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Disentangling drivers of reproductive performance in urban great tits: a food supplementation experiment.

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SUMMARY STATEMENT

This study aims at estimating wild urban great tits breeding performance and at testing experimentally how food resources influence it, using food supplementation.

ABSTRACT

Worldwide urban expansion induces degradation of the natural environment, resulting in new constraints in terms of breeding sites, anthropogenic disturbances as well as food resources. The alteration of resource abundance and type may induce non-adaptive investments in reproduction from urban dwellers. Food availability and quality have been identified as potential drivers of the decline in passerine body mass and fledging success in urbanized landscapes, particularly if birds misinterpret cues of food abundance used to adjust their reproductive investment. In a previous study, we demonstrated in urban great tits, *Parus major* that highly-preferred larger cavities have larger clutches with lower breeding success, leading to a maladaptive breeding investment. Previous studies also showed that urban great tits are smaller or thinner than rural ones, both at nestling and adult stages. Here we present the results of a food-supplementation experiment to examine whether food resources mediate this maladaptive breeding investment and constrain the reproductive performance of this urban bird population. We predicted higher performance in food-supplemented broods, especially in larger cavities, and stronger effects of the supplementation in more artificialized territories. Surprisingly, we found that food-supplemented nestlings and their parents had lower body mass and condition, especially in areas with more pedestrians. Supplementation was also associated with lower nestling survival until fledging in places presenting lower levels of naturalness, independently of cavity size. This work highlights a lack of knowledge on avian feeding behaviour in cities, a key element to understand how breeding performance is affected by human presence and habitat naturalness.

INTRODUCTION

Urbanization is a fast developing process worldwide, currently involving more than 50% of the human population (Marzluff, 2001; Gaston et al., 2015). This urban expansion induces degradation of the natural environments thereby promoting species able to adapt to cities (Clergeau et al., 2006; Adams and Lindsey, 2011) while often threatening native species (Aronson et al., 2014). Indeed, urban habitats involve new constraints in terms of breeding sites and anthropic disturbances (Hedblom and Söderström, 2011) but also offer new resources (Clergeau et al., 2006; Mackenzie et al., 2014). For birds, resource abundance and nature are drastically modified in urban habitats (Isaksson and Andersson, 2007; Gladalski et al., 2015). First, urban breeding habitats are notoriously poor in natural avian food such as caterpillars (Perrins 1965) because of the low density of trees. Second, exotic species of trees are often introduced in urban areas without their associated insect cohorts (Mackenzie et al., 2014) which also contributes to the low abundance of arthropod prey for birds. Third, human waste can represent a new resource type, although its nutritional properties, in particular fat-richness, can be detrimental for avian growth (Meillère et al., 2015). In urban environments, birds could switch to these novel food resources to compensate for the low abundance and poor quality of "natural" food, *e.g.* arthropods, less nutritive than caterpillars (Mackenzie et al., 2014) or other types of food provided by feeders (Robb et al, 2008; Chamberlain et al 2009). These constraints on resources could be worsened by a high density of birds in urban areas because of the attractiveness of breeding sites, the availability of food resources in winter, and a more favorable microclimate (Payevsky, 2006; Hedblom and Söderström, 2011). Such increased population density is thus likely to lead to higher competition for a poor quantity and quality of food (Shochat, 2004; Chace and Walsh, 2006).

Food resources available in urban areas could thus be expected to be detrimental for birds and to induce a non-adaptive investment in reproduction (Anderies et al., 2007). Resource acquisition is a key factor involved in the trade-off between breeding success and somatic maintenance of parents (Martin, 1987; Karell et al., 2009). During the avian breeding period, the timing and abundance of resources have a critical influence on avian breeding success, since they are strong determinants of nestling body condition and survival (Perrins, 1991; Hõrak et al., 1999; Reed et al., 2013). Hence it is not surprising that food availability and quality have been identified as major drivers of the decline in nestling mass and lower fledging success for passerines in urbanized landscapes (Solonen, 2001; Chamberlain et al 2009; Hedblom and Söderström, 2011; Seress et al., 2012; Meillère et al., 2017). In 1965,

Perrins' study of great tit nestlings (*Parus major*) in gardens revealed that they were lighter than nestlings from a nearby forest, and that urban chicks displayed all the signs of dying of starvation. Likewise, one of the drivers of the decline in house sparrow (*Passer domesticus*) populations in European cities could be the poor food conditions (Meillère et al., 2015). Great tits also face reduced breeding performance and nestling body condition in urban environments (Solonen, 2001; Chamberlain et al., 2009; Bailly et al., 2016; Demeyrier et al., 2016) possibly driven by constraints in food availability and accessibility (Remacha and Delgado, 2009), as well as a high heterogeneity of the urban habitat (Amrhein 2014).

The detrimental effects of low food resources could be exacerbated in urban areas because of artificial cues. In systems facing Human-Induced Rapid Environmental Change (HIREC, e.g. Sih, 2013; Hale and Swearer, 2016), cues exploited for habitat selection could be decoupled from the true quality of the habitat, potentially causing maladaptive responses. In a recent study (Demeyrier et al., 2016), we have demonstrated that urban great tits from the city of Montpellier (south of France) adjust their breeding investment to artificial breeding cavity size in a maladaptive way. We experimentally showed that great tits preferred settling in large breeding cavities relatively to smaller options. Individuals from these largest cavities also invested higher in egg production, yet had a lower fledgling success compared to those from medium-sized cavities. One hypothesis to explain these results is that females produce broods that are too large when choosing large cavities, and are then trapped because of a lack of food to rear these relatively large broods (Anderies et al., 2007; Robertson et al., 2013). Rytkönen and Krams (2003) proposed the same hypothesis to explain the lower breeding success in northern European compared to central European great tits.

The present study aims at testing experimentally the role of food abundance in mediating breeding performance in an urban system where maladaptive breeding investment associated with breeding cavity size has been previously reported (Demeyrier et al. 2016). We performed a feeding experiment over two years on wild urban great tits breeding in nest-boxes varying in size in the city of Montpellier.

Moreover, we investigated in detail variation in breeding performance according to the local breeding environment within our urban population. As highlighted by Amrhein (2014), urban habitats need "to be characterized by small-scale variation in environmental factors that include differences in vegetation and natural food resources", which is rarely the case since urban studies focus mainly on urban parks (e.g. Björklund et al., 2009 in Barcelona, Spain;

Hedblom and Söderström, 2011 in Uppsala, Sweden; Markowski et al., 2013, in Lodz, Poland). The nest-box distribution in our urban habitat was designed specifically to take into account the wide variety of urban landscapes composed of both parks and residential or commercial streets.

Previous avian studies with food supplementation experiments found contrasted results on bird reproduction performances. Indeed, some studies showed positive effects of food supplementation (Robb et al., 2008) while other studies showed no or even negative effects (Harisson et al., 2010; Sillanpäa et al., 2010; Ruffino et al., 2014). Following Robb et al.'s (2008) suggestion that food is a key limiting factor in the urban environment, we predicted that i) fledgling body condition and fledging success, as well as parental body condition, would be improved by food supplementation, ii) supplementation would be more beneficial in the most urbanized environments, and iii) supplementation would be more beneficial for broods reared in large nest-boxes, where birds are the most impacted by urban constraints (see Demeyrier et al., 2016). Overall, this study aims at understanding how food resources influence the reproductive performance of free-ranging urban great tits.

MATERIALS AND METHODS

Ethical Note

The current work was conducted under an agreement with the host research institute CEFE (Centre d'Ecologie Fonctionnelle et Evolutive, C34172-11) and the CNRS (Centre National de la Recherche Scientifique). In addition, the specific work was conducted following authorizations obtained from the national authorities in France: (1) authorization to work with and capture wild birds from the Centre de Recherches sur la Biologie des Populations d'Oiseaux (CRBPO, Permit number 1904, Museum Paris to A.G.); (2) authorization to work with and capture wild birds in the specific county (Authorization number 2012167-003 from Hérault department to A.G.); (3) personal certificates for animal experimentation (A34-470 & A34-496 from the Direction Départementale de la Protection des Populations de l'Hérault to A.G. & A.C.).

Study site and biological model

Since 2011, urban great tits (*Parus major*) have been monitored from March to July in the city of Montpellier, in the south of France (43°36'43''N, 3°52'38''E). This city counted 272 084 residents in 2013, with a density of 4 783 residents/km^2. It covered an area of around 57 km^2 with around 11 km^2 of green areas. The city was equipped in autumn 2010 with 243

nest-boxes placed along an urbanization gradient. At the beginning of the 2011 breeding season, only 168 nest-boxes were still there, because of human damaging. During the 2012-2015 breeding seasons the number of nest-boxes fluctuated between 163 and 180.

Food supplementation experiments were conducted during the breeding seasons of 2013 and 2014 in two wooden nest-box types that differed in their bottom floor area (medium=121 cm^2, *vs.* large=210 cm^2; Demeyrier et al. 2016 for details) The distance between neighbouring boxes was at least 100 meters, to limit intraspecific competition (Remacha and Delgado, 2009). All nest-boxes were visited once a week from the beginning of the breeding season (late March) until the fledgling phase, to collect data on occupation rate, clutch size and fledging success. Adults were trapped inside their nest-boxes with a mechanical trap when nestlings were *ca.* 9 days-old. They were ringed with unique leg rings provided by the CRBPO (Paris, France); their right tarsus length was measured with a calliper and their body mass measured with a Pesola balance. Nestling body mass was also measured at that time, corresponding to the peak of nestling food requirement (Perrins, 1991). At this age of 9 days, nestlings are still in their growth stage, hence tarsus length measures are not possible because of growth cartilage presence. When nestlings were 14 to 16 days post hatching, we measured again their body mass, as well as their right tarsus length to calculate a body condition index (*i.e.* body mass controlled for nestling size).

Urban environmental measures

To quantify local habitat characteristics per territory, we defined a disk with a radius of 50 meters around each box (Hinsley et al., 2002; Hedblom and Söderström, 2011; following Demeyrier et al. 2016). This allowed us to take six measures reflecting the level of artificialization inside the city: vegetation cover (oak cover, tree cover and global vegetation cover), motorized and non-motorized traffic, and light pollution. To provide a proxy of the abundance of insect prey for tits in each territory the three vegetation cover variables (Mackenzie et al., 2014; see Demeyrier et al., 2016) were measured from aerial pictures of the city of Montpellier, taken in summer 2011 and analysed with the software Delta (http://www.montpellier.fr/4053-cartographie-ancienne-de-montpellier.htm). We also measured, by five-minute counts: motorized, non-motorized and pedestrian traffic, at each nest-box during the nestling stage (Hedblom and Söderström, 2011; Katti and Warren, 2004; see Demeyrier et al., 2016). These counts were cross-validated with information available from measurements taken during longer periods by the Montpellier urban service (opendata.montpelliernumerique.fr/, R^2=0.96, $P < 0.001$, Demeyrier et al. 2016). Finally,

light pollution around each breeding cavity was quantified by the spatial surface overlap between the 50 m disk around each nest-box and a 50m disk around each street lamp (Mapinfo data, opendata.montpelliernumerique.fr/, Quantum GIs 1.7.4, Kempenaers et al., 2010; see Demeyrier et al., 2016 for details). Principal component analyses on these six urban factors (three types of vegetation cover, motorized traffic, pedestrian traffic, artificial lighting, ade4 R package, Dray et al., 2016) revealed two major axes of variation in these anthropic disturbance measures: PC1 was related to the degree of environment artificiality/naturalness (56.6% of the variance, with positive loadings for the three types of vegetation cover and negative ones for light pollution and traffic), called PC1 or "habitat naturalness" thereafter, while PC2 (or "human presence") was only related to the pedestrian frequency (16.6% of the variance) (details in Demeyrier et al., 2016).

Food supplementation experiment

We performed a supplementation experiment in 2013 and 2014, during the first seasonal breeding attempts, in order to follow a homogenous sample according to breeding stage. All medium ($n_{2013}=55$, $n_{2014}=52$) and large ($n_{2013}=50$, $n_{2014}=51$) nest-boxes were equipped with a feeder placed inside the box during this two-year experiment (Sillanpäa et al., 2010) (Fig. 1). We randomly assigned a control status (empty feeder) to half the large and half the medium-sized nest-boxes, the other half being experimental nest-boxes where supplementary food was provided in the feeder. All along the breeding season after hatching, the status of nest-boxes (empty/full) was attributed alternatively within nest-boxes of the same size. In line with the literature and previous feeding experiments on blue tits (*Cyanistes caeruleus*) and great tits (Eeva et al., 2009; Gienapp and Visser, 2006; Grieco, 2001; Sillanpäa et al., 2010), following hatching, feeders were refilled every second day with two species of live mealworms larvae (*Alphitobius diaperinus* and *Tenebrio molitor*) and a vitamin and carotenoid complement (Nutribird) (Sillanpäa et al., 2010). Mealworm larvae quantities were modulated according to the number of nestlings and their age (Gienapp and Visser, 2006; Grieco, 2001) (see supplemental material Table S1). From 0 to 8 days-old, nestlings were provided with small Tenebrio larvae (*Alphitobius diaperinus*) and from 8 to 15 days-old, nestlings were provided with miller Tenebrio larvae (*Tenebrio molitor*). These quantities represent 20-50% of daily required biomass for nestlings during the feeding period (Eeva et al., 2009; Grieco, 2001). We added a Nutribird complement in feeders, modulated by worm quantity (Sillanpäa et al., 2010; Koivula et al., 2011). The Nutribird complement was provided along the proportion of 5 gr of Nutribird (a full coffee spoon) for 15 gr of worms. The supply in carotenoids was

0.0015 mg per full coffee spoon. The Nutribird complement provided proteins (1.05 gr per full coffee spoon), oligo-elements (0.225 gr per full coffee spoon) and diverse vitamins (1.395 mg per full coffee spoon). Each time we refilled the feeder, we checked if larvae were eaten, although a small consumption was difficult to measure. In 21% of refills, at least half of the worms had disappeared and were not found the nest-box. Thus, in the remaining 80% of the refills less than half of the worms disappeared.

All the nests were handled in the same way with the following monitoring methods: i) weekly visits to control all nest-box contents (also during the experimental stage), ii) daily visits around the hatching date to determine the hatching date of all nests, iii) nestling manipulation at 9 and 15 days post-hatch of all nests, iv)-capture of parents and associated phenotypic measurements of all nests (between 9-15days post-hatch). Only the food supplemented nest-boxes were additionally briefly visited to fill-up the feeders (less than a minute per feeder visit).Thus, we assume that the additional visit to fill-up the feeders should have had a minor effect on the outcome of the experiment relatively to the potential impact (if any) of the above mentioned monitoring procedures.

Statistical analyses

We used the breeding monitoring data collected in 2013-2014 from all nest-boxes equipped with an inside feeder to test for a food supplementation effect on nestling body condition and fledging success, along the urbanization gradient within the city. In addition, we evaluated if the food supplementation affected parental physical condition.

We used General Linear Mixed Models (GLMM) run with R package lme4, to analyse nestling body condition (at 9 and 15 days-old) with a normal error distribution, nestling survival until fledgling with a binomial error distribution (0 or 1 for each nestling), and finally adult body condition with a normal error distribution. We followed a stepwise backward regression model selection (keeping fixed effects with a p-value<0.1).

To address our objective to investigate the role of resources on breeding performance using our supplementation experiment, we modeled nestling body condition, individual nestling survival as a proxy of fledging success, and adult body condition within the feeding experiment. Explanatory variables were: nest-box size, feeder status (food supplemented/control), PC1 (habitat naturalness), PC2 (human presence), year and all biologically relevant interactions (nest-box size*feeder status, year*feeder status, PC1*feeder status, PC2*feeder status, nest-box size*feeder status*PC1). All these interactions explore the potential effect of food availability in relation to the nest-box size (see Demeyrier et al.,

2016) and the local environment (PC1 and PC2) in an experimental framework. Additional explanatory factors were: number of nestlings in the brood when modeling nestling body condition, number of hatchlings when modeling fledging success and finally, number of nestlings, breeders' age (one-year old or more) and breeders' sex when modeling adult body condition. Nest-box number was included as a random effect since 60 % of nest-boxes were occupied more than once within the two-year experiment. Maternal identity (*i.e.* mother ring number) was also included as a random effect, to account for sibling relatedness and also environmental/genetic maternal effects. When modeling adult physical condition, ring number was included as a random effect since 8 % of adults were trapped twice.

RESULTS

Nestling body mass and condition negatively impacted by food supplementation and by human presence

Contrary to our predictions, there was no significant statistical interaction between food supplementation, nest-box-size and habitat naturalness, on nestling phenotype (Table 1). We found a significant negative effect of supplementary food on 9 days-old nestling body mass (body mass, in grams, in relation to feeder treatment: mean ± s.d. food supplemented=13.17±2.17; control=13.64±2.73; Table 1; Fig. 2).

As concerns the body condition of 15 days-old nestlings (*i.e.* body mass controlled for nestling tarsus length), we found a significant negative main effect of human presence and a marginally significant interaction between supplementary food and human presence (Table 1). Namely, in the food supplemented broods, nestling body condition was more negatively impacted in areas with higher human presence (Fig. 3). There was also a marginal effect of nest-box size, whereby nestlings from medium-size nest-boxes were in higher body condition than nestlings from large nest-boxes (Table 1). Finally, all three nestling traits (9 days old body mass, 15 days old condition and survival till fledging) showed significant annual variation. All three nestling traits were lower in 2014 than in 2013 (Table 1).

Nestling survival until fledging decreased with supplementation in highly urbanized areas

There was a significant positive association between individual nestling survival until fledgling (fledged=1, died=0) and the level of naturalness of the breeding environment (Table 1). However, there was also a significant interaction between the food supplementation treatment and the naturalness level on individual nestling survival until fledging (Table 1).

Nestling survival until fledging decreased overall in food supplemented broods (Fig. 4A), and this detrimental experimental effect was strongest in the less natural environments (Fig. 4B).

Adult body condition negatively impacted by supplementary food and by human presence

When exploring adult body condition (*i.e.* body mass controlled for tarsus length), we found a significant interaction between supplementary food and human presence as illustrated in Fig. 5 (Table 2). Adult body condition was negatively impacted by the supplementary feeding only in areas with high human presence. However, parents of food supplemented broods were in better body condition when human presence was lower (Fig. 5).

We also found that birds were marginally in worse body condition when they were one year old rather than older (body condition in relation to age, in grams: mean±s.d. 1year=16.46±0.78, >1year=16.95±1.02). The classic sexual size dimorphism was also confirmed, with males heavier than females (Body condition in relation to sex: mean±s.d.-male=17.25±0.83, female=16.33±0.87) yet the experimental effects were similar for both sexes (Table 2). Finally, adult body condition varied significantly between the two experimental years that is adult body condition was overall worse in 2014 than in 2013 (Table 2).

DISCUSSION

Breeding performance negatively impacted by artificial food

Contrary to our expectations, the experiment resulted in an overall negative effect of food supplementation for traits associated with breeding performance (nestling phenotype and survival). At least three possible explanations could account for this unpredicted experimental outcome, when contrasting our results with the literature.

The first explanation for the observed pattern is that the food supplemented was not of sufficient quality for a nestling diet, particularly in areas where food resources available are already penalizing their growth (e.g. Meillère et al., 2015). Our choice of diet (mealworms and Nutribird complement) was based on the most commonly used food for great tits in aviaries (Eeva et al., 2009; Gienapp and Visser, 2006; Grieco, 2001; Sillanpää et al., 2010). Negative effects of food supplementation have previously been found in at least two other great tit studies (Harrison et al., 2010; Sillanpää et al., 2010; Ruffino et al., 2014) and these negative effects have been attributed to a lack of nutrients other than proteins, in the supplementation diet (Eeva et al., 2009). Indeed, mealworms contain mostly protein, fat and

water. We anticipated this potential problem by adding a vitamin and carotenoid complement, however the results obtained support the idea that this may not be sufficient or may not be assimilated by birds when they eat the worms. Moreover, Simons et al. (2014) found that providing supplemental carotenoids to zebra finches (*Taeniopygia guttata*) could have negative effects on reproductive performances such as clutch size, in constraining environments. Adding nutrients to mealworms could hence also lead to a negative effect of our supplementation experiment.

Moreover parental body condition and hence feeding abilities could also mediate the negative effect of food supplementation. Perhaps the low body condition of parents classically found in urban areas (Bokony et al., 2009 ; Meillère et al., 2015) could impact their foraging capacities, resulting in a greater use of artificial food instead of natural food (Robb et al., 2008). However, parents involved in our experiment did not display decreased body condition along the naturalness level whereas they were impacted by the human presence (Fig. 5) and so were their nestlings (Fig. 3). Hence this hypothesis is unlikely to explain the detrimental effect of supplementation which is mostly revealed in areas with higher human presence (Fig. 3) and in most urbanized areas (Fig 4B).

Second, we could postulate that the experiment was conducted during exceptionally good years where supplementation with a low diverse food source might have negatively impacted breeding performance. However, nestling survival until fledging was not significantly different across the 6-year study period in this urban great tit population ($p=0.4$; 2011-2016) supporting the idea that the two years of food supplementation were not exceptionally good. A full understanding of the underlying environmental drivers (e.g. weather fluctuations) associated with the significant year effects for all traits (Table 1 & 2) will require multiyear studies.

Finally, we cannot rule out, as a third possible explanation, that experimental broods were negatively affected by the disturbance caused by the added food within the nest-box. Note that in our study, we provided supplementary food inside each nest-box in order to avoid negative effects of feeding due to increased competition for visible food sources present outside the boxes (Solonen 2001; Robb et al., 2008). In fact, in great tits, high competition in urban areas is most probably responsible for smaller clutch sizes and fewer fledglings per breeding attempt, because of a combination of food shortage and high bird density (Solonen, 2001; Hedblom and Söderström, 2011). Moreover, Briga et al., (2017) showed that in high competition areas, low food availability results in shorter lifespan for large-brooded zebra finches. Yet, the choice of placing feeders inside the boxes could induce

a stronger behavioral and possibly stressed-induced reaction from the breeding parents. Even though we habituated the birds with the presence of feeders inside the nest-boxes by adding them before the breeding season, similarly to Eeva et al. (2009) and Sillanpäa et al. (2010) studies, we cannot exclude that the novelty of the feeder might be perceived much stronger for a full rather than an empty feeder due e.g. to the worms' movement or smell. However, it is important to notice that feeder status (food supplemented or control) affected body condition of 15 days-old nestlings and adults as well as nestling survival until fledgling, always in interaction with another variable associated with local territory environment. Food supplementation was indeed found to have a positive effect in addition to certain levels of naturalness of the breeding sites. Thus, if perturbation due to items in the feeders was the prevailing effect, such interactions would not have been predicted.

These non-exclusive potential explanations for our main experimental results are probably not exhaustive. Indeed, we are aware that food supplementation effects could be modulated by environmental factors that are not controlled for in such experiments, such as natural resources or latitudes, but also by bird behavioral variation like food catching, or by the experimental design like timing of supplementation or accessibility of food (Ruffino et al., 2014).

Effect of cavity size on breeding performance in relation to our food supplementation experiment

An initial aim of the present work was to evaluate if food resources were the key parameter mediating the maladaptive breeding investment observed in association with large artificial cavity size (Demeyrier et al., 2016). Thus, we predicted an interaction between cavity size and food supplementation treatment, with a contribution of local conditions (naturalness level): we envisaged that when provided with additional food supply, larger broods in larger nest-boxes would no longer display lower fledgling numbers compared to smaller broods in medium-sized boxes. However, no interaction of the two key factors associated with food and cavity size was detected on nestling or parental traits. This absence of significant result did not necessary indicate that the role of food was not present but we were not able to detect it with our protocol.

Nestling survival until fledgling negatively impacted by food supplementation in less natural areas

Nestling survival until fledging was overall decreased in supplemented broods (Fig. 4A), and this detrimental experimental effect was strongest in the less natural environments (Fig. 4B). This result was exactly opposite to the positive effect of supplementary feeding expected.

In a highly constrained (*i.e.* more artificial) environment, as stated above the addition of poor quality food could be a penalty for birds rather than a 'bonus', *i.e.* nestling survival until fledging could be negatively impacted in highly artificialized habitat, whereas in a more natural environment, supplementary food, even in a poor quality, could improve the nestling survival until fledging (Fig. 4B). In fact, in natural environments, most studies have found a positive effect of food supplementation or no effect on nestling survival until fledging, according to the review of Robb et al. (2008). Following a feeding experiment in Spotless Starlings (*Sturnus unicolor*), Cuervo et al. (2011) suggested that artificial food could cause nestling satiety, which could lead to less begging for higher quality natural food. In this case, artificial food could not compensate completely the lack of natural food (Cuervo et al., 2011) and it could in fact worsen nestling survival until fledging. One element that is crucially missing in this puzzle is a fine knowledge on great tit diet in cities, and how this diet, and in particular nestling diet, varies across space in the heterogeneous urbanized landscape (see for instance Isaksson and Andersson, 2007; Andersson et al. 2015; Toledo et al. 2016).

Interactive effects of food supplementation and human presence

Interestingly, the experiment resulted in an interaction between the effects of food supplementation and the local pedestrian frequency on 15 days-old nestling body condition (Fig. 3) as well as on adult body condition (Fig. 5). Nestlings and adults from supplemented broods presented a decreased body condition (relatively to controls) only in areas with numerous pedestrians. Human presence could induce a temporary halt in nestling provisioning because of a behavioral reaction of parents, due to hormonal response to stress (Müller et al., 2006, on blue tits) or due to personality linked response (e.g. flight initiation distance and landing distance, Fernandez-Juricic et al., 2006). Our experiment might have added a disturbance too strong in areas where human presence is already high, resulting in a detrimental effect on nestling body condition. Dubiec (2011) suggested that the response of birds to human presence depends on parental body condition. Indeed, this author revealed a trade-off between reproduction and survival in great tits: parents with low body condition

deserted the nest more easily than parents in high body condition (Dubiec, 2011). This hypothesis could be worth considering in our population because in our experimental design, breeders' body condition was influenced by human presence in relation to our feeding experiment even if it was not influenced by urbanization level (Table 2).

In areas highly disturbed by human presence, birds could modulate their foraging strategy in relation to the predation risk and travel longer distances in order to provision their nestlings in safer or less stressful conditions (Sol et al., 2013; Voelkl et al., 2016). When facing a high predation risk, as human presence could be perceived, parents might also display longer latency periods before returning to their nest (Bokony et al., 2012), also affecting negatively their nestlings' condition. In poor habitats like urbanized areas, great tit parents might also be immune to human presence since previous studies have shown that great tit females plastically adjust their risk taking behavior according to the risk of nestling starvation (Quinn et al., 2012). When we provided food inside the nest-box, parents could reduce their foraging distance, and hence be more disturbed by pedestrian frequency as indicated by the interaction between human presence and feeding treatment. Indeed, although knowledge on foraging strategies in urban environments is very scarce (Amrhein, 2014), it is likely that parents forage further away from their nest-box in poor habitat, such as the more urbanized areas. Previous studies in several bird species support the idea that human presence can modify the spatial and temporal foraging patterns thereby modulting foraging costs (Fernandez-Juricic and Tellerian, 2000; Bokony et al., 2012; Sol et al., 2013; Voelkl et al., 2016) and that tolerance to disturbance is reduced with an increasing stimulus such as pedestrian presence (Fernandez-Juricic and Tellerian, 2000).

Concluding remarks

Our experiment was one of the first to explore experimentally the role of food availability on breeding performance in a city environment. The results we obtained in our Mediterranean study system highlights that the response to the question is potentially complex and that the presence of artificial food during the breeding season might exacerbate a negative effect of urbanization. In order to better understand processes associating food resources and nestling rearing conditions, an alternative approach that could be conducted is to manipulate brood size (Banbura et al., 2013; Hõrak, 2003). If breeders had to feed fewer nestlings and if food resources are a major constraint in urbanized areas, a brood size experiment would contribute to the same objective as this study, *i.e.* understanding how food resources influence the

reproductive success of wild urban great tits and whether the ecological trap effect described previously is due to a lack of food and/or to the low quality resources of urban environments. In light of the results from the present manipulation, such a brood size experiment would solve the issue of controlling for food quality in the alleviation of parental effort.

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Competing interests

The authors declare no competing interests.

Auhors contributions

V.D., M.L. and A.G. formulated and performed the research. V.D. analyzed the data and wrote the manuscript. AC., M.L. and A.G. discussed the results, commented on and edited the manuscript.

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Tables

Table 1: GLMM results of feeding experiment on 9 days-old nestling body mass (normal error distribution), 15 days-old nestling body condition (normal error distribution) and nestling survival until fledging (binomial error distribution). (Stepwise backward regression model selection, keeping fixed effects with a p-value<0.1)

	9 days-old body mass				15 days-old body condition				Nestling survival until fledging			
	Est	SE	t	p	Est	SE	t	p	Est	SE	t	p
Fixed effects												
Intercept	13.27	0.35	37.73	<0.001	-10.67	1.80	-5.91	<0.001	0.04	0.65	-0.06	0.95
Feeder - Control	1.05	0.44	2.40	0.02	-0.47	0.26	-1.81	0.07	1.27	0.62	2.04	0.04
Nest-box size - Medium		ns			0.60	0.34	1.77	0.08		ns		
PC1 - Naturalness	0.25	0.15	1.67	0.1		ns			0.76	0.28	2.70	0.007
PC2 - Human presence		ns			-0.60	0.28	-2.11	0.04		ns		
Year - 2014	-1.05	0.37	-2.82	0.008	-0.95	0.26	-3.58	<0.001	-1.75	0.56	-3.15	0.002
Number of hachtlings	-	-	-		-	-	-			ns		
Number of alive nestlings		ns			-0.18	0.08	-2.22	0.03	-	-	-	
Nestling tarsus Length	-	-	-		1.43	0.09	16.36	<0.001	-	-	-	
Feeder - Control * Nest-box size		ns				ns				ns		
Feeder - Control * Year		ns				ns				ns		
Feeder - Control * PC1		ns				ns			-0.83	0.36	-2.32	0.02
Feeder - Control * PC2		ns			0.61	0.32	1.87	0.06		ns		
Feeder * Nest-box size * PC1		ns				ns				ns		
Random effects	Var.	Std dev.			Var.	Std dev.			Var.	Std dev.		
Mother	0.66	0.81			1.33	1.15			9.12	3.02		
Nest-box	2.88	1.70			0.24	0.49			0	0		
Residual	2.59	1.61			0.93	0.97						
Sample size												
Mother		62				65				98		
Nest-box		52				56				71		
Observations		388				379				706		

Table 2: Results of GLMM on adult body condition (normal error distribution). (Stepwise backward regression model selection, keeping fixed effects with a p-value<0.1)

	Est	SE	t	p
Fixed effects				
Intercept	5.35	2.35	2.27	0.02
Feeder - Control	-0.13	0.09	-1.22	0.23
PC1 - Naturalness		ns		
PC2 - Human presence	-0.03	0.09	-0.35	0.73
Year - 2014	-0.69	0.12	-6.88	<0.001
Number of alive nestlings		ns		
Breeder tarsus Length	0.60	0.12	4.93	<0.001
Breeder sex - Male	0.43	0.15	2.93	0.004
Breeder age - 1 year-old	-0.25	0.13	-1.88	0.06
Feeder - Control * Nest-box size		ns		
Feeder - Control * Year		ns		
Feeder - Control * PC1		ns		
Feeder - Control * PC2	0.28	0.11	2.42	0.02
Random effects	Var.	Std dev.		
Ring number	0.45	0.67		
Residual	0.10	0.29		
Sample size				
Ring number		130		
Observations		140		

Figures

Fig. 1: Feeder inside each nest-box. (here a large nest-box)

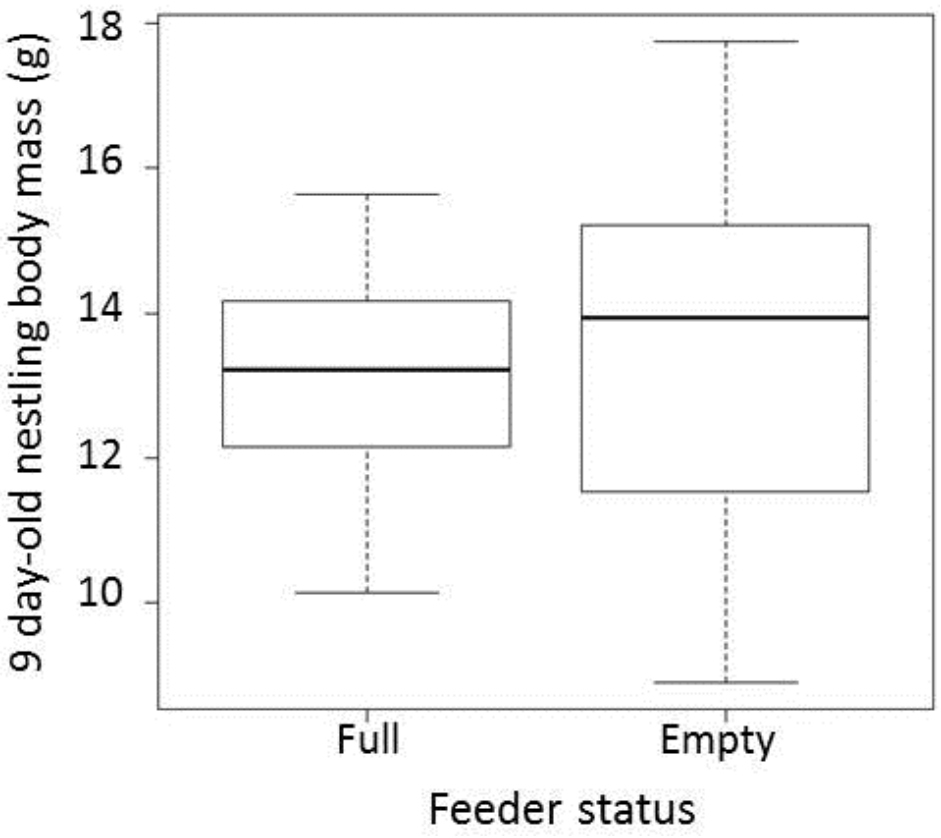


Fig. 2: 9 days-old nestling body mass in relation to food supplementation (from model residuals). (Median ± 1st and 3rd quartile ± min and max)

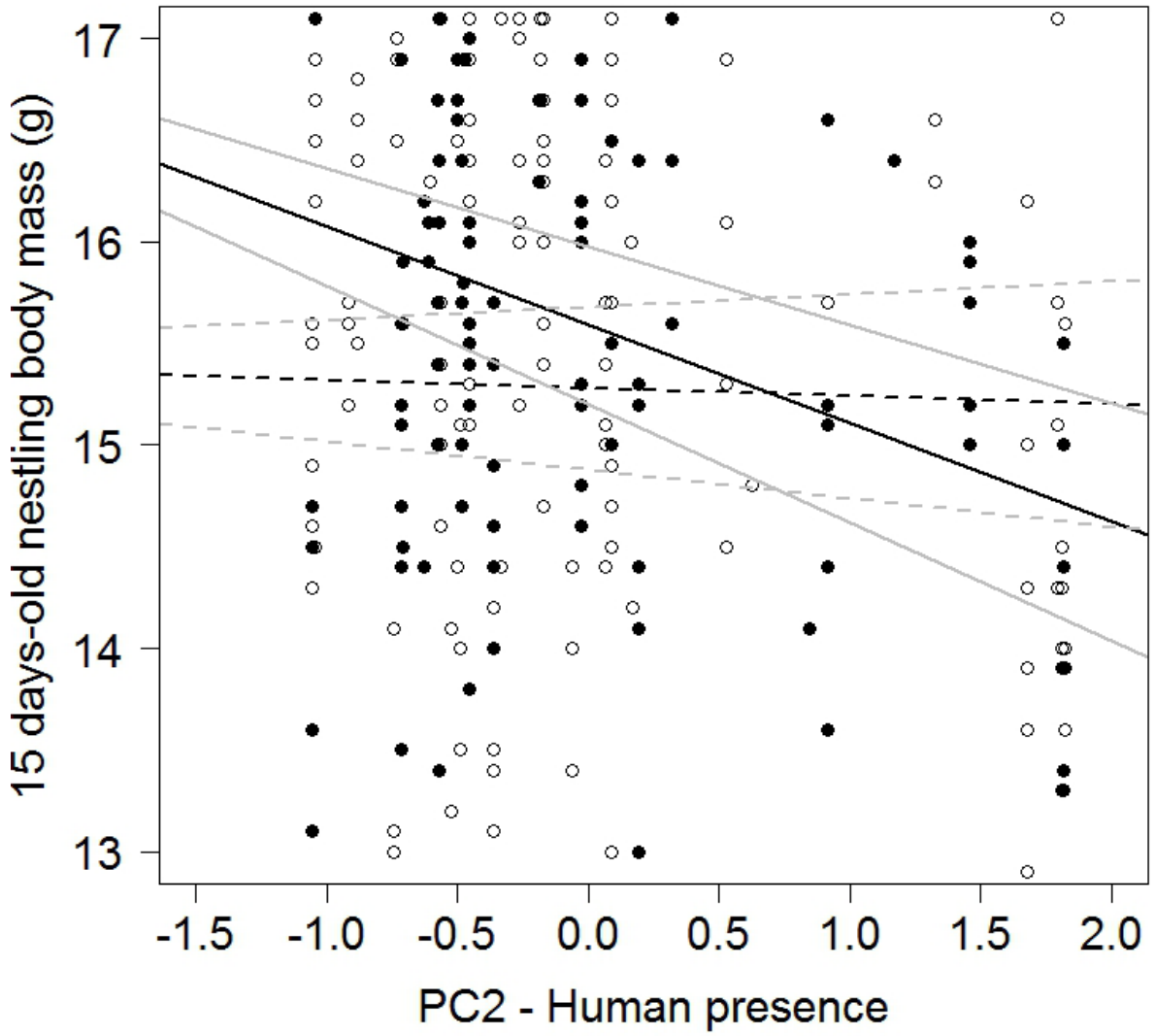
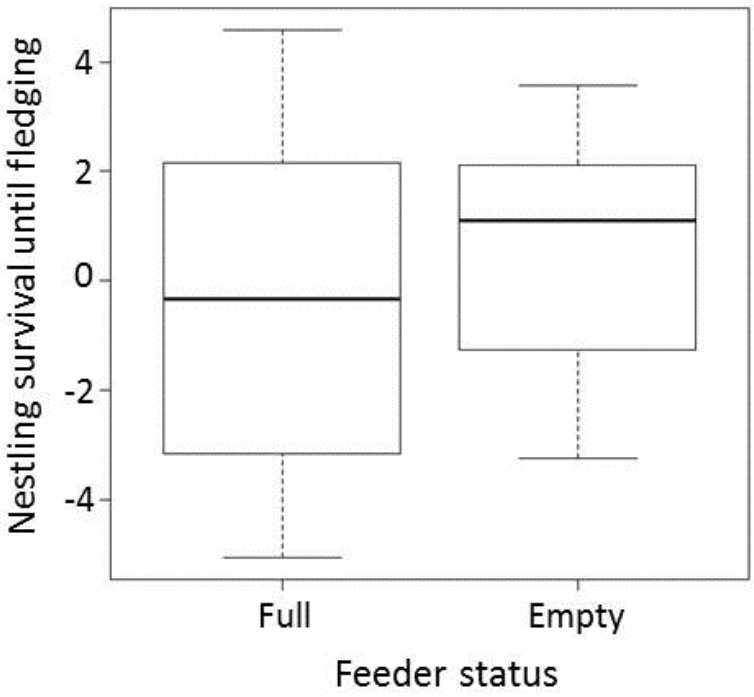


Fig. 3: 15 days-old nestling body condition (from model residuals) in relation to food supplementation and human presence. (mean: full line= full, dotted line=empty and grey lines: associated s.d; circles: control and full circles: supplemented)



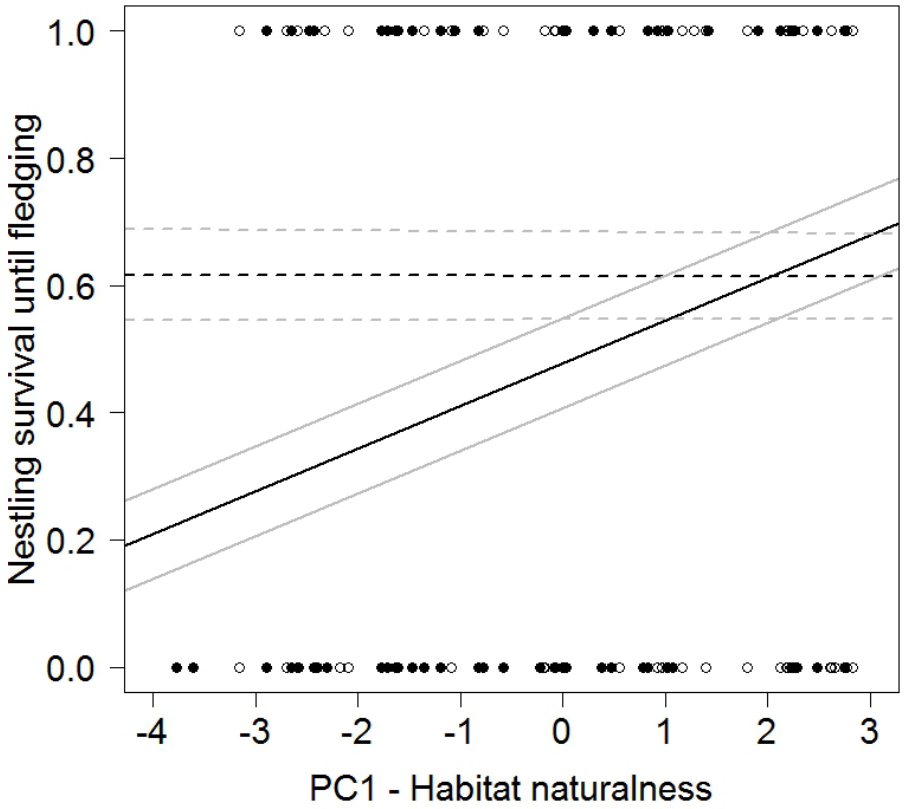


Fig. 4: Nestling survival until fledging (from model residuals) in relation to food supplementation (A) and in relation to supplementation along the habitat naturalness gradient (B). (A: Median ± 1st and 3rd quartile ± min and max; B: mean: full line= full, dotted lines=empty and grey line: associated s.d.; circles: control and full circles: supplemented)

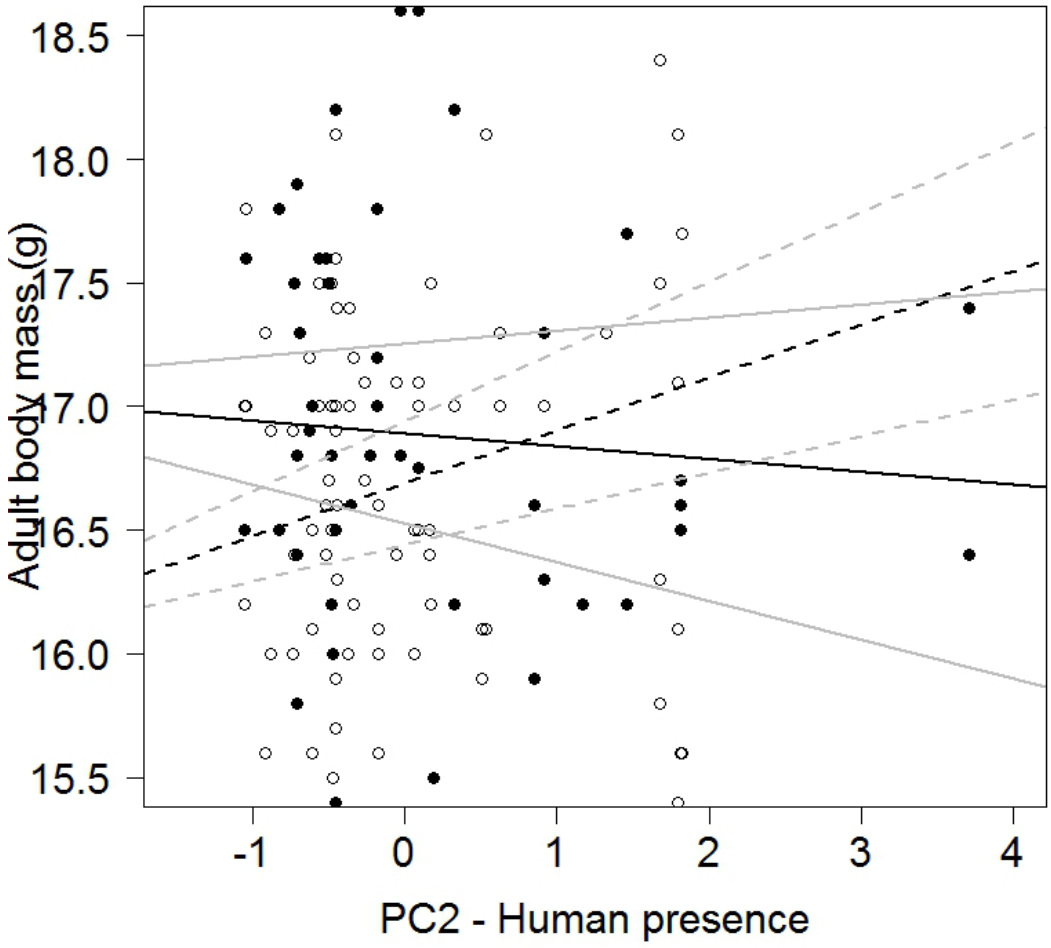


Fig. 5: Breeders' body condition (from model residuals) in relation to food supplementation and human presence. (mean: full line= supplemented, dotted line=control and grey lines: associated s.d; circles: control and full circles: supplemented)

Table S1. Proportion used to fill feeders in relation to age and number of nestlings.

Worms species	*Alphitobius diaperinus*					*Tenebrio molitor*		
Days after hatching	0 or 1	2	4	6	8	10	12	14 or 15
Nestlings number	Quantity / Nest (g)							
1	0.5	1	1.5	2.5	2.5	2.5	2.5	2.5
2	1	1.5	3	5	5	5	5	5
3	1	2.5	4.5	7.5	7.5	7.5	7.5	7.5
4	1.5	3	6	10	10	10	10	10
5	2	4	7.5	12.5	12.5	12.5	12.5	12.5
6	2.5	4.5	9	15	15	15	15	15
7	3	5.5	10.5	17.5	17.5	17.5	17.5	17.5
8	3	6	12	20	20	20	20	20
9	3.5	7	13.5	22.5	22..5	22.5	22.5	22.5

Nutribird complement was providing with worms larvae to be equivalent to 5 gr or 3 ml (a full coffee spoon) at day 6 for a 6-nestling brood. The supply in carotenoids was 0.0015 mg per full coffee spoon.