Efecto de la temperatura sobre el crecimiento, desarrollo y supervivencia de pollos de carbonero común *Parus major* en un entorno de cambio climático

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Tesis presentada por SAMUEL RODRÍGUEZ SORIANO para optar al grado de Doctor en Biología por la Universidad de Valencia

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"El camino más largo comienza con un paso"

(Proverbio hindú)

A mis padres

A mi hermano

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RESUMEN DE LA TESIS

En una población aviar, la supervivencia en el nido viene determinada por la interacción de factores bióticos y abióticos, entre los cuales la temperatura ambiental puede jugar un papel crucial sobre el devenir de los polluelos. Asimismo, la condición de los pollos en el momento de abandonar el nido (determinada por todos aquellos factores que afectan a su desarrollo previo) y el momento en que éste se produce, son dos de los aspectos más influyentes sobre la supervivencia durante el primer año de vida, periodo marcado por una alta tasa de mortalidad.

En esta Tesis Doctoral se analizan algunos de los factores que pueden afectar al crecimiento, desarrollo y supervivencia de los pollos de carbonero común (*Parus major*) en ambientes mediterráneos, particularizando en el efecto de las temperaturas dentro del presente contexto de cambio climático. Metodológicamente, se emplearon datos reproductivos y térmicos recopilados durante más de dos décadas en una población mediterránea de la especie, para determinar el conjunto de factores que influyen sobre la supervivencia de los pollos en el nido, así como sobre la supervivencia durante el primer año transcurrido tras el abandono del mismo. Además, se analizaron tendencias temporales en los diferenciales de selección en la fecha de puesta de la población a partir de los individuos reclutados anualmente, y se determinó su relación con las temperaturas experimentadas durante el periodo de cría. Por otra parte, y dentro de un enfoque experimental, se manipuló la temperatura en el interior de un conjunto de nidos para evaluar el efecto de ambientes térmicos sub-óptimos sobre el crecimiento y la supervivencia de los pollos durante la fase de nidificación, y la respuesta paterna frente a la alteración térmica. Asimismo, se recurrió al radio-seguimiento para analizar la supervivencia de una muestra de estos pollos durante los primeros días transcurridos después de volar.

Nuestros resultados evidencian la importancia del tamaño de la pollada sobre la supervivencia en el interior del nido y eventual producción de individuos volanderos. Nidos con un mayor número de pollos originaron más volantones, aunque el peso medio de éstos fue inferior. Por su parte, del conjunto de predictores analizados con posible efecto a largo plazo sobre la supervivencia después de volar, la fecha de eclosión y el tamaño de los pollos al abandonar el nido (i.e., su longitud de tarso) tuvieron un efecto significativo sobre la supervivencia durante el primer año de vida de los juveniles. De esta forma, la probabilidad de supervivencia sería menor para individuos más pequeños, así como para aquellos nacidos tanto demasiado pronto como demasiado tarde en la temporada de cría. Al analizar la evolución del inicio del periodo de cría en nuestra población, averiguamos que la fecha media de inicio de puesta no había avanzado significativamente en el periodo a estudio (1992-2013), pese a que en la mayoría de años puestas más tempranas produjeron un mayor número de reclutas. A nivel poblacional, se dio un adelanto de la fecha de puesta en años más cálidos, una respuesta explicada principalmente por plasticidad fenotípica individual. Además, las temperaturas alcanzadas durante los periodos de incubación y de estancia de los pollos en el nido influyeron en el establecimiento de presiones de selección sobre la fecha de puesta. Por otra parte, nuestros resultados experimentales muestran que la exposición de los pollos a temperaturas alejadas de su óptimo de tolerancia puede afectar a su desarrollo dentro del nido: pollos calentados alcanzaron un peso inferior a los quince días, mientras que pollos enfriados desarrollaron tarsos más pequeños. El debilitamiento de los pollos a consecuencia del estrés térmico padecido en el nido pudo repercutir negativamente sobre su supervivencia durante los días inmediatos al abandono del mismo, al menos en el caso de los individuos calentados. El comportamiento paterno, sin embargo, apenas se vio alterado por la manipulación térmica. Los resultados obtenidos en esta Tesis se integran en la situación actual de cambio climático, al poner de manifiesto la vulnerabilidad de las crías a temperaturas de nidificación adversas, y el posible impacto de ambientes térmicos sub-óptimos durante el desarrollo sobre la supervivencia futura de los juveniles.

CAPÍTULO 1

Introducción general

1.1. La supervivencia del pollo

Elucidar qué procesos influyen sobre la fecundidad es un punto central de la ecología aviar, y resulta imprescindible para el entendimiento de la dinámica de poblaciones y el desarrollo de actuaciones de conservación y gestión. Un aspecto que determina la eficacia biológica de un organismo es su capacidad para producir descendientes que, a su vez, logren reproducirse con éxito en el futuro (Howard 1979; Stahl & Oli 2006). La supervivencia de un pollo durante el periodo de inmadurez sexual pasa por dos estadios claramente diferenciados: el periodo de estancia en el nido y el periodo que transcurre desde el abandono del mismo hasta que el individuo es reclutado en la población reproductora. Lejos de resultar dos fases aisladas, todos aquellos factores que afectan al desarrollo previo del pollo en el nido determinan su condición al volar, lo cual puede repercutir decisivamente en su probabilidad de supervivencia futura (Magrath 1991; Green & Cockburn 2001; Blomberg et al. 2014). Por otro lado, el estudio de estos dos periodos conlleva una dificultad desigual, dado que cuanto más nos alejamos del momento en que los descendientes se independizan del organismo paterno, más difícil resulta el determinar su destino. Así, entre las aves, es relativamente fácil, y hay numerosos estudios sobre ello, determinar la supervivencia de los descendientes en la fase de huevo (e.g., si llega a nacer un pollo), e incluso la supervivencia de los polluelos hasta que abandonan el nido, pero es más complicado determinar cuántos de estos pollos llegan vivos hasta la edad reproductora y tienen, a su vez, descendientes.

1.1.1. La supervivencia en el nido

El periodo de estancia en el nido es uno de los momentos energéticamente más exigentes en la vida de un ave (Ricklefs 1983; Wellicome et al. 2013), por ser una fase de intenso desarrollo anatómico y rápida ganancia de peso (Lepczyk & Karasov 2000). La supervivencia durante esta etapa determina la fecundidad anual de numerosas especies insectívoras de aves (Nagy & Holmes 2005; Mattsson & Cooper 2007), y viene condicionada por la interacción de diversos factores, tanto bióticos como abióticos.

Aspectos tales como las dimensiones y la composición de los huevos influyen en el desarrollo y la supervivencia de los polluelos de una gran variedad de aves, con independencia de la habilidad de la hembra para la incubación o la cría de la nidada (Williams 1994; Tilgar et al. 2005; Grigg 2014). Huevos de mayor tamaño contienen más agua y nutrientes, lo cual favorece el desarrollo del pollo durante sus primeros días de vida al contar éste con mayores reservas energéticas (Williams 1994; Dzialowski & Sotherland 2004). Esta ventaja se hace especialmente evidente en situaciones de escasez de alimento al poco tiempo de nacer (Parsons 1970; Smith & Bruun 1998; Magrath et al. 2003). Por lo general, pollos procedentes de huevos más grandes poseen de inicio un mayor tamaño y pueden alcanzar una tasa de crecimiento más rápida, lo cual les otorga mejores perspectivas de supervivencia (Parsons 1970; Pelayo & Clark 2003).

Otro factor que puede tener un papel igualmente relevante a la hora de influir sobre el crecimiento de los polluelos y su supervivencia en el nido es el tamaño de la pollada. Esto puede afectar a la intensidad de la competencia entre hermanos, resultando en una disminución per cápita del alimento paterno (Sicurella et al. 2015), que puede llevar a la mortalidad por inanición de los individuos menos desarrollados (Werschkul 1979; You et al. 2009). Es por ello que, cuando la capacidad de los padres para proporcionar alimento a sus crías es limitada, el número de pollos presente en el nido puede condicionar su crecimiento y supervivencia (Gebhardt-Henrich & Richner 1998; Bowers et al. 2014). De esta forma, tanto la disponibilidad de recursos como la aptitud de los padres a la hora de aprovisionar a las crías determinan la posibilidad de criar con éxito polladas mayores.

La cantidad y calidad del alimento proporcionado juegan un papel primordial en el crecimiento y la supervivencia de estos organismos de metabolismo rápido (Martin 1987; Verboven 2001; Bowers et al. 2014), y el momento elegido para la cría debe coordinarse de tal forma que el periodo en el que los pollos necesitan más alimento coincida con el de máxima abundancia de recursos en el medio (Perrins 1979; Visser et al. 2006; McKinnon et al. 2012). En este sentido, la supervivencia de los pollos muestra una marcada variación estacional, tal que aquellos individuos que nacen antes durante la temporada de cría suelen crecer mejor y poseen mayores probabilidades de supervivencia que pollos más tardíos (Öberg et al. 2014; Low et al. 2015). Este hecho puede deberse a un declive en la disponibilidad de alimento, unido a un deterioro de las condiciones ambientales conforme avanza la temporada (Verhulst & Nilsson 2008), así como a diferencias en la calidad parental de individuos que crían antes respecto a aquellos que lo hacen más tarde (Price et al. 1988; Wilson & Nussey 2010).

Por otra parte, la incidencia de depredadores y parásitos también determina la supervivencia de los pollos durante su estancia en el nido. La depredación es una de las principales causas del fracaso de nidos en muchas especies de aves (Ricklefs 1969; Ibáñez-Álamo et al. 2015) y puede estar relacionada con la tasa de crecimiento de los polluelos, dado que cuanto más lento es su desarrollo, más largo es el periodo de nidificación y mayor es su exposición a posibles depredadores (Bosque & Bosque 1995; Stodola et al. 2010). Además, en periodos de escasez de recursos, las crías pueden solicitar alimento con más insistencia, facilitando así su detección por depredadores potenciales (McDonald et al. 2009), a la vez que los padres pueden verse obligados reducir su dedicación a la defensa del nido por tener que aumentar el tiempo de forrajeo (Mutzel et al. 2013). La presencia de parásitos también afecta negativamente al desarrollo y la supervivencia de los pollos, al alcanzar éstos una peor condición nutricional y aumentar su mortalidad en el nido (Møller et al. 1990; Richner et al. 1993; Segura & Reboreda 2011).

1.1.1.1. El desarrollo de la regulación térmica en pollos y efecto de las temperaturas

La capacidad termorreguladora de los pollos de aves altriciales aumenta con la edad. Al nacer, las crías presentan un comportamiento típicamente poiquilotermo: dado que son incapaces de regular la temperatura de su cuerpo, ésta varía directamente con la temperatura ambiente. A medida que van creciendo, los polluelos adquieren un mayor control sobre su temperatura corporal, y mejora su capacidad para contrarrestar o compensar las pérdidas de calor. Así pues, los cambios en la capacidad termorreguladora pueden ocasionarse ya sea (1) por desarrollo de la capacidad de los pollos para producir calor, o (2) por cambios en su capacidad para mantener el calor. De estas dos vías, la producción de calor es más importante en la práctica. Muchas crías logran alcanzar inicialmente temperaturas corporales estables manteniendo una elevada actividad metabólica. Posteriormente, conforme su aislamiento mejora, pasan a reducir dichos niveles metabólicos a tasas cercanas a las adultas. La transición hacia una termorregulación metabólica o química tiene lugar a diferentes edades en distintas especies, si bien, por lo general, la producción de calor comienza a darse de forma regulada entre 8 y 10 días después de la fecha de eclosión.

La segunda vía de desarrollo de la capacidad termorreguladora es el control de las pérdidas de calor. De las cuatro formas de pérdida de calor existentes, la convección y la radiación se encuentran determinadas en gran medida por la elección por parte de los padres del lugar y la estructura de nidificación. Ahora bien, la conducción depende de tres características de los pollos -el desarrollo del plumaje, su relación superficie/volumen, y su temperatura corporal-, mientras que las pérdidas evaporativas vienen determinadas por la capacidad de las crías para jadear. En pollos, el desarrollo del plumaje no es un factor decisivo durante las primeras etapas de desarrollo de la termorregulación: el papel de las plumas es simplemente el de reducir el coste energético de la regulación térmica una vez ésta ya se ha establecido (Shilov 1973). A medida que los pollos crecen, su aumento en tamaño provoca una disminución de la relación corporal superficie/volumen, lo cual reduce sus pérdidas de calor. En algunos paseriformes, esta reducción puede llegar a ser del 40-50% respecto a los valores registrados en la fecha de eclosión (O´Connor 1975). Por otra parte, dado que las pérdidas de calor por conducción son proporcionales a la diferencia de temperatura entre el cuerpo y el entorno, un pollo puede reducir sus pérdidas disminuyendo su temperatura basal. De esta forma, también se ven reducidos los costes energéticos asociados al mantenimiento de las crías durante la etapa de escaso aislamiento.

La temperatura ambiental es uno de los principales factores abióticos que pueden afectar al crecimiento y la supervivencia de los pollos durante su estancia en el nido. En sus primeras etapas de desarrollo, las crías de ave poseen una limitada capacidad de regulación térmica a consecuencia de su escaso plumaje e ineficiencia a la hora de generar calor metabólico, lo cual las hace especialmente vulnerables a condiciones térmicas sub-óptimas

(Takagi 2001; Dawson et al. 2005; Garvin et al. 2006). En este sentido, las bajas temperaturas parecen limitar el desarrollo de los pollos (Krijgsveld et al. 2003), al obligarlos a aumentar su tasa metabólica para contrarrestar las pérdidas de calor, lo cual provoca el consumo de reservas energéticas que de otro modo se invertirían en el crecimiento o en el desarrollo del sistema inmunológico (Weathers 1979; Dawson et al. 2005). Por otra parte, a elevadas temperaturas, los pollos tienden a perder apetito, por lo que el consumo de alimento, la tasa de crecimiento y la musculatura se ven reducidos (Murphy 1985; Geraert et al. 1996; Balnave & Brake 2005). Las demandas energéticas e hídricas de los polluelos también se ven incrementadas en condiciones de hipertermia (Cunningham et al. 2013), al tener que disipar el exceso de calor por vías evaporativas (Ardia 2013; Nilsson et al. 2016). Además, olas de calor pueden aumentar la mortalidad de los pollos o empeorar su condición corporal como consecuencia de la deshidratación (Nager & Wiersma 1996; Thomas et al. 2001; Salaberria et al. 2014), la mortalidad cardiovascular o las enfermedades respiratorias (Patz et al. 2005). Los efectos negativos de las altas temperaturas sobre el crecimiento y la supervivencia de los pollos pueden evidenciarse especialmente en aquellos hábitats en los que las temperaturas alcanzadas durante el periodo de cría son elevadas (Belda et al. 1995; Cunningham et al. 2013). Este es el caso del mediterráneo, donde las temperaturas máximas pueden llegar a los 30-40 °C con el avance de la temporada, unido a una progresiva reducción de la disponibilidad de alimentos propia de la mayoría de ambientes.

El efecto de la temperatura sobre la supervivencia de los pollos también puede manifestarse indirectamente, al ser capaz de condicionar la disponibilidad de presas en el ambiente (i.e., por alteraciones en su fenología) y, por tanto, la cantidad de alimento que va a estar presente para las crías (Visser & Both 2005; Gienapp et al. 2014). En el caso de aves insectívoras, por ejemplo, la disponibilidad de alimento depende de la abundancia, actividad y desarrollo de invertebrados (principalmente artrópodos, ver Razeng & Watson 2015), que a su vez se ven afectadas por el ambiente térmico (Avery & Krebs 1984; Visser & Both 2005; Logan et al. 2006). De igual forma, la temperatura también puede influir sobre la actividad de posibles depredadores, con la consecuente alteración de la tasa de depredación sobre los nidos (Cox et al. 2013).

El comportamiento paterno puede contribuir a mejorar las condiciones térmicas en el interior del nido, cuya composición y características contribuyen al mantenimiento de un microclima apropiado para el desarrollo de los polluelos (Collias & Collias 1984; Mainwaring et al. 2014). Ahora bien, la temperatura ambiente puede condicionar el cuidado suministrado, lo cual repercutiría indirectamente sobre los pollos: el aprovisionamiento de las crías impone sobre los padres importantes necesidades de recursos (Linden & Moller 1989; Canestrari et al. 2007), por lo que la tasa de alimentación y/o la calidad del alimento proporcionado decaerán bajo condiciones desfavorables (Barba et al. 2009). La inversión energética óptima durante cada etapa de la historia de vida de una especie está condicionada por compromisos en la asignación de recursos a otras etapas (Zera & Harshman 2001; Stoelting et al. 2015), así que, si bien los padres pueden mantener unas condiciones adecuadas en el nido gestionando sus periodos dentro y fuera del mismo, e incluso regulando la cantidad de calor transferida a los polluelos, deben ser capaces de equilibrar las demandas de su propio mantenimiento con el esfuerzo destinado a sus crías (Ardia 2005; Canestrari et al. 2007).

A pesar de su importancia, son pocos los estudios desarrollados hasta la fecha que establezcan una clara relación causa-efecto entre la temperatura y el crecimiento, desarrollo y/o supervivencia de los polluelos. En este sentido, las aproximaciones experimentales son más adecuadas para elucidar los efectos de la temperatura sobre el rendimiento reproductivo, si bien no son habituales (e.g., Nager & van Noordwijk 1992; Dawson et al. 2005; Nilsson et al. 2008; Pérez et al. 2008; Lynn & Kern 2014). La mayoría de estos trabajos se han centrado en manipular la temperatura durante las fases de puesta y de incubación de los huevos, buscando efectos posteriores sobre el comportamiento paterno y la condición de los pollos (e.g., Nager & van Noordwijk 1992; Nilsson et al. 2008; Pérez et al. 2008). Únicamente Dawson et al. (2005) y Lynn & Kern (2014) alteraron la temperatura durante el periodo de estancia de los pollos en el nido. Por todo lo expuesto anteriormente, se deduce la necesidad de un mayor número de aproximaciones manipulativas, dirigidas a analizar los efectos directos e indirectos de las altas y bajas temperaturas de nidificación sobre la condición y supervivencia de las crías.

1.1.2. La supervivencia después de volar

La condición de los pollos en el momento de abandonar el nido (determinada a su vez por todos aquellos factores que afectan a su desarrollo previo), y el momento en que éste se produce, son dos de los aspectos que más influyen sobre su supervivencia hasta alcanzar la madurez reproductora. Los primeros pollos en volar del nido generalmente se benefician de unas condiciones ambientales más benignas y de una mayor disponibilidad de alimentos (Middleton & Green 2008; Vitz & Rodewald 2010), mientras que individuos en mejor condición física tienen una mayor posibilidad de evitar depredadores y resistir periodos de escasez de recursos

(Sim et al. 2013). En paseriformes, la mortalidad en este primer año es muy elevada, llegando a alcanzar hasta un 70-95% (Naef-Daenzer et al. 2001), y las primeras semanas transcurridas después de dejar el nido son especialmente críticas (Cox et al. 2014). En los días inmediatos al abandono del nido, los juveniles todavía no han desarrollado su capacidad de vuelo y dependen del cuidado paterno para la provisión de alimento, lo cual los hace muy vulnerables a la presión depredadora (Naef-Daenzer et al. 2001; Wiens et al. 2006). En este sentido, la propia calidad de los padres influye en la supervivencia de los juveniles, ya que sus cuidados contribuyen a mitigar el alto riesgo de mortandad (Dybala et al. 2013). Transcurrida la fase de dependencia paterna, la mortalidad de los juveniles sigue siendo elevada, a consecuencia de su inexperiencia para el forrajeo y por apenas contar con mecanismos de evasión de depredadores (Lack 1954; Wiens et al. 2006). Otros factores también pueden influir en la supervivencia después de volar: el número de juveniles condiciona la competencia intraespecífica por los recursos limitados, lo cual puede suponer que polladas más numerosas alcancen una peor condición corporal media, viéndose así comprometida su supervivencia (Maness & Anderson 2013). De igual forma, la presencia de patógenos y parásitos puede mermar la capacidad inmunológica de los juveniles y aumentar su vulnerabilidad frente a los rigores del entorno (López-Rull et al. 2011).

Las condiciones experimentadas durante un periodo biológico pueden influir en el rendimiento (i.e., supervivencia o éxito reproductor) de los individuos en periodos subsiguientes (Blomberg et al. 2014). En el caso de las aves, como se ha comentado anteriormente, factores que afecten al desarrollo de los pollos durante su estancia en el nido y repercutan sobre su condición al volar, pueden a su vez afectar a su supervivencia tras el abandono del nido. Por lo que respecta a la influencia de la temperatura, Greño et al. (2008) han demostrado que las temperaturas ambientales durante el periodo de nidificación están correlacionadas con la supervivencia futura de los polluelos, tal que aquellos que crecen sometidos a elevadas temperaturas poseen menores perspectivas de supervivencia. Aparte de este trabajo, son pocos los estudios realizados hasta la fecha centrados en la incidencia de la temperatura sobre el periodo posterior al abandono del nido (e.g., Blomberg et al. 2014), puesto que los análisis implicados en el estudio de la supervivencia después de volar precisan, por lo general, de extensas series temporales de datos. Es necesario, por tanto, realizar un seguimiento a largo plazo del ave para poder esclarecer los principales factores que afectan, en el transcurso del tiempo, a la supervivencia de los juveniles desde el momento que abandonan el nido hasta que son reclutados en la población reproductora.

Teniendo en cuenta el impacto del reclutamiento sobre la dinámica poblacional (Arcese et al. 1992; Finkelstein et al. 2010), cabe esperar que en una población aviar se seleccionen aquellos caracteres fenotípicos que maximicen el número de descendientes reclutados y, por ende, el éxito reproductor. En este sentido, resulta clave la capacidad de las aves para optimizar el momento escogido para la reproducción, tal que éste tenga lugar cuando las condiciones del medio sean más propicias (Goodenough et al. 2010). En un contexto de cambio climático, por tanto, la mayor o menor flexibilidad de una población para hacer frente a la variación ambiental puede determinar su probabilidad de subsistencia a largo plazo.

1.2. El cambio climático y su impacto sobre las aves

La continua emisión de gases de efecto invernadero por parte del hombre y su progresiva acumulación en la atmósfera están provocando cambios globales en la temperatura terrestre, ocasionando alteraciones climáticas que pueden acarrear peligrosas consecuencias para la biodiversidad. En los próximos cien años se prevé un aumento en la temperatura mundial de entre 1 y 3,5 °C (Stocker et al. 2013), si bien los efectos pueden llegar a ser considerablemente mayores en una escala local (De Castro et al. 2005). Este incremento en las temperaturas está promoviendo cambios en las características de diversas especies, tales como su morfología, su comportamiento, sus densidades y rangos de distribución, así como en la ocurrencia de eventos como la cría y la migración (Root et al. 2005; Visser et al. 2010). Además, la alteración de una especie puede afectar indirectamente a otras en la cadena alimentaria, provocando desajustes entre la demanda y la disponibilidad de recursos esenciales (Stenseth & Mysterud 2002; Both et al. 2009; Jones & Cresswell 2010). En última instancia, todos estos factores pueden llevar a cambiar la historia de vida de un organismo (Boag & Grant 1981; Both & Visser 2005; Visser 2008).

Durante las últimas décadas, un creciente número de estudios ha evidenciado efectos ecológicos del cambio climático sobre las poblaciones de aves (Järvinen 1994; Sanz 2002; Parmesan 2006; Knudsen et al. 2011). Entre estos efectos, se incluyen cambios en la distribución geográfica, alteraciones en la abundancia relativa, cambios conductuales y fisiológicos, así como un aumento del riesgo de extinción de determinadas especies. El impacto del cambio climático sobre el rendimiento reproductivo aviar se ha observado tanto a nivel local como a escala continental (Sanz 2002; Devictor et al. 2012). Quizás uno de los efectos mejor documentados hasta la fecha es la alteración de la fenología de las aves, particularmente en lo referente al momento escogido para la migración y la cría (Visser & Both 2005; Gienapp et al. 2014). En respuesta al aumento general de la temperatura durante los meses primaverales, especies propias de climas templados están adelantando su periodo reproductivo (Dunn & Winkler 2010), lo cual a su vez podría acabar afectando a sus dinámicas poblacionales (Sæther et al. 2000; Wittger et al. 2015). En el caso de aves insectívoras, el avance en la fecha de puesta podría no ser suficiente como para hacer coincidir el momento de mayor abundancia de artrópodos con el de máxima demanda de alimento por parte de las crías, lo cual originaría un desajuste temporal entre los requisitos nutritivos de los pollos y su disponibilidad (Visser & Both 2005; Visser et al. 2006), con evidentes consecuencias negativas sobre la supervivencia tanto de crías como de adultos (Visser et al. 2004; Visser et al. 2006). Asimismo, el adelanto del periodo de cría también puede afectar a otros aspectos de la reproducción, tales como el número (Verboven et al. 2001; Husby et al. 2009) y tamaño (Both & Visser 2005) de las puestas, el comportamiento de incubación (Cresswell & McCleery 2003; Cooper et al. 2005) o el reclutamiento (Drent 2006; Wilson et al. 2007).

La plasticidad fenotípica, definida como la propiedad de un genotipo de producir más de un fenotipo cuando el organismo se halla sometido a diferentes condiciones ambientales (esto es, cualquier cambio en las características de un organismo en respuesta a una señal ambiental), es uno de los principales mecanismos con el que cuentan las aves para hacer frente a alteraciones a largo plazo en su entorno, tales como el cambio climático y la pérdida de hábitat. En este sentido, existen formas por las que un ave puede ajustar estratégicamente la fecha de eclosión de sus huevos una vez iniciada la puesta, respondiendo así a avances o retrocesos en el pico de abundancia de alimento ocasionados por la variación térmica anual (Cresswell & McCleery 2003; Ramsay & Otter 2007): pueden darse cambios en el tamaño de puesta (Klomp 1970; Cresswell & McCleery 2003), interrupciones en la frecuencia de puesta de los huevos (Lessells et al. 2002), variaciones en el momento de inicio de la incubación (Haftorn 1981; Hébert 2002), y pausas en la incubación (Haftorn 1988; Hébert 2002). Ahora bien, tanto el adelanto como la demora en la fecha de eclosión conllevan una serie de costes y beneficios. Un retraso en la fecha de eclosión puede dar lugar a puestas mayores, aunque si éste se consigue a costa de dilatar la incubación, podría aumentar la probabilidad de que el nido fuese depredado (Conway & Martin 2000; Basso & Richner 2015). Por otro lado, cuando se adelanta la fecha de eclosión iniciando la incubación antes de la puesta del último huevo, la eclosión de los huevos es asincrónica (Haftorn 1981; Bosman 2014), lo cual reduce la probabilidad de que todos los pollos nacidos salgan adelante y acaben abandonando el nido (Kim et al. 2010; Merkling et al. 2014). Así pues, los efectos del cambio climático sobre las poblaciones de aves dependen, en parte, de variaciones en la duración del periodo transcurrido entre el inicio de la puesta y el pico de abundancia de alimento, así como de la flexibilidad de los individuos para ajustar el momento de máxima demanda de recursos con el de mayor disponibilidad de los mismos.

Para entender en toda su extensión los impactos del cambio climático global sobre una especie aviar, es necesario contar con poblaciones en las que se haya realizado una exhaustiva recopilación de parámetros reproductivos a lo largo del tiempo, de tal forma que puedan elucidarse tendencias a largo plazo. Sin embargo, en la actualidad existen pocas poblaciones que cumplan dicho requisito, menos aún en la región mediterránea, donde se estima que los efectos del cambio climático pueden ser más severos (Giorgi & Lionello 2008; Diffenbaugh & Giorgi 2012). Por otra parte, son pocos los estudios que han examinado si la selección del periodo de cría u otras características propias de la historia de vida de un ave varían en función de la fenología del entorno o de las temperaturas durante el periodo reproductor (e.g., Visser & Both 2005; Ahola et al. 2009). En este sentido, la medida de las características fenotípicas de individuos marcados, y el seguimiento de su reclutamiento en la población local, permiten la estima de diferenciales de selección y la determinación de presiones de selección a favor de determinados caracteres (e.g., Ahola et al. 2009), lo cual podría explicar cambios observados a nivel poblacional. De cualquier forma, son imprescindibles nuevos estudios que analicen series temporales largas de datos de fecundidad, reclutamiento y supervivencia aviar desde un enfoque integrador (esto es, considerando la relación entre los distintos parámetros de la historia de vida del ave), para poder entender y predecir las consecuencias ecológicas de los cambios provocados por el calentamiento global.

1.3. Objetivos de la Tesis

El objetivo general de la presente Tesis es profundizar en el conocimiento de los factores que afectan al crecimiento, desarrollo y la supervivencia de los pollos de carbonero común (*Parus major*) en ambientes mediterráneos, haciendo especial énfasis en el efecto de las temperaturas dentro del presente contexto de cambio climático. Se distinguen dos periodos: el de estancia de los pollos en el nido, y el transcurrido desde el abandono del mismo hasta que los individuos son reclutados en la población reproductora al año siguiente. Dentro de este segundo periodo, se incide en la supervivencia durante los días inmediatos al abandono del nido, por ser una etapa crítica en la vida de los juveniles caracterizada por una elevada mortalidad.

A continuación, se indican los objetivos específicos de la Tesis, con los que se pretende alcanzar el objetivo general:

Objetivo 1: Analizar experimentalmente el efecto de las altas temperaturas sobre el crecimiento y la supervivencia de los pollos de carbonero común durante su estancia en el nido, y la repercusión sobre el cuidado paterno (i.e., tasa de ceba y estancia en el nido de las hembras).

Objetivo 2: Analizar experimentalmente el efecto de las bajas temperaturas sobre el desarrollo de los pollos de carbonero común durante su estancia en el nido, y la respuesta paterna a la manipulación térmica (i.e., estancia en el nido y condición corporal de las hembras).

Objetivo 3: Determinar qué conjunto de predictores, entre una serie de parámetros reproductivos básicos y variables de temperatura, tiene la mayor influencia sobre la producción local de volantones de una población mediterránea de carbonero común en el largo plazo.

Objetivo 4: Analizar el efecto de las temperaturas experimentadas en el nido sobre la supervivencia de los pollos de carbonero común durante los primeros días transcurridos tras el abandono del mismo, así como la influencia de la fecha de vuelo, la edad al volar, el peso y el tamaño de los juveniles.

Objetivo 5: Analizar el efecto de la fecha de eclosión, la condición física al volar (i.e., peso y tamaño), y las temperaturas experimentadas durante el desarrollo sobre la supervivencia de los pollos de carbonero común en su primer año de vida tras el abandono del nido.

Objetivo 6: Explorar tendencias temporales en los diferenciales de selección en la fecha de puesta de una población mediterránea de carbonero común a partir de los individuos reclutados anualmente, y su relación con las temperaturas experimentadas durante el periodo de cría.

1.4. Nota ética

Los protocolos de los experimentos descritos en los distintos artículos recopilados en la presente Tesis han sido sometidos a una evaluación ética como parte del proceso general de evaluación de los proyectos a los cuales pertenecen. El personal encargado del anillamiento y la biometría de las aves en la zona de estudio estaba en posesión del permiso para el anillamiento específico del carbonero común, concedido por el Ministerio de Agricultura, Alimentación y Medioambiente de España, y contaba con acreditación por parte de la Consellería de Agricultura, Pesca y Alimentación de la Comunidad Valenciana para el diseño y dirección de procedimientos experimentales con animales.

CAPÍTULO 2

Área de estudio y metodología general

2.1. Descripción de la zona de estudio

La totalidad del trabajo de la Tesis se ha desarrollado en una zona agrícola situada en el término municipal de Sagunto, al noreste de la provincia de Valencia (39°42’N, 0°15’W, 30 m s.n.m.; Figura 2.1). La parcela, actualmente de extensión total cercana a 470 ha, está dominada por plantaciones minifundistas de naranjos (*Citrus aurantium*). Existe un sistema de acequias de riego que atraviesa la zona y forman, usualmente, los límites entre huertos. En algunos casos, dichos límites los establecen caminos de tierra o carreteras asfaltadas.

Figura 2.1. Mapa de localización del área de estudio.

La distribución de los naranjos en la zona de estudio es casi siempre uniforme en malla cuadrangular, y su altura varía entre 3 y 5 m cuando los árboles alcanzan su altura máxima (Figura 2.2). El tipo de riego ha cambiado en los últimos años, pasando del sistema tradicional por inundación al actual de goteo (Andreu et al. 2005). Más información sobre las características de los huertos, la disposición de los frutales y las especies herbáceas presentes en el interior de los huertos y en sus márgenes pueden encontrarse en Gil-Delgado & Escarré (1977) y Gil-Delgado et al. (1979). Cabe indicar que parte de esta vegetación está desapareciendo con el cambio del sistema de riego.

Figura 2.2. Panorámica general de los campos de naranjos en Sagunto.

El clima en la zona de estudio se caracteriza por los inviernos suaves (con temperatura media por encima de los 0 °C en los meses más fríos, i.e., enero y febrero) y veranos cálidos, con temperaturas máximas superiores a 30 °C. La precipitación media anual es de aproximadamente 440 mm, siendo los meses de septiembre y octubre los más lluviosos (Figura 2.3; Fuente: es.climate-data.org).

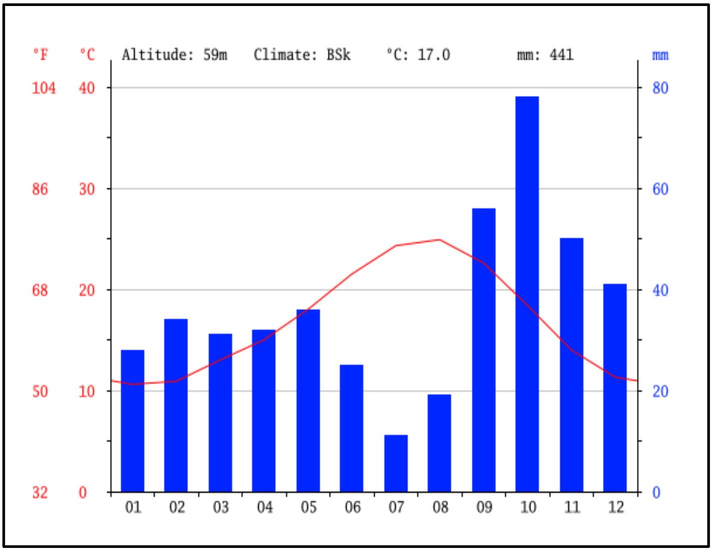

Figura 2.3. Climograma correspondiente al término municipal de Sagunto. En rojo, temperaturas. En azul, pluviometría.

El área de estudio ha sido empleada para el seguimiento a largo plazo de una población residente de carbonero común. Comenzando en 1986, durante el periodo de cría de la especie hay disponibles cajas de madera de pino diseñadas para páridos (dimensiones básicas: 126 cm^2 de superficie de base interna y 18,5 cm de altura; ver características detalladas en Lambrechts et al. 2010; Figura 2.4), en una densidad aproximada de una caja por hectárea. La ubicación de las cajas se ha ido manteniendo año a año, salvo casos puntuales en los que ha sido necesaria la recolocación de algunas unidades en parcelas cercanas por talado de huertos.

Figura 2.4. Detalle de una caja-nido.

Desde el comienzo de la monitorización, la extensión total de la zona de estudio ha ido aumentando, desde 50 ha en 1986 hasta 469 ha en 2013 (Figura 2.5). El carbonero común es la única especie de ave que en estos momentos se reproduce en las cajas-nido de la zona, si bien antiguamente

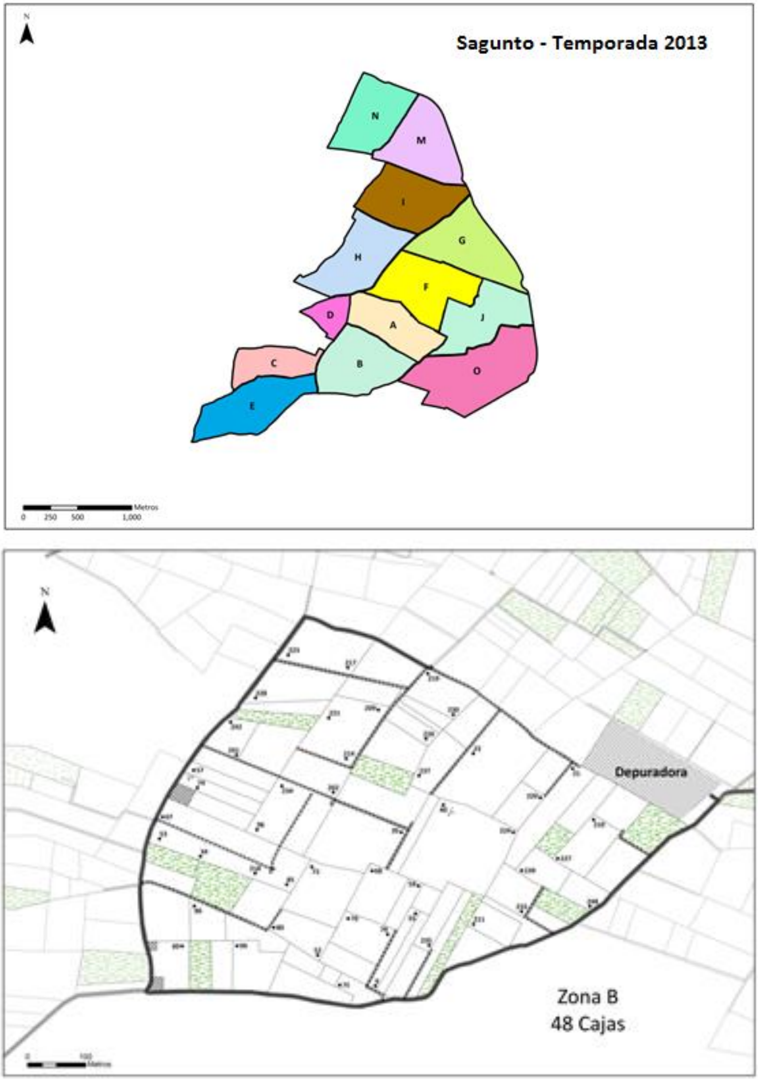

Figura 2.5. Mapa general del área de estudio de Sagunto (arriba) y detalle de una de las zonas de estudio, mostrando la ubicación de las cajas-nido (abajo).

también lo hizo el gorrión común *Passer domesticus* y algúna pareja ocasional de gorrión molinero *Passer montanus* (Barba & Gil-Delgado 1990; Gil-Delgado et al. 2009). Otros ocupantes de las cajas-nido son la rata negra *Rattus rattus* y el lirón careto *Elyomis quercinus*, que actúan a la vez como competidores y depredadores del carbonero común (Barba & Gil-Delgado 1990; Gil-Delgado et al. 2009).

2.2. Descripción de la especie objeto de estudio

Las características generales del carbonero común (Figura 2.6) están descritas ampliamente en Sáez-Royuela (1990), Cramp & Perrins (1993) y Atiénzar et al. 2012. A partir de dichos estudios, se resumen a continuación las más relevantes.

El carbonero común (*Parus major*, Linneo 1758) es un ave insectívora de pequeño tamaño perteneciente al Orden *Passeriformes* y la familia *Paridae*. Posee

Figura 2.6. El carbonero común *Parus major*

una longitud aproximada comprendida entre los 12 y 14 cm y un peso comprendido entre los 17 y 19 g. Su envergadura oscila entre los 22,5 y 25,5 cm. Posee un plumaje verde-oliváceo en sus partes superiores, algo más amarillento en la nuca y con el obispillo más grisáceo. Sus partes inferiores son amarillas, con una característica banda central negra que se origina en la cabeza y atraviesa longitudinalmente pecho y vientre. La cabeza es redondeada y negra, con manchas blancas en las mejillas. Las alas son negruzcas, si bien los bordes de las coberteras mayores, de color blanco amarillento, configuran una banda blancuzca en las alas. Posee una cola parda, con ribetes gris-azulados, y con los bordes externos de las plumas exteriores de la cola blanquecinos. Su pico es negro y las patas de un gris azulado. Los sexos son similares en plumaje, salvo que las hembras, además de ser ligeramente más pequeñas y ligeras, presentan tonos más apagados y una banda negra en el pecho más estrecha que los machos, que además se desdibuja en el vientre.

En España la especie se encuentra distribuida por casi toda la península e Islas Baleares. Únicamente falta en zonas deforestadas del interior del país, así como en las Islas Columbretes y Canarias. En general, puede encontrarse en la mayoría de ecosistemas desde el nivel del mar hasta altitudes superiores a los 2.100 m, pero con presencia arbórea y existencia de agujeros. Se les puede observar en bosques perennifolios (coníferas, carrascas) y caducifolios (hayas, robles), en zonas de frutales (naranjos) y parques urbanos. Llega a escasear en ambientes palustres carentes de arbolado, así como en praderas y matorrales alpinos. Se trata de una especie troglodita, que nidifica en huecos de árboles, si bien puede ocupar cualquier tipo de cavidad, incluyendo cajas-nido cuando éstas se proporcionan en el ambiente adecuado. Debido a su carácter generalista y a su capacidad de prosperar en hábitats humanizados, la especie no presenta problemas de conservación.

Se trata de una especie sedentaria. Forma bandos en invierno, en general inter-específicos, moviéndose por superficies más o menos extensas. Es territorial durante la época reproductiva, entre marzo y julio, siendo los individuos en general fieles al territorio de cría entre años. Durante el periodo reproductor, ambos miembros de la pareja comparten la tarea de la alimentación de los pollos, los cuales se nutren principalmente de orugas de lepidópteros, coleópteros, ortópteros, dípteros e himenópteros. Las hembras, en cambio, son las únicas encargadas de construir el nido, incubar los huevos y empollar, sobre todo cuando los pollos tienen pocos días.

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 constituido básicamente por musgo y suelen tardar una semana en construirlo. Desde ese momento, las hembras ponen un huevo diario hasta terminar la puesta, y acostumbran a enterrar los huevos entre el material del nido hasta que empieza la incubación. En la mayoría de poblaciones estudiadas, los individuos realizan con más frecuencia una única puesta por temporada, aunque en todas ellas se registran parejas que hacen dos puestas.

El tamaño de puesta puede variar entre los 4 y 13 huevos. Las hembras comienzan a incubar un día antes de que la puesta esté completa, y están incubando alrededor de 13 días. Durante este periodo, el macho aporta alimento a la hembra. Como norma general, los huevos eclosionan de forma asincrónica, extendiéndose el periodo de eclosión por dos o tres días. Los pollos permanecen el nido entre 18 y 20 días después de la eclosión, de ahí que la especie se considere altricial o nidícola.

En el caso concreto de nuestra zona de estudio, la dieta del carbonero común durante el periodo reproductor está basada fundamentalmente en polillas y larvas de lepidópteros (Barba et al. 1989; Atiénzar et al. 2009). Respecto a la composición de los nidos, destaca la baja proporción de musgo (23% del peso seco total) en comparación con materiales tales como las ramitas y el pelo (Álvarez & Barba 2009). La estación de nidificación en

Sagunto dura alrededor de tres meses. Las primeras puestas comienzan a finales de marzo, y los últimos pollos abandonan el nido a primeros de julio. La fecha media de puesta se sitúa en torno al 20 de abril, y el tamaño medio de puesta es de 8 huevos (Barba 1991). El volumen medio de los huevos es de 1,50 cm^3 (Encabo et al. 2001). El periodo de incubación dura aproximadamente 13 días y generalmente comienza el día en que se pone el último huevo (Álvarez & Barba 2014a). Los huevos generalmente eclosionan de forma asincrónica, extendiéndose el periodo de eclosión (i.e., el intervalo de días desde el nacimiento del primer pollo hasta el nacimiento del último) por aproximadamente dos días (Álvarez & Barba 2014a). El número medio de pollos que vuela por nido es de seis (Rodríguez et al. 2016).

2.3. Metodología general

2.3.1. Obtención de parámetros reproductivos y biometría de las aves

Las cajas-nido se colocaron cada año a finales de febrero y se retiraron al finalizar la estación reproductora, para evitar su ocupación durante el invierno por pequeños mamíferos tales como la rata negra y el lirón careto (Barba & Gil-Delgado 1990; Gil-Delgado et al. 2009). Asimismo, se pretendía evitar su deterioro o robo. Las revisiones de las cajas comenzaron a primeros de marzo, al principio una vez por semana. Posteriormente, las cajas con nidos pasaron a ser revisadas con la frecuencia necesaria (a veces diariamente) como para determinar con exactitud los parámetros reproductivos de interés, tales como la fecha de inicio de puesta, el tamaño de puesta, el tamaño de los huevos, el número de huevos eclosionados, y el número de pollos volanderos. Puntualmente, para estudios o experimentos concretos, se midieron otros parámetros o variables, y se visitaron nidos con distinta frecuencia, tal y como se detalla en el apartado de metodología de los artículos correspondientes.

Los adultos se capturaron cuando los pollos contaban con entre 10 y 14 días de edad. Para ello, se emplearon redes japonesas y/o trampas de resorte colocadas a la entrada de las cajas-nido. Una vez capturados, los ejemplares fueron anillados individualmente con anillas metálicas numeradas, y se determinó su sexo y clase de edad, diferenciando individuos reproductores de un año de edad de los nacidos con anterioridad a partir de diferencias en la coloración de su plumaje (Svensson 1992). Adicionalmente, se determinó la longitud del ala, la longitud del tarso y el peso de los ejemplares capturados.

Los pollos se anillaron y midieron (i.e., longitud del ala y peso) a los 15 días de edad, si bien para algún experimento fue necesario medirlos a diferentes edades (artículos 1 y 2). Los protocolos específicos empleados se detallan en los correspondientes artículos.

Para todos los estudios descritos en esta Tesis se trabajó únicamente con primeras puestas. Tanto las segundas puestas (puestas realizadas tras una primera puesta exitosa) como las puestas de reposición (puestas realizadas tras una primera puesta fracasada) son relativamente escasas, y en general poco exitosas, en nuestra población. Asimismo, los años implicados en cada estudio variaron en función de los objetivos marcados y de los datos disponibles para su implementación en el momento de ser redactados. De esta forma, al analizar los factores influyentes sobre la producción de volantones en nuestra zona de estudio (artículo 3), se emplearon datos de 25 años (i.e., desde 1986 hasta 2010). Por su parte, en el análisis de los factores determinantes de la supervivencia de los pollos en su primer año de vida (artículo 5) se emplearon datos de pollos anillados entre 1993 y 2010, ante la falta de medidas biométricas de las crías en años anteriores. A la hora de analizar la selección del inicio del periodo de cría en nuestra población (artículo 6), se aprovecharon datos de 22 años (i.e., desde 1992 hasta 2013), durante los cuales se contaba con registros completos de los parámetros de interés.

2.3.2. Metodología experimental básica

Para los trabajos detallados en los artículos 1, 2 y 4 de la presente Tesis, se manipuló la temperatura de un conjunto de nidos experimentales durante la fase de desarrollo de los pollos. Para ello, comenzando el día posterior al nacimiento del primer pollo de cada nido experimental, se colocaron paquetes calentadores o paquetes congelados en el interior de las cajas-nido, según fuese necesario someter a los pollos a temperaturas más cálidas o más frías (ver detalles en el apartado de metodología de los respectivos artículos). Estos paquetes se fueron reemplazando durante los primeros 14 días de vida de los pollos con a periodicidad necesaria para garantizar, en la medida de los posible, la efectividad de cada tratamiento térmico. Durante el transcurso del experimento, se registraron de forma continua las temperaturas en el interior de los nidos. Además, se analizaron cambios en el comportamiento paterno a consecuencia de la manipulación térmica. Para ello, se registró periódicamente la presencia o ausencia de las hembras en los nidos (artículos 1 y 2), y se estimó la tasa de ceba de ambos padres mediante la inyección subcutánea de transponders en el momento de su anillamiento y el posterior recuento de sus entradas y salidas de las cajas-nido por medio de lectores (artículo 1). El experimento de manipulación térmica se realizó en 2011 y 2012. Este segundo año, durante el anillamiento de los pollos (i.e., a los 15 días de edad de éstos), se seleccionó a los dos individuos más pesados de cada nido experimental y se les colocó un radio-emisor. De esta forma, una vez abandonaron el nido, fueron localizados diariamente por radio-seguimiento (ver detalles adicionales en el siguiente apartado, y en la metodología del artículo 4).

2.3.3. Radio-seguimiento

El radio-seguimiento es una técnica basada en la localización de animales utilizando ondas de radio. Para ello, el animal debe portar un dispositivo transmisor que emita dichas ondas, y que éstas sean localizadas por un receptor. Los transmisores deben pesar lo menos posible y, como norma, ningún transmisor debe superar el 5% del peso vivo del animal (López-López 2016). Un aspecto importante a considerar es dónde colocar el transmisor para que éste no suponga molestia alguna para el animal. En el caso de aves, en la mayoría de especies se coloca en el dorso. Para evitar equivocaciones, cada transmisor debe tener una frecuencia distinta cuando se siguen varios individuos. Por lo que respecta al receptor, éste debe ser capaz de localizar las distintas frecuencias sin confundirlas entre sí, y debe tener la suficiente potencia para diferenciar ondas de radio de animales distintos a distancias considerables.

El radio-seguimiento se suele emplear en animales que no realizan migraciones ni grandes desplazamientos, ya que por lo general los receptores poseen un campo de acción reducido (Fiedler 2009), aunque hay variaciones importantes entre distintos receptores y transmisores. El radio-seguimiento permite saber la localización del animal y, en muchos casos, la causa de su muerte si ésta se produce. Sus aplicaciones varían desde estudios de supervivencia hasta la determinación del área de campeo de especies de interés (e.g., Berkeley et al. 2007; Domenech et al. 2015). Como principales inconvenientes cabe destacar la dificultad de detectar los transmisores a grandes distancias, la influencia negativa de la rugosidad del terreno, y la limitada duración de la batería.

Para el estudio descrito en el artículo 4, se emplearon emisores Biotrack, de un peso y dimensiones adecuados para pequeños paseriformes (Naef-Daenzer et al. 2001). Los emisores se colocaron en el dorso de los juveniles mediante arneses Rappole (Rappole & Tipton 1991; Figura 2.7), permitiendo en todo momento el movimiento libre de las aves. Las localizaciones de los individuos se determinaron mediante receptores Sika con antenas Yagi (148-152 MHz de rango de frecuencia), de Biotrack. Para las pretensiones de nuestro estudio, fue suficiente con determinar el estado (vivo o muerto) de los juveniles.

Figura 2.7. Pollo de carbonero común con radio-emisor colocado mediante arnés.

CAPÍTULO 3

Resumen de los resultados de la Tesis

3.1. La supervivencia de los pollos de carbonero común en el nido (artículos 1-3)

El calentamiento artificial de los nidos hizo que los pollos alcanzaran un peso inferior a los 15 días en el más caluroso de los dos años de estudio (i.e., 2012). Su supervivencia en el nido, sin embargo, no se vio alterada (artículo 1). Ahora bien, teniendo en cuenta la ventaja que supone abandonar el nido con un peso mayor (e.g., para resistir periodos de escasez de alimento al poco tiempo de dejar el nido), es previsible que la exposición a altas temperaturas durante el desarrollo conlleve efectos negativos a largo plazo sobre el reclutamiento de los juveniles. Por lo que respecta al comportamiento de los adultos, éstos no alteraron su tasa de ceba para compensar el sobrecoste energético incurrido por los pollos calentados. Por otro lado, el calentamiento de los nidos hizo que las hembras invirtieran menos tiempo empollando a sus crías durante los primeros días de vida de éstas, comportamiento que ya ha sido detectado en estudios previos durante la incubación (Álvarez & Barba 2014b) y que apunta a una reducción en la dedicación a la pollada para favorecer el tiempo de estancia fuera del nido en atención de otras necesidades.

En relación al experimento de enfriamiento (artículo 2), las temperaturas en el interior de las cajas-nido enfriadas fueron, en promedio, 4,5 °C inferiores a las de las cajas control durante los primeros días de vida de los pollos, si bien no se detectaron diferencias entre unas cajas y otras cuando los pollos tenían una edad más avanzada. El enfriamiento artificial de los nidos hizo que las crías desarrollaran tarsos más pequeños a los 15 días. Ni su peso ni su supervivencia se vieron afectados. Pese a no haber hallado diferencias en la supervivencia de los pollos enfriados dentro del nido, no podemos descartar efectos negativos sobre su supervivencia una vez abandonado el mismo, ya que individuos de menor tamaño son más vulnerables a la depredación (De Laet 1985; Ragusa-Netto 1996), y tienen una capacidad limitada para acceder a los recursos del medio (Arcese & Smith 1985; Carrascal et al. 1998). Por lo que respecta al estado y comportamiento de los padres, el tratamiento térmico no alteró la condición de las hembras ni su presencia en el nido.

Al analizar los factores con posible influencia sobre la producción anual de volantones en nuestra población (artículo 3), únicamente el número de pollos nacidos tuvo un efecto significativo. Nidos con polladas más numerosas originaron un mayor número de volantones (i.e., el 86% de los pollos nacidos acabó abandonando el nido), aunque el peso medio de éstos fue inferior. El resto de parámetros analizados (i.e., tamaño medio de los huevos, tamaño de puesta, fecha de eclosión, número de huevos no eclosionados y temperaturas medias durante el desarrollo de los pollos) no afectó significativamente al número de pollos que volaron del nido en el global de los 25 años a estudio, aunque no podemos descartar que cobren importancia en determinados años, dependiendo de la variabilidad ambiental. Así pues, el escaso poder predictivo de estas variables sobre la producción de volantones podría ser consecuencia de que sus efectos, más o menos importantes, pueden variar en gran medida de un año a otro en función de las condiciones del medio.

3.2. La supervivencia de los pollos de carbonero común tras el abandono del nido (artículos 4-6)

La manipulación de las temperaturas en el interior del nido durante la etapa de desarrollo de los pollos reveló un peso menor de los pollos calentados a los 15 días y una supervivencia inferior que los no sometidos a ningún tratamiento (i.e., pollos control) durante los días posteriores el abandono del nido (artículo 4). Únicamente el 48% de estos últimos sobrevivió durante el periodo a estudio, siendo la depredación la principal causa de mortalidad. La supervivencia de los pollos control después de abandonar el nido aumentó con su tamaño, si bien esta relación se invirtió en el caso de los pollos calentados (i.e., los individuos de menor tamaño al volar tuvieron una mayor probabilidad de supervivencia). Por otra parte, no se detectaron efectos significativos del enfriamiento sobre la biometría de los pollos ni sobre su supervivencia en los primeros días tras el abandono del nido.

Del conjunto de predictores analizados con posible efecto a largo plazo sobre la supervivencia después de volar (artículo 5), la fecha de eclosión y el tamaño de los pollos al abandonar el nido (i.e., su longitud de tarso) afectaron significativamente a la supervivencia durante el primer año de vida de los juveniles. De esta forma, la probabilidad de supervivencia sería menor para individuos más pequeños, así como para aquellos nacidos tanto demasiado pronto como demasiado tarde en la temporada de cría. Por su parte, no hemos hallado evidencia de efectos *per se* de las temperaturas alcanzadas durante el desarrollo sobre la supervivencia futura de los pollos, más allá de los relacionados directamente con la fecha (i.e., el cambio en las condiciones ambientales a medida que avanza la temporada). Así pues, nuestros resultados apoyan la existencia de un periodo óptimo durante la temporada de cría en el que las condiciones son más propicias para la reproducción, de tal forma que desviaciones tanto positivas (i.e., individuos que crían tarde) como negativas (i.e., individuos que crían demasiado pronto) de dicho óptimo son perjudiciales para la supervivencia de los descendientes. Los resultados de nuestro estudio también muestran la idoneidad de la longitud de tarso como predictor de supervivencia futura de los juveniles, por ser un parámetro que permanece invariable tras el abandono del nido.

Al analizar la evolución del inicio del periodo de cría en nuestra población (artículo 6), averiguamos que la fecha media de inicio de puesta no había avanzado significativamente en el periodo a estudio, pese a que en la mayoría de años (i.e., en 16 de los 22 años analizados) puestas más tempranas produjeron un mayor número de individuos reclutados. A nivel poblacional, se dio un adelanto de la fecha de puesta en años más cálidos, una respuesta explicada principalmente por plasticidad fenotípica. Además, las temperaturas alcanzadas durante los periodos de incubación y de estancia de los pollos en el nido influyeron en el establecimiento de presiones de selección sobre la fecha de puesta.

CAPÍTULO 4

Discusión general

4.1. Visión global de la Tesis

En esta Tesis Doctoral se han estudiado algunos de los factores que pueden afectar al crecimiento, desarrollo y supervivencia de los pollos de carbonero común durante su estancia en el nido y tras el abandono del mismo, así como la presión de selección ejercida sobre la fecha de puesta poblacional en base a los individuos reclutados. Partiendo de un enfoque experimental insólito hasta la fecha, se manipularon las temperaturas en el interior de cajas-nido durante toda la fase de nidificación de los pollos para averiguar el efecto de la exposición a temperaturas sub-óptimas sobre el crecimiento y la supervivencia de las crías, y se recurrió al radio-seguimiento para determinar si, entre otros factores, la alteración del ambiente térmico del nido pudo afectar posteriormente a la supervivencia de los juveniles durante los primeros días después de volar. Por otra parte, el análisis de largas series temporales de parámetros reproductivos y térmicos recopilados en la zona de estudio durante más de dos décadas, unido al tratamiento de datos de captura-recaptura de la práctica totalidad de los pollos nacidos en este periodo de tiempo, han permitido dilucidar qué conjunto de variables tienen un mayor efecto a largo plazo en la producción anual de volantones, así como en la supervivencia de éstos desde que dejan el nido hasta que son reclutados al año siguiente. Además, se ha analizado la importancia de las temperaturas experimentadas durante el periodo de cría en el establecimiento de presiones selectivas en la fecha de puesta de la población, y su impacto sobre el reclutamiento de los ejemplares. Este tipo de tratamientos multiparamétricos no son comunes en aves, precisamente por ser pocas las poblaciones para las que se cuenta con una extensa base de datos reproductivos recogidos de manera constante durante un número razonable de años. Sin embargo, son de gran valía para esclarecer, de una forma integrada, los factores que determinan el éxito reproductor y las perspectivas de crecimiento de una población. Los resultados obtenidos en esta Tesis se integran en el presente contexto de cambio climático, permitiendo extraer una serie de conclusiones de relevancia para entender el impacto de la variación climática sobre la supervivencia de la especie en el ámbito mediterráneo.

4.2. Determinantes de la supervivencia en el nido

En nuestra población, la supervivencia de los pollos durante el periodo de nidificación resultó ser elevada (i.e., del 86%, ver artículo 3), siendo el número de individuos que nacen por nido el principal determinante a largo plazo de la producción de volantones. En este sentido, cabe incidir en que nuestras estimas de supervivencia parten únicamente de nidos exitosos, por lo que no se ha considerado el impacto de factores conducentes al fracaso total de los nidos, cuyo análisis constituiría la base para un futuro trabajo complementario al aquí expuesto.

Por su parte, la relación directa hallada entre el tamaño de la pollada y el número de pollos que abandona el nido sugiere un buen ajuste entre la disponibilidad de alimento presente en el medio y las necesidades de las crías (Gebhardt-Henrich & Richner 1998; Arnold 2011). No obstante, podría existir un compromiso entre la cantidad y la calidad de los juveniles (i.e., su peso medio al volar), con posibles repercusiones negativas sobre su supervivencia futura. Así, la posibilidad de los padres de sacar adelante polladas numerosas iría en detrimento de la condición de los pollos al volar, lo cual a su vez podría reducir su probabilidad de supervivencia tras el abandono del nido (artículos 4 y 5). En estos momentos, contamos con trabajos previos realizados en nuestra zona de estudio que apuntan a reducciones en la tasa media de ceba de los adultos por pollo, así como en la tasa de crecimiento de las crías, conforme aumenta el tamaño de puesta (Barba et al. 1993; Barba et al. 2009). Teniendo en cuenta la relación existente entre la disponibilidad de recursos y el número de pollos presentes en el nido (Gebhardt-Henrich & Richner 1998; Ruffino et al. 2014), y su efecto sobre el crecimiento y la supervivencia de las crías (Siikamäki 1998; Seward et al. 2014), un posible tema de estudio no contemplado en esta Tesis pasaría, por tanto, por cuantificar la cantidad de biomasa de alimento disponible para los pollos, y relacionarla con su tasa de crecimiento y supervivencia hasta el momento de abandonar el nido.

El resto de variables analizadas con posible incidencia sobre la supervivencia en el nido, como la fecha de puesta o las dimensiones de los huevos (Krist 2011; Polak 2016), no tuvo un efecto relevante a largo plazo sobre la producción de volantones en nuestra zona de estudio. El escaso poder predictivo de estas variables no implica que sean intrascendentes, sino más bien que sus efectos, pudiendo ser importantes determinados años, se diluyen al considerar una serie temporal larga por estar sujetos a variaciones interanuales en las condiciones del medio. Por ejemplo, la incidencia en años concretos de episodios puntuales de temperaturas adversas durante el periodo de estancia de los pollos en el nido podría comprometer su supervivencia (Takagi 2001; Salaberria et al. 2014). Frente a estas dificultades, el haber nacido un poco antes o después (y, por tanto, poseer un mayor o menor grado de desarrollo), o el hecho de contar con más o menos reservas energéticas por proceder de huevos de distinto tamaño, podría ser decisivo a la hora de determinar el número de pollos que acaban abandonando el nido. Otros años, en circunstancias más propicias, puede que estos factores resultasen de menor trascendencia.

4.3. Determinantes de la supervivencia hasta el reclutamiento

El tamaño de los pollos al volar (determinado por su longitud de tarso), junto con la fecha de eclosión, fueron las dos variables más influyentes sobre la supervivencia durante el primer año de vida de los juveniles en nuestra zona de estudio, siendo los ejemplares de mayor tamaño, nacidos en un periodo óptimo de la temporada (i.e., ni muy tarde, ni demasiado pronto), los que poseen mayores probabilidades de ser reclutados al año siguiente (artículo 5). Al igual que ya ha sido sugerido en estudios previos (Naef-Daenzer et al. 2001; Cox et al. 2014), creemos que las primeras semanas transcurridas tras el abandono del nido marcan un punto de inflexión en la vida de los juveniles, de tal forma que, los que logran sobrevivir a este periodo, tienen grandes posibilidades de alcanzar la edad reproductora. Así, el tamaño de los juveniles influiría en su supervivencia durante las primeras semanas fuera del nido, viéndose favorecidos los ejemplares más grandes (artículo 4), lo cual a su vez determinaría la probabilidad de llegar con vida al año siguiente.

Por lo que respecta al efecto de la fecha, resulta significativo que, en nuestra población, no solo los juveniles nacidos demasiado tarde, sino también los nacidos demasiado pronto, posean menores perspectivas de supervivencia futura. El declive en el rendimiento reproductivo conforme avanza la temporada de cría es común en poblaciones aviares, debido principalmente al empeoramiento de las condiciones ambientales (Öberg et al. 2014), a la menor disponibilidad de recursos (Naef-Daenzer & Keller 1999; Verhulst & Nilsson 2008) y al aumento de las tasas de depredación (Sim et al. 2012). Sin embargo, solo hemos encontrado referencia de un trabajo previo donde conste que criar demasiado pronto pueda también ser perjudicial (Norris 1993). Planteamos que este hecho pueda deberse a las particularidades de nuestra zona de estudio, caracterizada por la ocurrencia de tormentas de corta duración, pero fuerte intensidad, al comienzo de la temporada. Precisamente los costes potenciales de comenzar a criar demasiado pronto (Olofsson et al. 2009), unidos a la incapacidad de individuos en peor condición de criar en el periodo óptimo (Price et al. 1988; Verhulst & Nilsson 2008), podrían explicar la ausencia de respuesta a nivel poblacional a la presión de selección a favor de criar antes observada en la mayoría de años (artículo 6).

Por otra parte, la presente Tesis Doctoral evidencia la idoneidad de la medida de longitud de tarso en los días previos al abandono del nido como predictor de supervivencia de juveniles hasta su reclutamiento (artículo 5). Los resultados de nuestro trabajo muestran que, a la hora de proporcionar una estima de la condición física de los pollos al abandonar el nido, la longitud de tarso es un mejor predictor de la supervivencia futura de los juveniles, tal vez por el hecho de ser una medida relativamente invariable durante la transición a la edad adulta, en contraposición a la masa, que puede oscilar en función de la disponibilidad de alimento tras el abandono del nido (Monrós et al. 2002). Así pues, si bien es frecuente en estudios de supervivencia expresar el tamaño corporal de los individuos en base a su masa (Garnett 1981; Velando 2000; Dybala et al. 2013), sugerimos la conveniencia de contemplar, además, o alternativamente, la longitud de tarso como posible variable explicativa.

4.4. Efecto de la temperatura sobre el desarrollo y la supervivencia de los pollos

4.4.1. Efectos individuales *vs.* efectos a nivel poblacional

Las temperaturas alcanzadas en nuestra zona de estudio durante el periodo de crecimiento de los pollos no influyeron directamente a largo plazo en la producción local de volantones (artículo 3) ni en la supervivencia de los juveniles hasta su reclutamiento (artículo 5), si bien nuestros resultados experimentales demuestran que la exposición a temperaturas de nidificación sub-óptimas puede afectar al desarrollo de los pollos dentro del nido (artículos 1 y 2) y, en consecuencia, a su supervivencia inmediata tras el abandono del mismo (artículo 4). La ausencia de efectos directos a largo plazo de la temperatura ambiental sobre la población es indicativa de que, en general, en condiciones normales (i.e., en nidos no manipulados), no se alcanzan con frecuencia ambientes térmicos que puedan resultar nocivos para el desarrollo y posterior supervivencia de los pollos, o al menos no en suficientes nidos como para provocar un impacto a nivel poblacional. Al igual que sucede con otros factores analizados (ver apartado 4.2), el efecto directo que, determinados años, temperaturas adversas pudiesen tener sobre nidos concretos (a consecuencia de, e.g., fenómenos intempestivos), quedaría minimizado al contemplar una serie temporal larga. Es necesario, por tanto, diferenciar los efectos de las temperaturas sobre nidos puntuales hallados en un entorno experimental (donde se ha demostrado que, efectivamente, temperaturas demasiado altas o demasiado bajas tienen un efecto directo sobre el crecimiento de los pollos y su supervivencia después de volar; ver siguiente apartado), de los efectos de la temperatura ambiental sobre la población general a largo plazo. En este sentido, a nivel poblacional, hemos comprobado que las temperaturas alcanzadas durante la fase de crecimiento de los pollos pueden repercutir en los diferenciales de selección anuales en la fecha de puesta, lo cual respalda el impacto del ambiente térmico experimentado durante el periodo de mayor demanda de recursos por parte de las crías sobre la fenología de la especie (artículo 6).

En vistas a la ausencia de efectos poblacionales directos, *per se*, de las temperaturas sobre la supervivencia de los pollos en su primer año de vida, proponemos que dicho efecto sea indirecto (e.g., alterando el comportamiento de depredadores o la disponibilidad de presas) y esté, por tanto, íntimamente ligado a la fecha (i.e., al cambio en las condiciones ambientales con el avance de la temporada; artículo 5). En este punto cabría recordar que, en nuestros análisis de supervivencia, únicamente se ha trabajado con nidos exitosos, por lo que no se han tenido en cuenta aquellos nidos que han fracasado a lo largo de la temporada a consecuencia directa o indirecta de la temperatura (e.g., por abandono de los padres debido a bajas temperaturas, lluvias, o escasez de alimento). Así pues, cabe la posibilidad de que el impacto de la temperatura sobre la supervivencia de los pollos a nivel poblacional sea, en realidad, de una magnitud mayor que la aquí descrita.

4.4.2. Efectos en ambientes experimentales

En el presente trabajo se ha puesto de manifiesto cómo el efecto de las temperaturas sobre el crecimiento de los pollos en el nido puede variar en función de si éstos son sometidos a ambientes térmicos que, o bien sobrepasen, o estén por debajo de su óptimo de tolerancia: en nuestro experimento, los pollos calentados alcanzaron pesos menores (artículo 1), mientras que los enfriados desarrollaron tarsos más pequeños (artículo 2). Un aspecto que queda pendiente por esclarecer de cara a futuros estudios es el motivo de esta diferencia, aunque podemos suponer que esté relacionado con los distintos mecanismos termorreguladores implicados en la respuesta fisiológica a ambientes cálidos y fríos. En este sentido, tenemos constancia de estudios previos que muestran que la exposición de pollos a situaciones de estrés (e.g., desnutrición) no tiene por qué afectarlos morfológicamente de manera uniforme, sino que puede hacer que se priorice el desarrollo de determinados tejidos sobre el de otros (Schew &

Ricklefs 1998; Gil et al. 2008). Así pues, podría ser que, en respuesta al estrés por calentamiento se priorizara el crecimiento de estructuras esqueléticas, mientras que en respuesta al frío fuese más importante poseer mayores reservas de grasa a expensas de alcanzar un menor tamaño. Por otra parte, también habría que analizar si los efectos de la exposición a temperaturas sub-óptimas se limitan a la biometría de los pollos o si van más allá de ésta (e.g., afectando a su capacidad inmunológica, ver Dawson et al. 2005), teniendo, en consecuencia, una mayor incidencia si cabe sobre su condición física.

La supervivencia de los pollos en el nido, por su parte, no se vio afectada por el tratamiento térmico (artículos 1 y 2). Consideramos, por tanto, que la exposición a temperaturas dentro del rango de las alcanzadas en nuestro experimento, puede acarrear costes sobre la condición de las crías, pero sin llegar a resultar mortal. Ahora bien, nuestro estudio de radio-seguimiento demuestra que el debilitamiento de los pollos a consecuencia del estrés térmico padecido en el nido puede reducir sus posibilidades de supervivencia en los primeros días después de volar (artículo 4). Juveniles en peor condición son menos competitivos y resultan más vulnerables al ataque de depredadores (Sim et al. 2013), la principal causa de mortandad durante este periodo crítico en la vida de los polluelos. De esta forma, nuestro estudio sería el primero en demostrar experimentalmente el impacto de la temperatura durante la fase de nidificación en una etapa posterior de la historia de vida de la especie. Por otra parte, un resultado inesperado fue el comprobar que, entre los pollos calentados, fueron los individuos de menor tamaño los que tuvieron una mayor supervivencia en los días inmediatos al abandono del nido. Sugerimos que este hecho podría ser consecuencia de una relación corporal superficie-volumen más favorable para disipar el exceso de calor padecido en el nido, que les haría volar en mejor condición física, aunque sería un tema sobre el que profundizar en futuros trabajos.

Otra cuestión a tener en cuenta es el papel de los padres a la hora de mantener un microclima de nidificación propicio para las crías. En nuestro estudio, la manipulación térmica no pareció influir de forma significativa sobre su comportamiento (artículos 1 y 2). Únicamente se evidenció una disminución del tiempo invertido por las hembras dentro del nido durante los primeros días de vida de los pollos, en respuesta al calentamiento (artículo 1). Sugerimos que los adultos optaron por moderar el esfuerzo invertido en la ceba y acondicionamiento térmico de sus crías, a fin de garantizar su propia supervivencia y el éxito de futuras puestas. Ahora bien, diferencias anuales en la calidad de los individuos que crían, así como en la disponibilidad de recursos en el medio, podrían favorecer una mayor inversión parental en la pollada (Hainstock et al. 2010; Kidawa et al. 2016), lo cual podría amortiguar los efectos de las temperaturas. Este hecho podría ser especialmente evidente en el caso de las bajas temperaturas, ya que, si bien los padres cuentan con mecanismos eficaces para calentar a los pollos y preservarlos del frío cuando éstos son más vulnerables, su capacidad de actuación es limitada a la hora de paliar los efectos del calor (O´Connor 1984).

4.5. El cambio climático y sus consecuencias sobre la supervivencia de los pollos

Los resultados obtenidos en esta Tesis se encuadran en el presente contexto de cambio climático global. Hemos demostrado que los pollos de carbonero común son vulnerables a la variación térmica en su fase de estancia en el nido, y que la exposición a temperaturas sub-óptimas durante este periodo puede afectar a su supervivencia futura. Si tenemos en cuenta que el cambio climático va a acarrear alteraciones en la temperatura media durante la época de cría de las aves (Stocker et al. 2013) y, lo que es más importante, un aumento en la frecuencia de episodios de climatología extrema (Glądalski et al. 2014), se abre un escenario según el cual la supervivencia de las crías pueda resultar comprometida, con repercusiones sobre el rendimiento reproductivo de determinadas especies (e.g., aquellas con menor rango de distribución y capacidad de ocupación de hábitats) a medio o largo plazo. Máxime si tenemos en cuenta que la región mediterránea puede ser de las más afectadas por este fenómeno (Sanz 2002; Hallegatte et al. 2009; Stocker et al. 2013). Faltaría por ver hasta qué punto mecanismos tales como la plasticidad fenotípica dotan a las poblaciones aviares de la flexibilidad necesaria para reaccionar frente al cambio previsto en las temperaturas globales. Nuestros resultados apuntan a que la plasticidad fenotípica de los individuos podría explicar el avance en la fecha media de puesta poblacional en años más cálidos, lo cual estaría en consonancia con resultados previos obtenidos en otras poblaciones de carbonero común (e.g., Charmantier et al. 2008; Husby et al. 2010) y apoyaría la importancia de este mecanismo a la hora de garantizar la persistencia de la especie en un contexto de cambio climático (artículo 6). Resultan, por tanto, imprescindibles más trabajos como los descritos en esta Tesis, combinando los resultados de experimentos manipulativos con los obtenidos a partir del análisis de largas series temporales de datos, para poder prever la verdadera extensión de los efectos del cambio climático sobre las aves, y actuar en consecuencia.

CAPÍTULO 5

Conclusiones generales

A continuación, se enumeran las principales conclusiones obtenidas en esta Tesis Doctoral:

1. En hábitats mediterráneos, la exposición de los pollos a altas temperaturas durante su fase de estancia en el nido provoca que alcancen menor peso al volar. Los efectos del calor pueden ser más evidentes en hábitats templados y/o en años más calurosos.
2. Pollos sometidos a microclimas de nidificación más fríos durante su periodo de desarrollo en el nido alcanzan longitudes de tarso inferiores.
3. El tamaño de la pollada es el principal determinante de la producción de volantones en nuestra población. Polladas más numerosas originan un mayor número de volantones, aunque el peso de los mismos al abandonar el nido es menor.
4. La exposición de los pollos a elevadas temperaturas durante el periodo de nidificación reduce su probabilidad de supervivencia durante los primeros días tras el abandono del nido. Entre los

pollos calentados, los individuos de menor tamaño poseen una mayor probabilidad de supervivencia.

5. La fecha de eclosión y el tamaño de los pollos al volar determinan la supervivencia de los juveniles en su primer año de vida. Pollos más grandes nacidos en un periodo óptimo (i.e., ni demasiado pronto ni muy tarde en la temporada de cría) poseen mayor probabilidad de ser reclutados en nuestra población.
6. La medida de la longitud de tarso al volar es mejor predictor que la masa de la supervivencia futura de los juveniles.
7. De las distintas fases del periodo reproductor, las temperaturas alcanzadas durante las etapas de incubación y de presencia de los pollos en el nido tienen mayor influencia sobre la selección en la fecha de puesta de nuestra población, a consecuencia de su impacto sobre el reclutamiento de los juveniles.
8. La plasticidad fenotípica es el principal mecanismo responsable de la respuesta de nuestra población a la variación térmica, en consonancia con los resultados obtenidos en otras poblaciones mediterráneas de páridos.

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Anexo: Publicaciones

A continuación, se incluyen las seis publicaciones que constituyen esta Tesis Doctoral. Se agrupan en dos secciones: la primera recopila los trabajos que tratan de la supervivencia de los pollos de carbonero común en el nido, y la segunda los referidos a la supervivencia de los pollos después de volar.

Sección 1. La supervivencia de los pollos de carbonero común en el nido

Rodríguez, S. & Barba, E. 2016. Nestling growth is impaired by heat stress: an experimental study in a Mediterranean great tit population. *Zoological Studies* 55: 40. DOI: 10.6620/ZS.2016.55-40.

Rodríguez, S. & Barba, E. 2016. Effects of cool nest microclimates on nestling development: an experimental study with Mediterranean great tits *Parus major*. *Ardeola* 63: 251-260.

Rodríguez, S., Álvarez, E. & Barba, E. 2016. Factors affecting fledgling output of great tits, *Parus major*, in the long term. *Animal Biodiversity and Conservation* 39: 147-154.

Sección 2. La supervivencia de los pollos de carbonero común tras el abandono del nido

Rodríguez, S., Diez-Méndez, D. & Barba, E. Negative effects of high temperatures during development on immediate post-fledging survival in great tits *Parus major*. *Acta Ornithologica*. En prensa.

Rodríguez, S., van Noordwijk, A.J., Álvarez, E. & Barba, E. 2016. A recipe for post-fledging survival in great tits *Parus major*: be large and be early (but not too much). *Ecology and Evolution* 6: 4458-4467.

Rodríguez, S., Álvarez, E. & Barba, E. Phenotypic plasticity could account for temperature-related changes in timing of breeding of a passerine bird over 22 years. Enviado para publicación.

Sección 1: La supervivencia de los pollos de carbonero común en el nido

ARTÍCULO 1

Nestling growth is impaired by heat stress: an experimental study in a Mediterranean great tit population

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ABSTRACT

During the nestling stage, nestlings of small altricial birds face energetic limitations due to their rapid development and the need to maintain a stable body temperature once homeothermy is achieved. In Mediterranean habitats, high air temperatures reached during the breeding season could negatively affect the health and condition of the nestlings. The aim of this study was to determine the effect of an experimental increase of nest temperatures during the nestling stage on the growth and survival of great tit (*Parus major*) nestlings. Additionally, changes in parental brooding and feeding behavior as a result of the alteration of the nest microclimate were addressed. Increased nest temperatures affected nestling mass, as heated nestlings were lighter than controls on day 15 in the warmer of the two breeding seasons considered. Moreover, females from the heating treatment reduced their brooding time. Fledging success and parental feeding rates were not altered by the experimental treatment. The results of this study suggest that high nest temperatures may impair nestling development and therefore affect post-fledging survival probability. Negative effects are more likely to occur in warm habitats and/or warmer years, where juveniles are liable to suffer from thermal stress.

Keywords: brooding behavior, feeding behavior, hyperthermia, growth, survival.

RESUMEN

Durante su periodo de estancia en el nido, los pollos de aves altriciales deben hacer frente a limitaciones energéticas a consecuencia de su rápido desarrollo y la necesidad de mantener una temperatura corporal estable una vez lograda la homeotermia. En hábitats mediterráneos, las elevadas temperaturas ambientales alcanzadas durante la época de cría podrían afectar negativamente a la salud y la condición de los pollos. En este estudio pretendimos determinar el efecto de un aumento experimental de la temperatura del nido durante el periodo de nidificación sobre el crecimiento y la supervivencia de pollos de carbonero común (*Parus major*). Además, se analizaron alteraciones en el comportamiento parental, en cuanto al tiempo invertido en el empolle y la ceba de los pollos, a consecuencia de la modificación del microclima de nidificación. El aumento de la temperatura del nido afectó a la masa de los pollos en la más cálida de las dos temporadas consideradas, ya que los ejemplares calentados fueron más ligeros a los 15 días de edad que los controles. Además, las hembras de los nidos calentados redujeron el tiempo invertido empollando a las crías.

Por el contrario, ni la producción de volantones ni las tasas de ceba parentales se vieron alteradas por el tratamiento térmico. Los resultados de este estudio sugieren que temperaturas elevadas de nidificación pueden perjudicar al desarrollo de los pollos, y consecuentemente afectar a su probabilidad de supervivencia tras el abandono del nido. Los efectos negativos de las altas temperaturas son más factibles en hábitats y/o años cálidos, cuando los juveniles pueden ser más proclives a padecer estrés térmico.

Palabras clave: comportamiento de ceba, comportamiento de empolle, crecimiento, hipertermia, supervivencia.

INTRODUCTION

The nestling stage is one of the most energetically challenging periods of a bird’s lifetime (Lack 1968; Ricklefs 1983). The growth of developing chicks is optimum across a range of nest temperatures that meets their energetic needs, but deviations from this optimum may be harmful. In this sense, the effects of temperature on the metabolic demands of developing nestlings can vary with age, depending on their homeothermic abilities (McCarty & Winkler 1999). Newly hatched altricial young have very limited capacity for conserving heat, because of their high body surface-to-mass ratio (Dawson & Evans 1960) and absence of plumage (Jenni & Winkler 1994; Hinsley et al. 2003). During early development, it is therefore up to the parents to invest energy and resources in the provisioning and brooding of their young, at the expense of their own self-maintenance (Marsh & Wickler 1982; Starck & Ricklefs 1998; Visser 1998; Węgrzyn 2013). Temperature regulation in great tit (*Parus major*) nestlings begins when they are 4-6 days old, and consolidates rapidly once they are 10 days old (Mertens 1977a). Before reaching homeothermy, nestling altricial condition allows the allocation of available resources to tissue growth rather than maintenance (Ricklefs 1968, 1993; Olson 1992; Arendt 1997; Węgrzyn 2013). Once they begin to thermoregulate on their own, however, nestlings face energetic limitations associated with their rapid development and the need to sustain a stable body temperature (Ricklefs 1983; Schew & Ricklefs 1998).

Regardless of their developmental stage, heat loading could be a serious threat for nestlings. In Mediterranean habitats, high temperatures reached during the breeding season could exert negative effects on the health and condition of the nestlings (e.g., Belda et al. 1995). Hyperthermia has been shown to lower chick food intake, and consequently reduce their growth rate and muscle development (Murphy 1985; Geraert et al. 1996; Moreno et al. 2002; Cunningham et al. 2013). It also increases their energetic demands, causing them to lose weight (Routman et al. 2003; Catry et al. 2015). High environmental temperatures may impair nestling´s immune response, reducing the levels of total circulating antibodies or altering the phagocytic ability of macrophages (Lara & Rostagno 2013). In addition, chicks exposed to high ambient temperatures enter a stage of oxidative stress, leading to the production of heat shock proteins in several tissues, which have been shown to have negative effects on growth (Moreno et al. 2002). Further direct effects of elevated temperatures on nestling body condition include dehydration (a consequence of the activation of thermolytic mechanisms such as panting to promote evaporative heat loss), cardiovascular mortality and respiratory illnesses (Mertens 1977a; Belda et al. 1995; Patz et al. 2005). Additionally, the adverse effects of high temperatures on nestling fitness could also increase adult foraging costs, as chicks may require more food to compensate the energetic expenses of thermoregulation (Royama 1966; Barba et al. 2009).

Only a handful of experimental studies have shown a correlation between nest temperatures and nestling growth, development and/or survival. Most of these have sought to manipulate temperatures during the egg-laying and incubation stages, testing for subsequent effects on parental behavior and nestling condition (e.g., Nager & van Noordwijk 1992; Nilsson et al. 2008; Pérez et al. 2008; Álvarez & Barba 2014a). If we focus on the nestling stage, only Dawson et al. (2005) applied heat after hatching. They concluded that, in a temperate environment, increasing nest temperatures had overall positive effects on offspring fitness, benefits that could not be attributed to changes in parental behavior. It remains to be seen whether these results would reproduce under hotter conditions, where high air temperatures could act as potential stressors to which developing nestlings may be vulnerable (see Lobato et al. 2008). For example, Álvarez & Barba (2014a) showed that, in a Mediterranean great tit population, females incubating in nests where temperature was experimentally increased allocated less time to incubate. A similar response could be expected for females brooding small nestlings.

The aim of this study is to experimentally determine the influence of relatively high nest temperatures on great tit nestling growth and survival in a Mediterranean habitat. Additionally, we aimed to clarify whether these potential effects may lead to variations in parental care and/or provisioning behavior. We hypothesize that, under high air temperature regimes, a hot nest microclimate will have negative effects on nestling development, as the nestlings will have to allocate resources to thermoregulation rather than growth. Moreover, raising nest temperatures will likely modify parental behavior, as the extra heat provided may reduce female brooding constancy, and the higher energetic demands of the chicks may require an increase in adult provisioning effort.

MATERIALS AND METHODS

Study site and species

Data used for this study were collected from a great tit population breeding in nest boxes in Sagunto (Valencia, eastern Spain 39°42'N, 0°15'W, 30 m a.s.l.) during 2011 and 2012. The study area was located within an extensive orange monoculture, and wooden nest boxes [see Lambrechts et al. (2010) for details] were placed each year for the birds to breed (e.g., Rodríguez et al. 2016). Mean air temperatures at this site increase from 15.1 °C in April to 24.5 °C in July (Agencia Estatal de Meteorología, period 1986-2012). On average, mean maximum air temperatures during these months range from 21.0 °C in April to 30.0 °C in July, with extreme daily values that may be as high as 43.4 °C.

Great tits are uniparental incubators in which females incubate for about 66% of the day in the studied population (Álvarez & Barba 2014b). The average clutch size is around 8 eggs (Atiénzar et al. 2012). Incubation starts once the clutch is complete, and lasts for about 13 days (Álvarez & Barba

2014c). After hatching, both sexes take part in feeding the young. The nestling period in this species lasts for about 18 days (Atiénzar et al. 2012).

Experimental design

Nest boxes were visited weekly in order to obtain the laying date of the first egg (estimated assuming that one egg was laid per day; Encabo et al. 2001). After the fifth egg was laid, we made daily visits to determine the beginning of incubation as well as the final clutch size (see Álvarez & Barba 2014c). Once incubation started, we paired 20 nests according to clutch size and expected hatching date, with one nest from each pair randomly allocated to the heating treatment, and the other acting as control. Beginning on day 11 after the start of incubation, nests were visited daily until the first egg hatched.

On the date of hatching of the first egg (day 0), we took the nest and its contents (eggs and chicks) out of the nest box and measured the thickness of the nest base (see Álvarez & Barba 2008). We then prepared the nest box for the experiment by introducing a wire mesh 1 cm above the nest box floor (i.e., to standardize treatment conditions), thus creating a chamber where control and heat pads could be inserted (see Álvarez & Barba 2014a, for more details). Finally, we returned the nest and its contents to the nest box, positioning them on the wire mesh.

The temperature manipulation treatment started at 07:00 the next day (day 1), and lasted until 19:00 on day 14, when the heat and control pads, as well as the wire mesh, were permanently removed. Heat pads consisted of commercial warming units (UniHeat Packs, Chrosmack Ventures, Montana, USA, in 2011, and Mycoal warmpacks, Northbrook Industrial Estate, Southampton, UK in 2012), which produced elevated temperatures when exposed to air for 40 and 24 h respectively. We used pads kept at ambient temperature as controls. Heat pads were replaced as necessary to maintain continuously elevated nest temperatures during the experiment (every other day in 2011 and daily in 2012), and control nests were disturbed in a similar way (i.e., control pads were removed and replaced with the same frequency as the heat pads). Regardless of the different longevity of the pads used, their replacement in the field was done before they lost their warming capacity, in an effort to guarantee the effectiveness of the heating treatment in both years. The experiment was conducted between April 27 (i.e., day 1 of the first nest) and May 19 (i.e., day 14 of the last nest) in 2011, and between April 28 and May 23 in 2012.

The experimental nests were collected in sealed plastic bags after fledging, and stored at -20 °C. Once the breeding season concluded, the nests were dried in an oven (105 °C, for 12 h) and weighed with a digital scale (accuracy 0.01 g) to obtain dry nest masses (details in Álvarez et al. 2013). This parameter has been shown to be a good estimator of overall nest mass, regardless of the possible addition of nesting material during the course of the nesting cycle (Dubiec & Mazgajski 2013). Overall, we have data from 19 nests in 2011 (10 control, 9 heated) and 17 nests in 2012 (9 control, 8 heated). In 2011, one nest selected for heating failed during the incubation period (before the temperature manipulation experiment). In 2012, we lost three nests during the experiment: one control nest failed whereas two heated nests were preyed upon.

Nest and air temperature recording

Temperature was measured with data loggers in a sample of nests: 15 nests in 2011 (8 control, 7 heated), and 14 nests in 2012 (7 control, 7 heated). Temperature sensors were placed between the wire mesh and the nest base (see Dawson et al. 2005). In 2011, we used four thermocouple thermometers (Model HOBO U23 Pro v2, Onset Corporation, Bourne, MA, USA), programmed to make internal temperature readings every two minutes. The thermometers were moved between nests in order to obtain records of as many nests as possible. In 2012, we used 20 Thermochron iButton data loggers (Model DS1922L-F5, Embedded Data Systems, Lawrenceburg, KY, USA), programmed to make internal temperature readings every 32 seconds during the first four days, and every 95 seconds afterwards.

For each of the nests in 2011 we recorded nest temperature for 24 h, once between days 1 and 7, and again between days 8 and 14. These age categories were chosen so as to comprise two different stages of nestling thermoregulatory capabilities. In 2012 we were able to use continuous data recorded during the two periods. Then, we calculated, for each nest and age category, mean diurnal (07:00 – 20:00) and nocturnal (20:00 – 07:00) temperatures.

Ambient temperature during the experiment was estimated by calculating, for each nest, average maximum, mean and minimum air temperatures from day 1 to day 15 of age of the nestlings. Air temperature data were collected from a meteorological station located approximately at 4 km from our study site.

Female brooding and parental feeding rates

All the nests under study were visited four times a day after the beginning of the experiment (around 07:00, 11:00, 15:00 and 19:00) to record the presence of the female (i.e., we checked if the female was inside the nest box, without causing her to leave; see e.g. Álvarez & Barba 2014a, for a similar methodology during the incubation period). This pattern of visits allowed us to estimate female brooding constancy during the nestling period. We calculated, for each nest box, the proportion of visits with the female present during three age intervals: on days 1-4, 5-9 and 10-14.

Parents were captured at the nest using spring traps when nestlings were 10-12 days old. Adults were ringed with individually numbered metal rings at this time, and we measured their mass with a digital balance, and tarsus length with a digital caliper. In order to quantify their provisioning behavior, we subcutaneously injected them passive integrated transponder tags (PIT tags) specifically designed for small passerines (Álvarez & Barba, 2014a) in 2012. The day after both adults were fitted with transponders (on days 11-13), we attached a transponder reading system (Trovan Ltd, Isle of Man, United Kingdom) to the nest box entrance, which recorded the time when a tagged bird entered or left the nest, as well as its identity. The reader was removed the following day. Recording times varied between 4 and 15 h, which allows for a good estimation of feeding frequency (see Pagani-Núñez & Senar 2013). Previous studies in our site have shown that great tits feed their nestlings at a constant rate throughout the day (Barba et al. 2009), so we did not limit data collection to a specific time period.

From the collected data, we calculated the absolute number of feedings per hour of males and females. For analyses, transponder reader failure limited our sample size to seven heated and five control nests in the case of male provisioning data, and seven heated and seven control nests in the case of female provisioning.

Nestling biometry and survival

To allow for individual recognition of the nestlings during the nesting period, they were marked on day 5 on different parts of the body with a permanent non-toxic pen, remarked on day 7, and ringed on day 9 using individually numbered metal rings. We recorded the number of nestlings alive on days 5, 9 and 15 of age, and weighed them at these ages using a digital balance. On day 15, we also measured their tarsus length with a digital caliper. This age of measuring is a standard procedure in most great tit populations (e.g., Barba et al. 1993) and no nestling was force-fledged due to handling at this age. On day 20 we visited each nest box to check whether all the young had fledged (age of fledging in our population is approximately 18 days, per. obs.). Dead individuals were identified.

Statistical analyses

We tested for pre-experimental differences among treatments (control vs. heating) in nest dry mass, base thickness, laying date, clutch size, hatching date, number of hatchlings and proportion of eggs hatched (number of hatchlings/clutch size) using General Linear Models with normal distribution (nest dry mass, nest base thickness, laying date, hatching date) or Generalized Linear Models with Poisson (clutch size, number of hatchlings) and Binomial (proportion of eggs hatched) distributions. We considered including these variables as covariates in further analyses (i.e., nest dry mass in temperature model, see below) in case of significant pre-experimental differences between heated and control nests. We also tested for post-treatment differences in number of fledglings and proportion of young fledged (brood size at fledging/initial brood size) using Generalized Linear Models with Poisson (number of fledglings) and Binomial (proportion of young fledged) distributions. All models incorporated year, treatment group (control or heat), and treatment-by-year interaction term as fixed factors.

Differences between treatments in nestling survival to day 15 were tested with a Generalized Linear Mixed Model with Binomial distribution. We considered year, treatment, and treatment-by-year interaction term as fixed factors and nest box as a random factor. We used General Linear Mixed Models to examine whether nestling biometry differed among treatments and years. The variables examined individually in these analyses were nestling mass at ages 5, 9 and 15 days, tarsus length at age 15 days, and mass differences between days 15 and 9, and between days 9 and 5. The models included year, treatment group and treatment-by-year interaction term as fixed factors, and nest box as a random factor. Given that brood size may affect the growth and survival of each individual nestling (Pettifor et al. 2001), we initially included the number of nestlings present at each age as a covariate in the models, and eventually simplified them in case its inclusion did not lead to an improvement in model fit.

To test for differences among experimental treatments in parental feeding rates late in the nestling period, we used a Generalized Linear Model with Poisson distribution. We included sex, treatment group and treatment-by-sex interaction term as fixed factors. In addition, given that provisioning rates may vary with brood size (Rauter et al. 2000; Barba et al. 2009) and temperature (Rauter et al. 2000; García-Navas & Sanz 2012), we initially included the number of nestlings present on day 9 (as proxy of brood size during the recording period) and mean air temperature as covariates, and eventually simplified the model because their inclusion did not lead to an improvement in model fit.

We compared female body condition between heated and control nests using a General Linear Model. We included the body mass-to-tarsus ratio as dependent variable, the treatment group and treatment-by-year interaction term as fixed factors, and the number of nestlings on day 9 (as proxy of brood size during the analyzed period) as a covariate. Given that the inclusion of the covariate had no significant effect on the results of the model, it was eventually simplified.

Differences among treatments in the presence of brooding females in the nest boxes were analyzed with Generalized Linear Models with binomial distribution, including the proportion of visits with the female present during days 1-4, days 5-9 or days 10-14 of age of the nestlings as response variable, and year, treatment group, and treatment-by-year interaction term as fixed factors. As female attendance may be influenced by temperatures and brood size (Rauter et al. 2000; Leckie et al. 2008), we initially included mean air temperature during each period and the number of nestlings present on day 5, 9 or 15 (i.e., as proxy of the number of nestlings present during each of the analyzed periods) as covariates, and eventually simplified the models because their inclusion provided no significant improvement in model fit.

Variables affecting internal nest box temperatures were tested using a General Linear Mixed Model. We included nest temperature averages as dependent variable, treatment group, nestling age category (1-7 days or 8-14 days), time of day (day-time or night-time) and year (2011 or 2012) as fixed factors, and nest box as a random factor. Nest dry mass and mean air temperature were initially considered as covariates, although nest dry mass was eventually simplified from the final model, as it provided no improvement in model fit. Given that nest base thickness did not vary between treatments (see Results), it was not included into this analysis. For the sake of simplicity, we only considered in the model first-order interactions involving the treatment factor.

Nestling survival analysis was performed using package lme4 v. 1.1.9 (Bates et al. 2014) in software R v. 3.2.0 (R Development Core Team 2015). The remaining analyses were performed using IBM SPSS Statistics 22 software. We considered results significant at the 0.05 level.

RESULTS

There were no differences between heated and control nests in either the nest base thickness, laying date, clutch size, hatching date or number of hatchlings. We did find differences in nest dry mass ($F_{1,31}$ = 4.549, p = 0.041; Table 1), as heated nests were on average lighter than control nests.

Table 1. Breeding parameters and characteristics (± SE) of heated and control nests during the 2011 and 2012 field seasons. Sample sizes in parenthesis refer to number of nests. Statistics associated to the year, treatment and treatment-by-year interaction are provided, as computed by the Linear Models. Significant p values (≤ 0.05) are indicated in bold font.

	Year	Heated	Control	Factor	F	p
Nest base thickness (mm)	2011	7.07 ± 3.17 (9)	9.54 ± 5.04 (10)	Year	$F_{1,32}$ = 14.579	**0.001**
	2012	4.06 ± 3.10 (8)	3.00 ± 2.96 (9)	Treatment	$F_{1,32}$ = 0.318	0.577
				Year*Treatment	$F_{1,32}$ = 2.005	0.166
Nest dry mass (g)	2011	14.80 ± 2.30 (8)	19.95 ± 5.11 (10)	Year	$F_{1,31}$ = 3.392	0.075
	2012	14.34 ± 3.70 (8)	15.21 ± 4.58 (9)	Treatment	$F_{1,31}$ = 4.549	**0.041**
				Year*Treatment	$F_{1,31}$ = 2.293	0.140
Laying date	2011	11.33 ± 3.00 (9)	11.50 ± 2.88 (10)	Year	$F_{1,31}$ = 0.735	0.398
	2012	12.14 ± 2.80 (7)	12.56 ± 3.91 (9)	Treatment	$F_{1,31}$ = 0.071	0.792
				Year*Treatment	$F_{1,31}$ = 0.013	0.911
Clutch size	2011	8.44 ± 1.01 (9)	8.80 ± 1.48 (10)	Year	$F_{1,32}$ = 0.166	0.686
	2012	8.25 ± 1.39 (8)	8.67 ± 0.71 (9)	Treatment	$F_{1,32}$ = 0.918	0.345
				Year*Treatment	$F_{1,32}$ = 0.007	0.933

Hatching date	2011	30.22 ± 2.77 (9)	30.80 ± 3.08 (10)	Year	$F_{1,32} = 4.421$	**0.043**
	2012	32.63 ± 2.50 (8)	32.67 ± 3.61 (9)	Treatment	$F_{1,32} = 0.093$	0.762
				Year*Treatment	$F_{1,32} = 0.070$	0.793
Number hatched	2011	7.33 ± 1.41 (9)	7.50 ± 1.96 (10)	Year	$F_{1,32} = 2.509$	0.123
	2012	8.13 ± 1.36 (8)	8.33 ± 0.71 (9)	Treatment	$F_{1,32} = 0.133$	0.718
				Year*Treatment	$F_{1,32} < 0.001$	0.983
Proportion hatched	2011	0.87 ± 0.12 (9)	0.85 ± 0.19 (10)	Year	$F_{1,32} = 9.739$	**0.004**
	2012	0.99 ± 0.04 (8)	0.96 ± 0.08 (9)	Treatment	$F_{1,32} = 0.589$	0.449
				Year*Treatment	$F_{1,32} = 0.326$	0.572
Number fledged	2011	6.56 ± 2.24 (9)	7.10 ± 1.97 (10)	Year	$F_{1,32} = 0.005$	0.944
	2012	7.38 ± 1.41 (8)	6.22 ± 1.86 (9)	Treatment	$F_{1,32} = 0.199$	0.659
				Year*Treatment	$F_{1,32} = 1.529$	0.225
Proportion fledged	2011	0.88 ± 0.22 (9)	0.95 ± 0.10 (10)	Year	$F_{1,32} = 2.168$	0.151
	2012	0.91 ± 0.11 (8)	0.75 ± 0.22 (9)	Treatment	$F_{1,32} = 0.163$	0.689
				Year*Treatment	$F_{1,32} = 3.140$	0.086

Internal nest box temperatures varied significantly with year, treatment and time of day (Table 2). Average temperatures in 2012 were approximately 5 °C higher than in 2011. In both years, temperatures inside the heated nests were higher than in control nests. Additionally, temperatures in experimental nests were higher during day-time than during night-time (Figure 1). We also detected a significant treatment-by-time of day interaction (Table 2), implying that the effect of the heating treatment was greater during day-time than during night-time (Figure 1). Mean air temperatures in our study area during days 1-15 of age of the nestlings were 18.44 °C in 2011 and 19.10 °C in 2012. Maximum temperatures during this period were, on average, 2.05 °C higher in 2012 than in 2011.

Table 2. Factors affecting temperatures inside experimental nest boxes. Treatment group, nestling age category, time of day, and year are considered as factors, air temperature as a covariate, and nest internal temperature averages as dependent variable. Significant p values (≤ 0.05) are indicated in bold font.

Factor	**F**	**p**
Treatment	$F_{1,25} = 36.652$	**< 0.001**
Age	$F_{1,80} = 1.583$	0.212
Time of day	$F_{1,76} = 44.967$	**< 0.001**
Year	$F_{1,26} = 20.023$	**< 0.001**
Treatment*Age	$F_{1,79} = 0.156$	0.694
Treatment*Time of day	$F_{1,76} = 4.228$	**0.043**
Treatment*Year	$F_{1,25} = 1.178$	0.288
T_{air}	$F_{1,88} = 11.609$	**0.001**

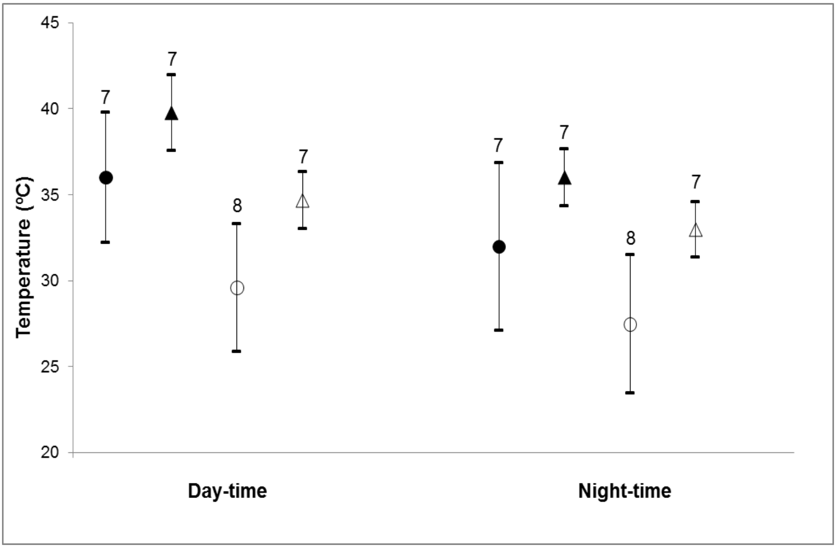

Figure 1. Temperatures of experimental nests in relation to time of day. Temperature means (± SE) for experimentally heated (black) and control nests (white) of great tits in relation to time of day (day-time: 07:00-20:00; night-time: 20:00-07:00). We show data for two consecutive years: 2011 (dots) and 2012 (triangles). Sample sizes above error bars refer to number of nests.

Nestling mass on day 15 differed significantly between experimental treatments and between years (Table 3). The treatment-by-year interaction effect was also close to significance (Table 3). We therefore analyzed both years separately, showing that heated nestlings were 1.56 g lighter than control ones in 2012 (Table 4, Figure 2). Tarsus length on day 15 also varied significantly with year (Table 3), but neither the treatment nor the treatment-by-year interaction factors were significant. Mass differences between days 15 and 9 and between days 9 and 5 did not differ significantly between treatments. Neither did nestling survival to day 15, the number of fledglings per nest, or the proportion of young fledged.

Table 3. Factors affecting nestling biometry. Year, treatment group, and the treatment –by-year interaction term are considered as factors, and nestling mass on day 15, tarsus length on day 15 or mass differences as dependent variable. Models include the number of nestlings present on day 5, 9 or 15 as a covariate in case of significance. Significant p values (≤ 0.05) are indicated in bold font.

	F	**p**
Mass day 5 (g)		
Year	$F_{1,29} = 0.351$	0.558
Treatment	$F_{1,29} = 1.423$	0.242
Year*Treatment	$F_{1,29} = 0.440$	0.512
Mass day 9 (g)		
Year	$F_{1,29} = 0.890$	0.353
Treatment	$F_{1,29} = 0.216$	0.646
Year*Treatment	$F_{1,29} = 0.208$	0.652
Mass day 15 (g)		
Year	$F_{1,33} = 14.360$	**0.001**
Treatment	$F_{1,33} = 4.172$	**0.049**
Year*Treatment	$F_{1,33} = 4.034$	0.053
Mass day 9 - Mass day 5 (g)		

Year	$F_{1,30} = 2.846$	0.102
Treatment	$F_{1,30} = 1.786$	0.191
Year*Treatment	$F_{1,31} = 0.794$	0.380
Mass day 15 - Mass day 9 (g)		
Year	$F_{1,29} = 2.126$	0.155
Treatment	$F_{1,30} = 1.567$	0.220
Year*Treatment	$F_{1,30} = 1.701$	0.202
Number of nestlings on day 9	$F_{1,31} = 5.328$	**0.028**
Tarsus length day 15 (mm)		
Year	$F_{1,31} = 12.002$	**0.002**
Treatment	$F_{1,31} = 0.015$	0.903
Year*Treatment	$F_{1,31} = 0.289$	0.595

Presence/absence data evidenced that heated females spent 15% less time in the nest than control females during the first four days of age of the nestlings (Table 5). There were no differences in female attentiveness between treatments either during days 5-9 of age of the nestlings or during days 10-14 (Table 5). Female body condition was not altered by the experimental treatment ($F_{1,31} = 0.048$, $p = 0.828$). We found no significant year ($F_{1,31} = 0.276$, $p = 0.603$) or treatment-by-year ($F_{1,31} = 0.016$, $p = 0.900$) effect.

Table 4. Biometrics (±SE) of heated and control nestlings during 2012. Least-squared means and standard errors are provided, as computed by the General Linear Mixed Models. Values in parenthesis refer to the degrees of freedom. Significant p values (≤ 0.05) are indicated in bold font.

	Heated	**Control**	**F**	**p**
Mass day 5 (g)	7.23 ± 0.26 (14)	6.76 ± 0.25 (15)	$F_{1,14} = 1.689$	0.214
Mass day 9 (g)	12.57 ± 0.40 (14)	12.93 ± 0.39 (15)	$F_{1,14} = 0.413$	0.531
Mass day 15 (g)	15.07 ± 0.52 (15)	16.63 ± 0.50 (15)	$F_{1,15} = 4.664$	**0.047**
Mass day 9 – Mass day 5 (g)	5.28 ± 0.38 (15)	6.05 ± 0.36 (15)	$F_{1,15} = 2.111$	0.167
Mass day 15 – Mass day 9 (g)	2.65 ± 0.68 (14)	4.19 ± 0.64 (15)	$F_{1,15} = 2.738$	0.119
Tarsus length day 15 (mm)	18.94 ± 0.16 (13)	19.00 ± 0.16 (14)	$F_{1,14} = 0.082$	0.779

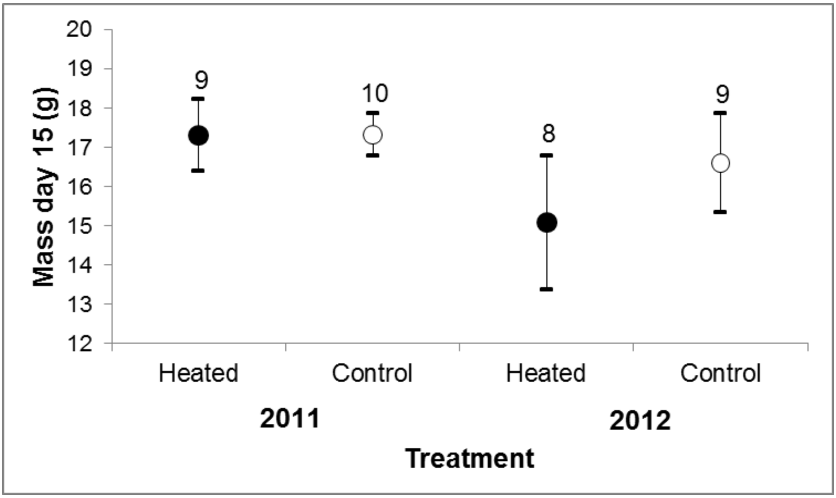

Figure 2. Nestling mass on day 15 in experimental nests. Average nestling mass on day 15 for experimentally heated (black) and control nests (white). We show data for two consecutive years: 2011, and 2012. Sample sizes above error bars refer to number of nests.

Table 5. Factors affecting female attentiveness. Year, treatment group, and the treatment –by-year interaction term are considered as factors, and the proportion of visits with the female present during days 1-4, days 5-9 or days 10-14 as dependent variable. Significant p values (≤ 0.05) are indicated in bold font.

	F	**p**
Female present days 1-4		
Year	$F_{1,32} = 0.456$	0.504
Treatment	$F_{1,32} = 5.959$	**0.020**
Year*Treatment	$F_{1,32} = 0.770$	0.387
Female present days 5-9		
Year	$F_{1,32} = 0.824$	0.371
Treatment	$F_{1,32} = 0.265$	0.611
Year*Treatment	$F_{1,32} = 1.996$	0.167
Female present days 10-14		
Year	$F_{1,32} = 0.864$	0.360
Treatment	$F_{1,32} = 0.881$	0.355
Year*Treatment	$F_{1,32} = 0.200$	0.658

In addition, the manipulation of nest microclimate did not significantly influence parental provisioning behavior ($F_{1,22} = 0.092$, $p = 0.765$). There was no significant effect of sex ($F_{1,22} = 0.323$, $p = 0.576$), although we did find a significant treatment-by-sex interaction ($F_{1,22} = 5.109$, $p = 0.034$), meaning that the heating treatment had a different, but non-significant effect on males than on females (i.e., heated males tended to increase their feeding rate in comparison with control males, whereas heated females tended to decrease it).

DISCUSSION

The experimental heating of the nests affected nestling condition in the warmer year, as heated nestlings were lighter on day 15 than control chicks in 2012. Nest temperatures reached in 2011 did not seem to have been high enough to cause significant impacts on the measured nestling parameters, although we cannot reject further effects in non-measured indicators of chick health, such as immune response or plasma osmolality (see Saito & Grossmann 1998; Mashaly et al. 2004; Dawson et al. 2005).

Increasing nest temperatures during chick development resulted in heated females spending less time in the nest during the first four days post-hatching. We are aware of the limitations of the data used to estimate female brooding constancy, which do not allow for a precise record of changing behavior patterns, but this effect was apparent despite the low resolution of the data. This decrease in parental attentiveness in response to experimental heating has already been shown by Álvarez & Barba (2014a) during the incubation stage, and it seems also to occur during the brooding period. The extra-heat provided during the first days of the experiment may have allowed adult females to increase their investment in self-maintenance activities outside the nest, although we did not detect a positive effect on female condition. This decrease in brooding time had no measurable effects on nestling mass on day 5, so it seems that the extra heat provided compensated the lower brooding attentiveness.

We predicted that increased nest temperatures would alter parental provisioning behavior at the time of greater food demand by the nestlings, a result of the adults having to intensify their feeding effort in order to compensate the higher thermoregulation costs incurred by the chicks. Contrary to expectation, the heating treatment had no significant impact on parental feeding rates on days 10-12 of age of the young. This agrees with the results of Dawson et al. (2005) with heated nestling tree swallows (*Tachycineta bycolor*), and suggests that the adults may have sacrificed investment in their young in favor of their own fitness. Food provisioning imposes high energetic demands on the parents (see Bryant & Tatner 1991), and they may trade-off current reproductive effort to guarantee their own survival and the success of future breeding attempts.

High nest temperatures have been shown to impose negative effects on nestling condition due to their greater vulnerability and restricted ability to regulate body temperature (Dunn 1979; Belda et al. 1995; Geraert et al. 1996). Nestlings must rely on evaporative cooling and the elevation of body temperature above normal levels as main defenses to cope with heat stress (O'Connor 1984), mechanisms that can be energetically-demanding and may lead to severe dehydration risks and lowered fitness (Du Plessis et al. 2012; Cunningham et al. 2013). Moreover, exposure of nestlings to elevated temperatures may eventually result in higher mortality, when the heat-loss mechanisms are inadequate to dissipate the total heat produced by the chicks and body temperature reaches lethal levels (Mertens 1977a; Warriss et al. 2005). In our case, increasing nest temperatures during nestling development did not alter nestling survival to day 15. Neither there were differences in the number of fledglings and proportion of young fledged. As far as we could tell, temperatures inside heated nests, although high enough to impose a net cost to developing nestlings in 2012 (see Dawson 1958; Quinteiro-Filho et al. 2010), did not reach lethal thresholds.

Our results are in agreement with previous experiments that have tried to determine the optimal thermal range in great tit nestlings (see Mertens 1977b). The upper and lower limits of this temperature range are dependent on factors such as brood size, nestling age and the dimensions and thermal properties of the nest (e.g., water content of the nest and heat conductance). For example, for broods consisting of six nestlings of 9 days of age, the upper and lower temperature limits in nests with a water content of 8% would be around 31 and 12 °C respectively. Temperatures inside this optimal range meet nestling metabolic requirements for growth, but values above and below it may have negative effects on nestling physiology, condition and survival (Salaberria et al. 2014). In his study, Mertens (1977a) distinguished between long-term and immediate risk of hyperthermia based on the maximum percentage of the basal heat production that nestlings could safely dissipate by water evaporation. Nestlings incurred in long-term risks of hyperthermia when they were forced to dissipate more water, leading to dehydration and eventual mortality if conditions persisted. Immediate risk of hyperthermia was evoked when the highest possible rate of water evaporation could not match the evaporation rate required to keep body temperature below lethal levels. Based on our experience, temperatures above 34 °C (i.e., temperatures reached in heated nest boxes in 2012) would be needed to start causing negative effects on nestling physiology. Other studies have reported similar upper-temperature thresholds for nestlings of altricial species (see Ardia 2013 and Cunningham et al. 2013 for studies with tree swallows and common fiscals *Lanius collaris* respectively).

The alteration of nest microclimate above reported optimal temperature levels led to reduced body mass in great tit nestlings. Although mass differences in the measured periods were not significant, it seems that the delay in mass gain occurred at older ages (i.e., between 9 and 15 days of age; see Table 4), when energy demands are higher. Nestling size, measured by tarsus length, was apparently unaffected. In this sense, previous studies have suggested the capacity of developing nestlings to selectively allocate resources towards growth of specific sets of tissues when exposed to limiting conditions, thus prioritizing those structures that maximize survival (Schew & Ricklefs 1998; Gil et al. 2008). This way, when exposed to suboptimal temperatures, the energetically-limited nestlings could have sacrificed fat accumulation in favor of skeletal growth or development of other non-measured morphological traits with direct effects on survival, such as muscle or wing length. Given the advantage of extra fat reserves at fledging to withstand periods of food shortage (Odum & Connell 1956; Perrins 1965; Blem 1990; Perrins & McCleery 2001), adverse temperatures encountered during nestling development could ultimately handicap post-fledging survival. Therefore, although we have been unable to detect an effect of experimental nest heating on immediate juvenile survival at fledging, we cannot discard possible implications on future recruitment (see Monrós et al. 2002; Greño et al. 2008). Further consequences of elevated nest temperatures on nestling physiology that may affect future survival include immunosuppression (e.g., reduced antibody response; see Mashaly et al. 2004), oxidative stress and alterations of thermoregulatory behavior (e.g., release of heat-shock proteins; see Lara & Rostagno 2013).

Recent studies have reached similar conclusions to those presented here regarding the negative effect of high temperatures on nestling fitness. Ardia (2013) evidenced a decrease in reproductive success of nestling tree swallows as a result of increased nest temperatures, and Salaberria et al. (2014) reported a reduction in nestling body mass and wing length when analyzing the effects of heat exposure late in the season on development of spotless starlings (*Sturnus unicolor*). Similarly, Cunningham et al. (2013) detected reductions in body mass gain of common fiscal nestlings in response to high nest temperatures, and Catry et al. (2015) evidenced a relationship between high maximum daily temperatures and mass loss of lesser kestrel (*Falco naumanni*) and European roller (*Coracias garrulous*) nestlings. Our work thus complements previous findings, being, to our knowledge, the first study providing experimental evidence on the negative impact of elevated nest temperatures on nestling fitness in warm environments. Deviations from the results of Dawson et al. (2005), where heating nest boxes caused positive effects on nestling growth and survival, could be attributed to differences in air temperatures (and, consequently, nest box temperatures) between study sites, considerably higher in our case (i.e., temperatures in our control nests where, on average, more than 6.8 °C higher than in Dawson's), which may have led to the heated nestlings being more often exposed to temperatures capable of inducing heat stress (i.e., temperatures above the upper limit of the optimal thermal range of the species). The negative effects of increased nest temperatures on offspring fitness are therefore more likely to manifest under warm air temperature regimes such as the Mediterranean, where birds are more frequently faced with energetically-challenging conditions.

In the current climate change scenario, bird species living in warm climates such as the Mediterranean are predicted to face hot-weather events of greater frequency (Pipoly et al. 2013), duration and intensity (IPCC 2013), which are likely to have a negative impact on breeding success (Sanz 2002). In this sense, individuals with small thermal ranges, such as developing young, are more susceptible to suffer the increased severity of climatic events (Jiquet et al. 2006). In this article we have shown experimentally how nestlings of a Mediterranean bird population are sensitive to nest temperatures surpassing their optimal thermal range, which may constrain their growth and eventually reduce their future recruitment probabilities. It remains to be seen whether these results would hold across the distribution range of the species, considering the possibility of local acclimation. Further manipulative approaches, as the one presented here, are therefore needed in order to effectively assess the effects of temperatures on breeding performance and, ultimately, predict the global effects of climate change on avian biodiversity.

CONCLUSIONS

In warm habitats, high temperatures experienced during the nestling period can affect nestling development. As a lower mass at fledging may limit post-fledging survival, the effects of adverse nest microclimates can eventually compromise offspring recruitment. This is the first study providing experimental evidence on the negative effects of elevated nest temperatures on fledgling mass.

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ARTÍCULO 2

Effects of cool nest microclimates on nestling development: an experimental study with Mediterranean great tits *Parus major*

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ABSTRACT

Suboptimal temperatures may exert negative effects on altricial nestlings, especially during their first days of life, when thermoregulation is not yet fully developed. We experimentally lowered nest temperatures by a mean of 4.5 °C during early development of great tit *Parus major* nestlings from the Mediterranean region. The thermal treatment only affected nestling size, as cooled nestlings had smaller tarsi by day 15 than controls. Female brooding constancy remained unaltered and female body condition was not negatively affected, so females did not incur additional energetic costs to ameliorate thermal conditions for nestlings. In conclusion, we found that colder nest microclimates may impair nestling growth, which may have negative consequences on future survival.

Keywords: nestling growth, nestling survival, thermal stress, thermoregulation.

RESUMEN

Temperaturas sub-óptimas pueden afectar negativamente a los pollos de aves nidícolas, especialmente durante sus primeros días de vida, cuando todavía no han desarrollado totalmente su capacidad termorreguladora. En este trabajo enfriamos experimentalmente nidos de carbonero común *Parus major* durante el periodo de desarrollo de los pollos (una media de 4,5 °C). El tratamiento térmico solo afectó al tamaño de los pollos, de modo que los enfriados alcanzaron tarsos menores que los controles a los 15 días de edad. Por su parte, tanto la condición corporal de las hembras como su tiempo de estancia en el nido se vieron inalterados, por lo que éstas no invirtieron un mayor esfuerzo en mejorar las condiciones térmicas de la pollada. En conclusión, encontramos que microclimas de nidificación más fríos pueden perjudicar el crecimiento de los pollos, lo cual puede repercutir negativamente sobre su supervivencia futura.

Palabras clave: crecimiento de los pollos, supervivencia de los pollos, estrés térmico, termorregulación.

INTRODUCTION

During development, young birds have to optimise the allocation of resources to attain a fully developed adult size that guarantees survival and future reproduction (Schew & Ricklefs 1998). Among the factors affecting offspring development, the thermal environment of the nest plays an important role in determining the energetic investment of the young (Dawson et al. 2005). Newly hatched altricial birds are ectothermic and eventually attain endothermy during the first days or weeks of life as a result of increased insulation, a more favorable heat loss/production ratio and development of thermoregulatory mechanisms (King & Farner 1961; Duchamp et al. 2002). Consequently, suboptimal temperatures may exert negative effects on developing nestlings, especially during their more vulnerable ectothermic stage (Takagi 2001; Bradbury et al. 2003). Hyperthermia may lead to dehydration or cardiovascular illnesses (Belda et al. 1995; Patz et al. 2005), whereas colder nest microclimates may require offspring to invest more energy in thermoregulation, at the cost of other processes such as growth or the development of the immune system (Dawson et al. 2005).

Nest temperatures can also affect parental care. Nestling attendance imposes high resource demands on the parents (Lindén & Moller 1989), so that their investment decisions may vary under unfavorable conditions (Barba et al. 2009). In altricial species, insufficient parental care may cause partial or even complete brood loss, especially when combined with high energetic demands as a result of low temperatures (O'Connor 1975; Mock et al. 1991; Sullivan & Weathers 1992; Rauter et al. 2000).

Despite its importance, few studies have addressed the relationship between reduced nest temperatures and offspring condition. Most of these have focused on precocial species living in cold climates (Krijgsveld et al. 2003), where low temperatures seem to have constrained nestling growth. Regarding altricial young, recent approaches have tried to decrease temperatures during the incubation stage and examine its effects on breeding performance (e.g., Nilsson et al. 2008; Ardia et al. 2010; Álvarez & Barba 2014). To the best of our knowledge, however, no study has examined the effects of experimentally reduced nest temperatures during the course of the nestling stage. The nearest attempt is that of Lynn & Kern (2014), who exposed eastern bluebird *Sialia sialis* nestlings to short cooling bouts, in an effort to simulate the drops in body temperature resulting when a brooding female leaves the nest. Experimental approaches are necessary to establish a clear cause-effect relationship between the thermal environment of the nest and nestling development, and/or survival. In the present study, we aimed to determine the response of great tit nestlings when exposed to lower temperatures in a Mediterranean habitat. Facing this stressful scenario, females could either spend more time brooding to compensate for the lowered nest temperature, and therefore themselves assuming the energetic costs, which could negatively affect their body condition, or could maintain their "normal" brooding schedule and let the nestlings pay the costs. The latter option could force nestlings to shift their energetic inversion towards thermoregulation rather than growth, therefore affecting their development and/or survival. An intermediate outcome (i.e., sharing costs) is possible, though it would be more difficult to detect.

MATERIALS AND METHODS

Study area and fieldwork

The study was conducted from February to July 2011, in an extensive orange monoculture located in Sagunto (Valencia, eastern Spain 39° 42'N, 0° 15'W, 30 m a.s.l.). By the end of February, wooden nest boxes were placed for the birds to breed (e.g., Andreu & Barba 2006). Mean ambient temperatures in our study site increase from 15.04 °C in April to 24.48 °C in July. Minimum temperatures vary, on average, from 8.99 °C in April to 19.00 °C in July (Agencia Estatal de Meteorología, period 1986-2011).

Weekly inspections allowed us to know the laying date of the first egg (we assumed that one egg was laid per day; see Encabo et al. 2001). Once the fifth egg was laid, we made daily visits to determine the beginning of incubation and final clutch size for each nest. After incubation started, we randomly selected ten pairs of nest boxes, matching as far as possible laying dates and clutch size within pairs. One nest of each pair was assigned to the "control" group and the other to the "cooled" group.

The exact hatching date of the first egg was determined by daily inspections beginning on day 11 after the start of incubation. On the hatching day (day 0 of the experiment), we took the nest and its contents out of the nest box, weighed the empty nest, and measured the thickness of its base (Álvarez & Barba 2008). To prepare each nest box for the experiment, we introduced a wire mesh about 1 cm above the nest box floor, creating a "second chamber" inside the nest box where cold and control packs could be placed. Cold packs consisted of frozen reusable refrigerating gel units (Cryopak, Edison, New Jersey, USA), empty refrigerating gel units serving as controls. The nest along with the nestlings was placed on the wire mesh.

A pilot study performed under field conditions showed that cold packs had to be replaced frequently in order to maintain sub-ambient temperatures inside the nest box. Therefore, all the study nests (control and cooled) were visited four times a day (at 07:00, 11:00, 15:00 and 19:00) either to replace the cold packs or just to disturb control nests in a similar way. The nest boxes had a removable door, which allowed packs to be changed without forcing females to leave the nest. We also recorded the presence or absence of the female during each visit as an estimate of brooding constancy during the nestling period. In order to standardise treatment conditions, the control and cold packs were introduced for the first time at 07:00 on the day after the wire mesh was placed, and were removed at 19:00 on day 14 post-hatching. The wire meshes were removed the following day (day 15), to avoid disturbing females late in the evening (we needed to force females out of the nest to remove the wire mesh).

Data loggers were placed between the wire mesh and the nesting material to monitor nest temperature. We used four thermocouple thermometers model HOBO U23 Pro v2 (Onset Corporation, Bourne, MA, USA), programmed to take internal temperature readings every two minutes (see Dawson et al. 2005). The thermometers were exchanged between experimental nests in order to obtain 24-hour recordings during two different periods of nestling thermoregulatory development: between days 1 and 7 and days 8 and 14 of post-hatching nestling age.

The cooling effect of the cold packs declined gradually with time. Therefore, in order to confirm that nestlings were experiencing different thermal regimes between treatments, at least during part of the day, we averaged the temperature values recorded in a sample of nests (8 control, 7 cooled) during two hours after the perturbation of each experimental nest box. As an estimate of external temperatures reached in our study area during the experiment, we averaged, for each nest, the maximum, mean and minimum ambient temperatures during days 1 to 15 of age of the nestlings. This could be estimated only for nests with nestlings that survived until day 15. Ambient temperature data were collected from a nearby (approx. 4.5 km) meteorological station.

Each nestling was ringed on day 9 using an individually numbered metal ring. Individual identification of each nestling before ringing was achieved by marking different parts of the body on days 5 and 7 with a non-toxic pen (re-marking nestlings on day 7 was necessary to keep the marks identifiable until day 9). We recorded the number of nestlings alive on days 5, 9 and 15 of age and measured their masses at these ages with a digital balance (accuracy ± 0.01 g). We also recorded their tarsus length on day 15 with a digital caliper (accuracy ± 0.01 mm). Females were caught at the nest using spring traps, and ringed with individually numbered metal rings, when nestlings were 10–12 days old. At this moment, we weighed them with a digital balance, and recorded their tarsus length using a digital caliper. On day 20 we visited each box to check whether all the young had fledged, and collected the nests in sealed plastic bags. Dead individuals were identified.

Experimental nests were stored in the laboratory at –20 °C. At the end of the breeding season, they were dried in an oven (105 °C, 12 h) and weighed with a digital caliper to determine dry nest masses (see Álvarez et al. 2013).

Overall, data from 17 nests (10 control, 7 cooled) were available. One nest assigned for cooling failed early in the season, whereas two cooled nests were lost during the temperature manipulation experience: one was abandoned and the other was lost to a predator.

Statistical Analyses

We tested for pre-treatment differences between nests (control vs. cooled) using General Linear Models (GLMs). We considered the following variables: dry weight, nest base thickness, laying date, clutch size, hatching date and number of hatchlings. We also tested for post-treatment differences in number of fledglings and proportion of young fledged (brood size at fledging/initial brood size). The latter ratio was arcsin square root transformed for normality in analyses. All these tests included treatment type (control or cooled) as fixed factor.

We examined factors affecting internal nest box temperatures using a General Linear Mixed Model (GLMM), with the two-hour temperature averages as the dependent variable; treatment type, nestling age category (1–7 days or 8–14 days), and time of day (07:00, 11:00, 15:00 or 19:00 hours) as fixed effects, and nest box nested within treatment as random factor. In order to analyse the treatment effect separately by age category, we used GLMMs, considering the two-hour temperature averages corresponding to each age category as the dependent variable, treatment type and daytime as fixed effects, and nest box nested within treatment as random factor. To simplify the interpretation of the results, we only included first-order interaction terms in the models. As there were no differences in nest dry weight and base thickness between treatments (see Results), these variables were not included in further analyses.

Effects of the experimental treatment on female attentiveness were analysed with a GLM, including the proportion of visits with the female present during days 1–4, days 5–9 or days 10–14 of nestling age as dependent variable, and the treatment type as fixed factor. Differences among treatments in female body condition were tested using a GLM. We included the body mass-to-tarsus ratio as the dependent variable, and the treatment type as the fixed factor.

The effect of the cooling treatment on the number of nestlings per nest on days 5, 9 and 15 was assessed using GLMs, introducing the treatment type as the fixed factor. We used GLMMs to test for differences among treatments in nestling biometry. We considered the following variables: nestling mass at ages 5, 9 and 15 days, mass differences between days 9 and 5, mass differences between days 15 and 9, and tarsus length at age 15 days. We included treatment type as the fixed factor and nest box nested within treatment term as the random factor.

Analyses were performed using IBM SPSS Statistics 19 software. We considered results significant at the 0.05 level.

RESULTS

We did not detect pre-treatment differences between control and cooled nests in laying date, clutch size, hatching date or number of hatchlings. There were also no significant differences between treatments in nest base thickness or dry weight (Table 1).

Table 1. Breeding parameters and characteristics (±SE) of control and cooled nests. Sample sizes in parentheses refer to number of nests.

	Control	**Cooled**	**F**	**P**
Nest base thickness (mm)	9.54 ± 5.04 (10)	8.32 ± 3.98 (7)	0.283	0.603
Nest dry mass (g)	19.95 ± 5.12 (10)	18.29 ± 5.31 (7)	0.423	0.525
Laying date	11.50 ± 2.88 (10)	10.29 ± 2.14 (7)	0.893	0.360
Clutch size	8.80 ± 1.48 (10)	8.43 ± 1.27 (7)	0.291	0.598
Hatching date	30.80 ± 3.08 (10)	29.57 ± 2.51 (7)	0.756	0.398
Number hatched	7.50 ± 1.96 (10)	7.71 ± 0.95 (7)	0.071	0.793
Number fledged	7.10 ± 1.97 (10)	7.14 ± 0.90 (7)	0.003	0.958
Proportion fledged	0.95 ± 0.10 (10)	0.93 ± 0.13 (7)	0.019	0.893

Temperatures inside the nest boxes varied with nestling age category and daytime. In addition, we found a significant treatment-by-nestling age category interaction term (Table 2, Figure 1). Temperatures inside the cooled nest boxes (24.40 ± 4.26 °C, n = 7 nests) were significantly lower than those of the control nest boxes (28.94 ± 3.17 °C, n = 7 nests) at early ages (GLMM: $F_{1,12} = 5.111$, p = 0.043), while they did not differ when nestlings were older (GLMM: $F_{1,11} = 1.272$, p = 0.283). Average ambient temperatures in our study area during the first 15 days of nestling age were 18.45 ± 0.28 °C, n = 15 nests. Average maximum and minimum temperatures during this period were, respectively, 23.28 ± 0.44 °C, n = 15 and 13.63 ± 0.22 °C, n = 15.

Table 2. Factors affecting temperatures inside experimental nest boxes. General Linear Mixed Model results, considering treatment type, nestling age category and daytime as factors, and nest internal two-hour temperature averages as the dependent variable.

Factor	F	P
Treatment	$F_{1,12} = 2.311$	0.154
Age	$F_{1,85} = 41.481$	<0.001
Daytime	$F_{3,81} = 5.973$	0.001
Treatment*Age	$F_{1,85} = 10.393$	0.002
Treatment*Daytime	$F_{3,81} = 0.664$	0.577
Age*Daytime	$F_{3,81} = 2.232$	0.091

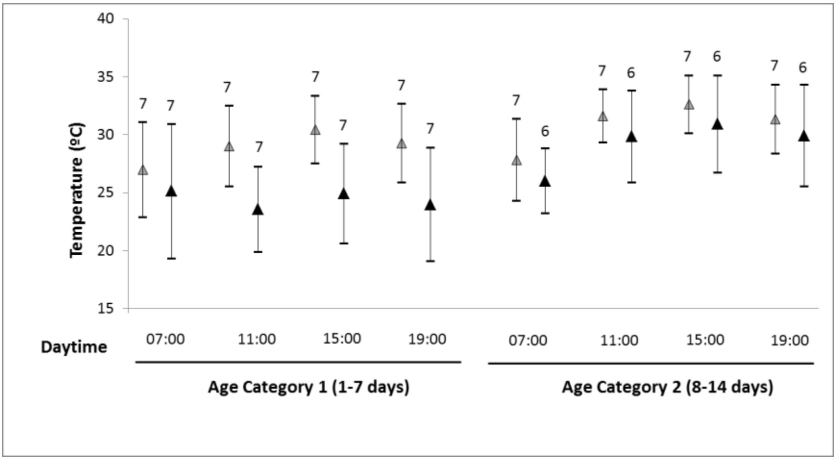

Figure 1. Temperatures of experimental nests in relation to daytime and nestling age category. Mean temperature (±SE) for control (grey) or artificially cooled nests (black) in relation to daytime and nestling age category. Sample sizes above error bars refer to number of nests.

The experimental treatment affected nestling mean tarsus length, with cooled nestlings having smaller tarsi by day 15 than control nestlings (Table 3, Figure 2). We found no significant effect of the cooling treatment on nestling mass at ages 5, 9 and 15 days, or on nestling mass differences. Similarly, there were no differences between control and cooled nests in the number of nestlings on days 5, 9 and 15, the number of fledglings, or the proportion of young fledged.

Female attentiveness, as shown by presence/absence data, was not affected by the experiment in any of the three periods considered [days 1–4 (GLM: $F_{1,15} = 1.559$, $p = 0.231$), days 5–9 (GLM: $F_{1,15} = 1.466$, $p = 0.245$), and days 10–14 (GLM: $F_{1,15} = 0.089$, $p = 0.769$)]. In addition, female body condition did not differ between treatments (GLM: $F_{1,15} = 1.688$, $p = 0.213$).

Table 3. Control and cooled nestling biometric parameters (±SE). Sample sizes in parentheses refer to number of nestlings. Significant differences in bold.

	Control	Cooled	F	p
Mass day 5 (g)	6.75 ± 1.49 (66)	6.25 ± 1.39 (52)	$F_{1,14} = 1.601$	0.227
Mass day 9 (g)	13.13 ± 1.69 (66)	12.42 ± 2.21 (52)	$F_{1,13} = 1.592$	0.229
Mass day 15 (g)	17.37 ± 1.00 (66)	16.68 ± 2.33 (52)	$F_{1,15} = 1.049$	0.321
Mass day 9 – Mass day 5 (g)	6.38 ± 0.70 (66)	6.17 ± 1.26 (52)	$F_{1,14} = 0.465$	0.507
Mass day 15 – Mass day 9 (g)	4.24 ± 1.37 (66)	4.27 ± 1.79 (52)	$F_{1,14} = 0.000$	0.996
Tarsus length day 15 (mm)	19.49 ± 0.67 (66)	18.75 ± 1.03 (52)	$F_{1,15} = 6.654$	**0.021**

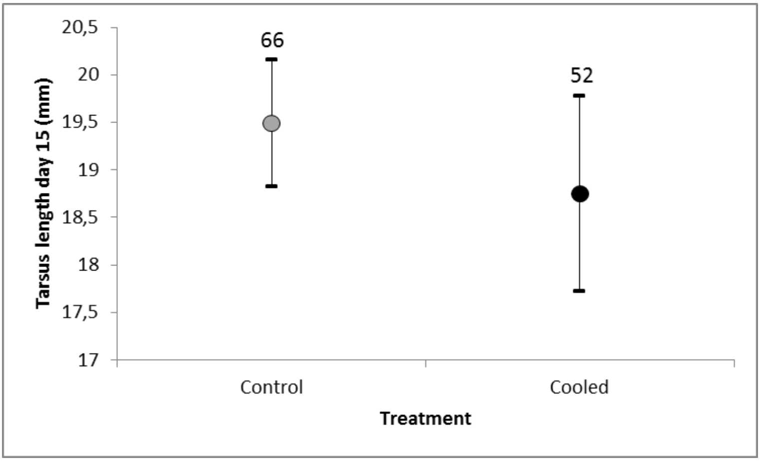

Figure 2. Nestling tarsus length on day 15 in experimental nests. Mean nestling tarsus length at day 15 (± SE) for control (grey) and artificially cooled nests (black). Sample sizes above error bars refer to number of nestlings.

DISCUSSION

We found that cooled nestlings were smaller by day 15 than control nestlings, while no effect was detected on nestling mass and survival to fledging. In addition, the experimental treatment did not affect female brooding constancy or female body condition.

Experimental designs to reduce nest box temperatures are limited by logistic difficulties in maintaining temperatures below natural levels, as the body heat of both nestlings and adults tends to buffer such thermal drops. In our case, we were able to significantly reduce nest temperatures by 4.5 °C on average during the first days of nestling development (i.e., during days 1–7 of nestling age). As nestlings grew older, however, the temperatures of cooled nest boxes did not differ from those of controls. This dependence of experimental nest temperatures on nestling age is probably associated with changes in nestling thermoregulatory capabilities. During early development, nestlings have a limited ability to compensate for heat loss, since they have little control of their own body temperature (Shilov 1973; O’Connor 1984). At this stage, parental behaviour may allow thermally-stressed nestlings to be kept in near homeothermic conditions, as adults can increase their brooding effort at the expense of reducing feeding frequencies or resources spent in their own self-maintenance (O’Connor

1984; Barba 2009; Álvarez & Barba 2014). By the time nestlings acquire a capacity for-chemical thermoregulation (i.e., between days 8 and 17 of age; see O'Connor 1975), adults spend less time inside the nest and it is therefore up to the nestlings to effectively deal with cold stress by huddling together and/or increasing metabolic heat production. We believe that the increased energetic costs associated with thermoregulation may have resulted in cooled nestlings being smaller than controls, a consequence of being forced to channel resources to maintenance rather than skeletal growth. Energy expenditure associated with cold resistance should have been greater during the first days of life of the young due to their greater vulnerability, especially considering that our manipulation had no apparent effect on female attendance. When faced with low nest temperatures, females did not increase their brooding effort, in effect maintaining their overall body condition, at the expense of exposing nestlings to the cold. As nestlings grew older, improved metabolic heat generation and control of heat losses may have allowed them to compensate for the experimentally-induced cooling bouts.

We have been unable to find immediate effects of the cooling treatment on nest survival, as there were no significant differences between control and cooled nest boxes in the number of nestlings present during the experiment. Neither there were differences in the number of fledglings that eventually left the nest. It seems that the temperatures reached during the experiment were not low enough to induce cold-related mortality in the nestlings. We do suspect, however, the existence of possible negative carry-over effects on post-fledging survival and future reproduction, as smaller-sized young have been shown to be more vulnerable against predators (De Laet 1985; Ragusa-Netto 1996) and to have restricted access to high-quality resources when compared with larger individuals (Arcese & Smith 1985; Carrascal et al. 1998).

Experimental approaches trying to determine the effects of low temperatures on breeding performance of altricial species are scarce (see Nager & van Noordwijk 1992; Nilsson et al. 2008; Álvarez & Barba 2014; Lynn & Kern 2014). Among these, only Lynn & Kern (2014) applied cold during the nestling stage. In their study, exposure of 5–7 day old eastern bluebird nestlings to significant bouts of cooling resulted in stimulated secretion of corticosterone, a hormone related to regulation of stress responses (Lindstrom 1999). The treatment was carried out under controlled conditions outside the nest and once the young had acquired at least a partial ability to thermoregulate. To our knowledge, the present study is the first to monitor the effect of experimentally-induced colder nest microclimates during the development of nestling thermoregulation. We are aware that our small sample size might not suffice to draw broad conclusions on the impact of colder nest temperatures on offspring condition but we believe that the finding of an effect with such a relatively limited number of experimental nests is in itself significant. We thus conclude that low nest temperatures acting during the first days of life of great tit nestlings, i.e., during the most vulnerable period in their development of temperature stability, may result in impaired growth, which can have negative consequences later in life.

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ARTÍCULO 3

Factors affecting fledgling output of great tits *Parus major*, in the long term

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ABSTRACT

Fledgling production has often been used as an estimator of avian reproductive success, and it is conditioned by factors affecting offspring development and/or survival during the nesting period. We aimed to determine which predictors influenced fledgling output among a set of basic breeding parameters and local temperature data collected over 25 years in a Mediterranean great tit (*Parus major*) population, using an information-theoretic approach for model selection. Of the studied variables, the number of hatchlings per nest was the single-most important predictor influencing fledgling production, with larger broods eventually yielding more fledglings, although mass prior to fledging may have been compromised. This result suggests an overall good adjustment between brood size and resource availability in the studied population.

Keywords: fledgling production, nestling survival, brood size, long-term study.

RESUMEN

La producción de volantones ha sido frecuentemente utilizada para estimar el éxito reproductor de las aves, y está condicionada por factores que afectan al desarrollo y/o a la supervivencia de los pollos durante su estancia en el nido. Nuestro objetivo en este trabajo fue determinar los predictores que influyen sobre la producción de volantones a partir de un conjunto de parámetros reproductivos básicos y temperaturas locales recopilados durante 25 años en una población mediterránea de carbonero común (*Parus major*), haciendo uso de criterios de información para la selección de modelos. De las variables estudiadas, el número de huevos eclosionados por nido resultó ser el predictor con mayor influencia sobre la producción de volantones, de tal forma que puestas en las que nacieron más pollos también originaron más volantones, si bien el peso de los pollos antes de abandonar el nido podría haberse visto comprometido en puestas grandes. Este resultado sugiere un buen ajuste general a largo plazo entre el tamaño de puesta y la disponibilidad de recursos en la población estudiada.

Palabras clave: producción de volantones, supervivencia en el nido, tamaño de puesta, estudio a largo plazo.

INTRODUCTION

Avian reproductive success is a recurrent topic in ornithological research. It depends on (1) the number of breeding attempts, with predation being the main cause of complete nest failure (see Martin 1995), and (2) the number of individuals surviving to become breeding adults per successful attempt. Among successful nests (i.e., those with at least one young fledged), the number of fledglings has often been used as a reliable estimator of the number of recruited young (Weatherhead & Dufour 2000; Wiens & Reynolds 2005), and is conditioned by factors influencing offspring development and/or survival during the nesting period.

Among the factors potentially affecting fledgling output, breeding date has proven to influence offspring fitness, with nestlings raised earlier in the season usually benefitting from higher resource availability (Catry et al. 1998), although in certain years, breeding too early could also be disadvantageous (Monrós et al. 2002). In this sense, hatching date could be a more accurate parameter than laying date when analyzing the optimal timing of reproduction in birds (Tomás 2015). Egg size, in turn, may affect nestling immune function and/or growth (Williams 1994; Hipfner 2000), as larger eggs provide the embryo access to higher quantities of energy (Birkhead & Nettleship 1982). The aforementioned factors (i.e., egg size and bird phenology), together with clutch size, may be indicators of the quality of the parents and their ability to raise the brood, which would have direct consequences on chick survival to fledging (Pettifor et al. 2001). Moreover, if parents optimize their clutch size based on resource availability (Cresswell & McCleery 2003; Naef-Daenzer et al. 2004), and some of these eggs fail to hatch, this could lead to the remaining young receiving greater care and thus improving their survival prospects. As a result, not only the absolute number of hatchlings, but also the number of unhatched eggs could affect fledgling production.

Temperature is one of the main abiotic factors influencing nesting conditions and eventual fledgling production. Nestlings have limited thermoregulatory abilities during their first days of life, which makes them especially vulnerable to suboptimal thermal conditions (Murphy 1985; McCarty & Winkler 1999; Takagi 2001; Bradbury et al. 2003). When exposed to high temperatures, nestlings loose appetite, and their growth rate and musculature decrease (Belda et al. 1995; Geraert et al. 1996). On the other hand, low temperatures also limit nestling condition (Krijgsveld et al. 2003), as colder nest microclimates require a higher investment in thermoregulation, at the expense of other processes such as growth or development of the immune system (Dawson et al. 2005; Rodríguez & Barba 2016).

Although many factors have been shown to affect fledgling production, they have seldom been studied simultaneously to determine their relative importance (Coulter & Bryan 1995; Martín-Vivaldi et al. 1999; Knight & Rogers 2004; Gullett et al. 2015; Herman & Colwell 2015). Moreover, their relative weight may vary from year to year, so that long-time series are needed to elucidate each factor's net effect on long temporal scales. Using reproductive and local temperature data collected over 25 years in a Mediterranean great tit (*Parus major*) population, we here aimed to determine the predictors with the greatest influence on the number of fledglings. We also assessed the relationships between the relevant predictors and condition at fledging (i.e., mass and size at fledging).

MATERIALS AND METHODS

Fieldwork

Data used for the present study were obtained during a long-term research project on a Mediterranean great tit population breeding near Sagunto (Valencia, eastern Spain 39º 42'N, 0º 15'W, 30 m a.s.l.). The study area was located within a homogeneous, extensive orange plantation (Andreu & Barba 2006). We used reproductive and thermal data collected from 1986 to

2010. Mean laying date of the first egg (given as April dates) for the studied population during this period was 15.92 ± 5.20.

Each year, we placed wooden nest boxes (see Lambrechts et al. 2010, for dimensions) by the end of February. They were removed after each breeding season. Nest boxes were visited with the periodicity necessary (daily at some stages) to accurately determine the following reproductive parameters: clutch size, hatching date (date of hatching of the first egg), number of hatchlings and number of fledglings (e.g., Greño et al. 2008). We measured the length and width of every egg of most clutches once it was considered to be complete (at least three days without the appearance of new eggs), using a caliper (± 0.1 mm). We determined the volume of each egg using the equation: $V = (0.4673 \times L \times B^2) + 0.042$, V being the egg volume in mm^3, 0.4673 the shape parameter, L the egg length in mm and B the egg width in mm (Ojanen et al. 1978). When nestlings were 15 days old, they were ringed with individually numbered metal rings and weighed (digital balance, ± 0.01 g), and their tarsus length was measured (caliper, ± 0.01 mm). We visited the nest boxes at least five days later to determine the number of fledglings.

Within-nest mean egg volume, mean nestling body mass and mean nestling tarsus length were used in analyses to avoid pseudoreplication (Hurlbert

1984). We only have data of nestling biometry since 1993. We used data from first clutches, of non-manipulated nests. As we were only interested in successful nests, we also excluded those nests where no nestlings fledged, and those for which data from any of the recorded reproductive parameters was missing. This led us to eventually discard data from three years (i.e., 1989, 2004 and 2005), either because of absence of a reasonable number of successful nests (i.e., less than five nests in 2004), or absence of data on egg size (1989 and 2005). Overall, we used data from 644 successful nests in the analyses.

Daily ambient temperatures were obtained from the Meteorological Station “El Pontazgo”, close to the study area. For each nest, we calculated average mean ambient temperatures during the first five and 15 days after hatching. We chose these periods so as to (1) encompass a period of high vulnerability to changes in ambient temperature (during their first five days of age, great tit nestlings lack the capacity to regulate their internal body temperature; see experiments in Shilov 1973), and (2) to account for overall temperatures experienced during nestling development.

Statistical analyses

We conducted Generalized Linear Models (GLMs) with a Poisson error distribution and log link function to determine which factors explained nestling survival, taking an information-theoretic approach to model selection (Johnson & Omland 2004; Whittingham et al. 2006). As dependent variable we considered the absolute number of fledglings. As explanatory variables we considered mean egg size, clutch size, hatching date, number of hatchlings, number of unhatched eggs (i.e., the difference between clutch size and the number of hatchlings), and mean ambient temperatures during the first five and 15 days after hatching. To simplify interpretation and limit the set of models considered, we did not include interaction terms. We also analyzed the relationship between the number of fledglings per nest and the number of hatchlings (see results for explanation) by fitting different regression curves and choosing the simplest model from among the significant ones. Additionally, we performed simple linear regressions to examine the relationship between the number of hatchlings per nest (see results for explanation) and mean nestling mass, and mean nestling tarsus length.

We assessed the relevance of incorporating the year as a factor by performing a Likelihood Ratio Test with the fully-parameterized model. As its addition did not result in a statistically significant improvement in model fit (χ^2 = 18.903, p = 0.5914), we rejected its inclusion in the models. We tested the validity of this general model by visually inspecting its residuals. Previous studies have shown that all of the analyzed predictors can affect nestling survival when considered individually, so we had no reason to select certain combinations of variables over others. Therefore, we generated 128 models considering all possible non-redundant combinations of predictive variables, ranking them using the small sample sizes' corrected Akaike Information Criterion (AICc, Burnham & Anderson 1998). We relied on model averaging to obtain a weighted average of predictor estimates from a subset of equally-plausible models (i.e., models with AICc value differing less than two units from the higher-ranked model), and determined each predictor's relative importance in this subset by adding the Akaike weights of those models where it appeared. To further contrast the influence of each parameter in the model subset, we examined their model-averaged weighted effect sizes or β estimates. When the 95% confidence intervals (CIs) of a model-averaged β estimate for a particular parameter overlapped zero, we considered it unlikely that the parameter had much influence on the response variable. Analyses were performed using the lmtest, MuMIn, and glmulti packages in R (R Development Core Team 2010; Zeileis & Hothorn 2002; Barton 2013; Calcagno 2013), as well as SPSS v. 22.

RESULTS

Overall, 89% of the eggs laid in the 644 nests included in this study produced live hatchlings, and 86% of these hatchlings eventually fledged. Moreover, in 48% of the nests, all the hatchlings eventually fledged and among the remaining nests (i.e., with at least one nestling lost prior to fledging), an average of 73% of the hatchlings left the nest. Mean annual number of fledglings per nest during the study period was 6.14 ± 0.88 (mean of yearly means; range: 4.25-7.71; n = 22 years).

Considering the initial model set (128 models), two predictor variables showed a higher probability of inclusion in the best approximating model of the number of fledglings, as given by the sum of Akaike weights of the models in which they appear: number of hatchlings ($\Sigma W_i = 0.784$) and clutch size ($\Sigma W_i = 0.648$). Egg size ($\Sigma W_i = 0.545$) had intermediate importance, whereas number of unhatched eggs ($\Sigma W_i = 0.432$), hatching date ($\Sigma W_i = 0.320$), and mean ambient temperatures during the first 15 days after hatching ($\Sigma W_i = 0.278$) and during the first five days after hatching ($\Sigma W_i = 0.273$) had lower importance.

Four models fitted the data equally well, as given by their AICc scores. This set of best-fitting models included clutch size, egg size, number of hatchlings and number of unhatched eggs as explanatory variables (Table 1, Models 1, 2, 3, and 4).

Table 1. Factors affecting fledgling production. Top–ranked models (i.e., with ΔAICc < 2), and last ranked model used to test the effect of temperature and reproductive parameters on the number of fledglings: CS, clutch size; ES, egg size; NH, number of hatchlings; NUE, number of unhatched eggs; T5, mean ambient temperatures during the first five days after hatching; T15, mean ambient temperatures during the first 15 days after hatching; AICc, corrected Akaike Information Criterion for small sample sizes; ΔAICc, difference in AICc values in relation to Model 1.

Model	Parameters included	AICc	ΔAICc	Akaike Weight
1	CS, ES, NH	2562.307	0	0.04166
2	CS, ES, NUE	2562.307	0	0.04166
3	ES, NH, NUE	2562.307	0	0.04166
4	CS, ES, NH, NUE	2562.307	0	0.04166
5	NH, NUE	2562.543	0.236	0.03702
6	CS, NH	2562.543	0.236	0.03702
7	CS, NUE	2562.543	0.236	0.03702
8	CS, NH, NUE	2562.543	0.236	0.03702
9	ES, NH	2563.106	0.799	0.02794
10	NH	2563.867	1.560	0.01770
11	CS, HD, ES, NH	2563.969	1.662	0.01814

12	CS, HD, ES, NUE	2563.969	1.662	0.01814
13	HD, ES, NH, NUE	2563.969	1.662	0.01814
14	CS, HD, ES, NH, NUE	2563.969	1.662	0.01814
15	CS, HD, NH	2564.200	1.893	0.01617
16	CS, HD, NUE	2564.200	1.893	0.01617
17	HD, NH, NUE	2564.200	1.893	0.01617
18	CS, HD, NH, NUE	2564.200	1.893	0.01617
19	T5, CS, ES, NH	2564.236	1.929	0.01588
20	T5, ES, NH, NUE	2564.236	1.929	0.01588
21	T5, CS, ES, NH, NUE	2564.236	1.929	0.01588
22	T5, CS, ES, NUE	2564.236	1.929	0.01588
23	T15, CS, ES, NH	2564.259	1.952	0.01569
24	T15, CS, ES, NUE	2564.259	1.952	0.01569
25	T15, ES, NH, NUE	2564.259	1.952	0.01569
26	T15, CS, ES, NH, NUE	2564.259	1.952	0.01569
128	T5, HD	2776.394	214.1	1.3527E-48

Overall, a total of 22 additional models had AICc values within two units of the best-ranked models. They generally explained 46-48% of the deviance of the null model. The combined Akaike weight of this subset of best-fitting models was 0.625. According to the model-averaged coefficients of the predictor variables (Table 2), the number of fledglings decreased with hatching date and number of unhatched eggs, and increased with egg size, number of hatchlings, temperatures during the first five and 15 days after hatching. The relative importance of the predictor variables in the model-averaged subset, calculated by the sum of the Akaike weights over all the models in which they appear, was high for number of hatchlings ($\Sigma W_i = 0.77$), clutch size ($\Sigma W_i = 0.69$), number of unhatched eggs ($\Sigma W_i = 0.69$), and egg size ($\Sigma W_i = 0.63$). Of these parameters, only the number of hatchlings had a strong effect size (i.e., β estimate), with CIs ranging from 0.105 to 0.184, whereas clutch size, number of unhatched eggs and egg size had CIs overlapping zero (Table 2). The remaining variables were of low importance and their 95% CIs overlapped zero (Table 2): hatching date ($\Sigma W_i = 0.22$), mean ambient temperatures during the first five and 15 days after hatching (both $\Sigma W_i = 0.10$). The simplest best-fitting function explaining the relationship between the number of fledglings (NF) and the number of hatchlings (NH) was linear ($NF = 0.8891NH – 0.1359$, $r = 0.983$, $p < 0.001$; Figure 1).

Table 2. Coefficients of predictors in the best-fitting models. Model-averaged coefficients of the predictor variables from the subset of best-fitting models. (For abbreviations, see Table 1)

Parameter	**Estimate**	**SE**	**Adjusted SE**	**95% CI**		**Z value**	**Pr(>|Z|)**
				Lower	**Upper**		
T15	0.003135	0.01118	0.01120	-0.01882	0.02509	0.280	0.780
HD	-0.001254	0.002065	0.002069	-0.005309	0.002802	0.606	0.545
ES	0.0002104	0.0001381	0.0001384	-0.00006084	0.0004816	1.520	0.128
NH	0.1448	0.02010	0.02012	0.1054	0.1843	7.197	<2E-16
T5	0.002941	0.009218	0.009235	-0.01516	0.02104	0.319	0.750
CS	0.02048	0.07555	0.07556	-0.1276	0.1686	0.271	0.786
NUE	-0.09400	0.06468	0.06469	-0.2208	0.03279	1.453	0.146

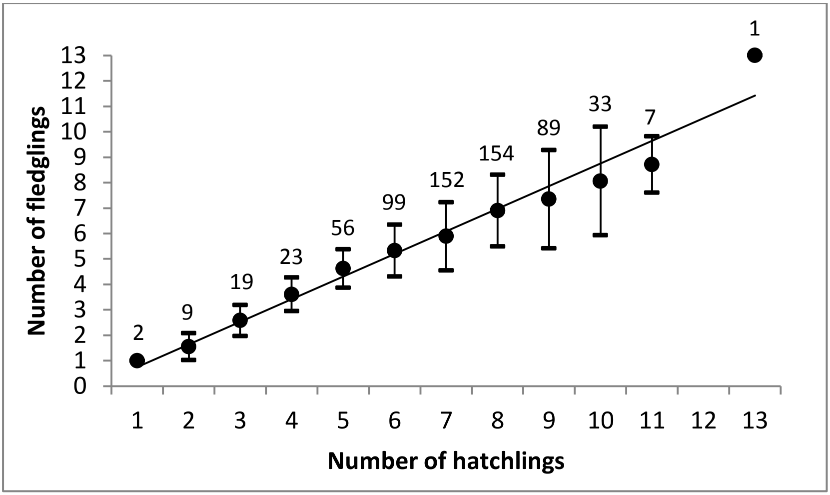

Figure 1. Fledgling production in relation to the number of hatchlings. Average number of fledglings (± SE) produced per nest in relation to the number of hatchlings. Sample sizes above error bars refer to the number of nests.

There was a significant negative relationship between mean nestling mass (M) and the number of hatchlings (NH) per nest ($M = -0.2318NH + 18.349$, $r = 0.282$, $p < 0.001$; Figure 2). We also found a non-significant trend for mean nestling tarsus length (T) to decrease with the number of nestlings ($T = -0.0269NH + 19.582$, $r = 0.074$, $p = 0.077$).

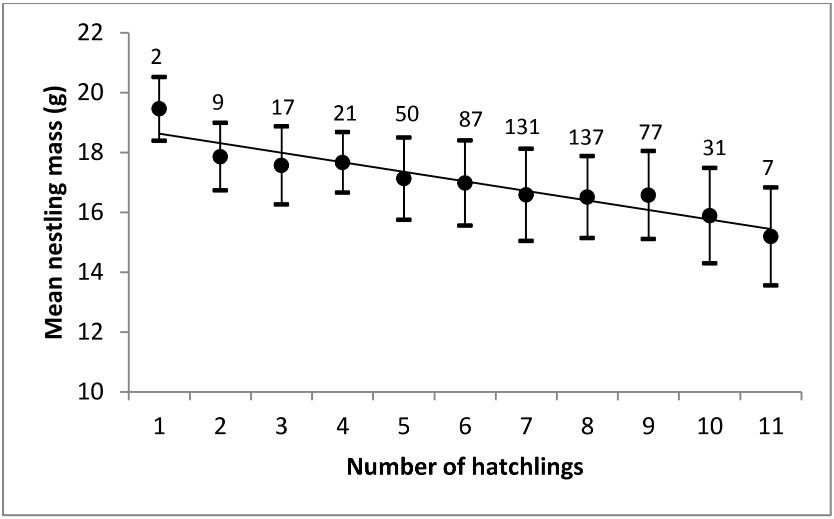

Figure 2. Nestling mass in relation to the number of hatchlings. Relationship between mean nestling mass at day 15 (± SE) and the number of hatchlings per nest. Sample sizes above error bars refer to the number of nests.

DISCUSSION

The number of hatchlings had a significant, positive effect on fledgling production, so that larger broods eventually yielded more fledglings. In this long-term approach, we did not find other significant predictors of the number of fledglings produced per nest in the studied population, although other predictors are likely relevant in certain years, depending on environmental conditions.

Several studies have analyzed the importance of brood size for nestling growth and survival (Groves 1984; Coulson & Porter 1985; Burness et al. 2000; Benharzallah et al. 2015), although its effect, either positive or negative, is dependent on parental quality and resource availability (Gebhardt-Henrich & Richner 1998). Taking care of large broods is energetically demanding, forcing parents to adjust clutch sizes based on their ability to rear the resulting chicks efficiently (Lack 1947; Murphy & Haukioja 1986; Wellicome et al. 2013). Parental age has been positively correlated with chick´s growth, either as a result of increased experience (Coulson & Porter 1985) or reproductive effort (Pugesek 1995). Moreover, parental breeding performance is necessarily linked to the ability to provide food to the developing chicks, so that limitations in food supply under resource-poor scenarios may carry over restrictions in nestling growth and/or survival inside the nest (Gebhardt-Henrich & Richner 1998; Wellicome et al. 2013). In our study, most chicks hatched per nest survived to leave the nest, suggesting a good adjustment between brood size and resource availability in the studied population in the long term. In addition, the mean annual number of fledglings was comparable to that of other areas within the same latitudinal range (Sanz 2002; Atiénzar et al. 2012).

The demands of raising large broods may have limited nestling growth, as revealed by the negative relationship between number of hatchlings and mean mass at fledging. In this study, nestling quality appeared to be compromised by nestling quantity. This result agrees with previous observational studies, where mean nestling mass has been shown to decline with increasing brood size (Perrins 1965; reviewed in Klomp 1970). Moreover, experimentally-enlarged broods produced lighter fledglings in several manipulative experiments (Smith et al. 1989; Tinbergen & Daan 1990; Pettifor et al. 2001; Hõrak 2003). In this population, previous studies revealed that adults reduced the number of feeding visits per nestling as brood size increased (Barba et al. 2009), and nestling growth rate decreased as clutch size increased (Barba et al. 1993).

None of the other predictors considered in this study had a significant effect on the number of fledglings in the long term. Therefore, nestling survival during the analyzed period seems to have been determined by brood size, regardless of the importance that, to a greater or lesser extent, other factors may have during certain years depending on particular environmental conditions. Adverse weather events prior to incubation, for instance, may negatively affect egg volume and clutch size, or bring about delays in hatching dates (Monrós et al. 1998). These breeding alterations may eventually affect nestling development and/or survival to fledging (Monrós et al. 1998; Krist 2011; Etezadifar & Barati 2015). Additionally, suboptimal nest temperatures during the nestling stage as a result of episodic hot or cold spells may directly handicap chick fitness and ultimately increase mortality (Belda et al. 1995; Takagi 2001). Based on our results, the weak predictive power of these factors could be explained by the annual variability in the intensity of their effects on fledgling production.

In conclusion, brood size emerged as the best predictor of the number of fledglings produced per nest in our Mediterranean great tit population. Larger broods produced more fledglings, although mass prior to fledging may have been compromised. The relatively weak effect sizes of the remaining potential predictors of fledging output could be a consequence, at least in part, of their dependence on environmental variation between years.

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Sección 2: La supervivencia de los pollos de carbonero tras el abandono del nido

ARTÍCULO 4

Negative effects of high temperatures during development on immediate post-fledging survival in great tits *Parus major*

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ABSTRACT

We analyzed the effect of nest temperatures, fledging date, age at fledging, fledgling mass and size on post-fledging survival of great tits *Parus major* in eastern Spain. We manipulated temperature during nestling development in 26 nests (average temperature was 39.8, 34.6 and 26.4 °C respectively, for heated, control and cooled nest boxes), and used radio-telemetry to monitor the survival of 48 nestlings (16 heated, 18 cooled, 14 controls) during the first 15 days after fledging. Heated chicks were lighter than control and cooled chicks. Estimated survival of heated fledglings was lower than that of controls. Additionally, survival of control fledglings increased with size, but this relationship was reversed for heated fledglings. Our results suggest that high temperatures experienced in the nest could have negative consequences on immediate post-fledging survival, and that smaller nestlings may deal more effectively with temperatures surpassing their optimal thermal range.

Keywords: great tit, heat stress, post-fledging survival, radio-telemetry.

RESUMEN

Analizamos el efecto de la temperatura durante el periodo de nidificación, la fecha de vuelo, la edad al volar, la masa y el tamaño sobre la supervivencia de juveniles de carbonero común *Parus major* al abandonar el nido. Manipulamos la temperatura durante la fase de desarrollo de los pollos en un total de 26 nidos (las temperaturas medias fueron de 39,8, 34,6 y 26,4 °C respectivamente, para los nidos calentados, control y enfriados), y empleamos radiotelemetría para monitorizar la supervivencia de 48 juveniles (16 calentados, 18 enfriados y 14 controles) durante los primeros 15 días después de volar. Los pollos calentados desarrollaron pesos menores que los controles y los enfriados. La supervivencia estimada para los juveniles calentados fue inferior que para los controles. Además, la supervivencia de los juveniles del grupo control aumentó con su tamaño, si bien esta relación se invirtió para los juveniles calentados. Nuestros resultados sugieren que las altas temperaturas sufridas en el nido pueden tener consecuencias negativas sobre la supervivencia inmediata después de volar, y que pollos más pequeños pueden hacer frente de forma más eficaz a temperaturas que superen su rango térmico óptimo.

Palabras clave: carbonero común, estrés por calentamiento, supervivencia después de volar, radiotelemetría.

INTRODUCTION

In passerine birds, mortality during the first year after hatching may be as high as 70-90% (Perrins 1986; Verboven & Visser 1998; Naef-Daenzer et al. 2001), and there is evidence that the immediate period following fledging (i.e., the post-fledging dependence period) is particularly critical (Perrins 1979; Drent 1984; Naef-Daenzer et al. 2001). However, for most bird species, following chicks after fledging is difficult due to their cryptic nature, and little is known about their behavior. The development of radio-tracking techniques and mark-based capture-recapture analyses has helped to shed some light into this stage (Lebreton et al. 1992; Skalski et al. 1993; White & Burnham 1999; review in Cox et al. 2014).

Among the factors which have been shown to be related to post-fledging survival, fledging date and body condition at fledging seem to be especially important, with heavier and early-fledged individuals generally having higher survival probabilities (Tinbergen & Boerlijst 1990; Naef-Daenzer et al. 2001; Monrós et al. 2002). Age at fledging could also be relevant, as remaining longer in the nest might improve flight capacity (Dial 2003). Consequently, factors affecting chick development during the nestling period could affect post-fledging survival through their effect on nestling condition at fledging (Greño et al. 2008). Suboptimal nest temperatures may also limit nestling development (Rodríguez & Barba 2016a, 2016b). Cold temperatures might require nestlings to invest in thermoregulation, at the expense of processes such as growth or the activation of a cell-mediated immune response (Dawson et al. 2005). High temperatures, in turn, can also affect nestling condition by leading to reduced food intakes, thereby affecting tissue growth (Murphy 1985; Geraert et al. 1996), or cause dehydration and respiratory illnesses (Belda et al. 1995; Patz et al. 2005). Additional consequences of heat exposure may include alterations of metabolic rate and oxidative stress, which may lead to the production of heat-shock proteins in different tissues (Salo et al. 1991). Moreover, the negative effects of high temperatures on nestling fitness may be accentuated depending on brood size and nest dimensions, as large broods constrained in small nesting spaces may have lowered ability to cope with heat stress (Mertens 1977a). If hyperthermia persists over long time periods, it may eventually lead to mortality (Mertens 1977b). In Mediterranean habitats, high temperatures reached during the breeding season may therefore cause negative effects on the health and condition of the chicks (Belda et al. 1995).

Greño et al. (2008) analyzed the relationship between temperatures experienced in the nest and post-fledging survival in a Mediterranean great tit *Parus major* population, and found that the effect of date on post-fledging survival could be, in part, a consequence of the seasonal pattern of temperature variation. In a climate-change scenario, fluctuating weather events may result in higher and/or lower than average temperatures during the breeding season (Winkler et al. 2002; Pachauri & Meyer 2014). In addition, the frequency of extreme weather events is also predicted to increase (Glądalski et al. 2014). Consequently, assessment of the direct (i.e., causing thermal stress in the chicks) and indirect (i.e., altering development of prey species and thus available food supply) effects of changing temperatures on nestling condition and subsequent fledgling survival could be of utmost importance.

The present study aims to check the effect of temperatures during the nestling stage on immediate (15 days) post-fledging survival of great tits, by experimentally increasing and decreasing nest temperature during nestling development. We expected that both types of manipulation (i.e., heating and cooling) would have negative consequences on post-fledging survival, as a result of the adverse effect of a suboptimal environment on juvenile condition at fledging (Greño et al. 2008). We controlled for the effect of other factors that may potentially influence juvenile survival, such as fledging date, age at fledging, and fledgling mass and size.

MATERIALS AND METHODS

Study area and experimental design

Data used for this study were collected during 2012 from a wild great tit population breeding in an extensive orange *Citrus aurantium* plantation near Sagunto (Valencia, eastern Spain; 39°42’N, 0°15’W, 30 m a.s.l.). The study site is a homogeneous habitat, where orange trees are uniformly distributed in their respective plots (Gil-Delgado & Escarré 1977; Andreu & Barba 2006). Clean wooden nest boxes [see Lambrechts et al. (2010) for details] were placed by the end of February for the birds to breed. Nest boxes were inspected with the frequency needed (at least weekly, and daily in specific phases of the nesting cycle) to accurately determine laying date (the date of laying of the first egg), clutch size and hatching date in each nest (e.g., Greño et al. 2008). For dates, we considered 1 = 1 April.

We randomly selected 30 nests from the breeding population (experimental nests hereafter), assigning 10 nests to each of a control, heated, or cooled groups, and matching as far as possible laying dates and clutch sizes between treatments. General Linear Models (GLMs) including treatment type (control, cooled or heated) as fixed factor revealed that there were no treatment-wise differences in reproductive parameters (Table 1). Neither there were differences in the thickness of the nest base (GLM; $F_{2,23} = 0.993$, p = 0.39; see Álvarez & Barba 2008 for details on measurement) or in nest dry mass (GLM; $F_{2,23} = 0.377$, p = 0.69; details in Álvarez et al. 2013), so we considered that nest size was similar between treatments and would not affect the experiment. A total of four experimental nests were lost during the season: a control nest failed prior to fledging, whereas two heated and one cooled nests were preyed upon.

Table 1. Reproductive parameters (±SE) of great tit nests involved in the temperature-manipulation experiment in Sagunto, Spain. Values in parenthesis refer to the number of nests. For dates, 1 = 1 April.

	Heated	Control	Cooled	Statistic	p
Laying date	11.89 ± 2.52 (9)	12.56 ± 3.91 (9)	13.40 ± 3.06 (10)	$F_{2,25} = 0.530$	0.60
Clutch size	7.90 ± 1.45 (10)	8.40 ± 1.08 (10)	8.30 ± 1.16 (10)	$F_{2,27} = 0.457$	0.64
Hatching date	32.20 ± 2.39 (10)	32.50 ± 3.44 (10)	33.10 ± 3.07 (10)	$F_{2,27} = 0.233$	0.79
Number of hatchlings	7.80 ± 1.40 (10)	8.10 ± 0.99 (10)	7.40 ± 1.17 (10)	$F_{2,27} = 0.856$	0.44

We altered temperatures inside heated and cooled boxes by introducing heat or cold pads on the nest box floor, ~1 cm below the nests, and replacing them periodically. Heat pads consisted of commercial warming units (Mycoal warmpacks, Northbrook Industrial Estate, Southampton, UK) that produced elevated temperatures for one day when exposed to air due to an exothermic oxidative reaction of iron powder. Cold pads consisted on frozen reusable refrigerating gel units (Cryopak, Edison, New Jersey, USA).

We used empty refrigerating gel units in control boxes. Cool pads were replaced four times a day (aprox. every four hours, beginning at sunrise; details in Álvarez & Barba 2014) to keep the cooling capacity, while heat pads were replaced once a day. However, to maintain the same degree of perturbation, all nests, including controls, were visited four times a day to replace (if necessary), or simulate the replacement, of the pads. In each box, the temperature manipulation started by 07:00 of the day after the date of hatching of the first egg and finished by 19:00 of day 14 of age of the nestlings (length of the nestling period in our population: 18-20 days; pers. obs.). Nestlings were ringed at day 15 with individually numbered metal rings, and we recorded their mass (to the nearest 0.1 g) and tarsus length (to the nearest 0.1 mm).

Temperatures inside the experimental nest boxes were recorded continuously every 32 seconds on day 1-4 post-hatching, and every 95 seconds on day 5-14 post-hatching, using Thermochron iButton data loggers (Model DS1922L-F5, Maxim Integrated) installed inside the nest cup, covered with a layer of nesting material and in the vicinity of the nestlings. The data loggers had to be replaced on the fourth day of the experiment in order to download their recordings in the laboratory. They were returned to the nest cup the following day, and remained there until day 14 post-hatching, when they were permanently removed from the nests. We checked the experimental nests during the first days following the installation of the data loggers to ensure they were properly located inside the nest cup. Average temperatures during this period were 39.78 ± 1.20 °C for heated nest boxes, 34.62 ± 1.16 °C for control nest boxes and 26.42 ± 3.80 °C for cooled nest boxes. Temperature records of heated nest boxes showed an approximately constant warming effect, whereas those of cooled nest boxes displayed temperature drops (after cool packs were changed) alternating with bouts of higher temperatures as ice melted between exchanges (see Appendix 1). Average ambient temperatures during the experiment, as provided by a nearby (i.e., approximately 4 km from our study site) meteorological station, were 18.65 ± 2.71 °C. As our study site is a homogeneous system (see description above), we did not expect there to be appreciable differences in nest box external temperatures.

Radio-tracking

In order to compare post-fledging survival of great tit juveniles across treatments (i.e., heated vs control vs cooled fledglings), we used a subset of individuals with *a priori* higher body condition. This way, when ringing the nestlings, we selected the two heaviest chicks at each nest and equipped them with a Biotrack radio transmitter (Biotrack, Wareham, Dorset, UK) of size 17x8x5 mm (without including the antenna) using a Rappole-type harness (Rappole & Tipton 1991). These transmitters weigh less than 0.5 g (2-3% of the great tit mass) and have been successfully used under field conditions with no observed differences in the behavior or maneuverability of the bird (e.g., Naef-Daenzer et al. 2001; Grüebler & Naef-Daenzer 2008). The radio transmitters carried a battery model Ag376, with an expected life of 21 days. Once the nestlings were radio-tagged, we visited the nests daily until they left the nest, to record fledging date. In these inspections, we approached the nest boxes and ensured signal detection of both nestlings inside the nest. Perturbation of nest boxes for visual confirmation was minimal, so no individual was forced to prematurely fledge during this period.

After fledging, each individual was radio-tracked once per day until it was found dead or the signal was no longer detected during at least three consecutive visits (in which case, the individual was censored from the study from that day onward, see statistical analyses). Homing was done using a Biotrack receiver model SIKA and a 3-element VHF Yagi-Uda antenna operating within the 148-152 MHz frequency range. We determined status as alive either by direct observation of radio-tagged fledglings or through changes in signal strength or direction. When we were unable to observe a particular juvenile, we estimated its location within 2-5 m radius. We recorded an individual as dead either if its carcass was located or if we located the transmitter with blood marks. In the latter case, we assumed mortality was due to direct predation, although we cannot discard other possible outcomes such as an individual dying from starvation and being eaten afterwards.

We radio-tagged a total of 48 chicks: 16 heated, 18 cooled, and 14 controls. Of these, 11 individuals (4 heated, 3 cooled and 4 controls) were excluded from the study, as we never received their signal after fledging. Moreover, the chicks pertaining to two control nest boxes were left un-tagged, due to technical difficulties.

Statistical analyses

We tested for post-treatment differences in the number of fledglings using a GLM, including treatment type (control, cooled or heated) as fixed factor. Differences between treatments in age at fledging, fledgling mass and tarsus length were analyzed using General Linear Mixed Models (GLMMs), including the treatment type as fixed factor and nest box as random factor. Pairwise comparisons between treatments in the GLMMs were implemented using the LSD test.

Previous studies with great tit fledglings have found no evidence of increased mortality within family parties after the first loss of a chick (Naef-Daenzer et al. 2001). Moreover, results obtained in our population support that survival probability of a fledgling is independent from that of its nest mates (Monrós et al. 2002). We thus considered juvenile mortality during the first days post-fledging as a random process with respect to nest origin, and treated individuals as independent units for analytical purposes. We used known-fate models within the program MARK (White & Burnham 1999) to estimate daily fledgling survival. This type of modelling, although similar to the Kaplan-Meier product-limit estimator, allows the inclusion of potential covariates affecting survival probability. Moreover, given that the status of all tagged animals is known at each sampling occasion, precision is high even with small sample sizes. Survival estimates provided by known-fate models account for the possibility of signal loss (e.g., by radio failure or dispersal of an individual outside detection range), as individuals may be coded at given time intervals as “censored”, in which case they do not take part in the survival estimation during those periods.

Preliminary data analysis showed that all chicks surviving to day 15 also survived to day 20 (when most of the transmitters stopped functioning due to battery depletion) independently of the experimental treatment received. Therefore, to simplify encounter histories, we focused our interest in survival during the first 15 days after fledging. In order to constrain the survival probability to a value between 0 and 1, we used a logit-link function in the models, the relationship between survival (ϕ) and covariates being:

$$Logit(\phi) = B_0 + B_1(covariate)$$

$$\phi = \frac{e^{B_0 + B_1(covariate)}}{1 + e^{B_0 + B_1(covariate)}}$$

where B_0 and B_1 are constants.

The model-fitting process consisted of two steps. First, we studied how daily fledgling survival varied with time by creating an initial set of three general models, where we considered a constant survival, an unspecified time-dependent survival, and a survival that varied with time following a linear trend. We then created a second set of models, by including the additive effect of potential covariates to the top-ranked model from the previous step. We only considered interactions between a maximum of two covariates to facilitate the interpretation of the results. We considered the effect of treatment type (introduced as a binary categorical dummy variable with three levels for “hot”, “cold” and “control” treatments, the first one being implicitly included in the intercept of all models with a treatment covariate), nestling age at fledging (considering the hatching date of the first egg as day 0), fledging date, nestling mass at day 15 and nestling tarsus length at day 15. Fledging date was included in the models to account for differences in the survival probability as a result of seasonal variation in environmental quality, e.g., food availability in the territory (see Ens et al. 1992; Verhulst et al. 1995; Sanz 2002), or predation (Naef-Daenzer et al. 1999). Moreover, its inclusion in the models was necessary for a proper codification of fledgling encounter histories, as required in known-fate designs (Cooch & White 2011).

We used Akaike's Information Criterion adjusted for small sample size (AIC_c) for model selection. The model with the lowest AIC was the one that best fitted the data. Models with a difference of $AIC_c < 2$ were considered to fit to data similarly (Burnham & Anderson 1998). We analyzed the 95% confidence intervals of the β parameters of the models with a similar fit. Given that the slope of the β parameter indicates the relationship between post-fledging survival and the covariate (Franklin 2001), we considered that when the 95% CI of the β parameter of a covariate included zero, it meant weak or no effect of that covariate on post-fledging survival.

Analyses were performed using IBM SPSS Statistics 19 and program MARK, version 6.2 (White & Burnham 1999). We considered results significant at the 0.05 level. Means are presented ± SE unless otherwise stated.

RESULTS

Breeding performance

There were no differences between experimental nests in the number of fledglings [means ± SE: 7.38 ± 1.40 (n = 8 heated nests), 6.22 ± 1.86 (n = 9 control nests), 7.00 ± 1.32 (n = 9 cooled nests); $F_{2,23}$ = 1.235, p = 0.31]. Fledgling mass differed between treatments (Least-squared means ± SE: 15.07 ± 0.50 g, 16.63 ± 0.48 g and 17.30 ± 0.47 g for heated, control and cooled chicks, respectively; $F_{2,23}$ = 5.430, p = 0.01), with heated chicks being lighter than control (LSD test, p = 0.034), and cooled chicks (LSD test, p = 0.004). We found no differences between experimental nests in either fledgling tarsus length (Least-squared means ± SE: 18.94 ± 0.13 mm, 18.99 ± 0.13 mm and 19.31 ± 0.13 mm for heated, control and cooled chicks, respectively; $F_{2,21}$ = 2.415, p = 0.11) or age at fledging (Least-squared means ± SE: 17.94 ± 0.31, 18.57 ± 0.33 and 18.94 ± 0.29 for heated, control and cooled fledglings, respectively; $F_{2,21}$ = 2.824, p = 0.08).

Fate of radio-tagged fledglings

Of the 37 radio-tracked fledglings, six survived until the end of the study, and 21 were documented as dead. We lost radio signal of the remaining 10 individuals before reaching day 15, and they were censored thereafter. Of the 21 dead individuals (6 heated, 10 cooled and 5 controls), 16 died during the first five days after fledging (4 heated, 8 cooled and 4 controls), and the remaining five during days 6-10. Deaths were probably mainly due to predation, as the transmitters were found apparently intact, with blood remains on the harness.

Post-fledging survival estimation and effect of potential covariates

The top general model describing a linear increase of survival probability with time had strong support (Akaike weight, $w_i = 0.978$; Table 2) and was selected as the reference model to which models including effects of candidate variables were compared.

Table 2. Model selection for time-dependent effects on post-fledging survival probabilities of great tit juveniles in Sagunto, Spain. For each model, the values for deviance (Dev), number of estimable parameters (K), Akaike's Information Criterion (AICc), difference between AICc of a model and the best performing model (ΔAICc), and AICc weights (w_i) are shown. Model notation is as follows: $S_.$, constant survival probability; S_t, time-dependent survival probability; S_{trend}, time-dependent survival probability following a linear trend.

	Dev	**K**	**AICc**	**ΔAICc**	**w_i**
S_{trend}	122.23	2	126.29	0.0	0.978
$S_.$	131.86	1	133.38	7.6	0.022
S_t	111.17	15	143.94	17.7	0.0001

We generated a total of 37 models including the additive effect of potential covariates to the reference time-dependent survival model (Table 3). The model that best fitted to data from our candidate set included the linear trend, the heated treatment type (as the intercept), the control treatment type, and the interaction between the experimental treatment and tarsus length ($S_{trend+control*tarsus}$). This interaction suggests that the effect of chick size on post-fledging survival is different for control and heated fledglings (see below).

Table 3. Top-ten ranked models used to test the covariates affecting post-fledging survival probabilities of great tit juveniles in Sagunto, Spain. For each model, the values for deviance (Dev), number of estimable parameters (K), Akaike's Information Criterion (AICc), difference between AICc of a model and the best performing model (ΔAICc), and AICc weights (w_i) are shown. Model notation is as follows: S, post-fledging survival probability; trend, time-dependence with a linear trend; control, control treatment; cold, cooling treatment; mass, mass at day 15 after hatching; tarsus, tarsus length at day 15 after hatching; age, age at fledging; date, fledging date.

	Dev	**K**	**AICc**	**ΔAICc**	**w_i**
$S_{trend+control*tarsus}$	114.33	5	124.66	0.0	0.207
S_{trend}	122.23	2	126.29	1.6	0.092
$S_{trend+cold}$	120.86	3	126.99	2.3	0.064
$S_{trend+control}$	121.56	3	127.69	3.0	0.046
$S_{trend+tarsus}$	121.66	3	127.79	3.1	0.043
$S_{trend+cold+tarsus}$	119.92	4	128.13	3.5	0.037
$S_{trend+mass}$	122.14	3	128.27	3.6	0.034
$S_{trend+age}$	122.19	3	128.32	3.7	0.033
$S_{trend+date}$	122.22	3	128.35	3.7	0.033
$S_{trend+cold*tarsus}$	118.13	5	128.45	3.8	0.031

The second best-supported model was the reference model (S_{trend}). Although these two models met our criteria for consideration as the best competing models ($\Delta AIC_c < 2$), the best-supported model ($w_i = 0.207$) was more than twice as plausible as the second-best model ($w_i = 0.092$). Moreover, the results of a Likelihood Ratio Test comparing the two models supported there being significant differences in model fit ($\chi^2 = 7.893$, $p = 0.048$). We thus decided to select the first model as the best alternative. The estimated effect (β) on the survival of fledglings, was strong and negative for the treatment type (evidencing a reduction in post-fledging survival for heated chicks relative to control chicks) and positive for the interaction term, but there was no evidence for an effect of either the tarsus length or the linear time trend, as their 95% CI overlapped zero (Table 4). As Figure 1 shows, survival differences between control and heated fledglings were greater during the first days after leaving the nest, and eventually leveled off by the end of the 15-day monitored period. Overall, we estimated a 48% accumulated survival probability for control fledglings and 26% for heated ones.

Table 4. Variables affecting post-fledging survival. Parameter estimates (β), standard errors, and 95% confidence intervals provided by model $S_{trend+control*tarsus}$, for the predictor variables hypothesized to affect survival of great tit juveniles in Sagunto, Spain.

Parameter	**B**	**SE(B)**	**95% CI**	
			Lower	**Upper**
Intercept	15.98	12.95	-9.41	41.37
Linear trend	0.16	0.08	-0.0006	0.33
Treatment	-48.82	19.85	-87.73	-9.91
Tarsus	-0.76	0.66	-2.06	0.54
Interaction	2.55	1.03	0.53	4.58

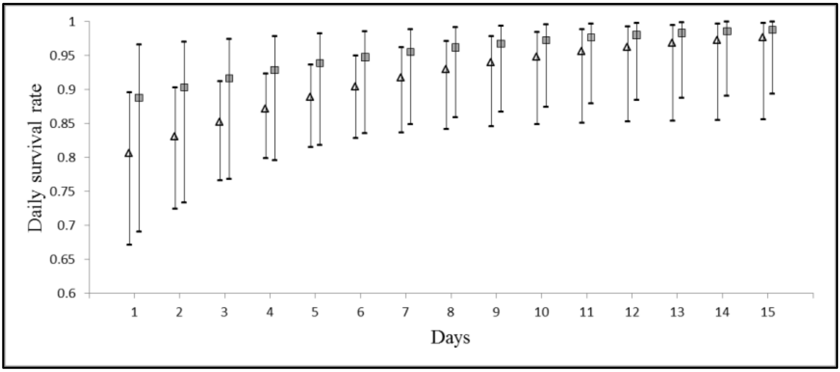

Figure 1. **Daily survival estimates of control and heated fledglings**. Comparison between daily survival rates for 15 days after fledging, and their 95% confidence intervals, of the control group (grey squares) and heated group (white triangles), as calculated by the program MARK model $S_{trend+control*tarsus}$.

As evidenced by the positive interaction between the experimental treatment and tarsus length, the effect of chick size on post-fledging survival depended on treatment type. In essence, juvenile post-fledging survival increased with tarsus length for the control group, with larger chicks having higher survival, as opposed to the heated group, where survival decreased with tarsus length (Figure 2).

We found no convincing support for an effect of the cold treatment on post-fledging survival. The ΔAIC of the best model that included the cold treatment, $S_{trend+cold}$, was 2.33. Additionally, in this model, the confidence interval of the β parameter of the cold treatment variable overlapped zero ($\beta = -0.56 \pm 0.48$, 95% CI = -1.50-0.38).

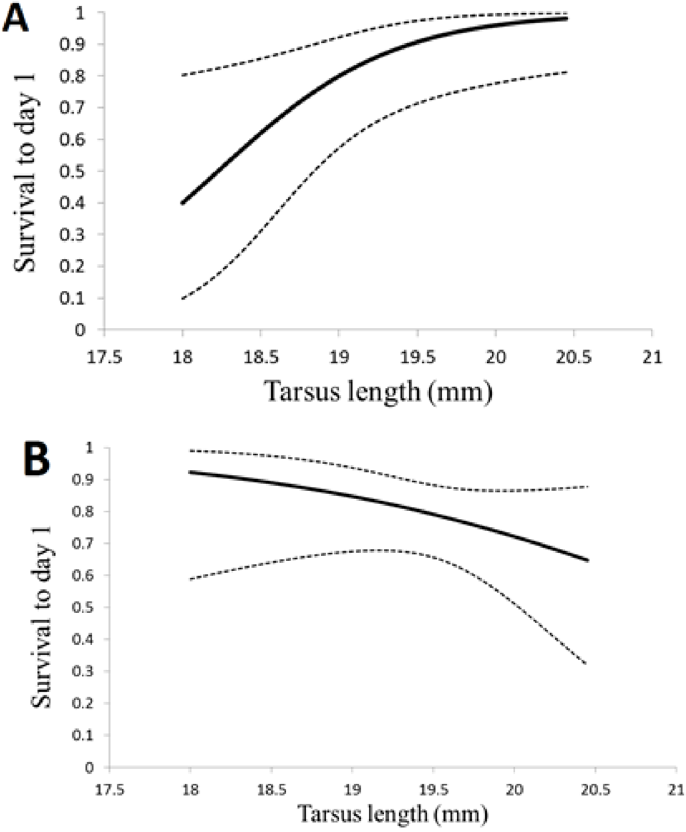

Figure 2. Post-fledging survival of control and heated fledglings in relation to tarsus length. Post-fledging survival to day 1 (i.e., from fledging to the first day post-fledging), of great tit juveniles as a function of tarsus length for (a) control and (b) heated fledglings, as calculated by the program MARK model $S_{trend+control*tarsus}$. The discontinuous lines represent the 95% CI. These trends were maintained during the 15-day study period (see text).

DISCUSSION

Immediate post-fledging survival probability of great tit fledglings was low, as only 48% of the juveniles from control nests survived during the first 15 days after leaving the nest. However, we have to keep in mind that we only radio-tracked the two heaviest nestlings of each brood. As there is evidence suggesting that heavier fledglings survive better than lighter ones (Monrós et al. 2002), actual survival rates should be, on average, lower than reported. To the best of our knowledge, the only comparable previous studies were those by Naef-Daenzer et al. (1999) and Naef-Daenzer et al. (2001), where similar low survival rates (e.g., 47-50%) for great tit juveniles during the first weeks after leaving the nest were reported.

Both in our case and in other studies (Naef-Daenzer et al. 2001), predation seems to be the main cause of juvenile mortality during the first weeks after fledging. During this period, juveniles are especially vulnerable to predation, as they are still developing their locomotion skills and lack predator avoidance strategies other than hiding or staying still when they hear warning calls from their parents (Gill 1995; Tome & Denac 2012).

The heating treatment affected nestling development, as heated nestlings were significantly lighter at day 15 than control and cooled nestlings. During their early development, great tit nestlings have limited thermoregulatory abilities, which make them more sensitive to suboptimal temperatures, especially higher ones (Mertens 1977b). In this sense, previous studies have estimated the upper and lower limits of the optimal thermal range to be set around 29 and 25 °C respectively, for broods consisting of six nestlings of 3 days of age. Above and below these limits, heat- or cold-related stress may be induced, respectively. This thermal range widens to around 29-12 °C when nestlings have 9 days of age (see Mertens 1977a). Even though these values are only indicative, we believe our heated nestlings could have been exposed to temperatures surpassing, on average, the optimal thermal range, which has been shown under controlled conditions to lower food intake, to alter energy and protein utilization, and reduce growth (Geraert et al. 1996). In addition, developing nestlings face the need to allocate their available resources to tissue growth and maintenance activities such as those involved in thermoregulation (Schew & Ricklefs 1998). In this sense, heated chicks could have allocated their energetic reserves to getting rid of body heat, leading to reduced mass gain (see Rodríguez & Barba 2016b).

On the other hand, we found no significant effect of cooling on nestling biometry. Intensive brooding by females could have kept nestlings from experiencing sustained temperature drops during early development (i.e., when average temperatures inside cooled nest boxes were closer to the lower limits of temperature tolerance), which would explain the observed pattern of our thermal records: temperature drops followed by bouts of higher temperatures (i.e., brooding bouts) during day time, and maintained, higher temperatures during night time (i.e., females might be increasing their brooding effort at night to compensate cooler nest temperatures during the day time). As nestling grew older, improvements in their own thermoregulatory ability as well as social thermoregulation (i.e., the possibility of nestlings to huddle together, reducing the individual heat production needed for body temperature stability) may have compensated the experimental cooling. Parental investment in thermoregulation to compensate adverse nest temperatures during the first days of nestling age (as a result of, e.g., episodes of environmental instability) may vary between years, as it is an energetically-demanding function that may compromise self-maintenance or future reproductive output (Ardia 2005; Canestrari et al. 2007). Inter-annual differences in factors such as food availability or parental quality could explain the results of previous studies, where the experimental cooling of nest boxes lead to reduced chick growth (Rodríguez & Barba 2016a).

The best fitting model of fledgling survival suggested a significant interaction between the experimental treatment and tarsus length. Survival of control chicks during the first day after leaving the nest (the moment of higher juvenile mortality of the 15-day study period) increased with tarsus length.

This effect of size on fledgling survival was reproduced on subsequent days, although with diminishing importance. Previous studies have demonstrated the relationship between chick body size and post-fledging survival (Garnett 1981; Sedinger & Flint 1995; van der Jeugd & Larsson 1998). Larger individuals have better survival prospects in part because they have more competitive foraging (Carrascal et al. 1998) and predator-avoidance skills (De Laet 1985), and are more aggressive and therefore tend to dominate over their smaller siblings (Garnett 1981). Similarly, chick mass at fledging has also proven to affect post-fledging survival, with heavier individuals surviving better than lighter ones (Both et al. 1999; Naef-Daenzer et al. 2001; Monrós et al. 2002). Heavier young may have extra fat reserves that can help them survive during the post-fledging period (Perrins & McCleery 2001), and may have a greater ability to cope with parasites and pathogens (Saino et al. 1997). In addition, heavier fledglings of improved condition may be harder to catch by predators than the lighter young (Naef-Daenzer et al. 2001). This could explain why the heavier control chicks had overall greater daily survival than heated chicks (Figure 1).

Surprisingly, the relationship between size and survival was reversed for heated fledglings, i.e., the experimental heating of nestlings favored post-fledging survival of smaller individuals. There is evidence suggesting a selection for smaller body size of individuals in response to warmer environmental conditions (Yom-Tov 2001, 2006; Gardner et al. 2014), one possible explanation being that smaller bodies of proportionally larger surface areas may be more efficient at shedding heat (Bergmann 1847). In this sense, the smaller chicks may have dealt more effectively with heat loading while confined in the nest, leading to a higher survival during the immediate days after nest abandonment. Although differences in body mass and size of our experimental nestlings were small, we believe that the prolonged exposure to temperatures well above their optimal thermal range may have made these small differences relevant. As we have been unable to test this assumption, we hope further experimental research such as the one we present here will help us clarify the implication of nestling size in the response to warm nest microclimates, taking into account alternative means of dealing with heat stress as temperatures increase (e.g., increases in peripheral blood circulation and evaporative cooling).

Finally, we are aware that the selection of the *a priori* better fitted pair of chicks from each nest could in part limit the conclusions of this study, as they cannot be considered representative of the whole brood, but this procedure has allowed us to compare post-fledging survival of a “similar” set of individuals (i.e., the heavier ones) across treatments, which was the main goal of the study. The results of this work therefore provide insight into the effect of adverse temperatures during the nestling period on a subsequent life-history stage, thus contributing to improve our understanding of the responses of birds to changing climates.

In conclusion, the alteration of the nest microclimate during nestling development might reduce post-fledging survival probability. Smaller nestlings may deal more effectively with heat stress, which would improve their survival prospects during the first days after leaving the nest. As far as we know, this is the first study providing experimental evidence on the existence of carry-over effects of suboptimal nest temperatures on a subsequent life-history stage of a Mediterranean hole-nesting passerine.

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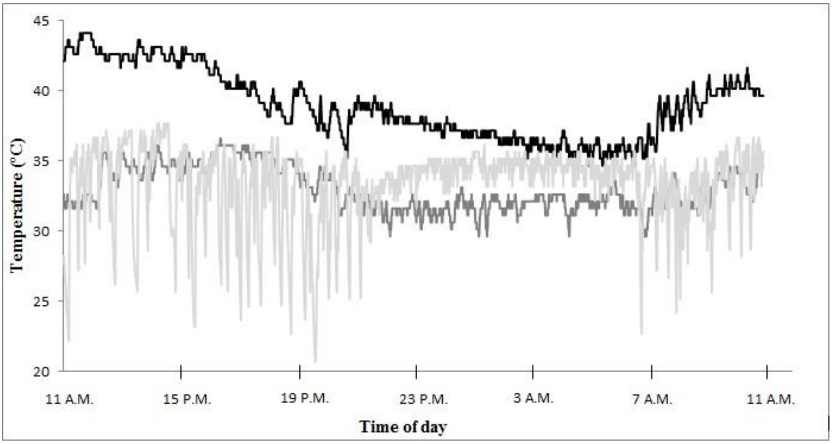
Appendix 1



Temperature records of experimental nest boxes. Examples of daily temperature records on day 1 of age of the nestlings, for a heated (black line), control (dark grey line) and cooled nest box (light grey line). Values were recorded on 1st (heated nest box), 2nd (cooled nest box) and 3rd of May (control nest box).

ARTÍCULO 5

A recipe for post-fledging survival in great tits *Parus major*: be large and be early (but not too much)

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ABSTRACT

Survival of juveniles during the post-fledging period can be markedly low, which may have major consequences on avian population dynamics. Knowing which factors operating during the nesting phase affect post-fledging survival is crucial to understand avian breeding strategies. We aimed to obtain a robust set of predictors of post-fledging local survival using the great tit (*Parus major*) as a model species. We used mark-recapture models to analyse the effect of hatching date, temperatures experienced during the nestling period, fledging size and body mass on first-year post-fledging survival probability of great tit juveniles. We used data from 5192 nestlings of first clutches ringed between 1993 and 2010. Mean first-year post-fledging survival probability was 15.2%, and it was lower for smaller individuals, as well as for those born in either very early or late broods. Our results stress the importance of choosing an optimum hatching period, and raising large chicks to increase first-year local survival probability in the studied population.

Keywords: breeding success, CJS models, fledging condition, hyperthermia, long-term study.

RESUMEN

La supervivencia de los juveniles durante el periodo posterior al abandono del nido puede ser muy baja, lo cual puede tener importantes consecuencias en la dinámica poblacional de las aves. Conocer cuáles de los factores que actúan sobre la fase de nidificación afectan posteriormente a la supervivencia después de volar es crucial para entender las estrategias de cría aviares. En este estudio obtuvimos un conjunto robusto de predictores de la supervivencia posterior al abandono del nido, empleando al carbonero común (*Parus major*) como especie modelo. Utilizamos modelos de captura-marcaje-recaptura para analizar el efecto de la fecha de eclosión, las temperaturas experimentadas durante la fase de nidificación, el tamaño al volar y la masa sobre la supervivencia local de los juveniles, una vez transcurrido su primer año tras el abandono del nido. Empleamos datos de 5192 pollos procedentes de primeras puestas, y anillados entre 1993 y 2010. La supervivencia media durante el primer año resultó ser de 15,2%, y fue menor tanto para individuos más pequeños, como para aquellos procedentes de puestas muy tempranas o demasiado tardías. Nuestros resultados ponen de manifiesto la importancia de elegir un periodo de cría óptimo, así como de sacar adelante pollos de mayor tamaño para aumentar la probabilidad de supervivencia en la población estudiada.

Palabras clave: éxito reproductor, modelos CJS, condición al volar, hipertermia, estudio a largo plazo.

INTRODUCTION

Variation in juvenile survival has profound effects on avian population dynamics (Arcese et al. 1992; Robinson et al. 2004; Finkelstein et al. 2010). First-year mortality after leaving the nest can be particularly high (Perrins 1979, 1980; Magrath 1991; Naef-Daenzer et al. 2001), which may have major consequences for the proportion of recruits into the breeding population (Starck & Ricklefs 1998). In spite of its importance, the post-fledging period has remained one of the least-studied components of avian demographics due to logistic difficulties in monitoring individuals after leaving the nest (Drent 1984; Hannon & Martin 2006; Vitz & Rodewald 2011). Consequently, many studies have often relied on pre-fledging characteristics to predict the survival of offspring, or have used local return rates to estimate survival (e.g., Ashcroft 1979; DiCostanzo 1980; Nisbet et al. 1984). In this sense, the development of capture-recapture models and their application to ringing data obtained from long-term studied populations have improved the reliability of the survival estimates, allowing the consideration of potential factors affecting post-fledging survival (Lebreton et al. 1992; Skalski et al. 1993; White & Burnham 1999).

A common pattern found in several studies with passerines is a selection for early breeding (e.g., Naef-Daenzer et al. 2001; Vitz & Rodewald 2011) and for a good condition at fledging, expressed through measures of fledging mass (e.g., Perrins 1965; Both et al. 1999; Monrós et al. 2002a) or skeletal body size (Brown & Brown 1998). Offspring fledging earlier in the season may benefit from milder environmental conditions, higher food availability (Krementz et al. 1989; Spear & Nur 1994; Naef-Daenzer et al. 2001), reduced intraspecific competition for resources (Kluyver 1971; Matthysen 1990; Verhulst et al. 1995), as well as lower predation rates (Newton 1978; Naef-Daenzer et al. 1999; Naef-Daenzer et al. 2001) and parasitism (Burtt et al. 1991; Merino & Potti 1995; Verhulst & Nilsson 2008). There may also be differences in parental quality between early and late breeders, so that early chicks may receive a greater investment from their higher-quality parents (Forslund & Pärt 1995; Hipfner 1997).

Deviations from the general pattern relating early breeding to high post-fledging survival have been observed in different populations. Anders et al. (1997) did not find evidence of a seasonal change in juvenile survival of wood thrushes (*Hylocichla mustelina*, Gmelin 1789), whereas survival of juvenile brown thornbills (*Acannthiza pusilla*, White 1790) and lark buntings (*Calamospiza melanocorys*, Stejneger 1885) increased as the season progressed (Green 2001; Yackel Adams et al. 2006). Additionally, at least one study showed that very early blue tit (*Cyanistes caeruleus*, Linæus 1758) hatchlings might experience a reduced post-fledging survival (Norris 1993). In the case of great tit (*Parus major*, Linæus 1758) fledglings, Monrós et al. (2002a) found that, depending on the year, either early, late, or mid-season nestlings had more post-fledging survival probabilities, and that temperatures experienced in the nest were in part responsible of these different patterns (Greño et al. 2008).

It is generally accepted that condition at fledging affects post-fledging survival. Larger juveniles may be better suited to escape from potential predators (De Laet 1985), and a larger body mass could be advantageous to endure periods of food limitation (Perrins 1965; Blem 1990; Perrins & McCleery 2001). This correlation between body size and condition at fledging and post-fledging survival implies that factors affecting chick development during the pre-fledging period may carry-over to subsequent biological phases and compromise future reproduction (van der Jeugd & Larsson 1998; review in Harrison et al. 2011). In this sense, temperatures experienced during the nesting period could affect fledgling condition through their effect on chick growth and immunocompetence (Geraert et al.

1996; Dawson et al. 2005). Newly hatched altricial nestlings might experience higher vulnerability to adverse cold nest microclimates due to their inability to regulate metabolic heat production (Shilov 1973; O’Connor 1984; Rodríguez & Barba in press), whereas high temperatures could affect grown nestlings of large broods, if they are unable to dissipate heat generated in excess (Mertens 1969; van Balen & Cavé 1970). Hyperthermia could be a serious issue in habitats such as those of the Mediterranean region, where maximum temperatures experienced during the breeding season may frequently exceed 30 °C, being thus liable to surpass the thermal tolerance of birds (Blondel et al. 1987; Belda et al. 1995; Greño et al. 2008). Previous manipulative studies in a Mediterranean great tit population have shown that exposition of nestlings to adverse high temperatures during development may not increase mortality in the nest, but rather lead to reduced mass at fledging, which could eventually lower first-year survival probability (Rodríguez & Barba, unpublished data).

Most analyses of juvenile post-fledging survival are based on relatively short-term data, which entails the risk of failing to account for all the variability in local survival trends, or ignoring certain factors affecting overall juvenile survival in favour of others that may only be relevant during specific years. Long-time series are therefore necessary to clarify the main factors affecting first-year post-fledging survival of juveniles across different years, leading to a robust set of predictors of post-fledging survival in a particular population.

Our aim here was to determine the effects of hatching date, temperatures experienced during nestling development, and fledgling mass and size on first-year post-fledging survival in a Mediterranean great tit population, using capture-recapture data from 21 years (1993-2013). Based on previous studies, we predicted that (1) both relatively high and relatively low ambient temperatures experienced during vulnerable periods of nestling development will have negative effects on juvenile survival, irrespective of dates; (2) the effect of dates *per se* will depend on the year (i.e., there will be years where early, late or mid-season hatchlings will have better survival prospects); and (3) large and/or heavier fledglings would have more post-fledging survival probabilities.

MATERIALS AND METHODS

We used data collected during a long-term study of a great tit population breeding in nest boxes within an extensive orange monoculture in Sagunto (Valencia), Eastern Spain (39°42’N, 0°15’W, 30 m a.s.l.). Wooden nest boxes were placed each year for the birds to breed, and were visited with the periodicity necessary (at least weekly, and daily at some stages) to record basic breeding parameters (Greño et al. 2008; Álvarez & Barba 2014). Relevant to this work were exact hatching dates, obtained for all nests through daily visits around the expected date of hatching (day 0), and fledgling mass (digital balance, 0.01 g accuracy) and tarsus length (digital calliper, 0.01 mm accuracy), obtained from 15 day-old nestlings. Nestlings were ringed with individually numbered metal rings at this date. For survival analyses (see below), we used data from 5192 nestlings of 876 first clutches fledged between 1993 and 2010. Between 1994 and 2013, 508 of these individuals were recaptured as adult breeders. Of these, 332 (65.4%) were recaptured for the first time in the first year after fledging. The total number of captures and recaptures, considering one capture event per breeding season, was 5995 (4684 birds were ringed and never recaptured, 318 individuals recaptured only once, 119 twice, 48 three times, 14 four times, 7 five times and 2 six times).

For each nest, we calculated average minimum ambient temperatures from hatching until nestlings were 5 days old, as well as average maximum ambient temperatures from day 10 to day 15. We considered these to be time periods of higher nestling vulnerability to low and high temperatures respectively (Mertens 1969; Shilov 1973). Temperature data were collected from the meteorological station “Sagunto Pontazgo” close to the study area.

Survival analyses

The general Cormack-Jolly-Seber modelling process

Previous studies with great tit fledglings have shown that the post-fledging survival probabilities of juveniles of the same nest were independent from each other (Naef-Daenzer et al. 2001). Moreover, results obtained in our population further support this finding (Monrós et al. 2002a). We thus considered individuals as independent units for analytical purposes. We used live recaptures models within the program MARK (White & Burnham 1999) to analyse post-fledgling survival data. Our first step in the modelling process was to obtain a reference Cormack-Jolly-Seber (CJS) model (Lebreton et al. 1992), incorporating time-dependency on local survival and recapture probabilities. Once we had this reference starting model, in a second step we incorporated the effect of individual covariates, as described in White & Burnham (1999). To ensure that the numerical optimization algorithm finds the correct parameter estimates, the values of individual covariates were standardized using the option “Standardized Individual Covariates” from MARK. Model selection was based on Akaike’s information criterion adjusted for sample size (AICc, Burnham & Anderson 2002). The model with the lowest AICc represents the best balance between loss of precision (due to over fitting) and bias of the estimates (due to under fitting;

Burnham & Anderson 2002). As general model selection criterion for analyses on post-fledging survival probability (i.e., tests for time dependence and effect of individual covariates, as described below), models with a difference in AICc of less than two units were considered to be similarly supported by the data. Although models ranked within two and seven units from the best-fitting model may also have some support (Burnham & Anderson 2011), likelihood ratio tests (LRTs) comparing these models with nested models from the selected subset were consistent with our more restrictive criterion, as none of the lower-ranked models contributed significantly to variation in the data. Among the models within two AICc units, we chose the one with the fewest parameters as the best one explaining the data (Burnham & Anderson 2002), and checked whether this decision originated a significant loss of fit using a likelihood ratio test.

Testing for time-dependence of local survival and recapture probabilities

We created a series of general models incorporating time-dependent effects on survival and recapture probabilities. These models were constructed using the sin-link function. Our initial model considered time-dependence in both survival and recapture probabilities, $\Phi(t)p(t)$. The validity of this simple model to the data was assessed by goodness-of-fit tests of program RELEASE in MARK (Burnham et al. 1987). The model fitted the data poorly (TEST 2 + TEST 3, $\chi^2 = 448.84$, d.f. = 61, $p < 0.001$). Results of TEST 3 ($\chi^2 = 394.67$, d.f. = 17, $p < 0.001$) suggested possible age-effects on survival probabilities. Given that an individual's first-year survival is likely to differ from that of older birds, we built a model incorporating two age classes: a1 (first-year survival) and a2 (adult survival). In this model, we considered first-year post-fledging survival to be time-dependent, and adult survival to be constant, as the analysis of time effects on older age classes was outside the objectives of our study. Recapture probability was considered to be time-dependent. The goodness-of-fit of this new model, $\Phi(a1t,a2)p(t)$, was tested using the parametric bootstrap approach implemented in MARK. The parameter estimates from the model were used to simulate data according to the assumptions of CJS models (i.e., no over-dispersion is included, animals are totally independent, and no violations of model assumptions are included). This process was repeated 1000 times and the deviance of each model was calculated to determine whether the deviance of the observed model exceeded that of simulated data. The probability of obtaining by chance a deviance value as large as or larger than the one observed was given by the ratio between the number of simulations with deviance larger than the one observed in our general model divided by the total number of simulations. We established a significance level $p < 0.05$ for rejecting the null hypothesis.

The bootstrap goodness-of-fit test indicated that the model had a good fit (p = 0.74), so we selected it as our reference model, and compared it with simpler nested models, using AICc values for model selection.

Testing for the effect of covariates on post-fledging local survival

We used an information-theoretic approach (Burnham & Anderson 2002) to examine first-year post-fledging survival of great tits in relation to hatching date, mass, tarsus length, average minimum temperatures during days 0 to 5 of age, and average maximum temperatures during days 10 to 15 of age. Starting with the best-fitting time-dependent general model from the previous step, we created a set of *a priori* hypothesized models where first-year post-fledging survival was dependent on different combinations of these individual covariates, never including in a single model both temperature variables. We also evaluated possible quadratic effects. Models including covariates were built with the logit-link function to constrain the survival probability to a value between zero and one. In order to limit the set of models analysed and simplify interpretation, we only included interaction terms in case we considered them relevant, in view of the results and/or their biological meaning. We created a total of 23 models and ranked them according to their AICc values. When estimating the effect of an individual covariate on a model, we assumed that when the 95% confidence interval of its β-parameter (as provided in program MARK output for each of the covariates included in a model, see Franklin 2001) included zero, it meant weak or no effect of that covariate on first-year post-fledging survival (e.g., Traylor et al. 2004).

RESULTS

Survival and recapture probabilities

The best-fitting general model had constant first-year and adult survival probabilities (Table 1, Model 1 vs. Model 3), and time-variation in recapture probabilities (Table 1, Model 1 vs. Model 2). Based on this model, first-year post-fledging local survival probability (± SE) was 15.2 ± 0.8%, whereas adult survival probability was 56.1 ± 1.6%. Estimated recapture probabilities ranged between 28.4% in 2004 and 72.8% in 2003, with a mean of 44.5 ± 11.8%.

Table 1. Model selection for time-dependent effects on recapture and first-year post-fledging survival probabilities of great tits breeding in eastern Spain. For each model, the values of Akaike's Information Criterion (AICc), difference of AICc values in relation to the best-fitting model (ΔAIC), AIC weights, number of estimable parameters (Np) and deviance (DEV) are shown. Model notation is as follows: Φ, survival probability; p, recapture probability; t, time dependence (year); a_1, first-year survival probability of fledglings; a_{2+}, survival probability of adults. Selected model in bold.

Models	AICc	ΔAIC	AIC weight	Np	DEV
Modelling recapture probability:					
1) Φ(a1,a2+)p(t)	5763.56	0.00	0.97873	22	523.31
2) Φ(a1,a2+)p(constant)	5791.36	27.80	0.00000	3	589.27
Modelling survival probability:					
1) Φ(a1,a2+)p(t)	**5763.56**	**0.00**	**0.97873**	**22**	**523.31**
3) Φ(a1t,a2+)p(t)	5771.22	7.66	0.02127	40	494.58

Covariates affecting post-fledging survival

In order to determine which of the studied covariates had a significant effect on first-year survival probability, we created separate models including the effect of each individual covariate on first-year post-fledging survival (Table 2, Models 9, 15, 17, 20 and 21), and compared them with the reference general model from the previous step (Table 2, Model 18). Models including an effect of tarsus length, hatching date and average minimum temperature during days 0-5 had a better fit to the data than the reference model, so we considered these covariates relevant. When compared individually, models with tarsus length fitted the data better than models with either hatching date or minimum temperature (Table 2, Model 9 vs. Model 15, Model 9 vs. Model 17). Contrasting the effect of temperature vs. that of date, a model including only the effect of hatching date on first-year post-fledging survival probability had a significantly lower AIC (Model 15 vs. Model 17, ΔAIC = 4), and received seven times more support than a model including T_{min}. The fit of these two latter models improved significantly with the introduction of quadratic effects (hatching date: Model 14 vs. Model 15, ΔAIC = 3.42; minimum temperature: Model 12 vs. Model 17, ΔAIC = 8.94). On the other hand, models including mass and average maximum temperature during days 10-15 received higher AICc scores than the reference model, and therefore we considered these covariates to have no significant direct effect on first-year post-fledging survival probability.

The inclusion of quadratic effects improved non-significantly the fit of the model in the case of maximum temperature (Model 19 vs. Model 21, ΔAIC = 0.89), and did not improve model fit in the case of mass (Model 20 vs. Model 22, ΔAIC = 1.93). Of the two biometrical covariates, a model including the effect of tarsus length on first-year post-fledging survival explained data 462 times better than a model including the effect of mass.

Table 2. Model selection for effects of covariates on first-year post-fledging survival probabilities of great tits breeding in eastern Spain. For each model, the values of Akaike's Information Criterion (AICc), difference of AICc values in relation to the best-fitting model (ΔAIC), AIC weights, number of estimable parameters (Np) and deviance (DEV) are shown. Model notation is as follows: Φ, survival probability; p, recapture probability; t, time dependence (year); a_1, first-year survival probability of fledglings; a_{2+}, survival probability of adults; +, additive factors; *, interaction; Tmax, average maximum temperatures during days 10-15 of age of nestlings; Tmin, average minimum temperatures during days 0-5 of age of nestlings; hd, hatching date; w, mass at fledging; tar, tarsus length at fledging. Covariates starting with sq mean squared effect of a covariate. Selected model in bold.

Models	AICc	ΔAIC	AIC weight	Np	DEV
1) $\Phi_{(a1(tar+hd+sqhd+tmin+sqtmin),a2+)}p_{(t)}$	5746.71	0.00	0.31746	27	5692.45
2) $\boldsymbol{\Phi_{(a1(tar+hd+sqhd),a2+)}p_{(t)}}$	**5747.32**	**0.61**	**0.23353**	**25**	**5697.10**
3) $\Phi_{(a1(tar+hd+sqhd+tmin),a2+)}p_{(t)}$	5748.42	1.71	0.13477	26	5696.18
4) $\Phi_{(a1(tar+hd),a2+)}p_{(t)}$	5749.54	2.84	0.07688	24	5701.34
5) $\Phi_{(a1(hd+sqhd+tmin+sqtmin),a2+)}p_{(t)}$	5749.67	2.97	0.07207	26	5697.44
6) $\Phi_{(a1(tar+hd+tmin),a2+)}p_{(t)}$	5750.48	3.77	0.04814	25	5700.26
7) $\Phi_{(a1(tar+tmin+sqtmin),a2+)}p_{(t)}$	5750.88	4.17	0.03940	25	5700.66
8) $\Phi_{(a1(tar*hd),a2+)}p_{(t)}$	5751.44	4.74	0.02975	25	5701.22
9) $\Phi_{(a1(tar),a2+)}p_{(t)}$	5752.97	6.26	0.01387	23	5706.78
10) $\Phi_{(a1(tar+tmin),a2+)}p_{(t)}$	5753.60	6.90	0.01010	24	5705.40
11) $\Phi_{(a1(tar+sqtar),a2+)}p_{(t)}$	5753.84	7.13	0.00896	24	5705.64
12) $\Phi_{(a1(tmin+sqtmin),a2+)}p_{(t)}$	5754.46	7.75	0.00658	24	5706.26
13) $\Phi_{(a1(tar*tmax),a2+)}p_{(t)}$	5755.41	8.70	0.00409	25	5705.19
14) $\Phi_{(a1(hd+sqhd),a2+)}p_{(t)}$	5755.98	9.27	0.00308	24	5707.77
15) $\Phi_{(a1(hd),a2+)}p_{(t)}$	5759.40	12.69	0.00056	23	5713.21
16) $\Phi_{(a1(hd+tmin),a2+)}p_{(t)}$	5759.67	12.97	0.00049	24	5711.47
17) $\Phi_{(a1(tmin),a2+)}p_{(t)}$	5763.40	16.69	0.00008	23	5717.21
18) $\Phi_{(a1,a2+)}p_{(t)}$	5763.56	16.85	0.00007	22	5719.39
19) $\Phi_{(a1(tmax+sqtmax),a2+)}p_{(t)}$	5764.43	17.73	0.00004	24	5716.23
20) $\Phi_{(a1(w),a2+)}p_{(t)}$	5765.08	18.38	0.00003	23	5718.90
21) $\Phi_{(a1(tmax),a2+)}p_{(t)}$	5765.32	18.61	0.00003	23	5719.13
22) $\Phi_{(a1(w+sqw),a2+)}p_{(t)}$	5767.01	20.30	0.00001	24	5718.81
23) $\Phi_{(a1(w*tmax),a2+)}p_{(t)}$	5768.25	21.54	0.00001	25	5718.03

Our next step in fitting models was to consider different additive combinations of the relevant covariates, and testing whether the results improved by including quadratic effects. Our three best-fitting models were similarly supported by the data, as their ΔAIC < 2 (Table 2, Models 1, 2 and 3). Together, their combined Akaike weight was 0.686. The three models incorporated tarsus length, hatching date and hatching date squared, and differed in the inclusion of minimum temperatures. The removal of T_{min} had no significant effect on the fit of the model, as judged by the LRT Test (Model 1 vs. Model 2: $\chi^2 = 4.650$, d.f. = 2, p = 0.0978; Model 3 vs. Model 2: $\chi^2 = 0.918$, d.f. = 1, p = 0.3380), and consequently the model with the fewer parameters (i.e., Model 2) was used to explain the effect of covariates on first-year post-fledging survival. In addition, we tested for a possible interaction between tarsus length and hatching date on first-year survival, but the resulting model (i.e., Model 8) received no convincing support, as its ΔAIC was 4.74 and the 95% confidence interval of the β-parameter of the interaction term included zero. Moreover, as the adverse effect of high temperatures on chick fitness may be aggravated during the late nestling stage depending on their size and overall ability to dissipate heat in excess (see van Balen & Cavé 1970), we also considered relevant to test for interactions between size (tarsus length or weight) and maximum temperatures. We found no convincing evidence to support these interactions, as the ΔAIC of the resulting models (Table 2, Models 13 and 23) was 8.70 and 21.54 respectively, and the 95% confidence interval of the β-parameter of the interaction terms overlapped zero. According to the best-ranked model, tarsus length and hatching date had a significant influence on first-year survival probability, as their β-terms did not overlap zero (Table 3). First-year post-fledging survival increased with nestling size (Figure 1), and varied with hatching date following a non-linear trend (Figure 2). The effect of date on first-year survival was such that hatching too early in the season, as well as hatching late, would have negative consequences on post-fledging survival (Figure 2). It is important to note that, regardless of the great dispersion in hatching dates in our study sample, the vast majority of chicks hatched during the "optimum" period leading to higher survival probability (i.e., April 21 to May 15), and that roughly less than 12% of the juveniles could be considered as being raised very early or late in the season. These marginal individuals also attained smaller sizes at fledging.

Table 3. Covariates included in the best-fitting models. β-parameters (±SE) and 95% CI (in brackets) for the covariates of the best-fitting models. Selected model in bold.

Model	Tarsus	Hd	Sqhd	t_{min}	Sqt$_{min}$
1) $\Phi_{(a1(tar+hd+sqhd+tmin+sqtmin),a2+)}p_{(t)}$	0.13 ± 0.06	-0.12 ± 0.06	-0.08 ± 0.04	0.21 ± 0.10	0.07 ± 0.04
	(0.01 – 0.24)	(-0.23 – -0.02)	(-0.159 – 0.002)	(0.02 – 0.40)	(-0.0005 – 0.1493)
2) $\boldsymbol{\Phi_{(a1(tar+hd+sqhd),a2+)}p_{(t)}}$	**0.17 ± 0.05**	**-0.14 ± 0.06**	**-0.08 ± 0.04**	-	-
	(0.07 – 0.28)	**(-0.24 – -0.03)**	**(-0.163 – -0.002)**		
3) $\Phi_{(a1(tar+hd+sqhd+tmin),a2+)}p_{(t)}$	0.17 ± 0.05	-0.13 ± 0.06	-0.08 ± 0.04	0.05 ± 0.05	-
	(0.06 – 0.27)	(-0.24 – -0.02)	(-0.1617 – -0.0003)	(-0.05 – 0.15)	

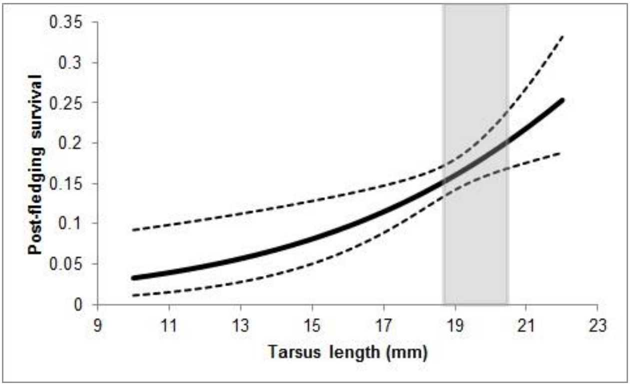

Figure 1. Post-fledging survival probability in relation to tarsus length. Effect of tarsus length on first-year post-fledging survival probability of great tits breeding in eastern Spain, as calculated by the program MARK model $\Phi_{(a1(tar+hd+sqhd),a2+)}p_{(t)}$. Dotted lines represent the 95% CI. Shaded area includes approximately 80% of chicks.

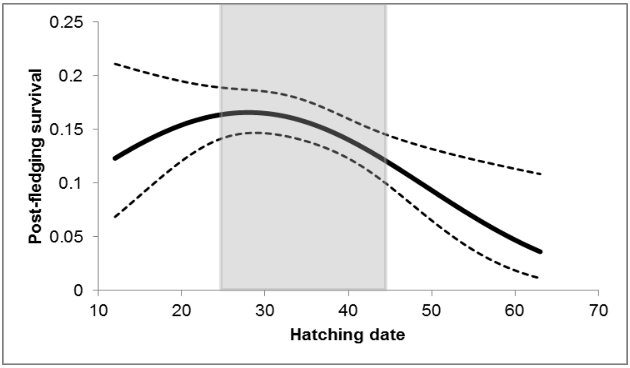

Figure 2. Post-fledging survival probability in relation to hatching date. Effect of hatching date on first-year post-fledging survival probability of great tits breeding in eastern Spain, as calculated by the program MARK model $\Phi_{(a1(tar+hd+sqhd),a2+)}p_{(t)}$. Dotted lines represent the 95% CI. Shaded area includes approximately 80% of chicks.

DISCUSSION

Our results suggest that hatching date and fledgling size (tarsus length) have a significant impact on first-year post-fledging survival probability. Smaller individuals, as well as those pertaining to either too-early or late broods would have lower survival prospects. The effect of other potential covariates affecting first-year survival, such as fledgling mass or temperatures experienced during the nestling stage, has not received convincing support. This way, of the two possible descriptors of body condition, fledgling size has proven to be a better predictor of first-year post-fledging survival than fledgling mass, and we have been unable to show the existence of carry-over effects of either maximum or minimum ambient temperatures experienced during vulnerable periods of nestling development on first-year survival probability.

Effect of date on local first-year survival probability

The effect of hatching date on first-year local survival was non-linear, suggesting there being an optimal range of breeding dates leading to a maximum first-year post-fledging survival probability, and that both positive and negative deviations from this range are not beneficial.

Birds have a limited period each year in which conditions for growth and reproduction are most suitable. In this sense, timing of breeding is essential, and individuals capable of adjusting their breeding schedule to match nestling development with the seasonal peak of prey availability will likely be able to raise larger fledglings of higher quality (van Noordwijk et al. 1995). Based on the results of this study (i.e., most of the chicks hatched during the optimum period), the majority of females in our great tit population were able to successfully track environmental change and raise their chicks when breeding conditions were finest.

The seasonal decline in breeding productivity is a common trend among avian populations (Perrins 1965; Nilsson & Smith 1988; Daan et al. 1989; Verhulst & Tinbergen 1991; Naef-Daenzer et al. 2001). It is argued that juveniles from later broods suffer higher predation rates (Newton 1978; Naef-Daenzer et al. 1999; Sim et al. 2012) and detrimental environmental conditions (Naef-Daenzer et al. 2001; Öberg et al. 2014). The causal relationship between poor breeding performance and late breeding has also been supported in our study site, as delayed great tit pairs have been shown to produce fewer fledglings, of lower quality, that were less likely to be recruited into the local breeding population (Barba et al. 1995). The present study reveals that not only late breeding, but also very early breeding, entails lower first-year post-fledging survival probability. This finding would be in agreement with previous findings in blue tits (Norris 1993). Though this is a relatively old study, we did not find other ones demonstrating that breeding too early was disadvantageous. We believe that very early broods may be more likely exposed to sudden episodes of environmental instability, which are frequent in our study site at the beginning of the spring. These episodes, although of short duration, are characterized by strong temperature drops and intervals of heavy rain, and may pose a serious threat to developing chicks, thus endangering future survival prospects. The influence of hatching date on post-fledging survival was also suggested by Monrós et al. (2002a), although its effect (either positive or negative) could vary from year to year, and no clear overall pattern emerged. The consideration of a longer data set has helped to highlight the advantage of early fledging on first-year survival, but also that juveniles hatching too early could be penalized as well.

On the other hand, Greño et al. (2008) took into account the potential effect of ambient temperatures experienced during the nestling stage on first-year post-fledging survival, and suggested the existence of both direct (i.e., increasing thermal stress) and indirect effects (i.e., through effects on food availability) of temperatures on first-year survival probability. We have been unable to find evidence for date-independent thermal effects, even after considering shorter periods of high nestling vulnerability to suboptimal temperatures. Maximum temperature was discarded in the first steps of model fitting and, although minimum temperature was a covariate included in two of the three best-scored models (Table 2, Model 1 and Model 3), its exclusion did not lead to a significant loss of fit. Our results support that the effect of ambient temperatures during the nesting period on post-fledging survival found in our study site is a consequence of their correlation with dates and more likely to be indirect, i.e., a result of changing environmental conditions at fledging as the season progresses.

Effect of fledgling size on local first-year survival probability

Juvenile size at fledging had a positive effect on first-year post-fledging survival probability. Larger individuals may be less vulnerable to diseases, parasites and predators during their first months of life (Ragusa-Netto 1996; van der Jeugd & Larsson 1998). They may also be favoured during severe weather conditions, due to their greater capacity to retain heat and store fat (Brown & Brown 1998). Additionally, body size has been shown to be directly related to the establishment of dominance relationships between juveniles during the post-fledging period, as larger fledglings tend to dominate over smaller ones (Garnett 1981). This superiority allows bigger fledglings to reach full independence in better physical condition than their weaker siblings (Vergara & Fargallo 2008; Kitowski 2005), thus improving long-term survivorship (Arcese & Smith 1985; Desrochers et al. 1988; Piper & Wiley 1990). Moreover, the absence of evidence for an interaction between date and body size on post-fledging survival probability suggests that large fledglings have higher first-year survival than their smaller siblings with independence of the date they were born. In this sense, it is important to note that, late in the season, few chicks eventually develop large body sizes at fledging in our population (e.g., only 14% of fledglings hatched after May 15 have tarsi greater than 20 mm).

The relationship between size and post-fledging survival has been documented in numerous studies, although it is common to express body size in terms of fledgling mass (Garnett 1981; Ragusa-Netto 1996; Velando 2000). In our case, tarsus length proved to be much better at predicting first-year post-fledging survival than mass (it was the single most important variable affecting first-year survival), probably because it is a more accurate indicator of overall chick size. In this sense, skeletal body size of juveniles at fledging is not likely to vary during their transition to adulthood; it is therefore a final measurement of juvenile size, whereas initial body mass differences between fledglings could be compensated during the post-fledging period depending on food availability. Monrós et al. (2002b) showed that great tit fledgling mass may vary during the immediate days after leaving the nest (i.e., lighter than average chicks will tend to gain mass, whereas heavier than average birds will tend to lose it). Our results suggest that measures of skeletal body size should be provided when analysing post-fledging survival in relation to fledging characteristics, as they are more consistent estimators of individual body size at fledging.

In conclusion, we highlight the importance of hatching date and body size as determinants of first-year survival in a Mediterranean great tit population. Large fledglings hatched between April 21 and May 15 have greater first-year post-fledging survival probabilities, most likely as a result of superior fitness and competitive skills, as well as more favourable environmental conditions at fledging.

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ARTÍCULO 6

Phenotypic plasticity could account for temperature-related changes in timing of a passerine bird over 22 years

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ABSTRACT

The adjustment of breeding time in response to changing environmental conditions may imply measurable differences in reproductive success of avian populations. Phenotypic plasticity is one of the major mechanisms allowing individuals to track changes in local temperatures and optimize breeding performance. In this study, we analyzed long-term annual trends of selection differentials for laying date in a Mediterranean great tit (*Parus major*) population and the contribution of individual phenotypic plasticity at explaining the observed population-level patterns in breeding initiation. Despite an overall selection for earlier breeding, mean laying date did not advance significantly in our population during the studied period. We found a significant advancement of breeding date in warmer years, a response that was mainly explained by phenotypic plasticity. In addition, temperatures experienced during the incubation and nestling stages had major influence on selection differentials. Our results suggest potential costs of beginning to lay too early in this population, and highlight the importance of two specific breeding periods (i.e., incubation and late nestling stages) in shaping selection pressures for breeding onset.

Keywords: great tit, laying date, Mediterranean, selection differentials.

RESUMEN

El ajuste del periodo de cría en respuesta al cambio en las condiciones ambientales puede implicar importantes diferencias en el éxito reproductor de las poblaciones aviares. La plasticidad fenotípica es uno de los principales mecanismos empleados por las aves para responder a cambios acaecidos en las temperaturas locales y optimizar el rendimiento reproductivo. En este estudio, analizamos tendencias a largo plazo en los diferenciales de selección anuales en la fecha de puesta de una población mediterránea de carbonero común (*Parus major*), así como la contribución de la plasticidad fenotípica a la hora de explicar los patrones observados a nivel poblacional en el inicio del periodo de cría. A pesar de haber, en general, una selección a favor de criar antes, la fecha media de inicio de puesta no avanzó de forma significativa en nuestra población durante el periodo estudiado. La fecha de puesta se adelantó en años más cálidos, respuesta explicada principalmente por plasticidad fenotípica. Además, las temperaturas alcanzadas durante las fases de incubación y de presencia de pollos en el nido tuvieron una gran influencia sobre los diferenciales de selección. Nuestros resultados sugieren la existencia de costes asociados a comenzar a criar demasiado pronto en esta población, y muestran la importancia de dos fases concretas del periodo reproductor (la fase de incubación y la fase de presencia de pollos) a la hora de determinar las presiones de selección en la fecha de puesta.

Palabras clave: carbonero común, fecha de puesta, Mediterráneo, diferenciales de selección.

INTRODUCTION

The timing of breeding is of utmost importance for avian species of temperate regions, as variation in breeding dates may imply measurable differences in reproductive success (Dunn & Winkler 2010). There is a narrow window in the annual cycle when conditions for reproduction are more suitable, and consequently birds have to optimize the laying of their clutches so that brood rearing takes place during the moment of greater food abundance (Perrins 1991; van Noordwijk et al. 1995; Cresswell & McCleery 2003). Spring temperature is usually associated with the timing of nestling food availability, as it may alter the phenology of key prey species (Visser & Both 2005). Moreover, temperatures may also have direct effects on bird phenology, as they may affect the energetic demands of females and, in turn, the timing of laying independently of food supply (Visser et al. 2009). In addition, adverse weather spells in early spring may reduce survival probabilities through direct effects of temperatures on individual fitness (Charmantier & Gienapp 2014; Rodríguez et al. 2016).

Many avian species have advanced their timing of breeding and migration in the past decades, in response to the advancement of optimal breeding conditions (Visser et al. 2004; Charmantier & Gienapp 2014). Contrasting with this general pattern, extreme weather events can bring about delays in breeding dates as a consequence of abnormally low early spring temperatures in atypical years (Glądalski et al. 2014). The analysis of long-term data on individually marked birds has allowed the estimation of selection differentials on the basis of differences in breeding success as the season progresses, and the documentation of selection pressures for either early or late breeding in a given population (Ahola et al. 2009; Goodenough et al. 2010).

Phenotypic plasticity, the capacity of a genotype to change the expression of a trait in response to environmental variation, may explain the means by which individuals are currently tracking variation in local temperatures (Charmantier et al. 2008; Porlier et al. 2012; Vedder et al. 2013). In this sense, although avian breeding schedule is relatively inflexible, birds do have certain mechanisms to alter the timing of their breeding activities to cope with changes in environmental conditions (Monrós et al. 1998; García-Navas & Sanz 2011; Álvarez & Barba 2014). The ability to respond to environmental fluctuations, however, may vary across the different stages of the breeding period. For instance, birds may show a higher flexibility to adjust their timing during the nest construction and laying stages but, once incubation starts, their scope for altering their breeding schedule may be more limited (van Noordwijk et al. 1995). These differences in the bird´s ability to cope with environmental change at different breeding stages may, in turn, affect the relationship between temperatures and selection differentials for breeding onset (van Noordwijk et al. 1995).

The question that arises is whether species are shifting adequately their phenology in response to the changing environment and consistent selection pressures for breeding initiation. In many cases, the observed shift is inadequate (i.e., either too little or too much), and this situation leads to mistimed reproduction (see review in Visser & Both 2005). There are, however, species that are not showing an apparent change in breeding time even when selection seems to favor an earlier reproduction (Goodenough et al. 2010; Townsend et al. 2013). Moreover, within the same study site, different coexisting species experiencing the same environmental conditions may display different responses, with some of them appropriately advancing their breeding time without selection for early laying becoming stronger, and others showing no significant advancement in laying date although selection differentials suggest selection for earlier breeding (Goodenough et al. 2010). Different theories have been proposed to explain why the absence of phenological responses can be expected from an evolutionary standpoint (see review in Johansson et al. 2015), although the mechanisms involved may be complex. For example, it has been put forward that a lack of phenological responses across trophic levels may be adaptive in systems where prior residency implies a competitive advantage (e.g., as a consequence of intraspecific competition for territories or dominance positions; see Johansson et al. 2014), or when life history trade-offs arise between survival and reproduction (Kristensen et al. 2015). Moreover, in highly stochastic environments, asymmetric fitness curves combined with temporal environmental variation may lead to apparently suboptimal strategies in the short term, but that are optimal in the long run (Lof et al. 2012). In any case, connecting recent findings in natural populations with some of these theoretical approaches may help disentangle some of the confounding interactions between temperature change, selection pressure, and phenological shift.

The magnitude of the phenological responses of bird species to changing environmental conditions has been shown to be greater in populations breeding at higher latitudes (Sanz 2003; Grosbois et al. 2006). In this sense, long-term studies have proven an essential tool to investigate the impacts of climate change, and allow predictions to be made across distribution ranges (Forchamer & Stenseth 1998; Parmesan & Yohe 2003; Grosbois et al. 2006). There is still much work to be done, however, as long-term studies relating climate variation and changes in bird phenology are currently scarce in regions located at relatively lower latitudes such as the Mediterranean (e.g., Sanz et al. 2003; Grosbois et al. 2006; Potti 2009; Porlier et al. 2012), where the effects of climate change are predicted to be severe (Diffenbaugh & Giorgi 2012). In this study, we analyze long-term annual trends of selection differentials for laying date in a Mediterranean great tit (*Parus major*, Linnaeus 1758: Passeriformes, Paridae) population, and determine whether phenotypic plasticity of individuals could account for the observed population-level patterns in laying dates. This way, we aim to find if there is a need for change (i.e., overall selection pressure for early or late reproduction) and, if so, whether (and how) our population is actually responding to this need. In addition, we aim to determine whether the effect of temperature on selection pressures varies across different stages of the reproductive phase, from nest-building to fledging.

MATERIALS AND METHODS

Study area and field methods

The data used for this study were collected during a long-term study of a great tit population breeding in nest boxes in Sagunto, eastern Spain

(39°42´N, 0°15´W, 30 m a.s.l.). The study area was located within an extensive orange *Citrus aurantium* monoculture, where wooden nest boxes (inner basal area 126 cm^2, height 18.5 cm; see Lambrechts et al. 2010 for details) have been available since 1986 for the birds to breed (Tomás et al. 2012; Álvarez & Barba 2014). Individual ringing of the nestlings, as well as trapping and individual ringing of parents, started in 1992, so we include here data from 22 years (1992 to 2013), for which we have available complete records of breeding parameters and identity of the parents. Data of the parents captured when breeding in 2014 were also used to estimate recruitment rates of the 2013 cohort (see below). The average clutch size in our population is around 8 eggs (Atiénzar et al. 2012). Incubation starts once the clutch is complete, and lasts for about 13 days (Álvarez & Barba 2014). The nestling period lasts for about 18 days (Atiénzar et al. 2012).

Beginning in late February, regular visits to the nest boxes allowed us to obtain the basic breeding parameters. Standard procedures have been described elsewhere (e.g., Tomás et al. 2012), so we detail here those relevant for the present work. Thus, we estimated the laying date of the first egg assuming that one egg is laid per day (given as April dates, where 1 = 1 April), and hatching date by daily visits to the nests around the expected date of hatching. Adults were caught at the nest using spring traps when nestlings were 10-12 days old and, if not already ringed, they were ringed using individually-numbered metal rings. Parents were sexed at this moment by the presence/absence of a brood patch and plumage characteristics, and we determined their age (i.e., distinguishing yearling breeders from adult breeders) by the color of the wing coverts (Svensson 1992). Nestlings were ringed when they were 15 days old. On day 20, we visited each nest box to determine if all the chicks had fledged, and identified dead nestlings. If they were still in the nest, we visited the nest box every other day until fledging. Only first clutches were used for this study.

Mean daily temperatures [i.e., (daily maximum temperature + daily minimum temperature)/2] from 1992 to 2013 were obtained from the Pontazgo Meteorological Station, located at approximately 4 km from the study site. We defined “spring temperature” as the annual mean of mean daily temperatures recorded during the first 15 days of March, as it has been shown to be highly correlated with breeding initiation in our great tit population (see results). Average temperatures during other time periods, as well as a phenological indicator based on temperature sums (i.e., spring warmth sum; see Charmantier et al. 2008), were also tested, but their correlation with laying dates were weaker (results not shown).

Analyses

We calculated yearly standardized selection differentials (Falconer & Mackay 1996) for the laying date (SSLD) by subtracting yearly average laying date (LD) from the average laying date weighted for the number of recruits produced per nest (LD_W), and dividing this difference by the standard deviation of the laying date (StdLD); SSLD = (LD_W – LD)/StdLD. Negative values imply that most recruits were born before the population-average date, and therefore there was a selection pressure for earlier breeding, while positive values imply that most recruits were born after the population-average date. These analyses were done using data from 6581 fledglings of 1097 different clutches, fledged between 1992 and 2013. Of these individuals, 648 (i.e., 9.8%) were recruited in subsequent years (i.e., between 1993 and 2014). Provided that recruitment is considered during every year following fledging (i.e., not just the first year), individuals fledged during the latter years have lower probability of being detected as recruits, as not all of them are captured in their first breeding year.

We examined temporal changes in laying date, spring temperature and SSLDs using linear regression models with year as the predictor. In order to test the assumption of no temporal autocorrelation across years (see Zuur et al. 2009), we re-ran the models including (1) an auto-regressive model of order 1 (AR-1) auto-correlation structure, establishing the “corAR1” correlation option with year; and (2) a compound symmetry auto-correlation structure, establishing the “corCompSymm” correlation option with year. Model fit (original model vs. model with an autocorrelation structure) was checked using Akaike´s Information Criterion (AIC). Models with a difference of AIC < 2 were considered to fit to data similarly (Burnham & Anderson 2011). Given that the inclusion of the auto-correlation structures did not improve model fit of either model, we did not incorporate them in subsequent analyses. In addition, the effect of temperature on laying date and SSLDs was analyzed using simple linear regression models with spring temperature as the predictor.

To determine the reproductive stages where temperatures have the greatest influence on selection differentials, we divided the breeding period in five 9-day intervals (see van Noordwijk et al. 1995 for details), i.e., the pre-laying stage (from LD – 9 days to LD – 1 day), the laying stage (from LD to LD + 8 days), the incubation stage (from LD + 9 days to LD + 17 days), the hatching stage (from LD + 18 days to LD + 26 days), and the nestling stage (from LD + 27 days to LD + 35 days). These intervals were selected to avoid the statistical problem of comparing correlations with temperatures over uneven time periods, and assume that each nest was at the specific stage during most of the selected period. The most inexact was the “hatching stage”, which usually lasts for only one or two days (Álvarez & Barba 2014), and therefore also includes the last days of the incubation period (which actually lasts about 13 days) and the first days post-hatching. We calculated mean annual temperatures during these time intervals, and correlated them with the SSLDs using simple linear regressions. In case of the model analyzing the relationship between selection differentials and temperatures during the nestling stage, we used data from 21 years, as we lacked temperature records during that period for 2002 due to technical problems at the meteorological station.

To analyze individual plasticity in laying date, we used a subset of the data including females for which we have data of at least two years. In total, we considered 753 observations of 299 individuals. We conducted a General Linear Mixed Model (GLMM), introducing laying date as dependent variable, age (i.e., yearling breeder or adult breeder) as fixed effect, year and individual identity as random effects, and spring temperature as covariate. In this model, age was included to account for age-dependence in breeding performance (Przybylo et al. 2000), whereas the effect of spring temperature determines its influence in producing changes in an individual´s laying date over its lifetime. On the other hand, we regressed mean within-female changes in laying date in two consecutive years against changes in spring temperature for the same pair of years, and compared the resulting slope (i.e., a direct estimate of phenotypic plasticity; see Charmantier et al. 2008) with that of the relationship between mean laying date and annual spring temperature at the population level. The similarity of these two slope values suggests that phenotypic plasticity in behavior accounts for an important amount of the population level response (see Charmantier et al. 2008; Thorley & Lord 2015).

Analyses were done using the function “gls” included the “nlme” package in R (R Development Core Team 2010), and software SPSS v.22.

RESULTS

Analysis of annual trends revealed that mean spring temperature (1-15 March) had not increased significantly in our population during the study period (t_{20} = 0.812, p = 0.427). In addition, we did not detect a significant bias towards earlier breeding (t_{20} = -1.951, p = 0.065), although we found a strong negative correlation between LD and spring temperature, indicating a significant advancement of laying dates in years when mean annual spring temperature was higher (r = 0.863; t_{20} = -7.634, $p < 0.001$; Figure 1).

There was an overall selection for earlier breeding in our study site, as selection differentials for laying date were negative in 73% of the years (i.e., 16 of 22 years, Figure 2). Moreover, mean annual selection differential

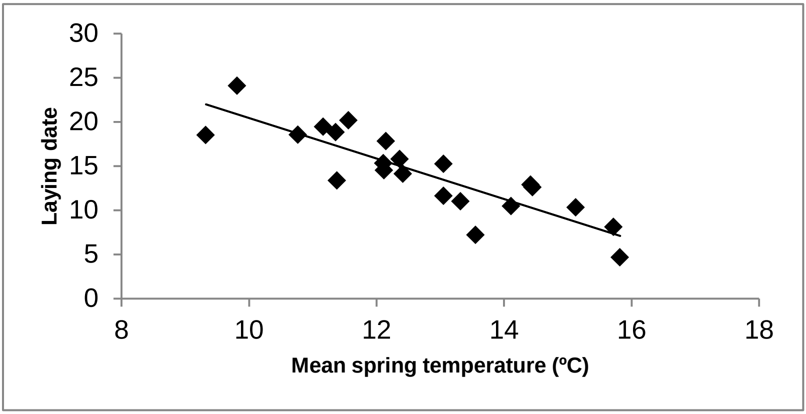

Figure 1. Laying date in relation to mean spring temperature. Variation of laying date (1 = 1 April) of Mediterranean great tits with mean spring (1-15 March) temperature. Regression line is shown.

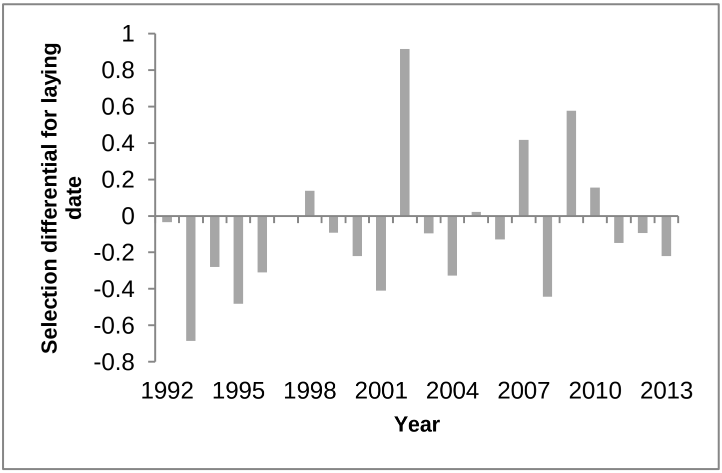

Figure 2. Annual selection differentials for laying date. Variation of standardized selection differentials for laying date in the years of study. Differentials close to zero suggest little selection pressure. Negative values suggest pressure for early laying, and positive values evidence pressure for late laying.

during the analyzed period was negative (-0.08 ± 0.36; range -0.68 - 0.91). The between-year correlation of the residuals of this model was -0.227, suggesting that the strength of the selection for earlier breeding fluctuated from high to low in consecutive years. On the other hand, we found no significant effect of spring temperature on the strength of selection for earlier breeding ($t_{20} = 1.226$, $p = 0.234$).

Of the reproductive stages analyzed, we found that low temperatures acting during the incubation ($r = 0.622$; $t_{20} = 3.555$, $p = 0.002$; Figure 3) and nestling stages ($r = 0.445$; $t_{19} = 2.166$, $p = 0.043$; Figure 4) were associated to

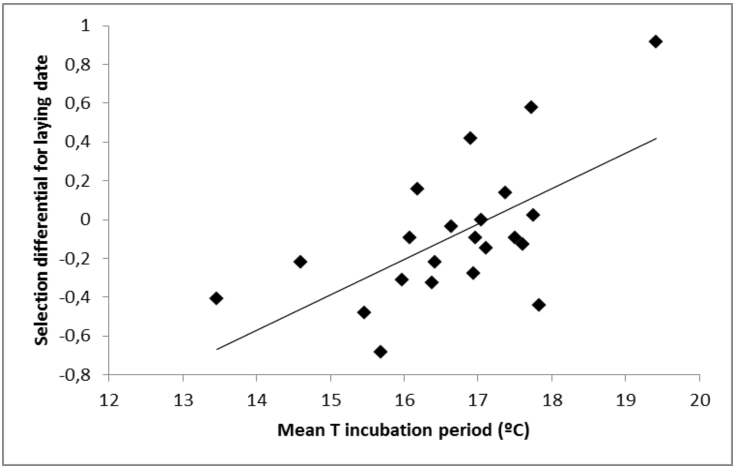

Figure 3. Selection differentials in relation to temperature during the incubation period. Relationship between standardized selection differentials for laying date and mean temperature during the incubation period (i.e., from mean annual laying date + 9 days to mean annual laying date + 17 days). Regression line is shown.

negative selection differentials, thus favoring earlier breeding. As there was no correlation between the temperatures during these two periods (t_{19} = 1.667, p = 0.112), they individually explained 39% and 20% of the variation in selection differentials, respectively. No significant correlations were found between selection differentials and temperatures during the pre-laying, laying, and hatching stages (p > 0.05 in all cases).

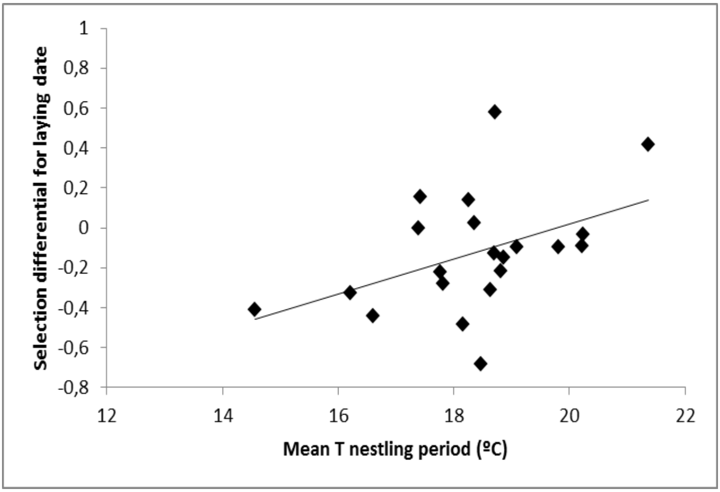

Figure 4. Selection differentials in relation to temperature during the nestling period. Relationship between standardized selection differentials for laying date and mean temperature during the nestling period (i.e., from mean annual laying date + 27 days to mean annual laying date + 35 days). Regression line is shown.

A general linear mixed model with spring temperature as a covariate revealed that individual females bred earlier in warmer years (parameter estimate for spring temperature: -2.01 ± 0.27, $t_{25} = -7.368$, $p < 0.001$). Moreover, the slope of the relationship between within-female changes in laying date and differences in spring temperature for consecutive years (i.e., -1.892 ± 0.370, Figure 5) was similar to that of the relationship between mean laying date and spring temperature at the population level (i.e., -2.292 ± 0.300, Figure 1). This finding suggests that the average population response of great tits to temperature variation was mainly explained by phenotypic plasticity.

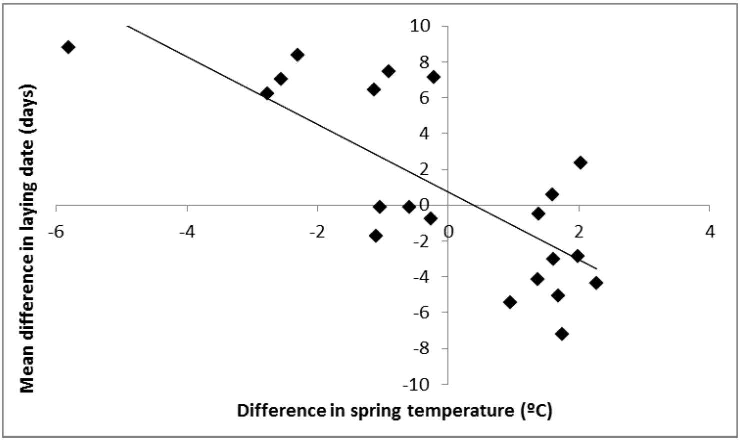

Figure 5. Difference in laying date in relation to difference in spring temperature. Relationship between the mean difference in laying date for females breeding in successive years and the difference in spring temperature in the same pair of years. Regression line is shown.

DISCUSSION

Mean spring temperatures did not increase significantly in the studied period. Likewise, our population showed no significant advancement in laying date, even when selection favored earlier breeding in most years. At the population level, birds began laying earlier in years with warmer spring temperatures, a response that was mainly explained by individual phenotypic plasticity in behavior. Of the reproductive stages analyzed, temperatures during the incubation and nestling periods seemed to have the greatest influence on selection differentials for breeding date, thus showing their importance in determining the timing of breeding in our population.

Our great tit population has not advanced its breeding time during the study period, although the negative selection differentials found in most years suggest that birds should be laying earlier. Moreover, the strength of the selection for early breeding fluctuated across years, suggesting that years with a stronger selection for advanced breeding (i.e., negative selection differentials) were followed by years with a weaker or even positive selection. This lack of response to apparent selection pressure has been shown in other studies (Goodenough 2010; Townsend et al. 2013), and three possible reasons could be put forward for our population. First, delayed breeding could be a case of “conservative bet-hedging” to prevent maladaptation (Slatkin 1974; Olofsson et al. 2009), due to the potential costs of beginning too early. Previous work in our study site has revealed that too-early breeding may be disadvantageous, likely as a result of exposure to unpredictable episodes of environmental instability, which are frequent at the beginning of spring (Rodríguez et al. 2016). This hypothesis would support current ecological theory explaining the existence of counterintuitive phenological responses in temporally-variable environments, where bet-hedging strategies may appear in order to maximize long-term fitness (see review of ecological mechanisms in Johansson et al. 2015). Moreover, in their study with great tits, Lof et al. (2012) modelled the timing of egg-laying in this species and analyzed the effects of environmental variation and fitness curve shape on optimal breeding time. One of their main conclusions was that any process leading to asymmetric fitness curves will lead to adaptive phenological mismatch in a changing environment. Although their model did not account for optimization of geometric fitness (i.e., necessary to consider bet-hedging strategies), it showed how environmental variation can have profound effects on optimal timing of breeding. Unpredictable climate events such as those found in our study site could therefore lead to alternative phenological outcomes.

Second, birds in lower nutritional condition may be unable to breed as early as better-fitted individuals, leading to an overall shift of the population-average laying date to later-than-optimum periods. This would be in accordance with Price et al. (1988), who showed how environmental factors (i.e., inclement weather acting early in the season and declining food resources as the season progresses) could lead to stabilizing selection favoring intermediate breeding dates.

Third, the apparent lack of reaction to selection pressure found here could suggest there being limited genetic variation for breeding time in our population. If this was the case, our sample size may simply not suffice to detect a response, even if it existed. In this sense, although it is generally assumed that the timing of breeding in birds shows heritability (e.g., Sheldon et al. 2003; Nussey et al. 2005), there is evidence suggesting that, at least in some populations, it may be lower than expected (Liedvogel et al. 2012). Further analyses would therefore be needed to provide a heritability estimate of the trait and ascertain whether this could be the case in our population.

Regardless of the reason behind the absence of response to selection differentials, several studies on climate change have shown that the magnitude of phenological changes of species varies geographically, with greater responses found at higher latitudes (see review in Dunn & Winkler 2010). Our results (i.e., limited or no variation in breeding time at a relatively low latitude) would be consistent with these findings.

In their work with black-throated blue warblers (*Setophaga caerulescens*, Gmelin 1789: Passeriformes, Parulidae), Townsend et al. (2013) found no significant effect of spring temperature on selection differentials. Similarly, we have not found this relationship in our great tit population. However, when splitting the breeding period in different sub-intervals, we have found a significant effect of temperatures during two distinct sub-stages on the strength of selection. This result stresses the importance of looking at the effects of temperatures during specific phases of the breeding activities, since some stages could be more sensitive than others, and therefore important relationships might be overlooked when considering temperatures over longer periods.

In our case, temperatures during the incubation stage were strongly correlated with selection differentials for laying date, with low temperatures favoring negative selection differentials (i.e., greater recruitment of early fledglings), in comparison with the pre-laying or laying stages, when a change in temperature had no apparent effect. This result is probably related to the existence of mechanisms enabling females to adjust the timing of breeding and, in particular, the time of egg hatching, in response to local environmental conditions, which differ across the reproductive phases. For example, during the pre-laying and laying stages the scope for adjusting hatching dates is relatively large: nest construction could be delayed or interrupted (Monrós et al. 1998; García-Navas & Sanz 2011), clutch size may vary (Klomp 1970; Cresswell & McCleery 2003) or interruptions in egg laying may occur (Monrós et al. 1998; Cresswell & McCleery 2003). However, once incubation has started, there is little scope to change the hatching date (e.g., van Noordwijk et al. 1995). The same could be said about the nestling period since, once hatched, the growth of the nestlings shows little flexibility and they would experience maximum food demands at an almost fixed period after hatching (about 10 days for great tits; Barba et al. 2009). It follows that, depending on the bird´s ability to strategically control the date of hatching in response to environmental variation, changes in ambient temperature may be compensated and thus may not lead to selection for laying earlier or later. We therefore suggest that the correlations found here between temperatures during the incubation and nestling periods and selection differentials reflect the limited ability of birds to compensate for environmental variation once these processes have been initiated.

The sign of the correlations merits a comment. In their study, van Noordwijk et al. (1995) also found an important effect of temperatures during incubation on selection differentials, although, in their case, the relationship was negative (i.e., with high temperatures favoring negative selection differentials). They did not find a correlation between temperatures during the nestling period and selection differentials. Contrary to most study sites, where tits try to adapt their reproductive phenology to a single seasonal peak of caterpillar availability (Betts 1955; Perrins 1965; Naef-Daenzer & Keller 1999), this prey type only accounts for 24% of the nestling diet in the Spanish orange groves (Barba & Gil-Delgado 1990; Barba et al. 2004). In this habitat, caterpillars (the preferred prey type; see Perrins 1979) are only available during a short time early in the season, and their rapid decrease in abundance forces great tits to switch to moth imagos as their primary diet (Barba & Gil-Delgado 1990; Barba et al. 2004). Consequently, prey availability in our study site shows two peaks: a caterpillar peak very early in the season and a moth peak afterwards. It follows that, in an average year, only very early nestlings might have access to caterpillars, and all the remaining nestlings would be mainly fed on moths. In a relatively cold year, caterpillar development rate may be delayed, and early breeding may be selected for, so as to adjust nestling presence to the relatively late caterpillar availability. On the other hand, in warmer years, great tits would be unable to advance so much their breeding time so as to catch up to the early caterpillar peak, and delayed breeding may be selected for, in order to match the moth peak.

On the other hand, our analyses show that individual plasticity can account for the reported population-level response towards earlier laying in warmer seasons, as birds breeding in successive years have been shown to adjust their laying date in accordance with changing spring temperatures. This result agrees with previous studies in other tit populations (Charmantier et al. 2008; Husby et al. 2010; Porlier et al. 2012), and highlights the importance of phenotypic plasticity as a mean to track environmental fluctuations and contribute to overall population persistence. Considering the high vulnerability of the Mediterranean region to global change in the coming century (Schröter et al. 2005; IPCC 2013), and the overall scarcity of long-term data on breeding parameters available in this area (Potti 2008, 2009), further studies such as the one we present here are needed to decipher the potential of Mediterranean bird populations to respond to a changing environment.

In conclusion, this work (1) provides evidence of lack of response to selection pressure for earlier laying in a Mediterranean great tit population, (2) shows the influence of temperatures during the incubation and nestling stages on selection for breeding date in this population, and (3) suggests the role of individual phenotypic plasticity in explaining population-level responses to temperature variation.

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