University of Natural Resources

and Life Sciences, Vienna

Comparison of nest composition in Great Tits (*Parus major*) in four different land-use types

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Declaration in lieu of oath

I herewith declare in lieu of oath that this thesis has been composed by myself without any inadmissible help and without the use of sources other than those given due reference in the text and listed in the list of references. I further declare that all persons and institutions that have directly or indirectly helped me with the preparation of the thesis have been acknowledged and that this thesis has not been submitted, wholly or substantially, as an examination document at any other institution.

H.Wilting

20.03.2017

Date

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Contents

List of Figures

List of Tables

Abstract

Urbanisation is an increasing process leading to a loss of natural environments. Thus, animals need to and seem to adapt to city habitats. This study aims to reveal new insights into the adaptation of Great Tits (*Parus major*) to urban environments by looking at nest composition as an important factor in reproduction. The objective was to investigate possible relationships between the degree of urbanisation and chosen nest materials. For this purpose, 75 nests of a Great Tit breeding population in Vienna were collected in 2015, and manually dissected into their constituents. In this study, in more urbanised areas, Great Tits used more artificial nest materials for nest construction. Moreover, the sealing of the nest site was positively correlated with the amount of artificial materials in the nest. Additionally, roe deer hair as an indicator of wildlife hair was only found in nests in lesser urbanised areas, and, nests in intermediate urbanised areas tended to contain a higher content of more natural wool. Those findings underline the high plasticity of Great Tits in their nesting behaviour (Mennerat et al. 2009). Great Tits seem to use material available at the nest site (Collias & Collias 1984, Britt & Deeming 2011), even if artificial, and thus, possibly, reallocate the energy needed for assembling nest material to breeding and raising the offspring.

Zusammenfassung

Urbanisierung ist ein fortwährender Prozess in der heutigen globalisierten Welt, der zum Verlust von natürlichen Habitaten führt. Daher ist es auch für Tiere notwendig, sich an die Stadt als Lebensraum anzupassen – ein Vorgang, der bereits stattfindet. Die vorliegende Studie gibt neue Einblicke in die Anpassung von Kohlmeisen (Parus major) an urbane Lebensräume. Dies geschieht durch eine Analyse der Nestzusammensetzung, da das Nest eine richtige Rolle bei der Reproduktion und damit bei der Populationsetablierung spielt. Das Ziel der Arbeit war die Untersuchung eines möglichen Zusammenhangs zwischen Urbanisierungsgrad und ausgewählten Nistmaterialien. In der Brutsaison 2015 wurden für diesen Zweck in Wien 75 Kohlmeisennester gesammelt und anschließend von Hand in ihre Einzelbestandteile zerlegt. Kohlmeisen in stärker urbanisierten Bereichen Wiens bauten mehr künstliche Materialien in ihre Nester ein als in weniger stark urbanisierten Bereichen. Außerdem korrelierten die Versiegelung der Brutplatzumgebung mit der Menge der künstlichen Materialien in einem Nest. Rehhaare, als Indikator für Wildtierhaare, konnten nur in Nestern aus weniger urbanisierten Gegenden (Wald und „mixed non-residential areas“) gefunden werden. Zusätzlich konnte eine Tendenz zu mehr natürlicher Wolle in Nestern aus gemäßigt urbanisierten Gegenden nachgewiesen werden. Die Ergebnisse

zusammengenommen unterstützen die Annahme einer hohen Plastizität im Nestbauverhalten von Kohlmeisen (Mennerat et al. 2009). Die Vögel nutzen Nistmaterialien, die sie in der direkten Nähe des Nistplatzes finden (Collias & Collias 1984, Britt & Deeming 2011), somit auch artifizielle Materialien, und nutzen dadurch effektiv ihre Energie, für den Nestbau, um ausreichend Reserven für die Aufzucht ihrer Jungen zu haben.

1 Introduction

This thesis is part of the project "Chick condition and reproductive success of great tits Parus major along an urban gradient" led by Priv.-Doz. Dr. Marcela Suarez-Rubio (Institute of Zoology) and Priv.-Doz. Dr. Sabine Hille (Institute of Wildlife Biology and Game Management) at the University of Natural Resources and Life Sciences, Vienna. The project aimed to link the urban gradient of the city of Vienna with chick condition and reproductive success of Great Tits (Parus major), and finally to provide new information about the reproductive success of Great Tits in urban environments.

In today's world, the process of globalisation is accompanied by increasing urbanisation. The term urbanisation is defined as "the combination of densification and outward spread of people and built areas" (Forman 2008, p.9), in which densification describes the increase in the "density of people and building units [...] by infilling greenspaces or by changing from low- to high-rise apartment buildings" (Forman 2008, p.9). Urban areas are highly attractive to people and thus, in the year 2007, for the first time, more people lived in cities than in rural areas (United Nations 2014). In 2014, already 54 % of all people lived in urban areas and the United Nations (2014) estimated that by 2050, 66 % of the global population will do so. Urban areas tend to increase in size faster than natural sites are being preserved (McKinney 2002). Consequently, urban areas grow every day and replace natural environments. Thus, animals need to adapt to cities as habitats and therefore, urban habitats attract notice to ecological research.

Urbanisation is one of the major reasons for local extinctions of species due to habitat loss (McKinney 2002). One effect of the expansion of urban areas is the loss of species richness (McKinney 2002, McKinney 2008), acknowledging a higher diversity in areas of an intermediate level of urbanisation (reviewed in Chace & Walsh 2006, Meffert & Dziock 2013). Urban areas constitute a special environment in many regards, for example regarding ambient temperature. Gaston et al. (2010) highlighted that inner urban environments show a "heat island" effect: the temperature rises proportionally to the increase of dense structures like buildings or roads, implicating that the temperature in the city centre is higher than in suburban areas with less dense infrastructure. This indicates that plants and animals might be forced to adapt to these special environments and also alter their behaviour, which might also be the case for the nesting behaviour of birds.

Particularly suitable as a study organism for studies along the urban gradient, such as investigations on nesting behaviour and nest construction is the Great Tit. The Great Tit depicts a common species occupying many different tree habitats, including city centres, giving researchers the possibility to obtain relatively large sample sizes. As a cavity nesting bird species, the Great Tit accepts nest boxes which are easily accessible and are, therefore, facilitating nest material sampling. Furthermore, Great Tits stay over winter in Central Europe. This might be beneficial, especially in urban areas, as they are still able to find enough food during winter and therefore, are in a good condition to compete with migratory returning to the breeding sites (reviewed in Chace & Walsh 2006). This possible advantage in the competition for breeding sites adds to the possibility for researchers to increase sample sizes when collecting nest material.

The bird's nest plays a crucial role in reproduction as it displays a protected place to lay eggs and raise offspring (Healy et al. 2008). Bird nests can be subdivided into four general zones (Ondrušová 2011, Hansell 2000): the attachment, the outer (decorative) layer, the structural layer, and finally the lining (Hansell 2000). The attachment zone holds the nest in its position. The outer (decorative) layer has usually lichen as the main component. It is arranged on the outer side of the nest and is decorating the nest without influencing the structure or the attachment. The structural layer displays the most important zone and gives the nest its shape, but might not be present in ground or cavity nests. The lining is not serving the integrity of the nest, but instead has other functions like insulation (Hansell 2000). However, not all four zones need to be present in every nest (Hansell 2000). Great Tit nests usually can be subdivided into the structural layer and the nest lining. The structural layer mostly consists of plant-derived material, such as little twigs and mostly moss (Harrison 1975). The nest lining is diverse in its composition and contains materials such as hair, wool, or artificial components (Mainwaring et al. 2012).

Britt & Deeming (2011) have analysed the nests of Great Tits and Blue Tits (Cyanistes caeruleus) and have weighed components from the following categories: mud, twigs, grass, leaves, moss, bark, plant stem, hair, wool, feathers, fur, artificial, and dust. Using this categorisation in order to investigate differences in nest material along the urban gradient might allow the comparison of quantitative results to the study of Britt & Deeming (2011), in which the compounds of Tit nests were analysed in combination with climatic conditions. Britt & Deeming (2011) did not find a correlation between mean air temperature leading up to the first egg date with nest mass or with nest composition. Schöll & Hille (2014) also did not find better insulated nests in Great Tits in colder environments. However, other studies (Deeming et al. 2012, Mainwaring et al. 2012) have shown that Great Tits adjust their nest composition regarding insulative materials to local temperature. The above described, partly contradicting results from literature indicate that investigations on nest composition and insulation among the urban gradient might be promising to further evaluate the adjustment to local temperatures. As it is generally warmer in high density and mixed residential areas than in sylvan and mixed non-residential (agricultural) areas ("heat island" effect: Grimm et al. 2008, Gaston et al. 2010), the need for insulation for the eggs and the chicks is reduced. This leads to the assumption that nests in areas with a higher ambient temperature contain fewer feathers than nests in colder areas, considering the amount of feathers in a nest as an indicator for insulation quality, and consequently, for adaption to temperature differences (Collias & Collias 1984). Long-Tailed Tits (Aegithalos caudatus), for example, show a positive influence of the mass of feathers in the nest on the quality of insulation (McGowan et al. 2004).

Additionally to feathers, Great Tits also use hairs in their nest lining (Ondrušová 2011). Ondrušová (2011) found mammalian hairs in bird nests in proportion to the local occurrence of mammalian species and she assumed the availability of hairs in the environment to be crucial for the birds' choice of material used in the nest lining and for insulation. Even though a higher density of the human population logically is accompanied with an increase in the appearance of pets, the availability of hairs for nest building is expected to be higher in mixed residential, mixed non-residential and sylvan areas than in high density areas. This is due to better possibilities to let cat roam freely and to walk dogs as well as a possibly higher density of mammalian wildlife in the three lesser urbanised clusters. As a consequence, birds might incorporate a higher amount of hairs in lesser urbanised areas (mixed residential, mixed non-residential and sylvan areas) rather than in high density areas, where availability might be reduced.

Furthermore, when analysing nest components, one needs to consider factors influencing the female when choosing the nest site. It is not only influenced by food availability, shelter possibilities from physical circumstances and from predators, but also by the availability of suitable nest materials (Collias & Collias 1984). However, Britt & Deeming (2011) could confirm the flexibility in the use of nest materials depending on availability. They found a difference in the use of wool between two years in which sheep were located in different areas of the study area. They conclude that Great and Blue Tits do not rely on specific nest materials, which are difficult to find at a site, but take what they discover (Britt & Deeming 2011). This leads to the assumption that Great Tits are able to find substitutes for missing nest materials at the nesting site. For example, if moss availability decreases with increasing coverage by buildings, birds may use hairs in high density and mixed residential areas as a substitute for moss.

The choice of nest material is species-specific, but nevertheless a high variation within species is possible to occur as well (Mennerat et al. 2009). Mennerat et. al (2009) emphasised that a high behavioural plasticity within a species can be responsible for an adaptation to new environments (see also Wang et. al 2009). This is of importance for this study, as one can suggest that Great Tits living in cities use human-derived materials, because that material is more abundant than in natural areas. That effect might be intensified when the nest site is surrounded by buildings or areas without vegetation. For example, a study carried out in China revealed an increase in human derived materials in nests of the Chinese Bulbul (Pycnonotus sinensis) with increased urbanisation (Wang et al. 2009). Birds may use artificial nest materials especially when they resemble natural materials (Collias & Collias 1984, Wang et al. 2009, Ondrušová 2011).

The aim of the study is to give an insight into the adjustment of Great Tits to urban environments by quantifiably investigating nest compositions. Even though nest building is an important part of the reproductive process, the analysis of nest composition has often been overlooked in research and mostly is only anecdotally reported. Most studies concentrate on the effect of urbanisation on biodiversity and species richness and thus, the results of this study are the first to give insight into nest compositions along an urban gradient, using Vienna as an example. Since animals react to their habitats on different geographic scales (Mayor et al. 2009), both, a larger scale of 2000 meter, but also a smaller scale of 50 meter around the nesting site were considered in the anaylsis. The results of this study might be valuable for further research on reproductive success of Great Tits in urbanised areas.

The above described differences of nest material choice in urban areas led to the research question of this thesis, which examines whether Great Tits in urban areas use different nesting materials than Great Tits in less urbanised areas of Vienna. In order to answer this question, I set up the following five hypotheses: 1) Nests in high human population density and mixed residential areas will contain more material derived from human origin, e.g. paper or cigarette stubs, than the nests in mixed non-residential and sylvan areas; 2) The sealing within a 50 meter buffer zone around the nest site is positively correlated with the amount of artificial nest materials; 3) Nests in high density and mixed residential areas will contain less roe deer hairs than nests in mixed non-residential and sylvan areas; 4) In mixed non-residential areas, the percentage of natural wool in nests will be higher than in high density, mixed residential, and sylvan areas; and finally, 5) Ambient temperature is negatively correlated with the number of feathers in the nest.

2 Materials and Methods

2.1 Study area

The study area compromises the city of Vienna, the capital of Austria (48° 07' 06''N to 48° 19' 23''N and 16° 10' 58''E to 16° 34' 43''E). Vienna inhabits about 1.8 million of people (Statistik Austria, calculation MA 23 2016, Magistrat der Stadt Wien 2016) and, noticeable for a large city, has a great percentage of green areas (45.1 %) and water bodies (4.7 %) (Magistrat der Stadt Wien 2016). The study area has an elevation range from 151 to 543 m.a.s.l. (Magistrat der Stadt Wien 2016). Due to national and international regulations, birds, including the Great Tit, are protected in Vienna (Wichmann et al. 2009).

The study area was divided into clusters of four land-use types, each provided with three nest boxes (Figure 1).

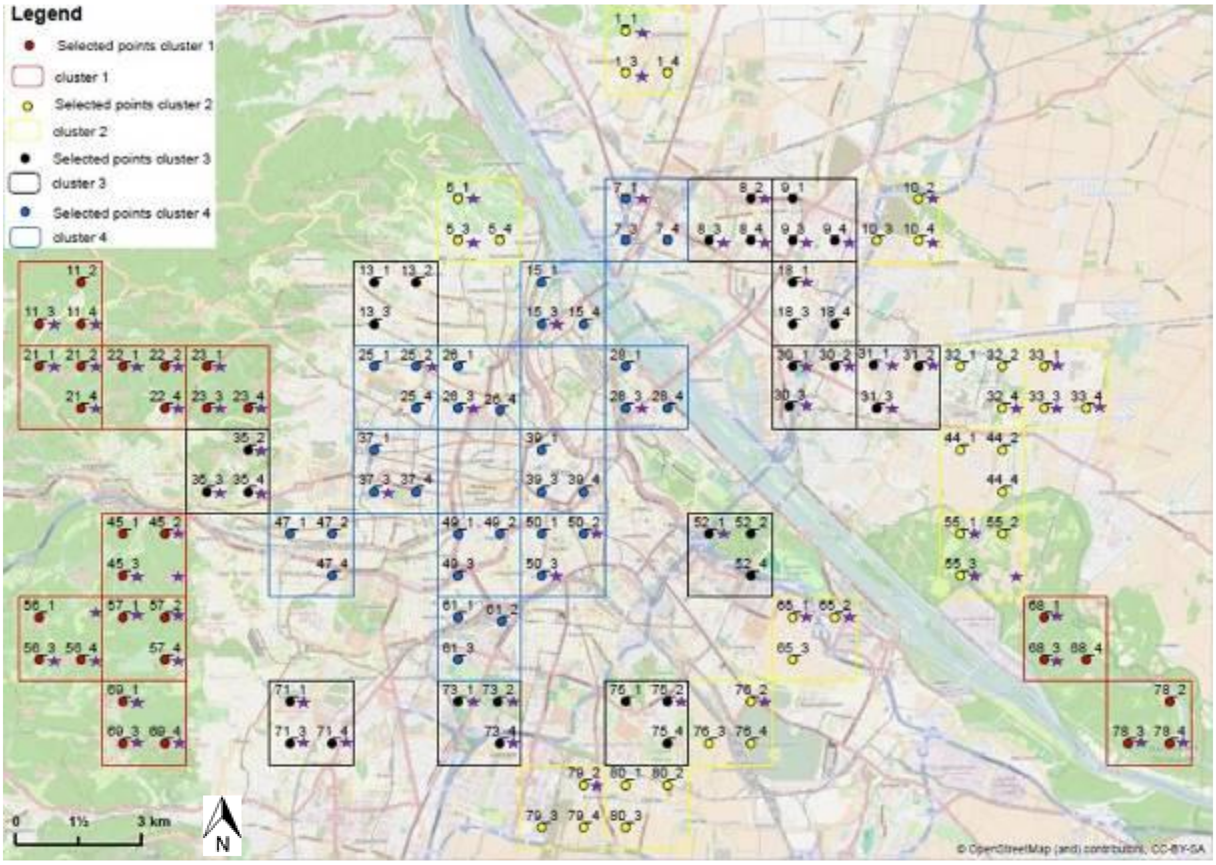

Figure 1: Overview of the study area and the nest sites in which nests were collected for analyses. Those nest sites at which nests were collected are marked with a violet star (75 in total). Colours of the clusters: red: sylvan areas, yellow: mixed non-residential areas, black: mixed residential areas, and blue: high density areas. Map was modified after Krenn (2015).

2.2 Studied species

The Great Tit (*Parus major*) is a common bird in Austria (Wichmann et al. 2009). In Vienna, the Great Tit is the second most occurring passerine bird species (only outnumbered by the Common Blackbird (*Turdus merula*)), inhabiting the whole city area throughout the year (Wichmann et al. 2009). There are about 25 000 – 39 000 breeding pairs in Vienna with the highest abundance in residential areas with gardens. The Great Tit is protected by the Nature Conservation Law of Vienna (Wichmann et al. 2009). They nest in secondary tree holes, but also use nest boxes and nest in settlements (Harrison & Castell 2004, Makatsch 1976). Consequently, the Great Tit is a desirable study species as it adapts easily to artificial nest boxes and thus, a greater sample size can be achieved. The Great Tit is not only living in natural habitats, but also occurs in urban landscapes (Solonen 2001), which makes it suitable for studying bird ecology along a rural-urban gradient as mechanisms of adaptation can only be investigated with species that are somehow adapted to new habitats. The Great Tit usually lays between 8 and 12 eggs in its nest, with a breeding period of 12 to 15 days (Hayman & Hume 2003, Bauer et al. 2005). Breeding in Central Europe takes place between March and July, including sometimes a second brood when feeding conditions are advantageous in a long-term (Bauer et al. 2005).

Great Tit nests are cup-shaped nests, defined as a “shape of a nest with a distinct or prominent concavity to hold the eggs” (Hansell 2000), which fill the space the hole is offering (Harrison & Castell 2004, Makatsch 1976). The outer structure is formed with moss, lichen, rootlets, and fine grass, while the lining contains wool, cobwebs, animal hairs, feathers (Harrison & Castell 2004), synthetic threads, silk, grass, leaves, and moss (Alabrudzińska et al. 2003). An example for a nest from the study site is shown in Figure 2. In Great Tits, the nest is built by the female, but the young get fed and raised by both parents (Harrison & Castell 2004).

Figure 2: Great Tit nest with eggs from the study site and the breeding season 2015. The nest in the picture belongs to the land-use type "mixed residential" (nesting site 8_3) and shows the typical structure of a nest described above. Some of the lining is built of artificial material. Picture credit: Christine Pech.

2.3 Dissection of the Nests

The 75 nests were collected from occupied nest boxes (Vivara, Salamanca WoodStone ™) after the breeding season of 2015 by three master students (Kathrine Schack Madsen, Christine Pech, and Martin Renner). Only first nests were collected. Afterwards, the nests were stored deep frozen in order to ensure that parasites are dead before the dissection began.

The dissection of all nests took place between October 2015 and March 2016.

Before dissection, the nests were dried at 40 °C for 18 hours (Memmert, Type UF 260, Schwabach, Germany). Then, the nests were manually and carefully dissected and components were sorted into 15 categories (Table 1).

Table 1: 15 categories of nest materials as used in the nest dissection. The grey colour represents the broader category "inorganic material" (containing solely natural, inorganic material), the violet colour stands for "animal materials", green symbolizes "plant materials", and blue "artificial materials" as used in Hansell (2000).

Dust	Feathers	Twigs and Plant stem	Bark	Paper
Other	Hair	Grass	Moss	Cigarette stubs
Non-nesting material	Natural wool	Leaves	Coloured wool	Other human-derived material

The category "natural wool" contains material that, during manual dissection of the nests, animal wool (e.g. sheep or other wild animals with dense fur) as well as undercoat of domesticated animals, such as dogs. This is due to the very difficult distinction between natural wool and undercoat once incorporated into the nest. The category "non-nesting material" was created in order to distinguish between feathers that were built into the nest on purpose and Great Tit feathers of which it cannot be said if they were built into the nest on purpose or if they were introduced by activities of the birds in the nest. Feathers from other bird species were included in the category "feathers". Further, insects and eggs that were not discovered before the drying process were included in the category "non-nesting material". The category "other" did contain any material that could not be sorted into any other category, for example little rocks. An example for each category is shown in picture in the Appendix (Figure 5 to Figure 19).

The categories were weighed (Mettler AE 200, Mettler-Toledo, Columbus, USA) in gram with four positions after the decimal point. Additionally, feathers, paper pieces, cigarette stubs and other human derived materials were counted.

2.4 Statistical analysis

All statistical analyses were performed using R (R x64 3.2.4 Revised, www.r-project.org) and the significance level was set to $p = 0.05$.

2.4.1 Amount of artificial materials in nests in relation to land-use types

The hypothesis aimed to test whether nests from more urbanised land-use types contain a higher amount of artificial, human-derived materials than nests from less urbanised land-use types.

The categories "cigarette stubs", "paper", "coloured wool" and "other human-derived materials" were added to be able to investigate all artificial materials combined and are in the following referred to as “artificial material”. The data was tested for normal distribution with a Shapiro-Wilk test. As the p-value lays under 0.0001, the data is not normally distributed. An arcsinus square root transformation could not achieve a normal distribution of the data. However, the data appears to be more homogenous when looking at outliers. Thus, the Kruskal-Wallis test was performed with the arcsinus square-root transformed data. The resulting p-value of the Kruskal-Wallis test ($p < 0.0001$) shows a difference in the amount of artificial materials in the nests of different land-use types. In order to bring to light where exactly the differences lie, a Dunn’s test with a Bonferroni correction was conducted.

2.4.2 Amount of artificial materials in nests in relation to the degree of sealing

Additionally to the first hypothesis, I aimed to test whether the amount of artificial materials in the nests is also correlated positively with the degree of sealing of the nest site at a small scale of 50 m.

The hypothesis was investigated using the variables “artificial material [%]” and “sealing [%]”. The data for the variable “sealing [%]” was recorded in the framework of the fieldwork for Renner (unpub.). The data is presented in the following intervals: 0 – 1 %, 1 – 5 %, 5 – 10 %, 10 – 20 %, 20 – 30 %, 30 – 40 % and, in 10 % steps, further on.

The variables were arcsinus square root transformed, which did not lead to a normal distribution. However, a homogenisation of the data was achieved and consequently, the transformed data were used for further investigation. The correlation coefficient by Spearman was chosen as both variables do not follow the normal distribution according to the Shapiro-Wilk test.

2.4.3 Amount of roe deer hair in nests in relation to land-use types

The hypothesis said that roe deer hair would be found more often in nests from sylvan and mixed non-residential areas than in nests from high density and mixed residential areas.

Solely nests with roe deer hair were taken into consideration as those were distinguishable from other hairs. By this, a rough distinction between hairs of wild animals and of domestic animals could be obtained. I took those nests into account which were marked by me with “high amount of roe deer hair”. These were ten nests, five each from sylvan areas and mixed non-residential areas.

The data (hair [%]) is normally distributed (Shapiro-Wilk test: $p = 0.828$) and has a homogeneous variance (Levene test: $p = 0.198$). This led to a classic unpaired, two-sample t-test to investigate differences of the amount of roe deer hair between nests from sylvan and nests from mixed non-residential areas.

2.4.4 Amount of natural wool in nests in relation to land-use types

The goal was to test whether natural wool was incorporated into nests in a higher amount in mixed non-residential areas than in the remaining three land-use types.

The variable “wool [%]” does not appear to be normally distributed according to the Shapiro-Wilk test. After an arcsinus square root transformation, the data was normally distributed. To investigate differences between the amounts of natural wool in nests of the different land-use types, an one-way ANOVA was performed.

2.4.5 Number of feathers in nests in relation to ambient temperature

The last hypothesis investigates the relationship between ambient temperature and the number of feathers in the nests, expecting a negative relationship.

During the breeding season 2015, temperature loggers were placed at several nest boxes. After selecting temperature loggers that were active during the nest building and egg laying phase, nine sites remained suitable for the analysis. Since the sample size is small and time spans from the first nest material until the first egg sighted differed greatly, I decided to divide the analysis into two parts: 1) the time span from the day with the first egg and the continuing two days was looked at separately from 2) the time span from the first nest material found until day with the first egg. Not only the mean temperature between 6 a.m. and 6 p.m. was studied, but also the lowest temperature in the time span in question. This differentiation allows us a look at the pattern more closely and reduce the risk of drawing false conclusions which could arrive from the small sample size. After checking for normal distributions with the Shapiro-Wilk test, the correlation coefficient after Pearson was chosen for analysis as it is suitable for normally distributed data.

3 Results

A total of 75 Great Tit nests were dissected manually. Out of those, 27 nests were collected in sylvan areas of the study area, 23 nests were originated in mixed residential areas, 17 nests in mixed non-residential areas, and finally, eight nests in high density areas (Fig. 1).

The nests did not significantly differ ($p = 0.272$) in their total mass when sorted by land-use types where they were collected. However, sylvan nests tend to be heavier than nests from other land-use types (Table 2).

Table 2: Overview over the mean masses of nests, sorted by land-use types. For each land-use type, a mean nest mass [g] and a standard deviation [g] is shown.

Land-use type	Mean mass [g]	Standard deviation [g]
sylvan	27.81	± 12.12
mixed non-residential	25.13	± 6.27
mixed residential	22.90	± 8.93
high density	22.34	± 7.41
all combined	25.11	± 9.70

Table 3 gives insight into the mean amount of materials in such a nest in order to convey a general overview of the nest composition in Viennese Great Tit nests of this study.

Table 3: Mean amount of nest materials in percent of total nest mass in a mean Viennese Great Tit nest (n = 75). The data are derived from all nests from all land-use types combined.

Nest material	Amount of the nest [%]	Nest material	Amount of the nest [%]
Moss	44.92	Grass	2.58
Dust	16.43	Human derived material	2.15
Twigs and plant stem	12.07	Non-nesting material	0.57
Natural wool	10.31	Bark	0.50
Hair	5.42	Leaves	0.30
Other	5.10	Feathers	0.08

3.1 Amount of artificial materials in nests in relation to land-use types

Firstly, the relationship between the amount of artificial materials and land-use types was investigated. The boxplot (Figure 3) reveals a first insight into differences of nests from different land-use types in terms of artificial materials. Materials from category "Other Human-derived Material" were, for example: plastic (glittery, tinsel, in stripes, wires, or other forms), yarn and strings, metallic wires, glue, and insulating wool.

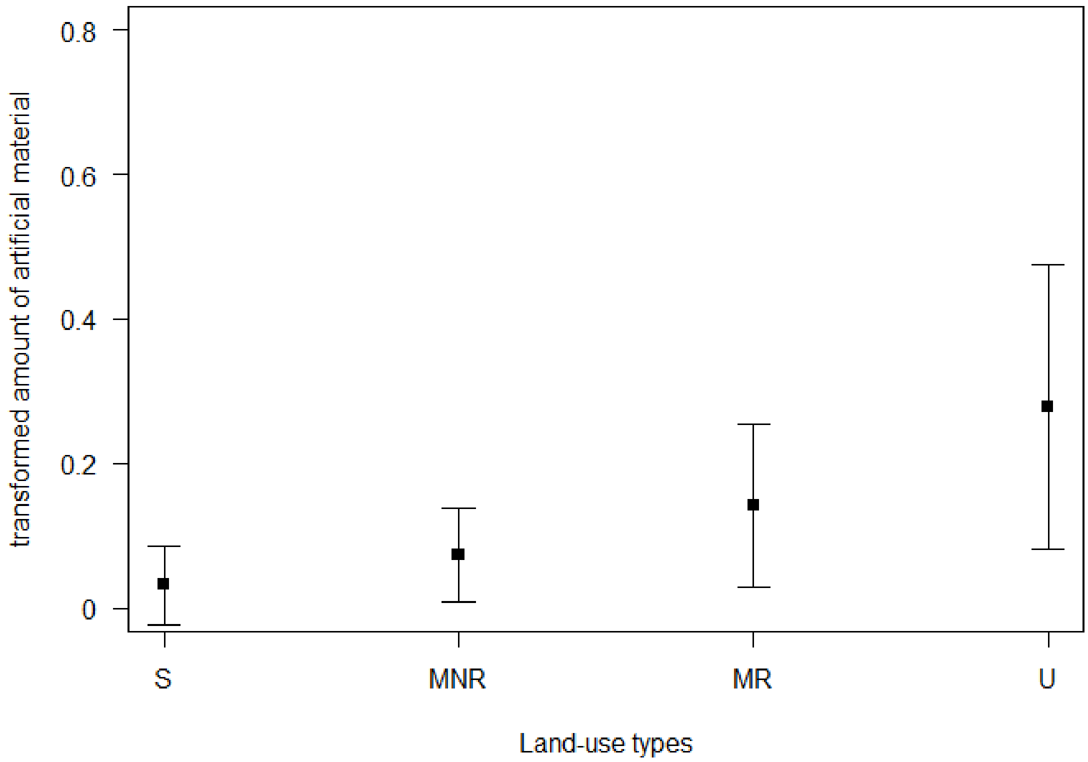

Figure 3: Mean values and standard deviations of artificial nest material depending on land-use types. The abbreviations of the land-use types mean the following: MNR – mixed non-residential areas, MR – mixed residential areas, S – sylvan areas, U – high density areas. Data is given in mass percentage of artificial nest material and was arcsinus square root transformed beforehand.

Nests in high density areas contained significantly more human-derived materials than nests in sylvan ($p < 0.0001$) and mixed non-residential areas ($p = 0.0067$). Nests in mixed residential areas contained less human-derived materials than nests in high density areas, however, this difference did not appear to be significant ($p = 0.2886$). Nests in mixed residential areas did contain significantly more human-derived materials than nests in sylvan areas ($p < 0.0001$).

3.2 Amount of artificial materials in nests in relation to the degree of sealing

In order to investigate the relationship between sealing of nest sites and the amount of artificial materials in the nest, the variables “sealing [%]” and “artificial materials [%]” were correlated against each other after being arcsinus square root transformed for reasons of homogeneity. As both variables did not follow a normal distribution (“sealing”: Shapiro-Wilk test: $p < 0.0001$; “artificial materials” Shapiro-Wilk test: $p < 0.0001$), the Spearman’s rank correlation test was performed. The rho value is positive (rho = 0.4213) and indicated a positive relationship between the sealing of the nest site and the amount of artificial materials in the nest, meaning that at nest sites with a higher percent of sealed ground, the nests contained more artificial nest materials. This relationship appears to be highly significant ($p = 0.0002$).

3.3 Amount of roe deer hair in nests in relation to land-use types

A total of ten nests were analysed to investigate the pattern of this hypothesis, five nests from sylvan areas and five nests from mixed non-residential areas.

The mean mass percentage of roe deer hair in mixed non-residential areas was 10.76 %, whereas the mean mass percentage of roe deer hair in sylvan areas was 13.27 %. The resulting p-value of the t-test did not show a significant difference between the nests in mixed non-residential and sylvan areas regarding the content of roe deer hair ($p = 0.5869$).

3.4 Amount of natural wool in nests in relation to land-use types

Natural wool was found in nests in all land-use types, as can be seen in Figure 4.

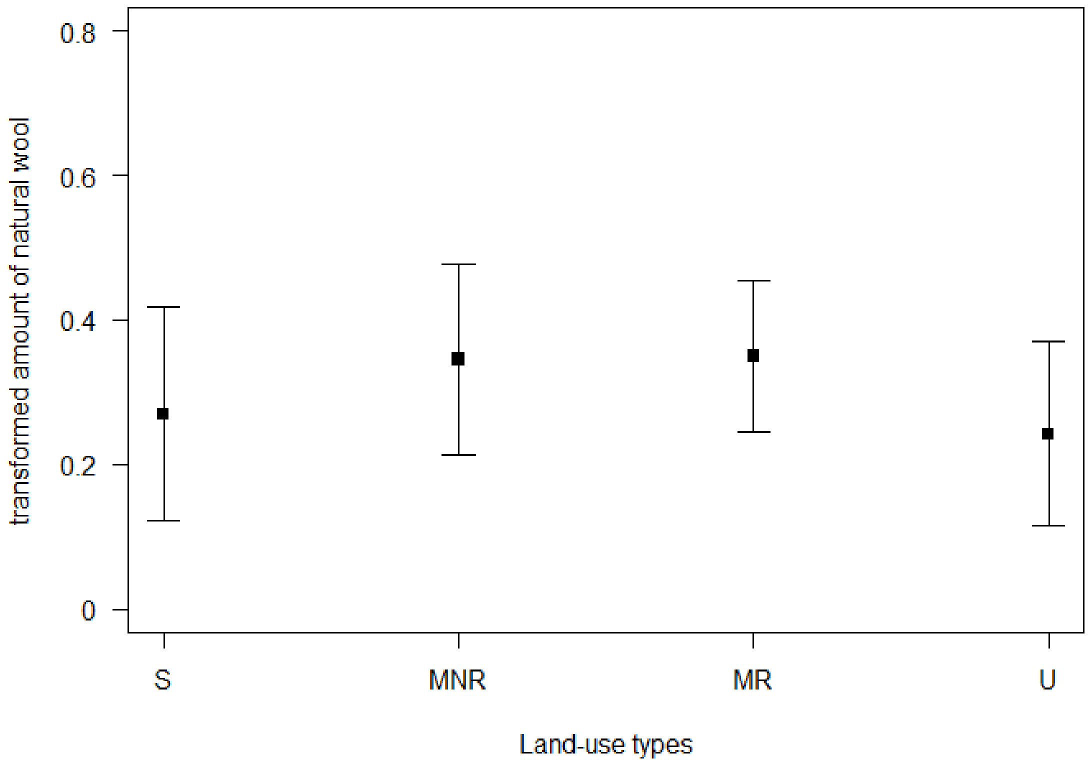

Figure 4: Mean values and standard deviations of the arcsinus-transformed percentage of natural wool of nests of different land-use types. Acronyms refer to Figure 3. The data of the mass percentage of natural wool was arcsinus square root transformed before the analysis.

After an arcsinus square root transformation, the data of natural wool content in the nests is normally distributed (Shapiro-Wilk test: $p = 0.1834$) and has a homogeneous variance (Levene test: $p = 0.6766$). Consequently, a one-way ANOVA was conducted to investigate significant differences between the nests of different land-use types. The resulting p-value ($p = 0.0517$) did not show significant differences, however, it showed a tendency that the nests of the land-use types do differ in their content of natural wool. As there was no significance found by the ANOVA, the Scheffe's test as a post hoc test is not applicable to detect further patterns. However, the boxplot gives an insight, showing a higher amount of natural wool in nests of mixed non-residential and mixed residential areas than in nests of sylvan and high density areas (Figure 4).

3.5 Number of feathers in nests in relation to ambient temperature

The hypothesis was tested with a set of variables derived from the temperature data received from the temperature loggers as well as with the number of feathers found in the nests. All variables included in the testing were normally distributed (Table 4).

Table 4: p-values of the Shapiro-Wilk test of all variables used in the testing of hypothesis five.

variable	p-value	normal distribution yes/no
Feathers [no] $_{n = 9}$	0.3026	Yes
Lowest temp. 3 days $_{\text{first egg}}$	0.2192	Yes
Mean temp. 3 days $_{\text{first egg}}$	0.5274	Yes
Lowest temp. $_{\text{first mat. to first egg}}$	0.2011	Yes
Mean temp. $_{\text{first mat. to first egg}}$	0.2732	Yes

As all variables were normally distributed, the Pearson's correlation coefficient was chosen to determine the relationship between the surrounding temperature at the nest site and the number of feathers in the nest. The results for each test are shown in Table 5.

Table 5: Results of correlation tests with the method by Pearson concerning the relationship between the number of feathers and ambient temperature. The table shows the tested variables, the resulting correlation coefficient and the resulting p-value.

tested variables	correlation coefficient	p-value
Feathers [no] – Lowest temp. 3 days $_{\text{first egg}}$	-0.04169518	0.9152
Feathers [no] – Mean temp. 3 days $_{\text{first egg}}$	0.04642929	0.9056
Feathers [no] – Lowest temp. $_{\text{first mat. to first egg}}$	-0.1616355	0.6778
Feathers [no] – Mean temp. $_{\text{first mat. to first egg}}$	0.2294148	0.5527

The lowest temperature was generally negatively correlated to the number of feathers in the nest, whereas the mean temperature was generally positively correlated to the number of feathers (Table 5). These patterns, however, appeared not to be significant since all p-values resulting from the correlation tests were higher than 0.05. Consequently, no relationship between the surrounding temperature at the nest site during the early stages of breeding and the number of feathers in the nest could be found.

4 Discussion

This study aimed to investigate differences in nest composition of Great Tits along the urban gradient in Vienna. Investigations were concentrated on artificial materials as well as on insulative materials like hair, natural wool, and feathers.

4.1 Amount of artificial materials in nests in relation to land-use types

In general, 59 of 75 nests contained artificial materials which represents 78.67 % of all nests. Compared to the study of Surgey et al. (2012) who found an average of 29.5 % of Great Tit nests with artificial, coloured wool, almost 80 % in Vienna might appear to be a very high number. However, one needs to consider that the study at hand took place in an area highly influenced by people whereas Surgey et al. (2012) analysed nests from woodland managed as a nature reserve. Consequently, a higher abundance of artificial materials in my study area can be presumed. Additionally, I did not only include coloured wool into the category, but all non-natural materials of any origin in contrast to Surgey et al. (2012) who disposed artificial wool and concentrated only on its usage in nest building. The high number of nests containing artificial materials in my study can therefore be explained.

Nests in high density areas did contain more human derived material than nests in the three other land-use types. However, only differences towards sylvan areas and mixed non-residential areas appeared to be significant. Nests in sylvan areas did significantly contain less human derived materials than nests in mixed residential areas and high density areas.

The results do reflect the expectations made *a priori*. Unfortunately, it was not possible within this study to obtain data on the availability of material in the environment of the nest sites, and thus, it appears to be difficult to link the observed pattern with the above-named availability of material. Nevertheless, the results show that Great Tits in Vienna do vary the amount of artificial materials in their nests in different land-use types. A study carried out in Spain showed altered nest compositions of Great Tits in four Mediterranean habitats (Álvarez et al. 2013) and therefore, complies with my results of varying nest compositions. However, the study did not include highly urban areas as it was the case in this project. My results support the hypothesis that Great Tits in less natural areas incorporate materials which can be found directly at the nest site and which are associated with a human-influenced environment, such as plastic, yarn, or metallic wires. The birds were able to build complete nests in all four land-use types investigated within the present study with an altered use of artificial materials in higher urbanised land-use types than in more natural habitats.

Nests from high density and mixed residential areas and nests from sylvan and mixed non-residential areas did not significantly differ in the amount of artificial materials, respectively. It can be suggested that those areas, respectively, do not show a significant difference in the availability of human-derived material. However, as mentioned before, this could not be investigated within this study. Alternatively, it might be that the birds in the two higher urbanised areas choose the same materials even though the availability may differ. A joint choice of materials then would also apply to birds from sylvan and mixed non-residential areas, respectively. Another reason could be that Great Tits choose natural materials over artificial ones and, when having the choice, might prioritise them. However, this deserves more detailed investigations as the behaviour of nest material choice remains unclear. It seems to be more plausible that the birds take the nest material opportunistically as was previously observed and demonstrated by Surgey et al. (2012). Consequently, the availability of material in the different land-use types seems to be more important for the nest composition as birds may predominately use those materials that they encounter more often (see also Britt & Deeming 2011).

4.2 Amount of artificial materials in nests in relation to the degree of sealing

The analysis revealed a significantly positive relationship between sealing of the nest site and the amount of artificial materials found in the corresponding nests. This result agrees with patterns observed in a study on Chinese Bulbuls (Wang et al. 2009). The study revealed a significant alteration in nest composition with different levels of urbanisation, showing an increase of artificial materials used in nest building with an augmented degree of urbanisation (Wang et al. 2009). Wang et al. (2009) also found an increase of availability of artificial nest material with increasing urbanisation, suggesting that this attributes to the pattern described above. The same could be assumed for the study at hand, even though no availabilities of materials were investigated here. However, it is convenient that the same pattern of availability, as observed in Hangzhou, occurs along the urban gradient of Vienna, assuming that the availability of materials is similarly distributed in both cases of urbanisation.

There are also myriad other studies reporting, mostly anecdotally though, the usage of artificial nest materials in urbanised areas. Collias & Collias (1984) address an example of a nest of a Rock Dove (*Columba livia*) near a factory in Michigan, USA, which was similar to other Rock Dove nests, but instead of twigs, iron wires were used. In Ludwigshafen, Germany, nests in industrialised areas could be observed in various bird species (e.g. Eurasian Collared Dove (*Streptopelia decaocto*), Black Redstart (*Phoenicurus ochruros*), Common Blackbird, European Greenfinch (*Chloris chloris*)). Those nests also contained artificial nest materials like paper, glass wool, binding wire, and other synthetic material (Stalla 1990). These examples show that the availability of materials plays a major role in nest building. Furthermore, it highlights that birds are flexible in the use of nest materials. Consequently, the usage of artificial materials in nest building in urbanised areas is not new to scientists. However, the quantification of artificial materials used by Great Tits, as in my study, was scarcely done until now. The positive relationship between the amount of artificial materials and the degree of sealing, as shown in the study at hand, indicates that, similar to Chinese Bulbuls, Great Tits adjust their nest building behaviour regarding material choice to the degree of urbanisation.

Another approach to interpret the results is that birds choose their nest site depending on the availability of suitable material, besides first and foremost considering the availability of food and predators (Collias & Collias 1984, Hansell 2000). This comes in hand with a lower occupation rate of the nest boxes in the city centre (Pech 2016, Schack Madsen 2016, Renner 2017). Probably, these birds find less suitable materials in urban areas and thus, occupy less nesting sites. However, the place they are finally choosing to breed, they have enough material, natural or artificial, to build nests. Further studies to evaluate the availability of materials in Vienna, as well as more specific studies on nest material choice by Great Tits are needed to either confirm or deny the suggestions made above.

4.3 Amount of roe deer hair in nests in relation to land-use types

Nests with almost exclusively roe deer hair could only be found in sylvan areas and mixed non-residential areas, concerning five nests from each of the two land-use types. Unfortunately, it was not possible to conduct a detailed hair analysis of all hairs in all nests. Thus, I decided to only use nests which contained almost exclusively roe deer hair, as it is very distinctive to other animals' hair, for the analysis. It surely would have been interesting to further discriminate between hair of wildlife and hair of domesticated animals, as well as humans. This would have given further insight into the adjustment to materials associated with human settlements.

Hair is used in Great Tit nests in the nest lining and serves insulation (Ondrušová 2011). Nests analysed for the amount of hair were exclusively collected in the two lesser urbanised land-use types investigated in this study. Those areas are less affected by the "heat island" effect of the city, making it possibly necessary to invest more energy to gather more insulative material for the nest in order to protect the eggs and the offspring from colder temperatures. However, it does not seem to be plausible to speak of an adjustment to local temperatures with increased insulation. Hairs do not solely contribute to the insulation of the nest, other materials, for example feathers and wool, need to be considered too. As only roe deer hairs were investigated here, it could not be analysed to what extend other hairs are used by the birds. Consequently, patterns of adjusted insulation to land-use types along an urban gradient could not reliably be detected in the study at hand.

The nests in sylvan and mixed non-residential areas did not significantly differ in their amount of roe deer hair. This result is not surprising as roe deer is abundant in forest habitats as well as in agricultural habitats (Wang & Schreiber 2001, Stadtwildtiere 2016), such as vineyards that can be found in the mixed non-residential areas of Vienna. Consequently, its hair can be found frequently in those sites. Even though no data about roe deer occurrence in the city of Vienna was integrated into this work, it is likely that roe deer abundance in mixed residential and high density areas is reduced compared to sylvan and mixed non-residential areas. This might be due to the high sealing and density of buildings and thus, lacking food and coverage availability in higher urbanised areas. Previous studies have shown that roe deer hair is one of the most abundant hairs in songbird nests when available (Henze 1962, Ondrušová 2011). The absence of nests with solely roe deer hair in the mixed residential and high density areas support the outcomes described above. The results regarding the amount of hairs support the conclusion drawn from hypothesis one and two that Great Tits do use material directly found at the nest site and its close surroundings.

4.4 Amount of natural wool in nests in relation to land-use types

Within this study, I could show a tendency for a higher amount of natural wool in nests of mixed non-residential and mixed residential areas (Figure 4). Those clusters display areas which enable keeping livestock, such as sheep. Additionally, the above-named land-use types lie in areas where people tend to walk and comb their dogs (personal observations). Consequently, I expected to find not only wool from sheep and possibly other wild animals with dense fur, but also undercoat of domesticated animals, which, after being incorporated into the nests, was no longer distinguishable from woolish material and thus, was also included into this category. My expectations do agree with the tendency shown here, supporting the conclusions drawn from previous hypotheses that material availability at the nest site is crucial for the choice of material used in the nests.

Woolish material is mostly used in the lining zone of the nest (Hansell 2000) and has an insulative impact (Hilton et al. 2004, Surgey et al. 2012). As discussed above (see 4.1), it appears to be difficult to determine the role of the analysed material in nest insulation and connect it to land-use. However, the separate quantification of insulative materials such as hairs and natural wool demonstrates that Great Tits can use both materials for nest lining and insulation (see also: Lamprecht & Schmolz 2004). As every nest, except for one, did contain material from the category “natural wool”, it can be conceived that it is very important for nest building of Great Tits and is not giving the birds the possibility to resemble it and significantly vary its amount in the nest. Consequently, the missing significance level could not only be explained by availability and land-use type. Instead, it also has to be taken into account that a considerable difference in the amount of woolish material might not be reasonable due to its great importance in nest lining.

Furthermore, material from the category “natural wool” might include undercoat from dogs or other carnivores and thus, might have an anti-predatory, deterrent effect. However, this suggestion could not be illuminated here and needs further investigations.

4.5 Number of feathers in nests in relation to ambient temperature

In the study at hand, no significant relationship between the ambient temperature and the number of feathers incorporated into the nest could be detected. This result, however, might be influenced by the study design as only feathers that are not of Great Tit origin were included into the category “feathers”, hazarding that this might create a shift in the pattern. Additionally, and essentially, the sample size was reduced to only nine nests due to logistical reasons while placing the temperature loggers. Therefore, relevant patterns might be masked by individual nest building behaviour of only nine breeding pairs. With a higher sample size, it might be possible to detect an adaptation to higher temperatures in city cores (1-2 °C difference: Wichmann et al. 2009). It would have been expedient to also investigate the first three days after the beginning of nest building as an important phase of the nest building process. However, due to logistic reasons, there was only data for four nests available for this time span and thus, conclusions from an analysis would be unreliable.

A study conducted in Lower Austria (Schöll & Hille, 2014) demonstrated that Great Tits do not appear to adapt their nest insulation with feathers to colder environments in a higher altitude. Instead, the birds seem to delay clutch initiation instead of adjusting their nest (Schöll & Hille, 2014). When having a closer look at the data, one can observe that the birds within the city seem to lay their eggs earlier than those in the colder, surrounding forest habitats, even though this was not statistically tested and demands further investigations, which would have gone beyond the scope of this thesis. The air temperature difference between Vienna and its surroundings reported by Wichmann et al. (2009) might be under a certain threshold for the birds to detect those temperature differences and, as a further step, to adapt their nest building behaviour.

Additionally, collecting insulating material like feathers is also more energy consuming than a greater commitment in incubation and nestling care and thus, it might not be ecologically efficient. Moreno et al. (2010) found an energetic trade-off for female Pied Flycatchers

(*Ficedula hypoleuca*) between nest building and nestling care, which results in an effect of energetic costs of nest building on the development and growth of the nestlings. This trade-off could also be applicable to Great Tits: The birds might not spend time and energy on building a very well insulated nest, but instead spend it on nestling care later in the season (Moreno et al. 2010). As flying is one of the activities of a bird that requires most energy (Collias & Collias 1984), it might be more efficient to collect nest materials nearby the nest site, rather than to fly further and search for possibly better and more natural material. To my knowledge, the energy costs of Great Tits during the time of nest building is not yet known. Surgey et al. (2012) were able to observe an average distance flown by Tits from an artificial material dispenser to the nest box with incorporated material of about 160 meter, with the greatest distance of a Great Tit of 860 meter. This implies that Great Tits stay near the nest site and their choice of material is opportunistic and depends on what the bird is able to find in the surrounding area (Surgey et al. 2012). Those findings support my conclusions described above.

Even though my data did not show a negative effect of urban environments on the birds' ability of complete nest construction, a similar breeding performance as in natural environments cannot be assumed. Possibly, younger individuals are forced into less natural environments as a result of competition for nesting sites in which older birds get to settle before first-year birds (Krebs 1971). This may result in a poorer breeding performance as observed in previous studies (Pech 2016, Schack Madsen 2016, Renner 2017).

However, the food availability for the nestlings is reduced in the high density and mixed residential areas (Chamberlain et al. 2009) and feeding the young consequently is energetically more demanding than in more natural habitats with a higher food abundance. As a result, the young birds fledge with a lower body mass and also in lower numbers compared to more natural habitats (Pech 2016, Schack Madsen 2016, Renner 2017). This might be due to post-hatching reasons and probably also stress levels of the adult birds (see Pech 2016), but effects of an altered nest composition cannot be excluded. It also needs further research to clarify whether the lower body mass of the fledglings in urban areas really is of disadvantage or if they can compensate for it compared to their conspecifics in more natural habitats due to milder winters in cities (Hinkel et al. 2003).

Although the nest plays a crucial role in reproduction, the birds do not seem to adjust the size of the nest (as bigger nests are associated with a positive impact on several fitness related parameters (Álvarez & Barba 2008)) or the use of insulating material in order to compensate for slower growth due to decreased food availability. A reason for that might be that, when collecting more nest material, the risks of predation during the increased amount of flights that are necessary are higher. With an increase of domestic cats along the urban gradient, the risk of direct predation and also sub-lethal effects also augmented (Lepczyk et al. 2003, Beckerman et al. 2007). Consequently, the birds might gather the fewest amount possible to build their nests to raise the offspring, accepting a lower reproductive success, measured in numbers and body mass of the fledglings (Pech 2016, Schack Madsen 2016, Renner 2017).

All in all, my study highlights that the nest composition in Great Tits varies in different habitat types and therefore reinforces the findings of various previous studies (e.g. Collias & Collias 1984, Wang et al. 2009, Britt & Deeming 2011, Ondrušová 2011). The amount of artificial materials in the nest lining is altered in high density and mixed residential areas, also coming in hand with a higher degree of sealing. This indicates that Great Tits opportunistically use any suitable materials they can find close by their nesting site. It confirms the findings of Álvarez et al. (2013), who found different nest compositions in four Mediterranean habitats. The plasticity in nest building behaviour, as demonstrated within this study by quantifiably investigating nest composition, reveals the ability of adjustment of Great Tits to a changing environment.

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7 Appendix

Figure 5: Example for material of the category "Twigs and Plant stem". The material shown is from nest 15_3 (land-use type: high density).

Figure 6: Example for material of the category "Grass". The material shown is from nest 68_3 (land-use type: sylvan).

Figure 7: Example for material of the category "Leaves". The material shown is from nest 09_3 (land-use type: mixed residential).

Figure 8: Example for material of the category "Bark". The material shown is from nest 56_2 (land-use type: sylvan).

Figure 9: Example for material of the category "Moss". The material shown is from nest 15_3 (land-use type: high density).

Figure 10: Example for material of the category "Feathers". The material shown is from nest 55_3 (land-use type: mixed non-residential). Great Tit feathers are not included into the category due to difficulties of differentiation of Great Tit feathers built into the nest on purpose and Great Tit feathers not built into the nest, but found due to bird activity.

Figure 11: Example for material of the category "Hair". The material shown is from nest 09_3 (land-use type: mixed residential).

Figure 12: Example for material of the category "Wool". The material shown is from nest 10_2 (land-use type: mixed non-residential).

Figure 13: Example for material of the category "Coloured wool". The material shown is from nest 15_3 (land-use type: high density).

Figure 14: Example for material of the category "Paper". The material shown is from nest 15_3 (land-use type: high density).

Figure 15: Example for material of the category "Cigarette stubs". The material shown is from nest 28_3 (land-use type: high density).

Figure 16: Example for material of the category "Other human-derived material". The material shown is from nest 30_1 (land-use type: mixed residential). In the picture, metallic, tinsel-like material is shown, however, other artificial materials, mostly plastic and metallic-like, were found in other nests.

Figure 17: Example for material of the category “Dust”. The material shown is from nest 10_2 (land-use type: mixed non-residential).

Figure 18: Example for material of the category “Non-nesting material”. The material shown is from nest 30_1 (land-use type: mixed residential) and contains Great Tit feathers as well as insects’ body parts.

Figure 19: Example for material of the category "Other". The material shown is from nest 56_2 (land-use type: sylvan) and contains mainly little rocks.