THE ECOLOGY OF RED FOXES (*VULPES VULPES*) IN ANTHROPOGENIC LANDSCAPES

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The ecology of red foxes (*Vulpes vulpes*) in anthropogenic landscapes

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“We must find time to stop and thank people who make a difference in our lives.”

John F. Kennedy

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TABLE OF CONTENT

SUMMARY

Human population is growing steadily (Goujon, 2019) and with this growth landscapes have been altered trough anthropogenic activities (Chase & Chase, 2016). An increasing amount of natural and semi-natural habitat is being transformed to provide residential space and associated infrastructure. Habitat transformation and loss of habitat connectivity exposes wildlife to new challenging conditions and novel environmental pressures including noise, chemical and light pollution (Grimm et al., 2008). Not all species can cope with these extensive and rapid changes. Urbanisation is identified as one of the main reasons for biodiversity loss (Altherr, 2007; Concepción et al., 2015; Luck & Smallbone, 2010; McKinney, 2006). Vertebrate loss is typically considered to be worst in urbanised areas because of intense and long-term disturbances that permanently alter habitats and depreciate food webs (Lombardi et al., 2017; McKinney, 2008). Nevertheless, there are always animal species that have adjusted to city life, so-called urban dwellers. An outstanding example of a successful urban dweller is the red fox (*Vulpes vulpes*). Foxes appear to be increasingly moving into human settlements throughout their range. Examples include Oslo, Norway (Christensen, 1985), Arhus, Denmark (Nielsen, 1990), Toronto, Canada (Adkins & Stott, 1998), Zurich, Switzerland (Gloor, 2002), or (in my focal area around) Berlin, Germany (Börner et al., 2009). Detailed knowledge of animal communities, food and competition relationships among the species, as well as species movement patterns and health status allows us to better understand the dynamics and predict the resilience of an ecosystem (Leibold et al., 2004). We need to know which characteristics allow species to persist in urban areas to prevent human-wildlife conflicts and promote biodiversity in cities. Identifying the biological traits favouring synurbisation is decisive to inform current management as well as to generate predictions for the future. In order to understand why the red fox is so successful in our anthropogenic world, we have to study different aspects of its ecology in, both, rural and urban settings. Therefore, this thesis investigates the diet, parasite spectrum and resting behaviour of red foxes along an urbanisation gradient in Berlin and Brandenburg (Germany). The diversity of these topics required the application of different analytical methods. For the investigation of the dietary strategies of rural and urban foxes, food niches were discovered using stable isotope analysis and compared with potentially available food items using Bayesian isotope mixing models (chapter 1). To study the diversity of helminths in the intestinal tract of foxes along a rural-urban gradient and to uncover environmental drivers of helminth communities I applied rarefaction curves, joint species distribution modelling (jSDM) and non-metric multidimensional scaling (NMDS) to helminth presence-absence data obtained by DNA metabarcoding. Finally, I compared the resting behaviour of foxes from Brandenburg and city foxes from Berlin using high resolution GPS and acceleration data. The assignment of behaviours based on the acceleration data served to determine temporal patterns of resting behaviour. Recurse analysis and the application of clustering algorithms allowed the identification of resting sites and their use. Our investigation on the red fox diet (Chapter 1) shows that dietary range of urban red foxes is smaller compared with that of rural conspecifics. Furthermore, higher δ13C values and lower δ15N values of urban foxes suggest relatively high input of anthropogenic food sources of urban foxes. Low within-individual variation compared to the between-individual variation lead to the conclusion that generalist fox populations consist of individual food specialists in urban and rural populations. Main results of the parasitological investigation (Chapter 2) show that the helminth diversity in the city Berlin is lower compared to surrounding rural Brandenburg and male red foxes tend to have higher helminth diversity than females. Diet features can drive helminth communities in red fox populations. Additionally, with increasing human population density, helminths transmitted via diet are less prevalent than pet-related helminths. Finally, I investigated habitat-dependent differences in resting patterns of red foxes from Berlin and Brandenburg (Chapter 3) and results revealed that urban foxes tend to rest more, with their resting behaviour concentrated during the day compared to rural red foxes. This increase in daily rest behaviour is reflected in an increased number of rest events. Moreover, the long-term resting events of city foxes last longer than those of foxes from Brandenburg. Even if rural foxes spend less time resting, rural red foxes of Brandenburg tend to have more resting sites compared to Berlin foxes. Overall, dietary specialisation and the use of anthropogenic food resources, in particular, have an overarching impact on the ecology of urban foxes. If proper food supply has such an extensive influence on the ecology, behaviour and lifestyle of red foxes, management strategies should focus on this topic. Reduced food availability would probably increase the competitive pressure within the fox population, reduce population density and thus also the contact rate between humans, domestic animals and foxes. Human-wildlife conflicts in the city could thus be reduced and the general acceptance of wild animals in the city increased. This could ultimately lead to the sharing of urban areas by humans and wildlife.

ZUSAMMENFASSUNG

Die menschliche Bevölkerung wächst stetig (Goujon, 2019) und mit diesem Wachstum haben sich die Landschaften durch anthropogene Aktivitäten verändert (Chase & Chase, 2016). Immer mehr natürlicher und halbnatürlicher Lebensraum wird umgewandelt, um Wohnraum und die dazugehörige Infrastruktur bereitzustellen. Die Habitattransformation und der Verlust der Habitatkonnektivität setzen Wildtiere vor neue Herausforderungen wie Lärm-, Chemikalien- und Lichtverschmutzung aus (Grimm et al., 2008). Nicht alle Arten können mit diesen umfangreichen und raschen Veränderungen umgehen. Die Verstädterung wird als einer der Hauptgründe für den Verlust der biologischen Vielfalt genannt (Altherr, 2007; Concepción et al., 2015; Luck & Smallbone, 2010; McKinney, 2006). Der Verlust an Wirbeltieren wird typischerweise in urbanisierten Gebieten am schlimmsten eingeschätzt, da intensive und langfristige Störungen die Lebensräume dauerhaft verändern und die Nahrungsnetze modifizieren (Lombardi et al., 2017; McKinney, 2008). Dennoch gibt es immer wieder Tierarten, die sich an das Stadtleben angepasst haben, so genannte Kulturfolger. Ein herausragendes Beispiel für die erfolgreiche Besiedelung der Stadt ist der Rotfuchs (Vulpes vulpes). Füchse scheinen in ihrem gesamten Verbreitungsgebiet zunehmend in menschliche Siedlungen zu ziehen. Beispiele hierfür sind Oslo, Norwegen (Christensen, 1985), Arhus, Dänemark (Nielsen, 1990), Toronto, Kanada (Adkins & Stott, 1998), Zürich, Schweiz (Gloor, 2002) oder (in meinem Schwerpunktgebiet) Berlin, Deutschland (Börner et al., 2009). Detaillierte Kenntnisse über Tiergemeinschaften, Nahrungs- und Konkurrenzbeziehungen zwischen den Arten sowie über die Bewegungsmuster und den Gesundheitszustand der Arten ermöglichen es uns, die Dynamik eines Ökosystems besser zu verstehen und seine Widerstandsfähigkeit vorherzusagen (Leibold et al., 2004). Wir müssen wissen, welche Faktoren das Fortbestehen von Arten in städtischen Gebieten ermöglichen, um Konflikte zwischen Mensch und Wildtieren zu verhindern und die biologische Vielfalt in Städten zu fördern. Die Identifizierung der biologischen Merkmale, die die

Synurbisierung begünstigen, ist entscheidend für die Entwicklung von Wildtiermanagementstrategien und um Vorhersagen für die Zukunft zu erstellen. Um zu verstehen, warum der Rotfuchs in unserer anthropogenen Welt so erfolgreich ist, müssen wir verschiedene Aspekte seiner Ökologie sowohl in ländlichen als auch in städtischen Gebieten untersuchen. In dieser Arbeit werden deshalb die Ernährung, das Parasitenspektrum und das Ruheverhalten von Rotfüchsen entlang eines Urbanisierungsgradienten in Berlin und Brandenburg (Deutschland) untersucht. Die Vielfalt dieser Themen erforderte die Anwendung verschiedener Analysemethoden. Zur Untersuchung der Ernährungsstrategien von ländlichen und städtischen Füchsen wurden Nahrungsnischen mit Hilfe der Stabilisotopenanalyse untersucht und mit potentiell verfügbaren Nahrungsressourcen verglichen (Kapitel 1). Zur Untersuchung der Diversität von Helminthen im Darmtrakt von Füchsen entlang eines ländlich-urbanen Gradienten und zur Identifizierung von Umwelteinflüssen auf Helminthen-Gemeinschaften wurden rarefaction Analysen, joint species distribution modeling (jSDM) und non-metric multidimensional scaling (NMDS) genutzt. Die Identifizierung der Helminthen im Darmtrakt von Rotfüchsen erfolgte mittels DNA Metabarcoding (Kapitel 2). Schließlich verglich ich das Ruheverhalten von Füchsen aus Brandenburg und Stadtfüchsen aus Berlin unter Verwendung von hochauflösenden GPS- und Beschleunigungsdaten. Die Zuordnung von Verhaltensweisen auf der Grundlage der Beschleunigungsdaten diente der Bestimmung zeitlicher Muster des Ruheverhaltens. Eine Recursanalyse und die Anwendung von Clustering-Algorithmen ermöglichten die Identifizierung von Ruheplätzen und deren Nutzung. Unsere Untersuchung zur Rotfuchsernährung (Kapitel 1) zeigt, dass die Nahrungsnische der städtischen Rotfüchse im Vergleich zu dem der ländlichen Artgenossen kleiner ist. Darüber hinaus deuten höhere δ13C Werte und niedrigere δ15N Werte darauf hin, dass städtische Füchsen einen relativ hohen Anteil an anthropogener Nahrung in ihre Ernährung integrieren. Geringe Variation der Isotopenwerte auf Individuenebene im Vergleich zur Variation zwischen den Individuen führt zu der Schlussfolgerung, dass generalistische Fuchspopulationen aus einzelnen Nahrungsspezialisten in städtischen und ländlichen Populationen bestehen. Die Hauptergebnisse der parasitologischen Untersuchung (Kapitel 2) zeigen, dass die Helminthendiversität in der Stadt Berlin im Vergleich zum umliegenden ländlichen Brandenburg geringer ist und männliche Rotfüchse tendenziell eine höhere Helminthendiversität aufweisen als Weibchen. Die Ernährung kann Helminthengemeinschaften in Rotfuchspopulationen beeinflussen. Darüber hinaus sind mit zunehmender menschlicher Populationsdichte über die Nahrung übertragene Helminthen weniger verbreitet als mit Haustieren assoziierte Helminthen. Schließlich untersuchte ich lebensraumabhängige Unterschiede in den Ruhemustern von Rotfüchsen aus Berlin und Brandenburg (Kapitel 3). Die Ergebnisse zeigen, dass städtische Füchse dazu neigen, mehr zu ruhen, wobei sich ihr Ruheverhalten im Vergleich zu ländlichen Rotfüchsen stark auf den Tag konzentriert. Diese Steigerung im täglichen Ruheverhalten spiegelt sich in einer erhöhten Anzahl von Ruheereignissen wider. Zudem dauern die Ruhephasen der Stadtfüchse länger als die der Füchse aus Brandenburg. Auch wenn ländliche Füchse weniger Zeit mit Ruhen verbringen, haben rurale Rotfüchse Brandenburgs im Vergleich zu Berliner Füchsen tendenziell mehr Ruheplätze.

Insgesamt haben vor allem die Spezialisierung der Ernährung und die Nutzung anthropogener Nahrungsressourcen einen umfassenden Einfluss auf die Ökologie der Stadtfüchse. Wenn die Ernährung einen so weitreichenden Einfluss auf die Ökologie, das Verhalten und die Lebensweise von Rotfüchsen hat, sollten sich Managementstrategien mehr auf dieses Thema konzentrieren. Ein vermindertes Nahrungsangebot würde wahrscheinlich den Konkurrenzdruck innerhalb der Fuchspopulation erhöhen, die Populationsdichte und damit auch die Kontaktrate zwischen Mensch, Haustier und Fuchs verringern. Mensch-Wildtier-Konflikte in der Stadt könnten so verringert und die allgemeine Akzeptanz von Wildtieren in der Stadt erhöht werden. Dies könnte letztlich dazu führen, dass sich Mensch und Wildtiere die städtischen Gebiete teilen.

GENERAL INTRODUCTION

Facing the Anthropocene

Human population is growing steadily (Goujon, 2019) and with this growth landscapes have been altered trough anthropogenic activities (Chase & Chase, 2016). An increasing amount of natural and semi-natural habitat is being transformed to provide residential space and associated infrastructure. Urban agglomerations are emerging, roads and railways are being built, rivers straightened, the soil searched for raw materials, forests managed and large areas transformed into agricultural land to feed livestock and people. This reaches extreme dimensions, especially in industrialised countries like Germany. Yet, the direct conversion of the land is only one side. At the same time, habitat transformation leads to fragmented landscapes and decreased habitat connectivity. Habitat transformation and loss of habitat connectivity exposes wildlife to new challenging conditions and novel environmental pressures including noise, chemical and light pollution (Grimm et al., 2008). Not all species can cope with these extensive and rapid changes. It is therefore no surprise that we are facing a biodiversity crisis. The current rate of global species extinction exceeds the assumed natural extinction rate by 100 to 1,000 times. Up to 50% of species are predicted to be lost in the decades (Pimm & Raven, 2000). And biodiversity is continuously decreasing worldwide. Humans - either directly or indirectly - are the main cause of this decline in diversity (Barlow et al., 2016; Hens & K Boon, 2005; Wood et al., 2013).

Urbanisation is identified as one of the main reasons for biodiversity loss (Altherr, 2007; Concepción et al., 2015; Luck & Smallbone, 2010; McKinney, 2006). Today, more people worldwide inhabit cities than rural areas (Mcdonald et al., 2008), in Germany it is more than three-quarters of the population. Cities are characterised by a high density of buildings, a dominance of sealed surfaces and of course a high human population density (Grimm et al., 2008). Existing cities are continuing to grow even bigger, but also the number of cities is increasing. This on-going urbanisation is an example of drastic transformation of natural habitats. Inevitably, pollution and noise are omnipresent in many areas of the world and wild animals must live in close contact with people.

Some wildlife species are able to cope with the on-going changes and avoid or adapt to urban areas. Studies show, however, that there is an overall decrease in animal diversity along the rural-urban gradient (reviewed by McKinney, 2006). For a large number of species, population density is declining or they may even become locally extinct. Animal species that depend on complex vegetation and plant species are particularly negatively affected by urbanisation (McKinney, 2008). Vertebrate loss is typically considered to be worst in urbanised areas because of intense and long-term disturbances that permanently alter habitats and depreciate food webs (Lombardi et al., 2017; McKinney, 2008). Mammal species, especially those with larger home ranges such as carnivores, have particular problems to cope with the conditions in cities (Bateman & Fleming, 2012). On the other side, several species show amazing adjustments to everyday challenges of city live. For example, some species of birds sing notably louder in cities than in natural habitats to drown out the everyday noise (Nemeth et al., 2013; Slabbekoorn & Peet, 2003). Kammanolis lizards (*Anolis cristatellus*) in the cities of Puerto Rico adapt to life on artificial surfaces: These city animals have longer limbs and more toe lamellae than their rural counterparts (Winchell et al., 2016).

At the same time, cities can form very diverse habitats. Within a small area one can find many different landscape elements and habitat types. Parks, city forests, cemeteries and garden settlements offer green islands. Cities therefore also hold a certain potential for wildlife, offering a variety of different types of shelters and weather protection. Very dense development, lack of vegetation and specific thermal conditions can lead to high average temperatures, low humidity and low wind speed in cities which is advantageous for some species (McKinney, 2008). In addition, cities offer a wide range of food sources. This includes discarded food, compost, fruit and vegetables grown in gardens, carcasses of road kills or direct supplementary feeding by humans (Contesse et al., 2004; Lowry et al., 2013). In an urban environment, hunting and predation pressures for many animal species are also noticeably lower than in natural habitats (Fischer et al., 2012). Whoever can profit from these advantages of the city has a decisive selection benefit. Some species have even adapted to the new environment and seem to benefit from us humans (McKinney, 2002).

The framework of urban dwellers

The discipline of urban ecology evolved in the 1990s motivated by the broad interest in documenting the abundance and distribution of flora and fauna in cities. As the field has developed the necessary terminology was also established by Blair (1996) and further developed by McKinney (2002). Recently, as a result of further developments in the field, the conceptual framework has been revised so that the terms urban avoiders, urban utilizer and urban dweller form now a gradient of responses to urbanization (Fischer et al., 2015). Urban avoiders are species that rarely occur in developed areas or just persist in natural areas embedded in urban habitats. Urban utilizers still depend on natural areas but may disperse into urban areas from neighbouring natural habitats. On the contrary, the persistence of urban dwellers in urban landscapes is totally independent of natural areas. Importantly, population responses to urbanisation can vary greatly within species of urban dwellers. Therefore, understanding population responses to urbanisation within the group of urban dwellers offers the possibility to support population stability of other species in urban areas to maintain and hopefully increase future biodiversity of cities. An outstanding example of a successful urban dweller is the red fox (*Vulpes vulpes*).

The red fox - a model of success

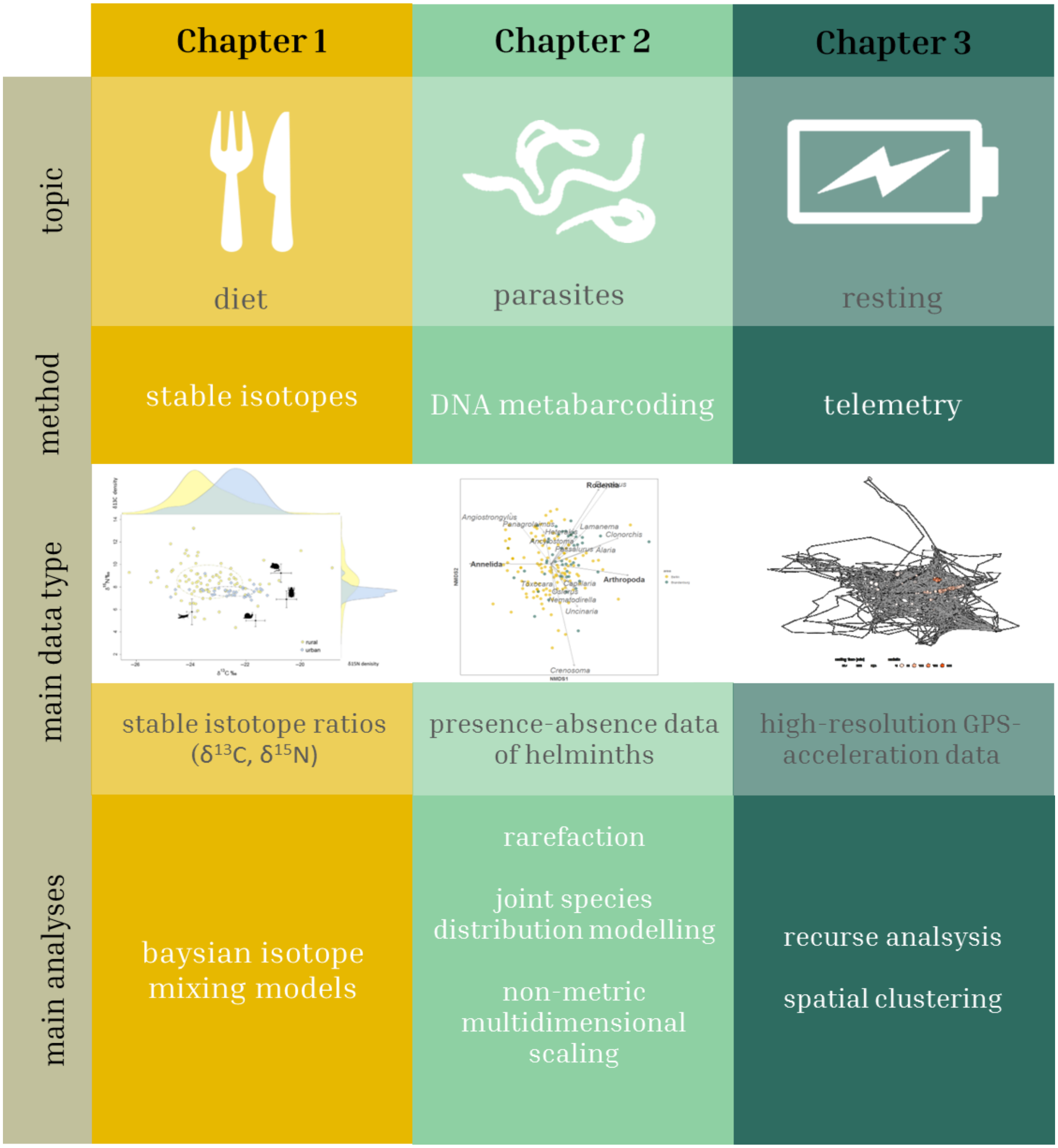
The red fox has the largest geographic range of all wild predators: its range covers almost the entire Northern Hemisphere from the Arctic Circle to North Africa, Central America and Eurasia (Macdonald, 2011). They can live both north of the Arctic Circle and in almost tropical areas. Originally, however, red foxes inhabited Eurasia. During glaciation they spread to America. In Australia and a number of Pacific islands, red foxes were released by settlers as game, but also to control rabbit infestations (G. R. Saunders et al., 2010). Since the marsupial fauna found there was not prepared for the adaptable newcomer as a predator, a lot of effort has been made for years to eliminate foxes in Australia (Gentle et al., 2007; Mahon, 2009; G. Saunders et al., 1995). This is why the red fox is also on the list of the "world's 100 most invasive species".

As widespread as the red fox is, it also inhabits a wide variety of landscapes. They are present in natural habitats characterised by forests and grasslands (Kurki et al., 1998; Meisner et al., 2014) and human-altered agricultural landscapes (Goldyn et al., 2003). They even live in deserts or coastal regions including sandy beaches and dunes (Cavallini & Lovari, 1994) and are comfortable in mountain landscapes up to 3000 m altitude (Meia & Weber, 1993; Weber et al., 1994). And increasingly foxes also discover the advantages of big city life. In Great Britain, the occurrence of foxes in cities like London, Bristol and Oxford has been known since the 1930s (Teagle, 1967). Since the mid-1980s, foxes have also been observed in cities in continental Europe, Canada and Japan. Foxes appear to be increasingly moving into human settlements throughout their range. Examples include Oslo, Norway (Christensen, 1985), Arhus, Denmark (Nielsen, 1990), Toronto, Canada (Adkins & Stott, 1998), Zurich, Switzerland (Gloor, 2002), or (in my focal area around) Berlin, Germany (Börner et al., 2009).

The first evidence of foxes in Berlin dates back to the 1950s (Saar, 1957), and since the 1990s Berlin has been widely populated with foxes (Börner et al., 2009). In recent decades there has been a substantial increase in the population density of red foxes in Germany (Börner, 2014; Keuling et al., 2011). In this context, successful rabies immunisation is mentioned as the main reason. Since rabies is a dangerous disease not only for animals but also for humans, efforts have been made to eradicate the pathogen from the fox population over a wide area (Müller et al., 2005). The campaign lasted several years and was a spectacular success in the control of animal epidemics: since 1991, Germany has been considered rabies-free and, according to the criteria of the World Organisation for Animal Health, terrestrial rabies has been officially eradicated from Germany since 2008. Most likely, this is the reason why the number of foxes has risen strongly, as evaluations of hunting records show. Stiebling (2000) reports for a region in the north-east of Brandenburg that during the outbreak of rabies, the highest population density of red foxes was 0.8 individuals/km^2. After the oral rabies immunisation campaign has started in 1991, the population rose to a maximum density of 1.6 individuals/km^2. Moreover, large predators such as wolf (*Canis lupus*) and lynx (*Lynx lynx)* were eliminated in Germany a long time ago. And even if some individuals are coming back to Germany again, competition and predation pressure for foxes is still low. All this, the adjustment to a life with humans, the eradication of rabies and the lack of predation pressure and competition, points to a strong increase in the population density of red foxes in Germany over the last decades. Even hunting cannot limit their steady expansion. But undoubtedly decisive for their success is their generalist nature, their extreme adaptability and flexibility. These characteristics certainly allowed red foxes to colonise artificial habitats such as cities but their spread is also influenced by patterns of human tolerance because the success of red foxes may also lead to human-wildlife conflicts.

Thesis outline

The red fox is both predator and hunted, therefore it experiences its habitat simultaneously as a "landscape of resources", where it finds food or mating partners, and as a "landscape of fear" (according to Laundré et al., 2010)), where it can be hunted by humans all year round. This trade-off constantly confronts it with new decisions and has a major impact on its land-use behaviour, especially in highly dynamic habitats that are subject to sudden and massive transformations and where food supply and cover can change very rapidly. Since the fox is a host and transmitter of the pathogens of various zoonoses and other diseases such as rabies, distemper, leptospirosis, echinococcosis or sarcoptic mange, which are also transmitted to humans and can sometimes be fatal (Lloyd, 1980), its high mobility is also of additional relevance for humans. Frequently, outbreaks of such diseases led to a collapse in the population size and density of foxes, which had a demonstrably decisive influence on predator-prey relationships and thus also on the dynamics and diversity of species communities in the studied ecosystems (e.g. Chautan et al., 2000; Forchhammer & Asferg, 2000). Detailed knowledge of animal communities, food and competition relationships among the species, as well as species movement patterns and health status allows us to better understand the dynamics and predict the resilience of an ecosystem (Leibold et al., 2004). Urban animals not only transmit zoonotic diseases, but also damage structures, attack domestic pets and humans, leave faeces, loot rubbish bins and dig in gardens or are the cause of traffic accidents (Soulsbury & White, 2015). We need to know why some species cope better than others with urban environments but also which characteristics allow them to persist to prevent human-wildlife conflicts and promote biodiversity in cities. Identifying the biological traits favouring synurbisation is decisive to inform current management as well as to generate predictions for the future. In order to understand why the red fox is so successful in our anthropogenic world and what distinguishes it from other species, we have to study its way of life in both rural and urban settings. In this way we can identify differences in the behaviour and ecology of this wildlife species and highlight which of these adjustments are due to specific environmental factors that vary between urban and rural environments. It is not enough to focus on a specific topic, as different ecological aspects determine whether or not a species can persist in one place. Therefore, this thesis investigates the ecology of red foxes along an urbanisation gradient in Berlin and Brandenburg (Germany) and applies an overarching approach (figure 1) to study different aspects of red fox ecology in anthropogenic landscapes.



	Chapter 1	Chapter 2	Chapter 3
topic	diet	parasites	resting
method	stable isotopes	DNA metabarcoding	telemetry
main data type	stable istotope ratios ($\delta^{13}C$, $\delta^{15}N$)	presence-absence data of helminths	high-resolution GPS-acceleration data
main analyses	baysian isotope mixing models	rarefaction joint species distribution modelling non-metric multidimensional scaling	recurse analsysis spatial clustering

Figure 1: Overview of the topics, methods, type of data and main analyses integrated in this thesis.

Chapter 1

One of the decisive factors for surviving in a place is food availability (Seward et al., 2013; Warner et al., 2015). Such considerations are more easily met for generalist species (i.e. that do not specialize in any particular food source) because if one resource is scarce, they can rely on other food sources. They are also able to tap into new food sources (Ducatez et al., 2015). The diet of red foxes is very diverse and varies between regions (Cavallini & Volpi, 1996; Contesse et al., 2004; Goldyn et al., 2003). In general, foxes adjust their diet to their habitat, the season and the prevailing resources (Dell'Arte et al., 2007). Thus, the fox's diet often reflects the resources availability in the landscape, with preference for easily captured food sources (Newsome et al., 2010). This probably explains why foxes in urban areas show a high proportion of anthropogenic food in their diet (Contesse et al., 2004; Harris, 1981; Scholz et al., 2020). Anthropogenic food includes discarded human food, compost, fruit and vegetables from gardens or pet food. Additional food sources may be (illegal) supplementary feeding from people or carcasses from animals killed by traffic. The establishment of such food sources inevitably leads to human-wildlife interactions and possibly conflicts (Soulsbury & White, 2015). The advantage of anthropogenic food in cities is the year-round availability and predictability. In addition, often only little energy needs to be expended to obtain these food resources. Nevertheless, prey animals such as mice, snails, insects and birds would of course also be available for foxes but they seem to play only a minor role for urban foxes. However, previous studies on the feeding behaviour of foxes along an urban-rural gradient have been carried out at the population level. Such studies were just a snapshot in time and need to be further investigated. To fill the lack of knowledge, Chapter 1 will investigate the extent to which the feeding niches of rural and urban foxes overlap and if the individual feeding behaviour changes over time. In particular, I further investigated whether foxes constantly and permanently integrated anthropogenic food into their diet and whether the nutritional strategies of urban foxes differed from those of their rural relatives. We used stable isotope ratios of red fox whiskers to quantify and compare the width of the isotopic niche and the feeding tactics of urban and rural red foxes at the population level and the individual level. To assess individual isotope specialisation, we used carbon (δ13C) and nitrogen (δ15N) stable isotope ratios of vibrissae increments, which provided a temporally continuous isotope record within the same individual. To differentiate the feeding habits of red foxes, we compared stable isotope ratios of red foxes with those of potential food using Bayesian isotope mixing models. Due to the individualised data basis, we gain insight into the nutritional strategy of the red fox for the first time.

Chapter 2

The red fox is known as a vector of various pathogens of diseases. Rabies, sarcoptic mange and canine distemper are the best known (Al-Sabi et al., 2014; Gras et al., 2018; Holmala & Kauhala, 2006). However, endoparasites such as gastrointestinal helminths also are common for red foxes, can be transmitted to humans or other animals and in some cases have dramatically health consequences for them (Brochier et al., 2007; Laurimaa et al., 2016; McCarthy & Moore, 2000). The risk associated with such zoonoses is often reflected in people's perception of wild animals. An extensive survey has shown that although the majority of city inhabitants are pleased to see a fox, more than half of them were afraid of red foxes because of the associated health risk (König, 2008). Health-risk is thus one of the major sources of conflict between humans and wildlife. In future, the risk for human health will probably increase as urban planning is focusing on the establishment of wildlife friendly initiatives such as an increase in urban greening, the establishment of rural–urban corridors and more urban–rural fringe habitats (Deplazes et al., 2004). The availability of greener infrastructure in urban areas may improve transmission opportunities for a number of zoonotic diseases (Santiago-Alarcon et al., 2013). If species richness and population densities of wild animals also increase, this has the potential to promote transmission rates and therefore increase fears of wildlife from residents and human-wildlife conflicts. The promotion of communication and education as well as the demonstration of willingness to address the issue will often have a positive impact on the attitudes of people in the context of conflict with wildlife (Baruch-Mordo et al., 2011; Espinosa & Jacobson, 2012; Madden, 2004). However, this requires appropriate and regularly updated data bases, if necessary at regional level. The need of education also highlights the necessity of monitoring wildlife population to allow a proper assessment of the current health status, parasitic burden and their role in zoonotic disease transmission. On the other side, intestinal parasites naturally also endanger the health of the fox itself and thus influence the survival of the individual and the population development (Eira et al., 2006). To understand whether the occurrence of certain helminth species and their diversity are influenced by certain traits and whether the parasites differ from urban and rural foxes, Chapter 2 relates presence-absence data of helminths identified by DNA metabarcoding to different environmental variables, traits and diet using non-metric multidimensional scaling and joint species distribution modelling. This will help to understand the occurrence and distribution of red fox parasites and to assess potential threats to humans and pets.

Chapter 3

Organisms that actively move and thus connect landscapes and habitats in space and time serve as vectors and are called "mobile links" in current scientific discussions (Lundberg & Moberg, 2003). The concept of 'mobile links' is embedded in the very modern and innovative area of "Movement Ecology". Movement in the sense of a change of location plays a central role in many ecological and evolutionary processes. The research of movement patterns of living organisms in connection with their physiology, or the interaction between them and their environment is of primary importance (Nathan & Giuggioli, 2013). ‘Movement Ecology' is a very young and emerging field that has incredibly benefited from the technical developments of the past years. The use of new, temporally and spatially high-resolution methods, which provide not only positional information but also activity patterns, makes it possible to map behavioural ecological reactions to anthropogenic interventions. Previous research has already provided surprising evidence that animal movements are influenced by human disturbances, e.g. hunting (Scillitani et al., 2010), landscape remodelling (Hilty et al., 2012) or road construction and traffic (Roedenbeck & Voser, 2008; D. B. Shepard et al., 2008) and to a lesser extent by food resources or interspecific competition. Investigation by telemetry is a method that has been practiced and established for years to investigate the movement patterns of animals (for an overview see Cooke et al., 2004). However, telemetry data can only provide information about the whereabouts at a certain time - information about specific behaviour of individuals remains inaccessible by this method alone. A novel method combines GPS and three-dimensional acceleration measurement using acceleration sensors (ACC). It promises a more precise analysis of behaviour and energy consumption in both temporal and spatial perspectives (Nathan et al., 2012; E. L. Shepard et al., 2008; Tanaka et al., 2001; Watanabe et al., 2005). Behaviour can be typified by body movement and posture, and by means of ACC, behavioural categories of varying complexity (e.g. resting, eating, walking, social interactions) can be distinguished and identified (Nathan et al., 2012; E. L. Shepard et al., 2008). Thanks to this novel method, it is now possible to obtain data sets on the behaviour of free-living animals that are difficult to observe because of their cryptic way of life (see Watanabe et al., 2005; Wilson et al., 2008). By combining ACC data with spatial information via GPS, 'maps of land use' can be produced, which visualise the significance of places. Roughly summarised: ACC data offer an insight into the behaviour, whereas GPS associates these data with observed tracks and environmental factors (Nathan et al., 2012). Thus, this method offers the possibility of an integrative analysis of movement within the movement ecology framework. In chapter 3 we used high-resolution GPS and ACC data to reveal habitat-dependent differences in the resting patterns of rural and urban red foxes. Recurse analysis was used to detect resting sites based on the movement and behavioural data. After spatial clustering, we uncovered transmission between those sites.

Objectives of the thesis

To recapitulate briefly: The overall aim of this work was to describe and understand different aspects of the ecology of red foxes in anthropogenic landscapes. For this purpose, I compared diet, parasite spectrum and resting behaviour of rural foxes from Brandenburg to urban foxes from Berlin. The diversity of these topics required the application of different analytical methods. For the investigation of the dietary strategies of rural and urban foxes, food niches were discovered using stable isotope analysis and compared with potentially available food items using Bayesian isotope mixing models (chapter 1). To study the diversity of helminths in the intestinal tract of foxes along a rural-urban gradient and to uncover environmental drivers of helminth communities I applied rarefaction curves, joint species distribution modelling (jSDM) and non-metric multidimensional scaling (NMDS) to helminth presence-absence data obtained by DNA metabarcoding. Finally, I compared the resting behaviour of foxes from Brandenburg and city foxes from Berlin using high-resolution GPS and acceleration data. The assignment of behaviours based on the acceleration data served to determine temporal patterns of resting behaviour. Recurse analysis and the application of clustering algorithms allowed the identification of resting sites and their use.

Chapter 1 - Individual dietary specialization in a generalist predator: A stable isotope analysis of urban and rural red foxes

At the population level, we hypothesize that isotope signatures of urban red foxes differ significantly from those of their rural counterparts. Taking into account previous studies on the food ecology of red foxes, we assume that the urban population consumes a larger proportion of anthropogenic food sources (e.g. leftover food, rubbish and pet food) consisting of a mixture of different foods that are in isotopic contrast to natural food sources. We, therefore, predict

(1) a smaller isotopic niche for urban foxes, since cities have a relatively constant supply of anthropogenic food over time and space.

In contrast, the abundance and availability of food resources in rural areas are habitat-dependent and variable over time and space, which should be reflected in

(2) a larger food niche (isotopic niche) for rural foxes compared to urban foxes.

If one assumes that foxes nevertheless focus on the best available and most easily obtainable food within their individual range, this should represent a large proportion of fox food intake and thus lead to

(3) low temporal variability of isotope signatures in rural and urban foxes (individual level).

Therefore, both rural and urban red fox individuals follow an (optional) specialised feeding tactic, even though foxes are a generalist species at a population level.

Chapter 2 - Lower helminth diversity in urban red foxes, but with higher association to pets

We postulate that urbanisation influences the population structure, land use and behaviour of the red foxes and thus their community. We predict that

(1) foxes in rural areas have a higher helminthic diversity, together with a higher diversity of their diet.

Males, which tend to have more extensive space use, will also

(2) have a higher helminth diversity than females.

As helminths with complex life cycles depend on multiple hosts (intermediate and primary host), occurrence of helminths depends on the frequency of their host and the uptake of this host by red foxes. We therefore assume that

(3) helminths transmitted through food are mainly found in natural environments, as the red fox diet in urban areas is based on anthropogenic rather than natural food resources.

As the contact rate between red foxes and humans or their pets increases with higher red fox densities, we predict

(4) foxes in close proximity to humans will show a higher prevalence of helminths associated with humans or pets.

Chapter 3 - The city never sleeps but city foxes do – habitat-dependent differences in spatial and temporal patterns of resting behaviour in a synanthropic species

We hypothesise that different living conditions in urban and rural areas, which lead to behavioural adjustments and different lifestyles in red foxes, influence the resting behaviour of red foxes. We predict that

(1) the daily resting amount and, number and duration of resting events will be higher in urban red foxes compared to rural red foxes, due to reduced foraging and increased consumption of anthropogenic food described for urban foxes.

In addition, we predict that

(2) greater home ranges of rural foxes compared to urban populations and increased availability of natural structures will lead to a higher number of resting sites and a higher transmission rate between resting sites in rural foxes compared to urban foxes.

Chapter 1 - Individual dietary specialization in a generalist predator: A stable isotope analysis of urban and rural red foxes

Individual dietary specialization in a generalistic predator: a stable isotope analysis of urban and rural red foxes

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Abstract

Some carnivores are known to survive well in urban habitats, yet the underlying behavioral tactics are poorly understood. One likely explanation for the success in urban habitats might be that carnivores are generalist consumers. However, urban populations of carnivores could as well consist of specialist feeders. Here, we compared the isotopic specialization of red foxes in urban and rural environments, using both a population and an individual level perspective. We measured stable isotope ratios in increments of red fox whiskers and in potential food sources. Our results reveal that red foxes have a broad isotopic dietary niche and a large variation in resource use. Despite this large variation, we found significant differences between the variance of the urban and rural population for $\delta^{13}C$ as well as $\delta^{15}N$ values, suggesting a habitat specific foraging behavior. Although urban regions are more heterogeneous regarding land cover (based on the Shannon index) than rural regions, the dietary range of urban foxes was smaller compared to that of rural conspecifics. Moreover, the higher $\delta^{13}C$ values and lower $\delta^{15}N$ values of urban foxes suggest a relatively high input of anthropogenic food sources. The diet of most individuals remained largely constant over a longer period of time. The low intra-individual variability of urban and rural red foxes suggests a relatively constant proportion of food items consumed by individuals. Urban and rural foxes utilized a small proportion of the potentially available isotopic dietary niche as indicated by the low within-individual variation compared to the between-individual variation. We conclude that generalist fox populations consist of individual food specialists in urban and rural populations at least over those periods covered by our study.

1 Introduction

Our environment is subject to constant anthropogenic influence, with urbanization being among the most outstanding examples of habitat transformation for wildlife species (Grimm et al.; Magle et al. 2012), including vital food sources. This transformation directly and indirectly affects wildlife at the individual (Newsome et al. 2015), population (Davison et al. 2009) and community level (Prange and Gehrt 2004). Although urbanization is one of the major threats to global biodiversity

(McKinney 2002), cities are growing, expanding into previously natural habitats, and becoming increasingly populated by a large number of wildlife species (McKinney 2008). Many of them have adjusted their behavior to these human-dominated, novel habitats others have been suppressed (Bateman and Fleming 2012).

The red fox (*Vulpes vulpes*) is one example for a successful synanthropic species and known for its wide distribution, its flexibility in habitat use, feeding, social organization, and thus for its high adaptability (Macdonald 2011). Successful breeding and flourishing fox populations in urban areas have been recorded in large cities and metropolitan areas, such as London (Page 1981), Bristol (Harris 1981), Toronto (Adkins and Stott 1998), Zurich (Hofer et al. 2000) and Berlin (Börner et al. 2009), among many others.

Access to food as vital bottom-up factor plays a key role in the success of animals, as it influences body condition and thus reproductive success and fitness. The utilization of a wide range of food resources can be advantageous in dynamic habitats with constantly changing food availability. Previous studies on red fox diet showed that its feeding behavior is highly flexible, spanning multiple trophic levels from berries to insects to small mammals (e.g. Harris 1981; Calisti et al. 1990; Leckie et al. 1998; Macdonald 2011). Usually, the most abundant and most accessible food source is used, which varies with resource availability (Calisti et al. 1990; Ferrari 1995; Cavallini and Volpi 1996; Leckie et al. 1998). In cities, food quality and obtainability are strongly influenced by human (Baker et al. 2000), and anthropogenic food in addition seems to play a major role in urban red fox diet (Harris 1981; Doncaster et al. 1990; Saunders et al. 1993; Contesse et al. 2004).

In our context, at the individual level, foraging specialists are individuals whose dietary niche (which represents the extent of the food spectrum) is clearly smaller than the total dietary niche width of the population. Foraging generalists in contrast, are individuals varying widely in their resource use and therefore represent the whole niche of the associated population (Bolnick et al. 2002a). Many species are commonly described as generalist foragers. However, generalist species can also enclose specialized individuals (see Fig. 1), each using only a small part of the entire feeding spectrum. The total dietary niche width of a species represents the sum of consumed prey species within the population and therefore population variation (Bolnick et al. 2002a).

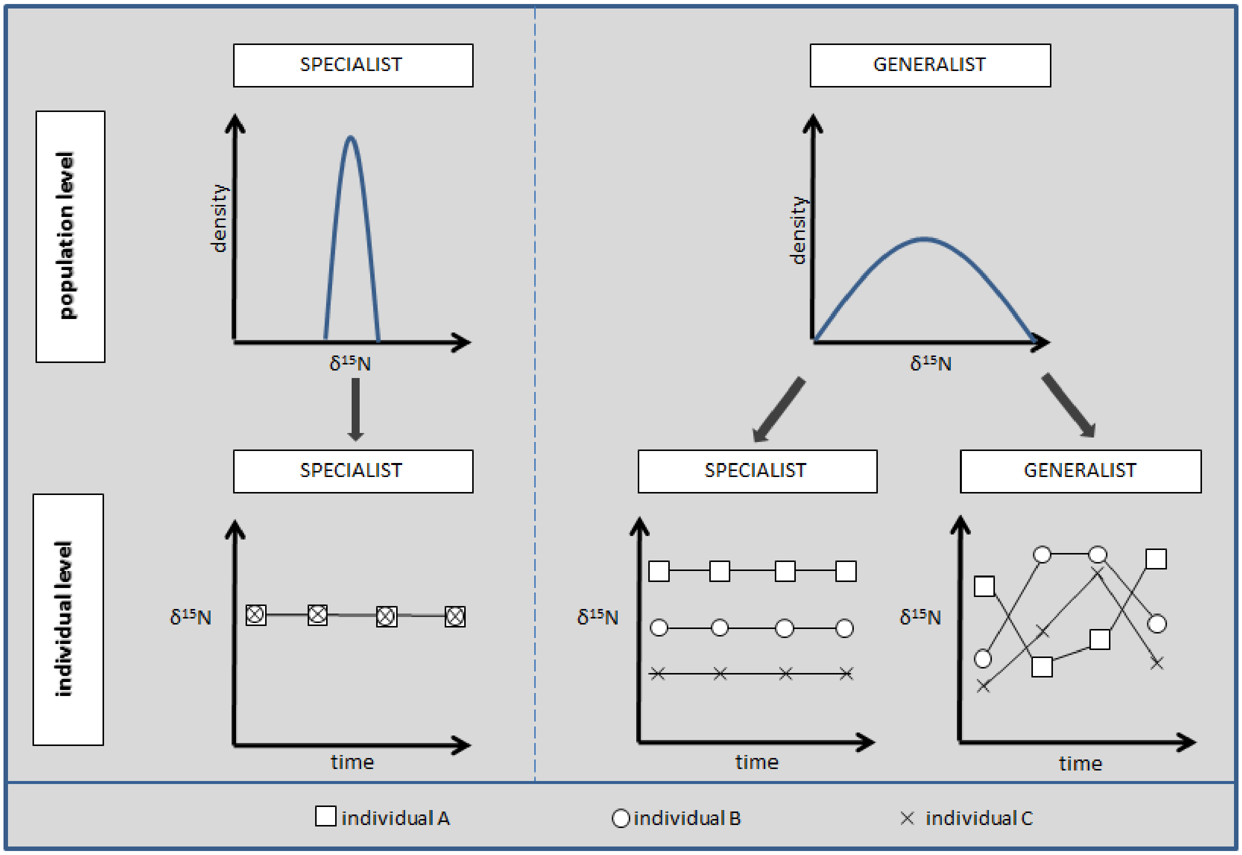

Figure 1: Conceptual diagram of how individuals can contribute to the population's dietary niche. Specialized populations consist of specialized individuals which all consume certain resources (left). Therefore, their total dietary niche represents a small dietary variation within and between individuals. In contrast, generalistic populations can consist either of specialized or generalistic individuals (right). In this case, specialized individuals show small dietary variation within individuals, but a large dietary variation between individuals leads to a broad overall resource spectrum and dietary variation at the population level. If individuals

of a generalistic population forage generalistic then those are characterized by a large within-individual dietary variation.

However, knowledge of individual feeding tactics (generalized or specialized food selection) and temporal tactic stability (in the sense of a constant tactic over time) is still limited. For red foxes, we are currently unaware of any study about individual feeding tactics, i.e. the level of individual dietary specialization. Neither do we know whether patterns of foraging decisions and feeding tactics of individuals are influenced by habitat, i.e. whether or not they differ among urban and rural populations of red foxes. A potentially individual dietary specialization could have complex consequences (Araújo et al. 2011). It is an important component in trophic interactions and food web structures, it enhances our understanding of selective pressure on prey and it has implications in the transmission and of parasites and pathogens (Bolnick et al. 2011). Furthermore, there is growing evidence that individual variation in resource use has implications for intra- and interspecific competition and population dynamics. Therefore, understanding the causes and importance of individual dietary specialization is a major goal in animal ecology (Araújo et al. 2011).

Stable isotopes have become a useful tool to study dietary niches, representing the trophic ecology of organisms (e. g. Layman et al. 2007). The power of the stable isotope method stems from the fact that isotope values measured of consumer tissues are related to the corresponding consumers' diet (DeNiro and Epstein 1978, 1981). Stable isotope analysis can be used to investigate resource use patterns across different organization levels and over different time scales, depending on the consumer tissue used. The analyses of carbon and nitrogen stable isotopes have also been successfully used as a proxy for diet specialization in predators (Lavin et al. 2003; Cherel et al. 2007; Woo et al. 2008; Anderson et al. 2009; Newsome et al. 2009, 2010; Matich et al. 2011; Voigt et al. 2014, 2018). Stable carbon isotopes reflect the baseline producers or the habitat whereas nitrogen isotopes are primarily influenced by the trophic position of the species. The stable isotope signatures of a tissue generally reflect the diet composition during the period of tissue synthesis (Hobson and Clark 1992; Bearhop et al. 2002) and the variance of isotopic values within these tissues is used as a measure of niche width (Bearhop et al. 2004). The more different prey species with different isotopic signatures are consumed the more variable is the isotopic signature, whereas dietary specialists focusing on a few prey items show a small variance in the isotopic signature of their tissues and therefore a narrower niche (Bearhop et al. 2004). In addition, individuals of populations consuming widely differing proportions of each prey over time will tend to show less variation in the C and N ratios than what is expected for constant proportions of each prey and therefore high evenness in the diet (Bearhop et al. 2004). However, feeding on many prey species may not necessarily lead to large isotopic variance in the consumer tissue, if prey species are isotopically similar (Martínez del Rio et al. 2009) or the amount of the consumed tissue varies. Finally, stable isotope analysis allows us to characterize the inter- and intra-individual variation of the diet. This could be an effective way to investigate dietary specialization, because the variance between and within individuals can then be compared to the associated population.

In this study, we used stable isotopic ratios of red fox whiskers (vibrissae) to quantify and compare the isotopic dietary niche width and feeding tactics of urban and rural red foxes at (I) the population level (single measurements of 119 red foxes) and (II) the individual level (each time 5 increments of 32 individuals reflecting five times 11 days). For assessing the individual isotopic specialization, we used carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios of vibrissae increments, which provided us with a temporally continuous isotopic record within the same individual. To delineate the feeding habits of red foxes, we compared stable isotope ratios of red foxes with those of potential food items using Bayesian isotope mixing models.

On a population level we hypothesize that isotopic signatures of urban red foxes differ noticeable from rural conspecifics. Taking into account previous studies on the feeding ecology of red foxes, we assume that urban populations consume a large proportion of anthropogenic food sources (e.g. food scraps, garbage and pet food), consisting of a mixture of different food items that is isotopically contrasting with natural food sources. Therefore, we predict a smaller isotopic niche for urban foxes, since cities have a relatively constant supply of anthropogenic food throughout space and time. In contrast, the abundance and availability of food resources in rural areas is habitat-dependent and variable over time and space, which should be reflected in a larger dietary (isotopic) niche compared to urban foxes. Assuming that foxes nevertheless concentrate within their individual range on the most available and easiest to obtain food item, this should take up a large proportion of the fox diet and thus result in a low variability in isotopic signatures over time in rural and urban foxes (individual level). Therefore, both rural and urban red fox individuals follow an (optional) specialized feeding tactic, even though foxes are a generalistic species at the population level.

2 Materials and Methods

2.1 Study area and sample collection

The study was conducted in Berlin and Brandenburg in the northeastern part of Germany (Fig 2). Berlin as a capital is characterized by highly urbanized areas, especially in the city center, whereas the surrounding federal state of Brandenburg is characterized by rural areas composed of small forests mostly embedded in agricultural landscapes. In the metropolitan area of Berlin, the density of humans increases towards the city center, forming a sub-urban area connecting the rural regions of Brandenburg and the highly urbanized areas of Berlin gradually.

Red foxes are found all over the study area, populating rural areas as well as highly urbanized regions. In cooperation with the state laboratory Berlin-Brandenburg

(LLBB), we collected a total of 119 whisker samples from dead red foxes originating from urban and rural environments. These samples stem from foxes that were either involved in accidents, were hunted or died of natural causes in the years of 2016 and 2017.

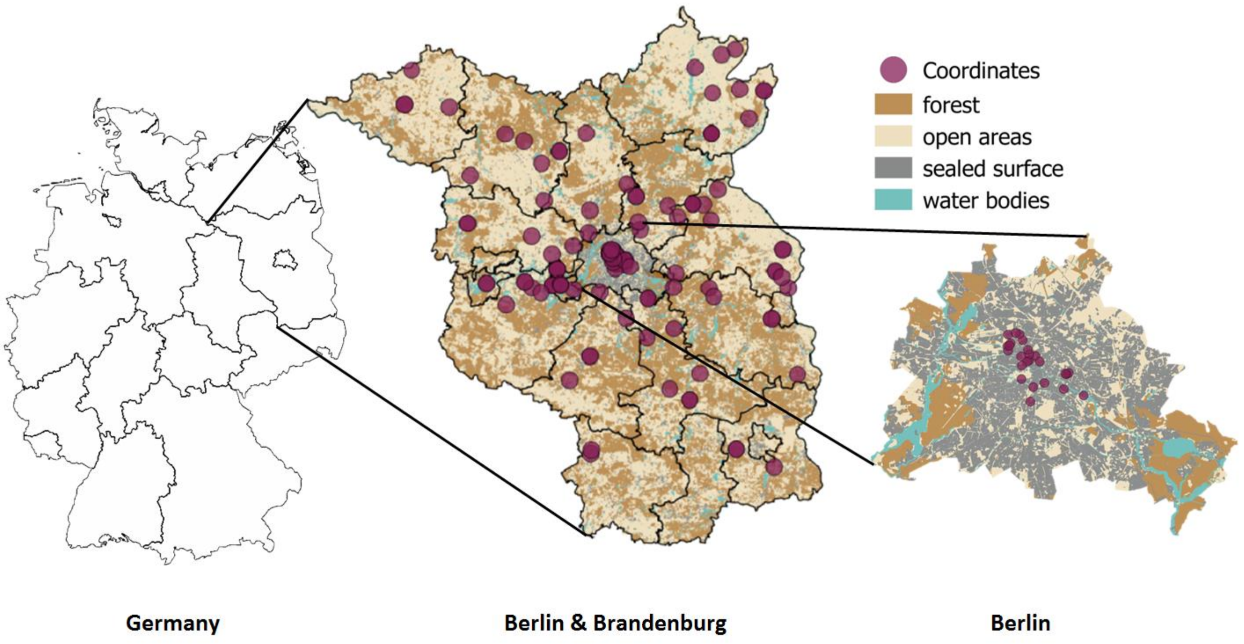

Figure 2: Location of the study area in northeastern Germany. The Berlin and Brandenburg map shows the landscape composition as well as the distribution of red fox samples (n=119).

To assign each fox sample to the “rural” or “urban” category, we calculated the percentage of imperviousness within a 1 km radius (reflecting approximately the size of a generously red fox home range) of each location of death. For this, we used a COPERNICUS imperviousness raster map of 2012 with 20 m resolution (http://land.copernicus.eu/pan-european/high-resolution-layers/imperviousness/ imperviousness-2012/ view) and extracted the mean of all raster cells within the buffer. Locations having a degree of imperviousness lower than 25% were categorized as “rural”, all other locations (>=25%) were assigned to the category “urban”. In the end, 85 of the individuals were assigned to the category “rural” and 34 to "urban". Imperviousness is considered to be a suitable proxy for urbanization, because it is also associated with factors such as human population density, light pollution, traffic and noise (Kuechly et al. 2012, Kasanko et al. 2006).

Besides, we characterized the heterogeneity of the landscape by using a land use map of Berlin (https://fbinter.stadt-berlin.de/fb/index.jsp; Umweltatlas Berlin/Stadtstruktur-Flächentypen differenziert 2015 (Umweltatlas)) and Brandenburg (https://lfu.brandenburg.de/cms/detail.php/

bb1.c.359429.de). Since each map has its own resolution regarding the land use categories, or names them partially differently, we have assigned all land cover types to the following in order to have a common basis. Nine land use categories were used: agriculture, forest, grassland, open areas, ruderal areas, shrubland, sealed surface, water bodies and others. As before, landscape diversity (Shannon diversity index of the nine land use categories) was calculated within a 1 km zone around each sample location (see Appendix).

To understand diet composition, we collected potential food sources as reference values for our analysis in order to confirm the availability of typical food resources over the entire study area (rural and urban) and to see whether the stable isotope values of food resources vary greatly between the contrasting habitats. Based on literature research and availability, we chose seven potential food sources at family level: dor beetle (*Geotrupidae*), earthworm (*Lumbricidae*), grasshopper (*Orthoptera*), land snail (*Helicidae*), land slug (*Limacidae*), house mouse (*Muridae*) and bramble (*Rosaceae*) (e.g. Harris 1981; Calisti et al. 1990; Leckie et al. 1998; Macdonald 2011). Ten samples for each food category were collected in six different locations in Berlin and seven sites in Brandenburg during July and October 2017 (see Table 1). All samples were frozen on the day of collection and stored at -80°C until analysis.

Table 1: Mean $\delta^{13}C$ or $\delta^{15}N$ values and standard error of the mean (SE) of red fox food items collected in Berlin and Brandenburg, Germany. $\delta^{13}C_{cor}$ values are corrected for a TEF of 4.31‰ and $\delta^{15}N_{cor}$ for a TEF of 3.05‰. * were pooled together, [!] was removed in further analyses.

			$\delta^{13}C$ (‰)			$\delta^{15}N$ (‰)		
Common name	**Family**	**n**	**mean**	**SE**	**$\delta^{13}C_{cor}$**	**mean**	**SE**	**$\delta^{15}N_{cor}$**
Earthworm*	Lumbricidae	10	-25.7	0.3	-21.4	2.0	0.9	5.0
Dor beetle	Geotrupidae	10	-24.8	0.4	-20.5	3.8	0.7	6.9
Grasshopper	Orthoptera	10	-28.3	0.2	-23.9	2.7	1.2	5.8
Land slug*	Limacidae	10	-25.9	1.0	-21.6	2.2	1.0	5.3
Land snail*	Helicidae	10	-26.4	0.6	-22.1	1.7	0.9	4.7
Bramble[!]	Rosaceae	10	-30.1	0.5	-25.8	-2.3	1.7	0.8
House mouse	Muridae	10	-25.0	0.4	-20.7	6.2	0.8	9.2

2.2 Sample preparation and analysis

We used whiskers because this body product has proved useful to delineate temporal changes in the isotopic data of mammals (Darimont and Reimchen 2002; Newsome et al. 2009, 2010; Voigt et al. 2018). Here, we assume that whiskers of red foxes grew at a constant rate (Robertson et al. 2013, Mutirwara et al. 2018). We selected whiskers with an average length of 4 cm, because they were sufficiently long and thick to guarantee repeated measurements of stable isotopes in whisker increments. Single whiskers put into a 1:2 methanol:trichlormethan solution in plastic tubes to cleanse them from surface contaminants. After shaking for 24 hours the liquid was removed and the clean whiskers were dried in an oven [*Heraeus Function Lab*] at 50°C for an additional 24 hours.

The metabolic rates between different organ tissues differ. Therefore, also the turnover rates and enrichment of stable isotopes differ as well (Tieszen et al. 1983; Hobson and Clark 1992). $\delta^{13}C$ and $\delta^{15}N$ values may vary systematically between an animal's tissues and its food, an offset called trophic enrichment factor (TEF; Tibbets et al. 2007; Parnell et al. 2010). Since TEF may vary across taxa, it is important to establish taxon-specific TEF for the specific study species. A study on stable carbon and nitrogen isotopic fractionation between diet and tissue of captive red foxes determined values for blood, fur, liver and muscle (Roth and Hobson 2000). Since the TEF and the growth rate of whiskers in red foxes are still unknown, we assumed that foxes show values comparable to other canids. Thus, we used data from a closely related species, the wolf (*Canis lupus),* as a reference (McLaren et al. 2015). For wolves a TEF δ value for carbon of 4.31‰, for nitrogen of 3.05‰ and an average growth rate of 0.43 mm/day were reported.

To access the diet niche of red foxes at the population level, we selected 119 foxes (males and females) and cut the basal 5 mm increment of the whiskers, using a scalpel. Assuming a growth rate of 0.43mm/day (McLaren et al. 2015) the sample represents food consumption of the last 11 days. To determine the diet niche of urban and rural red foxes at the individual level, we chose 19 adult urban foxes and 13 adult rural foxes (males and females) from these 119 individuals. We focused on adult foxes (>1 year), as they tend to remain stationary within a defined area throughout their lives. We cut further 5mm long increments of whiskers at 10, 15, 20 and 25 mm from the root, reflecting a time span of approximately 55 days in steps of 11 days each. All segments were weighed with an analytical microbalance (0.5 ±0.1mg), placed in tin capsules [*OEA Labs*, 6mm x 4mm], folded tightly, sealed and gently compacted into small cubes. The cubes were placed in a clean 96 position plastic culture tray [ELISA plate].

All food samples were defrosted and washed with distilled water. Indigestible parts such as chitin shells from beetles and grasshoppers or shells from land snails were removed. A small representative piece of each sample was cut, placed into a 2 ml tube and dried at 50°C for 48 hours [Heraeus Function Lab]. Afterwards, 110 ml of a 1:2 methanol:trichlormethan solution was added and the fat was extracted using a rapid extraction system [C. Gerhardt GmbH, SOXTHERM]. For extraction, sample solutions were boiled at 140°C for 30 minutes, distilled and re-added to the samples. We ran four extraction cycles of 25 minutes each. After extraction, samples were dried again at 50°C for 24 hours. Finally food samples were weighed and loaded into tin capsules following the protocol of whisker samples described above. Samples were combusted and analyzed using a peripheral elemental analyzer [*Flash EA 1112 Series, Thermo Fisher, Bremen, Germany*] coupled to a stable isotope ratio mass spectrometer [*Delta V Advantage, Thermo Fisher*] in continuous flow. For the calculation of isotope ratios, laboratory reference materials were used. The isotopic values for carbon are expressed in delta notation (in ‰ units) relative to Vienna Pee Dee Belemnite (VPDB). For the stable nitrogen isotopes atmospheric nitrogen was used as the standard.

2.3 Data analysis

All data analyses were performed with R Studio in R version 3.5.0 (R Core Team 2018).

2.3.1 Population level

We estimated and plotted the isotopic dietary niche metrics of urban and rural foxes based on stable isotope ratios of single individuals using Stable Isotope Bayesian

Ellipses in R (SIBER package: Jackson et al. 2011). The SIBER package is used to compare isotopic niches across communities by analyzing the isotopic distribution of consumer tissues. The metrics of SIBER take the uncertainty in the sampled data into account and naturally incorporate errors arising from the sampling process, propagating it through to the derived metrics. Therefore, calculated ellipses are unbiased with respect to sample size and allow robust comparison among data sets comprising different sample sizes (Jackson et al. 2011). Thus, the standard ellipse area (SEA) corrected for small sample size (SEAc) represents the trophic niche breadth. We calculated the SEAc for urban and rural fox data on the population level and the overlap between these two areas.

The relative contribution of the collected food sources to the diet of urban and rural red foxes was estimated with the Bayesian isotope mixing model MixSIAR (Stock and Semmens 2013). The isotopic signatures of tissues and food sources, fractionation of tissues and variability were used to estimate the isoscape plot and the contribution of the food sources to a mixture. The percentage of concentration of $\delta^{15}N$ and $\delta^{13}C$ values in the food sources was included in the analysis. This step is recommended in case of large variation of elemental concentrations among the sources (Phillips and Koch 2002). Rosaceae were excluded from the analysis after prior visualization of the isospace plot, as they lay well outside the source polygon. This could mean either that the source was not consumed by the sampled foxes or that the source is difficult to be found in whiskers. Berries are mainly composed by carbohydrates and therefore they should fuel immediate energy metabolism pathways rather than being incorporated in tissues. For this reason, they are more likely to be found in short-term samples, such as in breath exhaled CO_2 (Hobson and Stirling 1997; Hobson et al. 2009). Additionally, a PERMANOVA test was performed to test pairwise differences between food sources, which could be pooled together for *a-posteriori* combining before running the MixSIAR analysis. The Markov Chain Monte Carlo (MCMC) parameter estimates the probability density functions of variables of interest and the entire distribution for each variable. The MCMC parameter chosen to run the MixSIAR was "very long" (chain length of 1,000,000).

Then, we used a Kruskal-Wallis rank sum test to test for significant differences in isotopic values between males and females as well as between adults and juveniles. Finally, we tested whether the variance of $\delta^{13}C$ and $\delta^{15}N$ values differs among the urban and the rural fox population using an F-Test.

2.3.2 Individual level

We determined the SEAc of the 19 adult urban and 13 adult rural foxes of longitudinal measurements based on stable isotope ratios using Stable Isotope Bayesian Ellipses in R (SIBER package: Jackson et al. 2011). To calculate the degree of individual diet specialization within these two fox populations, we adjusted the mathematical model of Roughgarden (1972), which was discussed by Bolnick et al. (2002) as a possible index for individual specialization. Following that, a foraging specialist is an individual whose dietary niche is narrower than the total niche width (TNW) of the population. The TNW consists of two components, (1) the variation in resource use within individuals (WIC) and (2) the variance between individuals (BIC): TNW = WIC+BIC. To determine the TNW we used the trophic niche breadth represented by the total area (TA) of the urban and rural population. The WIC is represented by the TA of each individual. When the WIC/TNW ratio approaches 1, all individuals utilize the full range of the population's niche, whereas smaller values indicate an increasing degree of individual diet specialization. Additionally, we used that whisker, which covered the largest per mille range for $\delta^{13}C$ and $\delta^{15}N$ values as a reference to obtain the intra- and interspecific variation of the isotopic composition during a one-way ANOVA performed in R.

3 Results

3.1 Population level

After the PERMANOVA test, land snail, land slug and earthworm were pooled together as they did not differ significant in isotopic values between each other. As described in the method section, brambles were excluded from further analysis (Appendix C).

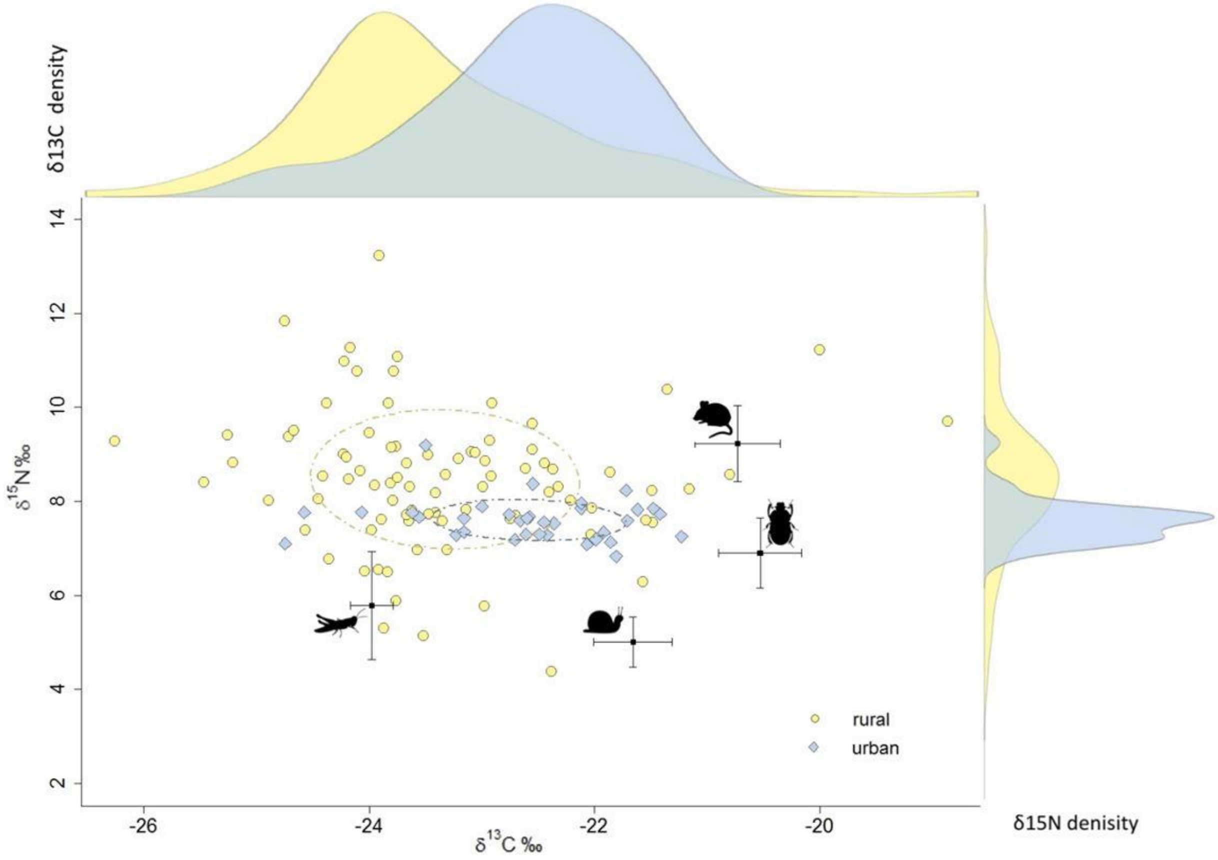

Figure 3: Isospace and density plot for raw $\delta^{13}C$ and $\delta^{15}N$ values of urban (blue diamonds) and rural (yellow circles) red foxes whisker samples (n=119) from Berlin and Brandenburg, Germany. Dashed ellipses represent SEAc of urban (blue) and rural (yellow) fox population. Black dots show trophic corrected mean (±SE bars) $\delta^{13}C$ and $\delta^{15}N$ values for the four prey taxa including (1) grasshopper, (2) land slug, land snail, earthworm (pooled together), (3) dor beetle and (4) house mouse.

Isotopic analysis of potential food items of red foxes indicated that the potential isotopic niche was broad for this species (Table 1, Fig. 3). The $\delta^{13}C$ values ranged from -30.1‰ to -24.8‰ and $\delta^{15}N$ values from 1.7‰ to 6.2‰. We observed an isotopic difference among most food sources (Table 1). The variation in isotopic values of individual food items is relatively small in comparison to variation between food items.

We tested the distribution of Shannon index values based on land use classes for both, urban (mean = 0.9 ± 0.16) and rural (mean = 0.5 ± 0.27) fox population (see also Appendix A). A Wilcoxon rank sum test revealed that the median Shannon index of rural regions is significant less than the median urban Shannon index (W=267, $p < 0.001$). Therefore, based on the Shannon index, our urban sites are more diverse on a landscape structure scale than the rural ones.

In contrast to population origin (rural vs. urban), sex had no effect on individual $\delta^{13}C$ values ($t_{(78,036)}=-1.48$, $p = 0.143$) or on $\delta^{15}N$ values ($t_{(61,966)} = -0.11$, $p = 0.914$). In addition, age had no effect on $\delta^{13}C$ values ($t_{(33.369)} = -1.41$, $p = 0.168$) and $\delta^{15}N$ values ($t_{(23,944)} = 0.11$, $p = 0.910$), thus we pooled data of both sexes and across age groups. The F-test confirmed a significant difference between the variance of the urban and rural population for $\delta^{13}C$ values ($F_{(84)} = 1.908$, $p=0.040$) as well as $\delta^{15}N$ values ($F_{(84)} = 11.394$, $p<0.001$).

The isotopic compositions of the 34 urban fox whiskers averaged 7.6 ± 0.4‰ for $\delta^{15}N$ (range 6.8 to 9.2‰) and -22.6 ± 0.9‰ for $\delta^{13}C$ (range -24.8 to -21.2‰), those of the 85 rural foxes 8.5 ± 1.5‰ for $\delta^{15}N$ (range 4.4 to 13.2‰) and -23.3 ± 1.2‰ for $\delta^{13}C$ values (range -26.3 to -18.9‰).

The stable isotope ratios of individual whiskers (n=119, Fig. 3) varied largely in their $\delta^{13}C$ and $\delta^{15}N$ values. $\delta^{15}N$ and $\delta^{13}C$ values of some red foxes fell outside the range of food stable isotope ratios, indicating that foxes might have consumed food resources of high $\delta^{15}N$ and low $\delta^{13}C$ values.

A Welch two sample t-test confirmed the significant difference between the means of the urban and rural population for $\delta^{13}C$ ($t_{(83,479)}=-3.77$, $p < 0.001$) as well as $\delta^{15}N$ values ($t_{(111,27)}=4.89$, $p < 0.001$). Additionally, the two populations differed in total area (TA) and SEAc indicating different isotopic niches. Rural foxes showed a TA of 36.8‰^{2} and a SEAc of 5.6‰^{2}, whereas the urban population had a narrower isotopic niche with a TA of 5.2‰^{2} and a SEAc of 1.2‰^{2}. The overlap between the SEAc of the two populations was 0.8‰^{2}, consisting of 66.7% of urban and 14.3% of rural SEAc size.

3.2 Individual level

The isotopic niche of rural foxes (TA=3.3‰^{2}) was broader than the isotopic niche of urban foxes (TA=1.7‰^{2}), with urban foxes averaging 7.4± 0.7‰ for $\delta^{15}N$ and -22.8± 0.9‰ for $\delta^{13}C$ values and rural foxes averaging 8.3± 1.0‰ for $\delta^{15}N$ and -23.5 ± 1.1‰ for $\delta^{13}C$ values. SEAc values of individual whiskers ranged from 0.1‰^{2} to 1.8‰^{2} in urban and 0.1‰^{2} to 5.0‰^{2} in rural foxes.

Rural red foxes differed in the consumption of isotopically different food items with regard to $\delta^{13}C$ (one-way ANOVA: $F_{(12)} = 6.094$, $p<0.001$) and $\delta^{15}N$ values ($F_{(12)} = 24.741$, $p<0.001$). This pattern was similar for urban foxes for both $\delta^{13}C$ ($F_{(18)} = 15.215$, $p<0.001$) and $\delta^{15}N$ values ($F_{(18)} = 9.697$, $p<0.001$). Looking at the results of the longitudinal analysis (Fig.4, Appendix B), the stable isotope values remain relatively similar over time and show little variance. Foxes in total used a wide range of the available isotopic dietary space, but individuals (apart from a few exceptions) utilized just a relatively small portion of the total range.

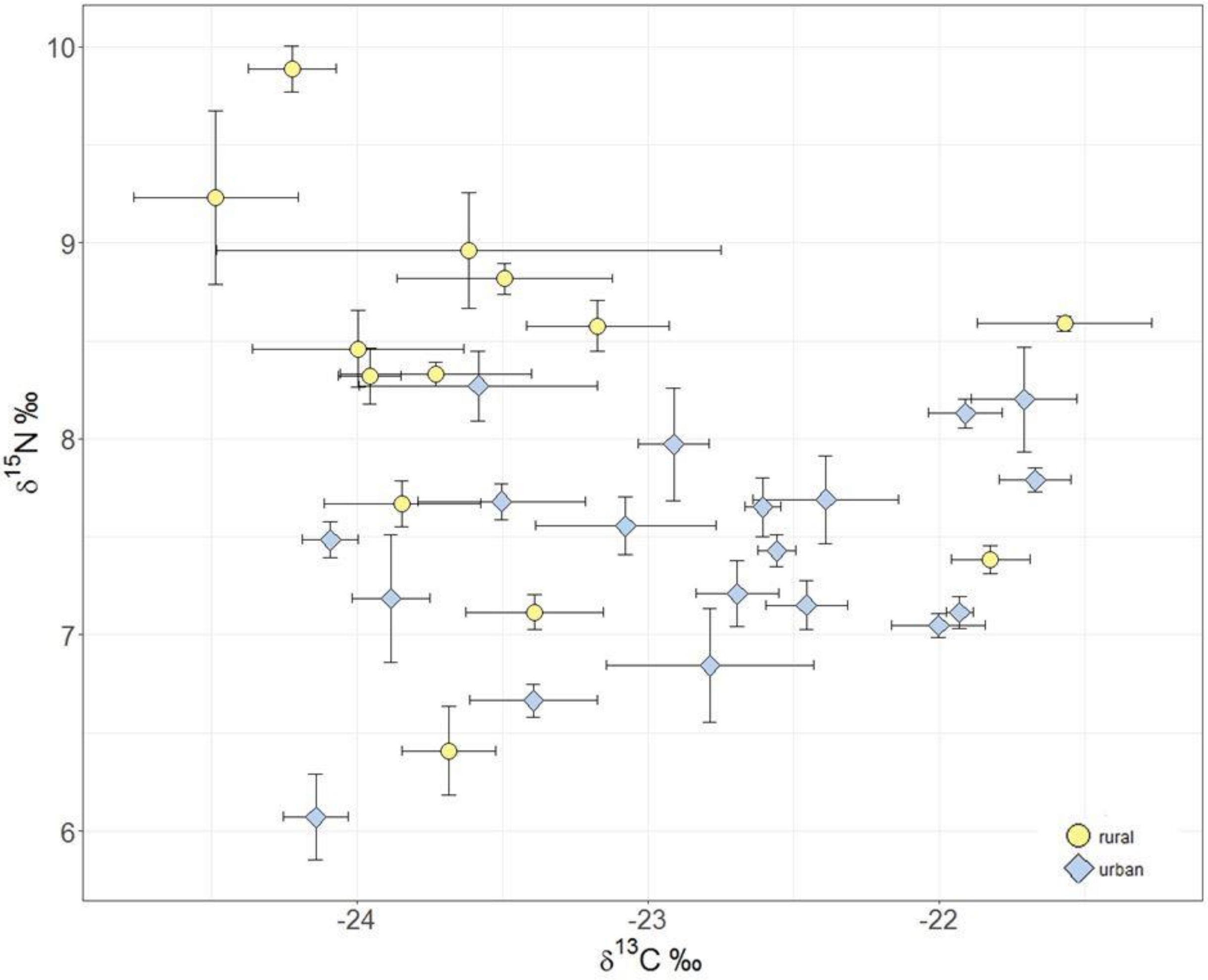

Figure 4: Mean C and N values over time of all segments sampled from 19 urban (blue diamonds) and 13 rural (yellow circles) red fox individuals from Berlin and Brandenburg, Germany. Error bars represent the standard deviation SD.

The WIC/TNW ratio as a measure of feeding specialization was very small in both populations (Fig. 5). The mean WIC/TNW was 0.05 (±0.08) and 0.05 (±0.04), respectively, for both, rural and urban foxes, indicating a highly specialized resource use at an individual level.

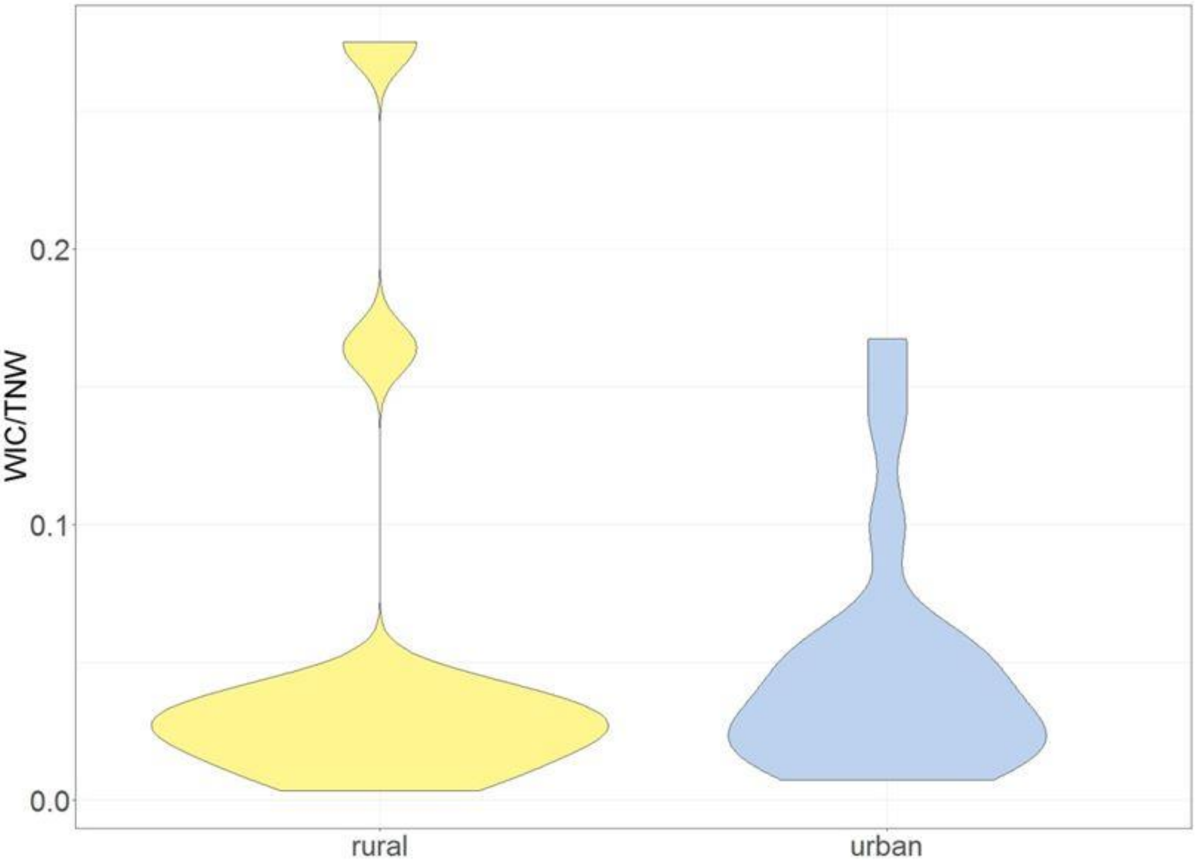

Figure 5: WIC/TNW ratios for 19 urban and 13 rural adult red foxes

4 Discussion

We investigated diet niche width and the feeding tactics of urban and rural red foxes at (I) the population level in space and (II) the individual level over space and time. For this purpose we used carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic signatures of red fox whiskers as well as longitudinal measurements with regard to these two isotopes. The diet of red foxes has been studied over decades (e.g. Englund 1965; Harris 1981; Calisti et al. 1990; Leckie et al. 1998; Contesse et al. 2004; Macdonald 2011; Díaz-Ruiz et al. 2013), but this is the first empirical investigation allowing conclusions about potential individual dietary specialization and comparing feeding tactics between urban and rural foxes. Thus, our study provides a more detailed insight into the foraging strategies of this successful predator in human-dominated landscapes. This is important in order to understand the complex interrelationships associated with increasing urbanization. Stable isotope analysis are the means of choice when it comes to deciphering the degree of food specialization (Crawford et al. 2008). Conventional methods such as fecal sample analysis are usually only a snapshot and do not provide longitudinal data over time. However, individual differences in resource use are crucial for understanding food webs, disease transmission and for an effective wildlife management. Nevertheless, stable isotope analyses also have their limitations. Thus, we cannot make an accurate statement about the food that is actually eaten. Since the metabolic turnover time (time of food intake to time of representation of the diet in organ of interest) for red fox vibrissae has not yet been investigated, we cannot make precise statements about the timing of food intake, which would be speculative to compare with potential seasonal food supply. Although nitrogen isotope signatures ($\delta^{15}N$) provide powerful measures of the trophic positions of individuals and populations, normally you have to apply baseline corrections to account for spatial variation (Woodcock et al. 2012). This is not meaningful for our study design. Nitzsche et al. (2016) have shown small-scale variations in plant, soil and sediment nitrogen isotope signatures even within the area of a typical red fox home range. Although foxes are locally bound when they have established their territory, they are nevertheless very mobile and generalistic feeder within its range. Therefore, an effort to investigate spatial variability at base level by collecting additional potentially suitable soil or plant samples is pointless. In contrast, we have collected potential food resources of different trophic levels at different locations within the study area along the urban-rural gradient. This gives us at least an impression of the variability of the stable isotope signatures of some typical food items and therefore the basis to correctly interpret the results of the red fox data. Although the food items were collected both in the highly urbanized city center of Berlin and in rural areas, the variance of isotopic values of the individual resources was small. Thus, a large variation in isotope values of the red foxes cannot be attributed to a large variance of single food items and can therefore be interpreted as variability in diet composition or food selection. In addition, the collection of potential food items along the rural-urban gradient reflects a general availability of these resources for urban and rural foxes, even though we cannot make any statements about the food density.

4.1 Population level

First of all, neither in the urban nor the rural population did we observe differences between sexes or age-classes on dietary choice. This confirms other studies showing that offspring consume the same food as adults (Kolb and Hewson 1979; Weber 1996), but contradicts (Panzacchi et al. 2008) who demonstrated significantly higher amount of large prey types fed to cubs in rural regions. A bigger prey size maximizes the energetic benefit and reduces the relative costs connected with returning to the den and feeding their offspring. Juveniles develop their hunting skills at the age of approximately 6 month (Harris and Trewhella 1988) and they focus on easy prey at the beginning. Since in our case "juveniles" are all animals < 1 year, our study design does not allow reconstructing such differences. In contrast to our results, Kidawa and Kowalczyk (2011) revealed sex-related diet preferences within adult red foxes. These differences are probably connected to the breeding period. A further explanation is that reproductive females probably monopolize superior food patches at the expense of younger nonreproductive animals.

In total, isotopic $\delta^{13}C$ and $\delta^{15}N$ values of rural and urban red foxes (n = 119) showed a broad range spanning multiple trophic levels and included all of the food items we examined specifically (Fig. 3). This corroborates current knowledge that the red fox is a food generalist (Englund 1965, Doncaster et al. 1990, Jędrzejewski & Jędrzejewska

1992). For a brief overview, a review of 55 studies from the Iberian Peninsula on red fox diet found a biogeographical relation between the consumption of lagomorphs and invertebrates as well as the intake of small mammals and fruits/seeds on the other hand (Díaz-Ruiz et al. 2013). Thus, the red fox showed that variation in feeding habits depending on environmental factors which determine the availability of their main food. Results of studies on red fox diet in agricultural landscapes yielded a diet mainly based on rodents and game birds (e.g. Leckie et al. 1998; Jankowiak et al. 2008). In contrast to that, food of vegetable origin (fruits and seeds) is important in the diet of red foxes from the Mediterranean coastal area (Calisti et al. 1990). In general, red fox diet seems to be highly divers. Nevertheless, our whisker samples of the rural population showed a higher variability in isotopic signature and therefore denote a broader isotopic dietary niche than urban foxes although overlapping with foxes of urban areas (Fig. 3). Bearhop et al. (2004) predicted that populations feeding on a wide range of prey species will exhibit wider variation in their tissue isotopic signatures, and populations where individuals consume prey over a broad spectrum of trophic levels will tend to show more isotopic variance than those which feed on the same number of prey species, but same trophic level. Accordingly, it is likely that the rural population consumes a wider range of different prey species spanning multiple trophic levels than the urban one.

However, an urban environment is a complex mosaic of different elements on a relatively small spatial scale, although consisting of much sealed area (high imperviousness value). Therefore, the mean Shannon diversity index of urban areas is higher than for rural regions (see Appendix A). Surprisingly, although thus urban areas are more heterogeneous on a habitat scale and should offer also a more diverse food spectrum for generalist species (Tews et al. 2004), the urban foxes don't feed as varied or broad as its rural conspecifics. Nevertheless, rural areas defined as area having less than 25 % sealed surface comprise more diverse habitat types in total, which together offer a wide range of prey species and food sources. Cities, on the other hand, provide an elementary and instantly available food source for urban foxes (Contesse et al. 2004), potentially explaining the narrower isotopic niche: anthropogenic food.

It is difficult to detect anthropogenic food items using traditional methods (e.g. macroscopic inspection of scats), as processed food usually does not contain identifiable, indigestible material such as exoskeletons, bones, feathers or hair. However, Newsome et al. (2010) were able to show that urban kit foxes (*Vulpes macrotis mutica*), had significantly higher $\delta^{13}C$ values (difference in mean = 2,4‰) and lower $\delta^{15}N$ values (difference in mean = 2,7‰) than non-urban individuals and isotopic values similar to human residents. Based on their findings they suggested a shared (anthropogenic) food source and similarities in their diet. Meaty anthropogenic food contains a noticeable amount of corn, because livestock reared for meat production is often fed a corn-based diet. Food crops like maize as well as sugar cane, millet and sorghum are typical C_4 plants, which differ in their $\delta^{13}C$ values (-12 to -14‰) from C_3 plants (-22 to -29‰) (Craig 1953; Farquhar et al. 1989). Urban wildlife that feed on anthropogenic sources show slightly higher $\delta^{13}C$ values, because of the direct or indirect influence of C_4 plants described before. As anthropogenic food also consists of food items of low trophic level (e.g. pastries, fruits and vegetables), individuals feeding on anthropogenic food sources also have lower $\delta^{15}N$ values than individuals which focus on natural prey animals (Lavin et al. 2003; Murray et al. 2015; Newsome et al. 2015). All this leads to the conclusion that also urban red foxes of our study area have established anthropogenic food in their diet.

4.2 Individual level

Our results of longitudinal data on individual level strengthened our previous findings. Since the TA of rural foxes is bigger in comparison to urban individuals, rural foxes cover a broader isotopic niche and therefore dietary spectrum. Again, mean $\delta^{15}N$ value is smaller and mean $\delta^{13}C$ value is bigger for urban foxes which confirm the difference in foraging behavior. Focusing on the SEAc values of individual whiskers, it can be seen that urban individuals have an even narrower isotopic dietary niche than individuals from rural areas.

At any time or location, the realized niche of a population represents the sum of all prey consumed by individuals belonging to this population (Bolnick et al. 2002a). Accordingly, there is a clear link between feeding tactic at the individual level and trophic interactions defined at the population or species level. Our results show that the stable isotope values of red foxes varied among and between urban and rural individuals (Fig. 4). Therefore, individuals feed on different prey items or have a different diet composition compared to conspecifics. The variability of the diet over time is represented by the standard deviation. Since the degree of variability was low, it is likely that the relative proportion of food items in the diets did not vary largely and individuals focused on the same diet. Moreover, urban as well as rural foxes may have consumed only a small proportion of potentially available isotopic dietary niche. Interestingly, the diet of a particular individual remained largely constant over a longer period of time (approximately 2 month, see Appendix B), since the variance of $\delta^{13}C$ and $\delta^{15}N$ values of the different whisker increments is low. Moreover, WIC/TNW ratio is very low in both populations (Fig. 5) and therefore the individual dietary niche is substantial narrower than the total niche with of the population. By definition (Bolnick et al. 2002a) and in addition to all other previous results of longitudinal analysis, generalistic fox populations consist of individual food specialists at least over medium time periods (here approximately 2 months) in our study area.

Differences in diet composition among conspecifics and dietary specialization have been documented across a broad range of taxonomic groups and habitats (reviewed in Bolnick et al. 2002a) and such variation at the individual level is increasingly recognized as an important component of diversity in trophic interactions.

Furthermore, the extent to which population-level dietary patterns are determined by the composition of similar or very different individuals has potentially important implications to behavioral and evolutionary ecology, ecosystem dynamics and conservation efforts (Tinker et al. 2008). However, the reasons leading to individual specialization can be very different. For example, a wide isotopic dietary niche of the population with heterogeneous use of the resources, combined with narrow individual niche widths, reduces intraspecific competition. If food specialization would only reflect food availability and thus the habitat, structurally very similar habitats with the same food disposability should result in overlapping isotopic food niches of different individuals. An avoidance of intraspecific competition could be necessary in case of decrease of habitat quality or quantity or an increase in population density (Araújo et al. 2011). Therefore, competitive and dominant individuals may monopolize territories of high quality and subordinate individuals will then be forced to resort to alternatives of lower quality (Morse 1974). In contrast, a very broad niche may increase interspecific competition (Vellend 2016) due to diet overlap with other species. It also reduces the impact of this overlap because only a subset of individuals in each species is affected when individuals of a generalist species feeding specialized. On the other hand, depending on resource availability, food generalists may nevertheless be factual individual foraging specialists because of different environmental and social factors such as social status, territory location or even trade-offs constraining the ability of individuals to forage. The variation between conspecifics in resource use can also reflect intrapopulation variation based on individual traits such as resource-specific preference and efficiency (Bolnick et al. 2002a). Therefore, the success of foraging behavior and prey capture bases on learning and experience; insufficiencies will restrict the handicapped individual to a limited range of prey (Kato et al. 2000). Individuals of (food-)generalist species often use the same resources if only this resource is available or a particularly high-quality food source is very abundant and easily accessible (Robinson and Wilson 1998).

Consequently, complex interactions of different factors affect individual resource use and feeding tactics.

4.3 General discussion

Different foraging and feeding habits can alter a cascade of direct and indirect effects. Identifying intraspecific trait variation, in our case the different foraging niches of conspecifics, is of enormous importance to understand ecological dynamics, because it will alter population densities, transient dynamics and persistence (Bolnick et al. 2011). At an individual level, the utilization of anthropogenic food subside are often predictable in space and time and can increase fitness (Oro et al. 2013). In contrast, different diets may incur different risks, for example, differences in parasite load of certain food items. Food generalists are more likely to encounter multiple parasite species because they consume various intermediate hosts. The exposure to a wider variety of different parasites at low frequencies may be worse than high exposure to a limited spectrum of parasites, when there are trade-offs in the immune response to several parasite species (Curtis et al. 1995). On the other hand, information about the diet at the individual level of food specialists within a generalistic population could be important to estimate the spread of specific parasite species within the host species. In this case looking only at the population level would lead to a wrong impression. Is a diet connected to a specific habitat, there could be also a difference in predation or mortality risk (Durell 2000) depending on the habitat. Moreover, individual feeding tactics also affect population dynamics as well as communities and ecosystems. They alter food webs, within-community competition and predator-prey dynamics, because food generalists have an impact on a diverse prey assemblage and food specialists influences only a limited assortment. Furthermore, they also promote the invasion of non-native species and increase human-wildlife conflicts (Oro et al. 2013). Identifying differences in feeding tactics on an individual level has also increasingly been recognized as an important part of population ecology, because it helps to create individual-based models for a mechanistic understanding of processes and patterns (Bolnick et al. 2002b). The investigation of foraging tactics is therefore of particular importance, because only then can we correctly interpret occurring patterns and relationships and ultimately develop appropriate strategies to promote biodiversity in cities and minimize human-wildlife conflicts.

Author Contributions

CScho, JF, SKS, CV and SO contributed conception and design of the study; CSchu organized the database and provided the samples; CScho and JF performed the statistical analysis and CScho wrote the first draft of the manuscript; JF wrote sections of the manuscript and was responsible for the laboratory work. SKS, CV and SO supervised the project. All authors contributed to data interpretation, manuscript revision, read and approved the submitted version.

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Chapter 2 - Lower helminth diversity in urban red foxes, but with higher association to pets

Lower helminth diversity in urban red foxes, but with higher association to pets

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Abstract

(1) Transmission of zoonotic diseases is one of the major human-wildlife conflicts of our age. Wildlife acts as a reservoir and important transmission pathway in this context. In areas of high wildlife and human population densities, like cites, this is of particular importance as contact rates and thus transmission probability increase. The red fox is a host species to a variety of helminths, including some of zoonotic potential. At the same time, it is a successful synanthropic species that occurs in many cities worldwide.

(2) Therefore, we wanted to investigate whether people and pets in urban areas are at higher risk with regard to the transmission of relevant helminths. We further aimed to identify possible causes that influence the prevalence of parasites in cities which may range from environmental factors to biotic factors such as inter- and intraspecific species interactions.

(3) We analysed the community composition of parasitic helminths in red foxes to study co-occurrence patterns of helminths and the abiotic drivers of helminth communities. We used a data set of 217 red foxes from Berlin and Brandenburg, Germany, sampled within urban and rural areas. The occurrence of helminths within these red fox populations was analysed using DNA metabarcoding.

(4) The main results show that the helminth diversity in the city Berlin is lower than in the surrounding rural Brandenburg, and male red foxes tend to have higher helminth diversity than females. Diet features can drive helminth communities in red fox populations. Additionally, with increasing human population density, helminths transmitted via diet are less prevalent than pet-related helminths.

(5) This suggests that pets, vice versa, might have an increased risk of being infested with red fox helminths.

Introduction

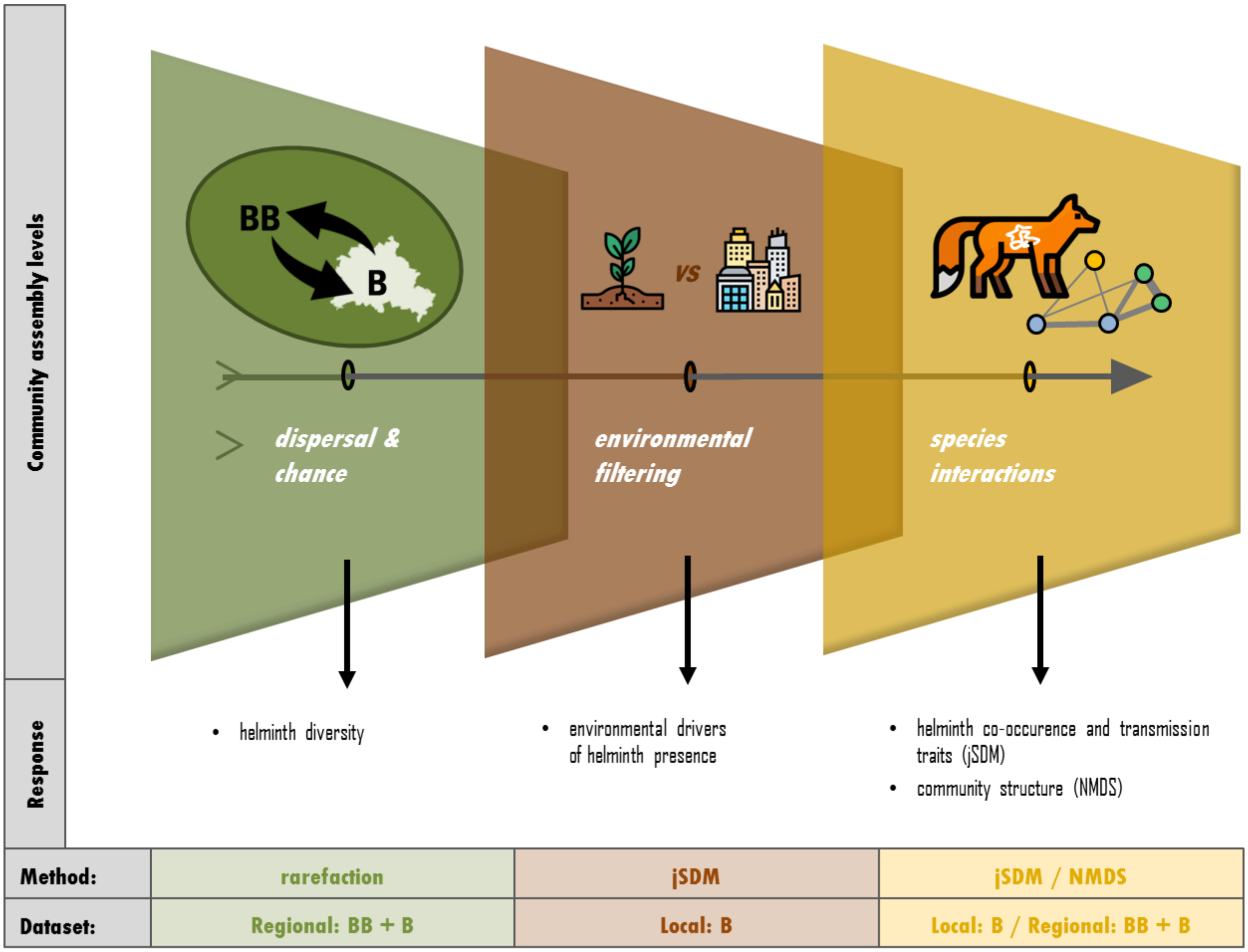
Approximately 75% of human diseases are of zoonotic origin and are thus transmitted directly or indirectly by wild or domestic animals (Taylor et al., 2001). Although the direct transmission of a disease from wild animals to humans is relatively rare, wildlife provides an important channel and sometimes may act as a reservoir to enable disease persistence (Bradley & Altizer, 2007; Mackenstedt et al., 2015). Pets often serve as a transmission link or reservoir of parasitic zoonoses and are important parts of the disease cycle (Deplazes et al., 2011). This fact is of particular importance in areas with a high probability of contact, for example in areas with high human and wildlife population densities and overlapping habitats of wildlife and humans, as it is the case in urban environments. Although urban areas reduce the number of wildlife species and biodiversity (McKinney, 2006), the species that inhabit cities appear to some extent at higher densities than in rural areas (Šálek et al., 2015).

The red fox (*Vulpes vulpes*) is a classic example of the successful colonisation of cities and of the adjustment to prevailing living conditions. This carnivore is a typical synanthropic species and nowadays lives in various cities worldwide (Adkins & Stott, 1998; Cignini & Riga, 1997; Contesse et al., 2004; Corlatti, 2014; Doncaster et al., 1990; Gloor, 2002; Kauhala et al., 2016; Macdonald, 2011; Page, 1981; Yimam et al., 2002) also in higher population densities than in the surrounding area (Börner et al., 2009; Šálek et al., 2015). Red foxes are known to spread infectious diseases that are highly dangerous for humans and their domestic animals. Some examples include rabies (Holmala & Kauhala, 2006; Müller et al., 2009), canine distemper (Gras et al., 2018) and sarcoptic mange (Al-Sabi et al., 2014; Bornstein et al., 2006; Devenish-Nelson et al., 2014; Nimmervoll et al., 2013). Diseases caused by intestinal helminths such as echinococcosis are also highly relevant (Deplazes et al., 2004). Some nematode species such as *Toxocara canis* or *Trichinella spiralis* are of medical importance as they can cause toxocarosis and trichinellosis, respectively (Criado-Fornelio et al., 2000; Davidson et al., 2006; Okulewicz et al., 2005; Reperant et al., 2007). Further, plathelminths including *Taenia* species infect farm live-stock (Boufana et al., 2012; Gicik et al., 2009).

Helminths are classified according to their life cycle. A complex (indirect, heteroxenous) life cycle requires more than one host to be completed. In contrast, parasites with simple (direct, homoxenous) life cycles exploit just one host species. Helminths with complex life cycles therefore have one or more intermediate hosts for growth and development but reproduction occurs just in the primary host (Poulin, 2007). In a recent meta-analysis, Werner and Nunn (2020) found that parasites with complex life cycles were less prevalent in urban carnivores like red foxes compared to rural conspecifics, without a difference in parasites with simple life cycles. Environmental changes associated with urban areas, such as increased average temperatures, human related disturbances or landscape structure dominated by sealed surfaces can alter survival of intermediate hosts and parasites itself (Parris, 2016). Therefore, some parasites are not expected to appear in cities along with their native hosts. Yet, as some urban species occur in higher population densities and forage in areas of clumped food resources, direct transmission of parasites could increase due to increases in contact rates and intra-species interactions (Kamiya et al., 2014; Marzluff, 2001).

While a number of studies have investigated the effect of urbanisation on single red fox parasites, they often show contradictory results. For example, the prevalence of the fox tapeworm *Ecchinococcus multilocularis* decreases with increasing urbanisation in Zurich, Switzerland (Fischer et al., 2005; Stieger et al., 2002), which was explained with rodent-related habitat factors, as rodents are intermediate hosts for the fox tapeworm. In contrast, such a pattern could not be detected in *Toxocara canis*, despite it being a helminth with a complex life cycle (Reperant et al., 2007). Possible causes that influence the prevalence of parasites in cities are very diverse and complex and may range from environmental factors to biotic factors such as inter- and intraspecific species interactions. Although common, it is therefore not sufficient to focus on the prevalence of single parasite species and specific explanatory variables (e.g. Davidson et al., 2006; Deplazes et al., 2004; Gicik et al., 2009; Gloor, 2002; Hofer et al., 2000; Reperant et al., 2007). In order to determine the occurrence of certain parasite species and how they potentially affect the occurrence of other helminths, it is necessary to study the full spectrum of parasites and the influence of various environmental factors and parasite traits, if possible, at the same time.

The pool of species, the meta-community, available and the forces that structure communities determine how many species occur in an intra-community within one host (Poulin, 1997). Community assembly is a process that leads to community formation (Barabás et al., 2018; HilleRisLambers et al., 2012; Letten et al., 2017) as a result of species colonisation ability, the suitability of the environment where these species arrive and interactions between species. In our case, those helminths are the species that colonise the intestinal tract of a red fox (Fig. 1).



Method:	rarefaction	jSDM	jSDM / NMDS
Dataset:	Regional: BB + B	Local: B	Local: B / Regional: BB + B

Figure 1: Study overview. Community assembly levels are addressed by application of different methods on different spatial scale.

Establishment of any ecological community requires that individuals of the species can reach the available habitat. Therefore, dispersal limitation is one of the main constrains on community assembly. In our study this corresponds to where a parasite can actually be transported, which is mainly driven by the host's (and potential intermediate hosts') movement capability and space use. In other words, parasite communities are special with regards to dispersal, as hosts, representing habitat patches, are mobile themselves. Therefore dispersal includes a likelihood of encounters between hosts belonging to the same or different species or prey species leading to exchange of parasites.

Next, still at the large spatial scale of the outside environment, the community assembly is largely defined by environmental filtering with regards to interactions between the host and parasites. This is determined by the suitability of the environment for the host, the host's resource use within its area of activity and the resources this provides to parasites. Therefore, parasites are crucially dependent on the lifestyle of their hosts and local environmental conditions (Toft & Karter, 1990). In terms of intestinal helminths, colonisation occurs through direct contact with contaminated surfaces, e.g. social interaction between hosts or contact with their faeces, or indirectly by ingesting infected final or intermediate hosts through their diet. The habitat and space use of the host species can have crucial influence on both, direct contact rates and diet. For example, urban red foxes tend to have smaller home ranges than rural conspecifics, live partly in social groups instead of being solitary and show higher population density (Šálek et al., 2015). Furthermore, urban red fox populations have a smaller dietary niche, whereas rural ones show a higher variability in their food resources (Scholz et al., 2020). A higher variability within the diet may lead to the ingestion of several different potential intermediate or paratenic hosts and, thus, parasite species, resulting in higher helminth diversity in the gastrointestinal tract of the red fox.

Finally, community assembly strongly depends on the species interactions, in our case at the level of different parasites. Often, the parasite fauna of a host is established to an important extent through biotic interactions (Holmes, 1973), i.e. competition within the gastrointestinal tract between parasites or facilitation. The latter happens when a parasite weakens the immune system of its host, allowing other parasites to infest its host (McSorley & Maizels, 2012).Studies on pairwise interactions find both facilitation and competitive suppression of e.g. helminths and Coccidia (Clerc et al. 2019). Whether these processes can be deduced from hierarchical analyses meta-communities and individual communities alone without treatment experiments, however, remains an open question (Rynkiewicz et al. 2019). We here ask the alternative question how such species interactions are embedded within a broader community assembly framework of dispersal and environmental filtering and focus on community structures rather than helminth interactions.

Our study presents the analysis of an epidemiological survey of intestinal helminths found in red foxes of Germany using DNA metabarcoding. We analysed the infestation of the red fox population with different relevant helminthic genera (nematodes and plathelminthes). We assessed the effects on helminths presence and their diversity patterns of an urban-rural gradient, defined by e.g. human population density and tree cover, foxes diet, and parasite transmission traits. With this, we aim to identify key environmental variables, helminth tratis and co-occurrence patterns that drive helminth community assembly. Further, we evaluate whether parasites with a zoonotic risk to humans and pets perform differently in the urban environment than non-zoonotic helminths.

We hypothesize that urbanisation affects the population structure, space use and behaviour of red foxes and hence also dispersal of helminth species. We predict that foxes in rural areas show higher helminth diversity, along with a higher diversity of their diet. Males that usually have a more extensive space use will likewise have higher helminth diversity than females. As helminths with complex life cycles depend on their multiple hosts (intermediate and primary host) helminth’s occurrence depends on the abundance of their host and the uptake of this host through the red fox's diet. Thus, we predict that helminths that are transmitted through food items are mainly found in natural environments, as diet of red foxes in urban areas based on anthropogenic instead of natural food resources. Since with higher densities of red foxes, humans and their pets, the contact rates also increase, we predict that foxes in close proximity to humans show higher prevalence of helminths associated with humans or domestic animals.

Methods

Study area

The study was conducted in the federal states of Berlin and Brandenburg in the north-east of Germany (Fig. 2). Berlin is the capital of Germany and is inhabited by 3.5 million people on an area of about 900 square kilometres. Compared to other big cities, Berlin has a relatively large number of green spaces. The urban structure is very heterogeneous, ranging from highly urbanised areas with high-rise buildings, multi-lane roads and areas of high proportion of sealed surfaces to districts dominated by lakes, forests and green spaces. Thus, about 2500 city parks, 160 square kilometres of forest (18 %) and several lakes characterise the city nature. In total, nearly 60% of city surface is sealed with housing and traffic. The federal state of Brandenburg completely surrounds Berlin. It is dominated by agricultural land (49 %) and small forests (37 %), and has just a few agglomerations (7 %). Both federal states together cover an area of 30.371 km^2 with a maximum diagonal extension of 291 km. Since several forests, lakes and green spaces stretch into the city and the periphery of the city is mainly characterised by single-family housing communities with gardens, there is a kind of suburban transition from the rural areas of Brandenburg to the highly urbanised city centre of Berlin (www.statistik-berlin-brandenburg.de, 2020).

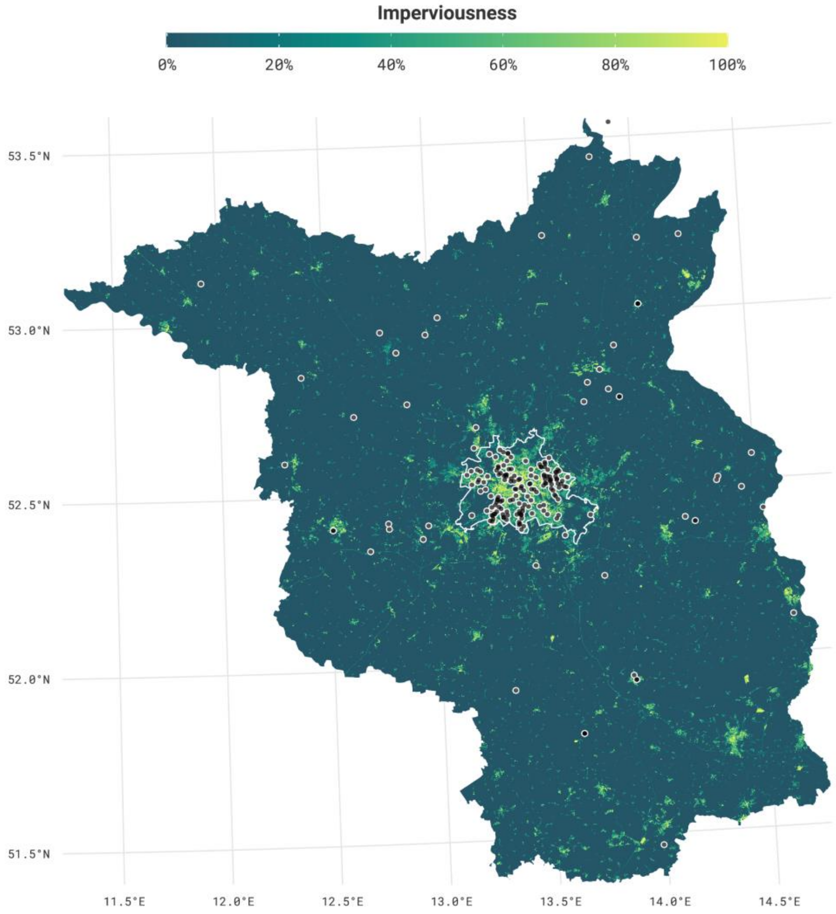


Figure 2: Study area. Degree of imperviousness over study area illustrated by a blue (low imperviousness) to yellow (high imperviousness) gradient. Circles represent sample locations (n = 200).

Red fox carcass sampling

In cooperation with the state laboratory Berlin-Brandenburg (LLBB), we collected a total of 620 faecal samples from the rectum of dead foxes originating from urban and rural environments of Berlin and Brandenburg. These samples stem from foxes that were either involved in road accidents, were hunted or died of natural causes in summer 2016 to spring 2018. No animal was killed with the aim of providing samples for this study. For most individuals, location, sex, age and (depending on state) weight were recorded. All samples were stored at -80 °C until further analysis.

DNA extraction and metabarcoding procedure

DNA was extracted from colon content of all 620 red foxes (thus, representing parasites and diet). The NucleoSpin© Soil kit (Macherey-Nagel GmbH & KG, Düren, Germany) was used following the instructions of the manufacturer. The quality and concentration of the extracts was determined by spectrophotometric measurements in a NanoDrop 2000c (Thermo Scientific, Walham, USA). All DNA extracts were normalized to a concentration of 30 ng/μL for further analysis. Multimarker amplification was perfomed with the Fluidigm Access Array 48 x 48 system (Fluidigm, San Francisco, California, USA. Library preparation is integrated into the amplification procedure and was performed according to the protocol Access Array Barcode Library for Illumina Sequencers (single direction indexing) as described by the manufacturer (Fluidigm, San Francisco, California, USA). The amplicon mixes were quantified (Qubit fluorometric quantification dsDNA High Sensitivity Kit, Thermo Fisher Scientific, Walham, USA) and pooled in equimolar concentration. The final library was purified using Agencourt AMPure XP Reagent beads (Beckman Coulter Life Sciences, Krefeld, Germany). Quality and integrity of the library was confirmed using the Agilent 2200 TapeStation with D1000 ScreenTapes (Agilent Technologies, Santa Clara, California, USA). Sequences were generated at the Berlin Center for Genomics in Biodiversity Research (BeGenDiv) in two runs on the Illumina MiSeq platform (Illumina, San Diego, California, USA) using v2 chemistry with 500 cycles. All sequencing raw data can be accessed through the BioProject PRJNA386767 in the NCBI Short Read Archive (SRA).

Sequencing reads processing from quality control to taxonomic assignment was performed using the package MultiAmplicon development version commit (Heitlinger, 2019). Custom scripts and the detailed pipeline is described in https://github.com/VictorHJD/AA_Fox/Fox_general_MA.R. In brief, sequencing reads were trimmed to a length of 250 bp allowing a maximum error of 2 nucleotides and zero ambiguous nucleotides (N=0). They were sorted into an amplicon based on the sequence of specific primer pairs. Forward and reverse sequencing reads were de-replicated, concatenated and chimaeras were removed using the package dada2 (Callahan et al. 2016). Sequences were trimmed in read pairs with zero mismatches in both forward and reverse reads. MultiAmplicon package assigns taxonomy to the inferred Amplified Sequence Variants (ASVs) based on BLAST+ against 18S sequence from the NCBI nt database unbiased for suspected target sequences. Taxonomy was assigned based on the single best hit or a last common ancestor in case multiple best hits. Assigned ASVs were compiled by sample ID and species annotation into a single occurrence matrix with the package phyloseq v1.28.0 (McMurdie and Holmes, 2013) for further analysis. We identified 82 helminth genera (including potential false positives from spurious annotations) and 8 diet groups (Annelida, Arthropoda, Aves, Insectivora, Lagomorpha, Mollusca, Pisces, Rodentia).

Data analysis and statistics

The final data set contained 217 observations (i.e. fox samples) with 82 helminth genus detections (0 for absence, 1 for detection), of which 200 metadata such as fox gender, age, location and weight could be assigned. These were used for further analysis. 183 samples were from adults and 17 from juveniles, respectively. 125 samples originated from male and 75 from female red foxes. The data set lacked samples for warmer months in our latitudes, especially May, June, July and August. Since dead foxes were also collected during these months, it is likely that the higher temperatures at this time of year caused the carcasses to decompose more quickly and thus no suitable DNA could be extracted in sufficient quantities for further analysis.

To determine the influence of environmental drivers on helminth communities of red foxes, we used a set of different raster layers related to red fox habitat use (for further information see Appendix A). We extracted the values of different environmental variables within a 1 km radius (reflecting approximately the size of a red fox home range) of each location of death and extracted the mean of all raster cells within the buffer.

If available, information on human or pet relationship (yes/no/unknown) and transmission type (diet/other/unknown) were assigned to helminth genera found in our data set based on literature research.

Helminth diversity

We compared helminth diversity, measured as species richness (Hill number q=0, Chao et al. 2014) of helminth genera in Berlin versus Brandenburg and male versus female red foxes using R-package 'iNEXT' (Hsieh et al., 2020). To make the samples comparable, we rarefied the data based on sample size.

Influence of environmental filtering on parasite community co-occurrence and trait distribution

We studied helminths biotic and abiotic interactions in our Berlin data set using the hierarchical model of species communities proposed by Ovaskainen et al. (2017), and implemented in the R package HMSC (Tikhonov et al. 2020). This model belongs to the class of joint species distribution models (Warton et al. 2015, Ovaskainen et al. 2017), which allow to test simultaneously individual helminths and helminth traits responses to a set of explanatory variables, as well as helminth-to-helminth association matrix after controlling for those variables' effects (Pollock et al. 2014, Ovaskainen et al. 2017). The response matrix consisted of the helminth genera presence/absence data on each of the sampling units (red fox). We used three traits to describe our helminth community: human-related (categorical: *yes, no, unknown*), pet-related (categorical: *yes, no, unknown*), and transmission type (categorical: *diet, others, unknown*). As explanatory variables we included red fox sex (categorical: *male, female*), *natural* environmental variables tree cover (continuous) and arthropod index (continuous), and as *urban* environmental variables noise (continuous) and human population density (continuous) measured at the sampling unit level. Sample location was included as random effect to control for potential spatial effects. Because important environmental variables like noise, human population density and arthropod index were only available for Berlin, we restricted jSDMs to this area. Since Berlin has a rural-urban gradient from the city border to the city centre, we expect transferable results for the whole study area.

To run the model, we used non-informative priors (default option) in the Hmsc package (Tikhonov et al. 2020), and performed 110 000 Markov chain Monte Carlo (MCMC) iterations with three chains. The first 1 000 iterations were removed as burn-in and we used a thinning of 10, obtaining 10 000 posterior samples per chain (30 000 posterior samples in total). We evaluated model convergence visually by plotting the chains and using Gelman-Rubin diagnostics (Gelman and Rubin, 1992), which was close to 1 in all cases. We assessed the explanatory power of the model by computing the AUC value for the occurrence of each helminth genus and averaged it across all helminths. The average AUC value for the model was 0.85. Additionally, we applied variance partitioning to calculate the relative importance of the environmental variables grouped as *natural* variables (tree cover, arthropod abundance), *urban* variables (noise, human population density), fox *sex*, and random effects (see Appendix B). Finally, we classified the estimated helminth associations (on genus level) as positive, negative, or neutral, based on a posterior 75 credible interval and the estimated value of the parameter (positive or negative). Association parameters with credible intervals overlapping zero were assigned to neutral.

Parasite community structure

We applied a non-metric multidimensional scaling (NMDS) to our full presence-absence data set of helminths using the ‘vegan’ R package (Oksanen et al., 2019), with ordination based on pair-wise Jaccard distance between fox samples as a first analysis of factors explaining the helminth community structure in the whole study area. We fitted the ordination with helminth genera and diet based on the presence-absence matrix of the metabarcoding (Annelida, Arthropoda, Aves, Insectivora, Lagomorpha, Mollusca, Pisces, Rodentia). Since the final configuration may differ depending on the initial (random) configuration and the number of iterations, we ran the NMDS multiple times and compared the interpretation from the lowest stress solutions. The repetition of the scaling (n = 10) led each time to a convergence of data and very similar solutions, so we obtained a robust result. Overall stress for the multidimensional scaling was 0.24.

Results

Metabarcoding analysis identified 150 different operational taxonomic units (OTUs) for nematodes and plathelminths within 217 samples. 111 OTUs had a taxonomy assigned at a species level, and 132 OTUs were identified at genus level. After collapsing OTUs based on taxonomical annotations at the species (where possible) and genus level, a total of 82 different genera were detected. 14 taxa appeared exclusively in Berlin, 26 taxa could only be found in Brandenburg. The remaining 45 species were detected throughout the entire study area. 93 % of all red foxes were infested with at least one helminth genus. Only 7 % of the samples were completely helminth-free.

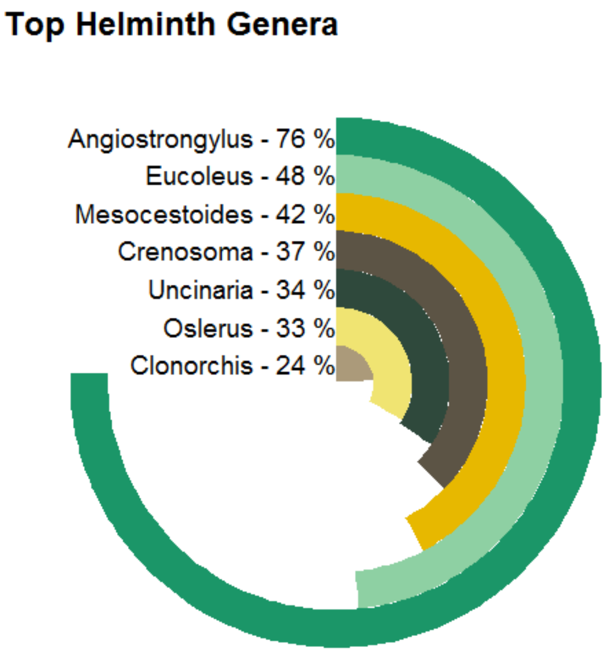


Figure 3: Most prevalent helminth genera (> 20 %). In total, 82 different helminth genera were identified during metabarcoding analysis within 217 red fox faeces samples.

In 75.6 % of samples Angiostrongylus was detected and thus is the most frequently occurring genus followed by *Eucoleus* (48.4 %) and *Mesocestoide* (42.4 %) (Fig. 3). *Trichinella spiralis* could not be detected in our samples. In addition, the fox tapeworm *Echinococcus multilocularis* was not identified in any single sample but in 5 cases the dog tapeworm *Echinococcus granulosus* was found. The infestation with *Teania* species was also very low (only 5 cases). On the other hand, *Toxocara canis* was detected in 26 samples (12 %).

Helminth diversity

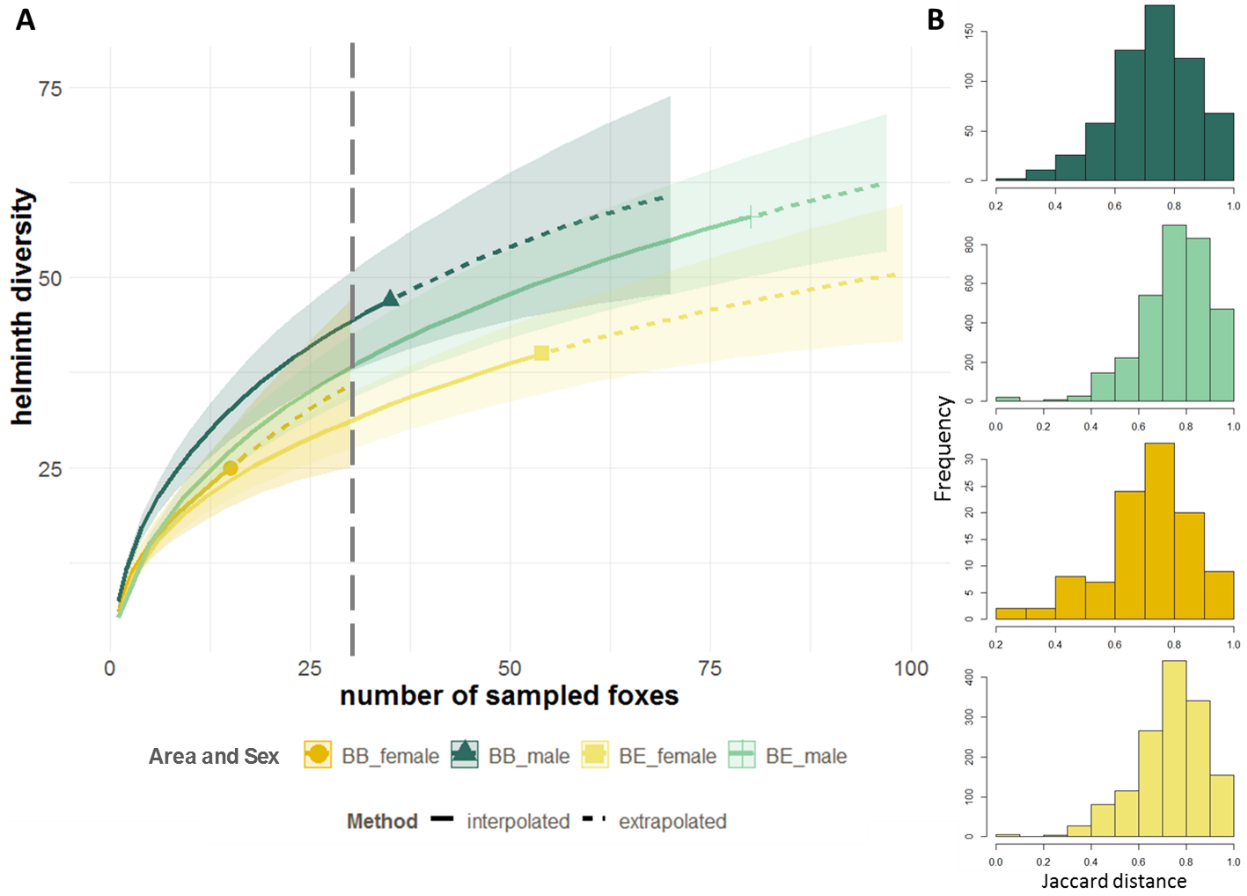


Figure 4: A – Rarefaction curve for helminth diversity (Hill number q = 0) at the genus level of Berlin (BE) and Brandenburg (BB) male (blue) and female (yellow) red fox samples. Shaded area represents 95 % confidence interval. Vertical dashed line represents reference of group comparison. Brandenburg red foxes tend to show higher helminth diversity compared to red foxes of the city Berlin and males tend to have higher helminth diversity than female red foxes. B – Jaccard distance is high in all groups (overall mean of 0.76 ± 0.15), thus, helminth composition differs between foxes (host level), indicating a high community turnover.

Rarefaction curves for helminth diversity point out that the complete parasite spectrum of red foxes in our study area has not yet been recorded as asymptotes of the curves have not yet been reached (Fig. 4, panel A). In addition, the analysis shows no significant difference in helminth diversity between groups of interest. Nevertheless, a trend can be seen that helminth diversity of red foxes of Brandenburg is higher than for red foxes of Berlin. Male foxes tend to have higher helminth diversity in comparison to female red foxes of the study area. This pattern between sexes is consistent for Berlin and Brandenburg. Overall, pairwise Jaccard distance of each pair of red fox samples was high in all groups (Fig. 4, panel B) with average dissimilarity of 0.76 ± 0.15. This means that the helminth composition on the host level differs greatly between sampled red foxes.

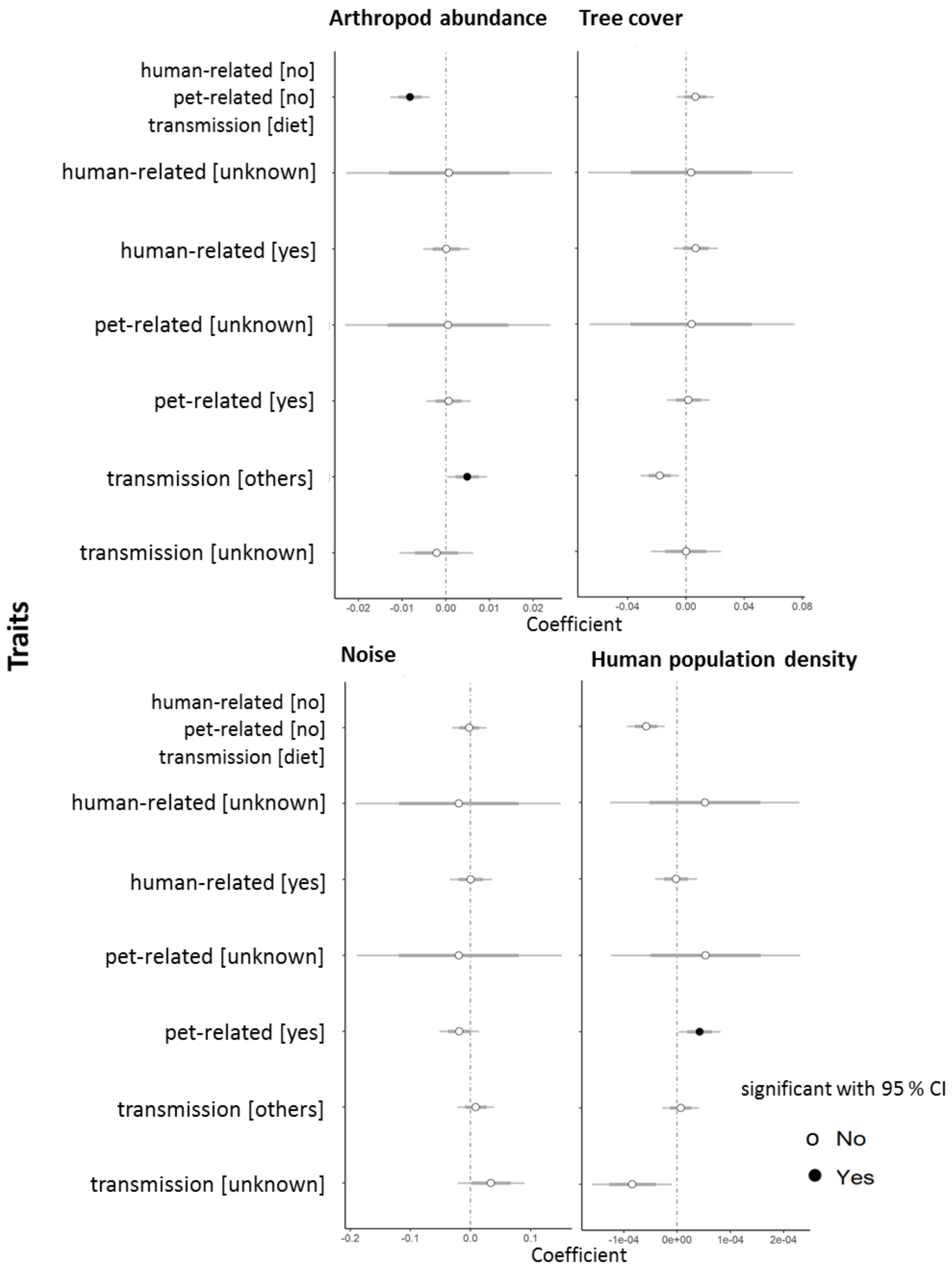

Arthropod abundance
Tree cover
human-related [no]
pet-related [no]
transmission [diet]
human-related [unknown]
human-related [yes]
pet-related [unknown]
pet-related [yes]
transmission [others]
transmission [unknown]
-0.02
-0.01
0.00
0.01
0.02
-0.04
0.00
0.04
0.08
Coefficient
Traits
Noise
Human population density
-0.2
-0.1
0.0
0.1
-1e-04
0e+00
1e-04
2e-04
Coefficient
significant with 95 % CI
○ No
● Yes

Figure 5: Helminth traits responses to a set of natural and urban explanatory variables of Berlin. The response matrix consisted of the helminth presence-absence data (genus level) on each of the sampling units (red fox). We used three traits to describe our helminth community: human-related (categorical: *yes, no, unknown*), pet-infecting (categorical: *yes, no, unknown*), and transmission type (categorical: *diet, others, unknown*). As explanatory variables we included red fox sex (categorical: *male*, *female*), natural environmental variables tree cover and arthropod abundance (upper row in the plot), and urban environmental variables noise and human population density (bottom row in the plot) measured at the sampling unit level. Association parameters with posterior 75 credible intervals (bold grey line) overlapping zero (dotted vertical line) were assigned to neutral, otherwise to positive (right side of dotted line) or negative (left side of dotted line). Results also significant with 95 % confidence interval are represented by a filled, black point. First row (human-related[no], pet-related[no], transmission[diet] represents intercept. Sex had no effect on investigated traits.

We used arthropod abundance and tree cover as descriptors of natural environments. With low arthropod abundance, i.e. low ‘naturalness’ of the area, there are more helminths associated with humans or pets (Fig. 5). Probability of presence of helminths transmitted by “others” (i.e. not via diet or unknown transmitter) is greater with higher arthropod abundance, i.e., more natural areas. In our case noise is not a suitable predictor for helminths with different traits. Probability of presence of pet-infecting helminths is positively related to human density. The sex of the red fox had no effect on the presence of helminths with specific traits.

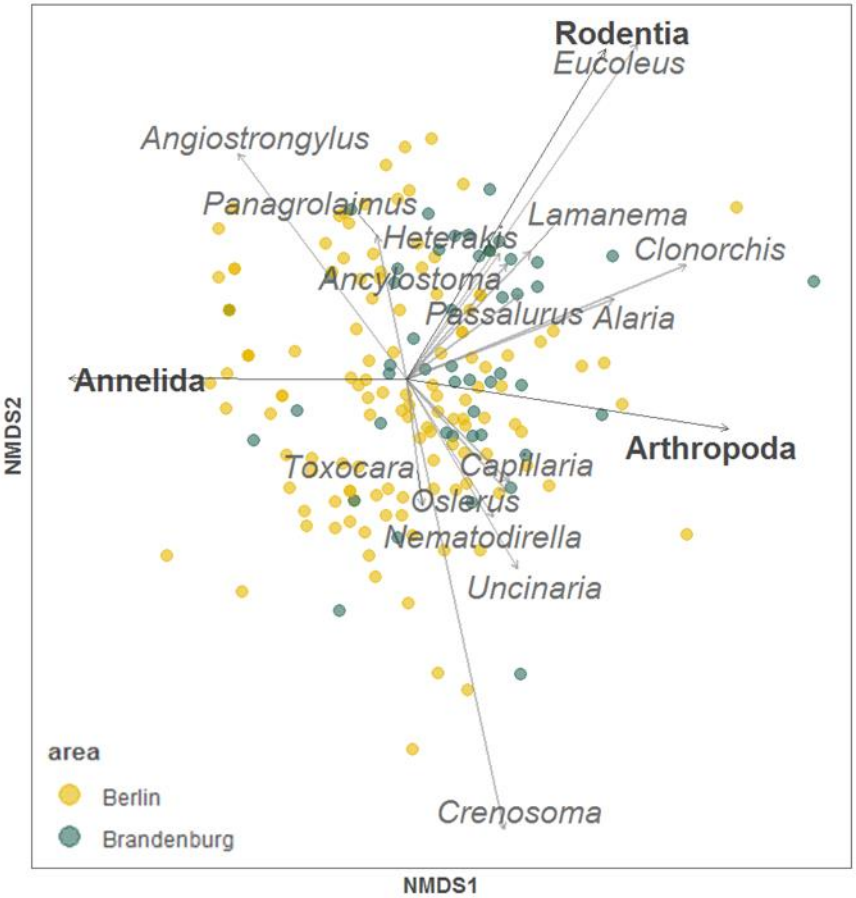


Figure 6: Non-metric multidimensional scaling plot. Sampling units (red fox samples) are displayed by circles, colour of circles (yellow = Berlin, blue = Brandenburg) represent area of red fox location. Closeness of circles indicates similarity in their composition. Significant diet features (Annelida, Arthropoda, Rodentia) are presented in bold, significant helminth genera in italic. Line lengths along ordination axes indicate correlation strengths.

We identified 15 helminth genera driving the helminth communities within red fox samples. (Fig. 6). 10 out of these genera are known for their potential transmission to either humans or pets or both. Additionally, the diet based on Rodentia, Annelida and Arthropoda determines the helminth composition. Importantly, there is an indication that helminth communities of the red foxes in Brandenburg represent a subset of the larger helminth community of the red foxes in Berlin.

Discussion

We examined mechanisms driving the helminth diversity of red foxes along the rural-urban gradient. Three main findings can be derived from our analyses. First of all, we found slightly higher helminth diversity in (more rural) Brandenburg compared to the (more urbanised) city Berlin. Additionally, males tend to have higher helminth diversity than female red foxes. Second, with increasing human population density, pet-infecting helminths are more prevalent. Third, diet features can drive helminth communities in red fox populations as well as interactions between helminths itself.

Red foxes, like many wild animals, are a reservoir and potential transmission pathway for various helminths. The high infestation rate of 93% in our study area illustrates this once again. However, they are often also transmitters of helminths with zoonotic potential. Helminths with zoonotic potential or associated with pets are also described for our most frequently detected genera, *Angiostrongylus*. This genus includes nematodes that parasitise in the lungs, pulmonary arteries, right atrium or mammalian or avian mesenteric arteries. The most commonly detected species within this genus is *Angiostrongylus vasorum* (identified in 67.7% of samples). Infection of dogs occurs by ingesting third larval stages while feeding on infected slugs or snails (intermediate hosts). Clinical signs of *A. vasorum* infestation are highly variable and can progress unnoticed or be fatal but has no zoonotic potential. The second most often detected genus in our study, *Eucoleus,* is assigned to the family *Capillariidae*. Since the taxonomy of the *Capillariidae* is disputed, the species are included in the single genus *Capillaria* or 22 different genera, including *Eucoleus*. This genus consists of parasitic hairworms. Hairworm infections also play a major role in veterinary medicine in dogs. For humans, the lung hairworm and liver hairworm are of particular importance. Within our data set only the lung hairworm

(*Capillaria aerophila*, 19.8%; Syn.: *Eucoleus aerophilus*, 5.5%) could be detected. Infection occurs through ingestion of food or water contaminated with eggs. The larvae hatch in the intestines and enter the lungs via the bloodstream. The lung hairworm rarely causes symptoms of disease; only in the case of more severe infestation does bronchitis with coughing occur, usually as a result of accompanying bacterial infections. *Mesocestoides* is a genus of parasitic flatworms that infests cats, dogs and wild canids including red foxes as final host. Some species affect very seldom also humans but human infections are extremely unusual in developed countries. The disease caused by *Mesocestoides* is called mesocesdoidosis but intestinal infections are usually benign and without clinical signs. Although often common for red foxes, *Trichinella spiralis* and the fox tapeworm *Echinococcus multilocularis* was not detected in any single sample. Infestation with different helminths varies greatly from region to region. For example, *Echinococcus multilocularis* was found in 20% of the Belgian red fox samples tested (Losson et al., 2003), in a study in Slovakia the prevalence was over 30% (Miterpáková et al., 2009) or up to 67% in parts of Zurich (Hofer et al., 2000). Results of helminth diversity show that not all potentially available helminths have been detected. Thus, helminth communities found at the host individual level are also just a subset of a larger pool of helminths within the host population or locality (here red foxes from Berlin vs Brandenburg) (Poulin, 1997). Therefore, we could not say with certainty that some helminth genera occur exclusively only in Brandenburg or Berlin, nor that some helminths are absent in our red fox populations. To answer this question requires a larger data set and a saturation of detected helminths for the respective host population.

Communities are determined by biotic and abiotic factors. The co-occurrence of certain species depends, among other factors, on whether they can coexist. We clearly found helminths that co-occurred, and also some that rarely occurred together. Certain helminths occur more often together. Other species form the opposite end of this continuum. However, gastrointestinal helminths are largely dependent on their hosts and their abundance, density and lifestyle (Ehlers & Kaufmann, 2010; Wells et al., 2019). For example, higher host densities can increase the contact rate and transmission rate of helminths or their developmental stages, e.g. via direct contact with contaminated faeces. High densities of intermediate or definitive hosts that serve as food for the red fox also increase the likelihood of them ending up in the red fox's intestinal tract. The red fox in particular is a generalist species that prefer food resources that are readily available and in high densities (Jedrzejewski & Jedrzejewska, 1992; Leckie et al., 1998). The fact that certain food items can determine the helminthic communities of red foxes has also been demonstrated. The more variable the diet, the higher the probability of being exposed to a large number of different helminths (Vitone et al., 2004). Brandenburg red foxes tend to have a higher helminth diversity compared to their Berlin counterparts. Since food has a decisive influence on helminth community assembly, it is likely that helminth diversity correlates with dietary variability. Rural fox populations in our study area have a larger dietary niche compared to urban animals, covering even several trophic levels (Scholz et al., 2020). Therefore, it can be assumed that the Brandenburg fox population have a higher dietary diversity, which ultimately explains the higher helminth diversity. Nevertheless, the detection of food items in the intestinal tract of red foxes in our study reflects food consumed recently, whereas helminths may have accumulated over a longer period of time and may not necessarily be related to food found. However, as the fox's diet usually reflects the availability in the habitat (Jedrzejewski & Jedrzejewska, 1992; Leckie et al., 1998) and at the individual level, red foxes often have a specialised diet (Scholz et al., 2020) food resources found may also have been eaten before. Our investigation has also shown that male red foxes tend to have higher helminth diversity in comparison to females. In the past, sex-specific differences in food niches have been observed (Englund, 1965; Kidawa & Kowalczyk, 2011). This is mainly explained by higher dispersal ability and larger home range size of males, whereas the smaller home ranges of females are a result of the energetic and behavioural shifts due to breeding activity (Travaini et al., 1993).

The transmission dynamics and stability of helminth communities are determined by an interplay between density, spatial distribution and processes regulating population growth of helminths and host species. In case of pet-related helminth systems, our results show that higher human population densities (associated with higher pet densities) lead to an increase in the prevalence of pet-related helminths in red foxes. In contrast to that, an increase in human-associated helminth presence could not be detected within our study. This suggests that the density of potential hosts such as humans and pets alone is not decisive for the establishment of associated helminths. Helminths are also dependent on successful transmission and infestation. This occurs mainly through contact with contaminated surfaces or through oral ingestion. Especially dogs and cats are therefore particularly at risk. Their natural behaviour, such as sniffing or rolling around in faeces and dead wild animals, but also hunting and feeding on potential helminth hosts such as small mammals, increases the likelihood of transmission. Infection via this route to humans is rather unlikely. The application of certain hygiene standards and the consumption of controlled, cleaned or even heated food rules out the possibility of helminth infection via this pathway. Nevertheless, pets themselves would be a potential transmission route if they cuddle or even lick their owners after contact with helminths. It is known that a major cause of zoonoses is the close contact between dogs and humans (Robertson et al., 2000).

Whether the increased presence of pet-associated helminths is also reflected in an increased prevalence of these helminths in pets in our study area cannot be analyzed with our data. However, other studies have shown that relevant helminth species were detected in higher numbers in, both soil samples and faeces from dogs in urban areas (Azian et al., 2008). It is difficult to assess whether the increased occurrence of pet-infecting helminths in urban red foxes also reflects a higher abundance of these helminths in general or only the increased transmission or more successful colonization within the red fox population. For example, the high red fox density in the cities could favour the transmission of helminths. Increased stress and the associated poorer physical condition of foxes or the increased occurrence of older foxes due to the lack of hunting pressure in the city could increase infestation and detectability. In this case we would expect a general increase in helminths in cities, which we cannot confirm. Pets, which serve as an additional reservoir for this type of helminths, therefore favour the establishment of pet-infecting helminths. Nevertheless, pet-infecting helminths do not necessarily have zoonotic potential. In addition, the use of DNA metabarcoding allows the identification of a broad spectrum of helminths, but we also detect fox non-specific helminths that are ingested only with food and would be excreted afterwards.

We demonstrate the diversity of helminths from the intestines of rural red foxes tend to be higher compared to urban red foxes. Nevertheless, helminth co-occurrence patterns that helminth communities on the red fox population level overlap. This may potentially be explained by the fact that Berlin provides lots of green areas, thus the urban conditions are perhaps not as prominent as they could be in other cities. Those green areas also promote connectivity between urban and rural regions. Yet, these findings are nuanced when looking closer at the descriptors of urban areas. Indeed, high population density was associated with higher probability of finding pet-related helminths with important implications for the disease management in the cities. The next step would be to include, in addition to the traits of helminths, niche differences of the helminths themselves (i.e. helminths which rely on ingested food by host vs. bloodsucking or helminths living in the colon vs intestine of the host) in the analysis and, based on detailed nutritional data of the foxes, to investigate the influence of dietary diversity on the diversity of helminths in the intestinal tract of the fox.

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Chapter 3 - The city never sleeps but city foxes do – habitat-dependent differences in spatial and temporal patterns of resting behaviour in a synanthropic species

The city never sleeps but city foxes do – habitat-dependent differences in spatial and temporal patterns of resting behaviour in a synanthropic species

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Abstract

The overall purpose of time allocation to different activities is to satisfy all vital needs and therefore ensure survival and maximise fitness. In theory, balancing energy intake and energy expenditure is the main goal. In this context, resting is a strategy to save energy for recharging. Many animals often spend most of their daily time in inactivity. Inter- and intraspecific differences in activity patterns are mainly explained by disturbance avoidance and food availability. In this context, cities represent special conditions differing from natural habitats. On the one hand, high human population density and associated increase in traffic and noise may be challenging for wildlife. On the other hand, the constant availability of anthropogenic food could be beneficial and is often implemented to high extent in the diet of urban animals. We investigated habitat-dependent differences in resting patterns of a typical and successful synanthropic species – the red fox (Vulpes vulpes) in urban (Berlin) and rural (Brandenburg) environments. Through the use of high-resolution GPS-acceleration data we provide detailed insight into when, how much, how long and how often red foxes rest. To determine the influence of position in the trophic chain on patterns of resting behaviour, we further compared red foxes with the coexisting species raccoon (*Procyon lotor)* and European hare (*Lepus europaeus*). Overall, urban foxes tend to rest more, with their resting behaviour concentrated

during the day and reflected mainly by an increased number and longer-lasting long-term resting events. Rural red foxes of Brandenburg tend to have more resting sites whereas especially the number of sporadically used resting sites is increased compared to Berlin foxes. The duration and number of resting events of foxes and raccoons are very similar, whereas field hares have significantly shorter resting events, which rarely last longer than 30 minutes and are compensated by an increased number of short-term resting events.

Introduction

For most animals, time is a key resource, which they have to economise every day. The overall goal of time allocation to different activities is to satisfy all vital needs and therefore ensure survival and maximise fitness. In theory, this means balancing energy intake and energy expenditure (Norberg 1977). A large number of scientific studies on time allocation of animals focus on costly behaviours such as foraging, travelling, vigilance, social behaviour and mating (Bancroft and Margolies 1996; Brown 1999; Korstjens et al. 2010; Kotler et al. 2010; Abáigar et al. 2018 but see Hooten et al. 2019). However, many animals spend most of their available time in inactivity (Herbers 1981). Inactivity reduces energy requirements and allows animals to be less active and therefore less exposed (Berger and Phillips 1990). Thereby inactivity can mean resting or sleeping. Although the benefits of resting are still under debate (Siegel 2009; Schmidt 2014; Charbonneau and Dornhaus 2015), it is clear that important physiological processes such as recovery or digestion, thermoregulation or cognitive processes occur also while animals rest (Siegel 2005). Real sleep fulfils various functions such as consolidation of memories, brain development or parasite resistance and is dependent on species body metabolism. Prolonged lack of sleep impairs cognitive performance (Horne 1988). Besides, severe sleep disorders are considered a health risk factor that can worsen disease processes (Miller and Bartus 1982) and even reduce longevity (Kripke et al. 1979). Therefore sleep is indispensable. Timing of sleep and resting is affected amongst others by digestive constraints, food availability and even activity patterns of coexisting species (Ball 1992). However, due to limited responsiveness (Bushey et al. 2015) sleep it is a very risky condition for many animals (Ramakrishnan and Coss 2001; Lima et al. 2005; Stuber et al. 2014) and should be kept to a minimum and be performed in a safe environment that provides shelter. In contrast, resting also serve behavioural functions and can be beneficial. Resting in terms of quiet wakefulness allows surveillance of the environment and predator detection (Capellini et al. 2008b). At the same time, the animal itself is less conspicious than in motion. Safety of the environment is described as the main factor influencing sleep quality (Allison and Van Twyver 1970). Mammals inhabiting relatively exposed environments have therefore shorter sleep cycles (Lima et al. 2005). Risk is often seen as an important feature of the background environment (Clark and Levy 1988; Werner and Anholt 1993; McNamara and Houston 1994) but we also have to consider temporal variation in risk (Lima and Bednekoff 1999). Besides predation, the disturbances by human activity, noise or severe weather conditions can be perceived as risky by wildlife. Thus, both, the selection of resting sites and the timing of resting events are crucial to avoid such disturbances.

Even though resting per se fulfills different functions for different species in different environments, timing and resting site selection is related to the prevailing ecological or physiological conditions of an animal (Roberts and Dunbar 1991; Siegel 2005; Korstjens et al. 2010). Thus, the ecological niche of a species could have a decisive influence on resting behaviour. Cities represent an artificial, unnatural habitat that confronts its wildlife with a variety of challenges and novel niches. Urban dwellers, who thrive in urban landscapes independently of natural areas (i.e. green spaces such as residual or restored habitats) and urban adapters, who can use urban landscapes but generally do not benefit from them and still rely primarily on more natural areas, have adjusted to these changed conditions (McKinney 2002; Fischer et al. 2015). This usually results in different lifestyles compared to their rural counterparts.

A typical urban dweller is the red fox (*Vulpes vulpes*) and it shows impressive adjustments to city life. For example, while it is common for foxes to raise their young in dens surrounded by natural vegetation (Goldyn et al. 2003; Macdonald 2011), breeding dens of city foxes are often associated with buildings (Baker et al. 2000;

Marks and Bloomfield 2006). Urban foxes are also described as more nocturnal than foxes in peri-urban or rural areas (Gil-Fernández et al. 2020). Another recognised adjustment of urban red foxes is that they base their diet mainly on anthropogenic food resources such as waste, compost or pet food (Harris 1981; Contesse et al. 2004; Scholz et al. 2020). Compared to their natural prey, this is often available and predictable regardless of season. As a result, little energy and time need to be spent on foraging, which could impact resting behaviour.

Historically, it has been challenging to precisely identify the different behaviour types of free-ranging wildlife, but the field of animal ecology is experiencing a rapid development of advanced technologies, especially for wildlife tracking. Modern tracking units can be equipped with additional sensors, which not only provide insights into the whereabouts of an animal but also link the location to physiological parameters or the animals' behaviour (Rutz and Hays 2009; Bograd et al. 2010; Fehlmann and King 2016). Animal behaviour can be inferred for example by acceleration sensors, which are often used as a proxy for animal activity and energy expenditure (Wilson et al. 2006; Qasem et al. 2012; Brown et al. 2013; Shepard et al. 2013), but also reveal different behavioural modes of animals including resting (Nathan et al. 2012). We used GPS-acceleration (ACC) sensors at high resolution (GPS: 4 minutes, ACC: 2 minutes) to describe spatial and temporal patterns of resting behaviour of red foxes from Brandenburg und the city Berlin. Additionally, we investigated temporal patterns of resting behaviour of two coexisting species from Brandenburg: the European hare (*Lepus europaeus*) and the raccoon (*Procyon lotor*). This helps to evaluate how trophic position of an animal and its body size may affect timing of resting behaviour. High resolution ACC data allows us to determine daily resting period, number of resting events and length of resting events of all three species. Coupling the resting behaviour assignment based on the ACC data with the high-resolution GPS data of red foxes also allows us to identify and characterise resting places. The term 'resting' is usually used to describe sleep or dormant states (e.g. torpor, hibernation), but could also mean that the animal just doesn't move (Siegel 2005, 2009). In the context of the present study, we define 'resting' as a state or basic behavioural mode of immobility and complete motionlessness independent of posture (e.g. staying, sitting, lying) including sleep.

We hypothesise that different living conditions in urban and rural areas, which lead to behavioural adjustments and different lifestyles in red foxes, influence the resting behaviour of red foxes. We predict that total daily resting time, number and duration of resting events of urban red foxes are higher in comparison to rural red foxes due to decreased foraging time and increased consumption of anthropogenic food described for city foxes (Baker et al. 2000; Contesse et al. 2004; Scholz et al. 2020). Furthermore, we predict that greater home ranges and increased availability of natural structures in rural red fox populations compared to urban ones (Harris and Rayner 1986; Gloor 2002; Börner et al. 2009; Corlatti 2014) provide a greater number of resting sites and higher transmission rate between resting sites in rural foxes compared to urban foxes. When comparing the temporal resting patterns of the red fox with those of raccoons and European hares, clear differences due to their position in the food chain can be expected. We predict raccoons to have a similar resting pattern as foxes, whereas hares as a typical prey species overall rest much less and subdivide resting into short resting periods.

Methods

Study area

The study was conducted in the northern part of the federal state of Brandenburg (BB) and in Berlin (BE), Germany. Berlin is the capital of Germany and is inhabited by 3.5 million people on an area of about 900 square kilometres. The urban structure is very heterogeneous, ranging from highly urbanised areas with high-rise buildings, multi-lane roads and areas of high proportion of sealed surfaces to districts dominated by lakes, forests and green spaces. Thus, about 2500 city parks, 160 square kilometers of forest (18 %) and several lakes characterise the city nature. In total, nearly 60% of the area is sealed with housing and traffic. Berlin is completely surrounded by the federal state of Brandenburg. It is composed of small forests (37 %)

and few agglomerations (7 %) but dominated by agricultural land (49 %). Within agricultural areas, comparatively large fields and monocultures are the rule. (www.statistik-berlin-brandenburg.de, 2020). The data collection in Brandenburg was part of the long-term research platform 'AgroScapeLab Quillow' of the Leibniz Centre for Agricultural Landscape Research (ZALF).

Data collection

We used data from eight raccoons, nine hares and two red foxes of Brandenburg from 2011 (for capturing details see Appendix 1 and 2) available and downloaded from movebank.org (Wikelski and Kays 2014). Additionally, we caught 11 red foxes between 2016 and 2018 (4 in Brandenburg and 7 in Berlin). For this later field campaign, we used wooden walk-through live box traps (comparable with model WEKA and Weisser, 200 x 40 x 35 cm) equipped with a guidance system on both sides of the trap. For several months, the traps were kept permanently open (not armed), regularly baited (Frolic® dry dog food) and monitored only with camera traps so that the wild animals could get used to the traps. When the trap was repeatedly visited by a fox, it was armed. All active traps were controlled twice a day (morning and evening hours) until an additional electronic trap monitoring system (Trapmaster, EPV Electronics GmbH) was used to check the status of the traps constantly. The tagging of captured target animals was done on the spot in the field. A separate cage was placed in front of the trap and waited for the captured animal to leave the trap independently. In the handling cage, the animals were first weighed using a hand scale. All animals that did not reach the required minimum weight (> 4 kg) were released directly on site. All suitable red foxes were immobilised by an intramuscular injection of a xylazine-ketamine combination ('Hellabrunner mixture') with a blowpipe or syringe. To keep the side effects as low as possible, respiratory accelerators were not used. If the desired sedation did not occur after 10 minutes, the anaesthetic was added again. An eye gel was used to prevent the cornea from drying out and the eyes were covered with a cloth. Afterwards, foxes were examined for injuries and ectoparasites, sexed and aged. All suitable animals were fitted with a GPS-collar including an acceleration sensor (e-obs®, Grünwald, model 1C-heavy, 180 g). On average, the handling time for tagging was 25 minutes. Thus, the animals, which were usually still completely immobilised, were put back into the wooden box trap so that they could wake up safe and undisturbed. All immobilised animals woke up independently and were able to leave the trap on their own. Animal handling permits were approved by the respective animal welfare licensing committee of Brandenburg ("Landesamt für Umwelt, Gesundheit und Verbraucherschutz", permit number: 2347-25-2015 and V3-2347 13-2011) and Berlin ("Landesamt für Gesundheit und Soziales", permit number: G 0211/15). The raw data were stored on the collars and regularly downloaded via a handheld receiver with a yagi antenna or fixed base stations. The collars of all tagged animals provided tri-axial ACC data every 2 minutes. Therefore, we included eight raccoons, nine hares, 6 red foxes from Brandenburg and 7 red foxes from Berlin to the temporal analysis. For spatial analysis we used just 14 days of 4 red foxes of Brandenburg and 6 foxes of Berlin providing GPS data in high resolution of every 4 minutes.

Data preparation and temporal analysis of acceleration data set

The accelerometer collected acceleration of three perpendicular axes that represent the sway, heave and surge of the animal (figure 1, panel C) and therefore show different acceleration patterns for different behaviours (figure 1, panel A and B). The loggers just record raw digital readings of the analogue digital converter without any unit. To distinguish resting behaviour from other behavioural modes we followed the workflow of Collins et al. (2015). The method includes the examination of histograms based on basic metrics (e.g. mean and standard deviation) calculated from the acceleration data and determines threshold values to distinguish simple behavioural modes. Since resting means no movement and therefore barely any acceleration, we assumed that the standard deviation of raw acceleration values during each acceleration sampling interval would be rather low (figure 1, panel A). Hence, low standard deviations for all three axes most likely point towards motionlessness. Therefore, we used the standard deviation of the summed axes as a suitable metric to determine resting behaviour.

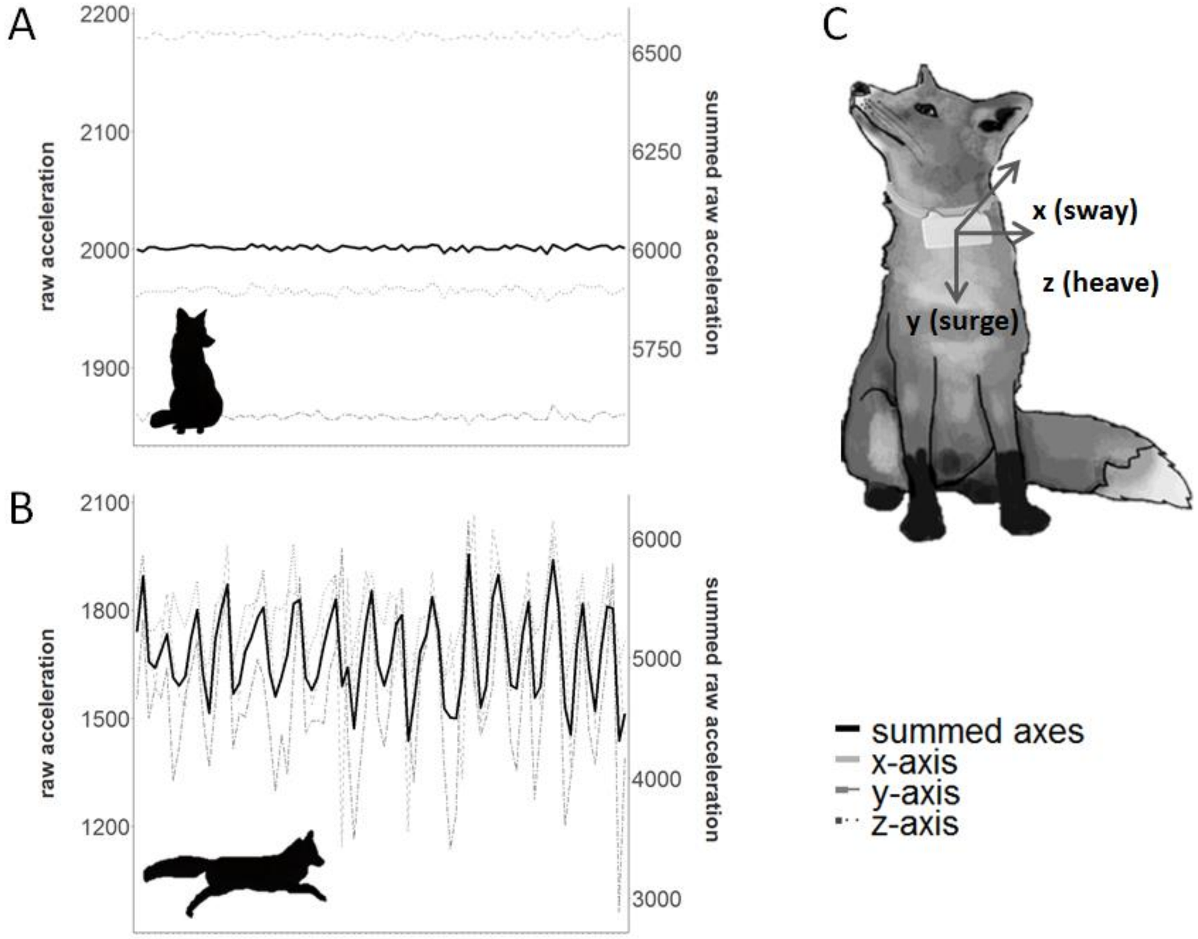

Figure 1: Example of Raw acceleration output for one burst of ACC sensor (A and B). Grey lines represent a single axis each with corresponding plot axis on the left side of the diagram ("raw acceleration"). The devices just record raw digital readings of the analogue digital converter without any unit. The accelerometer consists of three perpendicular axes that represent the sway, heave and surge of the animal (C). Since the patterns are dependent on the orientation and acceleration of the sensor, we can clearly distinguish resting (A) from non-non-resting behaviour (B).

The burst lengths (time during which the device records acceleration data) and sampling frequency differed between the studied individuals. Therefore, we calculated the mean and the standard deviation per burst for each axis and then summed up these values for all three axes within each burst. The resulting values do not interfere with the burst length.

As the standard deviation distribution was bimodal, the frequency minimum value between the two peaks was used as the threshold value for separating resting from movement, whereas everything < 10 corresponds to the state "resting". Please note that in this case it cannot be distinguished whether the animal is lying, sitting or standing. Outliers resulting from data gaps (temporary loss of data acquisition; > 600 min) were filtered out of the data set and the last resting event was removed, as this could potentially represent the death of the animal. We matched the behavioural mode (resting or non-resting) to the corresponding start time of the burst and were thus able to calculate the cumulative resting time for every resting event. A resting event is the successive occurrence of the assigned resting behaviour. As soon as the animal moves (non-resting) the resting event ends and a new resting event begins with the next resting behaviour. To calculate the daily proportion animals' are resting in comparison to non-resting, we summarized the number of assigned resting behaviours per hour (not to be confused with the cumulative resting events) in comparison to the total number of acceleration bursts for every individual. Afterwards, we computed the mean percentage of resting behaviour for a species per hour and summed them up over the entire day. However, the temporal indication of the duration of the resting behaviour per day is based on the duration of the resting event itself and is given in mean value ± standard deviation. Since a resting event can also reflect short-term inactivity of the animal that does not necessarily include resting but might be related to a break in current behaviour or being vigilant (since the data recording lasts just for seconds), we also highlighted long-term events in the evaluation and analysed them separately. We defined a long-term resting event as any event in which the animal had multiple consecutive individual resting assignments and thus rested for more than 30 minutes.

The ACC data of the four animal groups (hares, raccoons, Berlin red foxes and Brandenburg red foxes) originate from independent samples, therefore, do not affect each other and the data were not normally distributed. We tested for statistically significant differences in total daily resting, number and duration of resting events between groups using the Kruskal-Wallis test and for pairwise comparison of groups the Wilcoxon rank-sum test with correction for multiple testing.

Data preparation and spatial analysis of GPS data set

To investigate the number of resting sites, use and daily transmission between them of urban and rural red foxes we concentrated on animals with high-resolution data (every 4 minutes, n = 10 individuals). For comparability we just used random 14 days of continuous data recording for each individual.

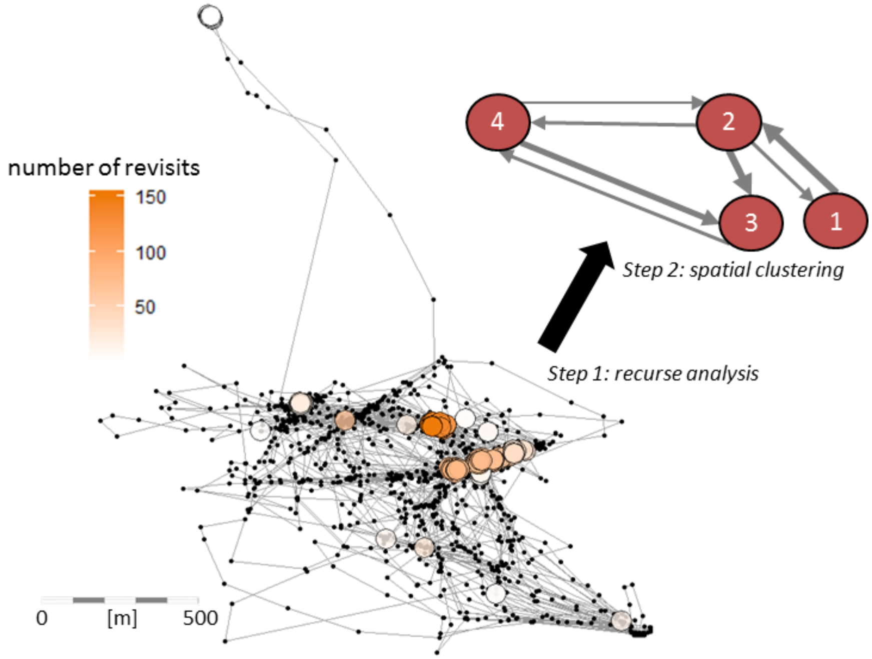

Figure 2: Exemplary illustration of the spatial analysis of resting places using the red fox "4142" as an example. Step 1 – Grey lines represent movement path, small black dots are GPS fixes and circles are resting sites (identified by ACC data). Colour of the circles represents the number of revisits over time within a 25 meter radius and used to distinguish permanent resting sites from sporadic resting sites. Step 2- Red circles (nodes) represent identified resting sites during spatial clustering using a k-mean approach. Grey arrows illustrate inter-site movement, thickness of arrows visualise the weight of connectivity based on number of transmissions.

For identification of resting sites we applied a recurse analysis (figure 2, step 1) using the 'recurse' package for R (Bracis et al. 2018). This package analyse revisitations based on trajectory data to identify ecological important sites. We set the radius to 25 meters to calculate number of revisits as maximum GPS accuracy error is 20 meters for our data set. Based on the resting behaviour assignment from the temporal analysis (ACC data), we identified sporadic and regular used resting sites. As a threshold we defined the mean number of revisits over all individuals. Observations assigned to 'resting' and with revisitation values greater than the threshold are categorised as 'permanent resting site', values less than threshold and assigned to 'resting beforehand resulted in the assignment 'sporadic resting site'. Afterwards we applied a clustering algorithm to locations of assigned resting sites to calculate number of resting sites for each individual (figure 2, step 2). To identify the optimal number of clusters we used the r package 'fpc' (Hennig 2018) to perform a partitioning around medoids clustering with the number of clusters estimated by optimum average silhouette width. For final clustering we used a k-mean approach.

All analyses were done in R (Version 3.5.1). Descriptive statistics represent mean ± standard deviation.

Results

We collected 1964918 behaviour events (including resting (n = 1088993) and non-resting events (n = 875925)) from 30 individuals in total for our ACC data set based on hares, raccoons and red foxes from Berlin and Brandenburg. After aggregating the individual data points to cumulated resting events, our results are based on 148983 resting events. Of these, 10801 events lasted longer than 30 minutes and were assigned to long-term resting events.

Final GPS data set for spatial analysis of red foxes consists of 10 individuals with 15622 data points in total.

Temporal analysis of resting behaviour

For all four studied groups, most of the resting behaviour occurred during light hours of the day (figure 3), demonstrating that all species are twilight and nocturnal active. Based on the simple resting state assignment (resting vs. non-resting) raccoons and hares rested about 5% less than foxes. Hares and raccoons spent only 40.9 % or respectively 41.5 % of the day resting, whereas foxes of Brandenburg spent 45.5 % of the day resting. City foxes rest the most (46.2 %). Looking at the daily resting time based on the duration of resting events, hares rest 6.2 (± 1.4) hours a day, raccoons rest longer with 11.5 (± 4.0) hours and foxes of Berlin (12.3 ± 2.3 hours a day) and Brandenburg (12.1 ± 1.6 hours a day) rest the most. All species differed significantly from each other in their daily resting time based on resting hours per day except the foxes themselves (Kruskal Wallis test: chi-squared = 30.92, df = 3, $p < 0.001$; pairwise Wilcoxon test, hare~raccoon: $p < 0.001$, hare~fox BB: $p < 0.001$, hare~fox BE: $p < 0.001$, raccoon~fox BB: $p = 0.002$, raccoon~fox BE: $p = 0.04$, fox BE~fox BB: $p = 0.07$).

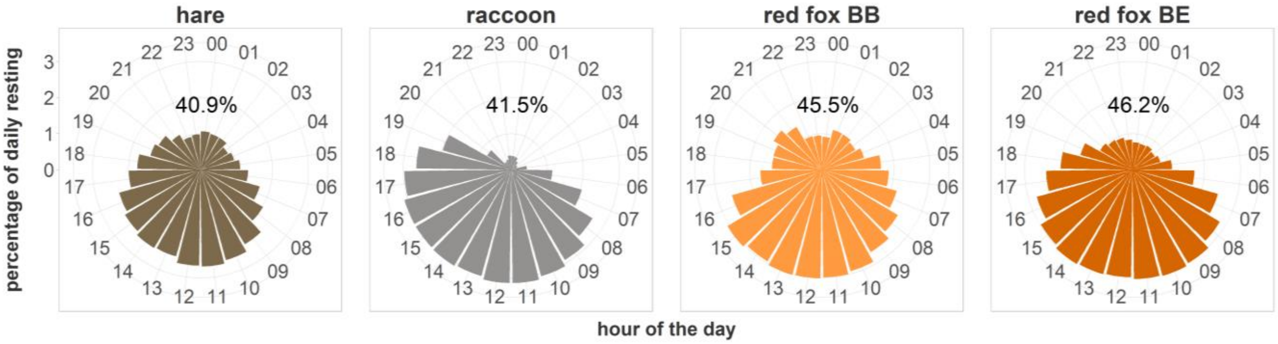

Figure 3: Percentage of resting over the day for hares (brown), raccoons (grey), red foxes of Brandenburg (light orange) and red foxes of Berlin (dark orange) based on resting assignments. The distribution of the coloured bars shows that resting behaviour takes place mainly during the day. Value in the middle of each plot is based on the sum of daily resting for each hour of the day and therefore refers to the total amount of time spent resting per day.

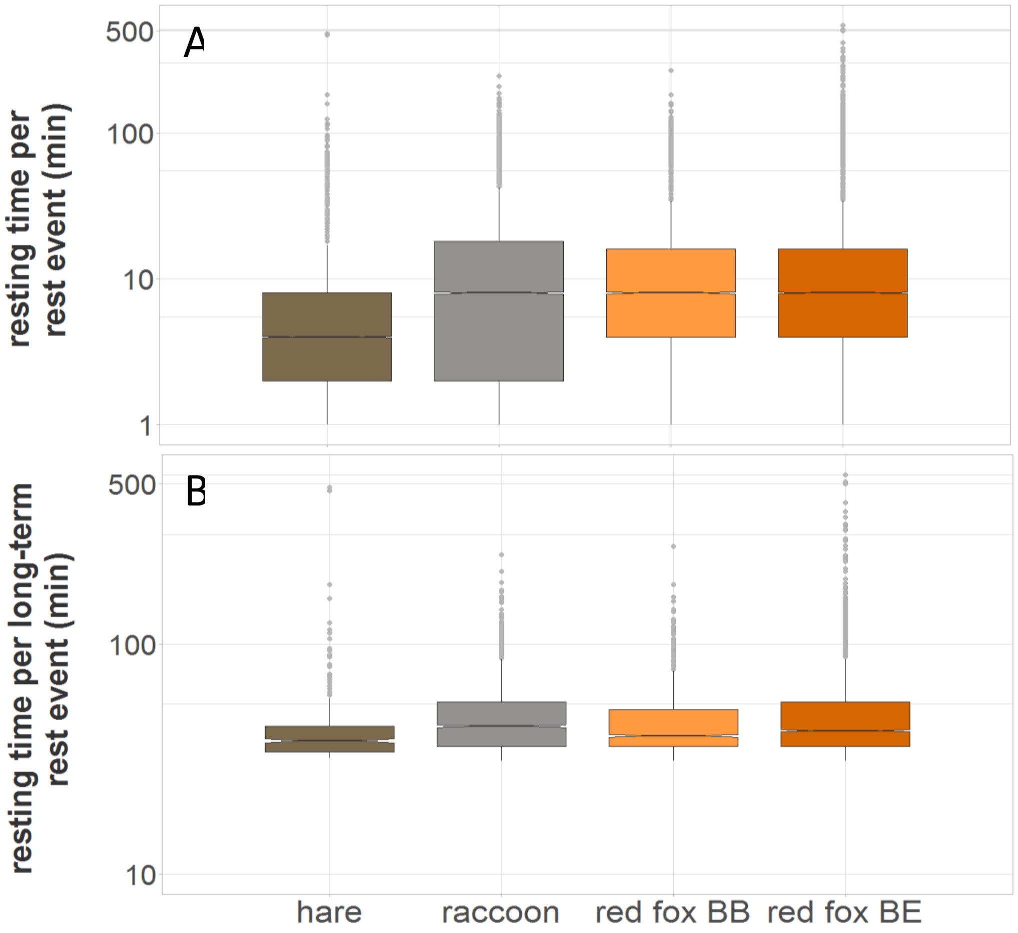

Figure 4: Rest time per resting event (duration of a single event) in minutes for hares (brown), raccoons (grey) Brandenburg red foxes (light orange) and Berlin red foxes (dark orange). The upper box plot (A) reflects all events, the bottom plot only long-term events with a minimum duration of 30 minutes (B). Y-axes are on a logarithmic scale.

When taking all resting events (figure 4, panel A) into account hares rested on average for 6.4 ± 2.2 minutes per event and therefore shorter than red foxes of Berlin with 12.4 ± 4 minutes and raccoons with 13.5 ± 2.4 minutes. Red foxes of Brandenburg rest on average longest (13.9 ± 3.8 minutes). All differences between groups are significant except between red foxes (Kruskal Wallis test: chi-squared = 7109.9, df = 3, $p < 0.001$; pairwise Wilcoxon test, hare~raccoon: $p < 0.001$, hare~fox BB: $p < 0.001$, hare~fox BE: $p < 0.001$, raccoon~fox BB: $p = 0.01$, raccoon~fox BE: $p < 0.001$, fox BE~fox BB: $p = 0.9$). When focusing on long-term resting events (figure 4, panel B), where only events longer than 30 min where considered, hares rested significantly shorter (42.0 ± 19.2 minutes), than raccoons (49.5 ± 3.8 minutes) and foxes of Brandenburg (47.7 ± 6.8 minutes) and Berlin (48.7 ± 19.4 minutes). Resting events of red foxes of Berlin last significantly longer than those of Brandenburg foxes (Kruskal Wallis test: chi-squared = 139.7, df = 3, $p < 0.001$; pairwise Wilcoxon test, hare~raccoon: $p < 0.001$, hare~fox BB: $p < 0.001$, hare~fox BE: $p < 0.001$, raccoon~fox BB: $p < 0.001$, raccoon~fox BE: $p = 0.02$, fox BE~fox BB: $p < 0.001$).

Hares rest on average with 75.8 ± 21.2 times a day considerably more often than raccoons, which rest 41.7 ± 11.9 times per day. The number of resting events of red foxes of Brandenburg (51.6 ± 15 times per day) and Berlin foxes (50.4 ± 14.9 times a day) do not differ significantly (Kruskal Wallis test: chi-squared = 930.16, df = 3, $p < 0.001$; pairwise Wilcoxon test, hare~raccoon: $p < 0.001$, hare~fox BB: $p < 0.001$, hare~fox BE: $p < 0.001$, raccoon~fox BB: $p < 0.001$, raccoon~fox BE: $p < 0.001$, fox BE~fox BB: $p = 0.06$). Long-term resting events (> 30 minutes) on the other hand only occur 1.9 ± 1.3 times a day for hares and significantly more often for raccoons (5.2 ± 2.3 times per day). The number of long-term resting events of red foxes of Brandenburg (4.5 ± 2.3 times a day) und Berlin (5.0 ± 2.6 times a day) showed intermediate values but differ significantly among groups with city foxes resting more often (Kruskal Wallis test: chi-squared = 332.56, df = 3, $p < 0.001$; pairwise Wilcoxon test, hare~raccoon: $p < 0.001$, hare~fox BB: $p < 0.001$, hare~fox BE: $p < 0.001$, raccoon~fox BB: $p < 0.001$, raccoon~fox BE: $p = 0.03$, fox BE~fox BB: $p = 0.01$).

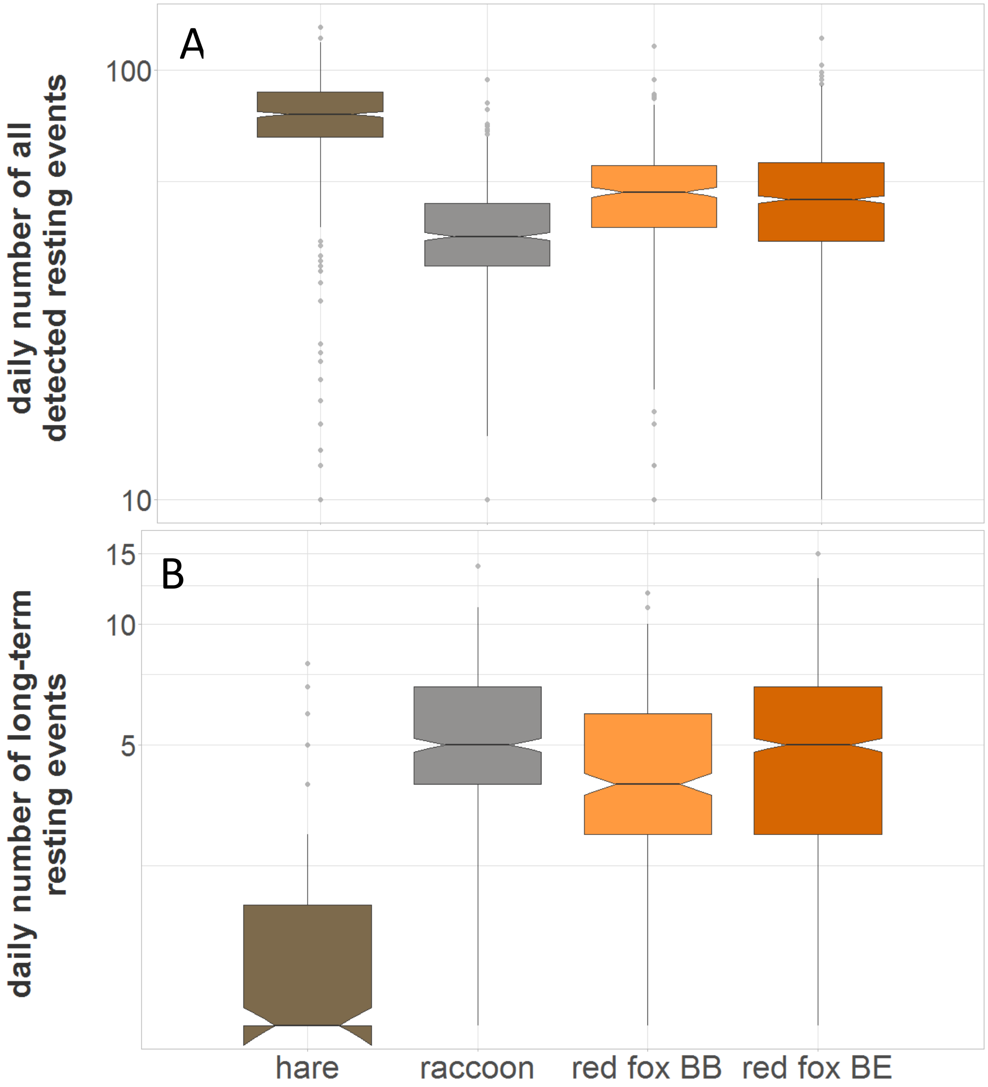

Figure 5: Number of resting events per day for hares (brown), raccoons (grey) Brandenburg red foxes (light orange) and Berlin red foxes (dark orange). The upper box plot (A) reflects all events, the bottom plot only long-term events with a minimum duration of 30 minutes (B). Y-axes are on a logarithmic scale.

Spatial analysis of resting behaviour

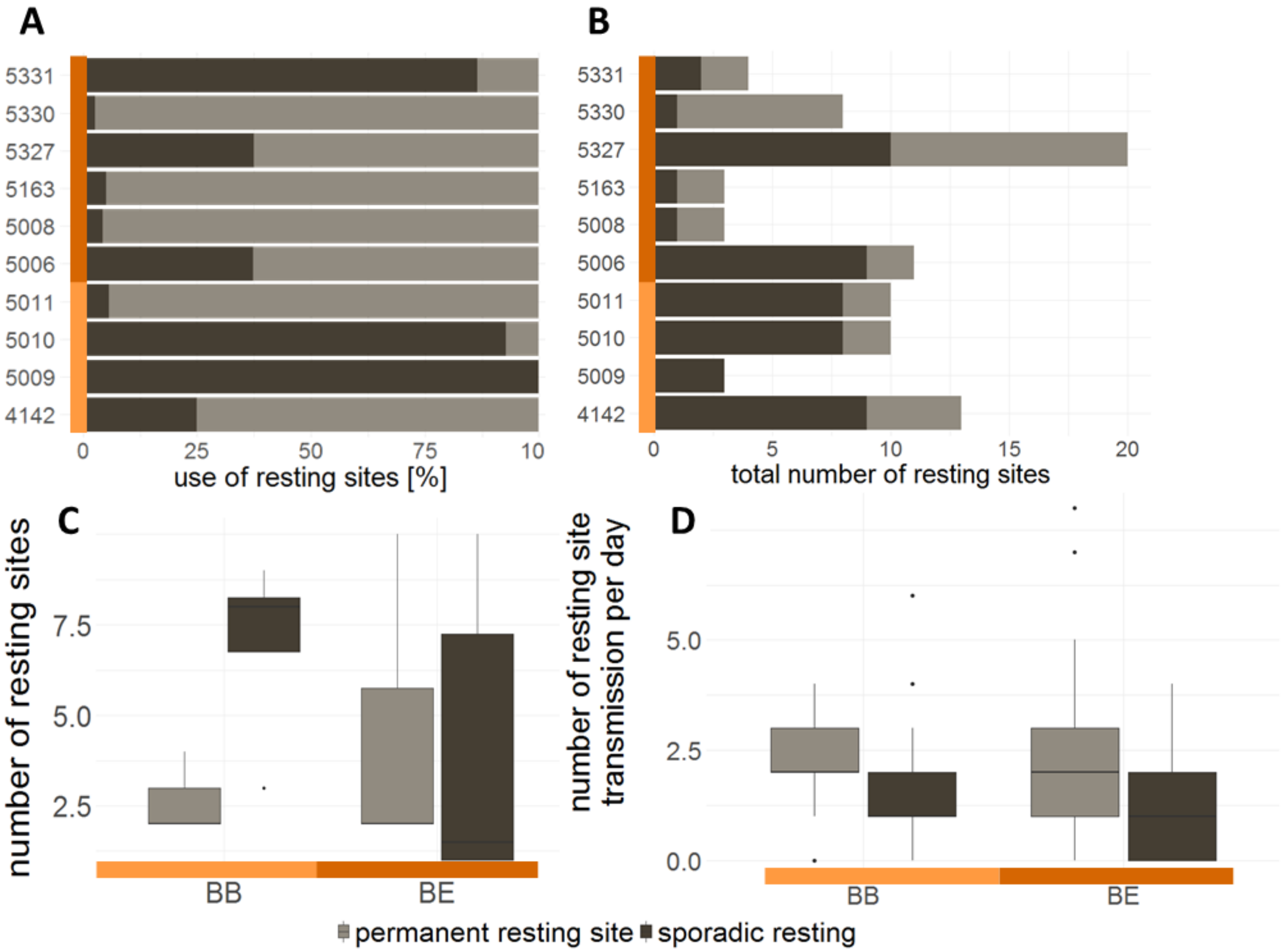

Figure 6: Use and number of permanent (grey) and sporadic (dark brown) resting sites of individual (four-digit number = ID) red foxes from Brandenburg (BB - light orange) and Berlin (BE- dark orange). A - Percentage of use of sporadic and permanent resting sites. B - Total number of identified resting sites by cluster analysis divided into sporadic and permanent resting sites. C - Number of resting sites of red foxes from Brandenburg and Berlin. D - Number of daily transmission between resting sites.

Analysis of resting sites reveal individual differences in the number and use of resting sites whereas a higher number of permanent resting sites (figure 6, panel A) do not necessarily mean a higher degree of use (figure 6, panel B) in comparison to sporadic resting sites. Foxes from Brandenburg tend to have more sporadic resting sites (6.1 ± 2.6) then city foxes of Berlin (5.2 ± 3.8) (figure 6, panel C), also variance among Berlin individuals is high. The number of permanent resting sites is less compared to sporadic resting sites in both populations (Brandenburg: 3.0 ± 1; Berlin: 4.5 ± 3.1). In total, Brandenburg red foxes use on average 7 (± 2.7) resting sites over 14 days of data exploration, whereas Berlin red foxes have 5.3 (± 3.8) resting sites.

Even if the number of sporadic resting sites of Brandenburg red foxes is high, transmissions between them per day are low (1.7 ± 1.3 times per day) (figure 6, panel D). Transmissions between permanent resting sites occur 2.0 ± 1.0 times per day. For Berlin red foxes transmission between permanent resting sites (2.1 ± 2.0 times per day) also to tend to be more often than between sporadic resting sites (1.3 ± 1.2 times per day). Berlin red foxes changed on average resting sites 3.4 ± 2.0 times a day and red foxes of Brandenburg 3.0 ± 1.9 times a day, regardless of resting site type (sporadic or permanent).

Discussion

The investigation of the resting behaviour of red foxes revealed habitat-dependent differences (more urban Berlin vs. more rural Brandenburg) in the temporal patterns of resting behaviour as well as in the number of resting sites. Overall, urban foxes tend to rest more, with their resting behaviour concentrated during the day, and reflected mainly by increased and longer-lasting long-term resting events. Foxes in Brandenburg are active earlier in the evening than their urban counterparts, but are also more likely to rest at night. Spatial analysis show, that red foxes of Brandenburg tend to have more resting sites with especially the number of sporadic resting sites being increased compared to Berlin foxes. Nevertheless, contrary to our expectations daily use based on the number of transmission between sites per day (~ 3 times) is similar.

Daily activity patterns are determined by species-specific endogenous clocks (Kronfeld-Schor and Dayan 2003) but individual variances are influenced by different external factors. Previous studies have shown that red foxes in natural habitats are mainly nocturnal-crepuscular (Blanco 1986; Cavallini and Lovari 1994; Baker et al. 2007; Monterroso et al. 2013). Human disturbance and food availability (often related to prey activity) seem to mainly affect timing of activity (Lovari et al. 1994; Weber et al. 1994). As human activity in cities is high during day, red foxes may be forced switching activity to the night. This pattern was also observed for red foxes of Oxford (Doncaster et al. 1990). Daily resting time of urban foxes is also achieved by a higher number of resting events. These also last longer compared to the resting events of Brandenburg red foxes. Urban foxes often concentrate their diet to a large extent on anthropogenic resources such as waste, compost, leftovers or pet food (Contesse et al. 2004; Murray et al. 2015; Scholz et al. 2020). These food items offer the foxes various advantages. They can be obtained with little energy effort, are often available in large quantities, occur in clumped form and are usually predictable in space and time. This makes it very easy for red foxes in the city to take in high-calorie food with minimal effort. The high energy content of anthropogenic food also reduces the amount of food items needed to cover the daily requirement. This means that less time has to be spent for foraging (Saunders et al. 1997; Kistler et al. 2009). As a result, the red fox in the city potentially has more time available for other activities or for resting. However, places with clumped food resources such as rubbish bins or compost are also accessible more undisturbed at night. Thus, for urban foxes, too, both human disturbance and food availability or accessibility could determine their nocturnal activity. Furthermore, if a large amount of food is ingested in a short period of time, as is the case with food appearing in clusters and large quantities in the city, it must be digested afterwards (Capellini et al. 2008b). Digestion is often associated with resting behaviour. Longer digestive phases due to increased food intake may be reflected in a longer resting phase, which would explain the increased duration of resting events of city foxes. In contrast, in natural and semi-natural habitats, rural red foxes have a wide food niche covering various trophic levels (Cavallini and Volpi 1995; Goldyn et al. 2003; Scholz et al. 2020). However, the availability of this prey varies in space and time. As a result, rural foxes have to spend more time for foraging to meet their energy requirements. Conversely, there is less time to rest resulting in a decrease in total rest amount, less resting events and shorter resting durations of red foxes of Brandenburg.

Studies on the population density and home range size of foxes from Berlin have shown that urban foxes have smaller home ranges than their rural relatives and are also found in higher population densities (Börner et al. 2009). In addition, cities offer only few natural vegetation structures which are delimited by many sealed or built-up areas and roads (McKinney 2002; Hunter 2007). This would explain the lower number of resting places for urban foxes. The few resting places that exist in their own territory must therefore be used more intensively. The limited availability of structurally rich cover and the high level of human activity results mainly in the lack of short-term resting places, which in contrast are increasingly used by Brandenburg foxes. Rural foxes therefore have a greater supply of resting places and change their places regularly. Their space use is therefore less predictable. Whether this is a result of increased hunting pressure in rural areas cannot be assessed with the available data. Nevertheless, Brandenburg foxes change their resting place as frequently as foxes in Berlin. This change can have several causes such as disturbances due to humans, pets or other wildlife, noise or adverse weather conditions but was not systematically investigated.

The comparison of temporal resting patterns of red foxes with coexisting medium-sized mammals, raccoons and European hares from Brandenburg, shows primarily the difference in resting behaviour between mesopredators and prey animals. For example, the duration and number of resting events of foxes and raccoons are very similar, whereas field hares have significantly shorter resting events, which rarely last longer than 30 minutes and are compensated by an increased number of short-term resting events. Whereas for predators, resting behaviour is more likely to serve the purpose of recovery and the avoidance of disturbance (Siegel 2011), the quiet wakefulness of prey animals is an anti-predatory behaviour (Carl and Robbins 1988; Capellini et al. 2008a). Their motionlessness reduces the predators' ability to detect them and at the same time enables them to observe their environment carefully to promptly react. At the same time, herbivorous prey animals have to spend a lot of time foraging, as plants supply comparatively little energy in comparison to protein-rich diet features and therefore have to be consumed in increased quantities (McNab 1986; Clauss et al. 2010). It is therefore obvious that short resting periods in which the environment is observed and monitored for safety alternate with phases of foraging. Predatory generalist species such as the fox and raccoon, on the other hand, benefit from the fact that they are not dependent on a specific food resource, which increases dietary breadth and thus makes foraging easier (Pineda-Munoz and Alroy 2014; Spencer et al. 2017). Time that does not have to be used for foraging and low predation pressure are thus reflected in longer resting periods and an increased daily resting time compared to the European hare.

During this study, we investigated and described the resting behaviour of urban and rural red foxes in particular. The analysis of high-resolution ACC data provided a detailed insight into when, how much, how long and how often red foxes rest. To our knowledge, the resting behaviour of foxes in semi-natural and urban habitats has not yet been described in this level of detail. In addition, combined analysis of ACC and GPS data revealed habitat-dependent differences in the number of resting sites. However, the in part high variance of results points to individual differences within the populations, which cannot be explained by this analysis. Further investigations based on the results of this study should therefore focus on biotic and abiotic explanatories. For example, gender could have an influence on resting behaviour, since male foxes usually have a greater space use (Kidawa and Kowalczyk 2011). The comparison of home range size and daily travel distance with daily resting amount at individual level could provide information on the relationship between space use and resting behaviour. Furthermore, correlation of energy expenditure, e. g. via overall dynamic body acceleration based on ACC data, and resting time may provide insight to which extent resting behaviour acts as part of energy management. To increase reliability an overall increase in sample size would be beneficial.

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GENERAL DISCUSSION

We are in the middle of the human age, the Anthropocene (McMahon et al., 2018). Industrialisation, climate change, urbanisation - the influence of humans on our earth is omnipresent (Chase & Chase, 2016). We are altering our landscapes, clearing forests, straightening rivers, converting natural habitats into agricultural landscapes. Remaining natural habitats are rare, especially in industrialised countries. The urban habitat, however, is growing. Urbanisation is increasing worldwide, with the result that important habitats for wildlife are disappearing (Altherr, 2007; Concepción et al., 2015; Hens & K Boon, 2005; McKinney, 2008). On the one hand, urbanisation is one of the greatest threats to biodiversity worldwide. On the other hand, structurally rich cities offer a wide variety of habitats through their small-scale use, which attracts many animals and plants(McKinney, 2002). Cities can therefore also provide habitats for wild animals. Adaptable animal species benefit from the expansion of our metropolises and the urbanisation of our settlements. For those species that have learned to find their way around, the city habitat is optimal with favourable climatic conditions, a plentiful supply of food and low predation pressure (McKinney, 2008). The red fox is one of these species that have successfully colonised the urban habitat and know how to use it for their own benefit (Adkins & Stott, 1998; Baker et al., 2007; Börner et al., 2009; Gloor, 2002). This study aims to understand the influence of changed living conditions in the city on different aspects of red fox ecology. The focus of this thesis is on the diet, parasite spectrum and resting behaviour of urban and rural red foxes from Berlin and Brandenburg. Our investigation on the red fox diet (**Chapter 1**) shows that

(1) dietary range of urban red foxes is smaller compared with that of rural conspecifics.

Furthermore, higher δ13C values and lower δ15N values of urban foxes suggest

(2) relatively high input of anthropogenic food sources of urban foxes.

Low within-individual variation compared to the between-individual variation lead to the conclusion that

(3) generalist fox populations consist of individual food specialists in urban and rural populations.

Main results of the parasitological investigation (**Chapter 2**) show that

(1) the helminth diversity in the city Berlin is lower compared to surrounding rural Brandenburg

and

(2) male red foxes tend to have higher helminth diversity than females.

(3) Diet features can drive helminth communities in red fox populations.

Additionally,

(4) with increasing human population density, helminths transmitted via diet are less prevalent than pet-related helminths.

Finally, I investigated habitat-dependent differences in resting patterns of red foxes from Berlin and Brandenburg (**Chapter 3**) and results revealed that

(1) urban foxes tend to rest more, with their resting behaviour concentrated during the day compared to rural red foxes.

This increase in daily rest behaviour is reflected in an

(2) increased number of rest events.

Moreover,

(3) the long-term resting events of city foxes last longer than those of foxes from Brandenburg.

Even if rural foxes spend less time resting,

(4) rural red foxes of Brandenburg tend to have more resting sites compared to Berlin foxes.

Even though red foxes are "carnivores", they do not feed on meat exclusively (Calisti et al., 1990). Rather, they are omnivores and are not particularly selective in their choice of food (Cavallini & Volpi, 1996; Contesse et al., 2004). Thus the spectrum spans several trophic levels. Plant resources such as fruits and nuts are consumed as well as earthworms or insects, but also smaller mammals or carrion, among others. This is also confirmed by the investigations in this work: the variance of isotope values for the foxes studied is great and reflects a large dietary niche. The comparison with isotope profiles of typical food resources shows that the red foxes investigated use plant and meat resources. Thus, looking at the species levels alone, foxes are generalists. At the population level, however, differences can already be observed between urban and rural foxes. The dietary niche of urban foxes is significantly smaller than that of rural foxes. Consequently, urban foxes do not feed as diverse as rural foxes. However, since typical food resources such as birds, mice, insects, worms and fruit would also be available in the city (seasonal fluctuations disregarded), it seems to be a conscious decision by urban foxes to restrict their range of food resources. The results of the stable isotope analysis indicate that urban foxes mainly focus on anthropogenic food. Anthropogenic food such as leftover food, waste, compost or pet food usually appears in the city in aggregated form and larger quantities. Besides, they are easily accessible, their availability is usually predictable in space and time throughout the year and the food is often very energy-rich (Contesse et al., 2004). As a result, foxes in the city no longer have to spend a lot of energy and time foraging or hunting to cover their daily requirements. Instead, the food only has to be collected by red foxes. Likely, this will also improve the supply of food to the offspring and, due to the resulting good (McMahon et al., 2018) the survival rate of young foxes may increase. Despite a limited food spectrum, urban foxes thus benefit from their diet. However, the results of the longitudinal data also show that red foxes are highly specialised in their diet at the individual level. This behaviour was observed for both urban and rural foxes. Thus, if we focus on the individual fox, the food spectrum of rural foxes is also very limited. Thus the feeding behaviour of urban foxes is not a special adjustment in itself, but merely reflects the local food availability in the landscape. As usual, preference is given to resources that are easy to capture and available in large quantities (Jedrzejewski & Jedrzejewska, 1992; Leckie et al., 1998). Not the feeding habits had to be adjusted to city life, but only the food. In the future, it would be interesting to determine whether focusing nutrition on anthropogenic food would also lead to physiological adaptations to better digest starch-rich food, for example, expressed in an increase in alpha-amylase gene copies like described for domestic dogs (Antkowiak 2020).

Whether a species can persist in an urban environment is not only a question of whether it can meet all its vital needs in this artificial habitat but also whether it is tolerated by the human inhabitants of the city. Even though the majority of urban residents are happy at the sight of a fox, there are still concerns about living directly with this wild animal (König, 2008). Above all, there is the fear that people could become infected with dangerous parasites or diseases transmitted by wild animals. Research into the parasite spectrum of red foxes shows that foxes in Berlin and Brandenburg can have a wide variety of different helminths. Which and how many different helminths a fox is infested with seem to depend on the use of space and nutrition. For example, certain helminths are associated with certain food resources, acting as intermediate or final hosts. However, since foxes in urban areas concentrate their food on anthropogenic resources and the proportion of natural food is significantly lower, it is unlikely that helminths will colonise the fox's gastrointestinal tract via their food (Vitone et al., 2004). This is also reflected in the helminth diversity of foxes from Berlin as diversity is lower in comparison to foxes from Brandenburg. Thus, the integration of this new food source, anthropogenic food, offers the advantage of decreased transmission rates of parasites through food. However, urban foxes are more likely to be hosts of helminths, which can also be transmitted to pets. Whether the increased prevalence of pet-associated helminths also leads to an increased infestation and ultimately disease rate in Berlin pets is speculative. Just because red foxes have more helminths in the city that can be transmitted to pets does not mean that they ultimately will be transmitted. Lower diversity of helminths does not, however, mean a lower infestation of the fox's intestinal tract. For example, it is quite possible that a city fox has only one species of helminth, but that this species occurs in large numbers in the fox's intestinal tract. Whether the lower helminth diversity in Berlin foxes also means a selective advantage for the foxes in the city has not yet been investigated.

As already described, the utilisation of anthropogenic food has one major advantage: the easy availability of large quantities of energy-rich food simplifies foraging (Bateman & Fleming, 2012). The fact that the fox population in the city as a whole concentrates on this food source suggests that they do not have to compete for the food and that there is enough available for all. The lower intraspecific competition also allows for the changes in the social structure of foxes in the city (Milinski, 1982). Studies have shown that urban foxes increasingly live in small family groups rather than solitary (Cavallini, 1996; Doncaster & Macdonald, 1991), and the offspring of the previous year help in raising young . The territories are smaller in the city compared to foxes from rural areas and the population density is higher. All this is ultimately possible due to the rich food supply in cities. However, the fact that less time has to be spent on foraging also means that more time is available for other things. If all basic needs are satisfied, this is expressed in increased rest time and increased and longer rest events. So foxes in the city rest more. However, smaller home ranges, which must also be shared with other relatives, could mean less availability of suitable resting places. For example, results have shown that foxes from Brandenburg have more resting places, even if they use only a subset of them every day. Berlin foxes, on the other hand, return to the same resting sites every day, which thus become hotspots of use in the urban landscape. Utilisation hotspots, whether resting or feeding sites, have the potential of transmission of parasites due to increased contact rates of the parasite (stages) with host species.

Overall, dietary specialisation and the use of anthropogenic food resources, in particular, have an overarching impact on the ecology of urban foxes. For example, the predominance of food leads to a lack of competitive structures and a good energy supply for foxes. This favours high population densities (Bateman & Fleming, 2012; McKinney, 2002). At the same time, the risk of infection with food-borne parasites, which can also pose a health risk to the fox, is reduced. However, since anthropogenic food often appears clumped, there are hotspots of use in the city, which in turn increases the probability of direct transmission of parasites and diseases from red fox to red fox. The amount of energy and time required to search for and obtain food for urban foxes is less than for rural foxes, which have to hunt a large part of their prey. This allows urban foxes to rest more than their rural counterparts. The availability of resting places (whether through increased population densities and thus increased demand for the population itself, or through the lack of suitable places due to dense building development and few green refuges) appears to be limited and is reflected in a lower number of used resting sites in the city compared to rural areas. If proper food supply has such an extensive influence on the ecology, behaviour and lifestyle of red foxes, management strategies should focus on this topic. Reduced food availability would probably increase the competitive pressure within the fox population, reduce population density and thus also the contact rate between humans, domestic animals and foxes. Human-wildlife conflicts in the city could thus be reduced and the general acceptance of wild animals in the city increased. This could ultimately lead to the sharing of urban areas by humans and wildlife.

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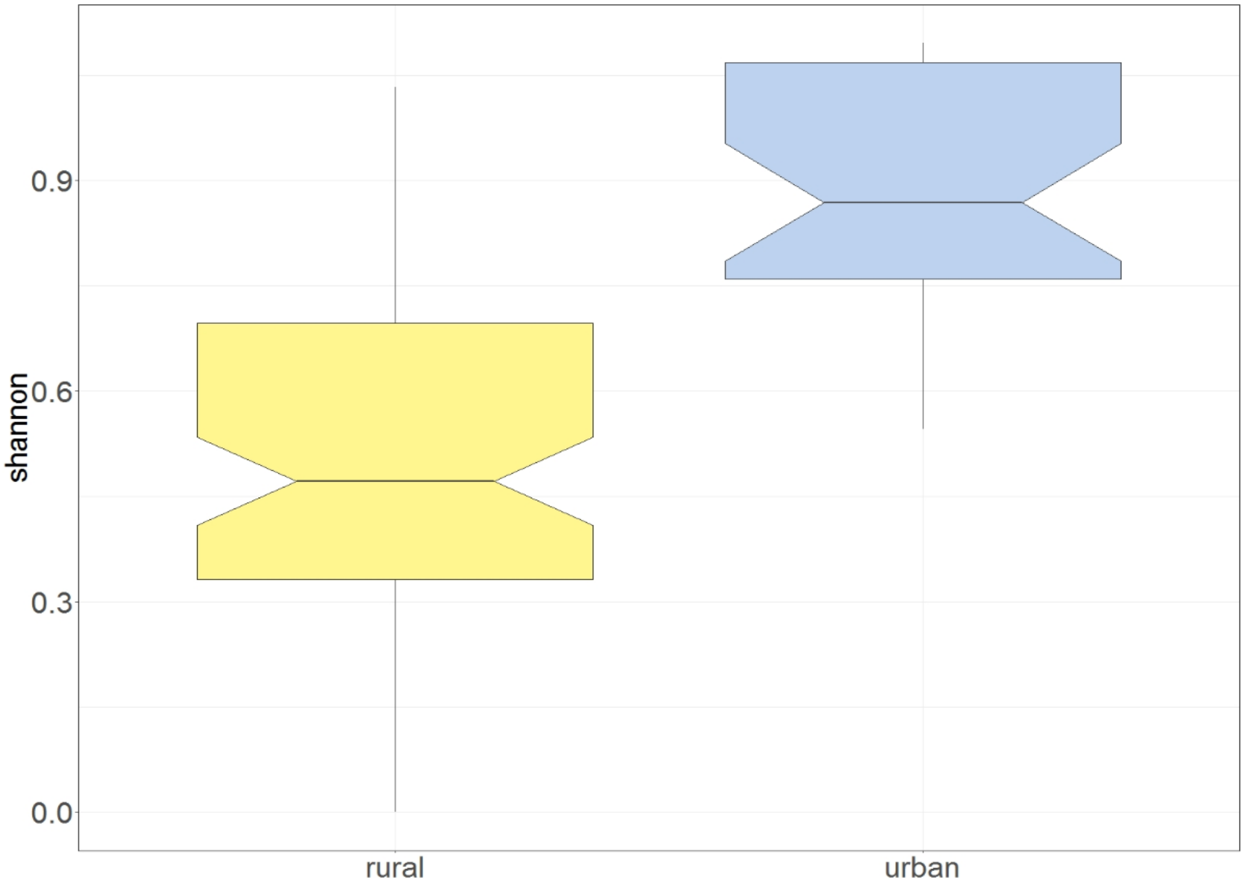
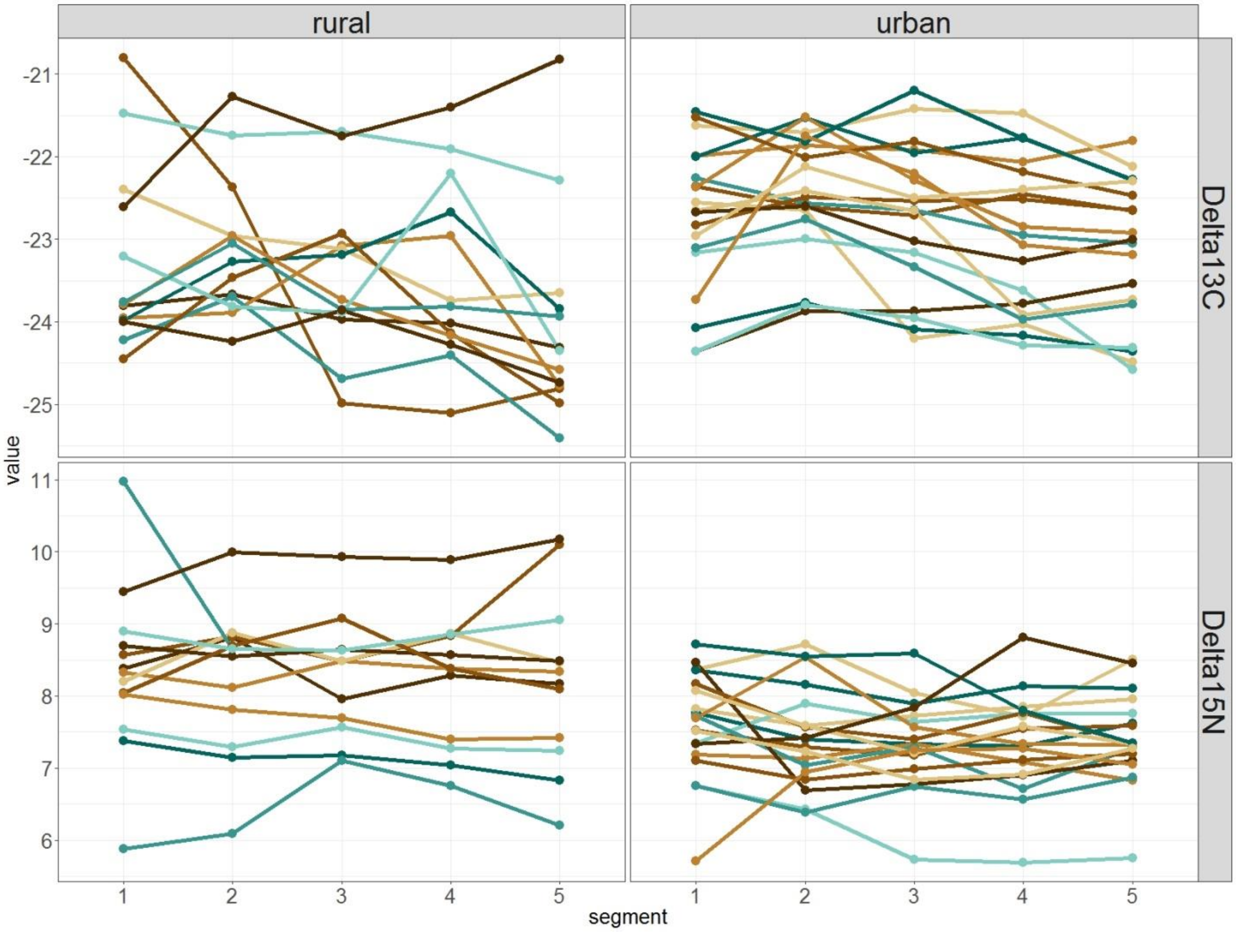
APPENDICES

Chapter 1



Appendix A: Boxplot of Shannon values for the rural (n=85) and urban fox population (n=34).



Appendix B: Stable nitrogen and stable carbon isotope ratios (‰) in increments of 19 urban and 13 rural red fox whiskers. Each line represents one individual.

Appendix C: Results of the PERMANOVA test to test pairwise differences between food sources.

	GEO	HEL	LIM	LUM	MUR	ORT	ROS
GEO		0.021	0.183	0.122	0.113	**0.002***	**0.001***
HEL			0.858	0.289	**0.002***	0.088	**0.003***
LIM				0.586	0.022	0.255	**0.004***
LUM					**0.006***	0.01	**0.001***
MUR						**0.001***	**0.001***
ORT							0.008
ROS							

Chapter 2

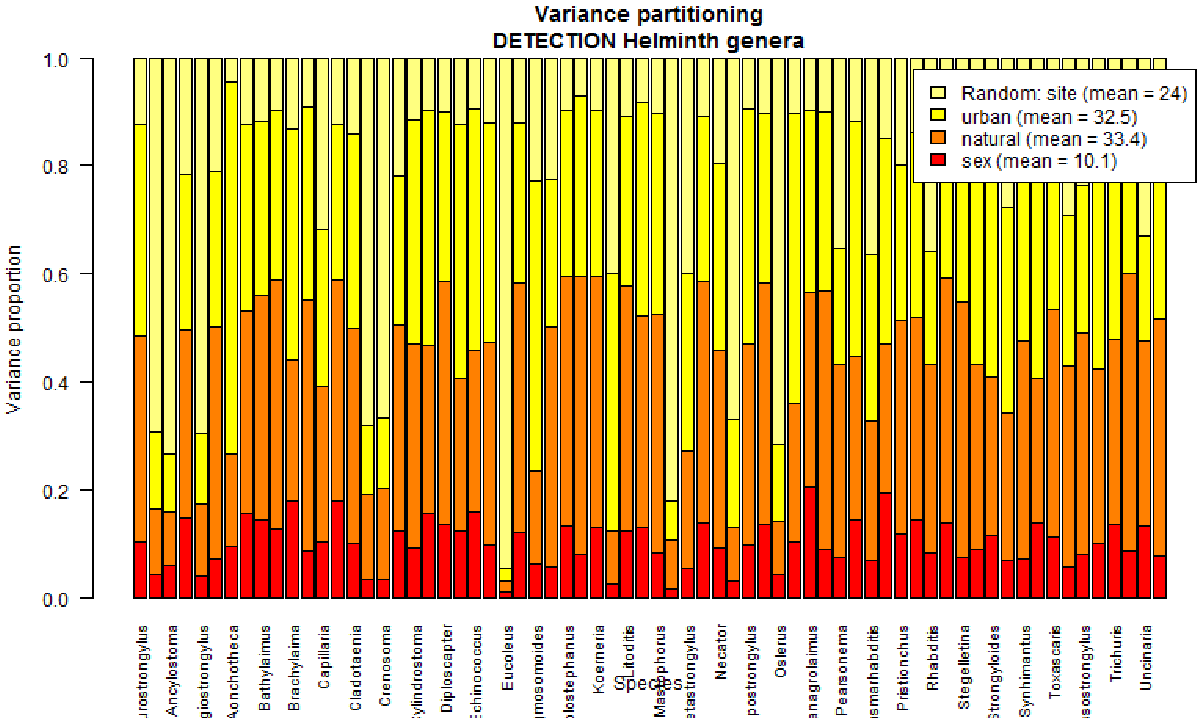
Appendix A: Environmental values: preparation of the 20 x 20 m resolution maps.

For the different maps:

- Original raster layers obtained from source 1 (tree cover) had a 20 m resolution.
- Human population density was obtained from source 2. The original data was a polygon vector layer. Data on inhabitants was extracted from each polygon and divided by polygon area to obtain number of citizen/m^2. Then data was aggregated and summed to obtain a raster with the number of citizens in each 20 x 20 m raster cell.
- Noise: raster layer of average day and night noise was obtained from source 2. The original resolution was 10 m, thus cells were aggregated to a 20 m resolution using the average value.

Sources:

1. European Union, Copernicus Land Monitoring Service, 2018; http://land.copernicus.eu/pan-european/high-resolution-layers/
2. Berlin Environmental Atlas, 2018 (https://www.stadtentwicklung.berlin.de/geoinformation/fis-broker/index_en.shtml)



Appendix B: Variance partitioning of helminth genera.

DECLARATION OF AUTHORSHIP

I hereby declare that I prepared this thesis independently under the guidance of my supervisor. All direct or indirect sources used are given as references. All contributions of my co-authors are acknowledged.

Neuenhagen, 30.09.2020 Carolin Scholz