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Short and long-term effects of variation in the breeding environment on behaviour and fitness traits in a colonial, cavity nesting raptor

Ph.D. Thesis

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Abstract

The heterogeneity of breeding environment leads the evolution of different behavioural strategies that individuals undertake to guarantee their fitness and survival. Moreover, the cyclic nature of animal life determines that each set of decision made by individuals during reproduction could severely affect behaviour and fitness traits during their successive stages of life cycle. Among different species that have been studied to investigate these relationships, migratory birds are optimal model species, since the rigid scheduling of their life cycle exacerbates the associations established among breeding environmental variations, fitness and behaviour. In the present thesis, I investigated the possible effects of variation in the breeding environment on fitness and behavioural traits of a colonial, migratory, avian species the lesser kestrel (*Falco naumanni*), assessing possible effects during two different temporal scales: in the current breeding season (short-term effects) and/or during successive life stages (long-term effects).

In the first part, I analysed short-term effects of breeding environmental variations, observing whether nest-site quality and food resource abundancy and distribution could affect fitness and behaviour of lesser kestrel. Firstly, I have investigated the strategy implemented by lesser kestrel during nest-site selection, and whether individual differences in the nest-site preference could affect fitness. Secondly, I have assessed whether food resource abundancy could limit lesser kestrel's breeding performance and whether the patchy prey distribution in the breeding environment could negatively affect their foraging behaviour and fitness.

In the second part, I have investigated whether variation in breeding environment could affect lesser kestrel during successive stages of the life cycle. I therefore analysed whether breeding area continental distribution affects migratory behaviour and strategy of five European populations of lesser kestrel.

The overall results reported in this thesis suggested that lesser kestrel suffers variations in the breeding environment that occur during the current breeding season, indeed I have assessed that individuals rely on public information gathered from the environment to choose the most suitable nest-site and to identify optimal foraging areas. Foraging strategies adopted by lesser kestrel during the breeding season have evolved to cope with the heterogeneous prey distribution and to limit intraspecific competition among individuals of the same and different colonies. Moreover, food resource abundancy mediates individual fitness according to parent body condition. Finally, despite the absence of any direct effects of lesser kestrel migratory connectivity on individual fitness, I observed that the continental distribution of the breeding site reflects the location of the non-breeding areas in Africa. This evidence highlighted that European populations remain separated during the African stay and thus are subjected to different abiotic and biotic traits with possible profound effects on population dynamics.

Riassunto

L'ambiente riproduttivo di molti organismi è caratterizzato da un'elevata eterogeneità, che ha spinto all'evoluzione di diverse strategie comportamentali adottate dagli individui al fine di garantire la propria *fitness* e sopravvivenza. Inoltre, le fasi vitali degli organismi possiedono una decorrenza ciclica, dalla cui interdipendenza ne consegue che qualunque decisione e azione intrapresa dagli individui durante il periodo riproduttivo possa comportare importanti conseguenze sul comportamento, sul successo riproduttivo o sulla loro stessa sopravvivenza durante le successive fasi del ciclo vitale. Tra le diverse specie protagoniste degli studi che hanno indagato queste relazioni, gli uccelli migratori rappresentano un'ottima specie modello grazie alla rigida tempistica del loro ciclo vitale, che esacerba le associazioni presenti tra ambiente riproduttivo, comportamento e *fitness* degli individui. Pertanto, nella presente tesi ho indagato i potenziali effetti delle variazioni dell'ambiente riproduttivo sul comportamento e le *performance* riproduttive di un uccello migratore coloniale, il grillaio (*Falco naumanni*). Questi sono stati indagati con due differenti scale temporali, indagando le conseguenze dell'eterogeneità ambientale nel breve e nel lungo termine.

Nella prima parte ho analizzato gli effetti a breve termine, osservando se e come la qualità del sito di nidificazione e l'abbondanza e la disponibilità di risorse nutritive possano avere ripercussioni sulla *fitness* e il comportamento del grillaio. Inizialmente ho indagato la strategia comportamentale seguita dal grillaio durante il processo di selezione del sito di nidificazione, analizzando come diverse preferenze manifestate dagli individui possano avere conseguenze sul loro successo riproduttivo. Successivamente ho indagato come l'abbondanza di risorse nutritive disponibili nel territorio circostante alle colonie sia un fattore limitante per la *fitness* degli individui. Ho inoltre osservato se tale particolare distribuzione abbia comportato l'implementazione di diverse strategie comportamentali volte al foraggiamento.

Nella seconda parte della tesi ho indagato se le variazioni nell'ambiente riproduttivo abbiano ripercussioni a lungo termine durante le successive fasi del ciclo vitale del grillaio. Pertanto, ho osservato se la posizione geografica delle aree riproduttive abbia o meno possibili conseguenze

sulla strategia migratoria seguita dai grillai alla fine del periodo riproduttivo, influenzando quindi la rotta migratoria, la scelta dell’area di svernamento e, più in generale, la qualità del periodo di svernamento, con possibili conseguenze sui futuri tentativi di riproduzione.

I risultati riportati dei diversi studi suggeriscono come il grillaio, come osservato in altre specie di uccelli migratori, sia soggetto alle variazioni ambientali durante il periodo riproduttivo. Gli individui basano infatti la scelta del sito di nidificazione e delle aree di foraggiamento sulle informazioni sociali raccolte nell’ambiente e dal comportamento dei propri conspecifici. Inoltre, le strategie di foraggiamento osservate nel grillaio sono il risultato di un processo evolutivo volto a limitare la competizione intraspecifica tra individui appartenenti alla stessa e a diverse colonie, a fronte dell’eterogeneità della distribuzione delle prede nell’ambiente riproduttivo. In aggiunta, ho osservato come l’abbondanza di risorse nutritive limiti le performance riproduttive del grillaio che, pertanto, modula il proprio investimento nella riproduzione basandosi sull’abbondanza di risorse nutritive disponibili durante tutto il periodo riproduttivo.

Per ciò che concerne i potenziali effetti a lungo termine, i risultati dei miei studi suggeriscono che la distribuzione geografica dei siti riproduttivi del grillaio influenza la posizione geografica delle aree di svernamento, occupate successivamente nel continente africano. Le diverse popolazioni europee, distinte durante la riproduzione, rimangono spazialmente segregate durante il periodo non riproduttivo e sono quindi soggette a differenti fattori biotici e abiotici, i quali potrebbero potenzialmente costituire la base delle differenze nella dinamica delle popolazioni europee.

Chapter 1.

General introduction and outline of the study

General introduction

The cyclic nature of life stages in organisms reflects the predictable and seasonal variation in the environment. To cope with such variation, organisms evolved several morphological and physiological changes that individuals carry out to arrange successfully each life-history phase during proper environmental conditions (Willmer et al. 2009). However, since virtually no environment on Earth is static, even within the same season, animals have evolved effective strategies to cope with unpredictable variations that characterized the environmental heterogeneity (Wingfield 2006).

Among the different stages that individuals experience during their life cycle, reproduction represents one of the most important phases due to the high-energy demands imposed to parents, spanning from breeding-site selection (Anderson and Harwood 1985; Martin and Roper 1988; Gavashelishvili and McGrady 2006) and territory defence (Nilsson 1984; Breitburg 1987) to gamete production (Sturmey et al. 2009) and offspring rearing (Bercovitch et al. 1988). Thus, fluctuations of different environmental conditions occurring during the breeding season have important implications on individual behaviour and current and future fitness (Willmer et al. 2009). During the breeding season, an individual could have to cope with sudden events, such as an attack by a predator or a dominant conspecific; otherwise, individuals must be ready to respond to slower environmental variations such as inclement weather conditions or changes in food resources availability. For terrestrial vertebrates, for example, storms are potentially disruptive. Prolonged heavy rains and low temperatures could reduce food resources availability, decreasing adult body condition and offspring food provisioning rate with possible reproductive failure (Kostrzewa and Kostrzewa 1990).

The close interdependency that exists among the life cycle stages implies that the set of choices undertaken by animals during breeding season can have profound consequences on the current (short-term effect) or future (long-term effect) breeding behaviour and performance (Harrison 2011).

Among different *taxa*, birds have been the subjects of a large number of studies focusing on short- and long-term effects of breeding environment variation on individual behaviour and fitness. This is especially the case for migratory birds whose time to exploit life stages is limited by restricted time windows (Newton 2008). Below, I reported a few instances of how major variations in environmental traits could affect fitness and behaviour of migratory bird species.

Breeding environment variations: short-term effects

A number of breeding environment variables could affect individual behaviour and fitness, and their effects can be observed during the current breeding event. Reproductive success in birds is influenced by a variety of environmental factors (Newton 1989); one of the major traits influencing breeding performance and behaviour in migratory birds is the quality of the nest-site. Indeed, nest-site quality affects individual fitness, implying that parents should be highly selective when making decisions about where to lay their eggs and rear their offspring (Refsnider and Janzen 2010). Birds therefore sample the environment to gather information for choosing the optimal breeding site, basing their preference on different types of direct or indirect information including presence of parasites (Rosenheim 1988) and/or conspecific behaviour and reproductive success ("public information"; Valone and Templeton 2002). Hence, there are a set of indirect cues of conspecific reproduction, such as tracks or signs of reproductive activity occurring in the past, used by breeders to choose the optimal nest-site. In cavity-nesting species, the presence of old nest material within suitable nest cavities (review in Mazgajski 2007) contains information about previous breeding activity. Thus, cavities containing such material may be preferred as they may be perceived as being more suitable than similar cavities where no sign of previous reproduction is evident (Brown and Shine 2005; Sumasgutner et al. 2014). At the same time, in species that do not add any material to line their nest (i.e. cavity-nester), the presence of organic material from previous breeding events may be a further cue to nest-site quality because it may contribute to increase thermal insulation and reduce egg heat loss (Hilton et al. 2004; Mazgajski 2007; Mainwaring et al. 2014), potentially improving incubation efficiency. For instance, experimental removal of old nest material decreased nestbox occupancy in the subsequent breeding season in burrowing owls *Athene cunicularia*, with birds returning from migration avoiding cleaned nestboxes (Riding and Belthoff 2015). Although public information is generally acquired from the activity of conspecific individuals, cues could also be provided from individuals of other species. The cues and signals produced by other species are thus part of the public information available to many bird species, through which individuals are able to assess habitat quality, the presence of resources or potential risks (Danching et al. 2004; Valone 2007). However, despite the potential benefits of choosing cavities with old nest material, some species/populations avoid breeding in previously used cavities since the organic substrate could be a favourable ground for the development of several nest-dwelling and pathogens species (e.g. Merino and Potti 1995; Mazgajski 2003; review in Mazgajski 2007).

Food resources availability represents another fundamental trait affecting fitness and behaviour. Since birds are exposed to fluctuations of ecological conditions, food is often limited during the breeding season (Lack 1954). Variation in food abundance and availability are commonly exploited by individuals as cues to modify their behaviour to contingent ecological conditions, providing information on when and where best to breed and how much resources to invest in producing and raising offspring to maximise fitness (Martin 1987). Whenever breeding individuals are exposed to poor environmental conditions, resulting in food limitation, parents are expected to trade self-maintenance against reproduction and offspring provisioning, with broad implications for their survival and breeding success (Lack 1966, Martin 1987). A number of different studies have experimentally investigated the importance of food limitation during reproduction manipulating food resources availability to breeders, assessing variation in reproductive investment and output of individuals receiving the extra food compared to unfed controls (reviews in Martin 1987, Boutin 1990). A considerable part of these studies has demonstrated that individuals receiving extra food generally achieve fitness benefits relative to controls (Ruffino et al. 2014). These benefits include advancing egg laying (Meijer and Drent 1999, Aparicio and Bonal 2002), laying heavier eggs and/or larger clutches (Wiebe and Bortolotti 1995, Korpimäki and Wiehn 1998, Karell et al. 2008, Saino et al. 2010), and enjoying improved nestling growth and survival (Dewey and Kennedy 2001, Hipkiss et al. 2002). The positive fitness effects of extra food may not be limited to breeding output. Extra food could positively affect parental condition, with food-supplemented individuals sparing energy resources that are otherwise required for self-maintenance and offspring provisioning, or using directly the extra food to enhance their survival prospects. Extra food may improve parental body condition (Garcia et al. 1993, Schoech 1996, Cucco and Malacarne 1997, Dewey and Kennedy 2001) and physiological state, in terms of e.g. body fat content, immune condition or oxidative status (Schoech 1996, Karell et al. 2008, Alan and McWilliams 2013, Fletcher et al. 2013, Giordano et al. 2015).

In addition, the natural distribution of food resources in the breeding area could affect behaviour and fitness of birds. It has been observed that, in habitats where food resource distribution is patchy, ephemeral and unpredictable, individuals gather information about the location of profitable foraging areas from the recent experience of conspecifics (Kuhn et al.2014; Lascelles et al. 2016; Cecere et al. 2018). Transfer of such social information may occur at the colony site, where individuals can actively (as proposed by the "Information Centre Hypothesis"; Ward and Zahavi 1973) or inadvertently (Lachmann et al. 2000; Richner and Danchin 2001) share information on foraging locations. Moreover, social information may be shared outside the colony site, which may occur by means of so-called "local enhancement" processes occurring at the foraging grounds, whereby individuals searching for food are attracted to feeding aggregations of other individuals (Machovsky-Capuska et al. 2014). Individuals from different colonies often show colony-specific and well-defined foraging areas, which do not overlap with those belonging to neighbouring conspecific colonies (Wanless and Harris 1993; Grémillet et al. 2004; Masello et al. 2010; Wakefield et al. 2011). As argued by the "diplomacy" hypothesis (Grémillet et al. 2004), spatial segregation of foraging individuals from different colonies may mitigate intraspecific competition for resources between conspecifics breeding in different colonies. By foraging in spatially segregated areas, conspecifics from different colonies may thus “diplomatically” avoid interference competition for food resources (Grémillet et al. 2004). For instance, it has been shown that inter-colony competition could be one of the main factors driving the at-sea distribution of pelagic foraging birds (Cecere et al. 2015). Both local enhancement and the transfer of information at the colony site have been hypothesized to be the most important mechanisms generating and maintaining specific foraging areas exploited by individuals belonging to the same colony (Wakefield et al. 2013). During the breeding period, colonial species are central-place foragers (Orians and Pearson 1979), with individuals foraging outside the colony, sometimes very far from the breeding site, and consistently returning to the colony (the “central place”) to egg incubation or nestling rearing. The progressive depletion of foraging areas around the breeding sites leads individuals to both increase foraging ranges (the “Ashmole’s halo” effect; Ashmole 1963) and, in the case of neighbouring colonies, to avoid moving towards adjacent colonies when searching for food. This, in turn, may generate and/or reinforce spatial segregation of foraging areas among individuals from neighbouring colonies (Wakefield et al. 2013). Moreover, differences in foraging areas and dietary preferences, aimed to reduce interspecific competition, could occurred also at the individual level (e.g. Woo et al. 2008; Ceia and Ramos 2015; Camprasse et al. 2017). In particular, dietary preferences may originate from spatio-temporal individual variation in foraging behaviour. For example, imperial shags *Phalacrocorax atriceps* are highly consistent within individuals in the maximum distances they reached from the shore and the colony, as well as in the time invested in flight and diving, both within a single breeding season and across years (Harris et al. 2014). In addition, individual differences in foraging behaviour can be conceptually considered as personality differences whenever these are consistent across environmental contexts (Patrick eta al. 2014).

Breeding environment variations: long-term effects

Variations in breeding environment could determine long-term consequences on individual fitness and behaviour during subsequent phases of the life cycle. Indeed, since every stage of the life cycle virtually depends on previous ones, any event occurred during one phase could provoke consequences during successive stages of the life cycle (for example the so-called “carry-over effect”; Harrison et al. 2011). Thus, any event occurred whenever in a given season influence the performance of an individual in the following season(s) or life cycle stages. For instance, two of the major drivers of long-term effects occurrence in migratory birds are the extent to which individuals have access to or are able to utilize food resources and the energy investment for offspring rearing (Norris 2005; Regular et al. 2014; Fayet et al. 2016). Several correlative and experimental studies underlined how an unusual increase in energy investment during reproduction could severely affect parent conditions with negative effects on post-breeding phases, such as autumn and spring migration timing, migrated distance and the ability to reach optimal non-breeding areas (see for example Marra et al. 1998; Newton 2008; Catry et al. 2013; Fayet et al. 2016). An experimental study conducted on the seabird Cory’s Shearwater (*Calonectris diomedea*) shows overall evidence that energetic and time-dependent costs of reproduction imply consequences on non-breeding and future breeding stages in a migratory bird. By removing nestlings at an early rearing stage, Catry et al. (2013) reduced parental investment of breeders during breeding season. Hence, manipulated individuals started autumn migration ca. 30 days sooner and, at the start of the following breeding season, returned to the colony ca. 20 days earlier, compared to controls (breeders that raised offspring until fledging). Moreover, late arrival individuals in the following year were more likely to fail the reproductive attempt (Catry et al. 2013).

Moreover, with regard to the migratory behaviour of individuals, the geographical distribution of the breeding areas could affect the migratory strategy adopted by individuals to entail the migratory journey, with consequences on migratory route and on the geographical distribution occupied in the non-breeding area (Marra et al. 2006). The migratory connectivity describes how individuals are spatially connected between seasons of the annual cycle (Webster et al. 2002; Marra et al. 2006). A weak connectivity between breeding and non-breeding areas occurs when individuals from every breeding population spread through several non-breeding grounds, therefore mixing together. Strong connectivity instead occurs when individuals from one breeding population move to a specific non-breeding location, so that the inter-population separation of non-breeding areas matches the inter-population separation of breeding areas (Webster et al. 2002). Long-term effects on individuals may depend on the strength of connectivity, particularly if changes in habitat quality occur (Webster and Marra 2005). For instance, declines in some independent breeding populations of Asian and North American migratory birds have been linked to quality decrease in Australian habitats exploited during the non-breeding period (Iwamura et al. 2013). Hence, it is crucial to understand if the geographical distribution in the breeding areas could affect where individuals travel and winter and what degree of individuals from a certain population used the same migratory route or migrate to the same non-breeding areas (i.e. how and to what extent breeding and non-breeding populations are connected; Marra et al. 1998; Webster et al. 2002; Webster and Marra 2005; Newton 2008; Trierweiler et al. 2014). When migratory connectivity is strong, individuals of different populations are exposed to different biotic and abiotic conditions during both breeding and non-breeding period. Otherwise, when migratory connectivity is weak, breeders share the same environmental conditions in non-breeding areas (Newton 2008). These two different circumstances entail variations in population density, with important consequences on successive population dynamics (Trierweiler et al. 2014). Thus, understanding the year-round geographical ranges of migratory species could be crucial to develop long-term conservation plans as well as for understanding other facets of the basic ecology and evolution of migratory species.

Outline of the study

The present thesis deals with short and long-term effects on behaviour and fitness traits mediated by breeding environment variations in the lesser kestrel (*Falco naumanni*). By means of both experimental and correlative approaches, I investigated how lesser kestrel deals with environmental variations in the breeding season and how these variations affect individual behaviour and fitness, both on a short and long-term temporal scale.

The present thesis is divided in two parts. In the first part (**Chapters 2 to 5**) I investigated the possible short-term effects of breeding environment variations on breeding performance and foraging behaviour, from a variety of perspectives, during the current breeding season. Firstly, I investigated the effects of nest-site selection on breeding performance (**Chapter 2**). Lesser kestrels, as other species, sample the environment to gather information about the breeding site for choosing the most suitable nest-site. Among the large number of information that individuals could evaluate to decide where to settle, lesser kestrels seem to exploit tracks and sings of reproductive activities occurred in the past, by observing the presence, inside the nest, of old organic material (Negro and Hiraldo 1993; Mazgajski 2007). Being a cavity-nesting species, the lesser kestrel lays eggs in holes and cavities available in the breeding site and individuals, during different breeding seasons, tend to reuse nests, which results in a compact organic layer that accumulates inside the nest. To assess whether the presence of an organic layer inside the nest is an important cue in lesser kestrel nest-site selection, I manipulated the nest substrate within nestboxes before the beginning of the breeding season. I performed a nest-site choice experiment whereby breeding pairs had the opportunity to select between two nestboxes settled as a dyad: one nestbox was lined with a clean gravel layer while the close paired nestbox was within an organic layer of the same thick from previous breeding attempts. According to a previous study conducted on a Spanish population (Negro and Hiraldo 1993; see also Sumasgutner et al. 2014 for a study conducted on a closely species), I expected that lesser kestrel parents preferred nests with old organic material from previous breeding attempts.

Moreover, by means of a correlative approach, I also evaluated possible effects of old vs. clean material on breeding performance.

In **Chapter 3**, I evaluated the consequences of variation of another critical trait that could affect breeding behaviour and performance: the abundance of food resources. Food is a key factor modulating life-history traits, and poor environmental conditions, resulting in limited food supplies, affect fitness and force parents to balance energy investment for self-maintaining and offspring rearing. To investigate possible effects of food availability on body conditions and breeding performance, I conducted a food supplementation experiment by means of which I provided extra food to a subset of individuals throughout the breeding season, from egg laying to early nestling rearing, while another group of unfed individuals was used as a control. I therefore assessed the effect of extra food on several short-term fitness components including adult body condition and oxidative status, egg mass and nestling growth. I expected an overall positive effect of extra food provisioning on adult conditions and breeding performance.

In Chapters 4 and 5 I examined the effects of environmental variability on lesser kestrel foraging behaviour. Lesser kestrels forage in farmland habitats surrounding colony sites (Catry et al. 2013), where the environment varies continuously during the breeding season because of seasonal processes and agricultural practices. Prey distribution is thus patchily distributed, highly ephemeral and unpredictable. Specifically, in **Chapter 4** we investigated, by means of GPS tracking devices, the spatial distribution of home ranges of lesser kestrel belonging to different but close colonies in two geographically distinct populations (Apulia and Sicily). Since the lesser kestrel is a colonial bird that often forages in groups (Cramp 1998), we expected that lesser kestrels should rely on social information acquired by other conspecific individuals at the colony (transfer of information at the colony site) or during the search of food (local enhancement) to target ephemeral productive foraging areas. Moreover, since the share of social information is the most important mechanism involved in maintaining specific foraging areas among individuals of the same colony (Wanless and Harris 1993; Grémillet et al. 2004; Masello et al. 2010; Wakefield et al. 2011), we expected a spatial segregation between home ranges of individuals belonging to different but close colonies.

In **Chapter 5**, we investigated the consequences of breeding environment heterogeneity on lesser kestrel prey searching behaviour. Consistent inter-individual differences in foraging tactics have been documented in several avian species (Woo et al. 2008; Ceia and Ramos 2015; Camprasse et al. 2017). These differences in behaviour may reduce the ecological niche overlap, resulting in a mitigation of intraspecific competition. We therefore analysed variations in individual foraging tactics according to weather conditions (i.e. gradients in solar radiation, rain and wind), sex and exploited habitat type. Information on prey searching behaviour was collected by means of GPS-accelerometer data logger. We expected foraging trips to be characterized by two main behavioural modes: specifically, individuals should mainly search for prey in flight (widely foraging tactic) whenever weather conditions are particularly favourable to soaring-gliding (Hernandez-Pliego et al. 2017) and with wind assistance (tailwind or crosswind) at departure from the nest site (Mellone et al. 2012; Klaassen et al. 2010). Under opposite weather conditions, we expected that birds mainly adopt a more static foraging tactic (sit-and-wait tactic), characterized by prolonged perching periods waiting for prey detection. According to previous studies analysing individual differences in movement patterns of colonial animals (e.g. Call et al. 2008, Votier et al. 2010, Patrick et al. 2013), we expected that individuals consistently differ in their tendency to adopt a given foraging tactic, but we also predicted such differences to be consistent across weather condition gradients. Finally, we predicted widely foraging tactic to be associated with higher energy expenditure compared to sit and wait one (Nadjafzadeh et al. 2016).

In the second part of my thesis, I investigated long-term effects of breeding environment on migratory phenology and non-breeding behaviour. In particular, in the **Chapter 6**, we conducted a large-scale study on the effects of geographic distribution of breeding areas on migratory strategies and wintering grounds in four European population of lesser kestrels. By means of a multi-population approach, we combined all the available data of lesser kestrel migration, obtained with different tracking devices (GLS and GPS), belonging to Spanish, Italian, Greek and Bulgarian populations. We investigated whether the continental configuration in the breeding grounds could have a role in determining the evolution of different populations' migration strategies. As observed in other long-distance Afro-Palearctic migratory bird species (Symes and Woodborne 2010; Trierweiler et al. 2014), we expected lesser kestrel populations that are spatially separated during the breeding season to share common non-breeding grounds and thus to show a weak migratory connectivity. Whether lesser kestrel form different population share the same overwintering areas, they could be affected to similar biotic and abiotic conditions, a circumstance that could have profound effects on population dynamics of this species.

To conclude, in the **Chapter 7** I present an overview of the main findings emerging from the studies reported in my thesis.

Part 1.

Short-term effects

Chapter 2.

Home, dirty home: effect of old nest material on nest-site selection and breeding performance in a cavity-nesting raptor

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Home, dirty home: effect of old nest material on nest-site selection and breeding performance in a cavity-nesting raptor

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Abstract

The quality of a breeding site may have major fitness consequences. A fundamental step to understanding the process of nest-site selection is the identification of the information individuals use to choose high-quality nest sites. For secondary cavity-nesting bird species that do not add nest lining material, organic remains (faeces, pellets) accumulated inside nest cavities during previous breeding events may be a cue for high-quality nest-sites, as they contain information about past successful breeding and may improve thermal insulation of eggs during incubation. However, cavities in which breeding was successful might also contain more nest-dwelling ectoparasites than unoccupied cavities, offering an incentive for prospective parents to avoid them. We exposed breeding cavity-nesting lesser kestrels (*Falco naumanni*) to nestbox dyads consisting of a dirty (with a thick layer of organic substrate) and a clean nestbox (without organic material). Dirty nestboxes were strongly preferred, being occupied earlier and more frequently than clean ones. Hatching success in dirty nestboxes was significantly higher than in clean ones, suggesting a positive effect of organic nest material on incubation efficiency, while nestbox dirtiness did not significantly affect clutch and brood size. Nestlings from dirty nestboxes had significantly higher ectoparasite load than those from clean nestboxes soon after egg hatching, but this difference was not evident a few days later. Nest substrate did not significantly affect nestling growth. We concluded that nest substrate is a key driver of nest-site choice in lesser kestrels, although the adaptive value of such a strong preference appears elusive and may be context-dependent.

Key words: *Carnus hemapterus*, ectoparasites, nestbox, nest substrate, nest-site selection

Breeding and oviposition site quality affects individual fitness, implying that parents should be highly selective when making decisions about where to lay their eggs and rear their offspring (Refsnider and Janzen 2010). As a consequence, animals continuously sample the environment to gather useful information for choosing the optimal breeding site. The type of information that animals can evaluate to decide where to settle and breed may be diverse, including nest substrate quality (e.g. in species where it provides direct fitness benefits, such as Lepidoptera; review in Renwick and Chew 1994), conspecific behavior, reproductive success (the so-called "public information"; Valone and Templeton 2002), perceived predation risk (Eggers et al. 2006), presence of parasites

(Rosenheim 1988), or a combination of those factors. Nest-site choice may also be context-dependent, with individuals choosing low-quality nest-sites if no better options are available in the surroundings (Stanback and Rockwell 2003).

Cues used by prospecting individuals for choosing their breeding site may be based on direct observations of conspecific presence, which may generate territorial aggregations ("conspecific attraction"; Stamps 1988), or conspecific behavior, such as offspring feeding effort by parents, which is expected to provide reliable information about breeding patch quality (Doligez et al. 2002; Pärt and Doligez 2003; Ward 2005). Moreover, prospecting individuals may directly assess conspecifics' breeding success (quantity/condition of offspring) in a given season and use this information to decide where to settle and breed subsequently (Boulinier and Danchin 1997).

Prospecting individuals may also exploit indirect cues of conspecific reproduction, such as tracks or signs of reproductive activity occurring in the past. In birds, these may include the density of old nests (e.g., Erckmann et al. 1990; Gergely et al. 2009; Ringhofer and Hasegawa 2014), or, in cavity-nesting species, the presence of old nest material within suitable nest cavities (review in Mazgajski 2007; see also Brown and Shine 2005 for a study of reptiles). The presence of old nest material in nest cavities (nest lining material, faeces, pellets, prey remains, feathers, etc.) does in fact contain information about previous breeding activity: cavities containing such material may be preferred as they may be perceived as being more suitable than similar cavities where no sign of previous reproduction is evident (Brown and Shine 2005; Sumasgutner et al. 2014). At the same time, in species that do not add any material to line their nest, the presence of organic material from previous breeding events may be a further cue to nest-site quality because it may contribute to increase thermal insulation and reduce egg heat loss (Hilton et al. 2004; Mazgajski 2007; Mainwaring et al. 2014), potentially improving incubation efficiency.

In line with the above, experimental removal of old nest material decreased nestbox occupancy in the subsequent breeding season in burrowing owls *Athene cunicularia*, with birds returning from migration avoiding cleaned nestboxes (Riding and Belthoff 2015). Similarly, female Eurasian kestrels *Falco tinnunculus* laid eggs later in experimentally cleaned nestboxes compared to uncleaned ones, indicating a preference for old nest material (Sumasgutner et al. 2014). A preference for nestboxes with old nest material was observed also in some passerine species, such as the pied flycatcher *Ficedula hypoleuca* (Orell et al. 1993; Mappes et al. 1994; Olsson and Allander 1995), the house wren *Troglodytes aedon* (Thompson and Neill 1991), and the eastern bluebird *Sialia sialis* (Davies et al. 1994).

In spite of the potential benefits of choosing cavities with old nest material, some species/populations avoid breeding in previously used cavities (e.g. Merino and Potti 1995; Mazgajski 2003; review in Mazgajski 2007). Breeding in previously used cavities may indeed entail non-trivial costs. Nests containing old nest material may be subjected to increased predation risk due to predators memorizing nest positions (e.g. Sonerud 1985; Nilsson et al. 1991). Importantly, organic nest material is a highly favourable ground for the development of nest-dwelling ectoparasites and pathogens (Rendell and Verbeek 1996). Nest-dwelling parasites infest adults and especially nestlings, eventually impairing individual growth, condition and fitness (Møller et al. 1990; Martínez et al. 2011). Nest parasites can impair fitness either directly (e.g. in the case of blood sucking by haematophagous species; e.g. Heylen and Matthysen 2008; Tomás et al. 2008) or indirectly, transmitting bacterial or viral pathogens and spreading disease (Møller et al. 1990).

On the whole, although some studies suggest the preference or avoidance of previously used nest cavities (see above), nest-site choice in secondary cavity-nesters appears rather insensitive to the presence of old nest material, with several studies not reporting any clear preference pattern (e.g., Olsson and Allander 1995; Tomás et al. 2007; review in Mazgajski 2007). Furthermore, the adaptive value of breeding in previously used versus non-used nest cavities has yet to be elucidated. In the majority of studies conducted so far, no significant impact of the presence of old nest material was found on clutch size, fledging success or nestling condition (review in Mazgajski 2007). Statistically significant fitness effects (mostly negative) of breeding in cavities with old nest material have been reported only occasionally (e.g., Tomás et al. 2007; González-Braojos et al. 2012; review in Mazgajski 2007).

Lesser kestrels *Falco naumanni* appear to make wide use of public, social, and environmental information for dispersal, colony-site settlement decisions, and nest-site selection, with breeding success of conspecifics being an important cue (Negro and Hiraldo 1993; Serrano et al. 2001, 2003; Aparicio et al. 2007). In lesser kestrel colonies, most successful breeding attempts take place in previously occupied cavities, which are also occupied earlier compared to seldom used cavities (Negro and Hiraldo 1993). However, to our knowledge, no study has experimentally addressed whether the presence of old nest material is used as a cue for choosing specific nest-sites within a breeding colony. We performed a nestbox choice experiment whereby breeding pairs had the opportunity to select either a nestbox without organic nest material (clean nestbox) or a paired nestbox with a thick organic layer from previous nesting attempts (dirty nestbox). Based on previous studies carried out in this species (Negro and Hiraldo 1993) and in the closely related Eurasian kestrel (Sumasgutner et al. 2014), we expected a preference for settling in dirty nestboxes. In addition, by exploiting a larger sample of unpaired dirty and clean nestboxes and adopting a correlative approach, we assessed whether breeding in dirty versus clean nestboxes was associated with variation in breeding performance and nestlings' mortality, ectoparasite load, and early growth patterns.

Materials and Methods

Study species, study area and general methods

The lesser kestrel is a small (~120 g), colonial breeding, Afro-Palearctic migrant raptor. European individuals reach breeding areas in February/March, and start laying eggs between late April and early May. Females lay 3–5 eggs (single brooded), which are incubated for ~30 days. Nestlings fledge when ~40 days old. Being a secondary cavity-nester, the lesser kestrel does not build its own cavity: it breeds in holes and cavities in rocks, ruins, roof tiles of buildings in urban areas or isolated abandoned farmhouses in the countryside, and it does not add any nest lining material (Cramp 1998). However, it readily settles in nest cavities containing an organic substrate resulting from previous breeding attempts, similarly to other secondary cavity-nesters (Cramp 1998; Negro and Hiraldo 1993).

The study was carried out during April–July 2016 in the city of Matera (Southern Italy; 40°67' N, 16°60' E), hosting a large colony of ~1,000 lesser kestrel pairs (La Gioia et al. 2017). Several hundreds of nestboxes were deployed in 2008–2010 within the framework of the LIFE Project "Rapaci Lucani" (LIFE05NAT/IT/00009), so that presently an unknown (but likely large) fraction of pairs breeds in nestboxes. We relied on 175 nestboxes that were placed on the roof terraces of two large buildings located ~500 m apart in the city center. Nestboxes were made by a hollow refractory brick (300 × 300 × 370 mm external size) closed by two wooden panels (300 × 300 × 20 mm), the frontal one with an entrance hole of 65 mm diameter. Ventilation of the nest chamber was provided by 9 small holes (~10 mm) on the wood panels. The front panel could be easily opened for nest inspection.

Upon deployment, the floor of all nestboxes was coated with a layer of sand and fine gravel to increase insulation towards the cement brick and reduce the probability of egg breakage during nest inspection or egg turning by the female.

In February 2016, before arrival of lesser kestrels at the colony site, nestboxes were organized in "dyads" of clean and dirty nestboxes ($N = 40$ dyads, see below) and "unpaired" nestboxes [24 old (dirty) nestboxes (all of which had been used for breeding and roosting in previous years) and 71 new (clean) nestboxes (deployed in February 2016 and never previously used by lesser kestrels)]. Both dyads and unpaired (dirty and clean) nestboxes were randomly positioned along the entire perimeter of each terrace, at a minimum distance of ~2 m from each other. Old nestboxes had never been cleaned after their original deployment (2008–2010). Hence, most old nestboxes had a thick (~5 cm), hard coating of organic material deriving from previous breeding events spread over the floor of the nestbox (see also section "Assessment of nest-site preference"). The position of all old nestboxes was randomly shuffled in February 2016 to accomodate deployment of new clean nestboxes and to form dyads, as well as to avoid nest recognition bias (see section "Assessment of nest-site preference").

All nestboxes were regularly checked throughout the breeding season to record breeding bird performance. Nestboxes were checked until the oldest nestling in the brood was ~16 days old (we refrained from checking nestboxes after that age because nestlings started wandering outside the nest and freely moved on the terraces, making monitoring difficult and increasing the risk of inducing premature fledging); over this period, each nestbox was checked five times (i.e., five monitoring sessions), with monitoring sessions occurring at an average of 0.8 (range 0–3), 3.0 (2–5), 5.3 (4–9), 7.9 (7–11), and 16.0 (14–18) days from hatching of the first egg in a nestbox, respectively.

Upon hatching, nestlings were individually marked with different combinations of small black dots on the down of the nape using a non-toxic black permanent marker, then ringed with metal rings when ~10 days old. Nestling body mass (accuracy of 0.1 g using an electronic scale) and ectoparasite load (see below) were recorded from the first to the fourth monitoring session, while tarsus (accuracy 0.1 mm with dial calliper) and forearm length we report in this study (accuracy 1 mm with a ruler) were recorded at the fourth monitoring session only. At the fourth monitoring session, a small (~200 μl) blood sample was collected in capillary tubes by puncturing the brachial vein with sterile needles in order to determine nestling sex. This was achieved by means of polymerase chain reaction amplification of the sex-specific avian CHD-1 gene, following standard protocols (Griffiths et al. 1998).

Each nestling in a given nestbox was ranked according to hatch order. When two or more nestlings were first found hatched on the same monitoring session, rank was assigned based on body mass (larger nestlings had higher rank). The first hatched nestling was assigned the highest rank (i.e. rank 1), while subsequent nestlings were assigned lower ranks (i.e. 2–5; no more than 5 nestlings were found in each nestbox). As there were no statistically significant sex differences in body mass at hatching (body mass recorded within 1 day of hatching, mixed model with nestbox identity as a random intercept effect, effect of sex: $F_{1,\ 167} = 0.01$, $P = 0.98$), sex did not confound nestling rank assignment.

As proxies of breeding performance, we used clutch size (number of eggs laid), hatching success (proportion of eggs hatched in a clutch), and brood size (number of nestlings in the nest), the latter being recorded at each monitoring session.

As a part of a parallel study, unrelated to the present one, in a sample of 44 nestboxes (20 belonging to dyads and 24 unpaired) out of the 98 where the clutch size was completed and incubation started, we performed a food supplementation by which we provided laying pairs with laboratory mice after the laying of the first egg and during the early nestling period. Pairs breeding in non-supplemented nestboxes served as controls. This concomitant experiment, whose results will be reported elsewhere (S. Podofillini et al., manuscript in preparation), could not alter nestbox occupation patterns because supplementation started after a given nestbox had been chosen by the kestrels (i.e., after the first egg had been laid).

Assessment of nest-site preference

Nest-site preference was experimentally investigated based on 40 nestbox dyads. A dyad consisted of two paired nestboxes placed side-by-side (the sides were touching each other), one of which was "dirty" while the other was "clean", with the two front panels with the entrance holes pointing towards the same direction (Figure 1). In this way, we aimed at forcing the choice between the dirty and the clean nestbox while eliminating any confounding effect due to nest orientation, position (e.g., shaded versus unshaded, disturbance level), nestbox wear (see below), predation risk, and surrounding habitat quality.

When assembling dyads, one old nestbox, in which clear signs of previous breeding attempts were obvious, was paired with an identical, brand-new nestbox. Old nestboxes, besides containing compressed organic material (mostly consisting of prey remains, regurgitated pellets, faeces, feathers, etc.), had a rather worn external appearance (i.e., faded colouration), including front panels. To remove any confounding effect of external nestbox wear on nest-site preference, we shuffled front panels and nest material between old and new nestboxes according to all eight possible combinations (Figure 1), each of which was applied five times (there were five dyads for each combination). The old nest material was carefully removed from any old nestbox included in a dyad, vigorously minced, shaken, and placed back either into the old or the new nestbox according to the predetermined combinations. To avoid any side bias, the old nestbox was placed alternately on the left or the right side. Hence, dirty nestboxes within a dyad were characterized by the presence of old, organic nest material (a cue of previous breeding attempts) while clean nestboxes did not have any organic nest material but only a thin layer of gravel and sand on the bottom of the nestbox (no cue of previous breeding attempts). Dyads were randomly interspersed among unpaired nestboxes along the perimeter of terraces, and were positioned at a minimum distance of 2 m from nearby dyads or unpaired nestboxes (see also section "Study species, study area and general methods").

Since lesser kestrels show a high natal and breeding philopatry (57% of first-time breeders recruit to the natal colony, and ~72% of adults return to the colony where they bred in the previous year; Negro et al. 1997; Serrano et al. 2001), nest-site preference could be affected by previous experience and recognition of previous year's nest-sites. To avoid this bias, in February 2016, all old nestboxes

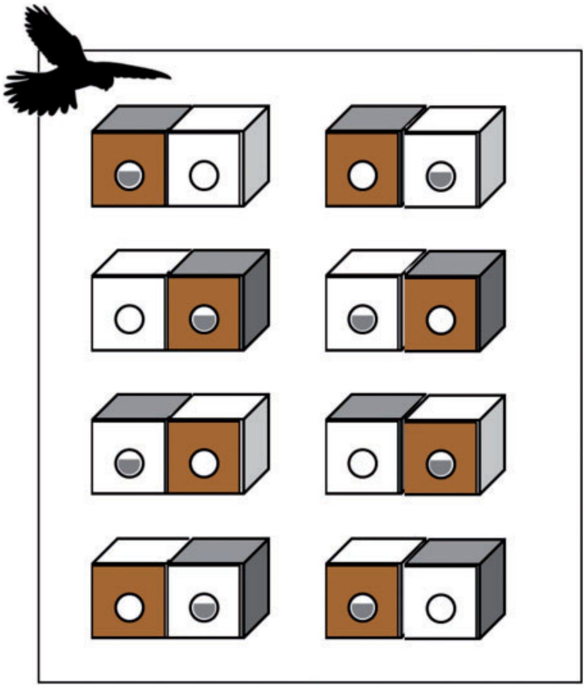

Figure 1. Schematic illustration of the different combinations adopted to randomize nest material, front panel, and cement block in dyads of adjacent clean and dirty nestboxes. The combinations were illustrated using white panels and white cubes for front panels and cement blocks installed for first time in 2016; brown panels and gray cubes for old front panels and cement blocks white holes: clean nestboxes; white and gray holes: dirty nestboxes. The dirty nestbox was alternately placed on the left or right side, to avoid any side bias. A dyad was interspersed in random order between unpaired nestboxes or other dyads along the perimeters of the terraces of two buildings, and was at a minimum distance of 2 m from any nearby dyad/unpaired nestbox.

(either included in dyads or not) were randomly shuffled along the perimeter of terraces.

Nest-site preference was determined by assessing the settlement of a breeding pair in each nestbox of the dyad (laying of eggs). Laying date of the first egg was used to establish which of the two nestboxes of a dyad was occupied first (in case both nestboxes of a dyad were occupied). Lesser kestrel females may occasionally start laying one egg in a nest and then lay the other eggs in nearby nests, especially when several identical nestboxes are placed nearby (authors' personal observation). This was not the case in our dyads, where occupancy mostly occurred in only one of the two nestboxes, and when both nestboxes of a dyad were occupied, we found different females in the nests. In one dyad, however, a single egg was laid in a clean nestbox and then abandoned. This dyad was considered in the analyses of nest site preferences, but excluding it did not alter our conclusions (see "Results" section).

Nestling ectoparasite load

We assessed ectoparasite load of nestlings by estimating infestation by a common, small (~2 mm) haematophagous ectoparasitic fly (*Carnus hemapterus*, Diptera: Carnidae), whose adults infest nestlings of several cavity-nesting bird species (Capelle and Whitworth 1973). Females lay eggs in the organic nest material and the saprophagous larvae thrive in the nest substrate, where they feed on detritus. The life-cycle of this ectoparasitic fly is synchronized with that of its hosts: the peak of emergence of adult parasites from the nest material coincides with the hatching of hosts' eggs (Roulin 1998). Pupae are able to overwinter inside nest organic material, waiting for potential hosts to settle (Roulin 1998; Valera et al. 2006).

Nestlings were inspected to estimate the number of adult flies on the *furcula* (interclavicular depression) and on the right and left *axillae* (underwings) from the first to the fourth monitoring session. We could not accurately count all flies as they were fast-moving and hid rapidly within the nestling down upon handling. Hence, nestling ectoparasite load was rapidly scored upon handling each nestling by estimating visible flies for each body district on a 0–3 scale (0: no ectoparasites, 1: 1–3 flies, 2: 4–6 flies and 3: > 6 flies) and then computing the mean value between all body districts before statistical analyses.

Statistical analyses

Nest-site preferences were assessed based on the sample of 40 dyads. The number of dyads with occupied dirty versus clean nestboxes was compared by means of a binomial test for deviation from equality.

The effects of nestbox dirtiness on laying date, breeding performance, nestling mortality, ectoparasite load, and growth patterns were assessed based on pooling data collected both from dyads and unpaired nestboxes. This was necessary because of the very low sample size of occupied clean nestboxes belonging to dyads (see "Results" section). The effect of nestbox dirtiness on proxies of breeding performance [clutch size, hatching success, brood size at 8 and 16 days from hatching of the first egg] was evaluated by generalized linear models (GLMs) with nestbox dirtiness (clean versus dirty) and laying date (day of laying of the first egg) as predictors (to control for seasonal variation in breeding performance). Hatching success was expressed as the proportion of eggs hatched on clutch size, and tested in a binomial GLM using the events/trials syntax. In models of clutch and brood size (count variables), we assumed a Poisson error distribution. To reduce noise in estimates of egg hatching success and nestling survival, we excluded from the analysis all 16 nests where clutch size was completed but no eggs hatched (likely deserted by parents; 16% of the 98 nestboxes where clutch size was completed; see "Results" section). This did not affect our conclusions concerning the effect of nestbox dirtiness on other breeding parameters because the proportion of nests abandoned before hatching did not significantly differ between clean (0.22) and dirty (0.12) nestboxes [binomial GLM: effect of dirtiness, estimate (*SE*): −0.39 (0.59), $Z = -0.66$, $P = 0.51$; effect of laying date, estimate (*SE*): 0.07 (0.04), $Z = 1.68$, $P = 0.09$], though there was a trend for clean nestboxes to be abandoned more frequently than dirty ones.

The effect of nestbox dirtiness on nestling mortality was investigated using a binomial mixed model whereby mortality of each nestling (0 = alive, 1 = found dead or disappeared) at the fifth monitoring session was the dependent variable, while nestbox dirtiness, nestling rank, brood size (maximum brood size across all monitoring sessions), laying date, and ectoparasite load (maximum ectoparasite load across all monitoring sessions) were included as covariates. Nestbox identity was included as a random intercept effect.

To assess the effect of nestbox dirtiness on ectoparasite load, we ran a linear mixed model with nestbox dirtiness, nestling rank, brood size, and laying date as predictors. We also included monitoring session as a four-level fixed factor to control for variation in ectoparasite infestation throughout the course of the nestling period. Two-way interactions between dirtiness and all other predictors were also included in the initial model. Nestling and nestbox identity were included as random intercept effects.

We evaluated the effects of nestbox dirtiness on body mass using a linear mixed model including nestbox dirtiness, nestling age, nestling rank, brood size (number of nestling in the nestbox at each check), laying date, ectoparasite load, and two-way interactions between dirtiness and nestling rank, brood size or ectoparasite load, as well as the two-way interaction between nestling rank and nestling age (to account for differential growth of nestlings differing in rank) as fixed effects; nestling and nestbox identity were included as random intercept effects. The models of tarsus and forearm length had a fixed effect structure identical to the model of body mass, but as we had a single measurement per nestling, we included only nest identity as a random intercept effect. Brood size and ectoparasite load referred to the maximum values recorded for that nestbox/nestling during the four monitoring sessions. Age effects on growth were controlled for by including the linear term of age only. Despite generally growth curves are sigmoidal-shaped (Starck and Ricklefs 1998), nestling growth of lesser kestrels up to 11 days (out of a nestling period of ~30 days) did not significantly deviate from linearity (details not shown for brevity).

In all models, two-way interaction terms were removed in a single step if non-significant ($P > 0.05$). Full models (including all non-significant interactions) are reported in Supplementary material.

Since the lesser kestrel is sexually size dimorphic, females being heavier and larger than males (Cramp 1998), we performed exploratory analyses on the subsample of 209 nestlings (out of 244 hatched) that were alive at the fourth monitoring session (when blood sampling was performed) to investigate possible effects of nestling sex (0 = female, 1 = male) on the response variables. Mixed models (with the same random intercept effects as detailed above) did not reveal any statistically significant difference in response variables according to sex [parasite load: estimate (SE): −0.07 (0.04), $F_{1,\ 169} = 3.71$, $P = 0.06$; body mass: −1.55 (1.65), $F_{1,\ 200} = 0.87$, $P = 0.52$; tarsus length: −0.20 (0.48), $F_{1,\ 185} = 0.18$, $P = 0.67$; forearm length: estimate (*SE*): −0.38 (0.84), $F_{1,\ 187} = 0.20$, $P = 0.65$]. Hence, for simplicity and to avoid sacrificing sample size for some of the analyses, we did not consider sex effects any further in the analyses. These results indicate that nestling parasite load is not significantly different between sexes and that sexual size dimorphism is not yet evident during the early nestling stage.

To check for the possible confounding effects of the food supplementation experiment on breeding performance traits, nestling ectoparasite load, body mass and skeletal growth, all relevant models were re-run while including food supplementation (supplemented versus control) as a fixed effect. The effect of food supplementation was never statistically significant (P-values always > 0.14; additional details not shown for brevity). Hence, for simplicity we did not consider this variable further.

Mixed models were fitted using the *lmer* or *glmer* function of the "lme4" library (Bates et al. 2014) for R 3.3.1 (R Core Team 2014). Degrees of freedom for linear mixed models were estimated using the Kenward–Rogers approximation ("pbkrtest" library; Halekoh and Højsgaard 2014). Non-Gaussian GLMs and mixed models were not overdispersed (see "Results" section; overdispersion for non-Gaussian mixed models was checked using the "blmeco" library; Korner-Nievergelt et al. 2015).

Results

Nestbox occupancy, nest-site preference, and laying date

Among unpaired nestboxes, old nestboxes were occupied significantly more often than new ones [old nestboxes: 20/24 (83.3%), new nestboxes: 34/71 (47.9%); $\chi^2 = 9.19$, $df = 1$, $P = 0.002$). In the nest-site selection experiment, 38 out of 40 dyads had at least one nestbox occupied (i.e., 95% of dyads had at least one nestbox occupied). Among the 38 dyads with at least one nestbox occupied, in 31 cases only the dirty nestbox was occupied, in 1 case only the clean nestbox was occupied (binomial test, $P < 0.001$), and in 6 cases both nestboxes were occupied. Among the latter 6 dyads, the dirty nestbox was occupied earlier in 5 out of 6 cases, the mean laying date in the dirty nestbox of the dyad being 12.0 (4.1 *SE*) days earlier than in the clean one (Wilcoxon matched-pairs test: $Z = 2.02$, $P = 0.043$). Considering both unpaired nestboxes and dyads, mean laying date in dirty nestboxes was May 13 (1.0 *SE*, $N = 57$), while it was May 18 (1.3 *SE*, $N = 41$) in clean ones ($t_{96} = 2.89$, $P = 0.005$).

Nestbox dirtiness, breeding performance, and nestling mortality

The effects of nestbox dirtiness (clean versus dirty) on clutch size, hatching success and brood size was analysed in the sample of 82 nestboxes where at least one egg hatched.

Clutch size did not significantly differ between clean and dirty nestboxes (Table 1), while hatching success of eggs laid in dirty nestboxes (percentage hatched = 86%) was slightly but significantly higher than that of eggs laid in clean nestboxes (76%) (Table 1). In spite of a significantly higher hatching success in dirty nestboxes, brood size did not significantly differ between clean and dirty nestboxes (Table 1). Breeding performance of lesser kestrels did not significantly vary across the breeding season, as shown by the lack of significant effects of laying date (Table 1).

The probability that a nestling had died by the last monitoring session was not significantly affected by nestbox dirtiness (Table 2), while it was significantly higher among low-ranking nestlings (Table 2).

Nestling ectoparasite load, body mass, and size in relation to nestbox dirtiness

Nestling ectoparasite load was recorded in 70 nestboxes (28 clean, 42 dirty). The model of ectoparasite load revealed a statistically significant nestbox dirtiness × monitoring session interaction (Table 3, Figure 2): *post-hoc* tests indicated that mean ectoparasite load was significantly higher in dirty nestboxes soon after the first eggs had hatched (i.e., in the first monitoring session) ($P = 0.003$), whereas

Table 1. Effect of nestbox dirtiness on breeding performance

	Clean	Dirty	Estimate (SE)	*Z*	*P*
Clutch size (*N* = 82)					
Dirtiness	4.10 (0.14)	4.34 (0.10)	0.05 (0.11)	0.47	0.64
Laying date	–	–	−0.01 (0.01)	−0.25	0.80
Hatching success (*N* = 82)					
Dirtiness	0.76 (0.04)	0.86 (0.03)	0.65 (0.29)	2.29	0.022
Laying date	–	–	0.01 (0.02)	0.04	0.97
Brood size, day 7 (*N* = 82)					
Dirtiness	2.59 (0.24)	3.16 (0.18)	0.21 (0.14)	1.54	0.12
Laying date	–	–	0.01 (0.01)	0.55	0.58
Brood size, day 15 (*N* = 82)					
Dirtiness	2.25 (0.21)	2.70 (0.17)	0.19 (0.15)	1.30	0.19
Laying date	–	–	0.01 (0.01)	0.38	0.70

Mean values (SE) of breeding parameters are reported (binomial SE for hatching success). Estimates are from Poisson or binomial GLMs (for hatching success). Models were not overdispersed (dispersion parameter always < 1.26).

Table 2. Binomial mixed model of the effect of nestbox dirtiness on the probability that a nestling had died by 15 days from start of egg hatching

Predictors	Estimate (SE)	*Z*	*P*
Dirtiness	−0.77 (0.78)	0.99	0.32
Nestling rank	1.30 (0.26)	4.95	<0.001
Brood size	−0.22 (0.36)	0.62	0.53
Laying date	−0.08 (0.05)	1.63	0.10
Ectoparasite load	−0.57 (0.48)	1.18	0.24

Nestbox identity was included as a random effect. The model was not overdispersed (dispersion parameter = 0.81).

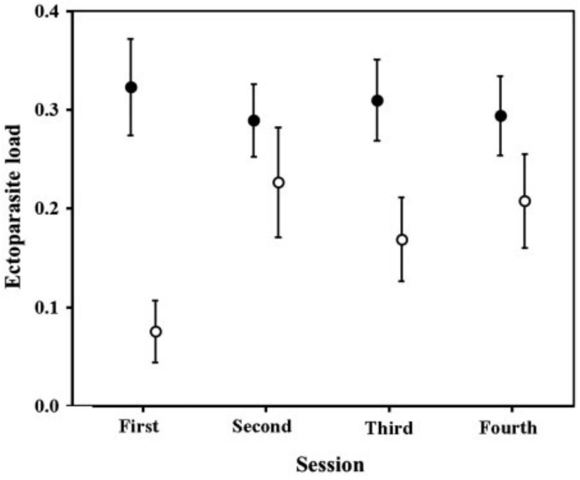

Figure 2. Nestling ectoparasite load in each of the four monitoring sessions. Filled dots represent the mean ectoparasite load of nestlings reared in dirty nestboxes while empty dots refer to nestlings reared in clean nestboxes (*N* = 70 nests, 244 nestlings). Error bars represent SE.

the effect of dirtiness on ectoparasite load became non-significant in all subsequent monitoring sessions (all $P > 0.40$). Moreover, ectoparasite load strongly decreased with nestling rank, high-ranking nestlings being more infested than low-ranking (smaller and late hatched) ones (Table 3). Finally, ectoparasite load markedly decreased in the course of the breeding season, late clutches being significantly less infested than early ones (Table 3). Two-way interactions between nestbox dirtiness and other predictors were not significant and were thus removed from the model (all $P > 0.33$; see Table S1 in Supplementary material for details).

Nestling body mass was not significantly affected by nestbox dirtiness (Table 3), while it significantly decreased in more parasitized nestlings, in low-ranking ones, and among nestlings reared in larger broods (Table 3). Moreover, early nestling growth was significantly lower in low-ranking nestlings, as shown by the negative sign of the significant age × nestling rank interaction (Table 3). Other two-way interactions with nestbox dirtiness were not significant and were removed from the model (all $P > 0.60$; see Table S1 in Supplementary material for details).

Tarsus and forearm length recorded at the last monitoring session were not significantly affected by nestbox dirtiness, while they were both lower in low-ranking nestlings (Table 3). Tarsus (but not forearm) length was significantly larger in nestlings reared in larger broods (Table 3). Two-way interactions between dirtiness and other predictors were not significant and were removed from the models (tarsus length, all $P > 0.30$; forearm length, all $P > 0.20$; see Table S1 in Supplementary material).

Discussion

Studies addressing the preference for dirty vs. clean nestboxes in secondary cavity-nesters have provided conflicting evidence, highlighting broad interpopulation and interspecific differences in preference patterns (see Introduction and review by Mazgajski 2007). Part of this variability may be due to different experimental designs that were not specifically aimed at testing the effect of cues of previous breeding attempts on nest-site choice (Mazgajski 2007). In our carefully designed nestbox choice experiment, lesser kestrels showed a strong preference for nestboxes previously used by conspecifics, breeding pairs settling earlier and more frequently in nestboxes with a dirty substrate. The preference for dirty nestboxes is consistent with two possible explanations. First, it is consistent with the idea that the breeders exploit cues about previous breeding attempts by conspecifics to choose their nest cavity or colony site (Negro and Hiraldo 1993; Serrano et al. 2001, 2003; Aparicio et al. 2007). Second, it may reflect preference for a more comfortable nest substrate by females. The organic material contained in old nests, being ~5 cm thick, may improve thermal insulation of the nest substrate, reducing heat loss, increasing incubation efficiency, and ultimately lowering the energetic costs of incubation (Mainwaring et al. 2014). Energy demands during incubation largely depend on the rate at which eggs lose heat (Deeming 2002). Incubating birds, especially those (as the lesser kestrel) that lay eggs directly on the substrate without lining their nest cavity, are therefore expected to preferentially lay eggs on those substrates that minimize the energetic costs of incubation (Deeming 2002; Mainwaring et al. 2014). Females may have been roosting in both nestboxes of a dyad before egg laying, and this might have promoted the choice for the likely more suitable organic nest substrate. Finally, earlier egg laying in dirty vs. clean nestboxes is in accordance with the hypothesis that the sequence of cavity occupation in lesser kestrels follows a despotic distribution (Negro and Hiraldo 1993; see also Sumasgutner et al. 2014), with early-settling individuals (likely older and experienced breeders; Catry et al. 2017) preferentially settling in dirty nestboxes compared to clean ones.

With regards to the fitness consequences of settling in a dirty nestbox, we envisage three possible explanations for the ~10% greater hatching success in dirty versus clean nestboxes. First, the organic material could allow establishing a favourable nest microclimate through improved thermal insulation and humidity stabilization (Hooge et al. 1999; Ardia et al. 2006), possibly increasing egg viability (Cook et al. 2003). Indeed, previous studies have shown that nest position and content are important factors in affecting thermal insulation and in buffering the potential negative effects of harsh environmental conditions on embryo development (Hilton et al. 2004; Mainwaring et al. 2014). Second, eggs laid on soft, organic rather than mineral substrate may suffer a lower risk of breakage and/or be more efficiently incubated, resulting in lower egg failure rates. Alternatively, a higher hatching success in dirty nestboxes may be due to a better incubation performance/higher phenotypic quality of early settling (older/more experienced; Catry et al. 2017) pairs occupying these nestboxes.

The higher *C. hemapterus* load of nestlings hatched in dirty versus clean nestboxes is likely due to the higher parasite load of dirty versus clean nestboxes. *Carnus hemapterus* flies undergo a

Table 3. Mixed models of the effects of nestbox dirtiness on nestling ectoparasite load, body mass, tarsus, and forearm length, while accounting for the concomitant effects of other predictors

Predictors	*F*	*df*	*P*	Estimate (SE)
Ectoparasite load (*N* = 70 nests and 244 nestlings)				
Dirtiness	1.95	1, 67	0.17	–
Session	0.44	3, 593	0.73	–
Nestling rank	11.29	1, 189	<0.001	−0.05 (0.01)
Brood size	0.11	1, 314	0.75	−0.01 (0.02)
Laying date	32.90	1, 77	<0.001	−0.02 (0.01)
Dirtiness × session	3.41	3, 581	0.017	–
Body mass (*N* = 70 nests and 244 nestlings; covariates centred on their mean value)				
Dirtiness	0.01	1, 51	0.82	–
Age	4960.8	1, 580	<0.001	6.96 (0.10)
Nestling rank	120.2	1, 144	<0.001	−4.01 (0.37)
Brood size	5.2	1, 294	0.023	−1.01 (0.44)
Laying date	3.4	1, 68	0.07	−0.15 (0.08)
Ectoparasite load	4.3	1, 697	0.038	−1.55 (0.75)
Age × nestling rank	123.5	1, 601	<0.001	−0.97 (0.08)
Tarsus length (*N* = 63 nests and 202 nestlings)				
Dirtiness	0.36	1, 53	0.55	–
Age	212.1	1, 168	<0.001	1.61 (0.11)
Nestling rank	63.4	1, 173	<0.001	−0.97 (0.12)
Brood size	4.73	1, 71	0.033	0.43 (0.20)
Laying date	0.03	1, 61	0.86	0.01 (0.03)
Ectoparasite load	0.65	1, 194	0.42	−0.21 (0.23)
Forearm length (*N* = 63 nests and 203 nestlings)				
Dirtiness	3.29	1, 145	0.08	–
Age	222.0	1, 123	<0.001	2.78 (0.19)
Nestling rank	63.6	1, 181	<0.001	−1.82 (0.23)
Brood size	3.89	1, 67	0.053	0.58 (0.30)
Laying date	1.49	1, 57	0.23	0.05 (0.04)
Ectoparasite load	0.01	1, 175	0.98	−0.01 (0.46)

Models for ectoparasite load and body mass included nestbox and nestling identity as random effects, while models for tarsus and forearm length included only nestbox identity as a random effect.

prolonged diapause when hosts are absent from the nest cavity, and adult emergence is synchronized with nestling hatching (Roulin 1998). However, ectoparasite load of nestlings raised in clean versus dirty nestboxes became very similar within a few days after hatching of the first egg, likely because of ectoparasite dispersal between nearby nestboxes to reduce competition for access to hosts (e.g., Dawson and Bortolotti 1997). Moreover, ectoparasite load strongly decreased over the course of the breeding season, late broods being significantly less parasitized than early ones. The seasonal decline of *C. hemapterus* load is in line with previous studies (e.g., Dawson and Bortolotti 1997; Sumasgutner et al. 2014), and may be due to natural variation in abundance through the parasite life-cycle (Roulin 1998).

The lack of significant effects of nestbox dirtiness on nestlings' early growth patterns suggests that the higher ectoparasite load of dirty nestboxes is of seemingly minor importance for nestling fitness (Sumasgutner et al. 2014), in spite of the higher *C. hemapterus* parasitism of nestlings hatched in dirty nestboxes that we observed soon after hatching. Together with the observation that breeding success in dirty nestboxes was not lower than in clean ones, this finding suggests that breeding in dirty nestboxes does not entail fitness costs (e.g., Sumasgutner et al. 2014).

On the whole, our results did not provide strong evidence that breeding in dirty nestboxes provides fitness payoffs in terms of improved reproductive output. Studies of nest-site or breeding habitat choice commonly assume that observed preference patterns are adaptive, implying that settlement decisions reflect fitness benefits (in terms of higher breeding success and/or survival; see Orians and Wittenberger 1991; Martin 1998; Chalfoun and Schmidt 2012), but this assumption has only seldom been tested (Brambilla and Ficetola 2012). In secondary-cavity nesters, the effects of nest dirtiness on reproductive parameters are unclear; the majority of studies have shown no obvious effects of nest material from previous breeding events on fitness traits, though some studies have documented weak statistically significant (mostly negative) effects (Mazgajski 2007). Our findings are thus in line with such previous evidence. We note however that the detection of significant fitness effects of nest-site preference for previously used nests may be context-dependent. It is known that lesser kestrels use conspecific presence as a major cue when deciding where to nest and when to breed (Serrano et al. 2003), and our study site may in fact act as a single huge colony of ~1,000 breeding pairs (La Gioia et al. 2017). In this context, selection of different nest-sites may not be so relevant in terms of fitness because the high number of individuals occurring at this colony may indicate favourable breeding conditions (for instance, larger colonies are mostly settled in sites that are less accessible to predators; Serrano et al. 2004). However, in a different context, with small colonies that are sparsely distributed through the landscape (thus more difficult to be detected by prospecting individual kestrels), the presence of organic material derived from previous breeding attempts in a cavity would be an important cue for settlement at a suitable breeding site and could have significant fitness consequences.

Other findings emerging from this study, unrelated to nestbox dirtiness, are briefly discussed below.

First, parasite load negatively affected body mass growth, suggesting that intense *C. hemapterus* parasitism may entail fitness costs for nestlings (e.g. Hoi et al. 2010). Alternatively, the negative effect of *C. hemapterus* parasitism on nestling body mass may be indirect, resulting from higher parasitism in clutches with low-quality nestlings (i.e. nestlings with a smaller cutaneous immune response; Bize et al. 2008), or from greater exposure to pathogens that may be transmitted through *C. hemapterus* blood meals.

Second, the higher *C. hemapterus* load in high- versus low-ranking nestlings is consistent with the idea that ectoparasites' host selection is non-random. *Carnus hemapterus* seem to aggregate in larger numbers on older/heavier nestlings, suggesting avoidance of smaller and/or poorer condition nestlings within broods (e.g. Dawson and Bortolotti 1997; Valera et al. 2004; Bize et al. 2008; Hoi et al. 2010; but see Roulin et al. 2003). This may occur because: 1) parasites can less easily obtain abundant/high-quality food resources from such hosts, decreasing their own fitness; 2) lesser kestrels show a relatively large hatching asynchrony [days between hatching of the first and the last egg in a clutch: 2 days (range 1–10); our unpubl. data], whereby early hatched hosts are the only target of parasites before hatching of their younger siblings; 3) smaller hosts simply provide less resources for parasites (in terms of total blood amount flow/feeding space available on the nestling skin). The fact that the per gram ectoparasite load (ectoparasite load/body mass) was not significantly predicted by nestling rank is in line with the third explanation (see Table S2 and Figure S1 in Supplementary material), though hatching asynchrony may also contribute to explain nestling rank effects on ectoparasite load.

Third, nestlings from larger broods had a lower body mass, but longer tarsi and forearm. This suggests that brood size may modulate early growth trajectories, perhaps via an effect on sibling competition (see also Gil et al. 2008). A larger skeletal size may provide competitive advantages in obtaining food items, as it may lead to dominance in sib–sib interactions once parents arrive at the nest with prey, and the payoff of a larger skeletal size may be greater in larger broods where sibling competition is higher (Schew and Ricklefs 1998).

In conclusion, we provide strong evidence that the presence of organic material from previous nesting attempts in the nest cavity is a key driver of nest-site choice, in line with lesser kestrels exploiting cues of conspecific presence for deciding where to settle and breed, and with the idea that organic nest material provides females with a comfortable substrate for egg laying and incubation. We emphasize that these findings may have bearings for projects aimed at improving the conservation status of the lesser kestrel, a species of European conservation priority that has suffered severe population declines and range contractions in the course of the 20th century (BirdLife International 2015). As the availability of suitable nest-sites has been identified as an important factor limiting population growth (Negro and Hiraldo 1993), many conservation projects rely on provisioning large numbers of nestboxes (Iñigo and Barov 2010; La Gioia et al. 2017). We propose that adding old nest material to newly deployed nestboxes may represent a cheap and effective way to enhance their occupation rate, hence increasing the effectiveness of conservation measures aimed at improving the conservation status of lesser kestrel breeding populations.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at https://academic.oup.com/cz.

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Author contributions

D.R., J.G.C., M.V., S.Po., and M.G. conceived the study and wrote the paper, with inputs from E.D.C. and N.S.; S.Po., A.C., E.F., S.Pi., L.S., J.G.C., E.D.C., M.G., and D.R. conducted fieldwork and collected the data; S.Po., D.R., J.G.C., and N.S. analyzed the data.

References

Aparicio JM, Bonal R, Muñoz A, 2007. Experimental test on public information use in the colonial Lesser Kestrel. *Evol Ecol* **21**:783–800.

Ardia DR, Pérez JH, Clotfelter ED, 2006. Nest box orientation affects internal temperature and nest site selection by tree swallows. *J Field Ornithol* **77**: 339–344.

Bates D, Maechler M, Bolker B, Walker S, 2014. Fitting linear mixed-effects models using lme4. *J Stat Softw* **67**: 1–48.

BirdLife International, 2015. *Falco naumanni. The IUCN Red List of Threatened Species 2015*. Available from: http://www.iucnredlist.org/details/biblio/22696357/0.

Bize P, Jeanneret C, Klopfenstein A, Roulin A, 2008. What makes a host profitable? Parasites balance host nutritive resources against immunity. *Am Nat* **171**:107–118.

Boulinier T, Danchin E, 1997. The use of conspecific reproductive success for breeding patch selection in terrestrial migratory species. *Evol Ecol* **11**: 505–517.

Brambilla M, Ficetola GF, 2012. Species distribution models as a tool to estimate reproductive parameters: a case study with a passerine bird species. *J Anim Ecol* **81**:781–787.

Brown GP, Shine R, 2005. Nesting snakes (*Tropidonophis mairii*, Colubridae) selectively oviposit in sites that provide evidence of previous successful hatching. *Can J Zool* **83**:1134–1137.

Capelle KJ, Whitworth TL, 1973. The distribution and avian hosts of *Carnus hemapterus* (Diptera: Milichiidae) in North America. *J Med Entomol* **10**: 525–526.

Catry T, Moreira F, Alcazar R, Rocha PA, Catry I, 2017. Mechanisms and fitness consequences of laying decisions in a migratory raptor. *Behav Ecol* **28**: 222–232.

Chalfoun AD, Schmidt KA, 2012. Adaptive breeding-habitat selection: is it for the birds? *Auk* **129**:589–599.

Cook MS, Beissinger G, Toranzos RR, Arendt W, 2003. Trans-shell infection by pathogenic micro-organisms reduces the shelf life of non-incubated bird's eggs: a constraint on the onset of incubation? *Proc R Soc Lond B* **270**: 2233–2240.

Cramp S, 1998. *The Complete Birds of the Western Palearctic on CD-ROM*. Oxford: Oxford University Press.

Dawson RD, Bortolotti GR, 1997. Ecology of parasitism of nestling American Kestrels by *Carnus hemapterus* (Diptera, Carnidae). *Can J Zool* **75**: 2021–2026.

Davies WH, Kalisz PJ, Wells RJ, 1994. Eastern bluebirds prefer boxes containing old nests. *J Field Ornithol* **65**:250–253.

Deeming C, 2002. *Avian Incubation: Behaviour, Environment and Evolution*. Oxford: Oxford University Press.

Doligez B, Danchin E, Clobert J, 2002. Public information and breeding habitat selection in a wild bird population. *Science* **297**:1168–1170.

Eggers S, Griesser M, Nystrand M, Ekman J, 2006. Predation risk induces changes in nest-site selection and clutch size in the Siberian jay. *Proc R Soc Lond B* **273**:701–706.

Erckmann WJ, Beletsky LD, Orians GH, Johnsen T, Sharbaugh S et al., 1990. Old nests as cues for nest-site selection: an experimental test with red-winged blackbirds. *Condor* **92**:113–117.,

Gergely Z, Mészáros LA, Szabad J, Székely T, 2009. Old nests are cues for suitable breeding sites in the Eurasian penduline tit *Remiz pendulinus*. *J Avian Biol* **40**:2–6.

Gil D, Bulmer E, Celis P, Lopez-Rull I, 2008. Adaptive developmental plasticity in growing nestlings: sibling competition induces differential gape growth. *Proc R Soc Lond B* **275**:549–554.

González-Braojos S, Vela AI, Ruiz-De-Castañeda R, Briones V, Cantarero A et al. 2012. Is nestling growth affected by nest reuse and skin bacteria in pied flycatchers *Ficedula hypoleuca*? *Acta Ornithol* **47**:119–127.

Griffiths R, Double MC, Orr K, Dawson RJ, 1998. A DNA test to sex most birds. *Mol Ecol* **7**:1071–1075.

Halekoh U, Højsgaard S, 2014. A Kenward-Roger approximation and parametric bootstrap methods for tests in linear mixed models: the R package pbkr test. *J Stat Softw* **59**:1–30.

Heylen DJA, Matthysen E, 2008. Effect of tick parasitism on the health status of a passerine bird. *Funct Ecol* **22**:1099–1107.

Hilton GM, Hansell MH, Ruxton GD, Reid JR, Monaghan P, 2004. Using artificial nests to test importance of nesting material and nest shelter for incubation energetics. *Auk* **121**:777–787.

Hooge PN, Stanback MT, Koenig WD, 1999. Nest-site selection in the acorn woodpecker. *Auk* **116**:45–54.

Hoi H, Krištofík J, Darolová A, Hoi C, 2010. Are parasite intensity and related costs of the milichiid fly *Carnus hemapterus* related to host sociality? *J Ornithol* **151**:907–913.

Iñigo A, Barov B, 2010. *Species Action Plan for the Lesser Kestrel Falco Naumanni in the European Union*. Bruxelles: European Commission.

Korner-Nievergelt F, Roth T, Felten S, Guelat J, Almasi B et al., 2015. *Bayesian Data Analysis in Ecology Using Linear Models with R, BUGS and Stan*. London: Academic Press.

La Gioia G, Melega L, Fornasari L, 2017. *Piano d'Azione Nazionale per il grillaio Falco naumanni: Quaderni di Conservazione della Natura 41*. MATTM – ISPRA, Roma.

Mainwaring MC, Hartley IR, Lambrechts MM, Deeming DC, 2014. The design and function of birds' nest. *Ecol Evol* **4**:3909–3928.

Mappes T, Mappes J, Kotiaho J, 1994. Ectoparasites, nest site choice and breeding success in the pied flycatcher. *Oecologia* **98**:147–149.

Martin TE, 1998. Are microhabitat preferences of coexisting species under selection and adaptive? *Ecology* **79**:656–670.

Martínez de. P J., Merino S, Tomás G, Moreno J et al. 2011. Nest ectoparasites increase physiological stress in breeding birds: an experiment. *Naturwiss* **98**:99–106.

Mazgajski TD, 2003. Nest site choice in relation to the presence of old nests and cavity depth in the starling *Sturnus vulgaris*. *Ethol Ecol Evol* **15**: 273–281.

Mazgajski TD, 2007. Effect of old nest material on nest site selection and breeding parameters in secondary hole nesters, a review. *Acta Ornithol* **42**: 1–14.

Merino S, Potti J, 1995. Pied flycatchers prefer to nest in clean nest boxes in an area with detrimental nest ectoparasites. *Condor* **97**:828–831.

Møller AP, Allander K, Dufva R, 1990. Fitness effects of parasites on passerine birds: a review. In: Blondel J, Gosler A, Lebreton JD, McCleery R, editors. *Population Biology of Passerine Birds*. Berlin: Springer, 269–280.

Negro JJ, Hiraldo F, 1993. Nest-site selection and breeding success in the lesser kestrel *Falco naumanni*. *Bird Study* **40**:115–119.

Negro JJ, Hiraldo F, Donazar JA, 1997. Causes of natal dispersal in the lesser kestrel: inbreeding avoidance or resource competition? *J Anim Ecol* **66**: 640–648.

Nilsson SG, Johnsson K, Tjernberg M, 1991. Is avoidance by black woodpeckers of old nest holes due to predators? *Anim Behav* **8**: 439–441.

Olsson K, Allander K, 1995. Do fleas and/or old nest material, influence nest site preference in hole-nesting passerines? *Ethology* **101**:160–170.

Orell M, Rytkönen S, Ilomäki K, 1993. Do pied flycatchers prefer nest boxes with old nest material? *Ann Zool Fenn* **30**:313–316.

Orians GH, Wittenberger JF, 1991. Spatial and temporal scales in habitat selection. *Am Nat* **137**:S29–S49.

Pärt T, Doligez B, 2003. Gathering public information for habitat selection: prospecting birds cue on parental activity. *Proc R Soc Lond B* **270**:1809–1813.

R Core Team, 2014. *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing. Available from: http://www.R-project.org/.

Refsnider JM, Janzen FJ, 2010. Putting eggs in one basket: ecological and evolutionary hypotheses for variation in oviposition-site choice. *Annu Rev Ecol Evol Syst* **41**:39–57.

Rendell WB, Verbeek NA, 1996. Are avian ectoparasites more numerous in nest boxes with old nest material? *Can J Zool* **74**:1819–1825.

Renwick JAA, Chew FS, 1994. Oviposition behavior in Lepidoptera. *Ann Rev Entomol* **39**:377–400.

Riding CS, Belthoff JR, 2015. Removal of old nest material decreases reuse of artificial burrows by burrowing owls. *Wildlife Soc Bull* **39**:521–528.

Ringhofer M, Hasegawa T, 2014. Social cues are preferred over resource cues for breeding-site selection in Barn Swallows. *J Ornithol* **155**: 531–538.

Rosenheim JA, 1988. Parasite presence acts as a proximate cue in the nest-site selection process of the solitary digger wasp, *Ammophila dysmica* (Hymenoptera: sphecidae). *J Insect Behav* **1**:333–342.

Roulin A, 1998. Cycle de reproduction et abondance du diptère parasite *Carnus hemapterus* dans les nichées de chouettes effraies *Tyto alba*. *Alauda* **66**:265–272.

Roulin A, Brinkhof MW, Bize P, Richner H, Jungi TW et al., 2003. Which chick is tasty to parasites? The importance of host immunology vs. parasite life history. *J Anim Ecol* **72**:75–81.

Schew WA, Ricklefs RE, 1998. Developmental plasticity. In: Starck JM, Ricklefs RE, editors. *Avian Growth and Development*. New York: Oxford University Press, 288–304.

Serrano D, Tella JL, Forero MG, Donázar JA, 2001. Factors affecting breeding dispersal in the facultatively colonial lesser kestrel: individual experience vs. conspecific cues. *J Anim Ecol* **70**:568–578.

Serrano D, Tella JL, Donázar JA, Pomarol M, 2003. Social and individual features affecting natal dispersal in the colonial lesser kestrel. *Ecology* **84**:3044–3054.

Serrano D, Forero MG, Donázar JA, Tella JL, 2004. Dispersal and social attraction affect colony selection and dynamics of lesser kestrels. *Ecology* **85**: 3438–3447.

Sonerud GA, 1985. Nest hole shift in Tengmalm's owl *Aegolius funereus* as defence against nest predation involving long-term memory in the predator. *J Anim Ecol* **54**:179–192.

Sumasgutner P, Vasko V, Varjonen R, Korpimäki E, 2014. Public information revealed by pellets in nest sites is more important than ectoparasite avoidance in the settlement decisions of Eurasian kestrels. *Behav Ecol Sociobiol* **68**: 2023–2034.

Stanback MT, Rockwell EK, 2003. Nest-site fidelity in eastern bluebirds *Sialia sialis* depends on the quality of alternate cavities. *Auk* **120**:1029–1032.

Starck JM, Ricklefs RE, 1998. *Avian Growth and Development: Evolution within the Altricial-Precocial Spectrum*. Oxford: Oxford University Press.

Stamps JA, 1988. Conspecific attraction and aggregation in territorial species. *Am Nat* **131**:329–347.

Tomás G, Merino S, Morales J, 2007. Consequences of nest reuse for parasite burden and female health and condition in blue tits *Cyanistes caeruleus*. *Anim behav* **73**:805–814.

Tomás G, Merino S, Martínez-de la Puente J, Moreno J, Morales J et al., 2008. Determinants of abundance and effects of blood-sucking flying insects in the nest of a hole-nesting bird. *Oecologia* **156**:305–312.

Thompson CF, Neill AJ, 1991. House wrens do not prefer clean nestboxes. *Anim Behav* **42**:1022–1024.

Valera F, Hoi H, Darolova A, Kristofik J, 2004. Size versus health as a cue for host choice: a test of the tasty chick hypothesis. *Parasitology* **129**: 59–68.

Valera F, Casas-Crivillé A, Calero-Torralbo MA, 2006. Prolonged diapause in the ectoparasite *Carnus hemapterus* (Diptera: *Cyclorrhapha Acalyptratae*). How frequent is it in parasites? *Parasitology* **133**:179–186.

Valone TJ, Templeton JJ, 2002. Public information for the assessment of quality: a widespread social phenomenon. *Phil Trans R Soc Lond B* **357**:1549–1557.

Ward MP, 2005. Habitat selection by dispersing yellow-headed blackbirds: evidence of prospecting and the use of public information. *Oecologia* **145**: 650–657.

Electronic supplementary material

Table S1 Mixed models of the effects of nestbox dirtiness on nestling ectoparasite load, body mass, tarsus and forearm length, while accounting for the concomitant effects of nestling rank, age, laying date and brood size. Models for ectoparasite load and body mass included nestbox and nestling identity as random effects, while models for tarsus and forearm length included only nestbox identity as a random effect. In this table, we report the full statistics for non-significant two-way interactions. The table is divided in sections for better layout

	F	***df***	***P***	**Estimate (SE)**
Ectoparasite load (n = 70 nests and 244 nestlings)				
Dirtiness	0.37	1, 84	0.54	-
Session	0.46	3, 592	0.71	-
Nestling rank	12.03	1, 189	< 0.001	-0.07 (0.02)
Brood size	0.15	1, 299	0.70	-0.03 (0.03)
Laying date	23.36	1, 83	< 0.001	-0.02 (0.01)
Dirtiness × nestling rank	0.88	1, 189	0.35	0.03 (0.03)
Dirtiness × laying date	0.47	1, 83	0.49	-0.01 (0.01)
Dirtiness × brood size	0.56	1, 299	0.45	0.03 (0.05)
Dirtiness × session	3.83	3, 592	0.0097	-

	F	***df***	***P***	**Estimate (SE)**
Body mass (n = 68 nests and 223 nestlings; covariates centered on their mean value)				
Dirtiness	0.1	1, 50	0.81	-
Age	4936.4	1, 580	< 0.001	6.96 (0.1)
Nestling rank	103.2	1, 143	< 0.001	-4.14 (0.66)
Brood size	4.7	1, 296	0.031	-0.89 (0.70)
Laying date	3.4	1, 67	0.07	-0.14 (0.08)
Ectoparasite load	3.7	1, 716	0.054	-1.66 (1.38)
Dirtiness × nestling rank	0.1	1, 142	0.81	0.18 (0.79)
Dirtiness × brood size	0.0	1, 313	0.82	-0.19 (0.88)
Dirtiness × Ectoparasite load	0.0	1, 712	0.92	0.15 (1.63)
Age × nestling rank	123.0	1, 599	< 0.001	-0.97 (0.08)

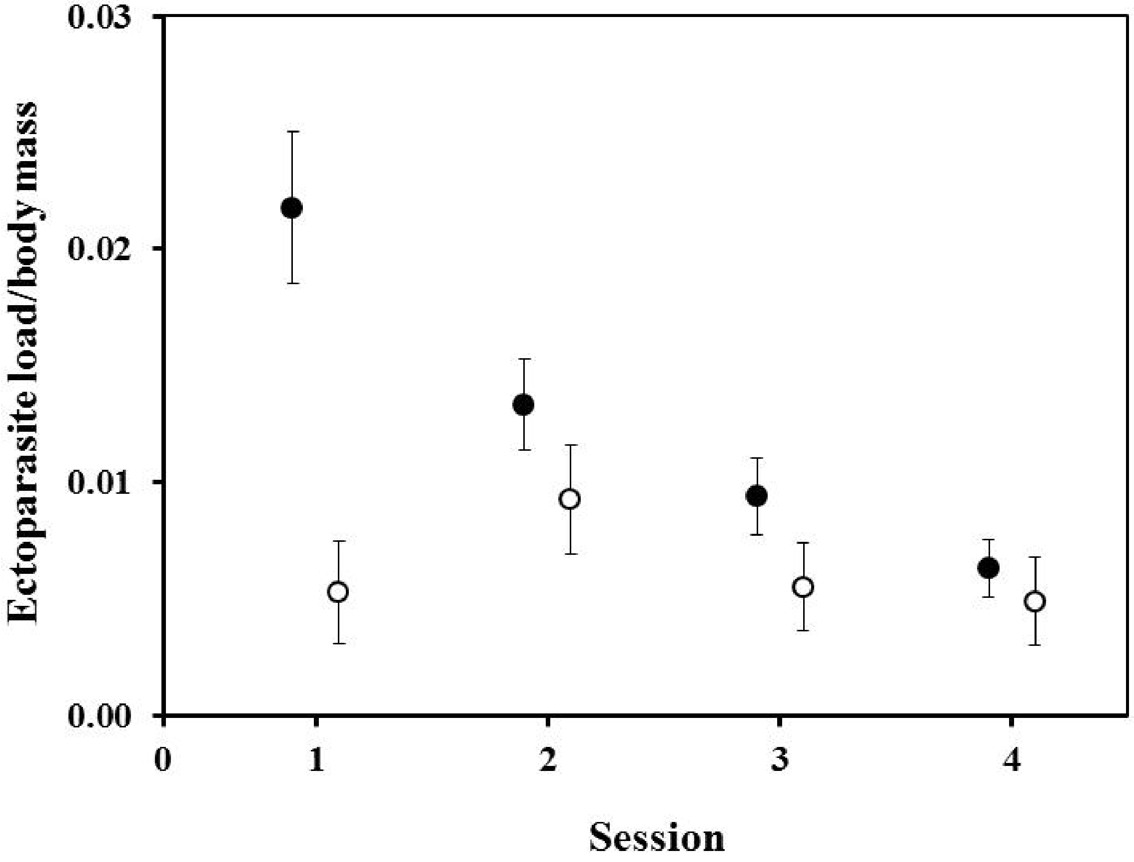
	F	*df*	*P*	Estimate (SE)
Tarsus length (n = 63 nests and 202 nestlings)				
Dirtiness	0.24	1, 93	0.63	-
Age	35.02	1, 179	< 0.001	1.51 (0.25)
Nestling rank	5.44	1, 186	0.02	-1.39 (0.60)
Brood size	4.9	1, 74	0.03	0.40 (0.30)
Laying date	0.01	1, 59	0.95	0.01 (0.01)
Age × nestling rank	0.24	1, 179	0.62	0.03 (0.07)
Dirtiness × nestling rank	1.06	1, 144	0.30	0.24 (0.24)
Dirtiness × brood size	0.11	1, 75	0.74	0.13 (0.40)
Dirtiness × ectoparasite load	0.02	1, 189	0.89	0.01 (0.6)
Ectoparasite load	0.32	1, 187	0.57	0.13 (0.49)

	F	*df*	*P*	Estimate (SE)
Forearm length (n = 63 nests and 203 nestlings)				
Dirtiness	0.34	1, 91	0.56	-
Age	56.68	1, 170	< 0.001	0.33 (0.43)
Nestling rank	0.42	1, 190	0.52	-0.32 (0.96)
Brood size	4.10	1, 75	0.046	0.95 (0.47)
Laying date	1.98	1, 56	0.16	0.06 (0.04)
Age × nestling rank	1.67	1, 184	0.20	-0.17 (0.13)
Dirtiness × brood size	1.23	1, 69	0.27	-0.67 (0.60)
Dirtiness × ectoparasite load	0.10	1, 191	0.74	-0.32 (0.10)
Ectoparasite load	0.11	1, 190	0.74	0.95 (0.47)

Table S2 Mixed model of the effects of nestbox dirtiness on a nestling per gram ectoparasite load (ectoparasite load/body mass), while accounting for the concomitant effects of different predictors. The model included nestbox and nestling identity as random effects. Sample size is 70 nests and 244 nestlings. The statistically significant interaction between dirtiness and session is illustrated in Fig. S3

	F	*df*	*P*	**Estimate (SE)**
Dirtiness	1.77	1, 66	0.18	-
Session	11.42	3, 585	< 0.001	-
Nestling rank	0.35	1, 198	0.55	-0.05 (0.10) × 10^{-2}
Brood size	0.15	1, 338	0.69	-0.04 (0.02) × 10^{-2}
Laying date	23.97	1, 76	< 0.001	-0.02 (0.01) × 10^{-2}
Dirtiness × session	8.71	3, 571	< 0.001	-

Figure S3 Nestling per gram ectoparasite load (ectoparasite load/body mass) in each of the four monitoring sessions. Filled dots represent the mean ectoparasite load of nestlings reared in dirty nestboxes while empty dots refer to nestlings reared in clean nestboxes (*N* = 70 nests, 244 nestlings). Error bars represent 1 SE



Chapter 3.

Benefits of extra food to reproduction depend on maternal condition

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Benefits of extra food to reproduction depend on maternal condition

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Declarations

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Abstract

The amount of food resources available to upper-level consumers can show marked variations in time and space, potentially resulting in food limitation. The availability of food resources during reproduction is a key factor modulating variation in reproductive success and life-history trade-offs, including patterns of resource allocation to reproduction vs. self-maintenance, ultimately impacting on population dynamics. Food provisioning experiments constitute a popular approach to assess the importance of food limitation for vertebrate reproduction. In this study of a mesopredatory avian species, the lesser kestrel (*Falco naumanni*), we provided extra food to breeding individuals from egg laying to early nestling rearing. Extra food did not significantly affect adult body condition or oxidative status. However, it increased the allocation of resources to flight feathers moult and induced females to lay heavier eggs. Concomitantly, it alleviated the costs of laying heavier eggs for females in poor body condition, and reduced their chances of nest desertion (implying complete reproductive failure). Extra food provisioning improved early nestling growth (body mass and feather development). Moreover, extra food significantly reduced the negative effects of ectoparasites on nestling body mass, while fostering forearm (a flight apparatus trait) growth among highly parasitized nestlings. Our results indicate that lesser kestrels invested the extra food mainly to improve current reproduction, suggesting that population growth in this species can be limited by food availability during the breeding season. In addition, extra food provisioning reduced the costs of the moult-breeding overlap and affected early growth trade-offs by mitigating detrimental ectoparasite effects on growth and enhancing development of the flight apparatus with high levels of parasitism. Importantly, our findings suggest that maternal condition is a major trait modulating the benefits of extra food to reproduction, whereby such benefits mostly accrue to low-quality females with poor body condition.

Keywords: body condition, egg size, food limitation, food provisioning, income breeding, moult-breeding overlap, offspring development, oxidative status, sex allocation

Introduction

Seasonal or stochastic fluctuations of ecological conditions often result in limited availability of food resources for upper-level consumers. Variation in food availability is one of the key factors modulating variation in life-history traits related to reproduction among individuals, ultimately affecting population dynamics (Lack 1954). Food availability is commonly exploited by individuals as a cue to adjust reproductive decisions to contingent ecological conditions, providing 'biological information' (*sensu* Wagner and Danchin 2010) on when and where best to breed and how much resources to invest in producing and raising offspring to maximise fitness (Martin 1987). Whenever breeding individuals are exposed to poor environmental conditions, resulting in food limitation, parents are expected to trade self-maintenance against reproduction and offspring provisioning, with major implications for their survival and breeding success (Lack 1966, Martin 1987).

The relevance of food limitation during reproduction for population dynamics can be assessed by analysing the effects of resource availability on breeding success, or via experimental manipulation (removal or addition) of food resources. The most widespread experimental approach to investigate the extent to which reproduction is limited by food resources, and to highlight the resulting life-history trade-offs, is to provide extra food to breeders, while concomitantly assessing variation in reproductive investment and output of individuals receiving the extra food compared to unsupplemented controls (reviews in Martin 1987, Boutin 1990). Among vertebrates, birds have been the favourite subject of food supplementation experiments during breeding (Martin 1987, Boutin 1990, Ruffino et al. 2014). In spite of broad differences in food provisioning protocols and of the heterogeneity of effects among studies, which may be partly due to variable background ecological conditions (in terms of e.g. food availability) during the experiments (Ruffino et al. 2014), individuals receiving extra food generally achieved reproductive benefits relative to controls, indicating that most bird populations are limited by food availability during the energy-demanding reproductive period (Martin 1987, Boutin 1990, Ruffino et al. 2014). These benefits include advancing egg laying (Meijer and Drent 1999, Aparicio and Bonal 2002), laying heavier eggs and/or larger clutches (Wiebe and Bortolotti 1995, Korpimäki and Wiehn 1998, Karell et al. 2008, Saino et al. 2010), and enjoying improved nestling growth and survival (Dewey and Kennedy 2001, Hipkiss et al. 2002).

Besides breeding output, extra food could affect parental condition. Food-supplemented parents may indeed spare energy resources that are otherwise required for self-maintenance and offspring provisioning, or directly use extra food to enhance their own survival prospects. Extra food may improve parental body condition (Garcia et al. 1993, Schoech 1996, Cucco and Malacarne 1997, Dewey and Kennedy 2001) and physiological state, in terms of e.g. immune system functioning or oxidative status (Karell et al. 2008, Alan and McWilliams 2013, Fletcher et al. 2013, Giordano et al. 2015). For instance, extra food may lower oxidative damage by reducing physical activity for self-provisioning (Giordano et al. 2015). Moreover, food provisioning may affect the timing and extent of feather moult, a highly energy-demanding process in the avian life cycle (Murphy 1996), and a reduction of the costs of the overlap between moult and competing activities, such as reproduction and migration (Siikamäki 1998, Danner et al. 2014).

In general, it may be expected that the advantages provided by extra food vary according to the resource allocation and consumption decisions that parents adopt during the breeding season (Roff 1992, Stearns 1992). For instance, breeding success of ‘capital breeders’ (whose reproduction relies on energy stored in advance of breeding; Jönsson 1997) may show limited sensitivity to extra food provisioning during the reproductive period, whereas that of ‘income breeders’ (which do not accumulate reserves prior to breeding and fuel reproduction with concurrent energy intake) should be more positively affected (Meijer and Drent 1999). Similarly, the behavioural response to food supplementation by parents may vary between species, populations or individuals, depending on which strategy maximises lifetime reproductive success under specific environmental contexts (e.g. high or low background prey availability) or physiological constraints (Kacelnik and Cuthill 1990, Ydenberg 1994, Markman et al. 2002). On the one hand, providing parents with extra food may reduce the amount of resources delivered to the progeny, reducing reproductive costs and enhancing parental residual reproductive value, while offspring quality and breeding success are unaltered (Dawson and Bortolotti 2002). On the other hand, extra food may not reduce offspring provisioning rates with natural food items, markedly increasing fledging success (Gonzalez et al. 2006).

Finally, whenever sexes differ in their susceptibility to harsh rearing environments, parents may adaptively tune the sex ratio of their offspring in relation to extrinsic conditions (West et al. 2000, West and Sheldon 2002). Indeed, nutritional constraints may affect avian sex allocation (Wiebe and Bortolotti 1992, Korpimäki et al. 2000, Saino et al. 2010), with mothers biasing the sex ratio of their clutches towards the larger sex, which is more susceptible to harsh rearing environments, when food resources are abundant (Wiebe and Bortolotti 1992, Nager et al. 1999, Korpimäki et al 2000).

In this study of the lesser kestrel (*Falco naumanni*), a small (ca. 120 g), sexually dimorphic, cavity-nesting diurnal raptor with biparental care of the progeny (Cramp 1998), we provided extra food to breeders from the onset of egg laying to the early nestling-rearing period, and assessed the effects of food supplementation on different short-term fitness components, such as: 1) parental body condition and oxidative status (in terms of plasma non-enzymatic total antioxidant capacity, TAC, and total oxidant status, TOS; Erel 2004, 2005); 2) moult timing and extent (lesser kestrels may initiate the annual moult of primary feathers during reproduction; Cramp 1998, Zuberogoitia et al. 2018); 3) breeding performance, including egg and clutch characteristics (egg mass, clutch size, hatching success, duration of the incubation period), biparental nest desertion (i.e. the abandonment of eggs and/or nestlings by both parents, leading to reproductive failure for the current breeding season; Székely et al. 1996), offspring growth and mortality; 4) patterns of primary sex allocation and sex-biased offspring mortality. We expected: 1) an overall positive effect of food supplementation on parental condition and oxidative status (increased TAC and/or decreased TOS); 2) earlier onset of annual moult and/or faster primary feathers growth among food-supplemented individuals compared to controls; 3) extra food to have an overall positive effect on breeding performance (Ruffino et al. 2014) and to shorten incubation (Sanz 1996), as reduced self-provisioning needs of food-supplemented parents may induce them to spend more time incubating eggs compared to controls. In the sexually-size dimorphic lesser kestrel (males are ca. 15 % lighter than females; Cramp 1998, Donázar et al. 1992), we might also expect 4) food-supplemented females to lay female-biased clutches and/or raise female-biased broods (Korpimäki et al. 2000; but see Aparicio and Cordero 2001).

Importantly, we investigated whether female body condition modulated the effects of extra food on breeding performance. To our knowledge, no previous study has investigated whether individual quality modulates the fitness benefits of extra food provisioning. We expected low-quality, poor condition females to obtain greater fitness benefits from extra food provisioning compared to high-quality, better condition females.

Material and methods

Study species, study area and general field procedures

The lesser kestrel is a long-distance Afro-palearctic migrant (Cramp 1998). European populations spend the winter mainly in the Sahel and reach the breeding areas in March-April (Cramp 1998). Egg laying takes place in late April-early May, and a previous food provisioning experiment beginning before egg laying revealed that food-supplemented females advanced first egg laying date by ca. one week compared to control ones (Aparicio and Bonal 2002), suggesting that egg laying may be food-limited. Females lay clutches of 3-5 eggs with a 2-3 days laying interval between consecutive eggs. Eggs are incubated for ca. 30 days by both parents. Altricial nestlings hatch asynchronously, generating strong size hierarchies among nestmates, with last-hatched, low-ranking nestlings often dying when resources are insufficient (Aparicio 1997). Fledging occurs at ca. 40 days post-hatching. Lesser kestrels feed mainly on invertebrates (Orthoptera, Coleoptera), lizards (chiefly *Podarcis siculus*) and small rodents (voles *Microtus* spp.) (Cramp 1998, Rodríguez et al. 2010, Catry et al. 2016, Di Maggio et al. 2018) that are captured in open farmland or grassland areas surrounding breeding colonies (Cecere et al. 2018). Both parents contribute to rearing nestlings (Cramp 1998). During breeding, some adults initiate their complete annual moult by shedding a few primary feathers (usually between 1 and 3 feathers, mostly P4-P6; primaries numbered descendantly) (Cramp 1998; see also Zuberogoitia et al. 2018).

The study was carried out during April-July 2016 in the Matera (Southern Italy; 40°67’ N, 16°60’ E) colony (ca. 1000 pairs, La Gioia et al. 2017). In this colony, many pairs breed in specially designed concrete nestboxes (external size: 30 cm height × 30 cm width × 37 cm length; entrance hole diameter 65 mm) with wooden front and rear panels (see Podofillini et al. 2018 for further details of nestboxes). For the present study, we relied on 209 nestboxes that were checked three times a week during the entire breeding season (late April - end July) to determine the onset of egg laying, egg hatching, duration of the incubation period, nestling body mass, morphology, and mortality. Eggs were weighted using a digital scale (accuracy 0.1 g) and individually marked using a non-toxic black marker to record the laying sequence. In those cases when two (or more) eggs were found in a same nestbox during the same monitoring session, we coded each egg using their mean laying order value (e.g. in the case of uncertainty between the fourth and the fifth egg, we coded both eggs as 4.5). Duration of the incubation period was expressed as the difference (in days) between the day of hatching of the first egg and the day of laying of the first egg in a clutch (laying date hereafter).

After hatching of the first egg, each nestbox was checked five times to assess nestling growth. Over this period, nestbox monitoring sessions occurred at an average of 0.8 (range 0-3), 3.0 (2-5), 5.3 (4-9), 7.9 (7-11) and 16.0 (14-18) days after hatching of the first egg. We did not check nestboxes after the fifth session because nestlings may start wandering outside nestboxes when ca. 15 days old, making monitoring difficult and increasing the risk of inducing premature fledging (Podofillini et al. 2018). Nestling body mass was recorded during all monitoring sessions, tarsus and forearm length (the latter measured from the front of the folded wrist to the proximal end of the ulna) were recorded at the fourth monitoring session, and the length of primary feather P8 (a measure of nestling feather growth) was recorded at the fifth monitoring session (it could not be accurately recorded at earlier sessions). Nestlings were ranked according to hatch order, or to body mass (assigning the higher rank to the heavier nestling) when two or more newly hatched nestlings were found on the same monitoring session (see Podofillini et al. 2018). The first hatched nestling was assigned the highest rank (i.e. rank 1). During the first four monitoring sessions, we recorded the intensity of infestation by *Carnus hemapterus*, a common blood-sucking dipteran ectoparasite of cavity-nesting birds (Capelle and Whitworth 1973). Ectoparasite infestation was assessed on each nestling for three body districts (interclavicular depression and right and left underwings) on a 0-3 scale (0: no ectoparasites, 1: 1–3 flies, 2: 4–6 flies and 3: > 6 flies). Nestling ectoparasite load was expressed as the mean value of ectoparasite infestation across the three districts (see Podofillini et al. 2018). At the fourth monitoring session, a small (ca. 200 µl) blood sample was collected in capillary tubes by puncturing the brachial vein with sterile needles. Blood was kept at -20°C and later used to molecularly determine sex (according to Griffiths et al. 1998).

A nest was considered as deserted if no eggs from a complete clutch hatched (clutch desertion) or if all nestlings were found dead from one session to the next (brood desertion) (Székely et al. 1996), leading to complete reproductive failure for the current breeding season (lesser kestrels are single-brooded; Cramp 1998). Although in our case nest desertion occurs because both parents abandon the clutch/brood (biparental desertion, Székely et al. 1996), the process likely begins with desertion by one parent (the female in other raptor species with a similar breeding ecology; Newton and Marquiss 1984, Kelly and Kennedy 1993), rapidly followed by desertion of the other parent because the costs of reproduction for the remaining parent would be unsustainable (Székely et al. 1996).

Starting from ca. 10-15 days before the expected time of hatching, adults were captured opportunistically by hand in the nestbox or by nestbox traps while brooding their eggs or feeding newly hatched nestlings. We captured ca. 80 % of the adults breeding in experimental nestboxes (see Statistical analyses). Upon capture, birds were individually marked, and body mass (0.1 g) and keel length (using a dial calliper, accuracy 0.1 mm) were recorded. As an index of body condition, we used the scaled mass index (SMI hereafter), which standardizes body mass at a fixed value of a linear body measurement (keel length in our case) based on the scaling relationship between mass and length (Peig and Green 2009, 2010). Body mass and keel length were moderately positively correlated in both sexes; females: $r = 0.27$, $p = 0.016$, $n = 82$; males: $r = 0.33$, $p = 0.004$, $n = 74$). As the scaling exponent significantly differed between the sexes (Supplementary material Appendix 1, Table A1), SMI was computed for each sex separately. We recorded moult status by inspecting flight feathers of the right wing (moult was mostly symmetrical; our unpubl. data). When growing/newly grown primary feathers were found, we measured their length using a ruler (accuracy 1 mm); in case of a shed primary feather with no signs of quill growth, we recorded a value of 0. Moult status was expressed as moult initiation (i.e. whether an individual had begun moulting, including the presence of shed feathers, or not) and total moult investment. Total moult investment was assumed to reflect the total amount of resources invested in the synthesis of new feathers. It was expressed as the sum of the length of all growing feathers, and was assigned a value of 0 if no sign of moult was detected or if feathers were shed but had not yet grown (i.e. no resources allocated yet to new feather synthesis). Finally, for each individual we collected ca. 500 µl of blood into microhematocrit capillary tubes by puncturing the brachial vein using a sterile needle. To separate plasma from blood cells, two capillary tubes per individual were centrifuged (11500 rpm × 10 min) within 4 hours of sampling. Plasma was then stored at – 20° C for later biochemical assays.

Food supplementation

We alternately assigned each nestbox where an egg was found to a food supplementation or a control treatment. Breeding pairs were supplemented with commercial white laboratory mice (*Mus musculus*, ca. 20 g each) [Rapax Mangimi, Santo Stino di Livenza (VE), Italy] that were placed within the nestbox (at the rear end). We provided three mice every two days during the egg laying period and after hatching, and one mouse every two days during the incubation period (visits were reduced during incubation to limit disturbance to brooding adults). In the vast majority of cases, mice disappeared between consecutive monitoring sessions. Although we could not directly assess mice consumption by the target breeding pair, the consumption of extra food was confirmed by regularly observing regurgitated pellets containing white fur within nestboxes where mice were placed. Food supplementation ceased when nestlings were ca. 8 days old (i.e. at the fourth monitoring session) because by the time of the subsequent nest visit (14-18 days old) they may start wandering outside nestboxes (see above), where they are fed by parents, and we could not provide food outside nestboxes (as we could not control whether it was actually consumed by the target individuals or by other birds). Overall, each food-supplemented pair received ca. 40 mice (ca. 800 g of extra food). To standardize disturbance, control nestboxes were inspected in exactly the same way and with the same frequency as those receiving the extra food, simulating mice insertion into the nestbox. The lesser kestrel performs intensive mate-feeding (Cramp 1998, Donázar et al. 1992): before and during egg laying/incubation, the male feeds its partner, and deposits prey within the nest cavity for later consumption by the female if mate is absent (pers. obs.; Cramp 1998). Hence, placing extra food within the nestbox mimicked a natural condition and assured that only the target breeding female received most of the extra food, at least during egg laying and early incubation, avoiding common pitfalls of food provisioning experiments (where target individuals may not actually consume the extra food; Ruffino et al. 2014). During nestling rearing, parents may have used the extra food both to feed themselves or to provision their nestlings.

Oxidative status of breeding adults

The oxidative status of individuals reflects the balance between circulating antioxidants and pro-oxidants (e.g. free radicals), deriving from normal physiological activity, pathological states, or the external environment (Halliwell and Gutteridge 2007). Pro-oxidants may cause oxidative damage to biomolecules, cells and tissues, impairing organismal functions (Halliwell and Gutteridge 2007). Oxidative stress arises whenever an organism’s antioxidant defences are insufficient to counteract oxidative damage (Halliwell and Gutteridge 2007). High levels of antioxidants and low levels of pro-oxidants are regarded as indicators of good health state and positively predict fitness (e.g. survival; Bize et al. 2008, Saino et al. 2011). Food is a major source of antioxidants such as vitamins and carotenoids, as well as of substances (e.g. proteins) that do not have direct antioxidant activity, but may affect an organism’s resistance to oxidative stress (Halliwell and Gutteridge 2007, Costantini 2014). Moreover, high food availability might reduce the oxidative costs of foraging for self and offspring provisioning (Costantini 2014, Giordano et al. 2015). Hence, extra food may positively affect oxidative status through different mechanisms, leading to high levels of antioxidants/low levels of pro-oxidants. As proxies of the oxidative status of breeding adults, we measured TAC through a global test of blood non-enzymatic antioxidant capacity, and TOS, reflecting the overall concentration of circulating pro-oxidants in the blood flow. High TAC indicates high antioxidant capacity, while high TOS indicate high levels of circulating pro-oxidants and an increased risk of oxidative damage.

TAC was measured according to Erel (2004), with some modifications. Briefly, 7 µl of plasma were added to 230 µl of the 2, 2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^{*+}$) solution, which bleaches depending on the concentration of non-enzymatic antioxidants in the sample. The reaction was monitored at an absorbance of $\lambda = 750$ nm by a spectrophotometer. The final absorbance is inversely related to TAC of samples. The reaction was calibrated by drawing a standard curve with serial dilution of Trolox and the results were expressed as µM Trolox equivalent.

TOS was measured according to Erel (2005). Briefly, 20 µl of plasma were added to 237 µl of a ferrous ion-*o*-dianisidine and Xylenol Orange solution. Oxidant molecules oxidise the ferrous ion to the ferric ion, which reacts with Xylenol Orange to give a coloured (blue) complex. Colour intensity was measured by a spectrophotometer at an absorbance of $\lambda = 535$ nm and it is proportional to the total amount of oxidant agents in the plasma. The assay was calibrated by drawing a standard curve with serial dilution of hydrogen peroxide (H_2O_2) and the results were expressed as nM H_2O_2 equivalent ml^{-1}.

The mean intra- and inter-plate coefficients of variation of TAC, measured on a pool of plasma assayed twice in all plates, were 2.7 (0.5 s.d.) % and 5.3 (0.7 s.d.) %, while the same figures for TOS were 2.9 (0.9 s.d.) % and 4.8 (1.1 s.d.) %, respectively. We measured TAC for all available plasma samples, while TOS could be only measured for a subsample of these because of plasma amount limitations.

Statistical analyses

To investigate the effects of food supplementation (0 = control, 1 = food-supplemented) on fitness, we relied on generalized linear, linear mixed or generalized linear mixed models (GLMs, LMMs or GLMMs, respectively), as summarized in Table 1. Below we provide details on reasons for including specific predictors in models and on coding of dichotomous variables.

To control for intra-seasonal variation in SMI, oxidative and moult status (birds were captured over 38 days, during both incubation and nestling-rearing phases), in GLMs of these variables we included either breeding stage (0 = incubation, 1 = nestling rearing) or sampling date (Julian date; day 1 = January 1) as predictors, choosing the one which better fitted the data (Supplementary material Appendix 1, Table A2) (selected predictors are shown in Table 1). Due to sex differences in body mass [mean body mass: females = 155 g (11 s.d.), males = 135 g (10 s.d.), n = 82 and 76, respectively; $t_{156} = 12.1$, $p < 0.001$; see also Donázar et al. 1992], and consequently in SMI (see Results), in GLMs where SMI was included as a predictor together with sex (0 = female, 1 = male), we centred SMI within each sex category to eliminate the possibility of spurious SMI/sex effects on dependent variables (e.g. Lewin and Mitchell 1999). In GLMs of body condition and oxidative status we included total moult investment among predictors, as moult might affect physiological state (e.g. Hemborg and Lundberg 1998, Rubolini et al. 2002, Costantini 2014). Although adults were food-supplemented for a variable number of days before sampling [mean value = 27 days (s.d. 7)], there was no evidence that such variation affected food supplementation effects on SMI, oxidative and moult status (Supplementary material Appendix 1, Table A3).

Owing to the intrinsic association between laying order and clutch size (only large clutches can have large values of laying order), in LMMs of egg mass and hatching success we coded laying order as relative laying order, assigning value 1 to the first egg and 3 to the last one. Intermediate eggs were assigned values between 1 and 3 according to clutch size (e.g. eggs from a 3-egg clutch were coded 1, 2 and 3; eggs from a 5-egg clutch were coded 1, 1.5, 2, 2.5 and 3). In the egg mass LMMs we included the squared term of relative laying order because the change in egg mass along the laying sequence was best described by a quadratic function of relative laying order (Supplementary material Appendix 1, Table A4). Because food supplementation began after laying of the first egg, the mass of the first egg could not be affected by food supplementation. The effect of food supplementation on egg mass was therefore evaluated as the food supplementation × relative laying order interaction.

The GLM of nest desertion (0 = nest not deserted, 1 = nest deserted) was fitted by including both female and male SMI as a predictor (in separate models by sex): although desertion in related species is always initiated by females (see '*Study species, study area and general field procedures'*), we cannot rule out the possibility that it was initiated by males and depended on male (rather than female) condition.

Primary sex ratio (PSR) was expressed as the ratio between number of sons and brood size, computed for the subset of nests where all eggs hatched. To investigate whether sex allocation varied along the laying sequence according to food supplementation, we fitted a binomial GLMM of nestling sex and tested the food supplementation × rank interaction. Because we could not assign most of the nestlings to their egg of origin, we assumed that laying order was closely reflected by nestling rank, an assumption supported by the strong correlation between these variables (for nestlings from eggs with known laying order; r = 0.86, n = 49).

In LMMs of nestling body mass (recorded at four monitoring sessions) and morphology (tarsus, forearm and feather length recorded at a single session), we included ectoparasite load among predictors (see Podofillini et al. 2018). Age effects were controlled for by including the linear term of age, as growth is mostly linear during the sampled age range (see Podofillini et al. 2018). Nestling mortality was evaluated as a nestling being alive (0) or dead (1) by the fifth monitoring session. Nestlings that disappeared before they were able to move outside nestboxes were assumed to be dead, even if no remains were found (likely removed/eaten by parents/nestmates). In tarsus, forearm and feather length LMMs, and in the mortality GLMM, brood size and ectoparasite load were the maximum values recorded across all monitoring sessions (Podofillini et al. 2018).

Due to weak sex differences in body size (Supplementary material Appendix 1, Table A5; see Podofillini et al. 2018), we did not consider sex in the analyses of nestling body mass, morphology, and mortality to ensure the largest possible sample size (some nestlings could not be sexed due to premature death/disappearance).

Differential effects of food supplementation on target traits according to other model predictors were tested by including two-way interactions between food supplementation and additional predictors in initial models. In the nestling body mass LMM, we also included the rank ×

age interaction (accounting for differential growth of nestlings according to rank; see Podofillini et al. 2018). Final models included all main effects and significant interaction terms, while non-significant ($p > 0.05$) interactions were removed in a single step. Analyses were run in R 3.3.3 (R Core Team 2017). LMMs/GLMMs were fitted using the *lme4* package (Bates et al. 2014). Non-Gaussian models were not overdispersed (see Results). GLMMs overdispersion was computed using the *blmeco* package (Korner-Nievergelt et al. 2015). When GLM residuals showed a highly skewed distribution, significance was calculated by randomization (Manly 1991) (*permuco* package; Frossard and Renaud 2018) (see Table 1). For all models, we report R^2 as computed by the *rsq* (GLMs) and *r2glmm* (GLMMs) packages (Dabao 2017, Jaeger et al. 2017). To facilitate comparisons of food supplementation effects between different models, and to compare effects between different predictors, we report the absolute value of Pearson's r (obtained from the partial correlation/semi-partial R^2 values returned by the *rsq* and *r2glmm* packages).

We considered data from 100 nestboxes (50 food-supplemented, 50 controls), in which we found 423 eggs and captured 160 adults [80 food-supplemented (36 males, 44 females), 80 controls (42 males, 38 females)]. To ensure the largest possible sample size in egg and clutch characteristics models, if SMI was not significant we reported results while excluding this variable. Twenty-two clutches/broods (out of 100) were deserted (13/50 controls, 9/50 food-supplemented). Among these, 16 were deserted at the clutch stage. We recorded data for 288 nestlings from 42 food-supplemented and 43 control broods. Sample size may vary between analyses because of missing data.

Results

Effects of food supplementation on body condition, oxidative and moult status

Extra food did not significantly affect adult body condition and oxidative status (TAC and TOS) (Table 2). Because of large sexual dimorphism in body mass, females had considerably larger SMI than males (Table 2). Moreover, antioxidant defences progressively improved during the breeding season and birds in better body condition had better antioxidant defences, as TAC significantly increased with both sampling date and SMI (Table 2, Fig. 1).

Food supplementation increased resource allocation to primary feather moult. Although the proportion of individuals initiating moult did not significantly differ between treatments [controls = 0.27 (19/70); food-supplemented = 0.38 (28/74), Table 2], the increase of total moult investment with sampling date was significantly larger for food-supplemented individuals [estimate = 4.17 (0.50 s.e.) mm/day] than controls [1.71 (0.43 s.e.) mm/day] (food supplementation × sampling date interaction, Table 2, Fig. 2). Besides, females initiated moult much more frequently (0.52) than males (0.13) (Table 2), and the probability of initiating moult markedly increased with sampling date (Table 2). However, the total moult investment, after accounting for the strong sampling date effect, did not significantly differ between the sexes (Table 2).

Effects of food supplementation on egg mass and hatching success

Food supplementation significantly mitigated the (non-linear) decline in egg mass along the laying sequence observed in control clutches (food supplementation × relative laying order interaction, Table 3), food-supplemented females producing larger last-laid eggs compared to controls (Fig. 3) (see Supplementary material Appendix 1, Table A6 for details of egg mass models selection). The predicted egg mass among last eggs laid by food-supplemented females was 14.58 g, while it was 13.93 g among control females (Fig. 3). Egg mass peaked at relative laying order 0.96 among control (implying a continuous decline within the actual relative laying order values) and 1.54 among food-supplemented females, respectively (Fig. 3).

Extra food allowed females in poor body condition to lay heavier eggs compared to poor condition control females: egg mass significantly increased with SMI among control females [estimate: 0.032 (0.013 s.e.)], whereas no significant association emerged among food-supplemented ones [-0.011 (0.014 s.e.)] (food supplementation × female SMI interaction, Table 3, Fig. 4). Finally, eggs belonging to larger clutches were significantly lighter than those belonging to smaller ones (Table 3). A similarly supported (according to the Akaike Information Criterion value) model of egg mass variation further revealed that food supplementation had stronger positive effects on egg mass among early-breeders than among late-breeders (Supplementary material Appendix 1, Table A7 and Figure A1; see also Supplementary material Appendix 1, Table A6 for more details about fitting of the egg mass models).

Hatching success was not significantly affected by food supplementation (Table 3): the proportion of hatched eggs was 0.82 (146/178) in control clutches and 0.84 (146/173) in food-supplemented ones. In addition, hatching success significantly decreased among late-laid eggs in the laying sequence (Table 3) (see Supplementary material Appendix 1, Table A8 for further details about fitting of the egg hatching success model).

Effects of food supplementation on clutch size, duration of the incubation period, and nest desertion

Extra food did not significantly affect clutch size [food-supplemented females = 4.3 (0.7 s.d.), controls = 4.2 (0.7 s.d.), n = 50 in both groups] and duration of the incubation period [food-supplemented clutches = 32.6 d (2.4 s.d.), controls = 32.5 (2.3 s.d.), n = 41 and 43, respectively], the latter becoming significantly shorter in late-laid clutches compared to early-laid ones (Table 3).

Body condition significantly mediated the effects of food supplementation on the likelihood of nest desertion (food supplementation × female SMI interaction, Table 4): control females in good body condition were less likely to abandon their nests than those with poor body condition [estimate

= -0.147 (0.065 s.e.)], while this was not the case among food-supplemented females [estimate = 0.009 (0.044 s.e.)] (Fig. 5). Hence, in the food-supplemented group, females with low SMI were as likely to desert their nest as those with high SMI (Fig. 5). This analysis could not be performed by including male (instead of female) SMI because we could obtain male SMI data for 5 deserted nests only, all of which were controls. However, when restricting the analyses to control clutches/broods, female SMI significantly negatively predicted the probability of nest desertion, while male SMI did not (Supplementary material Appendix 1, Table A9).

Effects of food supplementation on sex allocation

PSR was slightly male-biased (0.57, n = 72 males and 54 females from 31 complete clutches) but did not significantly deviate from 0.5 (intercept-only binomial GLM, Z = 1.60, p = 0.11). Sex allocation was not significantly affected by food supplementation nor by other predictors (Table 4) (see Supplementary material Appendix 1, Table A10 for additional details of PSR model fitting).

When considering the entire set of sexed nestlings, the proportion of males was 0.48 (n = 123 males and 130 females), again not significantly deviating from 0.5 (intercept-only binomial GLMM with clutch identity as a random effect, Z = 0.46, p = 0.64). Food supplementation did not significantly affect sex allocation along the laying sequence [food supplementation × nestling rank interaction, estimate = -0.31 (0.24 s.e.), Z = -1.29, p = 0.20]. The final model disclosed a strong sex bias along the laying sequence (effect of rank, Table 4), with a female bias among high-ranking nestlings (hatched from first-laid eggs) and a male bias among low-ranking nestlings (hatched from last-laid eggs). A similar tendency emerged also when analysing those nestlings whose egg of origin was known (Supplementary material Appendix 1, Table A10). In this subset, there was no significant difference in mass between male and female eggs, and no significant difference in mass allocation to male and female eggs according to food supplementation (Supplementary material Appendix 1, Table A11).

Effects of food supplementation on nestling body mass, morphology, and mortality

Food supplementation significantly improved nestlings' body mass growth, and significantly mitigated body mass loss induced by haematophagous ectoparasites (Table 5, Fig. 6): the mass increase of food-supplemented nestlings was ca. 10 % greater than controls, a significant difference (food supplementation × age interaction) [food supplemented: 7.17 (0.13 s.e.) g/d; controls: 6.47 (0.12 s.e.) g/d; Table 5], and body mass significantly decreased with parasite load among control nestlings [estimate: -2.62 (0.89 s.e.)], whereas this was not the case among food-supplemented ones [0.16 (0.85 s.e.)] (food supplementation × ectoparasite load interaction, Table 5, Fig. 6). Besides, high ranking nestlings were significantly larger and grew faster than low ranking ones (Table 5), nestlings from late clutches were significantly lighter than those from early clutches, and those from larger clutches were lighter than those from smaller clutches (Table 5).

Skeletal growth was not significantly affected by food supplementation (Table 5). However, food supplementation enhanced forearm growth under highly parasitized conditions (significant food supplementation × ectoparasite load interaction, Table 5): in control nestlings, forearm length did not significantly change with ectoparasite load [estimate: -0.71 (0.57 s.e.)], whereas it significantly increased with ectoparasite load among food-supplemented nestlings [1.11 (0.53 s.e.)] (Fig. 6). Both skeletal traits showed a tendency to be larger in nestlings from larger clutches, after accounting for age and rank effects (Table 5).

Food-supplemented nestlings grew significantly longer feathers than controls at day 16 of age, after accounting for age and rank effects (Table 5).

Overall, 31 % (44/143) control nestlings and 23 % (33/145) food-supplemented ones died by 16 days of age, a non-significant difference (Table 5). Mortality was considerably higher among low ranking nestlings (strong positive effect of rank on mortality) and among those growing in larger clutches (Table 5). Results were qualitatively similar if deserted broods were excluded (Supplementary material Appendix 1, Table A12).

Discussion

In this study of a mesopredatory avian species, we simulated favourable environmental conditions during reproduction by providing extra food to breeders, and assessed the short-term consequences of food supplementation for adult conditions, resource allocation to an energy-demanding process (wing feather moult) competing with breeding, breeding output, and early offspring growth. Below we discuss the main findings.

Moult-breeding overlap and extra food provisioning

Although moult is mostly temporally separated from competing activities such as reproduction and migration (Jenni and Winkler 1994, Barta et al. 2008), in several species, including the migratory lesser kestrel, tight annual scheduling of the yearly cycle has promoted the evolution of moult-breeding overlap (Hemborg and Lundberg 1998, Hemborg 1999, Zuberogoitia et al. 2018). Similarly to other raptors, lesser kestrels start their annual wing feather moult when incubating, females being more likely to do so than males (review in Zuberogoitia et al. 2018). Males, as in most falcons, are smaller, more agile, perform extensive mate-feeding and carry most of the prey to their progeny (Donázar et al. 1992, Krüger 2005): they are thus likely to pay a greater cost than females for the moult-breeding overlap (Espie et al. 1996). Extra food resulted however in greater moult investment in both sexes, after statistically controlling for seasonal effects on timing of moult. The positive effect of food supplementation on wing feather renewal supports the idea that moult-breeding overlap is costly (Hemborg and Lundberg 1998, Saino et al. 2014) and that favourable environmental conditions promote a greater allocation of resources to feather renewal (Espie et al. 1996, Siikamäki 1998, Danner et al. 2014). A greater resource allocation to moulting feathers may either be a direct consequence of food supplementation, with birds investing extra resources in new feathers' synthesis, or a consequence of lower mobility of fed individuals compared to controls, which may have reduced maintenance costs and promoted feather renewal.

Extra food effects on egg traits, nest desertion, and nestling body mass and morphology

Females invested part of resources gained from the extra food in producing heavier last-laid eggs. This result is in line with evidence that egg size is a plastic trait that rapidly responds to the ecological conditions to which the mother is exposed to (Wiebe and Bortolotti 1995, Karell et al. 2008, Saino et al. 2010). Extra food led to a ca. 4 % increase in the mass of last-laid eggs compared to control eggs (see also Christians 2002). Although egg size is a critical trait for offspring fitness soon after hatching (Williams 1994), such a relatively minor increase in egg size did not produce any significant survival/growth advantage of last hatched (low ranking) food-supplemented nestlings compared to controls. The lack of detectable egg mass effects on offspring fitness could be due to the moderately favourable ecological conditions in the study year (in terms of food supplies and weather; authors' pers. obs.), as the effects of extra food provisioning on fitness may vary according to contingent ecological conditions (e.g. Hipkiss et al. 2002, Karell et al. 2008, Ruffino et al. 2014).

Furthermore, extra food allowed poor-condition females to lay eggs that were as large as those laid by good condition ones. Among controls, ca. 16 % of the variation in mean egg mass of a clutch was explained by female body condition (correlation between mean egg mass and female SMI, r = 0.40), in line with previous studies (reviewed by Christians 2002). However, this correlation was heavily modified when females could consume extra food, with variance in egg mass explained by body condition dropping to 1.6 % (r = -0.13). Overall, we conclude that food provisioning positively affected female egg production, and that the benefits of extra food in terms of egg size increase were greater for poor condition females.

A similar finding emerged for the likelihood of nest desertion, which was higher for poor condition control females compared to poor condition food-supplemented ones. Nest desertion is widespread in birds, especially among long-lived species (Székely et al. 1996). It occurs whenever the perceived costs of current reproduction for parents outweigh the expected fitness payoffs of future reproduction (Kelly and Kennedy 1993, Székely et al. 1996), which may be the case under harsh ecological conditions (Anderson et al. 1982, Hörnfeldt et al. 1990, Wiggins et al. 1994, Oppliger et al. 1994) or among low-quality parents with poor body condition (Kelly and Kennedy 1993, Yorio and Boersma 1994, Wiggins et al. 1994). It may also follow from mortality of one parent (Roche et al. 2010, Santema and Kempenaers 2018), which may dramatically increase the costs of current reproduction for the remaining parent in biparental species (Székely et al. 1996). Our results suggest that extra food alleviated the costs of reproduction for females of low phenotypic quality, reducing their probability of completely failing reproduction, in accordance with the hypothesis that nest desertion is the outcome of an adaptive life-history decision conditional on maternal state (Székely et al. 1996).

Among nestlings, extra food resulted in significantly higher mass gain and increased feather growth. Such an effect was most likely due to parents using the extra food to feed their nestlings, as by 10-15 days post hatching nestlings are not able to swallow or split to pieces a relatively large food item (mice) by themselves (Cramp 1998). It also suggests that parents did not markedly reduce nestling provisioning with natural prey items. Furthermore, extra food provisioning affected nestling growth dynamics in combination with ectoparasite infestation. First, it significantly alleviated the negative effects of an haematophagous ectoparasite on mass gain, whereby body mass significantly decreased with ectoparasite load among control nestlings but not among food-supplemented ones. Second, it increased resource allocation to flight apparatus development in highly parasitized nestlings compared to controls. These two results were not due to differences in ectoparasite load between food-supplemented and control nestlings (Supplementary material Appendix 1, Table A13). Haematophagous ectoparasites are well known to exert detrimental effects on early growth dynamics, either by directly withdrawing resources (blood) or by activating the immune system (Møller 1993, Merino and Potti 1995, Saino et al. 1998, Lochmiller and Deerenberg 2000, Nilsson 2003, Tschirren et al. 2003). Under food limitation, parasites may impose a trade-off between allocation of resources to somatic growth and immunity, diverting limiting resources from somatic growth to immune defences (Saino et al. 1998, Lochmiller and Deerenberg 2000, Soler et al. 2003, Brommer 2004, Tschirren and Richner 2006). Extra food may relax such constraints, resulting in weaker direct/indirect negative ectoparasite effects (Merino and Potti 1998, Brommer et al. 2011). Furthermore, growing nestlings may respond to ectoparasites by differentially allocating resources to growth of specific traits in order to increase their short-term fitness prospects (Mainwaring and Hartley 2012). For instance, ectoparasite infestation may promote investment in growth of feathers at the expense of mass or other skeletal traits, because an earlier maturation of the flight apparatus may facilitate escape from the highly parasitized nest environment to avoid detrimental ectoparasite effects (Saino et al. 1998). The observation that ectoparasites significantly promoted resource allocation to growth of the flight apparatus (forearm length) when provisioned with extra food is coherent with the latter suggestion.

Other findings unrelated to extra food provisioning

Non-enzymatic antioxidant defences increased during the breeding season, which may be due to variation in external conditions, diet, or physiological state (Costantini et al. 2010). For instance, the strong increase of ambient temperature during the breeding season (ca. 10°C increase between April and July; data from http://www.ssabasilicata.it/) may improve flight efficiency in the lesser kestrel (Hernández-Pliego et al. 2017) and reduce the oxidative costs of locomotion (e.g. Costantini et al. 2008), promoting mobilization/redistribution of non-enzymatic antioxidants. Seasonal changes in hormone profile (Meijer and Schawbl 1989, Pereira et al. 2010) may also play a role (Costantini et al. 2011, Costantini 2014), whereas this is unlikely for seasonal dietary changes (see Rodríguez et al. 2010), because extra food did not significantly affect oxidative status. In addition, the strong positive covariation of antioxidant defences with body condition indicates that levels of non-enzymatic antioxidants may represent a reliable indicator of general physiological state and individual quality (e.g. Costantini and Bonadonna 2010).

Irrespective of food provisioning, the duration of the incubation period strongly decreased with laying date, a common pattern in birds (e.g. Runde and Barrett 1981, Hipfner et al. 2001, Weiser et al. 2018). In our case, it might be due to seasonal increase in ambient temperature (e.g. Ardia et al. 2006), or differences in incubation behaviour between early- and late-breeders (late-breeders partly compensating for delayed timing by more intense incubation; Hipfner et al. 2001).

Offspring sex ratio was female-biased among early- and male-biased among late-laid eggs. This may suggest that females (the larger sex in terms of adult mass) are more susceptible to harsh rearing environments, and that mothers may adaptively bias sex along the laying sequence in order to provide daughters with a competitive advantage over their sons (due to earlier hatching of early laid eggs; Magrath 1990). In spite of this, no sex difference in nestling mortality was detected, and nestling sex ratio was unbiased, in line with previous studies (Tella et al. 1996, Aparicio and Cordero 2001).

Finally, nestling pre-fledging mortality significantly decreased in birds raising larger broods, suggesting that parents of high quality (in terms of e.g. nestling provisioning or resource acquisition ability) may be able to raise more offspring (van Noordwijk and de Jong 1986).

Concluding remarks

Our comprehensive analysis of the benefits of extra food provisioning to reproduction under natural conditions suggests that parent lesser kestrels invested the extra food mainly in improving current reproduction, similarly to most of the bird species studied so far (Ruffino et al. 2014), rather than using it to accumulate resources (in terms of e.g. body fat or muscle fibres) for improving their residual reproductive value. Indeed, parents mainly used the extra resources for laying larger eggs and raising heavier/larger offspring, while the extra food did not improve their own body condition or oxidative status. The increase of egg size following extra food provisioning suggests that egg formation by females relies partly on resources acquired during the laying period, besides pre-laying food provisioning by the male partner through courtship feeding (Donázar et al. 1992), in line with an ‘income breeding’ strategy of energy storage for reproduction (Jönsson 1997, Meijer and Drent 1999). Our findings indicate that food availability during the breeding season can limit population growth of this species. Importantly, maternal condition appears to be a major trait modulating the benefits of extra food provisioning to reproduction, whereby such benefits accrued mostly to low-quality females with poor body condition. Moreover, extra food reduced the costs of the moult-breeding overlap, and affected early growth trade-offs by fostering development of the flight apparatus traits in response to ectoparasite infestation. To sum up, our results illustrate the pervasive consequences of food limitation in natural environments for resource allocation to competing energy-demanding activities (e.g. moult and reproduction), breeding output and offspring development, at the same time highlighting that the negative consequences of food limitation on reproductive output may be disproportionately larger for individuals of low phenotypic quality.

Data policy/repositories – Data used in statistical analyses will be archived on Dryad upon acceptance of the manuscript.

Legend to figures

Figure 1. Plasma non-enzymatic total antioxidant capacity (TAC) of breeding adults markedly increases with a) sampling date and b) body condition (scaled mass index, SMI; values centered within each sex category; see Material and Methods), irrespective of food supplementation. Black dots: food-supplemented individuals; grey dots: control individuals. The fitted lines (with 95 % confidence bands) are derived from the corresponding model reported in Table 2.

Figure 2. Food supplementation promoted the allocation of resources into renewal and growth of primary feathers (total moult investment; see Material and Methods) in breeding adults. Dot size is proportional to the number of overlapping datapoints, with the smallest dots corresponding to single data, and larger dots proportional to sample size according to the function: dot size = 1 + ln(sample size). The fitted lines (with 95 % confidence bands) are derived from the corresponding model reported in Table 2. Black line and dots: food-supplemented individuals; grey line and dots: control individuals. Data from the two treatment groups were represented with slightly modified x-axis values to reduce overlap and improve clarity.

Figure 3. Egg mass significantly declined along the laying sequence (relative egg laying order, see Material and Methods) in a quadratic fashion in both control and food-supplemented females, but the quadratic function had significantly different peak values for eggs laid by control and food-supplemented females, resulting in heavier last-laid eggs among food-supplemented females compared to controls. The fitted lines (with 95 % confidence bands) are derived from the corresponding model reported in Table 3. Black dots and black line: food-supplemented females; grey dots and grey line: control females. Data from the two treatment groups were represented with slightly modified x-axis values to reduce overlap and improve clarity.

Figure 4. Egg mass significantly increased with body condition (scaled mass index, SMI) among control females (a), while no significant association emerged among food-supplemented females (b). Partial plots accounting for other model effects are shown. The fitted lines (with 95 % confidence bands) are derived from the corresponding model reported in Table 3. Full line: significant slope; dotted line: non-significant slope.

Figure 5. The probability of nest desertion significantly declined with female body condition (scaled mass index, SMI) among control females (a), but not among food-supplemented ones (b). The fitted lines (with 95 % confidence bands) from the corresponding binomial model reported in Table 4 are shown (full line: significant slope; dotted line: non-significant slope). Dots represent original data.

Figure 6. Variation of a) body mass and b) forearm length according to ectoparasite load in control and food-supplemented nestlings (see Material and Methods). Partial plots accounting for other model effects are shown. Black dots and black line: food-supplemented nestlings; grey dots and grey line: control nestlings. The fitted lines (with 95 % confidence bands) are derived from the corresponding models reported in Table 5. Data from the two treatment groups were represented with slightly modified x-axis values to reduce overlap and improve clarity.

Figure 1

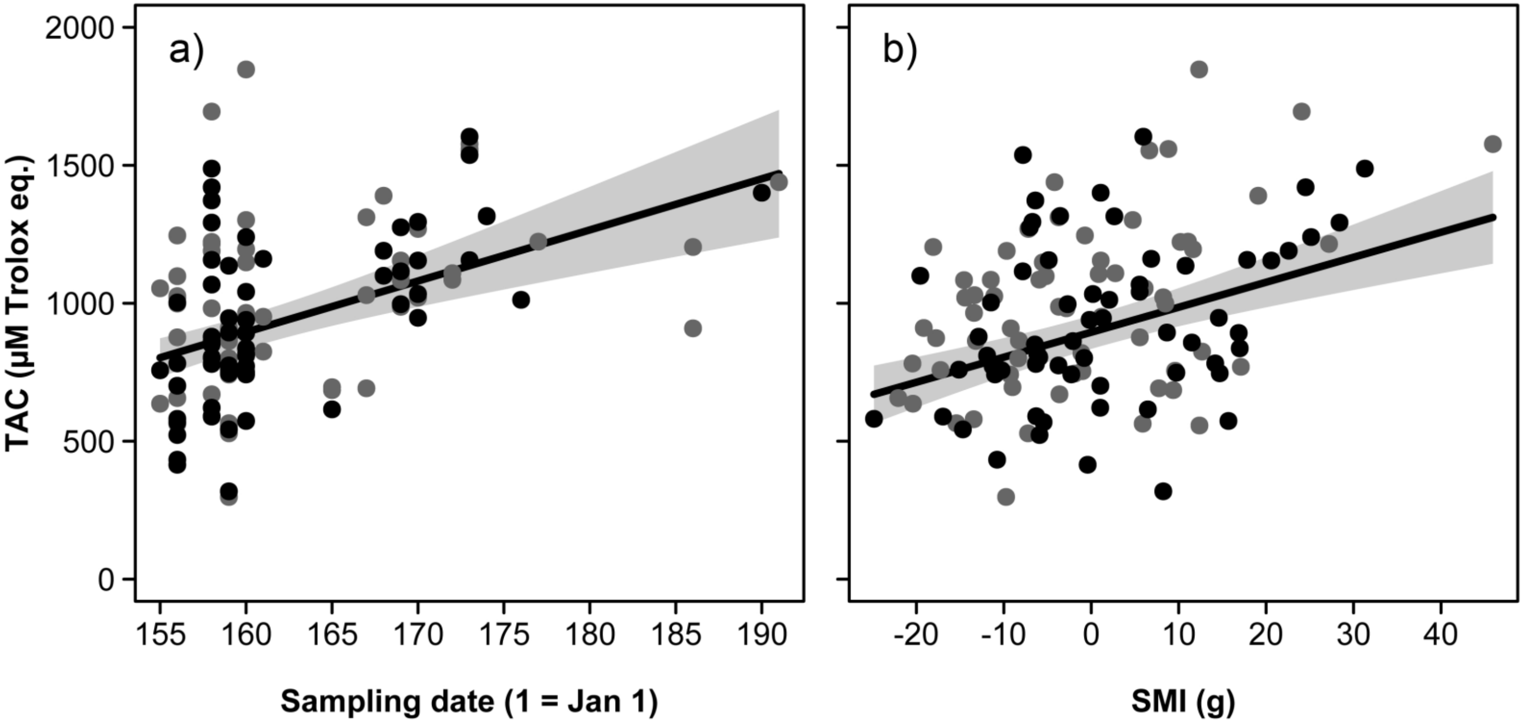

a)
b)
TAC (µM Trolox eq.)
2000
1500
1000
500
0
155
160
165
170
175
180
185
190
Sampling date (1 = Jan 1)
-20
-10
0
10
20
30
40
SMI (g)

Figure 2

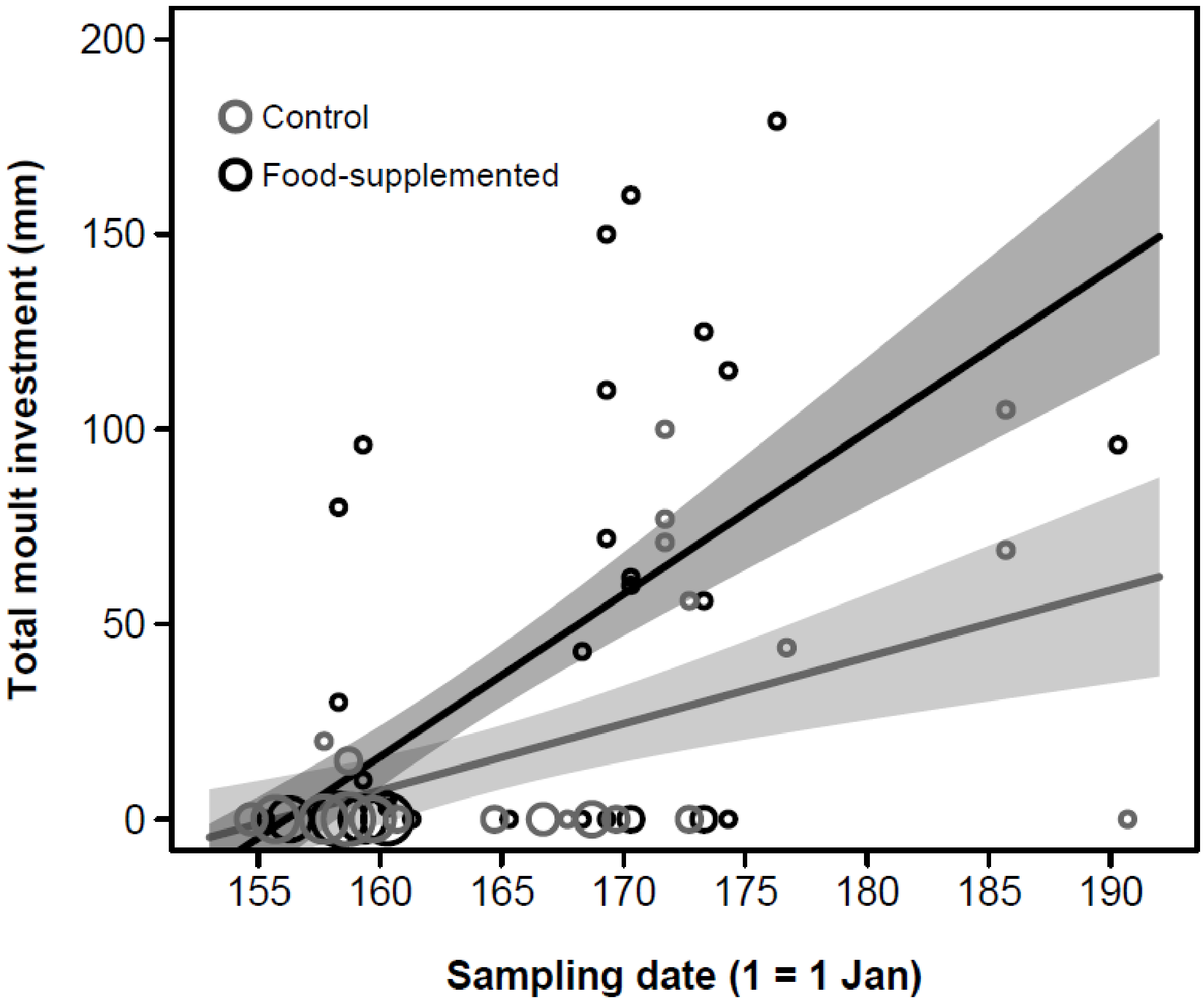

200
150
100
50
0
Total moult investment (mm)
Control
Food-supplemented
155
160
165
170
175
180
185
190
Sampling date (1 = 1 Jan)

Figure 3

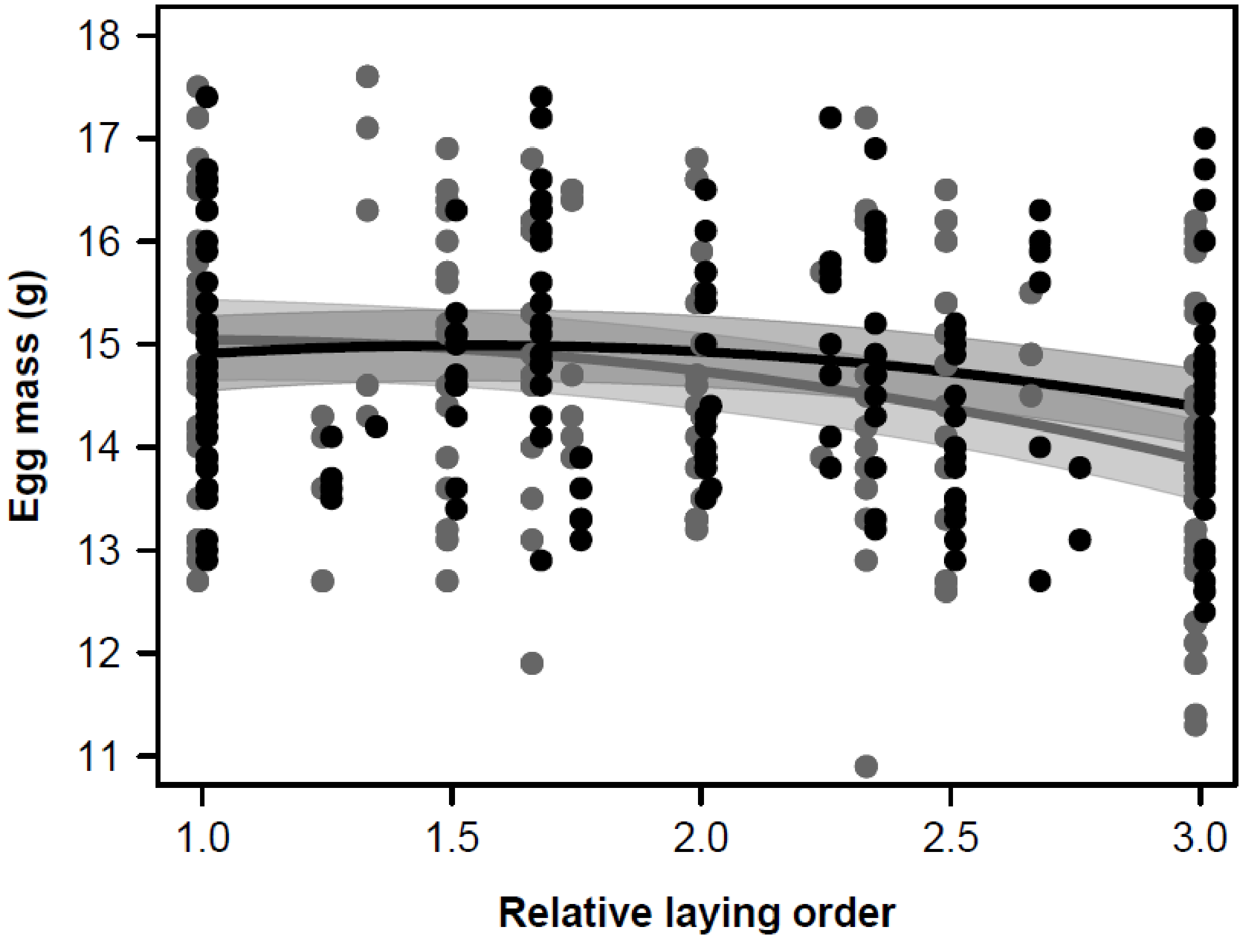

Egg mass (g)
18
17
16
15
14
13
12
11
1.0
1.5
2.0
2.5
3.0
Relative laying order

Figure 4

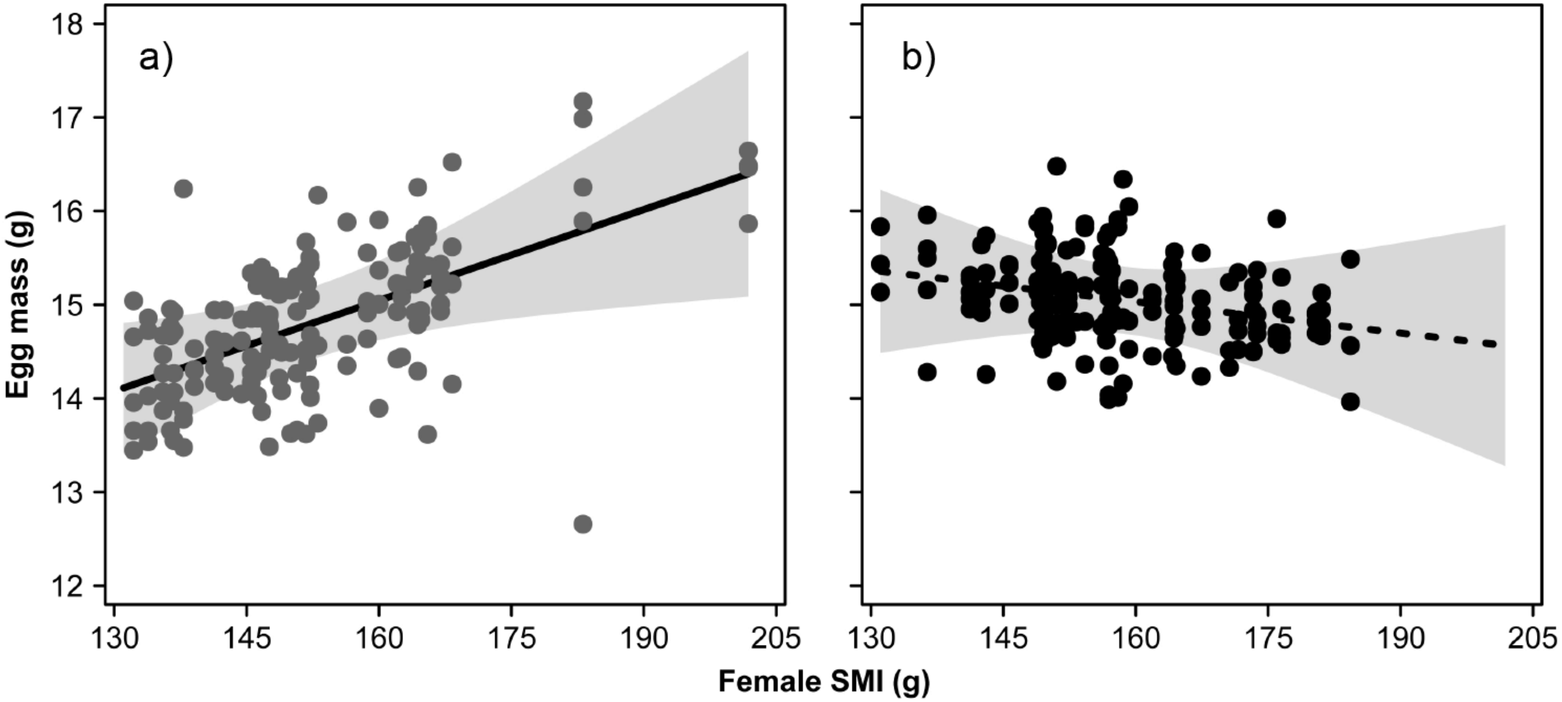

a)
b)
Egg mass (g)
18
17
16
15
14
13
12
130
145
160
175
190
205
Female SMI (g)

Figure 5

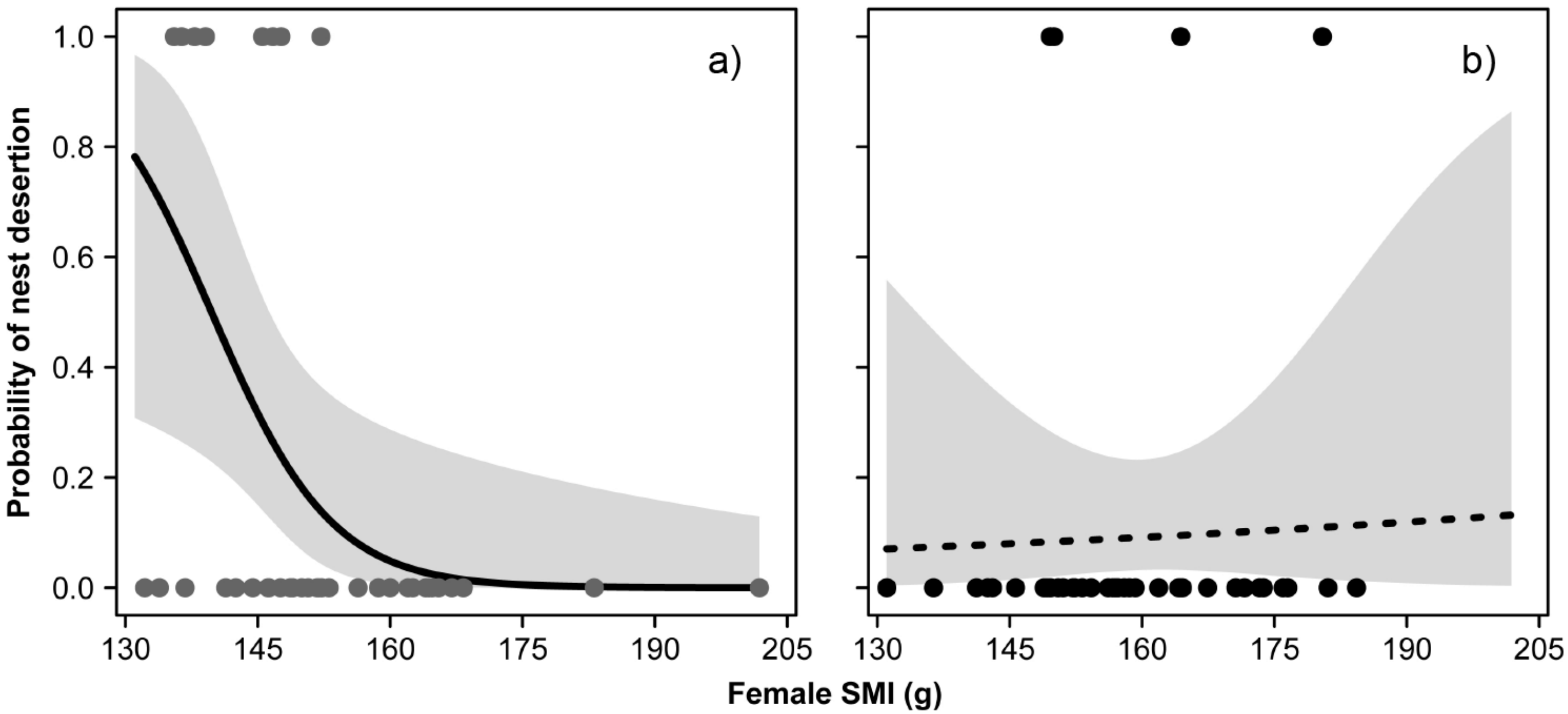

Probability of nest desertion
1.0
0.8
0.6
0.4
0.2
0.0
a)
b)
130
145
160
175
190
205
Female SMI (g)

Figure 6

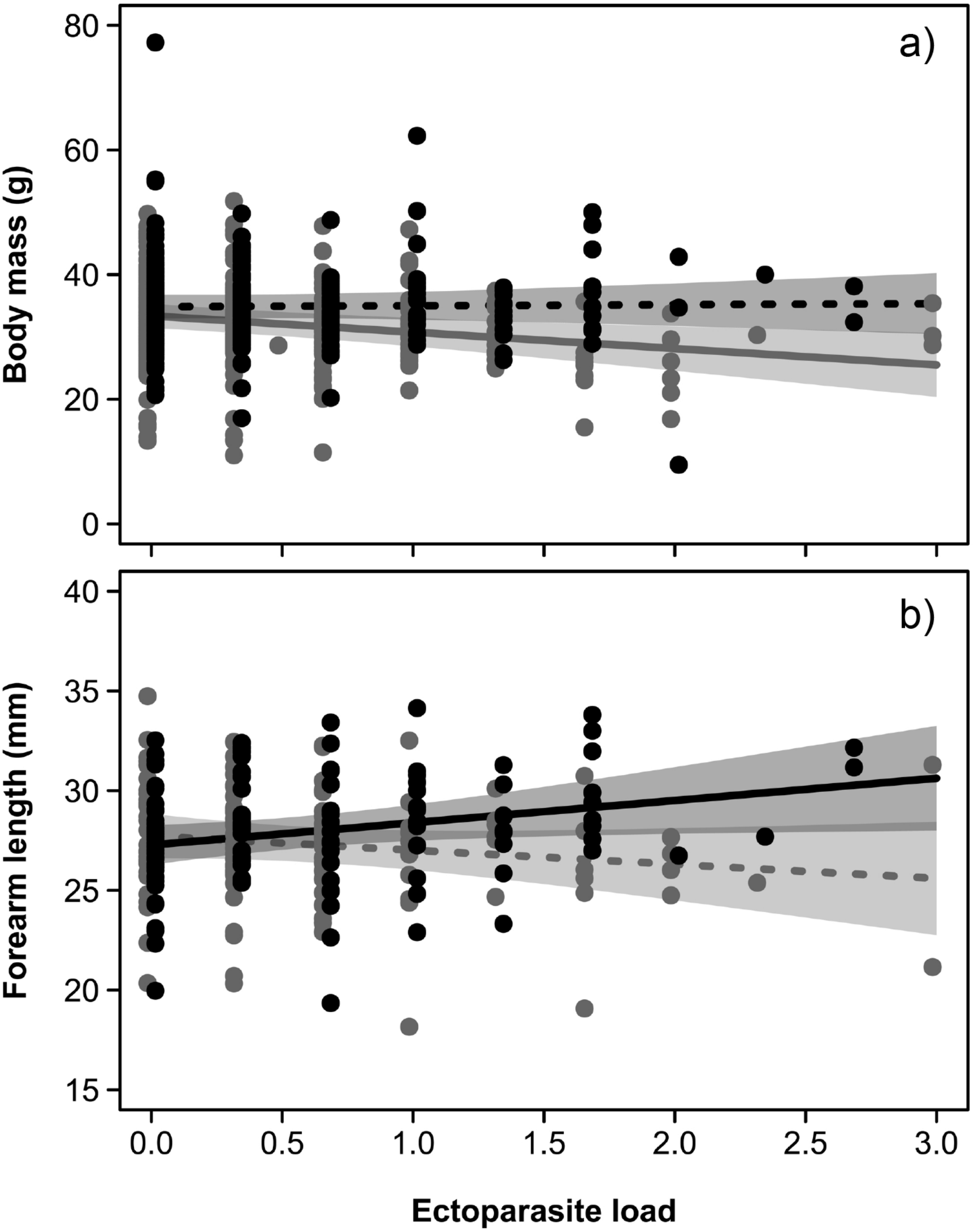

a)
Body mass (g)
0
20
40
60
80
b)
Forearm length (mm)
15
20
25
30
35
40
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Ectoparasite load

Table 1. Summary of the generalized linear (mixed) models fitted to the data to investigate the effects of food supplementation on different fitness components. For consistency, the same sequence of analyses is followed in the Results section. All models included the main effect of food supplementation (0 = control, 1 = food supplemented). Initial models included all two-way interactions between food supplementation and each additional predictors (see Statistical analyses for further details). Non-significant ($p > 0.05$) interactions were removed from initial models in a single step. The final models reported in Tables 2-5 thus included all main fixed effects and any statistically significant interaction. Random intercept effects were included in LMMs/GLMMs in order to account for non-independence of data belonging to the same clutch/brood and for repeated sampling of the same individual (in the nestling body mass model).

Table 1

Dependent variable	Additional predictors (confounding variables)	Random intercept effects	Error distribution	Details of fitted model	Notes
Effects of food supplementation on body condition, oxidative and moult status					
Body condition (SMI)	Sex, breeding stage, total moult investment	-	Gaussian	Table 2	
Total antioxidant status (TAC)	Sex, sampling date, SMI, total moult investment	-	Gaussian	Table 2	
Total oxidant status (TOC)	Sex, sampling date, SMI, total moult investment	-	Gaussian	Table 2	
Moult initiation	Sex, sampling date, SMI	-	Binomial	Table 2	
Total moult investment	Sex, sampling date, SMI	-	Gaussian	Table 2	Skewed residuals
Effects of food supplementation on egg mass and hatching success					
Egg mass	Relative laying order, (relative laying order)^2, laying date, clutch size, female SMI	Clutch identity	Gaussian	Table 3	
Egg hatching success	Relative laying order, laying date, clutch size, female SMI	Clutch identity	Binomial	Table 3	Deserted nests excluded
Effects of food supplementation on clutch size, duration of the incubation period, and nest desertion					
Clutch size	Laying date, female SMI	-	Gaussian	Table 4	
Duration of the incubation period	Laying date, clutch size, female SMI	-	Gaussian	Table 4	Skewed residuals
Nest desertion	Laying date, clutch size, female or male SMI	-	Binomial	Table 4	
Effects of food supplementation on sex allocation					
Primary sex ratio (PSR)	Laying date, clutch size, female SMI	Clutch identity	Binomial	Table 4	
Nestling sex	Rank, laying date	Clutch identity	Binomial	Table 4	
Effects of food supplementation on nestling body mass, morphology, and mortality					
Body mass	Age, rank, laying date, brood size, ectoparasite load	Brood identity, nestling identity	Gaussian	Table 5	
Tarsus length	Age, rank, laying date, brood size, ectoparasite load	Brood identity	Gaussian	Table 5	
Forearm length	Age, rank, laying date, brood size, ectoparasite load	Brood identity	Gaussian	Table 5	
Feather length	Age, rank, laying date, brood size, ectoparasite load	Brood identity	Gaussian	Table 5	
Mortality	Rank, laying date, brood size, ectoparasite load	Brood identity	Binomial	Table 5	

Table 2. Generalized linear models of the effects of food supplementation on body condition (scaled mass index, SMI), oxidative (TAC, TOS) and moult status (probability of initiating moult, total moult investment); the coding of the sex variale is 0 = female, 1 = male; t-values are reported as test statistics for Gaussian models, Z-values for the binomial model of moult initiation.

Predictors	Estimate (s.e.)	t/Z	p	Effect size r
SMI (n = 144) (R^2 = 0.43)				
Food supplementation	2.86 (2.16)	1.32	0.19	0.11
Sex	-21.72 (2.21)	9.83	< 0.001	0.64
Breeding stage	-3.66 (2.60)	1.41	0.16	0.12
Total moult investment	-0.02 (0.03)	0.58	0.56	0.05
TAC (n = 126) (R^2 = 0.38)				
Food supplementation	-65.62 (45.74)	1.43	0.15	0.13
Sex	5.30 (45.47)	0.12	0.91	0.01
Sampling date	18.52 (3.66)	5.04	< 0.001	0.42
SMI[a]	9.06 (1.74)	5.20	< 0.001	0.43
Total moult investment	0.23 (0.75)	0.31	0.76	0.03
TOS (n = 71) (R^2 = 0.04)				
Food supplementation	0.41 (0.42)	0.99	0.33	0.12
Sex	-0.123 (0.41)	0.32	0.75	0.04
Sampling date	-0.03 (0.04)	0.74	0.47	0.09
SMI[a]	-0.01 (0.02)	0.88	0.38	0.11
Total moult investment	0.003 (0.008)	0.34	0.74	0.04
Probability of initiating moult (n = 144)[b] (R^2 = 0.30)				
Food supplementation	0.64 (0.44)	1.46	0.15	0.14
Sex	-1.83 (0.45)	4.04	< 0.001	0.35
Sampling date	0.13 (0.04)	3.71	< 0.001	0.38
SMI[a]	-0.01 (0.02)	0.73	0.47	0.07
Total moult investment (n = 144)[c] (R^2 = 0.43)				
Food supplementation[d]	14.05 (4.61)	3.05	0.003	0.25
Sex	-7.35 (4.67)	1.57	0.12	0.13
Sampling date[d]	2.97 (0.34)	8.84	< 0.001	0.60
SMI[a]	-0.28 (0.18)	1.57	0.12	0.13
Food supplementation × sampling date	2.46 (0.65)	3.76	< 0.001	0.30

a: variable group-centered within sex categories (see Statistical analyses)
b: binomial GLM
c: p-values from randomization test (see Statistical analyses)
d: estimate for mean-centered covariate

Table 3. Mixed models of the effects of food supplementation on egg mass and hatching success. Degrees of freedom for F-tests of the egg mass linear mixed model (LMM) were estimated according to the Kenward-Roger's approximation; Z-values are reported as test statistics for the egg hatching success binomial generalized linear mixed model.

Predictors	Estimate (s.e.)	F/Z	d.f.	p	Effect size r
Egg mass (n = 349 eggs, n = 82 clutches) (R^2 = 0.30)					
Food supplementation[a]	0.19 (0.25)	0.59	76	0.45	0.08
Relative laying order[a]	0.72 (0.31)	5.21	265	0.023	0.06
(Relative laying order^2)[a]	-0.28 (0.08)	13.29	265	< 0.001	0.10
Laying date	-0.01 (0.02)	0.31	77	0.58	0.05
Clutch size	-0.50 (0.18)	7.48	77	0.008	0.25
Female SMI[a]	0.01 (0.01)	0.76	76	0.39	0.09
Food supplementation × relative laying order	0.33 (0.09)	13.12	264	< 0.001	0.09
Food supplementation × female SMI	-0.04 (0.02)	5.14	75	0.026	0.23
Egg hatching success (n = 351 eggs, n = 83 clutches)[b] (R^2 = 0.03)					
Food supplementation	0.12 (0.39)	0.31	-	0.76	< 0.01
Relative laying order	-0.69 (0.23)	3.04	-	0.002	0.17
Laying date	0.01 (0.03)	0.30	-	0.77	< 0.01
Clutch size	0.03 (0.30)	0.09	-	0.93	< 0.01
Female SMI[c]	-0.01 (0.01)	0.39	-	0.69	< 0.01

a: estimate for mean-centered covariate
b: dispersion parameter = 0.88
c: estimate from a different model (sample size: n = 302 eggs, n = 71 clutches; see Statistical analyses)

Table 4. Generalized linear models (GLMs) of the effects of food supplementation on clutch size, duration of the incubation period, nest desertion, and primary sex ratio (PSR, proportion of males in a brood), and binomial generalized mixed model (GLMM) of the probability of a nestling being male; t-values are reported as test statistics for Gaussian GLMs, Z-values for binomial models.

Predictors	Estimate (s.e.)	t/Z	p	Effect size r
Clutch size (n = 100 clutches) (R^2 = 0.01)				
Food supplementation	0.10 (0.14)	0.74	0.46	0.07
Laying date	-0.01 (0.01)	0.79	0.43	0.08
Female SMI[a]	-0.01 (0.01)	0.62	0.54	0.07
Duration of the incubation period (n = 84 clutches)[b] (R^2 = 0.15)				
Food supplementation	0.07 (0.48)	0.14	0.89	0.02
Laying date	-0.11 (0.03)	3.40	0.001	0.36
Clutch size	0.53 (0.35)	1.50	0.14	0.17
Female SMI[c]	-0.01 (0.02)	0.23	0.82	0.03
Probability of nest desertion (n = 82 clutches) (R^2 = 0.19)				
Food supplementation[d]	-0.04 (1.01)	0.04	0.97	0.03
Laying date	0.07 (0.05)	1.37	0.17	0.25
Clutch size	0.10 (0.57)	0.18	0.86	0.08
Female SMI[d]	-0.06 (0.04)	1.58	0.11	0.17
Food supplementation × female SMI	0.16 (0.08)	2.07	0.038	0.23
PSR (n = 31 broods)[e] (R^2 = 0.06)				
Food supplementation	-0.36 (0.38)	0.95	0.34	0.16
Laying date	-0.01 (0.03)	0.37	0.71	0.07
Clutch size	-0.17 (0.27)	0.62	0.54	0.12
Female SMI[f]	0.01 (0.02)	0.18	0.86	0.02
Probability of a nestling being male (n = 253 nestlings, n = 80 broods)[g] (R^2 = 0.05)				
Food supplementation	-0.31 (0.27)	1.15	0.25	0.07
Rank	0.38 (0.12)	3.23	0.001	0.21
Laying date	-0.01 (0.02)	0.22	0.82	0.01

a: estimate from a different model with smaller sample size (n = 82 clutches; see Statistical analyses)
b: p-values are from a randomization test (see Statistical analyses)
c: estimate from a different model with smaller sample size (n = 72 clutches; see Statistical analyses)
d: estimate for mean-centered covariate
e: dispersion parameter = 1.23; PSR expressed as n males/brood size
f: estimate from a different model with smaller sample size (n = 25 broods; see Statistical analyses)
g: dispersion parameter = 1.15

Table 5. Mixed models of the effects of food supplementation on nestling body mass (g), morphology [tarsus, forearm and feather length (mm)], and mortality. Degrees of freedom for F-tests were estimated according to the Kenward-Roger’s approximation.

Predictors	Estimate (s.e.)	F/Z	d.f.	p	Effect size r
Body mass (n= 288 nestlings, n = 85 broods) ($R^2 = 0.87$)					
Food supplementation[a]	2.27 (1.22)	3.50	1, 79	0.07	0.16
Age[a]	6.47 (0.12)	5836.60	1, 767	< 0.001	0.92
Rank[a]	-3.99 (0.33)	147.80	1, 238	< 0.001	0.46
Laying date	-0.23 (0.08)	7.60	1, 95	0.007	0.18
Brood size	-1.38 (0.43)	10.30	1, 519	0.001	0.16
Ectoparasite load[a]	-1.20 (0.62)	3.70	1, 902	0.05	0.07
Food supplementation × age	0.69 (0.17)	16.30	1, 754	< 0.001	0.12
Food supplementation × ectoparasite load	2.78 (1.22)	5.20	1, 901	0.023	0.09
Age × rank	-1.00 (0.08)	171.50	1, 806	< 0.001	0.37
Tarsus length (n = 249 nestlings, n = 79 broods) ($R^2 = 0.66$)					
Food supplementation	0.62 (0.39)	2.56	1, 74	0.11	0.15
Age	1.54 (0.11)	179.32	1, 227	< 0.001	0.70
Rank	-0.96 (0.12)	62.66	1, 214	< 0.001	0.41
Laying date	-0.03 (0.03)	1.16	1, 81	0.28	0.10
Brood size	0.37 (0.19)	3.86	1, 95	0.052	0.16
Ectoparasite load	0.21 (0.24)	0.73	1, 238	0.39	0.06
Forearm length (n = 250 nestlings, n = 79 broods) ($R^2 = 0.70$)					
Food supplementation[a]	0.64 (0.54)	1.36	1, 72	0.24	0.09
Age	2.74 (0.19)	213.55	1, 197	< 0.001	0.72
Rank	-1.64 (0.21)	58.31	1, 224	< 0.001	0.42
Laying date	0.01 (0.04)	0.11	1, 83	0.74	0.03
Brood size	0.57 (0.28)	4.23	1, 102	0.042	0.15
Ectoparasite load[a]	0.26 (0.40)	0.41	1, 192	0.52	0.05
Food supplementation × ectoparasite load	1.82 (0.76)	5.61	1, 195	0.019	0.17
Feather length (n = 186 nestlings, n = 69 broods) ($R^2 = 0.61$)					
Food supplementation	2.10 (1.04)	4.05	1, 59	0.049	0.19
Age	4.60 (0.45)	103.59	1, 124	< 0.001	0.66
Rank	-2.75 (0.40)	47.68	1, 150	< 0.001	0.42
Laying date	-0.06 (0.09)	0.42	1, 77	0.52	0.06
Brood size	0.53 (0.51)	1.07	1, 82	0.30	0.09
Ectoparasite load	0.46 (0.79)	0.33	1, 154	0.57	0.05
Mortality (n = 285 nestlings, n = 85 broods)[b] ($R^2 = 0.17$)					
Food supplementation	-0.68 (0.54)	1.25	-	0.21	0.09
Rank	1.32 (0.24)	5.50	-	< 0.001	0.37
Laying date	-0.02 (0.04)	0.47	-	0.64	0.04
Brood size	-0.61 (0.28)	2.19	-	0.029	0.18
Ectoparasite load	-0.63 (0.40)	1.56	-	0.12	0.10

a: estimate for mean-centered covariate
b: dispersion parameter = 0.83

References

Alan, R. R. and McWilliams, S. R. 2013. Oxidative stress, circulating antioxidants, and dietary preferences in songbirds. – Comp. Biochem. Physiol. 164: 185-193.

Anderson, D. W. et al. 1982. Brown pelicans: influence of food supply on reproduction. – Oikos 39: 23-31.

Aparicio, J. M. 1997. Cost and benefits of surplus offspring in the lesser kestrel (*Falco naumanni*). – Behav. Ecol. Sociobiol. 41: 129–137.

Aparicio, J. M. and Bonal, R. 2002. Effects of food supplementation and habitat selection on timing of lesser kestrel breeding. – Ecology 83: 873-877.

Aparicio, J. M. and Cordero, P. J. 2001. The effects of the minimum threshold condition for breeding on offspring sex-ratio adjustment in the lesser kestrel. – Evolution 55: 1188-1197.

Ardia, D. R. et al. 2006. Warm temperatures lead to early onset of incubation, shorter incubation periods and greater hatching asynchrony in tree swallows *Tachycineta bicolor* at the extremes of their range. – J. Avian Biol. 37: 137-142.

Bates, D. et al. 2014. Fitting linear mixed-effects models using lme4. – J. Stat. Softw. 67: 1–48.

Barta, Z. et al. 2008. Optimal moult strategies in migratory birds. – Phil. Trans. R. Soc. B 363: 211–229.

Bize, P. et al. 2008. Fecundity and survival in relation to resistance to oxidative stress in a free-living bird. – Ecology 89: 2584–2593.

Boutin, S. 1990. Food supplementation experiments with terrestrial vertebrates: patterns, problems, and the future. – Can. J. Zool. 68: 203-220.

Brommer, J. E. 2004. Immunocompetence and its costs during development: an experimental study in blue tit nestlings. – Proc. R. Soc. B 271: S110-S113.

Brommer, J. E. et al. 2011. Body size and immune defense of nestling Blue Tits (*Cyanistes caeruleus*) in response to manipulation of ectoparasites and food supply. – Auk 128: 556-563.

Capelle, K. J. and Whitworth, T. L. 1973. The distribution and avian hosts of *Carnus hemapterus* (Diptera: Milichiidae) in North America. – J. Med. Entomol. 10: 525–526.

Catry, I. et al. 2016. Sexual and parent-offspring dietary segregation in a colonial raptor as revealed by stable isotopes. – J. Zool. 299: 58-67.

Cecere, J. G. et al. 2018. Spatial segregation of foraging areas between neighbouring colonies in a diurnal raptor. – Sci. Rep. 8: 11762.

Christians, J. K. 2002. Avian egg size: variation within species and inflexibility within individuals. – Biol. Rev. 77: 1-26.

Costantini, D. et al. 2008. Long flights and age affect oxidative status of homing pigeons (*Columba livia*). – J. Exp. Biol. 211: 377-381.

Costantini, D. and Bonadonna, F. 2010. Patterns of variation of serum oxidative stress markers in two seabird species. – Polar Res. 29: 30-35.

Costantini, D. et al. 2010. Relationships among oxidative status, breeding conditions and life-history traits in free-living Great Tits *Parus major* and Common Starlings *Sturnus vulgaris*. – Ibis 152: 793-802.

Costantini, D. et al. 2011. A meta-analysis of glucocorticoids as modulators of oxidative stress in vertebrates. – J. Comp. Physiol. B 181: 447-456.

Costantini, D. 2014. Oxidative stress and hormesis in evolutionary ecology and physiology. A marriage between mechanistic and evolutionary approaches. – Springer.

Cramp, S. 1998. The complete birds of the western Palearctic on CDROM. – Oxford University Press.

Cucco, M. and Malacarne, G. 1997. The effect of supplemental food on time budget and body condition in the black redstart *Phoenicurus ochruros*. – Ardea 85: 211-221.

Dabao, Z. 2017. A coefficient of determination for Generalized Linear Models. – Am. Stat. 71: 310-316.

Danner, M. R. et al. 2014. Winter food limits timing of pre-alternate moult in a short-distance migratory bird. – Funct. Ecol. 29: 259-267.

Dawson, R. and Bortolotti, G. 2002. Experimental evidence for food limitation and sex-specific strategies of American kestrel (*Falco sparvierus*) provisioning offspring. – Behav. Ecol. Sociobiol. 52: 43-52.

Dewey, S. R. and Kennedy, P. L. 2001. Effects of supplemental food on parental-care strategies and juvenile survival of Northern Goshawks. – Auk 118: 352-365.

Di Maggio, R., Campobello, D. and Sarà, M. 2018. Lesser kestrel diet and agricultural intensification in the Mediterranean: An unexpected win-win solution? J. Nat. Conserv. 45: 122-130.

Dietz, M. W. et al. 1992. Energy requirements for molt in the kestrel *Falco tinnunculus*. Physiol. Zool. 65: 1217-1235.

Donázar, J. A. et al. 1992. Functional analysis of mate-feeding in the Lesser Kestrel *Falco naumann*i. – Ornis Scand. 23: 190-194.

Erel, O. 2004. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. – Clin. Biochem. 37: 277-285.

Erel, O. 2005. A new automated colorimetric method for measuring total oxidant status. – Clin. Biochem. 8: 1103–1111.

Espie, R.H. et al. 1996. Ecological correlates of molt in Merlins (*Falco columbarius*). – Auk 113: 363-369.

Fletcher, Q. E. et al. 2013. Oxidative damage increases with reproductive energy expenditure and is reduced by food-supplementation. – Evolution 67: 1527-1536.

Frossard, J. and Renaud, O. 2018. Permutation tests for regression, ANOVA and comparison of signals: the permuco package. – https://cran.r-project.org/web/packages/permuco/index.html

Garcia, P. F. J. et al. 1993. Energy allocation to reproduction and maintenance in Mountain Bluebirds (*Sialia currucoides*): a food supplementation experiment. – Can. J. Zool. 71: 2352-2357.

Giordano, M. et al. 2015. Female oxidative status, egg antioxidant protection and eggshell pigmentation: a supplemental feeding experiment in great tits. – Behav. Ecol. Sociobiol. 69: 777-785.

Gonzalez, L. M. et al. 2006. Supplementary feeding as an effective tool for improving breeding success in the Spanish imperial eagle (*Aquila adalberti*). – Biol. Conserv. 129: 477-486.

Griffiths, R. et al. 1998. A DNA test to sex most birds. – Molec. Ecol. 7: 1071–1075.

Halliwell, B. and Gutteridge, J. M. C. 2007. Free Radicals in Biology and Medicine. – Oxford University Press.

Hemborg, C. 1999. Sexual differences in moult–breeding overlap and female reproductive costs in pied flycatchers, *Ficedula hypoleuca*. – J. Anim. Ecol. 68: 429-436.

Hemborg, C. and Lundberg, A. 1998. Costs of overlapping reproduction and moult in passerine birds: an experiment with the pied flycatcher. – Behav. Ecol. Sociobiol. 43: 19-23.

Hernández-Pliego, J. et al. 2017. Combined use of tri-axial accelerometers and GPS reveals the flexible foraging strategy of a bird in relation to weather conditions. – PloS ONE 12: e0177892.

Hipfner, J. et al. 2001. Seasonal declines in incubation periods of Brunnich's guillemots *Uria lomvia*: testing proximate causes. – Ibis 143: 92-98.

Hipkiss, T et al. 2002. Year-dependent sex-biased mortality in supplementary-fed Tengmalm’s owl nestlings. – J. Anim. Ecol. 71: 693-699.

Hörnfeldt, B. et al. 1990. Effects of cyclic food supply on breeding performance in Tengmalm's owl (*Aegolius funereus*). – Can. J. Zool. 68: 522-530.

Jaeger, B. C. et al. 2017. An R^2 statistic for fixed effects in the generalized linear mixed model. – J. Appl. Stat. 44: 1086-1105.

Jenni, L. and Winkler, R. 1994. Moult and ageing of European passerines. – Academic Press.

Jönsson, K. I. 1997. Capital and income breeding as alternative tactics of resource use in reproduction. – Oikos 78: 57-66.

Kacelnik, A. and Cuthill, I. C. 1990. Central place foraging in starlings (*Sturnus vulgaris*). II. Food allocation to chicks. – J. Anim. Ecol. 59: 655–674.

Karell, P. et al. 2008. Maternal effects on offspring Igs and egg size in relation to natural and experimentally improved food supply. – Funct. Ecol. 22: 682-690.

Kelly, E. J. and Kennedy, P. L. 1993. A dynamic state variable model of mate desertion in Cooper's hawks. – Ecology 74: 351-366.

Korner-Nievergelt, F. et al. 2015. Bayesian data analysis in ecology using linear models with R, BUGS, and Stan. – Academic Press.

Korpimäki, E. and Wiehn, J. 1998. Clutch size of kestrels: seasonal decline and experimental evidence for food limitation under fluctuating food conditions. – Oikos 83: 259–272.

Korpimäki, E. et al. 2000. Environmental-and parental condition-related variation in sex ratio of kestrel broods. – J. Avian Biol. 31: 128-134.

Krüger, O. 2005. The evolution of reversed sexual size dimorphism in hawks, falcons and owls: a comparative study. – Evol. Ecol. 19: 467-486.

Lack, D. 1954. The natural regulation of animal numbers. - Oxford Univ. Press.

Lack, D. 1966. Population studies of bird. – Clarendon Press.

La Gioia, G. et al. 2017. Piano d’Azione Nazionale per il grillaio (*Falco naumanni*). – MATTM-ISPRA.

Lewin, A. C. and Mitchell, M. N. 1999. Using group mean centering for computing adjusted means by site in a randomized experimental design: the case of California's Work Pays Demonstration Project. – Evaluation Rev. 23: 146-161.

Lochmiller, R. L. and Deerenberg, C. 2000. Trade-offs in evolutionary immunology: just what is the cost of immunity? – Oikos 88: 87-98.

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

Mainwaring, M. C. and Hartley, I. R. 2012. Causes and consequences of differential growth in birds: a behavioral perspective. – Adv. Stud. Behav. 44: 225-277.

Manly, B. F. J. 1991. Randomization, Bootstrap and Monte Carlo Methods in Biology. – CRC Press.

Markman, S. et al. 2002. The manipulation of food resources reveals sex–specific trade–offs between parental self-feeding and offspring care. – Proc. R. Soc. B 269: 1931-1938.

Martin, T. E. 1987. Food as a limit on breeding birds: a life-history perspective. – Annu. Rev. Ecol. Syst. 18: 453-487.

Meijer, T. and Schwabl, H. 1989. Hormonal patterns in breeding and nonbreeding kestrels, *Falco tinnunculus*: field and laboratory studies. – Gen. Comp. Endocrinol. 74: 148-160.

Meijer, T. and Drent, R. 1999. Re-examination of the capital and income dichotomy in breeding birds. – Ibis 141: 399–414.

Merino, S. and Potti, J. 1995. Mites and blowflies decrease growth and survival in nestling pied flycatchers. – Oikos 73: 95-103.

Merino, S. and Potti, J. 1998. Growth, nutrition, and blow fly parasitism in nestling Pied Flycatchers. – Can. J. Zool. 76: 936-941.

Møller, A. P. 1993. Ectoparasites increase the cost of reproduction in their hosts. – J. Anim. Ecol. 62: 309-322.

Murphy, M. E. 1996. Energetics and nutrition in molt. – In: Carey, C. (ed.), Avian Energetics and Nutritional Ecology. Chapman and Hall, pp. 158–198.

Nager, R. G. et al. 1999. Experimental demonstration that offspring sex ratio varies with maternal condition. – Proc. Natl Acad. Sci. USA 96: 570–573.

Newton, I. and Marquiss, M. 1984. Seasonal trend in the breeding performance of Sparrowhawks. – J. Anim. Ecol. 53: 809-829.

Nilsson, J.-Å. 2003. Ectoparasitism in marsh tits: costs and functional explanations. Behav. Ecol. 14: 175–181.

Oppliger, A. et al. 1994. Effect of an ectoparasite on lay date, nest-site choice, desertion, and hatching success in the great tit (*Parus major*). – Behav. Ecol. 5: 130-134.

Peig, J. and Green, A. J. 2009. New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. – Oikos 118: 1883-1891.

Peig, J. and Green, A. J. 2010. The paradigm of body condition: a critical reappraisal of current methods based on mass and length. – Funct. Ecol. 24: 1323–1332.

Pereira, R. J. G. et al. 2010. Annual profile of fecal androgen and glucocorticoid levels in free-living male American kestrels from southern mid-latitude areas. – Gen. Comp. Endocrinol. 166: 94-103.

Podofillini, S. et al. 2018. Home, dirty home: effect of old nest material on nest-site selection and breeding performance in a cavity-nesting raptor. – Curr. Zool. (doi: 10.1093/cz/zoy012).

R Core Team, 2017. R: A Language and Environment for Statistical Computing. – R Foundation for Statistical Computing.

Roche, E. A. et al. 2010. Apparent nest abandonment as evidence of breeding-season mortality in Great Lakes Piping Plovers (*Charadrius melodus*). – Auk 127: 402-410.

Roff, D. A. 1992. The evolution of life histories: theory and analysis. – Chapman and Hall.

Rodríguez, C. et al. 2010. Temporal changes in lesser kestrel (*Falco naumanni*) diet during the breeding season in southern Spain. – J. Rapt. Res. 44: 120-128.

Rubolini, D. et al. 2002. Replacement of body feathers is associated with low pre-migratory energy stores in a long-distance migratory bird, the barn swallow (*Hirundo rustica*). – J. Zool. 258: 441-447.

Ruffino, L. et al. 2014. Reproductive responses of birds to experimental food supplementation: a meta-analysis. – Front. Zool. 11: 80-93.

Runde, O. J. and Barrett, R.T. 1981. Variations in egg size and incubation period of the Kittiwake *Rissa tridactyla* in Norway. – Ornis Scand. 12: 80-86.

Saino, N. et al. 1998. Effects of a dipteran ectoparasite on immune response and growth trade-offs in barn swallow (*Hirundo rustica*) nestlings. – Oikos 81: 217–228.

Saino, N. et al. 2010. Sex allocation in yellow-legged gulls (*Larus michahellis*) depends on nutritional constraints on production of large last eggs. – Proc. R. Soc. B 277: 1203-1208.

Saino, N. et al. 2011. Antioxidant defenses predict long-term survival in a passerine bird. – PLoS ONE 6: e19593.

Saino, N. et al. 2014. A trade-off between reproduction and feather growth in the barn swallow (*Hirundo rustica*). – PloS ONE 9: e96428.

Santema, P. and Kempenaers, B. 2018. Complete brood failure in an altricial bird is almost always associated with the sudden and permanent disappearance of a parent. – J. Anim. Ecol. (doi: 10.1111/1365-2656.12848).

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

Schoech, S. J. 1996. The effect of supplemental food on body condition and the timing of reproduction in a cooperative breeder, the Florida scrub-jay. – Condor 98: 234-244.

Serra, L. et al. 2012. Seasonal decline of offspring quality in the European starling *Sturnus vulgaris*: an immune challenge experiment. – Behav. Ecol. Sociobiol. 66: 697-709.

Siikamäki, P. 1998. Limitation of reproductive success by food availability and breeding time in pied flycatchers. – Ecology 79: 1789-1796.

Soler, J. J. et al. 2003. Trade-off between immunocompetence and growth in magpies: an experimental study. – Proc. R. Soc. B 270: 241-248.

Stearns, S. C. 1992. The evolution of life histories. – Oxford Univ. Press.

Székely, T. et al. 1996. An evolutionary approach to offspring desertion in birds. – In: Nolan, V. and Ketterson, E. D. (Eds.), Current Ornithology, Vol. 13. Springer, pp. 271-330.

Tella, J. L. et al. 1996. Seasonal and interannual variations in the sex-ratio of lesser kestrel *Falco naumanni* broods. – Ibis 138: 342–345.

Tschirren, B. et al. 2003. Sexual dimorphism in susceptibility to parasites and cell-mediated immunity in great tit nestlings. – J. Anim. Ecol. 72: 839-845.

Tschirren, B. and Richner, H. 2006. Parasites shape the optimal investment in immunity. – Proc. R. Soc. B 273: 1773-1777.

van Noordwijk, A. J. and de Jong, G. 1986. Acquisition and allocation of resources: their influence on variation in life-history tactics. – Am. Nat. 128: 137-142.

Wagner, R. H. and Danchin, E. 2010. A taxonomy of biological information. – Oikos 119: 203-209.

Weiser, E. L. et al. 2018. Life-history tradeoffs revealed by seasonal declines in reproductive traits of Arctic-breeding shorebirds. – J. Avian Biol. 49: e01531.

West, S. A. and Sheldon, B. C. 2002. Constraints in the evolution of facultative sex ratio adjustment. – Science 295: 1685–1688.

West, S. A. et al. 2000. The benefits of allocating sex. – Science 290: 288–290.

Wiebe, K. L. and Bortolotti, G. R. 1992. Facultative sex ratio manipulation in American Kestrels. – Behav. Ecol. Sociobiol. 30: 379-386.

Wiebe, K. L. and Bortolotti, G. R. 1995. Egg size and clutch size in the reproductive investment of American kestrels. – J. Zool. 237: 285–301.

Williams, T. D. 1994. Intraspecific variation in egg size and egg composition: effects on offspring fitness. – Biol. Rev. 68: 35-59.

Wiggins, D. A. et al. 1994. Correlates of clutch desertion by female collared flycatchers *Ficedula albicollis*. – J. Avian Biol. 25: 93-97.

Ydenberg, R. C. 1994. The behavioral ecology of provisioning in birds. – Ecoscience 1: 1–14.

Yorio, P. and Boersma, P. D. 1994. Causes of nest desertion during incubation in the Magellanic Penguin (*Spheniscus magellanicus*). – Condor 96: 1076-1083.

Zuberogoitia, I. et al. 2018. Moult in birds of prey: a review of current knowledge and future challenges for research. – Ardeola 65: 183-207.

Supplementary material

Appendix 1

Table A1. Summary of the scaling relationships between body mass and keel length in male and female lesser kestrel used for computing the scaled mass index (SMI). SMI for individual *i* was computed according to the formula $SMI_i = BM_i \times (L_0/L_i)^{b_{SMA}}$, where BM_i is the body mass of the individual *i*, L_0 is a reference values of the linear body measurement for the population, L_i is the linear body measurement (in our case keel length) for individual *i*, and b_{SMA} is the scaling exponent of the relationship between body mass and the linear measurement (Peig and Green 2009, 2010) [slope of the standardized major axis (SMA) regression between body mass and the linear measurement (both natural log-transformed) in the reference population]. As scaling exponents were significantly different between males and females (likelihood ratio test: $\chi^2 = 5.58$, d.f. = 1, p = 0.018), SMI was computed separately for each sex. As the reference value L_0 for the population we used mean keel length of each sex. Because mean keel length of males and females was very similar [females: 31.9 mm (1.9 s.d.), males: 31.9 mm (1.6 s.d.); $t_{154} = 0.01$, p = 0.99], SMI values of both sexes were comparable. Scaling exponents and test statistics of SMA regressions were computed by the *smatr* R package (Warton et al. 2012).

Sex	n	b_{SMA} (95% c.i.)	Intercept (95% c.i.)	p^a	R^2
Females	82	1.037 (0.839; 1.281)	1.453 (0.687; 2.220)	0.011	0.08
Males	74	1.496 (1.202; 1.863)	-0.278 (-1.243; 0.867)	0.003	0.11

a: p-values of the null hypothesis that b_{SMA} was equal to zero

Table A2. Fit statistics (Akaike Information Criterion value, AIC, and R^2) of body condition (scaled mass index, SMI), oxidative (TAC, TOS) and moult status GLMs (full models) including either breeding stage or sampling date as predictors. These two variables were intrinsically correlated (r = 0.39) and could thus not be included together in the same model. To control for intra-seasonal variation in body condition, oxidative and moult status (birds were captured over 38 days, during both incubation and nestling-rearing phases; see e.g. Donázar et al. 1992 for body mass decline from incubation to nestling rearing), we therefore included in models either breeding stage or sampling date as a predictor, choosing the one which better fitted the data. The best-fitting model (lowest AIC value) is highlighted in boldface. List of abbreviations for predictors: FS = food supplementation, SE = sex, BS = breeding stage, TM = total moult investment, SA = sampling date, SMIc = scaled mass index, centred within sex categories (see Statistical analyses).

Model predictors	AIC	R^2
SMI (n = 144)		
FS + SE + BS + TM + (FS × SE) + (FS × BS) + (FS × TM)	**1146.5**	**0.44**
FS + SE + SA + TM + (FS × SE) + (FS × SA) + (FS × TM)	1149.5	0.43
TAC (n = 126)		
FS + SE + BS + SMIc + TM + (FS × SE) + (FS × BS) + (FS × SMIc) + (FS × TM)	1763.3	0.35
FS + SE + SA + SMIc + TM + (FS × SE) + (FS × SA) + (FS × SMIc) + (FS × TM)	**1755.3**	**0.39**
TOS (n = 71)		
FS + SE + BS + SMIc + TM + (FS × SE) + (FS × BS) + (FS × SMIc) + (FS × TM)	286.7	0.06
FS + SE + SA + SMIc + TM + (FS × SE) + (FS × SA) + (FS × SMIc) + (FS × TM)	**285.3**	**0.08**
Moult initiation (n = 144)		
FS + SE + BS + SMIc + (FS × SE) + (FS × BS) + (FS × SMIc)	160.6	0.24
FS + SE + SA + SMIc + (FS × SE) + (FS × SA) + (FS × SMIc)	**147.2**	**0.32**
Total moult investment (n = 144)		
FS + SE + BS + SMIc + (FS × SE) + (FS × BS) + (FS × SMIc)	1421.5	0.19
FS + SE + SA + SMIc + (FS × SE) + (FS × SA) + (FS × SMIc)	**1371.3**	**0.43**

Table A3. Summary of body condition, oxidative and moult status GLMs accounting or not for the duration of food supplementation (time to sampling). Because birds were captured at different times after start of food supplementation [mean value = 27 days (s.d. 7)], we checked whether the duration of food supplementation, rather than food supplementation *per se*, affected adult traits, by re-running final models while expressing food supplementation as the interaction between food supplementation and the number of days elapsed between sampling date and laying date (time to sampling hereafter; corresponding, for food-supplemented individuals, to the duration of food supplementation). The food supplementation × time to sampling interaction hence tests for any differential effect of food supplementation on measured adult traits according to the duration of food supplementation. In these models, breeding stage and sampling date were replaced by time to sampling, because the former two variables were intrinsically correlated with the latter (r = 0.70 and 0.44, respectively). Since these models provided a similar or worse fit than those including food supplementation only, and the results concerning food supplementation effects were qualitatively unaltered, for simplicity we report in the main text the results of models not accounting for time to sampling effects (see Table 1). List of abbreviations for predictors: FS = food supplementation, SE = sex, BS = breeding stage, TM = total moult investment, SA = sampling date, SMIc = scaled mass index, centred within sex categories (see Statistical analyses), TS = time to sampling. Estimates for the food supplementation effect are reported (upper model: FS effect; lower model: FS × TS effect).

Model predictors	Estimate (s.e.)	t/Z	p	AIC	R^2
SMI (n = 144)					
FS + SE + BS + TM	2.86 (2.16)	1.32	0.19	1146.5	0.44
FS + SE + TS + TM + (FS × TS)	-0.25 (0.29)	0.87	0.39	1141.4	0.45
TAC (n = 126)					
FS + SE + SA + SMIc + TM	-65.62 (45.74)	1.43	0.15	1750.8	0.38
FS + SE + TS + SMIc + TM + (FS × TS)	-6.43 (6.34)	1.01	0.31	1767.6	0.30
TOS (n = 71)					
FS + SE + SA + SMIc + TM	0.41 (0.42)	0.99	0.33	280.1	0.04
FS + SE + TS + SMIc + TM + (FS × TS)	0.06 (0.06)	1.00	0.32	279.7	0.07
Moult initiation (n = 144)					
FS + SE + SA + SMIc	0.64 (0.44)	1.46	0.15	145.5	0.30
FS + SE + TS + SMIc + (FS × TS)	-0.003 (0.056)	0.05	0.96	163.7	0.20
Total moult investment (n = 144)					
FS + SE + SA + SMIc + (FS × SA)	2.46 (0.65)[a]	3.76	< 0.001	1367.8	0.43
FS + SE + TS + SMIc + (FS × TS)	1.54 (0.74)	2.07	0.028	1418.4	0.19

a: estimate refers to the FS × SA interaction (see Table 2).

Table A4. Fit statistics for LMMs of egg mass (with clutch identity as a random intercept effect) with different codings of laying order. Abbreviations: LOc = laying order (continuous variable); LOf = laying order (5-level factor); RLO = relative laying order. Models were fitted on the subset of eggs whose laying order was certain (n = 217 eggs and 65 clutches) and are sorted according to AIC value [lowest through highest; AIC values computed according to Maximum Likelihood estimation (Zuur et al. 2009)]. The best-fitting model is highlighted in boldface.

Model predictors	AIC	R^2
RLO + RLO^2	**589.4**	**0.11**
LOc + LOc^2	600.1	0.10
LOf	601.3	0.11
RLO	603.3	0.08
LOc	603.8	0.09

Table A5. Summary of sex differences in nestling body mass, morphology, and mortality (LMM or GLMM, see Table 1). Even though tarsus and forearm length were slightly significantly larger in males than in females (see Table footnotes), body mass and mortality were not significantly different between the sexes, and there were no significant food supplementation × sex effects. Estimates for main effects of food supplementation and sex were from models with the same structure as the corresponding models reported in Table 5, whereas the food supplementation × sex effect was from the corresponding full model. Degrees of freedom for F-tests were estimated according to the Kenward-Roger's approximation.

Predictors	Estimate (s.e.)	F/Z	d.f.	p	Effect size r
Body mass (n= 253 nestlings, n = 80 broods) (R^2 = 0.88)					
Food supplementation[a]	1.84 (1.22)	2.30	1, 74	0.14	0.12
Sex	0.37 (0.84)	0.20	1, 220	0.66	0.02
Food supplementation × sex[b]	-1.12 (1.71)	0.40	1, 215	0.52	0.04
Tarsus length (n = 243 nestlings, n = 77 broods) (R^2 = 0.68)					
Food supplementation	0.57 (0.37)	2.35	1, 71	0.13	0.14
Sex[c]	0.50 (0.24)	4.10	1, 209	0.044	0.12
Food supplementation × sex[b]	-0.82 (0.48)	2.92	1, 209	0.09	0.10
Forearm length (n = 244 nestlings, n = 77 broods) (R^2 = 0.71)					
Food supplementation[a]	0.62 (0.54)	1.33	1, 69	0.25	0.09
Sex[d]	0.94 (0.42)	4.84	1, 224	0.029	0.14
Food supplementation × sex[b]	-0.87 (0.86)	1.02	1, 220	0.31	0.06
Feather length (n = 181 nestlings, n = 67 broods) (R^2 = 0.60)					
Food supplementation	1.99 (1.07)	3.42	1, 58	0.07	0.18
Sex	-0.32 (0.81)	0.15	1, 161	0.70	0.03
Food supplementation × sex[b]	-1.76 (1.64)	1.13	1, 160	0.29	0.08
Mortality (n = 250 nestlings, n = 80 broods) (R^2 = 0.15)					
Food supplementation	-0.01 (0.52)	0.02	-	0.98	< 0.01
Sex	-0.31 (0.46)	0.68	-	0.49	0.04
Food supplementation × sex[b]	-0.01 (0.95)	0.01	-	0.99	0.01

a: estimate from mean-centered covariate
b: estimate from the full model, or from a model without other non-significant interactions
c: estimated mean values: males = 20.7 (0.22 s.e.) mm, females = 20.2 (0.22 s.e.) mm
d: estimated mean values: males = 28.3 (0.34 s.e.) mm, females = 27.3 (0.34 s.e.) mm

Table A6. Fit statistics for LMMs of egg mass including different interaction terms, with clutch identity as a random intercept effect. The non-significant food supplementation × (relative laying order^2) interaction (FS × RLO^2, p = 0.14) was removed first. In exploratory analyses, we observed that including in the egg mass model both the food supplementation × laying date (FS × LD) and the food supplementation × female SMI (FS × SMI) interactions at the same time negatively affected model performance because it increased correlations among fixed effects. We therefore fitted two separate models and reported in Table 3 the results of model M6 (final model after removing non-significant interactions), which included the test of differential body condition effects on egg mass according to food supplementation (one of the main hypotheses being tested in the study). AIC values were computed according to Maximum Likelihood estimation (Zuur et al. 2009). List of abbreviations for predictors: FS = food supplementation, RLO = relative laying order, LD = laying date, CS = clutch size, SMI = female scaled mass index.

Model predictors	AIC	R^2
M1: Full model (including all 2-way interactions) FS + RLO + RLO^2 + LD + CS + SMI + (FS × RLO) + (FS × RLO^2) + (FS × LD) + (FS × CS) + (FS × SMI)	860.0	0.33
M2: Excluding FS × RLO^2 FS + RLO + RLO^2 + LD + CS + SMI + (FS × RLO) + (FS × LD) + (FS × CS) + (FS × SMI)	860.2	0.33
M3: Excluding FS × RLO^2 and (FS × SMI), including (FS × LD) FS + RLO + RLO^2 + LD + CS + SMI + (FS × RLO) + (FS × LD) + (FS × CS)	861.4	0.30
M4: Excluding FS × RLO^2 and (FS × LD), including (FS × SMI) FS + RLO + RLO^2 + LD + CS + SMI + (FS × RLO) + (FS × CS) + (FS × SMI)	861.3	0.30
M5: Final model (M3 excluding non significant interactions) (Table A7) FS + RLO + RLO^2 + LD + CS + SMI + (FS × RLO) + (FS × LD)	860.6	0.30
M6: Final model (M4 excluding non significant interactions) (Table 3) FS + RLO + RLO^2 + LD + CS + SMI + (FS × RLO) + (FS × SMI)	860.6	0.30

Table A7. Linear mixed model of the effects of food supplementation on egg mass (model M5 from Table A6). Degrees of freedom for F-tests were estimated according to the Kenward-Roger's approximation. See Fig. A1 for graphical representation of the food supplementation × laying date interaction.

Predictors	Estimate (s.e.)	F	d.f.	p	Effect size r
Egg mass (n = 349 eggs, n = 82 clutches) (R^2 = 0.30)					
Food supplementation[a]	0.17 (0.25)	0.48	76	0.49	0.07
Relative laying order[a]	0.71 (0.31)	5.17	265	0.024	0.10
(Relative laying order^2)[a]	-0.28 (0.08)	13.23	265	< 0.001	0.22
Laying date	-0.02 (0.02)	0.97	77	0.33	0.10
Clutch size	-0.48 (0.18)	6.96	77	0.01	0.24
Female SMI[a]	0.02 (0.01)	2.23	76	0.36	0.15
Food supplementation × relative laying order	0.33 (0.09)	13.13	264	< 0.001	0.09
Food supplementation × laying date	-0.07 (0.03)	5.36	76	0.023	0.22

a: estimate for mean-centered covariate

Table A8. Fit statistics of GLMMs of hatching success including the squared term of clutch size (which significantly predicted hatching success in a previous study; Serrano et al. 2005). The squared term of clutch size was not significant ($p = 0.24$) in the model with main effects and did not significantly improve model fit according to AIC values. Clutch identity was included as a random intercept effect. See final model in Table 3 for the (non-significant) effect of female SMI on hatching success.

Model predictors	AIC	R^2
Full model FS + RLO + LD + CS + CS^2 + (FS × RLO) + (FS × LD) + (FS × CS) + (FS × CS^2)	315.2	0.07
Excluding FS × CS^2 FS + RLO + LD + CS + CS^2 + (FS × RLO) + (FS × LD) + (FS × CS)	313.3	0.07
Excluding non-significant interactions FS + RLO + LD + CS + CS^2	314.5	0.04
Final model excluding CS^2 (Table 3) FS + RLO + LD + CS	313.9	0.03

Table A9. Binomial GLMs (restricted to control clutches/broods) testing the effect of female/male SMI on the probability of nest desertion.

Predictors	Estimate (s.e.)	Z	p	Effect size r
Model including female SMI (n = 38 clutches) ($R^2 = 0.32$)				
Laying date	0.16 (0.09)	1.86	0.06	0.41
Clutch size	1.27 (0.98)	1.30	0.20	0.29
Female SMI	-0.16 (0.07)	2.28	0.023	0.53
Model including male SMI (n = 31 clutches) ($R^2 = 0.21$)				
Laying date	-0.12 (0.10)	1.23	0.22	0.33
Clutch size	1.73 (1.10)	1.57	0.12	0.43
Male SMI	0.03 (0.06)	0.44	0.66	0.11

Table A10. Binomial models of sex allocation: a) testing whether PSR was predicted by the squared term of female SMI (see Aparicio and Cordero 2001); b) assessing whether the analysis of factors affecting the probability of a nestling being male (Table 4) was affected by excluding those broods with one sexed nestling only (final model); c) testing whether the probability of a nestling being male varied along the laying sequence, fitted on data from those nestlings with known egg of origin; the model-predicted proportion of males in the first-laid egg was 0.18, whereas it was 0.80 in the fifth-laid egg. Brood identity was included as a random intercept effect in GLMMs.

Predictors	Estimate (s.e.)	Z	p	Effect size r
a) Binomial GLM of PSR (n = 25 broods) (R^2 = 0.10)				
Food supplementation	-0.29 (0.45)	0.65	0.51	0.12
Laying date	-0.02 (0.03)	0.52	0.60	0.10
Clutch size	-0.31 (0.30)	1.00	0.31	0.20
Female SMI	0.01 (0.02)	0.02	0.99	< 0.01
Female SMI^2	0.01 (0.01)	0.20	0.84	0.04
b) Binomial GLMM of the probability of being male (n = 248 nestlings, n = 75 broods) (R^2 = 0.05)[a]				
Food supplementation	-0.37 (0.27)	1.35	0.18	0.09
Rank	0.36 (0.12)	3.08	0.002	0.20
Laying date	0.01 (0.02)	0.05	0.96	< 0.01
c) Binomial GLMM of the probability of being male (n = 51 nestlings, n = 41 broods) (R^2 = 0.14)[b]				
Laying order	0.65 (0.32)	1.99	0.047	0.37

a: dispersion parameter = 1.15
b: dispersion parameter = 1.05

Table A11. LMM of egg mass fitted on the subset of eggs which could be associated to sexed nestling (n = 51 eggs from 41 clutches), with clutch identity as a random intercept effect. The squared term of relative laying order was not included in the model as it did not significantly improve model fit in this subset. Degrees of freedom were estimated according to the Kenward-Roger's approximation. R^2 of the final model (excluding the two non-significant interactions) = 0.25.

Predictors	Estimate (s.e.)	F	d.f.	p	Effect size r
Food supplementation	0.71 (0.41)	2.97	1, 37	0.09	0.26
Relative laying order	-0.64 (0.19)	9.03	1, 21	0.007	0.34
Sex	-0.64 (0.36)	2.78	1, 41	0.10	0.22
Food supplementation × relative laying order[a]	0.07 (0.47)	0.02	1, 20	0.88	0.02
Food supplementation × sex[a]	0.44 (0.85)	0.24	1, 37	0.63	0.07

a: excluded term

Table A12. Binomial GLMM of nestling mortality (final model) excluding deserted broods (sample size: n = 266 nestlings from 78 broods) (with brood identity as a random intercept effect). R^2 of the final model = 0.18.

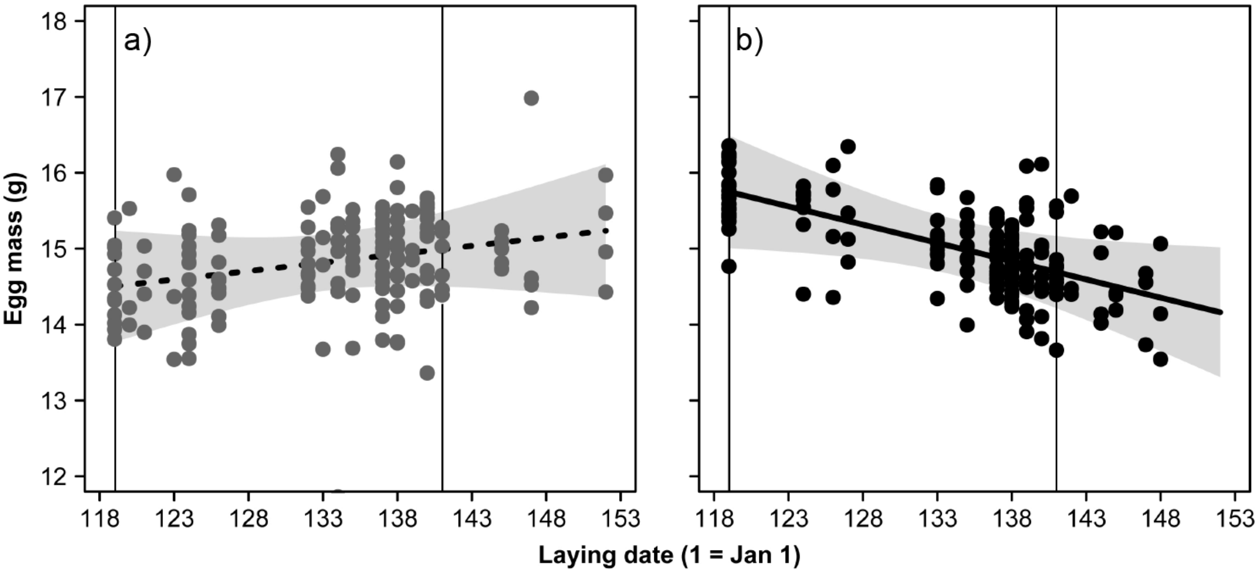
Predictors	Estimate (s.e.)	Z	p	Effect size r
Food supplementation	0.10 (0.44)	0.22	0.83	0.02
Rank	1.38 (0.25)	5.61	< 0.001	0.41
Laying date	-0.01 (0.03)	0.12	0.90	0.01
Brood size	-0.44 (0.26)	1.69	0.09	0.13
Ectoparasite load	-0.15 (0.36)	0.41	0.68	0.05

a: dispersion parameter = 0.85

Table A13. LMM of nestling ectoparasite load (final model), with brood and nestling identity as random intercept effects. Monitoring session was included in the model as a 3-level factor (see Podofillini et al. 2018, which see also for discussion of significant rank and laying date effects on ectoparasite load). Degrees of freedom were estimated according to the Kenward-Roger's approximation. R^2 of the final model = 0.12.

Predictors	Estimate (s.e.)	F	d.f.	p	Effect size r
Food supplementation	0.01 (0.06)	0.01	1, 80	0.92	0.01
Session	-	1.62	3, 723	0.18	< 0.05
Rank	-0.05 (0.01)	12.42	1, 219	< 0.001	0.12
Laying date	-0.02 (0.004)	24.13	1, 90	< 0.001	0.32
Brood size	0.01 (0.02)	0.01	1, 384	0.96	0.01

Figure A1. Egg mass did not significantly vary with laying date among control females [0.02 (0.02 s.e.)] (a), while it significantly decreased among food-supplemented females [estimate: -0.05 (0.02 s.e.)] (b). Partial plots accounting for other model effects are shown. The fitted lines (with 95 % confidence bands) are derived from the corresponding model reported in Table A7. Full line: significant slope; dotted line: non-significant slope. Estimated marginal means (derived from the Table A7 LMM) computed at the 10th and 90th percentiles of the distribution of laying date (day 119 and 141, respectively; shown as thin vertical lines within each panel) revealed that egg mass of food-supplemented females was significantly larger than control ones early in the season [controls: 14.23 g (0.37 s.e.); food-supplemented: 15.48 g (0.38 s.e.), $t_{77} = 2.39$, p = 0.019], whereas no significant difference in egg mass between groups emerged among late-laid clutches [$t_{76} = 0.92$, p = 0.36].



References

Aparicio, J. M. and Cordero, P. J. 2001. The effects of the minimum threshold condition for breeding on offspring sex-ratio adjustment in the lesser kestrel. – Evolution 55: 1188-1197.

Donázar, J. A. et al. 1992. Functional analysis of mate-feeding in the Lesser Kestrel *Falco naumanni*. – Ornis Scand. 23: 190-194.

Peig, J. and Green, A. J. 2009. New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. – Oikos 118: 1883-1891.

Peig, J. and Green, A. J. 2010. The paradigm of body condition: a critical reappraisal of current methods based on mass and length. – Funct. Ecol. 24: 1323–1332.

Podofillini, S. et al. 2018. Home, dirty home: effect of old nest material on nest-site selection and breeding performance in a cavity-nesting raptor. – Curr. Zool. (doi: 10.1093/cz/zoy012).

Serrano, D. et al. 2005. Proximate causes and fitness consequences of hatching failure in lesser kestrels *Falco naumanni*. – J. Avian Biol. 36: 242-250.

Warton, D.I., Duursma, R.A., Falster, D.S. and Taskinen, S. 2012. smatr 3–an R package for estimation and inference about allometric lines. – Methods Ecol. Evol. 3: 257-259.

Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A. and Smith, G. M. 2009. Mixed effects models and extensions in ecology with R. – Springer.

Chapter 4.

Spatial segregation of home ranges between neighbouring colonies in a diurnal raptor

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Spatial segregation of home ranges between neighbouring colonies in a diurnal raptor

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Enhancement of information transfer has been proposed as a key driver of the evolution of coloniality. Transfer of information on location of food resources implies that individuals from the same colony share foraging areas and that each colony can be associated to a specific foraging area. In colonial breeding vertebrates, colony-specific foraging areas are often spatially segregated, mitigating intercolony intraspecific competition. By means of simultaneous GPS tracking of lesser kestrels (*Falco naumanni*) from neighbouring colonies, we showed a clear segregation of space use between individuals from different colonies. Foraging birds from different neighbouring colonies had home ranges that were significantly more segregated in space than expected by chance. This was the case both between large and between small neighbouring colonies. To our knowledge, the lesser kestrel is the only terrestrial species where evidence of spatial segregation of home ranges between conspecifics from neighbouring colonies has been demonstrated. The observed spatial segregation pattern is consistent with the occurrence of public information transfer about foraging areas and with the avoidance of overexploited areas located between neighbouring colonies. Our findings support the idea that spatial segregation of exploited areas may be widespread among colonial avian taxa, irrespective of colony size.

Coloniality occurs when conspecifics gather in groups to reproduce close to one another and exploit shared resources, often showing reduced territoriality[1]. The ecological factors promoting the evolution of colonial breeding have long been puzzling to evolutionary biologists[2]. Evans *et al.*[3] argued that enhancing information use is the main advantage of colonial breeding and that it may have contributed more than any other factor to the evolution of coloniality. The transfer of information may allow individuals to gather experience during high-energy demanding life-cycle stages, such as reproduction, reducing costly trials and errors. In a colony environment, there are indeed several kinds of information that can be either intentionally or inadvertently shared among colony members[3]. The "public information" that can be exploited by conspecifics may be disparate, including for example cues useful for sexual choice[4], nesting habitat selection[5], foraging[6,7] and anti-predator defence[8–10].

In habitats where food resource distribution is both spatially and temporally predictable, individuals - particularly in long-lived species - can rely on memory and cognitive maps for targeting food[11]. In the case of patchy, ephemeral and unpredictable food resources, the use of cognitive maps may not be sufficient for efficiently targeting food and individuals can greatly benefit from the recent experience of conspecifics[12,13]. Transfer of social information about the location of profitable foraging areas may occur at the colony site, where individuals can actively (as proposed by the "Information Centre Hypothesis"[6]) or inadvertently[14,15] share information on foraging locations. Moreover, social information may be shared outside the colony site, which may occur by means of

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so-called "local enhancement" processes occurring at the foraging grounds, whereby individuals searching for food are attracted to feeding aggregations of other individuals[16].

Most of the studies on foraging areas exploited by colonial central-place foragers concerns marine top-predators, especially pinnipeds[17] and seabirds (e.g. albatrosses, shearwaters, cormorants and gannets), the vast majority of which (>90%) breed colonially[18]. Individuals from different colonies often show colony-specific and well-defined foraging areas, which do not overlap with those belonging to neighbouring conspecific colonies[19–24]. As argued by the "diplomacy" hypothesis[20], spatial segregation of foraging individuals from different colonies may mitigate intraspecific competition for resources between conspecifics breeding in different colonies. By foraging in spatially segregated areas, conspecifics from different colonies may thus "diplomatically" avoid interference competition for food resources[20]. For instance, it has been shown that inter-colony competition could be one of the main factors driving the at-sea distribution of pelagic foraging birds[25]. Both local enhancement and the transfer of information at the colony site have been hypothesized to be the most important mechanisms generating and maintaining specific foraging areas exploited by individuals belonging to the same colony[23]. Such a spatial arrangement pattern of foraging areas is peculiar of colonial species (*sensu* Danchin *et al.*[1]) and does not occur, for instance, in eusocial insects, where space partitioning between nests originates and is maintained by aggressive interactions[26–28]. In the latter case, spatial arrangement of neighbouring nests often results from the destruction of the newer nest by killing or ejecting founding queens[27].

During the breeding period, colonial species are central-place foragers (*sensu* Orians & Pearson[29]), with individuals foraging outside the colony, sometimes very far from the breeding site (e.g. seabirds[30], seals[31] and bats[32]), and consistently returning to the colony (the "central place") to perform parental duties. The progressive depletion of foraging areas around the breeding sites leads individuals to both increase foraging ranges (the "Ashmole's halo" effect[33]) and, in the case of neighbouring colonies, to avoid moving towards adjacent colonies when searching for food. This, in turn, may generate and/or reinforce spatial segregation of foraging areas among individuals from neighbouring colonies[23].

We analyzed the spatial distribution of home ranges of individuals of a landbird species breeding in neighbouring colonies. We focused on the lesser kestrel (*Falco naumanni*), a small (ca. 120 g) diurnal colonial raptor. The lesser kestrel mainly nests in holes and crevices of anthropogenic structures (roofs, ancient monuments, buildings) and forages in farmland habitats surrounding breeding sites[34], where it targets invertebrates and small vertebrates (mice, lizards)[35,36]. In such farmland landscapes, lesser kestrel prey can be patchily distributed, highly ephemeral and unpredictable during the species' breeding season, since habitat characteristics change continuously as a function of seasonal processes (changes in primary productivity affecting prey distribution/availability) and agricultural practices (including pesticide applications, harvesting, stubble burning, ploughing)[37]. Hence, information gathered during previous years or during the pre-breeding period might not be sufficient to identify profitable foraging areas, leading us to hypothesize that lesser kestrels should exploit social information to target profitable hunting grounds. This is corroborated by the observation that lesser kestrels, similarly to other colonial raptors such as vultures (e.g. family *Aegypiinae*) and the Eleonora's falcon (*Falco eleonorae*), commonly forage in groups, both during the breeding and the non-breeding season[35].

By analysing GPS information collected during the entire nestling-rearing stage (ca. 30 days) from simultaneously tracked individuals, we investigated the occurrence of spatial segregation between birds from neighbouring colonies in two geographically distinct lesser kestrel populations (Apulia and Sicily, both in Southern Italy). According to the "diplomacy" hypothesis, we predicted spatial segregation of home ranges (assessed by means of the utilization distribution[38]) between lesser kestrels from neighbouring colonies to occur because: 1) resources are expected to be depleted in the surroundings of colony sites (Ashmole's halo)[39] and 2) lesser kestrels forage in groups, suggesting that they are highly likely to rely on social information to target ephemeral productive foraging areas.

Results

Individual home range size largely differed between the two geographical populations (Table 1), being ca. one order of magnitude larger among Apulian compared to Sicilian birds [95% Kernel Density Estimation (95% KDE), Apulia: 138.8 km^2 (84.5 s.d., n = 18 individuals); Sicily: 13.0 km^2 (59 s.d., n = 6 individuals); Mann-Whitney U test, $Z = 3.6$, $p < 0.001$]. No significant sex differences in home range size emerged among Apulian birds [95% KDE, males: 121.7 km^2 (73.7 s.d., n = 12); females: 172.8 km^2 (101.1 s.d., n = 6); Mann-Whitney U test, $Z = 0.84$, $p = 0.40$)], while sex differences could not be tested for Sicilian birds because a single male was tracked.

Representativeness of tracked individuals from the two Apulian colonies, Gravina in Puglia and Altamura, was very high (95% KDE: >94%; Fig. 1), indicating that we captured most of the variability in space use by individuals from the target colonies. Moreover, the steep curves of the representativeness analysis (Fig. 1) indicated that birds belonging to same colony showed highly overlapping home ranges (see also Fig. 2). Although we could not test for representativeness of tracked individuals from the Sicilian colonies due to the small sample size, Fig. 1 suggests that even a few individuals can well represent the space use of the target colony (e.g. with 2 individuals representativeness is >75%, a relatively high value[40].

Individual home ranges of birds from the two different Apulian colonies showed a very limited overlap (Fig. 2). The overlap between home ranges of individuals from neighbouring colonies, computed by means of the Utilization Distribution Overlap Index (UDOI)[41,42], was very low, varying between 0 and 0.11 (mean value = 0.01). At the same time, the UDOI between individuals from the same colony varied between 0.03 and 1.53 (mean value = 0.53). By randomly rotating individual home ranges, we showed that birds from the two Apulian colonies had home ranges that were significantly more spatially segregated than expected by chance according to UDOI values ($r_{\mathrm{obs}} = -0.71$, $p_{\mathrm{rand}} = 0.024$; Fig. 3). The pattern for the Sicilian colonies, albeit based on a very small sample size, was even more striking ($r_{\mathrm{obs}} = -0.96$, $p_{\mathrm{rand}} = 0.057$; Fig. 3): UDOI values between individual home ranges of birds belonging to the three neighbouring colonies were indeed 0 or close to 0 in all

Bird identity	Study area	Colony	Sex	N GPS positions	Hatching date	End tracking date	Days tracked	95% KDE area (km^2)
H207147	Apulia	GRA	F	245	161	191	22	266.34
H207149	Apulia	GRA	M	927	170	200	29	205.29
H207151	Apulia	GRA	M	803	167	197	28	62.88
H207154	Apulia	GRA	M	1195	165	195	26	69.94
H207155	Apulia	GRA	F	424	165	188	19	109.77
H207156	Apulia	GRA	F	987	171	198	26	206.08
H207200	Apulia	GRA	F	409	168	198	24	48.35
H207204	Apulia	GRA	M	993	168	198	24	307.05
H208463	Apulia	GRA	M	514	171	194	20	149.75
H207172	Apulia	ALT	M	681	175	199	23	79.86
H207174	Apulia	ALT	M	295	173	187	13	122.4
H207175	Apulia	ALT	F	733	169	199	29	303.13
H207176	Apulia	ALT	M	1137	178	208	29	90.97
H207180	Apulia	ALT	M	1253	172	202	29	67.98
H207181	Apulia	ALT	F	363	160	190	20	103.38
H207216	Apulia	ALT	M	436	173	203	28	133.66
H207220	Apulia	ALT	M	148	177	188	10	45.51
H207222	Apulia	ALT	M	1192	172	202	27	125.25
T58200	Sicily	TOR	F	281	135	152	16	11.19
T67675	Sicily	TOR	F	879	135	165	29	11.78
TK7884	Sicily	CAN	F	515	140	170	29	18.58
TK7885	Sicily	CAN	M	1055	137	167	29	4.09
T69718	Sicily	SAN	F	651	142	172	29	11.59
TK7978	Sicily	SAN	F	804	148	177	28	20.54

Table 1. Summary information of GPS tracking data for each individual included in the study. In Apulia, data were collected during the 2016 breeding season, whereas in Sicily data were collected during the 2015 breeding season. Within a given study area, birds from different colonies were tracked simultaneously (see also Methods). Colony size was ca. 1000 pairs for both Gravina in Puglia (GRA) and Altamura (ALT), 16 for Torrevecchia (TOR), 11 for Canalotto (CAN) and 32 for San Gregorio (SAN); sex: M = male; F = female; dates are expressed in days since January 1.

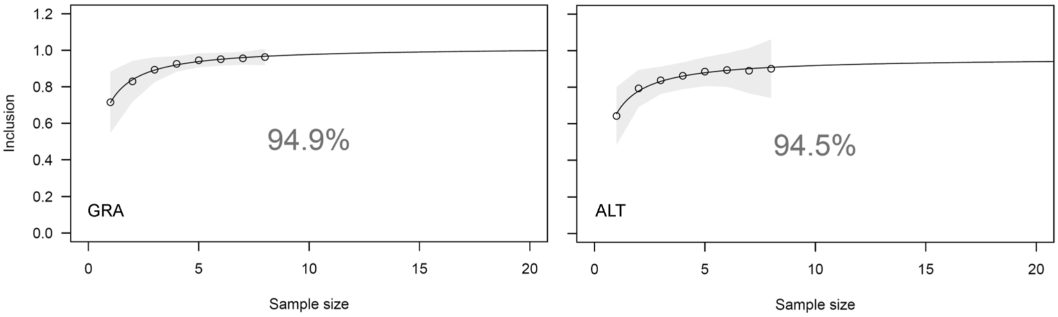


Figure 1. Results of the representativeness analysis showing that the sample of tracked individuals reliably represents the variability in space use of birds from each Apulian colony (GRA: Gravina in Puglia, n = 9 individuals; ALT: Altamura, n = 9 individuals). Circles indicate the average proportion of out-of-sample GPS positions located within the 95% KDE areas estimated from sampled positions (Inclusion) for 100 random draws of sample sizes, from 1 to 8 individuals. Grey bars indicate variability of inclusion value for 100 random draws of tracked individuals, and the solid line represents the fitted nonlinear regression line. Inclusion rate (and thus representativeness of the tracking dataset) is based on the estimated asymptote of the nonlinear regression[40].

comparisons (a single comparison had a value of 0.0002), while those between birds from the same colony varied between 0.63 and 1.17 (mean value = 0.89). For Apulia, results of the randomization procedure for assessing home range segregation were strengthened when considering only GPS positions located outside the urban area of the cities where lesser kestrels breed (see Methods) ($p_{\text{rand}} = 0.004$).

We could rule out that the observed patterns of spatial segregation resulted from the presence of unsuitable foraging habitats in the inter-colony areas. Indeed, the proportion of the main lesser kestrel foraging habitat (arable land), which is the main land use in both study areas, was very similar between the inter-colony and the outer-colony areas both in Apulia and Sicily (Figs 4 and S1).

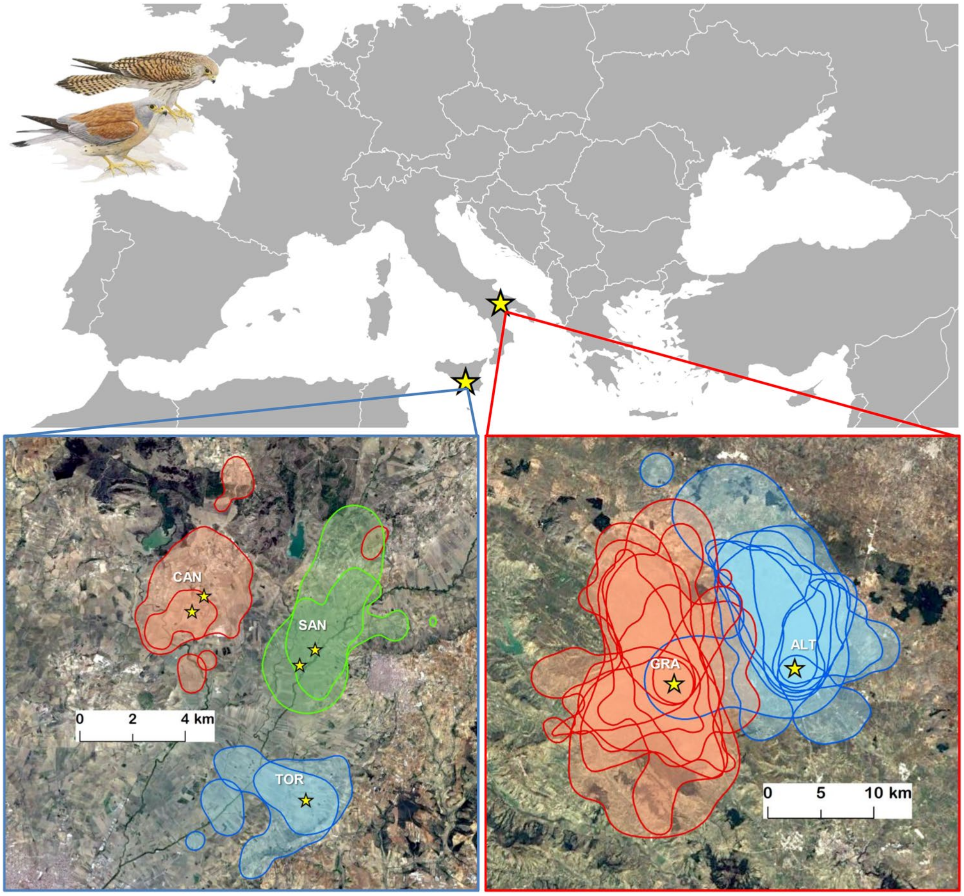


Figure 2. Home ranges (95% KDE) of lesser kestrels breeding at three colony sites in Sicily (bottom left; n = 6 individuals) and at two colony sites in Apulia (bottom right; n = 18 birds). Within each panel, home ranges of the same colour denote birds from the same colony (GRA = Gravina in Puglia, ALT = Altamura, CAN = Canalotto, SAN = Sangregorio, TOR = Torrevecchia) and breeding sites of tracked birds are marked with yellow stars. Satellite images were downloaded from Google EarthPro ver. 7.3.0.3832 3832 (sources: "Gravina in Puglia and Altamura", coordinates 40.82°N - 16.39°E, 12 March 2016–14 August 2017, Map data © 2018 Google; "Gela", coordinates 37.14°N - 14.31°E, 12 March 2016–14 August 2017; Map data © 2018 TerraMetrics) and elaborated with ArcGIS ver. 10.2.1 for Desktop. Lesser kestrel drawing is by U. Catalano and has the ISPRA copyright.

Discussion

Our findings clearly showed that home ranges of lesser kestrels from neighbouring colonies were spatially segregated during the nestling-rearing period, with home ranges of birds from different colonies overlapping less than expected by chance, resulting in space partitioning. This pattern of spatial segregation was observed in two geographically distinct populations (Apulia and Sicily) and occurred both between two very large (Apulia) and three small (Sicily) neighbouring colonies. Moreover, colonies were associated to specific exploited areas (the colony "hinterland"[43], with individuals from the same colony showing overlapping home ranges, supporting the idea that individuals belonging to the same colony share information on the location of profitable foraging grounds.

In colonial species, areas surrounding the colonies are likely to rapidly become resource-depleted (Ashmole's halo), and increasing colony size is expected to translate into faster resource depletion and/or progressive expansion of foraging ranges in the course of the breeding season[33]. If colonies are physically close by, intraspecific competition between colonies may then arise, because individuals from different colonies may target the same foraging areas located between colonies. Due to the relatively higher density of foraging individuals, those areas may rapidly become resource-depleted. Such areas may thus become progressively avoided, possibly leading to spatial segregation of foraging areas between birds from different colonies. Segregation may result from individuals preferentially performing foraging trips directed away from any neighbouring colony. For instance, this has been clearly shown for northern gannets (*Morus bassanus*) breeding in 12 neighbouring colonies fringing the coastline of the British Isles and Northern France, whose trips towards at-sea foraging areas were directed away from closely neighbouring colonies[23].

The avoidance of overexploited foraging areas between neighbouring colonies is a density-dependent process: spatial segregation is in fact expected to be reinforced with increasing size of neighbouring colonies, which imply a greater local density of foraging individuals in the area that is lying between colonies. Our observation of spatial segregation occurring also between birds from neighbouring small colonies may at first seem surprising,

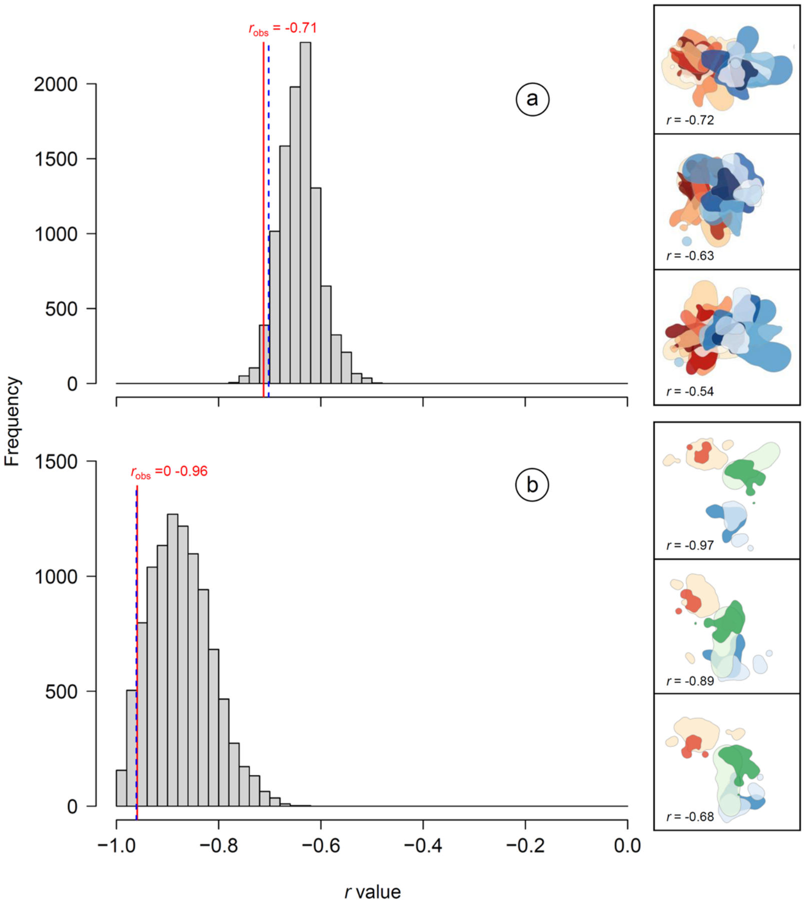
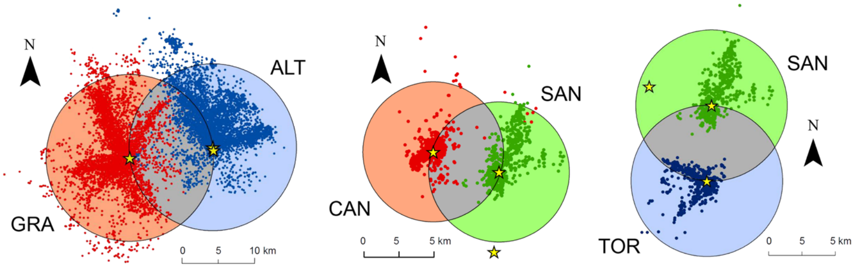


Figure 3. Frequency distribution of randomized *r* values obtained from random rotations of home ranges (with breeding site as the anchor point) in a) Apulia and b) Sicily. *r* values were computed by correlating the matrix of Utilization Distribution Overlap Index (UDOI) values with the matrix of colony membership (0 = individuals belonged to the same colony; 1 = individuals belonged to different colonies) (see Methods for details). More negative *r* values denote greater spatial segregation of home ranges between lesser kestrels from neighbouring colonies (see Fig. 2). The observed *r* value (r_{obs}), resulting from the spatial distribution of home ranges shown in Fig. 2, is highlighted with a (continuous) red line within each panel. The 95% empirical quantile of the frequency distribution of randomized *r* values is shown with a (broken) blue line. Representative examples of random rotations of home ranges (and the corresponding *r* value) for each study population are shown on the right insets of each panel (home ranges of birds from different colonies are depicted with colour shadings corresponding to those used in Fig. 2); for simplicity, overlapping home ranges are represented with 95% KDEs.

since it may be hypothesized that density-dependent spatial segregation should be detectable only between large neighbouring colonies, whereas competition between colonies should be relaxed when colony size is small[23]. This would be the case if the distance between pairs of large and small neighbouring colonies is similar and/or the area of potential overlap between home ranges of foraging individuals from different colonies is comparable in both large and small colonies. In our case, the pairwise distances between small neighbouring colonies are considerably smaller than those between large colonies, and the area of potential overlap between home ranges among small neighbouring colonies is considerably smaller than among large ones (Fig. 2). Although there may be a much smaller absolute number of individuals potentially targeting the inter-colony areas between small than between large neighbouring colonies, the density of foraging conspecifics in such inter-colony areas (number of kestrels foraging per unit area) may be similar in either case. Hence, overexploitation of areas located between colonies is likely to occur also between small colonies.

The comparison of land use of outer-colony areas with that of inter-colony areas did not reveal any differences for both Apulian and Sicilian colonies. Arable land, which is the main foraging habitat of the lesser kestrel, was the predominant land use class in all cases. Hence, we can safely rule out that the spatial segregation patterns we observed resulted from the presence of unsuitable foraging habitats in the inter-colony areas.

Despite several studies investigating the spatial ecology of colonial landbirds and mammals, such as vultures[44,45] and bats[46,47], to our knowledge evidence for spatial segregation of home ranges between individuals from neighbouring colonies has been lacking so far in terrestrial animals, with the single exception of the lesser kestrel[48]. Our findings support the idea that mitigation of intraspecific competition between individuals from neighbouring colonies by means of spatial segregation of exploited areas is a general pattern among colonial species.



LAND USE CLASSES	GRA	Inter-colony	ALT	CAN	Inter-colony	SAN	TOR	Inter-colony	SAN
Urban areas	0.02	0.08	0.02	0.02	0	0.06	0	0.04	0.02
Arable land	**0.74**	**0.68**	**0.70**	**0.54**	**0.89**	**0.63**	**0.71**	**0.72**	**0.67**
Permanent crops	0.05	0.03	0	0	0	0.01	0.07	0	0.01
Grasslands	0.04	0.07	0.20	0.07	0	0.01	0.01	0.02	0
Heterogeneous cultivations	0.03	0.11	0.05	0.15	0	0.28	0.13	0.21	0.18
Wooded areas	0.12	0.03	0.02	0.21	0.09	0	0.07	0	0.10
Water bodies	0	0	0	0.02	0.02	0	0	0	0.01

Figure 4. Proportion of land use classes within outer- (orange, blue or green areas) and inter-colonies areas (grey areas). Points represent all recorded GPS positions for a given colony (different colours for neighbouring colonies) and stars represent breeding sites of GPS-tagged birds in each colony (GRA = Gravina in Puglia, ALT = Altamura, CAN = Canalotto, SAN = Sangregorio, TOR = Torrevecchia). The radius of each buffer around colonies (centered on the mean of breeding sites positions) was calculated as the distance between the two neighbouring colonies. Land use classes were identified by means of ArcGIS 10.2.1 for Desktop according to the Corine Land Cover 2012 classification. Arable land (in bold) represents the main foraging habitat for the lesser kestrel in the study areas.

Methods

Target species and study areas.

European populations of the lesser kestrel breed mostly around the Mediterranean Sea in pseudo-steppe and open farmland landscapes. Lesser kestrels mostly overwinter in sub-Saharan Africa, returning to the breeding areas in February/March[35]. Between late April and early May, pairs are formed and females lay 3–5 eggs (single brooded). Incubation lasts ca. 30 days and nestlings fledge at ca. 35–40 days. After hatching, at least one pair member spends the night inside the nest until the late nestling-rearing stage, when both pair members shift to frequenting large communal night roosts.

The study was carried out in two geographically distinct populations, both in Southern Italy: one in Apulia and the other in Sicily. In Apulia, we collected data at two large urban colonies that are ca. 10 km apart, Altamura (40°49′N; 16°33′E) and Gravina in Puglia (40°49′N; 16°25′E). Altamura and Gravina in Puglia are small cities (ca. 50–70000 inhabitants) hosting large colonies of ca. 1000 breeding pairs each[48]. Both cities are surrounded by extensive pseudo-steppe farmland landscapes (mostly cereal steppe habitats) where lesser kestrels forage. In both colonies, we relied on birds nesting in nestboxes placed on the terraces of large buildings located in the old towns (see also Podofillini *et al.*[49]. In Sicily, the study was conducted in the Gela Plain (37°07′N; 14°20′E) at three small colonies (ca. 11–32 breeding pairs), which are located ca. 5 km apart (Fig. 2). Colonies are settled on rural buildings, often abandoned and partly decaying, which are surrounded by croplands mainly represented by wheat (*Triticum* spp.) and artichoke (*Cynara* spp.) alternated with grassland and other cultivations[50]. We relied on birds nesting both in nestboxes and crevices of rural buildings.

Nests were checked twice per week from 15 April to 30 July (both in Apulia and in Sicily), recording information about laying date, brood size, hatching date, hatching success and nestling survival at 20 days from hatching of the first egg (it was difficult to follow the fate of nestlings after 20 days because most left their nest to wander around, sometimes mixing with nestlings from nearby nests[49]).

GPS deployment.

All birds were captured by hand within their nestbox or nest cavity, and equipped with GPS tags during the late incubation stage, mostly a few days before hatching. The study was conducted in accordance with relevant guidelines and regulations. Specifically, captures in Apulia were carried out by Istituto Nazionale per la Protezione e la Ricerca Ambientale (ISPRA) under the authorization of Law 157/1992 [Art.4 (1) and Art. 7 (5)] and in Sicily by the University of Palermo under authorization n. 1616/2014 issued by Regione Sicilia. We equipped with GPS tags 25 lesser kestrels from 25 different nests in Apulia (2016 breeding season), and 12 individuals from 11 nests in Sicily (2015 breeding season). We deployed solar-driven, remote-downloading GPS-UHF tags (NanoFix GEO + RF, PathTrack Ltd., UK, in Apulia and customized Pica, Ecotone, PL, in Sicily) using a backpack Teflon harness[51]. Tags were programmed to record 1 GPS position every 15 min. However, tags automatically adjusted the GPS sampling rate according to the actual battery level, preserving battery power and allowing UHF data transmission to base stations that were deployed at breeding sites. The weight of tags (NanoFix

GEO + RF: 4 g; Pica: 5 g; plus 1 g of Teflon harness) was always below 5% of body mass [NanoFix GEO + RF: 3.46% (0.41 s.e.m.), range 2.77–4.20%; Pica: 3.49% (0.25 s.e.m.), range 3.33–3.92%].

Data from 18 simultaneously tracked individuals breeding in Apulia (9 from Altamura and 9 from Gravina in Puglia) and from 6 simultaneously tracked individuals breeding in Sicily (two for each colony site) were available for statistical analyses (Table 1). We excluded birds with largely malfunctioning devices (that in a few cases stopped transmitting data a few days after deployment) and those that failed reproduction and did not fledge any nestling (as they were no longer tied to the colony site and started wandering far from the colony site; our unpubl. data).

Home range determination. To identify areas exploited by tracked birds during the nestling-rearing stage, we calculated for each individual the Utilization Distribution (UD) using the fixed kernel density estimation (KDE) with reference bandwidth (href) by means of the R package adehabitatHR[52]. To this end, we selected GPS positions according to the following criteria: 1) we considered positions collected during the 29 days after hatching of the first egg (a few individuals were tracked for a shorter period because of tag failure; see Table 1); 2) we excluded all positions collected within 50 m of the nest site (to eliminate all instances when the birds were perching close to the nest); 3) we avoided the inclusion of roosting sites, used by males and by females only during the late nestling-rearing stage, considering only GPS positions recorded between 5:00–17:00 h UTC (7–19 h local time, approximately 2 hours after sunrise and 2 before sunset); this time window was identified after exploring high-frequency tracking data (1 GPS position every minute for both day- and night-time) of lesser kestrels in southern Italy (our unpubl. data).

Because a small fraction of the individuals from each colony was tracked, we can draw inferences at the colony level only if the tracked individuals are representative of the variability of space use by colony members. To assess representativeness, we investigated for each colony how the total 95% KDE area increased with sample size, performing a bootstrap analysis according to Lascelles *et al.*[40]. For each sample size (from 1 to n – 1 individuals), we plotted a random selection of individual 95% KDEs and calculated the proportion of positions from non-selected individuals that overlapped with the sum of selected individual 95% KDEs. This process was iterated 100 times and the average overlapping proportion ("inclusion") was calculated for each sample size. Then, we fitted a non-linear regression to inclusion values (see details of fitted function in Lascelles *et al.*[40]) and the representativeness of the tracked individuals was computed as the percentage of the estimated asymptote value reached by the highest predicted inclusion value. This test was not performed for Sicilian colonies due to the small sample size (see "*GPS deployment*"). Computations were performed in R 3.3.1[53].

In order to rule out possible sources of bias when comparing home ranges between colonies, we checked for variation in the duration of the tracking, sampling periods, and breeding success between colonies and sexes (comparisons were made within each study area, Apulia and Sicily; sex effects were not tested for Sicilian birds since only one male was tracked). There were no statistically significant differences between colonies (or sexes in Apulia) in the number of days tracked for each individual (linear models; Apulia, colony: $F_{1,15} = 0.21$, $p = 0.66$; sex: $F_{1,15} = 0.07$, $p = 0.79$; Sicily, colony: $F_{2,3} = 0.92$, $p = 0.48$), in the end date of tracking (Apulia, colony: $F_{1,15} = 0.23$, $p = 0.64$; sex: $F_{1,15} = 1.27$, $p = 0.28$; Sicily, colony: $F_{2,3} = 3.86$, $p = 0.15$) and in the number of nestlings at day 20 (Apulia, colony: $F_{1,15} = 0.02$, $p = 0.89$; sex: $F_{1,15} = 0.39$, $p = 0.54$; Sicily, colony: $F_{2,3} = 0.20$, $p = 0.83$). We could therefore rule out the possibility that systematic differences between colonies and sexes in tracking effort and breeding success biased our findings concerning the spatial distribution of home ranges.

Statistical analysis of home range segregation. We estimated the magnitude of spatial segregation between home ranges of individuals belonging to different neighbouring colonies separately for each study population (i.e. the two neighbouring Apulian colonies and the three neighbouring Sicilian colonies) by means of a randomization procedure. We first built a home range overlap matrix between individuals belonging to both the same colony and neighbouring colonies according to the UD. The UD overlap between a pair of individuals *i,j* was calculated using the Utilization Distribution Overlap Index (UDOI), as recommended by Fieberg & Kochanny[41], by means of the *kerneloverlap* function of the adehabitatHR R package[52]. The UDOI is an home range overlap index which assumes that different individuals use space independently of one another[41,42]. UDOI values range from zero (no overlap) to 1 (uniformly distributed and have 100% overlap; it can however be >1 when UDs are non-uniformly distributed and have a high degree of overlap)[41]. To compute the UDOI home range overlap matrix, we specified a grid extent equal to 1 and a grid size equal to 200 in the *kerneloverlap* function. We then built a second matrix of colony membership, whereby each pair of individuals *i, j* was coded as 0 if both individuals belonged to the same colony, and 1 if they belonged to different colonies. After removing diagonals from both matrices, we computed a correlation coefficient (Pearson's r; r_{obs} hereafter) between the two matrices. Because of the coding of colony membership, highly negative values of r_{obs} indicate that 1) home ranges of individuals belonging to the same colony are highly overlapping, and that 2) those of individuals belonging to different colonies are deeply segregated. We then randomly and independently rotated each individual set of positions (by anchoring it to the coordinates of its own breeding site; see stars in Fig. 2) 9999 times and calculated each time a new home range overlap matrix, which was correlated with the colony membership matrix. By this way, we obtained a distribution of r values representing the null hypothesis of random spatial distribution of home ranges around the breeding site, assuming that individuals were free to move in the space surrounding the colonies while remaining tied to their breeding site. In the analysis of data from the three Sicilian colonies, we deleted from the overlap matrix the data referring to the two most distant colonies, which were non-neighbouring (see Fig. 2). Significance of r_{obs} was calculated as the probability (p_{rand}) of obtaining a more negative value than r_{obs}. All computations were performed in R 3.3.1[53].

Because nestling-feeding lesser kestrels search for food in areas that can be widely scattered in the surroundings of the breeding site but frequently return to the breeding site to deliver food to their progeny, the UD had invariably higher values on the breeding site (see Supplementary Figs S2 and S3). The UD might thus overemphasize the exploitation of the areas in the immediate surroundings of the breeding site (where the birds did not forage), at the same time underestimating the importance of the use of foraging areas located away from the breeding site (see Supplementary Figs S2 and S3), which are important in an inter-colony foraging competition perspective. To assess the robustness of our conclusions, we therefore repeated the analyses by using only the GPS positions located outside the urban area of the cities where tracked birds breed in Apulia (see Fig. 2; these urban areas are not used for foraging; urban areas identified by Corine Land Cover 2012, CLC12 hereafter; https://land.copernicus.eu/pan-european/corine-land-cover/clc-2012; code 111, continuous urban habitat). Such a procedure could not be applied to data from the Sicilian colonies, where birds breed on isolated buildings in open farmland landscapes.

Comparison of land use in the areas surrounding colonies. To rule out that spatial segregation between neighbouring colonies is actually due to uneven distribution of suitable foraging habitats in the areas surrounding colonies, we described land use in these areas. Because the inter-colony areas appeared to be avoided in both Apulia and Sicily (Fig. 2), we assessed whether the availability of pseudo-steppe and open farmland landscapes (i.e. the main lesser kestrel foraging habitat[35]) was lower in the inter-colony areas than in the rest of the areas surrounding each colony. For each pair of neighbouring colony we created a buffer area centred on the nesting site whose radius was equal to the distance between the two nesting sites. The intersection of the two buffers created three areas for each pair of neighbouring colonies: one inter-colony area and two different areas surrounding colonies (outer-colony areas), one for each colony (see Fig. 4). We then calculated the proportion of each land use type from CLC12 within each of these three areas by means of ESRI ArcMap 10.2.1 for Desktop (see Supplementary Fig. S1). We pooled together CLC12 land use categories that were similar in habitat and structure, hence obtaining 7 land use classes: urban areas (urban fabric; industrial, commercial and transport units), arable land, permanent crops (vineyards; fruit tree and berry plantations; olive groves), grasslands (pastures; natural grasslands), heterogeneous cultivations (heterogeneous agricultural areas), wooded areas (forests; scrub and/or herbaceous vegetation associations), water bodies.

Data Accessibility. The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

References

1. Danchin, E., Giraldeau, L.-A. & Cézilly, F. *Behavioural Ecology* (Oxford University Press, 2008).
2. Rolland, C., Danchin, E. & de Fraipont, M. The evolution of coloniality in birds in relation to food, habitat, predation, and life-history traits: a comparative analysis. *Am. Nat.* **151**, 514–529 (1988).
3. Evans, J. C., Votier, S. C. & Dall, S. R. Information use in colonial living. *Biol. Rev.* **91**, 658–672 (2015).
4. Griggio, M., Hoi, H., Lukasch, B. & Pilastro, A. Context-dependent female preference for multiple ornaments in the bearded reedling. *Ecol. Evol.* **6**, 493–501 (2016).
5. Aparicio, J. M., Bonal, R. & Muñoz, A. Experimental test on public information use in the colonial lesser kestrel. *Evol. Ecol.* **21**, 783–800 (2007).
6. Ward, P. & Zahavi, A. The importance of certain assemblages of birds as "information-centres" for food-finding. *Ibis* **115**, 517–534 (1973).
7. Barta, Z. & Giraldeau, L. A. Breeding colonies as information centers: a reappraisal of information-based hypotheses using the producer—scrounger game. *Behav. Ecol.* **12**, 121–127 (2001).
8. Clode, D., Birks, J. D. & Macdonald, D. W. The influence of risk and vulnerability on predator mobbing by terns (*Sterna* spp.) and gulls (*Larus* spp.). *J. Zool.* **252**, 53–59 (2000).
9. Danchin, E., Giraldeau, L. A., Valone, T. J. & Wagner, R. H. Public information: from nosy neighbors to cultural evolution. *Science* **305**, 487–491 (2004).
10. Di Maggio, R., Campobello, D. & Sarà, M. Nest aggregation and reproductive synchrony promote Lesser Kestrel *Falco naumanni* seasonal fitness. *J. Ornithol.* **154**, 901–910 (2013).
11. Weimerskirch, H. Are seabirds foraging for unpredictable resources? *Deep Sea Res. Part II* **54**, 211–223 (2007).
12. Weimerskirch, H. Seabirds - Individuals in Colonies. *Science* **341**, 35–36 (2013).
13. Tóth, Z., Tuliozi, B., Baldan, D., Hoi, H. & Griggio, M. The effect of social connections on the discovery of multiple hidden food patches in a bird species. *Sci. Rep.* **7**, 816 (2017).
14. Richner, H. & Danchin, E. On the importance of slight nuances in evolutionary scenario. *Anim. Behav.* **61**, 17–18 (2001).
15. Lachmann, M., Sella, G. & Jablonka, E. On the advantages of information sharing. *Proc. R. Soc. B.* **267**, 1287–1293 (2000).
16. Machovsky-Capuska, G. E., Hauber, M. E., Libby, E., Amiot, C. & Raubenheimer, D. The contribution of private and public information in foraging by Australasian gannets. *Anim. Cognit.* **17**, 849–858 (2014).
17. Carter, M. I. D., Bennett, K. A., Embling, C. B., Hosegood, P. J. & Russell, D. J. Navigating uncertain waters: a critical review of inferring foraging behaviour from location and dive data in pinnipeds. *Mov. Ecol.* **4**, 25 (2016).
18. Coulson, J. C. C*olonial breeding in seabirds*. In: Schreiber, E. A. & Burger, J. (eds) *Biology of marine birds* (CRC Press, 2002).
19. Wanless, S. & Harris, M. P. Use of mutually exclusive foraging areas by adjacent colonies of blue-eyed shags (*Phalacrocorax atriceps*) at South Georgia. *Col. Waterbirds* **16**, 176–182 (1993).
20. Grémillet, D. *et al.* Offshore diplomacy, or how seabirds mitigate intra-specific competition: a case study based on GPS tracking of Cape gannets from neighbouring colonies. *Mar. Ecol. Prog. Ser.* **268**, 265–279 (2004).
21. Masello, J. F. *et al.* Diving seabirds share foraging space and time within and among species. *Ecosphere* **1**, 1–28 (2010).
22. Wakefield, E. D. *et al.* Habitat preference, accessibility, and competition limit the global distribution of breeding Black-browed Albatrosses. *Ecol. Monogr.* **81**, 141–167 (2011).
23. Wakefield, E. D. *et al.* Space partitioning without territoriality in gannets. *Science* **341**, 68–70 (2013).
24. Ainley, D. G. *et al.* Geographic structure of Adélie Penguin populations: Overlap in colony-specific foraging areas. *Ecol. Monogr.* **74**, 159–178 (2004).
25. Cecere, J. G. *et al.* Commercial fisheries, inter-colony competition and sea depth affect foraging location of breeding Scopoli's Shearwaters *Calonectris diomedea*. *Ibis* **157**, 284–298 (2015).
26. Cushman, J. H., Martinsen, G. D. & Mazeroll, A. I. Density- and size-dependent spacing of ant nests: evidence for intraspecific competition. *Oecologia* **77**, 522–525 (1988).
27. Ryti, R. T. & Case, T. J. The role of neighborhood competition in the spacing and diversity of ant communities. *Am Nat.* **139**, 355–374 (1992).
28. Adler, F. R. & Gordon, D. M. Optimization, conflict, and nonoverlapping foraging ranges in ants. *Am Nat.* **162**, 529–543 (2003).

29. Orians G. H. & Pearson N. E. On the theory of central place foraging. In: Horn D.J., Mitchell R. & Stair G.R. (eds) *Analysis of ecological systems*. Columbus, Ohio: Ohio State University Press; 155–177 (1979)
30. Pinaud, D. & Weimerskirch, H. Scale-dependent habitat use in a long-ranging central place predator. *J. Anim. Ecol.* **74**, 852–863 (2005).
31. Staniland, I. J., Boyd, I. L. & Reid, K. An energy–distance trade-off in a central-place forager, the Antarctic fur seal (*Arctocephalus gazella*). *Mar. Biol.* **152**, 233–241 (2007).
32. Rainho, A. & Palmeirim, J. M. The importance of distance to resources in the spatial modelling of bat foraging habitat. *PLoS One* **6**, e19227 (2011).
33. Ashmole, N. P. The regulation of numbers of tropical oceanic birds. *Ibis* **103**, 458–473 (1963).
34. Catry, I. *et al*. Foraging habitat quality constrains effectiveness of artificial nest-site provisioning in reversing population declines in a colonial cavity nester. *PLoS One* **8**, e58320 (2013).
35. Cramp, S. *The complete birds of the Western Palearctic on CD-ROM* (Oxford University Press, 1988).
36. Rodríguez, C., Tapia, L., Kieny, F. & Bustamante, J. Temporal changes in lesser kestrel (*Falco naumanni*) diet during the breeding season in southern Spain. *J. Raptor Res.* **44**, 120–128 (2010).
37. Catry, I., Amano, T., Franco, A. M. & Sutherland, W. J. Influence of spatial and temporal dynamics of agricultural practices on the lesser kestrel. *J. Appl. Ecol.* **49**, 99–108 (2012).
38. Van Winkle, W. Comparison of several probabilistic home-range models. *J. Wildl. Man.* **39**, 118–123 (1975).
39. Bonal, R. & Aparicio, J. M. Evidence of prey depletion around lesser kestrel Falco naumanni colonies and its short term negative consequences. *J. Avian Biol.* **39**, 189–197 (2008).
40. Lascelles, B. G. *et al*. Applying global criteria to tracking data to define important areas for marine conservation. *Divers. Distrib.* **22**, 422–431 (2016).
41. Fieberg, J. & Kochanny, C. O. Quantifying home-range overlap: the importance of the utilization distribution. *J. Wildl. Manag.* **69**, 1346–1359 (2005).
42. Walter, W. D. & Fischer, J. W. *Manual of applied spatial ecology*. *Walter Applied Spatial Ecology Lab*, *Pennsylvania State University*. University Park, Pennsylvania, USA. (2015).
43. Cairns, D. K. The regulation of seabird colony size—a hinterland model. *Am. Nat.* **134**, 141–146 (1989).
44. Holland, A. E. *et al*. Fine-scale assessment of home ranges and activity patterns for resident black vultures (*Coragyps atratus*) and turkey vultures (*Cathartes aura*). *PLoS ONE* **12**, e0179819 (2017).
45. García-Ripollés, C., López-López, P. & Urios, V. Ranging behaviour of non-breeding Eurasian Griffon Vultures *Gyps fulvus*: a GPS-telemetry study. *Acta Ornithol.* **46**, 127–134 (2011).
46. Arlettaz, R. Habitat selection as a major resource partitioning mechanism between the two sympatric sibling bat species *Myotis myotis* and *Myotis blythii*. *J. Anim. Ecol.* **68**, 460–471 (1999).
47. Oleksy, R., Racey, P. A. & Jones, G. High-resolution GPS tracking reveals habitat selection and the potential for long-distance seed dispersal by Madagascan flying foxes *Pteropus rufus*. Glob. *Ecol. Conserv.* **3**, 678–692 (2015).
48. La Gioia, G., Melega, L., & Fornasari, L. Piano d'Azione Nazionale per il grillaio (*Falco naumanni*). (Quad. Cons. Natura, 41, MATTM - Istituto Superiore per la Protezione e la Ricerca Ambientale - ISPRA, 2017).
49. Podofillini, S., *et al*. Home, dirty home: effect of old nest material on nest-site selection and breeding performance in a cavity-nesting raptor. *Curr. Zool.* https://doi.org/10.1093/cz/zoy012 (2018).
50. Sarà, M., Campobello, D. & Zanca, L. Effects of nest and colony features on lesser kestrel (*Falco naumanni*) reproductive success. *Avian Biol. Res.* **5**, 209–217 (2012).
51. Limiñana, R., Romero, M., Mellone, U. & Urios, V. Is there a different response to winds during migration between soaring and flapping raptors? An example with the Montagu's harrier and the lesser kestrel. *Behav. Ecol. Sociobiol.* **67**, 823–835 (2013).
52. Calenge, C. The package adehabitat for the R software: a tool for the analysis of space and habitat use by animals. *Ecol. Model.* **197**, 516–519 (2006).
53. R Core Team. R: *A language and environment for statistical computing*. (R Foundation for Statistical Computing, 2016).

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Author Contributions

J.G.C. and D.R. conceived the study and wrote the paper with inputs from M.S., S.I., U.M., M.G. and N.S., S.B., S.P., E.F., M.S., A.C., M.G., L.S., D.R. and J.G.C. carried out fieldwork; D.R., S.I., S.P., J.G.C. and D.M. analysed the data.

Additional Information

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Competing Interests: The authors declare no competing interests.

SUPPLEMENTARY INFORMATION

Spatial segregation of home ranges between neighbouring colonies in a diurnal raptor

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Figure S1. Land use map (according to land use classes defined in the Methods and Corine Land Cover 2012) of outer- (coloured buffers) and inter-colonies areas (grey areas). Top panel: Apulian colonies, red line = Gravina in Puglia (GRA), blu line = Altamura (ALT). Low panel: Sicilian colonies, red line = Canalotto (CAN), green line = San Gregorio (SAN), blue line = Torrevecchia (TOR). Images elaborated with ESRI ArcGIS 10.2.1. for Desktop.

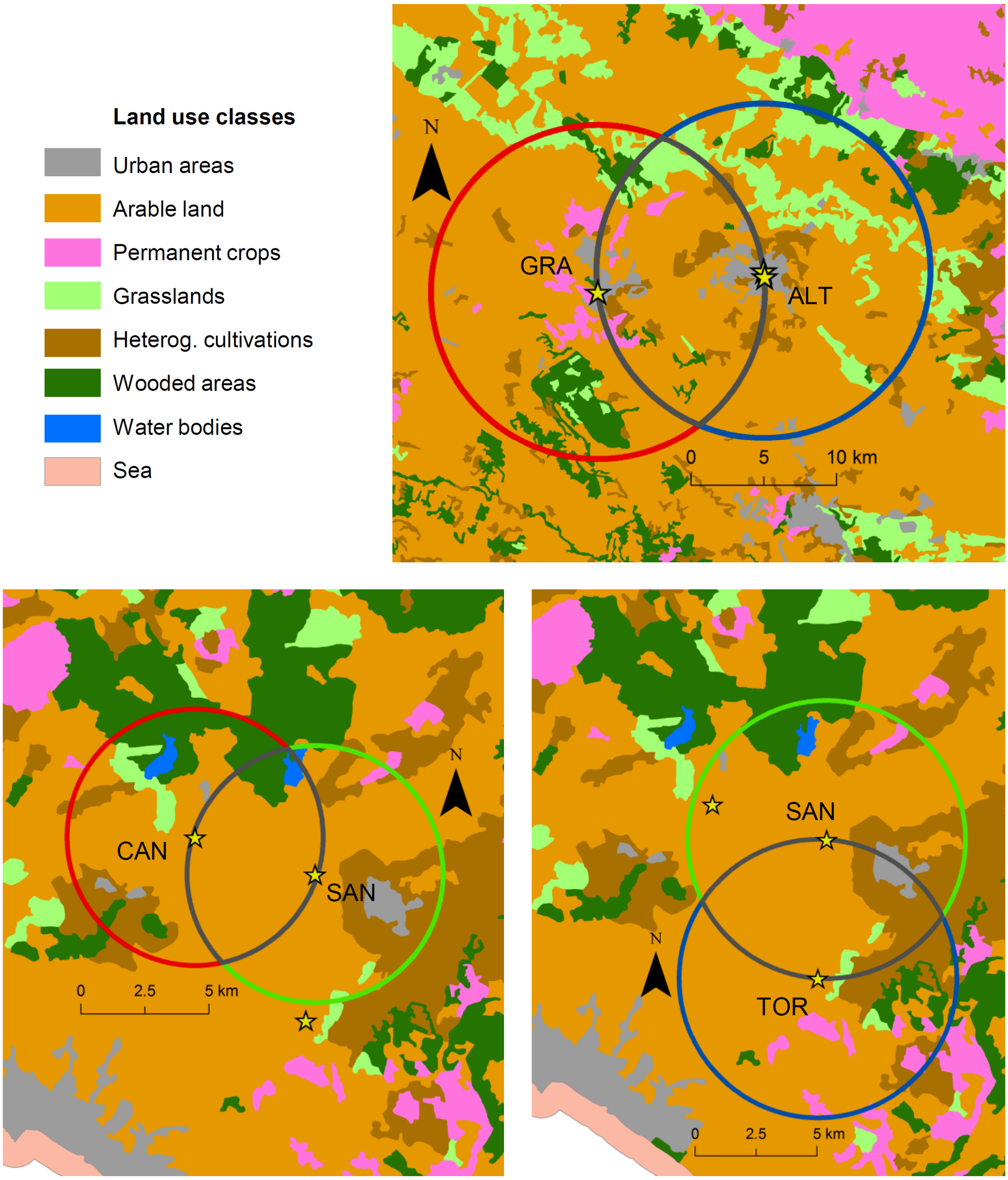
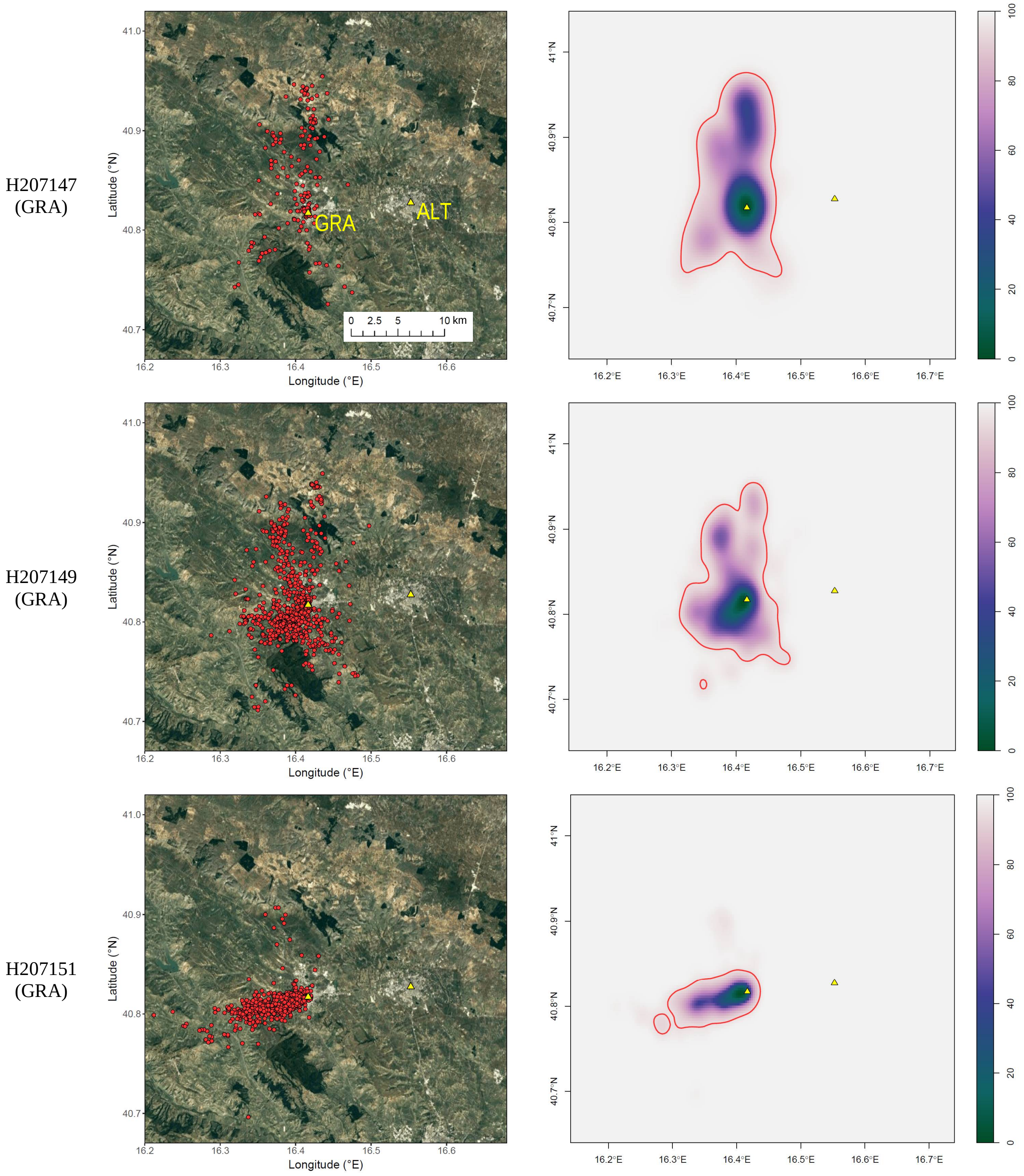


Figure S2. Maps of selected GPS positions (left panels) and volume UD (with 95% KDE) (right panels) for each tracked individual in Apulia. The position of the breeding sites are marked with yellow triangles. Individuals (see list in Table 1) are sorted according to colony site (GRA = Gravina in Puglia, red dots; ALT = Altamura, blue dots). The corresponding metric unit is shown in the first map.

Map data: Google, TerraMetrics 2018, plotted with the R package *ggmap* (Kahle & Wickham 2013).



Kahle D. & Wickham H. ggmap: Spatial Visualization with ggplot2. *The R Journal*. **5**, 144–161 (2013).

Figure S2. Continued

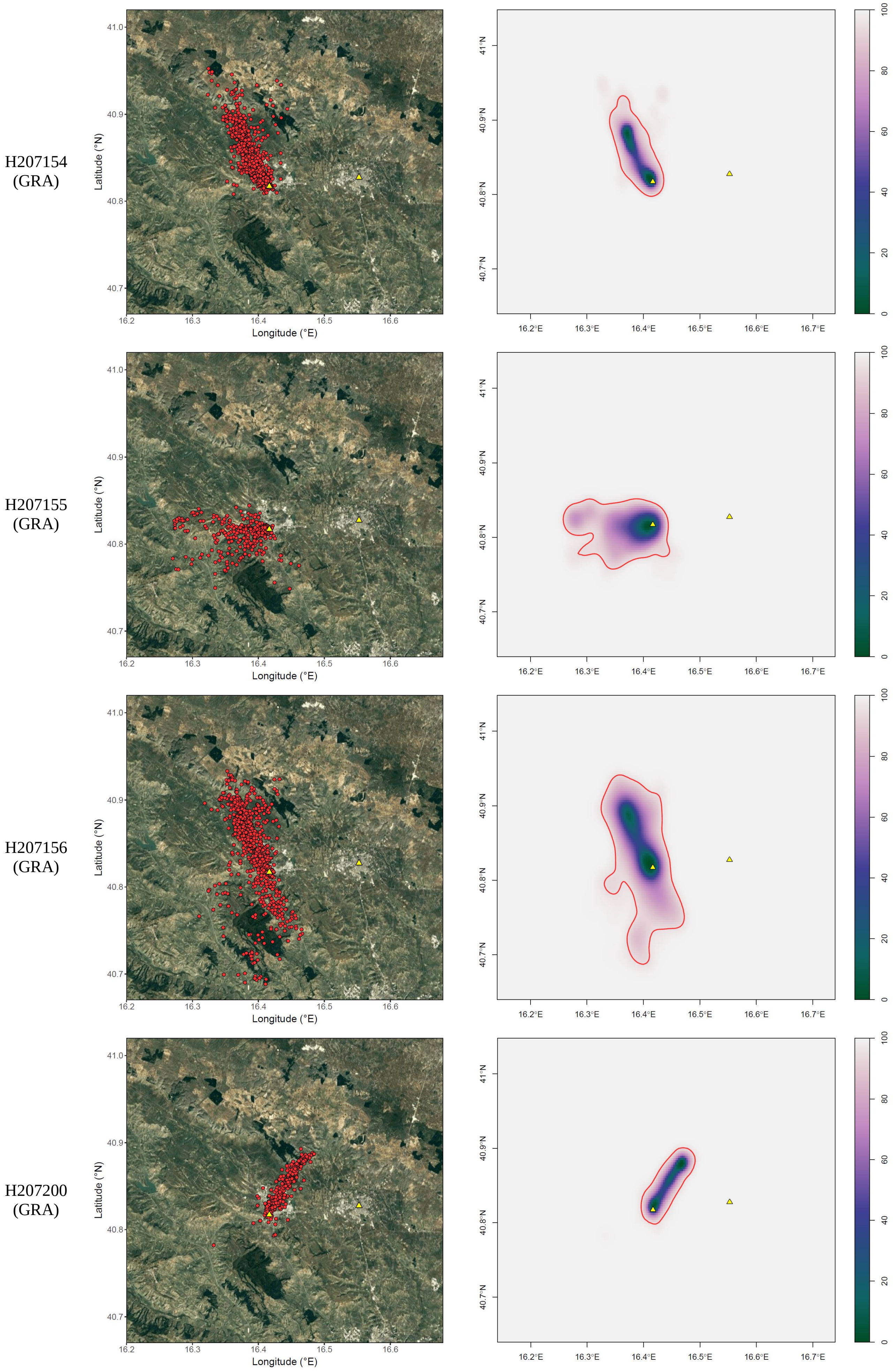


Figure S2. Continued

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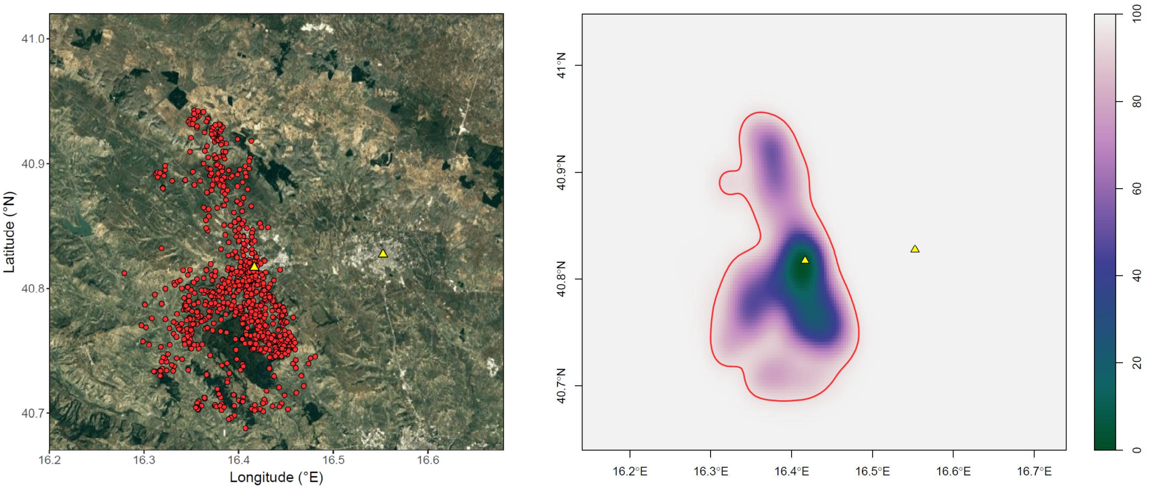

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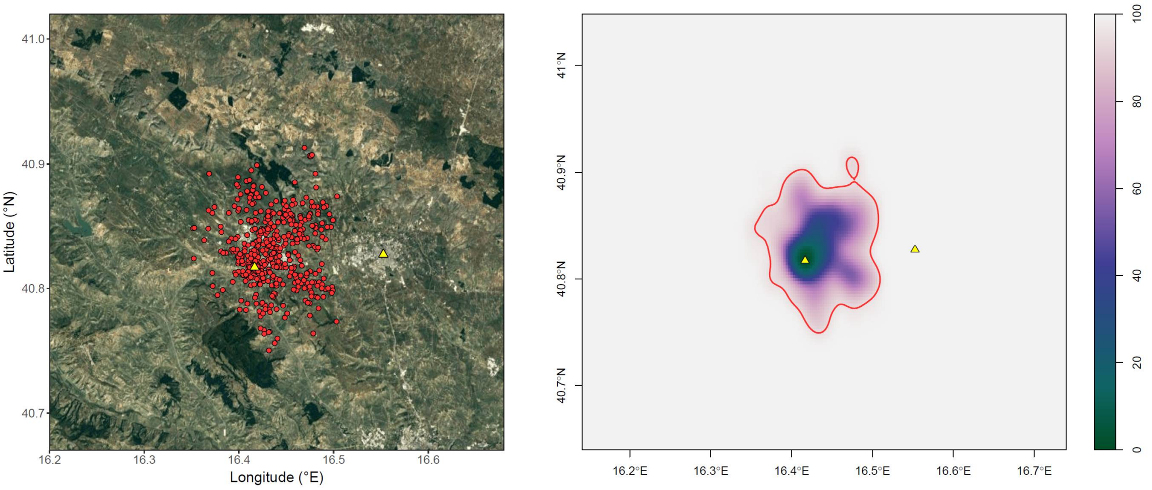

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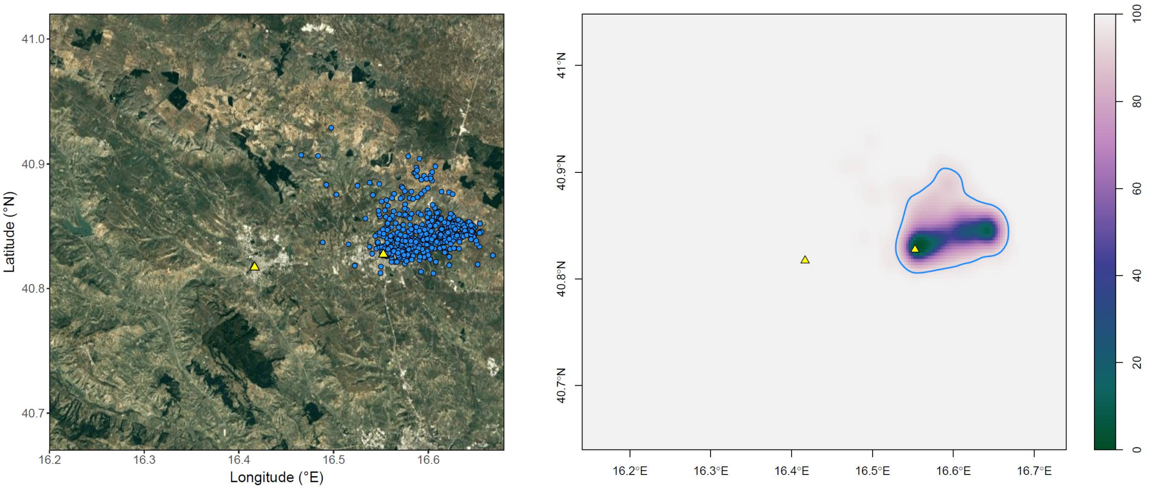

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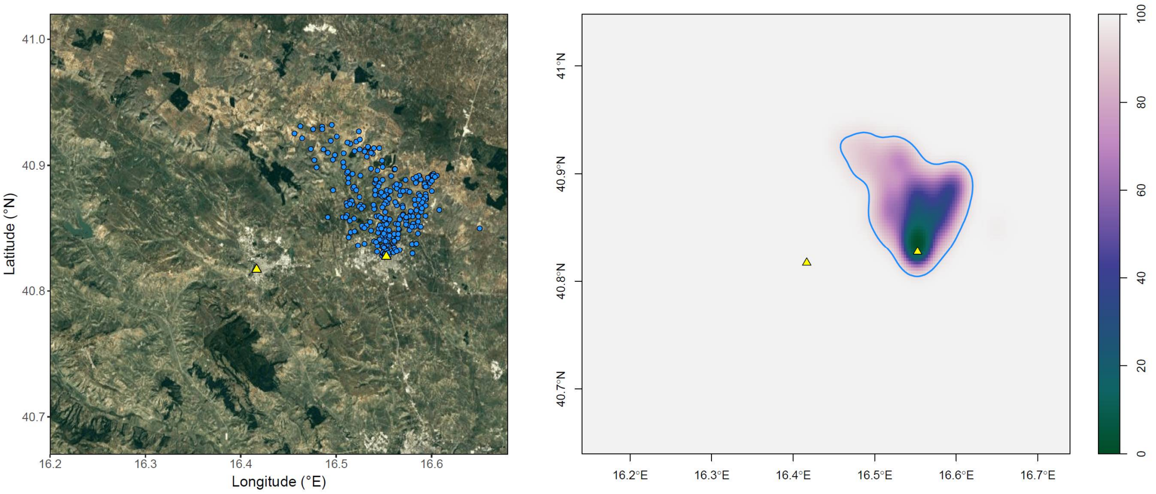

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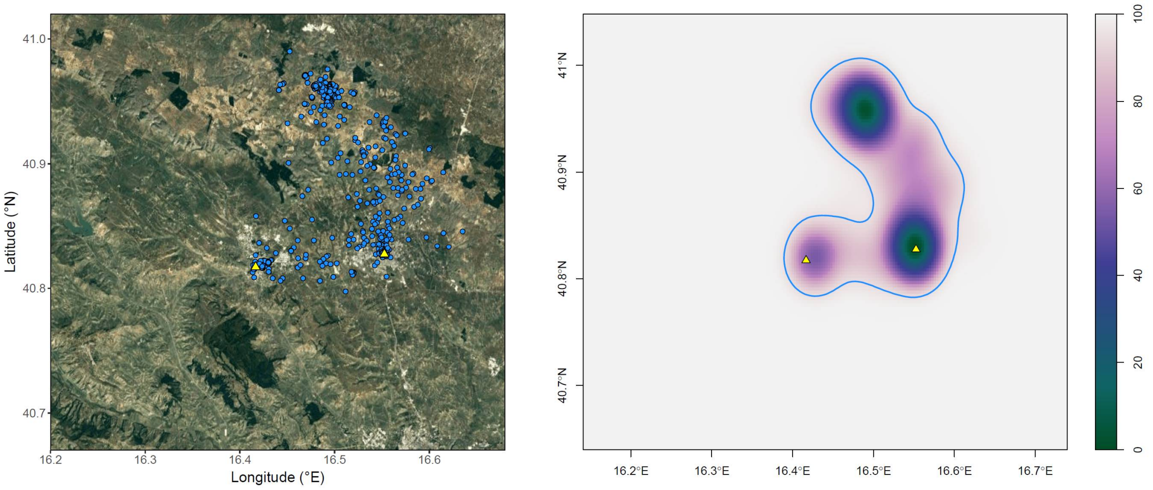

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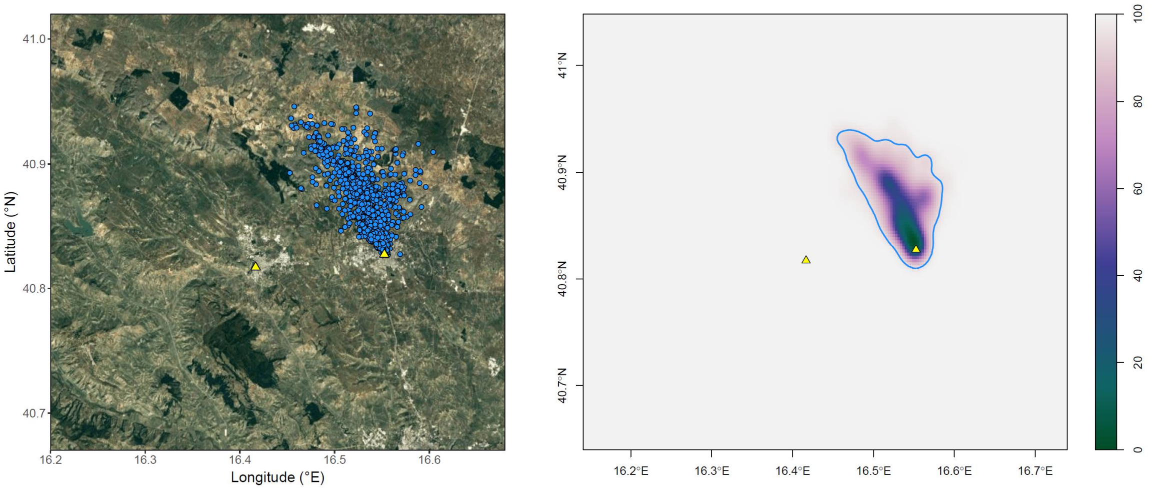

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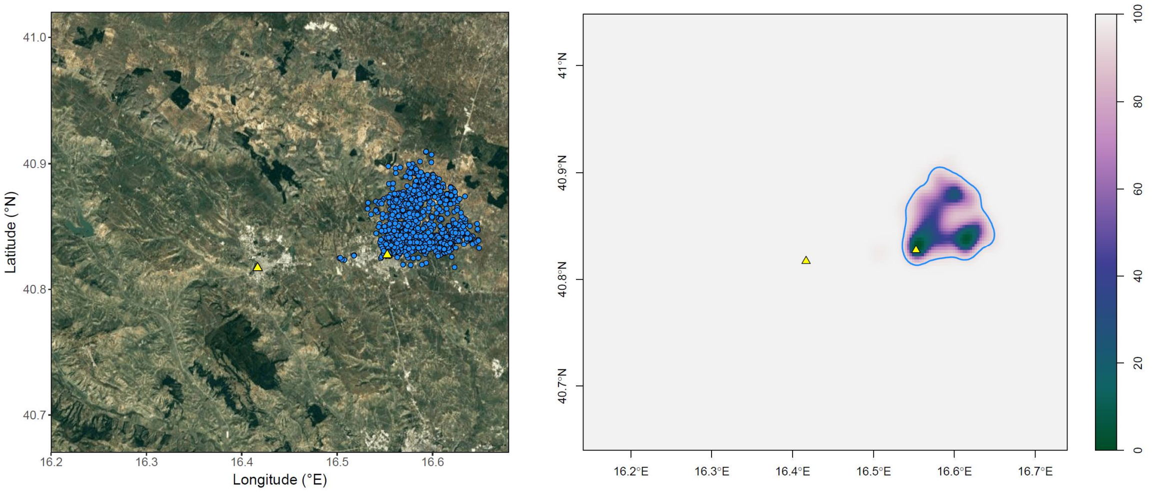

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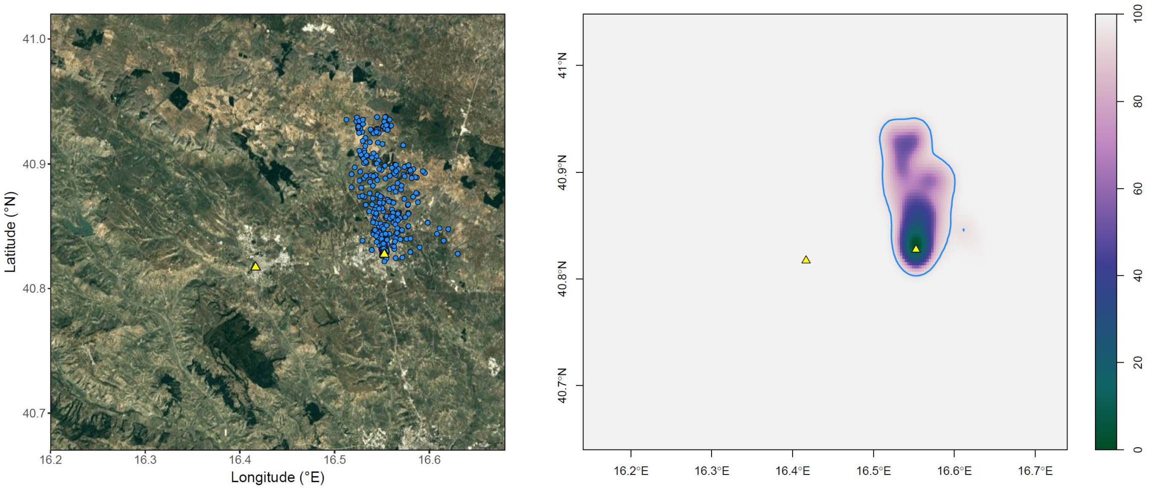

Figure S2. Continued

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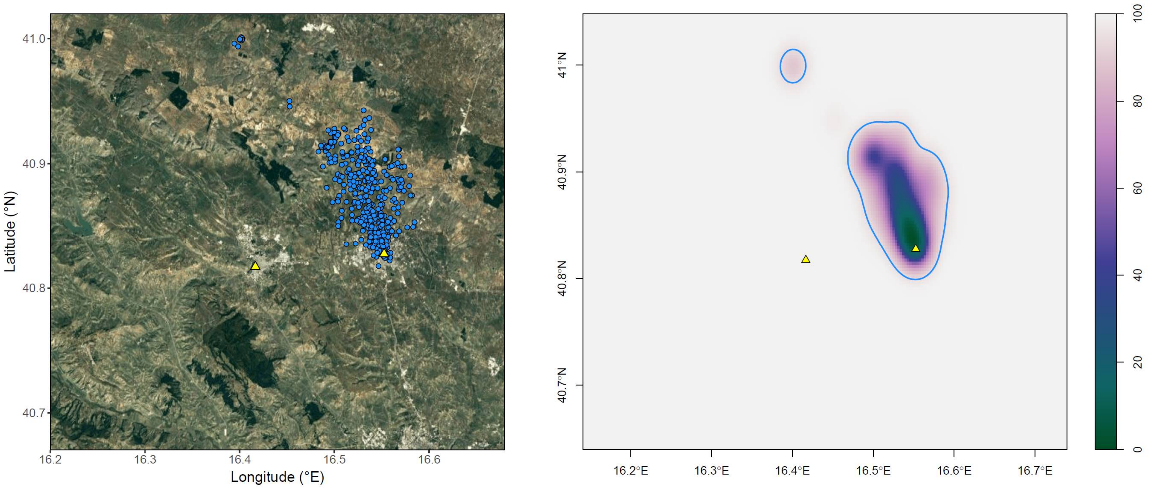

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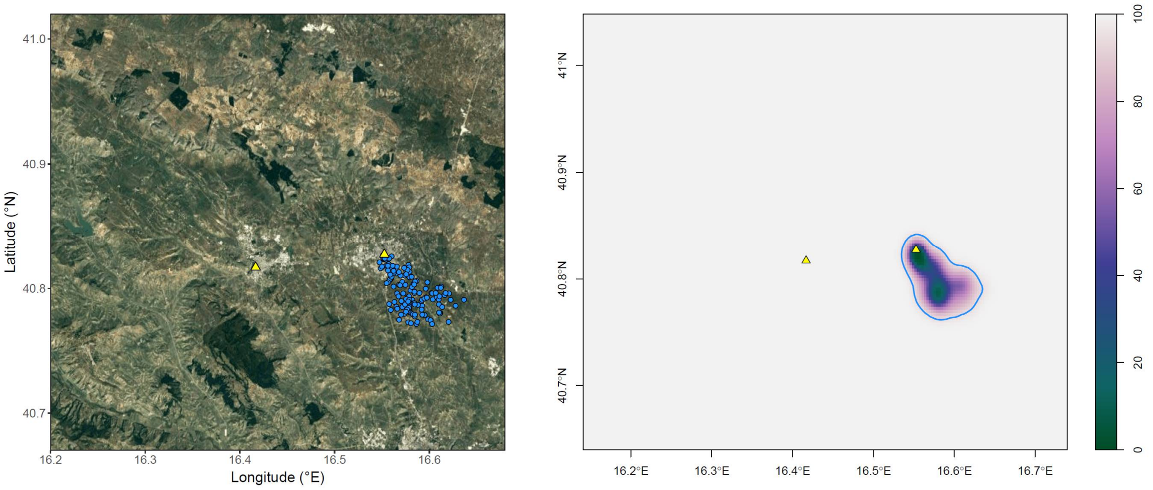

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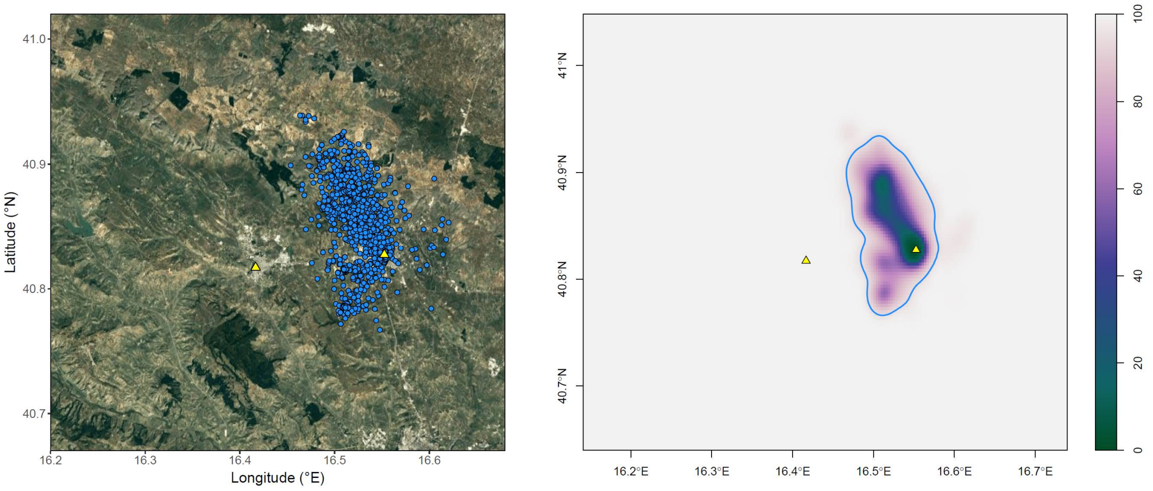

Figure S3. Maps of selected GPS positions (left panels) and volume UD (with 95% KDE) (right panels) for each tracked individual in Sicily. The position of the breeding sites are marked with yellow triangles. Individuals (see list in Table 1) are sorted according to colony site (CAN = Canalotto, blue dots; SAN = San Gregorio, green dots; TOR = Torrevecchia, red dots). The corresponding metric unit is shown in the first map.

Map data: Google, TerraMetrics 2018, plotted with the R package *ggmap* (Kahle & Wickham 2013).

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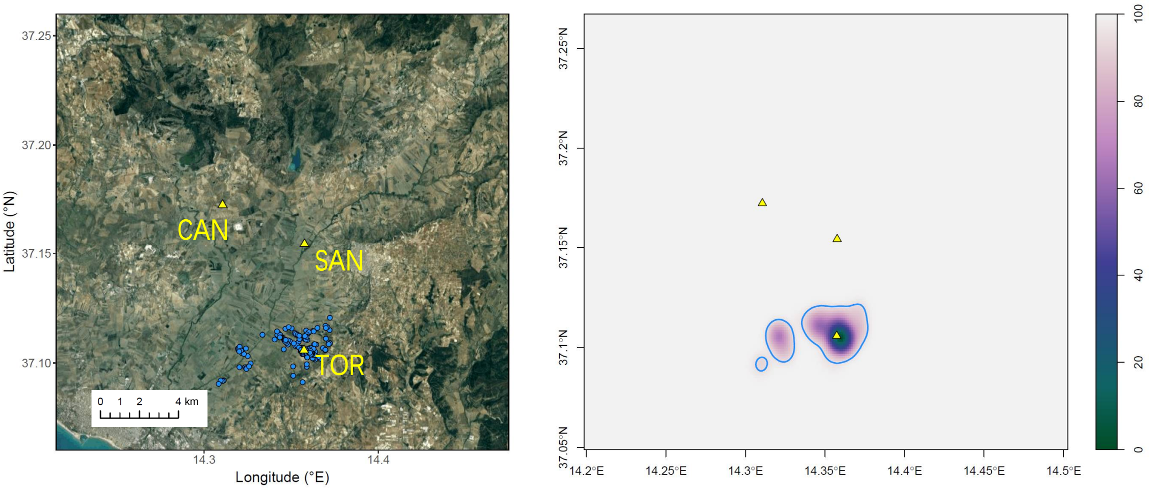

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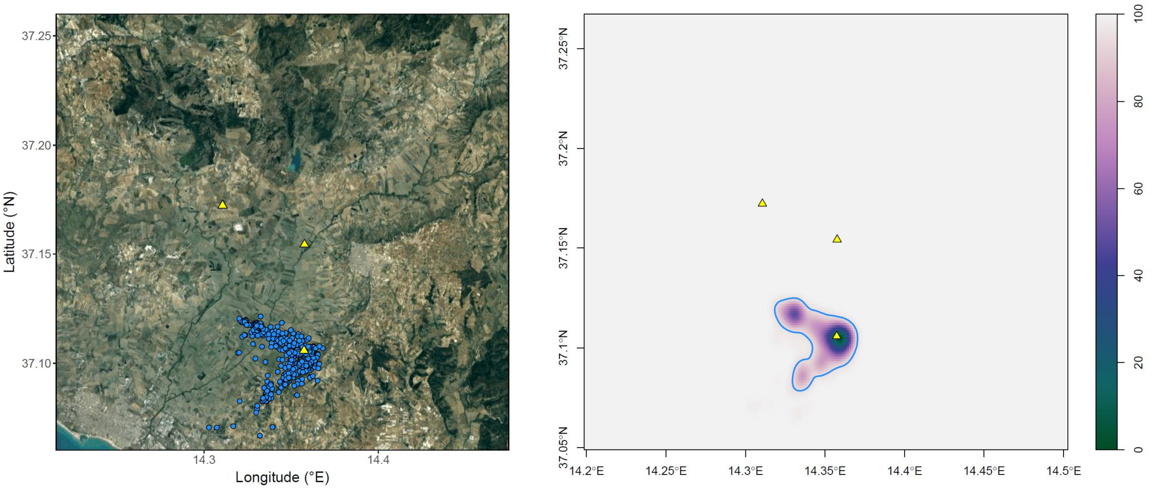

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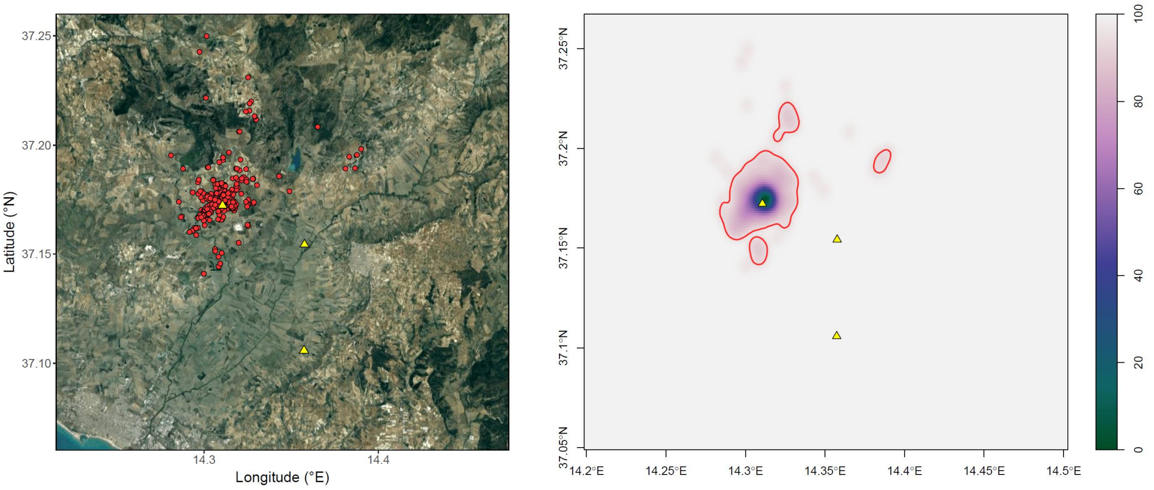

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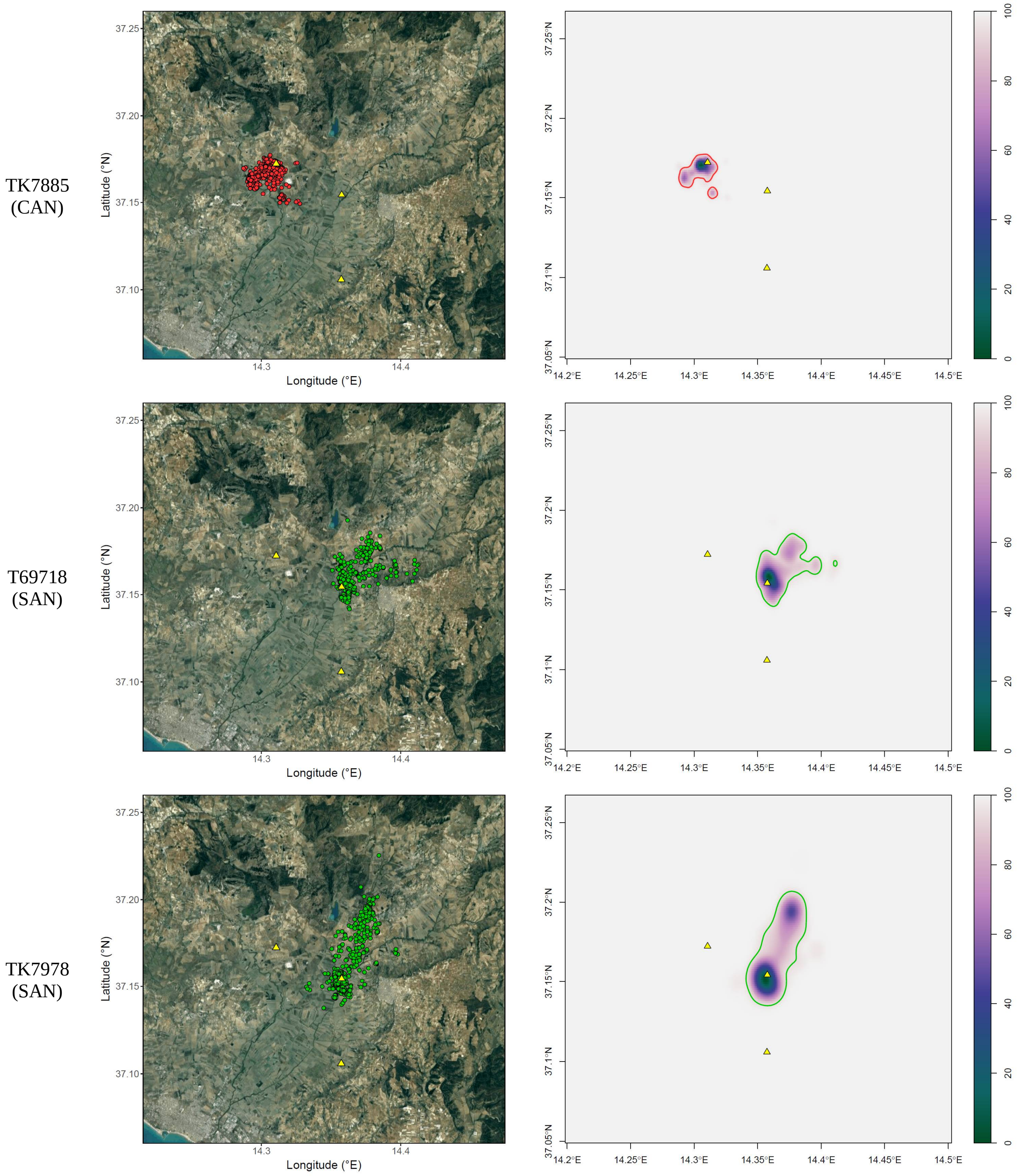
Figure S3. Continued



Chapter 5.

Foraging tactic of a colonial raptor differs among individuals and varies according to weather conditions

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Abstract

1. Consistent inter-individual differences in behavioural phenotypes and their fitness correlates have been documented in several animal species. Diversification in foraging behaviours may reduce ecological niche overlap, resulting in a mitigation of intraspecific competition. This can be particularly advantageous for colonial species breeding in large aggregations and exploiting shared foraging areas. At the same time, different foraging behaviours may vary in their efficiency and energy expenditure, with different fitness implications.
2. We investigated individual differences in foraging tactics in a colonial raptor species, the lesser kestrel (*Falco naumanni*), and assessed their implication in terms of energy expenditure and fitness.
3. We analyzed 279 foraging trips from breeding individuals equipped with GPS-accelerometer data-loggers by means of expectation–maximization binary clustering to infer behavioural mode at each GPS position. Cluster analysis of the relative duration of each behavioural mode within a given foraging trip identified two distinct foraging tactics: widely-foraging and sit-and-wait. Using behavioural reaction norms we investigated differences in individual tendency to adopt a specific foraging tactic across weather condition gradients. Energy expenditure for each foraging trip was estimated by means of accelerometer data. Finally, we assessed the relationship between the individual tendency to adopt a given foraging tactic and nestling growth rate (a proxy for fitness).
4. Overall, lesser kestrels preferred to pursue widely foraging tactic over sit-and-wait as solar radiation and cross-wind-component intensity increased, with the individual tendency to adopt a specific foraging strategy being consistent across weather condition gradients. Energy expenditure in widely foraging trips was markedly higher than in sit-and-wait trips, but nestling feeding rates of parents that were more prone to adopt the more energy demanding tactic were higher and their nestlings grew faster.

5. Our study provided major insights into the intraspecific variability in foraging behaviour of a colonial species, and how it is modulated by weather changes. We further highlighted broad energy expenditure differences associated with alternative foraging tactics. Importantly, the fitness benefits for parents adopting the most energy expensive foraging tactic suggest that individual differences in foraging behaviour may play a key role in maintaining life-history trade-offs between reproduction and self-maintenance.

Introduction

Inter-individual differences in behavioural phenotypes, that are consistent over time and across environmental contexts, have been frequently documented in animals (Réale et al. 2010). Besides, individuals can also consistently differ in how they modulate specific behaviours in accordance to external stimuli, the so-called contextual plasticity (Stamps & Groothuis 2010). Ultimately, inter-individual differences in behavioural phenotypes and in contextual plasticity have evolved because they may have major fitness effects (Dingemanse & Réale 2005; Smith & Blumstein 2008), which may be translated at the population level (Brown 1996; Hilton et al. 1999; Briffa et al. 2008; Refsnider et al. 2012). For instance, individual differences in foraging behaviour may favour foraging specialization, resulting in a reduction of intraspecific competition by limiting niche overlap (Bolnick et al. 2003; Swanson et al. 2003; Bolnick et al. 2007, Araújo et al. 2011; Navarro et al. 2017;).

In colonial species, where several conspecifics gather in groups to reproduce close to each other and exploit shared foraging areas (Kuhn et al.2014; Lascelles et al. 2016; Cecere et al. 2018), resource depletion around colony sites commonly occurs (Ashmole 1963). Such depletion may thus favour the evolution and maintenance of individual foraging specialization, which may be important in limiting the negative fitness effects of intraspecific competition. Indeed, individual differences in dietary preferences have been documented in several colonial vertebrates, including birds (e.g. Woo et al. 2008; Ceia & Ramos 2015; Camprasse et al. 2017), pinnipeds (e.g. Cherel et al. 2009; Hückstädt et al 2012) and a few terrestrial mammals (Cryan et al. 2012). Such dietary preferences may originate from spatio-temporal individual variation in foraging behaviour. For example, female northern fur seals *Callorhinus ursinus* show consistent individual differences in foraging trips during the lactating phase (Call et al. 2008). Imperial shags *Phalacrocorax atriceps* are highly consistent within individuals in the maximum distances they reached from the shore and the colony, as well as in the time invested in flight and diving, both within a single breeding season and across years (Harris et al. 2014). The analysis of foraging trips of northern gannets *Morus bassanus* breeding in two large colonies revealed consistent individual preferences in foraging areas during the nestling-rearing period (Patrick et al. 2013). In the latter species, the analysis of both food boluses and blood isotopes, combined with the analysis of at-sea foraging behaviour, have allowed documenting individual differences in foraging tactics, with some birds exploiting consistently and more frequently than others discards from fishing vessels (Votier et al. 2010). In addition, some studies have found that individual differences in foraging behaviour may also arise from sexual (Lewis et al. 2005; Patrick et al 2014a), age (Field et al. 2007; Votier et al. 2017), reproductive stage (Focardi & Cecere 2014) or physiological status differences (Watanabe et al. 2006).

Individual differences in foraging behaviour can be conceptually considered as personality differences whenever these are consistent across environmental contexts (Patrick eta al. 2014b). However, in spite of the large number of studies investigating foraging behaviour and diet choice, these traits are rarely examined from an animal personality perspective (Dall et al. 2012).

The lesser kestrel *Falco naumanni* is a small (ca. 120 g) colonial diurnal raptor, which mostly breeds in holes and crevices of buildings in towns and cities, and forages in farmland areas surrounding breeding sites (Cramp 1998; Cecere et al. 2018), where it targets a wide spectrum of prey including vertebrates (mice and lizards) and invertebrates (mainly Orthoptera but also Coleoptera and Scolopendromorpha) (Cramp 1998). Lesser kestrels show a flexible foraging behaviour, whereby both flight mode and hunting strategy vary in accordance to weather conditions (Hernandez-Pliego et al. 2017): flapping flight is more frequently adopted than soaring-gliding when solar radiation is high, and perch-hunting is more frequently used than flight-hunting when both wind speed and solar radiation are lower. However, it is as yet unknown whether individuals consistently differ in their foraging tactic across weather condition gradients, and whether there are individual differences in the reaction norms to weather conditions.

In this study, we first defined the foraging tactics adopted during each foraging trip by lesser kestrels breeding in a large colony and tracked over multiple foraging trips with miniaturized bio-loggers (GPS and tri-axial accelerometer). By means of expectation–maximization binary clustering

(EMbC) (Garriga et al. 2016), which is particularly suitable to investigate behavioural responses to environmental cues (Benisson et al. 2017), we inferred behavioural modes (i.e. perching, relocation, intensive and extensive search) associated to each 1-min GPS position during foraging trips. Foraging tactics were identified through cluster analysis of the relative duration of each behavioural mode within a given foraging trip (Louzao et al. 2014). This approach allowed us to identify foraging tactics considering the entire behavioural pattern shown by individuals during a given foraging trip. Subsequently, using behavioural reaction norms estimated from mixed effect models (Dingemanse et al. 2010), we investigated variation in foraging tactics according to weather condition gradients (i.e. gradients in solar radiation, rain and wind), controlling for breeding stage (incubation vs. nestling-rearing), sex and exploited habitat type. More specifically, we investigated whether individual differences in foraging tactics were consistent across environmental contexts, i.e. whether individuals showed consistent prey searching personalities (*sensu* Réale et al. 2010), and assessed the extent of individual differences in the behavioural responses to weather condition gradients (contextual plasticity *sensu* Stamps & Groothuis 2010). Since differences in energy expenditure according to foraging tactics were suggested for different birds of prey (e.g Nadjafzadeh et al. 2016), we estimated the energy expenditure of each foraging trip by means of tri-axial accelerometer data and assessed the energy expenditure of different foraging tactics. Finally, we explored the fitness correlations of foraging strategies by assessing the association between offspring growth rates and parental foraging tactics.

Considering the flexible foraging behaviour of the lesser kestrel (Hernandez-Pliego et al. 2017), we expected foraging trips to be characterized by two main behavioural modes: specifically, birds should mainly search for prey in flight, i.e. adopting a “widely foraging” (WF) tactic, whenever weather conditions are particularly favourable to soaring-gliding (high solar radiation; Hernandez-Pliego et al. 2017) and with wind assistance (tailwind or crosswind) at departure from the nest site (Mellone et al. 2012; Klaasen et al. 2010). Under opposite weather conditions (i.e. low solar radiation, headwind at departure), we expected that birds mainly adopt a more static foraging tactic, i.e. “sit-and-wait” (SAW) foraging, characterized by prolonged perching periods waiting for prey detection. According to previous studies analyzing individual differences in movement patterns of colonial animals (e.g. Call et al. 2008, Votier et al. 2010, Patrick et al. 2013), we expected that individuals consistently differ in their tendency to adopt a given foraging tactic, but we also predicted such differences to be consistent across weather condition gradients. Finally, we predicted WF to be associated with higher energy expenditure compared to SAW (Nadjafzadeh et al. 2016.).

Materials and methods

Target species and study area

The lesser kestrel is a sexually size dimorphic species, females being ca. 15% heavier than males (Cramp 1998; our unpubl. data). Females lay up to five eggs that both parents incubate for ca. 30 days. After hatching, both parents feed the nestlings until fledging, which takes place at 35-40 days of age. The study was carried out in the lesser kestrel colony of the city of Matera (S. Italy; 40°39′ N, 16°36’ E), hosting ca. 1000 breeding pairs (La Gioia et al. 2017). We relied on nest-boxes placed on buildings roofs in the old town (Podofillini et al. 2018), which allowed us to easily capture breeding individuals (by hand or by specially designed nest traps) during both incubation and nestling-rearing stages. Data were collected in the first two decades of June of the breeding seasons 2016 and 2017, when pairs were in the late incubation or early nestling-rearing stage.

GPS deployment and identification of foraging trips

We equipped 25 breeding lesser kestrels (7 females and 18 males) with Axy-Trek loggers by TechnoSmArt S.pA., Rome, Italy. The Axy-Trek devices, consisting of a GPS logger and a tri-axial accelerometer logger, were deployed on the back using Teflon harness. For all devices, the tri-axial accelerometer was set to record data at 25 Hz and the GPS to record one position per minute from

05:00 to 21:00 local time (i.e. ca. 20 min before sunrise and ca. 30 after sunset). Since our focus was on foraging activity, we preserved battery power by stopping collecting data during night-time roosting. The total weight of the equipment (including the harness) varied between 6.0 and 7.2 g, which on average accounted for 4.6% of the birds' body mass (range: 3.9 - 5.3%). Nest-boxes were monitored three times per week to obtain detailed data about reproduction stage (i.e. laying dates, incubation, hatching, nestling growth). Birds were tracked for 2-5 days, after which batteries were exhausted. They were thus recaptured and the devices were removed. Birds were tagged in the morning and devices were set to start the following day, in order to collect data when the tagged birds were likely inured to the device.

Foraging trips were identified as those tracks starting and ending within a 50-m buffer around the nest site, and heading to the rural surroundings, by means of ESRI ArcMap 10.2.1. We did not consider as foraging trips all the excursions which only covered the urban area, identified by means of the 2012 CORINE Land Cover (CLC) map (codes 111 and 112, respectively continuous and discontinuous urban habitat). Each trip was classified as occurring during incubation if only eggs were present in the nest of the target individual on the date when the foraging trip was performed, or as occurring during the nestling-rearing stage if at least one nestling was present in the nest on the date when the trip was performed.

Identification and characterisation of foraging tactics

To identify and characterise foraging tactics adopted during each foraging trip, we first identified behavioural modes from GPS data by applying the Expectation Minimization binary Clustering (EMbC) algorithm using the "EMbC" package (Garriga et al. 2016) for R ver. 3.3.2 (R Core Team 2016). The EMbC is a state-space model based on maximum likelihood which assigns a behavioural mode to each GPS position according to the instantaneous speed and the turning angles between successive positions. The algorithm identifies four behavioural modes: perching (characterised by low speed and low turns), intensive search (low speed and high turns), relocation (high speed and low turns) and extensive search (high speed and high turns). After clustering, we applied a post-processing smoothing implemented in the EMbC package to minimize incorrect labelling of single localizations. For this procedure, that is based on the temporal behavioural correlations, we used the default value of the maximum likelihood difference to accept a relabelling, δ_w=1, i.e. “accept all changes”.

Then, to identify the main foraging tactics, we performed a hierarchical clustering of all recorded trips based on the proportion of the four behavioural modes observed during each trip, using the “Pvclust” R-package (with Euclidean distance and Ward’s aggregation method; Suzuki & Shimodaira 2015). To find the optimal number of clusters, we used three different methods:1) the ‘elbow’ method, selecting the number of clusters that minimizes the total intra-cluster variation; 2) the ‘silhouette’ method, which measures the quality of a clustering by assessing the similarity of an object compared to the others; and 3) the gap statistics for hierarchical clustering, which compares the total within intra-cluster variation with their expected values under null reference distribution of the data (Tibshirani et al. 2001). All three methods coherently identified two clusters as those optimally grouping foraging trips. These two clusters represent two different foraging tactics.

To characterize differences between the two foraging tactics, we compared, by means of linear mixed models (LMMs, with individual identity as a random factor), the percentage of the four behavioural modes (as identified by the EMbC method) in each foraging trip and the following descriptors calculated for each trip using the “trip” R-package (Summers et al. 2016): (1) duration; (2) total trip length; (3) maximum distance from the nest site; (4) tortuosity, calculated as the ratio between the total trip length and the maximum distance from the breeding site.

Finally, we calculated the overall dynamic body acceleration (ODBA) for each foraging trip using tri-axis accelerometer data. ODBA provides a measure of dynamic acceleration mainly resulting from the movement of body parts, i.e. excluding the static acceleration resulting from body angle with respect to gravity (Wilson et al. 2006; RopertCoudert et al. 2007, Halsey et al. 2008) and it is considered a proxy of energy expenditure in birds (Halsey et al. 2011; Elliot et al.

2013). ODBA was shown to be positively correlated to O_2 consumption rates and CO_2 production in great cormorants (*Phalacrocorax carbo*) (Wilson et al. 2006) and to heart rate in two griffon vulture species (*Gyps fulvus* and *G. himalayensis*) (Duriez et al. 2014). According to Wilson et al. (2006), we computed ODBA for each foraging trip as the sum of the instantaneous dynamic acceleration values, obtained subtracting the total acceleration data from each of the three axes to the static acceleration (total acceleration smoothed with a 1 s running). Finally, we investigated differences in energy expenditure according to foraging tactics by running LMM of ODBA with individual identity as a random intercept effect, and foraging tactic, sex.

Both LMMs and LMs also included all possible interactions between variables as predictors, which were then removed if non-significant ($P > 0.05$) in the final models.

Environmental variables

To assess whether the probability to adopt a given foraging tactic was affected by weather conditions, each trip was associated to: (1) solar radiation at departure, which seems to be determinant for performing soaring-gliding flight in lesser kestrel (Hernandez-Pliego et al. 2017); (2) presence/absence of rain at the trip departure, which we hypothesized may affect the decision of flying; (3) tail-wind (TWC) and (4) cross-wind components (CWC), both of which are known to affect movement activity in soaring raptors (Mellone et al. 2012; Klaasen et al. 2010). We also included sex, breeding stage (incubation vs. nestling-rearing) and the proportion of each trip spent in arable lands (i.e. the main habitat used for foraging) as covariates.

Solar radiation and rain information were recorded at a weather station located at 8 km from the nest sites (Matera, Contrada Matinelle, 40°41' N; 16°31' E). Wind information (speed and direction) were recorded at a different weather station, located at 15 km from the nest sites (Grottole 40°37’ N; 16°26’ E).

TWC and CWC were calculated for each trip from the mean speed and direction of the wind (WS and WD respectively) associated to each GPS position, and the mean direction of the trip (TD), as follows:

$$TWC = WS \times \cos(TD - WD)$$

$$CWC = |WS \times \sin(TD - WD)|$$

The mean direction of the trip was calculated as the angle between the N-S axis (directed northwards) and the position of the furthest point of the trip. A positive TWC implies that the bird flew globally with tail-wind on its way out of the colony towards the foraging grounds, whereas a negative TWC indicates the opposite (outgoing flights with headwinds). A large CWC value means that the bird flew with a high global side-wind during the foraging trip.

To evaluate whether foraging tactics were affected by habitat, we assigned all GPS positions, excluding those identified as relocation by the EMbC, to the corresponding habitat type from CLC by means of ESRI ArcMap 10.2.1. We pooled together those CLC habitat types that were similar in habitat and structure, obtaining 6 habitats classes: artificial landscape (continuous and discontinuous urban fabric, infrastructures, industrial areas), arable lands, permanent crops (tree plantations, olive groves, vineyards), grasslands (pastures and natural grasslands), heterogeneous agricultural areas (annual crops associated with permanent crops, complex cultivation patterns, agro-forestry areas), and wooded areas (forests and bushes). Each trip was then characterised by the percentage of positions occurring in each habitat class. Arable land was the main used habitat during foraging trips (60.22% SE: 2.08) and it was negatively correlated with grassland, the second most frequently used habitat (16.92% SE: 1.66) (r = -0.67, p < 0.0001, n = 279 trips).

Analysis of factors affecting foraging tactics

The probability to adopt a given foraging tactic identified by the cluster analysis was modelled by means of a binomial generalised linear mixed model (GLMM) with foraging tactic as the binary dependent variable (0 = SAW, 1 = WF) and solar radiation, TWC, CWC, and presence/absence of rain as predictors, controlling for sex, breeding stage, habitat (% of arable land) and sampling year. Because sexually differences in body size (Cramp 1998), we cannot exclude different behavioural response of sexes to weather conditions; we hence included in the model all the interactions between sex and each of weather variables (i.e. solar radiation, TWC, CW and rain) as predictors. The non-significant ($P > 0.05$) interactions were then removed in a single step. All predictors were standardized to have mean = 0 and standard deviation = 1. Bird identity was included as a random intercept effect in all models. Binomial GLMMs were checked for overdispersion by means of the “blmeco” R-package (Korner-Nievergelt et al. 2015).

Analysis of individual differences and fitness consequences

The random intercept effect of the above-mentioned binomial GLMM describes the extent to which individuals preferentially adopt one of the two foraging tactics (i.e. whether foraging tactics can be regarded as a personality trait; Biro 2010). To investigate individual differences in the response to environmental gradients (behavioural reaction norms), which represent the extent of contextual plasticity (see Dingemanse et al. 2010 for more details), we re-run the final binomial GLMM by including a random slope effect at the individual level. Random slopes were fitted only for weather variables significantly affecting the modulation of the foraging tactics, as resulting by the binary GLMM described above, to avoid model overparametrization and lack of convergence. Significance of random intercept and slope effects were tested by means of parametric bootstrapping (using the “pbnm” R-package, with n = 10000 resamplings; Banghart 2015).

To assess whether the individual tendency to adopt one tactic over the other one was associated with fitness, we tested the relationship between the individual intercept, derived by binomial GLMM described above using the *ranef* fuction in “lme4” R-package (Bates et al. 2015), and offspring growth rate. The latter was defined as the difference between the mean body mass of all nestlings of a given nest at 12 days and that at 3 days after hatching of the first egg of the clutch. Given the coding of the foraging tactic in the binomial GLMM (0 = SAW, 1 = WF), higher values of the individual intercept imply a higher propensity of individuals to adopt the WF tactic. The fitness consequence of the individual tendency to adopt WF tactic was hence tested by means of a linear model (LM) with offspring growth rate as dependent variable and individual intercept as a predictor, controlling for sex, sampling year and brood size at 12 days from hatching of the first egg. This analysis only regarded the 15 successful breeders that were tracked (i.e. those whose eggs hatched and whose offspring were alive at 12 days from hatching of the first egg).

Finally, to assess whether individuals with a higher tendency for WF tactic feed their nestlings more frequently than individuals with a lower tendency for WF, we modelled the number of foraging trips per hours as a function of the individual tendency to adopt WF, controlling for sex. For this latter analysis we only considered foraging trips performed during the nestling-rearing stage (from 11 individuals). Because of the small sample size we did not include in the model brood size and sampling year which, however, did not significantly affect the number of foraging trips per hours in single-variable LMs ($P > 0.91$).

Results

Identification and characterization of foraging tactics

We obtained 281 foraging trips (110 in 2016 and 171 in 2017) from 25 breeding birds. Details about sub-samples and trip characteristics related to breeding stages and sampled years are reported in Table 1. Foraging trips performed during incubation stage were significantly longer than those performed during the nestling-rearing stage, with no significant effects of sex and year (Table 2).

Consequently, birds covered significantly greater distances during incubation trips than during nestling-rearing trips (Table 2).

Each trip was characterised by the percentage of time spent performing each of the four behavioural modes (Fig. S1 and S2). Two trips, which only included relocations, were removed from the analyses. The 279 remaining trips were then subjected to hierarchical cluster analysis, which identified two distinct clusters (Fig 1). The first cluster included trips characterised by high frequency of perching positions, and low frequency of intensive search and relocation positions, representing a SAW tactic. The other cluster included trips characterised by high frequency of relocation and intensive search positions, and a low frequency of perching positions, representing WF tactic, with bird mostly searching for food while flying. On average, SAW foraging trips lasted longer than WF trips, whereas all other trip descriptors did not significantly vary according to foraging tactic (Table 3). Accelerometer data were available for 23 out of 25 tagged birds. ODBA, computed for 219 trips, was markedly higher for WF foraging trips than for SAW foraging with no effects of sex and brood size (Table 2).

Environmental gradients affecting foraging tactics, individual differences in foraging behaviour and their effects on nestling growth rate

The probability of adopting WF was positively affected by solar radiation, CWC and breeding stage (Table 4): with low CWC and low solar radiation, birds mostly adopted SAW, whereas as CWC and solar radiation increased, the probability of adopting WF increased (Fig. 2). Birds adopted significantly more WF trips during the nestling-rearing stage compared to the incubation stage (Table 4). The random intercept effect was significant ($P < 0.01$), indicating that individuals consistently differed in foraging tactic across solar radiation and CWC gradients (Fig. 3). The variance estimate for the random slope in response to changes in weather conditions was not significant for both solar radiation ($P = 0.92$) and CWC ($P = 0.77$), indicating that tracked individuals responded in a similar way to weather condition changes.

Offspring growth rate was positively affected by the individual tendency to perform WF, with no significant effects of brood size, sex, and sampling year (Table 2). Finally, the number of nestling-rearing foraging trips performed per hour was marginally significantly higher among parents showing a stronger tendency to adopt WF, with males performing more foraging trips per hour than females (Table 2).

Discussion

We analyzed the foraging behaviour of lesser kestrels to investigate the degree of individual specialization in foraging tactic during the breeding period in a colonial bird species. Foraging trips performed during incubation were more extended in time and space than those performed during early nestling-rearing stage, a common pattern in central place foraging bird species (Weimerskirch et al. 1993; Guilford et al. 2008; Cecere et al. 2013). Like many other raptor species (Andersson & Norberg 1981), male lesser kestrels perform more frequent foraging trips than females during nestling-rearing stage. By means of hierarchical cluster analysis based on the relative duration of each behavioural mode identified with EMbC algorithm, we identified two distinct tactics adopted by breeding lesser kestrels tracked over multiple foraging trips: one was strongly characterized by perching, lower proportion of intensive search and relocation and it was associated to long lasting trips (SAW tactic); the other one was characterized by a higher proportion of both relocation and intensive search, lower perching and it mainly referred to short lasting trips (WF tactic). WF was associated to 1.6-fold higher energy expenditure compared to SAW. The probability of adopting WF tactic over SAW increased with increasing solar radiation and CWC, and it was higher during nestling-rearing stage.

On top of this, individuals consistently differed in their preference for adopting a given foraging strategy, while no significant contextual plasticity emerged (i.e. all individuals modified their foraging tactic in a similar way in relation to weather gradients). Finally, the more individuals showed a tendency to adopt the most energy demanding foraging tactic (i.e. WF) tactic, the higher the growth rate of their offspring was.

The occurrence of both SAW and WF tactics has been documented for many predator species, including reptiles (e.g. Perry 1999; McLaughlin 1989), fish (e.g. East & Magnan 1991; Grant & Noakes 1988), birds (e.g. McLaughlin 1989; Nadjafzadeh et al. 2016) and mammals (Williams et al. 2014). However, to our knowledge, the alternation of the two foraging tactics has seldom been analyzed at the individual level (Wakeley 1978). The lesser kestrel relies on thermal soaring when foraging, especially during periods of high solar radiation (Hernández-Pliego et al. 2015). Here we show that foraging individuals of lesser kestrel mainly adopted SAW when weather conditions were not ideal for soaring-gliding fights, i.e. with low solar radiation (Hernández-Pliego et al. 2015) and low crosswinds, a wind condition which is known to affect movement activity in soaring raptors (Mellone et al. 2012; Klaasen et al. 2010). Accordingly to what was predicted by a number of studies of raptors (Wakeley 1978; Nadjafzadeh et al. 2016), we found that SAW is a low-energy expenditure foraging tactic compared to WF. However, as weather conditions get better for soaring-gliding individuals preferred to switch to the more energy demanding WF tactic. This was likely the case because WF allowed birds to perform shorter lasting foraging trips compared to SAW, thus increasing offspring feeding rates. Indeed, WF was more frequently adopted during nestling-rearing, when breeders are constrained to frequently come back to the nest for feeding their offspring, compared to incubation, when foraging trips are aimed at self-feeding.

We envisage at least two positive fitness effects of adopting the most energy demanding foraging tactic for breeding adults. First of all, we were able to show that birds that were more prone to adopt WF were able to increase the feeding rate of their offspring with respect to parents mainly adopting SAW tactic. Although this finding should be viewed with caution because we could assess the behaviour of a single partner, it suggests that, even when considering the uniparental contribution to nestling growth, an increased energy expenditure during foraging could result in positive effects on fitness and faster offspring growth due to the higher frequency of foraging trips. An additional possible benefit of adopting WF compared to SAW may be the increased opportunity to forage in group by means of local enhancement processes, whereby individuals searching for food are attracted by feeding aggregations of other individuals (Machovsky-Capuska et al. 2014). It has been predicted that social foraging increases individual foraging efficiency, particularly when birds exploit unpredictable opportunities (Overington et al. 2008). The higher opportunity of foraging in groups when adopting the WF tactic may explain the shorter duration of foraging trips respect to the one associated to SAW trips. In the study area, we regularly observed large aggregations of lesser kestrels following harvesting machines to catch large orthopterans flushed during harvesting operations or social foraging around burning cereal stubbles, with individuals waiting for prey escaping from fires. It is likely that such unpredictable and profitable social foraging opportunities can be better exploited by adopting WF rather than SAW.

Besides the general figure about the alternation of the two foraging tactics according to weather conditions, our results also showed an individual component to foraging tactic, with individuals being consistently more prone to adopt a specific foraging tactic, and that these differences were consistent across different environmental gradients of solar radiation and wind assistance. Such individual differences in foraging tactics may reflect foraging specialization, and may contribute to reduce intraspecific competition at foraging grounds. By adopting different foraging tactics, lesser kestrels may indeed forage in different sites and/or target different prey items. Although we did not find any difference in the main used habitats (arable lands and grasslands) between SAW and WF trips, it is anyhow possible that birds targeted on different prey items accordingly to foraging tactic. According to Greene (1986), indeed, sit-and-wait predators generally catch larger prey compared to those taken by active predators (see also de Arruda Bueno and Motta-Junior 2008). We hence may hypothesize that lesser kestrel mainly target on lizard and small mammals during SAW foraging trips and that WF is mainly used by lesser kestrels to forage on slow-moving and flying large insects.

Overall, such individual difference in foraging behaviour, which was consistent across contexts, represents a personality difference (Patrick et al. 2014b; Dall et al. 2012). Our study supports the idea that individual differences in foraging behaviour widely occur in colonial species, likely resulting in a mitigation of intraspecific competition by limiting niche overlaps. Moreover, it reveals that foraging tactics are not equivalent in term of energy expenditure and fitness, ultimately suggesting that interindividual differences in foraging tactics may contribute maintaining key life-history trade-offs, such as those between reproduction and self-maintenance.

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References

Andersson, M., Norberg, R. Å. (1981). Evolution of reversed sexual size dimorphism and role partitioning among predatory birds, with a size scaling of flight performance. Biological Journal of the Linnean Society, 15: 105-130.

Araújo, M. S., Bolnick, D. I., Layman, C. A. (2011). The ecological causes of individual specialisation. Ecology letters, 14: 948-958.

Ashmole, N. P. (1963). The regulation of numbers of tropical oceanic birds. Ibis, 103: 458-473.

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. Journal of Statistical Software, 67: 1-48.

Banghart, M. (2015). pbnm: Parametric Bootstrap Test of Nested Models. R package version 0.3.0.9003.

Bennison, A., Bearhop, S., Bodey, T. W., Votier, S. C., Grecian, W. J., Wakefield, E. D., Jessopp, M. (2017). Search and foraging behaviors from movement data: A comparison of methods. Ecology and Evolution.

Biro, P. A., Beckmann, C., Stamps, J. A. (2010). Small within-day increases in temperature affects boldness and alters personality in coral reef fish. Proceedings of the Royal Society of London B: Biological Sciences, 277: 71-77.

Bolnick, D. I., Svanbäck, R., Fordyce, J. A., Yang, L. H., Davis, J. M., Hulsey, C. D., & Forister, M. L. (2003). The ecology of individuals: incidence and implications of individual specialization. The American Naturalist, 161: 1-28.

Bolnick, D. I., Svanbäck, R., Araújo, M. S., & Persson, L. (2007). Comparative support for the niche variation hypothesis that more generalized populations also are more heterogeneous. Proceedings of the National Academy of Sciences, 104: 10075-10079.

Briffa, M., Rundle, S. D., & Fryer, A. (2008). Comparing the strength of behavioural plasticity and consistency across situations: animal personalities in the hermit crab *Pagurus bernhardus*. Proceedings of the Royal Society of London B: Biological Sciences, 275: 1305-1311.

Brown, A. C. (1996). Behavioural plasticity as a key factor in the survival and evolution of the macrofauna on exposed sandy beaches. Revista Chilena de Historia Natural: 69, 469-474.

Call, K. A., Ream, R. R., Johnson, D., Sterling, J. T., & Towell, R. G. (2008). Foraging route tactics and site fidelity of adult female northern fur seal (*Callorhinus ursinus*) around the Pribilof Islands. Deep Sea Research Part II: Topical Studies in Oceanography, 55: 1883-1896.

Camprasse, E. C., Cherel, Y., Arnould, J. P., Hoskins, A. J., Bost, C. A. (2017). Combined bio-logging and stable isotopes reveal individual specialisations in a benthic coastal seabird, the Kerguelen shag. PloS one, 12: e0172278.

Cecere, J. G., Catoni, C., Maggini, I., Imperio, S., Gaibani, G. (2013). Movement patterns and habitat use during incubation and chick-rearing of Cory's shearwaters (*Calonectris diomedea diomedea*) (Aves: Vertebrata) from Central Mediterranean: influence of seascape and breeding stage. Italian journal of zoology, 80: 82-89.

Cecere, J. G., Bondì, S., Podofillini, S., Imperio, S., Griggio, M., Fulco, E., Curcio, A., Ménard, D., Mellone, U., Saino, N., Serra, L., Sarà, M. & Rubolini, D. (2018). Spatial segregation of home ranges between neighbouring colonies in a diurnal raptor. Scientific reports, 8: 11762.

Ceia, F. R., Ramos, J. A. (2015). Individual specialization in the foraging and feeding strategies of seabirds: a review. Marine Biology 162: 1923−1938.

Cherel, Y., Kernaléguen, L., Richard, P., & Guinet, C. (2009). Whisker isotopic signature depicts migration patterns and multi-year intra-and inter-individual foraging strategies in fur seals. Biology letters, 5: 830-832.

Cramp, S. (1998). The complete birds of the Western Palearctic on CD-ROM. Oxford University Press, Oxford.

Cryan, P. M., Stricker, C. A., Wunder, M. B. (2012). Evidence of cryptic individual specialization in an opportunistic insectivorous bat. Journal of Mammalogy, 93: 381-389.

Dall, S. R., Bell, A. M., Bolnick, D. I., Ratnieks, F. L. (2012). An evolutionary ecology of individual differences. Ecology letters, 15: 1189-1198.

de Arruda Bueno, A., & Motta-Junior, J. C. (2008). Small mammal prey selection by two owl species in southeastern Brazil. Journal of Raptor Research, 42: 248-255.

Dingemanse, N. J., Réale, D. (2005). Natural selection and animal personality. Behaviour, 142: 1159-1184.

Dingemanse, N. J., Kazem, A. J., Réale, D., Wright, J. (2010). Behavioural reaction norms: animal personality meets individual plasticity. Trends in Ecology & Evolution, 25: 81-89.

Duriez, O., Kato, A., Tromp, C., Dell'Omo, G., Vyssotski, A. L., Sarrazin, F., & Ropert-Coudert, Y. (2014). How cheap is soaring flight in raptors? A preliminary investigation in freely-flying vultures. PLoS One, 9: e84887.

East, P., Magnan, P. (1991). Some factors regulating piscivory of brook trout, *Salvelinus fontinalis*, in lakes of the Laurentian Shield. Canadian Journal of Fisheries and Aquatic Sciences, 48: 1735-1743.

Elliott, K. H., Le Vaillant, M., Kato, A., Speakman, J. R., Ropert-Coudert, Y. (2013). Accelerometry predicts daily energy expenditure in a bird with high activity levels. Biology letters, 9: 20120919.

Field, I., Bradshaw, C., van den Hoff, J., Burton, H. Hindell, M. (2007) Age-related shifts in the diet composition of southern elephant seals expand overall foraging niche. Marine Biology, 150: 1441–1452.

Focardi, S., & Cecere, J. G. (2014). The Lévy flight foraging hypothesis in a pelagic seabird. Journal of animal ecology, 83: 353-364.

Garriga, J., Palmer, J. R. B., Oltra, A., Bartumeus, F. (2016) Expectation-Maximization Binary Clustering for Behavioural Annotation. Plos one, 11: e0151984.

Grant, J. W., & Noakes, D. L. (1988). Aggressiveness and foraging mode of young-of-the-year brook charr, *Salvelinus fontinalis* (Pisces, Salmonidae). Behavioral Ecology and Sociobiology, 22: 435-445.

Greene, C. H. 1986. Patterns of prey selection: implications of predator foraging tactics. American Naturalist 128: 824–839.

Guilford, T., Meade, J., Freeman, R., Biro, D., Evans, T., Bonadonna, F., Perrins, C. M. (2008). GPS tracking of the foraging movements of Manx Shearwaters *Puffinus puffinus* breeding on Skomer Island, Wales. Ibis, 150: 462-473.

Halsey, L. G., Shepard, E. L., Hulston, C. J., Venables, M. C., White, C. R., Jeukendrup, A. E., Wilson, R. P. (2008). Acceleration versus heart rate for estimating energy expenditure and speed during locomotion in animals: tests with an easy model species, *Homo sapiens.* Zoology, 111: 231-241.

Halsey, L. G., Shepard, E. L., Wilson, R. P. (2011). Assessing the development and application of the accelerometry technique for estimating energy expenditure. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 158: 305-314.

Harris, S., Raya Rey, A., Zavalaga, C., Quintana, F. (2014). Strong temporal consistency in the individual foraging behaviour of Imperial Shags *Phalacrocorax atriceps*. Ibis, 156: 523-533.

Hernández-Pliego, J., Rodríguez, C., & Bustamante, J. (2015). Why do kestrels soar?. PloS one, 10: e0145402.

Hernández-Pliego, J., Rodríguez, C., Dell'Omo, G., Bustamante, J. (2017). Combined use of tri-axial accelerometers and GPS reveals the flexible foraging strategy of a bird in relation to weather conditions. PloS one, 12: e0177892.

Hilton, G. M., Ruxton, G. D., Cresswell, W. (1999). Choice of foraging area with respect to predation risk in redshanks: the effects of weather and predator activity. Oikos, 295-302.

Hückstädt, L. A., Koch, P. L., McDonald, B. I., Goebel, M. E., Crocker, D. E., Costa, D. P. (2012). Stable isotope analyses reveal individual variability in the trophic ecology of a top marine predator, the southern elephant seal. Oecologia, 169: 395-406.

Klaassen, R. H., Hake, M., Strandberg, R., Alerstam, T. (2010). Geographical and temporal flexibility in the response to crosswinds by migrating raptors. Proceedings of the Royal Society of London B: Biological Sciences, rspb20102106.

Korner-Nievergelt, F., Roth, T., von Felten, S., Guelat, J., Almasi, B. Korner-Nievergelt, P. (2015). Bayesian Data Analysis in Ecology using Linear Models with R, BUGS and Stan. Elsevier, Oxford, UK.

Kuhn, C. E., Ream, R. R., Sterling, J. T., Thomason, J. R., Towell, R. G. (2014). Spatial segregation and the influence of habitat on the foraging behavior of northern fur seals (*Callorhinus ursinus*). Canadian journal of zoology 92: 861-873.

La Gioia, G., Melega, L. Fornasari, L. (2017). Piano d'Azione Nazionale per il grillaio (*Falco naumanni*). Quad. Cons. Natura, 41, MATTM - Istituto Superiore per la Protezione e la Ricerca Ambientale (ISPRA).

Lascelles, B. G., Taylor, P. R., Miller, M. G. R., Dias, M. P., Oppel, S., Torres, L., Weimerskirch, H. (2016). Applying global criteria to tracking data to define important areas for marine conservation. Diversity and Distributions, 22: 422-431.

Lewis, S., Schreiber, E. A., Daunt, F., Schenk, G. A., Orr, K., Adams, A., Wanless, S. Hamer, K. C. (2005) Sex-specific foraging behaviour in tropical boobies: does size matter? Ibis, 147: 408–414.

Louzao, M., Wiegand, T., Bartumeus, F., Weimerskirch, H. (2014) Coupling instantaneous energy-budget models and behavioural mode analysis to estimate optimal foraging strategy: an example with wandering albatrosses. Movement Ecology, 2014: 2:8.

Machovsky-Capuska, G. E., Hauber, M. E., Libby, E., Amiot, C., Raubenheimer, D. (2014). The contribution of private and public information in foraging by Australasian gannets. Animal Cognition, 17: 849–858.

McLaughlin, R. L. (1989). Search modes of birds and lizards: evidence for alternative movement patterns. The American Naturalist, 133: 654-670.

Mellone, U., Klaassen, R. H., García-Ripollés, C., Limiñana, R., López-López, P., Pavón, D., Alerstam, T. (2012). Interspecific comparison of the performance of soaring migrants in relation to morphology, meteorological conditions and migration strategies. Plos one 7: e39833.

Nadjafzadeh, M., Hofer, H., Krone, O. (2016). Sit-and-wait for large prey: foraging strategy and prey choice of White-tailed Eagles. Journal of Ornithology 157: 165-178.

Navarro, J., Grémillet, D., Ramirez, F. J., Afán, I., Bouten, W., & Forero, M. G. (2017). Shifting individual habitat specialization of a successful predator living in anthropogenic landscapes. Marine Ecology Progress Series.

Overington, S. E., Dubois, F., Lefebvre, L. (2008). Food unpredictability drives both generalism and social foraging: a game theoretical model. Behavioral Ecology, 19: 836-841.

Patrick, S. C., Bearhop, S., Grémillet, D., Lescroël, A., Grecian, W. J., Bodey, T. W., Votier, S. C. (2013). Individual differences in searching behaviour and spatial foraging consistency in a central place marine predator. Oikos, 123: 33-40.

Patrick, S. C., Weimerskirch, H. (2014a). Consistency pays: sex differences and fitness consequences of behavioural specialization in a wide-ranging seabird. Biology letters, 10: 20140630.

Patrick, S. C., Weimerskirch, H. (2014b). Personality, foraging and fitness consequences in a long lived seabird. PLoS One 9: e87269.

Perry, G. (1999). The evolution of search modes: ecological versus phylogenetic perspectives. American Naturalist 153: 98–109.

Podofillini, S., Cecere, J. G., Griggio, M., Curcio, A., De Capua, E. L., Fulco, E., Pirrello, S., Saino, N., Serra, L., Visceglia, M., Rubolini, D. (2018). Home, dirty home: effect of old nest material on nest-site selection and breeding performance in a cavity-nesting raptor. Current Zoology https://doi.org/10.1093/cz/zoy01 2.

R Core Team. R: A language and environment for statistical computing. (R Foundation for Statistical Computing, 2016).

Réale, D., Dingemanse, N. J., Kazem, A. J., Wright, J. (2010). Evolutionary and ecological approaches to the study of personality. Phil. Trans. R. Soc. B 365: 3937-3946.

Refsnider, J. M., Janzen, F. J. (2012). Behavioural plasticity may compensate for climate change in a long-lived reptile with temperature-dependent sex determination. Biological Conservation, 152: 90-95.

Ropert-Coudert, Y., Wilson, R. P., Gremillet, D., Kato, A., Lewis, S., Ryan, P. G. (2007). Electrocardiogram recordings in free-ranging gannets reveal minimum difference in heart rate during flapping versus gliding flight. Marine Ecology-Progress Series 331: 309

Smith, B. R., Blumstein, D. T. (2008). Fitness consequences of personality: a meta-analysis. Behavioral Ecology, 19: 448-455.

Stamps, J. Groothuis, T. G. G. (2010a). The development of animal personality: relevance, concepts and perspectives. Biological Reviews 85: 301–325.

Summers, M. D., Luque, S., Fishbach, A. (2016). Package 'trip' – Tools for the Analysis of Animal Track Data URL: https://github.com/mdsumner/trip.

Suzuki, R., Shimodaira, H., Suzuki, M. R., Suggests, M. A. S. S. (2015). Package 'pvclust'. R topics documented, 14.

Swanson, B. O., Gibb, A. C., Marks, J. C., Hendrickson, D. A. (2003). Trophic polymorphism and behavioral differences decrease intraspecific competition in a cichilid, *Herichthys minckleyi*. Ecology, 84: 1441–1446.

Tibshirani, R., Walther, G., Hastie, T. (2001). Estimating the number of clusters in a data set via the gap statistic. Journal of the Royal Statistical Society: Series B (Statistical Methodology), 63: 411-423.

Votier, S. C., Bearhop, S., Witt, M. J., Inger, R., Thompson, D., Newton, J. (2010). Individual responses of seabirds to commercial fisheries revealed using GPS tracking, stable isotopes and vessel monitoring systems. Journal of Applied Ecology, 47: 487-497.

Votier, S. C., Fayet, A. L., Bearhop, S., Bodey, T. W., Clark, B. L., Grecian, J., Wakefield, E. (2017). Effects of age and reproductive status on individual foraging site fidelity in a long-lived marine predator. Proceeding of the Royal Society B, 284: 20171068.

Wakeley, J. S. (1978). Activity budgets, energy expenditures, and energy intakes of nesting Ferruginous Hawks. The Auk, 667-676.

Watanabe, Y., Baranov, E.A., Sato, K., Naito, Y. & Miyazaki, N. (2006) Body density affects stroke patterns in Baikal seals. Journal of Experimental Biology, 209: 3269–3280.

Weimerskirch, H., Salamolard, M., Sarrazin, F., & Jouventin, P. (1993). Foraging strategy of wandering albatrosses through the breeding season: a study using satellite telemetry. The Auk, 325-342.

Williams, T. M., Wolfe, L., Davis, T., Kendall, T., Richter, B., Wang, Y., Wilmers, C. C. (2014). Instantaneous energetics of puma kills reveal advantage of felid sneak attacks. Science, 346: 81-85.

Wilson, R. P., White, C. R., Quintana, F., Halsey, L. G., Liebsch, N., Martin, G. R., Butler, P. J. (2006). Moving towards acceleration for estimates of activity-specific metabolic rate in free-living animals: the case of the cormorant. Journal of Animal Ecology, 75: 1081-1090.

Woo, K. J., Elliott, K. H., Davidson, M., Gaston, A. J., Davoren, G. K. (2008). Individual specialization in diet by a generalist marine predator reflects specialization in foraging behaviour. Journal of Animal Ecology, 77: 1082-1091.

Author Contributions

J.G.C. conceived the study and wrote the paper with inputs from D.R., S.I. and D.M.; D.M., S.I. and J.G.C. analysed the data; S.P., J.G.C., D.M., C.C., D.R. and M.G. carried out fieldwork.

Data Accessibility

Data will be upload on Movebank upon acceptance.

Table 1 – Foraging trip characteristics and sample size (N). For each variable the mean values ± S.E. (minimum and maximum value) are reported.

	Duration (h)	**Max distance from breeding site (km)**	**Total distance covered (km)**
2016 Incubation trips (N = 76)	2.4 ± 0.18 (0.21 - 9.76)	5.26 ± 0.28 (0.89 - 17.62)	18.22 ± 1.21 (3.85 - 55.59)
2017 Incubation trips (N = 91)	2.02 ± 0.16 (0.37 - 8.61)	6.63 ± 0.26 (0.61 - 13.33)	20.79 ± 0.89 (2.79 - 74.79)
All incubation trips (N = 167)	2.19 ± 0.12 (0.21 - 9.76)	6 ± 0.19 (0.61 - 17.62)	22.93 ± 1.3 (2.79 - 74.79)
2016 Nestling-rearing trips (N = 34)	1.37 ± 0.14 (0.2 - 3.2)	4.75 ± 0.42 (1.43 - 8.18)	14.68 ± 1.4 (3.21 - 29.34)
2017 Nestling-rearing trips (N = 78)	1.09 ± 0.07 (0.24 - 2.93)	6.03 ± 0.25 (2.45 - 13.98)	16.87 ± 0.83 (5.77 - 39.84)
All nestling-rearing trips (N = 112)	1.17 ± 0.06 (0.2 - 3.2)	5.66 ± 0.22 (1.43 - 13.98)	16.21 ± 0.72 (3.21 - 39.84)

Table 2 – Models 1 and 2: linear mixed models (LMM) of the effect of breeding stage (incubation and nestling-rearing) and sex (male and female) on trip duration and distance covered during foraging trips, respectively, while accounting for concomitant effect of sampling year (2016 and 2017). Model 3: LMM of the effect of foraging tactic (SAW and WF) on overall dynamic body acceleration (ODBA), accounting for sex effects. Models 4 and 5: linear models (LMs) of the effect of individual tendency to adopt WF (individual intercept from GLMM reported in Table 4) on offspring growth rate and number foraging trips per hours during nestling-rearing stage respectively, while accounting for the concomitant effects of other predictors. Non-significant interactions were removed in the final models.

Predictors	**Estimate ± SE**	***t***	***P***
1. *Trip duration (N = 279 trips from 25 birds)*			
Breeding stage	-0.97 ± 0.16	-6.07	< 0.0001
Sex	0.29 ± 0.21	1.34	0.18
Sampling year	-0.30 ± 0.16	-1.80	0.07
2. *Distance covered (N = 279 trips form 25 birds)*			
Breeding stage	-5.26 ± 1.28	-4.10	< 0.0001
Sex	0.77 ± 1.71	0.45	0.65
Sampling year	4.00 ± 1.35	2.95	0.003
3. *ODBA (N = 219 trips from 23 birds)*			
Foraging tactic	0.14 ± 0.01	12.10	< 0.0001
Sex	-0.004 ± 0.03	-0.32	0.89
4. *Offspring growth rate (N = 15 birds)*			
Individual tendency	15.31 ± 6.50	2.36	0.04
Brood size	-1.48 ± 3.51	-0.42	0.68
Sex	5.67 ± 7.71	0.74	0.48
Sampling year	5.66 ± 7.71	0.74	0.48
5. *Nestling rearing foraging trips/hour (N = 11 birds)*			
Individual tendency	0.22 ± 0.1	2.26	0.05
Sex	-0.30 ± 0.1	-3.17	0.01

Table 3 – Differences between trips belonging to the two foraging strategies (SAW and WF) in behaviour and movement parameters, assessed by means of linear mixed models with individual identity as a random intercept. For each variable the mean values ± S.E. are reported.

Variable	**SAW** **n = 123**	**WF** **n = 156**	**t**	**df**	***P***
Behavioural modes					
Perching (%)	31.27 ± 2.47	19.34 ± 1.91	-4.21	1	< 0.001
Relocation (%)	38.16 ± 1.73	45.30 ± 1.59	3.51	1	< 0.001
Intensive search (%)	23.36 ± 1.32	28.38 ± 1.30	2.78	1	0.005
Extensive search (%)	7.21 ± 0.79	6.99 ± 0.53	-0.12	1	0.91
Movement descriptors					
Duration (h)	2.52 ± 0.14	1.20 ± 0.07	-8.51	1	< 0.001
Total distance (km)	19.28 ± 0.96	18.69 ± 0.81	-0.65	1	0.52
Tortuosity	3.33 ± 0.08	3.15 ± 0.08	-0.4	1	0.7
Maximum distance (km)	5.74 ± 0.23	5.96 ± 0.19	-0.15	1	0.9
ODBA	0.25 ± 0.002*	0.41 ± 0.009**	12.14	1	< 0.0001

* *n = 107 foraging trips*
** *n = 112 foraging trips*

Table 3 – Binomial generalized linear model of foraging tactic (SAW vs. WF). The binomial dependent variable was coded as 0 for SAW and 1 for WF. Estimates refer to standardized variables. The model includes individual identity as a random intercept effect. The model was not overdispersed (ϕ = 1.0).

Predictors	**Estimate ± SE**	**Z**	***P***
Solar radiation	0.52 ± 0.16	3.31	0.001
CWC	0.57 ± 0.17	3.43	< 0.001
TWC	-0.17 ± 0.16	-1.03	0.30
Rain	-0.16 ± 0.16	-0.97	0.33
Arable lands	-0.02 ± 0.16	-0.11	0.91
Breeding stage	0.46 ± 0.19	2.51	0.01
Sampling year	0.64 ± 0.23	2.82	0.005
Sex	-0.07 ± 0.21	-0.36	0.72

Figure 1 – Cluster analysis of the foraging trips according to the percentage of the four behavioural modes identified by the EMbC analysis (extensive search, intensive search within each trip, n = 279 trips). Left: dendrogram of foraging trips derived from the cluster analysis. Right: percentage of positions in each of the four behavioural classes for each trip; red rectangles delimit the two clusters (cluster 1: 156 trips referred to WF tactic; cluster 2: 123 trips, referred to SAW tactic).

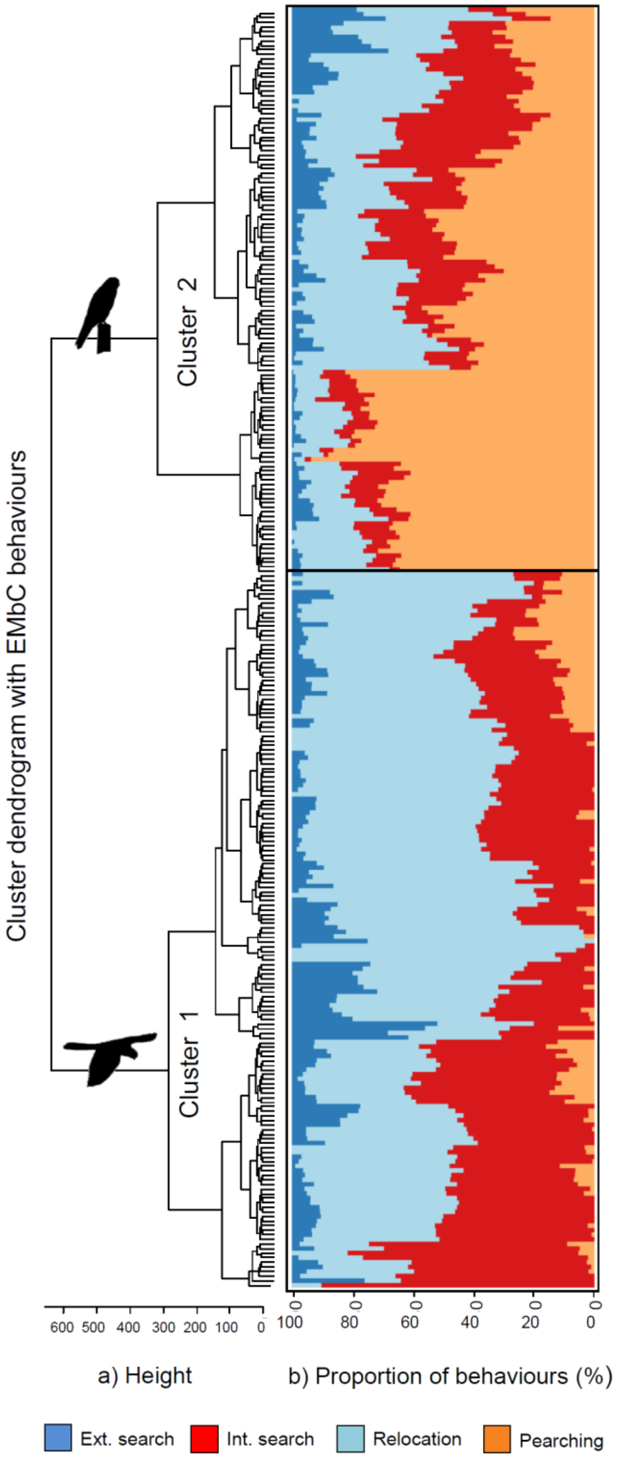


Figure 2 – Variation in the probability of adopting widely foraging (WF) vs. sit-and-wait (SAW) foraging tactics according to solar radiation and CWC (cross-wind component). Bold lines represent the values predicted by the model shown in Table 3 and the grey area indicates the 95% confidence interval.

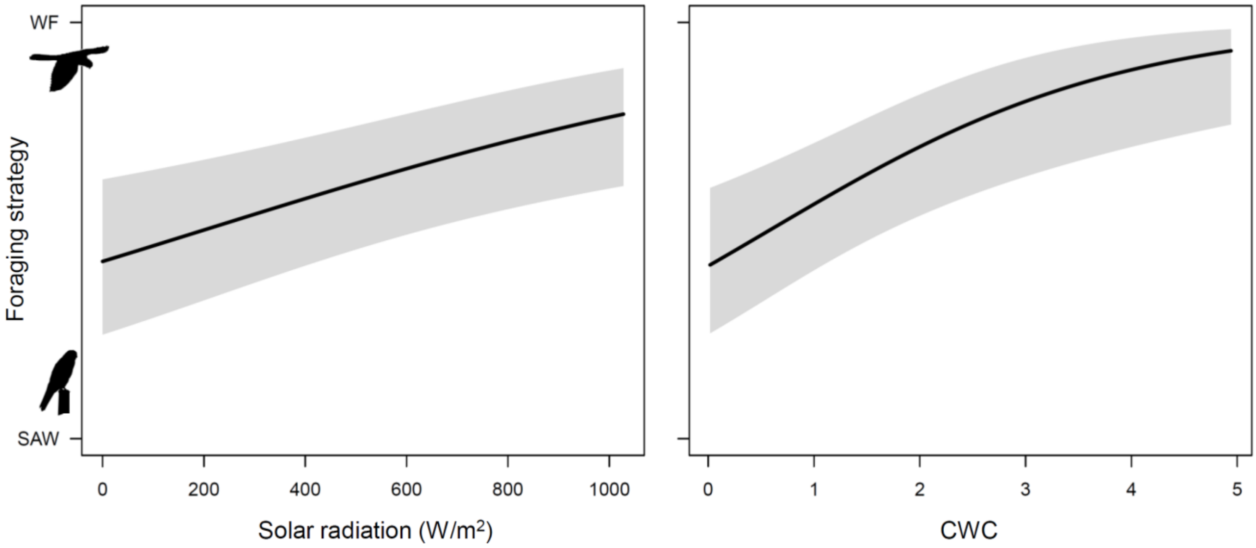
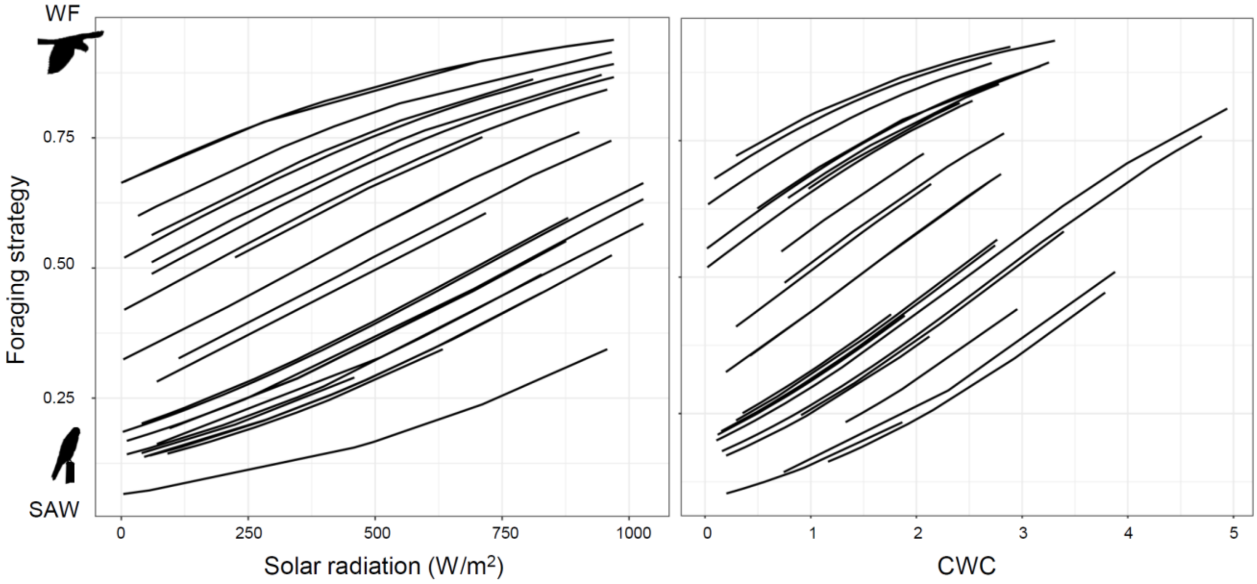


Figure 3 – Probability of adopting WF vs. SAW according to solar radiation and CWC (cross-wind component). Lines are model-predicted values for each individual assuming a random intercept, fixed slope effect.



Supplementary materials

Figure S1 – Scatter-plot of GPS positions in relation to speed and turning angle, showing with different colours the four behavioural modes identified by the EMbC algorithm (Garriga *et al.* 2016), before (upper panel) and after (lower panel) post-processing smoothing. Grey lines depict the values of the binary delimiters (a set of parameters that split input data into high and low values and define the binary regions of the input space, Garriga et al. 2016). LL = low speed and low turns (perching); LH = low speed and high turns (intensive search); HL = high speed and low turns (relocation); HH = high speed and high turns (extensive search); NC = not classified data points (e.g. the last point of the trajectory).

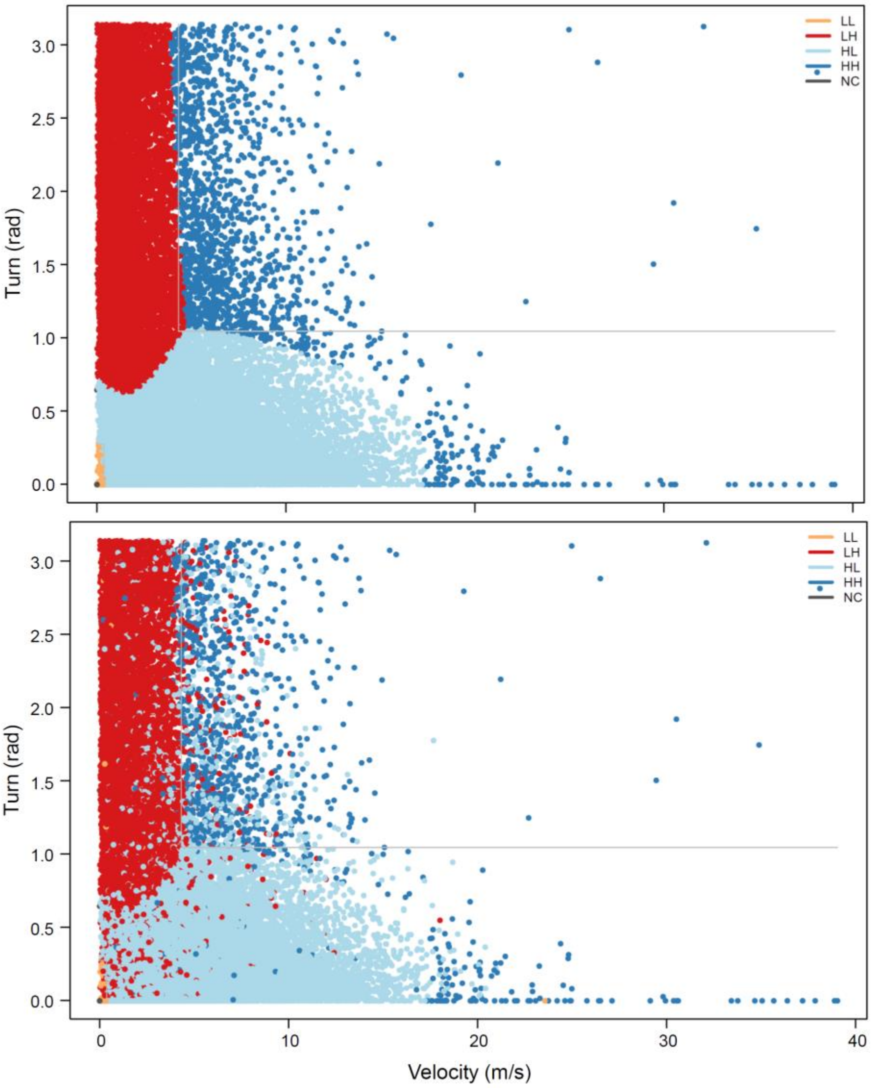
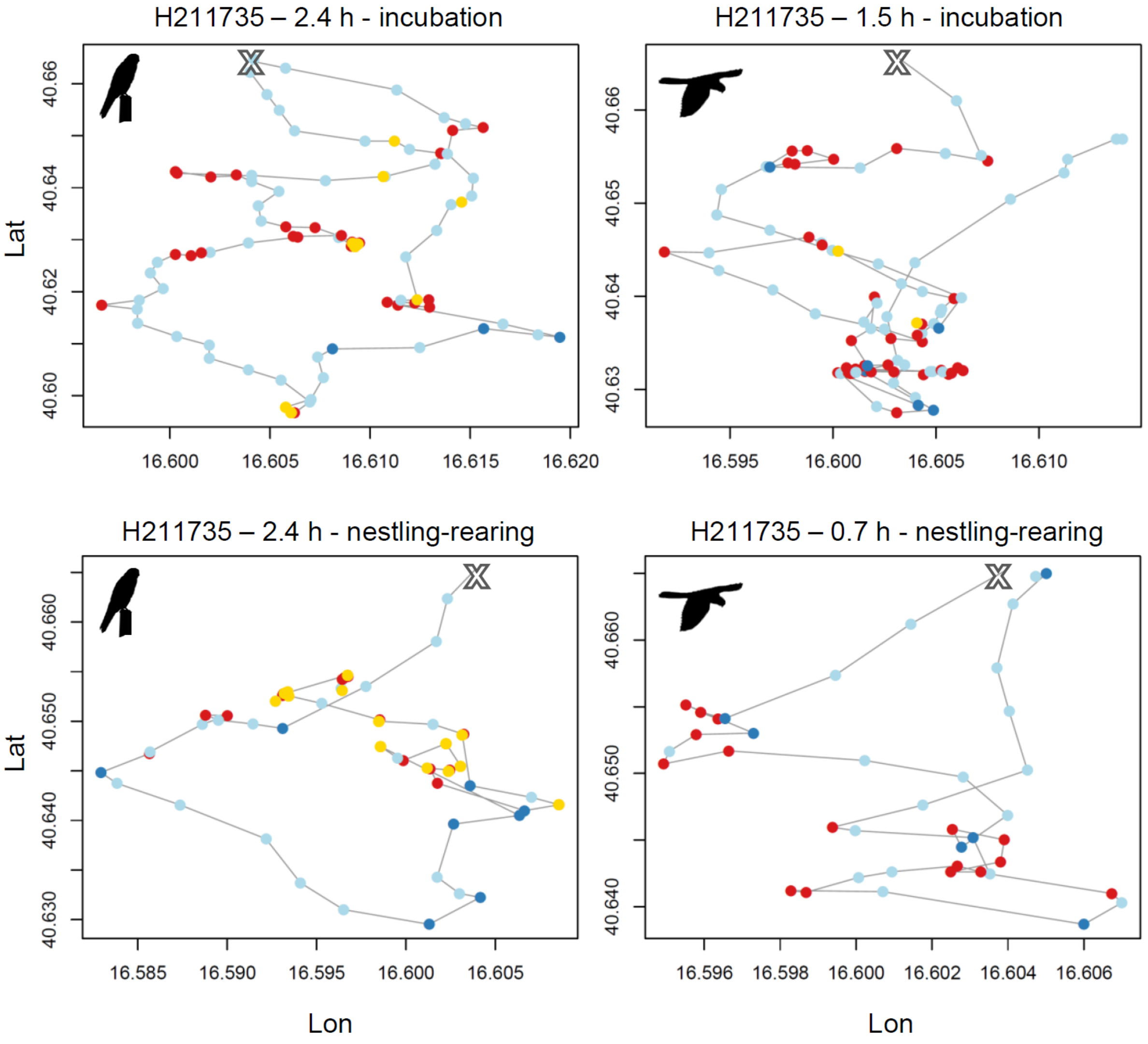


Figure S2 – Pairwise comparison of SAW (left panels) and WF (right panels) trips performed by the same individual during incubation and nestling-rearing stages. Colours represent behavioural modes: perching (yellow), intensive search (red), relocation (light blue) and extensive search (dark blue). Bird identity, trip duration and breeding stage of trip are reported on the top of each panel. Perching points always represent multiple consecutive 1-min GPS-positions with same location. Large “X” shows the nest site position.



Part 2.

Long-term effects

Chapter 6.

Interpopulation differentiation in migratory routes leads to strong migratory connectivity in an Afro-Palearctic migratory raptor, the lesser kestrel (*Falco naumanni*)

Salvatore Bondì[a,m], Ana Bermejo[b], Mathieu Bourgeois[c], Mathias Bouzin[d], Jacopo G. Cecere[e], Angelos Evangelidis[f], Gradimir Gradev[g], Matteo Griggio[h], Panagiotis Kordopatis[f], Simeon Marin[g], Ugo Mellone[i], Philippe Pilard[j], Stefano Podofillini[k], Marta Romero Gil[i], Diego Rubolini[k], Nicolas Saulnier[d], Lorenzo Serra[e], Athanassios Sfougaris[l], Vicente Urios[i], Konstantinos Vlachopoulos[l], Laura Zanca[m], Maurizio Sarà[a]

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Abstract

Environmental conditions and trade-off between costs and benefits of migration led the evolution of different migratory strategies. Among all the strategies observed, migratory connectivity describes the continental configuration of the breeding areas and how it affects the continental distribution of the overwintering grounds, at species and population level. It has been observed that, in several long-distance migratory bird species, migratory connectivity is weak with individuals, that belonged to different populations during the breeding season, shared the same non-breeding grounds. Since environmental conditions occurred during non-breeding period (i.e. food abundancy, intraspecific competition and weather conditions) could severely affect individuals, understanding broad patterns of migratory connectivity is the first step to evaluate causes of intraspecific population dynamics. Following a multi-population approach, in this study we combined all the existing data, obtained with different tracking devices (GLS and GPS), of lesser kestrel migration belonged to Spanish, Italian, Greek and Bulgarian populations. Aim was to assess whether the continental configuration in the breeding grounds could have a role in determining population migration strategy. Irrespective to other long-distance migratory bird species, we found that European lesser kestrels show strong migratory connectivity and thus the continental configuration in the breeding grounds significantly affected overwintering ground locations. Despite lesser kestrel winter grounds are spread out over extensive areas, and thus is expected to be more resilient to environmental changes, the spatial segregation that occurs among European populations underlined the importance to gather information about the associations between breeding and overwinter areas to implement successful conservation strategies and deeply understand population dynamics.

Key words: biogeography of migration, migratory connectivity, migratory strategy, satellite telemetry

Introduction

Avian migration is a naturally plastic trait that has fascinated generations of researchers for its evolutionary, ecological, neural and physiological implications (Berthold, 2001, Greenberg & Marra, 2005, Alerstam 1990, Newton 2008). During migration, bird populations move from their breeding quarters to their non-breeding residence areas, implying that avian migration is essentially a geographic process during which species' ranges become spatially and ecologically separated through seasons (Joseph et al., 1999). A consistent part of biogeographic investigation has been devoted to the comprehension of the evolution of bird migration, trying to solve the long-standing debate about the 'northern-home' or 'southern-home' origin of migration (e.g. Joseph et al., 1999, Salewski & Bruderer, 2007, Louchart, 2008, Zink & Gardner, 2017). Other biogeographic aspects of avian migration have been almost overlooked, due to the known difficulties of reconstructing both ancestral and current breeding and non-breeding ranges. In the last twenty years, comparative phylogenetic analyses have been used to infer the biogeographic history (including ancestral range reconstruction) of migratory lineages (Joseph, 2005, Winger et al., 2014), while technological advances have revolutionised the study of migration (Bridge et al., 2011) allowing the identification of current non-breeding ranges even in species that migrate to remote areas, by means of biologging systems (Rutz & Hays, 2009), intrinsic biological markers like nucleotide sequences (e.g. Wink et al., 2004, Irwin et al., 2011), stable isotopes (Hobson 2005, Bearhop et al., 2005), which were integrated with the traditional analyses of ringing data (e.g. Boulet et al., 2006, Bensch et al., 2009). The growing body of individual tracking studies has revealed, or described in more detail than ever before, the movement ecology of species within and across continents (Nathan et al., 2008). Explicit spatial information on the geographic position of populations in non-breeding areas is therefore no longer a limiting factor, and is giving strong impulse to the analysis of migratory patterns in a biogeographic perspective. One useful approach in this direction is the study of migratory connectivity (e.g. Calandra et al., 2014, Ouwehand et al., 2015) and of migratory divides (Delmore et al., 2012).

Migratory connectivity is the link between breeding and non-breeding areas of animals moving during migrations. Studies of migratory connectivity focus on the retention of breeding population structure on the non-breeding grounds, and vice versa (Marra et al., 2006, Cohen et al. 2018). A 'weak' or 'diffuse' connectivity occurs when individuals from every breeding population spread through several non-breeding grounds, therefore mixing together (Webster et al., 2002). Strong connectivity instead occurs when individuals from one breeding population move to a specific non-breeding location, so that the inter-population separation of non-breeding areas matches the inter-population separation of breeding areas. Strong connectivity is a pre-condition for the establishment of migratory divides (Bearhop et al., 2005). These latter are contact zones between divergent populations promoted by differences in migratory behaviour that favour reproductive isolation and speciation (Irwin & Irwin, 2005). Typical case studies are the geographically isolated inland and coastal Swainson's thrushes (*Catharus ustulatus*) populations, that expanded from separate eastern and western American regions after the Last Glacial Maximum, and have current migratory pathways that trace the post-glacial colonization routes (Ruegg et al., 2006, Delmore et al., 2012), and the Scandinavian willow warbler subspecies (*Phylloscopus trochilus trochilus* and *P. t. acredula*) (Chamberlain et al., 2000), that breed in adjacent ranges but use different migratory routes to reach distinct non-breeding grounds (Helbig, 1996, Bensch et al., 1999). Migratory divides are considered to maintain genetic differentiation, local adaptation and reproductive isolation in many Holarctic species (Chamberlain et al., 2000, Bearhop et al., 2005, Rolshausen et al., 2009, Delmore et al., 2012).

Biologging technologies are providing major insights into the potential contribution of seasonal migration to reproductive isolation, and hence in speciation (Turbek et al., 2018).

At continental scales, comparing non-breeding distribution of conspecifics populations that differ in migratory behaviour will be a fundamental step to assess the degree of migratory connectivity.

Understanding patterns of migratory connectivity and population differentiation in migratory behaviour is of fundamental importance to improve our understanding of ecological processes affecting populations of migratory species through their life cycle. For instance, discrete breeding populations sharing non-breeding areas (i.e. weak connectivity) would be similarly affected by biotic and/or abiotic factors occurring in their non-breeding grounds, whereas the opposite would be the case for populations that show strong connectivity (Webster et al., 2002). We know that avian migrants are flexible enough to adjust their migration in response to environmental conditions en route (e.g. Tøttrup et al., 2008, Vansteelant et al., 2017), yet physical factors and geographic configuration can shape the general framework of migration routes (Irwin & Irwin, 2005, Mellone et al. 2011a), and in many cases ecological barriers and migratory distances determine the evolution of migratory flyways and connectivity links (e.g. Henningsson & Alerstam, 2005). Heterogeneity of environmental factors and trade-offs between costs and benefits associated to migration have thus shaped migration strategies among populations, and even among individuals within species (O'Reilly & Wingfield, 1995, Sergio et al., 2014, Shamoun-Baranes et al., 2017, Monti et al. 2018). For Afro-Palaearctic migrant species, the Mediterranean Sea and the Sahara Desert constitute major ecological barriers separating breeding and non-breeding ranges. The hazards of long journeys, often across hostile habitats and the dependence upon resources and habitats in areas distant thousands of kilometres make long-distance migrants more susceptible to anthropogenic impacts and global change (Newton, 2008), such that species breeding in Eurasia and spending the boreal winter in sub-Saharan Africa are experiencing stronger population declines than short-distance migrants or resident species (Sanderson et al., 2006).

Delineating broad patterns of migratory connectivity at continental scale can thus be a primary step in evaluating causes of differential intra-specific population trends among breeding populations (Marra et al., 2006).

In this study we investigate patterns of migratory behaviour and route differentiation among breeding populations, and patterns of migratory connectivity in the long-distance migratory lesser kestrel (*Falco naumanni*), a small (ca. 120 g) diurnal raptor. This species is distributed across the Palaearctic region, with populations breeding across southern Europe, North Africa, until to Middle East and Central China. During the boreal winter, populations migrate almost entirely to the Afrotropical region, south of Sahara (Ferguson-Lees & Christie, 2001). We focus on populations breeding in the northern Mediterranean region, whose breeding range is fragmented in three distinct main geographic areas (Bounas et al. 2018) corresponding to the main south European peninsulas (west to east: Iberia, Italian, and the Balkan peninsulas). Available data suggest that western European populations spend the non-breeding period in West Africa (Rodríguez et al., 2009, Limiñana et al., 2012), whereas those from eastern Europe, the Middle East and Asia move to non-breeding areas located in eastern and southern Africa (Pepler & Matin 2001). Analyses of mtDNA suggest that west European populations have diverged from east European and Asian populations during the Pleistocene (Wink et al., 2004). Rodríguez et al. (2011) further supported this divergence by showing that non-breeding individuals from western Africa had the same genetic structure of western European breeding populations, while non-breeding birds in South Africa were genetically differentiated from western European breeding populations, likely originating from the eastern distribution range. Pleistocene glaciations have left a strong biological legacy to the Eurasian biota (Hewitt, 2000). It is generally agreed that migratory divides originated after a secondary contact between populations that were isolated in different glacial refugia (Møller et al., 2011, Rohwer & Irwin, 2011). Populations within these refugia could have diverged in many traits, including migratory orientation, so that their current migratory routes probably reproduce those used by the related ancient populations (Newton, 2008). The distribution of Eurasian refugia overlays the current breeding range of lesser kestrel and it is therefore expected that the investigation of migratory connectivity in this species might shed light into its genetic structuring (Wink et al., 2004, Rodríguez et al., 2011).

A broad-scale approach encompassing as many as possible populations within a species' range is the preferred approach to study migratory connectivity (e.g. Trierweiler et al., 2014, Finch et al., 2015). Here we combined published and original data about lesser kestrel migration and non-breeding distribution of birds breeding in three distinct geographic regions of the Mediterranean basin (Iberian, Italian, and Balkan regions), that are representative of the whole European distribution of the species (collectively, populations from sampled countries encompass ca. 82 % of the total European population size; BirdLife International, 2017; La Gioia et al., 2017), and are at least partly genetically differentiated (Bounas et al. 2018).. Based on the genetic and migration data recalled above, we expected a relatively strong differentiation of migratory routes, behaviour and non-breeding areas between birds from these three different regions, leading to a relatively strong migratory connectivity.

Materials and methods

Data collection and general methods

Previous studies have reported migration data of breeding birds from Spain (e.g. Rodríguez et al., 2009, Limiñana et al., 2012), Portugal (Catry et al., 2010) and France (Pilard et al., 2017) using a variety of techniques differing in accuracy (light-level global location sensing devices, geolocators or GLS: 17 individuals from Portugal and France; Catry et al., 2010, Pilard et al., 2017; Argos Platform Transmitter Terminals, Argos PTTs: 5 individuals from Spain; Limiñana et al., 2012). We report new data from 29 individuals tracked with GLS, 14 individuals equipped with remote-downloading archival GPS tags (GPS-UHF devices), and 5 individuals tracked with Argos PTTs, which were combined with the above mentioned published data, some of which were partly reanalysed (GLS data from Pilard et al., 2017). All devices were deployed on individuals captured at or near the respective nest sites, mostly at the end of incubation or during nestling rearing. The full list of data used in the study is reported in Table 1. A single track was available for each individual. Overall, devices were deployed in 18 breeding colonies from 6 countries (1 Portugal, 2

Spain, 3 France, 3 southern Italy, 4 Sicily, 1 Bulgaria, 4 Greece). For the analyses, birds were grouped in the three distinct regions, Iberia (Portugal, Spain and France), Italy (southern Italy and Sicily) and Balkans (Bulgaria, Greece). Birds from the small French population were considered as closely tied to the nearest Iberian core populations. Migration tracks from Bulgaria were collected from breeding birds originating from a re-introduction project involving Spanish individuals. However, as their migratory behaviour appear similar to the Greek birds (see Results), they were treated as belonging to the Balkan group.

Our sample incorporated a composite set of devices, with GPS-UHF tags and Argos PTTs allowing precise inference about migration routes, whereas this information could not be obtained from geolocators, because latitudinal uncertainty of geolocator data during movement periods may prevent the reconstruction of migratory movements (Lisovski et al., 2018, but see Rakhimberdiev et al. 2017). Main parameters and patterns of large-scale migratory flights do not vary conditionally of tracking data systems and devices (Kuhn et al., 2009), and multi-population studies employing different devices have already been successfully carried out (e.g. Terraube et al., 2012, Chevallier et al., 2013, Trierweiler et al., 2014, Finch et al., 2015). We therefore treated data according to the protocols detailed below, assuming that heterogeneity of protocols and devices originating from the different studies would produce non-systematic errors in the precision of our analyses.

GLS data collection and analysis

Breeding birds were equipped with GLS (models Mk5 and Mk7, Biotrack Ltd., UK) using a backpack wing-loop harness made of braided nylon rope. The total weight of devices (including harness) was ca. 2.5 g, corresponding to 1.5-2.0 % of body mass. We processed downloaded data according to manufacturer’s specifications, and visually inspected twilight events, removing those twilights when the light change profile at sunrise/sunset was unclear (i.e. whenever unexpected darkness events, due to light shading or bird behaviour, confounded the light change pattern around sunrise/sunset), and those which were obvious outliers, using the TransEdit software (Biotrack Ltd., UK). Latitude and longitude for each twilight event were estimated by means of the R package GeoLight v. 2.0 (Lisovski and Hahn 2012), using device-specific estimates of sun elevation angles (as recommended by the manufacturer) obtained from pre-deployment calibration performed at the breeding site. Due to the uncertainty of latitudinal estimates around equinoxes, we removed all latitude estimates in a period spanning 21 days before and after each equinox. Stationary and non-stationary periods during the non-breeding season were identified using a customized version of the R script provided by Liechti et al. (2015). Briefly, stationary periods were identified based on patterns of change in the timing of consecutive twilight events, considering that stationary periods are expected to show a smooth seasonal variation of timing of sunrise and sunset, whereas whenever a bird makes significant movements, this smooth trend is broken, resulting in detectable change points of the seasonal trend of sunset/sunrise (Liechti et al. 2015). Timing of departure/arrival from/to the breeding area were estimated by visual inspection of light profiles (assuming that abrupt occurrences of dark periods corresponded to visits of nest cavities) and from concomitant changes in the temporal trend of twilight events. Timing of arrival and departure from the sub-Saharan non-breeding residence areas were identified according to Liechti et al. (2015) as the first day of the first stationary period or the last day of the last stationary period south of the Sahara, respectively. The geographic position of the non-breeding residence area was estimated as the centre of density (modal value) of all stationary positions between arrival and departure (Liechti et al. 2015). Hence, the geographic position of the non-breeding residence area corresponds to the location that has been more frequently used by birds during the non-breeding period (considering that birds may move between different non-breeding residence locations, as observed in satellite tracked birds; our unpubl. data). Migration routes could not be reconstructed because of the uncertainties in latitudinal estimates around the equinoxes and because few reliable twilight events were identified during the migration periods, preventing the application of advanced route reconstruction methods, such as those provided by the R package FlightR (Rakhimberdiev et al. 2017) (E. Rakhimberdiev, pers. comm.). We also reanalysed the original geographic positions of the geolocator data reported in Pilard et al. (2017) (which see for methods of calculating arrival/departure dates and daily locations) to compute the modal values of all positions between arrival/departure to/from the non-breeding residence area.

Satellite tracking devices data collection and analysis

Three models of solar-powered satellite tracking devices (Argos PTT devices: 5 g PTT 100, Microwave Telemetry Inc., USA; GPS-UHF devices: 5 g Pica, Ecotone, Poland, 4 g nanoFix-Geo+RF, PathTrack Ltd., UK) have been deployed in 2010-2017 in several populations (Table 1). From these tracking efforts, we obtained post-breeding migration data and non-breeding residence areas for 24 individuals, and pre-breeding migration data for 18 individuals (Table 1). Satellite transmitters were mounted as a backpack using a Teflon harness, which added some further 0.9-1.2 g to the total weight of device. Total mass of tag plus harness was in all cases within the recommended 3-5% limit of birds' body mass (Kenward, 2001). Argos PTTs were programmed with an 8-h ON ⁄ 15-h OFF duty cycle and collected on average one fix every 15 minutes during the activity period (Limiñana et al., 2012) while GPS-UHF devices were programmed with a 17-h ON / 7-h OFF duty cycle and were programmed to collect one fix every 15 minutes during wintering and one fix every 30 minutes during the migration months, though sampling frequency could actually vary according to battery power (Bermejo et al., 2016). Data from GPS-UHF devices was retrieved the year after deployment via passive or active UHF base station. Location data were processed by QGIS 2.16 (QGIS Development Team, 2016). We filtered and cleaned every location according to the accuracy measures provided by the satellite-tracking systems. In the case of Argos PTT devices, we used the 0-3 location classes (LC), which have an accuracy of ca. 1.5 km (ARGOS, 2011). In the case of GPS-UHF devices, the accuracy of locations was expected to be within 15-50 m in 95% of occasions. Outliers with unrealistic geographic locations were removed by visual inspection of data and maps. To establish migration routes, distances and migratory phenology, we relied both on visualization of movement data and on net displacement values (ND, Kareiva & Shigesada, 1983, Turchin, 1998). ND measures the Euclidean distance between the initial location and each subsequent relocation of each individual. Interpretation of ND values varies as a function of season and depends from the timing and location of marking (Bunnefeld et al., 2011). When tagging occurs during the breeding season, like in our study, increasing ND values represent the onset of autumn migration, while the ND will become stable near its maximum value once individuals have reached their wintering grounds. Afterwards, the ND values will decrease at the onset of spring migration and will stabilize approaching zero when birds arrive again in their breeding colony. We used plots of ND values, together with visual maps, to identify both the abrupt change of movement patterns at the onset of migration (e.g. the movement of a birds flying from southern Italy to North African coasts corresponds to a very large ND value) and the directionality and stability of progressive travel movements towards and from the non-breeding residence areas (e.g. the movement of the same bird immediately after its arrival to the African coast and flying southbound across North Africa corresponds to a very flat ND plot-line). This procedure (cfr. Limiñana et al., 2008, 2012) allowed us to accurately classify onset and end dates of migration, duration of migration and migration distances of all individuals. Migration distances were the shortest distance between the breeding and non-breeding locations for each individual, taking into consideration the curved surface of Earth (i.e. orthodromic distance, see *Migratory connectivity*). Besides, the average distance covered in a day was calculated as the migration distance ⁄ number of travelling days (excluding stopovers). Duration of the non-breeding residence period was calculated as the interval in days occurring from date of arrival to the date of leaving the wintering areas. As the birds could move between different nearby non-breeding areas (our unpub. data), the position of the non-breeding residence area (see Migratory connectivity) was calculated as the modal latitude and longitude from all the positions obtained during the non-breeding residence period. This also allowed comparisons with data retrieved from GLS (see below)

Analysis of migratory connectivity

Examination of migratory connectivity postulates that individuals move between two separated and clearly identifiable geographic ranges (Marra et al., 2006), that in the case of lesser kestrel have been assumed to correspond with the south European breeding areas and the sub-Saharan non-breeding residence areas (Ferguson-Lees and Christie 2001). Migratory connectivity arises through both the spreading and mixing of breeding populations across wintering areas, with two major components, i.e. the 'population spread' and the 'inter-population mixing' (Finch et al. 2017). In the first case, we considered the spreading of individuals from every region by computing pairwise distances between the wintering sites of all individuals from the same breeding region, with high values indicating high population spread (Finch et al., 2017). We first checked by a one-way ANOVA test whether the mean wintering distances of lesser kestrels coming from any single macro-area (intra-distances) differed from the pair-wise combinations of macro-areas (inter-distances). We then calculated the population spread of lesser kestrels with respect to the country of provenance (i.e. breeding area) by a clustering procedure using the Ward's method. This procedure employs an algorithm based on Euclidean distance measure, and joins the clusters such as that the increase in within-groups variance is minimized (Hammer et al., 2001). Clustering nodes were supported after resampling with a bootstrapping procedure (n = 999 replicates).

To assess the value of inter-population mixing (i.e. migratory connectivity *sensu lato*), we used orthodromic distances, following the approach of Ambrosini et al. (2009). We obtained two matrices of orthodromic distances by using the geographical coordinates of all pair-wise combinations of individuals both in the breeding and wintering areas. The breeding orthodromic matrix corresponded to the geographical coordinates of colonies, while the wintering one was created using the centroids of the overwintering areas. The centroids of individuals equipped with GLS and GPS devices were obtained by calculating the arithmetic mode of latitude and longitude values of all positions obtained during the non-breeding residence period (i.e. all positions recorded between arrival to and departure from the non-breeding areas). In the case of the two ring recoveries, the non-breeding area were a single location and corresponded to the geographical coordinates of the roost where the two lesser kestrels were recorded, respectively. We analysed whether orthodromic distances differed between macro-areas by means of a linear mixed model with a normal error distribution. In such a GLMM, orthodromic distance was the dependent variable, macro-area was the fixed factor. We introduced sex as a random factor to check whether its unbalanced distribution among locations (Table 1) could potentially confound the geographic effects. Then we computed the Mantel correlation coefficient (r_M) between the two matrices (i.e. breeding and wintering) of orthodromic distances. The r_M value is simply the Pearson's correlation coefficient between all the entries in the two symmetric matrices. It ranges from -1 to +1 and determines the value of migratory connectivity. The significance of r_M was assessed by a randomization procedure in which a permutation test compared the original r_M to the distribution of r_M from 9999 random permutations, and is the probability of obtaining a more extreme value than the observed one.

Results

Geographic distribution of non-breeding residence areas

All individuals migrated to non-breeding areas within the Sahel region (Fig 1). Individuals from a given breeding region tended to migrate to a specific non-breeding residence area, as expected. Iberian individuals had their non-breeding areas in Senegal, Mauritania and western Mali (western Sahel), while most of the Balkan individuals went to the central-eastern Sahel countries (Niger, Nigeria and Chad). Italian lesser kestrels spread over a broad area of the central Sahel belt, from eastern Mali to Niger and Nigeria. A minority of individuals escaped this general pattern, with an Iberian kestrel that went Burkina Faso, in the area where most Italian birds spend the non-breeding period, while an Italian individual went to Senegal (Fig. 2).

Differences in migratory behaviour and routes between birds breeding in different regions

Detailed information of autumnal migration routes has been outlined by the GPS devices alone (Fig. 1 above) the only tool allowing the track of birds across their journey. Populations of the western European peninsula (WEU), for which we had Spanish and French GPS-equipped lesser kestrels, headed to south Iberia to cross the Mediterranean Sea. Only one out 8 birds crossed the sea at the Strait of Gibraltar, while the others crossed in a broad front to reach the Moroccan and Algerian coasts. Four birds followed a more or less straight route to reach their wintering ground. The most remarkable route was that followed by the French lesser kestrel, that passed over Ibiza, then crossed the sea to land in Algeria near the salt lakes region of Bougtob, and continued with only a small eastern detour to reach the Mauritanian-Mali wintering area. When crossing the Sahara deserts either from Morocco and Algeria most WEU kestrels did a detour to adjust the route towards west at the moment of reaching the wintering latitudes. The Central European peninsula (CEU) birds, coming from both south Italy and the island of Sicily crossed the sea also on a broad front. The south Italians travelled more eastbound over the Ionian Sea or approaching the eastern coasts of Sicily; while the Sicilians went more westerly over the Sicilian Channel. All Italians landed in Tunisia and Libya and all, but one making a large western detour in Libya, did a fairly straight flight from north-east to south-west. The Eastern European peninsula (EEU) population represented by GPS-equipped birds from Bulgaria behaved similarly to lesser kestrels of the other two peninsulas, although more consistent with EEU birds. They crossed the Mediterranean with a broad front, arrived to the African coasts in Libya and Egypt and maintained a fairly northeast-southwest trajectory to cross the Sahara Desert, until their final destination in Sahel region. Indeed, one of the four individuals did a large western detour, while another passed over Naxos (Aegean islands) and Crete before to fly to African coasts. During last days of autumnal migration, 46% of the GPS-equipped individuals stopped their latitudinal descent and made a definite east-west turn to fly straight to their wintering quarters. Mean departures data from the breeding sites was 20 September (range: 22 August – 23 October). After and average travel of 13 days (range: 3-37 days) spent in autumn migration, the lesser kestrels arrived to their wintering grounds on average the 3 of October (range: 17 September – 5 November). Regardless to the macro-area of breeding colony location ($F_{2,58}$ = 1.33, p = 0.27), males tend to leave the breeding areas a week later than females ($F_{1,58}$ = 6.05, p = 0.02), and therefore again irrespective to their macro-area ($F_{2,58}$ = 0.13, p = 0.88) arrive a week later than females in the wintering areas ($F_{1,58}$ = 7.56, p = 0.008). This sex difference does not affect the length of autumn migration, as males and females cover the autumnal travel in the same number of days ($F_{1,58}$ = 0.02, p = 0.89), regardless to the macro-area of provenance ($F_{2,58}$ = 1.57, p = 0.22), (Table 2). The average (± SE) distance covered during the autumnal travel is 3294.64±115.79 km (range: 2534.50 – 4469.28, n = 23). There are no statistically significant differences in the length (km) of autumnal travel between both sex ($F_{1,19}$ = 1.04, p = 0.32) and macro-areas ($F_{2,19}$ = 0.13, p = 0.88). Eventually, during autumnal migration lesser kestrels cover an average daily distance of 356.14 ± 44.65 km (range: 131.84 – 1092.187, n = 17), without sex ($F_{1,19}$ = 0.01, p = 0.92), but with macro-area differences ($F_{2,19}$ = 3.59, p = 0.05).

Pattern of spring tracks is quite similar to that of autumn migration, with differences mostly for WEU birds (Fig. 1 below). Their directions of flight are more westbound than the autumnal tracks and WEU lesser kestrels fly along the Atlantic coasts and over the ocean to converge later to north-east. Interestingly, the 50% of the WEU individuals used the Strait of Gibraltar to cross the Mediterranean and reach the Iberian Peninsula, with respect to the 12.5% during the autumn migration. Also most of south Italian lesser kestrels drifted toward west and made the conversion toward east, at some 29°-31° latitudes, to rectify their route to Tunisia, in order to cross the Mediterranean Sea towards continental Italy (Fig 1 below). Yet, one Sicilian bird had a contrary track, going east until southern Libya and then making a western detour to rectify the route and fly straight to Sicily. Another south Italian and the remaining Sicilians made a relatively straight fly from south-west to north-east, left Africa from Libyan coasts and reached eastern Sicily.

Bulgarian birds, the only representative of EEU populations, can either fly straight from south Niger to Serbia and then adjust in the Balkans, at some 42° latitudes, the route to the breeding colonies, or fly eastbound and then adjust the route very early, making the western detour at varying North-

African latitudes (one in Libya, another in Egypt), and from there flying straight to their breeding colony (Fig. 1 below). The same bird, passing over Crete and Naxos islands during autumn, used as well the same Aegean islands as spring stopover before reaching its breeding colony.

The mean date of spring departures is the 9 of March (range: 4 February - 23 April), and the mean date of arrival to the colony is the 27 of March (range: 14 February – 14 May), after an average travel of 18 days (range: 3 – 49), a flight time that lesser kestrels do without sex ($F_{1,45} = 3.75$, $p = 0.06$) and macro-area differences ($F_{2,45} = 1.29$, $p = 0.28$), (Table 2). There are no sex differences also in the dates of departure from wintering areas ($F_{1,46} = 0.14$, $p = 0.71$) and arrival to breeding areas ($F_{1,45} = 2.42$, $p = 0.13$). WEU Lesser kestrels tend to depart earlier from wintering grounds than CEU and EEU conspecifics ($F_{2,46} = 8.69$, $p = 0.001$) and, as consequence to arrive earlier to breeding areas ($F_{2,45}= 8.79$, $p = 0.001$), (Table 2). The average ± SE distance of the spring migration is 3572.88±177.98 km (range: 2192.59 – 4890.52, n = 17), a not significantly longer return travel with respect to the autumnal one ($t_{39} = 1.487$, $p = 0.21$). There are no statistically significant differences in the length (km) of spring travel between both sex ($F_{1,13} = 4.24$, $p = 0.06$) and macro-areas ($F_{2,13} = 2.16$, $p = 0.15$). Eventually, during spring migration lesser kestrels cover on average a daily distance of 165.93 ± 14.65 km (range: 82.89 – 305.79, n = 17), without sex ($F_{1,13} = 0.08$, $p = 0.78$) and macro-area differences ($F_{2,13} = 1.33$, $p = 0.30$), but this daily average distance is significantly shorter than the autumnal one (i.e. 348.18 km/day; $t_{39} = 3.420$, $p < 0.001$).

Wintering quarters fall within western and central pre-desert areas of Sahel, including eight different African countries (Senegal, Mauritania, Mali, Algeria, Burkina Faso, Chad, Niger, Nigeria). Lesser kestrels distributed across a huge potential wintering belt extended 5.243.060 km^2 between 20° 44’ 24” and 8° 57’ 35” N of latitude, and -16° 44’ 54” and +20° 44’ 34” E of longitude (Fig. 2). However, most of individual records are within a much restricted area (Fig. 2) and data from GPS-equipped kestrels show that individuals make small-scale movements across Sahel habitats, having multi home-ranges (2.32±0.22 range: 1-4, n =, 19) without sex ($F_{1,15} = 0.54$, $p = 0.47$) and macro-area ($F_{2,15} = 0.46$, $p = 0.64$) differences, but moving usually in a clock-wise direction through the winter (Cecere, Rubolini, Saràat al. in prep.). Restricting to individuals with a complete wintering period, the average extension of winter areas is 7925.31±2230.37 km^2 (range: 362.53-42511.06, n =, 19), without sex ($F_{1,15}$ = 2.56, p = 0.13) and macro-area ($F_{2,15}$ = 1.23, p = 0.32) differences. Total permanence in Sahel is on average 209 days (range: 155 – 261), without sex differences ($F_{1,46}$ = 1.47, p = 0.23), but WEU lesser kestrels spend in Sahel significantly more time than EEU (>, 19 days) and CEU (> 17 days) conspecifics ($F_{2,46}$ = 4.95, p = 0.01), (Table 2).

There are remarkable differences in the stopover strategy of lesser kestrels during the autumnal and spring migrations. Only 29.2% of the 24 individuals, six females and one male, stopped during the autumnal travel doing each a single stopover of 3.29 ± 0.81 days (range: 1 – 5). Contrariwise, the 94.4% of 18 lesser kestrels stopped during the spring migration on average 1.33 ± 0.18 times (range: 0 – 3) and for 8.82 ± 2.29 days (range: 1 – 26). Besides, the time of stopover was significantly longer for males than for females (t_{22} = 2.75, p = 0.01), on average 11.7 ± 7.83 vs 4.9 ± 4.39 days, respectively. The few autumn stopovers are mostly localized into the desert (n = 5), near fresh water gullies, locally named “wadi”, like those located inside the Tassili N’Ajjer National Park, in Algeria, and in coastal agriculture areas (n = 2). Contrariwise the majority of spring stopovers (20 out of 24) is made in coastal agricultural areas, when the lesser kestrels approached North African coasts (n = 11) or just few hundred kilometres before (n = 9). These latter coincide often with circular irrigated fields created by pumping water from underground aquifers into the Sahara in Algeria and Libya. Interestingly, one of the remainder spring stopovers was along the Nile River and other two were made in Aegean islands (Naxos, Crete), soon before reaching the breeding areas. Indeed, lesser kestrels used to fly over islands without stopping there, as most of CEU individuals did over Malta and Lampedusa; and EEU did over the Ionian islands. Few individuals also did short pauses of few hours (hence not computed as stopovers), like the WEU individual that stayed 5 hours on Lanzarote (Canary Islands), before its long flight over the Atlantic Ocean, or the CEU individual that stayed 2 hours on Ischia island, in the Naples gulf, near the Italian coasts. The general linear mixed model of 2282 travelling segments indicates a significant effect of the random factor ‘individual’ nested in macro-area ($F_{21,2251} = 14.728$, $p = 0.000$), hence a strong individuality in flight speed, with some individuals flying fastest than others. One South-Italian kestrel maintained the highest (35.64±18.49 km/h), and a Sicilian the lowest average speed (8.45±5.22 km/h), while all remainder individuals flew in-between these two extreme speeds. Total average flight speed resulted 17.22±0.28 km/h (Table 3). The other random factor, the macro-area of provenance does not play any role in flight speed variation ($F_{2,2251} = 3.028$, $p = 0.066$). Indeed, Lesser kestrels adjust their flight speed in response to the sector ($F_{1,2251} = 19.10$, $p = 0.000$) and time of day ($F_{1,2251} = 28.44$, $p = 0.000$) and to sector*time of day ($F_{1,2251} = 5.88$, $p = 0.015$), and season*time of day ($F_{1,2251} = 7.65$, $p = 0.006$). Essentially (Table 3), lesser kestrels flight faster over sea than over desert (Tukey’s post hoc HSD test $p = 0.000$) and during night-time than daytime (Tukey’s post hoc HSD test $p = 0.000$). Besides all the flight speeds during season and time of day, but one (spring during daytime vs spring during night-time), are statistically different as flight speeds over desert during daytime (Tukey’s post hoc HSD, details not showed for brevity).

Migratory connectivity

Preliminary GLM indicates how orthodromic distances vary among macro-areas ($F_{2,59} = 4.290$, $p = 0.018$), but not between sex ($F_{1,59} = 0.496$, $p = 0.484$). Lesser kestrels breeding in the WEU peninsula are closer than CEU and EEU conspecifics to their overwintering areas (Fig. 3), as the above macro-area effect revealed (Table 4 and Fig. 3). Maximum spread among wintering lesser kestrels is 3939.59 km (average ± SE = 1183.94 ± 17.51). However, the population spread of lesser kestrels coming from each macro-area is significantly lower than the corresponding as calculated by mixing distances between macro-areas ($F_{5,2074} = 258.26$, $p < 0.001$). The inter-distance of spreading between WEU and EEU individuals provided the highest relative contribution in the prediction of population spread (WEU-EEU: beta estimate = 0.499±0.017, $t = 28.77$, $p < 0.001$), while the WEU intra-distances provided the lowest (WEU-WEU: beta estimate = -0.312±0.017, $t = -17.92$, $p < 0.001$). Consistently with model results, lesser kestrels coming from WEU spread in Sahel at an average distance of 564.70±34.81 km between them, while combining WEU kestrels with EEU and CEU birds, the population spread becomes 2266.92±38.93 km and 1293.87±22.86 km, respectively. In addition, lesser kestrels from CEU spread over an average distance of 774.37±31.15 km, and EEU individuals averaged 999.92±93.57 km; while their respective inter-distance of spreading was much larger (EEU-CEU = 1223.11±36.86 km). Overall, the total average of intra-distance of population spread (699.64±17.20, CL 95% = 665.87-733.40, n = 776) is nearly half lower than the corresponding inter-distance (1472.14±22.47, CL 95% = 1428.07-1516.20, n = 1304), and this difference is statistically significant ($F_{1,2078} = 582.58$, $p < 0.001$). The Mantel correlation coefficient of orthodromic distances was $m_R = 0.58$ and significant ($p < 0.001$). Individuals from a given breeding macro-area tend to migrate to different wintering areas, as most of the western European (Portugal, Spain and France) lesser kestrels winter in Senegal, Mauritania and Mali, hence in western Sahel. Accordingly, most of the eastern (Bulgaria and Greece) lesser kestrels winter in Central Sahel countries (Niger, Nigeria and Chad). Italian lesser kestrels spend the winter in the middle of this large Sahel belt, as they are present from Mali and Mauritania to the west, where they overlap with Iberian and French kestrels, to Niger and Nigeria in central Sahel where they overlap with Greeks and Bulgarians. Only a minority of individuals (n = 3) escapes this general pattern, as a French kestrel overwintered in Burkina Faso, in the same area where the most western EEU individual from Greece did, while a CEU kestrel from Sicily overwintered in the coast of Senegal (Fig. 2).

Discussion

Our sample of 65 lesser kestrels allowed gathering adequate data to sketch out the species' migratory phenology and strategy. Although satellite devices were heterogeneous, the migratory information provided by 39 GLS-equipped lesser kestrels were consistent with the more detailed routes provided by the 24 GPS-equipped birds. For instance, during autumnal migration all the 13

GLS-equipped birds left French coasts to pass over Balearics and arrive to Africa landing in Algeria (see Fig. 2 in Pilard et al., 2017), as the single GPS-equipped French bird did (Fig. 1 below). Alike other *Falco* species which use powered flight during migration (e.g. Strandberg et al., 2009, Mellone et al., 2011, Dixon et al., 2012, Prommer et al., 2012) also lesser kestrels tend to not use Straits (i.e. Gibraltar, Sicily) to cross the Mediterranean Sea, but fly in broad fronts over the open sea. Spring travels are generally more western than autumn ones, and eastern detours are made later, around 29-31° of latitude to rectify the route towards the northern breeding areas. This is a general pattern shown by all trans-Saharan migrants that during their return travels are drifted by eastern tailwinds (e.g. Vansteelant et al., 2017). During spring migration lesser kestrels fly at the same speed than autumn, nonetheless the travel is longer in time and with many more stopovers than autumn migration. These travel breaks made in agricultural areas on African coasts, or immediately before, are likely necessary for waiting the optimal conditions (weather, rest and foraging, etc.) before the flight over Mediterranean Sea. Besides straits are relatively more used than in autumn. These results suggest that lesser kestrels during their spring migration have a more conservative strategy to save energy and make a safer travel to reach the breeding grounds in the best conditions (Moore et al., 2005, Hahn et al., 2014).

Former GPS and GLS studies of WEU populations (Rodríguez et al., 2009, Limiñana et al., 2012, Pilard et al., 2017) recorded wintering only in western Sahel countries (Senegal, Mali, Mauritania). Our study extended notably the area showing how lesser kestrels winter in a Sahel area large over 5 millions square kilometres, and embedded in eight African countries, from Senegal to Chad. Indeed, the lesser kestrel was already recorded as winter visitor in Chad (e.g. Salvan, 1967), Niger (Pilard et al., 2004) and Nigeria (Elgood et al., 1964) but not in Burkina Faso (Pilard et al., 2004). Interestingly, the most southern record at around 9° latitude N coincides with presence in the Bauchi highlands of Nigeria already recorded in Pepler & Matin (2001). Despite the large population spread, reaching almost a maximum of nearly 4000 km within the sample of 65 individuals studied, we recorded a significant grouping of wintering populations dependent from the

European peninsula of origin. Indeed, continental configuration of breeding range has some influence on migration phenology of lesser kestrels. Birds from the western Iberian Peninsula are closer to their wintering grounds in western Sahel as the statistically significant orthodromic distances revealed. Yet, the length of the spring travel of a Portuguese or Spanish kestrel is on average some 800 and 1000 km less than that of an Italian or Balkan bird, respectively, a difference not reaching statistical significance perhaps for the limited sample so far available. Other phenological differences, like the longest permanence (i.e. > 2 weeks) in Sahel and the earlier departures from overwintering grounds and arrival to the breeding areas of WEU lesser kestrels than CEU and EEU individuals are very likely linked to such a different geographic configuration. Put simply, WEU lesser kestrels are nearest to Sahel than birds of other populations in the species' range and this may affect their timing of migration and creates temporal asynchrony between breeding populations with significant effects on populations' spread and mixing (Bauer et al., 2016). Longest permanence in the overwintering ground would likely mean better foraging and fattening before migration; whereas early arrival in the colony areas would likely mean early reproduction and potentially a better breeding success and productivity (e.g. Verhulst & Nilsson, 2008, Nisbet et al., 2016) than the late central- and eastern European conspecifics. Migratory connectivity has important implications for individual fitness (Alves et al., 2013) and we might argue whether the geographic position of Iberian kestrels could have elicited in the long-term the growth of species' abundance, as the Spanish population has always been the largest of Europe (Ferguson-Lees & Christie, 2001, Iñigo & Barov, 2011). It remains, however, an open question about the potential geographic features driving the species' abundance in the Iberian Peninsula.

Nonetheless, geography of Europe matters, as connectivity analysis revealed the spatial segregation of lesser kestrels wintering in Sahel. In our sample comprising lesser kestrel populations from the three major European peninsulas, the birds have a strong connectivity with their breeding areas paralleling quite exactly in latitude their wintering locations. The Iberian and French kestrels overwinter spreading in western Sahel (Limiñana et al., 2012, Pilard et al., 2017), the Balkan kestrels in central Sahel, whereas the Italians which breed in the central peninsula split half in Western and half in Central Sahel. Low levels of connectivity are common in long-distance migrant birds (Finch et al., 2017) and the strong connectivity of lesser kestrels would thus be an exception, although shared with many other birds (e.g. Calandra et al., 2014, Ouwehand et al., 2015, Ramos et al., 2016, Kramer et al., 2017) including raptors (e.g. Symes & Woodborne, 2010, Trierweiler et al., 2014). The relative homogeneity of habitats across the huge Sahel belt would have favoured the non-random spreading out of lesser kestrels in a large overwintering area (> 5 million km^2), and therefore would have reduced the mixing of populations, augmenting connectivity, as predicted by Finch et al. (2017).

The pattern of connectivity we have found ultimately arise through variation in the migratory trajectories of individuals coming from a partitioned, although large, breeding area. It can be the result of a conditional strategy followed by lesser kestrels to optimize the energetic constraints of their long-distance migration over the Mediterranean Sea and Sahara Desert, as the different timing of spring migration, with the major seasonal use of Gibraltar Strait, islands and stopovers would indicate. Migratory traits, including departure directions, are under strong genetic control and have high heritability (e.g. Berthold et al., 1992, Pulido, 2007), although this control can vary greatly between individuals (e.g. Thorup et al., 2007), and due to a vast array of contingent and proximate factors (e.g. Pulido 2007 and reference therein), including migratory bottlenecks (Newton, 2008), and more generally the geographic configuration of continents (Finch et al., 2017, Vansteelant et al., 2017). Thus, selection of the less-costly trajectories to reach the wintering areas from a breeding range extended from Portugal to Greece, could be invoked for the establishment of spatial segregation in Sahel, and in turn for the realization of connectivity between breeding and wintering areas. Winger et al. (2014) suggested that Plio-Pleistocene glaciations have clearly served to modify geographic ranges and migratory distances and routes. Indeed, modification of sea and land barriers are historical factors that have shaped during Ice Ages the current migratory routes of species (Pérez-Tris et al., 2004, Bensch et al., 2009) and might be advanced as a further explanation for the insurgence of connectivity in the lesser kestrel. It is currently becoming evident that variation in migratory behaviour of allopatric or co-occurring populations is often associated with genetic differentiation (Turbek et al., 2018), even in long-distance migrant species (e.g. Bensch et al., 2009, Lehtonen et al., 2009, Neto et al., 2012). Genetic structuring had already been revealed in the lesser kestrel at a cross-continental scale, with differences between European and Asian populations dating back to the Pleistocene glaciations (Wink et al., 2004, Rodríguez et al., 2011). Recently, Bounas et al. (2108) confirmed the genetic separation of European and Asian populations, besides finding a good level of genetic structuring internal to the European populations, particularly of the Central and Eastern Mediterranean region. Genetic divergence of Central and Eastern. Mediterranean populations correlated significantly with the geographical distance among these populations (Bounas et al., 2108). As most of the Bounas' et al. (2018) samples come from the same CEU and EEU populations of this study, it is plausible that the strong connectivity revealed by our study could be linked with such a genetic structuring. This suggests a scenario in which populations isolated in the three European peninsulas (i.e. the main Ice Ages refugia, Hewitt, 2000, 2004), would have formed independent migratory routes through historical times.

Our study reveals also the importance of multi-population approach for connectivity analysis, since considering most of the lesser kestrel range with the addition of CEU and EEU populations to the already known WEU (Rodríguez et al., 2009, Catry et al., 2011, Limiñana et al., 2012, Pilard et al., 2017), changed the species' connectivity level from the not statistically significant and weak degree (Finch et al., 2017) to the current statistically significant and strong degree. In conclusion, although species like the lesser kestrel that spread out over extensive nonbreeding areas should be more resilient and affected only by large-scale environmental changes (Gilroy et al., 2016); the strong connectivity we have found confirms that information about the precise link between breeding and overwintering areas is a pre-requisite to design optimal conservation strategies (Webster & Marra, 2005) and points out the urgency of addressing population-specific conservation actions.

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References

Agudo, R., Rico, C., Hiraldo, F. & Donazar, J. A. (2011). Evidence of connectivity between continental and differentiated insular populations in a highly mobile species. *Diversity and Distributions*, 17, 1–12.

Alves, J. A. Gunnarson, T. G., Hayhow, D. B., Appleton, G. F., Potts, P. M., Sutherland, W. J. & Gill, J. A. (2013). Costs, benefits, and fitness consequences of different migratory strategies. *Ecology*, 94, 11–17.

Ambrosini, R., Møller, A. P. & Saino, N. (2009). A quantitative measure of migratory connectivity. *Journal of Theoretical Biology*, 257, 203–211.

ARGOS, (2011). Argos User's Manual. Toulouse, CLS ⁄ Service Argos.

Bauer, S., Lisovski, S. & Hahn, S. (2016). Timing is crucial for consequences of migratory connectivity. *Oikos*, 125, 605–612.

Bearhop, S., Fiedler, W., Furness, R.W., Votier, S.C., Waldron, S., Newton, J., Bowen, G.J., Berthold, P. & Farnsworth K. (2005). Assortative mating as a mechanism for rapid evolution of a migratory divide. *Science*, 310, 502–504.

Beatty, W. S., Kesler, D. C., Webb, E. B., Raedeke, A. H., Naylor, L. W., & Humburg, D. D. (2013). Quantitative and qualitative approaches to identifying migration chronology in a continental migrant. *PLoS ONE* 8:e75673.

Bensch, S., Andersson, T. & Åkesson, S., (1999). Morphological and molecular variation across a migratory divide in willow warblers, *Phylloscopus trochilus*. *Evolution*, 53, 1925–1935.

Bensch, S., Grahn, M., Müller, N., Gay, L. & Åkesson, S. (2009). Genetic, morphological, and feather isotope variation of migratory willow warblers show gradual divergence in a ring. *Molecular Ecology*, 18, 3087–3096.

Bermejo, A., Bondì, S., Buonamassa, G., Carbonell, F., Cecere, J.G., Curcio, A., De la Puente, J., Del Moral, J.C, Fulco, E., Garcés, F., Griggio, M., Guzzo, E., Lorusso, M., Martín, Barranco, F.J., Martínez, J., Mellone, U., Parisi, F., Podofillini, S., Pulpillo, F.J., Romero, Gil, M., Sarà, M., Serra, L., Urios, V., Vidal-Mateo, J., Zanca, L. & Rubolini, D. (2016). Using miniaturized, remote-downloading solar GPS tags to investigate the spatial ecology of the Lesser Kestrel: a large-scale collaborative network. Abstract book of the International Lesser Kestrel Expert Workshop in Plovdiv, Bulgaria, 4-8.10.2016 [http://greenbalkans.org/birdsofprey/lesserkestrellife/en/International_Expert_Workshop_-c150, accessed 15.12.16].

Berthold, P. (2001). Bird migration: a general survey, (2nd ed.). Oxford, UK: Oxford University Press.

Berthold, P., Helbig, A.J., Mohr, G. & Querner, U. (1992). Rapid microevolution of migratory behaviour in a wild bird species. *Nature*, 360, 668–670.

Boulet, M., Gibbs, H.L. & Hobson, K.A. (2006). Integrated analysis of genetic, stable isotope, and banding data reveal migratory connectivity and flyways in the northern yellow warbler (*Dendroica petechia*; Aestiva group). *Ornithological Monography*, 61, 29–78.

Bounas, A., Tsaparis, D., Efrat, R., Gradev, G., Gustin, M., Mikulic, K., Rodriguez, A., Sarà, M., Kotoulas, G. & Sotiropoulos, K. (2018). Genetic structure of a patchily distributed philopatric migrant: implications for management and conservation. *Biological Journal of the Linnean Society*, 124, 633–644.

Brito, P.H. (2005). The influence of Pleistocene glacial refugia on tawny owl genetic diversity and phylogeography in western Europe. *Molecular Ecology*, 14, 3077–3094.

Bunnefeld, N., Borger, L., Van Moorter, B., Rolandsen, C.M. & Dettki, H. (2011). A model-driven approach to quantify migration patterns: individual, regional and yearly differences. *Journal of Animal Ecology*, 80, 466–476.

Calandra, Q., Stanley, E., McKinnon, A., Fraser, K. C. Macpherson, M. P., Casbourn, G., Friesen, L., Marra, P. P., Studds, C., Ryder, T. B., Diggs, N. E. & Stutchbury, B. J. M. (2014). Connectivity of wood thrush breeding, wintering, and migration sites based on range-wide tracking. *Conservation Biology*, 29,164-174.

Catry, I., Dias, M. P., Catry, T., Afanasyev, V., Fox, J., Franco, A. M. A. & Sutherland, W. J. (2010). Individual variation in migratory movements and winter behaviour of Iberian Lesser Kestrels *Falco naumanni* revealed by geolocators. *Ibis*, 153, 154–164.

Chamberlain, C. P., Bensch, S., Feng, X., Åkesson, S. & Andersson, T. (2000). Stable isotopes examined across a migratory divide in Scandinavian willow warblers (*Phylloscopus trochilus trochilus* and *Phylloscopus trochilus acredula*) reflect their African winter quarters. *Proceedings of the Royal Society of London*, 267, 43–48.

Chevallier, D., Baillon, F., Le Maho, Y., Blanc, S., Brossault, P.& Massemin, S. (2013). Importance of the connectivity of Spanish stopovers for Black Storks, *Bird Study*, 60, 550-554.

Delmore, K. E., Fox, J. W. & Irwin, D. E. (2012). Dramatic intraspecific differences in migratory routes, stopover sites and wintering areas, revealed using light-level geolocators. *Proceedings of the Royal Society of London B*, 279, 4582–4589.

Dixon, A., Sokolov, A. & Sokolov, V. (2012). The subspecies and migration of breeding Peregrines in northern Eurasia. *Falco*, 39, 4–9.

Bridge, E. S., Thorup, K., Bowlin, M. S., Chilson, P. B., Diehl, R. H., Fléron, R., Hartl, P, Kays, R., Kelly, J. F., Robinson, W. D. &Wikelski, M. (2011). Technology on the Move: Recent and Forthcoming Innovations for Tracking Migratory Birds. *BioScience*, 61, 689–698.

Elgood, J.H., Sharland, R.E. & Ward, P. (1964). Palearctic migrants in Nigeria. *Ibis*, 108, 98.

Ferguson-Lees J. & Christie D.A. (2001). Raptors of the World. London: Christopher Helm.

Finch, T., Saunders, P., Miguel Aviles, J., Bermejo A., Catry, I., de la Puente, J., Emmenegger; T., Mardega, I., Mayet, P., Parejo D., Račinskis E., Rodríguez-Ruiz, J., Sack P., Schwartz, T., Tiefenbach M., Valera, F., Hewson C., Franco A. & Butler, S.J. (2015). A pan-European, multipopulation assessment of migratory connectivity in a near-threatened migrant bird. *Diversity and Distribution*, 21,1051–1062.

Finch, T., Butler, S., Franco, A. & Cresswell, W. 2017. Low migratory connectivity is common in long-distance migrant birds. *Journal Animal Ecology*, 86, 662–673.

Gilroy, J.J., Gill, J.A., Butchart, S.H.M., Jones, V.R. & Franco, A.M.A. (2016). Migratory diversity predicts population declines in birds. *Ecology Letters*, 19, 308–317.

Greenberg, R. & Marra P. P. (2005). Birds of two worlds. The Ecology and Evolution of Migration. Baltimore, Massachusetts, USA: Johns Hopkins University Press.

Hahn, S., Emmenegger, T., Lisovski, S., Amrhein, V., Zehtindjiev, P. & Liechti. F. (2014).Variable detours in long-distance migration across ecological barriers and their relation to habitat availability at ground. *Ecology and Evolution*, 4, 4150–4160.

Hammer, Ø., Harper, D.A.T. & Ryan, P. D. (2001). PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontologia Electronica* 4, 1-9.

Helbig, A. (1996). Genetic basis, mode of inheritance and evolutionary changes of migratory directions in Palaearctic warblers (Aves: Sylviidae). *Journal of Experimental Biology*, 199, 49–55.

Hewitt, G. (2000). The genetic legacy of the Quaternary ice ages. *Nature*, 405, 907–913.

Hewitt, G. (2004). Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society of London, Bullettin of Biological Sciences*, 359,183–195.

Hobson, K. A. (2005). Stable isotopes and the determination of avian migratory connectivity and seasonal interactions. *The Auk*, 122, 1037–1048.

Hourlay, F., Libois, R., D'Amico, F., Sarà, M., O'Halloran, J., & Michaux, J. R. (2008). Evidence of a highly complex phylogeographic structure on a specialist river bird species, the dipper (*Cinclus cinclus*). *Molecular Phylogenetics and Evolution*, 49, 435-444.

Iñigo, A. & Barov, B. (2011). Actio*n plan for the lesser kestrel Falco naumanni in the European Union*, Madrid: SEO-BirdLife and BirdLife International for the European Commission.

Irwin, D.E. & Irwin, J.H. (2005). Siberian migratory divides: the role of seasonal migration in speciation. In R. Greenberg, & P. P. Marra (Eds.), *Birds of two worlds. The Ecology and Evolution of Migration*. (pp. 27-40). Baltimore, Massachusetts, USA: Johns Hopkins University Press.

Irwin, D. E., Irwin J. H. & Smith T.B. (2011). Genetic variation and seasonal migratory connectivity in Wilson's warblers (*Wilsonia pusilla*): species-level differences in nuclear DNA between western and eastern populations. *Molecular Ecology*, 20, 3102–3115.

Joseph, L. 2005. Molecular approaches to the evolution and ecology of migration. In R. Greenberg, & P. P. Marra (Eds.), *Birds of two worlds. The Ecology and Evolution of Migration*. (pp. 18-26). Baltimore, Massachusetts, USA: Johns Hopkins University Press.

Joseph, L., Lessa, E. P. & Christidis, L. (1999). Phylogeny and biogeography in the evolution of migration: Shorebirds of the *Charadrius* complex. *Journal of Biogeography*, 26, 329–342

Kareiva, P.M. & Shigesada, N. (1983). Analyzing insect movement as a correlated random walk. *Oecologia*, 56, 234–238.

Kassara C, Gangoso L, Mellone U, Piasevoli G, Hadjikyriakou TG, Tsiopelas N, Giokas S, López-López P, Urios V, Figuerola J, Silva R, Bouten W, Kirschel ANG, Virani MZ, Fiedler W, Berthold P, Gschweng M. (2017). Current and future suitability of wintering grounds for a long-distance migratory raptor. Scientific Reports 7: 8798

Kenward, R.E. (2001). A Manual for Wildlife Radio Tagging. London: Academic Press.

Klaassen, R.H.G., Strandberg, R., Hake, M., Olofsson, P., Tottrup, A.P. & Alerstam, T. (2010). Loop migration in adult Marsh Harriers Circus aeruginosus as revealed by satellite telemetry. J. Avian Biol 41: 200–207.

Kramer, G. R., Streby, H. M., Peterson, S. M., Lehman, J. A., Buehler, D. A., Wood, P. B., McNeil, D. J., Larkin, J. L. & Andersen, D. E. (2017). Nonbreeding isolation and population-specific migration patterns among three populations of golden-winged warblers. *Condor*, 119, 108–121.

Kuhn, C. E., Johnson, D. S., Ream, R. R. & Gelatt, T. S. (2009). Advances in the tracking of marine species: using GPS locations to evaluate satellite track data and a continuous-time movement model. *Marine Ecology - Progressive Series*, 393, 97–109.

Lehtonen, P.K., Laaksonen, T., Artemyev, A.V., Belskii, E.,Both, C., Bures, S., Bushuev, A.V., Krams, I., Moreno, J., MaGi, M., Nord, A., Potti, J., Ravussin, P.A., Sirkia, P.M., Sætre, G.P. & Primmer, C.R. (2009). Geographic patterns of genetic differentiation and plumage colour variation are different in the pied flycatcher (*Ficedula hypoleuca*). *Molecular Ecology*, 18, 4463–4476.

Limiñana, R., Soutullo, A., López-López, P. & Urios, V. (2008). Pre-migratory movements of adult Montagu’s Harriers *Circus pygargus*. *Ardea*, 96, 81–90.

Limiñana, R., Romero, M., Mellone, U., & Urios, V. (2012). Mapping the migratory routes and wintering areas of Lesser Kestrels *Falco naumanni*: new insights from satellite telemetry. Ibis, 154, 389-399.

Limiñana, R., Romero, M., Mellone, U. & Urios, V. (2013). Is there a different response to winds during migration between soaring and flapping raptors? An example with the Montagu’s harrier and the lesser kestrel . Behavioral Ecology and Sociobiology 67: 823-835

Lisovski, S., Schmaljohann, H., Bridge, E. S., Bauer, S., Farnsworth, A., Gauthreaux Jr., S. A., Hahn, S., Hallworth, M. T., Hewson, C. M. Kelly, J. F., Liechti, F., Marra, P. P., Rakhimberdiev, E., Ross, J. D., Seavy, N. E., Sumner, M. D., Taylor, C. M., Winkler, D. W., Wotherspoon, S. J. & Wunder, M. B. (2018). Inherent limits of light-level geolocation may lead to over-interpretation. *Current Biology*, 28, 89–102.

Lisovski S, Hahn S (2012). GeoLight – processing and analysing light-based geolocator data in R. Methods in Ecology and Evolution, 3, 1055–1059.

Louchart, A. (2008). Emergence of long distance bird migrations: A new model integrating global climate changes. *Naturwissenschaften*, 95, 1109–1119.

Marra, P. P., Norris, D. R., Haig, S. M., Webster, M., & Royle, J. A. (2006). Migratory connectivity In K. Crooks, & M. Sanjayan (Eds.) *Connectivity Conservation* (pp. 157-183). New York: Cambridge University Press.

Marra, P. P., Hunter, D. & Perrault, A.M. (2011). Migratory connectivity and the conservation of migratory animals. *Environmental Law,* 41,317-354.

Mellone U., Limiñana, R., Mallìa E. & Urios V. (2011a). Extremely detoured migration in an inexperienced bird: interplay of transport costs and social interactions. Journal of Avian Biology 42: 468-472.

Mellone, U., López-López, P., Limiñana, R. & Urios, V. (2011b). Weather conditions promote route flexibility during open ocean crossing in a long-distance migratory raptor. *International Journal of Biometeorology,* 55, 463–468.

Mellone, U., Klaassen, R. H. G., Garcia-Ripolles, C., Limiñana, R., Lopez-Lopez, P., Pavon, D., Strandberg, R., Urios, V., Vardakis, M., Alerstam, T. (2012). Interspecific comparison of the performance of soaring migrants in relation to morphology, meteorological conditions and migration strategies. PLoS ONE 7(7): e39833.

Møller, A. P., Garamszegi, L. Z., Peralta-Sánchez, J. M., Soler, J. J. (2011). Migratory divides and their consequences for dispersal, population size and parasite–host interactions. *Journal of Evolutionary Biology,* 24: 1744–1755.

Moore, F. R., Smith, R. J. & Sandberg, R. (2005). Stopover ecology of intercontinental migrants. En route problems and consequences for reproductive performance. Birds of two worlds. The Ecology and Evolution of Migration. (pp. 251–261). Baltimore, Massachusetts, USA: Johns Hopkins University Press.

Monti, F., Grémillet, D., Sforzi, A., Dominici, J. M., Triay, R., Antoni, B., Navarro, M., Fusani, L., Klaassen, R. H. G., Alerstam, T., Duriez, O. (2018). Migration distance affects stopover use but not travel speed: Contrasting patterns between long- and short-distance migrating ospreys https://doi.org/10.1111/jav.01839

Morganti, M., Preatoni, D. & Sarà, M. (2017). Climate determinants of breeding and wintering ranges of lesser kestrels in Italy and predicted impacts of climate change. *Journal of Avian Biology,* 48, 1595–1607.

Nathan, R.W. Getze, M., Revilla, E., Holyoak, M., Kadmon, R., Saltz, D. & Smouse P. E. (2008). A movement ecology paradigm for unifying organismal movement research. *Proceedings of the National Academy of Sciences,* 105,19052-19059.

Neto, J. M., Arroyo, J. L., Bargain, B., Monros, J.S., Matrai, N., Prochazka, P. & Zehtindjiev, P. (2012). Phylogeography of a habitat specialist with high dispersal capability: the Savi's Warbler *Locustella luscinioides*. *PLoS ONE* 7:e38497.

Newton, I. (2008). *The migration ecology of birds*. London: Academic Press.

Nisbet, I. C. T., Monticelli, D., Spendelow, J. A., & Szczys, P. (2016). Prebreeding survival of Roseate Terns *Sterna dougallii* varies with sex, hatching order and hatching date. *Ibis,* 158, 327–334.

O'Reilly, K. M. & Wingfield, J. C. (1995). Spring and autumn migration in Arctic shorebirds: same distance, different strategies. *American Zoologist,* 35, 222–233.

Ouwehand, J. M. P. Ahola, A. N., Ausems, M. A., Bridge, E. S., Burgess, M., Hahn, S., Hewson, C. M., Klaassen, R. H. G., Laaksonen, T., Lampe, H. M., Velmala, W. & Both, C., (2015). Light-level geolocators reveal migratory connectivity in European populations of pied flycatchers *Ficedula hypoleuca*. *Journal of Avian Biology*, 47, 69-83.

Pellegrino, I., Negri A., Cucco M., Mucci N., Pavia M., Salek M. & Randi E. (2014). Phylogeography and Pleistocene refugia of the Little Owl *Athene noctua* inferred from mtDNA sequence data. *Ibis*, 156, 639-659.

Pepler, D., & Matin, R. (2001). The lesser kestrel (*Falco naumanni*) in Africa: migration, conservation status and dangers: a review. In F.G. Toledano, & M. Corroto Matesanz (Eds.). *Proceedings of the IV Lesser Kestrel National Congress* (pp. 318-336) Madrid: Consejeria de Medio Ambiente y Grefa.

Pérez-Tris, J., Bensch, S., Carbonell, R., Helbig, A.J. & Tellería J.L. (2004). Historical diversification of migration patterns in a passerine bird. *Evolution*, 58, 1819-1832.

Pilard, P., Thiollay, J. M. & Rondeau, G. (2004). Données sur l’hivernage du Faucon crécerellette *Falco naumanni* en Afrique de l’Ouest. *Alauda*, 72, 323-328.

Pilard, P., Bourgeois, M. & Sylla, D. (2017). Localisation des quartiers d’hivernage et phènologie des migrations prènuptiale et postnuptiale chez la population francaise du faucon crecerellette *Falco naumanni* à l’aide de geolocateurs. *Alauda*, 85, 1-28.

Prommer, M., Bagyura, J., Chavko, J. & Uhrin, M. (2012). Migratory movements of the Central and Eastern European Saker Falcon (*Falco cherrug*) from juvenile dispersal to adulthood. *Aquila*, 119, 111-134.

Pulido, F. (2007). The genetics and evolution of avian migration. *Bioscience*, 57, 165–174

Qgis Development Team (2016). QGIS Geographic Information System. Open Source Geospatial Foundation Project.

Rakhimberdiev, E., Saveliev, A., Piersma, T., & Karagicheva, J. (2017). FLightR: An R package for reconstructing animal paths from solar geolocation loggers. Methods in Ecology and Evolution, 8, 1482-1487.

Ramos, R., Song, G., Navarro, J., Zhang, R., Symes, C. T., Forero, M. G. & Lei F. (2016). Population genetic structure and long-distance dispersal of a recently expanding migratory bird. *Molecular Phylogenetics and Evolution*, 99, 194-203

Rodríguez, A., Negro, J. J., Bustamante, J., Fox, J., & Afanasyev, V. (2009). Geolocators map the wintering grounds of threatened lesser kestrels in Africa. *Diversity and Distributions*, 15, 1010-1016.

Rodríguez, A., Alcaide, M., Negro, J. J. & Pilard, P. (2011). Using MHC markers to assign the geographic origin of migratory birds: examples from the threatened lesser kestrel. *Animal Conservation*, 14, 306-313.

Rohwer, S. & Irwin, D.E. (2011). Molt, orientation, and avian speciation. *The Auk*, 128, 419–425.

Rolshausen, G., Segelbacher, G., Hobson, K. A. & Schaefer, H. M. (2009). Contemporary evolution of reproductive isolation and phenotypic divergence in sympatry along a migratory divide. *Current Biology*, 19, 2097–2101.

Ruegg, K. C., Hijmans, R. J. & Moritz, C. (2006). Climate change and the origin of migratory pathways in the Swainson's thrush, *Catharus ustulatus*. *Journal of Biogeography*, 33, 1172–1182.

Rutz, C. & Hays, G. C. (2009). New frontiers in biologging science. *Biology Letters*, 5, 289–292.

Saino, N., Bazzi, G., Gatti, E., Caprioli, M., Cecere, J. G., Possenti, C. D., Galimberti, A., Orioli, V., Bani, L., Rubolini, D., Gianfranceschi, L. & Spina, F. (2015). Polymorphism at the Clock gene predicts phenology of long-distance migration in birds. *Molecular Ecology*, 24, 1758-1773.

Salewski, V. & Bruderer, B. (2007). The evolution of bird migration - a synthesis. *Naturwissenschaften*, 94, 68–279.

Salvan, J., (1967). Contribution à l'étude des oiseaux du Tchad. *L'Oiseau et R.F.O.*, 38, 53-85.

Sanderson, F.J., Donald, P.F., Pain, D.J., Burfield, I.J. & van Bommel, F.P.J. (2006). Long-term population declines in Afro-Palearctic migrant birds. *Biological Conservation*, 131, 93–105.

Sarà, M., Campobello, D., Zanca, L. & Massa, B. (2014). Food for flight: Pre-migratory dynamics of the Lesser Kestrel *Falco naumanni*. *Bird Study*, 61, 29–41.

Sergio, F., Tanferna, A., De Stephanis, R., Jiménez, L. L., Blas, J., Tavecchia, G., Preatoni, D. & Hiraldo, F. (2014). Individual improvements and selective mortality shape lifelong migratory performance. *Nature*, 515, 410–413.

Shamoun-Baranes, J., Burant, J. B., van Loon, E. E., Bouten, W. & Camphuysen, C. J. (2017). Short distance migrants travel as far as long distance migrants in Lesser Black-backed Gulls *Larus fuscus*. *Journal Avian Biology*, 48, 47–57.

Spina, F. & Volponi, S. (2008). *Atlante della Migrazione degli Uccelli in Italia*. *1. non-Passeriformi* [*Italian Bird Migration Atlas 1. Non-Passeriformes*]. Roma: Ministero dell'Ambiente e della Tutela del Territorio e del Mare - Istituto Superiore per la Protezione e la Ricerca Ambientale (ISPRA).

Strandberg, R., Klaassen, R. H. G., Hake, M., Olofsson, P. & Alerstam, T. (2009). Converging migration routes of Eurasian hobbies *Falco subbuteo* crossing the African equatorial rain forest. *Proceedings of the Royal Society of London B*, 276, 727–733.

Symes, C. T. & Woodborne, S. (2010). Migratory connectivity and conservation of the Amur Falcon *Falco amurensis*: a stable isotope perspective. *Bird Conservation International*, 20, 134-148.

Tanferna, A., López-Jiménez, L., Blas, J., Hiraldo, F. & Sergio, F. (2012). Different location sampling frequencies by satellite tags yield different estimates of migration performance: pooling data requires a common protocol. *PLoS One* 7: e49659.

Terraube, J., Mougeot, F., Cornulier, T., Verma, A., Gavrilov, A. & Arroyo, B. (2012). Broad wintering range and intercontinental migratory divide within a core population of the near-threatened pallid harrier. *Diversity and Distributions*, 18, 401–409.

Thorup, K., Rabol, J. & Erni, B. (2007). Estimating variation among individuals in migration direction. *Journal of Avian Biology*, 38, 182–189.

Trierweiler, C., Klaassen, R. H. G., Drent, R. H., Exo, K.-M., Komdeur, J., Bairlein, F., Koks, B. J. (2014). Migratory connectivity and population-specific migration routes in a long-distance migratory bird. *Proceedings of the Royal Society of London B*, 281, 2013-2897.

Turbek, S. P., Scordato, E. S. C. & Safran, R. J. (2018). The Role of Seasonal Migration in Population Divergence and Reproductive Isolation. *Trends in Ecology & Evolution*, 33, 164-175.

Turchin, P. (1998). Quantitative Analysis of Movement: Measuring and Modeling Population Redistribution in Animals and Plants. Sunderland, Massachusets: Sinauer Associates.

Vansteelant, W. M. G., Kekkonen, J., Byholm, P. (2017). Wind conditions and geography shape the first outbound migration of juvenile honey buzzards and their distribution across sub-Saharan Africa. *Philosophical Transactions of the Royal Society B*, 284, 20170387.

Verhulst, S. & Nilsson, J. A. (2008). The timing of birds' breeding seasons: a review of experiments that manipulated timing of breeding. *Philosophical Transactions of the Royal Society B*, 363: 399 -410.

Webster, M. S. & Marra P. P. (2005). The importance of understanding migratory connectivity and cross-seasonal interactions. In R. Greenberg, & P. P. Marra (Eds.), *Birds of two worlds. The Ecology and Evolution of Migration*. (pp. 199–209). Baltimore, Massachusetts, USA: Johns Hopkins University Press.

Webster, M. S., Marra, P. P., Haig, S.M., Bensch, S. & Holmes, R.T. (2002). Links between worlds: unravelling migratory connectivity. *Trends in Ecology & Evolution*, 17, 76–83.

Vidal-Mateo, J., Mellone, U., López-López, P., De La Puente, J., Garcia-Ripolles, C., Bermejo, A. & Urios, V. (2016). Wind effects on the migration routes of trans-Saharan soaring raptors: geographical, seasonal and interspecific variation. Current Zoology, 62, 89-97

Winger, B. M., Barker, F. K. & Ree, R. H. (2014). Temperate origins of long-distance seasonal migration in New World songbirds. *Proceedings Natural Academy Sciences USA*, 111, 12115–12120.

Wink, M., Sauer-Gürth, H. & Pepler, D. (2004). Phylogeographic relationships of the Lesser Kestrel (*Falco naumanni*) in breeding and wintering quarters inferred from nucleotide sequences of the mitochondrial cytochrome b gene. In R.D. Chancelor, & B.-U. Meyburg (Eds.), *Raptors Worldwide* (pp. 505–510). Berlin: The World Working Group on Birds of Prey and Owl.

Zink, R. M. & Gardner A. S. (2017). Glaciation as a migratory switch. *Sciences Advances*, 3: e1603133.

Table 1. List of the lesser kestrel migration data considered in the study of migratory phenology and connectivity. Year refers to the device's deployment time, na = not available.

Sex	**Macro area**	**Country**	**Colony**	**Device**	**Capture year**	**Reference**
na	WEU	France	Crau	ring	na	
M	CEU	Italy (Sicily)	Cerasaro	ring	2005	PRIN 2010 - 20108TZKHC
F	WEU	France	Saint-Michel	GPS-UHF	2016	LPO Hérault - EDF EN France
M	WEU	Spain	Pinto	GPS-UHF	2016	Migra - SEO BirdLife/GREFA
na	WEU	Spain	Los Alhorines	GPS-PTT	2012	CIBIO - Enerstar Villea, S.A.
M	WEU	Spain	Los Alhorines	GPS-PTT	2010	Limiñana et al. 2012
M	WEU	Spain	Los Alhorines	GPS-PTT	2010	Limiñana et al. 2012
M	WEU	Spain	Los Alhorines	GPS-PTT	2010	Limiñana et al. 2012
F	WEU	Spain	Los Alhorines	GPS-PTT	2010	Limiñana et al. 2012
F	WEU	Spain	Los Alhorines	GPS-PTT	2010	Limiñana et al. 2012
F	CEU	Italy (south)	Altamura	GPS-UHF	2016	LIFE11_NAT_IT068
F	CEU	Italy (south)	Altamura	GPS-UHF	2016	LIFE11_NAT_IT068
M	CEU	Italy (south)	Gravina	GPS-UHF	2016	LIFE11_NAT_IT068
F	CEU	Italy (south)	Gravina	GPS-UHF	2017	LIFE11_NAT_IT068
F	CEU	Italy (south)	Gravina	GPS-UHF	2017	LIFE11_NAT_IT068
F	CEU	Italy (south)	Altamura	GPS-UHF	2017	LIFE11_NAT_IT068
M	CEU	Italy (south)	Altamura	GPS-UHF	2017	LIFE11_NAT_IT068
M	CEU	Italy (south)	Altamura	GPS-UHF	2017	LIFE11_NAT_IT068
M	CEU	Italy (Sicily)	San Gregorio	GPS-UHF	2014	PRIN 2010 - 20108TZKHC
F	CEU	Italy (Sicily)	Canalotto	GPS-UHF	2015	PRIN 2010 - 20108TZKHC
M	CEU	Italy (Sicily)	Torrevecchia	GPS-UHF	2015	PRIN 2010 - 20108TZKHC
F	CEU	Italy (Sicily)	Canalotto	GPS-UHF	2015	PRIN 2010 - 20108TZKHC
F	EEU	Bulgaria	Sakar	GPS-PTT	2016	LIFE 11 NAT/BG/360
F	EEU	Bulgaria	Sakar	GPS-PTT	2015	LIFE 11 NAT/BG/360
F	EEU	Bulgaria	Sakar	GPS-PTT	2016	LIFE 11 NAT/BG/360
F	EEU	Bulgaria	Sakar	GPS-PTT	2015	LIFE 11 NAT/BG/360
M	WEU	France	Aude	GLS	2012	Pilard et al. 2017
F	WEU	France	Crau	GLS	2012	Pilard et al. 2017
F	WEU	France	Aude	GLS	2012	Pilard et al. 2017
F	WEU	France	Crau	GLS	2012	Pilard et al. 2017
F	WEU	France	Crau	GLS	2012	Pilard et al. 2017
F	WEU	France	Aude	GLS	2012	Pilard et al. 2017
F	WEU	France	Crau	GLS	2012	Pilard et al. 2017
M	WEU	France	Crau	GLS	2012	Pilard et al. 2017
M	WEU	France	Crau	GLS	2012	Pilard et al. 2017
F	WEU	France	Crau	GLS	2012	Pilard et al. 2017
F	WEU	France	Crau	GLS	2012	Pilard et al. 2017
F	WEU	France	Crau	GLS	2012	Pilard et al. 2017
F	WEU	France	Crau	GLS	2012	Pilard et al. 2017

Sex	Macro area	Country	Colony	Device	Capture year	Reference
F	WEU	Portugal	Castro-Verde	GLS	2008	Catry et al. 2011
F	WEU	Portugal	Castro-Verde	GLS	2008	Catry et al. 2011
F	WEU	Portugal	Castro-Verde	GLS	2008	Catry et al. 2011
F	WEU	Portugal	Castro-Verde	GLS	2008	Catry et al. 2011
F	CEU	Italy (south)	Matera	GLS	2016	Università statale di Milano
F	CEU	Italy (south)	Matera	GLS	2016	Università statale di Milano
F	CEU	Italy (south)	Matera	GLS	2016	Università statale di Milano
F	CEU	Italy (south)	Matera	GLS	2016	Università statale di Milano
F	CEU	Italy (south)	Matera	GLS	2016	Università statale di Milano
F	CEU	Italy (south)	Matera	GLS	2016	Università statale di Milano
F	CEU	Italy (south)	Matera	GLS	2016	Università statale di Milano
F	CEU	Italy (south)	Matera	GLS	2016	Università statale di Milano
F	CEU	Italy (south)	Matera	GLS	2016	Università statale di Milano
F	CEU	Italy (south)	Matera	GLS	2016	Università statale di Milano
F	CEU	Italy (south)	Matera	GLS	2016	Università statale di Milano
F	CEU	Italy (south)	Matera	GLS	2016	Università statale di Milano
F	CEU	Italy (south)	Matera	GLS	2016	Università statale di Milano
F	CEU	Italy (south)	Matera	GLS	2016	Università statale di Milano
F	CEU	Italy (south)	Matera	GLS	2016	Università statale di Milano
F	CEU	Italy (south)	Matera	GLS	2016	Università statale di Milano
F	EEU	Greece	Modestos	GLS	2014	LIFE11NAT/GR/001011
F	EEU	Greece	Modestos	GLS	2014	LIFE11NAT/GR/001011
F	EEU	Greece	Stefanovikeio	GLS	2014	LIFE11NAT/GR/001011
M	EEU	Greece	Stefanovikeio	GLS	2014	LIFE11NAT/GR/001011
M	EEU	Greece	Kokkina	GLS	2016	LIFE11NAT/GR/001011
F	EEU	Greece	Rizomylos	GLS	2016	LIFE11NAT/GR/001011

Table 2 (part one and two). Main average parameters of migratory phenology in the lesser kestrel. Departure and arrival dates expressed in Julian days (1st January = 1). Statistically significant differences of two-way ANOVA are marked in bold. Macro-areas group western breeding populations of Portugal, Spain, France (WEU), central breeding populations of South-Italy and Sicily (CEU) and eastern ones of Bulgaria and Greece (EEU).

		Macro-area			**Sex**	
	Total	**WEU**	**CEU**	**EEU**	**Male**	**Female**
Departure from breeding area						
Mean±SE	264±1.37	263±2.60	266±1.86	261±2.36	269±2.83	262±1.49
CL 95.00%	261.11-266.57	257.29-268.05	262.01 -269.63	255.76-266.44	263.38-275.62	259.19-265.19
N	62	24	28	10	14	48
F; p		1.33; 0.27			6.05; **0.02**	
Arrival to wintering area						
Mean±SE	277±1.30	277±2.19	277±1.96	275±2.32	283±2.81	275±1.32
CL 95.00%	274.16-279.23	272.63-281.71	272.79-280.85	269.95-280.45	276.86-288.99	272.21-277.54
N	62	24	28	10	14	48
F; p		0.13; 0.88			7.56; **0.008**	
Departure from wintering area						
Mean±SE	68±2.21	57±3.44	75±2.98	71±3.25	67±5.79	68±2.38
CL 95.00%	63.61-72.43	49.70-64.30	68.83-81.17	63.45-78.15	53.49-79-71	63.58-73.22
N	50	17	23	10	10	40
F; p		8.69; **0.001**			0.14; 0.71	
Arrival to breeding area						
Mean±SE	86±2.83	73±4.81	94±3.50	93±4.79	91±6.72	85±3.14
CL 95.00%	80.75-92.15	62.74-83.14	86.52-101.05	82.18-104.26	75.51-105.89	79.00-91.2
N	49	17	23	9	10	39
F; p		8.79; **0.001**			2.42; 0.13	
Length autumn migration (days)						
Mean±SE	13±0.94	14±1.83	11±0.91	14±2.77	13±1.13	13±1.16
CL 95.00%	10.97-14.74	10.71-18.29	9.12-12.87	7.84-20.36	10.43-15.41	10.34-15.03
N	62	24	28	10	14	48
F; p		1.57; 0.22			0.02; 0.89	
Length spring migration (days)						
Mean±SE	18±1.64	16±3.26	19±.91	22±4.35	24±3.33	17±1.83
CL 95.00%	15.17-21.77	9.03-22.85	14.83-22.74	12.41-32.47	16.56-3.64	13.33-20.72
N	49	17	23	9	10	39
F; p		1.29; 0.28			3.75; 0.06	
Length autumn migration (km)						
Mean±SE	3294.64±115.79	3165.98±147.56	3344.24±188.30	3371.00±291.70	3124.82±122.30	3225±177.32
CL 95.00%	3054.50-3534.79	2804.92-3527.04	2929.79-3758.70	2442.68-4299.33	2842.79-3406.84	3035.80-3771.83
N	23	7	12	4	9	14
F; p		0.13;0.88			1.04; 0.32	

		Marco-areas			**Sex**	
	Total	**WEU**	**CEU**	**EEU**	**Male**	**Female**
Length spring migration (km)						
Mean±SE	3572.88±177.98	2996.50±184.64	3834.00±241.10	4029.31±442.46	3059.11±44.04	3932.51±244.81
CL 95.00%	3195.57-3950.19	2521.86-3471.14	3263.87-4404.12	2125.58-5933.05	2951.35-3166.87	3378.71-4486.32
N	17	6	8	3	7	10
F; p		2.16;0.15				4.24;0.06
Length permanence in Sahel (days)						
Mean±SE	209±2.88	221±4.83	202±4.05	204±4.25	218±7.16	207±3.05
CL 95.00%	203.14-214.70	211.06-231.53	193.33-210.15	193.78-214.02	202.09-234.51	200.39-212.75
N	50	17	23	10	10	40
F; p		4.95; **0.01**			1.47; 0.23	

Table 3. GLMMs testing whether season (autumn, spring), time of day (daytime, night-time) and sector (Mediterranean Sea, Sahara Desert) are important predictors lesser kestrel flight speeds (km/h) during of migration. Individual and macro-area are included as random factors. The best levels of predictors are presented in bold and are compared to the respective baseline levels of every fixed factor (e.g. Spring vs Autumn, etc.). The descriptive statistics (mean±SE, 95% coefficient limits) of fixed factors and their interactions employed in the general linear mixed model have here been reported for comparison of flight speeds.

	Mean±SE	**CL 95.00%**	**N**	**Estimate±SE**	**p**
Total	17.22±0.28	16.66-17.78	2282		
Autumn	17.27±0.33	16.62-17.92	1306		
Spring	17.15±0.49	16.18-18.12	976	0.745+0.629	0.237
Desert	16.36±0.30	15.78-16.95	1981		
Sea	22.86±0.80	21.28-24.43	301	8.343±1.157	**<0.001**
Daytime	15.89±0.28	15.35-16.44	1915		
Night-time	24.14±0.91	22.35-25.93	367	5.284±0.986	**<0.001**
Autumn*Desert	16.30±0.34	15.62-16.98	1140		
Autumn*Sea	23.93±0.94	22.08-25.78	166		
Spring*Desert	16.45±0.53	15.41-17.48	841		
Spring*Sea	21.53±1.36	18.85-24.22	135	-2.644±1.675	0.114
Autumn*Daytime	16.36±0.35	15.68-17.04	1086		
Autumn*Night-time	21.78±0.90	20.01-23.55	220		
Spring*Daytime	15.28±0.46	14.39-16.18	829		
Spring*Night-time	27.68±1.81	24.11-31.25	147	5.382±1.525	**<0.001**
Desert*Daytime	15.02±0.29	14.46-15.59	1674		
Desert*Night-time	23.67±1.02	21.66-25.69	307		
Sea*Daytime	21.94±0.87	20.23-23.65	241		
Sea*Night-time	26.53±1.92	22.69-30.37	60	-5.114±2.364	**0.030**
Autumn*Desert*Daytime	15.45±0.36	14.75-16.16	963		
Autumn*Desert*Night-time	20.90±1.00	18.92-22.89	177		
Autumn*Sea*Daytime	23.43±1.07	21.30-25.55	123		
Autumn*Sea* Night-time	25.37±1.92	21.49-29.25	43		
Spring*Desert*Daytime	14.44±0.47	13.51-15.36	711		
Spring*Desert*Night-time	27.44±1.95	23.58-31.30	130		
Spring*Sea*Night-time	20.39±1.37	17.67-23.11	118		
Spring*Sea*Night-time	29.46±4.76	19.37-39.55	17	0.511±4.081	0.900

Table 4. Orthodromic distances (in km) between breeding and overwintering areas of European lesser kestrels. The statistical significance (marked in bold) of GLM model with macro-area as fixed factor and sex as random factor depends from the shortest orthodromic distances between the WEU and CEU breeding grounds and respective overwintering grounds in Sahel with respect to the EEU areas.

	Mean±SE	**CL95.00%**	**N**	**Estimate±SE**	**p**
Male	3198.90±100.08	2984.25-3413.56	15	0.086±0.122	0.484
Female	3151.81±45.39	3060.49-3243.13	48		
WEU	3085.61±70.48	2940.45-3230.77	26	-0.517±0.177	**0.005**
CEU	3156.30±57.87	3037.75-3274.86	29	-0.388±0.176	**0.032**
EEU	3410.88±86.40	3215.43-3606.33	10		
Total	3167.19±42.15	3082.99-3251.40	65		

Figure Captions

Figure 1.

Migratory routes of lesser kestrel (*Falco naumanni*) from the breeding Euroepan grounds to the overwintering Sahel areas in Africa. Above autumnal travel, below spring travel. Star = stopover site of ≥ 1 day.

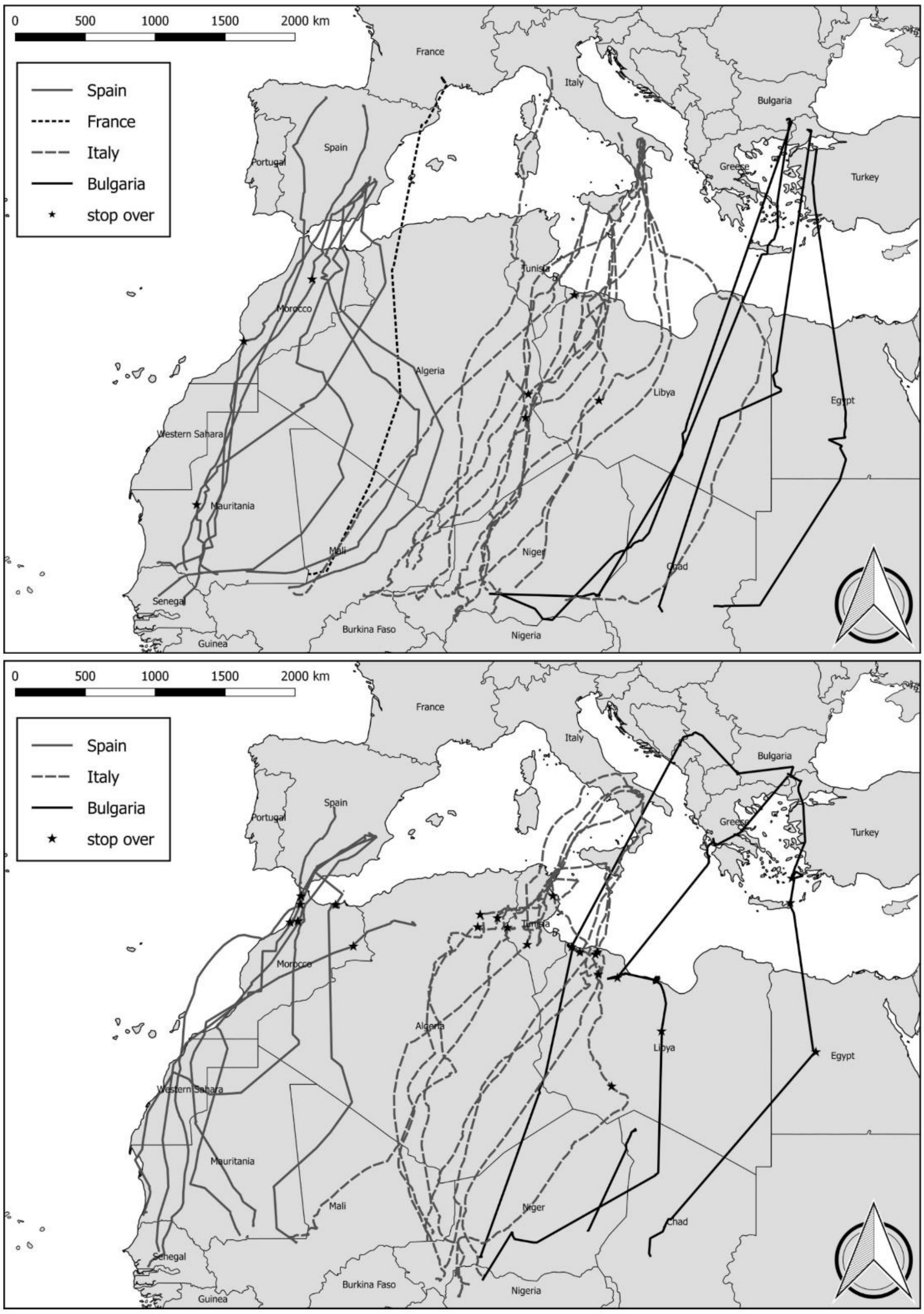
Figure 2.

The overwintering area of lesser kestrel (*Falco naumanni*) in Sahel. Symbols indicate the geographic European origin and colours the device deployed on the 65 individuals.

Figure 3.

The increasing orthodromic distances from breeding areas in the Western European countries, mostly corresponding to the Iberian Peninsula (WEU), to the Central European countries (CEU), corresponding to the Italian peninsula and the Eastern European countries (EEU), corresponding to the Balkan peninsula.

Figure 1.


0
500
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Spain
France
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Turkey
Tunisia
Morocco
Algeria
Libya
Egypt
Western Sahara
Mauritania
Mali
Niger
Chad
Senegal
Burkina Faso
Nigeria
Guinea
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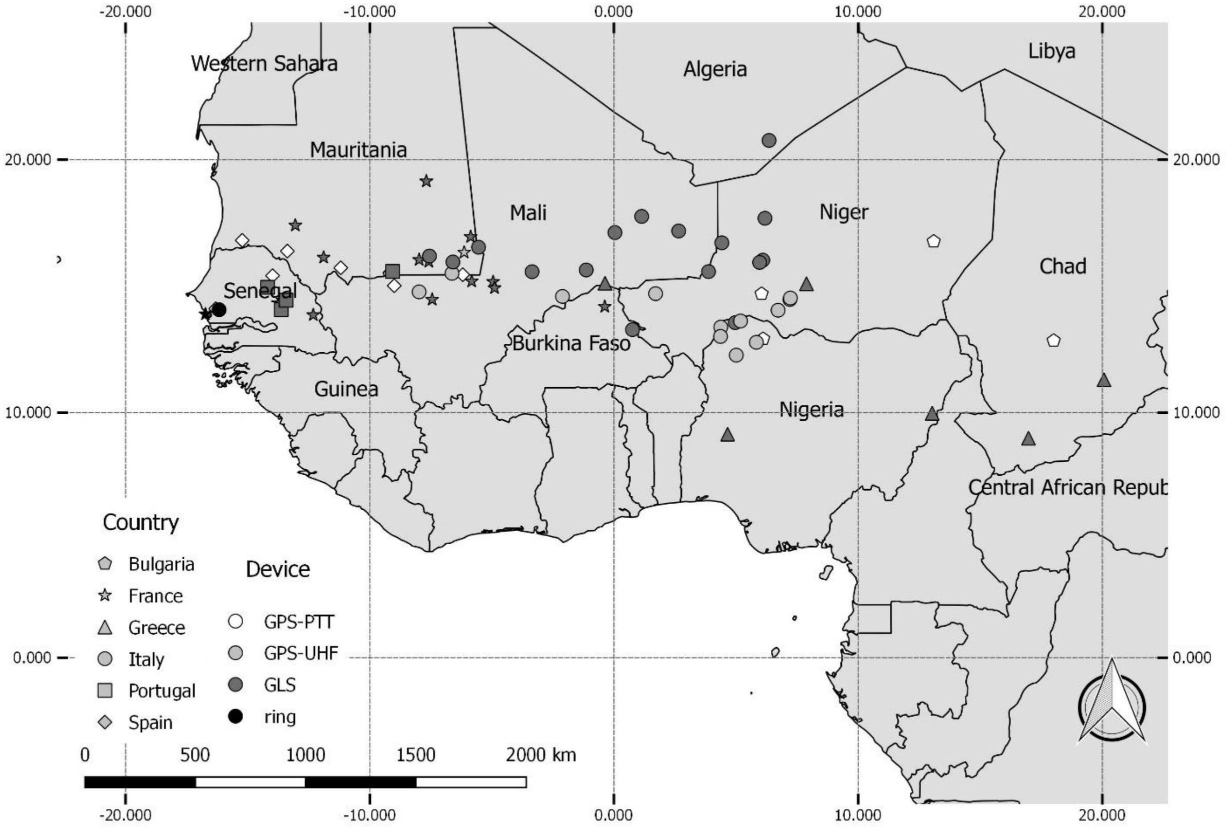
Guinea

Figure 2.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Western Sahara
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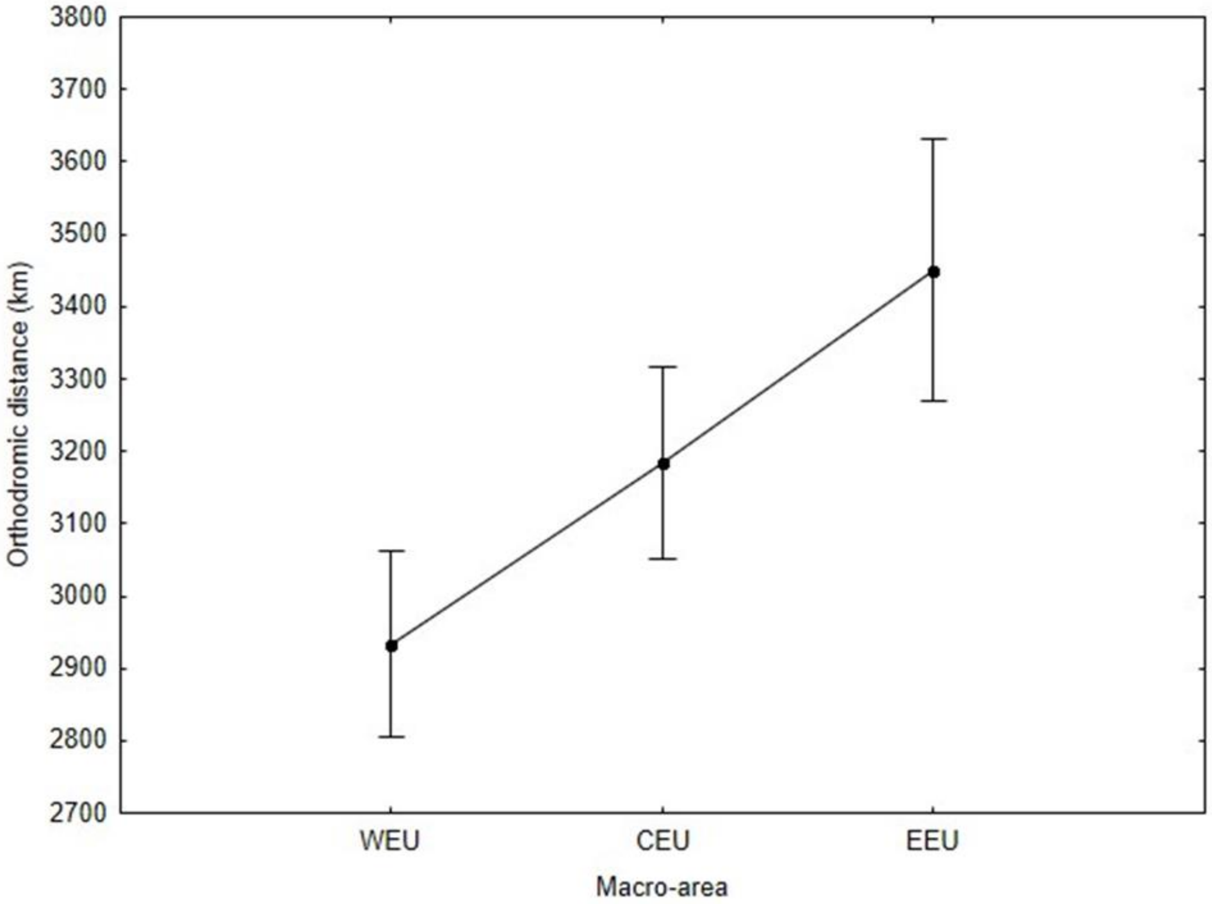
Niger
Chad
Senegal
Burkina Faso
Guinea
Nigeria
Central African Repub
Country
Bulgaria
France
Greece
Italy
Portugal
Spain
Device
GPS-PTT
GPS-UHF
GLS
ring
0
500
1000
1500
2000 km

Figure 3.


3800
3700
3600
3500
3400
3300
3200
3100
3000
2900
2800
2700
Orthodromic distance (km)
WEU
CEU
EEU
Macro-area

Chapter 7.

Synthesis and concluding remarks

Synthesis

Aim of the present thesis was to investigate the short and long-term effects of variation in the breeding environment on behavioural and fitness traits in the colonial breeding lesser kestrel, using both experimental and correlative approaches. Environmental traits I considered in my studies were the nest-site quality and consequences for lesser kestrel breeding performance, the availability of food resources and the short-term effect of these on fitness and foraging behaviour, the geographic distribution of lesser kestrel European populations and its consequences on migratory behaviour.

In the first part, I analysed the short-term effects of variation in the breeding environment on lesser kestrel breeding behaviour and performance. Starting from nest-site selection, results reported in **Chapter 2** showed that lesser kestrel exhibited a strong preference for previously used nestboxes lined with an organic substrate accumulated during past breeding attempts. However, I did not find any strong effects of nest substrate on fitness. A significantly higher occupation rate of nestboxes containing old nest material may have several explanations. First, lesser kestrels, as observed in a Spanish population (Negro and Hiraldo 1993), may rely on previous breeding attempts to identify the most suitable nest-sites within colony sites. Second, it is possible that the preference for nestboxes within old nest organic material could be led by differences in the features of the organic substrate that is more comfortable and softer. Moreover, the increase (~10%) of hatching success observed in eggs laid on the old nest material could be due to better microclimatic conditions within the nestbox. Indeed, in species that do not add any material to line their nests, the presence of an old organic layer may be a further cue of the nest-site selection since it may contribute to increase thermal insulation, reduce egg loss and improve incubation efficiency (Hooge et al. 1999; Cook et al. 2003; Ardia et al. 2006). Moreover, eggs laid on the soft mineral material may suffer a lower risk of accidental breakage. As expected, nestlings reared in nests with old organic material have a higher level of infestation by a common nest-dwelling blood-sucking ectoparasite (*Carnus hemapterus*). However, differences between nestlings reared in clean vs dirty nestboxes disappear soon after hatching. I did not find any significant difference in survival between nestlings reared in new vs. old nest substrate, suggesting that breeding in old nest does not entail fitness cost for lesser kestrels. It is also possible that benefits from laying on old material could be context-dependent: the huge number of individuals composing our study colony (~ 1000 breeding pairs) may indicate favourable breeding conditions (availability of food resources surrounding the colony or the absence of a relevant predation risk) and thus nest-site selection could be less important in the specific environmental context of our study, while it may be more important in other contexts (e.g. isolated small colonies, as is the case in other areas of the distribution range of the species).

Results described in **Chapter 3** showed that in the lesser kestrel population breeding in the city of Matera food is a limited resource during the breeding season. Indeed, extra food provided benefits to breeding performance and adult moult. I found that food supplemented individuals, despite the absence of an improvement of body conditions and antioxidant capacity, invested extra food resources into the synthesis of new flight feathers. This result suggested that moult, especially during the breeding season, is costly and that only individuals supported by highly favourable environmental conditions could promote a greater allocation of resources to feather renewal. Regarding breeding performance, I observed that food supplemented females laid heavier last-laid eggs, reared nestling with higher body mass and with larger feather growth compared to controls. Moreover, nestlings reared by food-supplemented parents were more likely to successfully cope with ectoparasite infestation whereas controls showed a decrease in body mass in associations with high ectoparasite load. Importantly, I observed that maternal conditions are the major trait modulating the benefits of extra food provisioning. Ca. 16% of the variation in egg mass was explained by female conditions, with females in better conditions laying heavier eggs. This association arose only in the control group while food supplemented females did not show any variation in egg mass according to their conditions. I observed a similar association considering the premature nest desertion: once again, in the control group, females in poor conditions were more likely to abandon the nest, probably due to high perceived costs of the current breeding attempt in association with their conditions, while this association did not emerge among females belonging to the food-supplemented group. My comprehensive analysis of the benefits of food supplementation on breeding performance and adult traits suggest that lesser kestrels may thus conform to an income breeding model of energy storage for reproduction.

The importance of food availability as an environmental variable affecting lesser kestrel behaviour arose also in **Chapter 4**. Analysing home ranges of individuals belonging to five different colonies (two in Apulia and three in Sicily), we observed that, in both populations studied, individuals from the same colony forage in colony-specific areas, likely because they shared information about the location of profitable foraging grounds. Our study showed that home ranges of lesser kestrels from different neighbouring colonies are spatially segregated. Segregation may result from resource depletion in the inter-colony areas, that hence become progressively avoided by individuals of the two colonies in the course of the breeding season (Wanless and Harris 1993; Grémillet et al. 2004; Masello et al. 2010; Wakefield et al. 2011).

The analysis of foraging tactics conducted in the **Chapter 5** revealed that, as in other central place foraging bird species, foraging trips are more extended in time and space during incubation rather than during nestling rearing stage (Woo et al. 2008; Ceia and Ramos 2015; Camprasse et al. 2017). Moreover, I observed that males performed more frequent foraging trips than females during nestling rearing. As expected, two distinct foraging tactics were observed. The ‘sit-and-wait’ tactic was associated to long lasting trips and it was characterized by perching, lower proportion of intensive search and relocation, while the ‘widely foraging’ tactic was characterized by a higher proportion of both relocation and intensive search, lower perching and it mainly referred to short lasting trips. The widely foraging tactic was ca. two-fold more energetically expensive than the sit-and-wait tactic; indeed, lesser kestrels adopted the most expensive foraging tactic when weather conditions were favourable for soaring-gliding (high solar radiation for soaring flight and with high cross-wind conditions) and during nestling-rearing phase, when offspring provisioning requirements are higher. Moreover, despite individuals consistently differed in their preference for adopting a given foraging strategy, individuals were consistently more prone to adopt a specific foraging tactic across different environmental gradients of solar radiation and wind assistance. Such individual differences in foraging tactics may reflect foraging specialization, and may contribute to reduce intraspecific competition at foraging grounds. Furthermore, it is possible that birds targeted on different prey items according to foraging tactic. For instance, it has been hypothesized that sit-and-wait predators generally catch larger prey compared to those taken by active predators (de Arruda Bueno and Motta-Junior 2008). We hence suggest that lesser kestrel targets different preys during sit-and-wait or widely foraging trips. Moreover, we observed that nestlings reared by parents preferring high-energy foraging tactic have a higher growth rate.

In the second part of my thesis, I analysed possible long-term effects of variation in breeding environmental traits on lesser kestrel migration. In **Chapter 6**, we investigated whether the geographic distribution of breeding areas could affect migratory behaviour, influencing wintering areas in sub-Saharan quarters. We found that, contrary to our expectation, European lesser kestrel populations show a strong migratory connectivity. Indeed, we observed that grouping of wintering populations depended on the breeding area. Therefore, continental configuration of breeding range has an influence on migration patterns of lesser kestrels: breeding areas were matched in latitude with non-breeding locations. It has been observed that long-distance migratory species generally show a weak migratory connectivity (Symes and Woodborne 2010; Trierweiler et al. 2014). However, the relative homogeneity of habitats across the huge Sahel belt (> 5 million km^2, the wintering area of all lesser kestrel populations analysed) would have favoured the non-random spread out of lesser kestrels, and therefore would have reduced the mixing of populations, increasing connectivity. Different migratory strategies have evolved as a trade-off between costs and benefits of migration; it is possible that the overwinter segregation of lesser kestrel population observed in sub-Saharan quarters is led by the different migratory trajectories that individuals follow during the migratory journey. To conclude, birds from a given European population occupied shared areas during the overwinter period and thus were subjected to the same biotic and abiotic conditions, with important consequences on future individual performance during successive stages of the life-cycle and, more generally, on population dynamics.

Concluding remarks

This thesis provides novel information about the effect of several environmental traits on breeding performance and behaviour of the European lesser kestrel. Results presented show that lesser kestrels are profoundly affected by variations of the breeding area and that such variations could also affect several stages of life-cycles. I observed that during the early phases of the breeding season lesser kestrel preference for nest-site selection is strongly influenced by public information provided by conspecifics. Thus, individuals gather cues about the quality of nest-site from the past presence of other individuals in the area. It is possible that this behaviour has evolved to avoid the occupancy of inappropriate nest-sites (i.e. cavities that are more exposed to adverse weather conditions or easily accessible for predators). The communication between individuals and the share of information is also fundamental to face the patchy and heterogeneous distribution of the preys around the colonies. Sharing information among individuals belonging to same or different colonies could be fundamental for the location of productive foraging areas. Moreover, the search of food resources has a major role in determining the relationships among individuals from same and different colonies. Hence, both the spatial segregation of neighbouring colonies and the implementation of different foraging strategy among individuals belonging to the same foraging areas underlined that, in lesser kestrel, the distribution of preys in the breeding environment has led to the evolution of strategies to limit intraspecific competition. Finally, breeding habitats affect the migratory strategy adopted by lesser kestrels at the end of the breeding season. Therefore, I observed that the geographic distribution of European colonies determines the non-breeding areas location occupied by individuals in Africa, during the winter. The strong migratory connectivity showed by the lesser kestrel could imply severe effects on European population dynamics, since individuals are exposed to different biotic and abiotic conditions during the non-breeding stage.

To conclude, despite the potential effects of variations in the environment on current and future fitness and behaviour of individuals has been largely investigated in different species, the presented scenario is generally contrasting and species-specific (see for example Kruuk et al. 2015; Schipper et al. 2016; review in Vázquez et al. 2017). Further investigations are necessary to better understand how breeding habitat variation could affect current and future breeding performance and behaviour of bird.

References

Anderson S. S., Harwood J. (1985). Time budgets and topography: How energy reserves and terrain determine the breeding behaviour of grey seals. Animal Behaviour 33: 1343–1348.

Alan R. R., McWilliams S. R. (2013). Oxidative stress, circulating antioxidants, and dietary preferences in songbirds. Comparative Biochemical Physiology 164: 185–193.

Aparicio J. M., Bonal R. (2002). Effects of food supplementation and habitat selection on timing of lesser kestrel breeding. Ecology 83: 873–877.

Ardia D. R., Pérez J. H., Clotfelter E. D. (2006). Nest box orientation affects internal temperature and nest site selection by tree swallows. Journal of Field Ornithology 77: 339–344.

Arnold J. M., Nisbet I. C., Oswald S. A. (2016). Energetic constraint of non-monotonic mass change during offspring growth: a general hypothesis and application of a new tool. Journal of Animal Ecology 85: 476–486.

Ashmole N. P. (1963). The regulation of numbers of tropical oceanic birds. Ibis 103: 458–473.

Bercovitch F. B., Lebron M. R., Martinez H. S., Kessler M. J. (1998). Primigravidity, body weight, and costs of rearing first offspring in rhesus macaques. American Journal of Primatology 46: 135–144.

Boutin S. (1990). Food supplementation experiments with terrestrial vertebrates: patterns, problems, and the future. Canadian Journal of Zoology 68: 203–220.

Breitburg D. L. (1987). Interspecific competition and the abundance of nest sites: factors affecting sexual selection. Ecology 68: 1844–1855.

Brown G. P., Shine R. (2005). Nesting snakes (*Tropidonophis mairii*, Colubridae) selectively oviposit in sites that provide evidence of previous successful hatching. Canadian Journal of Zoology 83: 1134–1137.

Call K. A., Ream R. R., Johnson D., Sterling J. T., Towell R. G. (2008). Foraging route tactics and site fidelity of adult female northern fur seal (*Callorhinus ursinus*) around the Pribilof Islands. Deep Sea research Part II: Topical Studies in Oceanography 55: 1883–1896.

Camprasse E. C., Cherel Y., Arnould J. P., Hoskins A. J., Bost C. A. (2017). Combined bio logging and stable isotopes reveal individual specialisations in a benthic coastal seabird, the Kerguelen shag. PloS One 12: e0172278.

Catry I., Franco A. M. A., Rocha P., Alcazar R., Reis S., Cordeiro A., Ventim R., Teodósio J., Moreira F. (2013). Foraging habitat quality constrains effectiveness of artificial nest-site provisioning in reversing population declines in a colonial cavity nester. PLoS One 8: e58320.

Cecere J. G., Catoni C., Gaibani G., Geraldes P., Celada C., Imperio S. (2015). Commercial fisheries, inter-colony competition and sea depth affect foraging location of breeding Scopoli's Shearwaters *Calonectris diomedea*. Ibis 157: 284–298.

Cecere J. G., Bondì S., Podofillini S., Imperio S., Griggio M., Fulco E., Curcio A., Ménard D., Mellone U., Saino N., Serra L., Sarà M., Rubolini D. (2018). Spatial segregation of home ranges between neighbouring colonies in a diurnal raptor. Scientific reports 8: 11762.

Ceia F. R., Ramos J. A. (2015). Individual specialization in the foraging and feeding strategies of seabirds: a review. Marine Biology 162: 1923−1938.

Cook M. S., Beissinger G., Toranzos R. R., Arendt W. (2003). Trans-shell infection by pathogenic microorganisms reduces the shelf life of non-incubated bird's eggs: a constraint on the onset of incubation? Proceeding of the Royal Society of London B 270: 2233–2240.

Cramp S. (1998). The complete birds of the Western Palearctic on CD-ROM. Oxford University Press, Oxford, UK.

Cucco M., Malacarne G. (1997). The effect of supplemental food on time budget and body condition in the black redstart *Phoenicurus ochruros*. Ardea 85: 211–221.

Danchin E., Giraldeau L-A., Valone T. J., Wagner R. H. (2004). Public information: from nosy neighbors to cultural evolution. Science 305: 487–491.

de Arruda Bueno A., Motta Junior J. C. (2008). Small mammal prey selection by two owl species in southeastern Brazil. Journal of Raptor Research 42: 248–255.

Dewey S. R., Kennedy P. L. (2001). Effects of supplemental food on parental-care strategies and juvenile survival of Northern Goshawks. The Auk 118: 352–365.

Fayet A. L., Freeman R., Shoji A., Kirk H. L., Padget O., Perrins C. M., Guilford T. (2016). Carry-over effects on the annual cycle of a migratory seabird: an experimental study. Journal of Animal Ecology 85: 1516–1527.

Fletcher Q. E., Selman C., Boutin S., McAdam A. G., Woods S. B., Seo A. Y., Leeuwenburgh C., Speakman J. R., Humphries M. M. (2013). Oxidative damage increases with reproductive energy expenditure and is reduced by food-supplementation. Evolution 67: 1527–1536.

Garcia P. F. J., Merkle M. S., Barclay R. M. R. (1993). Energy allocation to reproduction and maintenance in Mountain Bluebirds (*Sialia currucoides*): a food supplementation experiment. Canadian Journal of Zoology 71: 2352–2357.

Gavashelishvili A., McGrady M. J. (2006). Breeding site selection by bearded vulture (*Gypaetus barbatus*) and Eurasian griffon (*Gyps fulvus*) in the Caucasus. Animal Conservation 9: 159–170.

Giordano M., Costantini D., Pick J. L., Tschirren B. (2015). Female oxidative status, egg antioxidant protection and eggshell pigmentation: a supplemental feeding experiment in great tits. Behavioural Ecology and Sociobiology 69: 777–785.

Grémillet D., Dell'Omo G., Ryan P. G., Peters G., Ropert-Coudert Y., Weeks S. J. (2004). Offshore diplomacy, or how seabirds mitigate intra-specific competition: a case study based on GPS tracking of Cape gannets from neighbouring colonies. Marine Ecology Progress Series 268: 265–279.

Harris S., Raya Rey A., Zavalaga C., Quintana F. (2014). Strong temporal consistency in the individual foraging behaviour of Imperial Shags *Phalacrocorax atriceps*. Ibis 156: 523–533.

Harrison X. A., Blount J. D., Inger R., Norris D. R., Bearhop S. (2011). Carry-over effects as drivers of fitness differences in animals. Journal of Animal Ecology 80: 4–18.

Hernández-Pliego J., Rodríguez C., Dell'Omo G., Bustamante J. (2017). Combined use of tri axial accelerometers and GPS reveals the flexible foraging strategy of a bird in relation to weather conditions. PloS One 12: e0177892.

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

Hipkiss T., Hörnfeldt B., Eklund U., Berlin S. (2002). Year-dependent sex-biased mortality in supplementary-fed Tengmalm's owl nestlings. Journal of Animal Ecology 71: 693–699.

Hooge P. N., Stanback M. T., Koenig W. D., (1999). Nest-site selection in the acorn woodpecker. The Auk 116: 45–54.

Iwamura T., Possingham H. P., Chadès I., Minton, C., Murray N. J., Rogers D. I., Treml E. A., Fuller, R. A. (2013). Migratory connectivity magnifies the consequences of habitat loss from sea-level rise for shorebird populations. Proceedings of the Royal Society of London B: Biological Sciences 280: 20130325.

Karell P., Kontiainen P., Pietiäinen H., Siitari H., Brommer J. E. (2008). Maternal effects on offspring Igs and egg size in relation to natural and experimentally improved food supply. Functional Ecology 22: 682–690.

Klaassen R. H., Hake M., Strandberg R., Alerstam T. (2010). Geographical and temporal flexibility in the response to crosswinds by migrating raptors. Proceedings of the Royal Society of London B: Biological Sciences rspb20102106.

Korpimäki E., Wiehn J. (1998). Clutch size of kestrels: seasonal decline and experimental evidence for food limitation under fluctuating food conditions. Oikos 83: 259–272.

Kostrzewa A., Kostrzewa, R. (1990). The relationship of spring and summer weather with density and breeding performance of the Buzzard *Buteo buteo*, Goshawk *Accipiter gentilis* and Kestrel *Falco tinnunculus*. Ibis 132: 550–559.

Kruuk L. E., Osmond H. L., Cockburn, A. (2015). Contrasting effects of climate on juvenile body size in a Southern Hemisphere passerine bird. Global change biology 21: 2929–2941.

Kuhn C. E., Ream R. R., Sterling J. T., Thomason J. R., Towell R. G. (2014). Spatial segregation and the influence of habitat on the foraging behavior of northern fur seals (*Callorhinus ursinus*). Canadian journal of zoology 92: 861–873.

Lack D. (1954). The natural regulation of animal numbers. Oxford University Press, Oxford, UK.

Lack D. (1966). Population studies of bird. Oxford University Press, Oxford, UK.

Lachmann M., Sella G., Jablonka E. (2000). On the advantages of information sharing. Proceeding of the Royal Society B 267: 1287–1293.

Lascelles B. G, Taylor P. R, Miller M. G. R., Dias M. P., Oppel S., Torres L., Hedd A., Le Corre M., Philips R. A., Shaffer S. A., Weimerskirch H., Small C. (2016). Applying global criteria to tracking data to define important areas for marine conservation. Diversity and Distributions 22: 422–431.

Liker A., Freckleton R. P., Remeš V., Székely T. (2015). Sex differences in parental care: gametic investment, sexual selection, and social environment. Evolution 69: 2862–2875.

Machovsky-Capuska G. E., Hauber M. E., Libby E., Amiot C., Raubenheimer D. (2014). The contribution of private and public information in foraging by Australasian gannets. Animal Cognition 17: 849–858.

Mainwaring M. C., Hartley I. R., Lambrechts M. M., Deeming D. C. (2014). The design and function of birds' nest. Ecology and Evolution 4: 3909–3928.

Marra P. P., Hobson K. A., Holmes R. T. (1998). Linking winter and summer events in a migratory bird using stable-carbon isotopes. Science 282: 1884–1886.

Marra P. P., Norris D. R., Haig S. M., Webster M., Royle J. A. (2006). Migratory connectivity In K. Crooks, M. Sanjayan (Eds.) Connectivity Conservation (pp. 157-183). New York: Cambridge University Press, NY, USA.

Martin T. E. (1987). Food as a limit on breeding birds: a life-history perspective. Annual Review of Ecological Systems 18: 453–487.

Martin T. E., Roper, J. J. (1988). Nest predation and nest-site selection of a western population of the Hermit Thrush. The Condor 51–57.

Masello J.F. Mundry R., Poisbleau M., Demongin L., Voigt C.C., Wikelski M., Quillfeldt P. (2010). Diving seabirds share foraging space and time within and among species. Ecosphere 1: 1–28.

Mazgajski T. D. (2003). Nest site choice in relation to the presence of old nests and cavity depth in the starling Sturnus vulgaris. Ethology Ecology and Evolution 15: 273–281.

Mazgajski T. D. (2007). Effect of old nest material on nest site selection and breeding parameters in secondary hole nesters, a review. Acta Ornithology 42: 1–14.

Mellone U., Klaassen R.H., García-Ripollés C., Limiñana R., López-López P., Pavón D., Strandberg R., Urios V., Vardakis M., Alerstam T. (2012). Interspecific comparison of the performance of soaring migrants in relation to morphology, meteorological conditions and migration strategies. PLoS One 7: e39833.

Merino S., Potti J., (1995). Pied flycatchers prefer to nest in clean nest boxes in an area with detrimental nest ectoparasites. The Condor 97:828–831.

Meijer T., Drent R. (1999). Re-examination of the capital and income dichotomy in breeding birds. Ibis 141: 399–414.

Murren C. J., Auld J. R., Callahan H., Ghalambor C. K., Handelsman C. A., Heskel M. A., Kingsolver J. G., Maclean H. J., Masel J., Maughan H., Pfennig, D. W., Relyea R. A., Seiter S., Snell-Rood E., Steiner U. K., Schlichting C. D. (2015). Constraints on the evolution of phenotypic plasticity: limits and costs of phenotype and plasticity. Heredity 115: 293.

Nadjafzadeh M., Hofer H., Krone O. (2016). Sit-and-wait for large prey: foraging strategy and prey choice of White-tailed Eagles. Journal of Ornithology 157: 165–178.

Negro J. J., Hiraldo F. (1993). Nest-site selection and breeding success in the lesser kestrel Falco naumanni. Bird Study 40:115–119.

Newton I. (1989). Lifetime reproduction in birds. Academic Press, London, UK.

Newton I. (2008). The migration ecology of birds. Elsevier, London, UK.

Nilsson S. G. (1984). The evolution of nest-site selection among hole-nesting birds: the importance of nest predation and competition. Ornis Scandinavia 167–175.

Norris D. R. (2005). Carry-over effects and habitat quality in migratory population. Oikos 109: 178–186.

Orians G. H., Pearson N. E. (1979). On the theory of central place foraging. In: Horn D. J., Mitchell R. and Stair G. R. (Eds) Analysis of ecological systems (pp 155–177). Columbus, Ohio State University Press, Ohio, USA.

Patrick S. C., Bearhop S., Grémillet D., Lescroël A., Grecian W. J., Bodey T. W., Votier, S. C. (2013). Individual differences in searching behaviour and spatial foraging consistency in a central place marine predator. Oikos 123: 33–40.

Patrick S. C., Weimerskirch H. (2014). Personality, foraging and fitness consequences in a long-lived seabird. PLoS One 9: e87269.

Podofillini S., Cecere J. G., Griggio M., Curcio A., De Capua E. L., Fulco E., Pirrello S., Saino N., Serra L., Visceglia M., Rubolini D. (2018). Home, dirty home: effect of old nest material on nest-site selection and breeding performance in a cavity-nesting raptor. Current Zoology 1–10.

Refsnider J. M., Janzen F. J. (2010). Putting eggs in one basket: ecological and evolutionary hypotheses for variation in oviposition-site choice. Annual Review of Ecology, Evolution and Systematics 41:39–57.

Richner H., Danchin E. (2001). On the importance of slight nuances in evolutionary scenario. Animal Behaviour 61: 17–18.

Riding C. S., Belthoff J. R. (2015). Removal of old nest material decreases reuse of artificial burrows by burrowing owls. Wildlife Society Bulletin 39:521–528.

Rodríguez C., Tapia L., Kieny F., Bustamante J. (2010). Temporal changes in lesser kestrel (*Falco naumanni*) diet during the breeding season in southern Spain. Journal of Raptor Research 44: 120–128.

Rosenheim J. A., (1988). Parasite presence acts as a proximate cue in the nest-site selection process of the solitary digger wasp, *Ammophila dysmica* (Hymenoptera: sphecidae). Journal of Insect Behaviour 1:333–342.

Ruffino L. Salo P., Koivisto E., Banks P.B., Korpimäki E. (2014). Reproductive responses of birds to experimental food supplementation: a meta-analysis. Frontiers in Zoology 11: 80–93.

Saino N. Romano M., Caprioli M., Ambrosini R., Rubolini D., Fasola M. (2010). Sex allocation in yellow-legged gulls (*Larus michahellis*) depends on nutritional constraints on production of large last eggs. Proceedings of the Royal Society B 277: 1203–1208.

Schipper A. M., Belmaker J., de Miranda M. D., Navarro L. M., Böhning-Gaese K., Costello M. J., Dornelas M., Foppen R., Hortal J., Huijbregts M. A. J., Martín-López B., Pettorelli N., Queiroz C., Rossberg A. G., Santini L., Schiffers K., Steinmann Z. J. N., Visconti P., Rondinini C., Pereira M. H. (2016). Contrasting changes in the abundance and diversity of North American bird assemblages from 1971 to 2010. Global change biology 22: 3948–3959.

Schoech S. J. (1996). The effect of supplemental food on body condition and the timing of reproduction in a cooperative breeder, the Florida scrub-jay. The Condor 98: 234–244.

Sturmey R. G., Reis A., Leese H. J., McEvoy T. G. (2009). Role of fatty acids in energy provision during oocyte maturation and early embryo development. Reproduction in Domestic Animals 44: 50-58.

Sumasgutner P., Vasko V., Varjonen R., Korpimäki E. (2014). Public information revealed by pellets in nest sites is more important than ectoparasite avoidance in the settlement decisions of Eurasian kestrels. Behavioural Ecology and Sociobiology 68: 2023–2034.

Symes C. T., Woodborne S. (2010). Migratory connectivity and conservation of the Amur Falcon *Falco amurensis*: a stable isotope perspective. Bird Conservation International 20: 134–148.

Trierweiler C., Klaassen R. H. G., Drent R. H., Exo K-M., Komdeur J., Bairlein F., Koks B. J. (2014). Migratory connectivity and population-specific migration routes in a long-distance migratory bird. Proceedings of the Royal Society of London B 281: 2013–2897.

Valone T. J. (2007). From eavesdropping on performance to copying the behavior of others: a review of public information use. Behavioural Ecology and Sociobiology 62: 1–14.

Valone T. J., Templeton J. J. (2002). Public information for the assessment of quality: a widespread social phenomenon. Philosophical Transactions of the Royal Society of London B 357: 1549–1557.

Vázquez D. P., Gianoli E., Morris W. F., Bozinovic, F. (2017). Ecological and evolutionary impacts of changing climatic variability. Biological Reviews 92: 22–42.

Votier S. C., Bearhop S., Witt M. J., Inger R., Thompson D., Newton, J. (2010). Individual responses of seabirds to commercial fisheries revealed using GPS tracking, stable isotopes and vessel monitoring systems. Journal of Applied Ecology 47: 487–497.

Wanless S., Harris M. P. (1993). Use of mutually exclusive foraging areas by adjacent colonies of blue-eyed shags (*Phalacrocorax atriceps*) at South Georgia. Colonial Waterbirds 16: 176–182.

Wakefield E.D., Phillips R.A., Trathan P.N., Arata J., Gales R., Huin N., Robertson G., Waugh S.M., Weimerskirch H., Matthiopoulos J. (2011). Habitat preference, accessibility, and competition limit the global distribution of breeding Black-browed Albatrosses. Ecological Monographs 81: 141–167.

Ward P., Zahavi A. (1973). The importance of certain assemblages of birds as “information-centres” for food-finding. Ibis 115: 517–534.

Webster M. S., Marra P. P., Haig S. M., Bensch S., Holmes, R. T. (2002). Links between worlds: unravelling migratory connectivity. Trends in Ecology & Evolution 17: 76–83.

Webster, M. S., Marra, P. P. (2005). The importance of understanding migratory connectivity and seasonal interactions. Birds of two worlds: the ecology and evolution of migration 199–209.

Wiebe K. L., Bortolotti G. R. (1992). Facultative sex ratio manipulation in American Kestrels. Behavioural Ecology and Sociobiology 30: 379–386.

Willmer P., Stone G., Johnston I. (2009). Environmental physiology of animals. John Wiley & Sons, Oxford, UK.

Wingfield J. C. (2006). Control of behavioural strategies for capricious environments. Essays in animal behaviour celebrating 50 years of Animal Behaviour 115–133.

Woo K. J., Elliott K. H., Davidson M., Gaston, A. J., Davoren G. K. (2008). Individual specialization in diet by a generalist marine predator reflects specialization in foraging behaviour. Journal of Animal Ecology 77: 1082–1091.

Ph.D. Course in Environmental Sciences

Ph.D. Student Final Report

Ph.D. Student:	Stefano Podofillini
Ph.D. Course Cycle:	XXXI
Scientific Tutor:	Prof. Diego Rubolini
Scientific co-tutor:	Dr. Jacopo Giuseppe Cecere
Thesis project title:	Short and long-term effects of variation in the breeding environment on behaviour and fitness traits in a colonial, cavity nesting raptor
Project performed at:	University of Milan; Department of Environmental Science and Policy; Laboratory of Behavioural and Evolutionary Ecology

List of Scientific Publications

Cecere J.G., Bondì S., **Podofillini S.**, Imperio S., Griggio M., Fulco E., Curcio A., Ménard D., Mellone U., Saino N., Serra L., Sarà M., Rubolini D. (2018). *Spatial segregation of home ranges between neighbouring colonies in a diurnal raptor*. Scientific reports, 8: 11762.

Podofillini S., Cecere J.G., Griggio M., Curcio A., De Capua E.L., Fulco E., Pirrello S., Saino N., Serra L., Visceglia M., Rubolini, D. (2018). *Home, dirty home: effect of old nest material on nest-site selection and breeding performance in a cavity-nesting raptor*. Current Zoology.

Corti M., **Podofillini S.**, Cecere J.G., Griggio M., Gianfranceschi L., Ducrest-Roulin A., Roulin A., Saino, N., Rubolini D. (2018). *Sequence variation in MC1R and TYRP1 genes and their relationship with melanin-based plumage trait expression in lesser kestrel (*Falco naumanni*) males*. Journal of Ornithology 159: 587-591.

Corti M., Romano A., Bazzi G., Costanzo A., **Podofillini S.**, Saino N., Rubolini D. (2017). *Behavioural stress response and melanin-based plumage colouration in barn swallow nestlings*. Behaviour 154: 853-874.

Bazzi G., **Podofillini S.**, Gatti E., Gianfranceschi L., Cecere J.G., Spina F., Saino N., Rubolini, D. (2017). *Candidate genes have sex-specific effects on timing of spring migration and moult speed in a long-distance migratory bird*. Current zoology 63: 479-486.

Bazzi G., Cecere J.G., Caprioli M., Gatti E., Gianfranceschi L., **Podofillini S.**, Possenti C.D., Ambrosini R., Saino N., Spina F., Rubolini, D. (2016). *Clock gene polymorphism, migratory behaviour and geographic distribution: a comparative study of trans-Saharan migratory birds*. Molecular ecology 25: 6077-6091.

Bazzi G., Ambrosini R., Caprioli M., Costanzo A., Liechti F., Gatti E., Gianfranceschi L., **Podofillini S.**, Romano A., Scandolara C., Saino N., Rubolini D. (2015). *Clock gene polymorphism and scheduling of migration: a geolocator study of the barn swallow* Hirundo rustica. Scientific reports 5: 12443.

Cecere J.G., Ménard D., Imperio S., **Podofillini S**., Catoni C, Griggio M, Rubolini D. (2018). *Foraging tactic of a colonial raptor differs among individuals and varies according to weather conditions*. Submitted to Journal of Animal Ecology.

Podofillini S., Cecere J.G., Griggio M., Corti M., De Capua E.L., Parolini M., Saino N., Serra L., Rubolini D. *Benefits of extra food to reproduction depend on maternal condition*. Under review in Oikos.

Podofillini S., Bazzi G., Matyjasiak P., Saino N., Rubolini D. *Flight performance of migrating juvenile barn swallows in relation to fat load and wing aerodynamics*. In submission.

Podofillini S., Corti M., Costa I., Pirrello S., Griggio M., Serra L., Saino N., Cecere J. G., Rubolini, D. *Parental quality and protoporphyrin-based eggshell pigmentation in a biparental raptor species: a food supplementation experiment*. In submission.

Bondì S., Bermejo A., Bouzin M., Cecere J.G., Romero Gil M., Gradev G., Griggio M., Marin S., Mellone U., **Podofillini S**., Rubolini D., Saulnier N., Zanca L., Sarà M. *Continental configuration induces the migratory connectivity of European lesser kestrel (*Falco naumanni*).* In submission.

Podofillini S., Cecere J.G., Griggio M., Quilici S., Saino N., Rubolini D. *Long-term effects of a food supplementation experiment on migratory phenology, breeding dispersal and breeding performance of a colonial raptor species*. In preparation.

Podofillini S., Cecere J.G., Griggio M., Pezzo F., Saino N., Rubolini D. *Conspecific and intraspecific signs of previous breeding attempts influence nest-site selection in the cavity nester lesser kestrel*. In preparation.

Meeting and Congress Contributions

Podofillini S., Rubolini D., Griggio M., Quilici S., Curcio A., Soravia C., Cecere J.G. Oral presentation: *Spuntini di riflessione: effetti a lungo (e breve) termine dell'integrazione alimentare sul ciclo vitale del grillaio*.
BEM (Behavioural Ecology Meeting), February 2018

Podofillini S., Corti M., Costa I., Cecere J.G., Rubolini D., Griggio M. Poster presentation: *Analysis of eggshell maculation in lesser kestrel*.
CIO (Convegno Italiano di Ornitologia), September 2017

Podofillini S., Curcio A., Griggio M., Rubolini D., Cecere J.G. Oral presentation: *Contextual effects of food supplementation on lesser kestrel breeding output*.
CIO (Convegno Italiano di Ornitologia), September 2017

Podofillini S., Curcio A., de Capua E.L., Fulco E., Griggio M., Pirrello S., Rubolini D., Serra L., Visceglia M., Cecere J.G. Oral presentation: *Home dirty home, lesser kestrel prefers to breed in previous used nest sites*.
International Lesser Kestrel Expert Workshop, October 2016

Podofillini S., Bazzi G., Matyjasiak P., Saino N., Rubolini D. Oral presentation: *Flight performance of migrating juvenile barn swallows in relation to fat load and wing aerodynamics*.
BEM (Behavioural Ecology Meeting), February 2016

Podofillini S., Bazzi G., Matyjasiak P., Saino N., Rubolini D. Poster presentation: *Flight performance of migrating juvenile barn swallows in relation to fat load and wing aerodynamics*. CIO (Convegno Italiano di Ornitologia), September 2015

List of Attended Seminars

Mandatory courses of *Transferable skills* – different authors. 2015, 2016 and 2017

Bonisoli-Alquati A., December 2017. *Environmental disasters and their ecological consequences. A short course on how to study them*.

Ficetola F., November 2016. *Environmental DNA to understand biodiversity changes*.

Epis S., November 2016. *On symbionts arthropods and vector-borne diseases*.

Romano A., March 2016. *Evolution of prenatal sex allocation strategies in a sexually promiscuous passerine birds*.

Della Torre C. February 2016. *Do carbon based nanoparticles act as carrier for benzoαpirene. An investigation on Danio rerio embryos*.

Manenti R., November 2015. *Cave colonization by the first salamander (*Salamandra salamandra*): zoological, ecological and evolutionary insights*.

Rubolini D., March 2015. *Unraveling climate change effects on migration birds: a comparative approach*.

Bandi C., February 2015. *Gendercide symbionts...e altre storie di sesso, simbiosi e parassitismo*.

List of Attended PhD Courses

December 2017. Environmental disasters and their ecological consequences. A short course on how to study them (Dr. Andrea Bonisoli-Alquati).

November-December 2017. Statistic course organized by the PhD course in Environmental Sciences (Prof. Roberto Ambrosini)

October 2015. Molecular methodologies applied to environmental research (Dr. Diego Fontaneto)

February 2015. Tree rings as archives to understand past and present environmental conditions. (Dr. Paolo Cherubini)