Effects of urbanisation on the ecology, foraging behavior and energy expenditure of the Eurasian red squirrel (*Sciurus vulgaris*)

Dissertation

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II **Wist B**, Stolter, C & Dausmann KH (2022) Sugar addicted in the city: Impact of urbanisation on food choice and diet composition of the Eurasian red squirrel (*Sciurus vulgaris*). *Journal of Urban Ecology* 8(1), juac012. https://doi.org/10.1093/jue/juac012

III **Wist B**, Montero BK & Dausmann KH (2023) City comfort: weaker metabolic response to changes in ambient temperature in urban red squirrels. *Scientific Reports* 13, 1393. https://doi.org/10.1038/s41598-023-28624-x

Authors' contributions	**I**	**II**	**III**
Study conception & design	BW, KHD	BW, CS, KHD	BW, KHD
Field work	BW	BW	BW
Data preparation	BW	BW	BW
Statistical analysis	BW	BW (with advice by CS)	BW, BKM
Writing the manuscript	BW	BW	BW
Commenting	BW, KHD	BW, CS, KHD	BW, BKM, KHD

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Summary

Increasing urbanisation worldwide is a major conservation problem in the Anthropocene. Urban habitats represent evolutionarily novel environments that challenge wildlife at various levels. The multitude of urban stressors leads to biodiversity loss and species homogenisation. Yet, cities are home to a few apparently successful, so-called ‘synurban’, species that occur in higher densities than in their natural habitats, supported by factors such as supplemental food availability and buffered climatic conditions. However, consequences of living in urban habitats and particularly in core areas of increasingly larger cities might be detrimental in the long-term and are still poorly understood. Deeper insight into species responses and underlying mechanisms is essential though to mitigate the effects of present and future urbanisation by reaching informed and timely actions in urban planning and nature conservation.

Against this background, my thesis combined a field study on the effects of urbanisation on a common synurban mammal, the Eurasian red squirrel (*Sciurus vulgaris*), in small centrally located parks with subsequent experiments under semi-natural conditions. It provides insight into species responses to urbanisation and associated changes in food supply with regard to population parameters, nutrient ecology, foraging behaviour and ecophysiology. Parks in city centres are surrounded by a dense urban matrix, but seem to be important habitats for wildlife, such as squirrels, given the comparably high population densities there. However, my findings document pronounced differences between parks of comparable size and location with regard to food abundance and composition as well as squirrel population density. This underlines the small-scale urban heterogeneity and demonstrates that environmental conditions and population parameters can differ even in nearby comparable urban patches.

In general, I found a great variety of non-natural foods, such as bakery products, in the parks along with limitations in natural foods and a possibly maladaptive response in the foraging behaviour of urban squirrels living in a park with high conspecific density and high supplemental food abundance. My findings indicate concerning changes in the dietary composition of urban squirrels, as they frequently chose non-natural food items and high sugar

foods in possibly detrimental amounts compared to conspecifics from the forest in an experiment where they were free to choose. Similar indications have been found in other urban dwelling species. The results underline that the availability of inappropriate supplemental food sources increases the risk of malnutrition and health problems.

Apart from dietary aspects, relevant for energy and nutrient intake, urban wildlife are affected by the urban heat island. The resulting warmer ambient temperatures reduce the need of endogenous heat production in endothermic mammals and influence an animals' physiology. My study documented a less sensitive thermoregulatory response in urban squirrels compared to forest individuals, possibly driven by a lower diurnal temperature fluctuation than in rural habitats. The less steep slope of metabolism in relation to ambient temperature in urban individuals though indicated lower energetic costs at the colder range of temperatures, but comparably higher expenses in warmer conditions. In the light of climate change and rising temperatures globally, this is concerning given the fact that endothermic species are not only challenged by the need of heat production, but also by heat dissipation. This is even more true since climate change interacts with urbanisation and increase its negative effects, particularly in city centres. Mammals in urban core areas thus might get closer to their thermal limits, though they possibly might have better access to water or use adaptations that enhance cooling. However, knowledge about physiological responses and adaptations in urban environments in endothermic species remains scarce and little is known about thermal tolerance in squirrels.

At present, we cannot fully answer the question whether or not urban green habitat patches act as source or sink habitats on the long-term and choosing an appropriate spatial scale for urban wildlife studies is fundamental for informed conclusions and conservation actions. Nonetheless, my results indicate maladaptive responses in synurban species. Abundant supplemental food sources likely act as potentially misleading environmental cue that increases the number of individuals at a given patch, but are not necessarily beneficial for fitness-relevant traits, such as nutrition or body condition. However, my study highlights the need of protecting and increasing urban vegetation. This would not only be beneficial for urban

wildlife with regard to factors such as natural food supply, but can also provide crucial cooling to mitigate the impact of the global temperature rise for animals and humans. On top of this, the findings of this thesis emphasise that public guidance in supplemental feeding in combination with impeded access to refuse are crucial measures to increase the diet quality for urban dwelling species. With a view to future research, it is vital to consider that other factors, such as digestive constraints or the intake of widespread pollutants in cities likely interact with behaviour and physiology and this needs further investigation. To date, we are still in the beginning of understanding the full mechanisms and long-term consequences of living in urban habitats across the globe.

Zusammenfassung

Die zunehmende weltweite Urbanisierung ist ein großes Naturschutzproblem im Anthropozän. Städtische Lebensräume stellen evolutionär neuartige Umgebungen dar, die die Tierwelt auf verschiedenen Ebenen herausfordern. Die Vielzahl städtischer Stressfaktoren führt zum Verlust der Artenvielfalt und zu einer Homogenisierung der Arten. Dennoch beherbergen Städte einige scheinbar erfolgreiche, sogenannte „synurbane“ Arten, die in größerer Dichte als in ihren natürlichen Lebensräumen vorkommen, was durch Faktoren wie die Verfügbarkeit zusätzlicher Nahrung und abgefederte klimatische Bedingungen unterstützt wird. Allerdings könnten die Folgen des Lebens in städtischen Lebensräumen und insbesondere in Kerngebieten immer größerer Städte langfristig schädlich sein und sind noch immer kaum verstanden. Ein tieferer Einblick in die Reaktionen der Arten und zugrunde liegenden Mechanismen ist jedoch unerlässlich, um die Auswirkungen der gegenwärtigen und zukünftigen Urbanisierung durch fundierte und rechtzeitige Maßnahmen in der Stadtplanung und im Naturschutz abzumildern.

Vor diesem Hintergrund kombinierte meine Dissertation eine Feldstudie zu den Auswirkungen der Urbanisierung auf ein weit verbreitetes synurbanes Säugetier, das Eurasische Eichhörnchen (*Sciurus vulgaris*), in kleinen, zentral gelegenen Parks mit anschließenden Experimenten unter naturnahen Bedingungen. Sie bietet Einblicke in die Reaktionen der Art auf Urbanisierung und damit verbundenen Veränderungen im Nahrungsangebot im Hinblick auf Populationsparameter, Nahrungsökologie, Futtersuchverhalten und Ökophysiologie. Parks im Stadtzentrum sind von einer dichten städtischen Matrix umgeben, scheinen aber angesichts der dort vergleichsweise hohen Populationsdichten wichtige Lebensräume für Wildtiere wie Eichhörnchen zu sein. Meine Ergebnisse dokumentieren jedoch deutliche Unterschiede zwischen Parks vergleichbarer Größe und Lage hinsichtlich der Nahrungshäufigkeit und -zusammensetzung sowie der Eichhörnchenpopulationsdichte. Dies unterstreicht die kleinräumige städtische Heterogenität und zeigt, dass Umweltbedingungen und Populationsparameter selbst in nahegelegenen

vergleichbaren Stadtgebieten unterschiedlich sein können.

Ich fand in den Parks grundsätzlich eine große Vielfalt an nicht-natürlichen Nahrungsmitteln, wie z. B. Backwaren, zusammen mit Einschränkungen bei natürlicher Nahrung und einer möglicherweise maladaptiven Reaktion im Futtersuchverhalten von urbanen Eichhörnchen, die in einem Park mit hoher Artgenossendichte und hohem Vorkommen von Zufütterung leben. Meine Ergebnisse deuten auf besorgniserregende Veränderungen in der Nahrungszusammensetzung urbaner Eichhörnchen hin, da sie in einem Experiment, in dem sie die freie Wahl hatten, im Vergleich zu Artgenossen aus dem Wald häufig unnatürliche Nahrungsmittel und Lebensmittel mit hohem Zuckergehalt in möglicherweise schädlichen Mengen wählten. Ähnliche Hinweise wurden bei anderen Arten, die in Städten leben, gefunden. Die Ergebnisse unterstreichen, dass die Verfügbarkeit ungeeigneter Nahrungsergänzungsmittel das Risiko von Mangelernährung und Gesundheitsproblemen erhöht.

Abgesehen von diesen Ernährungsaspekten, die für Energie- und Nährstoffaufnahme relevant sind, ist die Tierwelt auch von der städtischen Wärmeinsel betroffen. Die daraus resultierenden wärmeren Umgebungstemperaturen verringern den Bedarf an endogener Wärmeproduktion bei endothermen Säugetieren und beeinflussen die Physiologie der Tiere. Meine Studie dokumentierte eine weniger empfindliche thermoregulatorische Reaktion bei städtischen Eichhörnchen im Vergleich zu Waldhörnchen, was möglicherweise auf eine geringere tägliche Temperaturschwankung als in ländlichen Lebensräumen zurückzuführen ist. Der weniger steile Anstieg des Stoffwechsels im Verhältnis zur Umgebungstemperatur bei Eichhörnchen aus der Stadt deutet zwar auf niedrigere Energiekosten im kälteren Temperaturbereich, aber vergleichsweise höhere Kosten bei wärmeren Bedingungen hin. Angesichts des Klimawandels und weltweit steigenden Temperaturen ist dies besorgniserregend, da endotherme Arten nicht nur durch die Notwendigkeit der Wärmeerzeugung, sondern auch durch die Wärmeableitung herausgefordert werden. Dies gilt umso mehr, als der Klimawandel mit der Urbanisierung interagiert und negative Auswirkungen, insbesondere in Innenstädten, verstärkt. Säugetiere in urbanen Kerngebieten könnten daher näher an ihre thermischen Grenzen stoßen, obwohl sie möglicherweise einen besseren Zugang zu Wasser haben oder Anpassungen nutzen, die die Kühlung verbessern. Das Wissen über physiologische Reaktionen und Anpassungen endothermer Arten in urbanen Lebensräumen ist jedoch nach wie vor dürftig und es ist wenig über die thermische Toleranz bei Eichhörnchen bekannt.

Die Frage, ob urbane Grünflächen langfristig als Source- oder Sinkhabitate fungieren, lässt sich derzeit noch nicht abschließend beantworten. Die Wahl eines geeigneten räumlichen Maßstabs für Studien zur städtischen Tierwelt ist für fundierte Schlussfolgerungen und Schutzmaßnahmen von grundlegender Bedeutung. Meine Ergebnisse deuten jedoch auf maladaptive Reaktionen bei synurbanen Arten hin. Reichlich vorhandene Zufütterung fungiert wahrscheinlich als potenziell irreführender Umweltreiz, der die Individuenzahl an einem Ort erhöht, aber nicht unbedingt vorteilhaft für fitnessrelevante Merkmale wie Ernährung oder Kondition ist. Diese Studie unterstreicht jedoch die Notwendigkeit, die städtische Vegetation zu schützen und auszuweiten. Dies würde nicht nur der städtischen Tierwelt im Hinblick auf Faktoren wie natürliche Nahrungsversorgung zugutekommen, sondern kann auch für entscheidende Abkühlung sorgen, um die Auswirkungen des globalen Temperaturanstiegs für Tiere und Menschen abzumildern. Darüber hinaus unterstreichen diese Arbeit, dass öffentliche Leitlinien zur Zufütterung in Kombination mit einem erschwerten Zugang zu Müll entscheidende Maßnahmen zur Verbesserung der Ernährungsqualität städtischer Arten sind. Im Hinblick auf zukünftige Forschungen ist es jedoch wichtig zu berücksichtigen, dass andere Faktoren wie Verdauungseinschränkungen oder die Aufnahme weit verbreiteter Schadstoffe in Städten wahrscheinlich mit Verhalten und Physiologie interagieren und dies weiterer Untersuchungen bedarf. Bis heute stehen wir noch am Anfang des Verständnisses der vollständigen Mechanismen und langfristigen Folgen des Lebens in städtischen Lebensräumen auf der ganzen Welt.

Chapter 1:

General introduction

Urbanisation: a major conservation challenge in the Anthropocene

Human activities and associated global change are growingly seen as driver of a new geological epoch – the Anthropocene (Steffen et al. 2011, Corlett 2020, Waters and Turner 2022). The major challenges of this epoch are climate change and biodiversity loss (Corlett 2020). Globally and rapidly increasing urbanisation is amongst the most profound man-made environmental modifications and impacts global wildlife in multiple ways (Grimm et al. 2008, Johnson and Munshi-South 2017, McDonald et al. 2019). It is projected that more than two third of the world's population will live in urban areas by 2050 (United Nations 2018, Fig. 1.1). Cities reflect the strongest form of urbanisation and are predicted to further increase in number and size (Grimm et al. 2008, United Nations 2018, Fig. 1.2). The effects of urbanisation on species and wildlife communities are most severe in urban core areas (McKinney 2002).

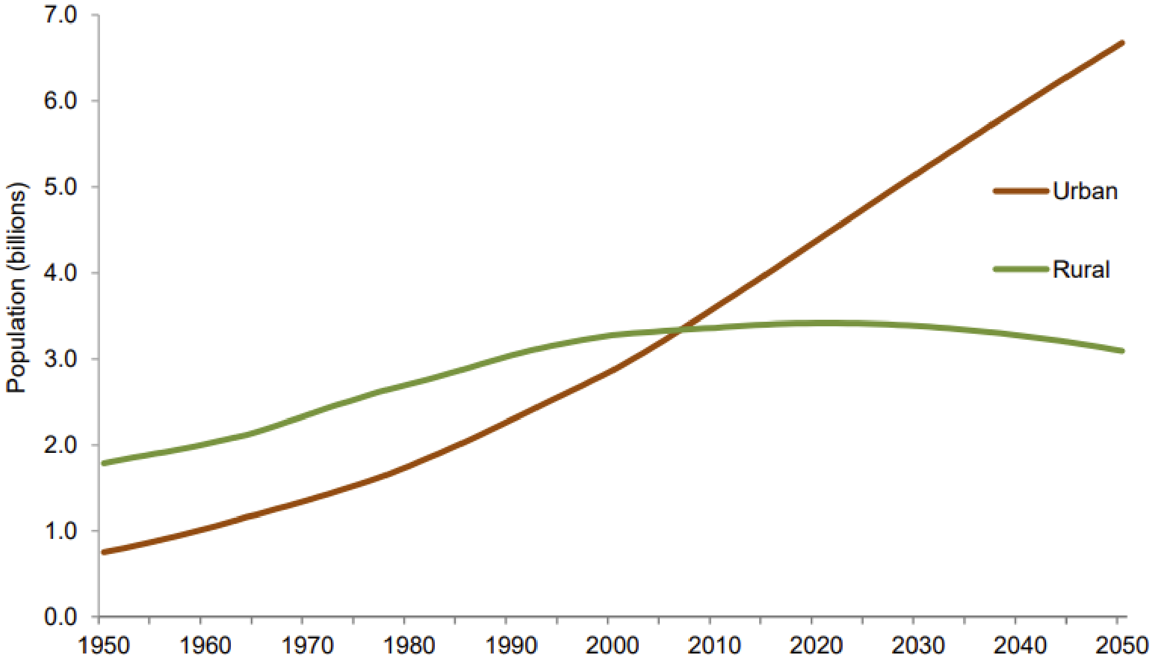

Figure 1.1 Urban (brown line) and rural populations (green line) of the world (1950 – 2050) with more than two thirds of the world's population projected to live in urban settings by 2050 (graph modified from United Nations, Department of Economic and Social Affairs, Population Division (2018a), *World Urbanization Prospects 2018*)

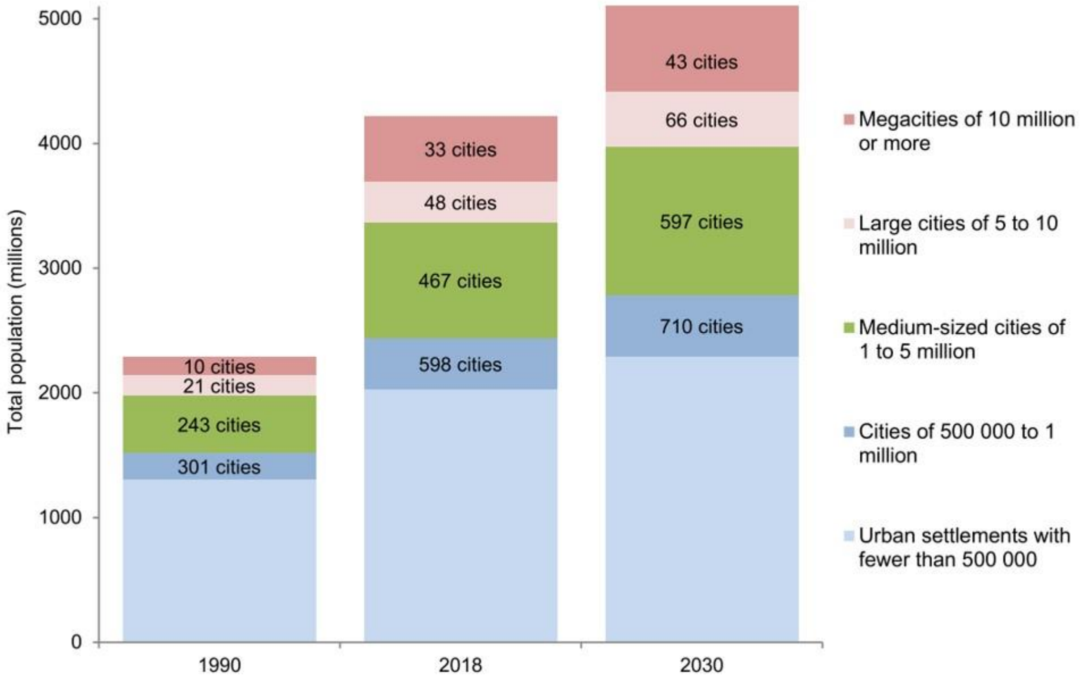

Figure 1.2 Population and number of cities of the world, by size class of urban settlement (years 1990, 2018 and 2030, graph modified from United Nations, Department of Economic and Social Affairs, Population Division (2018a), *World Urbanization Prospects 2018*)

There is no uniform definition of when a habitat is considered urban. Countries, institutions and researchers use different approaches, such as categorising sites by variables like human population density, amount of impervious surface or distance from a city centre (McIntyre et al. 2008, Liu et al. 2014, Short Gianotti et al. 2016). Furthermore, urban habitats are extremely heterogeneous (McIntyre et al. 2008, Birnie-Gauvin et al. 2016, Rivkin et al. 2019). Various factors, such as vegetation cover or socio-economic status can differ between or within a city. However, it was found that small mammal populations become effected by urbanisation when human population densities exceed 1,000 people per km^2 (Łopucki et al. 2020).

Environmental change & urban wildlife

Urban settings are characterised by numerous abiotic and biotic environmental changes and a severe loss of natural habitats like forests (Birnie-Gauvin et al. 2016, Alberti et al. 2017, Johnson and Munshi-South 2017, McDonald et al. 2019). Land development and surface sealing result in vast changes of landscape structure and vegetation cover (Grimm et al. 2008, Seress and Liker 2015). Remaining vegetation is often concentrated in managed public parks or private gardens and includes high numbers of non-native or ornamental plants (Rebele 1994, McKinney 2006). Dense human populations and their activities cause altered chemical or nutrient cycles, pollution of air and soil as well as noise and artificial lights (Grimm et al. 2008, Kight and Swaddle 2011, Johnson and Munshi-South 2017). Buildings and roads with dense traffic cut the landscape into fragmented habitat patches and can create movement barriers for wildlife (Johnson and Munshi-South 2017, DeCandia et al. 2019).

Furthermore, buildings and impervious surfaces contribute to the urban heat island effect with higher air and soil temperatures compared to surrounding rural areas, as well as changes in precipitation and wind speed (Rizwan et al. 2008, Pickett et al. 2011, Han et al. 2014, Droste et al. 2018). Global climate change and associated extreme weather events are predicted to be most severe in cities (IPCC 2022). Consequently, wildlife species able to successfully colonise urban settings or persist on a long-term basis have to be resilient to various stressors (Fig. 1.3). Numerous animal species cannot cope, resulting in severe declines in biodiversity, species richness and abundance in cities (Faeth et al. 2011, McDonald et al. 2019, Łopucki et al. 2020). Moreover, species composition and consequently trophic structures and biotic interactions are changed (Faeth et al. 2005, Grimm et al. 2008, Berger-Tal and Saltz 2019). This is accompanied by a higher chance of successful establishment and spread of non-native or invasive species, thereby further exacerbating the problem (McKinney 2006).

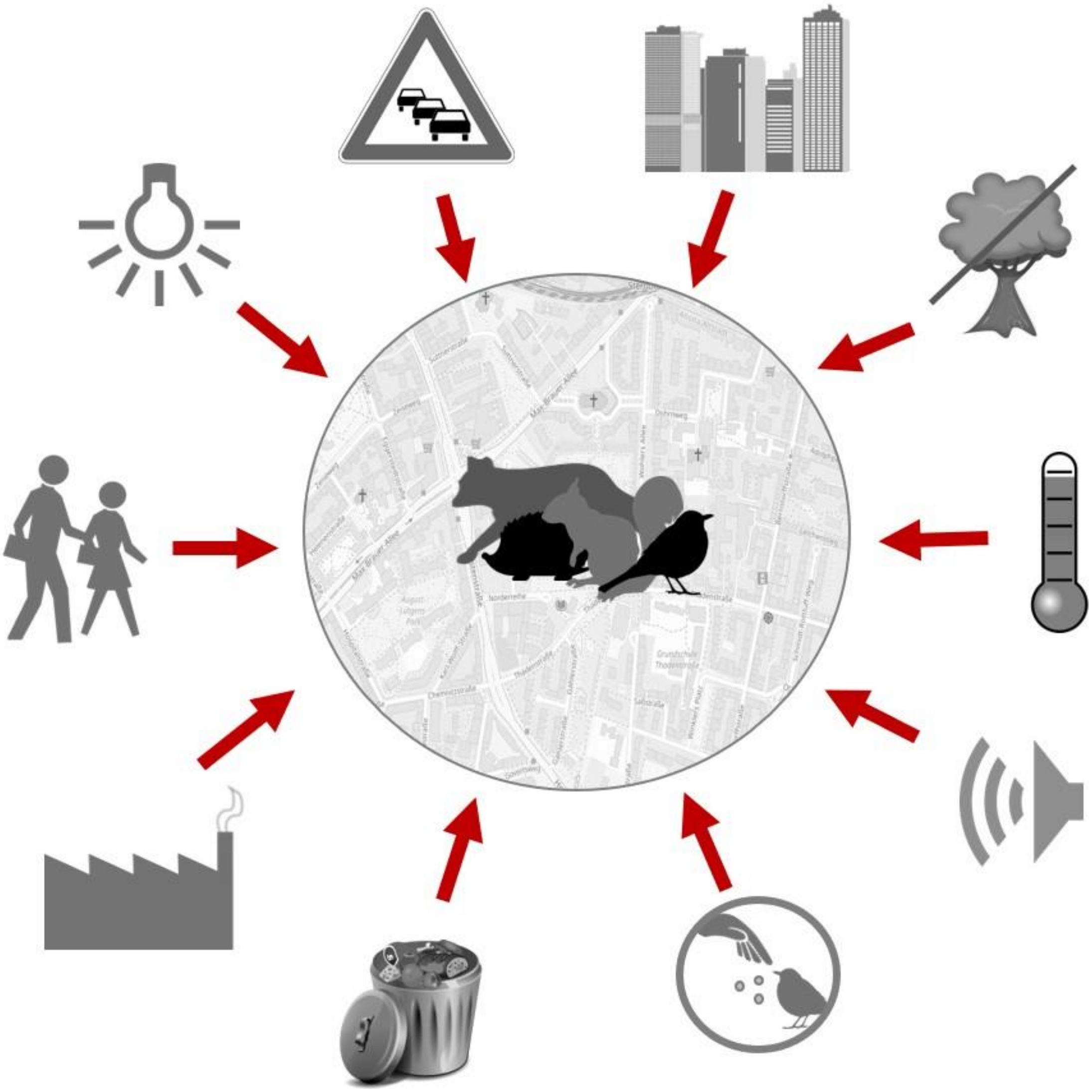

Figure 1.3 Examples of human induced habitat modifications for urban wildlife: changes vary from buildings, loss of natural vegetation, elevated temperatures and pollution (noise, artificial lights, chemicals and toxins) to high contact rates with humans and abundant supplemental resources from human refuse or active feeding (Picture Credit: Pixabay, background map: OpenStreetMap 2023).

Costs and benefits of an urban life

Urban habitats challenge wildlife species at numerous levels, yet they also offer advantages that can attract animals to settle in a completely altered environment. Supplemental feeding and easily accessible refuse contribute to a higher and more stable year-round food availability and urban structures can create a higher abundance of shelter for certain species (Lowry et al. 2013). Furthermore, natural predators are usually reduced in cities, even when this reduction can be outcompeted to a certain degree by domestic pets or roadkill (Shochat et al. 2004).

Beyond this, the urban heat island in combination with snow removal in winter or the availability of non-natural water sources in summer (e.g., bird baths, fountains) produce buffered climatic conditions and extended growing seasons (Shochat et al. 2006). Consequently, natural seasonal variation in ambient temperature (T_a) and resource availability is decreased, and thereby the occurrence of unfavourable periods (Shochat et al. 2006, Lowry et al. 2013). These aspects are particularly interesting from a physiological perspective since ambient temperature and resource availability strongly influence an animals' energetic supply and demands (Speakman 1999, Birnie-Gauvin et al. 2017, Diamond and Martin 2021). Urban endothermic species might benefit from relaxed environmental constraints through a lower need of metabolic heat production compared to the conditions in their natural habitats (Ricklefs and Wikelski 2002).

For those reasons, some species, such as black-birds (*Turdus merula*), hedgehogs (*Erinaceus europaeus*), foxes (*Vulpes vulpes*) or Eurasian red squirrels (*Sciurus vulgaris*) apparently thrive in urban systems (Luniak 2004). Strikingly, some populations even display higher densities than in their natural habitats (McCleery 2010, Hubert et al. 2011). There are different terms for species that occur in urban habitats that refer to traits, such as population density or degree of utilisation of supplemental foods sources: from synanthrop or synurban to urban adapters, exploiters, utilisers or dwellers (Francis and Chadwick 2012, Fischer et al. 2015). Independently of which term is used, phenotypic plasticity seems to be a key trait for urban species (Lowry et al. 2013). These are usually generalists and opportunists concerning their food and / or habitat preferences (McCleery 2010). Moreover, they display higher degrees of boldness or reduced neophobia, characteristics that support them to successfully exploit urban resources (Uchida et al. 2019).

Notwithstanding the beneficial factors for urban wildlife, several urban stressors, such as pollution, noise or malnutrition can have profound impacts on urban dwelling animals (Kight and Swaddle 2011, Birnie-Gauvin et al. 2016) and lead to modifications of several biological traits (McCleery 2010). Studies document changes in morphology, behaviour or reproduction of urban populations when compared to rural conspecifics (de Satgé et al. 2019, Austin et al. 2022, Caspi et al. 2022). Those changes can be beneficial, neutral or negative (Seress and Liker 2015, Lambert and Donihue 2020). A range of studies found negative effects on reproduction in urban bird species (Demeyrier et al. 2017, de Satgé et al. 2019, Grabarczyk et al. 2022). Particularly the presence of supplemental food sources might represent a misleading cue of habitat suitability which masks the diverse urban stressors (Birnie-Gauvin et al. 2016). Individuals could then be attracted by false cues to settle in a low-quality environment with adverse effects on their long-term survival, reproduction and fitness (Lepczyk et al. 2017, de Satgé et al. 2019). Animals in urban habitats forage on significant amounts of human provisioned foods (Contesse et al. 2004, Evans and Gawlik 2020, Dasgupta et al. 2021). However, anthropogenic impacts on food availability and foraging behaviour are usually negative and the quality of food available in urban habitats might not equal its quantity (Murray 2015, Birnie-Gauvin et al. 2017, Schulte-Hostedde et al. 2018). Hence, we do still not know, whether urban habitats act as sink habitats or ‘ecological traps’ on a long-term basis (Lepczyk et al. 2017, Zuñiga-Palacios et al. 2021).

Studying consequences of urbanisation

Studies about the effects of urbanisation have generally increased within the last few decades (McDonnell and MacGregor-Fors 2016). Nonetheless, urban ecology still remains a comparably young field of biology. We still lack basic understanding about underlying mechanisms of living in an urban environment and about how and why urban individuals differ from their rural counterparts (Ouyang et al. 2018). Moreover, research on urban wildlife is still biased towards certain geographic locations, taxa and research fields (Faeth et al. 2011, Magle et al. 2012, Fusco et al. 2021). Behaviour and physiology are stated to be particularly important for urban conservation actions (Cooke et al. 2014, Ouyang et al. 2018). However, many studies concentrate on arthropods or birds and behavioural traits are far more often studied than physiology (Zuñiga-Palacios et al. 2021, Tranquillo et al. 2023). With regard to nutrient ecology, studies on anthropogenic effects often focus on food quantity rather than quality (Birnie-Gauvin et al. 2017). We further lack physiological data of urban versus rural populations in endotherms since, to date, studies mostly concentrate on ectotherms (Birnie-Gauvin et al. 2016, Miles et al. 2020, Diamond and Martin 2021). Population ecology, nutrition and energy expenditure form a crucial baseline for the fitness of urban animals though. Therefore, a better understanding of these aspects is essential to predict about urban population dynamics as well decision making in urban planning and conservation.

The Eurasian red squirrel: an iconic urban-dwelling species

The Eurasian red squirrel (*Sciurus vulgaris*, henceforth named “squirrel”) naturally lives in forests, but seems to be a success story in urban habitats, considering the apparently high population densities occurring there (Jokimäki et al. 2017, Fingland et al. 2021). As an arboreal species (Lurz et al. 2005), squirrels can easily climb onto houses or other man-made structures and also use non-natural roosts (pers. obs.). The species is welcomed by humans and provided with supplemental foods in many countries (Krauze-Gryz and Gryz 2015). Squirrels favour energy-rich foods, i.e. seeds and nuts, but as generalists and opportunists, they feed on a variety of other things, from fruits, flowers and buds to mushrooms or insects (Lurz et al. 2005, Krauze-Gryz and Gryz 2015). They therefore have the prerequisites to utilise the urban food supply. Studies document behavioural adaptations to life in the city in squirrels, such as reduced antipredator behaviour, altered activity patterns or home range shifts towards artificial feeding sites (Reher et al. 2016, Thomas et al. 2018, Uchida et al. 2020b, Beliniak et al. 2021). However, while the species is well-studied in its natural range in terms of various aspects of its biology and ecology, several aspects, such as physiology, are still unknown in urban populations, especially in city core areas and in direct comparison to rural populations (Jokimäki et al. 2017).

The species is currently listed as Least Concern by the IUCN (Gazzard 2023). However, the population in GB, Ireland and Italy is threated by the invasive grey squirrel (*Sciurus carolinensis*), which can outcompete red squirrels in different ways (Gurnell et al. 2004, Lurz et al. 2005). For example, grey squirrels are bigger and more competitive at feeding sites, especially in deciduous forests and urban habitats (Bryce et al. 2001, Lurz et al. 2005). They also transfer a parapoxvirus that is highly lethal to *S. vulgaris* (Tompkins et al. 2002). It is likely that grey squirrels will reach other European countries such as Germany within decades (Bertolino et al. 2008, Di Febbraro et al. 2016). In particular, cities and highly disturbed core areas are at higher risk of being colonised by non-native species that exploit the imbalance of ecosystems (McKinney 2002, Alberti et al. 2017). Of particular concern is the assumption that urban habitats even serve as source habitats for grey squirrels (Wauters et al. 2023). Therefore, greater knowledge of urban red squirrels is essential to equip conservation decision-makers with the appropriate knowledge to take timely action. Finally, Eurasian red squirrels are widespread and can act as a model system for other urban mammals.

Study set-up and overview

My project aimed to study the effects of urbanisation on a typical synurban mammal, i.e., a species that occurs in higher densities in cities than in natural habitats. Therefore, I focused on Eurasian red squirrels and investigated whether and how they respond to urbanisation in their population and nutrient ecology, foraging behaviour and physiology. I studied populations from highly contrasting sites: urban core areas, characterised by an intense surrounding urban matrix and large distances to the city boundaries versus a forest in a rural area outside the city. As study sites, I chose three small and heavily disturbed parks in the centre of Hamburg, the second largest city in Germany, and a forest area under nature protection close to the city (Fig. 1.4, Fig. 1.5).

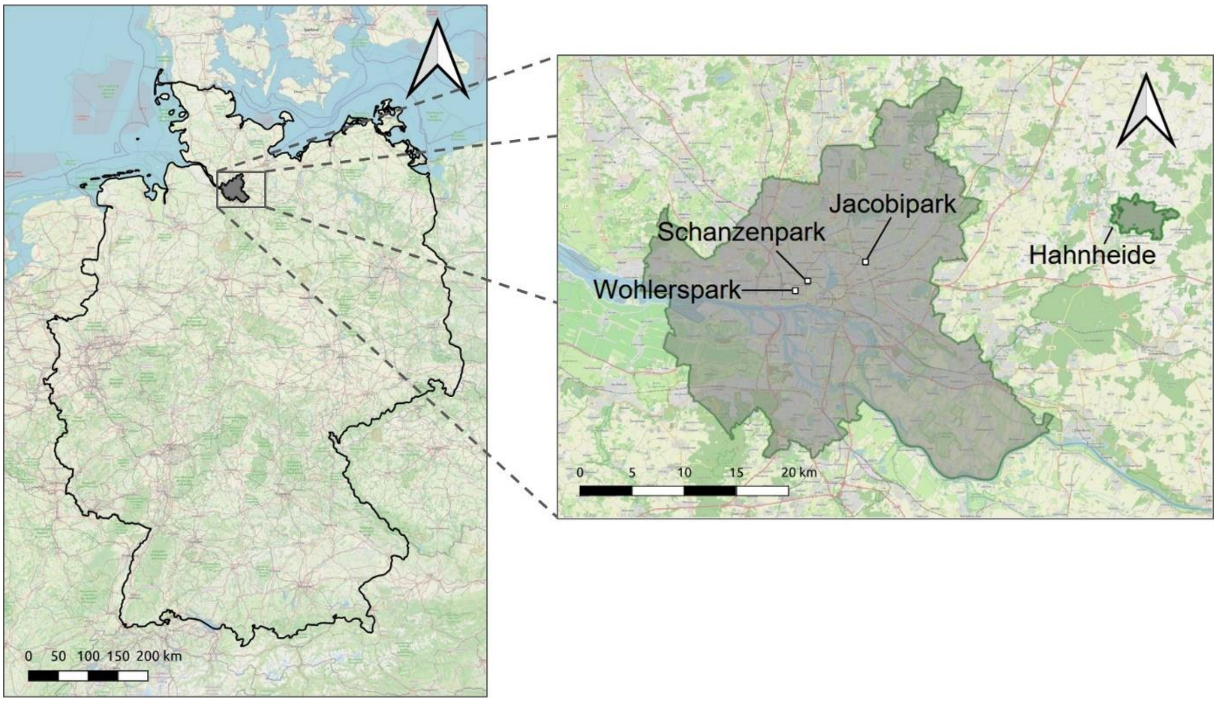

Figure 1.4 Map of Germany (left) and a close-up of the city of Hamburg (right), showing the three small urban parks in city centre as well as the rural forest study site Hahnheide (source: Open StreetMap 2023 / Natural Earth 2023)

Figure 1.5 Impressions from the Wohlerspark urban study area (A & B) and Hahnheide forest area (C), where stripped spruce cones indicated the presence of squirrels (D). Photo credits: Bianca Wist

Based on initial observations and the background described above, I divided my project into a field study and a subsequent experiment with wild-caught individuals. For my dissertation, I focussed in particular on the following topics (Fig. 1.6):

1. Studying natural and supplemental food availability as well as population parameters of squirrels at the different study sites (field study)
2. Examining food choice behaviour and diet composition of urban versus forest squirrels in outdoor enclosures (experiment under semi-natural conditions)
3. Investigating energy expenditure of urban versus forest squirrels (experiment under semi-natural conditions)

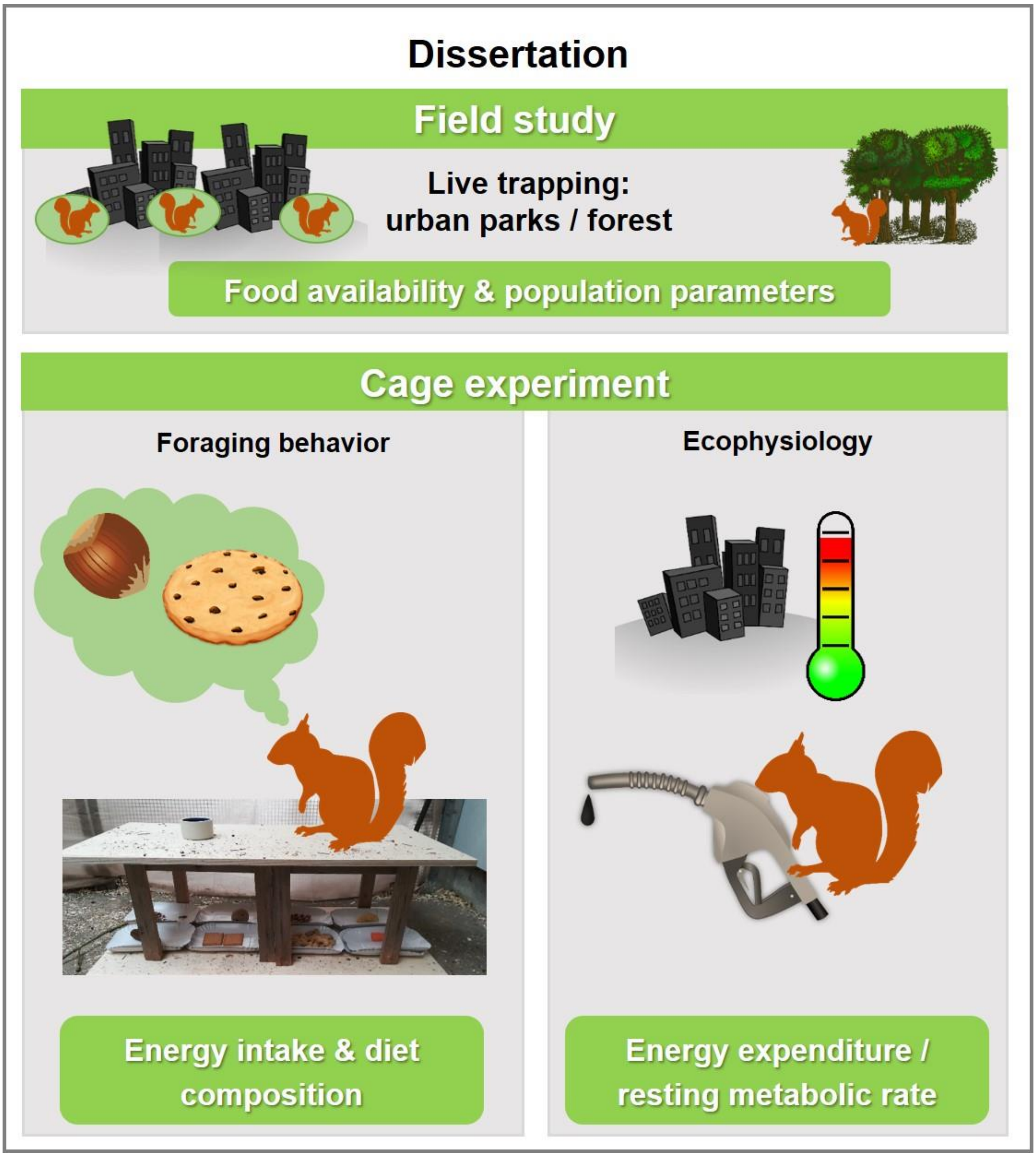

Figure 1.6 Project overview with the three main research topics of this dissertation: A field study investigating food availability and population parameters of urban versus forest squirrels formed the basis for the project (upper panel). A subsequent experiment under semi-natural conditions with wild-caught squirrels in outdoor enclosures included a food choice experiment (left lower panel) and investigation of energy expenditure in relation to ambient temperature (right lower panel). (Photo Credit: Bianca Wist; Picture Credit: Pixabay)

The topics of my dissertation are dealt with in the following three chapters:

Chapter 2: Food availability and population parameters

Published as: **Wist B** and Dausmann KH (2023) Food availability and population parameters for squirrels differ even in neighbouring urban parks. *Urban Ecosystems*. https://doi.org/10.1007/s11252-023-01468-z

Key findings:

- Urban parks offer higher tree diversity and various supplemental foods compared to a forest site, yet nearby urban parks differed in abundance and composition of natural and anthropogenically provided food sources
- Squirrel density was considerably higher in urban parks compared to the forest, but the highest density was found in the smallest park, which had highest number of trees and the highest availability of supplemental foods
- Local population parameters and environmental conditions may differ at the smallest scale, which should be considered when drawing conclusions from studies or making decisions about urban conservation actions

Chapter 3: Foraging behaviour

Published as: **Wist B,** Stolter C & Dausmann KH (2022) Sugar addicted in the city: Impact of urbanisation on food choice and diet composition of the Eurasian red squirrel (*Sciurus vulgaris*). *Journal of Urban Ecology* 8(1), juac012. https://doi.org/10.1093/jue/juac012

Key findings:

- Urban squirrels:
  - had a higher kJ intake and higher weight gain throughout the experiment
  - consumed more non-natural foods and more sugar
  - had poorer body condition before and after the experiment
- Urban populations with high access to non-natural or sugary foods may therefore be at risk of health-related side effects from malnutrition

Chapter 4: Ecophysiology

Published as: **Wist B,** Montero BK & Dausmann KH (2023) City comfort: weaker metabolic response to changes in ambient temperature in urban red squirrels. *Scientific Reports* 13, 1393. https://doi.org/10.1038/s41598-023-28624-x

Key findings:

- Urban squirrels showed a less steep increase of metabolic rate relative to ambient temperature compared to woodland squirrels, possibly due to a smaller daily T_a variation
- This indicates lower metabolic costs for urban individuals in the colder T_a range, but higher costs at warmer T_a
- The results are concerning in light of global warming and the increase in heat waves, as higher metabolic rates at warmer T_a levels might reduce the ability of urban individuals to dissipate heat

Further projects affiliated with this thesis

As a side project to this dissertation, I aimed to determine the genetic diversity of the species at the study sites with regard to possible urban-related effects, such as genetic differentiation or inbreeding. In addition, I have joined in an international project investigating anti-predator behaviour in urban versus rural squirrels from four different countries to assess whether squirrels show a general response to urbanisation and increased contact to humans in their flight behaviour (Fig. 1.7).

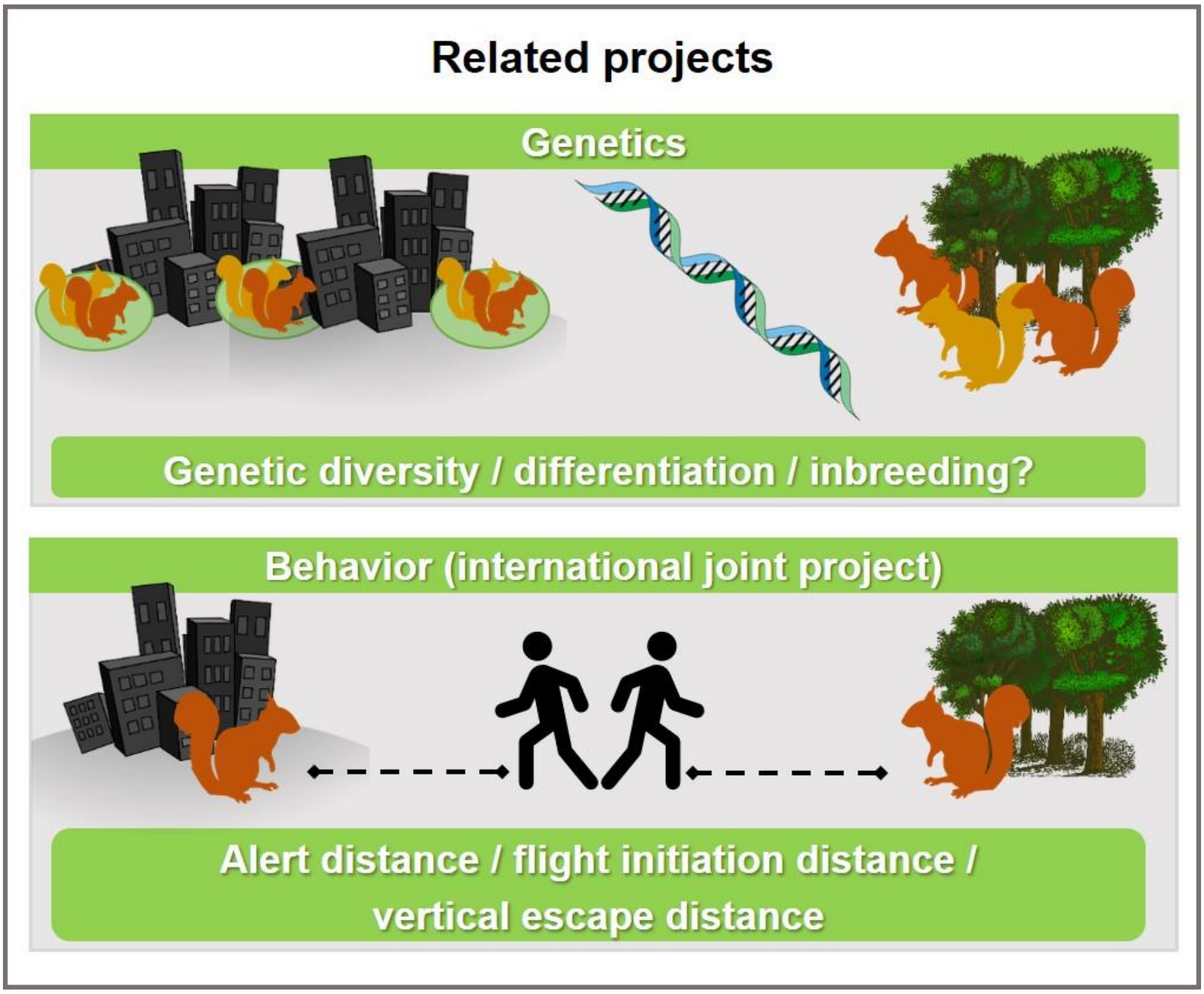

Figure 1.7 Illustration of further projects related to this thesis. Using hair samples collected in the field, we analyse the genetic diversity in urban and rural squirrels (top panel). A joint project with researchers from different countries furthermore investigated anti-predator behaviour, i.e., alert distance, flight initiation and vertical escape distances (bottom panel) (Picture Credit: Pixabay)

Affiliated project 1: Genetic diversity of urban versus rural squirrels

Ongoing: **Bianca Wist,** David Lohmann, Mathilde Cordellier, Heike Feldhaar und Kathrin Dausmann

Suitable refuges for urban wildlife require not only features that are beneficial for the survival and fitness of individuals, such as adequate food sources or an favourable thermal environment, but also those, that allow populations to maintain genetic diversity. This is a prerequisite for adaptation and resilience to environmental change (Rezouki et al. 2014, Berger-Tal and Saltz 2019, Dakos et al. 2019). Urban stressors, such as fragmentation or pollutants yet can influence gene flow, genetic drift, mutation and selective pressures. As a result, they can alter allele frequencies within populations and drive genetic differentiation between them (Munshi-South et al. 2016, Johnson and Munshi-South 2017, Brans et al. 2018, Beans 2019). Accordingly, it is assumed that cities represent a strong evolutionary driver within a short period of time (Alberti et al. 2017, Johnson and Munshi-South 2017, Miles et al. 2020). For example, urbanisation does not only reduce the amount of suitable green habitat patches, but also increases the distance between them (Merckx et al. 2018). Suitable habitat patches that are sufficiently large are often scarce and poorly connected, and the meaning of “sufficient” can depend on several other factors, such as distance from city boundaries or the composition of the urban matrix (Lepczyk et al. 2017). Green patches can therefore resemble islands at long distance to source habitats outside the city, as surrounding streets and buildings can act as movement barriers (Adducci et al. 2020, Alberti et al. 2020, Hardouin et al. 2021). This is likely to affect small parks in the centre of large cities. In addition, the dense urban matrix combined with abundant supplemental food sources reduces dispersal rates, resulting in offspring settling in close proximity to their parents (McCleery 2010, Selonen et al. 2018). Accordingly, I recaptured a high percentage of juveniles as adults in following seasons in a small park (Chapter 2).

To date, the results of studies on the genetic diversity of urban species are not yet clear and vary depending on the species and site (Miles et al. 2019, Fusco et al. 2021). Genetic structuring between different sites within a city has been found in great tits (*Parus major*), red foxes (*Vulpes vulpes*) or brown rats (*Rattus norvegicus*) (Björklund et al. 2009, Combs et al. 2018). On the other hand, urban hedgehogs (*Erinaceus europaeus*) or common opossums (*Didelphis marsupialis*) showed no genetic differentiation within the same city (Barthel and Berger 2020, Henao-Sáenz et al. 2023). However, our knowledge about genetic diversity in urban vertebrate populations, especially in urban core areas and in relation to environmental factors such as supplementary feeding, is still limited (Ouyang et al. 2018, Fusco et al. 2021).

This additional part of my project therefore focused on the genetic diversity of urban squirrel populations in small green habitat patches in the city centre. Previous studies on urban squirrel genetics are rare and found no evidence for reduced genetic diversity or genetic isolation (Rezouki et al. 2014, Selonen et al. 2018, Tranquillo et al. 2023). However, they were conducted in a comparably small city using an urban-rural gradient in Turku, Finland (Selonen et al. 2018) or in one large park (184 ha) outside Paris, France (Rezouki et al. 2014). The conditions may differ for populations in centrally located parks of a large city since effects are likely scale-dependent (Łopucki et al. 2020, Uchida et al. 2020a). I therefore collected hair samples from captured individuals for DNA extraction, and we studied microsatellite loci already established for squirrels (Todd 2000, Hale et al. 2001, Rezouki et al. 2014) to investigate their genetic diversity and signs for genetic differentiation and inbreeding. The samples collected in the field were supplemented with samples from squirrel nursing stations located in or near other major German cities (Berlin, Munich and Stuttgart). In this way, we aim to compare our results from the greater Hamburg area in northern Germany with other urbanised regions in the east and south of the country. The final analysis of the results is still ongoing, but will contribute to answer the question of whether small urban fragments act as source or sink habitats and to assess the long-term persistence of synurban populations.

Affiliated project 2: Joint study on anti-predator behaviour

Behavioural changes, such as increased boldness, are among the best-studied species responses in urban habitats (Magle et al. 2012, Lowry et al. 2013, Caspi et al. 2022). However, there are few studies investigate whether animals show respond similarly across the globe and how their response depends on local factors such as human population density or availability of supplemental food sources. For these reasons, we took the opportunity to participate in an international study comparing anti-predator behaviour in squirrels from Japan, Finland, UK and Germany. Local alert distances, flight initiation distances and vertical escape distances were studied in each country using similar methodology at various sites with different degrees of urbanisation. The fieldwork in Germany resulted in two master's theses (see below in the section "Further publications affiliated with this thesis") and the results of the entire study are currently being prepared for submission to Urban Planning:

In preparation: Kenta Uchida, Kathryn Fingland, **Bianca Wist,** Rachel Cripps, Marja-Liisa Kaisanlahti-Jokimäki, Mark-André Kampmann, Maira-Lee Lindtner & Jukka Jokimäki (2023): Region-dependent tolerance to humans: a multi-country comparison of horizontal and vertical escape distance in arboreal squirrels

Further publications affiliated with this thesis

Bachelor's theses

Björn Probst (2018) Verbreitungsmuster Eurasischer Eichhörnchen (*Sciurus vulgaris*) in einem stark anthropogen beeinflussten Habitat mit hoher Individuendichte

David Lohmann (2020) Genetische Diversität von *Sciurus vulgaris* in Hamburger Stadtparks

Sarah Wodtke (2021) Einfluss der Urbanität auf die Endoparasiten des Eurasischen Eichhörnchens (*Sciurus vulgaris*)

Master's theses

Janine Köthe (2018) Einfluss anthropogener Störungen auf die Aktivitätsmuster des Eurasischen Eichhörnchens (*Sciurus vulgaris*)

Marc-André Kampmann (2019) Factors influencing flight and vigilance behaviour of the European red squirrel (*Sciurus vulgaris*)

Maira-lee Lindtner (2019) Fluchtverhalten von europäischen Eichhörnchen (*Sciurus vulgaris*) unter dem Einfluss von Urbanisierung und Saisonalität

Gina Völkers (2020) Futterplatzanalyse von *Sciurus vulgaris* anhand von Fraßspuren – Entwicklung eines Schulversuches

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Chapter 2

Food availability and population parameters for squirrels differ even in neighbouring urban parks

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Abstract

Some species occur in higher densities in cities than in natural habitats, despite the variety of urban stressors. Urban fragments can be extremely heterogeneous though, and species' responses might vary between urban patches. However, only few studies examine mammalian populations from different fragments in the core of cities, where stressors are at their maximum. The Eurasian red squirrel (*Sciurus vulgaris*) is highly abundant in urban green spaces and readily makes use of supplemental food sources. We examined natural and supplemental food sources as well as squirrel populations in three small parks in an urban core. We found pronounced differences in natural and supplemental food availability between the parks. Squirrel population densities were generally higher at all three urban sites when compared to a geographically close located natural forest, but with variation between the parks. The squirrel density in the smallest park in the summer season was 42 times higher (index of minimum number alive: 8.8 individuals/ha) than in the forest, whereas it was only 20 or respectively 16 times higher in the two other parks. Individuals from the park with the highest population density had access to the highest amount of supplemental food sources. However, side effects from non-natural food items, e.g., related to nutrition, also need to be taken into consideration and might counteract the advantages of supplemental food sources. This study shows that urban effects can vary even at the smallest scale between nearby urban habitat patches and this needs to be considered when drawing conclusions about the effects of urbanisation and deciding about conservation actions.

Keywords

Urbanisation, *Sciurus vulgaris*, population density, city centre, supplemental feeding

Introduction

Urbanisation is a rapidly increasing threat for global biodiversity (Seto et al. 2012, McDonald et al. 2019, Simkin et al. 2022) and urban wildlife have to tolerate multiple stressors. Yet some species have successfully established dense populations in urban habitats (Luniak 2004, Hubert et al. 2011, Francis and Chadwick 2012). Urban stressors are most intense in the city centre (Grimm et al. 2008, Bateman and Fleming 2012). However, cities are composed of a heterogeneous mosaic of habitat patches and spatial scales can have considerable influence on wildlife responses (de Satgé et al. 2019, Alberti et al. 2020, Uchida et al. 2020). An important driver of high urban population densities is the higher availability of food (Shochat et al. 2006, McCleery 2010). Natural food sources in urban environments are strongly modified by humans due to vegetation cover being reduced and distributed in patches; primarily concentrated in green spaces such as parks and private gardens (Singh et al. 2018). However, urban habitats and vegetation can also offer benefits for wildlife. For example, planting of ornamental plants and neophyts as well as the heat island effect lead to higher plant diversity, earlier flowering and extended growing seasons. These processes can result in a more continuous natural food availability (Shochat et al. 2006, Pickett et al. 2011, Singh et al. 2018).

Urban habitats also provide access to various intentional or unintentional food sources from humans, further contributing to a more stable food availability throughout the year (Shochat et al. 2006). Supplemental feeding can have diverse impacts on wildlife ecology and biology – both on the individual and the community level (Robb et al. 2008, Birnie-Gauvin et al. 2016), which are not necessarily advantageous. Abundant food sources in urban systems might not meet the nutritional needs of a species or even be detrimental (Birnie-Gauvin et al. 2017). Vertebrate species that receive supplementary food show higher population densities and body masses in general (Boutin 1990). The former is likely a result from increased birth rates, litter size and survival of juveniles and adults, affecting not only density, but also structure of populations (Dantzer et al. 2020). However, possible negative effects range from increased disease transmission and loss of natural behaviour to reductions in gut microbiome or community restructuring (Robb et al. 2008, Galbraith et al. 2015, Gillman et al. 2022). Artificially increased food abundance thus might act as false cue of habitat quality, thereby creating an ecological trap for urban species (Reynolds et al. 2017, Zuñiga-Palacios et al. 2021).

Given the local heterogeneity of urban green spaces and the high proportion of non-native and ornamental plants in parks and gardens, the availability of suitable natural energy rich and nutritionally adequate food can vary substantially between patches (Pickett et al. 2011, Seress and Liker 2015, Lepczyk et al. 2017). Moreover, utilization of urban green spaces by humans and their willingness to feed wildlife can also differ between urban patches, e.g., in relation to socio-economic status (Fuller et al. 2008, Hassell et al. 2021). An increasing number of studies investigate urban wildlife, however deeper insights into how green spaces within the same city core area differ in availability and composition of food sources in combination with mammalian population parameters, remain scarce.

The Eurasian red squirrel (*Sciurus vulgaris*, henceforth named “squirrel”) is a *synurban* species; able to successfully inhibit urban habitats (Luniak 2004, Jokimäki et al. 2017, Fingland et al. 2021). This arboreal small mammal is naturally forest dependent and favours seeds and nuts in its diet. However, as a food generalist, the species can successfully exploit urban resources (Reher et al. 2016, Fingland et al. 2021, Wist et al. 2022). Food availability is known to influence population demography in squirrels, as well as several other traits, such as home range, body mass and reproduction (Wauters et al. 2007, Wauters et al. 2008, Di Pierro et al. 2010, Reher et al. 2016, Selonen et al. 2016). Urban squirrels also show behavioural adaptations to the urban environment and the ever-abundant presence of humans, for example, by altering their anti-predator behaviour or activity patterns (Uchida et al. 2019, Beliniak et al. 2021, Fingland et al. 2021). Urban green spaces, such as parks, are expected to act as suitable refuges for squirrels (Rezouki et al. 2014, Kopij 2015, Beliniak et al. 2022). However, most of these results were obtained from comparably large parks or small cities. We therefore focused on three small urban parks located in the centre of a big city approaching 2 million inhabitants. We deliberately chose parks that were similar in many environmental conditions and investigated the availability of natural and anthropogenically provided food sources. Furthermore, we examined population parameters of Eurasian red squirrels within the parks to explore whether they were influenced by heterogeneity in food abundance. Our results help to elucidate the interplay of urbanisation and local urban food abundance with mammalian population parameters.

Methods

Study sites

We conducted field work in northern Germany across three urban parks in the centre of Hamburg (Wohlerspark, Schanzenpark and Jacobipark; Fig. 1, Tab. 1). With around 1.9 million inhabitants (2,455 per km^2) and an area of 755 km^2, Hamburg is the second largest city in Germany. All parks were similar in several environmental factors. They were all small, with a size ranging from 4.6 to 7.4 ha, located in area with very high human population density and surrounded by a similar type of urban matrix (Tab. 1). In detail, we chose these parks using following criteria: 1) located in the city centre (6 – 9 km from the closest city limits), 2) area < 10 ha, 3) surrounded by a dense urban matrix (at least 2 large streets closer than 250 m plus large residential or commercial buildings directly adjacent to the park), and 4) high degree of diverse human utilization, such as sports, dog walking and various other outdoor activities (own observations prior to this study). In order to better assess regional conditions and population parameters, we also captured squirrels in a mixed forest about 30 km away (Hahnheide) for comparison purposes (Fig 1, Tab. 1). The forest site was a nature conservation area with a high proportion of conifers and old trees (Bundesamt für Naturschutz 2019).

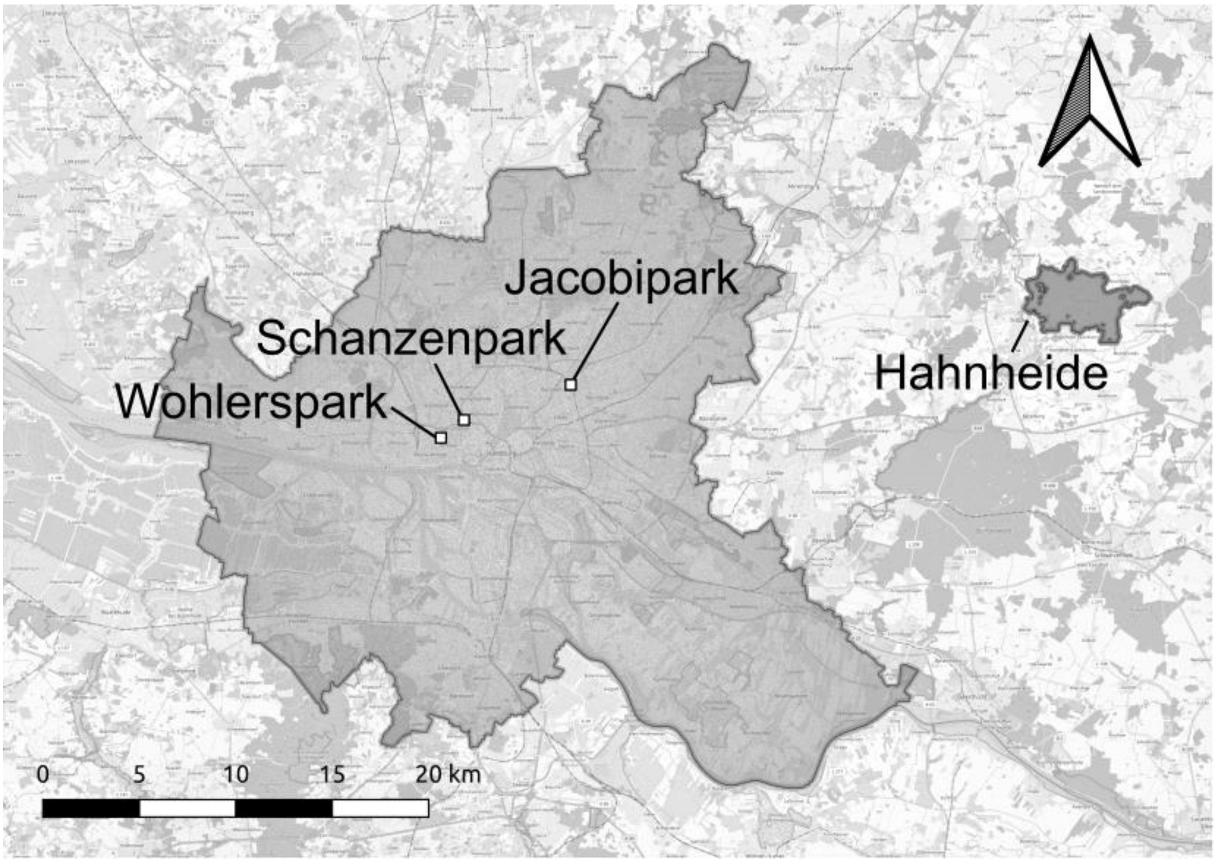

Fig. 1 Map of the city of Hamburg and the four study sites: Wohlerspark, Schanzenpark and Jacobipark located in the city centre and the forest site Hahnheide outside the city (source: Open StreetMap 2023 and Natural Earth 2023)

Tab. 1 Overview of study sites with total size (ha), human population density (including a ~ 1 km^2 catchment area around the site[A]) and trap days (product of number of traps by number of capture days) per site and season

Site	Hahnheide	Jacobipark	Schanzenpark	Wohlerspark
classification	rural forest	urban park	urban park	urban park
size (ha)	1,401	6.1	7.4	4.6
human population density*	212	13,125	11,557	15,784
trap days				
winter	200			54
spring	120			80
summer	57	36	48	64
autumn	51			32

[A] Statistisches Bundesamt (DESTATIS)

Natural food abundance

We compared tree species abundance and diversity per study site by using tree data obtained from Hamburg authorities (tree cadastres 2017, Freie und Hansestadt Hamburg), and the forestry office of Schleswig-Holstein (tree ledgers 2012, Schleswig-Holsteinische Landesforsten) respectively. Furthermore, we counted and classified tree seeds within two 1 x 1 m random seed plots at each trap location (20 x 20 m around the trap) at each study site in the summer season. This included fallen seeds as well as seed remains, as a measure of relative seed abundance per site (Wauters and Lens 1995). We further used literature values from the species or genus (Suppl. Tab. 1) for assessment of available energy-content in kJ per seed.

Supplemental food sources

For assessment of supplemental food sources, we counted and weighed anthropogenically provided food items per trapping period at each study site. We used mean values from three different days per season whenever possible as human activities varied with weather conditions and weekday. To collect these samples, we walked all paths of each park, observing the shrubbery on both sides and inspected open lawns. In the forest, we walked the whole trapping grid, but never observed supplemental food. Food was weighed to the nearest 0.5 g with a spring balance (KERN & SOHN GmbH, Balingen-Frommern, Germany). If weighing was not feasible (e.g. due to non-accessible position), the approximate weight was estimated by either comparing the amount with already measured food items (e.g. ~half fat ball) or by reproducing the set in the lab and then weighing it (e.g. a thin layer of oats 20 x 30 cm in size). We included all kinds of anthropogenically provided foods, since initial observations and a food choice experiment revealed that urban squirrels forage on a broad range of supplemental foods (Wist et al. 2022). We assigned the different food items to broader categories (e.g. bakery products or vegetables) for further analysis. The bird food category included all kinds of bird foods, such as seed mix, fat balls or scattered oats. Peanuts as high energy food were included into the “nuts or peanuts” category. We used literature values (Suppl. Tab. 2) to estimate the kJ content of the anthropogenic food items.

Trapping and animal handling

We trapped squirrels in all sites in summer 2017 or 2018 respectively. Furthermore, we trapped squirrels in the two most contrasting sites Wohlerspark and Hahnheide from winter 2016/2017 to autumn 2017/2018. We baited cage traps (20 x 20 x 50 cm; Tomahawk Live Trap, Hazelhurst, Wisconsin, USA) at 07:30 hours with a mixture of seeds and nuts and kept them open for approximately 7 h. A longer trapping effort per day was not feasible due to the high degree of human activities in the later afternoons in the parks. Traps were placed around 100 m apart (Magris and Gurnell 2002), at approximately 1.2 - 1.5 m height in the park shrubbery or on trees and checked regularly. Park traps were prebaited for 1 – 2 days prior to each trapping period. Forest traps were prebaited every few days for one month as forest individuals are not as accustomed to search for foreign food sources.

We used a cloth cone for handling non-anesthetized trapped squirrels (Koprowski 2002). Individuals were weighed with a spring balance to the nearest 5 g (KERN & SOHN GmbH, Balingen-Frommern, Germany), sexed and aged (adult, subadult, juvenile) depending on their body mass, length and inspection of reproductive organs (Magris & Gurnell 2002, Wauters et al. 2007). The nose-anus length was determined to the nearest 0.5 cm by using a measuring tape (Wauters & Dhondt 1989a, b, Magris & Gurnell 2002, Wauters et. al 2007). Finally, the individuals were marked via subcutaneous implantation of a passive integrated transponder (ID-100B; Trovan Ltd., East Yorkshire, UK) between the shoulder blades. Animals were released immediately at the capture site after around 10 min of handling time.

Population density

We used the minimum number of individuals known to be alive (MNA) per site in one season as estimate of population density, a common method for squirrels (Magris and Gurnell 2002, Gurnell et al. 2004, Wauters et al. 2008). Furthermore, we calculated an index of relative abundance (MNA_{ind}) per site and season for adequate visualization and comparison, as exact estimates were not possible (Jokimäki et al. 2017):

$$MNA_{ind} \frac{MNA}{trapping\ days * number\ of\ traps * ha * 100}$$

Statistics

Statistics were performed in R 4.3.1 (R Core Team 2023) within the "RStudio" environment (RStudio Team 2023). Graphical data exploration was performed with the packages 'lattice' (Sarkar 2008) and 'ggplot2' (Wickham 2016). We used the 'psych' package (Revelle 2023) for descriptive statistics and Shapiro-Wilk test or Levene's test respectively for testing normality and equality of variance.

We compared tree species diversity at the four study sites by using the relative abundance in percent per tree species and site since absolute tree numbers were not available for the forest. We then calculated the Shannon-Wiener Index (H') per site (package 'vegan', Oksanen et al. 2013). Despite data transformation, kJ content from fallen seeds in the counted seed plots ($n_{Jakobipark} = 18$, $n_{Schanzenpark} = 12$, $n_{Wohlerspark} = 19$, $n_{Hahnheide} = 38$) did not follow a normal distribution or equality of variance (Shapiro-Wilk test and Levene's test, both $p < 0.05$). We therefore used the non-parametric Kruskal Wallis test with kJ content of fallen seeds per seed plot as dependent variable to investigate differences in natural food availability between sites. We used Dunn's test with adjusted p values for post-hoc comparisons (package 'FSA') (Ogle et al. 2023). Unless otherwise stated n represents the number of individuals / seed plots per site or group. A level of $p < 0.05$ represents statistical significance.

Results

Natural food sources

Number of tree species was highest in the smallest park (Wohlerspark) with 45 species in total (10 per ha), followed by Schanzenpark with 36 species (5 per ha) and Jacobipark with 32 species (5 per ha). The forest study site had 11 tree species (0.3 per ha). Among the urban sites, Wohlerspark also had the highest number of trees per ha (136), which was around 1.5-times the trees per ha of Schanzenpark and 3 times that of Jacobipark. All parks were higher in tree diversity when compared to the forest site (Tab. 2). However, among the parks, Wohlerspark had the lowest tree diversity (Shannon-Wiener-Index H' 2.1 vs. 2.7 in the other two parks). See Tab. 2 and Suppl. Tab. 3 for tree details. With regard to tree composition, Wohlerspark was dominated by lime (*Tilia* spec.) which accounted for around 55 % of the park trees, whereas total proportion of conifers was only 8 %. Schanzenpark was dominated by maple (*Acer* spec., 27 %) and lime trees (*Tilia* spec., 26 %), with only 3 % conifers in the park. The most common trees in Jacobipark were European white birch (*Betula pendula,* 20 %), maple trees (*Acer* spec., 20 %) and oaks (18 %, mostly *Quercus robur*). Conifers accounted for 9 % of the Jacobipark trees. In the forest, European beech (*Fagus sylvatica,* 36 %) and spruce (34 %, mostly *Picea abies*) were the most abundant trees and the proportion of conifer trees was 59 %.

Tab. 2 Overview of trees at the four different study sites

Site	Hahnheide	Jacobipark	Schanzenpark	Wohlerspark
no. of tree species	11	32	36	45
no. of tree species/ha	0.3	5	5	10
no. of trees	nA	266	684	625
no. of trees/ha	nA	44	92	136
total share of conifers in %	59	9	3	8
tree diversity (Shannon-Wiener-Index H')	1.5	2.7	2.7	2.1

kJ availability from seeds in summer differed significantly between sites ($Chi^2 = 41.45$, df = 3, $p < 0.001$). Pairwise post-hoc Dunn's test revealed higher kJ availability in the forest vs. Schanzenpark ($p < 0.001$), but also vs. Wohlerspark ($p < 0.001$). Furthermore, among the urban sites, Jacobipark had higher kJ availability from fallen seeds than Schanzenpark ($p < 0.001$) or Wohlerspark ($p < 0.001$, Fig. 2).

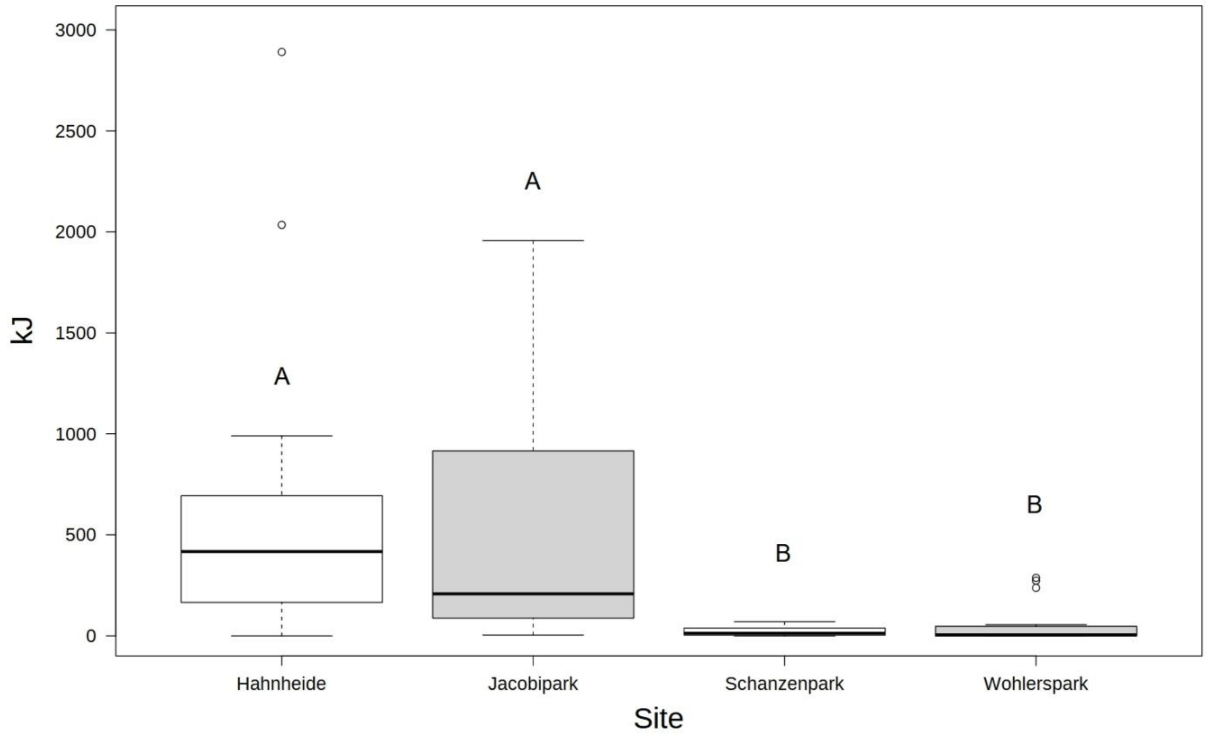

Fig. 2 Available kJ from fallen seeds in the seed plots ($n_{Jakobipark} = 18$, $n_{Schanzenpark} = 12$, $n_{Wohlerspark} = 19$, $n_{Hahnheide} = 38$) at the four different study sites. Boxes show the upper and lower quartiles (box limits) with the median as centre line. Whiskers indicate the 5 and 95 % percentiles and outliers are represented as circles. Different letters indicate significant differences between sites (Dunn's test: $p \leq 0.001$)

Supplemental food sources

Anthropogenically provided food sources at the four study sites in summer ranged from zero supplementation in the forest to 1,683 g found in total on an average day in the highest supplemented park, corresponding to an available energy of 6,537 kJ (Tab. 3). In total, we found 13 different food items in Wohlerspark, three in Jacobipark and two in Schanzenpark (Suppl. Tab. 4). The availability of supplemental food sources in Wohlerspark, which was sampled throughout the year, clearly peaked in winter and was lowest in spring (Suppl. Tab. 5). Additionally, we observed squirrels from Wohlerspark repeatedly feeding on various foods, such as fat balls, watermelon, grapes, cupcakes, bread, rice crackers and BBQ leftovers during field work. We also observed hand feeding with walnuts, hazelnuts and peanuts in Wohlerspark on most trapping days, but not in the other two parks. This manifested itself in active begging behavior from the squirrels at Wohlerspark.

Tab. 3 Overview of available anthropogenic food items in g and kJ at the four different study sites in the summer season; given are mean values from three days of observation. Peanuts have been included in a common category with nuts

	Hahnheide		**Jacobipark**		**Schanzenpark**		**Wohlerspark**	
	g	kJ	g	kJ	g	kJ	g	kJ
bird food	0	0	80	1,616	0	0	0	0
nuts or peanuts	0	0	0	0	0	0	20	492
fruits (fresh/canned)	0	0	275	349	57	124	1,010	1,876
vegetables (raw/boilded/grilled)	0	0	17	16	0	0	317	522
bakery products	0	0	0	0	36	407	283	3,134
fast food	0	0	0	0	0	0	53	514
total in g / kJ	**0**	**0**	**372**	**1,981**	**93**	**531**	**1,683**	**6,537**

Squirrel population density and structure

In total, we trapped 67 squirrels at the urban sites (314 trap days) and 10 squirrels (428 trap days) in the forest. Within the urban sites we trapped 46 individuals in Wohlerspark (230 trap days), 12 in Jacobipark (48 trap days) and 9 in Schanzenpark (36 trap days). Summer population densities calculated as MNA_{ind} were substantially higher in the three urban parks compared to the forest. The smallest park (Wohlerspark) housed a 42-fold higher squirrel density than the forest. The density in this park was also higher than in the other two parks: 2.2 times higher compared to Jacobipark and 2.6 times higher compared to Schanzenpark. Compared to the forest, Jacobipark had a 20-fold higher and Schanzenpark a 16-fold higher population density. Specifically, we found a MNA_{ind} of 0.21 individuals per ha in summer in the forest, 4.10 in Jacobipark, 3.38 in Schanzenpark and 8.83 in Wohlerspark. We did not trap any individuals in the forest in winter.

We never trapped subadults or juveniles in the forest, whereas they were trapped in all seasons in Wohlerspark (Tab. 4). In summer, 23 % of the trapped individuals in Wohlerspark were juvenile or subadult and in Jacobipark they accounted for 25 % of the individuals. We trapped more juveniles or subadults than adults in Schanzenpark (56 %), but absolute trapping numbers were low. Since all juveniles were trapped in the same corner of the park, they might have been siblings from the same litter. Apart from one exemption (Wohlerspark in winter), we always trapped more males than females, however, the proportions were neither statistically different between the four sites in summer (p = 0.40) nor between the forest and Wohlerspark in spring (p = 1.00, odds ratio 0.51, CI 0.01-6.63) or autumn (p = 0.49, odds ratio 0.00, CI 0.00-6.75). We did not recapture any of the forest individuals from one season to another during this study, but three males were recaptured in the years 2018/2019 during another study. In Wohlerspark, recapture rate was fairly high with 20 (54 %) out of the 37 adult individuals being recaptured at least once in another season, thereof five individuals in two seasons, nine in three seasons and six in all four trapping seasons. Ten (53 %) of the subadults were recaptured as adults in following seasons. See Tab. 4 as well as Fig. 3 – 5 for full details on population structures and trapping success.

Tab. 4 Overview of trap days (product of no. of traps by no. of capture days per site and season) and population structure per study site and season. MNA: minimum number alive per ha; MNA$_{\text{ind}}$: Index of MNA, calculated to account for differences in trapping effort (i.e., varying no. of trapping days and used traps)

Site	**Hahnheide**			**Jacobipark**			**Schanzenpark**			**Wohlerspark**		
Sex	**f**	**m**	**total**	**f**	**m**	**total**	**f**	**m**	**total**	**f**	**m**	**total**
Winter	**2016/2017**									**2016/2017**		
trap days			200									54
individuals	0	0	0							13	11	24
adults	*0*	*0*	*0*							*9*	*6*	*15*
subadults	*0*	*0*	*0*							*4*	*4*	*8*
juveniles	*0*	*0*	*0*							*0*	*1*	*1*
MNA			0.00									5.22
MNA$_{\text{ind}}$			0.00									9.66
Spring	**2017**									**2017**		
trap days			120									80
individuals	1	4	5							7	14	21
adults	*1*	*4*	*5*							*7*	*13*	*20*
subadults	*0*	*0*	*0*							*0*	*1*	*1*
juveniles	*0*	*0*	*0*							*0*	*0*	*0*
MNA			0.20									4.57
MNA$_{\text{ind}}$			0.17									5.71
Summer	**2017**			**2018**			**2017**			**2017**		
trap days			57			48			36			64
individuals	1	2	3	5	7	12	1	8	9	10	16	26
adults	*1*	*2*	*3*	*4*	*5*	*9*	*1*	*3*	*4*	*8*	*12*	*20*
subadults	*0*	*0*	*0*	*0*	*2*	*2*	*0*	*3*	*3*	*1*	*4*	*5*
juveniles	*0*	*0*	*0*	*1*	*0*	*1*	*0*	*2*	*2*	*1*	*0*	*1*
MNA			0.12			1.97			1.22			5.65
MNA$_{\text{ind}}$			0.21			4.10			3.38			8.83
Autumn	**2018**									**2017**		
trap days			51									32
individuals	0	2	2							11	12	23
adults	*0*	*2*	*2*							*9*	*11*	*20*
subadults	*0*	*0*	*0*							*1*	*0*	*1*
juveniles	*0*	*0*	*0*							*1*	*1*	*2*
MNA			0.06									5.00
MNA$_{\text{ind}}$			0.11									15.63

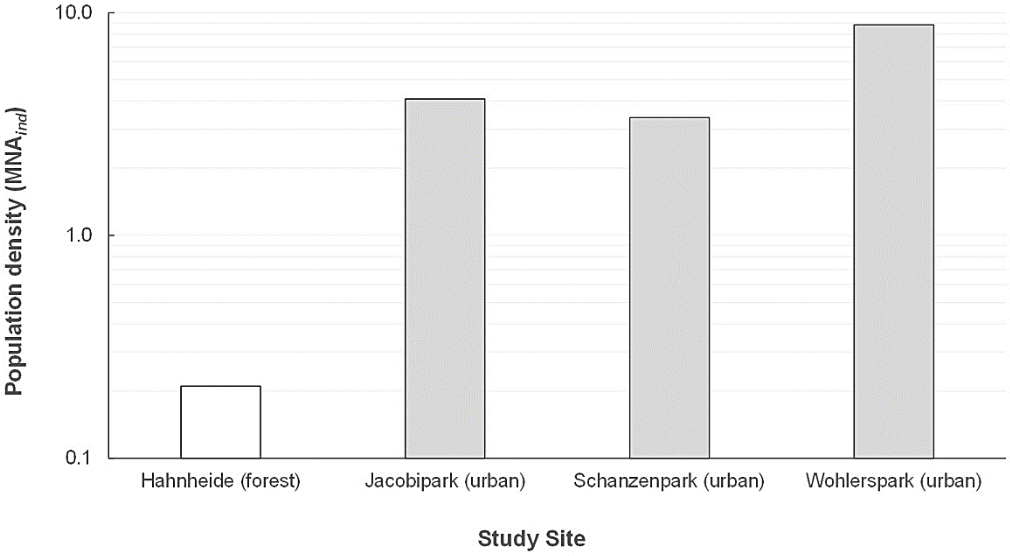

Fig. 3 Squirrel density in summer at the four different study sites; shown as index of MNA (MNA$_{ind}$; note the logarithmic scale)

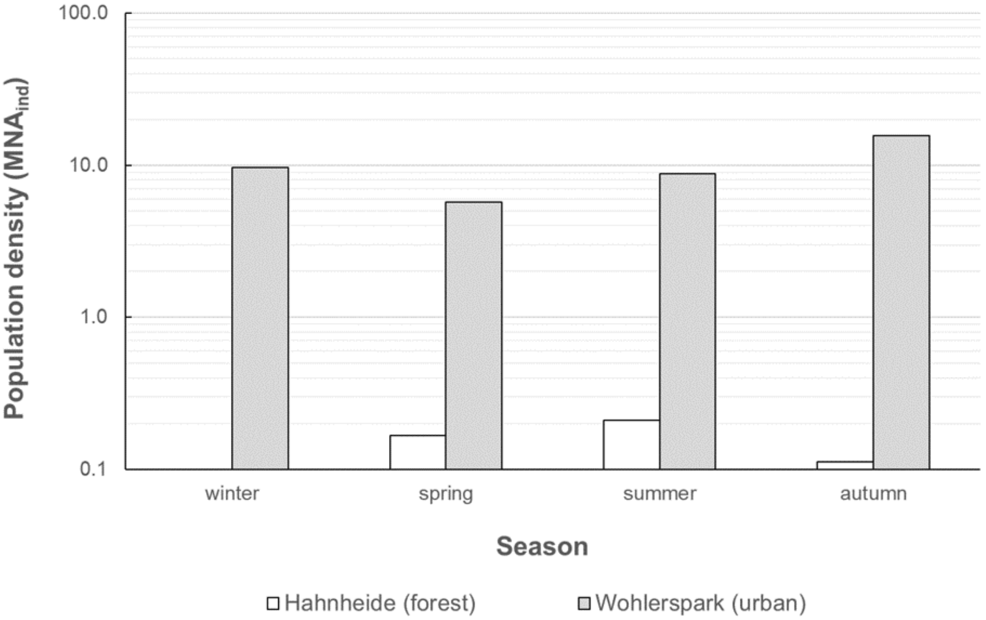

Fig. 4 Squirrel density across the year for the forest site (Hahnheide, open bars) and the most contrasting urban site with regard to size and food supplementation (Wohlerspark, grey bars); shown as index of MNA (MNA$_{ind}$; note the logarithmic scale). We did not trap any individuals in winter in the forest despite extensive pre-baiting, 10 trapping days and squirrel feeding signs (stripped spruce cones)

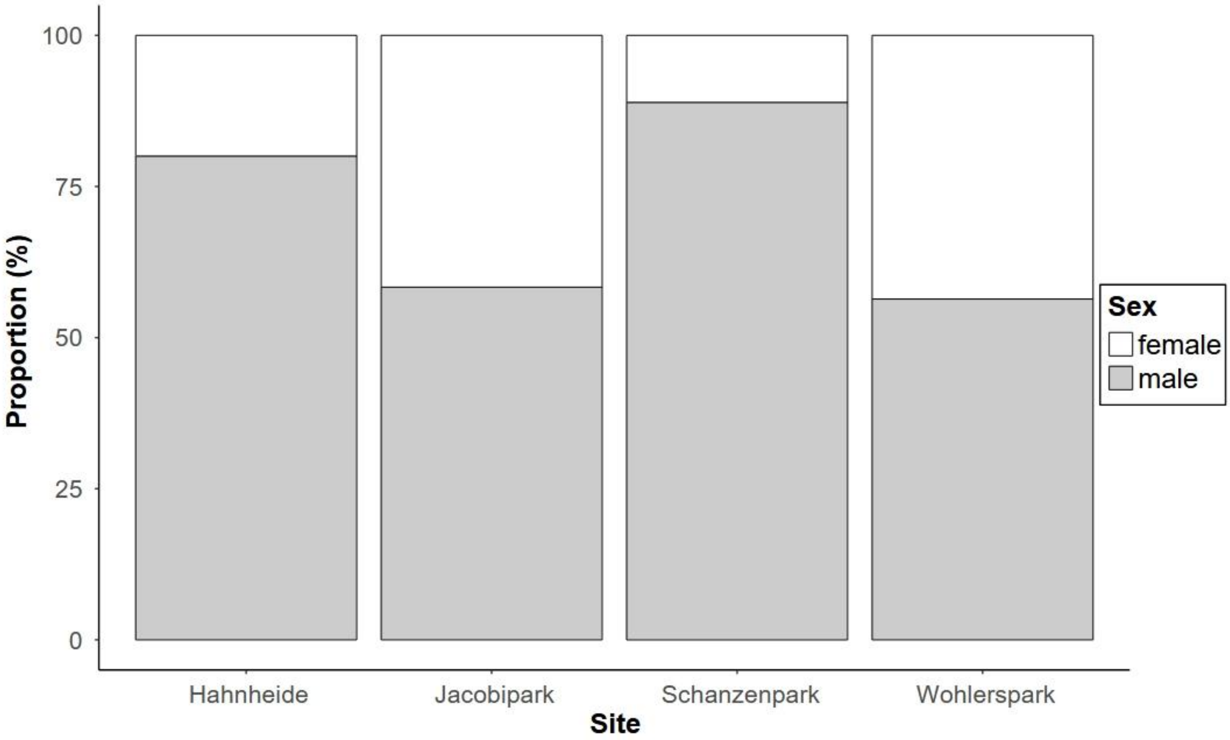

Fig. 5 Proportions (%) of female (white) and male (grey) squirrels trapped in summer at all four study sites. Proportions were not statistically different (Fisher's exact test: p = 0.40)

Discussion

Our study highlights the heterogeneity of urban habitat patches that appear to be very similar. Urban stressors peak at urban centres, yet the spatial heterogeneity of cities can lead to distinct environmental conditions for wildlife in different green urban fragments even when located closely together (Alberti et al. 2020). The urban parks we studied showed pronounced differences in natural and supplemental food availability, though being located in the core area of the same city and sharing similar environmental conditions in terms of human population density, park size or type of urban matrix. Tree species richness as well as number of trees in total and per ha were highest in the smallest park (Wohlerspark), which might indicate higher availability of seeds and shelter for wildlife, compared to the other two parks. However, this park had the lowest tree diversity among the parks and was strongly dominated by lime trees. Lime tree seeds are not favored by squirrels, but represent secondary food items (Bertolino et al. 2004, Krauze-Gryz and Gryz 2015). Conifers, an important primary food source for squirrels, were scarce in all parks, accounting for only 3 to 9 % of the local trees, thus suitability as permanent habitat could be disputable. Availability of kJ from fallen tree seeds was lower in Wohlerspark and Schanzenpark compared to the third park and the forest, but this could change throughout the year with different types of food ripening at different times. Natural seed availability might have been influenced by gardening activities in the parks where measures such as cutting of trees and cleaning of paths etc. occurred regularly during the trapping seasons. These activities might also impact the possibilities to retrieve hoarded food items.

Besides natural food sources, anthropogenically provided foods also differed between the parks in amount and variety in the summer season and were highest in Wohlerspark. Throughout the year, we observed multiple feeding spots in this park with intentionally provided wildlife food, for example, bird food or nuts. However, supplemental foods in the parks also included leftovers, e.g., processed foods or bakery products, both inappropriate food for squirrels with regard to nutrients. We received reports and witnessed first-hand that squirrels in the park foraged on various food items, e.g., fat balls, rice crackers, cookies and even cupcakes. Moreover, peanuts – a non-native food – were frequently offered to squirrels in Wohlerspark. The chemical composition of peanuts, such as a high phosphorus content, is likely to restrict the peanut intake or can lead to nutrient deficiencies in squirrels (Shuttleworth 2000). We did not take any data on trash bin content in this study, but squirrels often inspected them and food intake from trash bins was documented for grey squirrels (Rimbach et al. 2022).

Supplemental foods can make up a significant part of the diet of urban wildlife and induce substantial shifts in the feeding repertoire (Dasgupta et al. 2021, Wist et al. 2022). For example, they accounted for more than half of the stomach content of urban foxes (Contesse et al. 2004). Consequently, abundant supplemental food sources in urban habitats can increase body mass or condition (relation between body mass and a measure of body length) in species that are able to exploit them successfully, as it is the case for, e.g., gulls, chipmunks and many carnivores (Auman et al. 2008, Bateman and Fleming 2012, Lyons et al. 2017). On the other hand, effects on health and fitness of wildlife can be ambiguous and an urbanized diet can have multiple side-effects (Andersson et al. 2015, Murray et al. 2015, Pollock et al. 2017). This depends on further parameters, such as supplemental food quality and the simultaneous availability and quality of natural foods sources (Shuttleworth 2000, Birnie-Gauvin et al. 2017). We have indication for lower body condition in the highly supplemented Wohlerspark (Suppl. Tab. 6, Suppl. Fig. 1), possibly due to unsuitable food types and a lower availability of natural food sources. Body mass in squirrels can be age dependent, but usually varies with season and food availability (Wauters and Dhondt 1989a). We do not have indications for seasonal food shortages, i.e., body mass changes over the year in the Wohlerspark population (Suppl. Tab. 7, Suppl. Fig. 2).

A study from Poland also documented lower body condition in urban squirrels when compared to forest individuals (Beliniak et al. 2022), though another recent study found higher body masses in urban squirrels (Tranquillo et al. 2023). These ambiguous results comply with studies on other species. House sparrows (*Passer domesticus*), for example, ranged in studies from no difference in body condition between urban and rural individuals to a significantly decreased body condition in urban individuals (Bókony et al. 2010, Meillère et al. 2017, Jiménez-Peñuela et al. 2019). We suggest that the heterogeneity of study sites, particularly in local food abundance and quality might have contributed to these findings, as we were able to show that even closely located patches can already differ substantially. Differences in city size, park area, human population density as well as socio-economical or cultural factors can influence natural food availability and also the amount of refuse or active wildlife feeding (Fuller et al. 2008, Aronson et al. 2017, Hassell et al. 2021). Furthermore, urban wildlife might also face different levels of intra- and interspecific competition for food sources. We frequently observed other species in high numbers in the parks, such as crows, jays and doves, but also rats. Most of these species interacted with the squirrels, particularly at feeding spots, and pillaged squirrel hoards.

Increased population density is a common feature in successful urban species, including squirrels, and strongly driven by the availability of supplemental food sources (Luniak 2004, McCleery 2010, Shochat et al. 2010, Jokimäki et al. 2017). Interestingly, species densities even seem to be highest in green patches surrounded by the densest urban infrastructure. Probably because the matrix does not offer enough other habitat options, supplemental feeding occurs and numbers of natural predators are often reduced (Luniak 2004, Parker and Nilon 2012). Local squirrel densities and habitat use at rural sites usually vary with forest stand structure, tree diversity and the proportion of conifers, representing food availability (Wauters et al. 2008, Rima et al. 2010, Flaherty et al. 2012, Dylewski et al. 2021). As expected, squirrel densities in this study were higher at all urban sites when compared to the forest, but with distinct variation between the parks. The park with the highest amounts and diversity of supplemental foods also had highest squirrel population density, despite natural food sources might have been limited.

So far, studies in rural habitats, forest fragments, or larger urban parks, have found that densities of *S. vulgaris* rarely exceed 2.0 individuals/ha (Wauters et al. 1994, Wauters et al. 1997, Magris and Gurnell 2002, Bosch and Lurz 2011, Rezouki et al. 2014, Haigh et al. 2015, Beliniak et al. 2021, Beliniak et al. 2022). Yet densities of grey squirrels (*Sciurus carolinensis*) and other synurban species can be up to 100 times higher in urban than in rural habitats (Rodewald and Gehrt 2014, Merrick et al. 2016). Our results for the Wohlerspark population even exceeded this level in the autumn trapping session. However, this might have been influenced by hoarding behaviour of squirrels in autumn, where foraging distances increase and thus individuals are more likely to be caught (Bertolino et al. 2004), together with a lower number of autumn trapping days in the forest location were trapping success was always low or even absent in winter.

Reliable conclusions about differences in population structures or breeding individuals were hampered by low absolute squirrel numbers in most sites. Generally, mammal species successful in urban habitats have larger litters (Santini et al. 2019). Yet, urbanisation can decrease the reproductive success within a species (de Satgé et al. 2019). However, the juvenile to adult ratio in all parks of our study was high and we assume that these individuals were locally born. Year-round access to abundant supplemental foods might enable squirrels to allocate more resources into reproduction (Wauters et al. 1995, Wauters et al. 1997), although this possibly might contribute to the observed lower investment into body condition in highly supplemented parks. Higher proportions of juveniles within urban populations were also found in other studies on urban squirrels (Beliniak et al. 2022). The high recapture rate of juveniles and subadults as adults in following seasons in Wohlerspark might indicate high offspring survival and successful establishment in the parks, again possibly supported by the high amounts of supplemental foods and higher tree numbers, i.e. higher shelter availability. Furthermore, urban fragmentation can limit dispersal, and dispersal distances in urban squirrels are rather short (Selonen et al. 2018).

Our results provide insight into differences between populations living in close proximity in the same urban core area, though we acknowledge the imbalance in trapping effort and success among the sites and seasons in our study. Living in an urban park with high access to supplemental food seems to support high population densities, however might not necessarily improve other fitness relevant traits. Urban grey squirrels, for example, showed poorer physiological condition represented by higher blood glucose levels and lower levels of, e.g., potassium resulting from an anthropogenic diet (Schmidt et al. 2019). We predict that there might be some kind of threshold where positive effects of urban conditions are outweighed by the costs of other urban factors, such as malnutrition of the urban diet or negative side-effects of high population densities.

Future studies should address long-term fitness consequences, since reproductive success and survival are influenced by food availability and body mass (Wauters and Dhondt 1989b, Wauters et al. 1995) and there might be different effects on different life stages. Urban planning and conservation actions should focus on ensuring sufficient natural food availability for urban wildlife such as squirrels, e.g., by increasing tree diversity and particularly conifer numbers. It is also crucial to inform the human population about suitable supplemental feeding and possible threats to urban wildlife. Reducing littering and limiting the access to trash bins might be beneficial to prevent animals from foraging on refuse and non-natural food items. These measures might also assist in preventing the spread of wildlife diseases at clumped feeding spots and onto humans.

Conclusions

Our study highlights the importance of studying the response of wildlife to urban conditions not only on a large, but also on a small scale. Differences in population parameters between different urban habitats within the same city can be substantial, even between parks with similar area and similar type of surrounding urban matrix. Small parks can hold surprisingly high population densities, likely dependent on vegetation structure and the availability of supplemental food sources and shelters. However, we cannot exclude that these habitats might also prove to be ecological traps on a long-term basis. Further studies are necessary investigating underlying mechanisms and consequences on long-term survival at those sites. It is also crucial to consider the urban heterogeneity when drawing conclusions from urban studies and deciding about appropriate conservation actions for a certain site.

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Ethical approval

We followed all applicable institutional and national guidelines for the care and use of animals. The authors complied with the ARRIVE guidelines. All procedures and animal handling were according to the German animal protection law and approved and authorized by the authorities of Hamburg and Schleswig-Holstein (“Hamburger Behörde für Gesundheit und Verbraucherschutz”, permit no. 87/16, 17 November 2016 and extension of the permit to Schleswig-Holstein by the “Ministerium für Energiewende, Landwirtschaft, Umwelt und ländliche Räume des Landes Schleswig-Holstein” (permit of 28 December 2016).

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Supplementary material

for

Food availability and population parameters for squirrels differ even in neighbouring urban parks

Bianca WIST, Kathrin H. DAUSMANN

Suppl. Tab. 1: kJ values per seed or average cone for tree seed species found in random seed plots at

Seed species	kJ per seed or cone	reference	comments
Abies alba	145.80	Salmaso et al. 2009	per cone
Acer sp.	1.70	Grodzinski 1970 and Jensen et al. 1985	calculated from *A. pseudoplatanus* und *A. plantanoides*
Aesculus hippocastanum	203.30	Papageorgiou (1978)	
Alnus glutinosa	0.03	Grodzinski 1970	
Betula pubescens	0.04	Jensen et al. 1985	calculated from *B. verrucosa* and *B. pendula*
Betula sp.	0.04	Jensen et al. 1985	calculated from *B. verrucosa* and *B. pendula*
Carpinus betulus	0.89	Grodzinski 1970	
Corylus avellana	18.71	Jensen et al. 1985	
Fagus sylvatica	3.71	Grodzinski 1970, Jensen et al. 1985, Wauters & Casale 1996	mean value
Fraxinus excelsior	0.88	Jensen et al. 1985	
Ilex sp.	11.07	Burns and Viers 1973 (*Ilex vomitoria*), Williams et al. 2000	calculated value using average seed weight
Lonicera xylosteum	1.30	Burns and Viers 1973; Williams 2001	calculated value using average seed weight
Picea abies	51.10	Salmaso et al. 2009	per cone
Pinus sylvestris	7.50	Salmaso et al. 2009	per cone
Quercus sp.	25.70	Wauters & Casale 1996	value taken from *Quercus robur*
Robinia pseudoacacia	0.26	Grodzinski 1970	
Rosa canina	27.40	USDA 2019, Esitken 2004	calculated value using average fruit weight
Taxus baccata	2.28	Burns and Viers 1973; Williams 2001	calculated value
Tilia sp.	0.63	Grodzinski 1970	value taken from *Tilia cordata*
Tsuga canadensis	1.37	Boone & Mortelliti 2019, Turgeon et al. 2011	calculated value using information from both references
Tsuga sp.	1.37	Boone & Mortelliti 2019, Turgeon et al. 2011	calculated value using information from both references

the four different study sites

Suppl. Tab. 2: kJ values (per g) and references for anthropogenically provided food items found in the three urban parks.

food item	kJ/g	reference	least assessed	comments
apple	2.6	USDA (2022)	21.06.2023	
bell pepper	1.3	USDA (2022)	21.06.2023	
bird seed mix	20.2	vivara.de	29.06.2023	mean value, calculated from six comercial products
bread (white)	11.2	USDA (2019)	21.06.2023	
bread and bread rolls (mix)	11.3	USDA (2019)	21.06.2023	
bread roll (white)	11.4	USDA (2019)	21.06.2023	
brussel sprouts	1.8	USDA (2019)	21.06.2023	
cabbage	1.0	USDA (2019)	21.06.2023	
carrot	1.7	USDA (2022)	21.06.2023	
carrot, boiled	1.5	USDA (2019)	21.06.2023	
chestnuts	20.3	Papageorgiou (1978)	21.06.2023	
cucumber	0.7	USDA (2022)	21.06.2023	
currants	2.6	USDA (2019)	21.06.2023	
egg, boiled with shell	6.5	USDA (2019)	21.06.2023	
fat and seed mix for birds	22.9	vivara.de	29.06.2023	mean value, calculated from six comercial products
Ferrero Küsschen	26.1	manufacturers specifications	21.06.2023	
foxtail millet	13.1	Tirajoh et al. (2014)	21.06.2023	
fruit salad	2.4	USDA (2022)	21.06.2023	
corn	2.8	USDA (2019)	21.06.2023	
grapes	3.3	USDA (2022)	21.06.2023	
hazelnut	26.8	USDA (2023)	21.06.2023	
home made seed dumpling	19.3	vivara.de	29.06.2023	mean value, calculated from six comercial products
honeydew melon	1.5	USDA (2019)	21.06.2023	
kohlrabi	1.1	USDA (2019)	21.06.2023	
leek	2.6	USDA (2019)	21.06.2023	
lettuce	0.7	USDA (2022)	21.06.2023	
mix for rodents	17.4	Alvarenga et al. (2017)		
nectarine	1.8	USDA (2019)	21.06.2023	
oats	15.9	USDA (2022)	21.06.2023	
orange	2.2	USDA (2019)	21.06.2023	
pancake	9.5	USDA (2019)	21.06.2023	
peanuts	24.6	USDA (2023)	21.06.2023	
pear peel	2.6	USDA (2019)	21.06.2023	
pineapple, canned	2.3	USDA (2022)	21.06.2023	
pistachios	23.9	USDA (2019)	21.06.2023	
"PommDöner" (kebab with fries and salad)	9.6	fddb.de	21.06.2023	
rice, boiled	4.1	USDA (2019)	21.06.2023	
salad	1.8	USDA (2022)	21.06.2023	
fat ball for birds	21.0	vivara.de	29.06.2023	mean value, calculated from six comercial products
seed ring for birds	22.9	vivara.de	29.06.2023	mean value, calculated from six comercial products
sunflower seeds	25.5	USDA (2023)	21.06.2023	
tomato	0.8	USDA (2019)	21.06.2023	
tomato, grilled	2.1	USDA (2022)	21.06.2023	
vegetable mix	1.2	USDA (2022)	21.06.2023	mean value from carrot, cabbage and lettuce (USDA)
walnuts	30.6	USDA (2022)	21.06.2023	
watermelon	1.3	USDA (2019)	21.06.2023	

Suppl. Tab. 3: Tree species abundance in % at the four different study sites

tree species	Hahnheide	Jakobipark	Schanzenpark	Wohlerspark
Abies spec.	0.8			0.2
Acer campestre		1.5	9.6	0.5
Acer ginnala		1.1		
Acer negundo				0.2
Acer palmatum				0.5
Acer platanoides		4.1	6.0	
Acer pseudoplatanus		12.8	11.0	5.4
Acer saccharinum			0.4	
Acer spec.		0.4		
Aesculus hippocastanum		2.6	1.5	2.9
Ailanthus altissima		0.4	0.7	
Alnus glutinosa		0.4		
Alnus rubra	2.2			
Betula pendula	0.1	19.9	1.9	9.9
Betula pubescens				0.3
Carpinus betulus		1.5	11.4	4.8
Catalpa bignonioides				0.2
Catalpa erubescens		0.4		
Cedrus atlantica				0.2
Cercis spec.		0.4		
Chamaecyparis lawsoniana				0.2
Cornus spec.			0.1	
Crataegus laevigata			0.3	0.2
Crataegus monogyna			0.4	
Fagus sylvatica	36.0	7.5	3.2	1.6
Fraxinus excelsior		7.1	0.9	0.2
Ginkgo biloba		0.4	0.3	0.2
Ilex aquifolium				0.2
Ilex spec.				1.9
Juniperus communis		0.4		
Larix decidua	0.2			
Larix kaempferi	6.7			
Liriodendron tulipifera				0.2
Magnolia spec.				0.3
Malus spec.		0.4	0.4	0.6
Parrotia persica		0.4		
Picea abies	33.7			0.2
Picea omorika				1.3
Picea pungens				0.5
Picea sitchensis	0.6			
Pinus nigra		0.4	2.8	
Pinus strobus		0.4		0.5
Pinus sylvestris	14.0			
Platanus acerifolia			2.0	
Platanus spec.		1.5		
Populus canadensis			0.6	

Populus canescens			0.3	
Populus lasiocarpa				0.6
Prunus 'Accolade'		1.9		
Prunus cerasifera			0.3	0.5
Prunus mahaleb			0.3	
Prunus padus			0.1	0.2
Prunus serotina			0.1	
Prunus serrulata				1.3
Prunus spec.			0.1	0.6
Pseudotsuga menziesii	4.4			0.5
Quercus palustris			0.1	
Quercus petraea			0.1	
Quercus robur		17.7	7.7	3.8
Quercus rubra		0.4	1.8	0.5
Quercus spec.	1.3			
Robinia pseudoacacia			9.1	0.3
Robinia spec.		1.9		
Salix caprea				0.2
Salix fragilis				0.2
Sorbus aria				0.2
Sorbus intermedia			0.6	
Taxodium distichum				0.5
Taxus baccata		1.5		2.1
Taxus spec.		5.6		
Thuja spec.				0.3
Tilia cordata			4.5	
Tilia euchlora			0.1	
Tilia platyphyllos			3.4	0.2
Tilia spec.		0.4	0.1	
Tilia tomentosa			0.3	
Tilia vulgaris		3.0	17.1	54.4
Tsuga canadensis				0.6
Tsuga heterophylla				0.2
Ulmus glabra		1.1		
Ulmus hollandica		1.9		0.2
unknown species		0.8		

Suppl. Tab. 4 Overview of food item details from the four different study sites in the summer season

	Hahnheide	Schanzenpark	Jacobipark	Wohlerspark
bird food			**x**	
bird seed mix			x	
nuts or peanuts			**x**	**x**
peanuts				x
fruits		**x**	**x**	**x**
apples		x		x
honeydew melon				x
watermelon			x	x
fruit salad				x
grapes				x
currants				x
vegetables			**x**	**x**
tomato				x
tomato (grilled)			x	
carrot				x
cabbage				x
bakery products		**x**		**x**
bread		x		x
pancake				x
fast food				**x**
kebab & fries				x

Suppl. Tab. 5 Overview of human-provided food items across the year in g and kJ in Wohlerspark. Spring and summer values show means from three days of observation, winter from eight days (autumn: one day). Peanuts have been placed into a common category with nuts

	winter		**spring**		**summer**		**autumn**	
	g	**kJ**	**g**	**kJ**	**g**	**kJ**	**g**	**kJ**
bird food	960	18,366	50	874	0	0	689	13,918
nuts or peanuts	303	7,841	0	0	20	492	0	0
fruits (fresh/canned)	4,633	11,817	230	367	1,010	1,876	15	50
vegetables (raw/boilded/grilled)	523	614	182	287	317	522	620	674
bakery products	78	877	63	708	283	3,134	40	447
fast food	0	0	50	203	53	514	0	0
egg (boiled)	0	0	23	151	0	0	0	0
sweets (chocolate)	0	0	6	157	0	0	0	0
total in g / kJ	**6,497**	**39,515**	**604**	**2,747**	**1,683**	**6,537**	**1,364**	**15,089**

Body condition index (BCI)

Methods

We assessed body condition by using a simple ratio index of the measured body mass in grams and the nose-anus length in centimeters (Labocha et al. 2014, Wist et al. 2022).

We tested for difference in BCI between sexes using a t-test prior to further analysis which was non-significant ($t = 1.00$, $df = 34$, $p = 0.32$). We performed a one-way analysis of variance (ANOVA) with one BCI per individual to examine differences in BCI group means between sites in the summer season. This was followed by post-hoc multiple comparisons (Tukey HSD). ANOVA assumptions were tested by using residual analysis. Furthermore, we used two-tailed Fisher's exact test to test for differences in sex proportions between sites. Repeated measures ANOVA (rmANOVA) including Mauchly's test of sphericity (Mauchly 1940) was used to investigate differences in BCI means across all four seasons in Wohlerspark. We used Greenhouse-Geisser correction since sphericity was violated (Girden 1992).

Results

A one-way ANOVA showed significant differences in BCI between sites ($F_{3,32} = 8.34$, $p < 0.001$; Suppl. Tab. 3, Suppl. Fig. 1). However, post-hoc multiple comparisons revealed that only Wohlerspark differed significantly from the other three sites ($p < 0.05$).

Tab. 6 Descriptive statistics for summer BCI at the four different study sites. Given are the numbers of adult individuals per site (n), BCI group means, standard deviation as well as minimum and maximum values per site.

Site	n	Mean	SD	min	max
Hahnheide	3	17.52	1.03	16.43	18.47
Schanzenpark	4	17.70	2.02	16.38	20.65
Jacobipark	9	16.94	1.36	14.52	19.52
Wohlerspark	20	15.37	0.83	13.92	17.40

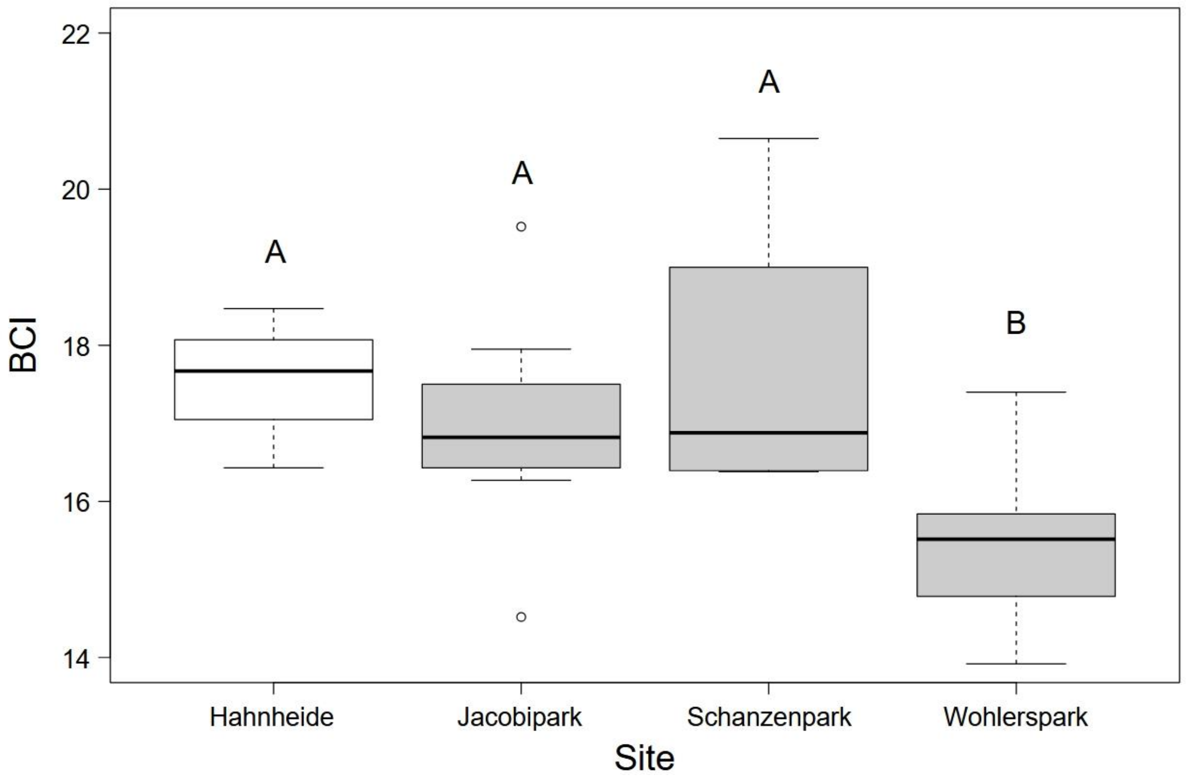
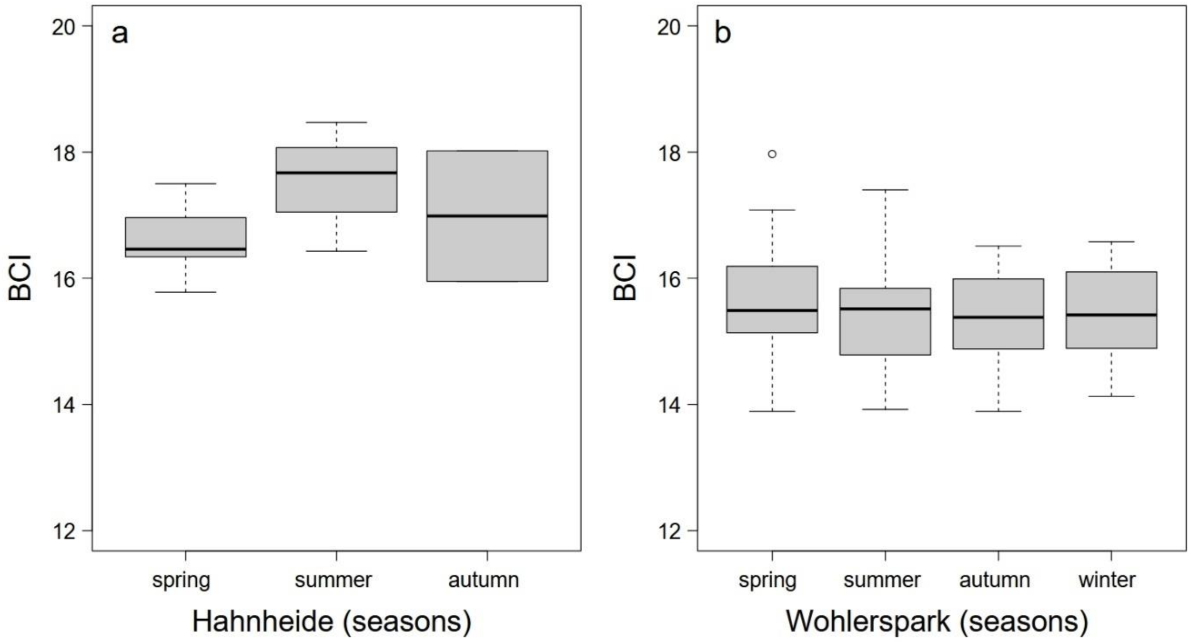


Fig. 1 Body condition index of adult individuals at the four different study sites in summer. N = 36 (thereof $n_{Hahnheide}$: 3, $n_{Jacobipark}$: 9, $n_{Schanzenpark}$: 4 and $n_{Wohlerspark}$: 20 individuals); different letters indicate statistical difference (Tukey HSD, $p < 0.05$)

Suppl. Tab. 7: Descriptive statistics for BCI of adult individuals at Hahnheide and Wohlerspark in all trapping seasons. Given are the number of individuals per site and season (n), BCI group means, standard deviation, standard error as well as minimum and maximum values per group

Site	season	n	mean	SD	min	max
Hahnheide	spring	5	16.61	0.65	15.78	17.50
Hahnheide	summer	3	17.52	1.03	16.43	18.47
Hahnheide	autumn	2	16.98	1.46	15.95	18.02
Wohlerspark	spring	20	15.67	0.93	13.89	17.97
Wohlerspark	summer	20	15.37	0.83	13.92	17.40
Wohlerspark	autumn	21	15.36	0.70	13.89	16.51
Wohlerspark	winter	17	15.49	0.78	14.13	16.58

BCI of individuals from Wohlerspark did not significantly differ between seasons (rmANOVA: $F_{1.21,\ 6.05} = 3.51$, $p = 0.11$, Suppl. Tab. 5, Suppl. Fig. 2). Low sample sizes in combination with no trapped individuals in winter did not allow for robust analysis of seasonal differences in BCI in the forest (Suppl. Tab. 5, Suppl. Fig. 2). However, lower body condition in squirrels from Wohlerspark in comparison with forest squirrels seemed to occur year-round and confirmed during trapping in spring for another study on male individuals in the years 2018/2019 (Wist et al. 2022).



Suppl. Fig. 2 Body condition index (BCI, of adult individuals at Hahnheide (a, $n_{spring} = 5$, $n_{summer} = 3$, $n_{autumn} = 2$) and Wohlerspark (b, $n_{spring} = 20$, $n_{summer} = 20$, $n_{autumn} = 21$, $n_{winter} = 17$) for all four seasons

Supplementary references

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Authors' contributions

I hereby confirm that Bianca Wist (BW) and Kathrin H. Dausmann (KHD) conceived and designed the study. BW conducted the field work and analysed the data. BW wrote the manuscript. All authors revised the manuscript and approved the final version of it.

Prof. Dr. Kathrin Dausmann

Chapter 3

Sugar addicted in the city: Impact of urbanisation on food choice and diet composition of the Eurasian red squirrel (*Sciurus vulgaris*)

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Abstract

Urban wildlife faces a great variety of human-induced habitat alterations, among others changes in resource availability and composition, often resulting in serious declines in biodiversity. Nevertheless, Eurasian red squirrels (*Sciurus vulgaris*) occur in high densities in urban areas and seem to benefit from supplementary feeding. However, we still lack knowledge about consequences of urbanisation on mammalian foraging behaviour and nutrient intake. Thus, we investigated body mass, food choice and diet composition in squirrels from an urban core area versus a forest population in a cafeteria experiment. Urban individuals were lower in initial body mass and condition, but consumed significantly more g and kJ per day and significantly gained weight over the course of the experiment (around 2 weeks); nevertheless, the difference in body mass and condition persisted. All squirrels preferred hazelnuts, but urban squirrels had a wider dietary range and consumed more non-natural food items. Both groups prioritised fat and there was no difference in protein intake. Urban squirrels though had a significantly higher sugar intake, mainly by eating biscuits. Our results demonstrate clear effects of urbanisation on foraging behaviour and preferences, which has the potential for nutritional mismatch or negative side effects due to consumption of non-natural food items. Our findings show that highly supplemented urban core fragments might not serve as adequate refuge for wildlife.

Keywords

urbanisation, dietary range, supplemental feeding, nutrient ecology

Introduction

Urban habitats are ever-faster growing in nearly all parts of the world (McDonald et al. 2020) and are therefore representing a severe global challenge for the survival of wildlife (Birnie-Gauvin et al. 2016). Animals in urbanised areas face multiple human-induced habitat alterations and this effect is strongest in cities, which represent the most intense type of urban settings (Grimm et al. 2008; Donihue and Lambert 2015; Alberti et al. 2017). Besides aspects such as, e.g. habitat fragmentation, high levels of disturbances through human activities, noise and pollution, urban wildlife experiences changes in landscape and vegetation structure and consequently in food availability. This is critical, since diet, i.e. energy and nutrient intake, is essential for all vital life processes and influences traits like body condition, physiology, reproduction and finally fitness (Baldwin and Bywater 1984; McNab 1986; Silva, Jaksic, and Bozinovic 2004; Perissinotti et al. 2009). In urban habitats, supplemental food sources, either intentional or inadvertently, can account for a substantial part of an animal's diet (Shochat et al. 2006; Coogan et al. 2018). This might be rather positive, e.g. through buffering of seasonality or negative, e.g. through non-natural food items of possible poor quality, which can have adverse effects for animals (Murray et al. 2015, Coogan et al. 2018, Isaksson 2018). Furthermore, urban habitats and large cities, in particular, are composed of an extremely heterogeneous habitat mosaic (Rebele 1994; Faeth et al. 2005; Faeth, Bang, and Saari 2011) with natural as well as human-provided resources being distributed very patchy, often in parks, private gardens or on balconies (Contesse et al. 2004). High densities of urban species increase competition for these patchy resources (Bowers and Breland 1996) and animals could be forced to feed on what they get rather than what might be part of their natural diet. Furthermore, species that naturally favour food items high in fat can be led to forage on detrimental amounts since the access to those food items might not be limited in cities with substantial supplemental resources. Therefore, food provided by humans is likely to influence energy intake, diet composition and foraging behaviour (McDonnell and Pickett 1990; Faeth et al. 2005; Coogan et al. 2018) and has the potential to alter diverse aspects of animals' life histories and ecologies (Contesse et al. 2004; Luniak 2004; Newsome and Rodger 2008; Robb et al. 2008; Lowry, Lill, and Wong 2013).

In general, vertebrate diversity and abundance are lower in urbanised habitats, as several species cannot cope with the challenging conditions that often have negative consequences on fitness and evolution (Faeth, Bang, and Saari 2011, Birnie-Gauvin et al. 2016; Johnson and Munshi-South 2017). However, some bird, mammal and arthropod species tend to thrive in urban settings and display higher densities than in their natural habitats, such as corvids (*Corvidae*), rats (*Rattus rattus*), red foxes (*Vulpes vulpes*) or Eurasian red squirrels (*Sciurus vulgaris*; Luniak 2004; Francis and Chadwick 2012; Feng and Himsworth 2014). This is a phenomenon known as ‘synurbanisation’ and refers to certain ecological and behavioural differences between urban populations and those living in natural habitats (Luniak 2004; Francis and Chadwick 2012). Successful urban species are usually generalists concerning their habitat or food preferences and show phenotypic plasticity in their behavioural responses (Lowry, Lill, and Wong 2013), enabling them to benefit from urban resources and supplemental feeding (Francis and Chadwick 2012). Foraging behaviour is driven by complex mechanisms that link physiological demands with environmental conditions. In contrast to earlier foraging models, there is growing evidence for a multidimensional nutritional ecology perspective (Simpson and Raubenheimer 2011; Coogan et al. 2018). Animals should select their food with focus on obtaining a nutritionally balanced diet (Raubenheimer et al. 2012) and in consideration of costs (e.g. processing time) and benefits (e.g. energy intake). Diet selection of several species aims at achieving a specific balance of target nutrients, i.e. individuals feed on different food items in varying quantities to a particular mix (Simpson et al. 2004; Felton et al. 2009; Simpson and Raubenheimer 2011; Raubenheimer, Simpson, and Tait 2012). This can be challenging in urban habitats with possibly limited access to high-quality natural food. Thus, it is likely that dietary balances are disturbed in cities. Moreover, the advantages of being a food generalist might be compensated by a higher risk to include non-suitable supplemental food items into the diet (Luniak 2004; Lefcheck et al. 2013).

In an evolutionary background, urbanisation is a comparably young phenomenon, but one with highly effective selective power on wildlife species (Donihue and Lambert 2015; Ouyang et al. 2018; Łopucki et al. 2020). As contrary to urban areas, natural habitats are constantly disappearing, species not being able to adapt successfully to urban habitats will be particularly at risk. Studying those species being apparently successful and seem to cope well with urban conditions, can give valuable insight in the mechanistic link between food availability, feeding behaviour and high urban abundances. Although research on urban wildlife has considerably increased over the past decades, many open questions remain and there is an urgent need to increase knowledge about mechanisms and consequences of urbanisation (Magle et al. 2012). Most notably, mammalian nutritional ecology in urban core areas is still poorly understood.

Among the species being apparently successful in urban habitats is the Eurasian red squirrel (henceforth 'squirrel'). The species is a food generalist and opportunist (Gurnell 1987) and occurs in high densities in urban and supplemented areas (Magris and Gurnell 2002; Jokimäki et al. 2017, Beliniak et al. 2021, Fingland et al. 2022). Squirrels change their feeding behaviour according to habitat type and environmental conditions. Despite favouring seeds and nuts (Moller 1983), the species' diet comprises several other food sources (Wauters, Swinnen, and Dhondt 1992; Krauze-Gryz and Gryz 2015) which might be one of the key traits enabling it to be successful in urban habitats. Especially in urban parks, squirrels feed on a great variety of human-provided food items (Krauze-Gryz and Gryz 2015) from nuts provided by hand-feeding, bird food, kitchen waste, as well as leftovers from picnics or at playgrounds (Wist, pers. obs.). As there is indication for higher food competition in urban habitats (Bowers and Breland 1996), not only with conspecifics, but also with other species such as corvids, urban squirrels might profit from those supplemental food sources. It has been shown, e.g. that the abundance of natural food sources alone is insufficient to explain the high densities of North American grey squirrels (*Sciurus carolinensis*) in urban parks, indicating the important role of supplemental foods for urban wildlife (Parker and Nilon 2008). Furthermore, semi-urban Eurasian red squirrels, i.e. squirrels living in an extensive cemetery park landscape within a city, shift their home range core areas closer to supplemental food sources in seasons of higher food provisioning (Reher et al. 2016). On the other hand, urban habitats can also act as ecological traps, if feeding on non-natural, possibly non-suitable, food items and subsequently on an imbalanced diet lead to negative effects as described above. Study results on the effects of human-provided food sources are still ambiguous in different species including squirrels (Shuttleworth 1996; Magris and Gurnell 2002; Lurz, Gurnell, and Magris 2005; Auman, Meathrel, and Richardson 2008; Rodewald and Shustack 2008). As long as natural food sources are available in sufficient amounts, species like squirrels and hedgehogs (*Erinaceus europaeus*) use human-provided food sources only as a supplement (Morris 1985; Shuttleworth 2000). However, this effect is questionable in highly urban fragments like parks where high competition occurs along with a restricted abundance of natural resources (Parker and Nilon 2008). It is not known how urban squirrels compose their diet in city core areas and whether they shift their foraging behaviour compared to rural populations.

Therefore, our study investigated habitat-related food choices and diet composition of the Eurasian red squirrel in a cafeteria experiment under semi-natural conditions. We compared body mass and body condition index (BCI) as well as energy and macronutrient intake (fat, protein and sugar) of different, natural and anthropogenic food items when offered *ad libitum* to squirrels adapted to two completely contrary habitat types: i.e. squirrels from a highly urban park versus forest individuals. We hypothesised that: (i) urban individuals would be higher in body mass and condition than their forest counterparts as found in other urban species (Auman, Meathrel, and Richardson 2008; McCleery 2010; Łopucki et al. 2019). (ii) With regard to diet composition, we hypothesised that a species favouring seeds and nuts should prioritise fat intake as macronutrient when being able to feed *ad libitum*, independent of its original habitat since it was shown that squirrels prefer high-energy foods when available (Shuttleworth 2000, Krauze-Gryz and Gryz 2015, Kostrzewa and Krauze-Gryz 2020). (iii) On the other hand, boldness and a lesser degree of neophobia seem to be crucial traits for urban exploiters (Lowry, Lill, and Wong 2013; Audet, Ducatez, and Lefebvre 2016, Krauze-Gryz et al. 2021).

Thus, we expected urban squirrels to be more used to supplemental foods and to show a wider dietary range. (iv) Finally, according to the nutrient balance theory, we expected all individuals to incorporate similar proportions of fat, protein and sugar in their diet, independently of their food item mix. The results of our study will contribute to the understanding how urbanisation influences and alters food choice behaviour and might detect mismatches in nutritional intake. Understanding nutritional ecology can be a powerful tool in conservation planning and urban park management (Raubenheimer, Simpson, and Tait 2012). Only detailed knowledge about mechanisms of urbanisation will enable us to conserve urban biodiversity appropriately and to decide whether urban parks can serve as suitable habitats for wildlife like squirrels as has been proposed by recent studies (Rezouki et al. 2014; Haigh et al. 2017).

Methods

Trapping and handling

We captured squirrels in a small park (Wohlers Park, 4.6 ha) in the core area of Hamburg, northern Germany (N53° 33′ 29.646″ E9° 57′ 11.459″), as well as in a forest study site close to the city (Hahnheide; N53° 37′ 14.146″ E10° 27′ 1.667″). The park is surrounded by a highly urban matrix with a mix of small and large roads, apartments and commercial buildings being located directly adjacent or near the park. Park vegetation had a mixed structure with 45 tree species, thereof many non-native. Many of the tree species (~75%) were suitable for squirrels with regard to their seed preferences. However, tree cover was dominated by linden trees (Tilia sp.), interspersed with lawn areas and high proportions of fallen seeds were removed by gardening activities. Despite a high degree of human disturbance, such as intensive use of the park for sports and parties, we found a very high density of squirrels in the park (average of 5.1 squirrels/ha, calculated as minimum number alive) as well as year-round intensive supplemental feeding (Wist et al., unpublished data). The latter consisted of intentional feeding with, e.g. peanuts and unintentional feeding with leftovers like fast food and butter biscuits or bird food that was frequently distributed throughout the park and in adjacent backyards or balconies (Wist et al. unpublished data). The forest study site is located ~30 km from the city centre. The mixed forest nature reserve comprises 1.460 ha with a high tree cover of eleven tree species (mainly Norway spruce and beech), and consisting mainly of old trees. The forest floor was covered with high amounts of fallen seeds and there was no supplemental feeding.

Trapping took place for 4–5 days per trapping run in spring 2018 and 2019 using Tomahawk live traps (20 × 20 × 50 cm; Tomahawk Live Trap, Hazelhurst, WI, USA) placed in the park shrubbery or at tree trunks at 1.5 m height in the core area of the forest. We used 6–8 traps in the park and 18–20 traps in the forest, placed in a trapping grid with a distance of about 100 m. We baited traps with a seed and nut mix (walnuts, hazelnuts, peanuts and sunflower seeds) when opened in the morning around 07:30 h and closed the traps after 5–8 h, depending on trapping success and study site. Traps were controlled every 30–60 min in the urban park and every 90–120 min in the forest. Trapped individuals were transferred into a cloth handling cone with zippers (Koprowski 2002) to enable handling of the non-anaesthetised individuals with a minimum of stress. Individuals were pit-tagged (ID-100B; Trovan Ltd., East Yorkshire, UK) and we recorded initial body mass to the nearest 5 g by using a spring balance (KERN & SOHN GmbH, Balingen-Frommern, Germany). Furthermore, we assessed age (juvenile/subadult/adult), sex, reproductive status (via inspection of the external reproductive organs, following Magris and Gurnell 2002), length of body (nose-anus to the nearest 0.5 cm, mean from multiple measurements whenever possible) and right hind foot (in mm, measured with a small transparent ruler). For the body length measurement, we positioned the animals with their snouts directed into the narrow tip of the bag. The animal was then restrained and a tape measure was passed from the tip of its snout to the base of the tail. We used only adult, obviously healthy males in this study to minimise the influence of sex, reproduction, disease or growth. Male adults were transferred into a cloth bag and transported to the Institute of Cell and Systems Biology of Animals, Universität Hamburg.

Study period and housing conditions

In total, we housed 20 adult male squirrels in outdoor enclosures under natural photoperiod and ambient temperature for 13–18 days in spring 2018 and 2019. Owing to limitations in housing capacities, we conducted the experiment in four different runs, each with a mix of urban and forest individuals (Table 1). The number of urban individuals per run was higher, as trapping success in the forest was very low (urban park: mean of 6.1 individuals, forest: 1.7 individuals per trapping day). We conducted all runs in a 2 months period (from end of March to end of May) in spring to minimise seasonal effects.

Table 1 Overview of housing periods and no. of individuals of *Sciurus vulgaris* per habitat (N = 20, urban: n = 13, forest n = 7), housed for the food choice experiment in outdoor facilities of Hamburg University, Germany.

Housing period	Date	No. of urban individuals	No. of forest individuals
A	04 – 24 April 2018	4	2
B	08 – 28 May 2018	4	1
C	28 March – 12 April 2019	3	2
D	02 – 22 May 2019	2	2
Total		**13**	**7**

Housing facilities were located at Hamburg University (53°34′ 02.2″ N 9°58′ 45.6″ E) in outdoor aviaries (2.3–2.7 m high and 4.4–7.9 m^2 floor area). We housed only one squirrel per aviary to account for their solitary lifestyle and used panels for some privacy screen. The aviaries were equipped with branches, some bedding material and a nest box (Elmato 10064 Großsittichkobel, Elmato GmbH, Holzheim, Germany, 30 × 22 × 20 cm), insulated with organic material (Pavatex Pavaflex, Soprema GmbH, Germany), and whole complex was covered with a roof. After the experiments, we recorded the final body mass of the individuals by weighing them three times in their nest boxes to the nearest 5 g (Kern PCB 3500 precision scale, KERN & SOHN GmbH, Balingen-Frommern, Germany). Afterwards, we released them at their trapping locations. Recapture of nine individuals (45%, thereof four forest and five urban individuals) during trapping sessions for the following runs proved them to be in good condition with no observable adverse effects of the treatment. Since we did not perform any trapping after the last run, there was no recapture opportunity for animals from the last run.

Food bars and food choice (cafeteria experiment)

Each aviary was equipped with a food bar (65 × 35 × 40 cm), constructed from plywood panels forming a floor and a roof. We placed the bars on the ground of the aviaries to allow access to the food bar from all sides and chose a location in the shade, away from the door and close to a 'rescue'-branch, to create a comfortable foraging site for the squirrels. We supplied food daily in paper food containers, which we placed into tacked containers to avoid sliding. A bowl with fresh water was positioned at the top of each food bar. Food items were placed in random order (using a random list generated in R 3.4.3), to avoid bias through side preferences by the individuals.

We used eight different food items, which we chose according to our field observations of supplemental food sources and the known natural diet for squirrels. For instance, we decided to include biscuits in the experiment, since we observed squirrels feeding at highly processed and sweetened foods like biscuits and cake leftovers in the park. We classified spruce cones, whole hazelnuts, dried grasshoppers and apples as 'natural' food, representing food items usually available in the wild. Whole peanuts, butter biscuits (Gut & Günstig Butterkekse, EDEKA Zentrale AG & Co. KG, Germany), fat balls (MultiFit Meisenknödel, MultiFit Tiernahrungs GmbH, Krefeld, Germany) and carrots were classified as 'urban'-related as these items are usually provided by humans and not (or very rarely in the case of carrots) available in natural squirrel habitats in Germany. Butter biscuits and insects were dried to constant weight in advance. Spruce cones were stored in the freezer after collection to avoid opening and seed loss under warm conditions. All other items were stored in closed plastic boxes at ambient temperature or in the fridge.

Food items were provided *ad libitum*. We collected leftovers every day at 14:00 h and weighed them with a scale (Kern PCB 3500 precision scale, KERN & SOHN GmbH, Balingen-Frommern, Germany) to the nearest 0.1 g. Given the low weight of dried grasshoppers and owing to the fact that the squirrels consumed only minor quantities of them (usually only heads or abdomen rather than whole insects), we provided 10 whole grasshoppers, to enable reliable determination of eaten amounts. All values were corrected for water loss or intake by using correction values taken from control items left in an empty aviary or by redrying leftovers of butter biscuits and insects. As many squirrels stayed in their nest boxes during food inspection, the nest boxes were only inspected for leftovers after release of the animals to avoid further disturbances. These, very rare, leftovers were weighed and evenly distributed over the days of the experiment.

Food intake in g eaten per day and food items were corrected as described above. We identified gross energy content of eaten food in kJ by bomb calorimetry (PARR 6100 bomb calorimeter, PARR Instruments Deutschland GmbH, Frankfurt, Germany) with benzoic acid as calibration standard. Crude protein was determined by using the standard Kjeldahl procedure for analysis of nitrogen content and the conversion factor of 6.25 (Association of Official Analytical Chemists 1984 in Ortmann et al. 2006). Additionally, we extracted fat with petroleum ether and analysed sugar content with the phenol-sulphuric acid-method (Kates, Work, and Work 1972; Jiang et al. 2014). We analysed each food type with at least two and up to six replicates. All results were converted from dry matter to fresh weight (i.e. weight of offered food items) by correcting the weight loss of the food items being dried for 24 h at 105°C. For the insects, we analysed whole animals versus heads and abdomen according to the squirrels' food choices. We excluded days with incomplete data from the analysis. On average 13 days per individual were analysed (range: 5–17 days). Food intake was corrected for body mass and therefore given in consumed kJ (total/fat/protein/sugar) per g of body weight. We used a simple ratio index with body weight (g)/body length (nose-anus length in cm) for assessment of body condition (BCI) which was validated to assess body condition (i.e. fat mass) of small rodents (Labocha, Schutz, and Hayes 2014).

Statistical analysis

We used the food intake from all measurement days to calculate a daily mean in g and kJ per individual over the housing period. Correspondingly, values are given as mean ± SD and n reports the number of individuals per group. Statistics were performed in R 3.4.3 (R Core Team 2017) within the “RStudio” environment (RStudio Team 2019) and the packages ‘lattice’ (Sarkar 2008), ‘ggplot2’ (Wickham 2016), ‘psych’ (Revelle 2016) and ‘vegan’ (Oksanen et al. 2019).

We used the Shapiro–Wilk-test to test for normality (Shapiro and Wilk 1965) and F-tests for homogeneity of variances (Duncan 1955). To test for differences in body weight, BCI and food intake (g, kJ, fat, protein, sugar and the single food items), we used (paired) t-tests, Welch-tests or Wilcoxon rank sum tests respectively. Non-metric multidimensional scaling (NMDS, e.g. Rabinowitz 1975) with Bray–Curtis as distance measure was performed to investigate dissimilarities in the diet composition between the individuals of different habitats and analysis of similarity (ANOSIM, Clarke and Green 1988) for testing of statistical significance. We used the significance level of $P \leq 0.05$ and a stress level < 0.2 as indication of goodness of fit in the NMDS. Statistic differences between the groups in the NMDS were tested with ANOSIM (Bray–Curtis coefficient and 9999 permutations) using the P-value and ANOSIM statistic R (−1 to +1) for hypothesis testing. We plotted the results of the NMDS as a spider diagram with centroids per group. We added polygons using the chull() function (base R) to create convex hulls connecting the outermost points per group.

Results

Body mass and condition

Initial and final body mass of urban individuals (i.e. body mass at trapping and after the experiment) was significantly lower compared to their forest counterparts (initial body mass: t-test, $t = 3.60$, $df = 18$, $n = 20$, $P < 0.005$; final body mass: t-test, $t = 3.37$, $df = 18$, $n = 20$, $P < 0.005$; Fig. 1 and Supplementary Fig. S1), as was BCI (initial BCI: t-test, $t = 3.85$, $df = 18$, n = 20, P < 0.005; final BCI: t-test, t = 2.99, df = 18, n = 20, P < 0.01; Fig. 1 and Supplementary Table S1). Mean daily mass change for forest individuals was 0.3 ± 1.2 and 0.7 ± 0.9 g for urban squirrels (Fig. 1 and Supplementary Fig. S1). In total, 15 out of 20 individuals maintained or increased their body mass during the experiment with mass gain ranging from 0.3% to 10.9%. Only five individuals showed mass loss (−0.6% to −4.8%). However, mass change was only statistically significant in the urban squirrels (paired t-test, t(13) = −2.52, n = 13, P < 0.05).

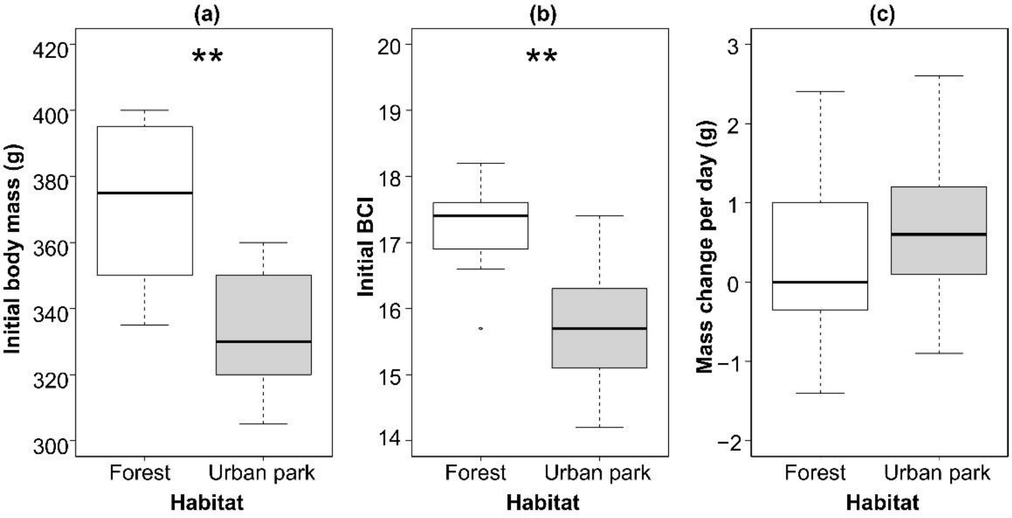

Figure 1 Box and whisker plots showing the initial body mass (a) and initial BCI (b) calculated from body mass g/nose-anus-length in cm as well as daily mass change (c), of forest (n = 7; open boxes) versus urban individuals (n = 13; grey boxes); each box represents 50% of the sample data around the median (indicated by the black bar); the whiskers show the 5% and 95% percentiles for each sample with outliers shown by circles; asterisks indicate significant differences between the groups (**P ≤ 0.01)

Energy intake and diet composition

Urban squirrels consumed significantly more energy with a daily mean of 1.51 ± 0.39 kJ (526.53 ± 148.90 kJ/day) versus 1.16 ± 0.16 kJ per g of body mass (411.37 ± 57.25 kJ/day) in forest individuals (Welch-test: t = −2.78, df = 17.17, n = 20, P-value < 0.05). We found no difference in protein or fat intake between the two groups, but the difference in daily sugar intake was highly significant (t-test: t = −4.40, df = 18, n = 20, P-value < 0.001; Fig. 2 and

Supplementary Table S2) with urban individuals consuming on average 0.12 kJ per g body mass from sugar (forest individuals 0.08 kJ).

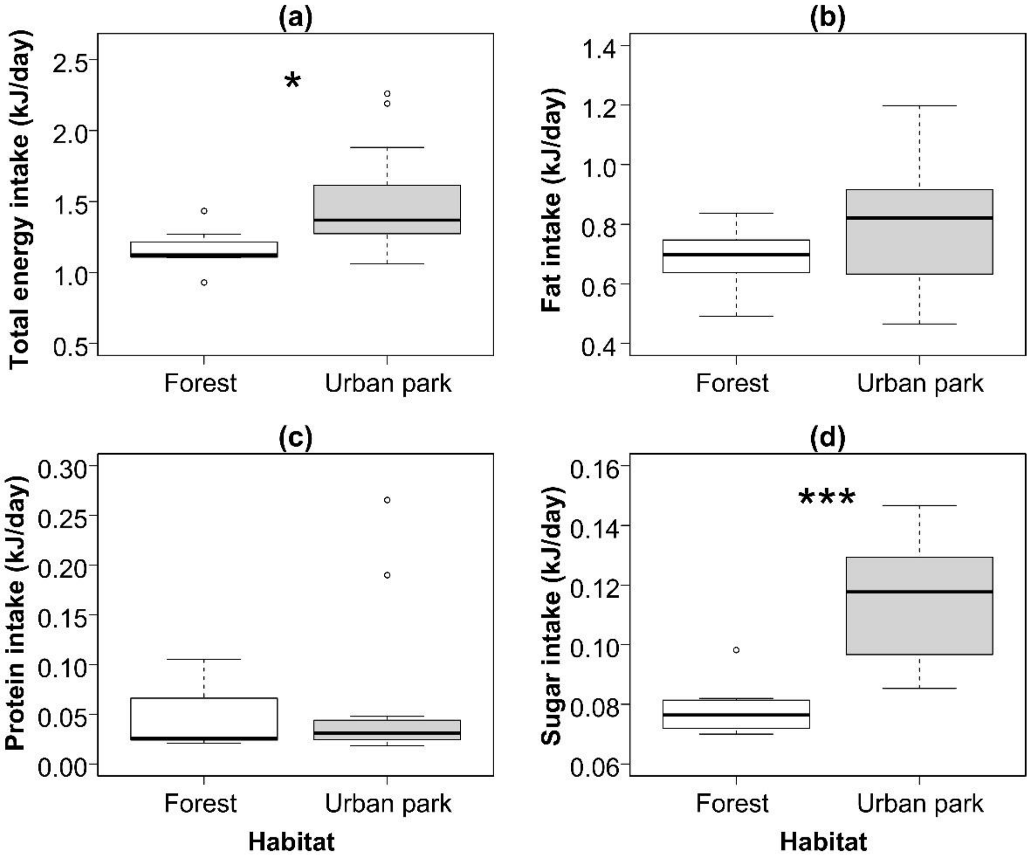

Figure 2 Box and whisker plots showing total daily energy intake in kJ per g body mass (a) as well as kJ intake from fat (b), protein (c) and sugar (d) for forest (open boxes, n = 7) and urban individuals (grey boxes, n = 13); note the differences in scales; each box represents 50% of the sample data around the median (indicated by the black bar); the whiskers show the 5% and 95% percentiles for each sample with outliers shown by circles; asterisks indicate significant differences between the groups (*$P \leq 0.05$, ***$P \leq 0.001$)

With regard to macronutrient intake per individual, all tested squirrels prioritised fat over protein intake, with fat accounting on average for 55.6% of their total kJ consumption (forest individuals 58.8%, urban individuals 52.4%; Fig. 3a and b). Interestingly, concerning sugar versus protein intake, we gained ambiguous results with some forest and urban individuals ingesting higher amounts of protein (Fig. 3c).

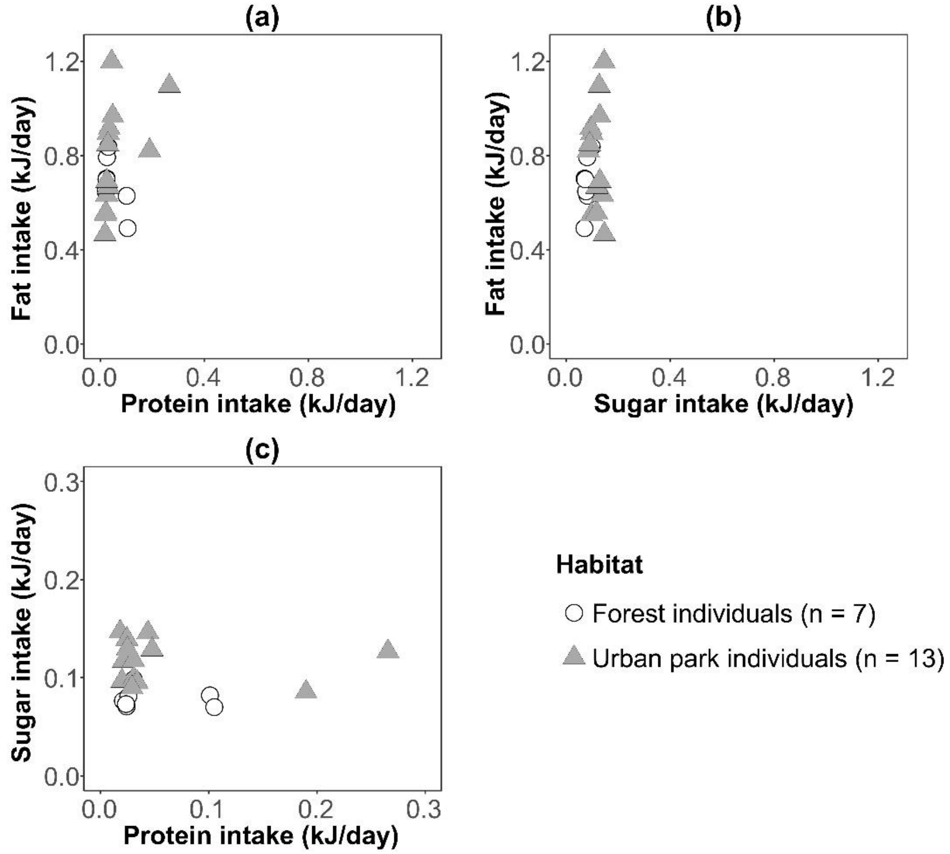

Figure 3 Scatterplots of mean daily energy intake in kJ per individual derived from fat versus protein (a), fat versus sugar (b) and sugar versus protein (c); note differences in scale; forest individuals are shown as open circles, urban individuals as grey triangles

There were no significant differences between urban and forest individuals (NMDS, stress = 0.08, ANOSIM R: −0.06, Significance: 0.69) when testing for dissimilarities in total diet composition (differential intake of the eight different food items). Possibly the results are skewed, because of the overlap in hazelnut consumption (highest energy intake from hazelnuts in both groups, Supplementary Fig. S2). However, the clustering in Fig. 4 clearly suggests differences between urban and forest squirrels, with urban squirrels being more dissimilar in their food choice, whereas the forest individuals are more uniform in their food preferences.

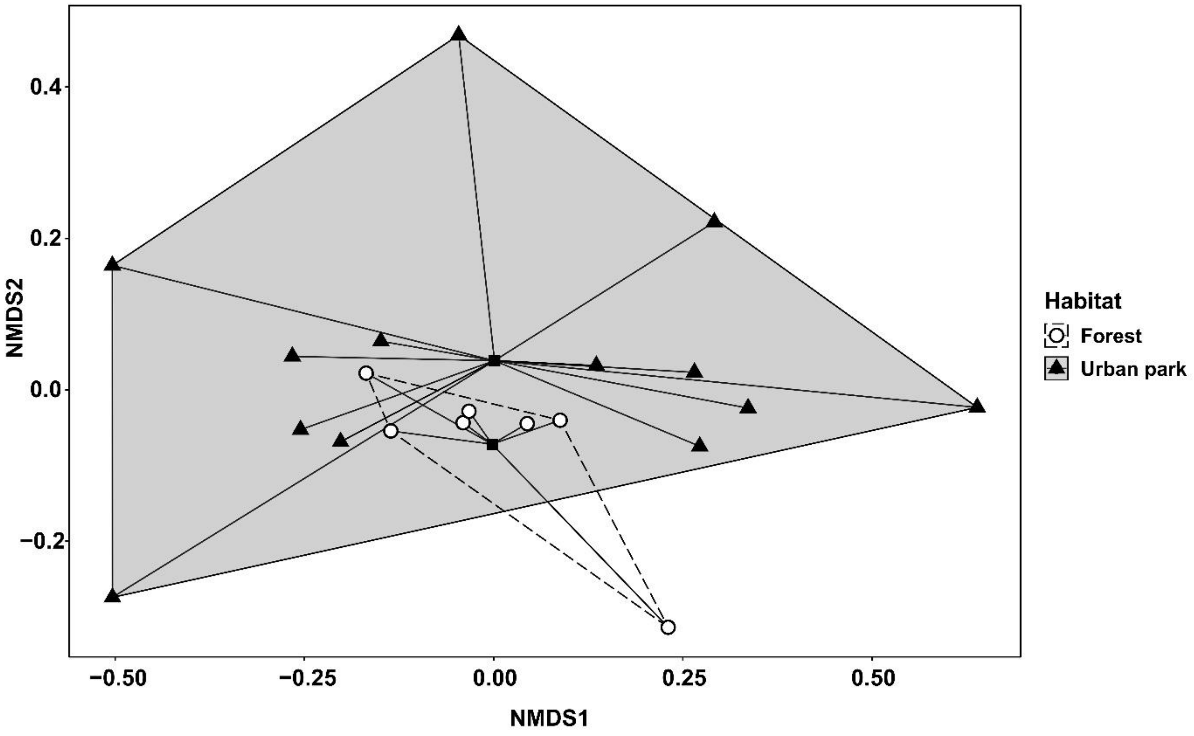

Figure 4 NMDS plot showing two dimensions of the diet diversity of forest (open circles, n = 7) versus urban squirrels (black triangles, n = 13) with regard to all eight food items used in the cafeteria experiment; both groups are marked with polygons (forest: dashed line, white filling, urban: solid line, grey filling), each sample is connected to its group centroid (black squares)

When comparing 'natural' with the 'urban' food items, there was no difference in the energy amount consumed from the four 'natural' food items with a mean of 1.12 kJ in forest and 1.21 kJ in urban squirrels (Welch-test, $t = -0.77$, $df = 16.84$, $P > 0.05$). The difference in kJ intake from the four 'urban' food items though was highly significant. Urban individuals gained an average of more than six times as many kJ from 'urban' food, accounting for 20% of their daily consumption, whereas forest squirrels got only 4% of their daily kJ intake through the 'urban' items (Wilcoxon rank sum test, $W = 6$, $P \leq 0.001$; Fig. 5, Supplementary Fig. S2 and Supplementary Table S2 for details per food item).

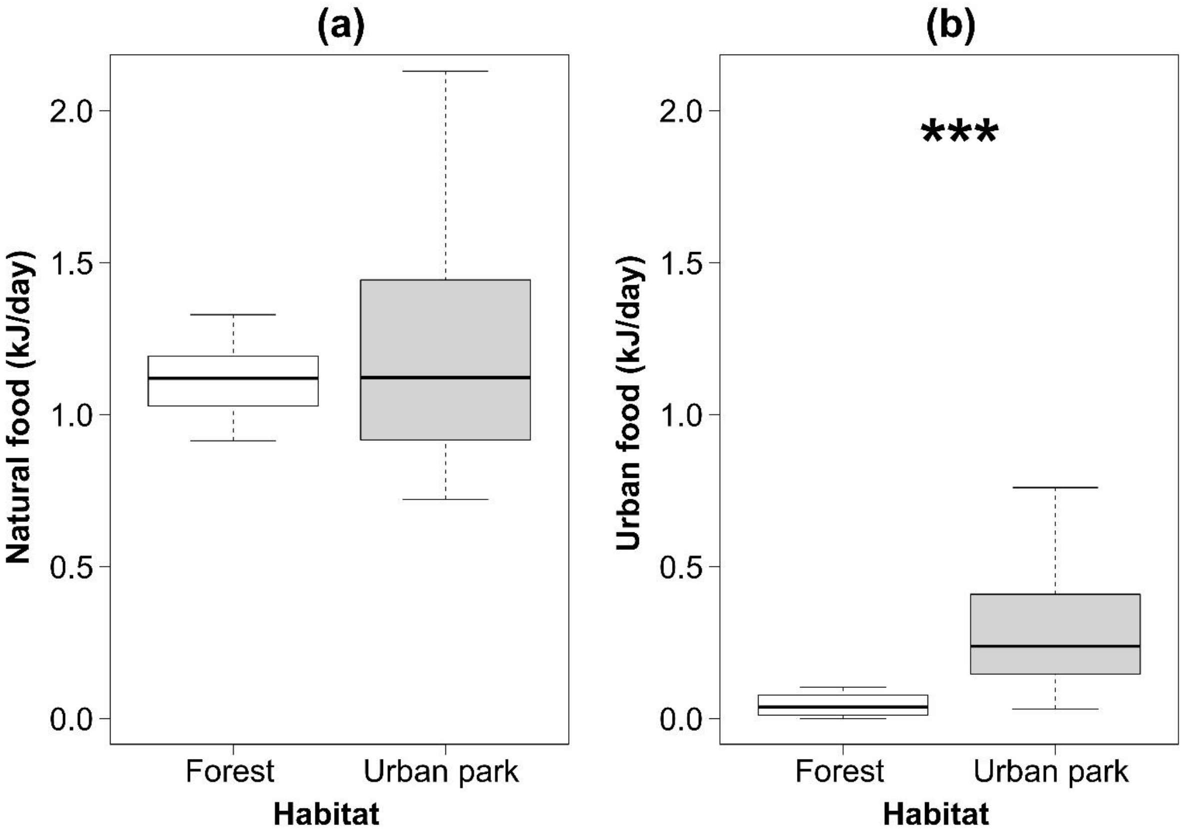

Figure 5 Box and whisker plots showing daily energy intake from 'natural' food in kJ per g of body mass on the left (a) and daily energy intake from 'urban' food items in kJ per day and g of body mass on the right (b) with forest individuals represented by open boxes (n = 7) and urban individuals by grey boxes (n = 13); each box represents 50% of the sample data around the median (indicated by the black bar); the whiskers show the 5% and 95% percentiles for each sample; asterisks indicate significant differences between the groups (***$P \leq 0.001$)

Discussion

We investigated how provenance of male Eurasian squirrels, adapted to very distinct habitat types, influences food choice behaviour when food is available *ad libitum*. More specifically, we studied the squirrels' body mass and condition as well as their energy intake and diet composition with regard to macronutrients when individuals from a highly urban park versus a natural forest were free to choose. We found significant differences in body mass, energy intake, consumption of non-natural food items and sugar intake between urban and forest squirrels. The lower body mass and condition of urban squirrels in our study contradict our assumptions and a number of studies that found comparable or even higher body mass or condition in urban birds and mammals including squirrels (Auman, Meathrel, and Richardson 2008; McCleery 2010; Meillère et al. 2017; Łopucki et al. 2019; Shimamoto et al. 2020). This is usually assumed to be strongly influenced by higher availability of human-provided resources throughout the year, buffering seasonal scarcities (Auman, Meathrel, and Richardson 2008; McCleery 2010). With squirrels, the absence of high-energy food sources such as seeds and nuts in spring usually leads to weight reductions in populations from natural habitats (Wauters and Dhondt 1989a). In urban habitats, this effect could be compensated by supplemental feeding (Reher et al. 2016; Turner et al. 2017). Nonetheless, supplemental feeding of wild animals, in general, is very controversially discussed and studies showed varying responses of different species to human-provided resources (Shuttleworth 2000; Auman, Meathrel, and Richardson 2008; Robb et al. 2008; Sorensen, van Beest, and Brook 2014; Starkey and del Barco-Trillo 2019). The topic is particularly relevant for cities and their core areas where species encounter the maximum expression of urbanisation and highest amounts of supplemental food sources (Grimm et al. 2008; Bateman and Fleming 2012). Human-provided food has the potential to familiarise wildlife with inappropriate foods (i.e. of poor quality or digestibility). Thus, despite the extensive supplemental feeding that we observed in the urban habitat, our findings might reflect a deficit in food quality from the perspective of the animal in the park, which might be caused by a lack of adaption of the digestive system to non-natural food. Another aspect is that high population densities and contact rates can, e.g. lead to higher stress levels or higher transfer of diseases and parasites (Bradley and Altizer 2007; McCleery 2010; Isaksson and Hahs 2015; Haigh et al. 2017) and therefore could negatively influence body mass. During park trapping, we found a very high population density (calculated as an index of minimum number alive, Wist et al., unpublished data) and observed numerous interactions among squirrels, but also with corvids and other bird species like blackbirds, city pigeons or great spotted woodpeckers. Those encounters were particularly common at feeding sites, indicating strong intra- and inter-specific competition for resources (Wist et al., pers. obs.). We cannot fully exclude that we might have trapped individuals from outside the park for this experiment. However, we strongly assume that those lived in close neighbourhood and experienced quite similar habitat conditions. Our results would be in line with some studies on urban mammals and birds that live in very high population densities, which seems to negatively influence body mass (Prange, Gehrt, and Wiggers 2003; Shochat 2004; Anderies, Katti, and Shochat 2007). Though, recent studies demonstrate that the interplay among densities, stress, parasites and body mass/condition in squirrels can be ambiguous and dependent on further factors (Romeo et al. 2013, Santicchia et al. 2022, Tranquillo et al. 2022). However, as body condition is fundamental for reproductive success in red squirrels (Wauters and Dhondt 1989b; Wauters et al. 2007), the differences found in our study could be an indication of negative effects in the urban parks.

When compared with the forest individuals, urban squirrels consumed significantly more kJ per g of body weight and day (+30%) and, contrary to forest individuals, significantly gained weight from the start to the end of the experiment (on average 3%). Mean daily consumption of urban squirrels in total was 527 ± 149 kJ per day [411 ± 57 kJ in forest individuals] and thus exceeded literature values from field studies, ranging from 340 to 420 kJ (Gurnell 1987; Wauters, Swinnen, and Dhondt 1992; Bosch and Lurz 2011). We are aware of that gross energy intake does not equal digestible and metabolisable energy (Ortmann et al. 2006). However, under the cafeteria experiment urban individuals were obviously able to consume more kJ and gain weight significantly, indicating some critical limitations in the urban habitat as mentioned above. Squirrels are seed specialists, i.e. their main food has a very high digestibility (up to 96%, Gurnell 1987). A diet high in other plant materials than seeds needs longer intestines for efficient digestion than usually found in squirrels (Gurnell 1987). Feeding on high amounts of other food items when seed availability is low might thus lead to less assimilation even when the intake is high, as the digestive system is not adapted to process it efficiently. On the other hand, urban squirrels are more habituated to interactions with humans and general disturbances (Uchida et al. 2016, 2019, Krauze-Gryz et al. 2021). Thus, forest individuals might have been more stressed by the housing situation and not taking full advantage of the food presented. Urban squirrels calmed down earlier when released into the aviaries, were more often observed during food change and in total spent more time outside their nest boxes (Wist et al. unpublished data). Accordingly, this might have increased both, their total energetic demands and foraging amounts. Additionally, high competition for food in their urban habitat might have trained urban individuals to maximise their food intake whenever they can, leading to a higher exploitation of the offered cafeteria compared with the forest squirrels.

In line with our hypotheses, we found no differences in fat or protein consumption between the study groups. Furthermore, both groups have prioritised fat in their diets. This met our expectations for a small homeothermic mammal favouring seeds and nuts, a food source that allows for comparably high metabolic costs without torpor use (McNab 1986; Dausmann et al. 2013). The percentage of protein intake on the other hand (urban: 3.7%, forest: 4.4%) was below existing literature values for minimum maintenance (10–12% crude protein; Pulliainen 1984). However, this value was obtained under very different conditions, in a study conducted in the winter of Lapland, i.e. at low ambient temperatures and with dense snow cover. It is likely that nutrient and energetic demands fluctuate on a seasonal basis and with latitude. Squirrels from both habitats fed mainly on hazelnuts, one of the primary food items in their natural diet (Krauze-Gryz and Gryz 2015). Consumption of spruce seeds was higher in urban squirrels, but in total, both groups ate only very few spruce seeds despite spruce cones being another one of their major food sources in the wild (Moller 1983; Gurnell 1987; Di Pierro et al. 2011, Rubino et al. 2012). However, squirrels have to invest more time for stripping cones than for cracking a hazelnut whereas the latter provides a higher amount of energy. It is known that squirrels compose their diet according to seasonal availability and possible energy intake rate versus processing time (Moller 1983; Gurnell 1987). As mentioned above, the digestibility of seeds in general is reported to be high for squirrels and further enhanced by a high-fat content (Gurnell 1987 and references therein), but, food quality is not only a matter of high crude fat or energy content. Interestingly, fat balls were consumed very rarely in both groups, despite several own and reported observations of squirrels feeding on fat balls in the park and surrounding areas. The low consumption in our study indicates that fat balls are not chosen preferentially, but rather used as a supplement when other food is limited. Peanut intake was also low in both of our study groups although urban squirrels seemed to be more open to peanut consumption. The chemical composition of peanuts can have detrimental effects, e.g. on amino acid absorption, and field studies showed that squirrels, even if provided *ad libitum*, restrict their peanut consumption (Shuttleworth 2000) and favour other nut types (Kostrzewa and Krauze-Gryz 2020). Quality of food always has to be seen in relation to the adaption of the digestive system of the investigated animal species.

Seen from a general perspective, even if the high amount of hazelnut intake in both study groups might have masked a statistical difference, our hypothesis that urban squirrels would show a wider dietary niche was confirmed. Urban squirrels were more willing to incorporate higher proportions of non-natural food items into their diet than forest squirrels. This is worrying as feeding on an unnatural and possibly poor quality or imbalanced diet can lead to possibly adverse effects on multiple traits like physiology, health status and reproductive success (Simpson and Raubenheimer 2011; Isaksson and Hahs 2015; Birnie-Gauvin et al. 2016; Pollock et al. 2017). Urban coyotes, e.g. were shown to feed on a more diverse diet than their rural counterparts by including higher amounts of anthropogenic food, but this was linked to lower protein intake and higher rate of diseases (Murray et al. 2015). Furthermore, despite a higher rate of food provisioning, urban blue-tits (*Cyanistes caeruleus*) fed their offspring with much fewer caterpillars than forest blue-tits did, indicating a dietary shift to urban food items like human-provided bird food, resulting in a reduced reproductive success (Pollock et al. 2017). A study on urban and rural house sparrows found higher plasma cholesterol and blood urea nitrogen in urban individuals, which reflected higher protein and fat intake (Gavett and Wakeley 1986). Additionally, there is indication for a negative effect on physiological important fatty acid composition in urban bird species through feeding on supplemental food sources, especially on bird food like sunflower seeds, which are often contained in fat balls (Andersson et al. 2015). Interestingly, an urban diet can also alter or reduce gut microbiota (Gillman, McKenney, and Lafferty 2022; Dillard et al. 2022). This reduction can be accompanied by a lower ability to gain weight, which has been shown very recently for urban sparrows (Teyssier et al. 2020). In our study, we found highest differences in consumption of single food items for butter biscuits with urban individuals consuming about seven times more kJ from butter biscuits than the forest group. In line with this, total sugar intake was significantly higher in the urban group. This corresponds to findings of other studies on sugar intake of species having high access to human-provided food. Urban grey squirrels (*S. carolinensis*) as well as raccoons (*Procyon lotor*), e.g. were shown to have higher blood glucose levels (Schulte-Hostedde et al. 2018; Schmidt et al. 2019). The fact that urban squirrels consumed so many butter biscuits while forest squirrels have not, might reflect a habituation to the taste of sugar by the urban squirrels, contradicting our assumption that all individuals should incorporate similar proportions of nutrients into their diet, independently of their dietary mix. This is alarming as high sugar consumption can lead to detrimental side effects. For example, sugar is linked to several diseases and dysfunctions (Johnson et al. 2007, 2013; Malik et al. 2010) as well as addiction properties through alterations in behaviour and neurochemistry, i.e. the brain reward system (Avena, Rada, and Hoebel 2008; Kendig 2014). It was, e.g. found to negatively influence behaviour and cognition in rodents by impairing memory and spatial learning (Kendig 2014). This might be particularly problematic for a hoarding species like squirrels, which relies on a successful recovery of cached food items. Although it is not known above what threshold sugar can cause detrimental effects, there is evidence of impairments starting from ~8% of the daily intake (Kendig 2014), which is less than what we found for the urban squirrels. We acknowledge the small sample size in our study and the possibility of urban individuals could have adjusted better to the housing situation, making higher use of the full cafeteria. However, prior data inspection showed that urban squirrels consumed cookies from the very beginning of the housing period whereas forest individuals rather refused them all through the experiment. We are thus convinced that our results are highly relevant in the background of intense supplemental feeding in urban settings and associated changes in feeding behaviour.

We recommend that local authorities should consider results from studies like ours as a basis for informing citizens about unhealthy food items and possible consequences for wildlife. People could be guided to provide natural foods, such as whole hazelnuts or even conifer cones collected elsewhere. Additionally, access to waste should be impeded. This could be done by, e.g. using bins that are not accessible to animals or even fines for littering since we often observed squirrels and birds feeding on waste items in the park.

Conclusion

The results from our cafeteria experiment are worrying and might reflect a lack of natural food alternatives for urban squirrels. Urban squirrels in our study were more willing to feed on non-natural food items and sugar. Thus, our results indicate a wider dietary range within urban individuals, whereas dietary flexibility within the forest group seems to be smaller. Plasticity in feeding behaviour and a wider dietary niche can be crucial assets for being successful under urban conditions where natural food sources might be less available and species seem to benefit from the plastic or adaptive ability to shift their food choice. On the other hand, food generalists like squirrels might have disadvantages on a long-term basis as they are at higher risk to incorporate inappropriate food items into their diet than specialists, which seems to be particularly true for urban habitats. Our results give valuable insight in potential nutritional mismatches and negative side effects on the health of urban wildlife in general. It remains questionable, whether urban parks represent adequate refuges for wildlife like squirrels. This might heavily depend on park structures, surrounding habitat types and availability of natural resources, providing food and shelter as well as restrictions in supplemental feeding. Future studies should investigate possible consequences on further aspects such as, e.g. the gut microbiome or long-term reproductive success. Only detailed knowledge about mechanisms of urbanisation will help to conserve urban wildlife successfully and to tackle appropriate conservation actions in the face of ongoing transformation of natural landscapes into urban areas.

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Ethical approval

We followed all applicable institutional and national guidelines for the care and use of animals. The authors complied with the ARRIVE guidelines. All procedures and animal handling were according to the German animal protection law and approved and authorized by the authorities of Hamburg and Schleswig-Holstein ("Hamburger Behörde für Gesundheit und Verbraucherschutz", permit no. 87/16, 17 November 2016 and extension of the permit to SchleswigHolstein by the "Ministerium für Energiewende, Landwirtschaft, Umwelt und ländliche Räume des Landes Schleswig-Holstein" (permit of 28 December 2016).

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Supplementary material

for:

Sugar addicted in the city: Impact of urbanisation on food choice and diet composition of the Eurasian red squirrel (*Sciurus vulgaris*)

Bianca Wist, Caroline Stolter and Kathrin Dausmann

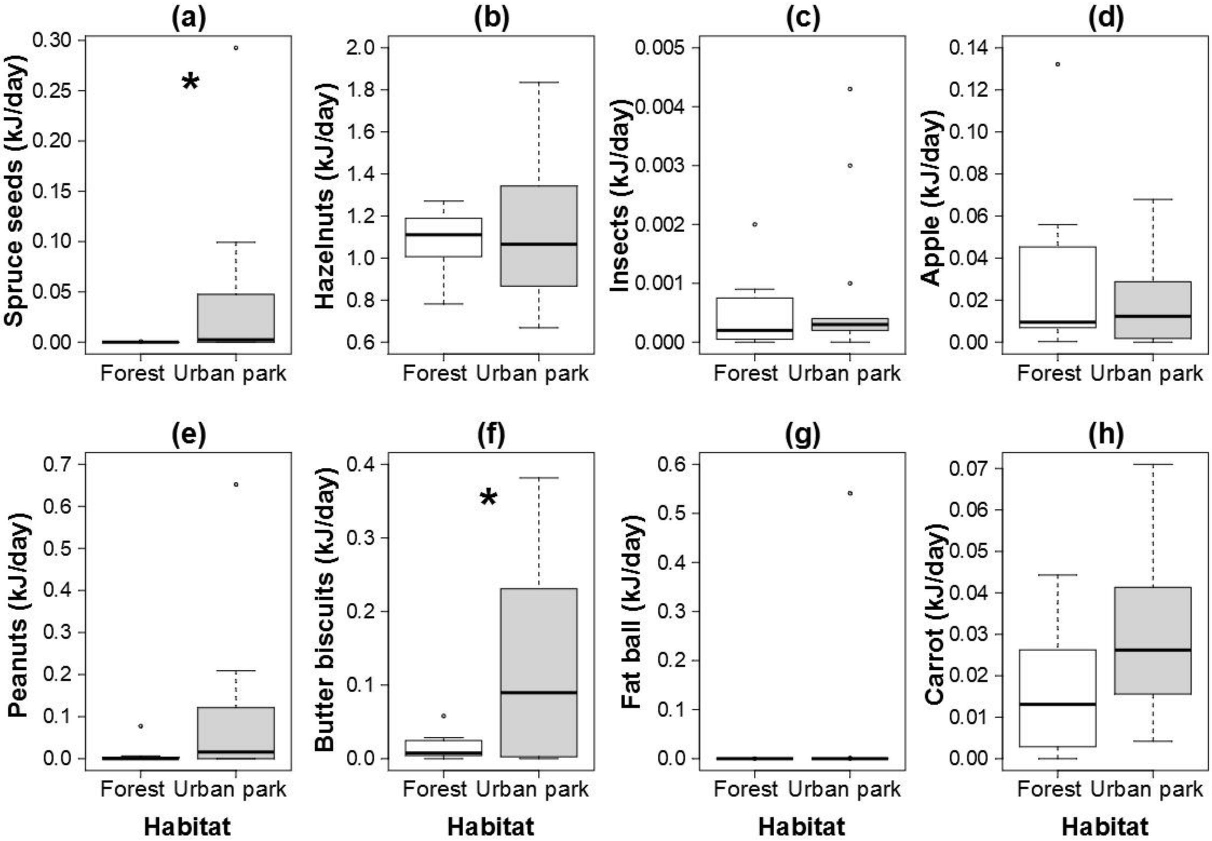
Supplementary figure 1 Example of a food bar (sheltered from rain) with eight different foods used within this experiment (left picture) and leftovers collected from one of the cages after 24 hours (right picture).

Supplementary table 1 Initial and final body mass / BCI per study group as well as total and daily change in body mass; values are given as mean ± SD and supplemented by t-test results

Habitat	n	Initial body mass (g)	Final body mass (g)	∆ Body mass (g)	Daily mass change (g)	Initial BCI	Final BCI
Forest	7	371.4 ± 27.0	376.3 ± 19.5	4.8 ± 17.6	0.3 ± 1.2	17.2 ± 0.8	17.4 ± 1.2
Urban Park	13	334.2 ± 19.0	343.8 ± 21.1	9.5 ± 13.6	0.7 ± 0.9	15.6 ± 0.9	16.1 ± 0.9
t		3.6	3.37	-0.66	-0.7	3.85	2.99
df		18	18	18	18	18	18
p-value		< 0.005	< 0.005	> 0.05	> 0.05	< 0.005	< 0.01

Results on single food items

All individuals fed mainly on hazelnuts, ranging from 56 to 99 % of the daily kJ intake (mean 82 %) with forest squirrels ranging from 84 to 99 % and urban squirrels from 56 to 92 % (see Supplementary fig. 1 and tab. 2 for details of daily consumption per food item). The differences in energy amount from hazelnut or peanut consumed between the groups were not significant (hazelnuts: t-test, t = - 0.49, df = 18, p > 0.05, peanuts: Wilcoxon rank sum test, W = 25.5, p > 0.05). Urban individuals had a significant higher kJ intake from butter biscuits (urban squirrels: 10.2 % of the daily kJ intake, forest squirrels 1.5 % of the daily kJ intake, Welch-test based on kJ intake: t= -2.98, df= 13.08, p < 0.05) as well as from spruce seeds (2.5 % of the daily kJ intake in urban squirrels, forest squirrels < 0.01 % of the daily kJ intake, Wilcoxon rank sum test: W = 17, p < 0.05, Fig. 5), but the latter was consumed to a very low degree by both groups. Insects and fat balls were also eaten only rarely and in very minor quantities (insects < 0.1 % in both groups, fat ball < 0.01 % in forest and 1.9 % of the daily kJ intake in urban squirrels, Fig. 5). When considering weight, apples and carrots made up for a substantial percentage of the total daily intake (forest squirrels: apples 21.6 %, carrots 14.9 %; urban squirrels: apples 10.3 %, carrots 21.5 %), but owing to their low energy content, their proportion of daily kJ intake was also in the low single digits (forest squirrels: apples 3.3 %, carrots 1.5 %; urban squirrels: apples 1.2 %, carrots 2.0 %) and not significantly different.



Supplementary figure 2 Box and whisker plots showing daily intake [kJ per g body mass] per food item and habitat (N = 20), open boxplots showing forest individuals (n=7), grey boxplots urban individuals (n=13); we found no difference in the energy amount consumed from hazelnuts (b), insects (c), apples (d), peanuts (e), fat balls (g) and carrot (h); spruce seed (a) and butter biscuit consumption (f) was significantly higher in urban individuals (t-tests with p ≤ 0.05); note the differences in scales; each box represents 50 % of the sample data around the median (indicated by the black bar); the whiskers show the 5 % and 95 % percentiles for each sample; outliers are represented by circles and significant differences between the groups by asterisks (*p ≤ 0.05)

Supplementary table 2 Daily consumption (g or kJ per g of body mass) per study group (mean ± SD) and percentage of fat, protein, sugar and the different food items; statistical test results refer to the total daily consumption in kJ per g of body mass

Habitat / test	**Forest**	**Urban park**	**t-test**	**Welch-test**	**Wilcoxon rank sum test**
n	7	13			
g / day	0.064 ± 0.020	0.078 ± 0.027	t = - 1.25, df = 18, p > 0.05	-	-
kJ / day	1.162 ± 0.156	1.505 ± 0.390	-	t = - 2.78, df = 17.17, p < 0.05	-
thereof:					
„natural“ food	1.115 ± 0.144	1.207 ± 0.380	-	t = - 0.77, df = 16.84, p > 0.05	-
„urban“ food	0.046 ± 0.043	0.298 ± 0.233	-	-	W = 6, p < 0.001
% of the daily kJ consumption:					
fat	58.8	52.4	t = - 1.19, df = 18, p > 0.05	-	-
protein	4.4	3.7	-	-	W = 42.5, p > 0.05
sugar	6.8	8.2	t = - 4.40, df = 18, p < 0.001	-	-
spruce	< 0.01	2.5	-	-	W = 17, p < 0.05
hazelnuts	92.7	76.3	t = - 0.49, df = 18, p > 0.05	-	-
peanuts	0.9	5.8	-	-	W = 25.5, p > 0.05
butter biscuits	1.5	10.2	-	t= -2.98, df= 13.08, p < 0.05	-
insects	0.04	0.05	-	-	W = 41, p > 0.05

fat ball	< 0.01	1.9	-	-	W = 40, p > 0.05
apple	3.3	1.2	-	-	W = 54, p > 0.05
carrot	1.5	2.0	t = - 1.55, df = 18, p > 0.05	-	-
% of the daily consumption in g:					
spruce	0.01	2.8			
hazelnuts	61.5	49.1			
peanuts	0.6	3.7			
butter biscuits	1.4	11.0			
insects	0.04	0.04			
fat ball	< 0.01	1.7			
apple	21.6	10.3			
carrot	14.9	21.5			

Authors' contributions

I hereby confirm that Bianca Wist (BW), Caroline Stolter (CS) and Kathrin H. Dausmann (KHD) conceived and designed the study. BW conducted the cage experiment tand analysed the data with support of Caroline Stolter (CS). BW wrote the manuscript with input from CS and KHD. All authors revised the manuscript and approved the final version of it.

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Prof. Dr. Kathrin Dausmann

Chapter 4

City comfort: weaker metabolic response to changes in ambient temperature in urban red squirrels

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Abstract

The ecophysiological responses of species to urbanisation reveal important information regarding the processes of successful urban colonization and biodiversity patterns in urban landscapes. Investigating these responses will also help uncover whether synurban species are indeed urban 'winners'. Yet we still lack basic knowledge about the physiological costs and overall energy budgets of most species living in urban habitats, especially for mammals. Within this context, we compared the energetic demands of Eurasian red squirrels (*Sciurus vulgaris*) from the core of an urban environment with those from a nearby forest. We measured oxygen consumption as a proxy for resting metabolic rate (RMR) of 20 wild individuals (13 urban, 7 forest), at naturally varying ambient temperature (T_a) in an outdoor-enclosure experiment. We found that the variation in RMR was best explained by the interaction between T_a and habitat, with a significant difference between populations. Urban squirrels showed a shallower response of metabolic rate to decreasing T_a than woodland squirrels. We suggest that this is likely a consequence of urban heat island effects, as well as widespread supplemental food abundance. Our results indicate energy savings for urban squirrels at cooler temperatures, yet with possible increased costs at higher temperatures compared to their woodland conspecifics. Thus, the changed patterns of metabolic regulation in urban individuals might not necessarily represent an overall advantage for urban squirrels, especially in view of increasing temperatures globally.

Keywords

Sciurus vulgaris, Eurasian red squirrels, RMR, metabolism, ecophysiology, urbanisation

Introduction

Animals have to carefully balance their energy budgets in response to environmental conditions, such as fluctuations in ambient temperature (T_a) or food availability [1]. The energetic demands of an animal in a certain environment are reflected by the metabolic rate (MR), which determines resource requirements and limits their allocation to body components, therefore directly influencing fitness [2, 3]. Studying drivers of variation in MR is fundamental for understanding ecological patterns [2]. In general, MR shows high variability and phenotypic plasticity, both among and within species. Even among populations from different locations, which is assumed to reflect an adaptation to the particular habitat conditions [4-6].

Urbanisation is a major driver of environmental change, and alongside the rapid loss of natural habitats poses a growing threat to biodiversity [7, 8]. Urban areas have more than doubled from 1992 to 2015 with an even larger increase forecast for 2030, where 60 % of the world’s population is projected to live in urban settlements [7, 9]. Urban wildlife experiences pronounced differences in environmental conditions compared to geographically close rural populations, even though they are located at similar altitudes and latitudes [10-12]. Cities in particular are challenging habitats for wildlife due to immense human induced alterations and disturbances, such as noise, pollution or impervious surfaces [13, 14]. However, some species, classified as synurban, seem to thrive in urban conditions displaying higher densities than in their natural habitats [15, 16]. Urban populations often exhibit changes in biology and ecology [8, 11, 17]. For example, they show shifted and / or extended breeding seasons [16, 18], differences in body mass or condition [18-20], and altered foraging and / or overall activity patterns [16, 18, 20, 21]. It is likely that these changes are associated with altered physiological processes.

However, despite the central role of physiology, our understanding of its contribution to the adaptability of wildlife to urban conditions is still limited. Urban heat islands, characterized by higher T_a, higher precipitation and altered wind velocity [22-24] are assumed to alter or negatively affect the metabolism of urban wildlife [11, 12, 25, 26]. Access to human-derived foods in urban habitats offer a more stable year-round food availability compared to rural or undisturbed natural environments [8]. As a consequence, animals in urban environments often differ in their seasonal fluctuations in body mass from their rural counterparts [20]. Furthermore, a diet that is supplemented by human foods and / or waste results in altered nutritional proportions, which can have knock-on effects on physiological and health-relevant traits [27-30]. Another factor that can influence MR are shifts in behaviour [1]. For example, exploratory behaviour is often elevated in urban settings [18]. Indeed, metabolism showed phenotypic correlation among populations of common voles (*Microtus arvalis*) [5].

Here, we explored the role of physiological plasticity in enabling Eurasian red squirrels (*Sciurus vulgaris*, henceforth “squirrels”) to cope with urbanisation. Squirrels naturally occur in coniferous or mixed and deciduous forests, but are also highly abundant in urban habitat patches [16, 31, 32]. Their ability to successfully colonize urban environments makes them a valuable study system to disentangle drivers of synurbanisation with regard to metabolism. Despite being a small endotherm, this species does not use physiological energy saving strategies like torpor and remains homeothermic throughout the year [33, 34]. This is remarkable, since small endothermic mammals are under strong pressure to maintain a careful balance between the costs of elevated body temperature and energy intake due to unfavourable surface area to volume ratios [35]. Previous work has demonstrated that squirrels from semi-urban environments show little seasonal variation in MR [34] and instead, appear to rely mainly on behavioural adjustments like reduced activity during the winter [33, 36-38]. As a food generalist and opportunist with a diverse diet, squirrels seem to benefit from urban food availability [32, 39]. They mainly feed on seeds and nuts, but use a large variety of other food items when these are unavailable [37, 40]. In urban areas, squirrels also feed on food sources provided by humans and exploit left-overs [39, 41, 42]. Akin with findings from other urban species [28, 43], the diet composition of urban squirrels can differ from their rural counterparts and they may feed on nutrient poor food items [44]. Furthermore, urban populations can be exposed to higher levels of intra- and interspecific contact rates, parasite transfer, stress, and exposure to environmental pollutants that might impact metabolism [11, 25, 45].

To gain a better understanding of the physiological mechanisms driving synurbanisation, we compared resting metabolic rates (RMR) between wild-caught squirrels from the core area of a major city and squirrels inhabiting a nearby forest. We used a common garden approach, housing both populations in semi-natural outdoor enclosures, measuring MR with nest boxes as metabolic chambers to enable exposure to the same natural climatic fluctuations and to minimize experimental disturbances. We explored mass-specific RMR with regard to ambient temperature, habitat of origin and inter-individual variability. Additionally, to explore the influence of diel cycle, we compared RMR of squirrels during their active (day-time) and non-active (night-time) phases. The results of our study will advance the knowledge on physiological plasticity in the Eurasian red squirrel. Moreover, our work on urban ecophysiology contributes to the understanding of physiological demands and possible constraints or benefits for wildlife in highly urbanised habitats in general.

Results

We captured 20 individuals (13 urban, 7 forest). Overall, we obtained a total of 57 measurement days (range of 1 – 5 per individual; $n_{forest} = 22$; $n_{urban} = 35$, Supplementary Table S2). Respirometry data and camera trap pictures showed that all squirrels left their nest box close to civil twilight to forage. The time spent outside the nesting boxes did not differ between urban and forest squirrels (Tab. 1, t-test: t = -1.48, df = 18, P = 0.157). Forest squirrels were heavier than urban squirrels and had higher body condition indices (body mass (g) / nose-anus length (cm)), at the beginning (t-test, body mass: t = 3.60, df = 18, P = 0.002; BCI: t = 3.85, df = 18, P = 0.001) as well as after the experimental period (t-test, body mass: t = 3.37, df = 18, P = 0.003; BCI: t = 2.99, df = 18, P = 0.008). However, urban squirrels showed significant weight gain over the course of the experiment (paired t-test, t = -2.53, df = 12, P = 0.027), whereas woodland individuals did not (see Tab. 1 and [44] for further details). Urban individuals consumed on average 0.44 ± 0.13 ml O_2 h^{-1} g^{-1} (0.33 – 0.52 ml O_2 h^{-1} g^{-1}) whereas RMR of forest squirrels was 0.48 ± 0.18 ml O_2 h^{-1} g^{-1} (0.24 – 0.64 ml O_2 h^{-1} g^{-1}) over the measured temperature range of 8 – 29° C. See Tab. 1 for a full overview of total and mass-dependent RMR results per group.

Table 1 Mean values (± SD) for body mass (before/after the housing period) and time spent outside the nesting box as well as mean values (± SD) and ranges (in square brackets) for mass-dependent and total RMR of forest (N_{forest} = 7, n_{forest} = 22) versus urban squirrels (N_{urban} = 13, n_{urban} = 35). Given are daily RMR averages (in ml O_2 and in kJ), as well as diurnal and nocturnal values.

	Forest	**Urban park**
Body mass (g), before / after the experiment	371.4 ± 27.0 / 376.3 ± 19.5	334.2 ± 19.0 / 343.8 ± 21.1
Time spent outside the nesting box (h/measurement day)	3.1 ± 2.5	4.7 ± 2.1
RMR (ml O_2 h^{-1} g^{-1})	0.48 (± 0.18) [0.24 - 0.64]	0.44 (± 0.13) [0.33 - 0.52]
RMR (ml O_2 h^{-1})	173.52 (± 67.85) [84.50 – 232.09]	149.84 (± 44.40) [109.35 – 202.99]
RMR (kJ h^{-1} g^{-1})	0.0097 (± 0.0038) [0.005 – 0.013]	0.0091 (± 0.0027) [0.007 – 0.011]
RMR (kJ h^{-1})	3.54 (± 1.38) [1.72 – 4.73]	3.05 (± 0.90) [2.23 – 4.14]
Diurnal RMR (ml O_2 h^{-1} g^{-1})	0.45 (± 0.18) [0.22 - 0.63]	0.42 (± 0.13) [0.30 – 0.50]
Diurnal RMR (ml O_2 h^{-1})	164.34 (± 67.65) [79.83 – 229.36]	140.32 (± 41.72) [99.73 – 193.25]
Nocturnal RMR (ml O_2 h^{-1} g^{-1})	0.53 (± 0.17) [0.26 - 0.68]	0.49 (± 0.13) [0.37 - 0.61]
Nocturnal RMR (ml O_2 h^{-1})	194.67 (± 65.35) [95.33 – 278.72]	166.60 (± 44.05) [125.48 – 213.71]

We found statistical support for the interaction between nest box temperature (T_{nest}) and habitat (ΔAIC_c = 4.79, Cohens f^2= 0.28, Tab. 2 and 3), whereby RMR increased with decreasing T_{nest} in both groups (Fig. 1), but with a steeper slope in forest squirrels (Fig. 1). Furthermore, we found support for an effect of the phase in the diel cycle (ΔAIC_c = 5.79, Cohens f^2= 0.16, Tab. 2 and 3). An effect of sampling period on RMR was marginally supported (ΔAIC_c = 2.02, Cohens f^2= -0.27 – 0.13, Tab. 2 and 3). Average RMR was higher during the night compared to daytime estimates (Fig. 1). We found no statistical support for an influence of the phase of the housing period (first/second half) nor the interaction of habitat and sampling period (Tab. 2 and 3). RMR was highly variable across individuals (ICC 0.42, Tab. 3). The marginal R^2 or conditional R^2 respectively for the top ranked model was 0.304 / 0.597 (Tab. 3).

Table 2 The top ranked candidate linear mixed models evaluating the relationship between habitat type and covariates on RMR of Eurasian red squirrels. The models M1 to M6 sum up to a cumulative Akaike weight of 0.95 (= 95% confidence set). Parameters included in the model are indicated by "**+**". AICc= Akaike's information criterion, corrected for multiple parameters and small sample size (AICc), ΔAICc= differences in AICc; $W_i(M)$= Akaike weights per model, variate weights ($W_i(V)$) for all predictors were calculated by summing up Akaike weights of the models containing the respective predictor ; ER= evidence ratio, T_{nest}: nest box temperature; habitat: habitat of origin; D/N: time of the day (day or night); run: sampling period (1 – 4), first/second: first or second half of the housing period

Model no.	**Intercept**	**T_{nest}**	**Habitat**	**D/N**	**Run**	**First/ second**	**Habitat: run**	**Habitat: T_{nest}**	**AICc**	**Δ AICc**	**$W_i(M)$**	**ER**
M1	0.610	-0.0094	+	+	+			+	-2941.849	0.000	0.488	
M2	0.612	-0.0095	+	+	+	+		+	-2939.834	2.015	0.178	2.738
M3	0.622	-0.0095	+	+				+	-2939.112	2.736	0.124	3.928
M4	0.570	-0.0094	+	+	+		+	+	-2937.938	3.911	0.069	7.068
M5	0.622	-0.0096	+	+		+		+	-2937.060	4.789	0.045	10.964
M6	0.550	-0.0069		+	+				-2936.062	5.787	0.027	18.054
M7	0.571	-0.0094	+	+	+	+	+	+	-2936.036	5.812	0.027	18.287
$W_i(V)$		**0.932**	**0.905**	**0.932**	**0.763**	**0.223**	**0.069**	**0.905**				

Table 3 Coefficients for the predictors in the top ranked model (model no. 1, Tab. 2) complemented by standard errors (SE), confidence intervals (CI), degrees of freedom (df), standardized effect sizes (Cohen's f^2) as well as the intraclass correlation coefficient (ICC) and the marginal / conditional R^2. Bold predictors indicate statistical significance with CI`s not overlapping zero.

Predictors	**Estimates**	**SE**	**CI**	**df**	**Cohen's f^2**
(Intercept)	0.612	0.050	0.510;0.714	26	0.000
Habitat (urban park)	**-0.103**	**0.048**	**-0.202; -0.005**	**32**	**-0.326**
T_{nest}	**-0.010**	**0.001**	**-0.012; -0.007**	**1679**	**-0.320**
D/N (night)	**0.051**	**0.006**	**0.040;0.062**	**1737**	**0.157**
Run (2)	0.050	0.051	-0.058;0.159	15	0.127
Run (3)	0.075	0.052	-0.034;0.185	16	0.223
Run (4)	-0.091	0.055	-0.208;0.026	15	-0.267
first_sec (first)	-0.003	0.020	-0.046; 0.039	17	-0.011
Habitat(urban park):T_{nest}	**0.005**	**0.002**	**0.002;0.008**	**1643**	**0.279**
ICC	0.422				
Marginal R^2 / conditional R^2	0.304 / 0.597				

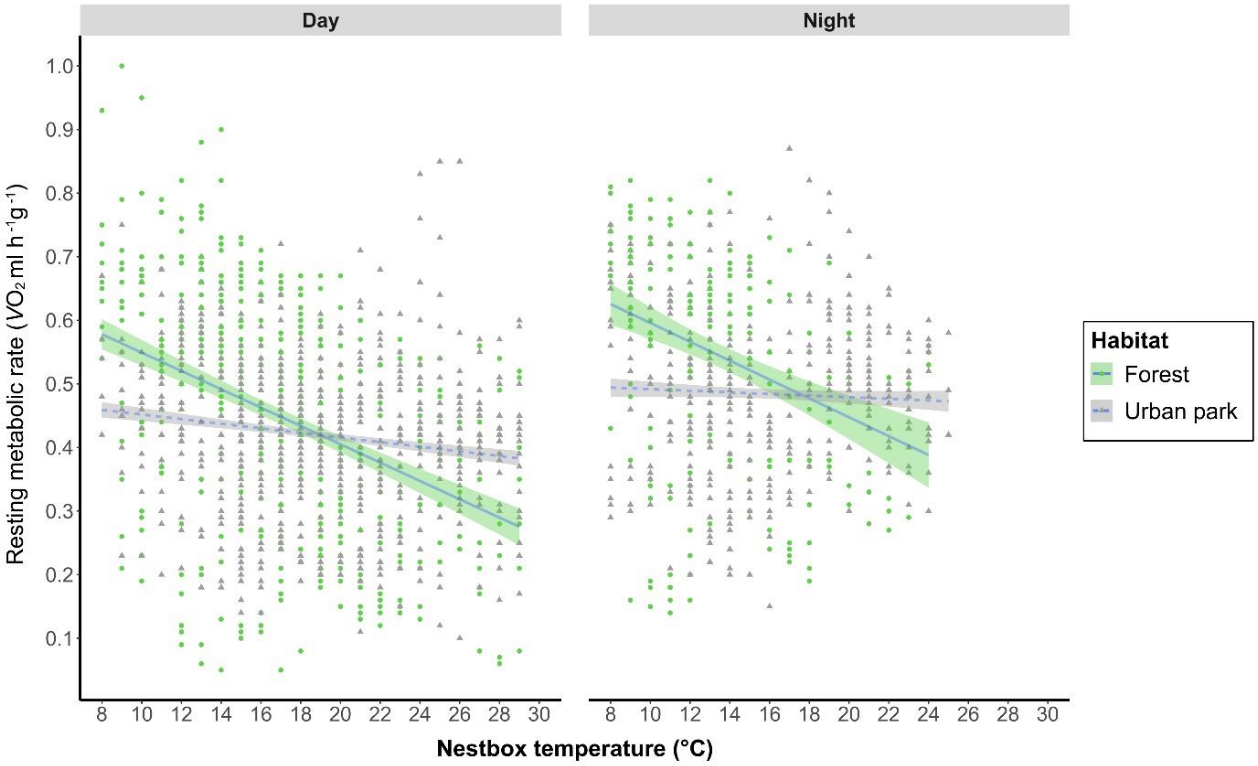

Figure 1 Fitted lines of model predictions and 95 % CI band generated from the top ranked

model as well as data points for diurnal (left panel) and nocturnal (right panel) mass specific resting metabolic rate (VO_2 ml h^{-1} g^{-1}) at measured ambient temperatures (diurnal range 8 – 29°C, nocturnal range 8 – 25°C) for forest (solid line, green band and green dots, N = 7) versus urban (dashed line, grey band and grey triangles, N = 13) squirrels (see Supplementary Figure S5 for graphs per individual).

Discussion

We used a common garden style experiment with semi-natural conditions to evaluate the energetic demands of Eurasian red squirrels from two contrasting habitats: an urban area and a nearby forest. Variations in energy expenditure were associated with T_{nest}, however, the strength of this relationship was different depending on the habitat of origin of individuals. Among endothermic species, T_a represents one of the main factors influencing metabolism [46-49] and several studies document physiological acclimatization to T_a over different seasons, latitudes or altitudes [4, 50]. Generally, urban populations experience higher T_a (on average 0.5 – 3.0°C) than their rural counterparts, especially at night [12, 23]. This is also the case for the core areas of Hamburg where temperatures are on average up to 1.1° C warmer than the surrounding areas, increasing up to 3° C in summer [51]. As expected, energy expenditure in both of our experimental groups increased as T_{nest} decreased below the thermal neutral zone [34, 52-54]. However, we did not find an overall lower RMR in urban individuals. Instead, we found that the magnitude of the effect of T_{nest}, i.e. the increase in RMR per 1°C change, was habitat-dependent.

We found that forest individuals showed a steeper slope of metabolic regulation, indicating a higher thermal sensitivity of RMR compared to urban squirrels. This supports previous findings of cold adaptation or temperature compensation in populations from colder climates, expressed by a higher RMR or a steeper relationship of RMR to T_a [55]. Conversely, the apparently lower responsiveness of the urban squirrels indicates metabolic acclimatization to the warmer, local urban microclimate. Besides warmer T_a, urban habitats display smaller diurnal urban T_a ranges [56, 57], which might have further contributed to the lower responsiveness in urban squirrel. Changes in MR driven by the thermal environment are usually closely linked to changes in thermal conductance, i.e., differences in insulation [58]. We assume that the urban squirrels differed in insulation-effective body components, e.g., in fur density or body fat compared to their forest counterparts. Seasonal changes in fur density have been documented in semi-urban squirrels [34]. Higher T_a combined with more stable resource availability leads to a buffering of seasonality in urban habitats [8] and this might lead to different fur densities in urban vs forest squirrels. Interestingly, urban and rural bird nestlings differ in their number of feathers [59] and similar insulation effective differences might occur in mammals.

Alterations in activity as found in several urban species including squirrels [18, 60-62] as well as changes in the diet could also lead to modifications in body composition, such as an increase in body fat and / or a decrease in muscle mass [63], which may help explain our findings of different conductance between the groups. In urban areas, the scarcity of natural food items is often compensated by supplemental feeding [31, 32, 39]. We observed massive year-round supplementation in the urban habitat, but none in the forest (Wist et al., unpublished data). Surprisingly, body mass and condition were lower in the urban squirrels though. Despite higher food availability, urban wildlife often experiences low-quality diets, a shift in nutrient composition, or the ingestion of toxins and pollutants [20, 28, 30, 43]. This can entail decreased digestibility of foods or lower processing efficiency, as well as other functional alterations, relevant for body mass and MR, such as changes in organ size (e.g., of the gut or liver), fat deposits or muscle mass [63-65]. Metabolic processes, such as efficiency in food digestion or in ATP generation, directly influence or even limit energy expenditure [1, 66]. Moreover, diet quality and digestive efficiency together affect MR [67]. For instance, yellow bellied marmots (*Marmota flaviventris*), exhibit higher MR when fed a diet deficient in essential fatty acids [68]. The Talas tuco-tuco (*Ctenomys talarum*) was found to have lower MR when on a low-quality diet, in combination with a lower digestibility and higher gut transit [67]. South American foxes (*Pseudalopex cupaeus*) displayed increased MR when fed a mixed diet containing rats and fruits compared to a diet of only rats [69]. We found that urban squirrels chose food items higher in sugar content and more non-natural food items [44]. In combination with high supplementation, this could also have contributed to an increased insulation-effective fat layer in urban individuals and / or a different distribution of fat deposits between urban and forest squirrels, influencing conductance [63].

Interestingly, as a result of the lower thermal sensitivity of RMR to changes in T_{nest}, urban individuals spent less energy at the colder end of the T_{nest} range, whereas this pattern was reversed at higher T_{nest} (below/above ~19°C during the day and ~ 18°C at night). These findings contradict assumptions that higher urban temperatures are linked to a general increase in MR, e.g. by pollution induced metabolic costs [11]. However, this effect was mostly found in ectotherms and eco-physiological studies on urban mammals remain scarce. Notably, striped field mice (*Apodemus agrarius*) showed a reduced oxygen-carrying capacity in urban habitats, which was assumed to be caused by higher pollutant levels [70]. As the oxygen-carrying capacity limits the scope of MR [1], this would also imply a decreased, rather than an increased MR in urban habitats. The comparably higher MR of urban squirrels at higher T_{nest} was an unexpected outcome. Endothermic mammals are not only challenged by the need for heat production at low T_a, but by heat dissipation at high T_a [52, 71, 72]. Despite possible shifts in thermal tolerance of urban wildlife [12, 73], urban mammals might be pushed towards their upper critical limits, suffering from over-heating and water loss, especially in the light of global warming. This could be further exacerbated by additional negative stressors such as urban noise or pollution [11, 74, 75]. Temperatures exceeding the above-mentioned threshold of ~18°C for at least one hour occurred on 40 % of the days in 2018 – a comparably hot year and on 32 % of the days in 2019 (Meteorological Institute, Universität Hamburg, Germany). However, urban squirrels might be less limited by heat dissipation, as they are less active and humans frequently provide year-round drinking water for birds and squirrels in surrounding gardens and on balconies (citizen survey data, Wist et al., unpublished). In addition, as urban squirrels respond less strongly to fluctuations in T_a, the more stable rate of metabolism might be advantageous at the cellular level, as the body does not have to cope with pronounced, recurrent changes in homeostasis, which could possibly lead to e.g. cell stress, as is known from torpor-arousal-cycles [76].

Independent of habitat of origin, phase in diel cycle was a relevant predictor of RMR in our model. As diurnal endotherms, squirrels usually display slightly lower body temperatures during the night [33, 77] and this is usually accompanied by a lower MR in an animal's inactive phase [4]. Surprisingly, nocturnal RMR in our study was consistently higher in both urban and forest individuals than diurnal RMR at the same T_{nest}. The drivers of this unexpected finding remain unclear. We assume that squirrels have to be able to elevate their metabolism radically as a prerequisite for quickly and suddenly climbing trees or jumping, e.g., in order to escape from predators. Diurnal periods of activity bursts might be followed by compensatory periods of extremely low RMR when resting. As we were only able to obtain MR data while the squirrels were in the nest boxes comparably low RMR values probably contributed disproportionately to the dataset although we statistically accounted for imbalances in the data. Moreover, the stress response in vertebrates seems to be highest during their inactive phase [78], which might have contributed to elevated metabolism during the night in this study.

Besides the habitat-related differences, mass-specific RMR was highly variable across individuals. This is a well-known phenomenon, likely explained by diverse extrinsic and intrinsic factors, such as developmental conditions or genotype [45, 79, 80]. Historically, squirrels from both of our study sites had to adapt to similar environmental conditions due to their close geographic location. It is also plausible that gene flow occurred between them since an urban matrix does not always represent a barrier [81-83]. Nevertheless, there was still a clear effect of habitat on the thermoregulatory response of our two study groups. We are aware of the small sample size, particularly within the forest group and acknowledge that our results should be viewed with some caution. Our study also differs from many other studies in that we used a semi-natural set-up. Natural variability in environmental factors is often deliberately removed in physiological studies to reduce the effect of confounding variables. However, we aimed to expose the squirrels to as natural conditions as possible to express natural behaviour, using freshly caught individuals and largely undisturbed nest boxes with natural fluctuations in T_a and other climatic parameters to obtain biologically relevant results applicable to the field situation [84].

Conclusions

Our study gives valuable first insight into energetic demands of urban versus forest squirrels and thereby contributes to the understanding of ecophysiological consequences of urban heat islands on mammalian wildlife. This is particularly important in the light of rapid global urbanisation and climate change. Since resting metabolism sets limitations for resource intake and allocation to fitness components, physiological plasticity can be a key trait making squirrels successful colonizers in urban environments. However, we did not find an overall lower or higher RMR in the urban population, but more stable response to T_a-fluctuations. The higher energy expenditures at higher T_a might indicate increased costs at temperatures that are expected to occur more frequently with the ongoing climate change [85]. There is an urgent need for studies exploring the interrelation of additional factors such as intrinsic processes related to diet or pollution with metabolism of urban mammals to provide a more comprehensive picture of the physiological consequences of urbanisation. Urban populations might be composed by “many losers and few winners” [86, 87] and we still do not know if synurban species thrive or rather persevere, even when occurring in high densities.

Methods

Trapping and handling

We trapped squirrels in a small park (Wohlerspark, 4.6 ha) located in the core city area of Hamburg, Germany (N53° 33' 29.646'' E9° 57' 11.459'') and in a forested site (Hahnheide, 1.450 ha) located approximately 30 km from the city centre (N53° 37' 14.146'' E10° 27' 1.667''). The park is characterized by a dense urban matrix of a city with 1.8 million inhabitants, resulting in very high human disturbance and various supplemental foods. The forest site is a nature reserve of mixed forest stands with many conifers and old trees. The mean year-round squirrel density in the park was much higher than in the forest site, (park= 5.1 squirrels/ha; forest = 0.1 squirrels/ha, minimum number alive, Wist et al., unpublished data). We used live traps (20 x 20 x 50 cm; Tomahawk Live Trap, Hazelhurst, Wisconsin, USA) with a seed and nut mix for bait. We opened the traps at 07:30 and checked them regularly until closing after 6 – 8 h. We used a cloth handling cone [88] and individually marked captured animals using PIT-tags (ID-100B; Trovan Ltd., East Yorkshire, UK). We recorded body mass (spring-balance ± 5 g, KERN & SOHN GmbH, Balingen-Frommern, Germany), body length (nose-anus-length ± 0.5 cm, tape measure), sex, reproductive status and age (juvenile, subadult or adult, classified via body mass and reproductive status, following [89]). To minimize confounding effects on metabolism, such as sex or growth, we only selected adult males with no signs for disease. We transported squirrels to the Institute of Cell and Systems Biology of Animals, Universität Hamburg (53°34'02.2"N 9°58'45.6"E).

Housing conditions

We held squirrels individually in large outdoor enclosures (average floor area 5 m^2), under natural photoperiod, T_a and humidity. Housing facilities allowed us to house a maximum of six individuals at one time, resulting in four sampling periods (i.e. runs) that took place between the end of March and end of May during two consecutive years (2018/2019). We equipped each enclosure with branches to enable natural climbing behaviour and a nest box (Elmato 10064 Großsittichkobel, Elmato GmbH, Holzheim, Germany, 30 x 22 x 20 cm, ~12 L), insulated with organic material (Pavatex, Pavaflex, Soprema GmbH, Germany). Individuals were housed between 13 and 18 days. Food (mix of foods usually encountered in their habitats) and water were offered *ad libitum* and changed every day at 2 p.m. (see [44] for further details and Supplementary Figure S1 for a schematic sketch of the experimental set-up). We weighed all individuals again after the experiment to record possible changes in mass and released them back into their habitats at their capture sites.

Ethical approval

All applicable institutional and national guidelines for the care and use of animals were followed. The authors complied with the ARRIVE guidelines. All procedures and animal handling were according to the German animal protection law and approved and authorized by the authorities of Hamburg and Schleswig-Holstein (general and housing permission by "Hamburger Behörde für Gesundheit und Verbraucherschutz", permit no. 87/16, 17 November 2016 and permit no. 1/2018, 7 February 2018; exemption for the keeping of wild animals by the "Hamburger Behörde für Energie und Umwelt" and extension of the permits to Schleswig-Holstein by the "Ministerium für Energiewende, Landwirtschaft, Umwelt und ländliche Räume des Landes Schleswig-Holstein" (permit of 28 December 2016).

Measurements of energy expenditure and temperature

We quantified RMR via oxygen consumption as ml O_2 h^{-1} using open flow respirometry. Air from each animal was drawn directly from the nest boxes through airtight tubing (Tygon, Saint-Gobain, Paris, France). Oxygen content was quantified using portable oxygen analysers (OxBox 1 - 4, designed and constructed by T. Ruf & T. Paumann, FIWI, University of Veterinary Medicine Vienna, Austria), powered by a standard 12 V car battery [34, 90-92]. By placing the measurement devices in a storage room next to the enclosures and using the nest boxes as respirometry chambers, disturbances to the animals were avoided since only the air tube already connected to the particular nest box had to be plugged into the oxygen analyser. We used a non-toxic modelling clay for sealing crevices and connections to minimise outflow contamination. Airflow was monitored by the flowmeter integrated in the set-up and set to 80 l/h. Oxygen content of the sample air was determined every ten seconds. As a reference, an hourly zero check was performed, i.e. oxygen content of the ambient air was analysed in the same interval for five minutes. We used silica gel to dry the air before entering the measurement devices. Calibration of the oxygen sensors was performed for each sampling period with calibration gas mixed by a gas mixing pump (Wösthoff Messtechnik GmbH, Bochum, Germany).

We measured RMR of the individuals after ~3 days of acclimation to the enclosures. We took measurements for periods of 24 h, starting in the early afternoon directly after food change, to enable recording of the complete inactive period at night as well as diurnal resting phases. Individuals were measured for a second 24 h period at the end of the housing period (day 12 – 17). Therefore, we obtained at least two measurement days for 16 out of 20 individuals to account for potential variation in MR throughout their time in captivity. Four individuals refused to use their nest box in the beginning, therefore, impeding the initial measurement. We measured a subset of individuals also in the middle of the housing period (see Supplementary Table S2). Ambient temperatures in the nest boxes (T_{nest}) as well as T_a and humidity in the enclosures were measured in intervals of 10 minutes with loggers (Thermochron iButtons DS 1922 / Hygrochron iButtons, DS1923L, resolution ± 0.5°C, Maxim Integrated Products, San Jose, CA, USA). We also monitored some of the squirrels (n=7) with camera traps (Snapshot Mini 5.0MP, DÖRR GmbH, Germany) to validate periods in which the individuals were outside and inside the nest box with the respirometry data.

Data processing and statistics

We processed data-files from the oxygen analyser with Clampfit 10.3.1.4 (Molecular Devices, Sunnyvale, USA) to account for zero checks and exclusion of periods where the individuals were outside of the nest boxes (Supplementary Figure S6). We corrected measured values to standard temperatures and pressure and calculated the rate of oxygen consumption with the following equation [93], which is applicable for our set-up [52, 94]: $V'O_2 = FR_e \frac{(FiO^2 - F'eO^2)}{[1 - F_i O_2(1 - RQ)]}$. FR_e represents the excurrent flow rate and F_iO_2–F'_eO_2 accounts for the difference in fractional O_2 concentration when entering and leaving the nest box. We assumed a substrate utilization composed by 50 % fat and 50 % carbohydrates and thus used a respiratory quotient (RQ, ratio of CO_2-production to O_2-consumption) of 0.85 [1, 91, 92, 95].

We used the energy equivalence of 20.37 J/ml O_2 to convert oxygen consumption into energy units [1, 91].

We included only the lowest 30 % of RMR values per hour for analyses (RMR) to ensure excluding activity peaks [90]. Additionally, we categorized the data as day-time or night-time using civil twilight times. We then excluded the hour before and after official sunrise/sunset, since they represent potential transition periods from diurnal to nocturnal metabolism. To control for pseudo-replication, we then calculated RMR means per individual and hour of measurement day for T_{nest} rounded to the nearest integer. Clear upper outliers in T_{nest} (>3° C above T_a), indicating an animal sitting close to or on the temperature logger were discarded. In those cases, we used the median of T_{nest} of the other nest boxes at the same time for the analysis. Due to the natural fluctuations of T_a, and thus T_{nest}, not all temperature integers were represented sufficiently frequently for robust analyses and were thus excluded. Therefore, we focused on RMR values for a temperature range between 8°C and 29°C. To obtain mass-specific RMR, we divided the values by individual body mass in g. We assumed a steady mass change from the start to the end of the experiment and used the estimated body mass for the particular day of measurement.

Data processing and statistics were performed in Excel (MS Office 2016) and R 4.1.2 (R Core Team 2021), respectively. We used the “RStudio” environment (RStudio Team 2021) and the packages “lubridate’ [96], “dplyr” [97], “zoo” [98] and “lattice” [99] for initial data processing. We used linear mixed-effects models (LME) (lmer function within the package “lme4” [100]) and the package “lmertest” [101] with RMR (ml oxygen consumption per hour and g of body mass; ml O_2 h^{-1} g^{-1}) as response variable. We tested all predictors for pair-wise correlations to avoid multicollinearity. We modelled T_{nest}, habitat, phase in diel cycle (day/night), sampling period and time of housing period (first or second half of the housing period) as fixed factors in the full model. Graphical exploration (“ggplot2” [102], “effects” [103]) revealed an interaction of habitat and T_{nest}, as well as habitat and sampling period on RMR, therefore interactions were included in our model. Differences in sampling success per individual were corrected by using a standardized weight in the global model with weighting individuals according to the number of data points. We further allowed different intercepts and slopes per individual by including individual nested in phase of housing period as random factor to account for individual differences, repeated measurements across the individuals and the effect of the length of housing. See Supplementary Methods, Supplementary Table S3 and Supplementary Figure S4 for detailed information of the full model.

We used an information theoretic approach for our data analysis and performed model selection based on Akaike information criterion for small sample size (AIC_c) and Akaike weights [104-106]. We further used the dredge function from the MuMIn package [107] and model comparison with maximum likelihood. Normality and homoscedasticity were assessed by visual inspection of residual plots [108]. Since we included interactions in our models, model averaging was not feasible. Thus, we present the 95 % confidence set of all possible models, i.e., models with sum of AIC weights ≤ 0.95 [105, 106]. To further obtain a measure of relative importance for each predictor, we summed up the AIC weights from all models of the confidence set containing the respective predictor [109, 110]. Finally, we refitted the top ranked model with REML and report predictor estimates, confidence intervals (CIs) and local effect sizes (standardized coefficients or Cohen's f^2) for the predictors retained in the model [111, 112] as well as the marginal and conditional R^2 (table created via "sjPlot" [113]). We interpreted estimates with CIs that do not overlap zero as evidence of model support and statistical significance [106]. Furthermore, we estimated the intra-class correlation coefficient (ICC) for the top ranked model as a measure of differences in RMR among individuals [114-116]. Unless otherwise stated, we present the data as mean ±1 SD, N reports the number of individuals and n the number of measurement days.

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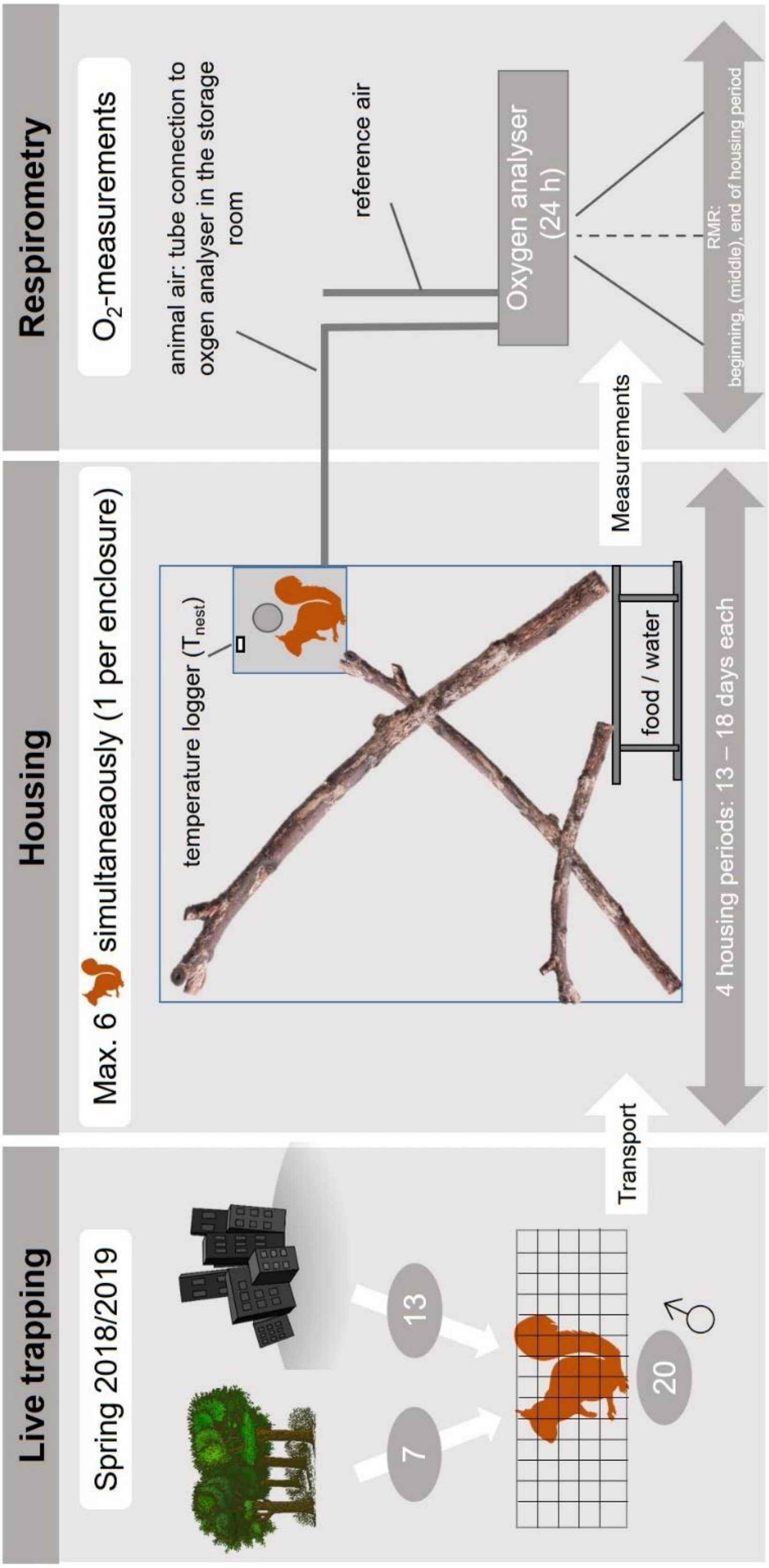
Supplementary material

for:

City comfort: weaker metabolic response to changes in ambient temperature in urban red squirrels

Bianca Wist, B. Karina Montero and Kathrin H. Dausmann

S1



Supplementary Figure S1 Overview of experimental design: live trapping (left panel), housing conditions and length (middle panel) and respiromentry set-up (right panel)

S2

Supplementary Table S2: Overview of sampling effort per sampling period

Run	Year	Month	Individual no.	Habitat	Measurements (24 h)
1	2018	April	1	forest	2
			2	forest	1
			3	urban	2
			4	urban	2
			5	urban	2
			6	urban	2
2	2018	May	7	forest	2
			8	urban	2
			9	urban	2
			10	urban	3
			11	urban	3
3	2019	April	12	forest	5
			13	forest	3
			14	urban	1
			15	urban	4
			16	urban	3
4	2019	May	17	forest	5
			18	forest	4
			19	urban	4
			20	urban	5
Total			**20**		**57**

Supplementary Methods (statistical details)

Our full model was fitted with restricted maximum likelihood (REML) and described by the following structure (Supplementary Tab. 2):

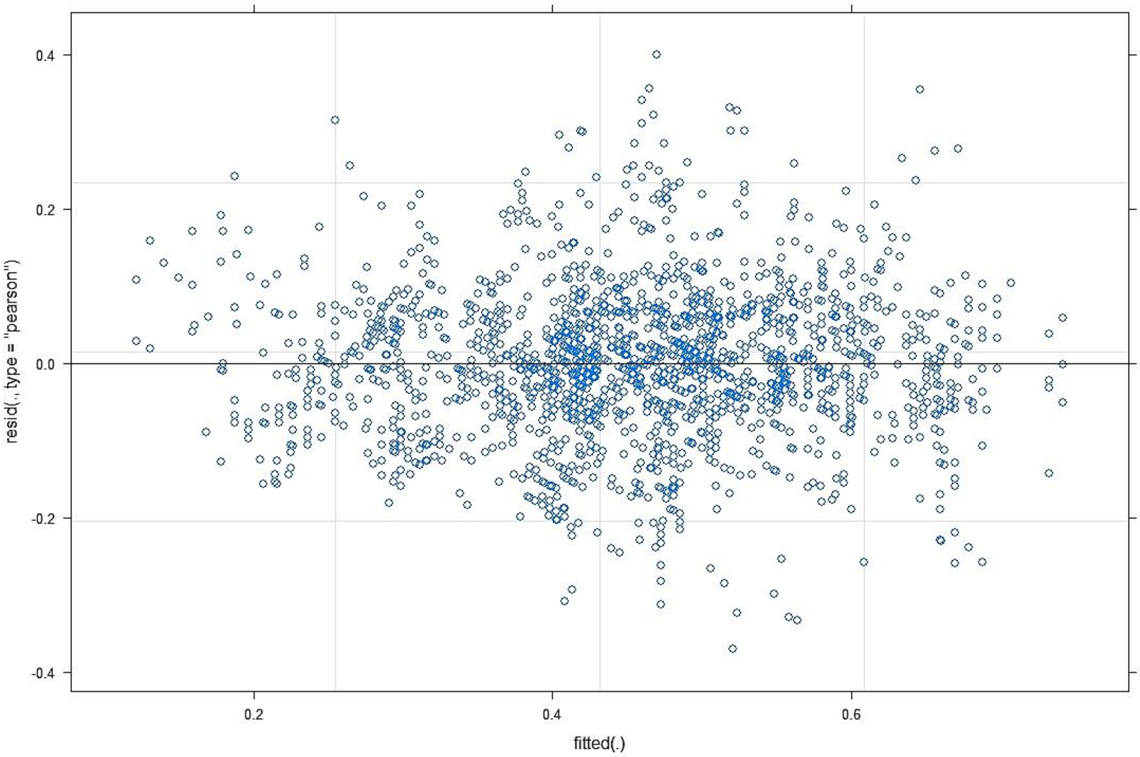
ml O_2 h-^{1} g^{-1} ~ habitat + T_{nest} + D/N + run + first_sec + habitat:run + habitat:T_{nest} + (1+first_sec | individual), data = eich, REML = T

S3

Supplementary Table S3: Overview of predictors included in the full model

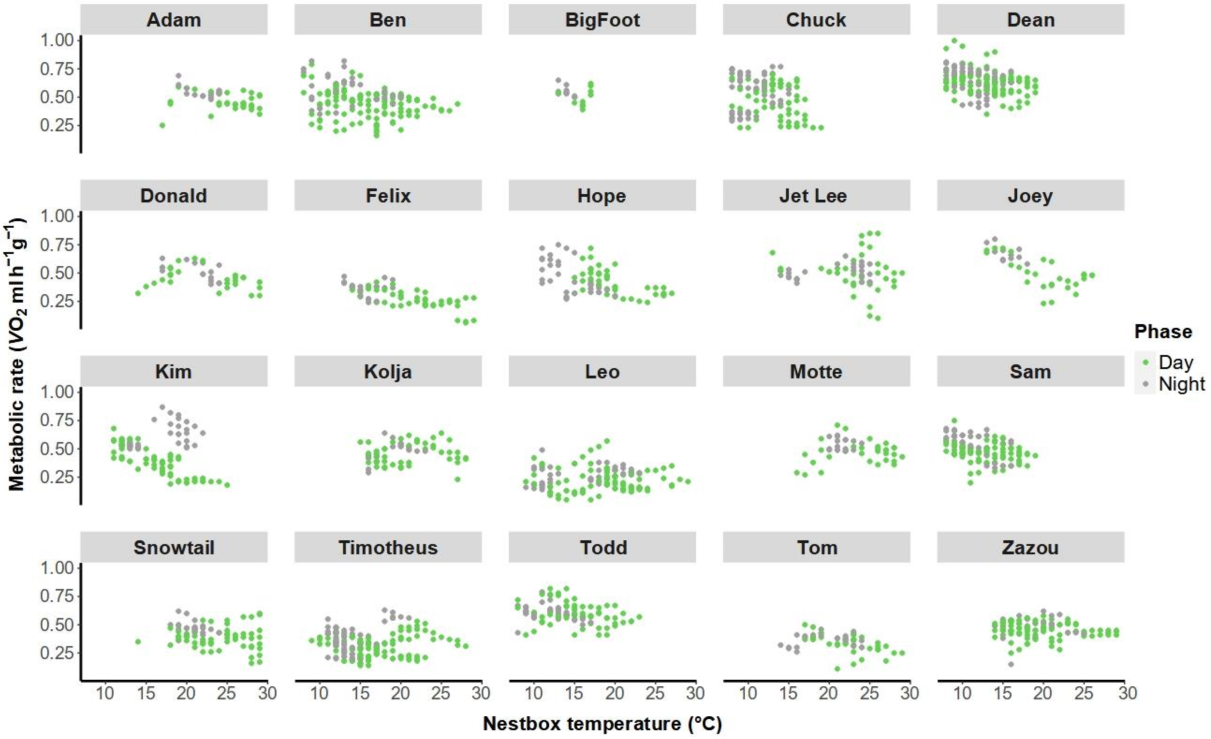
Variable	Type of predictor	Abbreviation
Nest box temperature	fixed effect, continuous	T_{nest}
Habitat of origin (forest/urban park)	fixed effect, factor with 2 levels	habitat
Phase in diel cycle (day/night)	fixed effect, factor with 2 levels	D/N
Time of housing period (first/second half)	fixed effect, factor with 2 levels	first_sec
Sampling period	fixed effect, factor with 4 levels	run
Habitat and sampling period	fixed effect, interaction	habitat:run
Habitat and nest box temperature	fixed effect, interaction	habitat:T_{nest}
Individual nested in first/second half of the housing period	random effect, nested	first_sec|individual

S4



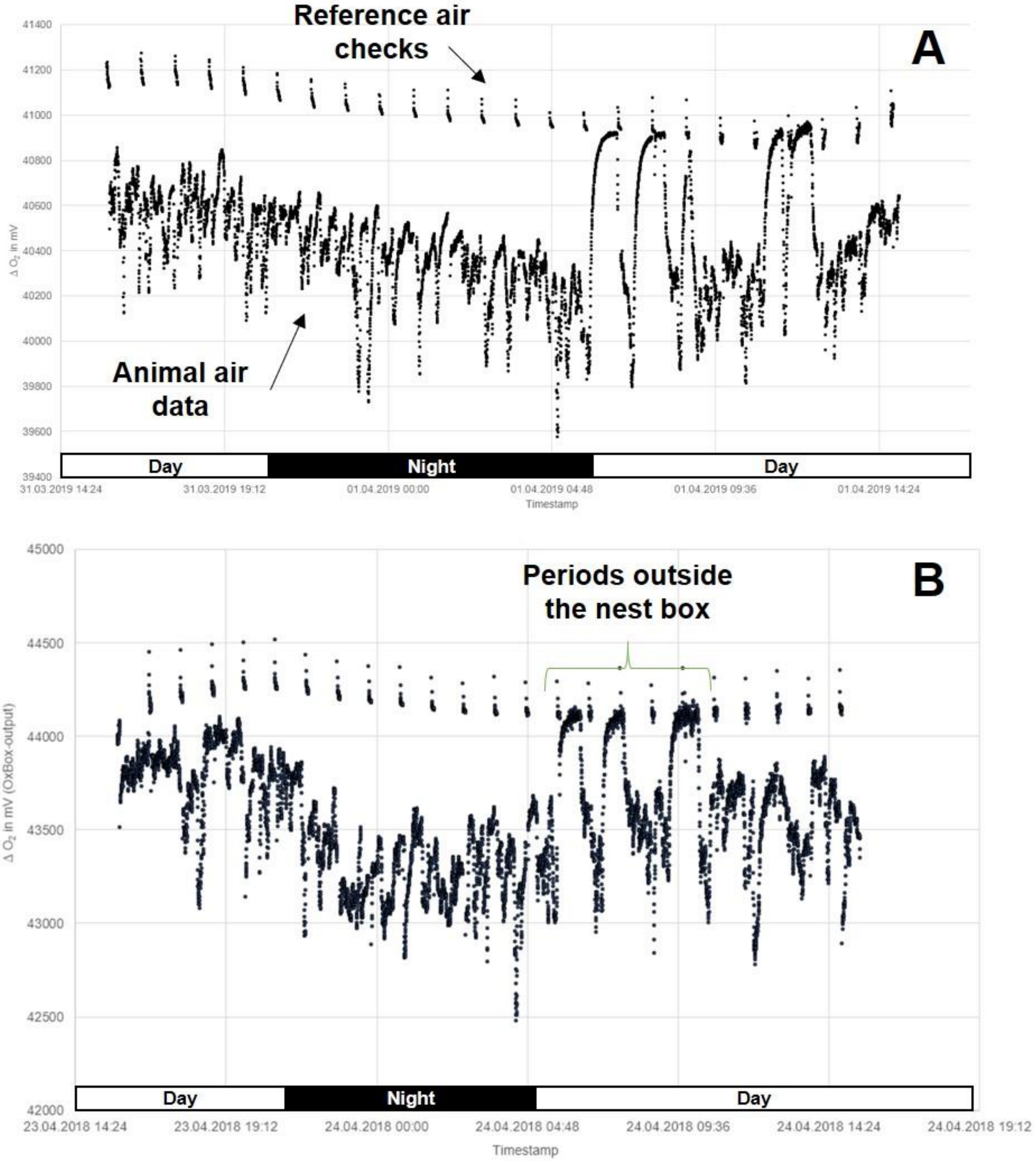
Supplementary Figure S4 Diagnostic plot with fitted values versus residuals created from the full model.

S5



Supplementary Figure S5 Data points from all measurement days per individual for diurnal (green dots) and nocturnal (grey dots) mass specific metabolic rate (*VO*^{2} ml h^{-1} g^{-1}) at measured ambient temperatures (diurnal range 8 – 29°C, nocturnal range 8 – 25°C).

S6



Supplementary Figure S6 Two examples of the raw data output from the urban individuals Sam (A) and Kim (B). Difference in oxygen content of the animal air/reference air in mV are plotted over time. The measurement curve includes hourly reference air checks as well as periods where the animals left their nest boxes (usually around sunrise to forage). In these cases, the data points generated from the animal rapidly dropped to the level of reference air checks (ambient air). These timespans were discarded prior to analysis. Day and night are shown by the white or black bars and correspond to local civil twilight data. Higher nocturnal values were already detectable within these raw data outputs (indicated by an average higher distance to the reference air checks). Double checking these outputs was part of our steps to validate the data and the unexpected outcome of a higher nocturnal RMR.

Authors' contributions

I hereby confirm that Bianca Wist (BW) and Kathrin H. Dausmann (KHD) conceived and designed the study, BW conducted the measurements in the cage experiment, BW and B. Karina Montero (BKM) analysed the data, BW wrote the manuscript with input from KHD and BKM. All authors revised the manuscript and approved the final version of it.

Prof. Dr. Kathrin Dausmann

Chapter 5

General discussion & Conclusions

The urgent need to study urban wildlife species

Urbanisation, a major part of the ongoing human induced global change, significantly drives biodiversity loss and species homogenisation (McKinney 2006, Grimm et al. 2008, Li et al. 2022). Unlike other forms of land conversion, urbanisation is usually a non-reversible transformation with lasting impacts on wildlife (Gibbs et al. 2019, Gao and O'Neill 2020). Future land conversion to urban habitats is predicted to further decrease local within-site species richness of amphibians, birds and mammals by around one third and their within-site abundance by more than 50 % (Li et al. 2022). Yet, animals' sensitivity to environmental change varies, and a couple of usually generalist species apparently successfully colonises urban habitats (Alberti et al. 2017). However, being a successful urban adapter or even exploiter is not without costs (Birnie-Gauvin et al. 2016). Although our knowledge of the consequences of urbanisation for wildlife has increased in recent decades (Collins et al. 2021), we still lack basic information about mechanisms and consequences, particularly in taxa such as mammals (Ouyang et al. 2018, Zuñiga-Palacios et al. 2021). Greater insight into species response to urbanisation is crucial though for informed urban planning and nature conservation to mitigate effects of urban stressors in already existing and rapidly emerging urban systems around the world.

Human activities in urban habitats change the availability and quality of food as well as an animals' thermal environment, all of which are fundamental to various aspects of animal biology and fitness (Birnie-Gauvin et al. 2017). In my dissertation, I therefore investigated three research topics, important for a better understanding of the presence, long-term survival and fitness of urban wildlife populations (Birnie-Gauvin et al. 2017, Ouyang et al. 2018). First, I investigated natural and human provided food resources as well as population parameters of the common urban mammal *Sciurus vulgaris* in three small parks in a city core area in comparison to a rural forest. Second, I aimed to get a deeper understanding of its nutrient ecology and associated consequences by examining whether and how squirrels respond to an urbanised food availability in terms of their food choice behaviour and nutrient intake. Third, I investigated the metabolism of squirrels to assess whether and to which degree they adjust their energetic expenses to the urban habitat. Here, I summarise and discuss the findings of my project and conclude with conservation implications and future research questions.

Urban environmental variability influences local wildlife populations

Cities consist of a great variety of different habitat patches. Built-up areas are generally interspersed with urban green spaces. These are, in turn, divided into subcategories and green spaces can comprise remnant vegetation, urban wasteland, private gardens, managed parks, green roofs, etc. (Aronson et al. 2017, Lepczyk et al. 2017). The resulting habitat mosaic leads to a strong heterogeneity of environmental factors in space and / or time that asymmetrically affects urban wildlife (Faeth et al. 2005, Alberti et al. 2020, Szulkin et al. 2020). Modified vegetation cover and structure, extended growing seasons or changes in nutritional quality of plant material, as well as abundant supplemental food sources significantly alter the food supply of urban populations (Birnie-Gauvin et al. 2017, Anders et al. 2021), yet the magnitude of this modification likely varies across scales (Blumstein et al. 2023). Deeper knowledge of the variability of natural and supplemental food availability within the same urban matrix, i.e. at small spatial scale, and its consequences for the spatial ecology of wildlife populations is still poor though (Alberti et al. 2020).

The findings from the first part of this project (Chapter 2) demonstrate that pronounced heterogeneity in factors, such as food availability and animal population densities, can not only occur between urban and rural habitats or different types of urban habitat patches, but also between nearby urban fragments of the same type, in this case small parks in the urban centre. As confirmed here, tree diversity is often increased at urban sites resulting from management activities and active planting of many different species, whereas managed forests are usually characterised by comparably few tree species relevant for timber production (Wiersum 1997, Grimm et al. 2008, Zhao et al. 2023). At the park scale, local vegetation varies with historical and current management plans since cities usually do not have an overarching plan for their green areas. Local decision makers can be influenced by individual preferences and optical appearance of plants. Moreover, they have to balance between several factors and consider local financial and human needs while nature conservation is usually not a priority (Aronson et al. 2017). Consequently, urban wildlife encounters a novel environment with an artificial vegetation assembly that can vary substantially in composition and nutrient suitability at small scale across the urban matrix. Accordingly, tree numbers and diversity differed between the parks in this project. These variables as well as share of conifers naturally represent food and shelter availability for squirrels (Koprowski 2005a, Flaherty et al. 2012, Krauze-Gryz and Gryz 2015). Thus, they influence survival, reproduction or dispersal and finally squirrel population parameters (McCleery 2010, Hubert et al. 2011, Selonen et al. 2018). Strong dominance of one tree species as found in Wohlerspark or low conifer numbers in all three parks indicate limitations in natural food supply or quality for squirrels.

Supplemental food sources that were absent in the forest, but available at all urban sites in this study, might compensate for natural food limitations, yet they also differed in abundance and composition between parks. I did not collect data on human visitors, but observations during field work indicated differences in recreational use, littering and wildlife feeding by humans that likely contributed to this finding. Particularly, Wohlerspark was visited by high numbers of younger people and intensively used for picnics and parties. Visitors frequently left refuse on the lawns or in the shrubbery and waste bins were overfilled in the summer season. Some people visited the park on a daily basis and provided hand-feeding or bird food in the park shrubbery. Accordingly, observations as well as data collection documented a great variety of human-related food items and we observed urban squirrels to forage on diverse supplemental foods, from watermelon to even processed foods, such as cupcakes or biscuits (Fig. 5.1).

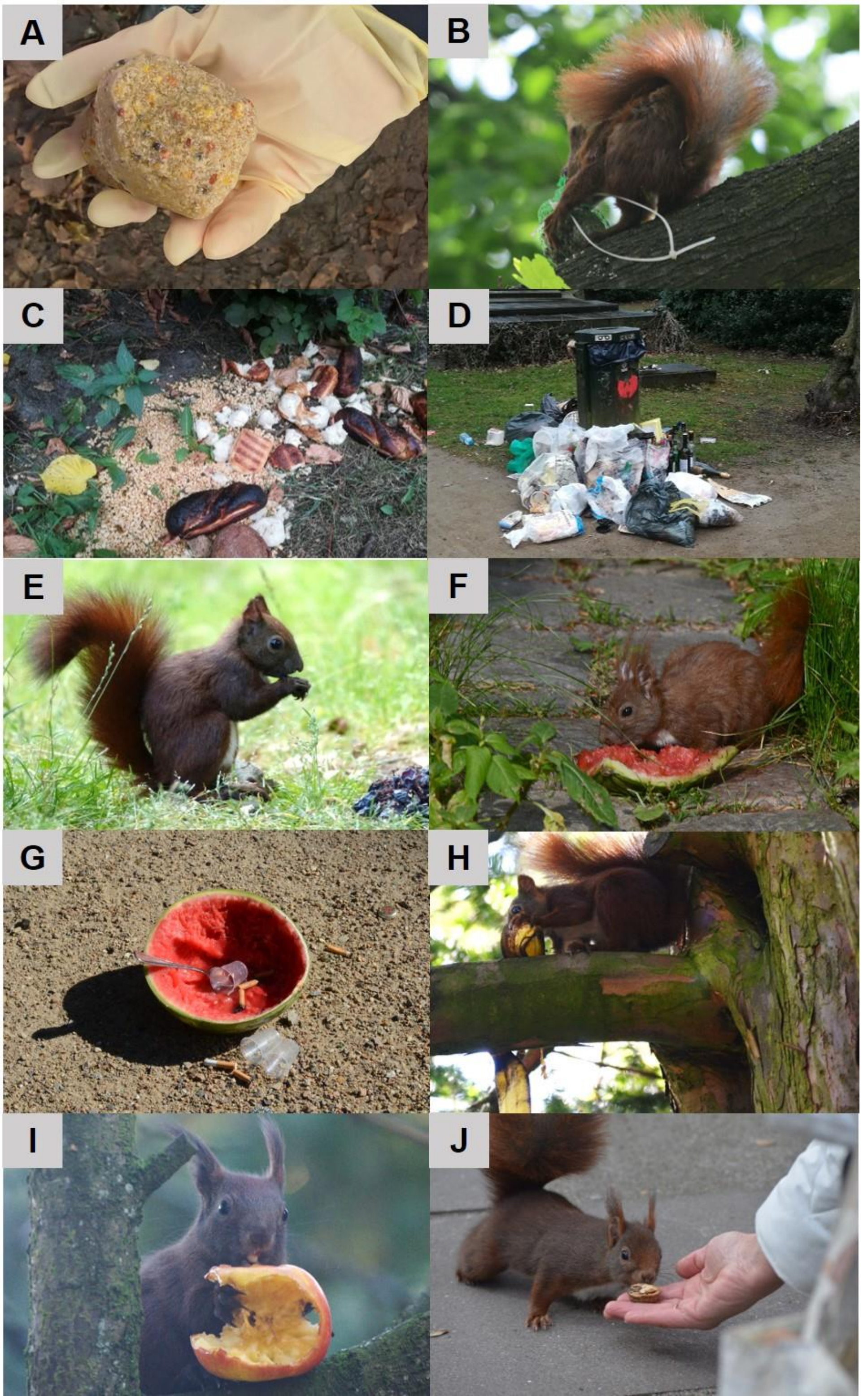


Figure 5.1: Examples of supplemental food sources available and / or foraged by squirrels in Wohlerspark: fat balls (A, B), scattered bakery products (C), refuse (D), picnic leftovers: e.g., grapes (E), watermelon (F, G), banana (H), apple (I) and walnuts provided by hand feeding (F). Photo credits: L. Thomas (A), B. Wist (photos C, D, E), B. Probst (photo F), R. Schädlich (photos B, G, H, I, J)

These differences in food supply are an environmental factor that acts on the individual as well as on the population, affecting species abundance and population dynamics (Rodewald and Gehrt 2014, Ouyang et al. 2018). Supplemental foods generally drive high population densities in some species since they decrease food limitations and increase resource predictability (Luniak 2004, Anderies et al. 2007, Evans and Gawlik 2020). They occur unevenly clumped across the urban matrix and can act as environmental cue that shortens dispersal, decreases home ranges or attracts individuals (Rodewald and Shustack 2008, McCleery 2010). Thereby, supplemental feeding accumulates individuals around feeding spots (Alvey 2006, Rodewald and Gehrt 2014). Consequently, wildlife population density estimates might rarely apply to a whole city (Rodewald and Gehrt 2014, Ouyang et al. 2018). Density estimates from different patches within a city thus represent a fundamental basis for a better understanding of the mechanisms of synurbanisation. Particularly the ratio of urban to rural densities is considered a crucial metric for assessment of species responses (Evans et al. 2011). My findings from Chapter 2 confirmed increased population densities of a common urban mammal, but showed that density ratios in comparison to a rural forest can considerably vary between nearby parks. Variability in densities across urban sites was also found in other urban species, such as raccoons (Graser III et al. 2012). However, these differences are often detected when comparing different types of urban habit patches, such as parks versus surrounding matrix or sites with increasing distance from the city core.

Interestingly, the smallest park in this project had the lowest tree diversity among the parks, but highest tree numbers, highest supplemental food abundance and an unexpectedly high squirrel density. In rural areas, tree squirrels can maintain high densities in small forest fragments, but this only applies to *S. vulgaris* when supplemental feeding and connectivity to other fragments are high (Koprowski 2005b). The former was confirmed here, but the latter remains questionable since all parks in this project were surrounded by a dense urban matrix and had main roads and railway lines in short distance to the park limits. My findings highlighted the relevance of food supply for local wildlife population parameters. They further emphasise that choosing an appropriate spatial scale for urban wildlife studies is fundamental for informed decision about urban species persistence and conservation actions since the range and composition of available foods change in space or time and population parameters from one spot might not tell the full story (Shochat et al. 2006, Birnie-Gauvin et al. 2017, Alberti et al. 2020). My field study enabled a more comprehensive understanding of local conditions in highly urbanised sites and represented an important prerequisite for the following experiments.

The relevance of nutrient ecology in urban research

The type of available non-natural food items documented in Chapter 2 questioned how they influence squirrels' diet composition and whether the supplemental foods meet their nutritional requirements, particularly in light of the comparably lower body condition in the park with highest food supplementation (Chapter 2 / Supplementary material, Chapter 3). Furthermore, increased population densities and associated resource competition can have subsequent effects on behaviour and this likely applies to park populations (Parker and Nilon 2012). Hence, studying not only the urban food availability in the field, but also exploring how species respond behaviourally, i.e., how they compose their diet with regard to energy intake and macronutrients, contributes to the understanding of urban wildlife patterns and appropriate in-situ conservation, since energy and a well-balanced nutrient intake are fundamental for whole body functioning, reproduction and fitness (Raubenheimer et al. 2012, Simpson et al. 2015, Birnie-Gauvin et al. 2017, Coogan et al. 2018).

With that in mind, I conducted a food choice experiment under semi-natural conditions with male squirrels from the park with highest food supplementation (Chapter 3). Urban squirrels resembled forest individuals in their fat prioritisation and protein intake, but showed a wider dietary range with higher consumption of non-natural foods and thereby sugar. In principle, plasticity in foraging behaviour allows animals to exploit urban resources and is a prerequisite for the high individual numbers of synurban species (Luniak 2004, Lowry et al. 2013). Thus, it is likely one of the key tools that enable squirrels to inhibit small urban parks with apparently limited natural food supply. A broadening or shift of the dietary niche was also found in other urban species (Murray et al. 2015, Anders et al. 2021, Dasgupta et al. 2021). This response is likely supported by higher ‘boldness’ of urban individuals that enables exploitation of novel resources and faster acclimation to the housing conditions of the experiment (Uchida et al. 2019). Moreover, urban species loss and homogenisation towards more generalist species in high abundance can ease interspecific competition while intraspecific competition is increased. This might result in intraspecific niche partitioning or further increase in the generalising of food consumption (Manlick and Newsome 2021). Thus, the higher readiness of the urban individuals in the experiment to make full use of the offered food range fits the picture and matched the field observations.

Having said that, the results are worrying since the individuals were free to choose in a cafeteria experiment without current competitive forces or food limitations. In theory, animals should aim for a nutritionally balanced diet and adjust their foraging behaviour to their physiological demands and environmental conditions (Raubenheimer et al. 2012, Simpson and Raubenheimer 2012, Simpson et al. 2015). Dietary shifts towards human-provisioned resources might yet entail nutritional imbalance and are related to diverse health- and fitness-relevant aspects depending on food composition and quality (Simpson and Raubenheimer 2012, Murray et al. 2016, Birnie-Gauvin et al. 2017, Pollock et al. 2017, Dasgupta et al. 2021, Gillman et al. 2021). Urban animals show alterations in gut microbiome community (Anders et al. 2021), protein deficits (Murray et al. 2015) elevated blood glucose levels (Schulte-Hostedde et al. 2018, Schmidt et al. 2019, Anders et al. 2021), poor dental health (Hungerford et al. 1999) and higher immune responsiveness (Peneaux et al. 2021) or lower breeding success (Pollock et al. 2017). Some of these effects, such as higher blood glucose levels or dental health issues are linked to higher sugar consumption. Similar to our knowledge from human diets (Johnson et al. 2007), sugar can have strong addictive and detrimental effects on animals, even without body weight change (Kendig 2014, DiNicolantonio et al. 2018). In lab studies, rats favoured sugar even over cocaine (DiNicolantonio et al. 2018) and consequently one might not be surprised that urban individuals with access to sugary or processed foods, as found in Chapter 2, developed a higher preference for sugar. However, in Chapter 3, I discussed the potential negative effects of sugar consumption. Sugar can impair spatial learning and memory and might thereby reduce the ability to find hoarded foods (Kendig 2014). I frequently observed hoarding behaviour in the park, but items were often not well hidden and partly removed by other species or by gardening activities. An impairment in memory abilities that additionally reduces the retrieval of hoarded food items might be detrimental. On the other hand, year-round supplemental foods might generally lower the relevance of hoarded foods for urban squirrels (Brzeziński and Zalewski 2023).

The lower body condition of park squirrels in the experiment confirmed indications gained in in the field (Chapter 2, Supplementary material) and suggest the presence of influences that counteract high supplemental abundance. Possibly, the confined availability of natural food sources in combination with low quality supplemental foods and high intra- and / or interspecific competition might cause food or nutritional limitations despite high supplementation. My results indicated the potential for high resource competition in the parks since I observed additional species, such as crows (*Corvus corone*), jays (*Garrulus glandarius*), domestic pigeons (*Columba livia* forma *domestica*), great tits (*Parus major*), rats (*Rattus norvegicus*) and yellow-necked mice (*Apodemus flavicollis*) feeding on supplemental sources and these also frequently interacted with squirrels at feeding spots. Urban related food limitations were assumed to explain foraging behaviour of urban grey squirrels which found provided food pans and removed sunflower seeds at higher rate and number than rural conspecifics (Bowers and Breland 1996). Possibly, urban squirrels developed an “eat as much as you can from whatever you can get” behaviour which might explain their higher kJ intake and weight gain over the course of this experiment, though in the end, their body condition was still below the forest individuals.

Shifts in diet composition and exploitation of novel resources require flexibility in digestive traits, such as the production of digestive enzymes (Manlick and Newsome 2021). It is unknown if the digestive adaptations of synurban species can keep up pace with their plasticity in foraging behaviour. To my knowledge there are no studies on digestive capacity of urban squirrels, but the gut physiology of this seed and nut favouring species might simply not be adapted to digest high amounts of processed or sugary foods. Besides this, urban species likely ingest health-relevant amounts of chemical pollutants, heavy metals or toxins that can impact the digestive physiology and a broad range of other biological traits (Birnie-Gauvin et al. 2017, Isaksson 2018, Samuel et al. 2023).

Otherwise, stable urban food availability and buffered conditions might lower the need for fat reserves (Shochat 2004) and I did not find differences in body condition across seasons in the highly supplemented Wohlerspark (Chapter 2, Supplementary material). The very high population densities or the survival of more subdominant individuals might be further explanatory factors for the observed lower body condition here (Prange et al. 2003, Shochat 2004, Anderies et al. 2007, Beliniak et al. 2022). Other factors, such as genetic depletion, higher stress levels or parasite load are rather unlikely in view of previous findings (Shimamoto et al. 2020, Werner and Nunn 2020, Fusco et al. 2021, Santicchia et al. 2022, Tranquillo et al. 2022). I did not investigate stress hormones in this project, but have no indication for differences in parasite load between sites (Wodtke et al., unpublished data). However, results about the influence of urbanisation on body mass or condition are generally ambiguous (Bókony et al. 2010, Meillère et al. 2017, Jiménez-Peñuela, 2019, Beliniak et al. 2022, Tranquillo et al. 2023). I strongly assume that local differences in food availability and / or quality in combination with changes in foraging behaviour are one of the main reasons for this. However, details of the underlying processes remain unclear and further research is necessary to explore the influence and interaction of factors such as digestive constraints or the ingestion of pollutants.

Ecophysiological aspects of an urbanised world

My findings from Chapters 2 and 3 show that urban-related changes in resource availability and foraging behaviour influence the energy and nutrient intake in urban dwelling species. This likely has physiological consequences for endothermic animals, which must energetically costly generate their own heat to maintain a stable body temperature. As a prerequisite for all biological processes and ultimately fitness, they need to carefully balance their energy expenditure in relation to environmental conditions, particularly to T_a and resource availability (Brown et al. 2004). Ambient temperature is increased in cities due to urban heat island effects, and importantly, these occur within evolutionarily short timescales and in relatively short spatial distances from surrounding colder environments (Diamond and Martin 2021). Beyond this, higher T_a as well as year-round supplemental food availability act as a buffer against seasonal resource fluctuations or periods of scarcity (Shochat et al. 2006). Urban conditions therefore have the potential to relax energetic constraints by reducing the need of endogenic heat production and / or reducing natural resource limitations and thereby in energy availability to cover energy needs (Sumasgutner et al. 2023).

For those reasons, studying the ecophysiology of a species inhibiting urban and geographically proximate rural habitats represents an important framework for a deeper understanding of its physiological flexibility or adaptations to an urbanised life, as well as the associated metabolic costs or benefits. Species with physiological flexibility might be better prepared to take full advantage of beneficial urban conditions. This may be an important mechanism contributing to higher winter survival or reproduction rates observed in some urban populations (Prange et al. 2003, Luniak 2004, McCleery 2010, Bateman and Fleming 2012). Urban stressors, such as altered nutrition as described in Chapters 2 and 3, yet have the potential to increase physiological costs (Birnie-Gauvin et al. 2016, Isaksson 2020, Samuel et al. 2023). However, studying intraspecific variations in the physiological response of endothermic taxa to urbanisation has been neglected to date.

In this context, I examined energy expenditure of Eurasian red squirrels from an urban and a nearby rural habitat (Chapter 4). The species is widespread throughout the Palearctic from Europe to Asia and occurs at altitudes from 0 to 3,100 m (Lurz et al. 2005, Gazzard 2023), therefore inhibits and physiologically copes with a wide range of climatic conditions. It is not known to use hibernation or torpor to overcome adverse periods and likely relies on behavioural or subtle physiological adaptations (Dausmann et al. 2013, Turner et al. 2017). My project demonstrated intraspecific physiological differences in squirrels from urban versus rural populations, which are likely primarily explained by acclimatisation to the warmer urban temperatures, as well as the dietary differences described in Chapters 2 and 3. Urban squirrels showed a weaker thermoregulatory response to a decrease in T_a in comparison to forest squirrels. Remarkably, urban ants also showed reduced acute plasticity in their metabolic rate, i.e. they were less sensitive to a change in temperature than rural ants (Chick et al. 2021). This lower responsiveness could be related to reduced daily T_a variance in urban habitats (Gallo et al. 1996, Wang et al. 2012, Chick et al. 2021). It is possible that use of well-insulated dreys as well as hoarding behaviour decouple squirrels' energy expenditure from environmental conditions to some extent (Menzies et al. 2020). This effect could be enhanced by the urban heat island, a more stable food supply or better insulated urban nesting sites. Higher availability of shelter is likely one of the benefits for some urban animals (Bateman and Fleming 2012, Andersson et al. 2015). I observed and was reported drey building, e.g. on house walls or balconies (Fig. 5.2). In these cases, individuals might benefit from thermal radiation or wind protection, although this is speculative as I have no information about drey sites of the park squirrels in the experiment.

Figure 5.2: Urban squirrel in a drey, located in a protected window area in the streets of Hamburg Altona (Photo credit: B. Wist)

Dietary changes such as those documented in in this project have the potential to alter an animals' physiology, e.g., by changing the amount and location of fat layers or general metabolic and digestive processes (Perissinotti et al. 2009, Birnie-Gauvin et al. 2017, Jarman et al. 2020). Food supplementation has been shown to alter physiological traits such as body temperature and heart rate in squirrels (Menzies et al. 2020). In particular, the nutrient composition of the food available in cities seems to be relevant factor for metabolic changes: white-footed mice (*Peromyscus leucopus*) in New York City showed genetic patterns that indicate local adaptation of metabolic processes to the novel urban resources high in fat, carbohydrates and sugar (Harris and Munshi-South 2017). An anthropogenic diet and exposure to pollutants or oxidative stress have also been assumed to trigger epigenetic changes and differences in abundance of fatty acids involved in metabolic and immune response pathways in urban versus rural great tit populations (Andersson et al. 2015, Watson et al. 2017, Watson et al. 2020). Pollutants and toxins can reduce appetite or digestive efficiency, but can also interact with other urban features while influencing metabolic rate and further physiological mechanisms (Birnie-Gauvin et al. 2016, Birnie-Gauvin et al. 2017, Watson et al. 2017, Watson et al. 2020, Samuel et al. 2023, Sumasgutner et al. 2023).

Food supplementation can also alter activity patterns or space of urban dwelling species including squirrels and this may further contribute to metabolic changes (McNab 2002, Luniak 2004, Careau et al. 2008, Lowry et al. 2013, Thomas et al. 2018, Oliveira et al. 2020, Beliniak et al. 2021). Scattered supplementary foods, which were highly available in Wohlerspark, can cause squirrels to spend more time on the ground (Thomas et al. 2018, Krauze-Gryz et al. 2021). Additionally, we have indication for very small squirrel home ranges in this park (~ 1.5 ha based on telemetry estimates, Probst et al., unpublished data) and urban squirrels have shorter flight initiation distances (Uchida et al. 2016, Uchida et al. 2019). Therefore, I speculate that urban squirrels move less, perform less vertical climbing and thereby accumulate less muscle mass or more fat in certain parts of their bodies or have smaller organ size, all of which would be relevant to their metabolism (Burton et al. 2011).

Interestingly, a study on semi-urban squirrels in a controlled climate chamber determined mean RMR of 0.75 ml O_2 h^{-1} g^{-1} within the measured thermal neutral zone, a range of T_a which started at 31.7°C and in which heat of basal body functions is sufficient to maintain body temperature (Speakman and Król 2010, Kobbe et al. 2014, Turner et al. 2017). The temperatures during our experiment did not reach this level. Nevertheless, RMR for both groups (0.47 / 0.45 ml O_2 h^{-1} g^{-1} for forest/urban squirrels), even at the lowest measured T_a (0.66 / 0.57 ml O_2 h-^{1} g^{-1} for forest / urban squirrels at 8°C) remained well below this value. Possibly, this is due to the different set-ups of our studies. Metabolic measurements are often conducted under controlled conditions, i.e. by using a climate chamber and a controlled temperature regime. However, insights gained from this method might differ from species response in the wild (Auer et al. 2016, Morash et al. 2018). Here, I aimed to study energy expenditure of the individuals without further disturbance and under semi-natural conditions.

Regardless of the reasons for observed metabolic differences, the results from Chapter 4 suggest intraspecific physiological plasticity and energetic advantages for urban squirrels in temperate regions with limited occurrence of hot days. However, the comparatively higher costs at warmer T_a are alarming given the assumption that heat dissipation is a major challenge for urban endotherms in the face of climate change (Speakman and Król 2010, Tattersall et al. 2012, Ohrnberger et al. 2020, Bateman et al. 2023, Sumasgutner et al. 2023). Mammals in city centres may be more constrained by the need to lose heat and closer to their thermal limits, as temperature increases due to climate change will be higher in urban habitats and especially in urban core areas (Sumasgutner et al. 2023). Notably, climate change and urban heat islands have the potential to affect body condition and fitness of urban rodents, triggered by heat stress or seed desiccation (Bateman et al. 2023). On the other hand, lower fat reserves or condition of urban individuals, as found in some studies including mine, yet might be beneficial for heat loss. Additionally, higher urban water availability or fewer movements as described above might counteract overheating to a certain extent (Sumasgutner et al. 2023).

Interestingly, urban nestlings of *Parus major* were less affected by heat events in terms of body mass and survival than rural conspecifics, presumably due to adaptations that increase heat dissipation or a smaller impact of heat on resource availability in cities (Pipoly et al. 2022). Urban greater white-toothed shrews (*Crocidura russula*) showed lower resting metabolism than rural individuals, a finding that I would have expected in the urban squirrels in this project. However, contrary to our study, the shrews were only measured at one single T_a within their thermoneutral zone. Notably, and similar to the urban individuals in my experiment, they also gained more body mass during the housing period (Oliveira et al. 2020). Further physiological studies of urban versus rural populations have been carried out primarily on ectotherms and demonstrate thermal adaptations, i.e., a higher heat tolerance, not also to the higher urban temperatures, but also to the occurrence of stronger heat-waves (Angilletta et al. 2007, Brans et al. 2017, Miles et al. 2020, Diamond and Martin 2021b).

A possibly maladaptive response has been shown for urban acorn-dwelling ants (*Temnothorax curvispinosus*). They had elevated metabolic rates at a T_a of 25°C compared to rural ants, possibly influenced by urban effects such as pollution, yet the increase was less pronounced under acute heat stress (Chick et al. 2021). However, the generally low number of studies investigating the physiology of urban endotherms hampers a broad comparison of their responses to urban conditions across species. It remains unclear how a comparably higher heat production at high T_a is compensated for in urban squirrels and whether their response might be maladaptive, as I am not aware of any studies examining their heat tolerance and associated fitness effects. We are just beginning to understand the subtle interplay of metabolism with urban heat islands, climate change, diet and behaviour in urban dwelling species.

Are synurban squirrels urban ‘winners’?

The intraspecific variations in foraging behaviour and physiology shown in this project are likely prerequisites for the success of squirrels in contrasting and evolutionarily new habitat types (Beliniak et al. 2021, Cordeschi et al. 2021, Beliniak et al. 2022). From a broader perspective, the ability to respond plastically, e.g., to novel resources, gives a species the time to adapt genetically to a changing environment (Diamond and Martin 2021a). It is assumed that several urban species are still in the process of adaptation to the new environment and this could also apply to squirrels (Diamond et al. 2022). However, plastic trait variation is not always beneficial or neutral (Lambert and Donihue 2020) and being a generalist that is able to successfully colonise urban habitats and exploit any available food is not without risks and costs, as shown in this project.

At first glance, high population densities of synurban species suggest positive effects for reproduction and / or survival in the urban habitat and accordingly, populations densities of urban squirrels in this project were many times higher than in the forest. Yet, the different squirrel densities in nearby urban parks suggest that effects vary greatly within a city, likely dependent on local factors, such as food availability and quality. There might be thresholds above which negative side effects from urbanisation, such as high competition and / or nutrient deficiency counteract the benefits of high supplemental food abundance (Anderies et al. 2007, Parker and Nilon 2012, Alberti et al. 2020). For example, it has been assumed that density thresholds above which a population can be considered synurban can subsequently lead to, e.g., synurbic-related behavioural changes (Parker and Nilon 2012). In view of the very high population density and the differences in foraging behaviour, I consider the Wohlerspark population to be a truly synurban.

My results though showed that urbanisation and low-quality food supply may have negative effects on body condition, foraging behaviour, nutrition and energy expenditure of squirrels from this park with highest supplementary feeding. Their lower body condition, higher sugar consumption and higher energy expenditure at higher T_a indicate maladaptive responses. Body mass and condition are highly relevant for survival and reproduction in squirrels, for example (Wauters and Dhondt 1995, Wauters et al. 1995). Notably, reduced body condition can even occur in successful urban species, but increases vulnerability to unexpected changes in their environment (Anderies et al. 2007). In addition to climate change or recurring urban planning-related changes to the urban habitat, the predicted spread of the grey squirrel (*Sciurus carolinensis*) from northern Italy across the Alpes in the coming decades could represent a new stress factor for urban *S. vulgaris*, as urban sites are highly likely to serve as source habitat for this invasive species (Bertolino et al. 2008, Wauters et al. 2023).

The results on the genetic diversity of squirrel populations at the different sites are still pending, but will help to answer the question of whether urban green habitat patches act more as isolated islands or refugees for squirrels. Initial data exploration indicated population structuring between urban parks, as known from some other species (Björklund et al. 2009, Munshi-South et al. 2016, Hurtado and Mabry 2019). Genetic structuring is not necessarily synonymous with adaptation to the urban habitat and could also be due to other effects, such as genetic bottlenecks or impediments in gene flow through the urban matrix (Donihue and Lambert 2015, Lambert and Donihue 2020). Notably, maladaptive behaviours such as a high sugar consumption as found in park squirrels, may serve as drivers of urban adaptation by promoting the evolution of genes that counteract negative effects (Caspi et al. 2022). However, small green spaces can only serve as suitable habitats for squirrels if gene flow is preserved and increased in future. Although squirrels can easily climb built structures, arboreal mammals are generally more isolated by fragmentation than terrestrial ones, and species movement seems to be impaired by the urban matrix (Danneck et al. 2023).

Finally, information on population structures can provide valuable insights into population health in terms of sink or source dynamics. I almost always caught more males than females, and the ratio of juveniles to adults was higher at urban sites. The former may indicate a sink population, while the latter may also indicate a source population, as has been assumed for urban fox squirrels (McCleery 2009, Rodewald and Gehrt 2014). Unfortunately, the sometimes low trapping success made it difficult to reliably compare population structures between the sites. Therefore, I cannot rule out the possibility that urban core fragments serve more as sink habitats or ecological traps (Zuñiga-Palacios et al. 2021). As indicated in this thesis, the abundant supplemental food sources could simulate a more valuable habitat, although they are of poor-quality and have negative effects on health-relevant traits or reproduction (Shipley et al. 2013). Hence, current high population densities may not equate to ‘urban winners’ in the long term, and further research is needed to assess whether or not these populations can persist over time. Furthermore, given the results from Chapter 2, experiments with individuals from different parks would be needed to clarify whether their behavioural and physiological responses depend on population density as well as food availability and composition in their park.

General conservation implications

Studying the response of urban wildlife to urbanisation is vital to reduce biodiversity loss and species homogenisation by mitigating negative factors through appropriate urban planning. My findings highlight the importance of increasing natural food sources for urban wildlife. Although interacting socioeconomic and cultural factors have to be considered in management decisions (Aronson et al. 2017), increasing plant richness and cover, e.g. by protecting or newly creating green habitat patches with high connectivity, is a key factor for urban biodiversity (McKinney 2002, Berthon et al. 2021). These measures can also support gene flow between park populations (Beninde et al. 2015, Lepczyk et al. 2017, Lambert and Donihue 2020). Moreover, urban planning should focus on planting diverse and preferably native species to ensure high quality food supply throughout the year (Alvey 2006, Berthon et al. 2021). A strong dominance of one tree species as found in one of the parks, may increase the vulnerability of the vegetation to plant pests, which in turn would also affect wildlife (Alvey 2006). On top of this, common management or horticultural practices in urban green spaces, such as tree pruning or leaf litter removal, which were common in the parks, can hinder nature conservation (Aronson et al. 2017). They can be very disruptive and probably also interfere with squirrels finding and hoarding natural food sources. Thus, reducing these practices in urban parks would be beneficial. Informing the public about 'wildlife-friendly' gardening could be another helpful tool, as residential gardens can also provide a valuable habitat opportunity for small to medium-sized mammals (Van Helden et al. 2020). Such wildlife-friendly management measures not only improve urban ecosystems for wildlife, but also can help to fulfil the social and ecological needs of humans (Luniak 2004).

Contact with and feeding of wildlife can even bring benefits to human health and well-being (Soulsbury and White 2016), but can also harbour risks (Dubois and Fraser 2013). Many people enjoy to offer supplemental food to wildlife, and it is probably not a realistic scenario to completely prevent human feeding (Dubois and Fraser 2013). However, my findings highlight the possible negative consequences for urban species and emphasise the importance of ecological education of human urban dwellers, e.g., through the use of information boards, so that they provide adequate food or limit their feeding (Cerri et al. 2020). In addition, a more widely dispersed food supply and the avoidance of clumped feedings sites, which increase intra- and interspecific contact rates, are likely recommendable. Besides this, my results stress the need for conservation-oriented waste management in cities to prevent people from littering and animals from having easy access to refuse (Cerri et al. 2020).

Finally, urban parks and mixed tree stands in particular provide several ecosystem services relevant to health and nature conservation, such as carbon sequestration or air purification

(Mexia et al. 2018). As urbanisation and global warming likely interact, increased urban greening measures are of great importance with regard to rising temperatures, as they can entail cooling effects (Li and Bou-Zeid 2013, Saaroni et al. 2018, Leal Filho et al. 2021, Sumasgutner et al. 2023). Mitigating the effect of climate change and associated heat waves in cities is essential for human well-being (Li and Bou-Zeid 2013, Ward et al. 2016, Zhao et al. 2018). My findings of a possibly maladaptive physiological response at high T_a in a synurban mammal highlight that this may be also crucial to reduce heat stress in urban wildlife species. This is particularly true my study region, since cities located in cooler climates of northern Europe are predicted to be more affected by heat waves than cities in southern Europe (Ward et al. 2016).

Project limitations and future research

This thesis provides insight into the responses of a common synurban mammal to living in the centre of a large city, although I acknowledge the limited sample size for some of the results and the fact that the influence of urbanisation on, for example, body condition can vary by sex, as I used only male individuals in my experiments (Auman et al. 2008, Lyons et al. 2017). However, my results are robust, fit with findings from other studies and add to the knowledge about urban mammal populations. The findings are only one piece of the puzzle though, as animals have to cope with multiple urban stressors simultaneously, which can lead to cumulative effects (Chen et al. 2023). Urbanisation and climate change are key challenges for global biodiversity, in particular the likelihood of their interactive effects and the importance of a conservation focus on both factors simultaneously have recently been highlighted (Haight et al. 2023, Sumasgutner et al. 2023). Importantly for this project, these two factors, together with other urban stressors, affect an animals' food supply, foraging behaviour and (digestive) physiology, thereby influencing a broad range of subsequent biological traits (Fig. 5.3, modified from Birnie-Gauvin et al. 2017). Future research should therefore investigate urban mammal populations with a focus on the possible interaction of urbanisation and associated diet with other factors, such as sex, climate change, pollution or digestive constraints as well as resulting fitness consequences to get a more comprehensive understanding of synurban species and their persistence over time.

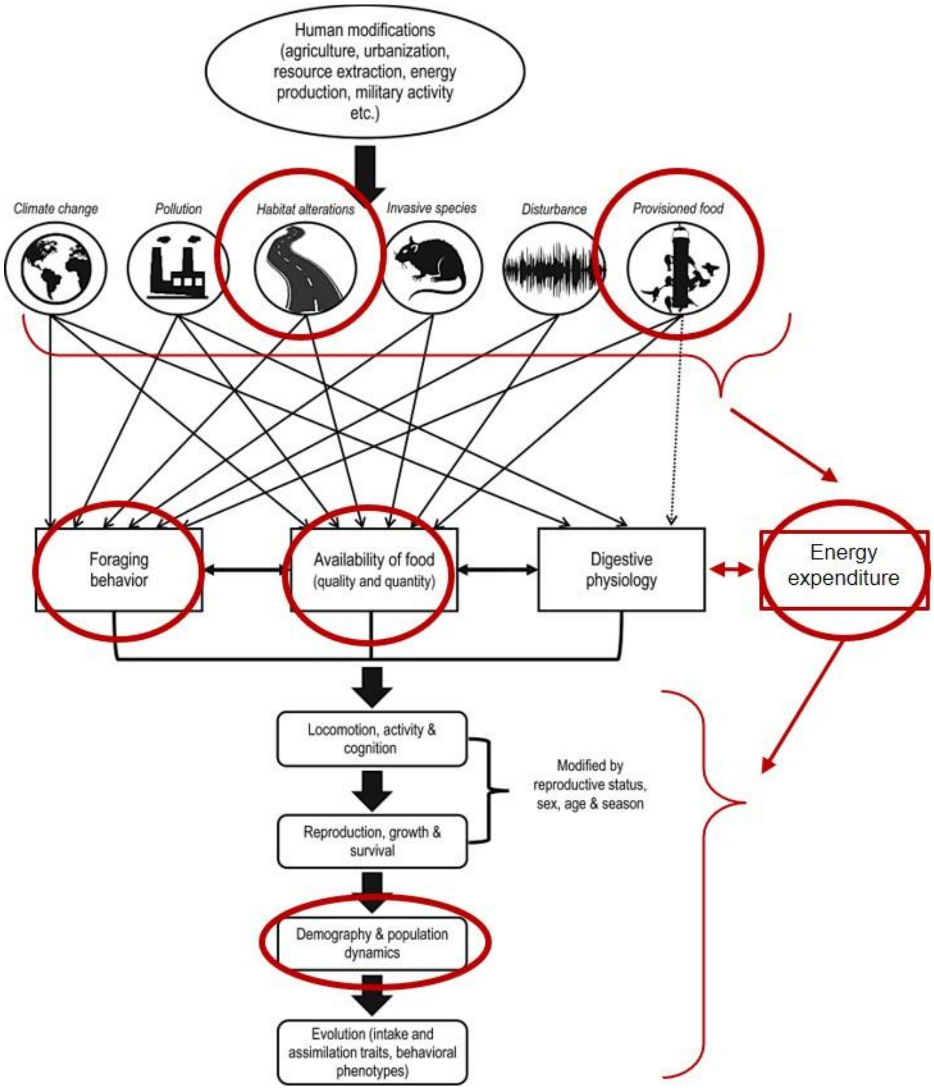


Figure 5.3: Graph modified from Birnie-Gauvin et al. 2017 (Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/)), showing the human induced effects on the components of animal nutrition, their interaction and consequences at different biological levels. Red circles mark the main variables and responses relevant to this thesis. To illustrate the whole project, energy expenditure has been added as additional relevant factor, which is likely to be affected by all environmental changes shown and is another important basis for all aspects of an animals' biology.

Beyond this, the trait variations observed in my project do not allow us to decide whether the intraspecific differences are related to developmental (irreversible) or (flexible) phenotypic plasticity or genetic adaptation. This question is not trivial and often remains unanswered

(Ouyang et al. 2018, Winchell et al. 2022). If individuals were housed over longer periods of time and in different T_a patterns, this could potentially show whether differences between groups persist or diminish over time, which would then indicate a flexible plastic response to habitat conditions rather than developmental plasticity or genetic adaptation. However, we would need translocation or 'common garden style' experiments to fully address this question (Lambert and Donihue 2020).

In addition, it is important to recognise that the response of species can vary across cities (delBarco-Trillo and Putman 2023, Haight et al. 2023). Urban stressors exert selective pressures on urban wildlife that trigger plastic responses, with the magnitude of the effect likely to vary by location (Dunn et al. 2022). The results from Chapter 2 demonstrate that this can already be the case at the local level, but this might be even more true across different cities. So far, studies comparing the response from urban populations around the world and especially in different climate zones are lacking. Studies from high-income and temperate countries are heavily overrepresented (McDonald et al. 2019, Zuñiga-Palacios et al. 2021). Wildlife in tropic or desert areas, for example, where the effects of heat waves can be amplified, may be exposed to different thermal constraints.

Cities are also shaped by geography, history, the age of the city, surrounding matrix, human population density as well as socio-politic, economic and cultural factors (Birnie-Gauvin et al. 2016, Ouyang et al. 2018, Miles et al. 2020, Dunn et al. 2022). For example, some cities can invest more money into biologically relevant structures like green corridors, simply have more space available or a different attitude of people towards wildlife. Thus, there can be significant differences in environmental conditions, such as the type and amount of vegetation cover, accessible waste or the willingness to feed wildlife (Aronson et al. 2017, Miles et al. 2020). In addition, the type and amount of food that can be found in refuse likely varies depending on local diet or the level of littering accepted within a society (Szulkin et al. 2020). Finally, it should be noted that *S. vulgaris* is a rather iconic species in Germany. Therefore, the extent of dietary changes may be different for a species that is less welcomed by humans and does not receive deliberately provided supplementary food (Perry et al. 2020, Egerer and Buchholz 2021).

General Conclusions

Global biodiversity is being severely impacted by rapidly increasing urbanisation. Studying the response of wildlife to a completely altered environment, distinct from the natural habitats in which they evolved, is of urgent importance for nature conservation. Despite living in high population densities that at first glance indicate suitable habitat, urban species respond to environmental conditions at different scales and experience multiple sublethal effects (Birnie-Gauvin et al. 2016). This thesis highlights the small-scale heterogeneity of urban habitats as well as the influence of urbanised diets and the urban heat island on wildlife. My findings show that urban squirrels show clear responses in terms of their population density, body condition, food choice behaviour, nutrient intake and physiology (Fig. 5.4). Not all results can be interpreted as neutral or beneficial in the long term, so there is a risk that they are maladaptive. Yet, responses may depend on the extent of local habitat modifications. Furthermore, species may also have delayed responses to environmental changes and only long-term studies can fully elucidate their long-term resilience and determine whether populations follow sink or source dynamics (Tilman et al. 1994, Hanski 1998, Isaksson 2018). However, this project contributes to a better mechanistic understanding of synurbanisation and its various consequences, which may help to mitigate the effects for a broader range of species. Urban greening measures, preventing waste disposal in public spaces or restricting wildlife access to rubbish bins and the like, as well as advising the public on supplementary feeding are essential conservation measures for urban wildlife in terms of diet quality and climate change.

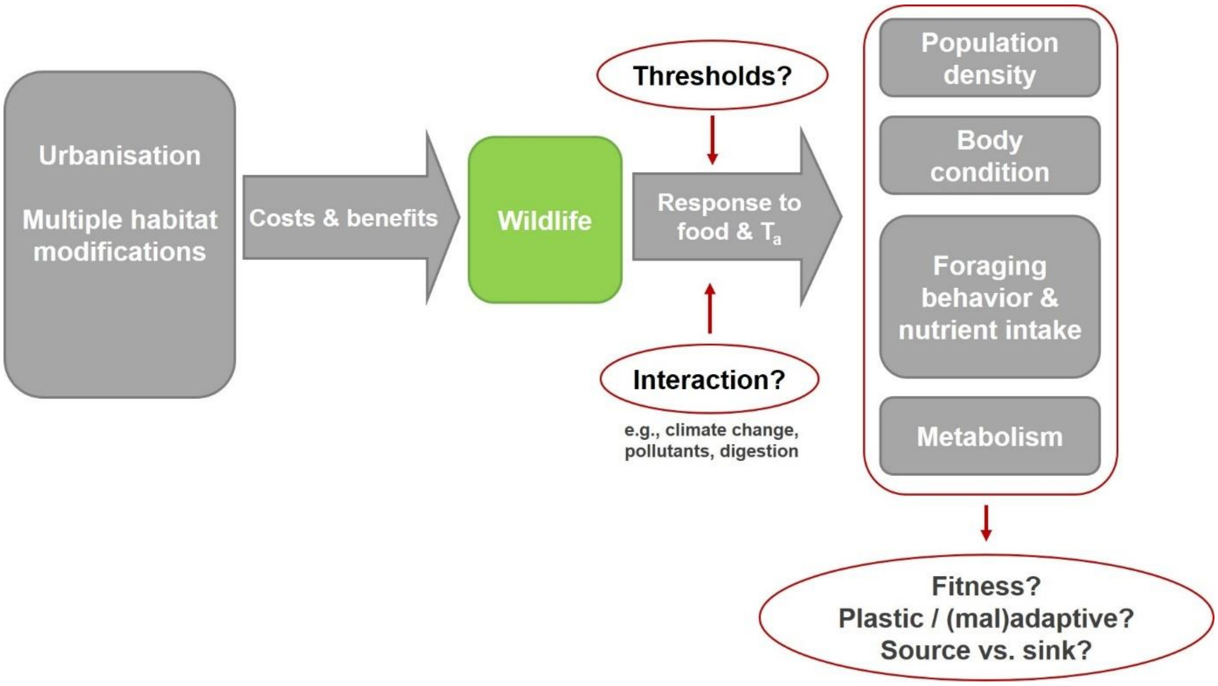


Figure 5.4: Infographic with the project’s key findings: Changes in urban habitat, e.g., in terms of food availability, affect urban wildlife populations and lead to changes in density, body condition, foraging behaviour and nutrient intake, as well as metabolism. However, local heterogeneity could influence the magnitude of changes at a given site and wildlife responses and / or their consequences might depend on whether or not a threshold is crossed. Further studies are needed to understand the long-term consequences on fitness and the interaction with covariates, such as climate change.

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Declaration of Oath

I hereby declare upon oath that I have written the present dissertation independently and have not used further resources and aids than those stated in the dissertation.

Bianca Wist

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“Mühsam ernährt sich das Eichhörnchen”

(Herkunft unbekannt)

Photo credit: Bianca Wist

I, the undersigned, declare that this bound copy of the dissertation and the dissertation submitted in electronic form (via upload) and the printed bound copy of the dissertation submitted to the faculty for archiving are identical.

Place, Date	First name and surname, signature