aunque las temperaturas medias del nido fueron más altas en los nidos calentados que en los controles, el hecho de calentar no afectó al éxito reproductor, al tamaño o condición de los pollos, o a la condición parental o al comportamiento cuando cebaban a los pollos. Por otro lado, las hembras cuyos nidos fueron enfriados pudieron compensar el tratamiento, y las temperaturas en dichos nidos fueron similares a los controles. El éxito reproductor fue similar en los nidos enfriados y en los controles, y ni los pollos ni los padres mostraron efectos negativos del tratamiento de enfriamiento. Otros estudios han enfriado o calentado nidos de diferentes especies durante la incubación, y las consecuencias para los padres y embriones o pollos difirieron enormemente entre ellos. Concluimos que es necesario un estudio a gran escala para determinar si esas diferencias son atribuibles a los protocolos experimentales, a diferencias en el comportamiento o el tipo de nido de las diferentes especies estudiadas, o a diferencias en las condiciones ambientales.

# 2. INTRODUCCIÓN GENERAL

## DEFINICIÓN DE INCUBACIÓN Y ASPECTOS GENERALES

La incubación la realizan los animales ovíparos (sobre todo las aves) y, en sentido estricto, sería "el proceso por el cual se transfiere al huevo, después de que haya sido puesto, el calor necesario para el desarrollo embrionario" (Beer 1964; Tullet 1985). La palabra "incubación" deriva del latín *incubatio*, *incubationis*, y es la acción del verbo *incubare* (estar acostado o acostarse; ver Jayakar y Spurway 1965). La gran mayoría de las aves dan el calor necesario a los huevos echándose sobre ellos, de ahí que se haya adoptado esta palabra para describir, en general, el proceso de transferencia de calor entre adultos incubantes y huevos (Drent 1975). Durante este proceso, el ave incubante mantiene los huevos dentro de un estrecho rango de temperaturas (generalmente 37-38 ºC), en un ambiente húmedo, renovado regularmente para permitir intercambios de gases respiratorios, y durante el cual los huevos son volteados regularmente (Ar y Sidis 2002). La mayoría de las aves incuba los huevos en un nido más o menos complejo, y es la unidad ave-nido la que consigue mantener el ambiente adecuado para el desarrollo embrionario (Ar y Sidis 2002).

La transferencia de calor a los huevos por contacto corporal se encuentra sólo en algunos reptiles (Benedict 1932), unos pocos mamíferos (Hawkins y Battaglia 2009), y, en su forma más desarrollada, en la gran mayoría de las aves. La incubación por contacto precisa la presencia de un padre para proporcionar la suficiente energía calorífica para elevar la temperatura del huevo a un nivel apropiado para el desarrollo embrionario, por lo que el ave se ve en el compromiso de equilibrar sus propios requerimientos con las necesidades del embrión en desarrollo. Skutch (1957; ver también Williams 1996) clasificó la incubación en tres categorías, basándose en la presencia y actividad de las aves adultas:

1. Incubación biparental continua: ambos padres incuban, turnándose en la atención a los huevos. La presentan casi el 50% de todas las familias de aves, y casi el 80% de las familias de no-paseriformes.
2. Incubación gineparental: sólo la hembra incuba. La presentan el 37% de las familias de aves, y el 62% las familias de paseriformes.
3. Incubación androparental: sólo el macho incuba. La presentan el 6% de las familias de aves.

En los casos en los que sólo incuba la hembra, ésta puede ser alimentada por el macho durante la incubación (incubación gineparental asistida; e.g. Kendeigh 1952; Kemp 1995; Williams 1996). No hay ejemplos registrados de hembras alimentando a los machos que incuban (Deeming 2002a). Es probable que el patrón de incubación adoptado por diferentes especies sea el reflejo de restricciones impuestas por su ambiente (Deeming 2002a).

En la mayoría de las especies de aves, el calor necesario para el desarrollo embrionario se transmite al huevo desde el ave a través de una región, llamada comúnmente placa incubatriz (ver Galería Fotográfica), generalmente, pero no siempre, situada en la zona ventral del cuerpo (Jones 1971; Drent 1975). La placa incubatriz se desarrolla durante la estación reproductora, y su grado de desarrollo difiere entre especies y sexos. Está caracterizada por la ausencia de plumas y un aumento de la vascularización, así como un engrosamiento de la piel, que adquiere una apariencia arrugada. Estos cambios permiten una transferencia más eficaz de calor a los huevos (Drent 1975; Lea y Klandorf 2002).

Estudios observacionales y experimentales han mostrado que la actividad de la incubación conlleva costes energéticos significativos para los padres (Reid et al. 2002a; Tinbergen y Williams 2002; Ardia et al. 2009; DuRant et al. 2013), que son mayores si sólo incuba uno de los padres y éste tiene que abandonar los huevos periódicamente para alimentarse (Reid et al. 2002b). Los padres gastan energía manteniendo la temperatura de los huevos y recalentándolos a la vuelta de sus salidas para alimentarse, y la cantidad de energía invertida puede afectar negativamente a fases posteriores de la reproducción presente, o a eventos reproductivos futuros (Reid et al. 2000a; Chaplin et al. 2002; Hanssen et al. 2005; Ardia et al. 2009). Para atender estas demandas energéticas de la incubación, los padres utilizan la estrategia de acumular recursos antes de la reproducción y depender de ellos para satisfacer sus necesidades en las diferentes actividades reproductoras ("*capital breeders*"), o la de ajustar su alimentación a sus necesidades actuales, alternando actividades de forrajeo y reproductivas durante la estación reproductora ("*income breeders*"; e.g. Stephens et al. 2009). En las especies que adoptan esta última estrategia, el ave que incuba tiene que estar constantemente decidiendo si invertir en sí misma o en el cuidado de sus huevos (Williams 1996; Bryan y Bryant 1999; Tinbergen y Williams 2002; Turner 2002). Modificando experimentalmente los costes de incubación se pueden evaluar las decisiones de inversión de la hembra, y sus consecuencias para los padres (especialmente las hembras), los embriones o los pollos, siendo la manipulación directa de la temperatura del nido la mejor aproximación (Reid et al. 2002b; Nilsson et al. 2008). Sin embargo, los resultados de los experimentos realizados hasta la fecha son en parte contradictorios (e.g. Davis et al. 1984; Reid et al. 1999;

Bryan y Bryant 1999; Reid et al. 2000; Cresswell et al. 2004; Magrath et al. 2005; Londoño et al. 2008; Nilsson et al. 2008; Pérez et al. 2008; Ardia et al. 2009, 2010).

## EL NIDO

Algunas revisiones sobre la incubación de las aves se han concentrado en aspectos específicos de la reproducción, incluyendo el nido (Hansell 2000) o la atención al nido (Kendeigh 1952). Los nidos son estructuras multifuncionales, más o menos elaboradas, construidas para proporcionar aislamiento térmico y protección frente a los depredadores, ayudar a mantener la posición óptima de los huevos durante la incubación, y facilitar el cuidado de los pollos, mejorando así su supervivencia (Collias y Collias 1984). Pero, además de conferir importantes beneficios reproductivos (e.g. Quader 2006; Møller 1982; Soler et al. 1998, 2001), la construcción de un nido conlleva costes (e.g. Tomás et al. 2006; Moreno et al. 2008; Mainwaring y Hartley 2009). Estos costes son más elevados de lo que se pensaba, comparados con los costes de la producción del huevo o de la incubación, y son especialmente relevantes si las tareas de construcción son llevadas a cabo sólo por la hembra (e.g. Mainwaring y Hartley 2009).

La mayoría de especies de aves construyen algún tipo de nido, siendo éste característico de la especie y fácilmente diferenciable del de otras especies (e.g. Welty 1982; Hansell 2000). Las aves “delegan” en el nido algunos de los roles del cuidado parental (Marchant 1985; Deeming 2002a). En especial, el nido contribuye a mantener la temperatura de los huevos dentro del rango adecuado para el desarrollo embrionario. Así, las aves pequeñas, con altas demandas metabólicas específicas, construyen nidos más cerrados que especies de tamaño corporal mayor (Hansell 2000). Estos nidos cerrados protegen mejor los huevos durante sus frecuentes ausencias para alimentarse (Ricklefs 1974).

Varios trabajos han descrito los nidos de las aves y el comportamiento de construcción de los mismos (Skutch 1976; Collias y Collias 1984; Hansell 2000). Las aves pueden “diseñar” su ambiente de incubación a tres niveles: (1) mediante la elección de la ubicación del nido, (2) mediante la forma del nido, y (3) mediante la selección de materiales de construcción. Hansell (2000) describe ocho amplias categorías de ubicaciones del nido: (1) sobre ramas de árboles o arbustos, (2) sobre herbáceas o carrizo, (3) sobre el suelo, (4) en cavidades de árboles, (5) en cavidades naturales o excavadas en el suelo, (6) sobre paredes o muros, (7) en salientes rocosos, y (8) flotantes.

En cuanto a la forma, Hansell (2000) reconoce ocho distintas: (1) de cuenco, (2) de domo, (3) de domo y tubo, (4) de plato, (5) de cama, (6) de pequeña depresión en el suelo, (7) de montículo, y (8) de madriguera.

Por último, aunque existe un infinito número de clases de materiales que podrían ser reconocidos, Hansell (2000) considera que existen sólo tres en el nivel más básico: animal (plumas y pelo), "vegetación" (plantas, hongos y líquenes, incluyendo varios tipos de hojas), y mineral (piedras y barro). La cantidad y/o proporción de un material en el nido puede depender de su disponibilidad y accesibilidad en el hábitat circundante (Moreno et al. 2009; Wang et al. 2009), sus propiedades físicas y/o químicas (Hilton et al. 2004; Mennerat et al. 2009), las condiciones ambientales (McGowan et al. 2004; Mainwaring et al. 2008; Deeming et al. 2012), y la calidad de los constructores, ya que algunos materiales, costosos de encontrar o de transportar (piedras, plumas, ciertas plantas), pueden ser utilizados por el ave que los porta para señalizar su calidad (Moreno et al. 1994; Veiga et al. 2005; Polo y Veiga 2006).

Los materiales, además de otorgar propiedades características a las estructuras que las aves construyen, dictan en gran medida el método de construcción. Hansell (2000) identificó las siguientes técnicas de construcción: esculpir, moldear, colgar, amontonar, entretejer, y tejer. En la mayoría de nidos pueden diferenciarse cuatro zonas funcionalmente diferentes (Hansell 2000): (1) la capa de unión (materiales empleados para asegurar el nido al lugar donde se ubica), (2) la capa periférica (material colocado alrededor de la estructura, con función únicamente decorativa), (3) la capa estructural (provee integridad a la estructura del nido, previniendo la distorsión o el desbaratamiento), y (4) el forro o revestimiento (recubre la parte de la capa estructural en contacto con los huevos).

El tamaño del nido, y su variación intraespecífica, ha sido bien estudiada (Palomino et al. 1998; Quader 2006; Moreno et al. 2009; Crossman et al. 2011). En general, el tamaño del nido puede estar afectado por las características de los padres (e.g. Slagsvold 1989; De Neve et al. 2004; Tomás et al. 2006; Mainwaring et al. 2009; Broggi y Senar 2009), y por las condiciones ambientales, como la temperatura ambiental, el alimento disponible, los parásitos, o las características de la cavidad donde construyen el nido en el caso de las aves trogloditas (Nager y Van Noordwijk 1992; Heeb et al. 1996, 2000; Mazgajski y Rykowska 2008; Mainwaring y Hartley 2009; Britt y Deeming 2011).

Las propiedades térmicas del nido afectan a la tasa de pérdida de calor (Collias y Collias 1984; Hilton et al. 2004), y consecuentemente al equilibrio energético de los padres (Fast et al. 2007; Ardia et al. 2010) y al desarrollo fisiológico de los pollos (Chaplin et al. 2002; Olson et al. 2006; Ardia et al. 2010; DuRant et al. 2013). A su vez, las propiedades térmicas del nido dependen de su tamaño, composición, humedad y aislamiento (Whittow y Berger 1977; Møller 1984; Lombardo et al. 1995; Ar y Sidis 2002; Hilton et al. 2004; Lamprecht y Schmolz 2004; Szentirmai et al. 2005; Pinowski et al. 2006; Windsor et al. 2013).

Un aspecto fundamental es el mantenimiento de un microclima adecuado en el nido. La Figura 1 resume y agrupa la mayoría de factores directos e indirectos que están involucrados en la determinación del microclima del nido.

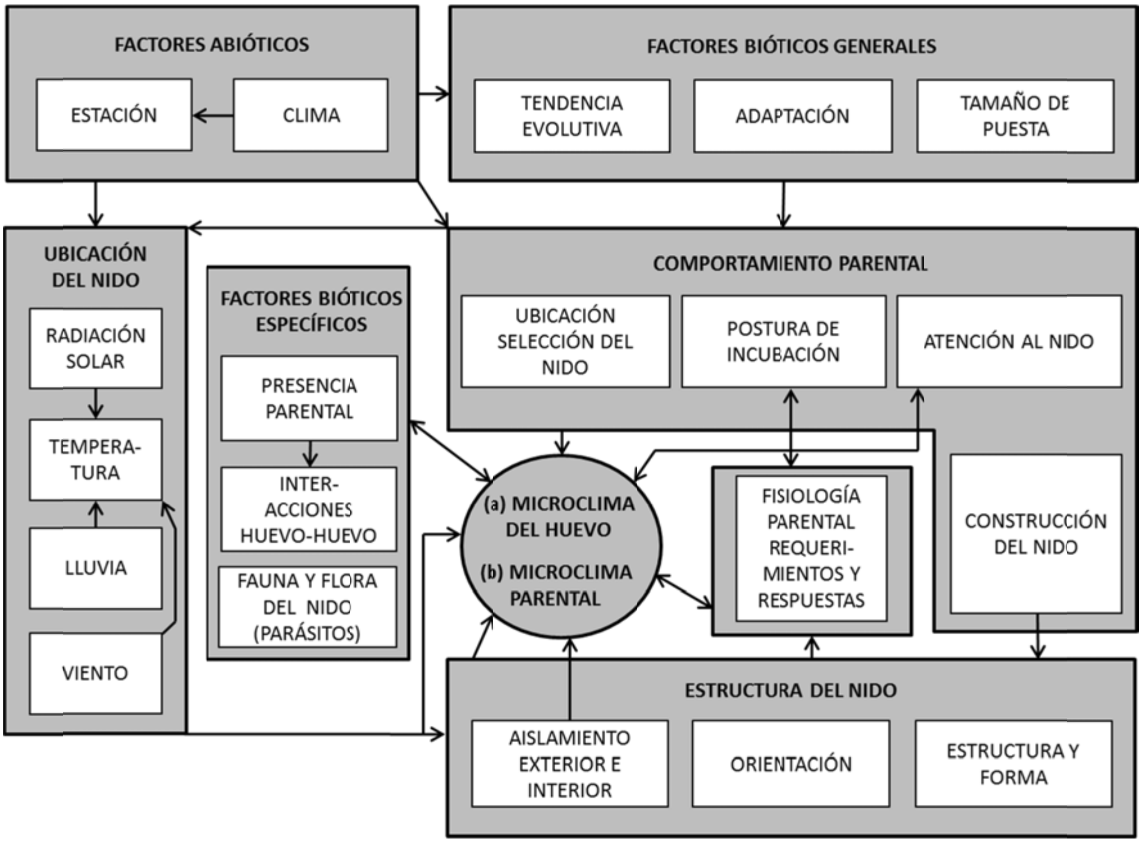

**Figura 1.** Una representación esquemática de los principales factores que interactúan, los cuales determinan el microclima del nido antes y durante la incubación, para embriones y/o adultos. Figura tomada de Ar y Sidis (2002).

## CONDICIONES DE INCUBACIÓN

Existen cuatro requerimientos necesarios para que los embriones se desarrollen adecuadamente: la temperatura, la humedad, y el ambiente gaseoso deben ser adecuados, y los huevos tienen que ser volteados regularmente.

La temperatura en el centro del huevo está comprendida entre 32 y 38 ºC (Huggins 1941; Drent 1975; Webb 1987). Hacia el final del periodo de incubación, la circulación sanguínea del embrión distribuye el calor más homogéneamente dentro del huevo, lo que provoca un incremento en la pérdida de calor; este aumento es sólo parcialmente compensado por la producción de calor metabólico del embrión, por lo que se requiere una mayor inversión parental para mantener la temperatura del huevo (Turner 1987, 1991, 2002). Aunque la temperatura del huevo debe mantenerse relativamente alta y constante durante la incubación, muchas especies, sobre todo aquéllas en las que sólo un miembro de la pareja incuba, son capaces de soportar variaciones importantes en la temperatura del huevo causadas por las ausencias periódicas del ave incubante (Webb 1987). La temperatura durante la incubación puede afectar directamente a la viabilidad del huevo (Romanoff y Romanoff 1972; Deeming y Ferguson 1991), a los patrones y eficacia de incubación (Stoleson 1999; Hepp et al. 2006; Parker y Andrews 2007), al éxito en la eclosión, al fenotipo del neonato (Hepp et al. 2006), y a la salud y supervivencia de los pollos (Geraert et al. 1996).

La humedad también es crítica, y la pérdida de agua excesiva o insuficiente interfiere con el desarrollo embrionario normal (Robertson 1961; Ar y Rahn 1980; Meir y Ar 1986), poniendo en peligro, o incluso impidiendo, que se produzca la eclosión. Los factores que determinan la pérdida de agua de los huevos son la humedad absoluta del nido (Ar y Sidis 2002), la temperatura del huevo, y la conductancia del vapor de agua de la cáscara (Ar 1991a, 1991b). Se ha sugerido que las aves regulan la humedad del nido (Rahn et al. 1976, 1977; Morgan et al. 1978), disminuyéndola al calentar los huevos y aumentándola mediante su respiración y la evaporación de su piel (Brown 1994).

Los huevos deben mantenerse en un ambiente gaseoso adecuado durante la incubación. Se ha comprobado que una composición de gases similar a la de la atmósfera al nivel de mar es adecuada para el correcto desarrollo de los embriones (Visschedijk 1980). La composición de gases que entra en el huevo depende de las presiones parciales de gases en el nido, que a su vez están determinadas por todos los "habitantes" del nido: los huevos, los pollos, si alguno ha nacido, posibles comensales o parásitos que pueda haber en el nido y, fundamentalmente, el padre que está incubando (Howe y Kilgore 1987; Ar y Piontkewitz 1992). Cuando no está el ave incubante, la convección y la acción del viento contribuyen a airear el nido, reponiendo niveles de oxígeno adecuados (White et al. 1978), aunque la acción de los padres puede tener gran influencia, sobre todo en especies que crían en cavidades (Ar y Piontkewitz 1992).

Por último, voltear los huevos durante la incubación es una parte fundamental para el éxito de la eclosión en casi todas las especies de aves (Poulsen 1953; Deeming 1991). Las funciones de este volteo son tanto redistribuir el calor desde la placa incubatriz (Caldwell y Cornwell 1975) como, sobre todo, prevenir que el embrión se adhiera a la membrana interna de la cáscara (New 1957; Drent 1975; Freeman y Vince 1974; Skutch 1976; Wilson 1991).

## COMIENZO DE LA INCUBACIÓN

El cuidado parental a la descendencia tiene importantes consecuencias para la eficacia biológica de los individuos y los compromisos de la historia de vida, pero se conocen relativamente poco los factores que influyen en la variación en el comienzo de la atención parental (Clutton-Brock 1991). En las aves, el cuidado parental comienza con la incubación de los huevos, y su inicio puede afectar al tamaño de puesta, la sincronización en el momento de nacimiento de los pollos, el éxito en la eclosión de los huevos, la mortalidad de los pollos en el nido por competencia entre hermanos, y las tasas de depredación sobre los padres y los pollos (Stoleson y Beissinger 1995, 1999; Hanssen et al. 2002; Cam et al. 2003; Bitton et al. 2006; Wang y Beissinger 2009).

El desarrollo embrionario no comienza hasta que la temperatura del huevo está por encima del cero fisiológico (25-27 ºC; Drent 1975), pero la temperatura óptima de incubación está entre 34-36 ºC (Drent 1975, Wang y Beissinger 2011). Se considera que la incubación “efectiva” típica comienza cuando la temperatura de los huevos se mantiene de forma constante por encima del umbral para el correcto desarrollo del embrión. Aunque muchas veces se asume que la incubación comienza con la puesta del último huevo, puede comenzar antes o después de concluir la puesta (Clark y Wilson 1981; Stoleson y Beisinger 1995; Monrós et al. 1998; Stenning 2008, Rowe y Weatherhead 2009).

El comportamiento parental, incluido el de incubación de los huevos, está asociado a la secreción de prolactina. Aunque los niveles de prolactina circulante en sangre aumentan a lo largo de la estación reproductora como resultado de la fotoestimulación (Dawson y

Goldsmith 1982), se produce un incremento mayor a partir de la estimulación visual y táctil del nido, los huevos o los pollos (El Halawani et al. 1986; Hall 1987). La prolactina también estimula el desarrollo de la placa incubatriz (Jones 1971; Lea y Klandorf 2002).

Entre especies, el momento de inicio de la incubación puede verse afectado por diferencias en el modo de desarrollo, el tipo de nido, el modo de incubación (mono- o biparental), el riesgo de pérdida del nido, y efectos ambientales sobre la viabilidad de los huevos (Stoleson y Beissinger 1995; Hébert 2002; Cook et al. 2005). Entre individuos de la misma especie, el comienzo de la incubación puede estar afectado por factores ambientales, como la temperatura, las precipitaciones o la disponibilidad de alimento durante la fase de puesta de los huevos (Nilsson 1993; Nilsson y Svensson 1993; Wiebe y Bortolotti 1994), y por diferencias entre individuos en la edad, experiencia o condición física (Bortolotti y Wiebe 1993; Hanssen et al. 2002; Ardia y Clotfelter 2007; Wang y Beissinger 2009).

En la mayoría de especies, antes del comienzo de la incubación efectiva, y a veces desde la puesta del primer huevo, las aves llevan a cabo una “incubación parcial” (Putnam 1949; Seel 1968; Ashkenazie y Safriel 1979), también llamada “empollamiento” (Barth 1955), “incubación intermitente” (Samuel 1971), “incubación irregular” (Beer 1962), o “incubación arrítmica” (Morton et al. 1972; revisión en Wang y Beissinger 2011). Es una forma menos regular de incubación, caracterizada por una atención mucho menor a los huevos que durante la incubación efectiva. Se ha visto que esta “incubación” puede elevar, o no, la temperatura de los huevos por encima del cero fisiológico, y se han sugerido varias funciones en ambos casos (Wang y Beissinger 2011; Podlas y Richner 2013a). No está del todo claro si esta “incubación parcial” tiene efectos sobre el desarrollo embrionario (Wang y Beissinger 2009; Lord et al. 2011; Podlas and Richner 2013a).

## RITMOS DE INCUBACIÓN

El comportamiento parental para mantener un ambiente térmico estable es importante para el desarrollo normal de los embriones (White y Kinney 1974; Hainsworth et al. 1998). En especies donde un solo progenitor incuba, son típicos los patrones de incubación intermitentes. Este comportamiento es interesante porque, al abandonar la hembra regularmente el nido para alimentarse, los huevos sufren ciclos cortos de enfriamiento y calentamiento, lo que puede afectar a la tasa de desarrollo de los embriones y un incremento en el gasto energético de los padres (White y Kinney 1974; Tinbergen y Williams 2002;

Olson et al. 2006). Aunque la incubación continua es más beneficiosa para el desarrollo del embrión, la incubación intermitente aparece como un compromiso en el desarrollo, forzado por los requerimientos para la supervivencia parental (White y Kinney 1974; Hainsworth et al. 1998).

En los casos en los que la incubación es intermitente, es importante el ritmo de incubación, es decir, la longitud y el patrón temporal de las sesiones de incubación (periodos en los que el ave está sobre los huevos) y de los recesos (periodos en los que se ausenta del nido, para alimentarse o realizar otras actividades). Las longitudes de ambos son muy variables, comprendiendo entre unos pocos minutos, en el caso de los paseriformes en los que sólo incuba la hembra (Kendeigh 1952; Skutch 1962), y varios días (Warham 1990; Williams 1995). Para algunas especies, como paseriformes y pingüinos (Skutch 1962, Williams 1995) las longitudes de sesiones y recesos (constancia de incubación) pueden ser muy regulares, pero en otros muchos casos no lo son. Skutch (1957) distingue entre especies que (1) realizan sólo un largo receso durante el día; (2) realizan varios recesos durante el día y la noche; y (3) están sobre los huevos de forma continua durante varios días. Los factores que contribuyen a la distribución del tiempo durante la incubación incluyen las tasas de depredación al nido, la condición de la hembra al inicio de la incubación, la alimentación proporcionada por el macho, la temperatura ambiental, y la disponibilidad de alimento (Sanz 1997; Conway y Martin 2000a; Martin 2002; Tewksbury et al. 2002; Chalfoun y Martin 2007; Matysioková y Remeš 2010; Ricklefs 2010). Por otro lado, el peso del huevo es un factor significativo en determinar sus características térmicas y, en consecuencia, la atención de las aves durante la incubación, puesto que los huevos más pequeños pierden y ganan calor más rápidamente que los más grandes (Tazawa et al. 1988). De esta forma, las especies con huevos pequeños (e.g. paseriformes) pueden dejar sus huevos desatendidos más tiempo durante la incubación, ya que luego pueden recuperar su temperatura más rápidamente y sin mucho gasto energético.

Dentro de una especie, la determinación de los ritmos de incubación, y los factores que pueden afectarlos, es importante para estudiar los compromisos del ciclo vital (Conway y Martin 2000a; Martin 2004; Pérez et al. 2008; Ardia et al. 2009) y la inversión parental en la reproducción (Conway y Martin 2000a; Londoño et al. 2008; Wojczulanis-Jakubas et al. 2009), entre otros aspectos. El ritmo de incubación está afectado por los requerimientos térmicos de los huevos, compromisos entre demandas de recursos de los padres, y factores ambientales, incluyendo la calidad del nido y el riesgo de depredación (White y Kinney 1974;

Williams 1996; Conway y Martin 2000a; Deeming 2002a; Cresswell et al. 2004; Voss et al. 2006; Cooper y Voss 2013). Los ritmos de incubación pueden variar a lo largo del periodo de incubación (e.g. Yerkes 1998; Rompré y Robinson 2008; Ricklefs y Brawn 2013), durante el día (Weeden 1966; Wheelwright y Beagley 2005; Marín 2008) y a lo largo de la estación reproductora (Wheelwright y Beagley 2005; Zimmerling y Ankney 2005; Ardia et al. 2009). Las características de la hembra (Hegyi y Sasvári 1998; Joyce et al. 2001; Gorman y Nager 2003), e incluso las del macho, si éste la ceba, pueden afectar al ritmo de incubación. La longitud de las sesiones y los recesos puede verse también influida por la temperatura ambiente (e.g. Kluijver 1950; Haftorn 1978, 1984; Conway y Martin 2000b), y la tasa de enfriamiento de los huevos (Drent 1975; Kendeigh 1952) y su peso. La frecuencia de los recesos, y el tiempo pasado fuera del nido en cada receso, puede tener consecuencias importantes para el desempeño reproductor presente y futuro (e.g. Webb 1987; Joyce et al. 2001; Reid et al. 2002b; Ardia et al. 2009, 2010).

Independientemente de la distribución temporal y la duración relativa de sesiones y recesos, si los hay, es importante estimar el tiempo total en el que el ave está incubando de forma efectiva, lo que se ha denominado “atención” (*attentiveness*) o “constancia de incubación”, normalmente expresado como un porcentaje (Kendeigh 1952; Skutch 1962). En paseriformes (*n* = 272 especies) el porcentaje de atención es mayor si incuban ambos progenitores (89,0%), que si incuba sólo la hembra (73,4%; Deeming 2002). Conway y Martin (2000a) han demostrado que, dentro de los paseriformes, la variación en el tiempo de atención puede reflejar la estrategia reproductiva, la morfología del nido, el clima predominante, y las fuentes de alimento previo y durante la incubación de cada especie concreta. Dentro de una especie, el porcentaje de atención puede estar influido por las temperaturas ambientales (e.g. Yom-Tov et al. 1978), la lluvia (Skutch 1962), y el tamaño de puesta (Blagosklononv 1977).

## DURACIÓN DEL PERIODO DE INCUBACIÓN

El periodo de incubación puede variar de 11 días (en pequeños paseriformes) hasta 64 días en algunas especies (Tullet 1985). Diversos factores pueden afectar la variabilidad interespecífica en la duración del periodo de incubación, entre los que se encuentran el tiempo de atención que dedican los padres (Martin et al. 2007), la prevalencia de parásitos en los adultos (Ricklefs 1992), la longevidad de los adultos (Ricklefs 1993), o la velocidad del desarrollo embrionario (Ricklefs y Brawn 2013), o las tasas de depredación (Rompré y Robinson 2008).

Dentro de cada especie, la duración del periodo de incubación es relativamente constante (e.g Carey 1980), aunque diferentes factores pueden tener efectos significativos sobre él, como el tamaño del huevo (e.g. Parsons 1972; Rahn y Ar 1974), el tamaño de puesta (e.g. Moreno y Carlson 1989; Smith 1989), la temperatura (Price 1998; Martin 2002; Hepp et al. 2006), las condiciones ambientales (Kluijver 1950; Lowther 1979; Moreno 1989; Järvinen 1990; Cooper et al. 2011), incluyendo la presión de depredación (Bosque y Bosque 1995; Fontaine y Martin 2006), y la atención parental (Lifjeld y Slagsvold 1986; Nilsson y Smith 1988; Voss et al. 2006).

La atención del nido tiene importantes consecuencias para la eficacia biológica por su influencia sobre el número y calidad de los pollos que nacen y por los costes energéticos impuestos a los padres (Clark y Galef 1995; Reid et al. 2002b; Gorman y Nager 2004; Olson et al. 2006; Chalfoun y Martin 2007). Un periodo de incubación prolongado puede afectar desfavorablemente a los pollos, bien porque tengan que gastar más energía antes de eclosionar (Vleck y Hoyt 1980), empobreciendo por tanto su condición al nacer, e incrementando el tiempo en el que la puesta es más vulnerable a la depredación (Ricklefs 1993; Martin 2002; Tieleman et al. 2004), bien porque se retrasa la fecha de eclosión, haciendo que los pollos estén en desventaja cuando compitan por alimento o territorios (Pettifor et al. 1988; Barba et al. 1995; Visser y Verboven 1999).

## DEFINICIÓN Y TIPOS DE ECLOSIÓN

La palabra eclosión procede del francés *éclosion* (acción de salir del cascarón), y ésta del verbo *éclore*, tomado del latín *excludere*, con la idea de hacer salir (ex-) de lo cerrado (*claudere*). “La eclosión es la emergencia del pollo desarrollado desde el huevo después de la incubación” (Portmann y Stingelin 1985).

En vertebrados con fertilización y desarrollo embrionario interno (la mayoría de los mamíferos, y algunos peces, anfibios y reptiles), la atención parental comienza en el momento de la fertilización, y el nacimiento posterior de las crías ocurre de forma sincrónica (Stoleson y Beissinger 1995). En contraste, los huevos de las aves se desarrollan fuera del cuerpo de la hembra, y en general precisan ser calentados por uno o ambos padres para desarrollarse. Así, los padres tienen la opción de controlar los intervalos entre nacimientos, comenzando la incubación antes o después: el patrón de eclosión puede variar entre muy asincrónico, si la incubación comienza tras la puesta del primer huevo, a prácticamente sincrónico, si comienza tras la puesta del último huevo (Clark y Wilson 1981; Stoleson y Beissinger 1995). En general, la eclosión sincrónica suele ser típica de especies nidífugas (los pollos abandonan el nido al poco de nacer), mientras que la eclosión asincrónica es frecuente en especies nidícolas (los pollos permanecen en el nido varios días, o semanas, después de nacer; Deeming 2002a).

La asincronía con que nacen los pollos en diversas especies ha sido objeto de numerosas interpretaciones, oscilando entre conferirle un valor adaptativo, hasta considerarla una consecuencia "inevitable" de otras adaptaciones, o un efecto de limitaciones energéticas durante el periodo de puesta (revisiones en Magrath 1990; Nilsson 1993; Stenning 1996; Viñuela 2000; Sockman et al. 2006). Algunas hipótesis sugieren que la jerarquía de tamaño establecida por la eclosión asincrónica incrementa la eficacia biológica de los padres y de la progenie que eclosiona más pronto. Entre ellas, la mejor conocida y la más debatida es la hipótesis de reducción de la nidada propuesta por Lack (1947), que estipula que la eclosión asincrónica es ventajosa en ambientes impredecibles. Cuando el alimento es escaso, los pollos que han nacido más tarde, con menor capacidad que sus hermanos para competir por el alimento, crecen más lentamente (Stokland y Amundsen 1988; Nilsson y Svensson 1996; Rosivall et al. 2005) y tienen mayor mortalidad antes o después de volar (Magrath 1990; Forbes et al. 1997; Viñuela 2000). Se ha sugerido, sin embargo, que la eclosión asincrónica puede no ser esencial en facilitar la reducción de la pollada (Clark y Wilson 1981; Podlas y Richner 2013b). Así, otros estudios muestran que la inanición ocurre tanto en puestas asincrónicas como sincrónicas (Howe 1976; Hõrak 1995; Kontiainen et al. 2010), sugiriendo que otros factores, como el despotismo entre hermanos, pueden estar relacionados con la muerte de algunos pollos. Además, la eclosión asincrónica puede ser ventajosa para los padres (e.g. Slagsvold y Lifjeld 1989; Hõrak 1995). Por tanto, la relación entre las condiciones reproductoras prevalecientes y los beneficios de los patrones de eclosión es compleja (Amundsen y Slagsvold 1996; Forbes et al. 2002; Szöllősi et al. 2007).

El grado de asincronía en la eclosión puede estar determinado por múltiples factores, entre los que se encuentran el tamaño de puesta (Howe 1976; Haftorn y Reinertsen 1985; Hébert y Sealy 1992), la fecha de inicio de puesta (Stoleson y Beissinger 1995; Hébert 1999), la edad de la hembra, el tipo de hábitat, la temperatura ambiental durante el periodo de puesta

(Shaw 1985; Slagsvold 1986; Slagsvold y Lifjeld 1989), la disponibilidad de alimento durante la puesta, o la presión de depredación (Mitrus 2008).

El éxito de la eclosión (proporción de huevos que eclosionan) ha sido considerado uno de los principales factores asociados con la eficacia biológica en aves (Cabezas-Díaz y Virgós 2007), aunque los factores que afectan la eclosionabilidad son poco conocidos (Serrano et al. 2005). La eclosionabilidad podría jugar también un papel en la evolución de tamaño de puesta óptimo, por lo menos limitando el número máximo de huevos que los padres podrían incubar (Serrano et al. 2005).

El fracaso en la eclosión podría ser el resultado de dos sucesos independientes, infertilidad y muerte del embrión, los cuales no han sido considerados independientemente en muchos estudios y podrían ser causados por limitaciones fisiológicas de los padres (Cabezas-Díaz y Virgós 2007). El inicio de incubación en relación a la secuencia de puesta, y la consecuente asincronía en la eclosión inducida por los padres (Drent 1975; Wiebe et al. 1998) determinará el crecimiento de la progenie (Cotton et al. 1999; Clotfelter et al. 2000; Mainwaring et al. 2010) y su supervivencia (Forbes et al. 1997; Viñuela 2000), y puede tener consecuencias a largo plazo para los pollos (Forbes 2009; Mainwaring et al. 2012).

## ¿POR QUÉ ESTUDIAR LA INCUBACIÓN EN LAS AVES?

Esta Tesis Doctoral se centra en la incubación de las aves, así como en el patrón de eclosión de los huevos, consecuencia a su vez del patrón de incubación. ¿Por qué centrarnos en este tema? Podemos dar tres argumentos, que se inician en el mero interés por la historia natural, pasan por aspectos teóricos, y terminan en aplicaciones prácticas para la conservación de especies.

A lo largo del Siglo XX, los huevos de las aves y la incubación han sido un área de considerable esfuerzo investigador, aunque se ha centrado sobre todo en la incubación artificial en gallinas (*Gallus gallus*), con fines evidentemente comerciales (Webb 1987; Ricklefs y Stark 1998; Cooper et al. 2011). Estos estudios han sido muy exitosos, y la incubación artificial moderna puede conseguir altas tasas de eclosión dentro de grandes incubadoras. Sin embargo, el grado en que las conclusiones de los estudios en incubadoras pueden extrapolarse a condiciones naturales es reducido. El interés en la incubación en poblaciones naturales ha sido particularmente intenso en los últimos 40-50 años, y se ha estudiado en un amplio rango de especies. En estos años se ha avanzado mucho en el conocimiento de los diferentes aspectos relacionados con la incubación en poblaciones naturales (Deeming 2002b; Sockman et al. 2006; Angelier y Chastel 2009; Mennerat et al. 2009; Wang y Beissinger 2011; Husby et al. 2012; Moore y Rohwer 2012; Cooper y Voss 2013; Matysioková y Remeš 2013). No obstante, quedan muchas preguntas por resolver, y una gran cantidad de aspectos relacionados con la incubación, desde los mecanismos hormonales que desendadenan el inicio, mantenimiento y conclusión del comportamiento de incubación, hasta las consecuencias evolutivas de diferentes estrategias, se conocen relativamente poco, incluso en especies bien estudiadas (Deeming 2002b).

El segundo argumento es más teórico, y en él se utiliza la incubación como un proceso útil para desentrañar compromisos en las estrategias vitales. Las estrategias vitales son los conjuntos de rasgos asociados a la variación en fecundidad y supervivencia a lo largo de la vida. La teoría evolutiva predice que las estrategias vitales han sido seleccionadas para maximizar la eficacia biológica de los organismos (Roff 1992; Stearns 1992). La teoría de las estrategias vitales está interesada en explicar la diversidad de estrategias observadas en la naturaleza. El principal compromiso que moldea a las estrategias vitales es entre la fecundidad presente y futura, llamado coste de la reproducción. Un principio central es que los organismos tienen recursos finitos a su disposición, y la asignación de recursos en un aspecto de la vida reducirá los recursos disponibles para invertir en otros. La consecuente competencia por los recursos entre las diferentes actividades constreñirá la evolución del ciclo vital, de forma que la inversión realizada en una actividad particular refleja el compromiso que maximiza la eficacia biológica durante la vida del individuo.

En términos de distribución de recursos durante la reproducción, tales constricciones y compromisos se convierten en costes de reproducción, definidos como la reducción que la inversión en un evento reproductor, o componente o fase de ese evento, provoca en la capacidad de un progenitor para invertir en eventos reproductores futuros o en otras actividades. Así, para interpretar correctamente los patrones generales de distribución de recursos, y las consecuentes estrategias vitales, se requiere una comprensión de la limitación de recursos, y de los costes asociados a cada componente de la reproducción. Una elevada proporción de este tipo de estudios se han realizado con aves, y muchos de ellos en especies nidícolas (e.g. Clark y Wilson 1981; Magrath 1989; Mock y Parker 1997; Mainwaring et al. 2012). La razón más probable es que las aves ofrecen excelentes modelos para estudiar efectos parentales, ya que los embriones se desarrollan fuera del cuerpo de la madre, y facilitan la manipulación de los patrones de incubación, el orden de eclosión, y otros estadios del desarrollo de la progenie (Bernardo 1996; Groothuis y Schwabl 2008). Es decir, la reproducción aviar puede ser estudiada con relativa facilidad, las aves generalmente tienen la capacidad para reproducirse más de una vez, y cada evento reproductor está dividido en fases relativamente discretas de cortejo y emparejamiento, producción de huevos, incubación y crianza de pollos. Estos factores hacen de las aves organismos adecuados para investigar compromisos entre diferentes eventos reproductivos, e incluso entre diferentes fases del mismo evento (Lessells 1991; Heaney y Monaghan 1995, 1996; Monaghan y Nager 1997).

Sin embargo, hasta hace relativamente poco tiempo, tales estudios se han centrado casi exclusivamente en compromisos que involucraban las demandas de la crianza de los pollos (Lindén y Møller 1989; Dijkstra et al. 1990), asumiendo, como se ha comentado anteriormente, que los requerimientos de los estadios tempranos de la reproducción, tales como la incubación, eran relativamente triviales en comparación con la fase de crecimiento de los pollos (Walsberg y King 1978; Monaghan y Nager 1997). Como se ha visto, esto no es así, y los costes de la incubación representan unas elevadas demandas energéticas, y un riesgo de depredación, para las aves incubantes. Por ello, estudios en los que se estimen, o manipulen, los costes de la incubación son importantes para tener una idea clara de los compromisos a los que hacen frente las aves (Davis et al. 1984; Moreno y Sanz 1994, De Heij et al. 2006; Nilsson et al. 2008; Ardia et al. 2009).

Por último, el tercer argumento está relacionado con la conservación de especies. Veremos a lo largo de este trabajo la estrecha relación que existe entre las temperaturas a las que están expuestos nidos, huevos y adultos y el proceso de incubación. Y, precisamente, la temperatura ambiental es lo que está cambiando de forma dramática, a un ritmo sin precedentes en los últimos 65 millones de años (Diffenbaugh y Field 2013). Evidentemente, todos los procesos que dependen estrechamente de la temperatura ambiental están viéndose afectados, y la incubación no escapa a ello. Un aspecto crucial que determina el éxito reproductor de las aves insectívoras es la sincronía entre el momento de máxima demanda de alimento por parte de los pollos y la máxima disponibilidad de alimento en el hábitat (Van Noordwijk et al. 1995; Visser et al. 2004). La forma más sencilla de realizar este ajuste es comenzar a poner los huevos en la fecha adecuada para que, teniendo en cuenta la duración de los periodos de puesta, incubación, y desarrollo de los pollos, los dos máximos de oferta y demanda coincidan en el tiempo. Esto supone que las aves deben "predecir" cuándo ocurrirá el máximo de oferta con varias semanas de antelación. Pero, una vez comenzada la puesta, las aves tienen todavía algo de margen para acelerar o ralentizar el proceso, si la "predicción" que hicieron no fue muy precisa, o las condiciones cambian durante el proceso. Estas "correcciones" pasan por (1) dejar más de un día entre la puesta de huevos consecutivos (i.e. alargar el periodo de puesta); (2) reducir el tamaño de puesta, acortando el periodo de puesta; (3) empezar a incubar antes de cocluir la puesta, solapando los periodos de puesta e incubación; y (4) modificar el periodo de incubación, dedicando más o menos tiempo (Monrós et al. 1998; Cresswell y McCleery 2003; García-Navas y Sanz 2011). Los recientes cambios en el clima están desajustando de forma sistemática la sincronía entre demanda y oferta de alimento de muchas especies (Both 2010), por lo que es esperable que las aves utilicen todos los mecanismos disponibles, incluyendo cambios en el comienzo y duración de la incubación, para reajustar en lo posible oferta y demanda. Recientes estudios han mostrado que la duración del periodo de incubación está poco determinado genéticamente (Husby et al. 2012), por lo que los individuos tienen cierta capacidad de ajustarlo a las circunstancias prevalentes en cada momento. El estudio de los factores que pueden afectar al comienzo de la incubación, a su duración, y las consecuencias que todo ello tiene para la eficacia biológica de los individuos y, por extensión, para la viabilidad de las poblaciones, es un campo de mucho interés para la conservación de especies.

## OBJETIVOS

El objetivo general de la presente Tesis Doctoral es describir los patrones de incubación y eclosión de una población de carbonero común *Parus major* en un ambiente mediterráneo, profundizar en algunas de las causas que afectan a dichos patrones, centrándonos sobre todo en las características del nido, y describir sus posibles consecuencias en el desempeño reproductor. Partimos de la hipótesis general de que las características parentales, las del nido, y las condiciones ambientales pueden afectar a la duración de los periodos de incubación y eclosión, teniendo consecuencias en el desempeño reproductor. Para alcanzar el objetivo general de este trabajo, se han especificado los siguientes objetivos específicos:

*Objetivo 1*: Estudiar la relación entre la calidad parental, la calidad del nido y el desempeño reproductor en el carbonero común.

*Objetivo 2*: Estudiar las relaciones entre las características del nido y el desempeño reproductor, y las posibles variaciones entre años en dichas relaciones, y explorar las relaciones entre las características parentales y las variables relacionadas con el tamaño del nido y con el desempeño reproductor.

*Objetivo 3*: Describir el peso y la composición del nido del carbonero común en diferentes hábitats mediterráneos, y explorar sus posibles consecuencias sobre la eficacia biológica.

*Objetivo 4*: Determinar experimentalmente si la calidad del nido por sí misma, independientemente de la calidad de los padres, afecta al proceso de incubación.

*Objetivo 5*: Describir la variación de los periodos de incubación y eclosión de una población de carbonero común, y examinar los efectos de algunos factores sobre dichos periodos.

*Objetivo 6*: Describir el patrón de incubación de una población de carbonero común del Este de España y determinar algunos de los factores que afectan al ritmo de incubación.

*Objetivo 7*: Comparar los patrones de incubación y eclosión del carbonero común entre poblaciones europeas, a lo largo de un gradiente latitudinal.

*Objetivo 8*: Determinar experimentalmente los efectos de las condiciones térmicas durante la incubación sobre diferentes factores relacionados con la eficacia biológica, incluyendo la condición y el comportamiento parental, longitud del periodo de incubación, y el desempeño reproductor.

# 3. ÁREA DE ESTUDIO Y METODOLOGÍA GENERAL

## ÁREA DE ESTUDIO

Una gran parte de este trabajo se ha desarrollado en una zona del término municipal de Sagunto (Valencia, Este de España; 39º42’N, 0º15’W, 30 m s.n.m.; Figura 1) dominada por plantaciones naranjos (*Citrus aurantium*). Las explotaciones en esta zona son minifundistas, con pequeños huertos separados por acequias de riego, caminos de tierra y alguna carretera asfaltada. Algunos huertos se encuentran abandonados, o han sido transformados para cultivos hortícolas. Desde hace unos pocos años se está cambiando de sistema de riego, pasando del sistema por inundación tradicional al de goteo (Andreu et al. 2005). Gil-Delgado y Escarré (1977) y Gil-Delgado et al. (1979) realizan una descripción detallada de los tipos de huertos que se pueden encontrar en esta zona, así como de las especies vegetales que componen el estrato herbáceo en el interior de los huertos y márgenes de huertos y caminos, aunque gran parte de esta vegetación está desapareciendo al cambiar el sistema de riego (Figura 2).

El clima en esta zona está caracterizado por inviernos suaves (temperatura media sobre 0º C durante los meses más fríos: enero y febrero) y veranos cálidos, con temperaturas máximas sobre 30º C. La precipitación media anual está sobre 420 mm, siendo más abundante durante mayo y septiembre.

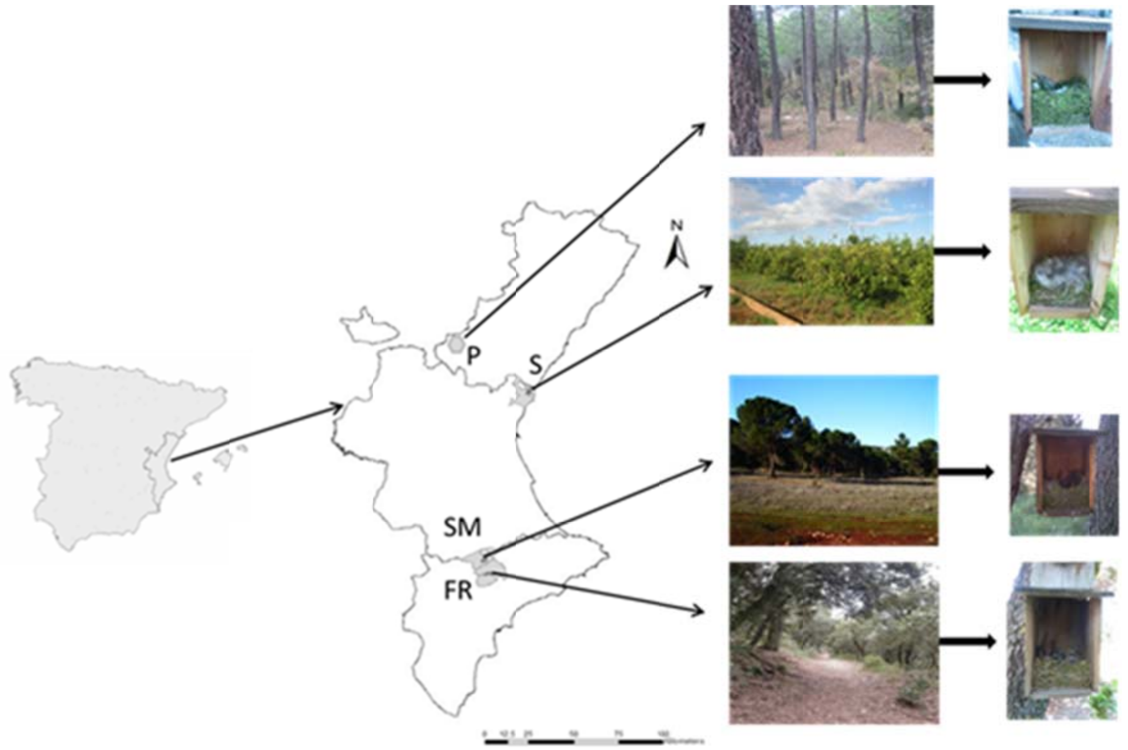

**Figura 1.** Localización de las cuatro áreas de estudio: Pina (P), Sagunto (S), Sierra Mariola (SM) y Font Roja (FR), mostrando una vista general del hábitat y un nido tipo de cada área.

Los datos tomados específicamente para este trabajo abarcan las temporadas reproductoras 2006-07 y 2009. En este periodo, la extensión del área de estudio ha sido de 485 ha. No obstante, para elaborar el Artículo 3, se han utilizado datos de 2005, y para el Artículo 5, datos de recolectados entre 1988 y 2010. En este periodo, el área de estudio ha ido creciendo desde las 50 ha en 1988, hasta las 485 de 2010.

**Figura 2.** Vista de uno de los campos de naranjos en el área de estudio de Sagunto y detalle de una caja-nido colgada en un naranjo.

En el área están disponibles cajas de madera de pino para páridos (ver características en Lambrechts et al. 2010; Figura 2), en una densidad aproximada de una por hectárea (Figura 3). Puesto que la densidad media de cajas se ha mantenido durante todos estos años, el aumento de la superficie no hemos considerado que haya tenido efecto sobre los parámetros reproductores tratados en este trabajo. El carbonero común *Parus major* es la única especie de ave que actualmente se reproduce en las cajas-nido dentro de esta área, aunque antiguamente también lo hacía el gorrión común *Passer domesticus* (Barba y Gil-Delgado 1990). Las cajas-nido también son ocupadas por la rata negra *Rattus rattus* y el lirón careto *Elyomis quercinus*, que son a la vez competidores y depredadores del carbonero común (Barba y Gil-Delgado 1990; Gil-Delgado et al. 2009).

Para uno de los trabajos (Artículo 3) incluimos datos de otras tres áreas de estudio (Figura 1). Por un lado, está la Sierra de Santa Bárbara de Pina, en la provincia de Castellón (40º01’N, 0º37’W, 1200 m s.n.m.), donde crece un bosque mixto de pino marítimo (*Pinus pinaster* Aiton), pino silvestre (*Pinus sylvestris*), melojo (*Quercus pyrenaica*) y quejigo (*Quercus faginea*). La dos áreas restantes están situadas en dos parques naturales en la provincia de Alicante: el Parque Natural de la Sierra de Mariola, en Bocairente (38º44’N, 0º33W, 900 m s.n.m.), dominado por un bosque de pino de Alepo (*Pinus halepensis*), y el Parque Natural del Carrascal de la Font Roja, en Alcoy (38º39’N, 0º32’W, 1090 m s.n.m.), donde domina la encina (*Quercus ilex*). Todas las áreas de estudio están localizadas en la región mediterránea del Este de España.

**Figura 3.** Mapa general del área de estudio de Sagunto (arriba) y detalle de una de las zonas de estudio, mostrando la ubicación de las cajas-nido (abajo).

## METODOLOGÍA GENERAL

Las cajas-nido están disponibles en el área de estudio de Sagunto desde 1986. Cada año se colocan a finales de febrero, y se retiran al terminar la estación reproductora (Monrós et al. 2002). Esto se realiza principalmente para evitar la ocupación invernal de pequeños mamíferos como la rata negra o el lirón careto (Barba y Gil-Delgado 1990; Gil-Delgado et al. 2006), así como para evitar el robo y el deterioro de las cajas (es un área de acceso público y, en algunas zonas, muy transitada). Siempre que es posible se colocan en los mismos lugares cada año, aunque a veces el huerto ha sido talado y se tienen que colocar en el lugar más cercano del huerto colindante. Cada temporada, las cajas se empezaban a visitar a primeros de marzo. La frecuencia de visitas ha sido muy variable, pero todas se visitaban al menos una vez por semana hasta que el último nido dejaba de estar activo. Con este patrón de visitas es posible determinar la fecha de inicio de puesta, el tamaño de la puesta, el número de huevos que eclosiona (los que no eclosionan quedan en el nido), y el número de pollos que abandona el nido con vida. A partir de esto datos, se estimó el éxito de eclosión (proporción de huevos eclosionados), el éxito de vuelo (proporción de pollos que vuelan) y el éxito reproductor (proporción de huevos que producen volanderos).

Para estudiar aspectos concretos, como las fechas exactas de comienzo de la incubación o la eclosión, algunos nidos eran visitados diariamente; esto se especifica en los artículos correspondientes. Por último, para algunos experimentos, como el realizado en el Artículo 7, se requería visitar algunos nidos varias veces al día. Detallamos en el apartado dedicado a la metodología, en cada artículo, el modo de proceder o las observaciones realizadas sobre distintos aspectos. En muchos casos el nido fue medido y pesado una vez iniciada la incubación, y se retiró tras volar los pollos para estimar, en el laboratorio, su peso seco y su composición.

La mayor parte de los adultos fueron capturados, utilizando trampas en el nido, normalmente cuando los pollos tenían 10-14 días de edad, pero en ocasiones (e.g. Artículo 6) antes de esa fecha. Cada ejemplar se anilló individualmente con una anilla metálica numerada, determinando su sexo y clase de edad (Svensson 1998). Para este trabajo diferenciamos únicamente individuos reproductores de un año de edad (nacidos la temporada anterior) de los más viejos, ya que esto se puede determinar con facilidad a partir de la coloración del plumaje (Svensson 1998). En general, tomaban medidas biométricas (longitud del ala y del tarso, y el peso), y se determinaba un índice de condición corporal (peso/longitud del tarso) para cada uno de los individuos capturados. En algunos casos, además, se les colocó un transponder en la pata opuesta a la de la anilla metálica, para detectar sus entradas y salidas de la caja-nido.

Los pollos fueron anillados y medidos principalmente el día 15 de edad, aunque en algún experimento se tomaron medidas a distintas edades. En algún caso se les inyectó subcutáneamente fitohemaglutinina, para poder determinar el nivel de respuesta inmune. Los protocolos específicos quedan detallados en cada artículo. En la mayoría de estudios trabajamos sólo con primeras puestas, ya que las segundas (puestas realizadas tras una primera puesta con éxito) o de reposición (puestas realizadas tras una primera puesta fracasada) son muy escasas.

## EL CARBONERO COMÚN COMO MODELO BIOLÓGICO

En este trabajo se ha utilizado como especie modelo al carbonero común (Figura 4). Es una especie de ave bien conocida, y sus características generales pueden encontrarse en Perrins (1979), Cramp y Perrins (1993), Gosler (1983) y Atiénzar et al. (2012). Tomando como base estas obras, resumimos a continuación las características más relevantes para el presente estudio.

El carbonero común es un ave del Orden Passeriformes, insectívora, y de pequeño tamaño (17-19 g). Es una especie troglodita, es decir, nidifica en huecos ya existentes, generalmente en árboles, pero también en muros u otros soportes, ya que no es capaz de excavarlos. Esta tendencia hace que ocupe las cajas-nido cuanto éstas son proporcionadas en el ambiente adecuado.

**Figura 4.** Carbonero común *Parus major*

Dada su amplia tolerancia a ocupar cualquier tipo de hueco, puede encontrarse criando en casi cualquier tipo de medio mínimamente arbolado, desde el nivel del mar hasta altitudes superiores a los 2100 m s.n.m. Es una especie de muy amplia distribución en las regiones paleártica y oriental, y en España se distribuye de modo continuo por prácticamente toda la Península. No presenta problemas de conservación, dado su carácter generalista en cuanto a la alimentación, y su capacidad de prosperar en ambientes humanizados.

Los carboneros comunes crían entre marzo y julio, y el inicio de la reproducción depende en gran medida de la temperatura primaveral y del pico de abundancia de orugas de lepidópteros, el principal alimento de los pollos. Es una especie territorial durante la época de cría. Las hembras son las encargadas de construir el nido, incubar los huevos y empollar, sobre todo cuando los pollos tienen pocos días. El macho aporta alimento a la hembra mientras está incubando.

Ambos sexos empiezan a criar con un año de edad. Es una especie típicamente monógama, manteniéndose la pareja al menos durante la estación reproductora, y muchas veces de un año a otro si los dos individuos siguen vivos. El nido está formado básicamente por musgo y suelen tardar una semana en construirlo. Ponen un huevo diario hasta terminar la puesta, y tienen la costumbre de enterrar los huevos entre el material del nido hasta que empiezan a incubar. En la mayor parte de las poblaciones estudiadas, lo más frecuente es que los individuos sólo realicen una puesta por temporada, aunque en casi todas ellas se registran parejas que hacen dos puestas.

El tamaño de puesta varía desde 4 a 13 huevos. Las hembras suelen comenzar a incubar un día antes de que la puesta esté completa, y la incubación dura en promedio 13 días. La eclosión es típicamente asíncrónica (i.e. los huevos eclosionan en un periodo superior a 24 h), y puede extenderse durante varios días en determinadas condiciones. Esto genera jerarquías de tamaño entre los pollos, y los últimos pollos nacidos a menudo mueren de inanición. Los pollos permanecen en el nido entre 18 y 20 días después de la eclosión, de ahí que se le considere como especie nidícola.

Utilizamos al carbonero común como especie modelo para este trabajo por varias razones:

1. Al criar a densidades relativamente elevadas, y aceptar de buen grado las cajas-nido cuando están disponibles, permiten la obtención de tamaños muestrales adecuados (Artículos 1, 2, 6), y disponer de nidos en los que realizar manipulaciones al tiempo que se mantienen áreas con parejas no manipuladas.
2. Otra ventaja, derivada del uso de cajas-nido, pero también de la tolerancia de la especie a las manipulaciones, es que se pueden visitar los nidos con gran frecuencia sin efectos aparentes sobre el comportamiento de los individuos o su éxito reproductor, e incluso realizar manipulaciones a veces bastante invasivas (cambios de nido, colocación de

termómetros o lectores de transponders, etc.), sin mucho riesgo de abandono del nido (Artículos 4 y 7).

3. También útil para el presente trabajo, y relacionado con el uso de las cajas, es que todos los individuos tenían las mismas condiciones iniciales (tamaño, forma y material de las cajas) para construir el nido, por lo que se pueden atribuir las diferencias en el tamaño y composición del nido a factores ajenos a las características del hueco donde lo construyen (Artículos 1-4).
4. Que tenga una gran variabilidad en el número de huevos que pone por intento reproductor (diferencias en la inversión reproductora) también facilita poder estudiar las consecuencias individuales de decisiones diferentes.
5. Una ventaja adicional, al tratarse de una población estudiada de forma ininterrumpida desde 1986 (Barba 1991; Tomás et al. 2012), es que se disponía de un registro de datos que permitió el estudio de la variabilidad interanual en la duración de los periodos de incubación y eclosión (Artículo 5).
6. Su amplia distribución geográfica, y el amplio seguimiento que realizan numerosos grupos de investigación en Europa (e.g. Van den Steen et al. 2009; Lambrecths et al. 2010), permiten comparaciones de los rasgos poblacionales, reproductores, morfológicos, genéticos, etc. entre poblaciones sometidas a muy diferentes presiones selectivas (Artículos 3 y 6).
7. De especial interés para este trabajo es que los carboneros comunes sean incubadores uniparentales (incuba la hembra sola), por lo que la hembra debe compaginar la atención a los huevos durante la incubación con su propia alimentación, para lo que debe abandonar los huevos con frecuencia. Esto permite el estudio, tanto de por qué toman las decisiones que toman, como de las consecuencias de esas decisiones para su eficacia biológica (Artículos 6 y 7).
8. Por último, como los carboneros comunes son aves residentes, y se asientan fielmente en el lugar de reproducción tras su primer asentamiento, los efectos a largo plazo de experimentos en padres y pollos pueden ser evaluados (Artículo 7).

A pesar de que el carbonero común es una de las especies de aves más estudiadas, con muchas poblaciones seguidas a lo largo y ancho de su amplio rango de distribución (e.g. Van den Steen et al. 2009; Lambrecths et al. 2010), sus patrones de incubación y eclosión han recibido relativamente poca atención (ver, por ejemplo, Lord et al. 2011; Podlas y Richner

2013b, y referencias citadas). Además, todos los estudios proceden de poblaciones del Norte y Centro de Europa, estando totalmente ausentes en la parte sur de su área de distribución (Atiénzar et al. 2012). Se han encontrado patrones latitudinales en la atención del nido durante el periodo de incubación entre especies (e.g. Martin 2002; Chalfoun y Martin 2007), pero a nivel intraespecífico la variación es mucho menos clara, y hay pocos estudios que la traten (e.g. Ardia et al. 2006). Casi lo mismo puede decirse del tamaño y composición de los nidos, de los factores que los determinan, y de sus consecuencias para padres y pollos. Aunque se suele asumir que los nidos están formados principalmente por musgo, muy pocos trabajos han descrito con detalle la composición de los nidos (Alabrudzińska et al. 2003).

En definitiva, aunque el carbonero común es una de las especies de aves mejor conocidas y más estudiadas a nivel mundial, los conocimientos sobre los temas abordados en esta tesis son relativamente escasos. Por ello, además del interés general en el estudio de los objetivos propuestos, creemos que también es significativa la aportación de este trabajo al conocimiento de esta especie concreta.

# 4. RESUMEN DE RESULTADOS, DISCUSIÓN Y CONCLUSIONES

## ARTÍCULO 1

No encontramos ninguna relación significativa entre la calidad de los padres y la del nido y sólo obtuvimos una débil relación positiva entre el tamaño de la hembra y la tasa de éxito reproductor. Puesto que estas relaciones se han descrito en algunos trabajos, quizás hiciera falta considerar otros componentes de la calidad parental (e.g. el estado de salud), del nido (e.g. su composición) y del desempeño reproductor (e.g. el peso y el estado de salud de los pollos). Como esperábamos, encontramos relaciones positivas entre la calidad del nido y el éxito reproductor. Concretamente, al aumentar el tamaño del nido, aumentó la tasa de éxito (una variable que incluye los éxitos de eclosión, de vuelo y reproductor), y nidos con cuencos más grandes contuvieron puestas que comenzaron más pronto y fueron más grandes. Parece ser que las hembras son capaces de construir un cuenco acorde a los huevos que van a poner. Una cuestión no resuelta en este trabajo es si las características del nido por sí mismas son importantes para el éxito del nido, o es la calidad de los padres la variable importante que está detrás de esta relación. Una clara conclusión de este trabajo es que los nidos de mejor calidad estuvieron relacionados con un mejor desempeño reproductor.

## ARTÍCULO 2

Encontramos una relación positiva entre la profundidad del cuenco del nido y el éxito de eclosión, y entre el tamaño total del nido y el éxito de vuelo en 2007, pero no en 2006. Esto está de acuerdo con la idea de que nidos más grandes pueden tener efectos positivos sobre el éxito reproductor, pero que esta relación puede depender de las condiciones ambientales, i.e. de la cantidad de recursos o las condiciones meteorológicas un año concreto. En este estudio encontramos que la fecha de puesta fue más temprana en los nidos con un diámetro de cuenco mayor en los dos años de estudio, pero no encontramos que el tamaño de puesta fuera mayor en estos nidos. En el trabajo anterior encontramos una relación positiva entre el tamaño de copa y el tamaño de puesta. Eso puede deberse a que en el presente estudio utilizáramos sólo primeras puestas, o que al ser más rigurosos en la selección de nidos, redujimos el tamaño muestral. Las hembras jóvenes construyeron nidos con cuencos más profundos, aparentemente de mejor calidad, pero el peso del nido fue similar entre las jóvenes y las adultas, lo que sugiere que las jóvenes puedan hacer lo nidos menos densos, en definitiva peores. Las hembras de tarso más largo y en peor condición (peso/tarso) comenzaron antes la puesta. El éxito de vuelo aumentó al disminuir el tarso de los machos, y la condición de los pollos fue mejor en nidos atendidos por machos mayores de un año. Encontramos por tanto relaciones positivas entre el tamaño de los padres y el éxito reproductor, aunque las relaciones con la condición no sean claras en el caso de las hembras. Esto puede deberse a que se hayan tomado las medidas de los padres en un momento inadecuado, es decir, al final del periodo de pollo. Concluimos que, en general, las características del nido estuvieron positivamente relacionadas con el éxito reproductor, aunque las relaciones específicas entre los distintos componentes variaron entre años. Estas relaciones fueron más evidentes en un año "bueno" (2007; mayor tamaño de puesta y pollos producidos en mejor condición) que en uno "malo" (2006) en términos de disponibilidad de recursos. La falta de relación entre las características parentales y las del nido, mientras que características del nido sí estuvieron positivamente relacionadas con el desempeño reproductor, sugiere que el nido por sí mismo podría, al menos parcialmente, afectar al éxito reproductor.

## ARTÍCULO 3

Los carboneros comunes utilizaron los mismos materiales para la construcción del nido en todos los hábitats, pero la cantidad y proporción de cada uno varió entre lugares. Los nidos fueron más pesados en Sagunto (naranjal) y Mariola (pinar), y más ligeros en Font Roja (carrascal), teniendo los de Pina (bosque mixto) pesos intermedios. Los nidos de Sagunto tuvieron menos cantidad de musgo, mayor cantidad y proporción de palitos, y mayor cantidad de pelo que el resto. La proporción de pelo fue mayor en Sagunto que en Mariola y Pina, y mayor en Font Roja que en Pina. La diferencia más notable es el relativamente bajo uso de musgo en Sagunto, lo que compensaron añadiendo palitos. El musgo es un componente fundamental en los nidos de carbonero común, como también lo apoyan nuestros resultados, por lo que probablemente la causa de su escasez en los nidos en Sagunto fue la dificultad de obtenerlo en este ambiente. El peso del nido puede variar entre las poblaciones de la misma especie, probablemente adaptándose a las condiciones prevalecientes y a la disponibilidad de materiales de cada sitio particular. El mayor peso en Sagunto es atribuible a un menor uso de musgo y mayor de ramitas, mientras en Mariola pudo ser un efecto acumulativo de pequeñas cantidades adicionales de varios materiales. No se detectaron diferencias entre años en el peso de los nidos, sugiriendo una relativa estabilidad en las condiciones reproductoras. No encontramos ninguna relación entre el peso total del nido y los parámetros reproductores. El tamaño de puesta difirió entre hábitats y estuvo relacionado negativamente con el peso del musgo en los cuatro hábitats. Esto sugiere que las hembras, que construyen y ponen los huevos, actividades ambas muy costosas, pueden tener que afrontar el compromiso de buscar material de alta calidad para construir el nido, y alimento para formar los huevos. Considerando la relación entre el éxito de eclosión y el peso del musgo separadamente entre los cuatro hábitats, sólo encontramos una relación positiva en Sagunto. Esto sugiere que disponer de la suficiente cantidad de musgo garantiza un buen desarrollo de la incubación, y allí donde es más escaso, nidos con poco musgo están penalizados. En cuanto a las características de los pollos, disponibles en todos los hábitats excepto en Pina, encontramos que la longitud del tarso difirió entre los tres hábitats, y que la condición de los pollos empeoró cuando el peso de los palitos y de las plumas y la proporción de plumas aumentaron en los tres hábitats. Este resultado enfatiza que los palitos fueran utilizados en Sagunto como material alternativo, y su uso pudiera ser considerado subóptimo. Es remarcable el hecho de que en Sagunto los nidos contuviesen la mayor cantidad de pelo, probablemente debido a que la falta de musgo fuerce a las aves a buscar materiales alternativos con mejores propiedades de aislamiento que los palitos. En general, encontramos que el peso y la composición del nido de carbonero común difirieron entre hábitats, que el peso del nido por sí mismo no estaba relacionado con las características reproductoras, pero que importantes parámetros reproductores como el tamaño de puesta y la condición de los pollos estuvieron relacionados con la cantidad y proporción de algunos materiales.

### ARTÍCULO 4

Al cambiar los nidos construidos por las aves por nidos artificiales de “buena” o “mala calidad” (8 por tratamiento), 13 de 16 fueron aceptados por las hembras, y continuaron atendiéndolos al menos por un tiempo. En casi todos los nidos experimentales se produjo una adición de materiales por parte de los padres, aunque la adición fue similar, en peso, entre tratamientos, por lo que el peso final siguió siendo mayor en los nidos de buena calidad. Se añadió musgo, pelo y plumas en casi todos los nidos. Los padres eliminaron lana de los nidos de buena calidad, mientras que la añadieron en los de mala calidad, de modo que la cantidad final no difirió entre ambos tratamientos. El añadir material durante la incubación no es algo habitual en el carbonero común, así que es algo excepcional que lo hagan al cambiarles el nido. Esto puede ser debido a que necesiten material específico (pelo, plumas), que no les proporcionamos. Además, casi todos añadieron musgo, probablemente debido a que las hembras, al encontrarse con un nido extraño, intentaran adecuarlo a sus necesidades. Un resultado inesperado fue que en todos los nidos de buena calidad eliminaron lana y en la mayoría de los de mala calidad la añadieron, de modo que al final la cantidad de lana en ambos tipos de nidos fue similar. La lana es un buen aislante térmico, por lo que debe ser beneficiosa para mantener un ambiente térmico adecuado en el nido. No obstante, las cantidades de lana observadas son en todo caso pequeñas, por lo que su significado biológico debería explorarse con más profundidad. El porcentaje de nidos que fracasan durante la incubación (no eclosiona ningún huevo) fue significativamente mayor en los nidos de mala calidad que en los de buena calidad o los nidos control. Entre los nidos que tuvieron éxito, la duración del periodo de incubación no difirió significativamente entre los nidos control y los de buena calidad, pero el éxito de eclosión fue mayor en los nidos de buena calidad que en los nidos control. En ambos análisis, no pudimos incluir los nidos de mala calidad por tener un bajo tamaño muestral. En el carbonero común, la parte básica del nido está compuesta típicamente de musgo. En nuestra área de estudio este material es escaso en los nidos, probablemente por su poca abundancia en los campos de naranjos. Es posible que la construcción de nidos de tamaño relativamente grande, en los que la composición era básicamente musgo, haya mejorado las condiciones medias de incubación en las parejas que criaron en nidos experimentales de buena calidad con respecto a la media de las parejas control. Existen numerosos trabajos que relacionan alguna propiedad del nido con alguna estima del éxito reproductor, pero en ninguno se desvincula a los padres de sus nidos, no quedando clara la contribución del nido *per se*. En nuestro estudio cambiamos aleatoriamente los nidos al principio de la incubación, desvinculando así la posible contribución de la calidad de los padres de la calidad de los nidos que atienden, por lo que los efectos encontrados podemos atribuirlos a la calidad del nido. En conclusión, nuestro trabajo sugiere que la calidad del nido *per se* puede contribuir de manera importante al éxito reproductor. Por un lado, las hembras tienen más tendencia a abandonar nidos de mala calidad, y por otro, la utilización de materiales adecuados es importante. Por lo que conocemos, este es el primer trabajo que muestra esta relación de forma experimental.

## ARTÍCULO 5

La incubación puede comenzar antes de acabar la puesta, con el último huevo, o algún día después de concluirla. El periodo de incubación fue más largo si la incubación se inició antes de terminar la puesta, quizás debido a que la placa incubatriz no está totalmente desarrollada en esta fase, lo que puede suponer una incubación menos eficiente. Además, encontramos que empezar la incubación antes de concluir la puesta implica una mayor asincronía en la eclosión. Esto puede tener desventajas a la hora de la supervivencia de los pollos, ya que se crea una jerarquía de tamaños que puede afectar a la capacidad de obtener alimento en competencia entre hermanos. Por el contrario, superponiendo las fases de puesta e incubación, se acorta la fase de huevo (desde la puesta del primer huevo hasta la eclosión del último), lo que puede ser una ventaja en ambientes donde el riesgo de depredación es elevado. Por otro lado, encontramos que antes de comenzar la incubación completa aparecen los huevos destapados y fríos, y las parejas que lo hacen tienen periodos de incubación más cortos. Esto sugiere que, antes de comenzar la incubación completa, realizan una mayor cantidad de incubación parcial, acortando así el periodo de incubación. Se ha sugerido que, el que los huevos estén tapados durante la puesta, podría evitar una posible depredación, pero el destaparlos antes se acorta el tiempo que están expuestos a ser depredados. Según el peso relativo de la presión de depredación en cada momento del periodo nidícola sería conveniente adoptar una u otra estrategia. El periodo de incubación medio fue de 13,3 días, similar al de otras poblaciones europeas. La duración media varió entre años, siendo más corta en años donde probablemente hubo más alimento disponible (mayor tamaño de puesta). No encontramos relación entre el volumen medio del huevo o el volumen medio de puesta y la duración del periodo de incubación, como ocurre en otros trabajos. Encontramos que, teniendo en cuenta que en las puestas mayores tienden a empezar a incubar antes de acabar la puesta, el periodo de incubación es más corto en puestas más grandes. Esto es contradictorio con los resultados de otros trabajos. Posibles causas son: que algunos trabajos son experimentales, e incrementaron artificialmente el tamaño de puesta, y que muchos calculan el periodo de incubación incluyendo el periodo de eclosión, por lo que en puestas más grandes, que suelen eclosionar de forma más asincrónica, se incrementa el periodo de eclosión. Además, si hay más huevos, pueden mantener mejor el calor cuando la hembra está fuera y no tiene que dedicar tanta energía a recalentarlos. En Sagunto, cuando avanza la estación reproductora se acorta el periodo de incubación. La posible explicación para nuestra área de estudio es que las temperaturas ambientales son generalmente más elevadas conforme avanza la estación reproductora. La duración del periodo de eclosión en nuestra área de estudio (1,7 días) se encuentra en el extremo inferior de lo encontrado en otras poblaciones europeas. Encontramos variación interanual en la duración del periodo de eclosión, pero no hemos encontrado ninguna relación con las variables consideradas. De acuerdo con lo encontrado en otras poblaciones, la asincronía aumentó con el tamaño de puesta, siendo la explicación potencial que la incubación suele empezar antes de concluir la puesta en puestas más grandes. No encontramos una variación a lo largo de la estación reproductora, y aunque sí se ha encontrado esta relación en otras poblaciones, en nuestra área de estudio sólo hemos tenido en cuenta las primeras puestas, con lo que no tenemos una visión de toda la estación. Tanto en Sagunto, como en otras poblaciones, la asincronía es mayor en nidos en los que se comenzó a incubar antes de terminar la puesta.

### ARTÍCULO 6

Durante el periodo de incubación, la hembra de media comienza su día activo (sale del nido) a las 07:21, ó 7 min después de salir el sol, y termina (entra al nido para pasar la noche) a las 19:49, ó 44 min antes de la puesta de sol. La duración media del día activo fue de 12:32 h, dejando 11:28 h para el descanso nocturno. Ninguno de estos parámetros difirió a lo largo de la incubación. Las hembras dejaron los nidos más pronto por la mañana al avanzar la estación, cuando la salida del sol fue más temprana, cuando las temperaturas ambientales máximas fueron más elevadas, y si no llovía. Las hembras entraron a la caja-nido por la noche más pronto cuando las temperaturas ambientales mínimas eran más bajas y si estaban emparejadas con machos jóvenes. La duración total del día aumentó con el número total de horas de luz diurna y con el peso de la hembra. La hembra dejó el nido de media 23 veces por día (1,8 veces por hora durante el día activo), siendo la duración media de las sesiones de 26 min y de 12 min la de los recesos. Los recesos fueron más largos cuando el tamaño de puesta fue más pequeño, y cuando el volumen de huevo y de puesta decreció. En total, la hembra estuvo sobre los huevos alrededor del 66% del tiempo de su día activo (12,5 h) y fuera del nido alrededor del 34% de su día activo. Considerando un periodo de 24 h, las hembras estuvieron sobre los huevos cerca de 20 h, o el 83% del día. Las hembras acortaron los recesos, pasando así más tiempo incubando, al avanzar el proceso de incubación. Las hembras con alas más largas dejaron los nidos más a menudo y realizaron sesiones más cortas. Hacia el comienzo de la incubación, las hembras jóvenes hicieron recesos más largos que las viejas. Durante la fase media de incubación, la proporción de tiempo que las hembras pasaron sobre los huevos fue mayor cuando la longitud de su ala disminuyó y cuando la longitud del tarso de su pareja fue más corto. Durante el estadio último de la incubación, las hembras hicieron recesos más largos cuando la longitud del ala de su pareja fue más larga. Finalmente, encontramos que la duración media de un receso particular fue más larga cuando la duración del previo decreció. Un resultado inesperado es que, en general, las hembras con mejor condición y/o las emparejadas con machos de mejor condición pasan más tiempo fuera del nido, ya que en general tanto en trabajos observacionales y experimentales muestran que las hembras en mejor condición pasan más tiempo sobre los huevos. Esto puede deberse a que la depredación de las hembras en el nido en Sagunto es relativamente alta, y las hembras que puedan ser más eficientes calentando los huevos intenten pasar el menor tiempo posible en el nido.

Se ha realizado, por primera vez, una comparación intraspecífica del patrón de incubación a lo largo de un amplio gradiente latitudinal. Considerando 7 poblaciones, que van desde Sagunto (España, 39º N) hasta Målsjøen (Noruega, 63º N), los carboneros comunes tienen entre 13,25 horas de luz diurna en Sagunto y 19,50 en Målsjøen, una diferencia de 6,25 horas. De las tres poblaciones donde hay información, las hembras incubantes dejan el nido más pronto en relación a la salida del sol, y entran al nido más tarde en relación a la puesta del sol cuando la longitud del día decrece – en Sagunto las hembras están moviéndose dentro y fuera del nido mientras hay luz. A pesar de esto, el día activo es al menos una hora más corto para las hembras incubantes en Sagunto. La duración de las sesiones es virtualmente el mismo en Sagunto (26 min) que en el resto de poblaciones (media 28,4 min), siendo 28 min en la población europea más norteña de la que hay datos (Målsjøen). La duración de los recesos parece decrecer desde Noruega hacia el sur (de 10 a 7 min), pero alcanza 12 min en Sagunto. El número de recesos por hora fue bastante similar en todas las poblaciones (entre 1,5-2,0, estando intermedio el de Sagunto), y el número de recesos por día activo estuvo entre 21-27, siendo 23 en Sagunto. Dado los errores potenciales en estimar algunos parámetros, la atención durante 24 horas es muy similar entre poblaciones, siendo del 83% en Sagunto y 88% en Bennekom (Holanda). Sin embargo, cómo es alcanzado este valor varía notablemente entre poblaciones, ya que en Sagunto pasan menos proporción de tiempo incubando durante el día (66% en Sagunto, 72-78% en el resto), y lo compensan con un periodo nocturno más prolongado. Por último, las hembras en Sagunto pasan más tiempo por día fuera del nido que las de las otras poblaciones, aunque, de nuevo, la diferencia con la población más norteña de Målsjøen es sólo de 12 min. En total, las hembras en Sagunto disponen de menos tiempo a lo largo del día activo para alimentarse. Sugerimos que esto se puede hacer por: (1) tener un menor tamaño corporal, lo que requiere menos recursos; (2) porque las condiciones ambientales son más cálidas y a igual tamaño necesitarán menos energía para mantenerse; y (3) porque al hacer más calor tienen que gastar menos energía en recalentar los huevos tras cada receso.

## ARTÍCULO 7

Tanto durante el día como durante la noche, la temperatura del nido fue mayor en los nidos calentados que en los enfriados y controles (una diferencia de 10 ºC entre calentados y controles), por lo que las hembras de nidos calentados dispusieron probablemente de más energía para actividades distintas de la incubación. Las temperaturas del nido durante el día no variaron a lo largo de la incubación, pero durante la noche fueron más altas conforme avanzó la incubación. Así pues, parece que el tratamiento fue efectivo en aumentar las temperaturas del nido en los nidos calentados, pero el tratamiento de enfriamiento fue compensado de forma efectiva por las hembras, así que las temperaturas medias fueron bastante similares entre nidos control y enfriados. No encontramos diferencias significativas entre tratamientos en la longitud del periodo de incubación, el número de huevos que eclosionaron o de pollos que llegaron a volar, y el éxito de eclosión, de vuelo o reproductor. El tamaño, el peso y las tasas de crecimiento de los pollos no estuvieron afectados por los tratamientos. Las tasas de reclutamiento fueron más altas en los pollos de nidos enfriados (20%), intermedio para los controles (13%) y más bajas para los de los nidos calentados (9%), aunque estas diferencias no fueron estadísticamente significativas. En total, 1 de 7 parejas de los nidos enfriados, 2 de 5 de los controles, y 2 de 7 de los calentados realizaron exitosamente una segunda puesta tras una primera con éxito, que produjeron 5, 6 y 8 volanderos respectivamente. Los datos de los termómetros mostraron diferencias entre tratamientos en la duración media de los recesos, con una duración media de 20,9 min en nidos calentados, 16,2 min en los nidos control, y 15,3 min en los nidos enfriados. Sin embargo, los tests *post hoc* no encontraron diferencias estadísticamente significativas. Los datos recogidos de presencia/ausencia durante el seguimiento sistemático del nido confirmó que las hembras de los nidos calentados estuvieron en los nidos menos frecuentemente (54%) que las de los control (69%) o las de los enfriados (66%). No encontramos diferencias entre los tratamientos en el número y duración de los periodos de sesiones a lo largo del día, ni en la duración del periodo nocturno. El peso y la condición de los padres cuando los pollos tenían entre 10-11 días de edad no difirió entre tratamientos. No hubo diferencias significativas en las tasas de ceba ni de machos ni de hembras, ni en la tasa combinada o en la tasa por pollo, entre tratamientos. Las hembras de nidos calentados pasan más tiempo fuera del nido, y esto puede deberse a que al no tener que estar tanto tiempo sobre los huevos puedan dedicar ese tiempo extra a otra cosa. En Sagunto, la depredación de las hembras en el nido en Sagunto es relativamente alta, por lo que quizás dediquen el tiempo extra a estar fuera del nido. Estudios previos y el presente han mostrado respuestas muy diferentes de las aves al estrés térmico durante la incubación. La idea de que "calentar es bueno" (porque provee energía extra), y que "enfriar es malo" (porque consume energía) no es apoyada, e incluso es refutada, en varios estudios. Esto puede deberse a diferencias en el protocolo experimental (el cual difiere entre estudios), el comportamiento de cada especie, el tipo de nido, y/o condiciones ambientales de cada lugar y año(s) del experimento. Es necesario un estudio a gran escala que intente separar el peso relativo de cada factor (e.g. trabajando con la misma especie y protocolo bajo diferentes condiciones ambientales).

# 5. CONCLUSIONES Y PERSPECTIVAS DE FUTURO

## CONCLUSIONES GENERALES

1. No encontramos relación entre la calidad de los padres y la calidad del nido.
2. Los nidos de mejor calidad estuvieron relacionados con un mejor desempeño reproductor.
3. La relación entre la calidad del nido y el éxito reproductor varió entre años, dependiendo de las condiciones ambientales – parece que es más evidente en años favorables.
4. El peso y la composición del nido difirieron entre hábitats, probablemente por una combinación de diferencias en la disponibilidad de materiales y en las condiciones ambientales.
5. Dentro de cada hábitat, no encontramos diferencias entre años en el peso de los nidos, sugiriendo una cierta estabilidad en las condiciones ambientales durante los años de estudio.
6. No encontramos relación entre el peso del nido y los parámetros reproductores, pero la cantidad y proporción de algunos materiales utilizados para la construcción del nido tuvieron relación con el tamaño de puesta y la condición de los pollos al volar.
7. Mostramos por primera vez, de forma experimental, que la calidad del nido *per se* puede contribuir de manera importante al éxito reproductor: las hembras tienen más tendencia a abandonar nidos de mala calidad, y el éxito de eclosión mejora en nidos de buena calidad.
8. Existe una gran variabilidad intrapoblacional en el momento del comienzo de la incubación en relación con la finalización de la puesta. Esto tiene consecuencias en la duración del periodo de incubación y en la asincronía en la eclosión de los huevos.
9. La duración media del periodo de incubación es menor en años en los que hay mayor disponibilidad de alimento.
10. La duración del periodo de incubación depende principalmente de la fecha (más corto al avanzar la estación) y del tamaño de puesta (más corto en puestas más grandes).
11. Mostramos por primera vez que, durante la fase más temprana de la incubación, la duración media de los recesos es más larga cuando el tamaño del huevo, el tamaño de puesta, y el volumen total de los huevos disminuyen.
12. La eclosión es más asincrónica en las puestas de mayor tamaño.
13. La atención al nido incrementa a lo largo del periodo de incubación.
14. Las hembras de mejor calidad, y las emparejadas con machos de mejor calidad, pasan más tiempo fuera del nido, probablemente debido a una presión de depredación relativamente alta en el nido.

15. Al comparar el ritmo de incubación de varias poblaciones europeas, mostramos, por primera vez, que la atención al nido es muy similar entre poblaciones, aunque debido a diferencias en la duración del día, en las poblaciones del sur pasan más tiempo sobre los huevos durante la noche y menos durante el día.
16. La duración media de las sesiones y recesos no tiene un patrón latitudinal claro, aunque la duración de los recesos parece ser más larga en las poblaciones extremas (España y Noruega).
17. Calentando experimentalmente algunos nidos, se observó que el único efecto aparente fue una reducción de atención a los huevos por parte de la hembra, que pasó más tiempo fuera del nido. Sugerimos que, en estos nidos en que la temperatura de los huevos se mantiene mejor en ausencia de la hembra, ésta pasa menos tiempo en el nido para reducir el riesgo de depredación, relativamente alto en esta población. No hubo un efecto positivo sobre los parámetros reproductores o la condición de los padres.
18. Las hembras consiguieron compensar de forma efectiva el enfriamiento experimental de los nidos, de modo que no se detectaron efectos sobre la temperatura media, y en consecuencia en los parámetros reproductores estudiados. Tampoco observamos un efecto negativo sobre las hembras, al menos a corto plazo.

## PERSPECTIVAS DE FUTURO

Cuando planteamos la presente Tesis Doctoral, la idea era abordar, tal y como se indica en el título, el estudio de los patrones de incubación y eclosión de la población de carbonero común de Sagunto, intentar identificar los factores que determinaban esos patrones, y explorar las consecuencias de los mismos para la eficacia biológica de padres y pollos. Pretendíamos combinar las observaciones, tanto realizadas durante el periodo de desarrollo de la tesis, como las almacenadas en la base de datos del grupo de investigación desde el año 1988, con experimentos para identificar relaciones relevantes. Llegado el momento de poner punto final al trabajo, estamos satisfechos de haber cubierto los puntos principales del proyecto inicial, pero con la espina de no haber tratado con más profundidad algunos de los temas de los que tenemos datos.

Uno de estos temas es la importancia de los nidos en el proceso de incubación. Lo que inicialmente iba a ser un trabajo, o como mucho dos, ha llegado a ocupar más de la mitad de la tesis. La característica composición de los nidos en la población de Sagunto, con muy poco contenido de musgo, materila que es típico en muchas otras poblaciones, nos llevó a plantear el estudio de la composición en otras poblaciones españolas, y sus efectos sobre el éxito reproductor. Y este trabajo, a su vez, a plantear un estudio a nivel europeo, que está en marcha actualmente. En él pretendemos comparar la composición del nido de poblaciones nidificantes bajo diferentes condiciones ambientales, y para ello solicitamos la colaboración de colegas que trabajan con poblaciones de carbonero común en diversos países. Disponemos ya de los nidos de algunas de las poblaciones, cada uno de ellos con datos sobre los parámetros reproductores, muchos con datos de las características de los padres, y se han recopilado también datos meteorológicos y del tipo de hábitat de cada población. El trabajo a realizar pretende: (1) describir el tamaño y la composición de los nidos en cada población; (2) identificar las especies de musgo utilizadas por el carbonero común en varios países; (3) estimar la capacidad aislante de los nidos bajo condiciones controladas de laboratorio; (4) relacionar la composición y la capacidad aislante con las condiciones meteorológicas y el tipo de hábitat en cada una de la poblaciones; y (5) dentro de cada población, relacionar el tamaño y la composición de los nidos con las características de los padres y con los parámetros reproductores. Además, en la población de Sagunto, nos hemos quedado con las ganas de saber si el musgo, como suponemos, es realmente escaso para las necesidades de los carboneros. Por ello pretendemos realizar un experimento de provisión de musgo, para ver el uso que las aves hacen de él, y las posibles consecuencias sobre la reproducción.

Al contrario que el punto anterior de los nidos, que estaba previsto ser más corto, y se ha extendido, un aspecto al que queríamos prestar más atención, y al final ha quedado reducido a un artículo, es la explotación de datos acumulados desde 1988 sobre la incubación y eclosión en la población de Sagunto. Algunas de las cosas que nos quedan por saber, y que esperamos poder abordar en el futuro, son: (1) el efecto de las temperaturas en el periodo de incubación de cada nido sobre la duración del mismo en ese nido concreto; (2) los efectos de la calidad de los padres sobre los patrones de incubación y eclosión; (3) las consecuencias de los patrones de incubación y eclosión sobre la eficacia biológica de padres y pollos; (4) la plasticidad fenotípica del patrón de incubación, comparando las mismas hembras en diferentes años; (5) la posible heredabilidad de la duración del periodo de incubación; (6) la posible heredabilidad del momento del comienzo de la incubación en relación con el momento de finalización de la puesta.

Y, por supuesto, muchas otras ideas que han ido surgiendo durante estos años y que no han podido abordarse, y cuyo tratamiento futuro sí dependerá de tener una financiación adecuada. Queremos destacar la aproximación experimental al estudio de los compromisos energéticos de las hembras durante el periodo de incubación. Aunque sólo recientemente hemos enviado el trabajo correspondiente (Artículo 7) para su publicación, el experimento se realizó en la temporada de cría de 2009. Alrededor de esas fechas se realizaron experimentos similares en diversas poblaciones de Europa y Norteamérica y, en conjunto, la diversidad de resultados es amplia, y a veces las conclusiones contradictorias. Al utilizar diferentes protocolos experimentales, en especies a veces muy diferentes, y bajo condiciones ambientales muy distintas, no es fácil atribuir las diferencias en los resultados a un solo factor. Por ello, el trabajo experimental con este enfoque sigue siendo necesario, y sería deseable estandarizar los protocolos, e incluso tomar una especie modelo (el carbonero común es una de las posibles) para tener una visión amplia de los efectos de calentar o enfriar los nidos bajo diferentes condiciones ambientales. Este tipo de trabajos cobra aún más importancia en el escenario de aumento global de las temperaturas, ya que permitiría predecir los efectos de estos cambios a nivel individual y poblacional.

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# 7. ANEXOS

# ANEXO I: PUBLICACIONES

En este Anexo se presentan las 7 publicaciones que componen esta Tesis Doctoral, agrupadas en dos Secciones, la primera con trabajos que hacen referencia al nido y su importancia como ambiente donde tiene lugar la incubación, y la segunda con trabajos que tratan los procesos de incubación y eclosión con más detalle.

## Sección I. El nido: el ambiente de la incubación.

Álvarez E, Barba E. 2008. Nest quality in relation to adult bird condition and its impact on reproduction in Great Tits *Parus major*. Acta Ornithologica. 43: 3−9.

Álvarez E, Barba E. 2011. Nest characteristics and reproductive performance in great tits *Parus major*. Ardeola. 58: 125−136.

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Álvarez E, Barba E. 2009. ¿Cómo afecta la calidad del nido *per se* al proceso de incubación? Una aproximación experimental. Revista Catalana d'Ornitologia. 25: 11−18.

## Sección II. Patrones de incubación y eclosión.

Álvarez E, Barba E. -- Incubation and hatching periods in a Mediterranean Great Tit population. Enviado para publicación.

Álvarez E, Barba E. -- Within and between population variations of incubation rhythm of great tits *Parus major*. Enviado para publicación.

Álvarez E, Barba E. -- Response of great tits to thermal stress during incubation. Enviado para publicación.

# SECCIÓN I. EL NIDO: EL AMBIENTE DE LA INCUBACIÓN

# ARTÍCULO 1

# *Nest quality in relation to adult bird condition and its impact on reproduction in Great Tits Parus major*

ELENA ÁLVAREZ Y EMILIO BARBA

ACTA ORNITHOLOGICA (2008), 43: 3-9

“La grandeza de un ideal es
luchar por él, conseguirlo es
simplemente la recompensa.”

ACTA ORNITHOLOGICA
Vol. 43 (2008) No. 1

# Nest quality in relation to adult bird condition and its impact on reproduction in Great Tits *Parus major*

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**Álvarez E., Barba E. 2008. Nest quality in relation to adult bird condition and its impact on reproduction in Great Tits *Parus major*. Acta Ornithol. 43: 3–9.** DOI 10.3161/000164508X345275

**Abstract.** Birds' nests are special structures built with reproductive aims. Size and structure of the nest can arise from evolutionary trade-offs between benefits such as the insulation from unfavourable conditions, maintenance of eggs or chicks, or security against predation, and costs such as energy spent in construction of the nest and the risk of predation in more visible nests. Therefore, building a good nest is beneficial in terms of reproductive output but expensive in terms of time and energy, so probably only "good" parents would be able to build "good" nests. Our objective was to study possible relationships between the quality of the parents and the quality of the nest, and between the quality of the nest and breeding performance in a Great Tit *Parus major* population. We found positive relationships between different components of the nest quality and components of breeding performance. However, we did not find any significant relationship between quality of the parents and that of the nest. A weak, though significant positive correlation was found between female size and breeding success rate.

**Key words:** Great Tit, *Parus major*, breeding performance, clutch size, nest size, parental quality

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## INTRODUCTION

Birds' nests are more or less elaborated structures built with reproductive aims. They form a receptacle which usually provides protection and an adequate microclimate for the development of eggs and nestlings (Collias & Collias 1984). The size and structure of the nest might arise from an evolutionary trade-off between benefits as the insulation from unfavourable weather conditions or protection of eggs and chicks against predation (Skowron & Kern 1980, Kern 1984, Quader 2006), and costs as the time and energy devoted to its building or the risk of predation in the most visible nests (Withers 1977, Slagsvold 1989b). Overall, nests seem to give important benefits, especially to females (Quader 2006, and references therein), so it might be expected that their characteristics fit adequately the needs for successful incubation and chick caring. For instance, some nest components (hair, feathers) may improve thermal insulation (Hilton et al. 2004), and others may have antiparasitic functions (Clark & Mason 1988). Nevertheless, some authors have stressed that very few studies consider the effects of the nest characteristics on reproductive performance (e.g. Tomás et al. 2006).

In many altricial species, where chicks stay at the nest for some time after hatching, and therefore build more elaborate nests, nest building is an important component of the parental effort. Thus, parents not only allocate a large amount of time and energy to nest building, but also expose themselves to a considerable predation risk (Martin et al. 2000). This effort might be mediated by their health status or their phenotypic quality, since bigger nests are associated to parents in better condition (Lens et al. 1994, Soler et al. 1998a, Tomás et al. 2006). In other words, nest characteristics might be a good indicator of the quality of the parents, and the nest size has been shown to be a sexually selected trait (Moreno et al. 1994, Soler et al. 1998a, b, Møller 2006, Polo & Veiga 2006).

Since the "quality" of the nests is something difficult to define and measure, nest size has been often used as a surrogate of nest quality. Thus, nest size has been shown to be related to the body condition of the parents (Soler et al. 1998a) and with some other estimates of parental quality (Palomino et al. 1998, Soler et al. 1999, De Neve & Soler 2002, De Neve et al. 2004, Mainwaring et al. 2008). Finally, nest size has been shown to be positively related to several fitness related parameters, such as clutch size (Møller 1982, Soler et al. 2001), the total number of eggs laid along the breeding season (Soler et al. 1998a), hatching success (Møller 1982) and breeding success (Slagsvold 1989a). For example, nests with thicker walls and nest bottom are probably better insulated from extreme ambient temperatures, allowing a better embrionic and nestling development (e.g. White & Kinney 1974, Kern 1984). Also, a thicker, soft nest bottom could prevent eggs from breaking during incubation. Sometimes, however, the positive relationship between breeding parameters and nest size is not perfect. For example, Wesołowski (2003) found small clutches in small nests, though the relationship between clutch and nest size disappeared in bigger nests. Furthermore, for hole nesting species, bigger nests might make eggs and nestlings more easy to be preyed upon, since they are closer to the nest entrance (Mazgajski & Rykowska 2008).

In the same sense, the "quality" of a bird is a parameter difficult to define, since many physiological, anatomical or behavioural aspects could influence it. In fact, many different variables have been used as correlates of bird quality when it has been tried to relate it with reproductive performance. Our "quality" measures were related to the age (experience) and size of the birds. Older birds use to be dominants over younger ones and bigger birds over smaller ones (Barluenga et al. 2000, Senar et al. 2001). Social dominance allows a better access to food and nest sites (e.g. Perrins 1979) and dominants use to have better body condition (Carrascal et al. 1998), so we assumed they would be in better condition to face the reproductive demands.

The aim of this work was to study the relationship between parental quality, nest quality and breeding performance in Great Tits *Parus major*. Since this species quickly accepts nestboxes for breeding, it is a good model to study variation in nest size, since all the individuals start from a cavity of the same size and general characteristics. Also, Great Tit nests are compact structures, so they can be removed from the nestbox to measure and weight them without damaging the nest. This allows measuring the nest at the early stages of incubation, avoiding the changes in nest characteristics that incubation and chick rearing might cause (e.g. Palomino et al. 1998). Overall, we expected a positive relationship both between parental and nest quality, and between nest quality and breeding success.

## STUDY AREA AND METHODS

The study was performed within extensive orange plantations in Sagunto, eastern Spain (39° 42′N, 0° 15′W, 30 m a.s.l.) in 2006. Nestboxes have been available in this area since 1986, and studies on Great Tits have been being carried on since then (e.g. Andreu & Barba 2006, Greño et al. 2007).

All nestboxes were visited at least once a week since late February. When a Great Tit nest was found, we visited the nestbox as frequently as necessary (daily in some periods) to estimate the parameters of interest (e.g. Barba et al. 1995). Thus, laying date of the first egg (laying date hereafter), clutch size, number of eggs hatched and number of fledglings were recorded. From these, we estimated hatching success (proportion of eggs hatched), fledging success (proportion of nestlings which fledged), and breeding success (proportion of eggs producing fledglings). These percentages were arcsin square root transformed for analyses (Zar 1996). Laying dates (earlier nests considered better), clutch size, and hatching, fledging and breeding success were considered as indicators of breeding performance. We used 47 nests, though sample sizes differed for different studied parameters as nests failed along the nesting period.

All nests were measured and weighed by the same author (EA) 2–3 days after incubation was started. Basal area was limited by the size of the nestbox. Nestboxes used, which proceed from different suppliers, measured between 110.5 and 116.5 mm long and between 106.2 and 121.2 mm wide, so the length and width of each nest was measured. The distance between the entrance hole and the nestbox bottom varied between 83.80 and 104.02 mm. After measuring the basal area, we took the nest out of the nestbox to take other measurements (see Fig. 1) and its weight. We used a digital calliper for linear measurements and a digital balance (accuracy 0.1 g) for weighing.

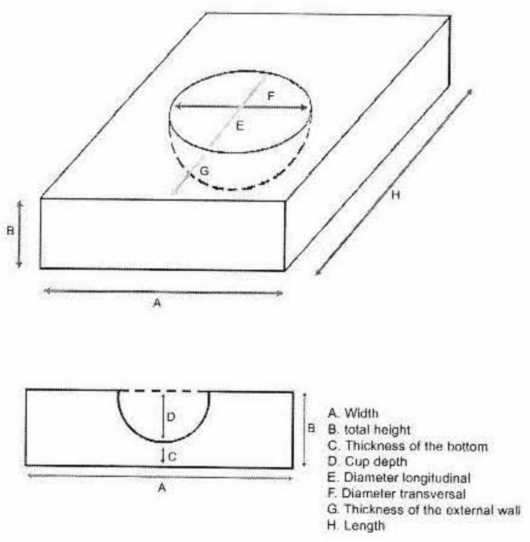


Fig. 1. Detail of the linear measurements taken from each nest. The bottom of the nest is always equal to the bottom of the nestbox.

Parents were captured at the nest, using traps, or around it, using mist nets, when nestlings were 12–14 days old. They were ringed with individually numbered metal rings, and their sex and age class (first year or older breeders) determined (Svensson 1992). Wing and tarsus length and weight were measured, and an index of body condition (weight/tarsus length) was computed. Most birds were measured by the authors using standardized protocols.

Our approach to cope with the difficulties in defining nest and parent "quality" has been taking into account several parameters measured on nests or birds and perform Principal Component Analyses to reduce the number of variables to a few components which might be better indicators of overall nest or bird quality. This is the same approach as the one used, for example, to estimate "body size" from a set of morphological parameters (e.g. Freeman & Jackson 1990, Wiebe & Swift 2001). We also followed this approach to extract the principal components of breeding performance from parameters generally used to define it. Factor scores were computed from factor loadings of the varimax rotation of the axes. We arbitrarily selected eigenvalues above 1, also taking into account that the selected components explained a large percentage of the variance. Each resulting component was regressed against the original variables to explore which were more related to each particular component.

We explored the relationships between selected components using multiple regressions with stepwise selection of variables, since at least two components were selected to describe each of the three aspects (see Results). We only show the final model which included significant variables. When no variables were entered into the model we report it as non-significant. We also explored the relationships between some original variables in those cases where previous studies (e.g. Slagsvold 1989a, Palomino et al. 1998, Alabrudzińska et al. 2003, Tomás et al. 2006) suggested a possible cause-effect relationship. Finally, we tested possible effects of parental age class on nest quality or breeding performance using General Lineal Models. Linear and quadratic relationships were explored in all cases, though quadratic relationships are only presented if they improved the linear ones. The SPSS 15.0 statistical package was used for all the analyses.

## RESULTS

The four variables used to define parental quality were reduced to two principal components. The first component explained 44% of the variance, and was related to the condition of the birds, while the second one explained a 43% of the variance and was related to the size of the birds (Table 1).

The nine variables used to define nest quality were reduced to three principal components. The first one explained 27% of the variance, and was related to the overall size of the nest (Table 2). The second one explained 25% of the variance and was related to the size of the nest cup. The third one explained 22% of the variance and was mostly related to the basal area of the nest. Since this last component was virtually fixed for all the nests

Table 1. Results of principal component analyses performed on four morphological variables measured on 33 female and 28 male Great Tits. Correlation coefficients between the components and the original variables are also shown. * — p < 0.05, ** — p < 0.01, *** — p < 0.001.

| | Female | | Male | |
|---|---|---|---|---|
| | PC1 | PC2 | PC1 | PC2 |
| Wing length | 0.15 | 0.63** | 0.24 | 0.78*** |
| Tarsus length | 0.15 | 0.90*** | 0.13 | 0.90*** |
| Weight | 0.87*** | 0.42* | 0.92*** | 0.33 |
| Condition | 0.97*** | 0.26 | 0.99*** | 0.17 |
| Eigenvalue | 1.74 | 1.46 | 1.89 | 1.56 |
| % variance accounted for | 43.45 | 36.37 | 47.35 | 38.89 |

Table 2. Results of the principal component analysis performed on 9 variables measured on 43 Great Tit's nests. Presentation as in Table 1.

| | PC1 | PC2 |
|---|---|---|
| Total height | 0.95*** | 0.09 |
| Width | 0.04 | 0.04 |
| Length | 0.19 | 0.25 |
| Cup depth | 0.62*** | 0.04 |
| Thickness of the external wall | 0.08 | 0.82*** |
| Thickness of the bottom | 0.73*** | 0.09 |
| Diameter longitudinal | 0.03 | 0.93*** |
| Diameter transversal | 0.04 | 0.77*** |
| Weight | 0.76*** | 0.09 |
| Eigenvalue | 2.42 | 2.21 |
| % variance accounted for | 26.94 | 24.55 |

(it was the basal area of the nestboxes), we did not consider it in further analyses.

The five variables used to define reproductive performance were reduced to two principal components. The first component explained 50% of the variance and was related to the three estimated success rates (Table 3). The second one explained 27% of the variance and was related to laying date and clutch size.

We found no significant relationship between the quality of the parents or their age (both for males and females) and the quality of the nest they build ($p > 0.05$ in all cases). On the other hand, the success rate of a nest was positively related to the size of the female ($F_{2,29} = 3.44$, $p = 0.046$) (Fig. 2), though this relationship was weak and disappeared when removing two outlying points. All the other relationship between parental quality and breeding performance were non-significant.

The success rate of the nest increased as the overall size of the nest increased ($F_{1,36} = 7.907$, $p = 0.008$) (Fig. 3). Also, nests with larger nest cup held clutches which started earlier and were larger ($F_{1,36} = 7.014$, $p = 0.012$) (Fig. 4). Success rate also tended to be higher in these nests, though the relationship was only marginally non-significant ($p = 0.067$).

Concerning the original variables, we explicitly tested some hypotheses based on results of previous studies (see Introduction). Thus, we found no significant relationship between laying date and nest weight ($r = 0.19$, $p = 0.21$, $n = 47$). Clutch size was not related to nest weight ($r = 0.18$, $p = 0.22$, $n = 47$), but it was positively related with the transversal diameter or the nest cup ($r = 0.36$, $p = 0.012$, $n = 47$). Neither nest weight ($r = 0.09$, $p = 0.62$, $n = 37$) nor the thickness of the nest bottom ($r = 0.05$, $p = 0.78$, $n = 37$) were related to hatching success. Finally, there was no significant relationship between nest weight and the number of fledglings ($r = 0.20$, $p = 0.20$, $n = 40$).

Table 3. Results of the principal component analysis performed on 5 variables of the breeding performance measured on 43 Great Tit's nests. Presentation as in Table 1.

| | PC1 | PC2 |
|---|---|---|
| Laying date | 0.04 | 0.82*** |
| Clutch size | 0.09 | 0.79*** |
| Hatching success | 0.83*** | 0.09 |
| Fledging success | 0.95*** | 0.18 |
| Breeding success | 0.95*** | 0.19 |
| Eigenvalue | 2.50 | 1.36 |
| % variance accounted for | 49.99 | 27.27 |

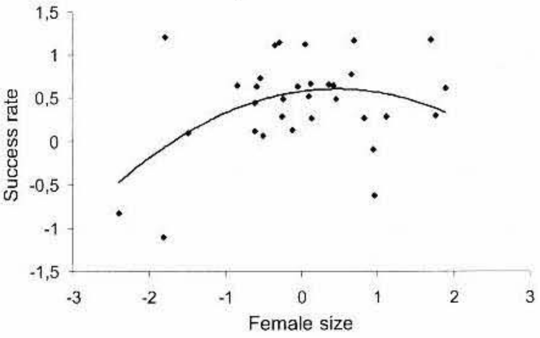


Fig. 2. Relationship between female size (PC 2 of bird quality) and success rate (PC 1 of reproductive performance, a variable including hatching, fledging and breeding success). The relationship disappeared when the two outlying points (lower success rate) were removed.

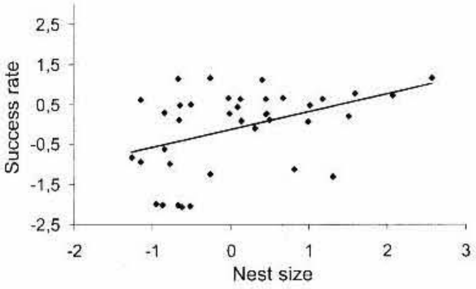


Fig. 3. Relationship between nest size (PC 1 of nest quality) and success rate (PC 1 of reproductive performance, a variable including hatching, fledging and breeding success).

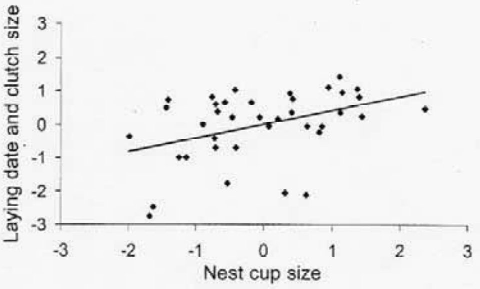


Fig. 4. Relationship between nest cup size (PC 2 of nest quality) and laying date and clutch size (PC 2 of reproductive performance).

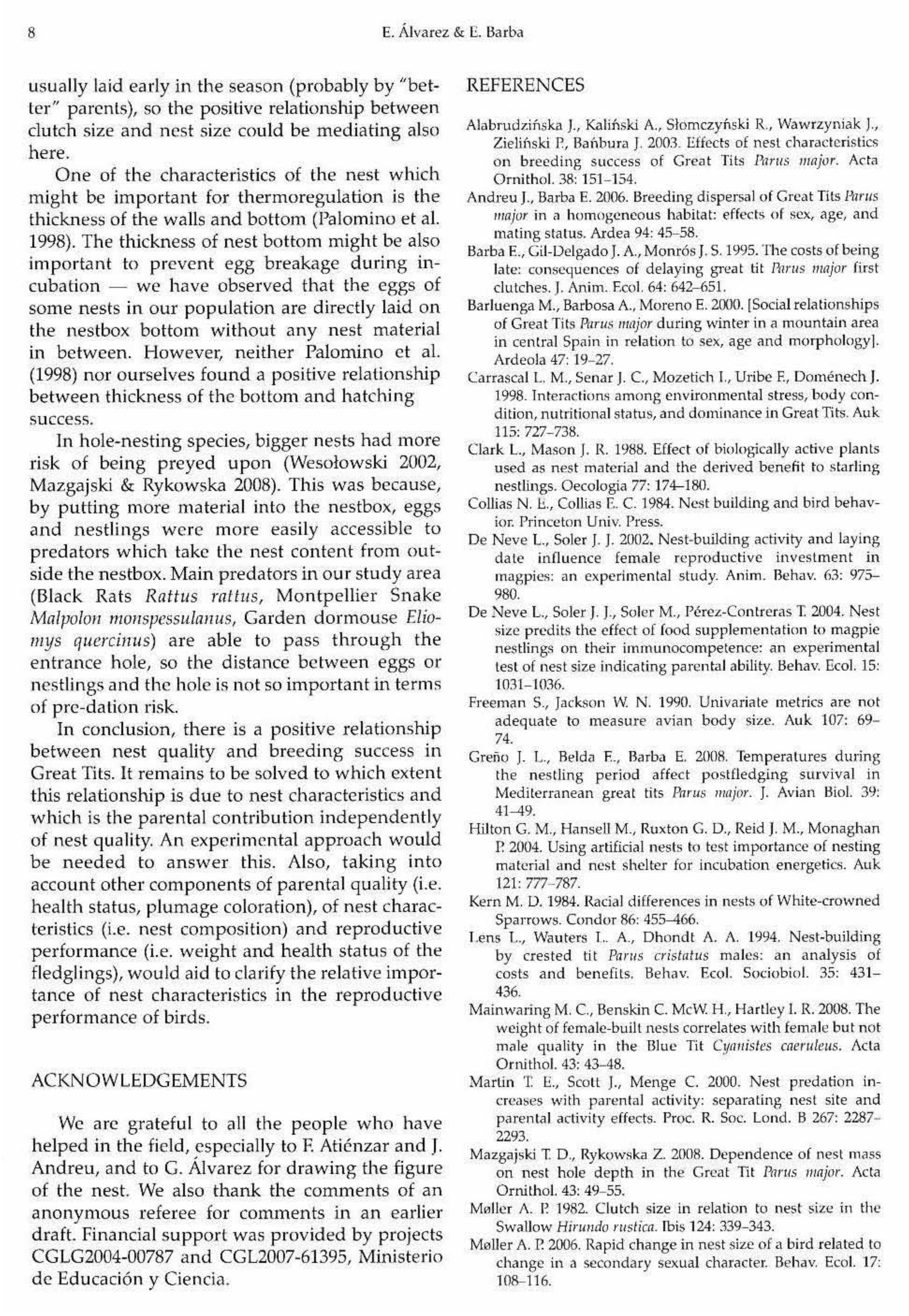
## DISCUSSION

A clear conclusion of our work was that better quality nests were related with better breeding performance. We have shown that the success rate of the nest (a variable including hatching, fledging and breeding success) increased as the overall size of the nest increased. The original variables more related to "overall size" were the weight of the nest and its height, the later related to a thicker nest bottom and a deeper nest cup. A related result was that nests with larger nest cups (wider cups) and with the cup farther from the nest entrance (thicker external wall) held larger clutches, started laying earlier and tended to have more success. Thus, the overall conclusion is that those nests in which the parents invested more effort were more successful.

An open question, not solved by our work, is whether nest characteristics themselves were important for success, or was the quality of the parents the important variable behind this relationship. What we could say is that none of the quality measures of the parents was positively related to the quality of the nest. On one hand, this might only reflect that we have not measured the adequate parental characteristics. For example, Tomás et al. (2006) found that age and size of the parents were not related to nest weight in Blue Tits *Cyanistes cyaneus*, while some measures of the health status, as prevalence of blood parasites or immunoglobulin levels were related to nest weight. However, this relationship was found only one out of two years, indicating that nest quality is not always clearly related to parental quality. Similar results were reported by Mainwaring et al. (2008), since they did not find significant relationships between parental age or size (except for head-bill length in females) and nest weight, while it was feather mite load of females the parameter positively related to nest weight. Second, the timing of measuring some characteristics might not have been the most appropriate. For example, parents were weighed by the end of the nestling period, when most effort was already done. On the other hand, the lack of relationship between parental quality and nest quality, while breeding success was positively related to nest quality, suggests that the nest itself might be directly affecting breeding success. The relative importance of this effect should be determined through experimental studies breaking the possible relationship between parental and nest quality.

The relationship between nest cup size and clutch size has been scarcely explored at an intraspecific level. Palomino et al. (1998) did not find a relationship between nest cup volume and clutch size in Rufous Bush Robins *Cercothichas galactotes*, while Møller (1982) found a positive relationship in Barn Swallows *Hirundo rustica*. At an interspecific level Slagsvold (1989b) showed that clutch size was related to the inner size of the nest cup. We found a positive relationship both between nest cup size and clutch size (using PCA) and, more explicitly, between the transversal diameter of the nest cup and clutch size, thus confirming this relationship at an intraspecific level. Slagsvold et al. (1989b) suggested that clutch size might therefore be limited by nest size. An alternative is that females build a nest big enough to hold the number of eggs they are going to lay (Møller 1982). Since, for the present study, all pairs had nestboxes of virtually the same bottom area, the positive relationship between nest cup size and clutch size support this later hypothesis, i.e. females are able to shape the nest cup to accommodate the eggs they are going to lay.

It is also noticeable the negative relationship between nest size and laying date, laying starting earlier in bigger nests. At least two studies have failed to find this relationship in cavity-nesting species, including the Great Tit (Alabrudzińska et al. 2003, Tomás et al. 2006). It is difficult to think about a direct cause-effect relationship, so this should be mediated by the quality of the parents. Though we found no relationship between our measures of parental quality and laying date, some relationship between laying date and parental quality might exist. Larger clutches are



usually laid early in the season (probably by "better" parents), so the positive relationship between clutch size and nest size could be mediating also here.

One of the characteristics of the nest which might be important for thermoregulation is the thickness of the walls and bottom (Palomino et al. 1998). The thickness of nest bottom might be also important to prevent egg breakage during incubation — we have observed that the eggs of some nests in our population are directly laid on the nestbox bottom without any nest material in between. However, neither Palomino et al. (1998) nor ourselves found a positive relationship between thickness of the bottom and hatching success.

In hole-nesting species, bigger nests had more risk of being preyed upon (Wesołowski 2002, Mazgajski & Rykowska 2008). This was because, by putting more material into the nestbox, eggs and nestlings were more easily accessible to predators which take the nest content from outside the nestbox. Main predators in our study area (Black Rats *Rattus rattus*, Montpellier Snake *Malpolon monspessulanus*, Garden dormouse *Eliomys quercinus*) are able to pass through the entrance hole, so the distance between eggs or nestlings and the hole is not so important in terms of pre-dation risk.

In conclusion, there is a positive relationship between nest quality and breeding success in Great Tits. It remains to be solved to which extent this relationship is due to nest characteristics and which is the parental contribution independently of nest quality. An experimental approach would be needed to answer this. Also, taking into account other components of parental quality (i.e. health status, plumage coloration), of nest characteristics (i.e. nest composition) and reproductive performance (i.e. weight and health status of the fledglings), would aid to clarify the relative importance of nest characteristics in the reproductive performance of birds.

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## STRESZCZENIE

### [Wielkość gniazd bogatki — jej związek z kondycją ptaków dorosłych i wpływ na reprodukcję]

Wielkość i struktura gniazd ptaków wynika z ewolucyjnego kompromisu między korzyściami takimi jak m. in. izolacja jaj oraz piskląt od niekorzystnych warunków i bezpieczeństwo lęgu a kosztami, np. czasem i energią poświęconymi na jego budowę i ryzyko drapieżnictwa w przypadku większych, bardziej widocznych gniazd. Stąd budowa gniazd jest korzystna dla reprodukcji, ale kosztowna z punktu widzenia poświęcanego czasu i energii. Można więc zakładać, że tylko "dobre" ptaki mogą budować "dobre" gniazda. Celem pracy było określenie związku między jakością ptaków dorosłych i charakterystykami gniazda oraz między charakterystykami gniazda a wynikami lęgu u bogatki.

Badania prowadzono we wschodniej Hiszpanii. Kontrolowano skrzynki lęgowe określając czas przystępowania do lęgów, wielkość zniesienia, liczbę wyklutych i opuszczających gniazdo piskląt. Gniazda były mierzone (Fig. 1) i ważone w pierwszych dniach wysiadywania jaj. Ptaki dorosłe były łapane podczas karmienia piskląt (ok. 12-14 dnia ich życia), określano ich płeć i wiek, mierzono długość skoku i skrzydła oraz ważono.

"Jakość" ptaków dorosłych jak i gniazd oraz wyniki lęgu opisywano grupując opisane cechy przy pomocy analizy składowych głównych (Tab. 1, 2, 3).

Nie stwierdzono związku między kondycją ("jakością") ptaków dorosłych a charakterystykami gniazda. Słaba, ale istotna pozytywna korelacja została stwierdzona między wielkością samic a sukcesem reprodukcyjnym (Fig. 2). Wykazano pozytywny związek między wielkością gniazda a sukcesem reprodukcyjnym (Fig. 3) oraz wielkością czary gniazdowej a składową łączącą termin przystępowania do lęgu i wielkość zniesienia (Fig. 4).

# ARTÍCULO 2

# Nest characteristics and reproductive performance in great tits *Parus major*

ELENA ÁLVAREZ Y EMILIO BARBA

ARDEOLA (2011), 58: 125-136

> "Si deseas algo con mucha fuerza, déjalo en libertad. Si vuelve a ti, será tuyo para siempre. Si no regresa, no te pertenecía desde el principio."

*Ardeola* 58(1), 2011, 125-136

# NEST CHARACTERISTICS AND REPRODUCTIVE PERFORMANCE IN GREAT TITS *PARUS MAJOR*

## CARACTERÍSTICAS DEL NIDO Y DESEMPEÑO REPRODUCTOR EN EL CARBONERO COMÚN *PARUS MAJOR*

Elena ÁLVAREZ[1] * and Emilio BARBA[1]

SUMMARY.—*Nest characteristics and breeding performance in great tits* Parus major.

For birds, building a nest entails a number of costs but it confers important benefits, so that nest quality is often positively associated with reproductive performance. Our main objective was to study the relationships between nest characteristics and breeding performance, and the possible between-year variability in these relationships. We used great tits *Parus major* as the model species and studied a population breeding in nestboxes within orange plantations in eastern Spain. The study only considered first clutches, in two consecutive years. Nests were visited as frequently as necessary to record basic breeding parameters. We measured and weighed all nests during the incubation period, took biometric characteristics of parents and nestlings (tarsus length, weight and condition, and parental wing length) and measured the immune response of chicks (phytohemagglutinin test). We used principal component analysis to reduce the number of nest variables to three relevant components. Overall, 2007 seemed to have been a more favourable year, with larger clutch sizes and heavier fledglings produced than in 2006. Laying date was earlier in nests with larger nest cup diameter, hatching success was higher as relative cup depth increased in 2007, and fledging success increased as overall nest size increased in 2007. The only significant relationship between parental traits and nest characteristics was that first year females built deeper nests than older ones. Females with longer tarsi and in poorer physical condition started laying earlier. Increased fledging success was associated with decreasing male tarsus length and fledgling condition was better in nests attended by older males. In general, nest characteristics were positively related to breeding success, although specific relationships between the different components could vary between years. These relationships have been more evident in a trophically "good" year than in a "bad" one.

*Key words*: breeding performance, immune response, nest size, parental traits, *Parus major*.

RESUMEN.—*Características del nido y desempeño reproductor del carbonero común* Parus major.

Para las aves, construir un nido implica una serie de costes, pero otorga importantes beneficios, ya que la calidad del nido está a menudo relacionada positivamente con el desempeño reproductor. Nuestro principal objetivo ha sido estudiar las relaciones entre las características del nido y los parámetros reproductores, y la posible variación interanual en estas relaciones. Utilizamos al carbonero común *Parus major* como especie modelo, y estudiamos una población que cría en cajas nido en plantaciones

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de naranjos en el este de España. Incluimos en este estudio sólo primeras puestas de dos años consecutivos. Visitamos los nidos con la frecuencia necesaria para determinar los parámetros reproductores básicos. Medimos y pesamos todos los nidos durante el periodo de incubación, tomamos las características biométricas de padres y pollos (longitud del tarso, peso y condición, y la longitud alar sólo en los padres), y medimos la respuesta inmune de los pollos (prueba de la fitohemaglutinina). Utilizamos análisis de componentes principales para reducir el número de variables del nido a tres componentes relevantes. En conjunto, 2007 parece que fue un año más favorable que 2006 (mayor tamaño de puesta y pollos producidos en mejor condición). La fecha de puesta fue más temprana en nidos con mayor diámetro de copa, el éxito de eclosión aumentó al aumentar la profundidad relativa del nido en 2007, y el éxito de vuelo aumentó con el tamaño global del nido en 2007. La única relación significativa entre las características de los padres y las del nido fue que las hembras de un año construyeron nidos relativamente más profundos que las de más edad. Las hembras de tarso más largo y en peor condición comenzaron antes la puesta. El éxito de vuelo aumentó al disminuir el tarso de los machos, y la condición de los pollos fue mejor en nidos atendidos por machos mayores de un año. En general, las características del nido estuvieron positivamente relacionadas con el éxito reproductor, aunque las relaciones específicas entre los distintos componentes varió entre años. Estas relaciones fueron más evidentes en un año "bueno" que en uno "malo" en términos de disponibilidad de recursos.

*Palabras clave*: características parentales, éxito reproductor, *Parus major*, respuesta inmune, tamaño del nido.

## INTRODUCTION

Although not all birds build nests (Marchant, 1985), most do so. Building a nest entails costs (e.g. Tomás *et al.*, 2006; Moreno *et al.*, 2008; Mainwaring and Hartley, 2009) and recent studies have shown that these are higher than previously thought, when compared with the costs of egg production or incubation. This is particularly relevant when these tasks are carried out only by the female (e.g. Mainwaring and Hartley, 2009). On the other hand, nests confer important benefits (Quader, 2006): they provide thermal insulation, they afford protection from predators, they help to maintain the eggs in an optimal position during incubation and they facilitate nestling care (Collias and Collias, 1984).

A positive relationship between relevant nest traits and breeding success would be expected if nest characteristics affect reproductive performance, and, indeed, positive relationships have been reported between nest size and clutch size (Soler *et al.*, 2001), the total number of eggs laid during the breeding season (Soler *et al.*, 1998a), immunocompetence of fledglings (De Neve *et al.*, 2004), and hatching and breeding success (Álvarez and Barba, 2008).

On the other hand, it has been demonstrated that nest size is related to body size (Slagsvold, 1989b; Mainwaring *et al.*, 2008), parental ability (Soler *et al.*, 1998b; De Neve and Soler, 2002; De Neve *et al.*, 2004), and condition or health status (Tomás *et al.*, 2006; Mainwaring *et al.*, 2008; Broggi and Senar, 2009). In contrast, direct relationships between age or size of the parents and nest size are difficult to find (e.g. Lombardo, 1994; Mainwaring *et al.*, 2008).

Finally, parental characteristics are frequently related to reproductive parameters such as clutch size, number of hatchlings, number of fledglings, breeding success (e.g. Pugesek and Diem, 1983; Slagsvold and Lifjeld, 1990; Vieyra *et al.*, 2009) and offspring quality (Wright *et al.*, 1998; Moreno *et al.*, 1999). Thus, relationships between nest characteristics and breeding performance could be at least partly mediated by parental traits.

The great tit *Parus major* is a widespread passerine that breeds in wooded areas across the Palearctic (Cramp and Perrins, 1993). At the beginning of the spring, the male defends a territory and chooses the nesting location, and the female builds the nest unaided by her mate (Gosler, 1993). The great tit nests in tree cavities and it readily accepts nestboxes. For cavity nesters nest size is obviously limited by the cavity dimensions (e.g. Slagsvold, 1989b). Thus, the use of nestboxes facilitates the study of nest size variation in these species, since different individuals build their nests in "cavities" of the same size and general characteristics, and this is important for standardising the initial conditions (Lambrechts *et al.*, 2010). Our main objective was to study the relationships between nest characteristics and breeding performance, and the possible between-year variation in these relationships. We also explored relationships between parental characteristics and variables related to nest size and breeding performance, since parental traits might affect both nest characteristics and breeding success.

## MATERIALS AND METHODS

The study was conducted within extensive orange plantations at Sagunto, Valencia, eastern Spain (39° 42' N, 0° 15' W, 30 m a.s.l.) in 2006 and 2007. Nestboxes have been available there since 1986, when studies of the local great tits began (e.g. Andreu and Barba, 2006; Greño *et al.*, 2008).

We visited all nestboxes at least once a week from late February. When we found a great tit nest, we visited the nestbox when the fifth egg was expected to have been laid (assuming the laying of one egg per day), then daily until no new eggs were found and incubation started, then 12 days after incubation started and daily thereafter until all the eggs hatched or no new eggs hatched during two consecutive days, then when nestlings were aged 13 and 14 days, and finally six days later, when nestlings were expected to have fledged. Other visits were made as necessary to trap parents and measure the nests. This protocol allowed us to estimate the laying date of the first egg (laying date hereafter): assuming one egg was laid per day, clutch size, number of eggs hatched and number of fledglings. From these, we estimated hatching success (proportion of eggs hatched), fledging success (proportion of nestlings that fledged) and breeding success (proportion of eggs that produced fledglings). These three measures of success, as well as the number of fledglings produced, were used as dependent variables in the relevant analyses. The percentages were arcsin square root transformed for analyses (Zar, 1996).

We considered 32 nests from 2006 and 24 from 2007, including only first clutches, though sample sizes differed for different parameters since some nests failed during the course of the nesting period. Thus, 10 nests failed before fledging in 2006 (2 depredated, 8 deserted in different phases), and 14 failed in 2007 (7 depredated, 7 deserted). There were no significant differences in nest characteristics (defined below) between nests that failed or succeeded (Student's *t*-test, $P > 0.05$ in all cases).

Nestlings were ringed with individually numbered metal rings when they were 13 days old. We then also injected 0.2 mg/0.04 ml concentration of phytohemagglutinin (a T-cell mitogen; PHA) dissolved in saline solution into the left wing web of each nestling. We measured the thickness in this place three times using a pressure-sensitive spessimeter (Mitutoyo, model PK-1012E; 0.01 mm accuracy) immediately before the injection. On day 14, we measured nestling tarsus length and weight, computing an index of body condition (weight/tarsus length). We also measured the thickness of the wing web at the point of injection three times, and calculated the difference in mean thickness on both occasions to determine the immune response level. For each nest, we calculated the average difference for all the nestlings present and we used this mean value in the analyses to assess nestling immunocompetence. The PHA test is an easy *in vivo* test which measures the T-cell mediated immune response of nestlings, this being a surrogate for immune function and indicator of the health of individuals (Tella *et al.*, 2008). Its use has no adverse effects on the nestlings (Merino *et al.*, 1999).

Parents were trapped at the nest, or mist-netted nearby, when nestlings were 12-14 days old. We ringed them with individually numbered metal rings, and determined their sex and age class (first year or older breeders; Svensson, 1996). Wing length was measured to the nearest mm with a wing rule and tarsus length to the nearest 0.01mm with digital callipers, and an index of body condition (weight/tarsus length) was calculated. Five females and one male were recaptured during both years and data from one year only was used in such cases to avoid pseudoreplication. For this, we took data from the year for which more parameters were available or selected one of the years randomly (data for 2 females and 1 male in 2006, and 3 females in 2007). Although the female alone builds the nest, it has been suggested that her nest-building activity is assessed by the male to modulate his own reproductive investment (Tomás *et al.*, 2006), so we also included male traits in our study.

All nests were measured and weighed by the same researcher (EA) 2-5 days after incubation started. The general dimensions of the nestboxes used are described by Lambrechts *et al.* (2010), although the basal area of each nestbox (length and width of the nest) was checked with a ruler. We measured nine nest variables (see table 1), using a digital calliper for linear measurements other than length and width, and a digital balance for weighing.

A Principal Component Analysis (PCA) was used to reduce the number of variables concerning nest characteristics to a few components. We computed factor scores from factor loadings of the varimax rotation of the axes. We arbitrarily selected eigenvalues above 1, also taking into account that the selected components explained a large percentage of the variance. We regressed each resulting component against the original variables to explore which were more associated with each particular component. The PCA reduced the nine variables used to define nest quality to four principal components. The first (PC1) explained 22% of the variance, and was related to overall nest size, its value increasing with the total height, thickness of the bottom and fresh weight. The second (PC2) explained 22% of the variance and was related to nest cup diameter. Its value increased when longitudinal and transverse diameters increased and the thickness of the external wall decreased. The third (PC3) explained 19% of the variance and was related to relative cup depth, increasing with the total height and cup depth (table 1). A fourth component (not shown in table 1) explained 20% of the variance and was mostly related to nest basal area. Since this last component was virtually constant for all the nests, being the basal area of the nestboxes, we did not consider it in further analyses.

We used General Linear Models to explore the relationships between parental, nest, and breeding performance traits, and between-year differences in them, including in each one the dependent variable, the independent variable as a covariate, year as a fixed factor, and the interaction between year and the covariate. When the interaction term was significant, a regression analysis was performed for each year. In the case of female age, both age and year were included as factors. In the case of males, the sample size was too small to permit an analysis for each year, so only age was included, pooling data from both study years.

TABLE 1

Results of the principal component analysis performed on nine variables measured for 56 great tit nests. Correlation coefficients between the components and the original variables are also shown. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

*[Resultados del análisis de componentes principales realizados sobre nueve variables medidos sobre 56 nidos de carbonero común. También se muestran los coeficientes de correlación entre los componentes y las variables originales. $*P < 0,05$, $**P < 0,01$, $***P < 0,001$.]*

| | **PC1**<br>(Overall nest size) | **PC2**<br>(Cup diameter) | **PC3**<br>(Relative cup depth) |
|---|---|---|---|
| Total height | 0.64*** | -0.05 | 0.74*** |
| Width | 0.05 | 0.05 | -0.11 |
| Thickness of external wall | 0.06 | -0.81*** | -0.11 |
| Thickness of base | 0.95*** | 0.01 | -0.13 |
| Cup depth | -0.04 | -0.06 | 0.98*** |
| Longitudinal diameter | 0.00 | 0.86*** | -0.01 |
| Transverse diameter | 0.05 | 0.72*** | -0.27* |
| Length | 0.09 | 0.10 | 0.18 |
| Fresh weight | 0.82*** | -0.01 | 0.21 |
| Eigenvalue | 2.00 | 1.94 | 1.71 |
| % variance accounted for | 22.19 | 21.57 | 18.94 |

The SPSS v. 17.0 statistical package was used for all analyses. Means ± SD are shown where appropriate.

## RESULTS

Mean values for the breeding parameters in 2006 and 2007 are shown in table 2. Overall, 2007 seems to have been a more favourable year for breeding in terms of food availability: both clutch sizes and fledgling weights were larger. However, hatching success was greater in 2006 and there was no between-year difference in the number of fledglings produced per nest. These differences are reflected in some of the analyses below, as year was introduced as a fixed factor. However, hereafter we only present results concerning between-year differences where these affected the relationship under study, i.e. when the interaction between year and the covariate was significant.

We found positive relationships between nest characteristics and such reproductive parameters as laying date, hatching success and fledging success. Thus, increased nest diameter was associated with earlier laying ($F_{1,52} = 11.75$, $P = 0.001$; fig. 1). The relationship between hatching success and relative cup depth differed between years (Interaction term: $F_{1,38} = 5.92$, $P = 0.02$): there was no significant relationship in 2006, but hatching success was higher as relative cup depth increased in 2007 ($F_{1,14} = 8.56$, $\beta = 0.62$, $P = 0.01$; fig. 2). Fledging success was re-

TABLE 2

Mean breeding parameters ± SD and sample size (in parentheses) for the two study years. Differences were tested with Student $t$-tests. For hatching, fledging and breeding success, means were computed with the transformed values.

*[Parámetros reproductores medios, DT y tamaño muestral (entre paréntesis) en los dos años de estudio. Las diferencias se contrastaron con tests* t *de Student. Para los éxitos de eclosión, vuelo y reproducción, las medias se calcularon sobre los valores transformados.]*

| | 2006 | 2007 | Statistics |
|---|---|---|---|
| Laying date (1 = 1 April) | 10.31 ± 4.73 (32) | 7.63 ± 5.98 (24) | $t_{54} = 1.88$, $P = 0.066$ |
| Clutch size | 8.22 ± 1.07 (32) | 9.13 ± 1.91 (24) | $t_{54} = 2.99$, $P = 0.004$ |
| Hatching success | 85.74 ± 9.03 (26) | 75.05 ± 14.41 (16) | $t_{22.35} = 2.66$, $P = 0.014$ |
| Number of fleglings | 6.73 ± 1.49 (22) | 6.60 ± 1.65 (10) | $t_{30} = 0.22$, $P = 0.829$ |
| Fledging success | 68.51 ± 14.57 (22) | 71.11 ± 13.78 (10) | $t_{30} = 0.48$, $P = 0.638$ |
| Breeding success | 67.39 ± 15.14 (22) | 61.99 ± 13.19 (10) | $t_{30} = 0.97$, $P = 0.339$ |
| Nestling tarsus length | 19.17 ± 0.63 (21) | 19.30 ± 0.37 (10) | $t_{29} = 0.61$, $P = 0.545$ |
| Nestling weight | 15.77 ± 1.49 (21) | 16.65 ± 0.49 (10) | $t_{26.94} = 2.45$, $P = 0.021$ |
| Nestling condition | 0.82 ± 0.07 (21) | 0.86 ± 0.03 (10) | $t_{29} = 1.90$, $P = 0.067$ |
| Nestling immune response | 0.61 ± 0.16 (18) | 0.48 ± 0.16 (9) | $t_{25} = 1.86$, $P = 0.074$ |

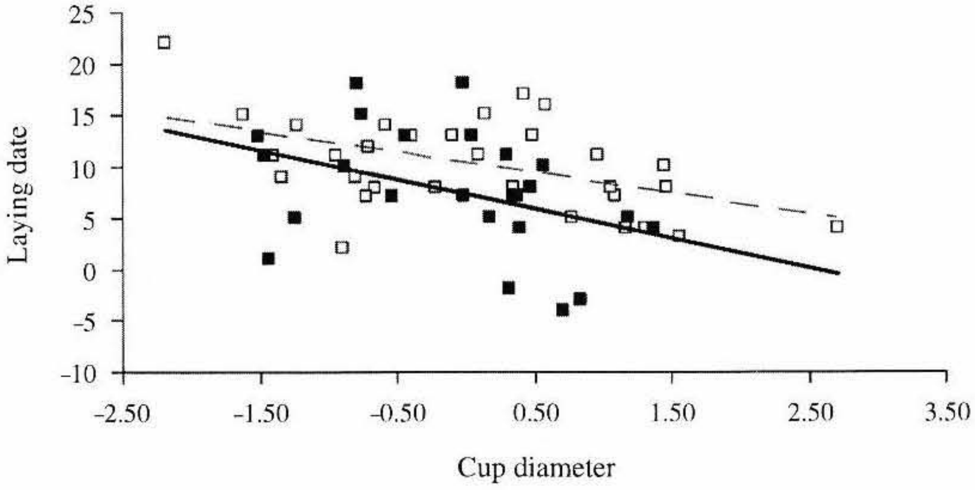

FIG. 1.—Relationship between laying date (1 = 1 April) and cup diameter (PC2) in 2006 (broken line and open squares; $N = 32$) and 2007 (solid line and black squares; $N = 24$).

*[Relación entre la fecha de puesta (1 = 1 de abril) y el diámetro del cuenco (PC2 del nido) en 2006 (línea discontinua y cuadrados vacíos; N = 32) y 2007 (línea continua y cuadrados negros; N = 24).]*

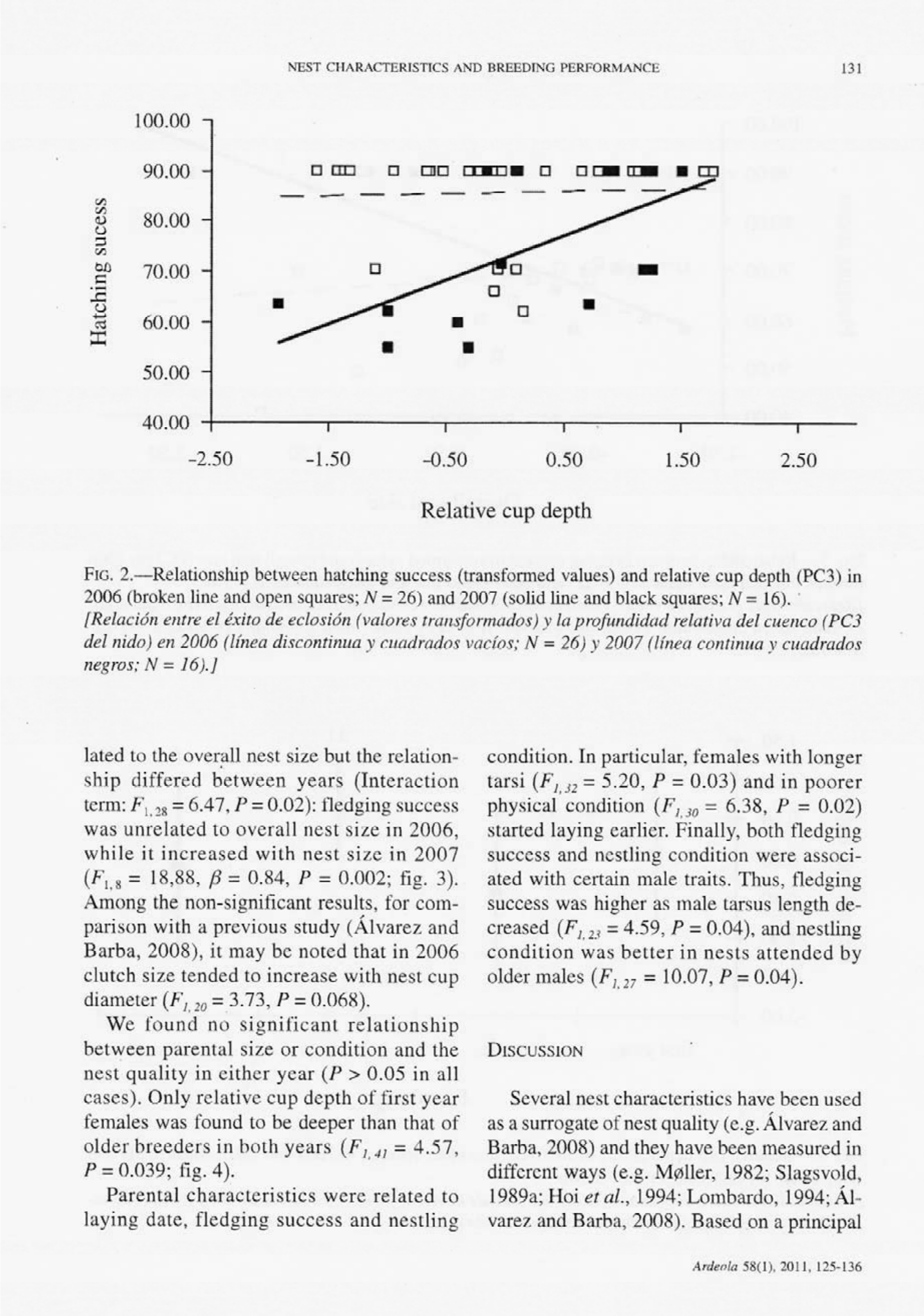

FIG. 2.—Relationship between hatching success (transformed values) and relative cup depth (PC3) in 2006 (broken line and open squares; $N = 26$) and 2007 (solid line and black squares; $N = 16$).
*[Relación entre el éxito de eclosión (valores transformados) y la profundidad relativa del cuenco (PC3 del nido) en 2006 (línea discontinua y cuadrados vacíos; N = 26) y 2007 (línea continua y cuadrados negros; N = 16).]*

lated to the overall nest size but the relationship differed between years (Interaction term: $F_{1,28} = 6.47, P = 0.02$): fledging success was unrelated to overall nest size in 2006, while it increased with nest size in 2007 ($F_{1,8} = 18{,}88$, $\beta = 0.84$, $P = 0.002$; fig. 3). Among the non-significant results, for comparison with a previous study (Álvarez and Barba, 2008), it may be noted that in 2006 clutch size tended to increase with nest cup diameter ($F_{1,20} = 3.73, P = 0.068$).

We found no significant relationship between parental size or condition and the nest quality in either year ($P > 0.05$ in all cases). Only relative cup depth of first year females was found to be deeper than that of older breeders in both years ($F_{1,41} = 4.57$, $P = 0.039$; fig. 4).

Parental characteristics were related to laying date, fledging success and nestling condition. In particular, females with longer tarsi ($F_{1,32} = 5.20$, $P = 0.03$) and in poorer physical condition ($F_{1,30} = 6.38$, $P = 0.02$) started laying earlier. Finally, both fledging success and nestling condition were associated with certain male traits. Thus, fledging success was higher as male tarsus length decreased ($F_{1,23} = 4.59$, $P = 0.04$), and nestling condition was better in nests attended by older males ($F_{1,27} = 10.07$, $P = 0.04$).

## DISCUSSION

Several nest characteristics have been used as a surrogate of nest quality (e.g. Álvarez and Barba, 2008) and they have been measured in different ways (e.g. Møller, 1982; Slagsvold, 1989a; Hoi *et al.*, 1994; Lombardo, 1994; Álvarez and Barba, 2008). Based on a principal

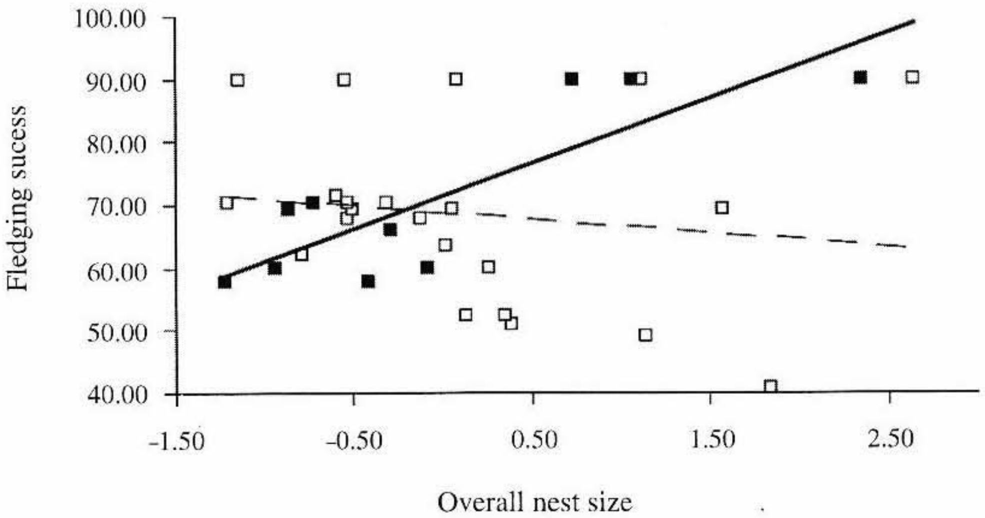

FIG. 3.—Relationship between fledging success (transformed values) and overall nest size (PC1) in 2006 (broken line and open squares; $N = 22$) and 2007 (solid line and black squares; $N = 10$).
*[Relación entre el éxito de vuelo (valores transformados) y el tamaño completo del nido (PC1 del nido) en 2006 (línea discontinua y cuadrados vacíos; N = 22) y 2007 (línea continua y cuadrados negros; N = 10).]*

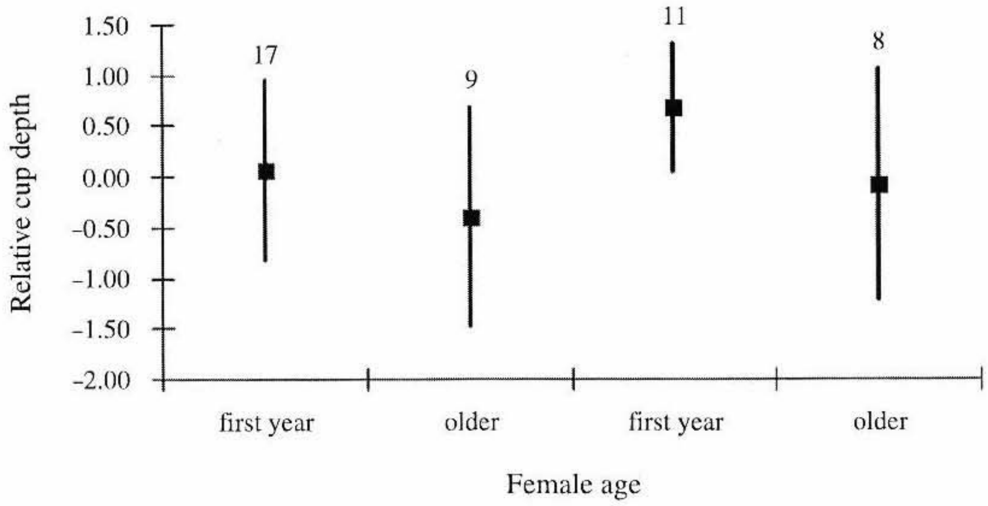

FIG. 4.—Relative cup depth (PC3) of first year and older females in 2006 and 2007. Mean ± SD and sample size for each category are shown.
*[Profundidad relativa del cuenco (PC3) del nido de hembras jóvenes y adultas en 2006 y 2007. Se indica la media ± DT y el tamaño muestral para cada categoría.]*

component analysis, we have "broken down" nest quality into three components: overall nest size, relative cup depth and nest cup diameter, and their possible relationships with great tit breeding parameters are discussed in turn below.

### *Overall nest size*

One of the most important functions of nests is the protection of eggs and nestlings (Collias and Collias, 1984). The "thermoregulatory" hypothesis suggests that nesting material may help to maintain egg temperature and to buffer temperature fluctuations. Bigger nests are better for these functions (Mertens, 1977) so we would expect eggs to develop better in bigger nests. For example, Møller (1982) found higher hatching success in large than in small barn swallow *Hirundo rustica* nests, and Álvarez and Barba (2009), in an experimental study, found higher hatching success in bigger great tit nests. However, this is not always so. For example, Alabrudzińska *et al.* (2003) and Álvarez and Barba (2008) found that hatchability of great tit eggs was unrelated to overall nest weight, and Tomás *et al.* (2006) found similar results for blue tits *Cyanistes caeruleus*. On the other hand, clutch size has been found to be positively (Soler *et al.*, 2001), or negatively (Alabrudzińska *et al.*, 2003) related to nest size, and a lack of any such relationship has also been reported (Tomás *et al.*, 2006; Álvarez and Barba, 2008). We did not find any relationships between overall nest size and clutch size or hatching success in either study year.

Higher fledging or breeding success have been reported in bigger nests in some species, including great tits (Lombardo, 1994; Álvarez and Barba, 2008), although negative relationships have also been found (e.g. Alabrudzińska *et al.*, 2003). We found here that fledging success was positively related to overall nest size in 2007, but not in 2006.

The immunocompetence of the nestlings could be affected by nutritional or thermal conditions during growth (Dietert *et al.*, 1994; Saino *et al.*, 1997; De Neve *et al.*, 2004). We did not find any relationship between overall nest size and the immune response of chicks in either year. We found no relationship between nest characteristics and number of fledglings, a factor which could have mediated the relationship between overall nest size and nestling immune response. It seems therefore that the degree of variation in overall nest size in our sample was insufficient to create a range of conditions that would allow potential effects on this aspect of nestling health status to be detected. Alternatively, parental investment may have been of greater importance than nest characteristics in determining nestling immunocompetence (e.g. Saino *et al.*, 1997). However, none of the parental characteristics considered here was associated with nestling immune capacity.

The "sexual display" hypothesis, suggests that nest size may reflect the health status or phenotypic quality of the builder (Soler *et al.*, 1998b; De Neve *et al.*, 2004; Tomás *et al.*, 2006). Thus, in species where only females build the nest, a relationship between nest quality and maternal characteristics would be expected, and this has been found in several studies (e.g. Tomás *et al.*, 2006; Mainwaring *et al.*, 2008). Thus, females could signal their quality to males via nest characteristics, so that males would invest more if nest quality (as a surrogate of female quality) was higher. We did not find any positive relationship between parental traits (size, condition and age) and overall nest size in either study year. Perhaps, components of parental quality other than those measured, such as immunocompetence or feather mite load (e.g. Tomás *et al.*, 2006; Mainwaring *et al.*, 2008), are more relevant. On the other hand, we measured and weighed the parents at the end of the nestling period, by when most of their work was already done, and this might not adequately reflect their conditions at the time of nest building. The lack of relationship between parental traits and overall nest size, while several indicators of breeding performance were positively related to overall nest size, suggests that the nest itself could be, at least partly, affecting breeding success.

### *Relative cup depth*

One of the components of nest size is relative cup depth. This may be an important nest characteristic, as deeper nests could provide better thermal conditions (higher insulation) to the nest contents (Calder, 1973), although this aspect remains largely unexplored. We found a positive relationship between relative cup depth and hatching success in 2007.

On the other hand, we also found that first year females built relatively deeper nests than older ones in both study years. As noted above, relationships between nest architecture and female age are infrequently found and, at first glance, the relationship found here suggests that first year females build better nests than older ones. However, nest weight was similar for first year and older females ($F_{1,41} = 0.02$, $P = 0.88$), so nests built by first year females seem to be less dense than those of older females. More compact nests would probably provide better insulation from ambient conditions (e.g. Mertens, 1977; Skowron and Kern, 1980). Therefore, future studies are needed to define cup depth and its relationships with such other nest characteristics as nest density more clearly, to determine which parental characteristics affect cup depth, and to determine the effects of cup depth on reproductive parameters.

### *Nest cup diameter*

Nest cup size has been also shown to be an important component of nest size. For example, clutch size has been shown to be positively related with nest cup size both at intra- (Møller, 1982) and at interspecific levels (Slagsvold, 1989b). Larger nest cups are associated with higher fledgling production in adult tree swallow *Tachycineta bicolor* (Lombardo, 1994). In the present study, we only found that laying date was earlier in nests with a larger cup diameter. However, in a previous study of the same great tit population (Álvarez and Barba, 2008), we found a positive relationship between nest cup size and both laying date and clutch size. This earlier study used 43 clutches from 2006, while we only considered 32 first clutches (only first clutches that provided all the necessary data). Thus we repeated the analysis using only first clutches included in the previous paper, but clutch size was still positively related to nest cup diameter in this subset ($F_{1,29} = 5.43$, $P = 0.027$). Another possible reason for the difference is that we used a lower number of nests in the present study since the selection criteria were more rigorous. We found that in 2006 clutch size tended to increase with nest cup diameter, so it seems that the lack of such a relationship found in the present study is probably due to the reduced sample size.

In conclusion, our results reveal that relationships between nest size and breeding performance may vary between years, being absent in some of them, so studies involving several contrasting years are necessary to reveal any such relationships (e.g. Lombardo, 1994; Tomás *et al.*, 2006). In this study, we found positive relationships between nest characteristics and breeding parameters mostly in 2007, when breeding conditions seemed to be better. It is therefore possible that environmental conditions could mediate these relationships. For example, having a good nest may be only important when other limiting factors, such as food supply or ambient temperature, are above a threshold, and may be relatively unimportant in more stressful years.

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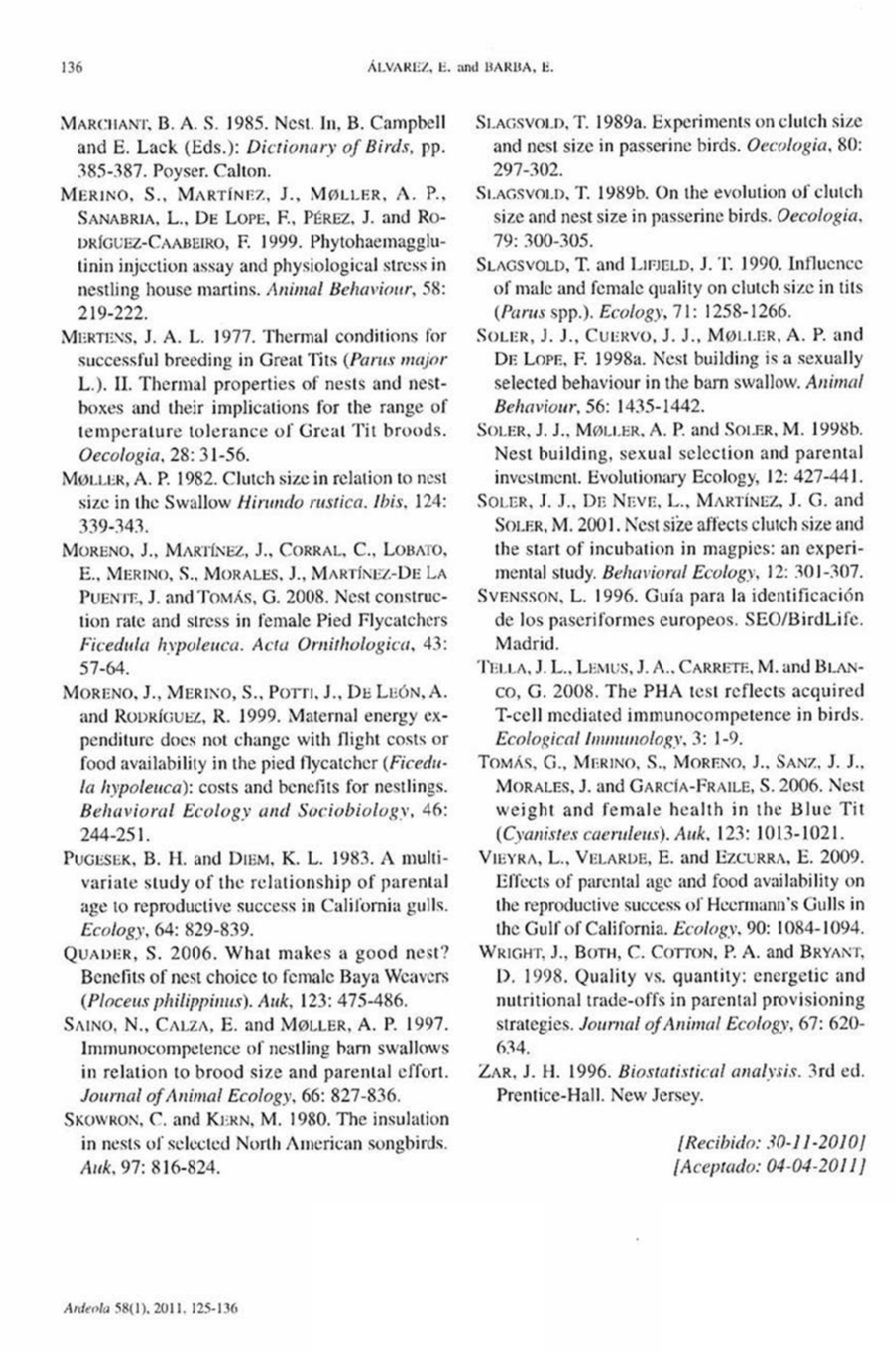

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# ARTÍCULO 3

## *Variation in Great Tit nest mass and composition and its breeding consequences: a comparative study in four Mediterranean habitats*

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*AVIAN BIOLOGY RESEARCH (2013), 6: 39-46*

*"Tropezando se aprende a caminar."*

AVIAN BIOLOGY RESEARCH 6 (1), 2013 39–46

# Variation in Great Tit nest mass and composition and its breeding consequences: a comparative study in four Mediterranean habitats

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## ABSTRACT

Bird nests are structures whose properties affect breeding performance. Thus, the vast majority of bird species build their own characteristic nests, selecting appropriate materials to do so. However, in habitats where the availability of "ideal" materials is low, some ability to use alternative ones would be necessary, even at the cost of having breeding success reduced. The Great Tit (*Parus major*) breeds under widely different environmental conditions. Its nests are althought to be composed mainly of moss, although very few studies have quantified nest composition. Our target here was describing Great Tit nest mass and composition in four different Mediterranean habitats, and exploring their fitness consequences. We recorded the basic breeding parameters, collected nests after fledging, and decomposed a sample of them, in four eastern Spanish populations: Pina (pines and oaks), Mariola (pines), Font Roja (holm oaks), and Sagunto (orange trees). Nests were heavier in Sagunto and Mariola and lighter in Font Roja, and those from Sagunto had the lowest amount of moss. Clutch size decreased as moss mass increased in the four sites. In Sagunto, hatching success increased as moss mass increased. In all habitats, nestling condition was lower when the amount of sticks and feathers was higher. We conclude that Great Tit nest mass and composition varies considerably between habitats, and the amount of different materials could affect different breeding parameters.

**Keywords:** clutch size, hatching success, moss, nest materials, nestling condition, *Parus major*

## 1. INTRODUCTION

Bird nests are multi-functional structures which provide thermal insulation, render protection from predators, help to maintain optimal position of eggs during incubation, and facilitate nestling care thus improving nestling survival (Collias and Collias, 1984). The size of the nests and its intraspecific variation has been well studied (Palomino *et al.*, 1998; Quader, 2006; Moreno *et al.*, 2009; Álvarez and Barba, 2011; Crossman *et al.*, 2011), and it has been shown to provide important reproductive benefits. For example, nest size has been shown to be positively related to several fitness related parameters, such as clutch size (Møller, 1982; Soler *et al.*, 2001; Álvarez and Barba, 2008), total number of eggs laid throughout the breeding season (Soler *et al.*, 1998), and hatching and breeding success (Møller, 1982; Álvarez and Barba, 2008, 2011).

By contrast, studies considering the relationship between nest composition and breeding traits are relatively scarce (Alabrudzińka *et al.*, 2003; Calvelo *et al.*, 2006; Britt and Deeming, 2011). Bird nests may be composed of a large number of diverse materials like plant, lichen, fungal, inorganic, animal and other materials, used to build, sometimes selectively, the four different zones of the nest: outer (decorative) layer, attachment, structural layer, and lining layer (Hansell, 2000). The amount and/or proportion of a particular material could depend on: (1) its availability and accessibility in the surrounding habitat (Moreno *et al.*, 2009; Wang *et al.*, 2009); (2) its physical and/or chemical properties (Hilton *et al.*, 2004; Mennerat *et al.*, 2009); (3) the environmental conditions (McGowan *et al.*, 2004; Mainwaring and Hartley, 2008; Britt and Deeming, 2011; Mainwaring *et al.*, 2012; Deeming *et al.*, 2012); and (4) the quality of the builders, since some materials, as stones, feathers or green plant material, could be brought to the nest to signal the quality of the bird who carried it (Moreno *et al.*, 1994; Veiga *et al.*, 2005; Polo and Veiga, 2006).

The relative importance of factors potentially affecting nest composition could vary among habitats, or between locations sharing the same habitat type but differing in environmental conditions. Therefore, species able to

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occupy different habitats, or with ample geographical ranges, should be able to adapt the size and composition of their nests to local conditions. This variation has been observed, for example, in American Robins (*Turdus migratorius*) and Yellow Warblers (*Dendroica petechia*), which constructed heavier nests, with thicker nest-walls, in colder habitats (Crossman *et al.*, 2011). To achieve larger nests, American Robins used the same materials, increasing their quantities, while Yellow Warblers increased the proportion of some materials, showing interspecific differences in the response against similar selective pressures.

Great Tits (*Parus major*) construct their nests inside natural or artificial cavities. As for other cavity-nesting species, there is an obvious limitation on nest size, inherent to cavity dimensions (Slagsvold, 1989). Thus, the use of nestboxes, easily accepted by some species, facilitates the study of nest size variation, since different individuals build their nests in "cavities" of the same size and general characteristics, and this is important to homogenise the initial conditions (Lambrechts *et al.*, 2010).

The Great Tit is one of the most widespread passerines, its many subspecies and races expanding along a wide range of environmental conditions and habitat types. For example, there are breeding populations from Lapland to North Africa, and from all kinds of natural forests and woodlands to orchards and city parks (Cramp and Perrins, 1993; Harrap and Quinn, 1996). In such a scenario, one would expect a great deal of variation in Great Tit nest size and composition. Thus, although its nests are typically composed of moss (Perrins, 1979), Alabrudzińska *et al.* (2003) suggested that there is a high variation in size and details of construction, and even breeding in open nests has been described (Monrós *et al.*, 1999). However, we are only aware of one study directly comparing Great Tit nest size and composition along a latitudinal gradient (Mainwaring *et al.*, 2012), but there is no mention to the habitat types included in the study, and nest components were pooled into "base" and "cup lining" materials, with no details of the quantity or proportion of each particular material type. Therefore, there is, to the best of our knowledge, no study explicitly comparing these characteristics among habitat types.

The aim of this work was to describe Great Tit nest mass and composition in different Mediterranean habitats, from homogenous orange plantations to a well preserved Mediterranean forest, and to explore their possible fitness consequences. We would expect that nest mass and composition would differ among habitats, and that the amount and/or proportion of key materials would affect breeding performance within habitats.

## 2. METHODS

Four sites in eastern Spain were selected for this study. The first one was situated in Sierra de Santa Bárbara de Pina, Castellón (40º01'N, 0º37'W, 1200 m above sea level), and it was mixed forest composed by Maritime (*Pinus pinaster* Aiton) and Scots Pines (*Pinus sylvestris* L.), and Pyrenean (*Quercus pyrenaica* Willd.) and Zeen Oaks (*Quercus faginea* Lam.). The second site was placed in Sagunto, Valencia (39º42'N, 0º15'W, 30 m above sea level), and it was located within an extensive Orange (*Citrus aurantium*) monoculture. And the last two sites were situated in two natural parks in Alicante: Sierra Mariola, in Bocairent (38º44'N, 0º33W, 900 m above sea level), dominated by an Aleppo Pine (*Pinus halepensis*) forest, and Font Roja, in Alcoy (38º39'N, 0º32'W, 1090 m above sea level), dominated by a Holm Oak (*Quercus ilex*) forest. We refer hereafter to these study sites as Pina, Sagunto, Mariola and Font Roja respectively. Nestboxes were available in all the study areas (Greño *et al.*, 2008; Atiénzar *et al.*, 2010). Nestbox characteristics were described in Lambrechts *et al.* (2010), the same model being used in all sites. A random sample of nests from each study area was selected for this study (see Table 1 for details of sample sizes per site and year).

We visited all nestboxes at least once a week, and daily in some periods, from late February to the end of the breeding season by mid July, and included here only data from first clutches. This pattern of visits allowed us to estimate the laying date of the first egg (assuming the laying of one egg per day; Perrins, 1979), and quantify clutch size, number of eggs hatched and number of fledglings. From these, we estimated hatching success (proportion of eggs hatched), fledging success (proportion of nestlings that fledged) and breeding success (proportion of eggs that produced fledglings). The percentages were arcsin square root transformed for analyses (Zar, 1996). These three measures of success, as well as clutch size and the number of fledglings produced per nest, were used as dependent variables in the relevant analyses. In Sagunto, Mariola and Font Roja, tarsus length and mass of nestlings were measured 15 days after hatching, and an index of body condition (mass/ tarsus length) was calculated for each nestling. A mean value was computed for each nest and used in the analyses.

At day 20 after hatching, when nestlings have already fledged, nests were removed from the nestboxes, placed into sealed plastic bag, and stored at –20 ºC. A sample of nests of one year per site (see Table 2 for details of sample sizes per site) were dissected and separated into their main components (moss, sticks, hair, feathers, and fabric). Within "fabric" we pooled plastic or canvas strings, wool, fluff and thread, which were found in very small quantities in some nests. The separate components of these dissected nests and the rest of collected nests, were dried in an oven at 105 ºC during 12 hours (Álvarez and Barba, 2009) and weighed (electronic balance, 0.01 g accuracy). Materials weighing less than 0.01 g were not considered in the analyses. Most nests were dissected by EA, being the rest initially dissected by trained students and finally supervised by EA.

We looked at differences in nest mass among sites and years, and in nest composition among sites. We then related nest mass and composition with reproductive parameters at each site. We used all the data available (nests, sites and years) for each particular analysis. We used generalised linear models to explore the relationships between nest characteristics and breeding performance traits, and between-year differences in them when possible, including in each one the dependent variable (breeding trait), laying date and the selected independent variable (nest characteristic) as covariates, year and site as fixed factors, and two-way interactions. Sample sizes did not allow including all the nests characteristics as independent variables in a single analysis. When site, but not year, was significant, we pooled years to compare among sites. When the interaction term between the covariate and year was significant, a regression analysis was performed for each year. No other interaction was significant. *Post-hoc* analyses (Tukey) were done when necessary. The SPSS v. 17.0 statistical package was used for all analyses. Means ± SE are shown where appropriate.

## 3. RESULTS

In a first analysis to compare nest mass between sites, we included data from one year per site (2010 for Pina, 2007 for the other three sites). Total nest mass differed among habitats ($F_{3,33} = 3.63$, $P = 0.023$; Table 1). Tukey tests revealed only significant differences between Sagunto, and Font Roja, with heavier nests in Sagunto.

We have the data of total nest mass from Sagunto, Mariola and Font Roja for 2005 and 2007. Including site and year as factors, only significant differences among sites were found ($F_{2,65} = 6.63$, $P = 0.002$). Since there were no between year differences ($F_{1,65} = 0.20$, $P = 0.65$), we pooled the data to explore differences among sites with a larger data set. Significant differences among sites were found again ($F_{2,68} = 6.19$, $P = 0.003$), being nests from Sagunto and Mariola of similar mass and both heavier than those from Font Roja.

Finally, we have 3 years of data from Sagunto and Font Roja. Results including one more year only confirmed those above: nests from Sagunto were significantly heavier than those from Font Roja ($F_{1,82} = 21.37$, $P < 0.001$), with no between-year differences ($F_{2,82} = 2.77$, $P = 0.07$).

Relationships between nest mass and reproductive parameters were first examined considering data of one year per habitat type (2007 for Sagunto, Mariola and Font Roja, and 2010 for Pina), including habitat as factor and laying date as a covariate. Then we included years 2005 and 2007 for Sagunto, Mariola and Font Roja, and then years 2005, 2006 and 2007 in Sagunto and Font Roja, with site and year as factors, and laying date as a covariate in both cases. No significant relationship was found between total nest mass and reproductive parameters ($P > 0.05$ in all cases).

In general, Great Tits used the same components to build their nests in all the studied sites (Table 2), although there was among-site variation in the amount and proportion of

**Table 1** Total mean nest dry mass (± SE) of four Mediterranean Great Tit populations in different years

| | Sagunto | Mariola | Font Roja | Pina |
|---|---|---|---|---|
| 2005 | 15.66 ± 1.63 ($n$ = 18) | 16.63 ± 1.68 ($n$ = 10) | 11.81 ± 1.09 ($n$ = 12) | |
| 2006 | 21.07 ± 1.38 ( $n$ = 26) | | 13.21 ± 1.87 ($n$ = 11) | |
| 2007 | 17.74 ± 1.59 ($n$ = 11) | 13.89 ± 1.69 ($n$ = 10) | 10.74 ± 0.80 ($n$ = 10) | |
| 2010 | | | | 10.65 ± 4.35 ($n$ = 6) |
| Total mean | 18.60 ± 0.94 ($n$ = 55) | 15.30 ± 1.20 ($n$ = 20) | 12.00 ± 0.77 ($n$ = 33) | 10.65 ± 4.35 ($n$ = 6) |

**Table 2** Mean dry mass (± SE) of the different nest materials in four Mediterranean Great Tit populations. Nests from Sagunto, Mariola, and Font Roja were collected in 2007, and those from Pina in 2010. Differences among sites in the amount and proportion of each material type were tested using oneway ANOVAs. Letters following each value indicate the results of the *post-hoc* analyses, being means with the different letter statistically different

| | Sagunto ($n$ = 11 nests) | Mariola ($n$ = 10 nests) | Font Roja ($n$ = 10 nests) | Pina ($n$ = 6 nests) | F | $P$ |
|---|---|---|---|---|---|---|
| Moss (g) | 2.54 ± 0.83 (A) | 8.13 ± 0.64 (B) | 7.87 ± 0.81 (B) | 6.55 ± 2.58 (A,B) | $F_{3,33} = 6.34$ | 0.002 |
| Sticks (g) | 8.62 ± 1.12 (A) | 1.67 ± 0.65 (B) | 0.47 ± 0.23 (B) | 2.20 ± 1.37 (B) | $F_{3,33} = 19.66$ | < 0.001 |
| Hair (g) | 5.59 ± 1.04 (A) | 1.50 ± 0.76 (B) | 1.51 ± 0.36 (B) | 0.17 ± 0.13 (B) | $F_{3,33} = 9.42$ | < 0.001 |
| Feathers (g) | 0.07 ± 0.02 | 0.14 ± 0.05 | 0.13 ± 0.05 | 0.03 ± 0.03 | $F_{3,33} = 1.38$ | 0.267 |
| Fabric (g) | 0.92 ± 0.30 (A) | 2.45 ± 0.57 (A) | 0.76 ± 0.32 (A) | 1.70 ± 0.80 (A) | $F_{3,33} = 2.96$ | 0.046 |
| Moss proportion | 0.16 ± 0.06 (A) | 0.63 ± 0.06 (B) | 0.73 ± 0.04 (B) | 0.68 ± 0.10 (B) | $F_{3,33} = 17.80$ | < 0.001 |
| Stick proportion | 0.49 ± 0.05 (A) | 0.10 ± 0.03 (B) | 0.05 ± 0.03 (B) | 0.18 ± 0.09 (B) | $F_{3,33} = 17.18$ | < 0.001 |
| Hair proportion | 0.29 ± 0.04 (A) | 0.08 ± 0.03 (B,C) | 0.15 ± 0.04 (A,B) | 0.01 ± 0.004 (C) | $F_{3,33} = 10.89$ | < 0.001 |
| Feather proportion | < 0.001 ± 0.00 | 0.01 ± 0.003 | 0.01 ± 0.006 | < 0.001 ± 0.00 | $F_{3,33} = 2.09$ | 0.121 |
| Fabric proportion | 0.06 ± 0.02 | 0.17 ± 0.04 | 0.06 ± 0.02 | 0.13 ± 0.04 | $F_{3,33} = 2.32$ | 0.093 |

each one. Specifically, there were among-site differences in the mass and proportion of moss, sticks and hair, and in the mass of fabric. *Post-hoc* analyses showed that the amount of moss was lower in Sagunto than in Mariola and Font Roja, while the amount of moss in Pina did not differ from all the other sites. The proportion of moss was lower in Sagunto than in the rest of sites. Sagunto also showed a higher amount and proportion of sticks, and higher amount of hair, than all the other sites, which did not differ between them. The proportion of hair was higher in Sagunto than in Mariola and Pina, and higher in Font Roja than in Pina. The amount and proportion of feathers, and the proportion of fabric did not differ between sites. Moreover, although we found marginally significant differences in the amount of fabric ($P = 0.046$), *a posteriori* analyses did not detect among-site differences. In summary, composition of nests in the three natural habitats was rather similar, while nests of Sagunto had a lower amount of moss and higher of sticks and hair.

Considering the relationships between nest size and composition and reproductive parameters, clutch size differed among habitats ($F_{3,33} = 10.31$, $P < 0.001$), and was negatively related to moss mass in all the four habitats ($F_{1,28} = 5.20$, $P = 0.03$; Figure 1). Also, the interaction term between moss mass and site was significant when looking at their relationship with hatching success ($F_{3,28} = 3.26$, $P = 0.036$). Considering this relationship separately in the four habitats, hatching success increased significantly with moss mass only in Sagunto ($F_{1,9} = 9.23$, $P = 0.014$; Figure 2), being non-significant its relationship in the other three habitats.

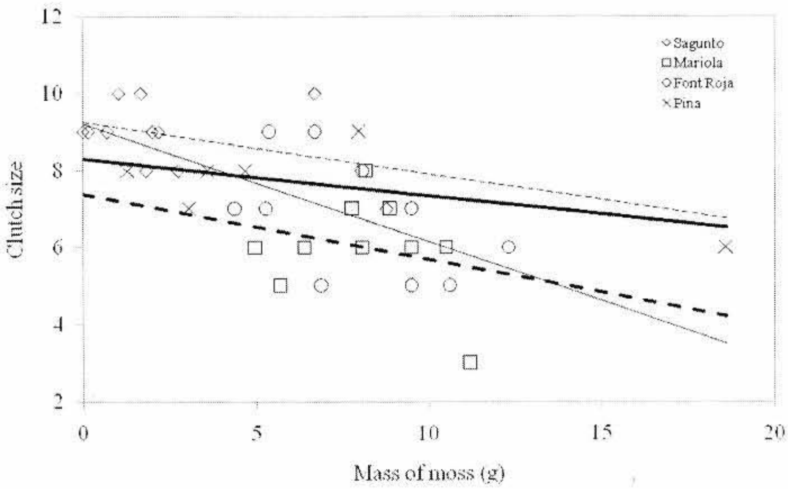

**Figure 1** Relationship between clutch size and the mass of moss in four Mediterranean Great Tit populations. Thick continuous line: Pina; thin continuous line: Font Roja; thick discontinuous line: Mariola; thin discontinuous line: Sagunto. The relationship between clutch size and mass of moss was significant, with no differences among sites.

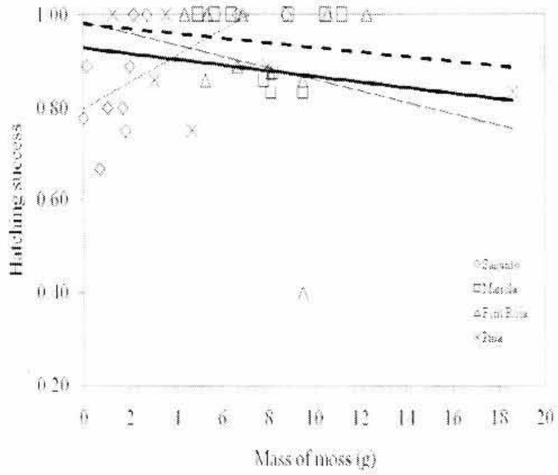

**Figure 2** Relationship between hatching success and the mass of moss in four Mediterranean Great Tit populations. Only the relationship in Sagunto was significant. Thick continuous line: Pina; thin continuous line: Font Roja; thick discontinuous line: Mariola; thin discontinuous line: Sagunto.

Nestling size, mass and condition was available for three populations (Sagunto, Mariola and Font Roja). Both tarsus length and mass differed among habitats ($P < 0.05$ in all cases), although nestling condition was similar. Nestling condition declined with increasing mass of sticks ($F_{1,22} = 4.72$, $P = 0.041$; Figure 3) and feathers ($F_{1,22} = 4.90$, $P = 0.038$; Figure 4), and with increasing proportion of feathers ($F_{1,22} = 5.72$, $P = 0.026$) in all the three habitats. No differences were found between sites, or interactions between sites and mass or proportion of nest components.

## 4. DISCUSSION

In general, we found that Great Tit nest mass and composition differed among habitats, that nest mass itself was not related to breeding traits, but that important breeding parameters such as clutch size and nestling condition were related to the amount and proportion of some materials.

Nest mass might vary among populations of the same species, probably adapting to prevailing conditions

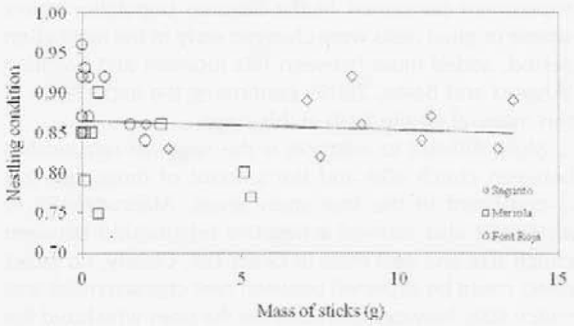

**Figure 3** Relationship between nestling condition and the mass of sticks in the nests of three Mediterranean Great Tit populations. The relationship was significant, with no significant differences among sites, so the regression line considering all the populations is presented.

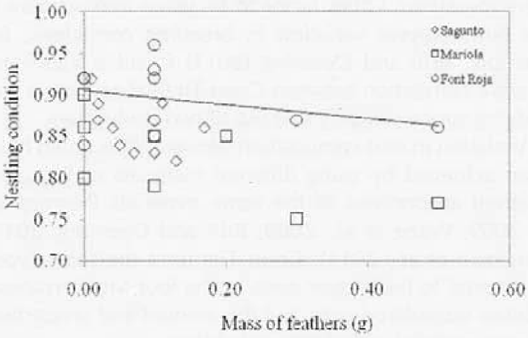

**Figure 4** Relationship between nestling condition and the mass of feathers in the nests of three Mediterranean Great Tit populations. As there were no significant differences among sites, the regression line considering all the populations is presented.

and availability of materials at each particular site. For example, nest mass varied between populations of Pied Flycatchers (*Ficedula hypoleuca*), due to differences in materials used (Moreno *et al.*, 2009). Total nest mass in our Great Tit studied populations varied between 10.65 g (Pina) and 18.60 g (Sagunto). Nests had similar mass in Sagunto and Mariola and were lighter in Font Roja. Mean nest mass of Pina did not differ from any other site, although values are relatively low and the lack of differences might well be caused by the small sample size. Another factor to be taken into account is that nests from Pina were collected in a different year. However, this difference *per se* is unlikely to be determinant. On one hand, we did not find significant differences in mean nest mass among years in sites where two or three years of data were available (Sagunto, Mariola, Font Roja). On the other hand, although sites were far apart, mean variation in local conditions seemed to occur synchronously. For example, mean April temperatures in Sagunto during the study years varied from 15.7°C in 2007 to 16.9°C in 2006, in Mariola between 10.5°C (2007) and 13.9°C (2006), and in Font Roja between 8.9°C (2007) and 12.2°C (2006; calculated from data provided by the Spanish Meteorological Agency; we do not have temperature data from Pina). 2010 was on the colder end in Sagunto (April mean: 15.3°C), so we could assume that it was a relatively cold year also in Pina (which is closer to Sagunto than Mariola and Font Roja). In any case, temperatures in 2010 were within the range of 2005–07. Anyway, data from Pina should be taken with caution, and an increase of sample size would be desirable for future studies.

In most species where they have been examined, nests are usually heavier in colder areas (Kern and Van Riper III, 1984; Briskie, 1995; Crossman *et al.*, 2011). In contrast to this general pattern, we found that the population of Font Roja, placed at higher elevation, where temperatures are lower during the breeding season, built lighter nests than Sagunto or Mariola. Differences with Sagunto might be attributed to the different use of moss and sticks – a higher use of sticks in Sagunto will probably end up with heavier, but otherwise smaller, nests. However, nests from Mariola were also heavier than those of Font Roja, when differences in altitude and mean temperatures would suggest, if any, a lower mass. Since the amount and proportion of materials was similar, differences could be attributed to the cumulative effect of slightly higher amount of some materials in Mariola.

We had data of nest mass in two (Mariola) or three (Sagunto, Font Roja) different years, and no between-year differences were detected. Variation in nest mass is sometimes reported, although the results are not consistent. For example, Moreno *et al.* (2009) found between-year differences in Blue Tit (*Cyanistes caeruleus*) nests, while Tomás *et al.* (2006) or Britt and Deeming (2011) failed to do so. This might depend on the prevailing conditions in the study years, but even 3°C of difference in mean ambient temperature in the study of Tomás *et al.* (2006) was unable to cause detectable differences in nest size. The lack of variation in our studied populations, some of them studied over 3 years, suggests a relative stability in breeding conditions.

We did not find relationships between total nest mass and reproductive parameters in any of the sites. although many studies have found positive relationships between nest mass or size and some reproductive traits (Møller, 1982; Soler *et al.*, 1998, 2001; Álvarez and Barba, 2008, 2011), others, including experimental ones, have failed to do so (Palomino *et al.*, 1998; Alabrudzińska *et al.*, 2003; Tomás *et al.*, 2006; Mainwaring and Hartley, 2008; Lambrechts *et al.*, 2010). Moreover, within the same habitat, the relationship might depend on the species or year studied (Britt and Deeming, 2011). Lambrechts *et al.* (2010) suggest that this variability could be caused by using different measures of nest size in different studies, and on where (laboratory or field) and when (*e.g.* before incubation starts, after fledging, *etc.*) the nests were measured. Other factor to be taken into account is the between-year variation in breeding conditions; for example, Britt and Deeming (2011) found a significant positive correlation between Great Tit total nest mass and fledging success in only one out of two study years.

Variation in nest composition between sites could have been achieved by using different materials or by using different proportions of the same materials (Moreno *et al.* 2009; Wang *et al.*, 2009; Britt and Deeming, 2011; Crossman *et al.*, 2011). Great Tits used the same types of material to build their nests in the four Mediterranean habitats considered here, but the amount and proportion of each material varied among habitats.

The most noticeable difference is the relatively low use of moss in Sagunto, which is basically compensated by sticks, finally achieving a similar (Mariola) or higher total nest mass (Font Roja) than other sites. Moss is a fundamental component of Great Tit nests in all the habitats where it has been studied (Cramp and Perrins, 1993), although precise quantitative studies are very scarce. Thus Alabrudzińska *et al.* (2003) found that moss amounted to 41% in a zoological and botanical garden in Poland, while Britt and Deeming (2011) found approximately 50% (estimated from Table 1 and Figure 1) at a University Campus in England with a small deciduous woodland. These proportions are notably lower than in our natural study areas (63–73%), our results supporting the preference of Great Tits for using moss as a basic component of their nests. The very low (16%) proportion of moss in nests from Sagunto is probably caused by difficulties in obtaining this material in orange plantations. However, experimental evidence, providing moss for the birds to choose freely, is lacking, so we cannot completely discard a preference for other materials in Sagunto. As stated above, a great deal of variation would be expected in such a widespread species, which is able to use different materials sometimes opportunistically (Britt and Deeming, 2011; Surgey *et al.*, 2012). The population of Sagunto has previously shown to adapt its nestling diet to local conditions, widely differing from that of many other populations (Barba *et al.*, 2004). We show here that this ability to adapt to local conditions also extends to nest-building behaviour.

It is interesting that the amount or proportion of moss is not related to hatching nor breeding success, nor with the quality of the nestlings produced, in any natural habitat, but hatching success was positively related to the amount of moss in Sagunto. This suggests that, in most habitats, Great Tits manage to bring enough moss to guarantee a good development of incubation and hatching, and/or that parents are able to compensate for small reductions in moss availability, but also highlights that moss is important in these processes and the effects could be detected below a threshold. Alabrudzińska *et al.* (2003) also showed a positive relationship between moss proportion and hatching success; this might suggest that moss was not available in necessary quantities for the birds. In an experiment performed in the Sagunto population, pairs whose original nests were changed early in the incubation period, added moss between this moment and hatching (Álvarez and Barba, 2009), confirming the importance of this material for the birds at this stage.

More difficult to interpret is the negative relationship between clutch size and the amount of moss, but this is consistent in the four study areas. Alabrudzińska *et al.* (2003) also showed a negative relationship between clutch size and nest mass in Great Tits. Clearly, no direct effect could be expected between nest characteristics and clutch size. However, females are the ones who build the nest and lay the eggs, and both activities are very energy-demanding (Visser and Lessells, 2001; Mainwaring and Hartley, 2009). We suggest a potential trade-off here: females investing more in nest-building, and especially in looking for a high-quality material, would have less resources left for egg formation. To our knowledge, this specific trade-off has not been found (Moreno *et al.*, 2008). However, Moreno *et al.* (2010) have reported that Pied Flycatchers show a trade-off between energy allocated to nest-building and that allocated to subsequent breeding activities: those pairs whose nest-building costs were reduced, increased nestling feeding rates thus improving nestling growth.

Nests in Sagunto contained higher amount and proportion of sticks than any other site. Great Tits used sticks, along with moss, to make the nest base. Actually, in some nests the proportion of moss was very low, and most of the nest base was made of sticks. The extreme was a nest (from the 10 dissected from Sagunto) with no moss at all, where 52% of the nest mass were sticks and 38% hair (total mass = 17.45 g). This might have negative consequences for nestling development. Notably, although mean nestling condition was similar in all the three habitats, nestling condition decreased with increasing amount of sticks in all sites, the decrease being quite sharp in the natural habitats (Font Roja and Mariola). This result emphasises that the use of sticks as an alternative material in Sagunto could be considered suboptimal.

Remarkably, nests from Sagunto also contain the highest amount of hair, with also higher proportion than Mariola and Pina. The probably low availability of moss could force the birds to look for alternative materials with better insulation properties than sticks, and hair might serve this purpose. Feathers might have been another candidate, since they have high insulative properties (Collias and Collias, 1984; Pinowski *et al.*, 2006), and its use have benefits to nestlings (Dawson *et al.*, 2011), but their amount and proportion did not vary among sites. Apart from their insulation properties, feathers in the nests might have signalling (Polo and Veiga, 2006; Sanz and García-Navas, 2011) and antiparasitic functions (Winkler, 1993), so perhaps its presence and abundance is governed by selective forces other than thermoregulation. Veiga and Polo (2005), for example, have suggested that the main function of feathers in Spotless Starlings (*Sturnus unicolor*) nests is signalling rather than insulation, and Sanz and García-Navas (2011) demonstrated that male Blue Tits carry feathers to the nests (otherwise built by females) to signal their quality. However, either as signal, antiparasitic, or thermoregulatory functions, we would have expected positive relationships between the amount of feathers and reproductive performance. However, a negative relationship was found, with consistent results in all the three habitats. We have no clear explanation for this. A potential, rather speculative, explanation is that birds bring feathers mostly as signal of their quality, but insulating properties of feathers would be detrimental for nestlings in Mediterranean habitats, where temperatures are high when nestlings are in the nests. High temperatures have been shown to negatively affect nestling development (Geraert *et al.*, 1996). In an experiment performed in the population of Sagunto, where nest composition was manipulated, birds removed wool, a good insulating material (Margalida and Bertran, 2000), from nests where this material was placed at a higher quantity (Álvarez and Barba, 2009). However, Sanz and García-Navas (2011) showed that male Blue Tits placed feathers outside the nest cup, thus having no contact with eggs or nestlings, therefore making this trade-off hypothesis unlikely in this case. This potential trade-off might be worth future study.

Nests might also have between-year variability in their composition, sometimes to take advantage of occasional availability of certain materials (Moreno *et al.*, 2009; Britt and Deeming, 2011), although we have no data to check this point in our populations.

## 5. CONCLUSION

In conclusion, we have shown that Great Tit nest mass and composition varies considerably between Mediterranean habitats, for example, the species is able to build nests with a very low amount of its preferred nest material (moss) in orange plantations. Within habitats, we showed an absence of relationships between total nest mass and basic reproductive parameters, while the mass and/or proportion of particular nest components were related to some breeding parameters, sometimes in counter-intuitive ways. Taken this and other studies into account, it seems necessary to develop experiments that might test some of the hypotheses which have been put forward so far.

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# ARTÍCULO 4

# ¿Cómo afecta la calidad del nido per se al proceso de incubación? Una aproximación experimental

ELENA ÁLVAREZ Y EMILIO BARBA

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"Los científicos ven lo que todos,
pero interpretan lo que nadie."

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# ¿Cómo afecta la calidad del nido *per se* al proceso de incubación? Una aproximación experimental

Elena Álvarez & Emilio Barba

**How does nest quality *per se* affect the incubation process? An experimental approach**
Given that a nest's thermal properties are affected by its composition and characteristics, and that nest temperature affects the incubation process, we would expect to find a relationship between nest characteristics and the incubation process. We tested this hypothesis in a Great Tit *Parus major* population, randomly substituting birds' original nests with artificial 'good' and 'bad' quality nests at the onset of incubation. Birds added material to almost all the replacement nests, although there was no difference between the good and bad nests in terms of the quantity of material added. The proportion of nests fledging at least one chick did not differ between control and good nests, but was significantly lower in bad nests. The length of the incubation period did not differ between good and control nests. Hatching success was higher in good than in control nests. Our results suggest that nest quality affects the incubation process, regardless of the quality of the parents. To our knowledge, this is the first study to have shown this relationship experimentally.

Key words: Great Tit, *Parus major*, nest size, incubation period, hatching success, Eastern Spain.

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Las aves construyen nidos para proporcionar protección y un microclima apropiado para el desarrollo de huevos y polluelos (Collias & Collias 1984). Por ejemplo, la composición y características del nido afectan a la temperatura, al flujo de calor y a la conductancia térmica del mismo (Mertens 1977, Skowron & Kern 1980, Hoi *et al.* 1994, Pinowski *et al.* 2006). Diversos trabajos han demostrado que cuando las temperaturas del nido son bajas los periodos de incubación son más largos (Haftorn 1983, Ardia *et al.* 2006, Hepp *et al.* 2006, Parker & Andrews 2007), el éxito de eclosión es menor, y el desarrollo de los embriones incluso puede llegar a ser anormal (Stoleson 1999, Hepp *et al.* 2006), aunque temperaturas demasiado altas también pueden afectar negativamente a algunos parámetros reproductores (e.g. Geraert *et al.* 1996). Por tanto, la "calidad" del nido (en este caso, la capacidad del nido de mantener una temperatura adecuada) tiene un impacto sobre el éxito reproductor.

En muchas especies de aves altriciales, el nido es un importante componente del esfuerzo parental. Los padres dedican una gran cantidad de tiempo y energía a la construcción del nido, lo cual puede estar mediado por su estado de salud o su calidad fenotípica; en general, nidos más grandes, o aquellos construidos en menos tiempo, están asociados a individuos en mejor condición (Lens *et al.* 1994, Soler *et al.* 1998, Tomás *et al.* 2006). Si la calidad del nido está relacionada con la de los padres, es posible que el mayor éxito observado en nidos de mejor calidad pueda deberse a que los padres sean mejores, a que el nido sea mejor, o a una interacción entre ambos factores. Que sepamos no existen trabajos que analicen el efecto de la calidad del nido sobre el éxito reproductor sin desvincular la calidad de los padres y la calidad o las características del nido, aunque la necesidad de estos estudios se ha puesto de manifiesto en algunos trabajos (e.g. Kim & Monaghan 2005a, b, Álvarez & Barba 2008).

El Carbonero Común *Parus major* es un paseriforme que anida en cavidades, y acepta de buen grado las cajas-nido para dicho propósito. Esto último hace que sea un modelo de estudio ideal para determinar la variación en el tamaño del nido, puesto que todos los individuos que crían en cajas-nido comienzan la construcción en una cavidad de dimensiones conocidas e idénticas. Esta especie construye un nido que está compuesto por una base principal de musgo y una parte superior que conforma la taza del nido compuesta básicamente por pelos y lana (Perrins 1979, Alabrudzińska *et al.* 2003), y son las hembras las que lo construyen (Gosler 1993). En nuestra área de estudio, la disponibilidad de musgo es baja, y la proporción de musgo en los nidos (23% ± 14%, n = 14), es notablemente menor que en otros ambientes en los que se ha estudiado (e.g. 41% ± 19%, n = 29; Alabrudzińska *et al.* 2003).

El objetivo de este trabajo fue determinar experimentalmente si la calidad del nido por sí misma afectaba al proceso de incubación. Para ello, manipulamos algunos nidos con objeto de aumentar o empeorar su calidad respecto a nidos control, desvinculando así la posible contribución de la calidad de los padres de la de los nidos que atienden. En base a esto, predecimos que, si el proceso de incubación depende de la calidad del nido, (1) la duración media del periodo de incubación será menor en los nidos experimentales de buena calidad y mayor en los de mala calidad respecto a los nidos control, y/o (2) el éxito medio de eclosión será mayor en nidos experimentales de buena calidad y menor en los de mala calidad respecto a los nidos control.

## Material y métodos

El estudio se realizó en un monocultivo extensivo de naranjos cerca de Sagunto, Este de España (39°42' N, 0°15' W, 30 m s.n.m.). Para el estudio de la biología reproductora del Carbonero Común se dispusieron cajas-nido durante la época reproductora (e.g. Greño *et al.* 2008).

Las cajas-nido se revisaron una vez por semana para determinar el inicio de la construcción de los nidos (día 1 = 1 de abril en todos los análisis). Conforme éstos se iban encontrando, se asignaron aleatoriamente a dos grupos: el grupo "experimental" (n = 16), en el que se realizaron las manipulaciones de los nidos, y el grupo "control" (n = 16), en el que se realizó el seguimiento de los nidos sin manipularlos. Todos ellos correspondían a primeras puestas. Ocho nidos del grupo experimental se cambiaron por nidos artificiales de buena calidad y ocho por nidos de mala calidad. Los nidos experimentales fueron construidos con una mezcla de musgo (en la base) y lana (en el cuenco), en una proporción de 20/0.6 g. (musgo/lana) en los de "buena calidad" y de 7/0.2 g. en los de "mala calidad" (peso fresco en todos los casos). La forma de construcción de los nidos experimentales fue similar, por lo que los nidos de "buena calidad" eran altos y con base más gruesa, mientras que los "malos" eran bajos y con base más fina. El peso de los nidos experimentales buenos y malos estuvo dentro del rango encontrado en la población (media = 20.6 ± 6.4 g., rango: 7.6 – 40.6 g., n = 47).

Todos los nidos fueron inspeccionados al menos una vez por semana. Se registró la fecha de puesta del primer huevo (estimada asumiendo la puesta de un huevo diario, Perrins 1979), el tamaño de puesta, la fecha de inicio de la incubación, la fecha de eclosión del primer pollo, y el número de huevos que eclosionaron.

El Carbonero Común suele comenzar la incubación, que realiza sólo la hembra, cuando la puesta está completa o con la puesta del penúltimo huevo (tamaño medio de puesta en la población: 7.7 ± 0.1 huevos, n = 4 años; Belda *et al.* 1998). En este trabajo hemos considerado como fecha de inicio de incubación el día de puesta del último huevo (e.g. Drent 1975). Así, el día 1 es el día de comienzo de la incubación determinado por este método.

Una vez determinado el día de inicio de incubación, se visitaron los nidos control de dos a cinco días más tarde para confirmar el tamaño de puesta. Los nidos experimentales se visitaron el día 3-4 de incubación para determinar el tamaño de puesta. En este momento se retiraron los nidos originales, junto con la caja en la que se encontraban, siendo sustituidos aleato-

riamente por cajas que contenían nidos artificiales de buena o de mala calidad. Los huevos fueron transferidos a estos nuevos nidos. Consideramos que los padres aceptaron inicialmente el cambio de nido si detectamos actividad tras el mismo: adición de material al nido (ver más abajo), continuación de la incubación y/o eclosión de los huevos.

El periodo de incubación (tiempo transcurrido desde la puesta del último huevo hasta el día anterior al nacimiento del primer pollo; e.g. Drent 1975) en esta especie dura aproximadamente 13 días (Perrins 1979). Por ello, los nidos se visitaron de nuevo el día 12 de incubación, y a partir de ahí diariamente, para registrar el día de inicio de la eclosión, definido como el día en que eclosiona el primer huevo.

Las visitas diarias continuaron hasta que nacieron todos los pollos o hasta que no se detectaron nuevas eclosiones, ya que algunos huevos no llegan a eclosionar. El periodo medio de eclosión en nuestra área de estudio es de 2.3 ± 0.57 días (n = 32; Barba 1991). Debido a que los huevos no eclosionados permanecen en el nido, el éxito de eclosión se estimó como el porcentaje de huevos que eclosionaron. Con el objeto de determinar si los padres modificaron el material de los nidos experimentales durante el periodo de incubación, al día siguiente de eclosionar el primer huevo, los nidos experimentales se cambiaron de nuevo por nidos hechos con musgo, y los polluelos fueron transferidos a estos nuevos nidos. Los nidos retirados se llevaron al laboratorio y se congelaron para su examen posterior.

Cuando los pollos tenían entre 10 y 14 días se intentó capturar a los padres mediante trampas colocadas en las cajas, o con redes japonesas rodeando éstas. Los individuos capturados se marcaron individualmente con anillas metálicas numeradas, y se determinó su sexo y edad (Svensson 1996). Diferenciamos dos clases de edad por diferencias en el plumaje: individuos de un año (que nacieron la temporada de cría anterior, y que llamaremos "jóvenes" a partir de ahora), y los que nacieron en años anteriores ("adultos"). De cada individuo medimos la longitud del ala y del tarso con una regla de tope (± 0.5 mm.) y un calibre digital (± 0.01 mm.) respectivamente.

Los nidos retirados se desmenuzaron, clasificando el material inicialmente en cuatro grupos: musgo, lana, pelo y plumas. En algunos fue difícil separar el pelo de las plumas por lo que se agruparon estos componentes en todos los casos para los análisis. Los diferentes componentes se secaron en una estufa de convección (105 °C, 12:00 horas) y determinamos su peso seco en una balanza digital portátil de precisión 0.01 g. Comprobamos en los primeros nidos que el peso se estabilizó tras 12:00 horas en la estufa. Además, se construyeron seis nidos experimentales, tres de buena y tres de mala calidad (que llamaremos nidos experimentales "iniciales"), a los que les aplicamos el mismo procedimiento de secado en la estufa y pesaje. Así, estos pesos se utilizaron de control para determinar el material añadido o eliminado por el ave de cada nido experimental (que llamaremos nidos experimentales "finales") colocado en el campo, calculando la diferencia entre las medidas de cada componente de los nidos experimentales finales con respecto a las medias de las medidas de los nidos experimentales iniciales.

### *Análisis estadísticos*

Para analizar el éxito de eclosión transformamos los datos calculando el arcoseno de la raíz cuadrada de los porcentajes para poder utilizar pruebas paramétricas (Zar 1996).

Para comparar porcentajes (padres de diferentes clases de edad, huevos que eclosionaron) utilizamos pruebas G, utilizando el valor ajustado en el caso de tablas de 2 x 2 (Zar 1996). Para el resto de análisis se emplearon modelos lineales generales univariados, y pruebas *a posteriori* (Tukey) cuando era necesario, utilizando el paquete estadístico SPSS 15.0. Presentamos los valores medios ± desviación típica.

## Resultados

No se encontraron diferencias significativas en la fecha de inicio de puesta, ni en las características de los padres (edad y biometría), entre los nidos control y los nidos experimentales de buena y mala calidad (Tabla 1). Sin embargo, el tamaño de puesta fue significativamente mayor en los nidos de mala que en los de buena calidad (prueba de Tukey, p = 0.004; Tabla 1).

De los 16 nidos experimentales, ocho por tratamiento, sólo en tres, que fueron de mala calidad, no tenemos evidencias de que las aves

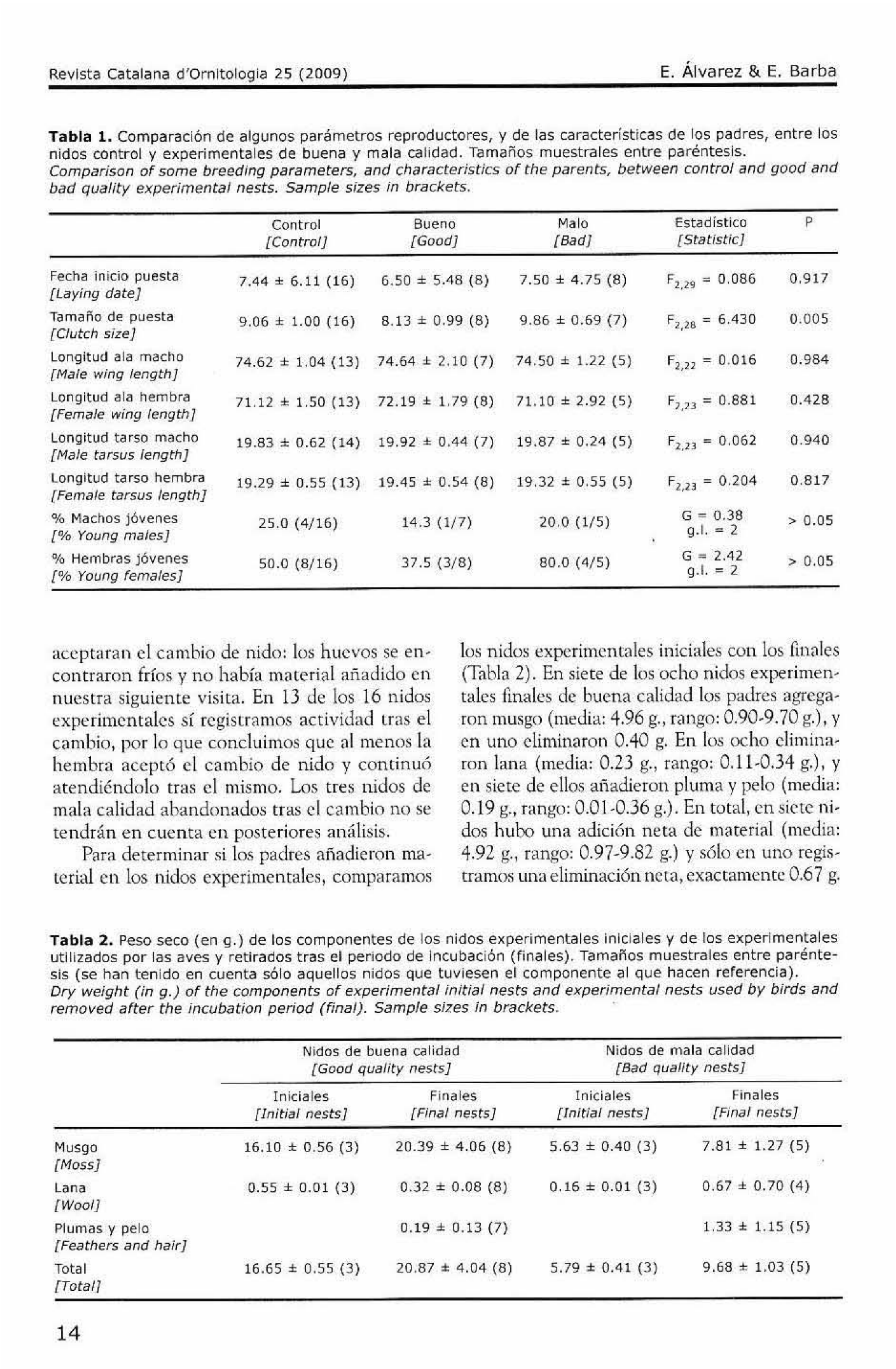

**Tabla 1.** Comparación de algunos parámetros reproductores, y de las características de los padres, entre los nidos control y experimentales de buena y mala calidad. Tamaños muestrales entre paréntesis.
*Comparison of some breeding parameters, and characteristics of the parents, between control and good and bad quality experimental nests. Sample sizes in brackets.*

| | Control *[Control]* | Bueno *[Good]* | Malo *[Bad]* | Estadístico *[Statistic]* | P |
|---|---|---|---|---|---|
| Fecha inicio puesta *[Laying date]* | 7.44 ± 6.11 (16) | 6.50 ± 5.48 (8) | 7.50 ± 4.75 (8) | $F_{2,29} = 0.086$ | 0.917 |
| Tamaño de puesta *[Clutch size]* | 9.06 ± 1.00 (16) | 8.13 ± 0.99 (8) | 9.86 ± 0.69 (7) | $F_{2,28} = 6.430$ | 0.005 |
| Longitud ala macho *[Male wing length]* | 74.62 ± 1.04 (13) | 74.64 ± 2.10 (7) | 74.50 ± 1.22 (5) | $F_{2,22} = 0.016$ | 0.984 |
| Longitud ala hembra *[Female wing length]* | 71.12 ± 1.50 (13) | 72.19 ± 1.79 (8) | 71.10 ± 2.92 (5) | $F_{2,23} = 0.881$ | 0.428 |
| Longitud tarso macho *[Male tarsus length]* | 19.83 ± 0.62 (14) | 19.92 ± 0.44 (7) | 19.87 ± 0.24 (5) | $F_{2,23} = 0.062$ | 0.940 |
| Longitud tarso hembra *[Female tarsus length]* | 19.29 ± 0.55 (13) | 19.45 ± 0.54 (8) | 19.32 ± 0.55 (5) | $F_{2,23} = 0.204$ | 0.817 |
| % Machos jóvenes *[% Young males]* | 25.0 (4/16) | 14.3 (1/7) | 20.0 (1/5) | G = 0.38<br>g.l. = 2 | > 0.05 |
| % Hembras jóvenes *[% Young females]* | 50.0 (8/16) | 37.5 (3/8) | 80.0 (4/5) | G = 2.42<br>g.l. = 2 | > 0.05 |

aceptaran el cambio de nido: los huevos se encontraron fríos y no había material añadido en nuestra siguiente visita. En 13 de los 16 nidos experimentales sí registramos actividad tras el cambio, por lo que concluimos que al menos la hembra aceptó el cambio de nido y continuó atendiéndolo tras el mismo. Los tres nidos de mala calidad abandonados tras el cambio no se tendrán en cuenta en posteriores análisis.

Para determinar si los padres añadieron material en los nidos experimentales, comparamos los nidos experimentales iniciales con los finales (Tabla 2). En siete de los ocho nidos experimentales finales de buena calidad los padres agregaron musgo (media: 4.96 g., rango: 0.90-9.70 g.), y en uno eliminaron 0.40 g. En los ocho eliminaron lana (media: 0.23 g., rango: 0.11-0.34 g.), y en siete de ellos añadieron pluma y pelo (media: 0.19 g., rango: 0.01-0.36 g.). En total, en siete nidos hubo una adición neta de material (media: 4.92 g., rango: 0.97-9.82 g.) y sólo en uno registramos una eliminación neta, exactamente 0.67 g.

**Tabla 2.** Peso seco (en g.) de los componentes de los nidos experimentales iniciales y de los experimentales utilizados por las aves y retirados tras el periodo de incubación (finales). Tamaños muestrales entre paréntesis (se han tenido en cuenta sólo aquellos nidos que tuviesen el componente al que hacen referencia).
*Dry weight (in g.) of the components of experimental initial nests and experimental nests used by birds and removed after the incubation period (final). Sample sizes in brackets.*

| | Nidos de buena calidad *[Good quality nests]* | | Nidos de mala calidad *[Bad quality nests]* | |
|---|---|---|---|---|
| | Iniciales *[Initial nests]* | Finales *[Final nests]* | Iniciales *[Initial nests]* | Finales *[Final nests]* |
| Musgo *[Moss]* | 16.10 ± 0.56 (3) | 20.39 ± 4.06 (8) | 5.63 ± 0.40 (3) | 7.81 ± 1.27 (5) |
| Lana *[Wool]* | 0.55 ± 0.01 (3) | 0.32 ± 0.08 (8) | 0.16 ± 0.01 (3) | 0.67 ± 0.70 (4) |
| Plumas y pelo *[Feathers and hair]* | | 0.19 ± 0.13 (7) | | 1.33 ± 1.15 (5) |
| Total *[Total]* | 16.65 ± 0.55 (3) | 20.87 ± 4.04 (8) | 5.79 ± 0.41 (3) | 9.68 ± 1.03 (5) |

Por otra parte los padres añadieron musgo en los cinco nidos experimentales finales de mala calidad (media: 2.18 g., rango: 0.67-3.47 g.). En cuatro añadieron lana (media: 0.51 g., rango: 0.01-1.53 g.) y en todos añadieron pluma y pelo (media: 1.33 g., rango: 0.13-2.93 g.). En total, en los cinco nidos registramos una adición neta de material (media: 3.89 g., rango: 2.57-5.40 g.). No se detectaron diferencias significativas en la cantidad de material añadido por los padres en los nidos experimentales finales de buena y mala calidad ($F_{1,11} = 0.031$, $p = 0.863$). Sin embargo se comprobó que en todos los nidos de buena calidad eliminaron lana y en la mayoría de los de mala calidad añadieron, por lo que la cantidad final de lana no difirió entre ambos tipos de nidos ($F_{1,11} = 0.851$, $p = 0.376$).

El porcentaje de nidos en los que al menos eclosionó un huevo fue del 94% (n = 16) en los nidos control, del 88% (n = 8) en los de buena calidad, y del 40% (n = 5) en los de mala calidad, siendo las diferencias significativas (G = 6.42, g.l. = 2, $p < 0.05$; Figura 1). No encontramos diferencias significativas entre los nidos control y los de buena calidad (G?= 0.19, g.l. = 1, $p > 0.05$; Fig. 1), por lo que los agrupamos para compararlos con los de mala calidad. El porcentaje de nidos que fracasaron durante la incubación (no eclosionó ningún huevo) fue significativamente mayor en los nidos de mala calidad que en los de buena calidad y los control juntos (G = 5.11, g.l. = 1, $p < 0.05$; Figura 1).

La duración del periodo de incubación (PI) no difirió significativamente entre los nidos control (PI = 12.93 ± 1.21 días, n = 14) y los de buena calidad (PI = 12.57 ± 0.98 días, n = 7; $F_{1,19} = 0.459$, p= 0.506). El éxito de eclosión fue mayor en los nidos experimentales de buena calidad que en los nidos control ($F_{1,20} = 4.788$, $p = 0.041$; Fig. 2). En ambos análisis, no pudimos incluir los nidos de mala calidad por falta de tamaño muestral (n = 2).

## Discusión

Existen numerosos trabajos que relacionan alguna propiedad del nido (volumen, tamaño, peso total o sólo del musgo, capacidad de aislamiento térmico, estructura) con alguna estima del éxito reproductor (tamaño de puesta, éxito de eclosión, desempeño reproductor; e.g. Møller 1982, Lombardo *et al.* 1995, Alabrudzińska *et al.* 2003, Quader 2006, Álvarez & Barba 2008). En ninguno de estos trabajos se desvincula a los padres de los nidos que atienden, por lo que es posible que la mejor calidad de los padres que presumiblemente atienden nidos de buena calidad influya positivamente en el éxito reproductor, no quedando clara la contribución del nido

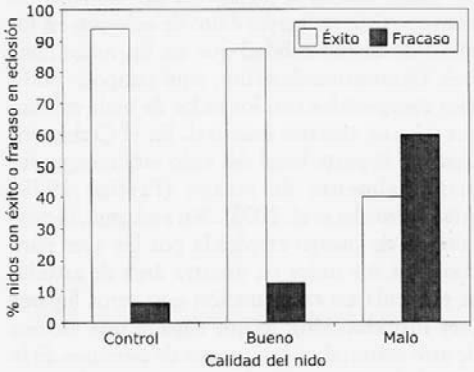

**Figura 1.** Porcentaje de nidos con éxito (en los que al menos eclosionó un huevo) y con fracaso (en los que no eclosionó ningún huevo) en función de la calidad del nido.
*Percentage of successful nests (at least one egg hatched) and unsuccessful ones (no eggs hatched) in relation to nest quality.*

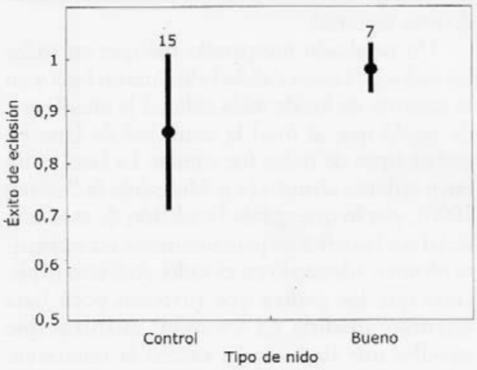

**Figura 2.** Éxito de eclosión en función de la calidad del nido. Se presenta la media ± d.t.; y el tamaño muestral sobre las barras.
*Hatching success in relation to nest quality. Mean ± s.d.; sample size above bars.*

*per se*. En nuestro estudio cambiamos aleatoriamente los nidos al principio de la incubación, desvinculando así la posible contribución de la calidad de los padres de la calidad de los nidos que atienden, por lo que los efectos encontrados podemos atribuirlos a la calidad del nido.

Pese a que la asignación de los nidos a uno u otro grupo fue aleatoria, el tamaño de puesta fue mayor en los nidos donde posteriormente realizamos el cambio por uno de mala que por uno de buena calidad. Este hecho no repercute en los resultados presentados, ya que para los nidos de mala calidad no pudimos analizar ningún factor relacionado con el número de huevos y pollos. Las características de los padres, y la fecha de inicio de puesta, no difirieron entre los nidos controles y los dos grupos experimentales.

El añadir material durante la incubación no es algo habitual en el Carbonero Común, o al menos no hemos encontrado referencia de ello (Perrins 1979, Cramp & Perrins 1993, Gosler 1993), así que es algo excepcional que lo hagan al cambiarles el nido. Esto puede ser debido a que necesiten material específico (pelo, plumas), que no les proporcionamos, puesto que se ha demostrado que este material es importante para el desarrollo de la incubación (Lombardo *et al.* 1995); prácticamente en todos los casos añadieron estos materiales en mayor o menor cantidad. Por otra parte, también casi todos añadieron musgo, probablemente debido a que las hembras, al encontrarse con un nido extraño, intentaran adecuarlo a sus necesidades añadiendo este material.

Un resultado inesperado fue que en todos los nidos de buena calidad eliminaron lana y en la mayoría de los de mala calidad la añadieron, de modo que al final la cantidad de lana en ambos tipos de nidos fue similar. La lana es un buen aislante térmico (e.g. Margalida & Bertran 2000), por lo que quizás la adición de este material sea beneficiosa para mantener un ambiente térmico adecuado en el nido. Así, esto explicaría que los padres que tuvieran poca lana intentan añadirla en los nidos mientras que aquellos que tuvieran en exceso la reiteraran, ya que en un ambiente mediterráneo esto podría comportar un sobrecalentamiento de la puesta. Por ejemplo, se han demostrado los efectos negativos de las altas temperaturas sobre el crecimiento de los pollos de algunas especies (Geraert *et al.* 1996). No obstante, las cantidades de lana observadas son en todo caso pequeñas, por lo que su significado biológico debería explorarse con más profundidad.

Las hembras añadieron la misma cantidad total de material en los nidos de buena y en los de mala calidad, por lo que se siguieron manteniendo nidos de ambas calidades durante todo el periodo nidícola estudiado.

La calidad del nido puede afectar a la duración del periodo de incubación, de manera que los nidos de peor calidad normalmente tienen periodos de incubación más largos (Lombardo *et al.* 1995). Al comparar el periodo de incubación entre los nidos control y de buena calidad no detectamos diferencias significativas. No pudimos compararlos con los de mala calidad por falta de tamaño muestral.

Las características del nido pueden influir para que la puesta finalmente eclosione con mayor o menor éxito (e.g. Lombardo *et al.* 1995). En nuestro estudio, al haber manipulado la calidad de los nidos, podemos concluir que el menor porcentaje de nidos de mala calidad en los que al menos eclosiona un huevo se debe a las características del nido. De los tres nidos que fracasaron durante el periodo de incubación, en dos de ellos la causa fue el abandono de la puesta por parte de la hembra. Es posible que, aunque inicialmente la hembra acepte un nido de mala calidad, posteriormente lo abandone si estima que las probabilidades de éxito van a ser reducidas.

Por otra parte, también se detectó que, en los nidos donde al menos ha eclosionado un huevo, existe un mayor éxito de eclosión en los nidos de buena calidad que en los nidos control. Desafortunadamente, aquí tampoco pudimos compararlos con los nidos de mala calidad por falta de tamaño muestral. En el Carbonero Común, la parte basal del nido está compuesta principalmente de musgo (Perrins 1979, Alabrudzińska *et al.* 2003). Sin embargo, la proporción de musgo empleada por las aves para construir sus nidos en nuestra área de estudio es reducida en comparación con otros lugares (ver Introducción), lo que sugiere una escasez de este material en los campos de naranjos. Si la cantidad y proporción de musgo mejora las condiciones de incubación y el éxito de eclosión (e.g. Alabrudzińska *et al.* 2003), es posible que la construcción de nidos de tamaño relativamente grande, en los que la composición era básicamente musgo, haya mejorado las condiciones

medias de incubación en las parejas que criaron en nidos experimentales de buena calidad con respecto a la media de las parejas control.

En conclusión, nuestro trabajo sugiere que la calidad del nido *per se* puede contribuir de manera importante al éxito reproductor. Por un lado, las hembras tienen más tendencia a abandonar nidos de mala calidad. Por otro, la utilización de materiales adecuados es importante – así interpretamos la manipulación de la cantidad de lana en el nido, y el mejor éxito de eclosión en los nidos experimentales de buena calidad, construidos básicamente con musgo, en relación con los nidos control, construidos con los materiales disponibles de forma natural en nuestra área de estudio.

## Agradecimientos

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## Resum

### Com afecta la qualitat del niu *per se* al procés d'incubació? Una aproximació experimental

La composició i característiques del niu afecten les seves propietats tèrmiques, i la temperatura del niu afecta al procés d'incubació. Aleshores, esperem una relació entre les característiques del niu i el procés d'incubació. Contrastem aquesta hipòtesi en una població de Mallerenga Carbonera *Parus major*, canviant aleatoriament els nius originals per nius artificials de "bona" i "mala" qualitat al començament de la incubació. Els ocells van afegir material en quasi tots els nius experimentals, però no es van trobar diferències en la quantitat de material afegit entre els nius bons i dolents. La proporció de nius on almenys un pollet va volar no va diferir entre els nius control i els bons. El període d'incubació no va diferir entre els nius control i els bons. L'èxit d'eclosió va ser major en els nius bons que en els control. Els nostres resultats suggereixen que la qualitat del niu *per se* afecta al procés d'incubació, independentment de la qualitat dels pares. Pel que sabem, aquest és el primer estudi que mostra aquesta relació de manera experimental.

## Resumen

### ¿Cómo afecta la calidad del nido *per se* al proceso de incubación? Una aproximación experimental

La composición y características del nido afectan sus propiedades térmicas, y la temperatura del nido afecta al proceso de incubación. Por tanto, esperamos una relación entre las características del nido y el proceso de incubación. Contrastamos esta hipótesis en una población de Carbonero Común *Parus major*, cambiando aleatoriamente los nidos originales por nidos artificiales de "buena" y "mala" calidad al comienzo de la incubación. Las aves añadieron material en casi todos los nidos experimentales, pero no hubo diferencias en la cantidad de material añadido entre los nidos buenos y malos. La proporción de nidos donde voló al menos un pollo no difirió entre los nidos control y buenos, siendo significativamente más baja en los nidos malos. El periodo de incubación no difirió entre los nidos control y los buenos. El éxito de eclosión fue mayor en los nidos buenos que en los control. Nuestros resultados sugieren que la calidad del nido *per se* afecta al proceso de incubación, independientemente de la calidad de los padres. Por lo que conocemos, este es el primer trabajo que muestra esta relación de forma experimental.

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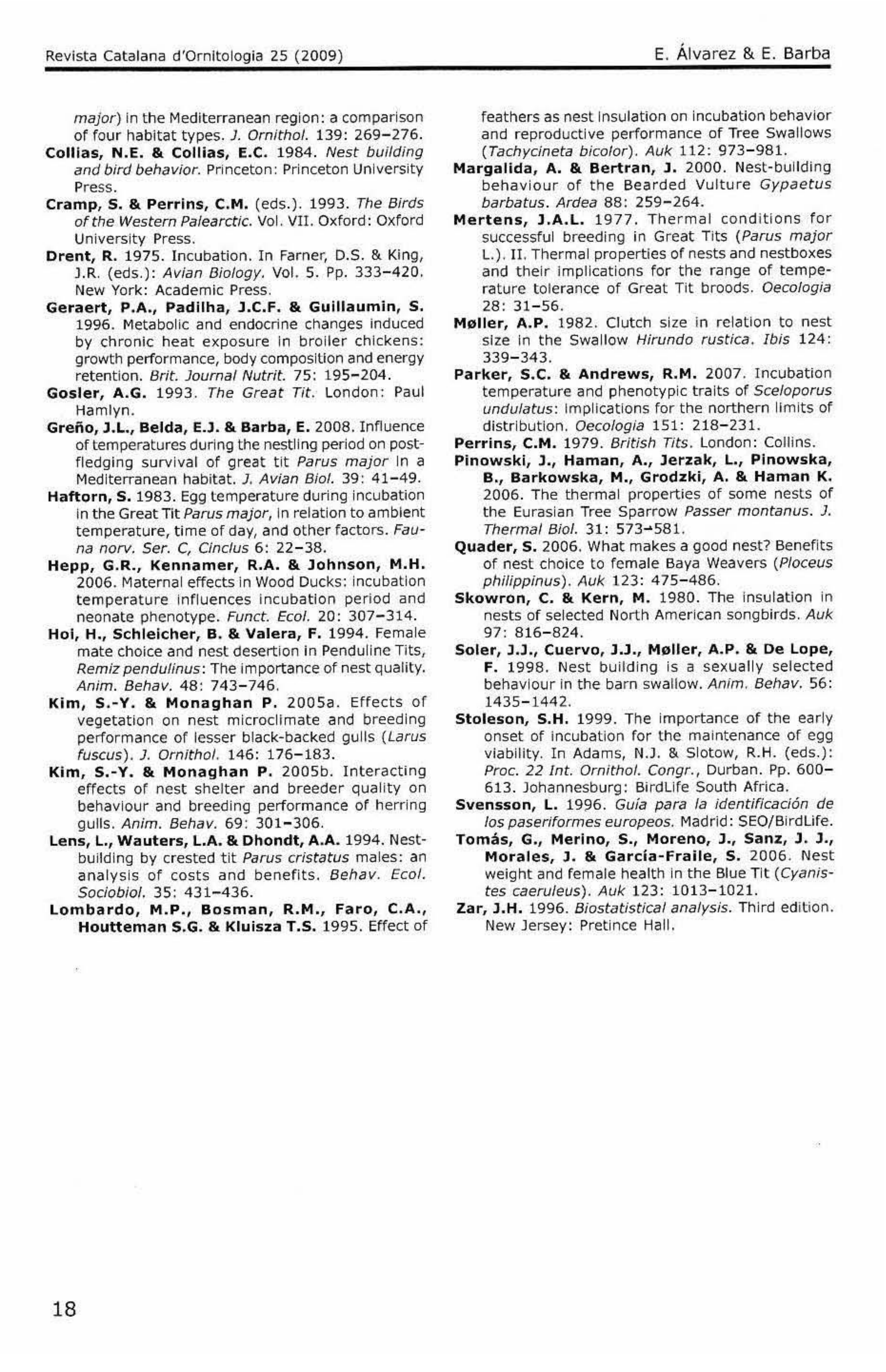

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# SECCIÓN II. PATRONES DE INCUBACIÓN Y ECLOSIÓN

## ARTÍCULO 5

# Incubation and hatching periods in a Mediterranean Great Tit population

ELENA ÁLVAREZ Y EMILIO BARBA

ENVIADO PARA PUBLICACIÓN

"El mejor remedio contra la
tristeza es aprender algo."

# Incubation and hatching periods in a Mediterranean great tit population

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**Short title**

Incubation and hatching periods in great tits

*Key words:* Egg covering, Full incubation, Hatching asynchrony, Laying sequence, Long-term studies

## ABSTRACT

When starting incubation relative to clutch completion, and how much time and energy investing in this activity, are important decisions that the incubating birds should make, since they would affect their breeding performance. The main consequences are related to the degree of hatching synchrony of the eggs, which affects the growth rates and survival of individual nestlings, and to the total length of the nesting period, which affects the exposure of the complete brood to predators or inclement weather. We used data from a long-term (1988-2010) study of a great tit *Parus major* population in Eastern Spain to investigate (1) when incubation started relative to clutch completion; (2) the length of the incubation period; (3) the degree of hatching asynchrony; and (4) which proximate factors affect all these traits. On average, incubation started with the laying of the last egg, though there were between year differences. The incubation period (from the date of onset of full incubation to the day previous to the hatching of the first egg) lasted 13.3 days. Mean incubation period was shorter in years where mean clutch size (as a proxy of food availability) was larger. The duration of the incubation period was shorter as incubation started later in the laying sequence, suggesting that incubation is less effective during the laying period. Contrasting with some previous studies, incubation was also shorter if clutch size was larger – perhaps larger clutches could keep warm easily when females leave the nest for foraging. The incubation period was also shorter if eggs were uncovered before the onset of full incubation, suggesting that females are applying some heat during these previous days. Mean hatching period was 1.7 days, though the means for individual years ranged between 1.0 and 2.9. As expected, hatching asynchrony was higher as the incubation period decreased, as clutch size increased, and as the onset of incubation was earlier relative to clutch completion.

## INTRODUCTION

The duration of the incubation period in birds not only varies between species (e.g. Martin et al. 2007; Deeming 2008; Ricklefs and Brawn 2013), but also between populations of the same species (e.g. Murphy 1983; Järvinen 1990; Zimmerling and Ankney 2005; Ardia et al. 2006; Ippi et al. 2012), and between individuals within populations. This within population variability is most probably caused by environmental factors, as the duration of the incubation period has a low heritability (Husby et al. 2012). Thus, egg size (e.g. Parsons 1972), clutch size (e.g. Moreno and Carlson 1989; Smith 1989; Hepp et al. 2005), environmental conditions (Kluijver 1950; Kendeigh 1952; Skutch 1962; Murphy 1978; Lowther 1979; Moreno 1989; Järvinen 1990; Ardia et al. 2006), or parental behaviour during incubation (Ricklefs and Smeraski 1983; Deeming 2002; Hébert 2002) have been shown to affect the duration of the incubation period within avian populations.

A problem with the study of the duration of the incubation period is that the way it is measured in field conditions differ. Strictly speaking, the incubation period is the time needed for the development of an embryo assuming a regular and constant attention by the parents (Drent 1975). In practice, it is usually measured as the time between the laying of the last egg and its hatching. However, in many species, incubation typically starts before the laying of the last egg (e.g. Clark and Wilson 1981; Haftorn 1981a; Hébert 2002; Lord et al. 2011), and even some time after clutch completion (Monrós et al. 1998; Stenning 2008; Rowe and Weatherhead 2009). There are further complications since, prior to full incubation onset, eggs might receive some incubation ("partial incubation"; Wang and Beissinger 2011), and the actual onset of full incubation might show a diversity of patterns (Wang and Beissinger 2009).

Both the onset of incubation, and the degree of attentiveness along this period, has important consequences for breeding success. Starting incubation before finishing the clutch implies that eggs will hatch asynchronously (i.e. hatching occurs over a period of 24 hours or more), although other factors affecting the duration of development of each particular embryo, as egg size and composition or offspring sex (Eising et al. 2001; Blanco et al. 2003), might also contribute to the duration of the hatching period. As hatching asynchrony increases, survival probabilities of later hatchlings decrease, as a consequence of important size differences between siblings (Lack 1947; Magrath 1990; Forbes et al. 1997; Viñuela 2000; Tilgar and Mänd 2006; but see Podlas and Richner 2013a). On the other hand, delaying the onset of the incubation implies increasing the time where nest is vulnerable to predators (Stoleson and Beissinger 1995), and also delaying hatching dates, thus decreasing survival probabilities (Perrins 1965; Barba et al. 1995). Hatching asynchrony has been the object of different interpretations, from assigning it an adaptive value to considering it a forced consequence of other adaptations or an effect of energetic limitations during the laying period (Magrath 1990; Stoleson and Beissinger 1995; Stenning 1996; Sockman et al. 2006; Podlas and Richner 2013a).

Several studies have shown relationships between different breeding and environmental parameters and the duration of the hatching period. Most frequent trends are an increase of hatching asynchrony with clutch size and along the season (e.g. Slagsvold 1986; Skagen 1987; Briskie and Sealy 1989; Moreno and Carlson 1989; Ardia et al. 2006; Stenning 2008). Also, hatching asynchrony is related to female quality, habitat type and ambient temperature during the laying period (Shaw 1985; Slagsvold 1986; Slagsvold and Lifjeld 1989; Hanssen et al. 2002), as well as to egg or nest temperature (Ardia et al. 2006, 2009).

The great tit *Parus major* is a widespread species in Europe. Its habit to occupy nestboxes has encouraged many research groups to use it as a model species for a wide range of ecological and evolutionary studies, some of them carried out during decades (e.g. Lambrechts et al. 2010). One of these is that performed in an extensive orange plantation in Sagunto, eastern Spain, where the breeding performance of great tits are followed since 1986 (e.g. Encabo et al. 2001; Tomás et al. 2012). In most of these years, we have data on the incubation and hatching periods of a sample of nests. Our objective here is describing within population variation of the incubation and hatching periods of this southern European great tit population, and checking the effects of some factors on these durations. To our knowledge, this is the first study on these aspects for a southern European great tit population, since previous information is restricted to very general figures or unpublished data (Barba 1991; Atiénzar et al. 2012).

## METHODS

### Study area and data selection

The study was performed on a great tit population breeding in nestboxes in an extensive orange plantation in Sagunto, Eastern Spain (39º42’N, 0º15’W, 30 m a.s.l.). Basic breeding parameters are recorded every year since 1986 (Encabo et al. 2001; Tomás et al. 2012) by periodic visits to the nests. Most pairs only attempt one clutch per year, but some lay a second one if the first one fails, and other could attempt a second one after a successful first clutch (Barba 1991).

For the present study, we include only first clutches laid between 1988 and 2010. As not all the parents were individually identified (none in early years), we considered first clutches those where the first egg was laid within 30 days since the laying of the first egg of that year. Some nests have been used for experiments in different years; we excluded data which might have been affected by any experimental procedure.

Also, we only included nests for which the laying date of the first egg was known or could be estimated assuming the laying of one egg per day. All clutches included were found during the laying period and eggs were covered by nest material when first found. Laying gaps (days during the laying period where no egg was laid) occurred occasionally under unfavourable environmental conditions (e.g. Monrós et al. 1998), but they are usually scarce, and they should have occurred before our first detection of eggs to go undetected, so we will consider here that the bias they could introduce is negligible.

Finally, we only included breeding attempts where clutch size was known. Laying was assumed to have finished, and then the clutch being complete, when no more eggs where laid during 2 consecutive days and female started full incubation (see below).

Considering all these restrictions, we excluded from the study data from years 1989, 1997, 1998, 2002-05, and included therefore data for a total of 16 years. Subsamples for analyses concerning the incubation period include 365 nests from 14 years, and for those concerning the hatching period 436 nests from 16 years. The date of onset of incubation relative to clutch completion (see below) could be calculated on 609 nests over the 16 years.
The length (L) and width (W) of the eggs of a sample of nests each year were measured with a caliper (± 0.01 mm) when the clutch was complete. The volume (V) of each egg was calculated as $V = (0.4673*L*W^2) + 0.042$ (Ojanen et al. 1978; Encabo et al. 2001). Mean egg volume and clutch volume (sum of the volumes of all the eggs in a clutch) were computed for each clutch.

Mean laying date of first clutches in Sagunto over the 23 years from 1988 to 2010 has varied between 5 and 24 April (mean 15 April; own unpubl. data). We therefore considered the mean temperature in April as a good approximation of the temperatures experienced by incubating birds. Data of ambient temperatures (maximum and minimum daily) of a nearby (c.a. 4 km) meteorological station were obtained from the Spanish Meteorological Agency. Mean temperatures were computed as the average of maximum and minimum.

Another factor which could affect the duration of the incubation period and, indirectly, that of the hatching period, is food availability. We used mean clutch size of first clutches as a proxy of food availability during this period, since mean clutch size has been shown to be related to food availability (Perrins 1991). Mean clutch size during this 23-year period varied between 7.2 and 9.0 (mean 8.0).

### The incubation period

In great tits, only females incubate, while males feed females during this period (Kluijver 1950; Perrins 1979). Females typically lay one egg per day until clutch completion. During the laying period, females spend the night in the nest, lay the egg early in the morning, usually cover the eggs with nest material, and leave the nest until late in the evening, when they return to overnight (Haftorn 1981a; Podlas and Richner 2013b). During this period, eggs can be raised above the physiological zero temperature for embryo development (25-27 ºC; Haftorn 1988; Pendlebury and Bryant 2005), though its effects on embryo development and its effects on hatching asynchrony are not clear (Wang and Beissinger 2009; Lord et al. 2011; Podlas and Richner 2013b). Full or effective incubation could be identified in the field because during this period eggs have to be kept at relatively high temperatures (Drent 1975; Haftorn 1983), so they are warm. We therefore considered that full incubation was started when eggs were seen uncovered and warm, or the female was seen incubating (e.g. Gibb 1950; Howe 1976; García-Navas and Sanz 2011). Unless otherwise stated, we will only refer to full incubation hereafter.

We defined the incubation period as the number of days elapsing from the day when full incubation was observed and the day previous to the hatching of the first egg. We only included nests where incubation period was determined precisely by daily visits to the nests from at least one day before incubation started and from at least one day before hatching started. For each nest we noted the day, in relation to the laying sequence, where full incubation started, being “0” if incubation started on the day of laying of the last egg, “-1” if it started with the penultimate egg, “+1” if it started the day after clutch completion, and so on (e.g. Loos and Rohwer 2004).

Some previous studies on Great Tits (Gibb 1950; Kluijver 1950) have calculated the incubation period in different ways. Thus, in order to compare our results with those of other populations we have estimated both the length of the period between the laying of the last egg and the hatching of the last egg, both included, and the length of the period between the observed onset of full incubation and the hatching of the last egg, both included.

Though great tits typically cover the eggs with nest material until full incubation started, we noticed that in some occasions eggs were uncovered and cold, probably meaning that full incubation was not yet started. Haftorn and Slagsvold (1995) found that when laying was about to finish, great tits cover the eggs more poorly. Here we classified nests into two groups, those in which eggs were uncovered the day of onset of full incubation, and those where eggs were uncovered, but cold, before starting full incubation. The objective was checking whether this behaviour had some effects on the duration of the incubation period, as would occur if uncovered eggs were partly incubated, even if we could not detect it.

### The hatching period

In a sample of nests each year, nests were visited daily from incubation day 12 until all the eggs had hatched, or remaining eggs did not hatch in two consecutive days (none of them hatched afterwards). We used these nests, excluding a few where hatching had started in our first visit, to estimate the hatching period, defined as the time in days from hatching of the first egg to hatching of the last successful egg (i.e. excluding those that did not hatch), both included (e.g Potti 1998). Great tits are unable of taking unhatched eggs out of the nest, but they do take out nestlings which die in their first days of life. As we made daily visits, we assumed that all the disappeared eggs had hatched, and missing nestlings had died and taken out of the nest by the parents.

### Statistical analyses

We first checked whether the duration of the incubation and hatching periods varied between years using ANOVAs. As they did (see results), year was introduced as a fixed factor in subsequent ANOVAs used to check the effect of other variables on the duration of both periods. Continuous independent variables were introduced as covariates in the models, and discrete variables as fixed factors. Interaction terms between year and the other variable were also included into the models. Models with more than two independent variables were used when appropriate, including all the first order interactions. We also used regression analyses to relate the yearly mean duration of the periods to yearly mean temperatures or clutch sizes.

As many of the predictor variables were correlated, we included all of them in a multiple regression model, with stepwise selection of variables, to find which of them contributed significantly to explain variability in the duration of the incubation and hatching periods. We pooled data from all years in these analyses, since the interaction terms between year and any of the other independent variables were significant (see results).

Means ± SD are presented when appropriate. Statistical analyses were carried out using SPSS v. 19.0.

## RESULTS

### Incubation period

In the studied population, females started incubation on average the date of laying of the last egg (mean = 0.04 ± 1.4; $n$ = 609). From these 609 clutches, 37% started incubation before the laying of the last egg, 35% the date of laying of the last egg, and 28% after the laying of the last egg. Incubation started from 3 days before to 6 days after cutch completion.

There were between year differences ($F_{14,594} = 5.09$, $P < 0.001$). *Post-hoc* analyses revealed three different groups, with years where incubation started relatively early (-0.67 to 0.37), others intermediate (-0.42 to 0.58), and others relatively late (-0.16 to 1.0). Yearly means were positive in 8 out of the 15 years, negative in 6 years, and 0.00 in 1988. The yearly mean of date of onset of incubation relative to clutch completion was not related with mean April temperature ($F_{1,13} = 2.61$, $P = 0.13$) or mean clutch size ($F_{1,13} = 1.51$, $P = 0.24$).

The incubation period, as defined here, lasted 13.3 ± 1.1 days ($n$ = 365) in the studied great tit population. The duration of the incubation period calculated between the date of laying of the last egg and the date of hatching of the last chick was 15.6 ± 1.4 days ($n$ = 176). If we consider the length between the observed start of full incubation and the date of hatching of the last egg, it was 15.7 ± 1.1 days ($n$ = 176).

Overall, the duration of the incubation period varied between years ($F_{13,351} = 3.5$, $P < 0.001$; Table 1). Removing from the analysis two years with less than 10 nests, differences persisted ($F_{11,344} = 3.9$, $P < 0.001$). 1992 and 2006 had the shortest incubation periods (12.6 days), while the longest was in 1996 (13.8 days), having the rest of the years intermediate values not differing from either group (Table 1). The two years excluded had incubation periods within the range of other years (Table 1), but we will remove them from further analyses to avoid low sample sizes.

**Table 1.** Incubation and hatching periods of great tits in Sagunto. Mean ± SD, and sample size in brackets, are offered. No data for the incubation period were available for 1990 and 2009, and neither for incubation or hatching periods in the missing years of the series. Mean values followed by the same letter were not significantly different (Tukey tests); years with simple sizes below 10 were not included into the analyses.

| Year | Incubation period | Hatching period |
|---|---|---|
| 1988 | 13.3 ± 1.0 (18) (ABC) | 2.4 ± 0.8 (23) (DE) |
| 1990 | --- | 1.0 ± 0.0 (10) (A) |
| 1991 | 12.7 ± 0.8 (6) | 1.3 ± 0.6 (16) (AB) |
| 1992 | 12.6 ± 0.9 (13) (A) | 2.2 ± 1.0 (12) (CDE) |
| 1993 | 13.4 ± 1.0 (27) (ABC) | 2.2 ± 0.7 (26) (CDE) |
| 1994 | 13.7 ± 1.1 (26) (BC) | 1.6 ± 0.7 (30) (ABC) |
| 1995 | 13.3 ± 1.1 (28) (ABC) | 1.6 ± 1.0 (37) (ABC) |
| 1996 | 13.8 ± 1.4 (54) (C) | 1.8 ± 0.7 (41) (BCD) |
| 1999 | 13.4 ± 1.0 (41) (ABC) | 1.1 ± 0.3 (40) (AB) |
| 2000 | 13.2 ± 1.1 (41) (ABC) | 1.1 ± 0.6 (46) (AB) |
| 2001 | 13.4 ± 0.8 (31) ABC) | 1.0 ± 0.2 (37) (A) |
| 2006 | 12.6 ± 0.7 (19) (A) | 2.4 ± 0.9 (26) (DE) |
| 2007 | 13.0 ± 0.9 (15) (ABC) | 1.5 ± 0.8 (29) (ABC) |
| 2008 | 13.3 ± 0.6 (3) | 1.9 ± 0.4 (7) |
| 2009 | --- | 1.1 ± 0.3 (10) (AB) |
| 2010 | 12.8 ± 1.0 (43) (AB) | 2.9 ± 0.9 (46) (E) |

Yearly means were not related to mean ambient temperatures in April ($F_{1,10} = 1.3$, $P = 0.29$), but mean incubation period decreased as mean clutch size increased ($r^2 = 0.366$, $F_{1,10} = 5.8$, $P = 0.037$; Figure 1).

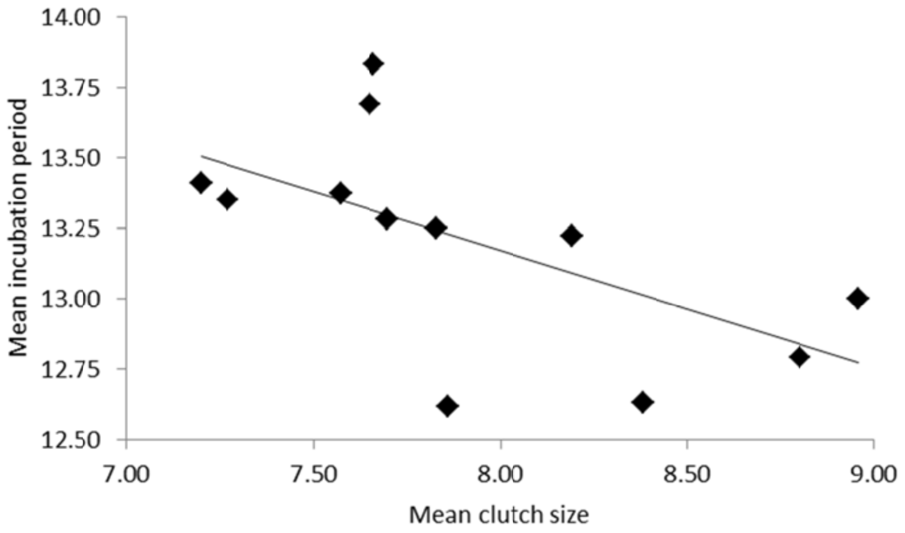

**Figure 1.** Relationship between mean incubation period and mean clutch size.

Including year as a fixed factor in the analyses, the incubation period decreased as the day of onset of incubation was delayed (year: $F_{11,332} = 1.8$, $P = 0.046$; day of start of incubation: $F_{1,332} = 4.3$, $P = 0.04$; interaction: $F_{11,332} = 1.2$, $P = 0.31$), and as incubation started later relative to clutch completion (year: $F_{11,332} = 4.15$, $P < 0.001$; onset of incubation relative to clutch completion: $F_{1,332} = 22.15$, $P < 0.001$; interaction: $F_{11,332} = 0.69$, $P = 0.75$ ; Figure 2). Laying date ($F_{1,332} = 0.7$, $P = 0.42$), egg volume ($F_{1,309} = 0.04$, $P = 0.85$), clutch size ($F_{1,332} = 0.3$, $P = 0.57$) and clutch volume ($F_{1,309} = 0.8$, $P = 0.38$) did not affect the duration of the incubation period. However, considering all these variables into a multiple regression model ($F_{2,330} = 11.70$, $P < 0.001$), those finally included were the date of start of incubation in relation to the laying sequence ($\beta = -0.20$) and clutch size ($\beta = -0.19$). Therefore, when the date of onset of incubation relative to clutch completion was taken into account, clutch size contributed more to explain the variation of the incubation period than the date of onset of incubation. Thus, the incubation period was shorter as incubation started later in the laying sequence and as clutch size increased.

In nests where the eggs were uncovered before full incubation started, the incubation period was shorter (13.0 ± 1.1 days, $n = 76$) than in those where the eggs were uncovered the day of onset of incubation (13.4 ± 1.1 days, $n = 280$; year: $F_{11,332} = 3.0$, $P = 0.001$; eggs uncovered: $F_{1,332} = 7.2$, $P = 0.008$; interaction: $F_{11,332} = 1.0$, $P = 0.50$).

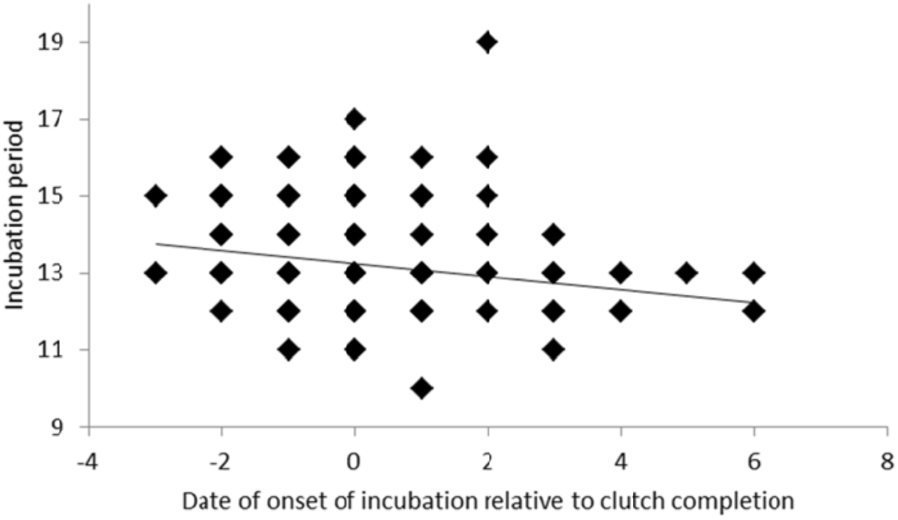

**Figure 2.** Relationship between incubation period and the date of the onset of incubation relative to clutch completion. "0" is the date of laying of the last egg, negative values mean that incubation started before clutch completion, and positive values that incubation started after clutch completion. Data for all years pooled ($n = 356$).

### Hatching period

Mean hatching period considering all data was 1.7 ± 0.9 days ($n = 436$). There were differences between years ($F_{15,420} = 21.1$, $P < 0.001$; Table 1). There was a single year with less than 10 samples (2008), and differences remained after removing it ($F_{14,414} = 22.3$, $P < 0.001$). Tukey tests grouped the years in 5 clusters, from years where hatching periods were relatively short (1.0-1.6 days) to those which were relatively long (2.2-2.9 days; Table 1). The year excluded had incubation periods within the range of other years (Table 1), but we will remove them from further analyses to avoid low sample sizes. Yearly means were not related to mean ambient temperatures in April ($F_{1,13} = 0.6$, $P = 0.44$) nor to mean clutch size ($F_{1,13} = 0.2$, $P = 0.64$), or to the mean duration of the incubation period ($F_{1,10} = 3.26$, $P = 0.10$).

Including year as a fixed factor in the analyses, the duration of the hatching period increased as incubation period decreased (year: $F_{11,149} = 0.9$, $P = 0.54$; incubation period: $F_{1,149} = 11.4$, $P = 0.001$; interaction: $F_{11,149} = 0.8$, $P = 0.66$; Figure 3), as clutch size increased (year: $F_{14,399} = 0.9$, $P = 0.59$; clutch size: $F_{1,399} = 6.0$, $P = 0.014$; interaction: $F_{14,399} = 1.17$, $P = 0.29$; Figure 4) and as the start of incubation was earlier relative to clutch completion (year $F_{14,364} = 15.5$, $P < 0.001$; incubation onset relative to clutch completion: $F_{1,364} = 7.6$, $P = 0.006$; interaction: $F_{14,364} = 0.6$, $P = 0.88$; Figure 5). The duration of the hatching period did not vary with laying date ($F_{1,399} = 1.4$, $P = 0.24$), date of start of incubation ($F_{1,367} = 1.1$, $P = 0.29$), mean egg volume ($F_{1,353} = 0.4$, $P = 0.53$), or clutch volume ($F_{1,353} = 2.3$, $P = 0.13$). When all the variables were entered into a multiple regression model ($F_{3,164} = 38.6$, $P < 0.001$), all the three variables which were independently related to the hatching period where included: the duration of the incubation period ($\beta = -0.48$), clutch size ($\beta = 0.14$) and the date of onset of incubation relative to clutch completion ($\beta = -0.39$).

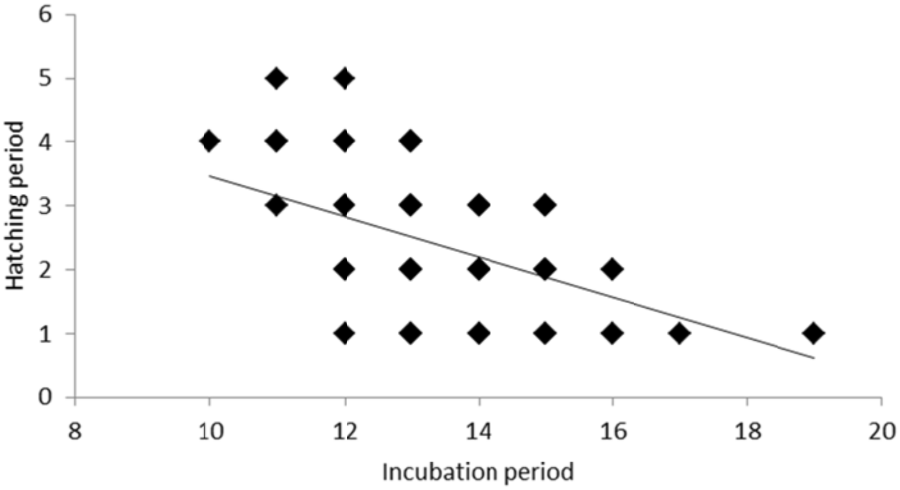

**Figure 3.** Relationship between hatching period and the incubation period. Data for all years pooled ($n = 175$).

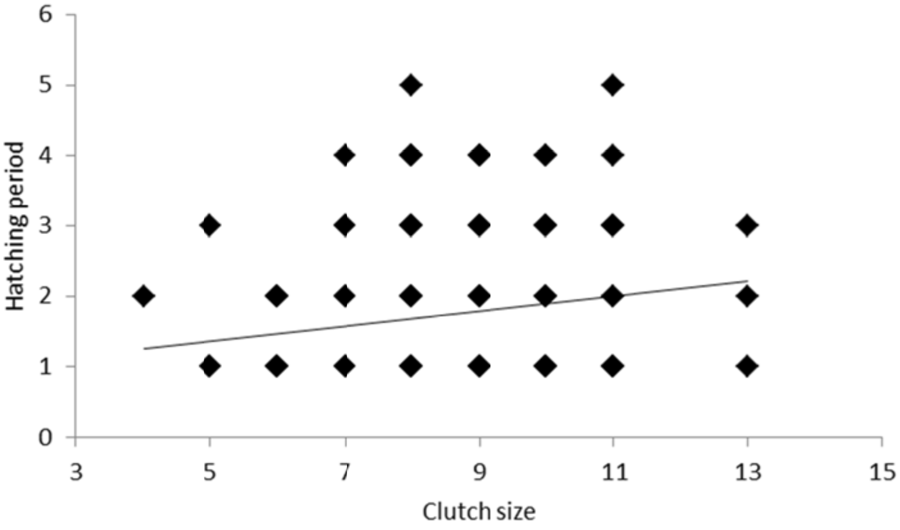

**Figure 4.** Relationship between hatching period and clutch size. Data for all years pooled ($n = 429$).

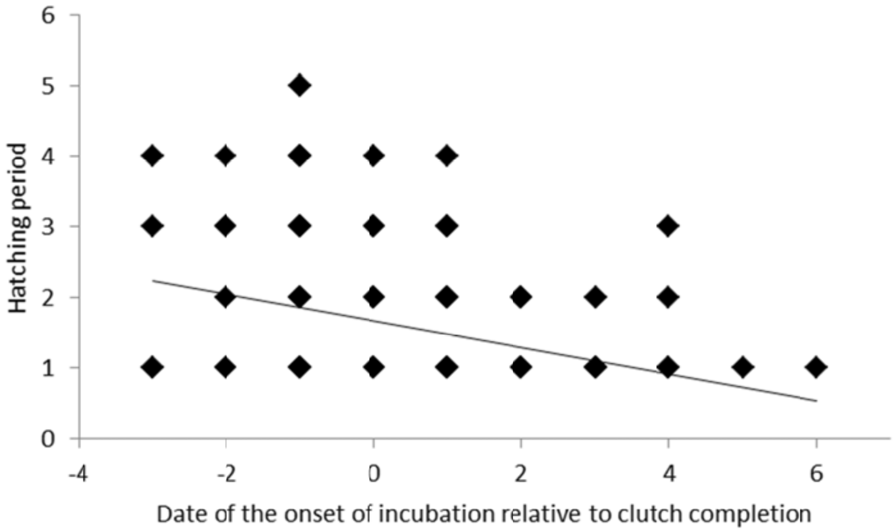

**Figure 5.** Relationship between hatching period and the date of the onset of incubation relative to clutch completion. Notation as in Figure 2. Data for all years pooled (*n* = 394).

The hatching period did not differ between nests in which eggs were uncovered before full incubation started (1.6 ± 0.9 days, *n* = 98) and those which were uncovered the day of onset of incubation (1.7 ± 0.9 days, *n* = 331; $F_{1,399} = 0.5$, $P = 0.49$).

## DISCUSSION

### Onset of the incubation

Rowe and Weatherhead (2009) described a “third incubation tactic” in the American robin *Turdus migratorius*, namely that 19.5% of females started incubation after (> 24 h) clutch completion. They did not find previous studies reporting this tactic in altricial birds. Actually, at least three studies (Monrós et al. 1998; Ardia et al. 2006; Stenning 2008) had reported this behaviour by these dates. Monrós et al. (1998) found that 40% (*n* = 43) of the great tit nests started incubation after clutch completion, and showed that, by a combination of delays in nest construction, laying gaps, and delays in the onset of incubation, great tits managed to hatch their eggs within the appropriate time window to improve fledgling survival. We confirm here, with a much larger sample size, that starting incubation after clutch completion is fairly common in this great tit population, and agree with Rowe and Weatherhead (2009) that this tactic should be common and it is probably under-reported, or reported in a way which is easy to overlook (e.g. García-Navas and Sanz 2011).

Incubation requires the transfer of heat from the incubating bird to the eggs, and in most species this is facilitated by the development of "brood patches" (featherless and highly vascularized areas) in the breast and belly of the incubating bird (Jones 1971; Drent 1975). Prolactin is one of the hormones triggering the formation of the brood patch and keeping incubation behaviour (Jones 1971; Lea and Klandorf 2002; Angelier and Chastel 2009). But this hormone also causes a regression of the gonads (Dawson et al. 2001), probably creating conflicts between producing new eggs and incubation behaviour (Haftorn 1981b; Sockman et al. 2000, 2006). It has been shown in many species, including great tits, that brood patch is not completely developed until some days after clutch completion (Bailey 1952; Jones 1971; Haftorn 1981b). It is therefore possible that, if incubation starts during the laying period, it would be less effective than starting once the physical structures and ethological repertory is fully developed, probably after clutch completion (see Runde and Barrett 1981; St. Clair 1998). In contrast Ardia et al. (2006) and García-Navas and Sanz (2011) have shown that tree swallow *Tachycineta bicolor* and blue tit *Cyanistes caeruleus* clutches where incubation started before clutch completion had shorter incubation periods than those where it started on or after laying of the last egg.

Our results show that the incubation period is longer in those nests where incubation started before clutch completion, supporting the idea of a lower efficiency. Moreover, starting incubation before clutch completion increased the hatching asynchrony, which could have disadvantages (Amundsen and Slagsvold 1998; Hébert and McNeil 1999; Mainwaring et al. 2012). A possible advantage, on the other hand, could be reducing the nesting period, if the effects of reducing efficiency are compensated by the overlapping between the laying and incubation periods, and therefore a reduction of the total nesting period. Thus, controlling for clutch size, the "egg phase" (i.e. from the laying of the first egg to the hatching of the last one), was shorter as the incubation started earlier in the laying sequence (multiple regression, $F_{2,173} = 188.69$, $P < 0.001$; clutch size: $\beta = 0.94$; date of onset of incubation relative to clutch completion: $\beta = 0.67$). Stenning (2008) also showed that the nesting period (from first egg to fledging) of blue tits was shortened as hatching asynchrony increased, i.e. as laying and incubation overlap increased.

## Uncovering the eggs before the onset of full incubation

During the egg laying period, great tit females typically cover the eggs with the nesting material when they leave the nest (Kluijver 1950; Perrins 1979; Haftorn 1981a). Why females do this is not clear. Haftorn and Slagsvold (1995) concluded that the function of protecting the eggs against unfavourable microclimate has little support, and that the most likely function is protecting the eggs against predators, as eggs are not visible at first glance. If this is so, uncover the eggs would mean a higher risk of clutch loss, so it is especially interesting the behaviour of uncovering the eggs before starting full incubation found in some pairs.

Our results show that pairs whose eggs were uncovered before the onset of full incubation shortened the incubation period by 0.4 days relative to those which did not perform this behaviour. This suggests that pairs which uncovered the eggs before starting full incubation were those giving more attention to their eggs during the “partial incubation” phase during the laying period (see e.g. Lord et al. 2011). This early onset has no significant effect on hatching asynchrony (a difference of 0.1 days), so this behaviour has the potential of shortening the nesting period. About 20% of the studied pairs ($n$ = 356) uncovered the eggs before full incubation, so it is not a rare behaviour in this population. It remains to be studied whether the possible increase in predation risk by uncovering the eggs compensates for the reduced risk associated to the shortening of the nesting period.

## Duration of the incubation period

In this study we defined the incubation period as the number of days elapsing from the onset of full incubation to the day previous to the hatching of the first egg. We find this more consequent with the definition of the incubation, since it is the minimum period which really takes to “incubate” (from onset of the incubation to hatch) an egg. The only bias is that hatching of the first egg could happen at any time within the first date of the hatching period, so, strictly speaking, the incubation period would be a bit longer (considering that we visited the nests during the morning). By including the hatching period within the “incubation period”, i.e. considering the incubation period until the hatching of the last egg, we are introducing the variability in hatching asynchrony, which depends more on factors different from the heat-transferring process, as when incubation started relative to clutch completion or clutch size. In some studies (e.g. Ardia et al. 2006), only the first hatching date was included in the estimation of the incubation period – doing this would increase in one day our estimate, but will not affect any of our analyses. Overall, including the hatching period, the duration of the incubation period was increased by about two days. As, on average, females started full incubation the date of laying of the last egg, the incubation period was roughly the same if it is computed from the date of laying of the last egg or from the date of observed onset of full incubation. This difference is, however, important when considering individual clutches.

Differences of one or two days in the duration of the incubation period between populations have been found in Haftorn 1981b some passerines (Murphy 1983; Järvinen 1990; Robinson et al. 2008). For great tits, the period between laying and hatching of the last egg lasted 15.6 days in Sagunto (present study), 13.5-13.9 days in Germany (Winkel 1970, 1975), 15 days in The Netherlands (Kluijver 1950), and 16.3 days in Norway (Haftorn 1981b). Gibb (1950), in England, defined the incubation period as the time between its onset (as defined here) or the laying of the last egg, whichever was later, and the hatching of the last egg; his "apparent incubation period" lasted 13.9 days. Matysioková and Remeš (2011) reported an incubation period of 11.8 days for a Czech Republic population, but they did not explain how it was calculated. Many studies have shown that incubation periods are generally longer in species breeding at lower latitudes (Ricklefs 1969; Martin et al. 2007). Ardia et al. (2006), however, did not find differences in the duration of the incubation period of two tree swallow *Tachycineta bicolor* populations breeding in Alaska and Tennessee (29 º of latitude apart). Being aware of the roughness of available estimates, and that they were derived using different criteria, it seems that the Sagunto population has a longer incubation period than northern populations, excluding that from Norway. Great tits would be a good model to explore the latitudinal variation of the duration of the incubation period within species, by using the same criteria in different populations.

Mean incubation period in Sagunto differed between years. Yearly variations in temperatures or food availability might affect the mean incubation period (e.g. Drent et al. 1985; Moreno 1989; Zimmerling and Ankney 2005). We found that, within the range prevailing during the study years, the duration of the incubation period was not related to mean temperatures in April, but it was shorter as food was more abundant (e.g. mean clutch size of the population was larger). In a species where only the female incubates, and she has to trade-off time allocated to incubation and time allocated to foraging, having more food available would mean making shorter foraging trips (Zimmerling and Ankney 2005; Londoño et al. 2008), and therefore more time for keeping eggs warm.

The duration of the incubation period is positively related to egg size across species (Rahn and Ar 1974; Deeming et al 2006), but data within species are contradictory (e.g. Parsons 1972; Runde and Barret 1981; Ricklefs and Smerasky 1983; Martin and Arnold 1991; Viñuela 1997; Hepp et al. 2006). Mean egg volume and clutch volume had no significant effects on the duration of the incubation period in the studied population. We are not aware of any study on passerines showing a significant relationship between these two variables and the duration of the incubation period, though non-significant trends in the expected direction (increase of the duration of the incubation period as egg volume increases) have been reported (Järvinen 1990).

Some authors have shown that larger clutches take longer to incubate (Baltz y Thompson 1988; Moreno y Carlson 1989; Smith 1989). However, these studies experimentally increased clutch size above the normal range for the studied species. Results in unmanipulated clutches show variability, some finding an increasing trend (e.g. Hepp et al. 2005; García-Navas and Sanz 2011), others a decreasing trend (e.g. Cooper et al. 2005; Ardia et al. 2006), and others no relationship (e.g. Moreno 1989). Within the natural range of clutch sizes, we did not find a significant relationship between clutch size and the duration of the incubation period, though the trend was negative. However, when controlling for other variables, particularly the date of onset of incubation relative to laying date, this negative trend was significant. Therefore, the incubation period seem to be shortened when clutches are large. Noteworthy, this was also the trend when we used yearly means of incubation period and clutch size. If food availability is behind these trends, perhaps pairs laying larger clutches, which might be in better condition or have more resources in their territory, are also more efficient incubators, so that both clutch size and incubation period are mediated by the quality of territories and/or individuals. Another possibility is that the egg cooling rate was lower in larger clutches, so mean egg temperature during female absences are higher in larger clutches (Frost and Siegfried 1977; Reid et al. 2000) and therefore incubation period is shorter. Finally, the way incubation period is computed could have also its weight. Thus, hatching asynchrony is usually longer in large clutches (present study; Potti 1998; Ardia et al. 2006), so including the hatching period in the calculation of the incubation period automatically will cause a positive relationship between incubation period and clutch size, even if the period where an egg needs to hatch is equal or even shorter than in a smaller clutch.

Although there are exceptions (Goldstein and Yom-Tov 1988; Järvinen 1990; Ippi et al. 2012), the duration of the incubation period uses to decrease as the season progresses (Slagsvold 1986; Nilsson and Smith 1988; Moreno and Carlson 1989; Smith 1989; Järvinen 1990; García-Navas and Sanz 2011). Our results agree with these observations, clutches where incubation started later in the season taking more time to incubate. Among the factors suggested to cause this seasonal decrease are a seasonal decrease in egg and clutch size, an increase in mean ambient temperature, and changes in female behaviour which increase incubation efficiency, though the evidences are sometimes contradictory (see e.g. Massaro et al. 2006). We have shown here that egg and clutch size did not affect incubation period in the studied population. Some studies have shown that ambient temperatures are negatively related to the incubation period of several species (MacRoberts and MacRoberts 1972; St. Clair 1998; Järvinen 1990), so the increase of ambient temperatures as the season progresses could cause a shortening of the incubation period. Looking at the relationship between the incubation period and the ambient temperatures during these dates will solve this question.

Something worth noting is that we did not find a significant relationship between laying date and the duration of the incubation period – seasonality was found using the date of onset of incubation as an independent variable. This variable was seldom used, as the incubation is assumed to start with the laying of the last egg. It is therefore important to determine the exact date of incubation onset (e.g. Howe 1976; Briskie and Sealy 1989).

### Hatching asynchrony

The duration of the hatching period in the studied population (1.7 days) was within the range of those found in other populations (1.8-4 days; Gibb 1950; Neub 1979; Haftorn 1981b; Orell 1983; Amundsen and Slagsvold 1998; Tilgar and Mänd 2006; Theofanellis et al. 2008; Matysioková and Remeš 2011; Podlas and Richner 2013a). This was not unexpected, taking into account that a mean female in Sagunto started incubation the day of laying of the last egg.

We found between-year variability in the duration of the hatching period, with mean ranging from 1.0 to 2.9 days. However, we were unable to explain this variability as a function of temperatures, clutch size (as a proxy of food availability) or duration of the incubation period. Stenning (2008) also found between year differences in hatching asynchrony in a blue tit population, and Potti (1998) found between-year differences in the proportion of pied flycatcher *Ficedula hypoleuca* females starting the incubation before clutch completion, which was associated to higher hatching asynchrony later on. Potti (1998) tried to relate incubation onset relative to clutch completion to ambient temperatures, but he also failed to find a significant relationship.

Many studies have found that hatching asynchrony increases with clutch size (Slagsvold 1982; Murphy 1983; Briskie and Sealy 1989; Moreno y Carlson 1989; Potti 1998; Ardia et al. 2006; Theofanellis et al. 2008). We also found this trend. A potential explanation was that the onset of incubation is earlier relative to the laying sequence as clutch size increases and, as hatching asynchrony increases as incubation starts earlier in the laying sequence, larger clutches would hatch over a longer period. However, we have shown that clutch size is important after controlling for the date of onset of incubation relative to laying date. Temperature variability among eggs is larger in larger clutches (Reid et al. 2000) and this might cause differential developmental rates among embryos.

Contrary to other studies, including some on great tits (e.g. Gibb 1950; Zink 1959; Haftorn 1981a; Slagsvold 1982; Orell 1983; Mead y Morton 1985; Skagen 1987; Potti et al. 1988; Moreno and Carlson 1989; Stenning 2008; Theofanellis et al. 2008), we did not find any seasonal variation in the duration of the hatching period. Potti (1998) did not find a relationship between the onset of incubation relative to the laying sequence and laying date in pied flycatchers, and Ardia et al. (2006) did not find a significant relationship between hatching asynchrony and clutch initiation date in tree swallows. We have only included first clutches in our sample, so perhaps potential effects affecting hatching asynchrony did not have enough seasonal variation to affect hatching patterns.

Potti (1998) did not find relationship between egg volume and onset of incubation relative to the laying sequence in the pied flycatcher. On the other hand, although Stenning (2008) found that haching was more asynchronous in blue tit clutches with denser eggs, this relationship disappeared when laying date was considered – late clutches happen to have denser eggs and hatch more asynchronously. We are not aware of other studies relating egg size, or clutch volume and hatching asynchrony, and we did not find a significant relationship between them.

We found that hatching asynchrony increased as incubation period decreased. The incubation period was shorter in nests where incubation started later relative to clutch completion and had larger clutches, but the negative relationship between incubation period and hatching asynchrony persisted after controlling for these effects. A potential explanation to this relationship is that partial incubation, occurring over several days (both prior and after clutch completion, before the onset of full incubation) started embryo development, resulting in both a shortening of the (full) incubation period and increased hatching asynchrony. Lord et al. (2011) have shown that differences in the amount of incubation received by individual eggs prior to clucth completion significantly affected the time they need to hatch after full incubation started. For example, Potti (1998) noticed that many pied flycatcher nests where incubation started after the last egg hatched asynchronously, being the amount of incubation prior to clutch completion probably involved.

Haftorn (1981a) found a positive relationship between the time allocated to incubation during the laying period and the duration of the hatching period. Lord et al. (2011) also found that individual eggs receiving more heat during the laying period experienced shorter incubation periods. Our results show that the hatching period increased as the onset of incubation was earlier relative to clutch completion, indicating that eggs receiving heat during the laying period hatched earlier.

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# ARTÍCULO 6

## *Within and between population variations of incubation rhythm of great tits Parus major*

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*ENVIADO PARA PUBLICACIÓN*

> “El que quiere hacer algo encuentra
> un medio, el que no una excusa.”

# Within and between population variations of incubation rhythm of great tits *Parus major*

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**Short title**

Incubation rhythm in great tits

*Key words:* Effective incubation, Incubation sessions, Incubation recesses, Latitudinal trends, Nest attentiveness, Nest predation

## ABSTRACT

Uniparental incubation frequently means that eggs remain unattended for periods where the incubating bird is foraging out of the nest. The determination of incubation rhythms (i.e. the length and temporal pattern of incubation sessions) and the factors which affect them are therefore important to understand life-history trade-offs. We studied how different factors affect the incubation rhythm of a southern European great tit *Parus major* population, and review previous studies to check for latitudinal trends. We show for the first time in a bird species that attentiveness (% of time incubating) is fairly similar (83-88%) between populations ranging from Norway to Spain. Mean duration of incubating sessions (on-bouts) and recesses (off-bouts) do not follow a clear latitudinal pattern, perhaps off-bouts being longer at the two extreme populations (10-12 min) than in central ones (7-8 min). Within the Spanish population, attentiveness increased along the incubation period, due to shorter off-bouts, and females left the nest earlier in the morning and retired later in the evening (thus prolonging their active day) when ambient temperatures were higher. We show for the first time that mean off-bout duration was longer as egg size, clutch size and clutch volume (total volume of the eggs of the clutch) decreased in the early incubation stage (days 3-6). Finally, better quality females, and/or those paired with better quality males, spend more time out of the nest, a probable cause being avoidance of predation at the nest.

## INTRODUCTION

Avian incubation is defined as the process whereby the heat necessary for embryonic development is applied to an egg after it has been laid (e.g. Tullet 1981; Wang and Beissinger 2011). Incubating birds regulate the temperature of their eggs by direct heating, transferring heat from the brood patch to the eggs. In most bird species, incubation requires almost uninterrupted parental attention to keep the eggs warm, and in many of them this is achieved through the sharing of incubation duties by the two parents (Deeming 2002). Other species, notably those where only one parent (usually the female) incubates, are able to tolerate periods where the incubating bird is out the nest ("egg neglect"), with a consequent dropping of egg temperature (Skutch 1962; Drent 1975; Deeming 2002).

Only two avian orders (Passeriformes and Apodiformes) show relatively low levels (< 75%) of nest attentiveness (proportion of time that the incubating bird is on the eggs) due, on one hand, to the uniparental incubation strategy and, on the other, to the need of the incubating bird to leave the nest to forage, which leads to an intermittent incubation pattern (Deeming 2002). Embryos develop optimally between 36–40 ºC, and development is suspended below 24–26 ºC (see e.g. Cooper et al. 2005), which is known as physiological zero temperature. Intermittent incubation imposes a variable temperature regime to the eggs, which have important implications for embryo development and for parental incubation behavior (Tinbergen and Williams 2002; Olson et al. 2006). Therefore, the determination of incubation rhythms, e.g. the length and temporal pattern of incubation sessions, and the factors which might affect them, are important to the study of life history trade-offs (Conway and Martin 2000a; Martin 2004; Pérez et al. 2008; Ardia et al. 2009) and parental investment in reproduction (Conway and Martin 2000b; Londoño et al. 2008; Wojczulanis-Jakubas et al. 2009), among other topics.

The incubation rhythm is determined by the decisions taken by the incubating bird of being in or out the nest (e.g. Joyce et al. 2001; Deeming 2002; Hainsworth and Voss 2002). These decisions are in turn affected by the thermal requirements of the eggs, trade-offs between resource demands on the parents, and environmental factors, including nest quality and predation risk (White and Kinney 1974; Williams 1996; Conway and Martin 2000a; Deeming 2002; Cresswell et al. 2004; Voss et al. 2006; Álvarez et al. 2013). As environmental conditions, parental condition, and needs of the embryos, vary on a diurnal and seasonal basis, as well as along the incubation period, birds should fine tune their behavior to cope with these variations (Cooper and Voss 2013). Thus, patterns of nest attendance might vary along the incubation period (e.g. Yerkes 1998; Rompré and Robinson 2008; Ricklefs and Brawn 2013), during the day (Weeden 1966; Wheelwright and Beagley 2005; Marín 2008) and along the season (Wheelwright and Beagley 2005; Zimmerling and Ankney 2005; Ardia et al. 2009), in response to variations of the relative weight of different factors. At the end, the length and frequency with which the incubating bird leaves the nest may have important consequences to its reproductive performance (e.g. Webb 1987; Joyce et al. 2001; Reid et al. 2002; Olson et al. 2006; Ardia et al. 2009, 2010).

Female characteristics might also affect the incubation rhythm (Hegyi and Sasvári 1998; Joyce et al. 2001; Gorman and Nager 2003). Furthermore, in some species with female-only incubation, females benefit from being fed by males, a behavior called “incubation feeding” (Lack 1940; Nilsson and Smith 1988). Incubation feeding could impose high energetic costs to the males (Wijnandts 1984, Moreno 1989), so the intensity of incubation feeding uses to be positively related to male quality (Lifjeld et al. 1987; Siefferman and Hill 2005). As a response, females which receive more food increase their nest attentiveness (Nilsson and Smith 1988; Halupka 1994). Therefore, male characteristics might also affect female incubation patterns.

Egg mass and clutch mass (sum of egg masses of a clutch) might affect the rate of egg cooling, and therefore the periods that eggs may remain unattended (Deeming 2002). Thus, when considering a wide range of species, a positive correlation between both egg mass and clutch mass and attentiveness during incubation has been found (Deeming 2002). However, we are not aware of studies testing this pattern within a species, though a positive relationship between clutch size and attentiveness has been found (e.g. Matysioková and Remeš 2010).

The great tit is a widespread passerine that breeds in wooded areas across the Palearctic (Cramp and Perrins 1993). They build their nests in tree cavities, readily accepting nestboxes. Females lay one egg per day until clutch completion (mean clutch size between 5-9 eggs in different Spanish populations; Atiénzar et al. 2012). Great tits are typically monogamous, with female-only incubation, therefore performing an intermittent incubation behavior (e.g. Kluijver 1950; Haftorn 1981a). During the egg-laying period, females devote some time during the night to incubate (Haftorn 1981b; Lord et al. 2011), but this does not seem to affect embryo development (Podlas and Richner 2013). Full (or effective) incubation [when egg temperature remains constantly well above the threshold for embryonic development, about 36 ºC (Haftorn 1981b; Wang and Beissinger 2011)] lasts around 13-14 days (Gibb 1950; Barba 1991), starting around clutch completion. Males do not take part in incubation, but feed the females during this period, both within and outside the nest (e.g. Kluijver 1950; Matysioková and Remeš 2010).

In spite of being one of the most studied bird species, with many populations under study across its distribution range (e.g. Lambrecths et al. 2010), its incubation patterns have received little attention. Only the study by Haftorn (1981a) deals with most aspects of incubation rhythm in some detail and with a reasonable sample size. Earlier observations by Kluijver (1950) and Hinde (1952) rely on one or very few pairs respectively, and more recent studies treat a limited number of aspects, and data were usually taken during a limited number of hours per day and/or only during part of the incubation period (Bryan and Bryant 1999; Cresswell and McCleery 2003; De Heij et al. 2006; Matysioková and Remeš 2010, 2011). Given the temporal variation in incubation patterns along the day and along the incubation period, samples taken in any part of them will not be representative of the whole incubation process (e.g. Cooper and Voss 2013).

Moreover, all these studies proceed from Central or Northern European populations (e.g. above 49º N), being completely lacking in the south part of the species' distribution range (e.g Atiénzar et al. 2012). Latitudinal patterns in nest attentiveness during the incubation period have been found between species (e.g. Martin 2002; Chalfoun and Martin 2007), but patterns within species are much less clear (see e.g. Ardia et al. 2006). One of the factors which might cause differences in attentiveness is the variation in incubation rhythms, but we are not aware of any study examining latitudinal variation of incubation rhythms within a species.

Our aims here were twofold. First, we aimed to describe the incubation pattern of an Eastern Spanish great tit population and determine some of the factors which might affect incubation rhythm within this population. Second, we reviewed published information on incubation patterns in other European great tit populations, and examine their variation along a latitudinal gradient. Noteworthy, our study, performed in Spain (39º N) will provide an interesting comparison with that of Haftorn (1981a), performed in Norway (63º N), near the two extremes of the species' distribution range.

## METHODS

### Study area and basic field procedures

The study area was located in Sagunto (Valencia), Eastern Spain (39º42'N, 0º15'W, 30 m a.s.l.), within an extensive orange *Citrus aurantium* monoculture where wooden nestboxes are available since 1986 (Greño et al. 2008; Tomás et al. 2012).

We visited all nestboxes at least once a week, and daily in some periods, from late February to the end of the breeding season by mid July, and included here only data from first clutches. This pattern of visits allowed us to estimate the date of laying of the first egg (assuming the laying of one egg per day), the date of start of full incubation (eggs uncovered and warm or female seen incubating; Haftorn 1981b), clutch size, and date of hatching of the first egg. The length (L) and width (W) of the eggs were measured with a caliper (± 0.01 mm) 2-5 days after incubation started. The volume (V) of each egg was calculated as $V = (0.4673*L*W^2) + 0.042$ (Ojanen et al. 1978; Encabo et al. 2001). Mean egg volume and clutch volume (sum of the volumes of all the eggs) were computed for each clutch.

We collected data on incubation behavior in 15 nests (5 in 2006, 10 in 2007). The first egg of these clutches was laid between 28 March and 15 April, and clutch size varied between 6 and 11 eggs. Incubation in these nests started between 6 and 23 April and finished (hatching of the first egg) between 18 April and 5 May, lasting between 13 and 15 days.

Data of ambient temperatures (daily maximum and minimum) and rain of a nearby (c.a. 4 km) meteorological station were obtained from the Spanish Meteorological Agency. Mean temperatures computed as the average of maximum and minimum, and days classified as rainy (> 10 mm) or not-rainy (≤ 10 mm). During the incubation period of the nests considered here, mean temperatures (± SD) were: 2006 (16 April to 5 May): maximum: 22.4 ± 1.6 ºC, range 19.0-26.0; minimum: 12.1 ± 1.9 ºC, range 8.5-15.0; mean: 17.2 ± 1.3 ºC, range 15.3-19.4; 2007 (6 April to 3 May): maximum: 20.8 ± 2.9 ºC, range 15.0-26.0; minimum: 11.6 ± 2.0 ºC, range 7.0-15.0; mean: 16.2 ± 1.6 ºC, range 13.3-19.3. Time of sunrise and sunset, and number of hours of light of each day (from sunrise to sunset) were obtained at: http://www.weatherimages.org/latlonsun.html.

## Incubation behavior

Different methods have been used to study incubation rhythms, from direct observations to temperature sensors, photo-resistors, videos or balances (see Joyce et al. 2001; Cooper and Mills 2005 and references therein). We used here PIT (Passive Integrated Transponder) technology to directly measure the entrances and leavings of incubating birds. This method has been successfully applied to the study of feeding patterns, especially in hole-nesting birds (e.g. Freitag et al. 2001, Nilsson et al. 2008), but its application to incubation studies has been, to the best of our knowledge, very limited (e.g. Wiebe 2008).

When nests were almost finished, we placed mist nets around the nestboxes to trap the females. Trapped females where individually ringed (numbered aluminium rings) or the ring number recorded if they were already ringed. Each bird was fitted with a passive integrated transponder (PIT) tag (Trovan ID 100; length: 11.6 mm, mass: 0.1 g) with unique codes (e.g. Wiebe 2008). The tag was glued to two colour rings and the ensemble was covered with adhesive tape and placed in the leg opposite to the one bearing the aluminium one (e.g. Freitag et al. 2001; Wiebe 2008). Males were trapped, and females retrapped, at the nests (nest-door traps) when feeding 10-12 days-old nestlings. Males were individually ringed (or the ring number recorded) and both parents were weighed (electronic balance, ± 0.1 g), and their wing (stopped rule, ± 0.5 mm) and tarsus length measured (digital caliper, ± 0.01 mm). An index of body condition for each individual was calculated as weight to tarsus length ratio. Parents were classified as either yearlings (one-year birds) or older birds (more than one-year old birds) based on plumage characteristics (Svensson 1996).

Female entrances to and leavings from the nests during the incubation period were recorded by means of a transponder reader system consisting of an antenna fitted to a nestbox door and connected to a datalogger (Trovan LID-650) and a 12 V battery mounted in a plastic box covered with camouflage fabric (e.g. Nilsson et al. 2008). When a tagged bird enters or leaves the nestbox, it interrupts the optical barrier, triggering the reading of the bird's corresponding PIT and a visit is stored in the memory, along with the exact time at which it occurred. Nestboxes used were opened at the front, so the original door could be removed and the one fitted with the antenna placed when setting the reader. The box with the reader was placed at the base of the tree (nestboxes were at most 1 m above ground). The installation of this device took about 2 minutes.

We had four readers, and these were moved between nests to take samples of as many days as possible starting at incubation day 2 and finishing at day 12. However, we have very few data from day 2, so only data from day 3 onwards were considered here. We divided the incubation period in 3 stages (early: days 3-6; middle: days 7-9; and late: days 10-12). Furthermore, to look for variation along the day, three periods were considered: morning (from first nestbox leaving in the morning to 11:59 h), afternoon (12:00 h to 15:59 h), and evening (16:00 h to last entering). First and last period were variable in length. Also, the overnight incubation period (from last entrance one day to first leaving next morning) was considered, and was also variable in length. The night was an extended period of attentiveness, including sleep (Weeden 1966). We tried to take samples from all periods within the 3 incubation stages at all sampled nests, though we missed some combinations in several nests. We considered having data for any particular period within a day if we had at least two hours of data.

The system was typically mounted either on the previous evening (18:00-21:00 h) or early (before 08:00 h) in the morning of the sampling day, and was left operating until this evening or next morning. Data for overnight incubation duration was only considered if readers were placed at least two hours before sunset and removed once the female has left the nest next morning. When placing the reader, we noted the presence or absence of the female, and therefore we know whether the first lecture was an entrance or a leaving event. In many occasions females were inside the nest, and they stayed there while removing the original nestbox door and placing the door with the antenna or *vice versa*. A sample started with the first record.

### Data from other populations

To study the latitudinal variation in incubation patterns, we reviewed studies offering this information for great tits. Some parameters were obtained directly from the text, tables and/or figures, while others were calculated by us using information on the paper. For example, the number of times that the female enters or leaves the nest could be computed knowing the mean time on and off the nest and the length of the active day. We acknowledge some limitations of this data set, since data ranged from detailed information of a single nest (Kluijver 1950) to intensive experimental studies (Bryant and Bryant 1999). Still, we think that, given the spatial scale considered (from Norway to Spain, with a difference of 24º in latitude), within population variation will have little effect on our large-scale comparison. The time of sunrise and sunset, and therefore the number of daylight hours, was obtained as above by entering the latitude and longitude of each site and an approximate (from data provided in the papers) mean incubation date.

### Statistical analyses

From data recorded, we estimated for each nest and period (as appropriate) the following parameters: time of first leaving in the morning (in absolute terms and in time after sunrise), time of last entrance at night (in absolute terms and in time before sunset), length of the overnight period, length of activity period (from first leaving to last entrance of the day, called “active day” hereafter), number of times that the female enters or leaves the nest per hour, mean duration of periods on (i.e. time spent incubating, called “on-bouts” hereafter) and off (time outside the nest, called “off-bouts” hereafter), total time on and off, and their proportions, both during the active day and during a complete 24-h day. Some of these parameters could be computed for each period along each day and for a complete day (e.g. mean duration of on-bouts during the morning, or during the whole active day), while others only have only one possible value per day (e.g. time of first leaving in the morning). In both cases, a mean value per incubation stage (early, middle or late incubation) could be calculated.

For those parameters which could vary along the day, we first checked whether this variation was significant. If there was, we kept them separately in further analyses; if not, a mean per day was computed and used later on. Similarly, variation along the three stages of the incubation period was checked. If there were differences, they were kept separately in further analyses; if not, a mean per nest was computed and used later on. For analyses where the independent variable varied depending on the day of sampling (e.g. time of sunrise, temperatures), we selected a random day per nest if there were more than one with available data within the appropriate period.

The following explanatory variables were used as appropriate: parental characteristics (age class, tarsus and wing lengths, weight and body condition), laying and incubation starting dates, mean egg volume, clutch size and clutch volume, maximum, minimum and mean temperatures, occurrence of rain, time of sunrise, time of sunset, and total number of daylight hours.

We have few data per year to sensibly consider the possible effect of year, so all the nests were pooled for analyses. Percentages were arcsin square root transformed for analyses (Zar 1996), but raw data are presented for greater ease of interpretation. Bout length data were approximately normally distributed, and results of Levene tests were nonsignificant, indicating homogeneity of variances, so they were not transformed for analyses. We used either generalized linear models (for categorical variables) or linear regressions (for continuous ones) to check for possible effects of independent variables on incubation rhythm related variables. Sample sizes were too small to include all independent variables in full models. To check for the relationship between on- or off-bout duration and duration of previous bout, we used ANCOVAs, where on- or off-bout duration was the dependent variable, nest identity was included as a random factor, and the duration of previous on- or off-bout was the covariate. The SPSS v. 19.0 statistical package was used for all analyses. Means ± SD are shown where appropriate.

## RESULTS

### Within population variation

During the incubation period, a mean female in our population started its active day at 07:21 local time, or 7 min after sunrise, and finished it at 19:49, or 44 min before sunset. Therefore, the duration of the active day averaged 12:32 hours, allocating 11:28 hours to the overnight rest (Table 1). None of these parameters differed between incubation stages ($P > 0.05$ in all cases). We therefore computed a mean value (mean of the means of the three stages) per each parameter per nest for some analyses, or took the value of a randomly selected day for others.

**Table 1**
Time of first leaving, of last entering, and duration of the active day, in the 3 incubation stages. Mean ± SD and sample size in brackets are shown.

| Incubation stage | Time of first leaving | Time of last entering | Active day |
|---|---|---|---|
| Early | 07:29:20 ± 00:18:23 (9) | 19:49:54 ± 00:49:44 (10) | 12:19:26 ± 00:53:30 (9) |
| Middle | 07:13:18 ± 00:04:53 (6) | 19:44:54 ± 00:58:53 (8) | 12:51:00 ± 00:44:56 (6) |
| Late | 07:16:32 ± 00:22:59 (9) | 19:53:12 ± 00:41:42 (6) | 12:26:07 ± 01:03:38 (9) |
| Mean | 07:19:43 ± 00:08:29 (3) | 19:49:20 ± 00:04:11 (3) | 12:32:11 ± 00:16:38 (3) |

Females leaved the nests earlier in the morning as the season progressed (laying date: $F_{1,13} = 4.93$, $\beta = -0.53$, $P = 0.045$; incubation starting date: $F_{1,13} = 6.26$, $\beta = -0.57$, $P = 0.027$), as sunrise was earlier ($F_{1,13} = 14.45$, $\beta = 0.73$, $P = 0.002$), as maximum ambient temperatures were higher ($F_{1,13} = 6.41$, $\beta = -0.58$, $P = 0.025$), and if there was no rain ($F_{1,13} = 5.27$, $P = 0.039$; though only 3 rainy days were included in this analysis). There were no significant relationships with the rest of the independent variables.

Females entered the nestbox to overnight earlier when minimum ambient temperatures were lower ($F_{1,13} = 7.03$, $\beta = 0.59$, $P = 0.020$) and if they were paired with young males ($F_{1,9} = 5.57$, $P = 0.043$). There was also a trend for females to enter earlier as her weight decreased ($P = 0.056$). There were no significant relationships with the rest of the predictor variables. In relation to the time of sunset, the results mirrored those above: females entered the nest early in relation to sunset when minimum temperatures were lower ($F_{1,13} = 6.50$, $\beta = -0.58$, $P = 0.024$) and if they were paired with young males ($F_{1,9} = 5.42$, $P = 0.045$), and there was also a trend for entering the nest early before sunset as her weight was lower ($P = 0.056$).

The total duration of the active day was positively related with the total number of daylight hours ($F_{1,13} = 5.00$, $\beta = 0.53$, $P = 0.044$; Figure 1) and with the weight of the female ($F_{1,6} = 8.52$, $\beta = 0.77$, $P = 0.027$), while the overnight period varied in an inverse way.

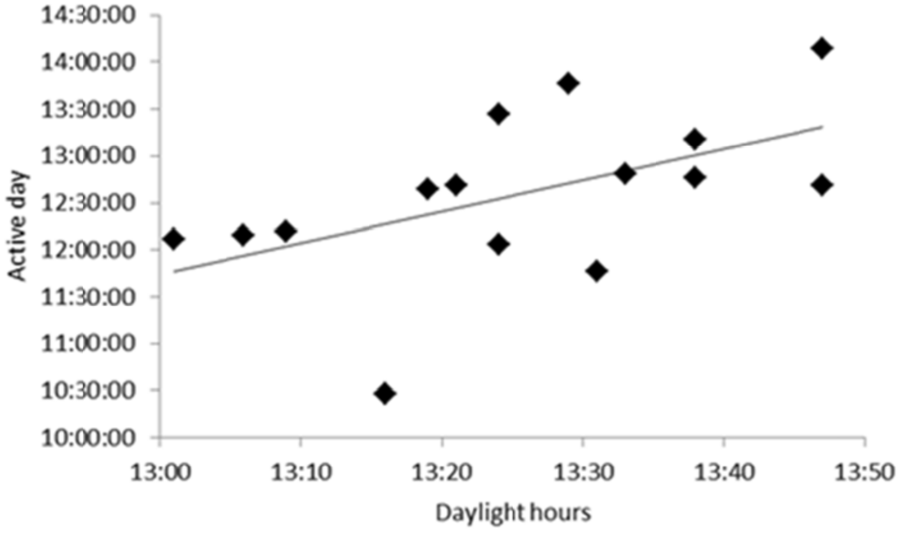

**Figure 1**. Relationship between the number of daylight hours and the duration of the active day (from first leaving to last entrance) of incubating females.

A mean incubating female in our population left the nest an average of 23 times per day (1.8 times per hour during the active day), performing on-bouts of around 26 min, and off-bouts of about 12 min (Table 2). This means that females stayed on the eggs about 66% of time of their active day (12.5 hours) and off the nest around 34% of their active day (Table 2). Considering a 24 h period, females were sited on the eggs nearly 20 h, or 83% of the day.

**Table 2**
Number of trips off the nest per hour, duration of on- and off-bouts, and attentiveness in each of the diurnal periods of each incubation stage. Mean ± SD and simple size in brackets are shown. We used all the nests available (one data point per nest) at each period and stage. The mean value per incubation stage was computed as the mean of each period, and the total mean was computed as the mean of the means of each stage.

| Stages | Period | Trips per hour | On-bout duration | Off-bout duration | Attentiveness (%) |
|---|---|---|---|---|---|
| Early | Morning | 1.36 ± 0.73 (7) | 00:36:04 ± 00:17:20 (7) | 00:18:58 ± 00:11:36 (7) | 63.03 ± 6.95 (7) |
| | Afternoon | 1.52 ± 0.51 (8) | 00:25:11 ± 00:13:17 (8) | 00:14:55 ± 00:03:45 (8) | 60.48 ± 11.22 (8) |
| | Evening | 2.00 ± 0.45 (7) | 00:21:17 ± 00:05:38 (7) | 00:12:11 ± 00:03:14 (7) | 61.31 ± 8.22 (7) |
| Mean Early | | 1.63 ± 0.33 (3) | 00:27:31 ± 00:07:40 (3) | 00:15:21 ± 00:03:25 (3) | 61.61 ± 1.30 (3) |
| Middle | Morning | 1.86 ± 0.44 (7) | 00:28:36 ± 00:17:29 (7) | 00:10:09 ± 00:06:20 (7) | 73.25 ± 2.25 (7) |
| | Afternoon | 2.01 ± 0.78 (6) | 00:22:57 ± 00:10:43 (6) | 00:11:38 ± 00:05:08 (6) | 64.81 ± 15.34 (6) |
| | Evening | 1.78 ± 0.88 (7) | 00:30:38 ± 00:16:46 (7) | 00:13:02 ± 00:06:22 (7) | 68.43 ± 10.40 (7) |
| Mean Middle | | 1.88 ± 0.12 (3) | 00:27:24 ± 00:03:59 (3) | 00:11:36 ± 00:01:27 (3) | 68.83 ± 4.23 (3) |
| Late | Morning | 2.10 ± 0.99 (9) | 00:24:31 ± 00:09:41 (9) | 00:07:27 ± 00:02:12 (9) | 72.36 ± 13.00 (9) |
| | Afternoon | 2.07 ± 1.10 (7) | 00:24:50 ± 00:12:46 (7) | 00:10:14 ± 00:02:49 (7) | 70.82 ± 12.90 (7) |
| | Evening | 1.85 ± 0.68 (6) | 00:24:17 ± 00:13:11 (6) | 00:10:58 ± 00:03:48 (6) | 65.72 ± 15.98 (6) |
| Mean Late | | 2.01 ± 0.14 (3) | 00:24:33 ± 00:00:17 (3) | 00:09:33 ± 00:01:51 (3) | 69.63 ± 3.48 (3) |
| Total mean | | 1.84 ± 0.19 (3) | 00:26:29 ± 00:01:41 (3) | 00:12:10 ± 00:01:02 (3) | 66.69 ± 1.52 (3) |

None of these five variables differed between periods along the day ($P > 0.05$ in all cases). The number of trips per hour, mean duration of on-bouts, and % of time off during the active day did not differ between incubation stages ($P > 0.05$ in all cases). However, females did shorter off-bouts ($F_{2,55} = 5.93$, $P = 0.005$; Tukey tests; Figure 2) and spent more time on the eggs during the active day ($F_{2,55} = 3.53$, $P = 0.036$; Tukey tests; Table 2) as the incubation progressed.

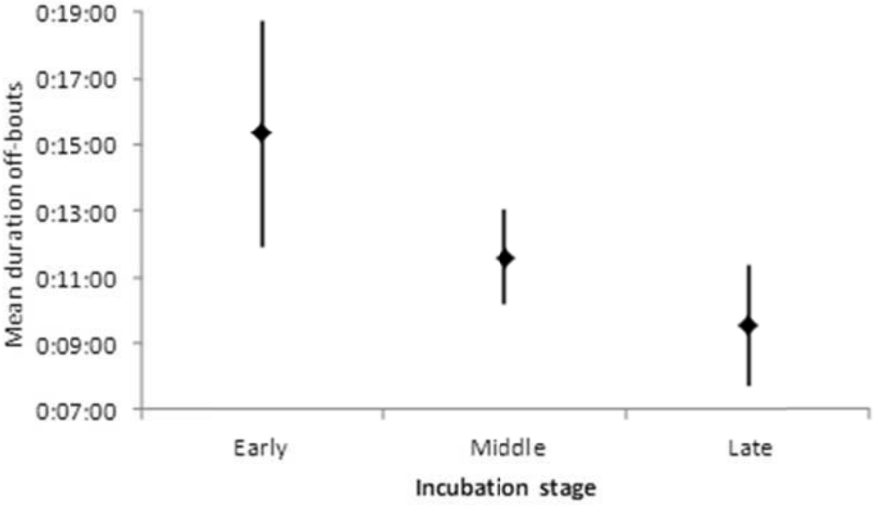

**Figure 2.** Mean (± SD) duration of off-bouts along the incubation period.

As there were no differences between periods along the day, we computed a single mean value for each incubation stage per nest for these five variables. Concerning the incubation stages, we computed a single mean per nest for variables whose values did not differ between stages, and a mean per incubation stage per nest for the mean duration of off-bouts and % of the active day on the nest.

Females with longer wings left the nests more often ($F_{1,10} = 6.26$, $\beta = 0.62$, $P = 0.031$), and had shorter on-bouts ($F_{1,10} = 8.18$, $\beta = -0.67$, $P = 0.017$; Figure 3). There was also a trend for females in better condition to spend a larger proportion of her active day out of the nest ($P = 0.05$).

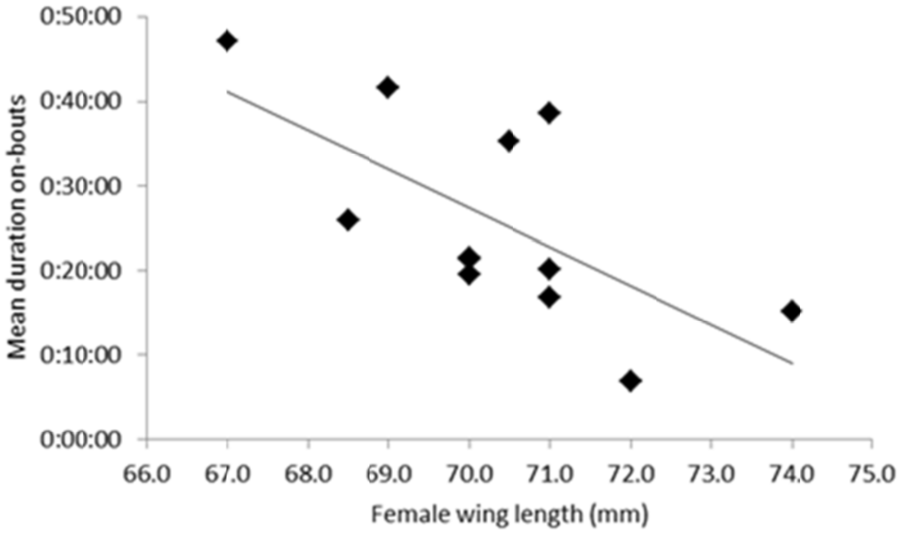

**Figure 3**. Relationship between female wing length and mean duration of on-bouts.

During the early incubation stage, young females make longer off-bouts than older ones [$F_{1,8}$ = 10.46, $P$ = 0.012; though sample size was low ($n$ = 3) for older females]. Off-bouts were longer as clutch size was smaller ($F_{1,8}$ = 5.64, $\beta$ = -0.64, $P$ = 0.045), and as egg ($F_{1,8}$ = 7.45, $\beta$ = -0.69, $P$ = 0.026) and clutch volume decreased ($F_{1,8}$ = 11.05, $\beta$ = -0.76, $P$ = 0.010, Figure 4).

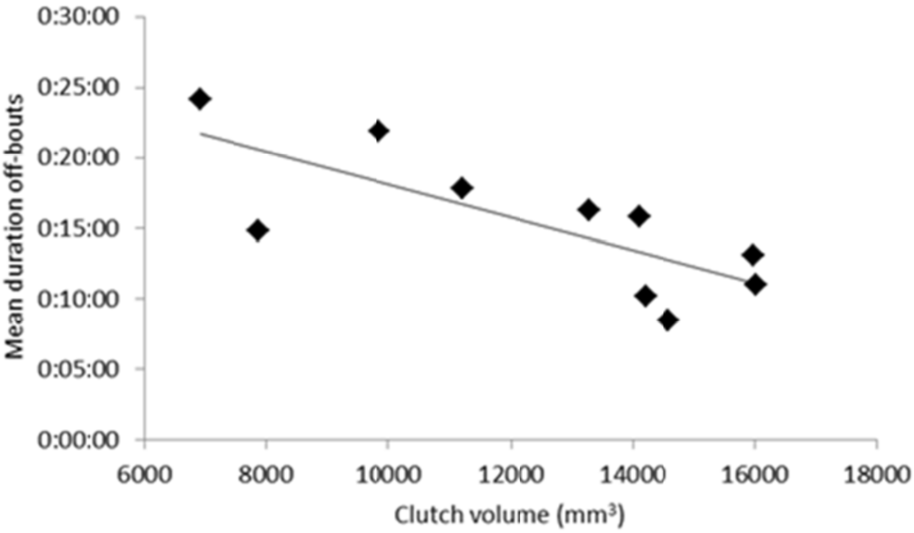

**Figure 4.** Relationship between clutch volume and mean duration of off-bouts in the early incubation stage.

During the middle incubation phase, the proportion of time that females spent on the eggs was higher as their wing length decreased ($F_{1,6} = 8.50$, $\beta = -0.77$, $P = 0.027$), and as the tarsus length of their mate was shorter ($F_{1,4} = 9.81$, $\beta = -0.84$, $P = 0.035$). There was also a trend for females to make longer off-bouts as the incubation started later in the season ($P = 0.058$).

During the late incubation stage, females made longer off-bouts as the wing length of their mate was longer ($F_{1,5} = 7.77$, $\beta = 0.78$, $P = 0.039$; Figure 5).

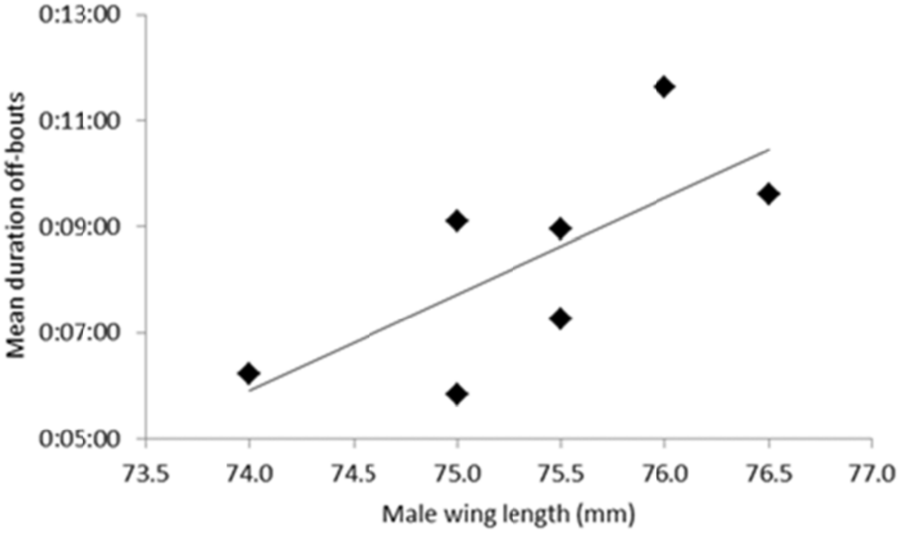

**Figure 5.** Relationship between male wing length and mean duration of off-bouts of its mate during the late incubation stage.

Finally, we found that the mean duration of a particular off-bout was longer as the duration of the previous one decreased ($F_{1,107} = 13.50$, $P < 0.001$; Figure 6). There were no significant relationships between the duration of off-bouts and that of previous on-bouts, and the duration of on-bouts and previous off- or on-bouts.

## Between population variation

At least part of the variables related to the incubation rhythm of great tits is available from 7 populations ranging from Sagunto (Spain, 39º N) to Målsjøen (Norway, 63º N; Table 3). Considering this latitudinal range, and the dates where incubation takes place at each site, great tits have between 13.25 hours of daylight in Sagunto and 19.5 in Målsjøen, a difference of 6.25 hours. The three populations from which information is available offer a clear response to this variation in daylength: incubating females leave the nest earlier relative to sunrise, and enter the nest later relative to sunset as daylength decreases or, in other words, females in Sagunto are moving in and out the nestbox as long as there is daylight, while those in northern populations remain in the nest, prolonging the overnight period, well after sunrise and retire well before sunset. In spite of this, the active day is at least one hour shorter for females incubating in Sagunto.

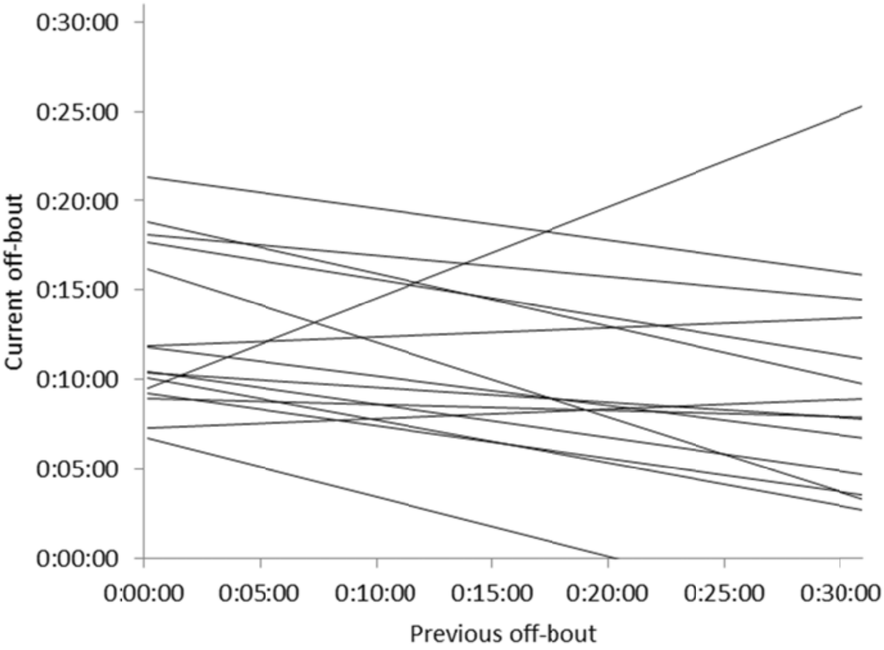

**Figure 6.** Relationship between the duration of an off-bout and that of the following one. Each line represents the relationship for a single nest. Individual data points are omitted for clarity.

The duration of on-bouts is virtually the same in Sagunto (26 min) than in the rest of populations (mean 28.4 min), being 28 min in the northernmost European population from which data are available (Målsjøen). The pattern of off-bouts are less regular: their duration seems to decrease from Norway to the south (from 10 to 7 min), but it reaches 12 min in Sagunto. Distributed along the active day, the number of foraging trips per hour was fairly similar in all populations (range 1.5-2.0, with that of Sagunto in between), and the number of trips per active day ranging between 21 and 27, being 23 in Sagunto.

**Table 3**
Incubation rhythm of great tit populations across Europe. Values were taken from the text, tables and/or figures of the references cited, or calculated by us (in italics) from these values and those of daylight hours. References: (1) Haftorn (1981a); (2) Bryan and Bryant (1999); (3) De Heij (2006; values calculated without considering the 2.25 hours of latency time); (4) Kluijver (1950); (5) Hinde (1952); (6) Cresswell and McCleery (2003); (7) Matysioková and Remeš (2010); (8) Matysioková and Remeš (2011); (9) Present study.

| Population | Latitude | Longitude | Time of sunrise | Time of sunset | Daylight hours | Leaving after sunrise (min) | Retiring before sunset (min) | Active day (min) | Trips per hour | Trips per day | Duration on-bouts (min) | Duration off-bouts (min) | Attentiveness during active day (%) | Attentiveness during 24 h (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Målsjøen (Norway)[1] | 63º15'N | 10º30'E | 3:30 | 23:03 | 19:33 | 91 | 264 | 816 | *1.5* | *21* | 28 | 10 | *72* | *84* |
| Stirling (Scotland)[2] | 56º8'N | 3º54'W | 5:45 | 22:41 | 16:56 |  |  | 850 | *1.6* | *22* | 30 | 8 | *78* | 86 |
| Lauwersmeer (The Netherlands)[3] | 53º20'N | 6º12'E | 5:35 | 21:29 | 15:54 |  |  | 820 | *2.0* | *27* | 22 | 8 | *76* | *87* |
| Bennekom (The Netherlands)[4] | 52ºN | 5º40'E | 6:04 | 21:06 | 15:02 | 20 | 100 |  |  |  | 28 | 8 |  | *88* |
| Wytham Woods (UK)[5] | 52ºN | 1ºW | 6:36 | 21:27 | 14:51 |  |  |  |  |  | 34 | 7 |  |  |
| Wytham Woods (UK)[6] | 52ºN | 1ºW | 6:36 | 21:27 | 14:51 |  |  |  |  |  |  |  |  | 92.5 |
| Velký Kosr (Czech Republic)[7] | 49º32'N | 17º04'E | 5:31 | 20:08 | 14:37 |  |  |  |  |  |  |  | 75 |  |
| Velký Kosr (Czech Republic)[8] | 49º32'N | 17º04'E | 5:31 | 20:08 | 14:37 |  |  |  |  |  |  |  | 77 |  |
| Sagunto (Spain)[9] | 39º42'N | 0º15'W | 7:24 | 20:39 | 13:15 | 7 | 44 | 750 | 1.8 | 23 | 26 | 12 | 66 | 83 |

Given the potential errors in estimating some parameters, it might be concluded from Table 3 that attentiveness during the 24 h is very similar among all populations, ranging from 83% in Sagunto and 88% in Bennekom (The Netherlands; excluding data from Wytham Woods – see Discussion). However, how this value is achieved is notably different among populations. In Sagunto, the overnight period contributes relatively more than in other sites, while attentiveness during the active day is lower (e.g. 66% in Sagunto, 72-78% in the rest), even considering the later start relative to sunrise and the earlier finish relative to sunset in northern populations.

Finally, is should be noted that, in spite of having the longest overnight period, small differences here and there make that, at the end, females in the population of Sagunto spend more time per day out of the nest than those of any other population, though, again, the difference with the northernmost population of Målsjøen is only of 12 min.

## DISCUSSION

### Latitude

Several studies have described latitudinal variation in incubation behavior and their underlying causes among species (e.g. Conway and Martin 2000a; Martin 2002; Chalfoun and Martin 2007; Ricklefs and Brawn 2013). Surprisingly, direct comparisons of incubation rhythm between populations of the same species breeding at different latitudes are virtually lacking, and the few studies which address this issue concentrate mainly on the start and duration of the incubation period (e.g. Ardia et al. 2006; Ippi et al. 2012). We report here, for the first time, that nest attentiveness of a small passerine during incubation is similar along a latitudinal gradient encompassing 24º of latitude, from Northern to Southern Europe.

Southern European female great tits remain active (entering and leaving the nest) during most of the available light hours: they leave the nest on average 7 min after sunrise, and enter the nest for the overnight rest on average 44 min before sunset. This contrasts with northern populations, where birds leave the nest much later after sunrise and enter it earlier before sunset. In spite of this, and due to the limited number of daylight hours compared with northern sites, the active day in Sagunto amounts to an average of 750 min, contrasting with the more than 800, and up to 850 min, of northern populations. This implies that females in Sagunto enjoy a longer overnight rest. Ricklefs and Brawn (2013), when comparing nest attendance between temperate and tropical species, suggested that longer attentive periods during the night in tropical species compensate for the lower dedication during the day, therefore making total attentiveness comparable between tropical and temperate species. Available data across Europe suggest that attentiveness varies little among great tit populations or, in other words, females spend a similar amount of time off the nest foraging or performing self-maintenance activities. As suggested by Ricklefs and Brawn (2013), the shorter days in Southern Europe are managed by increasing daytime activity out of the nest and compensating egg attendance during the longer night period.

A case which merits some attention is the attentiveness values reported by Cresswell and McCleery (2003) from Whytham Woods (England). We estimated an attentiveness of 92.5% from their Figure 1, and they stated that "…the percentage of the day spent incubating then (on day 3 after full incubation started) remained near 100% until hatch." Considering 272 species of Passeriformes, Deeming (2002) reported a mean attentiveness of 89% for species where male and female share incubation duties, and 73% for those where only females incubate. He recognizes that many of these values were calculated only during daylight hours, and that considering the continuous overnight incubation period they would increase. In fact, available values for great tits are all above 73%. However, that from Wytham is still strikingly higher than all the other values available for great tit populations recorded using different methods. Unfortunately, the other data set available from Wytham (Hinde 1952) does not provide information on attentiveness.

A consequence of the more limited number of daylight hours is that females in southern populations have less useful time to accommodate their trips off the nest to forage or performing other self-maintenance activities. A result supporting that short days might be a constraint is that the duration of the active day in Sagunto increased with increasing number of daylight hours. Several possibilities are open to cope with this reduction: (1) females need less food, or food is more easily available; and/or (2) eggs need less attention so females could spend more time foraging without risking the development of embryos. Egg and clutch sizes are generally smaller in southern latitudes (Sanz 1998, Encabo et al. 2002), and attentiveness decreases with clutch size and egg size (Deeming 2002, this study). On the other hand, temperatures are higher in the south, so energetic demands for the incubating females should be lower, and rates of cooling of the eggs slower (e.g Carey 2002); adjustments on nest composition could also contribute to maintenance of egg temperature (e.g. Deeming et al. 2012; Mainwaring et al. 2012). Finally, body size is smaller in southern populations (Encabo et al. 2002), so energetic costs of maintenance should be also lower. As said above, the level of attentiveness throughout the 24-h day is very similar among populations, and remarkably similar between Spain and Norway, the two extreme populations for which data are available. Considering only the active day, percent attentiveness is slightly lower in Sagunto than in northern populations. Altogether, females in our population seem to sacrifice a relatively low amount of time on the eggs, and basically cope with the reduction of daylight hours through a decrease in time allocated to foraging and self-maintenance. This might be achieved by their smaller size, lower energetic needs in a relatively mild environment, and perhaps savings in energy needed to rewarm eggs after off-bouts. Food availability might also play a role. We know that, in Sagunto, great tits feed their nestlings with an unusual food resource for the species (adult moths instead of caterpillars; Barba and Gil-Delgado 1990; Barba et al. 2004), but female diet and food abundance during incubation are unknown.

Total time allocated to warming eggs can be achieved through different combinations of periods on and off the nest (e.g. Conway and Martin 2000a; Martin et al. 2007; Cooper and Voss 2013). Therefore, independently of a potential latitudinal variation in nest attentiveness, incubation rhythms could also show latitudinal patterns. However, we are not aware of any study showing latitudinal variation in incubation rhythm within a species. In inter-specific comparisons, Conway and Martin (2000a) concluded that species breeding in colder environments made shorter off-bouts, to avoid excessive cooling of the eggs, and have therefore to perform many foraging trips, thus also reducing on-bout duration. This general pattern in across species comparisons does not seem to apply to the within species example presented here. On one hand, the duration of off-bouts is longer in the extremes of the distribution range, with less favorable habitat types [orange trees in Sagunto, spruces *Picea abies* in Målsjøen], which perhaps imply more difficulties in finding food during this period in unfavorable habitats. On the other hand, the latitudinal pattern of on-bout duration is not clear, being its average duration remarkably similar (26 *vs* 28 min) in Sagunto and Målsjøen respectively. Moreover, in spite of the large differences in daylight hours, the number of on- or off-bouts per day is remarkably similar for those populations with available data, including the two extremes of the latitudinal range considered.

On the whole, our analysis of latitudinal trends of incubation behavior of great tits agrees with the suggestion that “broad scale geographic differences in incubation behavior may be explained by individual-level responses to environmental conditions.” (Ardia et al.

2006). Perhaps "latitude" might capture many factors which could directly or indirectly affect incubation rhythm, from daylight or temperatures to individual size or reproductive investment, and broad latitudinal patterns could be found when considering many populations (as in the case of interspecific comparisons). But variability within these general potential trends might be large and dependent on local constraints, including some considered here and others as parasite loads, predation risk, availability of nest materials, etc.

## Time

Studies in several species with uniparental incubation (Weeden 1966; Joyce et al. 2001; Wheelwright and Beagley 2005; Marín 2008) have reported variation of the incubation rhythm along the day, though others have failed to do so (Reid et al. 1999; Maurer 2008). Haftorn (1981a), though finding daily variation in the incubation pattern in great tits, concluded that time of day *per se* was not determinant, being temperature the important factor determining incubation rhythm. We did not find a pattern of diurnal variation in any of the variables examined, perhaps suggesting that the range of temperatures experienced by incubating females does not condition their attentive patterns on a regular basis, though we have shown that temperatures might affect some behaviors.

It is known that both the thermal tolerance of avian embryos and the energy and time constraints of the parents vary along the incubation period (Webb 1987), and egg-cooling rates increase as embryos age (Cooper and Voss 2013). Birds use to respond with concurrent changes in some components of the incubation rhythm. Most studies reporting this variation found an increase in nest attendance as embryos aged (e.g. Voss et al. 2006; Rompré and Robinson 2008; Greeney 2009; Ricklefs and Brawn 2013). Cooper and Voss (2013) found that the duration of both on- and off-bouts in black-capped chickadees *Poecile atricapilla* decreased as the incubation progressed. The reduction of off-bouts along the incubation period has also been found in other species (e.g. Spaans et al. 2007). We also recorded a decrease in the duration of off-bouts in our great tit population. On-bouts were a bit shorter in the last incubation stage, but differences with previous stages were not significant.

The combination of times on and off the nest, and their relative frequencies use to end up with an increase in total attentiveness by the end of the incubation period (Spaans et al. 2007; Cooper and Voss 2013). For example, the simultaneous reduction of on- and off-bouts (on-bouts decreasing less than off-bouts), along with an increase in the frequency of off-bouts, produced and increase of nest attentiveness in black-capped chickadees (Cooper and Voss 2013). They suggested that birds keep egg temperatures within a narrow range by performing more, shorter, foraging trips. Our results also show that female great tits spend more time on the eggs as embryos aged, mainly due to shorter off-bouts. A more constant attention to the eggs as hatching approached is consistent with the idea of higher involvement of the parents as the fitness value of the eggs increased (Webb 1987).

Incubation patterns have been reported to vary along the breeding season (e.g. Wheelwright and Beagley 2005; Zimmerling and Ankney 2005; Ardia et al. 2009). Our data are restricted to first clutches laid during a relatively short period, so variation along the complete breeding season is missing. The only variable directly related to date was the time of first leaving of females, which was earlier as the season progressed. Wheelwright and Beagley (2005) also found that savannah sparrows *Passerculus sandwichensis* also leave the nest earlier in the morning as the season progressed. In our case, the advance of the time of nest leaving was also related to the time of sunrise and the increase of temperatures. Given the relationship between the duration of the active day and the number of hours of daylight, and the fact that many females leave the nest even before sunrise, it seems that the seasonal variation detected was due to the need of females to start their day as soon as possible, and it is the time of sunrise the main factor governing the decision of leaving the nest early in the morning.

### Weather

Though ambient temperature could be thought to be an important factor affecting nest attentiveness and duration of on- and off-bouts, either negative, positive, or no correlations have been found in observational studies (Conway and Martin 2000b). Experimental studies are also inconclusive. For example, heating nests may either cause an increase (Reid et al. 1999; Ardia et al. 2009) or a decrease (Londoño et al. 2009) in nest attentiveness, through different changes in the duration and frequency of on- and off-bouts. When considering the effect of ambient temperatures, changes in birds' behavior should depend on the actual values of temperature. For example, Conway and Martin (2000b) only found that the duration of on- and off bouts increased when ambient temperatures increased between 9-26 ºC, but not at lower or higher temperatures. In their review, they noticed that positive correlations between temperature and off-bout duration were found in studies performed in colder environments, negative correlations were found in hotter environments, and lack of correlation in those performed in environments with moderate temperatures. In our population, no relationship between temperatures and on- or off-bouts were found, suggesting that other factors are more important in governing incubation constancy. Considering the 14 cases in which no correlation between temperature and on- or off-bout duration (Conway and Martin 2000b, Appendix), mean temperature was 19.3 ºC (SD = 4.6, range: 10.8-26.5) and maximum 25.9 ºC (SD = 4.9, range: 16.3-33.0). During the incubation period, mean temperatures in Sagunto were around 16-17 ºC, and maximum around 21-22 ºC, therefore within the range considered as “moderate”.

It would be expected some degree of interdependence between the duration of consecutive on- and off-bouts. Conway and Martin (2000b) found that previous bout duration explained variation in both on- and off-bout duration in orange-crowned warblers *Vermivora celata*. However, this is not always found (e.g. Wheelwright and Ankney 2005). We did not find significant relationships between the duration of on-bouts and the duration of previous on- or off-bouts, nor between the duration of off-bouts and that of previous on-bouts. However, the duration of off-bouts was inversely correlated with the duration of the previous off-bout. It seems that birds which make a long foraging trip reduce the duration of the next one, or *vice versa*, thus compensating their absences from the nests in the short time.

Temperatures affected mainly to the length of the active day in our population: females left the nest earlier and retired later in the evening as ambient temperatures increased. There was no significant correlation between the length of the active day and temperature because hour of first leaving was more related to maximum temperatures, while the hour of retirement was more related to minimum temperatures. These results also support the idea that females have limited time for foraging. Reid et al. (1999) showed that female starlings *Sturnus vulgaris* whose nests were heated left the nest later in the morning than control ones, suggesting that those females with less energetic needs prolonged overnight attendance. In our case, higher temperatures probably reduce egg-cooling rates, so females could leave the nests early in the morning to look for food after the relatively long night at this latitude.

Rain during an incubation session appears to discourage the bird from leaving the eggs, leading to longer than normal sessions (Deeming 2002). Other studies have not found relationship between rain and nest attendance (e.g. Marín 2008), though the effect of rain would depend on its intensity and duration (Skutch 1962), and the concurrent variation on temperatures and food availability. We only found that, on rainy days, females leave the nest later in the morning, though only 3 rainy days could be included in this analysis.

### Clutch volume

Clutch size and volume are important parameters determining incubation patterns across species (Deeming 2002). In particular, attentiveness decreases with egg mass and with total clutch mass in a wide range of species, and this was also true when considering only species with female-only incubation. Thermal characteristics of the eggs (i.e. their cool or re-warming rates) vary with egg mass, and this would affect parental behavior to keep egg temperature within a narrow window. At this interspecific scale, it seems that lower egg masses allow longer off-bouts (Deeming 2002). However, we are not aware of intraspecific studies dealing with the effects of egg or clutch volume on incubation rhythms. We found that clutch size, egg size and clutch volume affected the duration of off-bouts during the early incubation stage in great tits, females making longer off-bouts as number and size of eggs decreased. We therefore show, for the first time, that, at least during part of the incubation period, attentiveness is also affected by egg and clutch volume within a species. There were, on the other hand, some previous studies showing relationships between clutch size and incubation rhythm. Thus, Blagosklononv (1977, in Deeming 2002) also found a positive relationship between clutch size and attentiveness in pied flycatchers *Ficedula hypoleuca*, while Cooper and Voss (2013) found that on-bout duration of incubating black-capped chickadees increased with clutch size, though the duration of off-bouts was not related to clutch size. Nevertheless, more within-species studies are necessary to show how general these patterns are.

### Parental quality

The physical condition of an animal could, to a great extent, condition the pattern of resource allocation to reproduction and self-maintenance (McNamara and Houston 1996). Maintaining an adequate egg temperature is a very energy-demanding activity for incubating females (e.g. Thomson et al. 1998; Tinbergen and Williams 2002). Consequently, traits related to female quality, as body condition (Hegyi and Sasvári 1998; Gorman and Nager 2003) or age (Joyce et al. 2001; Bogdanova et al. 2007) have been shown to affect incubation patterns. For example, Joyce et al. (2001) showed that older black-throated blue warblers *Setophaga caerulescens* make longer on-bouts than young ones. On the other hand, an experimental reduction of energy constraints on female great tits caused an increase in attentiveness (Bryan and Bryant 1999). In our case, heavier or bigger (wing length) females had a longer active day, left the nest more frequently, performing shorter on-bouts, and (during the middle incubation period) spending a lower proportion of time incubating. There was also a trend for females in better condition to expend a higher proportion of their active day out of the nest.

Male characteristics affected female incubation behavior. Thus, females paired with bigger males (either longer wing or tarsus) spend a higher proportion of their time out of the nest (during the middle incubation stage) and made longer off-bouts (during the late incubation stage). Furthermore, females paired with young males entered the nest in the evening earlier than those paired with adult ones.

On the whole, it seems that better quality females, and/or those paired with better quality males, spend more time off the nest, thus reducing attentiveness. This seems, at first glance, an unexpected pattern. There are two potential explanations for our results. First, that better quality females allocate more time to foraging or self-maintenance activities, perhaps being more effective in heating the eggs when they were at the nest. Second, that better quality females, by being more effective in rewarming egg during on-bouts, could spend more time out to avoid predation. Though Rompré and Robinson (2008) suggested that reducing nest attentiveness to reduce adult predation would not be a good strategy, since this would imply higher embryo mortality, the final outcome of this trade-off would depend on the relative pressure of adult predation at nests (e.g. Conway and Martin 2000a; Martin 2002; Fontaine and Martin 2006). Nearly half of the predation events detected in nests in our study area (48%, *n* = 147 between 1997 and 2012) involved the predation of the female, either during incubation or brooding (own unpubl. data), so being within the nestbox is a risky activity, and reducing the time inside would increase survival probability.

Two main hypotheses have been proposed to explain when an incubating bird should end an incubation bout. The “parental energy” model states that females should leave the nest when reaching an energy threshold (Chaurand and Weimerskirch 1994), while the “egg temperature” model suggests that females should leave when eggs reach a certain temperature (White and Kinney 1974). On the other hand, Deeming (2002) already stated that “for species with small eggs, variation in attentiveness may not necessarily reflect the need of the bird to forage, but rather indicates the ability of the bird to leave its egg for more time during incubation.” Within the general foraging-time constraint that relatively short days seem to impose to incubating females in Sagunto, the behavior of individuals in better condition also suggest that avoiding predation at the nest is an important factor in shaping the incubation rhythm. If this is so, it would imply that females would leave the nest when eggs reach a certain temperature threshold, not necessarily when their own energetic reserves force them to leave for foraging. Measuring egg temperatures would be necessary to solve this question.

Following this argument, we would have expected that young females (assumed of lower quality), would make shorter off-bouts than older ones. However, the opposite was found during the early incubation stage. Perhaps young females, during the early incubation period, need more time foraging to maintain an adequate body condition, probably at the cost of risking the maintenance of an adequate egg temperature. Nevertheless, as said above, this result was based on data for only 3 old females, so it should be taken with caution.

### Conclusions

Altogether, in the population studied, attentiveness increases along the incubation period, and better quality females spend more time off the nest. The quality of males also contribute in the same direction: females paired with better quality males spend more time off the nest. Ambient temperatures and time of sunrise are important in determining the length of the active day. Finally, clutch volume is also important in the early incubation stage, small-volume clutches allowing females to spend more time off the nest.

When comparing incubation rhythm of several European populations, we found that attentiveness is very similar between populations though, due to differences in daylight, southern populations spend more time on the eggs during the longer overnight period. Mean on- and off-bout duration had no clear latitudinal pattern, though duration of off-bouts seems to be longer in extreme populations.

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# ARTÍCULO 7

## *Responses of great tits to thermal stress during incubation*

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ENVIADO PARA PUBLICACIÓN

> "La vida, realmente no es el tiempo
> que pasa, sino aquellos momentos en
> los que el tiempo parece no pasar."

# Responses of great tits to thermal stress during incubation

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**Short title**
Thermal stress during incubation

*Key words:* Cooling nests, Heting nests, Incubation recesses, Incubation sessions, Intermittent incubation, Nest temperature

## ABSTRACT

In bird species when only one member of the pair incubates the eggs, it should trade-off its time between caring for the eggs and leave the nest to forage or perform other self-maintainance activities. Thus, daytime incubation is intermittent, and the number of “sessions” and “recesses”, and their length, condition egg temperatures and therefore embryo development. We evaluated female investment decisions, and their consequences, by experimentally heating or cooling great tit *Parus major* nest during the incubation period in a Mediterranean population. Heated females reduced their incubation time, by increasing the duration of their recesses. Even though mean nest temperatures were higher in heated than in control nests, heating did not affect breeding success, size or condition of the nestlings, or parental condition or behaviour when feeding nestlings. On the other hand, cooled females were able to compensate the treatment, and temperatures in cooled nests were similar to those in control ones. Breeding success was similar in cooled and control nests, and neither nestlings nor parents showed negative effects of the cooling treatment. Several other studies have cooled or heated nests of different species during incubation, and the consequences for parents and embryos or nestlings differed greatly among them. We conclude that it is time for a large scale study to ascertain whether these differences are attributable to the experimental protocols or to factors related to the behaviour of the species, nest type, or differences in environmental conditions.

## INTRODUCTION

Avian eggs need to be kept within a narrow range of temperatures (36-38 ºC; Drent 1975; Webb 1987) for embryos to develop adequately. Though many species build well-insulated nests, eggs are very frequently exposed to ambient temperatures outside this range, so parents should attend the eggs to prevent chilling or overheating. In most habitats, ambient temperatures during the incubation period are below those required for embryo development, so parents must transfer heat from their brood patch to the eggs to maintain adequate egg temperatures (Deeming 2002; Turner 2002). This heat transfer was formerly considered rather inexpensive for the incubating bird, though accurate measurements have made evident that incubation is energetically costly (Williams 1996; Tinbergen and Williams 2002). On the other hand, parents must spend time and energy to prevent eggs from overheating in hot environments by egg shading, egg wetting and/or egg burying (Carey 2002). Moreover, at least in some habitats, incubation behaviour might increase predation risk (Martin et al. 2000; Muchai and Plessis 2005).

To attend the energetic demands of incubation, birds might either store resources before breeding, and rely on these to satisfy the needs of the different breeding activities ("capital breeders"), or adjust food intake to the current needs, and therefore alternate foraging and reproductive activities during the breeding season ("income breeders") (Jönsson 1997; Meijer and Drent 1999). In bird species adopting an income-breeding strategy, and where only females incubate, females should be constantly deciding whether to invest in themselves, foraging or performing other self-maintenance activities, or in their eggs, taking care of keeping them within the narrow range of temperatures appropriate for embryo development (Williams 1996; Tinbergen and Williams 2002; Turner 2002). Thus, daytime incubation is intermittent, with incubation bouts ("sessions") followed by foraging trips out of the nest ("recesses"), and therefore eggs are experiencing a variable temperature regime.

A way to evaluate female investment decisions, and their consequences for parents (especially females) and embryos or nestlings, is modifying incubation costs. For example, a number of studies have altered incubation costs by increasing or decreasing clutch size (e.g. Moreno and Sanz 1994; Visser and Lessells 2001; Hanssen et al. 2005; de Heij et al. 2006). However, direct manipulation of the thermal environment would be a better approach (Reid et al. 2002; Nilsson et al. 2008). A handful of field studies have either provided extra heat (Davis et al. 1984; Haftorn and Reinertsen 1990; Reid et al. 1999; Bryan and Bryant 1999; Reid et al.

2000; Cresswell et al. 2004; Magrath et al. 2005; Londoño et al. 2008; Pérez et al. 2008; Ardia et al. 2009) or cool (Davis et al. 1984; Haftorn and Reinertsen 1990; Nilsson et al. 2008; Ardia et al. 2010) during incubation, and examined their consequences for parents, embryos and/or nestlings.

In spite of the experimental nature of these studies, relatively similar manipulations resulted in opposite results. Thus, providing extra heat could increase (Reid et al. 1999; Cresswell et al. 2004; Ardia et al. 2009), decrease (Davis et al. 1984; Haftorn and Reinertsen 1990; Londoño et al. 2008), or have no effect (Magrath 2005) on female attentiveness (percentage of time spent incubating). Similar variation could be found in the duration of incubation sessions and recesses. The effects on female condition seem also diverse, since Magrath et al. (2005) reported that heated starling *Sturnus vulgaris* females were lighter than controls after the heating treatment, Pérez et al. (2008) found that heated tree swallow *Tachycineta bicolor* females gained mass, and Cresswell et al. (2004) found no differences in mass or metabolic rate between heated and control pectoral sandpiper *Calidris melanotos* females. Concerning embryo and nestling development, results are also contradictory. Reid et al. (2000) examined a large number of parameters, finding only that breeding success (fledglings produced per egg laid) was higher in heated nests, and that females whose first clutch was heated had better hatching success in their second clutches. In contrast, Londoño et al. (2008) found that the size of 10-days old northern mockingbird *Mimus poliglottos* embryos was smaller in heated than in control nests. Pérez et al. (2008), using a cross-fostering design, showed that heating tree swallow nests mostly benefits females, so they could raise their nestlings more effectively, being the direct effects on embryos only apparent during the first week after hatching and disappearing later on.

Data from cooled nests are even scarcer – only four studies (Davis et al. 1984; Haftorn and Reinertsen 1990; Nilsson et al. 2008; Ardia et al. 2010) have attempted this approach. Nonetheless, results also differ. For example, Davis et al. (1984) and Haftorn and Reinertsen (1990) reported an increase of attentiveness during the incubation period in cooled nests, while Ardia et al. (2010) found a reduction, with different responses in the duration of sessions and recesses. There is at least some consensus in the two studies considering the effects of cooling on nestlings and parents (Nilsson et al. 2008; Ardia et al. 2010), both finding negative consequences, even though the details varied between them.

Summarizing, relatively few studies have manipulated thermal conditions during incubation. The duration and intensity of temperature modification and the response variables considered are different in each study, so results are sometimes difficult to compare. Though there is agreement on the negative consequences of cooling on parents and nestlings, there are only two studies to support these, both performed on relatively cold climates. The consequences of heating varied greatly among studies. Therefore, there is a clear need of more experiments, performed under diverse climate conditions, to look for general conclusions about the effects of thermal conditions on embryo and nestling development and survival, and on parental behaviour and fitness.

We present here an experimental study on the effects of thermal conditions during incubation on different fitness-related factors, including parental behaviour and condition, length of the incubation period, and breeding performance. For this, we heated and cooled great tit *Parus major* nests during the incubation period in a Mediterranean population breeding in nestboxes. This is, to our knowledge, the first study where both treatments are applied simultaneously in a bird population. It could be initially predicted that experimental cooling would increase incubation costs, while heating would decrease them, and females would allocate these extra costs or savings among themselves and/or their offspring (e.g. Bryan and Bryant 1999; Ardia et al. 2010). However, the study was performed in a relatively mild climate, and it could be also argued that some cold would be beneficial while some hot might cause disadvantages. For example, Greño et al. (2008) have shown in this population that hot ambient temperatures during the nestling period reduced recruitment probabilities. On the other hand, Bryan and Bryant (1999) found that experimental heating during the night had little effects on energy expenditure of incubating great tit females in mild nights. This, along with the contradictory results of previous studies, forced us to be cautious about our predictions.

## METHODS

The study was conducted on a great tit population breeding in nestboxes within an extensive orange *Citrus aurantium* monoculture in Sagunto (Valencia), Eastern Spain (39º42’N, 0º15’W, 30 m a.s.l.), in 2009. Wooden nestboxes (see characteristics in Lambrechts et al. 2008) are available in this area since 1986 (Encabo et al. 2001; Tomás et al. 2012). Great tits are uniparental incubators in which the female only incubates for about 66% of the daytime in the studied population (Álvarez and Barba, unpubl. data). Average clutch size is about 8 eggs (Atiénzar et al. 2012), the incubation period lasts around 13 days (Barba 1991), and males feed their mates during this period. After hatching, both sexes take part in feeding of the young.

### Experimental design

We visited all nestboxes at least once a week, and daily in some periods, from late February to the end of the breeding season by mid July. This pattern of visits allowed us to estimate the date of laying of the first egg (assuming the laying of one egg per day), the date of start of full incubation (eggs uncovered and warm or female seen incubating; Haftorn 1981) and clutch size. Only first clutches were used in this study.

When full incubation started (incubation day 1), nests where randomly assigned to one of the following treatments: heated (*n* = 12), control (*n* = 12) or cooled (*n* = 11). Sample sizes varied as nests were lost along the nesting period.

On incubation day 1, nests were prepared for the treatments. Eggs and nests were carefully taken out of the nestbox, an inverted U-shaped wire mess was placed on the nestbox floor, and the nest placed again on the mess. This leaved a "box" below the nest where cool, heat or control pads could be placed. There were about 1 cm between the pad and the bottom of the nest; this was especially convenient for a correct functioning of the heat pads, which need oxygen for chemical reactions. We used commercial warming pads (Uniheat 72 h) that, when exposed to the air, produced elevated temperatures for 3 days due to the oxidation of iron powder. Cool pads (9-cell Flexible Ice Blankets of 12 ml each) were stored at -20 ºC and taken into the field in a portable freezer with ice pads to keep them frozen. Control pads were cool pads at ambient temperature.

Control, cool or heat pads were placed below the nests around 07:00 on incubation day 2. Cool pads were replaced four times a day (around 07:00, 11:00, 15:00 and 19:00). Heat pads were changed every second day. Control pads were not changed. Nevertheless, to apply the same degree of perturbation to all the nests, they were visited, and pads removed and replaced or changed if necessary four times a day. Pads and wire messes were removed by 07:00 on incubation day 13. Thus, heated nests experienced a continuously elevated temperature, while cooled nests experienced bouts of cold temperature when cool pads were replaced, which were slowly disappearing as ice melted (see below).

On incubation day 2, when nests were taken out to place the wire mess, we measured the thickness of the bottom (digital caliper, 0.01 mm accuracy; see e.g. Álvarez and Barba 2008), as this might affect heat or cool transfer from pads to eggs. Nests were collected after fledging or failure, placed into sealed plastic bags, and stored at -20 ºC. After the breeding season nests were dried in an oven (105 ºC, 12 h) and weighed (digital balance, 0.01 g accuracy; see Álvarez et al. 2013 for details), so we are reporting here dry nest masses.

Two females (1 heated, 1 control) deserted after placing the wire mess, while 8 females (2 heated, 3 control, 3 cooled) abandoned the nest during the experiment (days 2-11).

## Breeding performance

The pattern of visits allowed us to estimate the date of hatching of the first egg, the length of the incubation period (from the date of start of full incubation to the date previous of the hatching of the first egg, both included), the number of eggs hatched and the number of fledglings. From these, we estimated hatching success (proportion of eggs which hatched), fledging success (proportion of nestlings which fledged), and breeding success (proportion of eggs producing fledglings). These percentages were arcsin square root transformed for analyses (Zar 1996).

We individually marked the nestlings on day 5 by painting different parts of the body with a permanent non-toxic felt pen, remarked them at day 7, and ringed them with individually numbered aluminum rings at day 10, so that they could be individually recognized during their nesting period. Nestlings were weighed (electronic balance, ± 0.1 g) and tarsus length measured (digital caliper, ± 0.01 mm), and an index of body condition for each individual was calculated as mass to tarsus length ratio when they were 5, 10 and 15 days old. We calculated tarsus (mm/day) and mass (g/day) growth rates between days 5 and 10, and 10 and 15, as a measure of nestling growth during two phases of the nestling period.

Parents were captured at the nest when nestlings were 10-11 days old using nest-door traps. We measured their wing (stopped rule, ± 0.5 mm) and tarsus length (digital caliper, ± 0.01 mm), weighed them (electronic balance, ± 0.1 g), and determined their sex and age (first year versus older birds) using plumage characteristics and the presence of brood patch (Svensson 1996). The index of body condition was also estimated. Males and females were ringed with individually numbered aluminum rings and fitted with a passive integrated transponder (see below).

Apart of the experimental ones, all the nestboxes in the study area (509 nestboxes in 2009) were checked once a week until early July, those containing nests following a different protocol for other studies. We tried to trap the parents from all of them when nestlings had 10-13 days. Thus, we could check which of the experimental pairs made a second breeding attempt that season, provided it was not lost before nestling day 10-13. For the present study, we recorded the number of fledglings produced in these second breeding attempts.

Finally, we tried to trap all the breeding pairs in the population during the 2010 and 2011 breeding seasons. This allowed us to estimate recruitment rates of fledglings produced in the experimental nests. Sample sizes were too low to have a sensible estimate for parental survival.

### Nest temperature and incubation behaviour

We used HOBO Pro v2 data-loggers to document nest and ambient temperatures. One of the probes was usually attached to the back of the nestbox or to a close branch, so that it was permanently in the shade, while the other was placed directly above the wire mess, but below the nesting material (e.g. Dawson et al. 2005). Temperature readings were recorded every 2 min during the sampling period.

We had four data-loggers, and these were moved between nests. We divided the incubation period in 2 stages (early: days 3-7, and late: days 8-12) and try to take samples from each active nest at least one day within each period. At each nest, data-loggers were placed at the second daily checking (by 11:00 h) and removed next day by the same time. Temperature data from each thermometer was downloaded to a shuttle when thermometers were moved from one nestbox to the other, so the thermometers were continuously into the field. Data were downloaded from the shuttle to the computer each day using the program HOBOware pro. These were converted into excel file to obtain temperature traces for each sample.

The times when females were on or off the nest generally showed as clear peaks and troughs on the resulting temperature traces, allowing times of arrival and departure to be deduced (e.g. Holcomb 1974; Reid et al. 1999). The traces showed a clear demarcation between the quiescent overnight incubation period, when the female remained on the nest, and the active daytime incubation period when the female was alternately incubating and foraging. During the daytime period, identification of the points of arrival and departure allowed us to calculate the number and duration of the incubation and foraging bouts, and hence the percentage of the daytime spent incubating. The duration of the female's overnight incubation period was also calculated. As an additional estimation of attentiveness, the presence or absence of the female on the nest was noted each of the four times per day that each nest was checked, thus amounting over 40 checkings per nest during the incubation period.

To summarize the temperatures experienced by nestlings, we averaged internal temperatures of each nest during each of the incubation stages and considering a "daytime" (from 08:00 to 17:59, where females were entering and leaving the nest) and a "night" period (22:00 to 04:59, where females were within the nestbox). Times were selected to avoid the approximate periods when females start and finish the overnight period. Temperatures from one random day were taken if more than one day was available from a certain incubation stage. We also computed the difference between external and internal temperatures at each 2 min interval, and averaged them for each nest and incubation stage.

### Parental feeding frequencies

We used here PIT (Passive Integrated Transponders) to study feeding frequencies (e.g. Freitag et al. 2001; Nilsson et al. 2008) when nestlings were 11-12 days old. When captured, each parent was fitted with an implantable PIT tag (Trovan ID 100; length: 11.5 mm, mass: 0.1 g) with unique codes. PIT tags were injected subcutaneously in the back of the birds in the featherless area above the scapula, following Nicolaus et al. (2008). Parent entrances to and leavings from the nests were recorded by means of a transponder reader system consisting of an antenna fitted to a nestbox door and connected to a datalogger (Trovan LID-650) and a 12 V battery mounted in a plastic box covered with camouflage fabric (e.g. Nilsson et al. 2008). When a tagged bird enters or leaves the nestbox, it interrupts the optical barrier, triggering the reading of the bird's corresponding PIT and a visit is stored in the memory, along with the exact time at which it occurred. Nestboxes used were opened at the front, so the original door could be removed and the one fitted with the antenna placed when setting the reader.

The box with the reader was placed at the base of the tree (nestboxes were at most 1 m above ground). The installation of this device took about 2 minutes. The system was typically mounted by 08:00 h in the morning of the sampling day (one day after parents were captured), and was left operating until next morning (about 08:00 h). For each nest, we estimated the feeding rates per hour of males, females, and the total, as well as the feeding rate per hour per nestlings (by dividing total feeding rate by the number of nestlings present).

### Statistical analyses

As there were no significant differences in initial conditions (laying date, clutch size, date of start of incubation, nest dimensions, parental characteristics; see Results) between treatments, there were no need to correct for these factors when comparing breeding performance between experimental groups.

We generally used univariate general lineal models to check for differences in parameters of interest between treatments (treatment as fixed factor), though there were some exceptions. To test for differences in nest temperatures and differences between internal and external temperatures, both treatment and incubation stage were included as fixed factors. Also, we used lineal mixed models, with nest identity as a random factor and treatment as a fixed factor, to check for differences in nestling tarsus length, mass, condition, and growth between treatments. Finally, we used chi-squared tests for looking at differences between the frequency of parental age classes and recruitment rates of fledglings.

Data were analysed by using SPSS v. 19.0 statistical package, according to Zar (1996). Means ± SD are shown where appropriate.

## RESULTS

### Pre-experimental data

The first egg of experimental clutches was laid between 30 March and 18 April and there was no difference in mean laying dates between treatments ($F_{2,32} = 0.08$, $P = 0.92$). Clutch size varied between 6 and 12 eggs, with no difference in mean clutch size between treatments ($F_{2,32} = 0.32$, $P = 0.73$). Finally, incubation in these nests started between 8 and 24 April, and there were no differences in mean starting dates between treatments ($F_{2,32} = 0.07$, $P = 0.93$).

Nest mass ($F_{2,28} = 0.05$, $P = 0.95$) and thickness of the bottom ($F_{2,32} = 0.02$, $P = 0.98$) did not differ among nests allocated to the different treatments.

Tarsus length (males: $F_{2,18} = 0.49$, $P = 0.62$; females: $F_{2,18} = 0.18$, $P = 0.84$), wing length (males: $F_{2,18} = 0.09$, $P = 0.92$; females: $F_{2,18} = 0.25$, $P = 0.78$) and age class (males: $\chi^2 = 0.92$, df = 2, $P > 0.05$; females: $\chi^2 = 0.34$, df = 2, $P > 0.05$) of the parents did not differ between treatments.

### Nest temperature

Nest temperatures during the day did not differ among incubation stages ($F_{1,34} = 2.46$, $P = 0.13$), but differed among treatments ($F_{2,34} = 42.42$, $P < 0.001$); the interaction was not significant ($F_{2,34} = 1.06$, $P = 0.36$). *Post hoc* Tukey tests revealed differences between heated and control, and between heated and cooled nests (Figure 1). The difference between nest and ambient temperatures also differed between treatments ($F_{2,34} = 40.97$, $P < 0.001$), being significantly higher (Tukey tests) in heated than in control and cooled nests, and marginally higher in control than in cooled nests ($P = 0.054$). There were no differences between incubation stages ($F_{1,34} = 1.06$, P = 0.31), and no interaction ($F_{2,34} = 1.10$, $P = 0.35$).

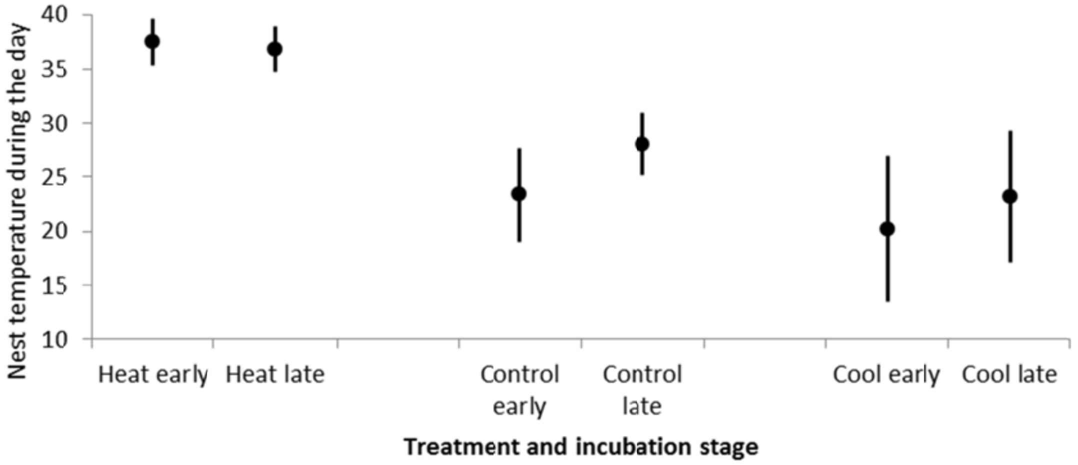

**Figure 1.** Mean temperatures (± SD) during the daytime (from 08:00 to 17:59) in heated, control and cooled nests during the early (days 3-7) and late (days 8-12) incubation stages.

During the night, nest temperatures differed between treatments ($F_{2,33} = 12.56$, $P < 0.001$) and incubation stages ($F_{1,33} = 5.83$, $P = 0.021$); the interaction term was not significant ($F_{2,33} = 1.46$, $P = 0.25$). Thus, nest temperatures in heated nests were higher than those in control and cooled nests, and higher during the late incubation stage. Also, the difference between nest and ambient temperatures differed between treatments ($F_{2,33} = 10.14$, $P < 0.001$), and marginally between incubation stages ($F_{1,33} = 3.97$, $P = 0.055$), with no interaction ($F_{2,33} = 1.42$, $P = 0.26$) . The difference in temperatures was higher in heated than in control or cooled nests (Tukey tests). Overall, it seemed that the treatment was effective in increasing nest temperatures in heated nests, but the cooling treatment was effectively compensated by females, so that mean temperatures were quite similar between control and cooled nests.

## Breeding performance

We did not find significant differences between treatments in the length of the incubation period, number of hatchlings or fledglings, and hatching, fledging or breeding success (Table 1). Size, mass and growth rates of nestlings were not affected by the treatments (Table 2). Recruitment rates were higher for cooled nestlings (20%), intermediate for controls (13%) and lower for heated ones (9%), though these differences were not statistically significant ($\chi^2 = 2.34$, $P > 0.05$).

**Table 1**
Breeding parameters of heated, control and cooled nests during incubation. Mean ± SD and sample size in brackets are shown.

| | Heated | Control | Cooled | *F* | *P* |
|---|---|---|---|---|---|
| Incubation period (days) | 12.44 ± 1.01 (9) | 13.00 ± 1.16 (7) | 12.29 ± 1.11 (7) | 0.85 | 0.4 |
| Number of hatchlings | 5.75 ± 3.96 (12) | 4.83 ± 4.32 (12) | 4.82 ± 3.95 (11) | 0.20 | 0.8 |
| Number of fledglings | 4.42 ± 3.90 (12) | 3.64 ± 4.20 (11) | 4.64 ± 3.91 (11) | 0.19 | 0.8 |
| Hatching success (%) | 91 ± 19 (9) | 95 ± 6 (7) | 92 ± 14 (7) | 0.01 | 1.0 |
| Fledging success (%) | 82 ± 26 (8) | 100 ± 0 (5) | 95 ± 9 (7) | 2.82 | 0.09 |
| Breeding success (%) | 77 ± 30 (8) | 95 ± 6 (5) | 88 ± 17 (7) | 0.93 | 0.4 |

In total, 1 out of 7 successful cooled , 2 out of 5 successful control, and 2 out of 7 successful heated pairs successfully raised a second brood after a successfully fledging the first one. They produced respectively 5, 6 and 8 fledglings.

**Table 2**
Mean ± SD biometric parameters of nestlings at day 5, 10 and 15 experimentally assigned a heated, control or cooled environment during incubation at day 2 and 12. Biometric parameters tested with ANOVA. Sample sizes for groups are presented within parentheses.

| Nestlings | Heated | Control | Cooled | *F* | *P* |
|---|---|---|---|---|---|
| Tarsus day 5 (mm) | 10.95 ± 1.31 (60) | 10.61 ± 1.37 (58) | 11.30 ± 1.53 (51) | 0.89 | 0.43 |
| Mass at day 5 (g) | 6.97 ± 1.43 (60) | 6.93 ± 1.31 (58) | 7.27 ± 1.43 (51) | 0.36 | 0.70 |
| Condition day 5 (g/mm) | 0.67 ± 0.09 (60) | 0.69 ± 0.06 (58) | 0.68 ± 0.07 (51) | 0.43 | 0.65 |
| Tarsus day 10 (mm) | 17.93 ± 1.20 (60) | 18.29 ± 1.06 (54) | 18.01 ± 0.89 (51) | 0.37 | 0.70 |
| Mass at day 10 (g) | 14.05 ± 1.87 (60) | 14.67 ± 1.37 (54) | 14.88 ± 1.56 (51) | 1.14 | 0.34 |
| Condition at day 10 (g/mm) | 0.81 ± 0.07 (60) | 0.82 ± 0.05 (54) | 0.85 ± 0.06 (51) | 1.50 | 0.25 |
| Tarsus at day 15 (mm) | 19.49 ± 0.76 (59) | 19.43 ± 0.63 (40) | 19.38 ± 0.68 (48) | 0.12 | 0.89 |
| Mass at day 15 (g) | 16.00 ± 1.83 (59) | 16.98 ± 1.56 (40) | 17.31 ± 1.29 (48) | 1.94 | 0.17 |
| Condition at day 15 (g/mm) | 0.84 ± 0.08 (59) | 0.89 ± 0.07 (40) | 0.91 ± 0.05 (48) | 2.83 | 0.09 |
| Tarsus growth rate between days 5 and 10 (mm/day) | 1.40 ± 0.18 (60) | 1.52 ± 0.18 (54) | 1.34 ± 0.23 (51) | 2.34 | 0.12 |
| Mass growth rate between days 5 and 10 (g/day) | 1.43 ± 0.25 (60) | 1.53 ± 0.19 (54) | 1.52 ± 0.16 (51) | 1.31 | 0.29 |
| Tarsus growth rate between day 10 and 15 (mm/day) | 0.31 ± 0.17 (59) | 0.27 ± 0.16 (40) | 0.27 ± 0.13 (48) | 0.41 | 0.67 |
| Mass growth rate between day 10 and 15 (g/day) | 0.35 ± 0.37 (59) | 0.49 ± 0.26 (40) | 0.48 ± 0.18 (48) | 1.03 | 0.38 |

### Parental behaviour

Data from thermometers showed differences between treatments in the mean duration of off-bouts ($F_{2,20} = 3.52$, $P = 0.049$), with a mean duration of 20.9 ± 5.9 min ($n = 9$) in heated nests, 16.2 ± 3.3 min ($n = 6$) in control nests, and 15.3 ± 3.6 min ($n = 8$) in cooled nests. However, *post hoc* Tukey tests did not find statistically significant differences. Presence/absence data collected during systematic nest checking confirmed that heated females stayed in the nests less frequently (54%) than control (69%) or cooled ones (66%; $F_{2,21} = 6.54$, $P = 0.006$; Figure 2). We found no differences between treatments in the number ($F_{2,20} = 0.98$, $P = 0.39$) and duration ($F_{2,20} = 1.51$, $P = 0.24$) of on-bouts periods along the day, nor in the duration of the overnight period ($F_{2,17} = 0.32$, $P = 0.73$).

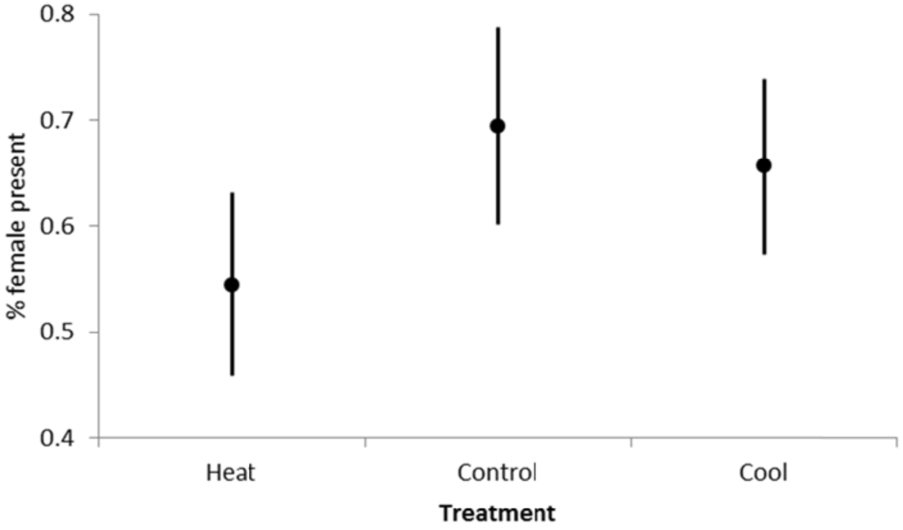

**Figure 2.** Percentage of presence at the nest (± SD) of females when nests were visited for the experimental treatments (4 times per day during the incubation period).

Mass and condition of the parents when feeding 10-11 days old nestlings did not differ between treatments (Table 3). There were no significant differences in the feeding rates neither of males or females, nor in the combined rate or in the rate per nestling, between treatments (Table 3).

**Table 3**
Mass and condition of adults at day 10-11, and feeding frequency at day 11-12 of heated, control and cooled nests. Mean ± SD and sample size in brackets are shown.

| | Heated | Control | Cooled | *F* | *P* |
|---|---|---|---|---|---|
| **Adults** | | | | | |
| Female mass (g) | 16.35 ± 0.39 (8) | 16.40 ± 0.66 (7) | 16.52 ± 1.07 (6) | 0.10 | 0.9 |
| Female condition | 0.85 ± 0.03 (8) | 0.87 ± 0.03 (7) | 0.87 ± 0.05 (6) | 0.36 | 0.7 |
| Male mass (g) | 17.43 ± 1.32 (8) | 17.37 ± 0.50 (6) | 17.34 ± 0.60 (7) | 0.02 | 1.0 |
| Male condition | 0.88 ± 0.06 (8) | 0.87 ± 0.03 (6) | 0.87 ± 0.04 (7) | 0.14 | 0.9 |
| **Feedings per hour** | | | | | |
| Female | 8.61 ± 3.41 (8) | 6.71 ± 2.38 (5) | 9.81 ± 4.26 (5) | 1.04 | 0.4 |
| Male | 10.48 ± 4.10 (6) | 8.54 ± 2.89 (5) | 7.64 ± 2.92 (5) | 1.01 | 0.4 |
| Total | 17.80 ± 5.69 (6) | 15.26 ± 3.88 (5) | 17.45 ± 5.68 (5) | 0.37 | 0.7 |
| Per nestling | 3.36 ± 1.32 (6) | 1.91 ± 0.46 (4) | 2.82 ± 1.46 (5) | 1.71 | 0.2 |

## DISCUSSION

### Effects of heating

The heating treatment was effective in increasing the nest temperature, both during the day and during the night, and therefore parents probably benefited from extra energy to allocate it into different activities. Options were increasing attentiveness, thus improving embryo development and/or shortening the incubation period, or allocating more time to foraging or self-maintaining activities out of the nest. It seems that they chose the second option, as they performed longer off-bouts, and reduced their presence at the nest, as shown by our regular nest checkings.

Our results agree with those of Davis et al. (1984), Haftorn and Reinertsen (1990) and Londoño et al. (2008), who also showed a decrease in attentiveness in heated nests of Belding's savannah sparrows *Passerculus sandwichensis beldingi*, pied flycatchers *Ficedula hypoleuca*, and northern mockingbirds respectively. Londoño et al. (2008) argued that females exposed to high temperatures would need to increase their foraging time, to cope with increasing metabolic rates. However, they did not find this increase in foraging activity in northern mockingbirds, and suggested that the increase in nest temperature might have not being enough to cause a significant thermal stress in females. If this is so, and considering that foraging bout duration depend on foraging efficiency (e.g. Drent et al. 1985; Weimerskirch 1994), which is supposed equal for heated and control birds, no changes in foraging time would be expected in heated females. Another factor which might be important in promoting longer off-bouts is avoiding predation at the nest. Álvarez and Barba (in prep.) have shown that the risk of being preyed upon is relatively high for incubating females in this population, and females in better condition spend more time off the nest than those in poorer condition, suggesting that predation avoidance could be an important factor shaping attendance patterns. Providing extra heat would allow females to leave the nest unattended for longer periods.

Opposite results, i.e. increasing attentiveness in heated nests, have been found in other species, including great tits (e.g. Bryan and Bryant 1999; Reid et al. 1999; Cresswell et al. 2004; Ardia et al. 2009). The argument is that females could allocate this extra energy into improving embryonic development, increasing on-bout duration during the day and duration of the overnight incubation session, and therefore mean egg temperature (e.g. Bryan and Bryant 1999; Reid et al. 1999; Cresswell et al. 2004; Ardia et al. 2009). Moreover, Magrath et al. (2005) did not find differences in attentiveness between heated and control nests, though here both males and females incubated, and heated females decreased attendance while heated males increased it relative to controls, ending up with similar total attendance.

The lower attention of females during the incubation period in heated nests was not clearly reflected in changes in breeding performance relative to controls. This means that the decrease in attentiveness was more or less related to the available extra heat provided by heat pads. If any, the trend was to having a reduced breeding performance (hatching, fledging and breeding success) relative to controls, suggesting that females left the eggs unattended a little more than they should have to, according with the extra energy provided. This trend was also present in the recruitment rates, since only 9% of fledglings from heated nests were present in the breeding population next years. Among studies which found a decrease of attentiveness when heating was applied, Londoño et al. (2008) only follow heated nests until the embryos were 10 days old, and they found that heated embryos were lighter than controls, while Davis et al. (1984) performed cooling and heating treatments in the same nests, so they could not follow the effects of either treatment on fitness related parameters. In a previous study (Greño et al. 2008), we found that high ambient temperatures during the nestling period negatively affect fledgling survival (local recruitment), thus suggesting that heat could have detrimental effects in this population.

On the opposite extreme, studies reporting increased attentiveness in heated nests generally found improvements in the breeding performance (e.g. Reid et al. 2000; Ardia et al. 2009). Pérez et al. (2008) found that the positive effects on tree swallow nestlings was mediated by differences in attendance by the parents, since the final size of nestlings heated as embryos, but raised by control parents, did not differ from control nestlings, even though they have some advantages shortly after hatching (higher residual body mass on days 4 and 7).

The probable savings in energy enjoyed by heated females was not reflected in their mass or body condition when feeding nestlings, so any possible improvement was very short term. Bryan and Bryant (1999) found that daily energy expenditure did not differ between heated and control great tit females in mild nights in Scotland (heating was applied only at night in this study). “Mild” conditions in Scotland mean minimum temperatures of 8-10 ºC. Minimum temperatures in Sagunto ranged between 3.7 and 15 ºC (mean 10.6, sd = 2.8 ºC) during the incubation period of the clutches considered here (8 April to 7 May), so the lack of effect on females could be explained. Results from other studies show also striking differences, from heated females losing (Magrath et al. 2005) or gaining mass (Pérez et al. 2008), to no differences in mass between control and heated females after the treatment (Bryan and Bryant 1999; Cresswell et al. 2004; Londoño et al. 2008).

The only study reporting feeding rates (Pérez et al. 2008) found that heated tree swallow females had higher feeding rates than control ones, while no differences were found for males. We found that feeding rates in heated nests were only a bit, but not significantly, higher than those in control nests.

### Effects of cooling

The cooling treatment failed in producing a significant decrease in nest mean temperature. Temperature traces showed clear droppings when ice pads were changed, and the difference between nest and ambient temperatures was marginally higher in control nests during the day, but females were able to rapidly recover nest temperatures almost to the level of control nests, and therefore mean nest temperatures did not differ between treatments. This was not achieved by increasing their presence in the nests, or the duration of on-bouts relative to control females, so perhaps they were more effective in transferring heat from the brood patch while they were within the nestbox. Temperatures during the night did not differ between control and cooled nests.

The two studies which have experimentally cooled the nest during incubation and examined female responses obtained opposite results, Davis et al. (1984) finding an increase of attentiveness, and Ardia et al. (2010) a decrease. Davis et al. (1984) found an increase of on-bout duration and a reduction of off-bout duration in cooled nests, while Ardia et al. (2010) found a decrease in on-bout duration in cooled nests, and no change in off-bout duration. Though the experimental protocols were rather different, there is no clear explanation for these opposite results. Our study suggest that a third response to a cooling treatment could be more related to female behaviour once she is within the nestbox than to changes in the duration of on- or off-bouts.

As expected, given the above results, breeding performance of cooled and control nests were very similar, confirming that cooled females effectively compensated the treatment. Previous studies have found negative consequences of experimental cooling on nestlings. Thus, tree swallow nestlings cooled as embryos had lower body mass and poorer body condition at fledging than control chicks (Ardia et al. 2010). Similarly, Nilsson et al. (2008) found that blue tit *Cyanistes caeruleus* nestlings cooled as embryos had lower growth rates than control ones, though differences in fledging mass were not significant. There were discrepancies between these two studies in the effects on the duration of the incubation period: Ardia et al. (2010) found a longer period in cooled nests, while Nilsson et al. (2008) did not find differences. Finally, hatching success, hatchling mass, and nestling survival did not differ between control and cooled nests (Nilsson et al. 2008).

The probable extra effort of increasing nest temperature after the replacement of cool pads had not consequences for female condition in the short time (when feeding nestlings), as mass and condition of cooled females were almost the same than those of control ones. The experiment of Nilsson et al. (2008) implied exchanging hatchlings between nests which had been cooled during incubation and control nests, so “cooled” nestlings were attended by control parents, and control nestlings by “cooled” parents. They do not find conclusive evidences of cooled parents being affected by the treatment (similar feeding rates than controls, and also similar, or even better, body condition), though they argued that females cooled during incubation dedicated more energy to themselves during the nestling period, and this had negative consequences for nestlings (lower body mass). Ardia et al. (2010), however, found clear negative effects on females (lower feeding rates and worse body condition than controls).

## Conclusions

Previous studies, along with the present one, reported very different responses of birds to thermal stress during incubation. The idea that “heating is good” (as it provides extra energy), and “cooling is bad” (as it drains energy) is not supported, and it is even contradicted, by some studies. These contrasting responses could have their origin in differences in the experimental protocol (which differs between studies), the behaviour of the species (from starlings to pectoral sandpipers), the nest type (from ground to cavity nesters), and/or the environmental conditions at the site and year of the experiment (all of them only lasted one year). A clear need exists of a large scale study trying to disentangle the relative weight of these factors (e.g. working with the same species and protocol under different environmental conditions) and ideally lasting more than one breeding season, to capture intra-site variability in the responses.

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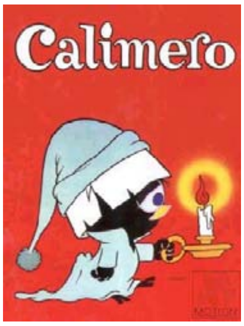
# ANEXO II: RECUERDOS DE CAMPO

En este Anexo se muestran algunos recuerdos de estos años de trabajo.

Siguiendo el orden de las páginas:

- Detalle de una parte del antiguo boletín semanal del grupo de trabajo, llamado “Parus newsletter”, y en la que se me daba la bienvenida.
- Detalle de una página de libreta de campo (arriba) y de una ficha donde se registran los datos de cada nido (abajo).
- Un ejemplo de protocolo general de campo (primero la parte de delante y después la parte posterior del mismo).
- Dibujo de la temporada de campo en Sagunto 2005 por Elena.
- Dibujo de la temporada de campo en Sierra Mariola y Font Roja 2005 por Elena.

# Parus newsletter



Año II, Número 61
29 de Febrero de 2004

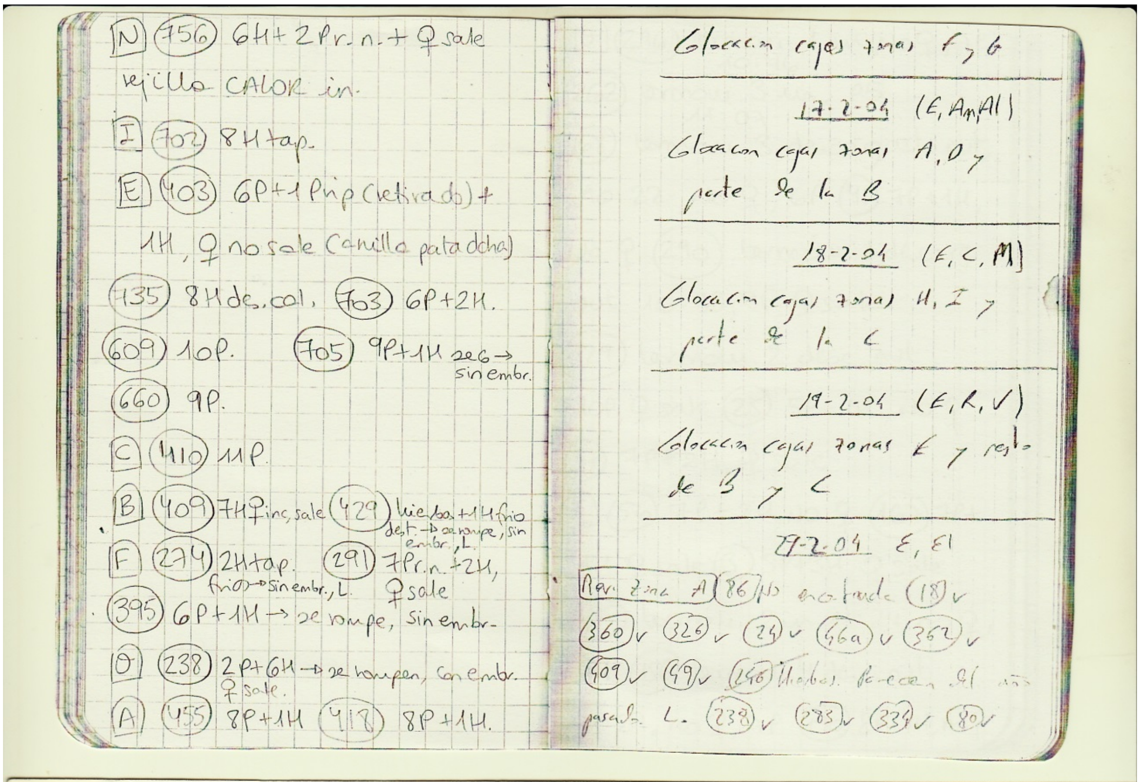
## Resumen de la semana

Semana bastante entretenida, cuyo resumen comenzamos con el apartado burocrático. Hemos firmado finalmente el **contrato con NaturLife Medio Ambiente S. L.** para hacer el trabajo del nacimiento del Río Verde, un *ullal* situado en Massalavés. Creo que todos sabéis ya de qué se trata y, como habéis visto más arriba, ya han comenzado los trabajos. Consiste en la caracterización del ecosistema a todos los niveles, por lo que incluye una descripción de las características físico-químicas del agua, de la vegetación, del plancton, del bentos, de los anfibios, reptiles y aves. Iremos informando de los trabajos y las salidas al campo previstas.

También ha sido una semana movidita en el campo de las nuevas incorporaciones. Cuatro nuevas aspirantes van a probar a ver qué tal se les da eso de deambular por los naranjales en busca de nidos. Por orden de solicitud, son: **Elena**, licenciada en Biología, antigua colaboradora de Rafa Oltra que se ha mudado a los pájaros; **Mónica**, estudiante de cuarto de Biología, compañera de anillamiento de Vero por la Font Roja; y **Amparo** y **Patricia**, estudiantes de tercero de Ciencias Ambientales. Bienvenidas a las cuatro y a ver qué tal se dan las jornadas saguntinas.

Y pasando al trabajo de campo, retomamos lo que quedó pendiente de la semana pasada, que acabó el domingo por la noche con la jornada de Pego-Oliva. Fran nos envía los reportajes pendientes, comenzando con los inicios de los trabajos en el **Río Verde**, en concreto, los censos de aves, que se realizaron el pasado martes:

> El día empezaba temprano para David y yo, ya que a las 4.45 había que levantarse para coger el tren hasta Carcaixent en donde Juanito nos recogió. A pesar de la hora y de no tomar café, no había sueño porque el ambiente refrescaba. Ese día se ejecutaron varias actividades, una de ellas los censos q realizó Juanito y, lo otro, el anillamiento. Los resultados del anillamiento fueron:
>
> -1 Alcedo atthis
> -2 Turdus merula
> -2 Serinus serinus
> -2 Erithacus rubecula
> -1 Parus major



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rejillo CALOR in.
I 702 8H tap.
E 403 6P+1 Pnp (retirado)+
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135 8H des. col. 703 6P+2H.
609 10P. 705 9P+1H se 6→ sin embr.
660 9P.
C 410 11P.
B 409 7H ♀inc, sale 429 huevos+1H frio dest.→ se rompe, sin embr., L.
F 274 2H tap. frio→ sin embr., L. 291 7Pr.n.+2H, ♀ sale
395 6P+1H → se rompe, sin embr.
O 238 2P+6H → se rompen, con embr. ♀ sale.
A 455 8P+1H 418 8P+1H.

Colocación cajas zonas F y G

17-2-04 (E, Am, Al)
Colocación cajas zonas A, O y parte de la B

18-2-04 (E, C, M)
Colocación cajas zonas H, I y parte de la C

19-2-04 (E, R, V)
Colocación cajas zonas E y resto de B y C

27-2-04 E, El
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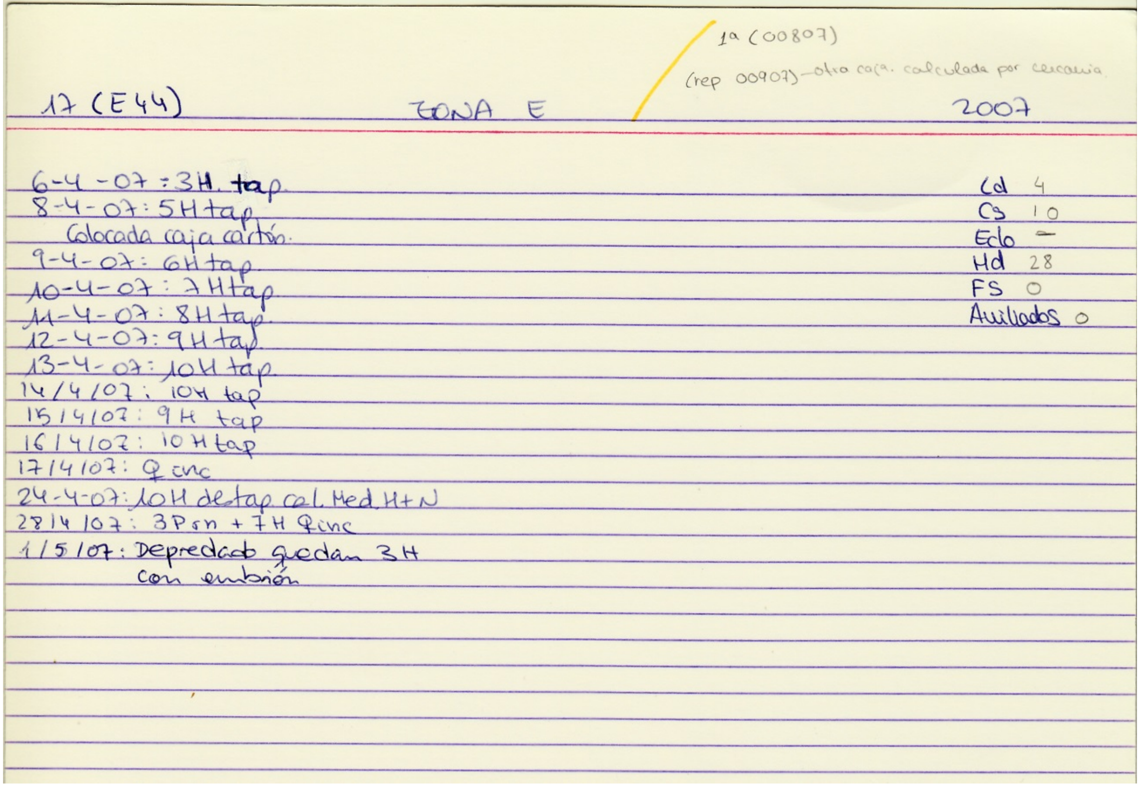

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12-4-07: 9H tap.
13-4-07: 10H tap.
14/4/07: 10H tap
15/4/07: 9H tap
16/4/07: 10H tap
17/4/07: ♀ inc
24-4-07: 10H destap col. Hed H+N
28/4/07: 3P s/n + 7H ♀inc
1/5/07: Depredado quedan 3H con embrión

Cd 4
Cs 10
Eclo –
Hd 28
FS 0
Auxiliados 0

## TEMPORADA DE CRÍA 2009 – OBJETIVOS Y ORGANIZACIÓN DEL TRABAJO DE CAMPO

### OBJETIVOS

**1 Seguimiento a largo plazo de la población.**
- Fecha de inicio de puesta
- Tamaño de puesta
- Tamaño del huevo
- Fecha de eclosión
- Eclosiones
- Volanderos
- Tarso y peso de los pollos; anillamiento con anillas metálicas
- Ala, tarso, peso y edad de los padres; lectura de anilla o anillamiento con anillas metálicas.

**2 Incubación y eclosión.**
- Determinación de los periodos de incubación y eclosión.

**3 Efectos de la temperatura en el nido sobre la incubación.**
- Manipulación de la temperatura en el nido.

**4 Dimensiones de los nidos**
- Medición del tamaño y peso de los nidos.

**5 Crecimiento de los pollos.**
- Medición de tarso y peso a los 5, 10 y 15 días.

**6 Tasas de estancia en la caja durante el periodo de incubación y de ceba.**
- Toma de muestras con transponders.

**7 Muda de las primarias en adultos.**
- Registrar el proceso de muda de las primarias al capturarlos el día 12-14.

### TRABAJO DE CAMPO

**Normas generales de organización y toma de datos**

Los responsables tendrán su **libreta de campo**, en la que registrarán toda la información que tomen. Habrá 2-3 libretas más para los colaboradores. Diariamente, toda la información se pasará a la libreta general. Las libretas quedarán depositadas en el fichero al acabar la temporada. El modelo de tomar notas será el mismo en todas:

- Cada día de trabajo se coloca la fecha (día, mes y año);
- Se identificará la zona de trabajo al principio de registrar las cajas, rodeando la letra con un cuadrado. Se comenzará una línea cada vez que se cambie de zona.
- Se identificará la caja con su número, rodeado con un círculo.
- Tras el número de la caja, se indicará su contenido, según las normas indicadas abajo.
- Al finalizar el día, se traza una línea horizontal, para separarlo del siguiente.

**Para los colaboradores**: Dispondremos previamente de un calendario con la disponibilidad de cada uno, pero cada día contaremos sólo con los que se lo hayan comunicado a Elena en vísperas de ese día **¡Avisad por favor cuanto antes de cualquier cambio!**

**Trabajo de campo y registro de datos**

Revisión **semanal** de todas las cajas (**zonas A-O, 509 cajas**), excepto de aquellas que contengan nidos y requieran otra periodicidad. En caso de no poder revisarse el día señalado, se revisarán **antes**, recuperando después la fecha de visita normal. Si, por cualquier circunstancia imprevisible (lluvia, retraso, enfermedad, etc.), todas o parte de las cajas de la zona a revisar no pueden visitarse, se realizará la visita lo antes posible. Cuando comiencen a aparecer los nidos, cada caja tendrá su propio patrón de visitas. En las **zonas J-M-N-O** sólo se seguirán 20 nidos experimentales (NE, 10 para enfriar y 10 para calentar) y en las **zonas A-I** se seguirán 10 nidos control (NC, a temperatura ambiente). En total 30 nidos que llamaremos Nidos Experimentales y Controles (NEC). Tras completar estos, sólo se les realizará un seguimiento a los nidos de las **zonas A-I** y será el de la forma habitual.

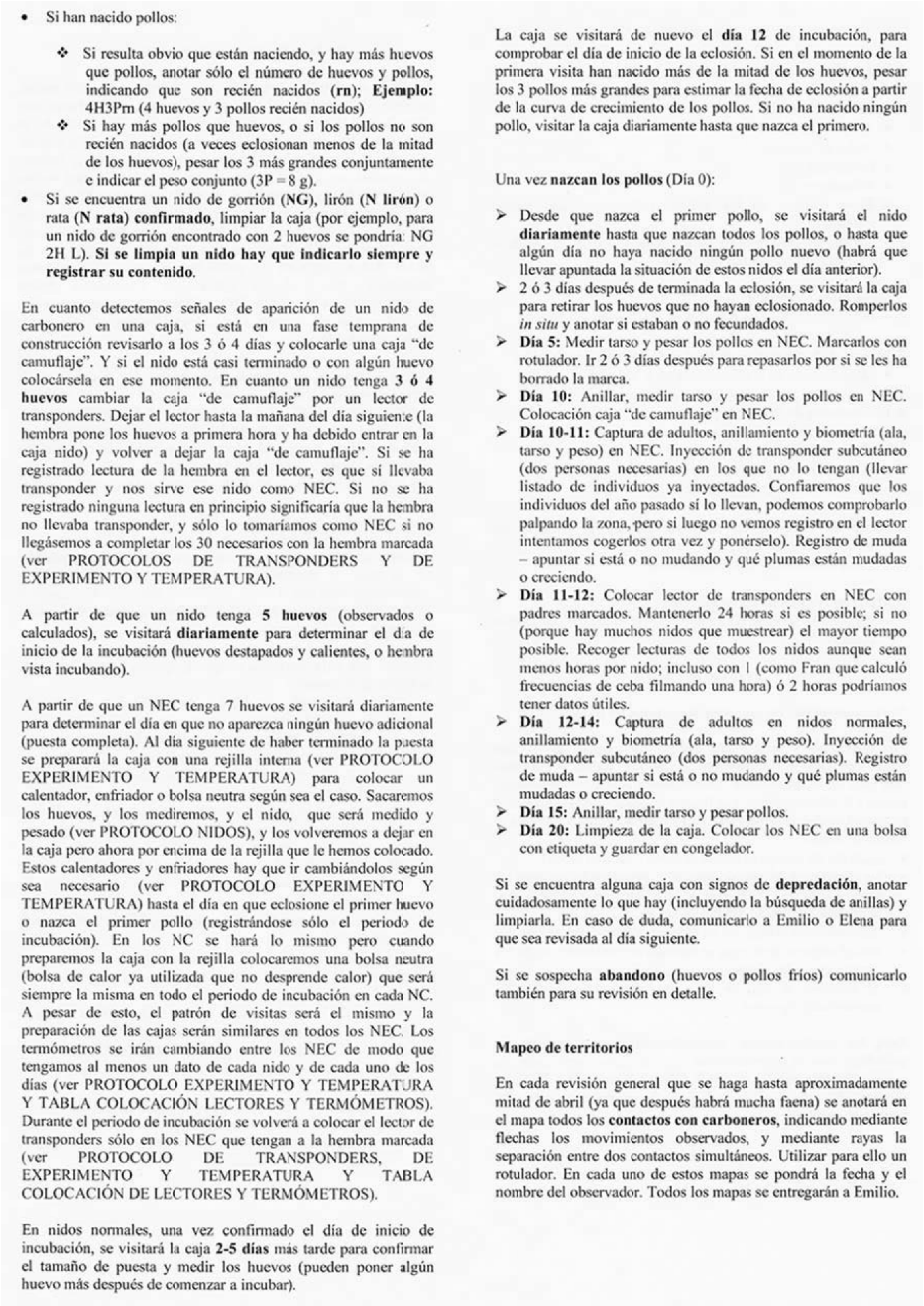
En las revisiones, se debe registrar **cada caja visitada** en la libreta de campo (**NO** en los mapas). Si una caja está vacía, se registra como "**vacía**" (v). Si una caja se busca y no se encuentra, se registra como "**no encontrada**". Si una caja no se visita por falta de tiempo, porque el huerto está regado, etc., se registra como "**no vista**", apuntando la causa. Las cajas robadas se repondrán lo antes posible mientras sigan apareciendo primeras puestas.

Si una caja no encontrada en la siguiente visita sigue sin aparecer, se dará por desaparecida y se repondrá lo antes posible. En la tapa se mantendrá el mismo número que tenía la desparecida, pero por si no se le hubiese cambiado el número y llevase otro distinto anotad **SIEMPRE** en la libreta el **número del mapa** y no el de la caja.

**Cuando una caja aparezca ocupada**, apuntar (entre paréntesis la forma de expresarlo):

- Estado de construcción del nido. Valorar los siguientes estados de construcción:
    - ❖ **Hierbas** – Hierbas amontonadas en la caja, sin ninguna estructura. Si hay musgo, indicarlo.
    - ❖ **1/4** – Un poco de material formando una base circular clara; se ve el fondo de la caja;
    - ❖ **1/2** – Nido con las paredes y la base (fondo de la caja recubierto de material), pero todavía sin recubrir de pelo, lana o musgo;
    - ❖ **3/4** – Nido casi completo, a falta de tapar el cuenco;
    - ❖ **4/4** – Nido acabado, con el cuenco tapado, pero sin huevos.
- A veces empleamos los valores de 1/3 y 2/3 para situaciones intermedias.
- Si se está seguro de que es de carbonero (tiene musgo), poner delante NC (e.g **NC 1/3**). Si no se está seguro, poner simplemente una N (e.g. **N 1/3**).
- Número de huevos – contar bien (mirar, no sólo tocar) -- expresado como el número seguido de H (**5H**).
- Si los huevos están tapados (**tap**), o destapados (**des**).
- En el caso de estar destapados, si están calientes (**cal**), o fríos (**fríos**).

    **Ejemplos**: 5H tap (5 huevos tapados); 7H des cal (7 huevos destapados calientes); 6H des fríos (6 huevos destapados y fríos) [se sobrentiende que si están tapados están fríos].



- Si han nacido pollos:
    - ❖ Si resulta obvio que están naciendo, y hay más huevos que pollos, anotar sólo el número de huevos y pollos, indicando que son recién nacidos (**rn**); **Ejemplo:** 4H3Prn (4 huevos y 3 pollos recién nacidos)
    - ❖ Si hay más pollos que huevos, o si los pollos no son recién nacidos (a veces eclosionan menos de la mitad de los huevos), pesar los 3 más grandes conjuntamente e indicar el peso conjunto (3P = 8 g).
- Si se encuentra un nido de gorrión (**NG**), lirón (**N lirón**) o rata (**N rata**) **confirmado**, limpiar la caja (por ejemplo, para un nido de gorrión encontrado con 2 huevos se pondría: NG 2H L). **Si se limpia un nido hay que indicarlo siempre y registrar su contenido.**

En cuanto detectemos señales de aparición de un nido de carbonero en una caja, si está en una fase temprana de construcción revisarlo a los 3 ó 4 días y colocarle una caja "de camuflaje". Y si el nido está casi terminado o con algún huevo colocársela en ese momento. En cuanto un nido tenga **3 ó 4 huevos** cambiar la caja "de camuflaje" por un lector de transponders. Dejar el lector hasta la mañana del día siguiente (la hembra pone los huevos a primera hora y ha debido entrar en la caja nido) y volver a dejar la caja "de camuflaje". Si se ha registrado lectura de la hembra en el lector, es que sí llevaba transponder y nos sirve ese nido como NEC. Si no se ha registrado ninguna lectura en principio significaría que la hembra no llevaba transponder, y sólo lo tomaríamos como NEC si no llegásemos a completar los 30 necesarios con la hembra marcada (ver PROTOCOLOS DE TRANSPONDERS Y DE EXPERIMENTO Y TEMPERATURA).

A partir de que un nido tenga **5 huevos** (observados o calculados), se visitará **diariamente** para determinar el día de inicio de la incubación (huevos destapados y calientes, o hembra vista incubando).

A partir de que un NEC tenga 7 huevos se visitará diariamente para determinar el día en que no aparezca ningún huevo adicional (puesta completa). Al día siguiente de haber terminado la puesta se preparará la caja con una rejilla interna (ver PROTOCOLO EXPERIMENTO Y TEMPERATURA) para colocar un calentador, enfriador o bolsa neutra según sea el caso. Sacaremos los huevos, y los mediremos, y el nido, que será medido y pesado (ver PROTOCOLO NIDOS), y los volveremos a dejar en la caja pero ahora por encima de la rejilla que le hemos colocado. Estos calentadores y enfriadores hay que ir cambiándolos según sea necesario (ver PROTOCOLO EXPERIMENTO Y TEMPERATURA) hasta el día en que eclosione el primer huevo o nazca el primer pollo (registrándose sólo el periodo de incubación). En los NC se hará lo mismo pero cuando preparemos la caja con la rejilla colocaremos una bolsa neutra (bolsa de calor ya utilizada que no desprende calor) que será siempre la misma en todo el periodo de incubación en cada NC. A pesar de esto, el patrón de visitas será el mismo y la preparación de las cajas serán similares en todos los NEC. Los termómetros se irán cambiando entre los NEC de modo que tengamos al menos un dato de cada nido y de cada uno de los días (ver PROTOCOLO EXPERIMENTO Y TEMPERATURA Y TABLA COLOCACIÓN LECTORES Y TERMÓMETROS). Durante el periodo de incubación se volverá a colocar el lector de transponders sólo en los NEC que tengan a la hembra marcada (ver PROTOCOLO DE TRANSPONDERS, DE EXPERIMENTO Y TEMPERATURA Y TABLA COLOCACIÓN DE LECTORES Y TERMÓMETROS).

En nidos normales, una vez confirmado el día de inicio de incubación, se visitará la caja **2-5 días** más tarde para confirmar el tamaño de puesta y medir los huevos (pueden poner algún huevo más después de comenzar a incubar).

La caja se visitará de nuevo el **día 12** de incubación, para comprobar el día de inicio de la eclosión. Si en el momento de la primera visita han nacido más de la mitad de los huevos, pesar los 3 pollos más grandes para estimar la fecha de eclosión a partir de la curva de crecimiento de los pollos. Si no ha nacido ningún pollo, visitar la caja diariamente hasta que nazca el primero.

Una vez **nazcan los pollos** (Día 0):

- ➢ Desde que nazca el primer pollo, se visitará el nido **diariamente** hasta que nazcan todos los pollos, o hasta que algún día no haya nacido ningún pollo nuevo (habrá que llevar apuntada la situación de estos nidos el día anterior).
- ➢ 2 ó 3 días después de terminada la eclosión, se visitará la caja para retirar los huevos que no hayan eclosionado. Romperlos *in situ* y anotar si estaban o no fecundados.
- ➢ **Día 5:** Medir tarso y pesar los pollos en NEC. Marcarlos con rotulador. Ir 2 ó 3 días después para repasarlos por si se les ha borrado la marca.
- ➢ **Día 10:** Anillar, medir tarso y pesar los pollos en NEC. Colocación caja "de camuflaje" en NEC.
- ➢ **Día 10-11:** Captura de adultos, anillamiento y biometría (ala, tarso y peso) en NEC. Inyección de transponder subcutáneo (dos personas necesarias) en los que no lo tengan (llevar listado de individuos ya inyectados. Confiaremos que los individuos del año pasado sí lo llevan, podemos comprobarlo palpando la zona, pero si luego no vemos registro en el lector intentamos cogerlos otra vez y ponérselo). Registro de muda – apuntar si está o no mudando y qué plumas están mudadas o creciendo.
- ➢ **Día 11-12:** Colocar lector de transponders en NEC con padres marcados. Mantenerlo 24 horas si es posible; si no (porque hay muchos nidos que muestrear) el mayor tiempo posible. Recoger lecturas de todos los nidos aunque sean menos horas por nido; incluso con 1 (como Fran que calculó frecuencias de ceba filmando una hora) ó 2 horas podríamos tener datos útiles.
- ➢ **Día 12-14:** Captura de adultos en nidos normales, anillamiento y biometría (ala, tarso y peso). Inyección de transponder subcutáneo (dos personas necesarias). Registro de muda – apuntar si está o no mudando y qué plumas están mudadas o creciendo.
- ➢ **Día 15:** Anillar, medir tarso y pesar pollos.
- ➢ **Día 20:** Limpieza de la caja. Colocar los NEC en una bolsa con etiqueta y guardar en congelador.

Si se encuentra alguna caja con signos de **depredación**, anotar cuidadosamente lo que hay (incluyendo la búsqueda de anillas) y limpiarla. En caso de duda, comunicarlo a Emilio o Elena para que sea revisada al día siguiente.

Si se sospecha **abandono** (huevos o pollos fríos) comunicarlo también para su revisión en detalle.

### Mapeo de territorios

En cada revisión general que se haga hasta aproximadamente mitad de abril (ya que después habrá mucha faena) se anotará en el mapa todos los **contactos con carboneros**, indicando mediante flechas los movimientos observados, y mediante rayas la separación entre dos contactos simultáneos. Utilizar para ello un rotulador. En cada uno de estos mapas se pondrá la fecha y el nombre del observador. Todos los mapas se entregarán a Emilio.


TEMPORADA
2005
SAGUNTO
por Elena ;)
VENGA CHICOS, QUE ES PA HOY
TERRANO
EMI
Je, Je, carbonerritos tiernosss...
Que salga mona, que está Fran grabando
17
Rápido mami, que vienen los biólogos pesaos...
Caja 17, 10:55 h. zona E grabando!!
Fran
♫ Perdóname...
Jeny


TEMPORADA DE CRÍA
MARIOLA - FONT ROJA
MAYO - JUNIO 2005
por. Elena ;)
¡¡VENGA, VUELVE A ENTRAR POR FAVOR!!!
EDU
CUÍDENME A MI ELENA, EIII!!!
PORTA
LA PURI, QUINA CALORRR...
PEP
IBERIS
¡JA, JA! NO ME COGEIS, SOMOS MÁS LISTOS QUE EN SAGUNTO...
¡QUÉ HAMBRE, UFF!
ZZZZ!!
¡¡¡TENGO SUEÑO!!! Y QUE LIGERA VOY...
CREO QUE ESTOY ADELGAZANDO UN POCO... ¿ME HAGO, QUÉ HAGO, ¿ME DUCHO O NO ME DUCHO? THAT'S THE QUESTION...
¿ENTRO O NO ENTRO?
PEPEEE
VEO UNA LINDA CAJITA, GG!!
¡Qué cómodo!, es super fácil ver la caja con mi gancheto brazo!
JENY
PEPEEEE, ¿ANDE ESTÁS QUE YA NO TE VEO...
FRAN
ELENA

# ANEXO III: GALERÍA FOTOGRÁFICA

En este Anexo se presentan algunas de las fotos más significativas que he realizado durante el desarrollo de esta tesis.

De izquierda a derecha y de arriba abajo:

- Con una hembra de carbonero común.
- Detalle de la placa incubatriz de una hembra de carbonero.
- Detalle de un transponder colocado en una pata del carbonero.
- Detalle de una caja-nido.
- Con el Terrano.
- Detalle de cómo quedaba colocaba una bolsa de frío, para enfriar los nidos, junto con el termómetro.
- Descargando los datos de temperatura en el campo.
- Sacando un nido, tras volar los pollos, para su posterior procesado.
- Desmenuzando nidos en el laboratotio.
- En la entrada de la Facultad de Ciencias Biológicas de la Universitat de València.
- Redactando la tesis en el despacho.
- En el XII Congreso de Etología organizado en Valencia por mi Director de tesis en 2008.
- Con Mike Hansell, autor del libro “Bird Nests and Construction Behaviour”.
- Con Charles Deeming, autor del libro “Avian Incubation: Behaviour, Environment, and Evolution”.
- Foto de grupo con algunos de mis compañeros de fatigas.
- Con Emilio Barba en el V Congreso de la Unión Europea de Ornitología celebrado en Estrasburgo.
- Elena en el campo.


XII Congreso Nacional
X Iberoamericano de Ecología


¡Ha sido un placer; nos vemos por el campo!

**CONTINUARÁ…**