Microclimate shifts in nest-boxes and natural cavities before, during and after nesting

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Abstract

Animals breeding in anthropogenic shelters such as nest-boxes experience nesting environment in which they did not originally evolve. Over the past decades, they are additionally challenged by climate change – a major environmental force influencing their reproductive ecology. Despite the central importance of nesting microclimate for offspring development and fitness, very little is known about the thermal properties of human-provided nests compared to natural ones. While it has been demonstrated that artificial nests provide poorer thermal insulation in comparison to natural breeding sites, there is no evidence on how these shifts are shaped along the reproductive stages. In particular, comparisons focusing on the time when offspring are in the nest are lacking. Here, we compare the microclimatic conditions (temperature and absolute humidity) along the nesting cycle (from nest-site choice in early spring until post-fledging) in natural cavities and nest-boxes used by several species of hollow-nesting passerines in a temperate deciduous forest. We confirm that across all nesting stages, nest-boxes are thermally unstable when compared to natural cavities, with higher temperature maximums, larger amplitudes and worse insulation from maximum ambient temperatures relative to natural cavities. Importantly, in the presence of young, and after they start to thermoregulate on their own, nest-boxes are also more humid than natural cavities. Artificial nest microclimate is likely to amplify the adverse effects of projected temperature increases modelled under climate change scenarios, specifically by compromising thermoregulation and increasing water requirements of developing animals. In contrast, internal microclimatic shifts were mitigated in natural cavities 3.0 times more effectively than in nest-boxes when offspring were in the nest (in terms of mean daily differences from ambient temperature). We stress that conservation efforts should focus on the protection of habitats offering natural breeding-hollows to reduce climate change impacts on breeding animals.

1. Introduction

The choice of nesting site can trigger major ecological and evolutionary consequences for breeding animals (Sudyka et al. 2021), and may be constrained by extreme and fluctuating environmental conditions (Salaberria et al. 2014). Nests protect and provide the optimal setting for the developing young and buffer from external climatic conditions by creating an internal microclimate, i.e. specific temperature and humidity. In endotherms, both play a vital role for energy and water budgets in the context of maintaining fixed core temperatures in largely varying thermal environments (Porter & Kearney 2009). Thus to be optimal, the nesting site microclimate should assure conditions as close as possible to the thermoneutral zone, i.e. the "comfort zone" in which the animal does not lose body water at basal metabolic rate (Porter & Kearney 2009). With climate change and the resulting extreme climatic events (United Nations 2021), many thermally specialized animals will be forced to invest more resources in maintaining their body temperature at the cost of energetic resources for other fundamental functions such as growth and reproduction (Boyles et al. 2011; Nord & Nilsson 2019; van de Ven et al. 2020), and survival (McKechnie & Wolf 2010; Cunningham et al. 2013; Bourne et al. 2020). The current reports on climate change use models factoring an increase in mean ambient air temperature by 1.5-4°C and in the frequency of extreme climatic events (United Nations 2021). During the reproductive period (spring and summer in temperate climates), this will be most likely manifested by extremely low temperatures early in spring during the nest-site choice stage, and increasing maximum temperatures and occurrence of heat waves with the progression of the breeding season (Ummenhofer & Meehl 2017). Animals are able to respond behaviourally to fluctuating temperatures (hibernation, migration, seeking protection against rain/wind or resting in shadow, reducing activity at the hottest day time), but young animals developing in nests have only limited possibilities for such adjustments. Thus nesting microclimate is in fact the best protection against thermal extremes, though in some species it is also additionally facilitated by parental care through nesting behaviours such as incubation or brooding (Arct et al. 2021).

Nesting microclimate across the reproductive cycle

Organisms that could be particularly harmed by climatic shifts during breeding and early development are birds. Their reproductive cycle (oviparity) allows climatic conditions to directly act on their development from the earliest embryonic stages, and much work has readily assessed avian far-reaching physiological responses to heat (detailed in Box 1). Since many bird species breed in hollows, parents may perceive favourable nest microclimate (temperature in a hollow higher than outside) as a cue to start nesting and egg laying (Dhondt & Eyckerman 1979) during nest-site choice, even before the onset of actual breeding. At pre-hatching stages,

BOX 1

Physiology of heat-response in endotherms/birds - in brief

In endotherms, heat-related mortality stems from a fundamental physiological conflict between evaporating water to maintain body temperature below lethal limits, and saving enough water to avoid dehydration (McKechnie & Wolf 2010). To cope with high temperatures, animals can increase their tolerance to hyperthermia and thus allow for greater passive heat dissipation (Weathers 1997; Boyles et al. 2011) to regulate evaporative water loss (EWL) (Eto et al. 2017). However, lethal levels of body temperature may be reached, even in excess water availability, because of the inability to dissipate heat at a sufficient rate (Mertens 1977a; McKechnie & Wolf 2010). Since EWL can be expressed as a function of water vapour pressure deficit, it is particularly ineffective in humid and hot environments (Weathers 1997). With extreme climatic events, the predicted increases in ambient temperatures will considerably increase water requirements. In small birds, these are estimated to increase by 150–200% of current values (McKechnie & Wolf 2010). Their small body size and diurnal activity patterns result in high rates of EWL of more than 5 % of body mass per hour (Wolf & Walsberg 1996), a strong prerequisite to dehydration. Dehydration is dangerous for prolonged periods of time because it distorts osmolarity and increases plasma Na^+ concentrations. This compromises survival, since, particularly in birds, blood volume decreases cannot be quickly restored even after water intake (Takei 2000).

during egg laying and incubation, the sole presence of incubating parents and eggs (incubated eggs diffuse water vapour to the nest air through the eggshell pores) may influence the nesting site microclimate of water vapor conditions [humidity increase above the ambient levels (Deeming 2011)]. At this stage, at least in temperate climates, a greater threat resulting from microclimate shifts is related to hypothermia of developing offspring, because it is currently more common in natural environments than hyperthermia, even if avian embryos are particularly vulnerable to high temperatures (Webb 1987). Several studies have readily demonstrated how critical are incubation temperatures for further development (Mueller et al. 2019) and survival (Ospina et al. 2018). As the breeding cycle progresses, the nesting microclimate may display additional shifts: post-hatching, it has been shown that the presence of nestlings increased internal temperature in natural cavities by 1.5–4.1 °C (Maziarz 2019). Such an increase can multiply the effects of abiotic conditions on nestlings development, which impacts on their growth, thermoregulation, parasite loads and survival (Cunningham et al. 2013; Salaberria et al. 2014; Rodríguez & Barba 2016; van de Ven et al. 2020).

Nestlings of small altricial birds are particularly prone to respond to non-optimal nest microclimate since their low development stage at birth (e.g. lack of feathers) in comparison to precocial birds and most mammals. They further need to maintain a stable body temperature once they start to thermoregulate, which occurs at 4-6 days of age (Mertens 1977a). Several studies have investigated the extent to which nest cavity microclimate – most often reported in terms of temperature - covaries with avian reproductive success. As young birds are faced with a trade-off between devoting energy to growth and thermoregulation, it is likely that nest thermal environment will influence the nature of this trade-off (Dawson et al. 2005). Thus, offspring in heated tree swallow (*Tachycineta bicolor*) nests survived better during nesting, had larger body mass and longer primary feathers 16 days after hatching (Dawson et al. 2005). The effects of increased temperatures in the nest can also be species-specific: prothonotary warblers

(*Protonotaria citrea*) experienced lower fledging success, whereas Carolina wrens (*Thryothorus ludovicianus*) had reduced body condition (Mueller et al. 2019). Finally, temperature variability in the nest of Crimson Rosella parrots (*Platycercus elegans*) tended to negatively affect fledging success, but in less extreme low temperatures nestlings were heavier (Larson et al. 2015).

All in all, these studies suggest that changes in nest temperatures may affect fitness in wild populations and induce life-history shifts in the long-term (Mueller et al. 2019). The nesting microclimate impact can be mitigated by parental care (Mueller et al., 2019; Ospina et al., 2018), but only to a certain extent, especially in the advanced nesting stages. For example, temperature and humidity can jointly influence the population dynamics of ectoparasites feeding on nesting birds, with important implications for parasite-host relationships (Griebel et al. 2020). Thus, lice load on captive pigeons (*Columba livia*) kept at different relative humidity levels was significantly reduced when ambient humidity was low (Moyer et al. 2002), while tick infestation in adult great tits (*Parus major*) was higher following a week of cool, humid conditions (Heylen et al. 2013).

Importantly, virtually all work to date on humidity variation in avian cavities (Maziarz et al. 2017; Griebel et al. 2020), or of its effect on birds (Moyer et al. 2002; Heylen et al. 2013) uses a measure of relative humidity. It has been stressed that relative humidity is repeatedly misused in ecological studies (Kurta 2014), because it does not predict evaporative water loss from animal’s body like absolute humidity does (see Box 2). In this context, the limited reporting of the role of absolute humidity in the avian literature (and beyond) is puzzling given its crucial role across reproductive stages and its alterations related to anthropogenic actions.

BOX 2

Relative humidity vs absolute humidity

Relative humidity (RH) is the partial pressure of water vapor that actually exists in the air (*Ea*), relative to the saturated vapor pressure (*Es*), which is the maximum pressure possible by water vapor at a given temperature (Kurta 2014):

(1) $RH = Ea / Es * 100$.

Temperature and humidity data loggers like i-Buttons record relative humidity. However as pointed out by Kurta (2014), this measure has limited biological relevance, because under conditions of constant relative humidity, evaporative water loss from the animal’s body can vary by more than 100%, depending on ambient temperature. In support of that, it has been demonstrated that changes in RH do not matter for body temperature, metabolic rate or thermal conductance (Eto et al. 2017). Consequently, there is no effect of RH for thermoregulation. This may be relevant not only for nestlings, but also for birds at the incubation stage due to differential ambient condition-dependent thermoregulation by the incubating parent. The importance of absolute humidity was also shown for the spread of pathogens as established in the biomedical literature [for example in the case of the influenza virus; (Shaman et al. 2010)] or for the spread of bacterial contamination on shell eggs in the poultry industry (Graves & MacLaury 1962). Thus, to identify biologically relevant differences in evaporative water loss between sites, habitats or treatments, absolute humidity measures, accounting for ambient temperature, are recommended. There are simple standard meteorological equations allowing to transform recorded temperature and relative humidity into absolute humidity. To do so, we first calculated saturated vapor pressure (after Alduchov & Eskridge 1996, eq. no.21):

(2) $Es(t) = 6.11 * e^{(17.625 * t)/(243.04 + t)}$,

t : temperature measured by i-Button in °C

We then calculated the actual vapor pressure (*Ea*), which is a measure of absolute humidity of our interest, by transforming the basic formula (1) to:

(3) $Ea= Es * RH/100$,

RH: relative humidity measured by i-Button in %.

Human impact on nesting microclimate

Anthropopressure can alter the reproductive ecology of animals in many ways, and climate change effects can be additionally enhanced by interacting with man-made landscape modifications. One such modification relates to the paucity of natural breeding sites in secondary forests and in urban environments. The default conservation measure initiated by wildlife managers and urban citizens alike are nest-boxes (Tomasevic & Marzluff 2017). They introduce additional opportunities for reproduction (Newton 1994; Mänd et al. 2009), but nest-level breeding success in such man-made structures is varied when compared to natural cavities (Purcell et al. 1997; Mitrus 2003; Czeszczewik 2004; Norris et al. 2018; Sudyka et al. 2021). Nesting microclimate is likely to underlie these differences (Sudyka et al. 2021), suggesting the role of a cavity's thermal properties in reducing the energy expenditure of cavity users (Jarolimek & Vierling 2019; Maziarz 2019). Surprisingly, these fundamental conditions for development have rarely been studied in the context of artificial, human-provided nesting sites. To date only several studies compared microclimate in artificial versus natural nesting sites in mammals (Rowland et al. 2017) and birds (McComb & Noble 1981; Lei et al. 2014; Maziarz et al. 2017; Strain et al. 2021) in various climates. All studies pointed to poor insulation in nest-boxes compared to natural nests, which is likely to be driven by wall thickness (Strain et al. 2021). It has been shown that natural cavities ascertained an optimal environment for incubation with the internal humidity matching well the water vapor eggshell conductance (Mersten-Katz et al. 2012). In contrast, maximum daily temperatures in nest-boxes were on average 8°C higher than in tree-hollows in summer, with a maximum temperature of 52°C recorded in nest-boxes and 38°C in tree-hollows. These thermal shifts brought consequences for maintaining body temperature, as estimated heat-loss required by marsupials was 1.5–2.4 times higher in nest-boxes than in natural tree-hollows (Rowland et al. 2017).

All studies report that natural hollows have larger humidity than nest-boxes (McComb & Noble 1981; Maziarz et al. 2017; Strain et al. 2021). However, none has evaluated absolute humidity at the stage when nestlings are present in the nest in the comparative context. Given the fact that hole-nesting birds currently use very different types of nesting cavities – such as plywood or woodcrete nest-boxes, natural cavities in trees – it is important to assess the microclimate of these different breeding cavities to provide a reference benchmark for reproductive success further recorded in such cavities. Knowledge on the potential of the different types of breeding cavities to provide a homeostatic environment, that is insulated from daily climate variation, is particularly pressing: indeed, ambient air temperature maxima tolerated by cavity-dependent species may soon be exceeded under current climate change projections (Strain et al. 2021; United Nations 2021), which can be further exacerbated in the urban space due to the heat-island effect.

Here, we report the results of a direct comparative study on internal nesting-site microclimate (temperature and absolute humidity) at various stages of the nesting cycle (nest-site choice, nest with offspring and nest post-fledging) in natural cavities and woodcrete nest-boxes within one habitat - an urban, seminatural forest with superabundant natural cavities. This type of nest-box is used by several species of small passerines, mainly blue tits (*Cyanistes caeruleus*) and great tits (*Parus major*), thus we included the natural cavities of both these species for comparisons. At the stage of nest-site choice in early spring, we also included another type of artificial nest-box – plywood, to test if the material used to construct artificial nesting site affects our observations. We predicted that the thermal conditions in nest-boxes of both types (woodcrete and plywood) will be unstable relative to conditions in natural cavities, with larger temperature amplitudes (higher maxima and lower minima) and poorer insulation from ambient conditions. In accordance with previous studies, we also predicted that nest-boxes will provide dryer nesting environment across all nesting stages.

2. Methods

The two types of nesting sites were located within the same study area: Bielany Forest (hornbeam-oak stands with >100 years succession), located in Warsaw, Poland. The measurements took place in 2018-2019 in a subset of randomly chosen natural cavities (mainly located in hornbeams and oaks) and woodcrete nest-boxes (Schwegler 1b) used for active breeding and standard plywood nest-boxes added for the purpose of the comparative measurements (see dimensions in Table 1). For details on fieldwork and study site, please refer to (Sudyka et al. 2021).

Measurements were taken automatically every hour by means of i-Buttons: temperature data loggers (Maxim Integrated DS1921G, range: -40°C to +85°C; precision ± 1°C; resolution: 0.5°C) and temperature and humidity loggers (Maxim Integrated DS1923-F5**,** range: -20 to +85 °C; 0 - 100% RH; precision: ± 0.5°C, ± 5% RH, resolution: 0.5°C, 0.6% RH). The measurements were taken simultaneously by paired data loggers of the same type, positioned inside and outside of each natural cavity/nest-box, to precisely test the buffering against ambient conditions at the nest level. The internal data logger was mounted with a thin transparent wire at the level of the nest. The external logger was hung in a protective white plastic tube to shade from direct sunlight and precipitation (double layer of plastic cups cut open at both sides to permit free air movement, Fig. S1) and placed at the same height and exposure as the cavity/nest-box entrance. In early spring, the set-up included four loggers in: a natural cavity, a woodcrete nest-box, a standard plywood nest-box and an external i-Button to measure ambient conditions precisely at the setup level (Fig. S1). During and after nesting, the set-up included an i-Button inside a natural cavity/woodcrete nest-box and an external i-Button to measure ambient conditions precisely at the setup level (Fig. S1). Since the number of i-Buttons was not sufficient to cover all locations at once, for before and after nesting measurements, we moved the loggers across locations and excluded the first measurement made at each location to allow the ambient conditions to settle (thus the first measurement considered in the analyses was taken between 61 to 119 mins after installation).

Microclimate during nest-site choice in early spring in three types of nesting sites: a natural cavity, a woodcrete nest-box and a plywood nest-box

To quantify internal microclimate conditions at a critical stage of nesting-site choice, we placed i-Buttons measuring temperature and humidity in cavities occupied in the previous breeding season, 2018, by blue tits and great tits (some of them were reused in the 2019 season). We installed the setup at 10 different locations from 7.03.2019 to 17.04.2019 (in 30 nests we analysed 2904 reads for temperature and 2904 for humidity).

Microclimate during nesting in two types of nesting sites: a natural cavity and a woodcrete nestbox

To measure internal ambient conditions at the most relevant time of the breeding cycle, we placed i-Buttons in cavities and nest-boxes at the beginning of the nesting period (at incubation) in May 2018 and May - early June 2019; the measurements lasted for the entire nesting period. The internal data logger was always placed outside of the nest cup, so it never came in direct contact with avian bodies (nestlings or adults), which would distort the microclimate readings. We evaluated nest microclimate in the phase of active nesting (with birds present), starting from the final days of incubation (median days of incubation 3.3, range 0.3 – 8.4), hatching day (day 0), up to 16 days of nestling presence in the nest (to keep the maximum number of nests in this analysis, as the earliest fledging in our subset was at 17 days and we excluded the fledging day). In one nest-box, we recorded microclimate only up to 5 days of nestlings' lives, because the nest was deserted the next day, and in one natural cavity we lost measurements from the stage of 14 days old nestlings up to the fledging day, because the logger got misplaced (no data for this nest from 14 days onwards). Clutch size did not vary between types of nests measured at this stage (mean ± SD: 10 ± 2.1 in natural cavities vs. 10 ± 3.2 in nest-boxes, species pooled as we did not differentiate for species in these analyses due to the low number of nests). In 9 nests we analysed 3906 temperature reads and in 5 nests 1968 humidity reads.

Microclimate in active nests measured soon after fledging

Evaluating microclimate variables during nesting was limited, especially in natural cavities, because of the difficulty of positioning a logger due to small entrances and internal cavity layout resulting in high risk of abandonment. Therefore, to measure nesting site type-specific microclimate in a larger number of replicates, and to allow for between species comparisons, we placed the loggers in nests that were active in the season, soon after fledging in two seasons: 2018 and 2019. To control for phenology and seasonal changes in environmental variables, nests of the same species with similar lay date and clutch size (±1) were matched in pairs (nest-box vs natural cavity) and were simultaneously measured with the same type of logger. In 90 nests we analysed 4198 temperature reads and in 45 nests 2102 humidity reads.

Statistical analysis

We modelled five response variables for both temperature and absolute humidity calculated from values recorded each day (24 hourly measurements see Fig. 1): mean daily (Tmean, Hmean), minimum daily (Tmin, Hmin), maximum daily (Tmax, Hmax), the difference between the daily maximum and minimum (amplitude: Tmax-Tmin, Hmax-Hmin) and minimum difference between conditions inside the nest and ambient subsequently called insulation (minimum daily difference for Tins-Tout and Hins-Hout calculated each hour). Analyses on insulation inform about the degree of buffering against maximum values of ambient conditions each type of nesting site provides (how much lower are the values inside the nest than outside of it at the hottest/most humid daytime).

We used linear mixed models for explaining variation of the abovementioned components of temperature and absolute humidity measured in natural and artificial nests at three nesting stages: at nest-site choice, with young in nest and post-fledging. We introduced nesting site type (as nest type) as a categorical response variable and the following ambient values were fitted as covariates: (i) mean ambient temperature and humidity for Tmean and

Hmean respectively, (ii) minimum ambient temperature and humidity for Tmin and Hmin respectively, (iii) maximum ambient temperature and humidity for Tmax and minimum Tins-Tout and Hmax and minimum Hins-Hout respectively, (iv) ambient temperature and humidity amplitudes for Tmax-Tmin and Hmax-Hmin respectively.

In models when young remained in the nest, we additionally introduced a categorical factor - nestling age - to account for variation in nesting microclimate experienced before the young start to thermoregulate on their own (≤ 6 days of age) and after (> 6 days) (Mertens 1977a). In models of microclimate at the post-fledging stage, we additionally accounted for species (blue tit or great tit), nest exposure (eight cardinal and intercardinal directions) and entrance height (above ground level), but these were retained only if significant as main factors or in interactions.

Additional tests were performed across the three nesting stages pooled together, to quantify the stage-wise differences in overall buffering abilities between the nesting site types (in terms of mean daily differences with ambient temperature and humidity in two separate models; please note that this variable is different than abovementioned insulation, which we describe as buffering against daily temperature maxima- less relevant at early nesting stages). As response variables, we fitted mean daily difference for Tins-Tout and Hins-Hout calculated each hour. We fitted nesting stage and nest type alongside their interaction as fixed factors. From this analysis we excluded plywood boxes, since these were only measured at one of the three nesting stages (therefore the interaction including the three types of nests with nesting stage could not be assessed).

In all models, we controlled for location (nest ID) and date of sampling as random factors (if a nest was used in two years we retained the same nest ID for both years; this was the case of 2 cavities and 8 nest-boxes in models on post-fledging stage). We log-transformed absolute humidity values in all analyses including it as a response to ensure normality of residuals. We checked all interactions of main factors in all models and removed the non-significant ones ($P > 0.05$). We checked all models for overdispersion and multicollinearity (VIF scores in all models never exceed 4) and performed Z-score scaling of all continuous variables for clarity of parameter estimates. Differences in basic nest dimensions of natural cavities and nest-boxes at each nesting stage (Table S2) and for blue tit and great tit nests in natural cavities during post-fledging stage (Table S3) were investigated with Kruskal-Wallis tests. All analyses were performed in R (v.4.0.4) (R Core Team 2021). Sample sizes for each analysis are shown in Table S1.

3. Results

Nesting site type (natural cavity vs. woodcrete nest-box, and in spring plywood nest-box) was found to have a pervasive effect on the internal temperature and humidity of nests throughout the reproductive cycle (Tables 1-3, S4-S6, Figs 2-3, S2-S4). In the main text we show models and figures for maximum values and amplitudes of temperature and humidity as these variables are the most biologically relevant for thermoregulation costs. Models and figures on mean, minimum and insulation are shown in Supplementary Material. The extent of microclimate variation triggered by nesting site type is further detailed below.

Microclimate in early spring during nesting-site choice. We detected considerable differences both in absolute humidity and temperature between natural cavities and artificial nests, regardless of the material they were made from (Tables 2A, 3A, S4A-S6A, Fig. 1A,B). Temperature values of Tmean, Tmax and insulation were higher in nest-boxes of both types relatively to natural cavities (Figs. S2A, 2A, S4A). In contrast, humidity values inferred as Hmean, Hmin, Hmax and insulation and also Tmin were lower in both types of nest-boxes in relation to natural cavities (Figs. S2B, S3A-B, 2B, S4B). Consequently, daily amplitudes of temperature and humidity were higher in nest-boxes (Table 3A), and nest-box temperature and humidity responded more strongly to changing environmental conditions than natural cavities (significant nest type*ambient conditions interaction; Tables 2A, 3A, S4A-S6A; Figs 2A-B, 3A, S2A-B, S3A-B, S4A, with the exception of humidity amplitude and insulation).

Microclimate during nesting - from incubation to fledging. The analysis of thermal profiles during nesting shows contrasting patterns to both remaining stages (Fig. 1C-D). Most importantly, nestling age influenced all temperature and humidity variables, with later nesting stage experiencing higher temperatures and humidity (Tables 2B, 3B, S4B-S6B). The significant interactions between nesting site type and nestling age showed that the increase in temperature and humidity values in nest-boxes after 6 days was higher than the increase in natural cavities (with exception of temperature amplitude and insulation), resulting in values for all temperature variables and Hmean and Hmax after day 6 higher in nest-boxes than in natural cavities (Figs 4, S5). Moreover, the formal analysis showed that there were no overall differences (regardless nestling age) between nesting site types in Hmean and humidity insulation (Table S4B, S6B) and also Tmin (Table S5B). Similarly to remaining nesting stages, temperatures (tested as Tmean, Tmax and insulation) were overall higher in nest-boxes relatively to natural cavities (Tables S4B, 2B, S6B). However, in case of Hmin and Hmax, the overall values (regardless of nestling age) were only slightly lower in nest-boxes relative to natural cavities (Tables 2B, S5B). Consequently, nest-boxes exhibited, compared to natural cavities, higher daily amplitudes in temperature and humidity (Table 3B). Importantly, nest-box temperatures responded considerably stronger to changing environmental conditions than natural cavities (significant nest type*ambient conditions interactions; Tables 2B, 3B, S4B, S6B, Figs. 2C, 3C, S2C, S4C, with the exception of Tmin). In terms of humidity, this response did not vary between nest types (non-significant nest type*ambient conditions interactions for all humidity values Tables 2B, 3B, S4B-S6B).

Microclimate in occupied nests measured soon after fledging. The variation in absolute humidity and temperature between natural cavities and nest-boxes detected at this stage was largely consistent with the differences during nest-site choice (when birds were not constantly present in nests; Tables 2C, 3C, S4C-S6C Fig. 1E,F). Values of temperature (Tmean, Tmax, insulation) were higher in nest-boxes relatively to natural cavities (Figs S2C, 2C, S4C). Likewise, humidity values (Hmean, Hmin, Hmax and insulation) and Tmin were lower in nest-boxes in relation to natural cavities (Figs. S2F, S3E-F, 2F, S4F). Moreover, daily amplitudes of temperature and humidity were higher in nest-boxes (Table 3C), and nest-box temperature and humidity responded more strongly to changing environmental conditions than natural cavities (significant nest type*ambient conditions interactions; Table 2C, 3C, S4C-S6C, Figs 2E,F, 3E,F, S2E, S3E, S4E, with exception of Hmean, Hmin and humidity insulation). Interestingly, we also detected species-specific differences for natural cavity thermal profiles in terms of temperature (Tmean, Tmax and temperature amplitude) and humidity amplitude. Thus, great tit cavities had greater buffering abilities than blue tit cavities: they temperature grew less in response to increasing ambient temperatures (significant interaction nest type*ambient conditions*species; Tables S4C, 2C, 3C, Fig. S6). Furthermore, the nest type*species interaction for humidity amplitude denotes that the increase of amplitude in nest-boxes was lower for great tits than for blue tits, pointing to a more stable humidity in great tit cavities (Table 3C). The height at which the entrance of the nest was located was always positively correlated with temperature and absolute humidity of nests (Tables 2C, 3C, S4C-S6C), but nest exposure did not show any effect on microclimate variables (for exposure in all models P>0.15)

Differences with mean daily ambient conditions across three nesting stages.

There were marked differences across the nesting stages and nest types in both temperature and humidity (Table S7, Fig. 5). The mean daily difference from ambient temperature was invariably higher in nest-boxes than in natural cavities, in particular when young remained in the nest, as shown by the significant interaction (nest type*nesting stage; Table S7A, Fig. 5A). Absolute humidity difference was on average lower in nest-boxes across all stages, but when young remained in the nest, the increase in difference from ambient humidity relative to other nesting stages was higher in nest-boxes than in natural cavities (significant nest type*nesting stage, Table S7B, Fig. 5B).

4. Discussion

Here, we simultaneously compared microclimatic conditions of natural cavities and artificial nest-boxes used by hole-nesting birds in a temperate climate across stages in the breeding cycle. For the first time, in the context of such comparisons, we report on internal microclimate conditions when chicks are present in the nest, and in terms of absolute humidity. Importantly, and in contrast to previous comparative studies that reported microclimatic conditions in empty nests (but see Lei et al. 2014) and focused on the biologically less relevant relative humidity, we show that active nest-boxes were not only invariably warmer but, after young start to thermoregulate, were also more humid than natural cavities in terms of maxima and means.

Our results confirm that nest-boxes were thermally unstable relatively to natural cavities (McComb & Noble 1981; Maziarz et al. 2017; Strain et al. 2021). Higher nest-box temperature maximums (Table 2), larger amplitudes (Table 3) and worse insulation from maximum ambient temperatures (Table S6) in comparison to natural cavities were observed across all nesting stages. Microclimate buffering against ambient conditions was dependent on nest-type: nest-box temperatures responded more strongly to changing environmental conditions than natural cavities across all nesting stages (Tables 2,3, S4-S6, Figs. 2, 3, S2-S4). When chicks were not present in the nest (irrespectively if during nest-site choice or post- fledging), the thermal profile of the nest-box interior closely followed the hourly variation of ambient temperature (Fig. 1A, B, E, F). Before nesting, the same pattern was observed in the two most widely used types of nest-boxes (plywood and woodcrete), suggesting the material used for construction did not greatly affect insulating qualities (Table 2A, 3A, S4A-S6A). However, when nestlings were present in the nest, we observed that the temperature profile of nest-boxes, whilst still mirroring ambient temperature variation (Fig. 1C), increased on average by over 5°C relatively to outside (Table 1, Fig. 5A). Absolute humidity was consistently higher at nest-site choice and post-fledging in natural cavities (Table 1, 2A,C, 3A,C, S4-S6A,C, Figs 1B, F). Surprisingly, humidity increased dramatically in nest-boxes during nesting (Fig. 1D, Fig. 5B), in particular after the nestlings start to thermoregulate on their own (Fig. 4B, S5B). Such a raise in both temperature and humidity can have profound biological consequences for nestling development. Despite the modest number of nests measured across the breeding stage when nestlings were present inside the nests (Table S1), the daily microclimatic patterns we noted were largely coherent within nesting site types and the differences were statistically marked (Table 2, 3, S4-S6), to the extent that it is not likely the result would be qualitatively affected if more nests were included.

Internal nest-site microclimate is important primarily due to varying metabolic and evaporative water requirements not only for young and adults, but also during incubation. Consequently, birds can be affected at all nesting stages, but at each stage the impact on fitness can act differently. Pre-hatching, at nest building and incubation, high relative temperatures during the day and low at night may provide erroneous cues for animals, resulting in shifts of breeding phenology. For example, earlier lay date was often reported in nest-boxes (Purcell et al. 1997; Czeszczewik 2004) but these were also frequently abandoned at building (Sudyka et al. 2021). The warm temperature in nest-boxes during the day provides a cue for building onset, but after sunset they cool down rapidly, which can be a signal to discontinue. As the breeding cycle progresses, the high temperature amplitudes in nest-boxes can be mitigated at the cost of increasing parental incubation or brooding. Incubation is also shorter in higher temperatures (Ospina et al. 2018; Mueller et al. 2019), and females reduce brooding in heated nests

(Rodríguez & Barba 2016). Indeed, in our study blue tits and great tits using nest-boxes started incubating earlier than in natural cavities, even before clutch completion (Sudyka et al. 2021). This can lead to increased hatching asynchrony with possible consequences on survival of the late hatched nestlings (Stenning 1996).

Different consequences of microclimate shifts may come to play post-hatching, especially after the chicks start to thermoregulate on their own. Indeed, we observed increases in internal nest temperature after 6 days of age in relation to ambient weather conditions in both types of nests (Figs 4, S5). The immediate risk of hyperthermia and dehydration is the most important factor shaping the upper limit of the range of temperature tolerance (Mertens 1977a) and high temperatures are a larger biological constraint than low ones (Andrew et al. 2017). Temperature tolerance in animals can be further lowered by high humidity, compromising effective conductance and evaporative water loss especially in nestlings, in which thermoregulatory mechanisms are still developing (Rodríguez & Barba 2016). This effect of heating up was further enhanced during the hottest part of the day (11:00-18:00, Fig. 1B-C) and in time (Figs 4, S5) with the development of thermoregulatory abilities and plumage of nestlings (Mertens 1977b). The higher absolute humidity in nest-boxes experienced in later nesting stages (Fig. 4B) may further compromise thermoregulation (Weathers 1997). Such unfavourable microclimate could be mitigated by adopting a thermoregulatory energy-saving behaviour, e.g. nestlings moving away from each other to facilitate evaporative water loss. However, this behaviour may be hampered in nest-boxes, because internal volumes are much smaller than in natural cavities (Sudyka et al. 2021). Our results may indicate that the lower fitness (body condition or mortality) of second broods (Salaberria et al. 2014) that was often observed in nest-boxes may also stem from the unfavorable nest-box microclimate as the season progresses (and ambient temperatures raise). Such deteriorated internal microclimates can also have developmental and even fitness consequences in species with lower tolerance for hyperthermia and lower ability to passively dissipate heat i.e. smaller body size (McKechnie & Wolf 2010). Thus, the fitness consequences of nest-box provisioning can be species-specific. In accordance with this, we observed a negative impact of nest-boxes in blue tits, which were found to have a lower hatching and fledging success in nest-boxes relative to those breeding in natural cavities and in consequence fewer young fledged, but no apparent effects in the larger great tits (Sudyka et al. 2021). Additionally, great tit cavities displayed better buffering qualities than the ones occupied by blue tits (Fig. S6), particularly when ambient temperatures were raising. The differences likely stem from the fact that great tit cavities were located lower above the ground (thus less exposed to insolation, Table S3), and in general have larger volumes than the blue tits cavities (Sudyka et al. 2021, Table S3).

Conclusion and outlook

Changing environmental temperatures will alter the selective pressures acting on all animals by distorting energy and water budgets (Boyles et al. 2011). As revealed in this study, this effect is likely to be amplified by inadequate conditions of nesting sites. The microclimatic conditions in nesting-hollows, still allowing to remain within physiologically tolerable thresholds in most temperate environments, may soon be exceeded under the current climate change predictions (United Nations 2021) – particularly in artificial cavities such as nest-boxes. Typically, evaporative water loss is relatively constant at low and moderate ambient temperatures, but increases rapidly at ambient temperatures approaching or exceeding body temperature (McKechnie & Wolf 2010). In Passeriformes, body temperature is 38.9 ± 0.87 (mean ± SD) for resting levels (Prinzinger et al. 1991). With the projected increase in ambient temperatures [ca. 4°C (United Nations 2021)] and increase in temperature within nest-boxes [more than 5°C in our study or more depending on the nest-box type (Griffiths et al. 2017; Rowland et al. 2017; Strain et al. 2021)], this threshold is likely to be exceeded on many occasions during nesting, in particular when nestlings have grown enough to thermoregulate on their own - and by doing so - actively altering the nest microclimate. We thus expect that projected temperature increases are likely to enhance non-selective mortality risks and resulting reproductive failure affecting entire populations of birds breeding in nest-boxes, phenomena that have been already observed in some monitored populations (Charmantier, pers. comm). As these mortality risks driven by temperature are highly dependent on humidity variation, more data on absolute humidity in breeding cavities is needed, if only to allow for the design of breeding cavities that are more resilient to the negative effects of temperature and humidity variation reported from regular nest-boxes (this study). To precisely quantify water requirements while nesting in a variety of climates and nest types, there is a need to evaluate absolute humidity and temperature alongside measurements of core body temperature and body surface temperature of nestlings to determine the vapor pressure of the body fluids (Kurta 2014).

In contrast to variation observed in artificial nest-boxes (plywood or woodcrete), it is important to point out that at all breeding stages, natural cavities provided a thermally stable microenvironment, as daily variations were similar at all stages. Importantly, even the presence of nestlings did not distort this pattern, which strongly contrasts with the shift of microclimate observed in nest-boxes with young in relation to remaining nesting stages (Fig. 1). Internal microclimatic shifts are mitigated in natural cavities three times more effectively than in nest-boxes (in terms of the mean daily difference from ambient temperature, Table 1, Fig. 5A). In other words, this property of natural breeding sites could be compared to natural air-conditioning.

Our results provide ground data of fundamental importance for the need to re-evaluate the use of nest-boxes as a default conservation tool, particularly in hot climates and in the face of climate change, also in the climates now considered as moderate. At the bare minimum, the careful design of nest-boxes that mitigates acute heat stress and dehydration is required. Other studies showed that thermal properties of nest-boxes can be ameliorated by large internal volumes, thicker walls (Maziarz 2019; Strain et al. 2021), avoiding direct sunlight, carefully choosing exposure [avoiding north and west, the orientations receiving the greatest amount of solar radiation during the hottest time of the day (Griffiths et al. 2017)] and surface reflectance [painting boxes to a colour ensuring higher reflectance e.g. white (Griffiths et al. 2017), and avoiding dark colours, especially in the face of temperature extremes (Rueegger 2019)]. However, it is not sufficient to manipulate nest-box size and shape, because these parameters have no detectable influence on the internal temperature fluctuations (Ellis 2016). For example, nest-boxes constructed of boles of aspen replicated the microclimate of natural cavities more closely and appeared to have slightly better insulating properties than traditional plywood nest-boxes (Griebel et al. 2020). Nevertheless, such a well-thought design requires large investments and careful planning, and the best nest-box will not provide the optimal nesting climate to such extent as a natural hollow will. Thus, the much simpler and cost-efficient solution is to protect ecosystems that provide natural nesting-hollows and old-growth tree stands, which are likely to be more effective in the long-run. This need is even more pressing in an urban context, as higher temperatures generated by the urban heat-island effect are likely to further accelerate risks generated by global warming.

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Author Contributions

JS and IDL developed the concept; JS and IDL collected the data in the field; JS analysed the data; JS led the writing of the manuscript with contributions from MS. All authors contributed critically to the drafts, agreed to be held accountable for the content therein and approved the final version of the manuscript.

Ethics

Permit numbers to work on the natural-cavity site: WPN-I.6401.80.2017.ŁM and WPN-I.6205.53.2017.AS and on the nest-box site: WPN-I.6401.515.2017.KZ and WPN-I.6205.227.2017.AS issued by The Regional Directorate for Environmental Protection in Warsaw (RDOŚ) and LM-W.LO.400.88.2017.DC1460 issued by Lasy Miejskie – Warszawa.

Conflict of Interests

We declare no conflict of interests.

Data Archiving Statements

Data are available in the online Supporting Information, and upon publication via fighshare.

TABLES

Table 1 Basic microclimate variables for the different types of nesting sites taken at three nesting stages – before, during and after breeding. Each stage was analysed separately (see Table 2-3, S4-S6 for results). Linear dimensions of each type of nest in cm on the graphs below; for natural cavities collective medians (and ranges) shown (for details on nest dimensions at each stage see Table S2 and S3). TEMP: temperature in °C and HUM: absolute humidity as vapor pressure in hPa.

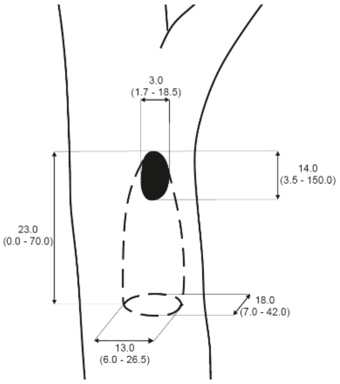
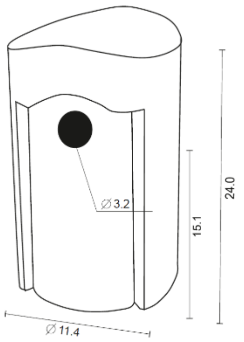
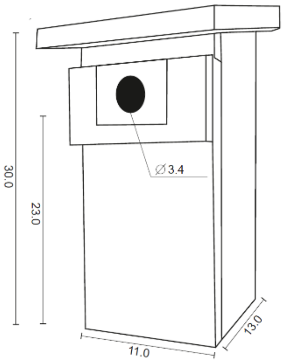
nesting stage	variable	natural cavity 		woodcrete box 		plywood box 		ambient	
		TEMP	HUM	TEMP	HUM	TEMP	HUM	TEMP	HUM
nest-site choice	Mean ± SD	7.2 ± 3.06	10.7 ± 2.28	7.6 ± 4.55	6.9 ± 1.82	7.7 ± 4.54	6.5 ± 2.06	7.4 ± 4.43	7.0 ± 1.71
	Minimum	-1.0	5.5	-1.9	3.4	-1.5	2.9	-2.0	3.5
	Maximum	16.6	17.4	21.1	12.7	21.6	13.1	19.6	11.7
	Mean difference with ambient ± SD (min-max)	-0.21 ± 3.549 (-11.0-8.6)	3.73 ± 2.502 (-2.2-10.7)	0.20 ± 0.845 (-6.5-4.1)	-0.07 ± 1.170 (-3.4-3.6)	0.27 ± 1.051 (-7.0-4.5)	-0.45 ± 1.623 (-5.1-4.4)	.	.
young in nest	Mean ± SD	16.5 ± 3.76	19.7 ± 4.62	20.3 ± 5.02	22.3 ± 6.45	.	.	15.0 ± 4.57	13.2 ± 4.16
	Minimum	5.5	9.2	3.0	7.7	.	.	1.0	4.6
	Maximum	29.8	36.6	33.5	40.1	.	.	26.9	23.4
	Mean difference with ambient ± SD (min-max)	1.72 ± 4.127 (-9.5-11.2)	7.07 ± 2.877 (-0.7-20.0)	5.20 ± 3.218 (-2.5-19.5)	8.17 ± 5.247 (-2.7-25.7)	.	.	.	.
post-fledging	Mean ± SD	19.4 ± 3.28	25.2 ± 5.85	20.5 ± 4.61	21.9 ± 6.36	.	.	20.5 ± 4.86	17.1 ± 4.11
	Minimum	11.5	13.8	8.0	9.8	.	.	7.0	5.2
	Maximum	27.8	42.8	31.6	41.6	.	.	33.8	28.5
	Mean difference with ambient ± SD (min-max)	-1.01 ± 3.582 **(-12.0-8.3)**	8.16 ± 4.603 **(-3.2-24.6)**	-0.08 ± 1.591 **(-3.5-16.5)**	4.83 ± 5.248 **(-4.6-22.0)**	.	.	.	.

Table 2 Linear mixed models inferring variation of daily maximum temperature and absolute humidity measured in natural and artificial nests across three nesting stages: (A) nest-site choice, (B) with young in nest and (C) post-fledging. At nest-site choice (A) two types of artificial nests-boxes were considered: woodcrete and plywood. When young in nest (B) nestling age was considered, and post-fledging (C) we additionally accounted for species, nest exposure and height. Natural cavities, nestling age less than 6 days and blue tit were used as a reference for parameter estimates and all estimates are shown after Z-score scaling of continuous variables. We present the final models with nonsignificant main factors and interactions removed (apart from the focal nest type). Sample sizes for each analysis are shown in Table S1. Significant differences ($P < 0.05$) are indicated in bold, and trends ($P < 0.20$) in italics.

Nesting stage	**Predictor**	**Effect**	**TEMPERATURE**				**HUMIDITY**			
			χ^2	**df**	**Pr(>** χ^2**)**	**Estimate ± SE**	χ^2	**df**	**Pr(>** χ^2**)**	**Estimate ± SE**
			Variance for random effects				***Variance for random effects***			
A) nest-site choice	nest type		**218.244**	**2, 102**	**<0.0001**		**187.793**	**2, 109**	**<0.0001**	
		woodcrete box				0.778 ± 0.060				-1.157 ± 0.101
		plywood box				0.752 ± 0.060				-1.240 ± 0.101
	ambient		**289.626**	**1, 56**	**<0.0001**		**43.843**	**1, 55**	**<0.0001**	
		maximum ambient				0.472 ± 0.056				0.124 ± 0.086
	type* ambient		**62.635**	**2, 102**	**<0.0001**		**24.879**	**2, 109**	**<0.0001**	
		woodcrete*ambient				0.403 ± 0.060				0.417 ± 0.101
		plywood*ambient				0.419 ± 0.060				0.457 ± 0.089
	Random effect	*date*	*0.030*				*0.046*			
		location	*0.052*				*0.154*			
		Residual	*0.091*				*0.261*			
B) young in nest	nest type		**21.454**	**1, 7**	**<0.0001**		**4.099**	**1, 3**	**0.043**	
		nest-box				1.055 ± 0.254				-0.073 ± 0.192
	ambient		**231.530**	**1, 54**	**<0.0001**		**71.456**	**1, 43**	**<0.0001**	
		maximum ambient				0.512 ± 0.052				0.604 ± 0.135
	nestling age		**183.767**	**1, 93**	**<0.0001**		**46.138**	**1, 59**	**<0.0001**	
		>6 days				0.699 ± 0.079				0.335 ± 0.193
	type* ambient		**19.822**	**1, 111**	**<0.0001**		0.185	1, 45	0.667	
		nest-box*ambient				0.286 ± 0.064				0.148 ± 0.155
	ambient* nestling age		**4.166**	**1, 113**	**0.041**		**7.069**	**1, 57**	**0.008**	
		ambient*>6 days				-0.130 ± 0.063				-0.073 ± 0.214
	type* nestling age		**5.554**	**1, 125**	**0.018**		**18.727**	**1, 45**	**<0.0001**	
		nest-box*>6 days				0.253 ± 0.107				1.141 ± 0.245
	type*ambient* nestling age						**5.105**	**1, 56**	**0.024**	
		nest-box*ambient*>6 days								-0.647 ± 0.286

	Random effect	*date*	*0.020*				*0.059*			
		location	*0.132*				*0.014*			
		Residual	*0.108*				*0.171*			
C) post-fledging	nest type		**40.261**	**1, 64**	**<0.0001**		**5.345**	**1, 20**	**0.021**	
		nest-box				0.510 ± 0.128				-0.271 ± 0.116
	ambient		**1020.80**	**1, 116**	**<0.0001**		**53.843**	**1, 72**	**<0.0001**	
		maximum ambient				0.336 ± 0.039				0.508 ± 0.096
	height		**16.910**	**1, 68**	**<0.0001**		**13.800**	**1, 32**	**0.0002**	
		height				0.198 ± 0.048				0.222 ± 0.060
	species		1.167	1, 103	0.280					
		great tit				-0.206 ± 0.136				
	type* ambient		**456.940**	**1, 177**	**<0.0001**		*3.497*	*1, 49*	*0.061*[a]	
		nest-box*ambient				0.622 ± 0.049				0.190 ± 0.102
	type*species		1.187	1, 96	0.276					
		nest-box*great tit				0.170 ± 0.156				
	ambient*species		0.034	1,196	0.855					
		ambient*great tit				-0.130 ± 0.054				
	type*ambient* species		**10.921**	**1,176**	**0.001**					
		nest-box *ambient*great tit				0.232 ± 0.070				
	Random effect	*date*	*0.011*				*0.224*			
		location	*0.174*				*0.071*			
		Residual	*0.044*				*0.215*			

a) we retained the interaction as it was close to significance, and the removal of this interaction did not change the model outcome for the remaining main factors: nest type: χ^2= 5.456, p = 0.019; ambient maximum humidity: χ^2= 54.487, p < 0.0001 and height: χ^2= 14.8719, p = 0.0001.

Table 3 Linear mixed models inferring variation of daily amplitudes of temperature and absolute humidity measured in natural and artificial nests across three nesting stages: (A) nest-site choice, (B) with young in nest and (C) post-fledging. At nest-site choice (A) two types of artificial nests-boxes were considered: woodcrete and plywood. When young in nest (B) nestling age was considered, and post-fledging (C) we accounted for species, nest exposure and height. Natural cavities, nestling age less than 6 days and blue tit were used as a reference for parameter estimates and all estimates are shown after Z-score scaling of continuous variables. We present the final models with nonsignificant main factors and interactions removed (apart from the focal nest type). Sample sizes for each analysis are shown in Table S1. Significant differences ($P < 0.05$) are indicated in bold, and trends ($P < 0.20$) in italics.

Nesting stage	**Predictor**	**Effect**	**TEMPERATURE**				**HUMIDITY**			
			χ^2	**df**	**Pr(>** χ^2**)**	**Estimate ± SE**	χ^2	**df**	**Pr(>** χ^2**)**	**Estimate ± SE**
			Variance for random effects				***Variance for random effects***			
A) nest-site choice	nest type		**625.410**	**2, 101**	**<0.0001**		**39.614**	**2, 103**	**<0.0001**	
		woodcrete box				1.128± 0.058				0.581 ± 0.131
		plywood box				1.215 ± 0.058				0.797 ± 0.131
	ambient		**257.440**	**1, 64**	**<0.0001**		*3.595*	*1, 70*	*0.058*	
		ambient amplitude				0.135 ± 0.050				0.159 ± 0.084
	type* ambient		**180.320**	**2, 101**	**<0.0001**					
		woodcrete*ambient				0.674 ± 0.058				
		plywood*ambient				0.676 ± 0.058				
	Random effect	*date*	*0.022*				*0.236*			
		location	*0.028*				*0.166*			
		Residual	*0.085*				*0.438*			
B) young in nest	nest type		**24.488**	**1, 7**	**<0.0001**		*3.611*	*1, 3*	*0.057*	
		nest-box				1.369 ± 0.277				0.682 ± 0.520
	ambient		**169.182**	**1, 60**	**<0.0001**		**8.684**	**1, 52**	**0.003**	
		ambient amplitude				0.248 ± 0.055				0.229 ± 0.078
	nestling age		**6.134**	**1, 95**	**0.013**		**12.294**	**1, 79**	**0.0005**	
		>6 days				0.149 ± 0.071				0.241 ± 0.200
	type* ambient		**83.480**	**1, 132**	**<0.0001**					
		nest-box*ambient				0.635 ± 0.070				
	ambient* nestling age		**6.503**	**1, 89**	**0.011**					
		ambient*>6 days				-0.191 ± 0.075				
	type* nestling age						**5.316**	**1, 78**	**0.021**	
		nest-box*>6 days								0.695 ± 0.302
	Random effect	*date*	*0.002*				*0.039*			
		location	*0.160*				*0.282*			
		Residual	*0.177*				*0.397*			

C) post-fledging	nest type		**338.239**	**1, 61**	**<0.0001**		**61.160**	**1, 25**	**<0.0001**	
		nest-box				1.123 ± 0.099				0.684 ± 0.209
	ambient		**295.211**	**1, 96**	**<0.0001**		**25.454**	**1, 107**	**<0.0001**	
		ambient amplitude				0.299 ± 0.051				0.180 ± 0.076
	height		**7.650**	**1, 72**	**0.006**		**9.144**	**1, 36**	**0.002**	
		height				0.099 ± 0.036				0.223 ± 0.074
	species		0.552	1, 102	0.457		0.523	1, 49	0.470	
		great tit				-0.186 ± 0.099				-0.466 ± 0.217
	type* ambient		**318.127**	**1, 161**	**<0.0001**		**10.448**	**1, 68**	**0.001**	
		nest-box*ambient				0.625 ± 0.068				0.295 ± 0.091
	type*species		*3.095*	*1, 94*	*0.079*		**5.433**	**1, 31**	**0.020**	
		nest-box*great tit				0.239 ± 0.129				0.645 ± 0.277
	ambient*species		*3.351*	*1, 218*	*0.067*					
		ambient*great tit				-0.193 ± 0.063				
	type*ambient* species		**7.050**	**1, 163**	**0.008**					
		nest-box *ambient*great tit				0.232 ± 0.087				
	Random effect	*date*	*0.035*				*0.202*			
		location	*0.064*				*0.115*			
		Residual	*0.100*				*0.230*			

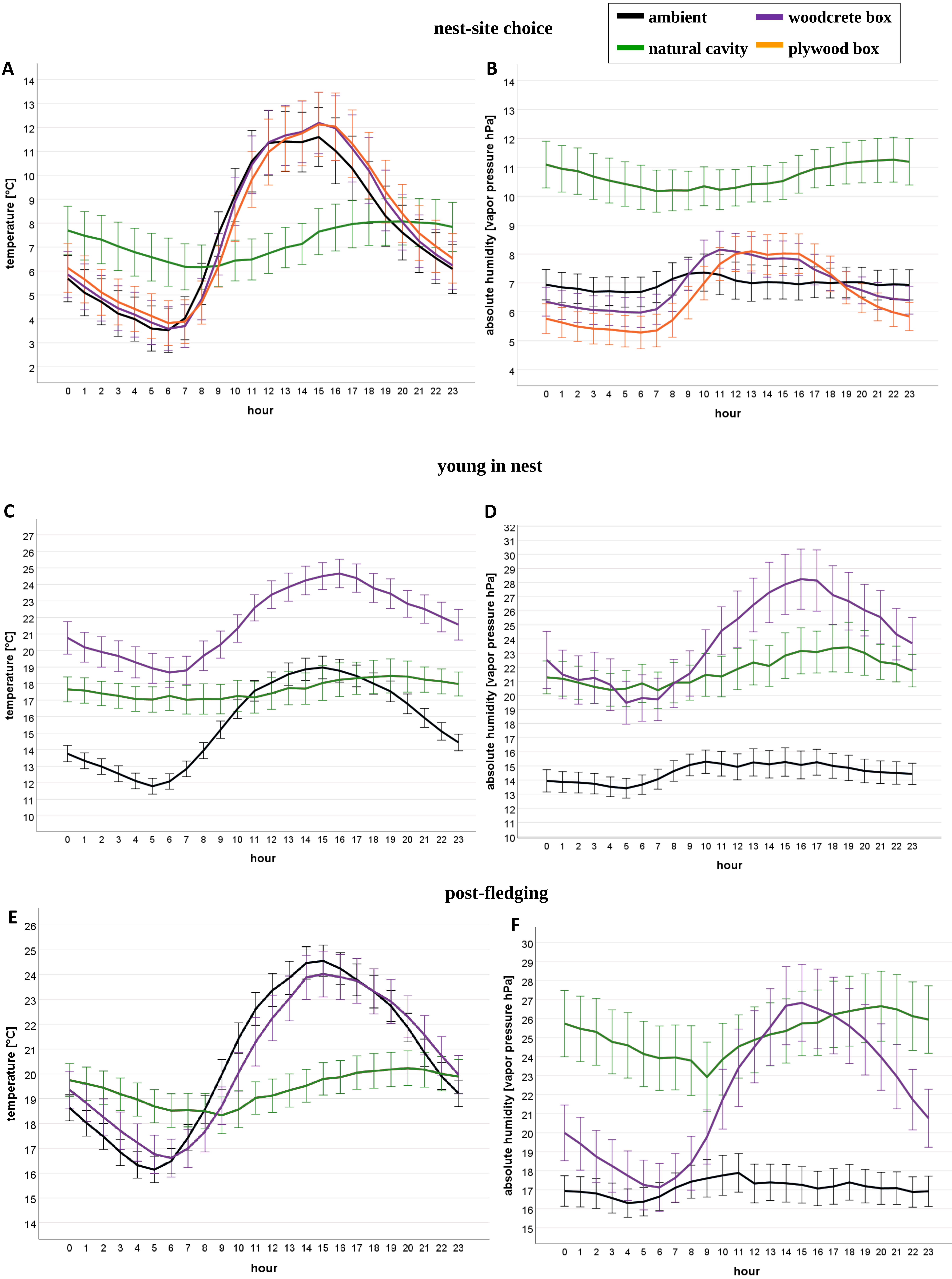


Figure 1 Daily changes in hourly mean temperature (A, C, E) and absolute humidity (B, D, F) across three nesting stages: at nest-site choice (A, B), in the presence of young in nest (C, D) and post-fledging (E, F). Raw data, mean ± 95%CI are shown, sample sizes for all graphs see Table S1. These data were used to calculate daily mean, maximum, minimum, amplitude and insulation.

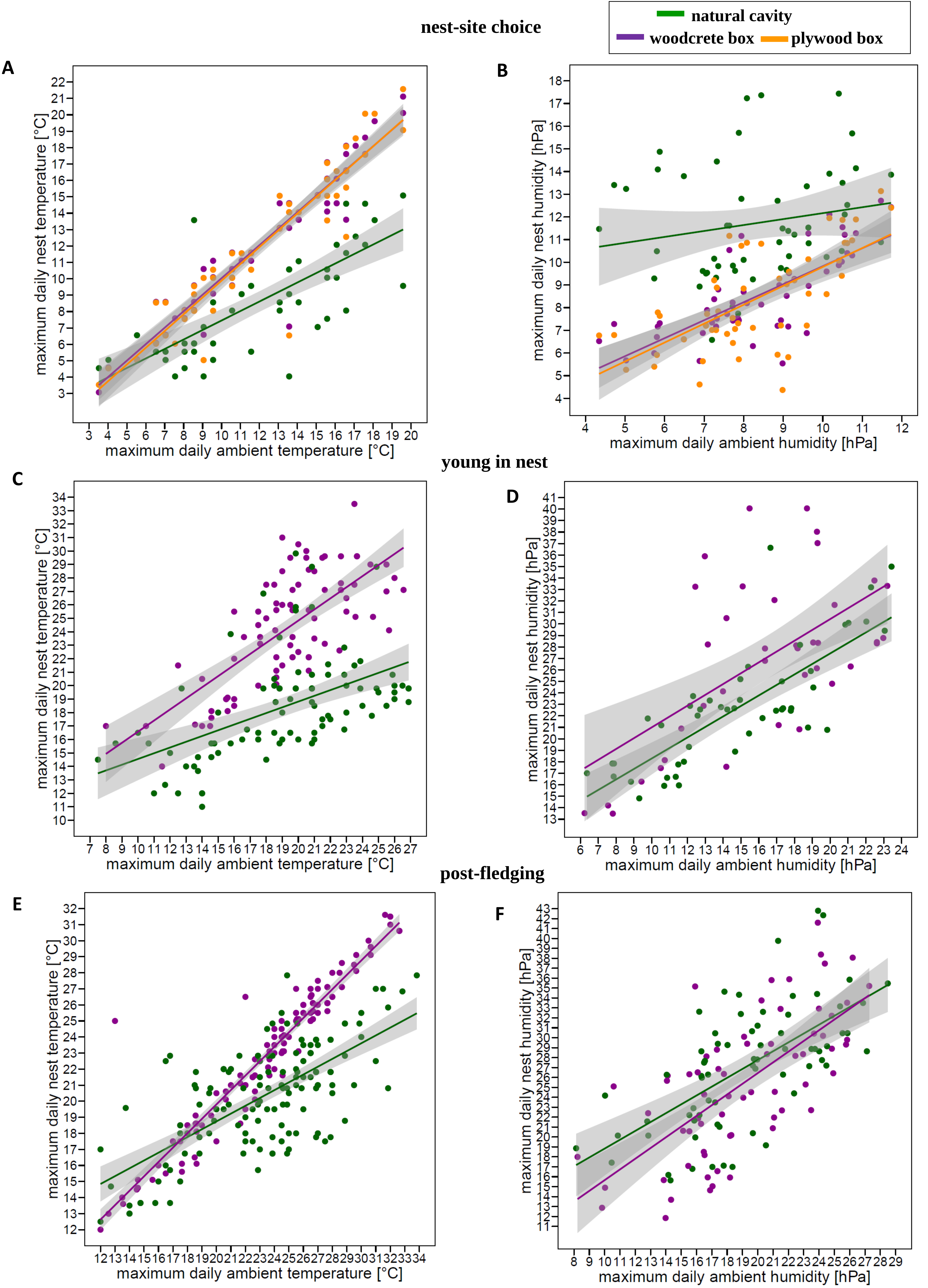


Figure 2 Variation in maximum daily nest temperature (A, C, E) and absolute humidity as vapor pressure (B, D, F) across three nesting stages: at nest-site choice (A, B), in the presence of young in nest (C, D) and post-fledging (E, F). Raw data points with regression lines ± 95%CI (shaded in grey) are shown. For statistical results see Table 2 and for sample sizes Table S1. In graph D the interaction of nest type with maximum ambient humidity was non-significant, but we show it for the consistency of data presentation.

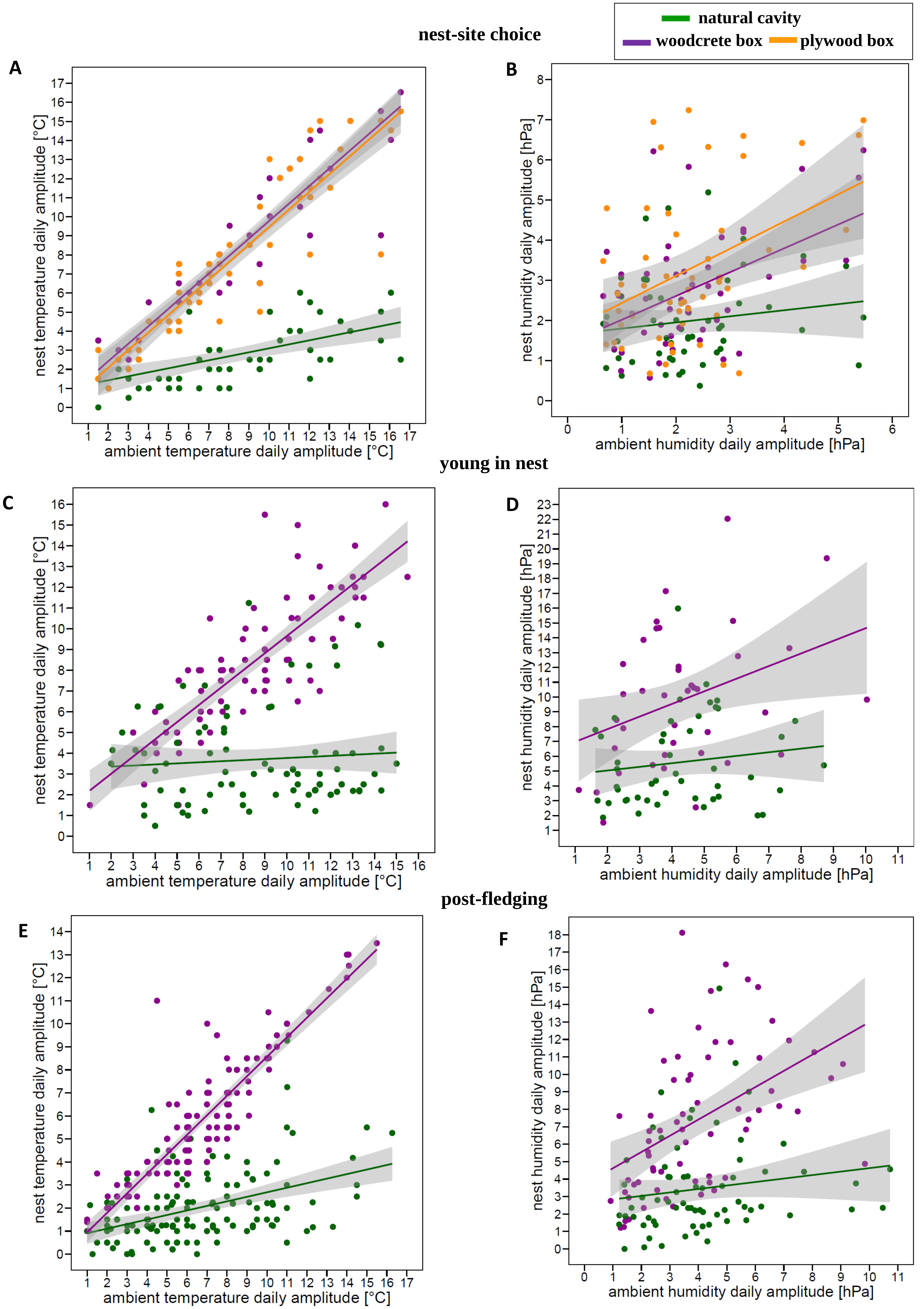


Figure 3 Variation in daily amplitudes of nest temperature (A, C, E) and absolute humidity (B, D, F) across three nesting stages: at nest-site choice (A, B), in the presence of young in nest (C, D) and post-fledging (E,F). Raw data points with regression lines ± 95%CI (shaded in grey) are shown. For statistical results see Table 3 and for sample sizes Table S1. In graph D the interaction of nest type with ambient humidity amplitude was non-significant, but we show it for the consistency of data presentation.

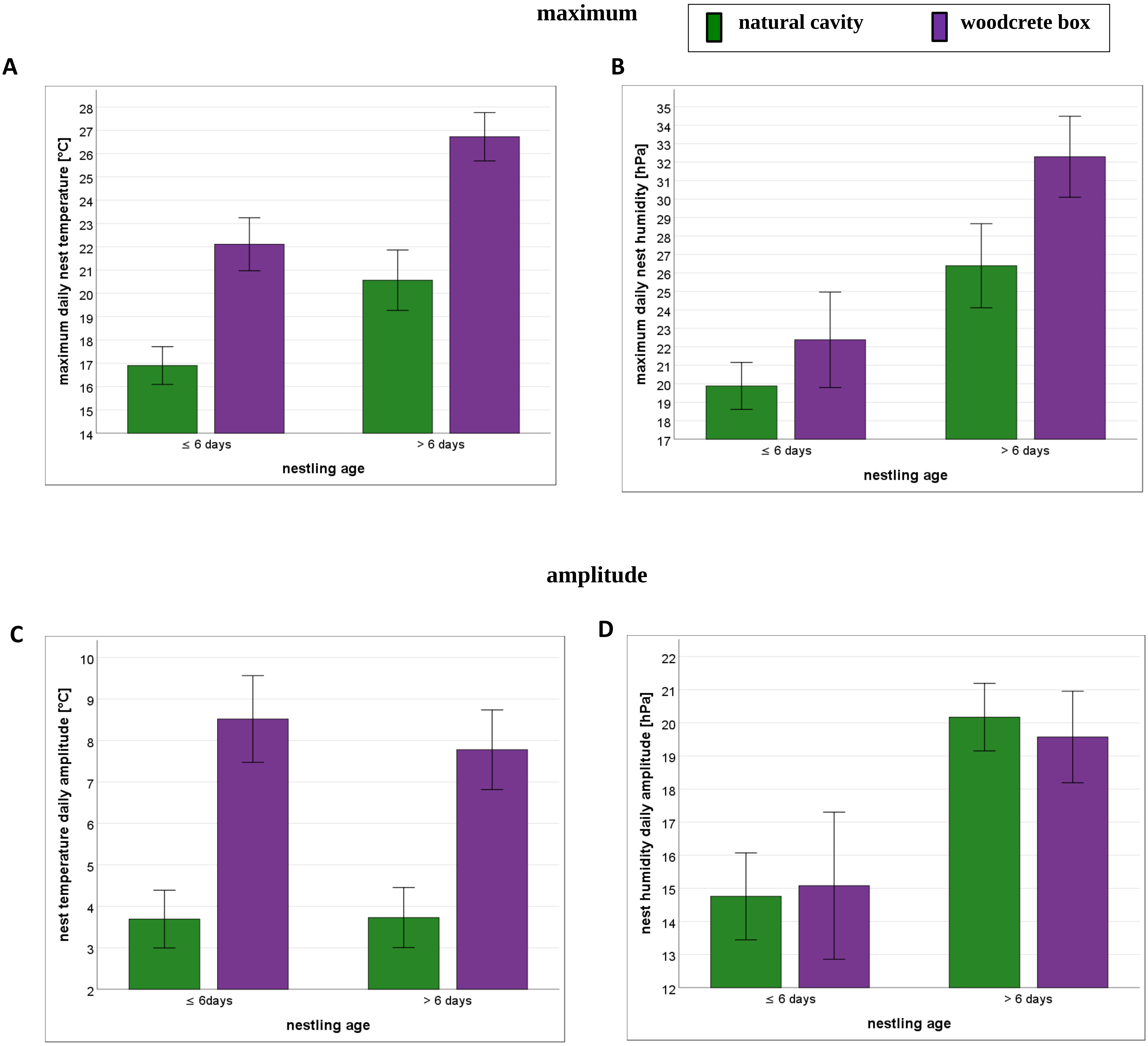


Figure 4 Maximum daily nest temperature (A) and absolute humidity (B) and daily amplitudes of nest temperature (C) and absolute humidity (D) with young in nest stage in natural cavities (green) and in woodcrete nest-boxes (dark magenta). The graphs show nest-type-wise differences according to nestling age: before 6 days when nestlings are not yet able to thermoregulate on their own and after 6 days when this mechanism is on. Raw data ± 95%CI are shown. For statistical results see Table 2B and 3B and for sample sizes Table S1. In graph C the interaction of nest type with nestling age was non-significant, but we show it for the consistency of data presentation.

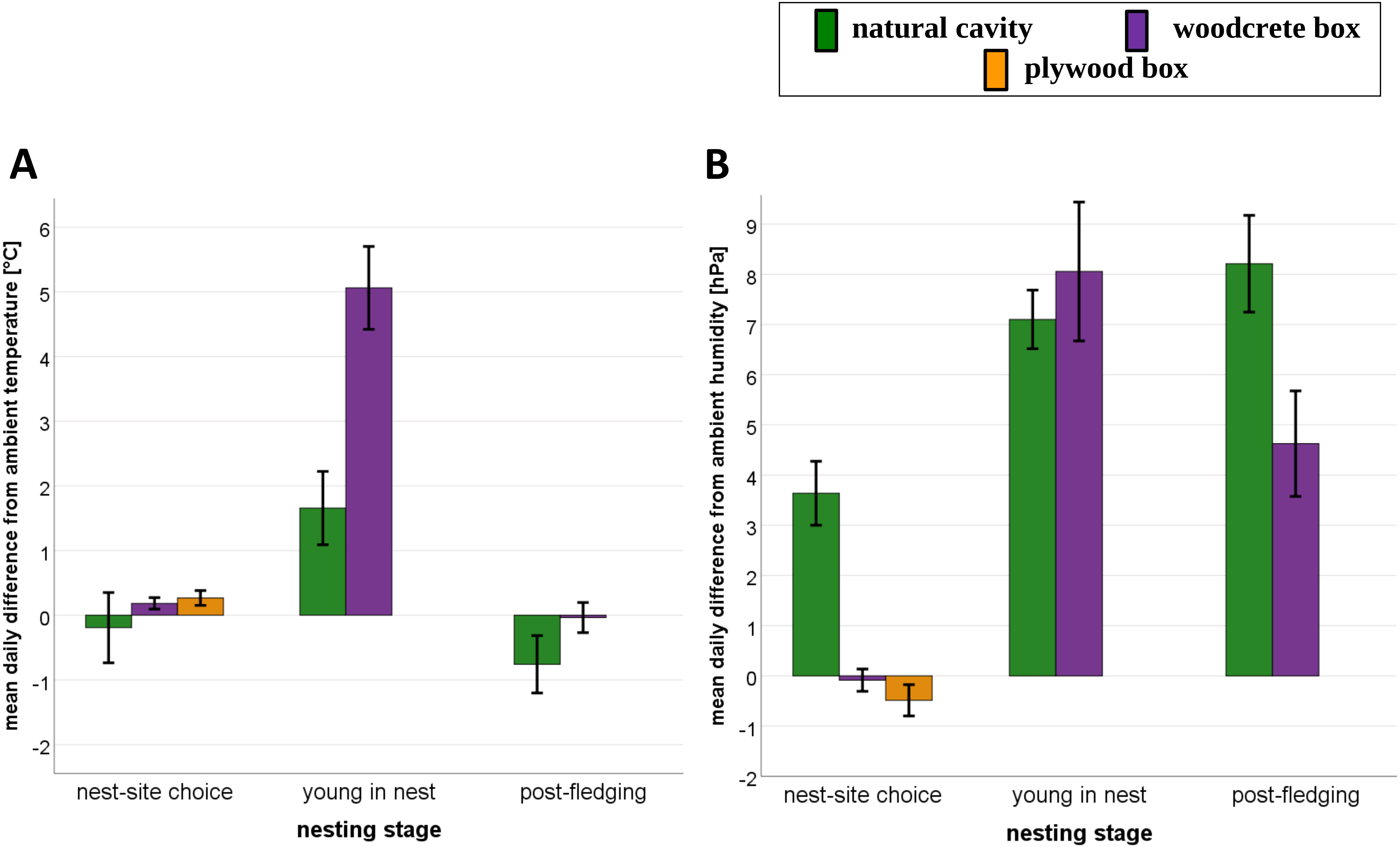


Figure 5 Mean daily difference from ambient temperature (A) and absolute humidity (B) at three nesting stages. Raw data, mean ± 95%CI are shown. Results of the comparison (with plywood boxes excluded) in Table S7.

Supporting Information

Microclimate shifts in nest-boxes and natural cavities before, during and after nesting

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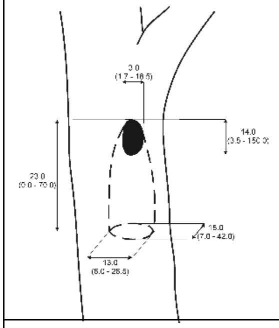
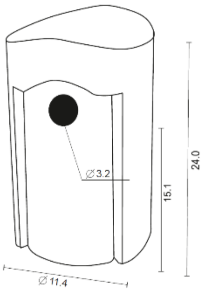
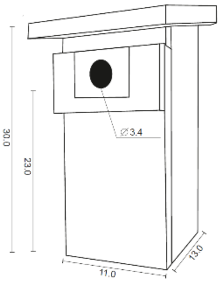
Table S1. Sample sizes for analyses of nest microclimate monitored in natural cavities and in two widely used types of nest-boxes: woodcrete and plywood (Temp: temperature, Hum: absolute humidity). These sample sizes are also of reference for data reported in Tables S2 and S3.

Stage	**Nesting site type**	**Blue tits** 				**Great tits** 				**Total**			
		Number of nests		**Number of reads**		**Number of nests**		**Number of reads**		**Number of nests**		**Number of reads**	
		Temp	**Hum**	**Temp**	**Hum**	**Temp**	**Hum**	**Temp**	**Hum**	**Temp**	**Hum**	**Temp**	**Hum**
nest-site choice	**Natural cavity**	-	-	-	-	-	-	-	-	**10**	**10**	**968**	**968**
	Woodcrete box	-	-	-	-	-	-	-	-	**10**	**10**	**968**	**968**
	Plywood box	-	-	-	-	-	-	-	-	**10**	**10**	**968**	**968**
young in nest	**Natural cavity**	2	1	1126	610	2	1	922	487	**4**	**2**	**2048**	**1097**
	Woodcrete box	2	2	450	450	3	1	1408	421	**5**	**3**	**1858**	**871**
post-fledging	**Natural cavity**	22	10	1016	466	24	13	1123	606	**46**	**23**	**2139**	**1072**
	Woodcrete box	21	10	971	460	23	12	1088	570	**44**	**22**	**2059**	**1030**

Cavities measured for the stage 'nest-site choice' were not separated by species, because for the early-season measurements we used cavities (natural or artificial) that were known to be occupied in the preceding season. However, these were not necessarily reoccupied in the current season and not necessarily by the same species.

Figure S1. Examples of experimental setup to measure temperature and humidity at nest-site choice (early spring) in the different types of nests. Loggers, used to measure ambient conditions, were covered with a thick white plastic cup, open on both ends, to avoid direct sunlight and precipitation.

Table S2. Basic parameters of nesting sites in natural cavities and nest-boxes used for measuring microclimate during the three nesting stages

Nesting stage	**Nesting site parameter**	**Natural cavity**  	**Woodcrete box**  	**Plywood box**  	**Kruskal-Wallis**	
		Median (range)			**H**	**P**
nest-site choice	**Height [m]**	2.9 (1.0-3.5)	2.9 (1.0-3.5)	2.9 (1.0-3.5)	-	-
	Entrance width [cm]	3.4 (2.5-18.5)	3.2 (-)	3.4 (-)	6.97	**0.031**
	Entrance height [cm]	36.0 (9.5-150.0)	3.2 (-)	3.4 (-)	27.86	**<0.001**
	Entrance area [cm^2]	95.2 (22.4-552.1)	8.0 (-)	9.1 (-)	27.85	**<0.001**
	Depth	29.5 (2.0-70.0)	15.1 (-)	23.0 (-)	17.55	**<0.001**
	Nest width [cm]	12.0 (6.0-24.0)	11.4 (-)	11.0 (-)	9.69	**0.008**
	Nest length [cm]	20.0 (9.0-42.0)	11.4 (-)	13.0 (-)	13.56	**0.001**
	Volume [cm^3]	7125.1 (728.8-20682.7)	1541.3 (-)	3289.0 (-)	12.95	**0.002**
young in nest	**Height [m]**	1.7 (1.3-2.1)	2.9 (-)		6.54	**0.011**
	Entrance width [cm]	4.2 (2.0-18.5)	3.2 (-)		1.80	0.180
	Entrance height [cm]	68.0 (30.0-150.0)	3.2 (-)		7.20	**0.007**
	Entrance area [cm^2]	397.0 (47.1-552.1)	8.0 (-)		7.20	**0.007**
	Depth	35.0 (28.0-70.0)	8.1 (8.0-9.6)		6.05	**0.014**
	Nest length [cm]	17.5 (12.0-21.0)	11.4 (-)		7.20	**0.007**
	Nest width [cm]	14.0 (12.21)	11.4 (-)		7.27	**0.007**
	Volume [cm^3]	7883.8 (3392.9-15833.6)	826.8 (816.6-979.9)		6.05	**0.014**
post-fledging	**Height [m]**	2.5 (0.3-6.5)	2.9 (2.9-3.0)		0.05	0.817
	Entrance width [cm]	3.0 (1.7-18.5)	3.2 (-)		3.64	0.056
	Entrance height [cm]	11.8 (3.5-150.0)	3.2 (-)		75.56	**<0.001**
	Entrance area [cm^2]	27.5 (5.9-552.1)	8.0 (-)		62.32	**<0.001**
	Depth	21.0 (0.0-70.0)	9.1 (6.6-10.6)		37.22	**<0.001**
	Nest length [cm]	14.2 (7.0-42.0)	11.4 (-)		10.68	**0.001**
	Nest width [cm]	13.0 (6.0-26.5)	11.4 (-)		16.70	**<0.001**
	Volume [cm^3]	2573.0 (643.2-41888.1)	928.8 (673.6-1081.9)		36.54	**<0.001**

All measurements were performed based on the methodology of Maziarz et al., (2016) and Wesołowski & Rowiński, (2012).

Height: measured at the bottom of entrance above ground level; Entrance width: largest horizontal dimension; Entrance height: vertical dimension; Entrance area: approximated to an ellipse area= π*1/2entrance width*1/2entrance height; Depth: distance from entrance to the nest measured vertically; Nest length: size of nest cup measured from entrance to the opposite wall; Nest width: size of nest cup measured perpendicularly to the nest length; Volume: nest size approximated to a cylinder volume= bottom area (approximated to an ellipse area= π*1/2 nest width*1/2 nest length) *depth. Significant differences ($P < 0.05$) marked in bold.

Table S3. Basic parameters for blue tits and great nests in natural cavities used for measuring microclimate during post-fledging stage. Significant differences between species ($P < 0.05$) marked in bold.

Nesting site parameter	**Blue tits** 	**Great tits** 	**Kruskal-Wallis**	
	Median (range)		**H**	**P**
Height [m]	3.3 (0.6-6.1)	1.9 (0.3-6.5)	4.27	**0.039**
Entrance width	2.5 (1.7-18.5)	3.5 (2.3-9.0)	13.95	**<0.001**
Entrance height	11.5 (3.8-45.0)	12.0 (3.5-150.0)	1.33	0.248
Entrance area [cm^2]	20.8 (5.9-552.1)	35.3 (9.6-447.7)	4.80	**0.028**
Depth	21.0 (7.2-70.0)	20.5 (0.0-64.3)	0.01	0.964
Nest length [cm]	11.2 (7.0-21.0)	20.5 (9.0-42.0)	11.10	**0.001**
Nest width [cm]	12.0 (6.0-24.0)	14.5 (6.0-26.5)	2.23	0.136
Volume [cm^3]	2118.0 (643.2-15833.6)	3887.7 (728.8-41888.1)	2.79	0.095

Table S4 Linear mixed models inferring variation of daily mean temperature and absolute humidity measured in natural and artificial nests across three nesting stages: (A) nest-site choice, (B) with young in nest and (C) post-fledging. At nest-site choice (A) two types of artificial nests-boxes were considered: woodcrete and plywood. When young in nest (B) nestling age was considered, and post-fledging (C) we additionally accounted for species, nest exposure and height. Natural cavities, nestling age less than 6 days and blue tit were used as a reference for parameter estimates and all estimates are shown after Z-score scaling of continuous variables. We present the final models with nonsignificant main factors and interactions removed (apart from the focal nest type). Sample sizes for each analysis are shown in Table S1. Significant differences ($P < 0.05$) are indicated in bold, and trends ($P < 0.20$) in italics.

Nesting stage	**Predictor**	**Effect**	**TEMPERATURE**				**HUMIDITY**			
			χ^2	**df**	**Pr(> χ^2)**	**Estimate ± SE**	**χ^2**	**df**	**Pr(> χ^2)**	**Estimate ± SE**
			Variance for random effects				***Variance for random effects***			
A) nest-site choice	nest type		**6.347**	**2, 114**	**0.042**		**377.222**	**2, 138**	**<0.0001**	
		woodcrete box				0.114 ± 0.059				-1.371 ± 0.089
		plywood box				0.140 ± 0.059				-1.589 ± 0.089
	ambient		**951.045**	**1, 75**	**<0.0001**		**85.258**	**1, 139**	**<0.0001**	
		mean ambient				0.720 ± 0.045				0.171 ± 0.071
	type* ambient		**31.830**	**2, 114**	**<0.0001**		**29.303**	**2, 138**	**<0.0001**	
		woodcrete*ambient				0.275 ± 0.059				0.416 ± 0.089
		plywood*ambient				0.302 ± 0.059				0.418 ± 0.089
	Random effect	*date*	*0.006*				*0.000 (removed)*			
		location	*0.008*				*0.089*			
		Residual	*0.089*				*0.200*			
B) young in nest	nest type		**13.520**	**1,6**	**0.0002**		*2.348*	*1, 39*	*0.125*	
		nest-box				0.559 ± 0.217				-0.335 ± 0.103
	ambient		**238.386**	**1,69**	**<0.0001**		**102.204**	**1, 57**	**<0.0001**	
		mean ambient				0.615 ± 0.050				0.686 ± 0.068
	nestling age		**169.464**	**1, 108**	**<0.0001**		**41.817**	**1, 75**	**<0.0001**	
		>6 days				0.545 ± 0.079				0.186 ± 0.133
	type* ambient		**5.250**	**1, 113**	**0.022**					
		nest-box*ambient				0.134 ± 0.059				
	type* nestling age		**33.574**	**1, 116**	**<0.0001**		**38.309**	**1, 41**	**<0.0001**	
		nest-box*>6 days				0.549 ± 0.095				1.003 ± 0.162
	Random effect	*date*	*0.049*				*0.125*			
		location	*0.096*				*0.000 (removed)*			
		Residual	*0.078*				*0.088*			

C) post-fledging	nest type		**5.267**	**1, 62**	**0.022**		**35.740**	**1, 20**	**<0.0001**	
		nest-box				0.154 ± 0.130				-0.621 ± 0.104
	ambient		**1118.93**	**1, 151**	**<0.0001**		**61.950**	**1, 67**	**<0.0001**	
		mean ambient				0.423 ± 0.041				0.602 ± 0.076
	height		**12.627**	**1, 66**	**0.0004**		**10.062**	**1, 32**	**0.002**	
		height				0.172 ± 0.048				0.172 ± 0.054
	species		1.299	1, 103	0.254					
		great tit				-0.175 ± 0.137				
	type* ambient		**281.409**	**1, 180**	**<0.0001**					
		nest-box*ambient				0.548 ± 0.052				
	type*species		0.576	1, 95	0.448					
		nest-box*great tit				0.114 ± 0.160				
	ambient*species		0.164	1,190	0.685					
		ambient*great tit				-0.110 ± 0.061				
	type*ambient* species		**9.126**	**1, 178**	**0.002**					
		nest-box *ambient*great tit				0.242 ± 0.080				
	Random effect	*date*	*0.009*				*0.183*			
		location	*0.172*				*0.041*			
		Residual	*0.054*				*0.224*			

Table S5 Linear mixed models inferring variation of daily minimum temperature and absolute humidity measured in natural and artificial nests across three nesting stages: (A) nest-site choice, (B) with young in nest and (C) post-fledging. At nest-site choice (A) two types of artificial nests-boxes were considered: woodcrete and plywood. When young in nest (B) nestling age was considered, and post-fledging (C) we additionally accounted for species, nest exposure and height. Natural cavities, nestling age less than 6 days and blue tit were used as a reference for parameter estimates and all estimates are shown after Z-score scaling of continuous variables. We present the final models with nonsignificant main factors and interactions removed (apart from the focal nest type). Sample sizes for each analysis are shown in Table S1. Significant differences ($P < 0.05$) are indicated in bold, and trends ($P < 0.20$) in italics.

Nesting stage	**Predictor**	**Effect**	**TEMPERATURE**				**HUMIDITY**			
			χ^2	**df**	**Pr(> χ^2)**	**Estimate ± SE**	**χ^2**	**df**	**Pr(> χ^2)**	**Estimate ± SE**
			Variance for random effects				***Variance for random effects***			
A) nest-site choice	nest type		**84.598**	**2, 113**	**<0.0001**		**422.094**	**2, 138**	**<0.0001**	
		woodcrete box				-0.645± 0.077				-1.434 ± 0.092
		plywood box				-0.581 ± 0.077				-1.776 ± 0.092
	ambient		**524.154**	**1, 79**	**<0.0001**		**46.453**	**1, 89**	**<0.0001**	
		minimum ambient				0.650 ± 0.058				0.118 ± 0.074
	type* ambient		**37.630**	**2, 113**	**<0.0001**		**19.553**	**2,138**	**<0.0001**	
		woodcrete*ambient				0.400 ± 0.078				0.363 ± 0.092
		plywood*ambient				0.422 ± 0.078				0.340 ± 0.092
	Random effect	*date*	*0.012*				*0.000 (removed)*			
		location	*0.005*				*0.089*			
		Residual	*0.152*				*0.200*			
B) young in nest	nest type		1.226	1,6	0.268		**5.414**	**1, 3**	**0.020**	
		nest-box				-0.092 ± 0.148				-0.814 ± 0.217
	ambient		**274.718**	**1,85**	**<0.0001**		**95.183**	**1, 54**	**<0.0001**	
		minimum ambient				0.660 ± 0.065				0.904 ± 0.088
	nestling age		**85.560**	**1, 111**	**<0.0001**		**25.134**	**1, 69**	**<0.0001**	
		>6 days				0.366 ± 0.098				0.317 ± 0.146
	type* ambient		*2.377*	*1, 127*	*0.123*					
		nest-box*ambient				0.217 ± 0.080				
	ambient* nestling age		1.312	1,137	0.252		**17.590**	**1,67**	**<0.0001**	
		ambient*>6 days				0.081 ± 0.110				-0.548 ± 0.131
	type* nestling age		**29.460**	**1, 124**	**<0.0001**		**15.979**	**1, 40**	**<0.0001**	
		nest-box*>6 days				0.685 ± 0.120				0.771 ± 0.193
	type*ambient* nestling age		**6.029**	**1, 144**	**0.014**					
		nest-box*ambient*>6 days				-0.345 ± 0.141				

	Random effect	*date*	*0.052*				*0.089*			
		location	*0.035*				*0.039*			
		Residual	*0.117*				*0.102*			
C) post-fledging	nest type		**15.645**	**1, 59**	**<0.0001**		**93.800**	**1, 19**	**<0.0001**	
		nest-box				-0.336 ± 0.086				-0.978 ± 0.101
	ambient		**835.336**	**1, 119**	**<0.0001**		**57.110**	**1, 70**	**<0.0001**	
		minimum ambient				0.479 ± 0.035				0.535 ± 0.071
	height		**14.563**	**1, 65**	**0.0001**		**4.633**	**1, 30**	**0.031**	
		height				0.158 ± 0.041				0.111 ± 0.052
	type* ambient		**189.871**	**1, 192**	**<0.0001**					
		nest-box*ambient				0.582 ± 0.042				
	Random effect	*date*	*0.020*				*0.161*			
		location	*0.128*				*0.057*			
		Residual	*0.058*				*0.151*			

Table S6 Linear mixed models inferring variation of temperature and absolute humidity insulation (daily minimum difference from ambient conditions) measured in natural and artificial nests across three nesting stages: (A) nest-site choice, (B) with young in nest and (C) post-fledging. At nest-site choice (A) two types of artificial nests were considered: woodcrete box and plywood box. When young in nest (B) nestling age was considered, and post-fledging (C) we accounted for species, nest exposure and height. Natural cavities, nestling age less than 6 days and blue tit were used as a reference for parameter estimates and all estimates are shown after Z-score scaling of continuous variables. We present the final models with nonsignificant main factors and interactions removed (apart from the focal nest type). Sample sizes for each analysis are shown in Table S1. Significant differences ($P < 0.05$) are indicated in bold, and trends ($P < 0.20$) in italics.

Nesting stage	**Predictor**	**Effect**	**TEMPERATURE**				**HUMIDITY**			
			χ^2	**df**	**Pr(> χ^2)**	**Estimate ± SE**	χ^2	**df**	**Pr(> χ^2)**	**Estimate ± SE**
			Variance for random effects				***Variance for random effects***			
A) nest-site choice	nest type		**211.606**	**2, 103**	**<0.0001**		**296.123**	**2, 140**	**<0.0001**	
		woodcrete box				1.298 ± 0.098				-1.311 ± 0.106
		plywood box				1.165 ± 0.098				-1.761 ± 0.106
	ambient		**39.690**	**1, 55**	**<0.0001**		**17.391**	**1, 148**	**<0.0001**	
		ambient amplitude				-0.868 ± 0.092				0.220 ± 0.053
	type* ambient		**53.402**	**2, 103**	**<0.0001**					
		woodcrete*ambient				0.647 ± 0.098				
		plywood*ambient				0.597 ± 0.098				
	Random effect	*date*	*0.087*				*0.000 (removed)*			
		location	*0.095*				*0.110*			
		Residual	*0.246*				*0.288*			
B) young in nest	nest type		**26.859**	**1, 7**	**<0.0001**		1.544	1,2	0.214	
		nest-box				1.299 ± 0.249				-0.333 ± 0.229
	ambient		**40.254**	**1, 63**	**<0.0001**					
		ambient amplitude				-0.442 ± 0.054				
	nestling age		**121.792**	**1, 117**	**<0.0001**		**10.049**	**1, 81**	**0.002**	
		>6 days				0.754 ± 0.068				0.241 ± 0.200
	type* ambient		**30.002**	**1, 118**	**<0.0001**					
		nest-box*ambient				0.340 ± 0.062				
	type* nestling age						**11.699**	**1, 54**	**0.0006**	
		nest-box*>6 days								0.695 ± 0.302
	Random effect	*date*	*0.057*				*0.403*			
		location	*0.132*				*0.039*			
		Residual	*0.098*				*0.396*			

C) post-fledging	nest type		**71.500**	**1, 53**	**<0.0001**		**70.885**	**1, 28**	**<0.0001**	
		nest-box				0.954 ± 0.114				-1.109 ± 0.132
	ambient		**465.770**	**1, 158**	**<0.0001**					
		ambient amplitude				-1.284 ± 0.043				
	height		**11.460**	**1, 61**	**0.0007**		**6.411**	**1, 39**	**0.011**	
		height				0.188 ± 0.056				0.171 ± 0.068
	type* ambient		**456.790**	**1, 166**	**<0.0001**					
		nest-box*ambient				1.026 ± 0.048				
	Random effect	*date*	*0.099*				*0.351*			
		location	*0.229*				*0.114*			
		Residual	*0.086*				*0.203*			

Table S7 Linear mixed models for explaining variation of mean daily differences from ambient conditions of (A) temperature and (B) absolute humidity measured at three nesting stages (at nest-site choice, when young remained in the nest and post-fledging) in natural cavities and woodcrete nest-boxes. Temperature and ambient absolute humidity in natural cavities and at nest-site choice are used as references for parameter estimates and all estimates are shown after Z-score scaling of continuous variables. Significant differences ($P < 0.05$) are indicated in bold.

Model	**Variable**	**Effect**	χ^2	**df**	**Pr(>** χ^2**)**	**Estimate** ± **SE**
			Variance for random effects			
D) temperature	nest type		**15.596**	**1,117**	**<0.0001**	
		nest-box				0.127 ± 0.116
	nesting stage		**108.668**	**2, 170**	**<0.0001**	
		young in nest				0.432 ± 0.166
		post-fledging				-0.246 ± 0.146
	nest type* nesting stage		**38.673**	**2,263**	**<0.0001**	
		box *young in nest				1.177 ± 0.214
		box *post-fledging				0.095 ± 0.153
	Random effect	*date*	*0.150*			
		location	*0.094*			
		Residual	*0.345*			
E) absolute humidity	nest type		**77.672**	**1,55**	**<0.0001**	
		nest-box				-0.854 ± 0.110
	nesting stage		**54.774**	**2,141**	**<0.0001**	
		young in nest				0.809 ± 0.232
		post-fledging				0.982 ± 0.171
	nest type* nesting stage		**9.652**	**2,98**	**0.008**	
		box *young in nest				0.955 ± 0.332
		box *post-fledging				0.007 ± 0.204
	Random effect	*date*	*0.108*			
		location	*0.226*			
		Residual	*0.309*			

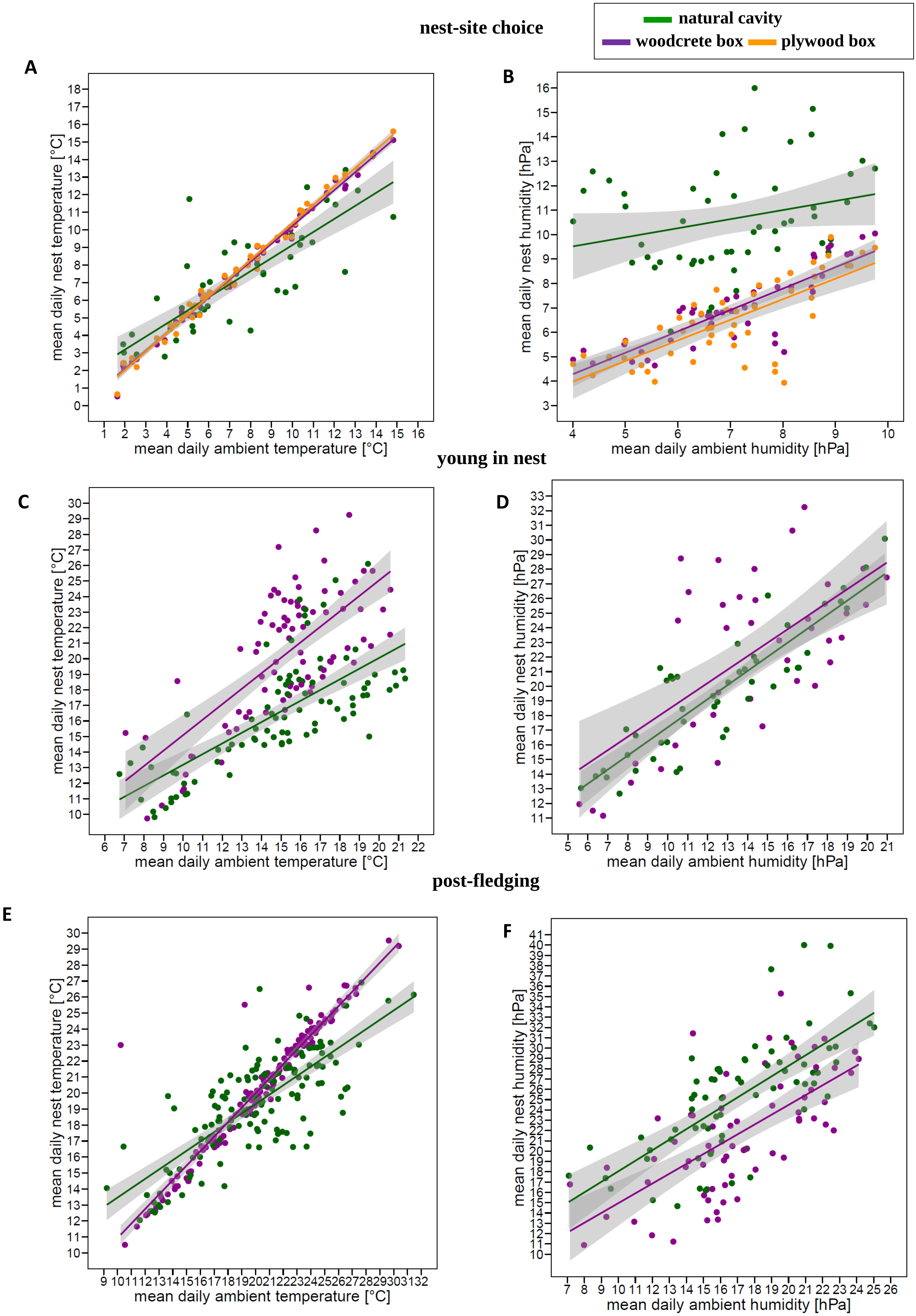


Figure S2. Variation in mean daily temperature (A, C, E) and absolute humidity (B, D, F) across three nesting stages: at nest-site choice (A, B), in the presence of young in nest (C, D) and post-fledging (E,F). Raw data points with regression lines ± 95%CI (shaded in grey) are shown. For statistical results see Table S4 and for sample sizes Table S1. In graph D and F the interaction of nest type with ambient humidity mean was non-significant, but we show it for the consistency of data presentation.

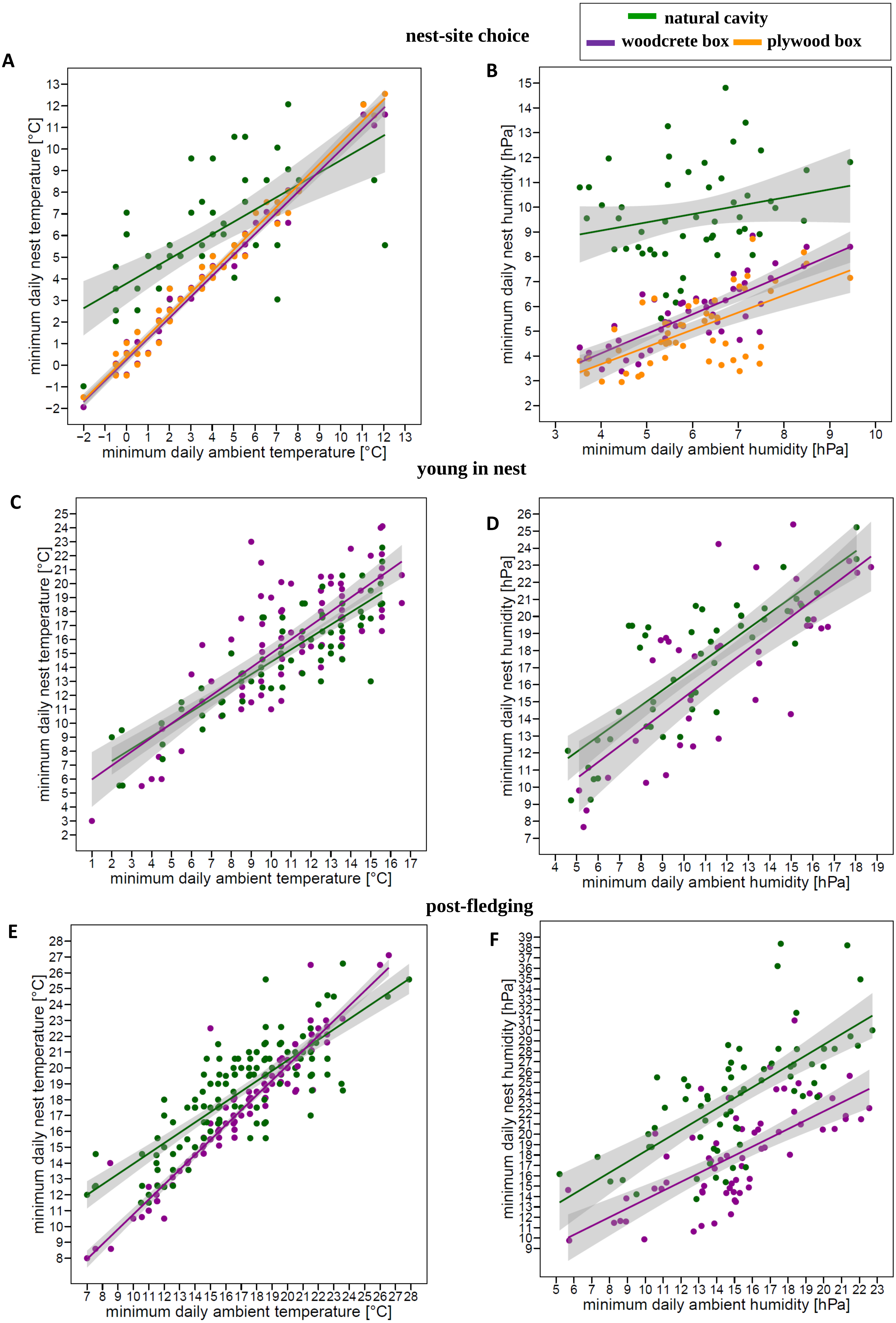


Figure S3. Variation in minimum daily temperature (A, C, E) and absolute humidity (B, D, F) across three nesting stages: at nest-site choice (A, B), in the presence of young in nest (C, D) and post-fledging (E,F). Raw data points with regression lines ± 95%CI (shaded in grey) are shown. For statistical results see Table S5 and for sample sizes Table S1. In graph C, D and F the interaction of nest type with ambient humidity mean was non-significant, but we show it for the consistency of data presentation.

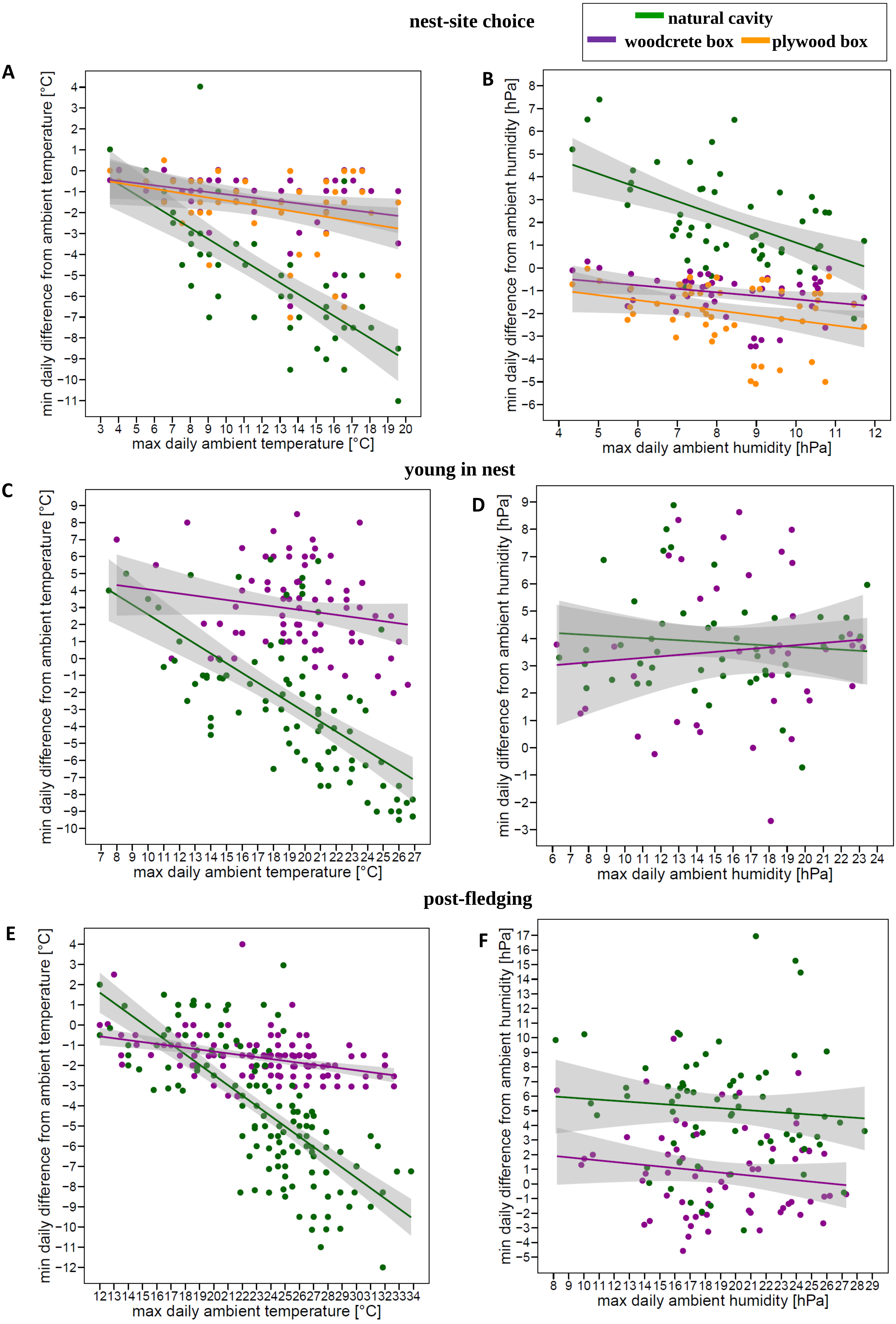


Figure S4. Variation in insulation (daily minimum difference with ambient conditions) of temperature (A, C, E) and absolute humidity (B, D, F) across three nesting stages: at nest-site choice (A, B), in the presence of young in nest (C, D) and post-fledging (E,F). Raw data points with regression lines ± 95%CI (shaded in grey) are shown. For statistical results see Table S6 and for sample sizes Table S1. In graph B, D and F the interaction of nest type with ambient humidity mean was non-significant, but we show it for the consistency of data presentation.

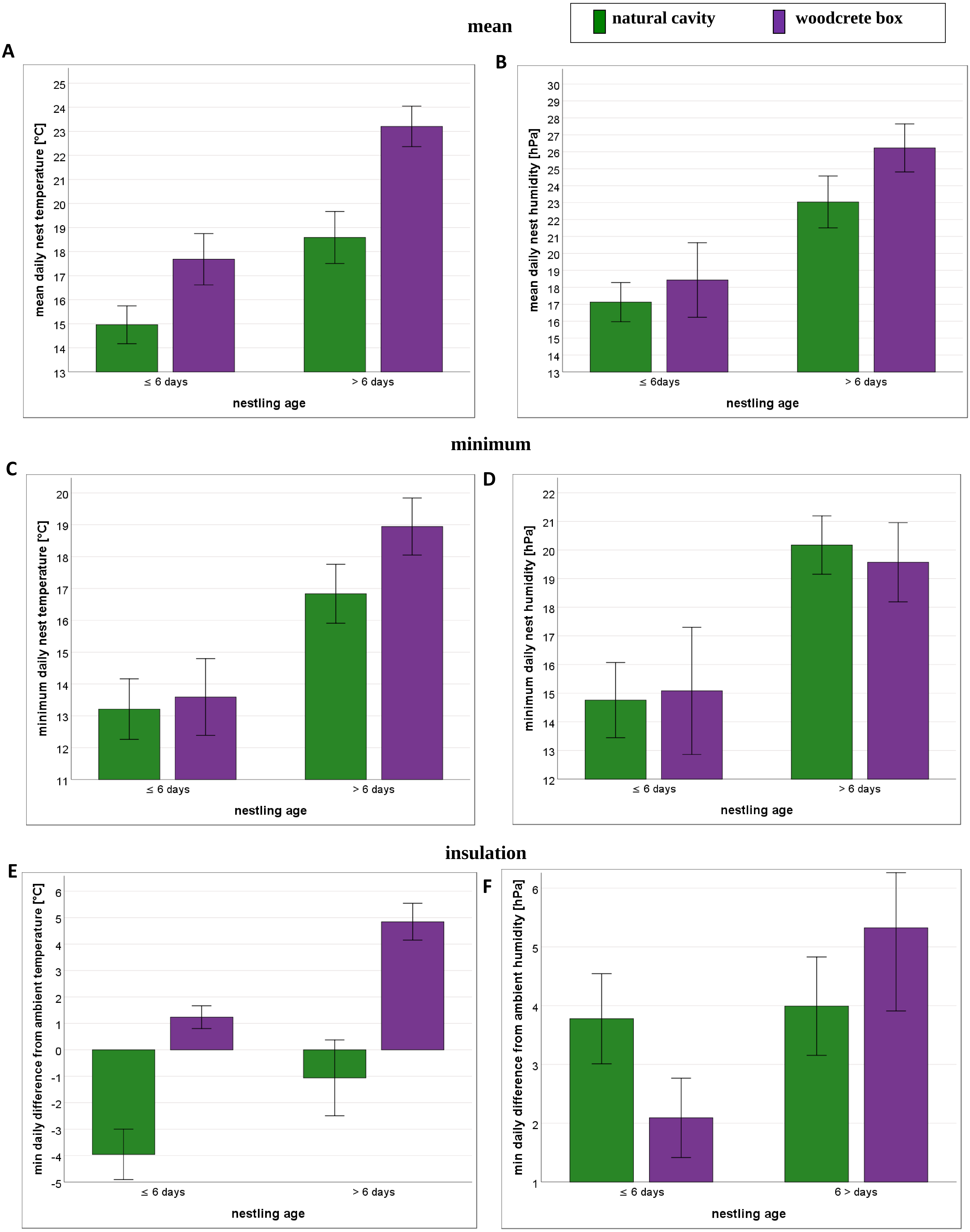


Figure S5. Mean daily nest temperature (A) and absolute humidity (B), minimum daily nest temperature (C) and absolute humidity (D) and temperature insulation (E) and humidity insulation (F) with young in nest stage in natural cavities (green) and in woodcrete nest-boxes (dark magenta). The graphs show nest-type-wise differences according to nestling age: before 6 days when nestlings are not yet able to thermoregulate on their own and after 6 days when this mechanism is on. Raw data ± 95%CI are shown. For statistical results see Table S4B, S5B, S6B and for sample sizes Table S1. In graph E the interaction of nest type with nestling age was non-significant, but we show it for the consistency of data presentation.

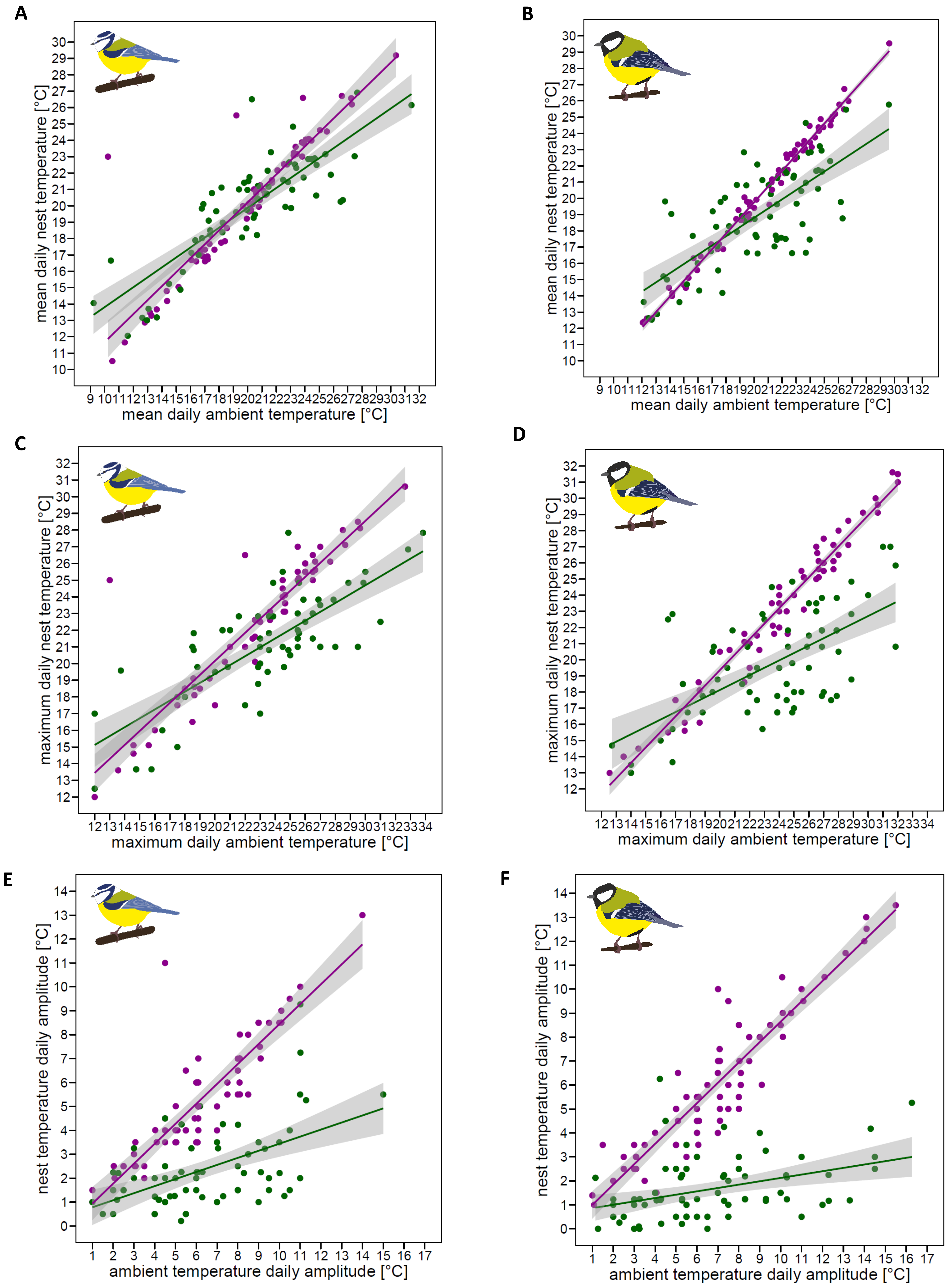


Figure S6. Species-specific variation in nest temperature during post-fledging stage in natural cavities (green) and in woodcrete nest-boxes (dark magenta) relative to ambient values. Mean daily (A, B), maximum daily (C, D) and daily amplitudes (E, F) in blue tits (A, C, E) and great tits (B, D, F). Raw data points with regression lines ± 95%CI (shaded in grey) are shown. For statistical results please see Table S4C, 2C, 3C and for sample sizes Table S1.