Effects of extreme hot weather on the reproductive output of great tits (*Parus major*, L.) in urban and natural habitats

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Abstract

Extreme weather events are rare, but can have high impact on human societies and biological systems. As the frequency of extreme events are increasing with current climate change, it is important to understand its effects on fitness of individuals and on long-term viability of populations. In this study, we investigated the effects of extreme high ambient temperatures on breeding success of great tits in two urban and two natural forest populations during six years. We found that the number of hot days had habitat-specific effects on nestlings during their development period (from hatching to 15-days age). Average body mass of broods decreased with increasing number of hot days in both forest populations and one city population. However, the negative effect of hot days on chick mass was significantly stronger in forest compared to urban populations, suggesting that forest populations are more vulnerable to extreme hot weather conditions. Unlike other study populations, average chick mass increased with the number of hot days in the hottest urban study site, suggesting adaptation in heat tolerance in this population. Tarsus length of chicks and their survival until fledging was not influenced significantly by hot weather. Hot weather conditions may affect the reproductive output of birds at least two ways: indirectly through food availability and directly through nestling physiology. As the tarsus size and survival of chicks were not affected by hot days in our study, we suggest that the negative effect of hot days on body mass may emerge more likely from the direct physiological effects of heat. These results are one of the first demonstrations that the effects of extreme weather events can differ between wild populations living in different environment.

Key words: heat, body mass, fledging success, climate change, avian reproduction

Introduction

Global climate change is expected to result in an increased incidence of extreme weather events, such as heat waves, cold spells and droughts (Field *et al.* 2012; Buckley & Huey 2016). When these events occur during the late spring and early summer, they can have detrimental effects on reproductive success in species for which this period is the peak breeding season, like many birds. A single event (usually) might not cause population-level decline, but can influence the short-term reproductive output of individuals e.g. due to loss of some of their actual offspring. If several extreme events occur or an extreme event takes long time in a single breeding season, majority of breeding individuals may die or may not successfully raise offspring which then can have population-level consequences (Møller 2011; Glądalski *et al.* 2018).

Heat-related mortality of animals can be significant (McKechnie, Hockey & Wolf 2012). Birds, because of their usually small body size and predominantly diurnal habits, are particularly vulnerable to extreme heat waves. For example, a "famous" heat wave occurred in southern and western Europe in 2003, when a prolonged and exceptionally intense heat wave resulted in 70,000 heat-related deaths of humans (Robine *et al.* 2008), and decreased populations of several bird species as well (Jiguet *et al.* 2006). In Australian passerine birds, adult survival was associated with temperature extremes more strongly than with average temperatures (Gardner *et al.* 2017), and in North American grassland birds nest survival rate was negatively associated with hot and dry weather both within and across breeding seasons in North American grassland birds (Conrey *et al.* 2016). A study found that offspring recruitment to the breeding population of long-tailed tits (*Aegithalos caudatus*) in the United Kingdom was associated with breeding season temperature (Gullett *et al.* 2015), so it seems that weather conditions during pre-fledging period can have long-term effects on offspring survival.

Compared to survival, a much less investigated phenomenon is the effect of extreme weather on the quality of reared offspring. Long-term fitness of individuals may be affected even if extreme weather events do not cause mortality but reduce the quality of offspring. As the size and body mass of nestlings are correlated with post-fledging survival (Perrins 1965; Tinbergen & Boerlijst 1990; Linden, Gustafsson & Part 1992), weather conditions during nestling development can influence fitness via affecting nestling size and/or body mass. A study found that morphometric traits of juvenile cliff swallows (*Petrochelidon pyrrhonota*) were not highly sensitive to weather conditions during a five-year investigation period, although nestlings were smaller in cooler years (Roche, Brown & Brown 2014). An other study on great tits showed that the body mass of offspring was lower in heated than control nests (Rodríguez & Barba 2016), but see also chapter 3.

Urban and natural areas are characterized by fundamental differences in their ecological features (see section 1.1). Urban areas are warmer than surrounding natural areas due to the urban heat island (UHI) effect, and there is some evidence in humans that heat waves have greater impact on mortality rates in highly built-up big cities than in rural settlements (Gabriel & Endlicher 2011). The UHI effect is especially important during the summer months when breeding of many birds occurs, and is usually more evident at night. The intensity of a city's heat island effect is dependent on the size of the city and its building density. During heat waves the local effect of an UHI is superimposed on the regional temperature, thereby the urban environment exacerbates extreme heat events. Thus, urban animals are more likely to reach their upper limit of thermal tolerance (Krause *et al.* 2017). However, to our knowledge, research comparing the effects of temperatures on bird populations living in urban and non-urban habitat types is completely lacking.

Constant exposure (i.e. over successive days) to high temperatures can lead to reductions in adult body mass, as well as affect nestling development. These phenomena can emerge for example through a combination of dehydration via increased rates of evaporative cooling, energetic constraints that result from reduced foraging opportunities or provisioning effort (Kruuk, Osmond & Cockburn 2015; Gardner *et al.* 2017), or through the cost of higher metabolic rate on higher temperatures (Merckx *et al.* 2018). Because urban environments are on average warmer, and the main food of insectivorous birds' offspring, e.g. caterpillars, may have much lower abundance in cities than in deciduous woodlands (Seress *et al.* 2018), extreme hot weather can be expected to exert stronger negative effects on the quality and survival of nestlings in urban areas than in more natural habitats.

In this study, our aims were 1) to investigate the effects of extreme high temperatures on breeding success of great tits measured as nestlings' size and survival, and 2) to assess if these effects differ between cities and non-urban forest habitats. We analysed breeding biology data of great tits from two urban and two forest populations from 2013 to 2018, and we took into account other, non-weather related factors that differ between the two habitat types. As urban areas are warmer due to UHI, and reproductive performance of urban animals is often lower in cities compared to more natural areas (Seress *et al.* 2012; Bailly *et al.* 2016), we predicted greater negative impact of extreme heat on reproduction in urban than natural habitats.

Methods

Measuring reproductive success

Nest-box colonies were set up for monitoring great tits from 2012 in urban (city of Veszprém 47°05'17"N, 17°54'29"E), and natural habitats (forests near Vilma-puszta 47°05'06.7"N, 17°51'51.4"E and Szentgál 47°06'39"N, 17°41'17"E), and additionally from 2013 in an other urban site (Balatonfüred 46°57'30"N, 17°53'34"E). Thus, we have two urban and two forest study sites. Urban nest-boxes are located mostly in public parks, university campuses and a cemetery, where vegetation contains both native and introduced plant species. Forest study sites are located in deciduous woodlands, characterized by beech *Fagus sylvatica* and horneam *Carpinus betulus* (in Szengtál) or downy oak *Quercus cerris* and South European flowering ash *Fraxinus ornus* (in Vilma-puszta).

Great tits usually rear maximum two broods per breeding season, thus, we recorded the number of eggs and nestlings in the nestboxes every 3-4 days from March to the end of July. In each study site, a clutch was regarded as first brood if it was initiated before the date of the first egg laid in the earliest second clutch at that site by an individually identifiable (i.e. colour-ringed) female that had a successful first breeding (i.e. fledged at least one young) in that year. We captured parent birds using a nest-box trap 6-15 days after their first nestling had hatched. We determined parents' sex based on their plumage characteristics, measured their tarsus length with a Vernier caliper, their wing length with a wing ruler, their body mass with a Pesola spring balance and ringed each bird with a unique combination of a numbered metal ring and three plastic colour rings. Breeding adults ringed on previous occasions were identified by observing their ring combination from recordings made by a small, concealed camera put on the nest boxes in the chick-rearing period (Seress *et al.* 2017). On these video samples we considered a colour ringed individual to be a parent bird if it was recorded to enter the nest box with food at least once. When the nestlings reached the age of 14-16 days we measured and ringed them using the same methods as with their parents.

For analysing the effects of weather on fledging success, we used broods where at least one offspring hatched and at least one offspring was alive on the third day after the hatching of the first nestling in that brood. We omitted the broods that failed within the first three days after hatching because the average interval between our nest monitoring visits was three days, so estimating the number of hatched chicks and their survival before the first nest check after hatching would be very uncertain. We could include n=760 broods for fledging success analyses, from which 385 had no chick mortality, 319 had partial mortality and 56 had complete brood loss during nestling rearing.

For analysing the effects of weather on the size of the nestlings, we used the average body mass and the average tarsus length of each brood as response variables, and we included broods where at least one offspring was alive at the age of 14-16 days (when the nestlings were ringed and measured). Throughout the study, "reproductive success" will be used as a collective term for both the survival and the size of the juveniles, however, the size of the nestlings is influenced by the genetic value of their parents as well. Because chick size varies with age, we omitted those few broods where the nestlings were measured before or after 14-16 days of nestling age (n=28).We had n=674 broods for nestling size analyses.

All procedures applied during our study were in accordance with the guidelines for animal care outlined by ASAB/ABS (www.asab.org) and Hungarian laws. We have all the required permissions for capturing, measuring of the birds and monitoring their breeding from the Balaton Upland National Park (permission number: 9135-2/2004, 2255/2008) and from the Government Office of Veszprém County, Nature Conservation Division (former Middle Transdanubian Inspectorate for Environmental Protection, Natural Protection and Water Management; permission number: 31559/2011).

Meteorological data

In March 2013, we installed a WH 2080 weather station (Ambient, LLC, AZ, USA) near each of our study sites, which record temperature, humidity, air pressure, precipitation and wind speed and direction. We also put one Voltcraft DL101T temperature and humidity data logger (Conrad Electronic SE, Germany) to an empty nestbox within each of our four nestbox colonies. Thus, we have hourly weather data for all of our four study sites. To analyse the effects of weather conditions on breeding success, we calculated weather variables for the nestling period (from the day of hatching of first chick to the day preceding the day of the last chick's death or the day of ringing and measuring the chicks) for each brood. We used the hourly temperature (°C) records of our weather stations at each study site to calculate the average temperature for each brood as the mean of our hourly temperature data over the nestling period. To quantify extreme heat, we defined a reference period from the earliest hatching date to the latest chick ringing date in the total of our six-year data set, so the reference period was from 9th April to 15th July. To estimate the typical temperatures in our geographic area, we used a 26-years dataset from an external reference weather station located in Szentkirályszabadja, a small village near Veszprém (47°57'06" É, 17°58'10" K, ca. 9.5 – 22 km from our study sites). The weather station was maintained by the Hungarian Meteorological Service and temperature data of this weather station was available from the NOAA database (www.ncdc.noaa.gov), recorded every three hours a day (0, 3, 6, 12, 15, 18, 21 UTC) in 1993-2018. We calculated the 90 % percentile of daily maximum temperatures for the reference period from this 26-year long dataset, and this percentile value (28.7 °C) was used as a threshold for defining extreme hot days during chick rearing. Thus, we calculated the number of hot days in each nestling period as the number of days when the daily maximum temperature was higher than 28.7 °C.

Statistical analyses

Investigating the effects of extreme weather is challenging. Extreme events are rare by definition, so their distribution is strongly skewed. Furthermore, weather variables can be correlated with each other and with other seasonal variables that can influence reproductive success, e.g. hatching date, so multicollinearity can be another problem in models containing multiple predictors. Statistical methods that can handle multicollinearity, such as covariance-based structural equation modelling (SEM), are less well suited for handling non-normal data distributions and the non-independence structure of ecological data (e.g. multiple broods per pair). Therefore, as a first approach to this problem, we used generalized linear mixed-effects (LME) models, which can be applied appropriately for non-normal and non-independent data, although less suitable for separating the effects of correlated predictor variables than the SEM approach.

For average nestling mass and average tarsus length of nestlings as response variables, we used LME models with Gaussian error. For fledging success (proportion of hatched chicks that were alive at the age of 14-16 days) as response variable, we used LME models with quasibinomial error distribution and logit link function. All of our models contained pair identity as random factor to control for the non-independence of broods that had the same parents (there were 111 pairs of parents that had more than one brood in our dataset, ranging 2-6 broods/ pair). Our main predictors of interest were the number of hot days (used as a numeric covariate) and study site (two cities and two forests), and their two-way interaction. We built two types of models for each response variable: 1) a simple model with no multi-collinearity that contained only our main predictors of interest, i.e. the number of hot days and study site and their two-way interaction, and

2) a complex model that contained further potentially important predictor variables. In the latter model, we included year as categorical variable (6 years), and hatching date as a numeric covariate defined as the number of days to the first hatched nestling in each brood from 1st January annually. For models of nestling size (i.e. average body mass and average tarsus length of nestlings) we also included two further numeric covariates: brood size as the number of offspring at fledging (i.e. number of measured nestlings), and brood age as the number of days from hatching of the first chick to measuring the fledglings (ranging 14 to 16 days). In the model of fledging success, we added the quadratic term of hatching date, because preliminary graphical inspection of the data suggested a non-linear seasonal change in fledging success. Variance inflation factor (VIF), a measure of multi-collinearity, ranged from 1.04 to 2.85 in these complex models. We did not incorporate the average temperature during each nestling period in the models, because average temperature correlated with both the number of hot days and date, leading to high multi-collinearity in some of the models (VIF: 1.04 – 6.24).

To statistically compare the effect of hot days between the two habitat types, we calculated a linear contrast from each model’s estimates (i.e. the difference between the two urban sites versus the two forest sites). We used this approach rather than including habitat type as a fixed effect and site as a random effect because variance estimations of random effects with few levels are unreliable (Piepho, Büchse & Emrich 2003; Bolker *et al.* 2008), whereas including both habitat type and site as fixed effect would have resulted in a model with high collinearity between these two factors (Dormann *et al.* 2012). Instead, we treated statistically the four sites similarly as if they were two control groups and two treatment groups in an experiment, and we used a pre-planned comparison to test the prediction that the two treatment (i.e. urban) groups would differ from the two control (i.e. forest) groups. Note that pre-planned comparisons are a powerful approach for testing a priori hypotheses (Ruxton & Beauchamp 2008). All analyses were implemented in the R 3.1.1 software environment (R Core Team 2014), using packages “nlme” (Pinheiro *et al.* 2013), “MASS” (Ripley et al. 2013) and “emmeans” (Lenth 2018).

Results

Urban and forest habitats had markedly different temperature profiles with an average 1.53 °C higher temperature in urban than in forest habitat over the whole 6 year study period. Average temperatures of the nestling periods were significantly different between habitat types and between study years, too (Figure 1). The number of hot days was also significantly different between habitat types and among years as well (Figure 2), with more frequent hot days in urban than in forest habitat. Out of the 390 urban nestling periods, at least one hot day occurred in 179 periods (45.9%), whereas out of the 370 forest nestling periods, at least one hot day occurred in 77 periods (20.8 %). Number of hot days ranged between 0 and 13 in urban nestling periods, and between 0 and 5 in forest nestling periods (Figure 2), and the highest maximum temperature was 40.1 °C in urban (in June 2013) and 33.6 °C in forest nestling periods (in May 2014).

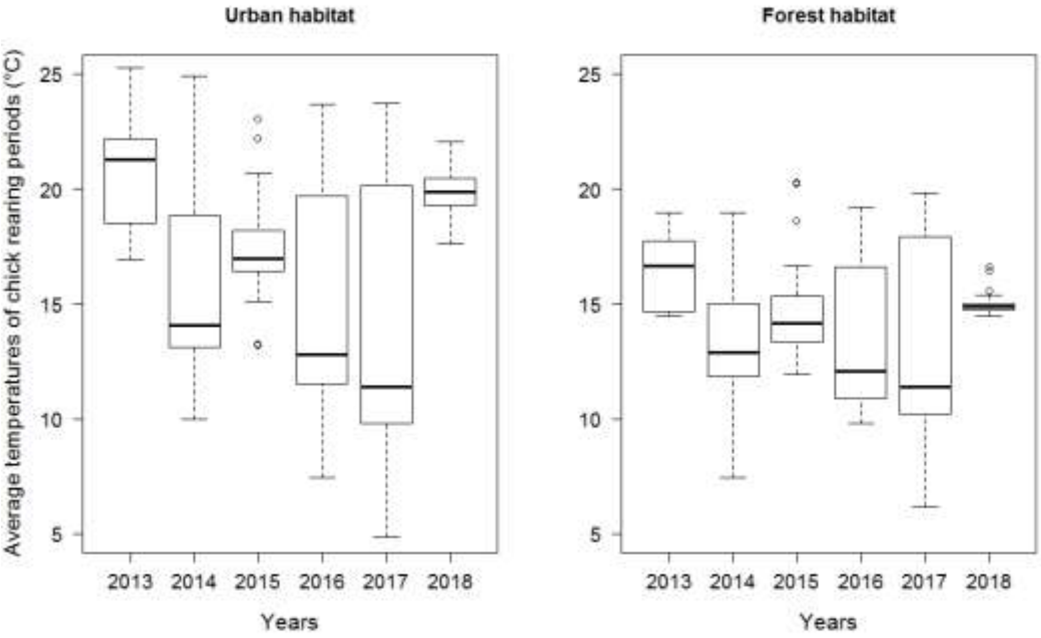

Figure 1. Distribution of the average temperature of nestling periods in each year in urban and forest habitats. Temperature significantly differed between years within habitat (ANOVA, urban: $F_{5,389} = 24.693$, $P < 0.001$; forest: $F_{5,369} = 11.772$, $P < 0.001$), as well as between habitats (ANOVA: $F_{1,759} = 41.993$, $P < 0.001$), with an average 1.53 °C higher temperatures in urban than in forest habitats.

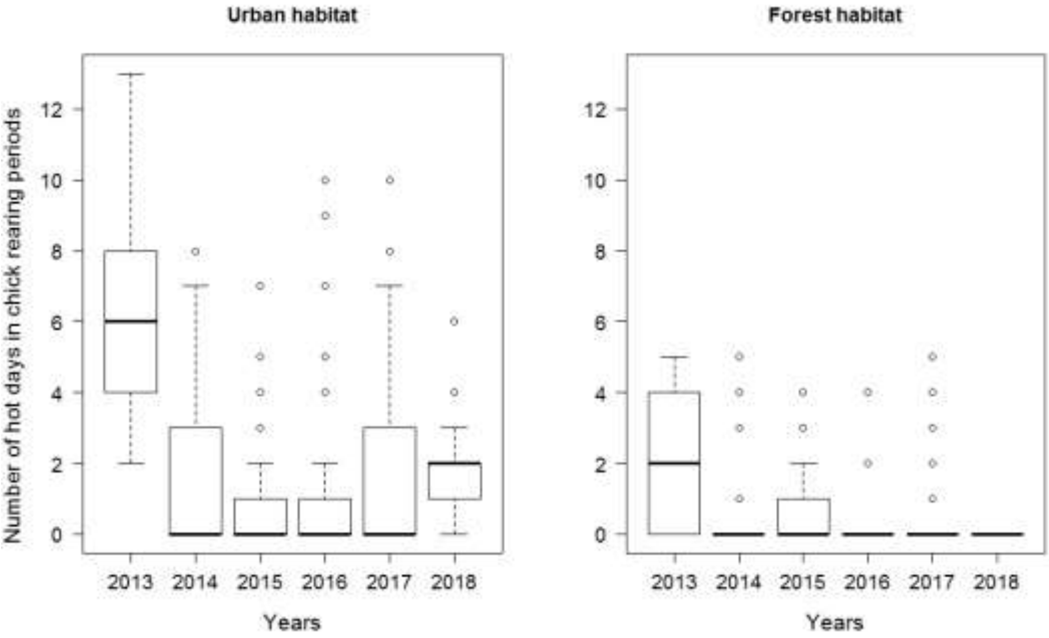

Figure 2. Distribution of the number of hot days during nestling periods in each year in urban and forest habitats. Number of hot days significantly differed between years within habitat (ANOVA, urban: $F_{5,389} = 25.915$, $P < 0.001$; forest: $F_{5,369} = 11.676$, $P < 0.001$), as well as between habitats (ANOVA: $F_{1,759} = 77.959$ $P < 0.001$), with an average 1.12 day difference between urban and forest habitats.

Our data suggest that the effect of extreme hot periods on reproductive success varies among sites. The number of hot days always showed positive relationship with the measured components of reproductive success in the urban population of Balatonfüred (the hottest site). In this study site, chick mass increased significantly with increasing number of hot days, and the other two reproductive components had non-significant positive relationships with the number of hot days. The reproductive success of the other three sites showed mostly negative relationships with number of hot days, although many of these relationships were not statistically significant (Table 1 & 2, Figure 1). The magnitude of negative effect of hot days varied among reproductive success components, and also between simple and complex models (Table 1 & 2). Chick mass decreased with increasing number of hot days according to both the simple and the complex models (Table 1 & 2, Figure 3), and this relationship was significant in all sites according to the complex models. The average tarsus length of chicks in a brood was only affected by number of hot days in the urban site Veszprém where it significantly decreased with increasing number of hot days according to the simple model (Table 1 & 2, Figure 3). Fledging success was significantly lower with increasing number of hot days at the urban site Veszprém and at the forest site Vilma-puszta according to simple models, although these relationships became non-significant in the complex models (Table 1 & 2, Figure 3).

Comparing the overall (average) effects of extreme heat between urban and forest populations with linear contrasts suggest significant habitat differences (Table 3). According to the simple models, the negative effect of increasing number of hot days on average chick mass was significantly stronger (Table 3) in forest compared to urban populations (which is consistent with the more negative slopes obtained for the two forest sites; Table 1). For the average chick tarsus length, we did not find significant habitat difference in the effect of hot days (Table 3). The decrease in fledging success with increasing number of hot days was significantly stronger in forest compared to urban habitat in the simple model (Table 3). Note that this latter difference is driven by Balatonfüred, since the negative slope for the other urban site (Veszprém) falls between the estimates for the forest sites (Table 1). In the complex model, the difference in effect of hot days on fledging success between urban and forest habitat was not significant, although it had similar direction as in the simple model (Table 3). All results remained qualitatively the same if we re-ran the analyses without the potential outlier in Balatonfüred (Figure 3). The effects of other predictor variables on chick size and fledging success are given in Table 4.

Table 1. Relationship between measures of reproductive success and the number of hot days during the nestling period, as estimated by marginal means from the *simple models* (including only the interaction between number of hot days and study site). Estimates with standard error (SE) refer to the slope of response variable with the number of hot days in each study site (the same slopes are shown in Fig. 3). Slopes significantly different from zero (i.e. zero not included between the lower and upper limit of the 95% confidence interval (CI) of the slope estimate) are highlighted in bold. Linear contrasts (Table 3) for habitat comparison were calculated from these parameter estimates.

Study sites	**Chick mass**				**Chick tarsus length**				**Fledging success**			
	Estimate	SE	lower CI	upper CI	Estimate	SE	lower CI	upper CI	Estimate*	SE	lower CI	upper CI
Veszprém city	-0.081	0.046	-0.172	0.010	-0.052	0.021	**-0.094**	**-0.011**	-0.107	0.049	**-0.204**	**-0.009**
Balatonfüred city	0.163	0.049	**0.065**	**0.260**	0.036	0.023	-0.009	0.081	0.002	0.055	-0.106	0.110
Szentgál forest	-0.106	0.085	-0.273	0.061	-0.050	0.038	-0.126	0.026	-0.029	0.130	-0.286	0.228
Vilma-puszta forest	-0.489	0.131	**-0.749**	**-0.230**	-0.059	0.060	-0.178	0.060	-0.507	0.150	**-0.802**	**-0.212**

For chick mass and chick tarsus length, number of pairs was 535 and number of broods was 674, df = 135. For fledging success, number of pairs was 600 and number of broods was 760, df = 156

* Estimates of fledging success are on the logit scale

Table 2. Relationship between measures of reproductive success and the number of hot days during the nestling period, as estimated by marginal means from the *complex models*. Estimates with standard error (SE) refer to the slope of response variable with the number of hot days in each study site. Slopes significantly different from zero (i.e. zero not included between the lower and upper limit of the 95% confidence interval (CI) of the slope estimate) are highlighted in bold. Linear contrasts (Table 3) for habitat comparison were calculated from these parameter estimates.

Study sites	**Chick mass**				**Chick tarsus length**				**Fledging success**			
	Estimate	SE	lower CI	upper CI	Estimate	SE	lower CI	upper CI	Estimate*	SE	lower CI	upper CI
Veszprém city	-0.123	0.057	**-0.236**	**-0.009**	-0.050	0.026	-0.102	0.001	0.079	0.069	-0.057	0.215
Balatonfüred city	0.116	0.053	**0.012**	**0.221**	0.029	0.024	-0.018	0.076	0.089	0.067	-0.044	0.221
Szentgál forest	-0.256	0.096	**-0.446**	**-0.066**	-0.070	0.043	-0.156	0.015	0.089	0.157	-0.222	0.400
Vilma-puszta forest	-0.401	0.130	**-0.659**	**-0.143**	0.011	0.059	-0.105	0.128	-0.225	0.153	-0.528	0.079

For chick mass and chick tarsus length, number of pairs was 535 and number of broods was 674, df = 127. For fledging success, number of pairs was 600 and number of broods was 760, df = 149

* Estimates of fledging success are on the logit scale

Table 3. Linear contrasts comparing the effect of the number of hot days between urban and forest habitats. Each contrast (d, with its standard error, SE) expresses the difference between the two urban and two forest populations in the slope of relationship between reproductive parameters and the number of hot days. Positive contrasts mean more positive slopes in the urban habitat or more negative slopes in the forest habitat.

	Simple model			Complex model		
Response	**d ± SE**	**t**	**p**	**d ± SE**	**t**	**p**
chick body mass	0.338 ± 0.085	3.978	< **0.001**	0.325 ± 0.082	3.978	< **0.001**
chick tarsus length	0.046 ± 0.039	1.187	0.237	0.018 ± 0.037	0.503	0.616
fledging success	0.215 ± 0.106	2.037	**0.043**	0.152 ± 0.108	1.401	0.163

Simple models: For chick mass and chick tarsus length, df = 135, for fledging success, df = 156
Complex models: For chick mass and chick tarsus length, df = 127, for fledging success, df = 149

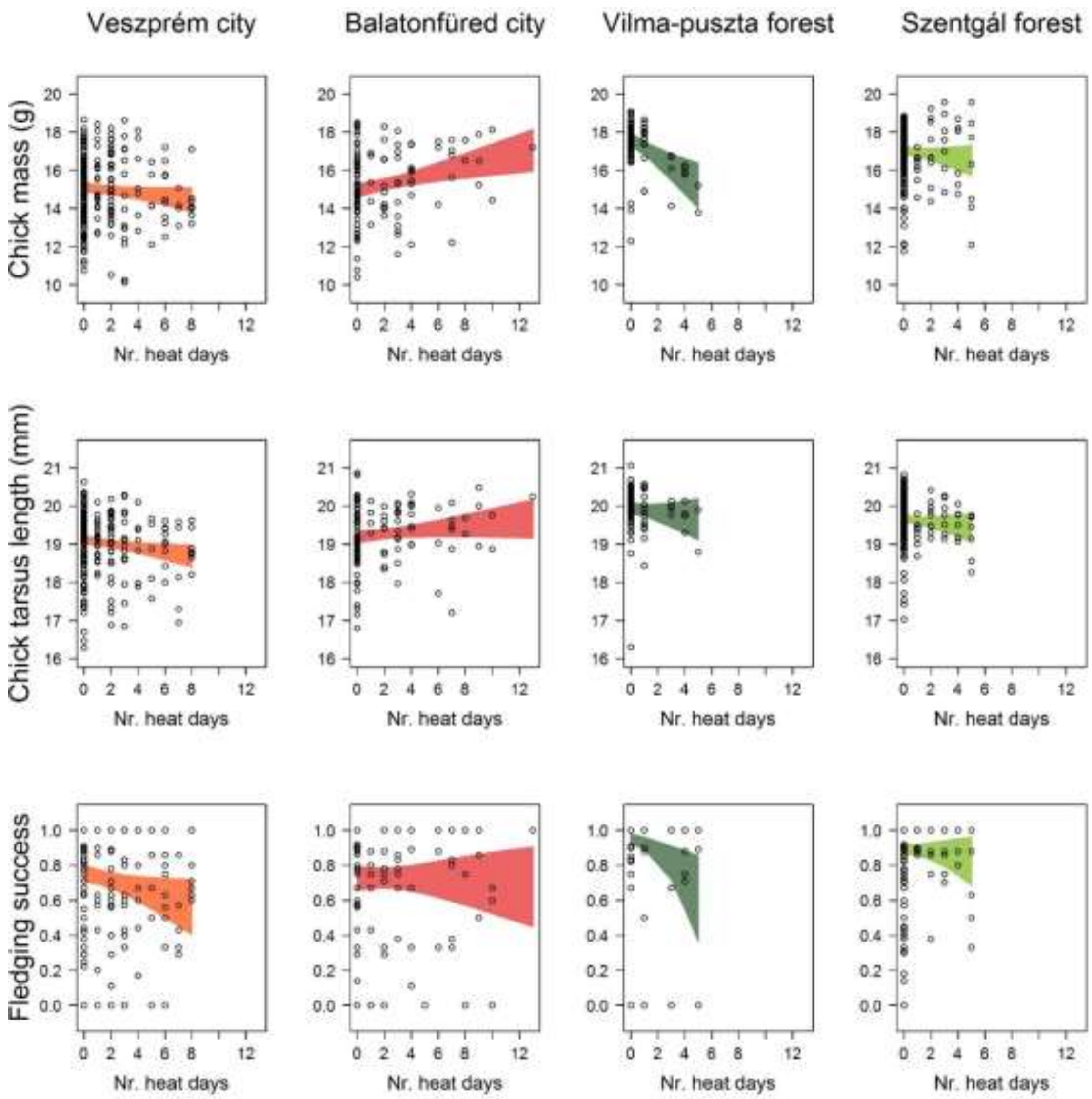

Figure 3. Relationship of average body mass (upper panels), average tarsus length (middle panels) and fledging success (bottom panels) of broods with number of hot days in each study site. Coloured stripes show the 95% confidence band of the slope of relationship estimated from the simple models (Table 1).

Table 4. Parameter estimates of the complex models of great tit reproductive success variables in relation to the number of hot days and other predictors. The intercept refers to the urban site Veszprém and year 2013 when all numeric predictors are zero; all other parameters refer to the difference from the intercept. ***ß*** is the parameter estimate, ***SE*** is the standard error, ***DF*** is the degrees of freedom. Sample size is n=674 broods with n=535 pairs for nestling size and n=760 broods with n=600 pairs for fledging success. Estimates of fledging success are on logit scale.

	Chick mass					**Chick tarsus length**					**Fledging success**				
	ß	SE	DF	t	p	ß	SE	DF	t	p	ß	SE	DF	t	p
(Intercept)	10.413	1.465	531	7.110	0.000	16.548	0.656	531	25.211	0.000	-10.094	3.988	596	-2.531	0.012
brood size	0.077	0.029	127	2.710	0.008	0.082	0.013	127	6.440	0.000	-	-	-	-	-
brood age	0.334	0.082	127	4.095	0.000	0.142	0.037	127	3.871	0.000	-	-	-	-	-
hatching date	0.004	0.005	127	0.777	0.439	0.003	0.002	127	1.263	0.209	0.207	0.061	149	3.370	0.001
hatching date^2	-	-	-	-	-	-	-	-	-	-	-0.001	0.000	149	-3.784	0.000
year 2014	-0.494	0.295	127	-1.673	0.097	-0.318	0.133	127	-2.396	0.018	-0.556	0.473	149	-1.175	0.242
year 2015	-0.915	0.311	127	-2.941	0.004	-0.219	0.140	127	-1.566	0.120	-0.800	0.506	149	-1.579	0.116
year 2016	-1.067	0.302	127	-3.530	0.001	-0.217	0.135	127	-1.605	0.111	-1.148	0.476	149	-2.410	0.017
year 2017	-1.479	0.323	127	-4.587	0.000	-0.448	0.144	127	-3.105	0.002	-2.226	0.466	149	-4.774	0.000
year 2018	-0.773	0.323	127	-2.394	0.018	-0.275	0.144	127	-1.910	0.058	-1.232	0.490	149	-2.512	0.013
nr. hot days	-0.123	0.057	127	-2.144	0.034	-0.050	0.026	127	-1.948	0.054	0.079	0.069	149	1.149	0.253
site Balatonfüred	-0.144	0.211	531	-0.682	0.496	0.082	0.093	531	0.878	0.380	-0.114	0.235	596	-0.487	0.626
site Szentgál forest	1.790	0.188	531	9.530	0.000	0.390	0.083	531	4.696	0.000	1.161	0.218	596	5.334	0.000
site Vilma-puszta forest	2.231	0.237	531	9.417	0.000	0.549	0.105	531	5.234	0.000	1.826	0.375	596	4.865	0.000
nr. hot days : site Balatonfüred	0.239	0.065	127	3.670	0.000	0.079	0.029	127	2.686	0.008	0.010	0.080	149	0.123	0.902
nr. hot days : site Szentgál	-0.133	0.094	127	-1.412	0.160	-0.020	0.042	127	-0.467	0.642	0.010	0.151	149	0.066	0.948
nr. hot days : site Vilma-puszta	-0.278	0.134	127	-2.073	0.040	0.062	0.061	127	1.017	0.311	-0.304	0.160	149	-1.900	0.059

Discussion

We found differences in the effects of extreme hot temperatures on different components of reproductive output, and also between urban and forest populations. Average body mass of broods was smaller when more extreme hot days occurred (except in the urban site of Balatonfüred), but average tarsus length was not affected by number of hot days. Fledging success decreased with number of hot days in one urban and one forest populations.

Heat might affect the reproductive output of birds at least two ways: indirectly through food amount and availability and directly through nestling physiology. According to studies about reactions to extreme high temperatures in lepidopteran larvae (which are the main source for nestling diet in great tits and many other birds), one of the most consistent result is that increasing temperature strongly decreases the time to pupation in several lepidopteran species (Kingsolver *et al.* 1997; Lee & Roh 2010; Lemoine, Capdevielle & Parker 2015), so optimal caterpillar food may be available for shorter time to insectivorous passerines when the temperature is hot due to frequent hot days. Additionally, growth and food consumption of caterpillars can decline rapidly above a critical temperature (Kingsolver *et al.* 1997) as well as their mortality can increase when temperature is constantly high (York & Oberhauser 2002; Lee & Roh 2010). So, it is possible that avian nestlings have lower body mass when there are extreme hot conditions due to shorter time when prey is available, and this effect can be stronger in forest where the amount of available caterpillar prey is much higher (Seress *et al.* 2018) and can have a stronger decrease because of high temperatures than in urban areas, where nestlings usually get greater proportion of other food types (Sinkovics *et al.* unpublished results). But, if it is true, one can expect reduced nestling growth and/or greater nestling mortality during such a food shortage under hot conditions, but in our study this was not the case as tarsus length of nestlings and fledging success were not affected robustly by number of hot days. In sum, we suggest that reduced caterpillar biomass due to hot weather may not be the major factor explaining our findings.

It seems more likely that the negative effect of hot days on nestling body mass emerged from the direct physiological effects of heat. Birds are homeothermic, meaning that individuals maintain stable internal body temperatures regardless of external influences. However, the nestlings cannot maintain stable body temperature in the first few days of their life, and their metabolic processes can alter from adults because they're growing organisms (Mertens 1977). So, offspring may more vulnerable to extreme heat than adults, but specific results about the heat response of young nestlings are scarce. Several studies found that even the body temperature of adult birds increased with increasing ambient temperature (Whitfield *et al.* 2015; Nilsson, Molokwu & Olsson 2016; Nilsson & Nord 2018), sometimes even exceeding 45°C, which is close to suggested lethal levels for birds. In hot environments, individuals could less effectively dissipate the excess heat as a consequence of increased metabolism, leading to hyperthermia. Increasing metabolism causes decreased utilization of food and faster mobilization of energy reserves, thus can cause lower body mass maybe without influencing other growth parameters. Additionally, during heat stress, the evaporative water loss is elevated as the organism tries to cool itself by evaporation to maintain body temperature in the physiologically normal range, causing dehydration (Weathers 1972; Arad *et al.* 1989). So, increased metabolic rate and water loss both can lead to decreased body mass. In broiler chickens (*Gallus domesticus*), weight gain and food intake were reduced under heat stress (Han & Baker 1993; Quinteiro-Filho *et al.* 2010), suggesting that the cost of heat stress can manifest in reduced body mass, as our study found. In a long-term study with increasing temperature and frequency of extreme heat on a cooperative breeder bird, the superb fairy-wren (*Malurus cyaneus*), heatwave events were associated with reduced chick mass (Kruuk *et al.* 2015). An experiment on great tits found that higher temperatures can impair nestling development, as offspring from heated nestboxes were lighter compared to controls, but the tarsus length of heat treated and control nestlings didn't differ significantly. Additionally, the manipulation of nest microclimate didn't influence parental provisioning behaviour, suggesting that body weight loss was independent from food intake (Rodríguez & Barba 2016). These results are similar to ours and suggest that heat stress has great costs for developing offspring. Additionally, extreme heat may have an effect on the body mass of nestlings indirectly through the physiological response of their parents, as provisioning rate in marsh tits (*Poecile palustris*) seems to potentially be limited by the rate of heat dissipation (Nilsson & Nord 2018).

We also found that the effect of hot days on fledglings' body mass differed between urban and forest populations. Although UHI caused 1.53 °C higher temperature on average and there were more number of hot days in urban compared to forest habitat (see Figure 1 & 2), hot days affected the reproductive components more negatively in forest populations than in cities. So, contrary to our predictions, non-urban populations seem more vulnerable to extreme heat than urban populations. Urban birds living in constantly warmer environment may have higher heat tolerance due to adaptive thermal evolution and/or phenotypic plasticity, similarly to some invertebrates. For example, in European diving beetles, positive relationship between upper thermal tolerance and adaptability was found (Calosi, Bilton & Spicer 2008). In the water flea (*Daphnia magna*), researchers found higher heat tolerance in animals living in urban compared to rural habitats and in animals reared at higher temperatures (Geerts *et al.* 2015; Brans *et al.* 2017), while urban acorn ants (*Temnothorax curvispinosus*) exhibited greater heat tolerance and a loss of cold tolerance compared with rural ants (Diamond *et al.* 2017). So, it is possible that urban birds have higher heat tolerance than conspecifics in natural habitats, but this should be further tested in wild bird populations. In our study, the positive effect of hot days in the hottest urban population, and the more negative effects of hot days in forest habitat suggest that urban birds might be adapted to UHI and high temperatures in cities, and thus tolerate the extreme heat better while individuals in natural habitat may suffer greater negative consequences of extreme heat when it occurs.

One other mechanism for heat adaptation in urban habitats may be reduced body size. Urban birds are usually smaller (Liker *et al.* 2008; Chamberlain *et al.* 2009; Bailly *et al.* 2016), and UHI may play a major role in forming this size difference between urban and rural individuals. The increased temperatures associated with UHI result in increased metabolic costs (Birnie-Gauvin *et al.* 2016) and are expected to drive shifts to smaller body sizes (Merckx *et al.* 2018). A study comparing the heat stress reaction of different-sized bird species found that small birds have a more advantageous water economy when environment is hot (Weathers 1972), but see Whitfield *et al.* (2015) where larger species tolerated higher ambient temperatures. In line with the principle of Bergmann's rule, animals with smaller size have higher surface-biomass ratio which facilitates heat loss, thus it can be beneficial against dehydration and overheating in a warmer urban habitat. This idea is supported by a study on several water flea populations which found that smaller individuals were more heat tolerant, and urban individuals were generally smaller than rural ones (Brans *et al.* 2017). In birds, a study on a population of white-browed scrubwrens (*Sericornis frontalis*) also found that smaller individuals survived better when more extreme hot and dry events occurred (Gardner *et al.* 2017). So, it is possible that in our study populations the lack/reduction of negative impact of extreme heat in urban populations emerged because of the smaller size of urban individuals. Further research is needed about long-term consequences of smaller size on recruitment, post-fledging survival and future reproductive success of nestlings that experienced different weather. Also, it remains to be tested if body size is genetically adapted to UHI in birds.

Our study experienced some difficulties of investigating weather effects in urban and natural ecosystems. Future studies about extreme weather effects on wild populations may want to take into account the following issues. First, weather variables can be significantly associated with other variables that affected breeding success, so it would be important to explore other relevant factors that interact with temperature and/or reproductive success, and these relationships should be taken into account when examining weather effects. Second, several factors other than temperature might have an influence on the lower reproductive output of urban populations, e.g. pollution, fragmentation and predation (Rodewald & Gehrt 2014; Seress & Liker 2015), so correlated characteristics and complexity of these systems should be taken into account when we want to compare weather effects in urban and natural habitats. Finally, extreme heat can have both short- and long-term consequences on populations' reproductive characteristics. There is a great importance of investigating physiological responses of organisms to extreme events and potential mechanisms that can help urban individuals to neutralize the negative effect of high temperatures, in order to predict the scope and degree by which populations will be able to resist future climatic stressors.

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Additional information

The authors declare that they have no conflict of interest.

Author Contributions

IP, VB, and AL designed the study. All authors collected data in the field. IP, VB and AL did the statistical analyses. All authors wrote the manuscript.

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