

Where to build a web? Interplay between microclimate and spider colour in urban and rural European garden spiders

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Introduction

Urbanisation

Humans are increasingly urbanising this planet, negatively impacting natural ecosystems and causing rapid human-induced environmental change (Seto et al., 2012). In 1900, 15% of the human population could be considered urban. In 2007, for the first time ever, global urban population crossed the rural population with just over 50% of the global population being urban. In the future this increase is expected to continue, and by the year of 2030, urban population will be 60% of the global population (Kundu & Pandey, 2020; H. Lowry et al., 2013; Seto et al., 2012; Zhang, 2016). Interestingly, cities can be considered as novel environments with altered abiotic and biotic components both in space and time. These include higher temperatures, elevated noise and light pollution (abiotic), but also higher habitat fragmentation and lower biodiversity (biotic) (Brandsma & Wolters, 2012; Concepción et al., 2015; McMahon et al., 2017; Swenson & Franklin, 2000). Consequently, these changes will greatly impact many aspects of species, leading to changes in community taxonomic and functional composition (Dahirel et al., 2017, 2019; Merckx et al., 2018), but also intraspecific phenotypic changes (M. T. J. Johnson & Munshi-South, 2017; Lambert et al., 2021). This way, cities can be seen as living labs for analyzing urban adaptations of organisms (M. T. J. Johnson & Munshi-South, 2017).

The urban heat island (UHI) effect (figure 1) is one of the most obvious characteristics of urban climate (Yang et al., 2016), which was first described by Luke Howard in his book 'The climate of London' (Howard, 1818). It can be described as a phenomenon where urban areas are several degrees warmer than the surrounding rural areas (Rizwan et al., 2008). Multiple factors contribute to UHI, such as: urban materials, air pollutants, wind blocking and lower tree cover (Nuruzzaman, 2015; Priyadarsini et al., 2008; Takebayashi & Moriyama, 2012). On average, the UHI effect is around 2°C (Taha, 1997). But depending on the location and weather, this effect can reach 10°C (Sharifi & Lehmann, 2014). Moreover, in the case of Ghent, Caluwaerts et al. (2020) found that the UHI effect is more pronounced at night than during the day. This can be explained by the uptake of heat by urban structures during the day that will be slowly released during the night. Conversely, Peng et al. (2012) found across 419 global big cities that the UHI effect is more pronounced during the day. Results may differ because of the choice of rural reference for the UHI effect, because ideally the rural zone is taken as close as possible to the city. In reality, there will always be a transition zone between rural and urban areas (W. P. Lowry, 1977). Additionally, key factors influencing UHI effect may also change with the location of the city on bigger scale, such as temperate cities compared to tropical cities (Wong, 2016). Moreover, city size can also influence the UHI effect, where it is expected that bigger and compacter cities experience a bigger UHI effect (Zhou et al., 2017). Finally, multiple papers have already analysed the UHI effect in Flemish cities. For the city of Ghent, the UHI effect is around 1 °C during the day and around 2 °C during the night (Caluwaerts et al., 2020). For the Brussels region, a nighttime UHI effect was found of 3,15 °C (Lauwaet et al., 2016). In the case of Antwerp, a UHI effect of 2,5 °C was found during the day (Lauwaet et al., 2015). In this thesis, urbanisation effects will also be analysed around these three cities and surroundings.

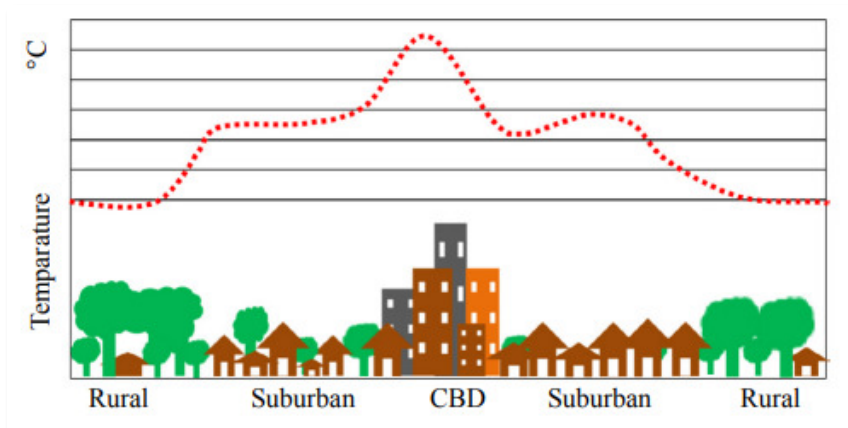


Fig 1: Example drawing of the UHI effect (Bhargava et al., 2017).

Cities pose new challenges to species as a novel environment, such as the UHI effect, lower humidity and higher habitat fragmentation (Huang et al., 2022; Swenson & Franklin, 2000; Yang et al., 2016). These different conditions will act as an external filter, and only those species that manage to pass this filter will be able to survive and reproduce in cities (Knop, 2016). This filtering will happen based on certain traits which will allow individuals to adapt to the city. Species will undergo phenotypic changes between urban and rural populations, either through local (genetic) adaptation or phenotypic plasticity, or a combination of both (Bókony et al., 2019; McLean et al., 2005; San Martín y Gómez & Van Dyck, 2012; Senar et al., 2014). Furthermore, these phenotypic changes have been found for many traits and across a lot of taxa. They include changes in behaviour, physiology, migration, morphology and colouration among others (Lambert et al., 2021). One of the first examples of rapid urban adaptation is that of the peppered moths (*Biston betularia*), which evolved darker colouration in response to polluted tree trunks (Kettlewell, 1958). Moreover, Hwang & Turner (2009) found that urban and rural blowflies (*Calliphora vicina*) differed in multiple life-history traits such as body size and growth rate using a common garden experiment. In these type of experiments, individuals from different populations and from early life stages will be reared under the same experimental conditions. In doing so, researchers are able to disentangle the effects of genetic and environmental variation on the phenotype (Lambert et al., 2021; Ljungfeldt et al., 2014; Schwinning et al., 2022).

Behaviour as adaptation to urbanisation

Urban adaptation can take many forms, one of them being behavioural changes. Numerous examples of behavioural changes in response to urbanisation exist in the literature, especially for birds. Urban blackbirds (*Turdus merula*) sing higher pitched songs in urban environments, possibly due to urban ambient noise (Nemeth & Brumm, 2009). In another study, Plummer et al. (2016) found that moths are more attracted into gardens with lamps that are spectrally more diverse and at higher densities. Urban bridge spiders (*Larinioides sclopetarius*) have higher measures of boldness and voracity when compared with rural individuals (Kralj-Fišer & Schneider, 2012). In another study on triangulate cobweb spiders (*Steatoda triangulosa*), they found that these spiders show less light avoidance from artificial light in urban areas. A phenomenon that can be explained by insects also being attracted to light and thus a higher prey abundance near light sources (Czaczkes et al., 2018). However, artificial light (ALAN) also influenced several life history traits in a nocturnal orb web spider (*Eriophora biapicata*). One of those being earlier maturation, which leads to smaller spider body size (length of the tibia as proxy) and lower amount of eggs produced by females (Willmott et al., 2018). Furthermore, the UHI also influences species' behaviour in cities. The black widow *Latrodectus Hesperus* showed an increase in web building, voracity and cannibalism under higher urban temperatures (J. C. Johnson et al., 2019, 2020).

Microclimate selection is a behaviour that can also be an important tool to an individual's survival, especially for ectothermic species. At smaller scales, animals select microhabitats depending on the cost-benefits: food availability, thermal performance, predation risks and more (Malishev et al., 2018; Sears et al., 2011). For example, different co-occurring

butterflies within the *Erebia* genus show a distinct microclimate selection for thermoregulation, depending on each species' thermal preference (Kleckova et al., 2014). Harwood et al. (2003) found that linyphiid spiders actively select their microhabitats for web building as a response to prey availability. Similarly, butterflies from different families will show different behaviours for thermoregulation, including microclimate selection and behaviour. Butterflies with a higher wing length showed better buffering ability (they are better at keeping their body temperature equal with different air temperatures) (Bladon et al., 2020). In this paper, both microclimate selection and behavioural thermoregulation was investigated. This was done by comparing values of butterfly body temperature (T_{body}) with the microclimate (T_{perch}) and local air temperature (T_{air}). In this thesis, this will also be investigated in terms of microclimate selection for web hub (Thub) and retreat (Tretreat) and also thermoregulation by measuring their body temperature (T_{spider}). Moreover, another study showed that spiders with three dimensional webs (tangle and sheet-and-tangle webs) prefer covered microhabitats to shelter themselves and their webs from heavy rains (Haberkern et al., 2020). Nonetheless, there is still few evidence on how species adapt to urbanisation in terms of their microclimate selection for thermoregulation.

Colour and size as adaptations to urbanisation

Colouration in animals results from a combination of many different functions, including thermoregulation, physical protection, signalling and background matching. Depending on the strength of selection on each function, there will be a different evolutionary 'solution' for these requirements (Storfer et al., 1999; Stuart-Fox et al., 2017). Consequently, Whether individuals will be lighter or darker in cities will therefor depend on intraspecific and interspecific selection pressures (Keinath et al., 2020; L. Leveau, 2021). For example, female ground beetles become more bronze coloured in polluted urban areas, while green colour persisted in males, likely due to sexual selection (Keinath et al., 2020). In cities, there might also be a selection for paler individual due to camouflage. One paper found that cities are dominated by grey coloured bird species as a response to the dominantly grey coloured buildings (L. M. Leveau, 2019).

One important potential adaptive function of colour is regulating one's internal temperature, especially important for ectotherms. Ectotherms depend on their environment for their biochemical and physiological pathways, because they cannot maintain a constant body temperature through homeostatic processes (Abram et al., 2017). It is expected that a dark surface will heat up faster than a light surface through higher absorption of solar energy, assuming an equal body size (Gates, 2012). In European butterfly and dragonfly species, research has shown that darker species are favoured in cooler climates and vice versa for light-coloured species in warmer climates (Zeuss et al., 2014). Moreover, urban land snails in the Netherlands are more likely to have yellow shells compared to rural snails, a response to thermal selection (Kerstes et al., 2019). Nevertheless, inferring thermoregulation as the main driver of colour differences should be done with caution. Besides colour, other factors such as behavioural changes and thermal mass may play a role in thermoregulation. In addition, most research focuses on the absorption of visible radiation (400-700 nm) for thermoregulation. Yet, 55% of solar radiation consists of wavelengths within the near-infrared (NIR; 700-2500nm), which is largely converted to thermal energy absorption by animals (Clusella Trullas et al., 2007; Nussear et al., 2000; Stuart-Fox et al., 2017). However, research on colouration changes in urban species for thermoregulation is still scarce, especially for ectotherms.

Size can also be important as adaptation for urbanisation. A typical pattern here is explained by the Atkinson's temperature-size rule, which states that at higher temperatures, arthropods are expected to be smaller because of an unequal response of growth and developmental rates to temperature (Atkinson, 1994; Forster & Hirst, 2012). Based on the UHI effect in cities, it thus expected that arthropods will generally be smaller in urban areas. Yet, this relationship is not always that straightforward, especially when dispersal capacity is also higher with body size. In that case, species might shift to bigger body sizes because it provides a dispersal advantage in highly fragmented urban environments (Merckx et al., 2018). Furthermore, prey availability might also play an important role in how body size may change with urbanisation. The orb weaving spider *Nephila plumipes* reached bigger sizes in more urbanised areas of Sydney due to higher prey abundances (Lowe et al., 2014). However, urban cross spiders were shown to be smaller than their rural counterparts, a result explained by lower prey biomass and the UHI (Dahirel et al., 2019).

Genetic Adaptations and phenotypic plasticity

One of the issues of researching urban evolution is disentangling genetically fixed urban adaption from phenotypic plasticity. Additionally, some phenotypic shift might also be the result of neutral evolution (non adaptive) (Lambert et al., 2021). Phenotypic plasticity is the capacity of one genotype to exhibit different phenotypes depending on the environment (Whitman & Agrawal, 2009). For example, common toads (*Bufo bufo*) in urban environments had larger toxin glands and secreted higher amounts of toxins in response to predators. Interestingly, when offspring of the same toads were raised in captivity, these changes were not present. This clearly shows that these changes in phenotype are the result of phenotypic plasticity, and not genetically based adaptations (Bókonyi et al., 2019). Phenotypic plasticity might both help or slow down organisms when adapting to cities, but this has not been analysed as of today (Lambert et al., 2021). Common garden experiments are a way of analyzing these phenotypic changes between urban and rural organisms.

The European garden spider

The European garden spider (*Araneus diadematus* Clerk 1757: Araneidae) is an arachnid that can be found both in urban and natural environments, around shrubs or trees (Bonte et al., 2008; Hänggi et al., 1995). It mostly resides in gardens and green spaces of cities, as well as natural systems (parks) (Van Keer et al., 2023). Females (10-18mm in length) reach adulthood in late summer (Nyffeler & Bonte, 2020). As a member of the orb weavers, they will trap (mostly) flying insects by building aerial webs (Nyffeler et al., 1989). They do this mostly by waiting in the web hub (centre of the web), but under unfavourable conditions, they will hide in their retreat (thesis Nel D'haenekint). Yet, this also means that they are fully dependant on these flying insects as a food source, which have shown a drastic decline in abundance due to agriculture, pesticides and fragmentation (Leather, 2018; Schuch et al., 2012). Consequently, this arachnid has also seen drastic declines in population numbers over the past half-century (99.3% decline in Switzerland; Nyffeler & Bonte, 2020), yet their population changes have not been analysed in Belgium. These spiders are able to maintain consistent population densities in urbanisation gradient, which points towards urban adaptation (Dahirel et al., 2017). Yet, these spiders also have smaller egg loads with urbanisation, which could point towards negative fitness effects. However, smaller sizes might also point towards adaptation for thermoregulation (UHI effect) and lower prey availability in cities (Dahirel et al., 2019). Interestingly, the design of their web is very plastic in response to the environmental influences (microclimate) and body condition (Vollrath et al., 1997). For example, web surface and mesh width decreased with urbanisation at a local scale, possibly compensating for lower prey biomass in cities (Dahirel et al., 2019). In this particular study, urbanisation effects were also analysed in 54 locations in Flanders in a hierarchical sampling design with varying local and regional urbanisation levels (see further for more details). Lastly, this spider also shows a distinct colour pattern in its abdomen with a darker background colour (possibly due to melanin) and highly reflective guanine crystals that lead to the white spots and cross (Levy-Lior et al., 2010). This spider is characterised by a remarkable continuous colour variation, ranging from very pale to very dark (figure 2). Because *A. diadematus* is found both in urban and rural habitats, and shows remarkable phenotypic and behavioural variation, it is an ideal species to study the role of colour and microclimate selection as adaptations to urbanisation.



Fig 2: Photos taken in the lab showing the remarkable colour variation in the European garden spider.

In a first part of this thesis, I measured spider microclimatic conditions for web building in different urbanisation levels according to a hierarchical sampling design in Flanders, more specifically around Ghent, Antwerp and Louvain. Additionally, spider phenotypic characteristics were also measured. This way I could link urbanisation with the spider's microclimate and phenotype. This was done according to the approach of Bladon et al., 2020. Here, Flanders can be seen as an interesting area to study effects of urbanisation, as it is one of the most densely populated regions in Europe with 6 million people living on an area of 13 500 km² (Cornelis & Hermy, 2004; Poelmans & Van Rompaey, 2009). An additional experiment was also conducted to test the background matching of these spiders. To verify whether spider phenotypic traits affect thermoregulation, I assessed how spider colour and abdomen size will affect its body temperature and heating rate in a controlled setting. Lastly, I also included a common garden experiment using the offspring of the spiders analysed in the field in the first step. I reared offspring from different urbanisation levels under low and high temperature and food regime. This way, I could find out whether urban and rural spiders develop faster under different temperatures and food availability, and whether this is a genetic adaptation or a result of phenotypic plasticity.

Objectives

The objectives of this thesis were split up in three parts regarding urban adaptations of the garden spider:

How do garden spiders adapt to urbanisation in terms of different phenotypic traits (size and colour) and microclimate selection for web building?

To explore potential adaptations of garden spiders to urbanisation, I will collect temperature measurements of the microclimate of web building location of female garden spiders in the field in different urbanisation levels. This provides info on whether behavioural adaptations (selection of suitable sites) play a role. In addition, these spiders will be collected and photographed in the lab to quantify spider colour and size and investigate whether phenotypic adaptations might contribute to adapting to an urban environment. This way both the microclimatic conditions of the spider's web and the spider's phenotypic characteristics can be linked with urbanisation. Finally, a site selection experiment will be performed to test the alternative hypothesis that spiders build webs on sites with a background colour that matches their own (background matching). The hypotheses are split up in three parts:

a) Phenotypic parameters:

- Urban spiders are smaller compared to rural spiders (UHI and prey availability).
- Urban spiders are paler than rural spiders (UHI).

b) Microclimatic conditions of web building:

- Urban spiders will select cooler microclimates (Thub/Tretreat) for web building relative to the ambient temperature compared to rural spiders.
- Darker spiders will select cooler microclimates (Thub/Tretreat) for web building compared paler spiders.
- Thermoregulation of urban spiders will be different compared to rural spiders.

c) Site selection experiment:

- Darker spiders will select a darker background as retreat than paler spiders and vice versa.

How does abdomen colour and area affect heating of the spider's abdomen?

To analyse how abdomen colour and area affects heating of the spider's body, we will conduct a heating experiment. We will construct heating curves for differently coloured spiders, and extract asymptotic temperatures and heating slopes from this.

Here, the hypotheses were as follows:

- Darker spiders reach higher asymptotic temperatures compared to lighter spiders.
- Larger spiders reach similar asymptotic temperatures compared to smaller spiders.
- Darker spiders heat up faster compared to pale spiders.
- Larger spiders heat up slower compared to smaller spiders.

How do spiders from different urbanisation levels develop under different temperature and food regimes? Can we find evidence for phenotypic plasticity or genetic adaptation?

To answer these questions, we set up a common garden rearing experiment in which we reared spiders from different urbanisation degrees under two temperature and food regimes: 20°C or 24°C and high food or low food. To quantify their development, we measured their weight at the beginning of the rearing and at the end of the rearing. This way, the bigger weight increase during the experiment, the faster they will develop.

I expect that:

- Under genetic adaptation, urbanisation background of the mother will influence their development. Urban spiders will develop faster under high temperature than rural spiders.
- Under phenotypic plasticity, urbanisation background of the mother will not influence their development. Urban and rural spiders will respond in the same way to the different treatments.
- In general, high food availability will have a positive effect on the spider's development.

Materials and methods

Spider sampling and temperature measurements

In order to find patterns in effect of urbanisation between urban and rural spiders, we measured temperature of adult female spiders and of several web characteristics (hub and retreat) in the field during September and October. Sampling was done according to an already existing sampling design of the 'SPEEDY' project (<https://bio.kuleuven.be/eeb/ldm/speedy> and figure 3), located in Flanders. This design consists of a crossed sampling strategy of high, intermediate and low levels of urbanisation and this at both large ($3 \times 3 \text{ km}^2$; *plots*) and smaller ($200 \times 200 \text{ m}^2$; *subplots*) spatial scales, where the last is nested in the first. The urbanisation levels were determined using the percentage of built up area (BU) in the (sub)plots (high: >15% of BU, intermediate: 5%-10% of BU and low: <3% of BU). This is an interesting sampling method because it provides objective information about effects of urbanisation within Flanders. Additionally, the hierarchical nature allows us to analyse these effects on multiple spatial scales.

For each region (Ghent, Antwerp and Leuven), three plots of each urbanisation level were selected for sampling (27 in total). Within each plot, one subplot of high urbanisation level and one subplot of low urbanisation level was selected for sampling (54 in total, appendix I table 1). We decided not to sample the intermediate level subplots because of time constraints and because we expected to find the clearest results when comparing the highest and lowest urbanisation levels.

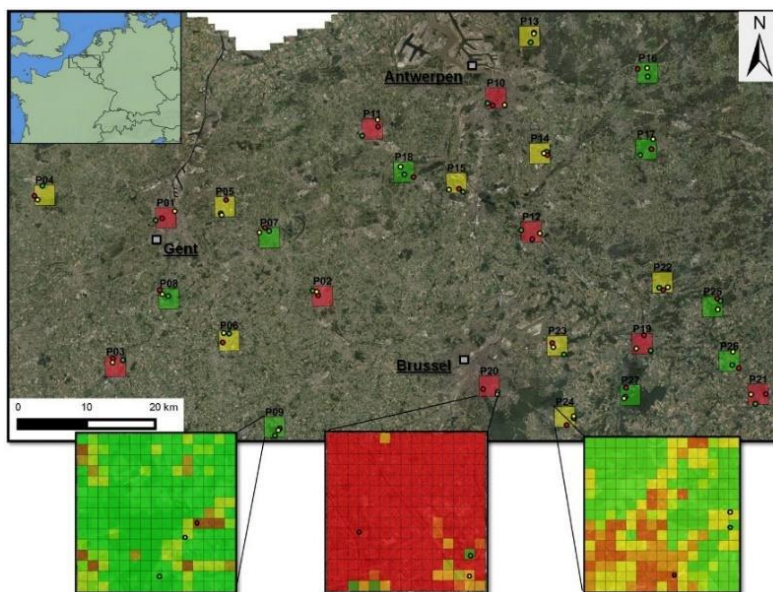


Fig 3. Map of the sampling area in northern Belgium with the 27 $3 \times 3 \text{ km}^2$ plots (fully coloured squares) in the triangle Ghent-Antwerp-Leuven. Their colour shows their level of urbanisation (green: low urbanisation with <3% of built-up area; yellow: intermediate urbanisation with 5%-10% of BU; red: high urbanisation with >15% of BU). Plots are divided into $200 \times 200 \text{ m}^2$ subplots with the same colour code. Light green and orange show subplots that are intermediately between the three levels. In each plot, one green and one red subplot was selected for sampling (Piano et al, 2017).

Once we arrived at a certain subplot, we started by randomly looking for cross spiders by locating their webs. We always aimed to measure and collect at least eight spiders. At this point in the season, only adult female spiders can be found. We focused on spiders located within their web hub, but always tried to collect a minimum of two spiders in retreat. When a spider was found, we first did all the measurements before collecting the spider and looking for the next one. First, some basic parameters were noted: local weather (cloudy or sunny), wind (yes/no), spider location (in retreat or in web hub), exposure of the spider to the sun (sun or shade) and local air temperature (T_{local}). Afterwards, we collected the spider and immediately measured its body temperature (T_{spider}) to have an accurate measurement. All temperature measurements were done using a thermocouple type T (Omega T-type 36 gauge thermocouple; SSC-TT-TI-36- 204 2M; accuracy = $\pm 1^\circ\text{C}$). We always placed the sensor of the thermocouple on the same spot of the abdomen: the left side of the cross (figure 4). Once this was done, we could collect the spider and finalize the temperature measurements: we measured the microclimatic temperature of the web hub (T_{hub}) and retreat (T_{retreat}) using the same thermocouple (figure 5 and 6).

Every temperature measurement was repeated three times, after which we eventually used the average of the three repeats.

In order to know how microclimate selection and thermoregulation change with urbanisation, I calculated some additional variables (following the approach in Bladon et al., 2020). **Microclimate selection** was calculated as the difference between T_{hub} and T_{local} and between $T_{retreat}$ and T_{local} . The first difference shows a possible microclimate selection in the web hub, and the second difference shows possible selection for retreats. These differences might thus indicate an active decision of the spider to make its web or retreat in a cooler place. Additionally, I also calculated some differences to analyse **thermoregulation** (behavioural, size or colour). Differences were calculated between T_{spider} and T_{hub} , T_{local} and $T_{retreat}$. These differences show whether the spider's body temperature is different from respectively the local air temperature, the temperature of their web hub and/or retreat on microclimate scale.



Fig 4. Placement of the sensor of the thermocouple (Omega® type T) on the spider's abdomen to measure its body temperature (T_{spider}). We always placed the sensor on the left side of the cross (credit: Katrien De Wolf).



Fig 5. Photo illustrating how the temperatures near the web were measured. An Omega thermocouple type T was used to measure the microclimatic temperature of the web hub (T_{hub}) and retreat ($T_{retreat}$). These measurements were repeated three times (credit: Pieter Vantieghe).

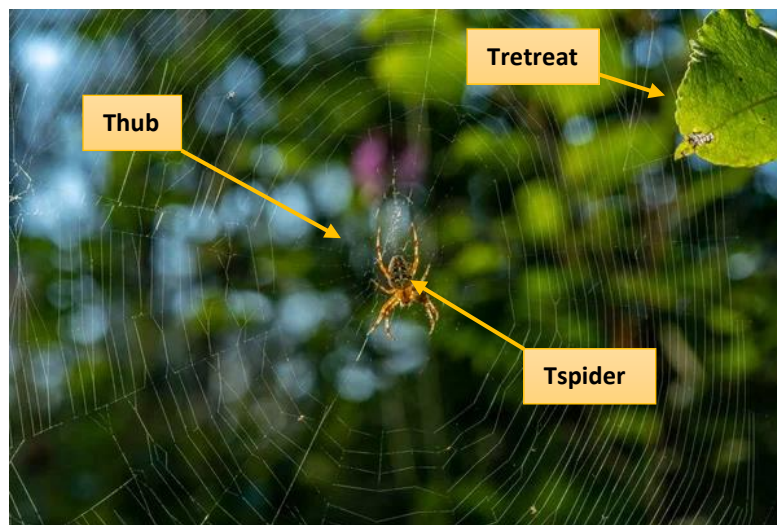


Fig 6: Location of different temperature measurements. Thub is the temperature of microclimate of the web hub (centre). Tspider is the body temperature of the spider's abdomen and Tretreat refers to the temperature of the microclimate of the retreat, which is the preferred hiding spot of the spider. As a last temperature measurement the local air temperature (T_{local}) was also quantified (credit: depositphotos.com).

After assessing these characteristics, every spider was brought to the lab for additional phenotypic measurements (i.e. body size and colouration) and for later use in the lab rearing experiment. The sampling of the spiders was conducted in September and October, because in this period female spiders are at the end of their life cycle and will have the highest chance of being fertilized. In doing so, we were able to maximize the amount of egg sacs laid by the spiders in the lab, which could later be used for the rearing experiment. Additionally, colouration is only complete in the adult stage of this spider as observed in previous lab rearing experiments (personal comm., Bram Vanthournout). It also makes sense to measure other parameters such as spider length or abdomen area only when the spider is fully mature.

Spider phenotypic traits

By measuring the spider's phenotypic traits, we are able to link urbanisation and microclimatic characteristics of the web with the phenotypic traits of the spider. After collection in the field, the spiders were taken to the lab and photographed using a Nikon camera. This was always done the same day of collection, to ensure that we quantified the correct size and colour from in the field. These photos were taken in a controlled setup with lights facing the same direction every time and a green background (figure 7a). In addition, we added a calibration line of 1 cm for the size measurements. Afterwards, the photos were analysed using ImageJ version 1.53t (Schneider et al., 2012). Before all quantifications were done, every picture was calibrated using the calibration line. This was achieved by first using the *straight* tool to select the calibration line, and then using *analyse* and *set scale*. We could then change *known distance* to 10 and *unit of length* to "mm" (figure 7b). Afterwards, the spider's full length (taken as the distance between the eyes and the end of the abdomen), abdomen length, cross length and cross width were measured by selecting the *straight* tool and then selecting *analyse* and *measure* (figure 7c and 7d). The cross length and width were divided by the abdomen length to find the proportion of cross in the abdomen, because these measures have more meaning than cross length and width alone (if spiders become bigger, their cross will also become bigger). Next, the abdomen area and mean gray value were measured by using *polygon selections* to select the abdomen, after which *analyse* and *measure* were used again (figure 7e). The mean gray value is a first measurement of the spider's abdomen colour (abdomen brightness) calculated as: $gray = 0,299 * red + 0,587 * green + 0,114 * blue$. Here, every pixel is converted to a gray value using this formula. Finally, a second colour measurement of the abdomen was taken (also expressed in gray values), being point measurements (repeated three times) of the cross (cross colour), first dark band of the leaf figure (leafdark colour) and first light band of the leaf figure (leaflight colour). This was done by using the *multi point* tool to select three points at each of these positions, after which *analyse* and *measure* were (figure 7f). Eventually, the average value of the three repeats was used. Since these colour

variables are expressed in gray values, higher values will mean the colour gets paler, and lower values means the colour gets darker.



Fig 7a. Example photo taken after collection. The photo is always accompanied with a calibration line of 1 cm (upper left corner).

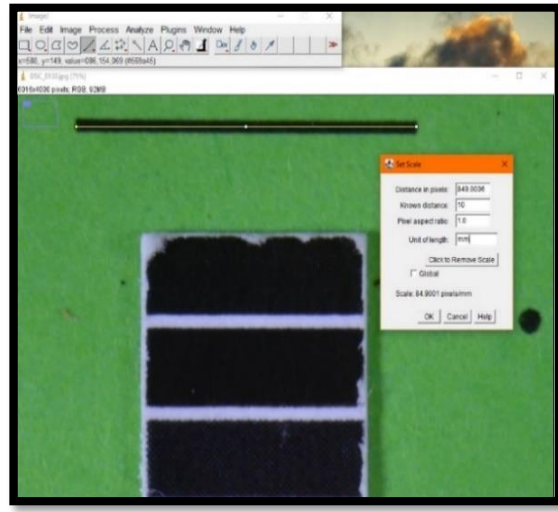


Fig 7b. First step in measurements of the spider. The 1cm calibration line was used to calibrate the photo.

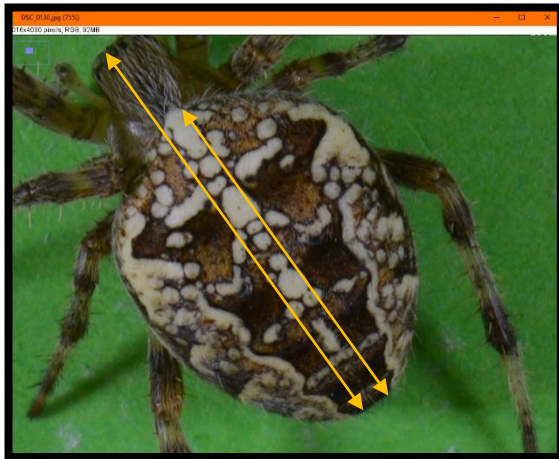


Fig 7c. First two size measurements of the the spider. The length of the spider and its abdomen are measured.

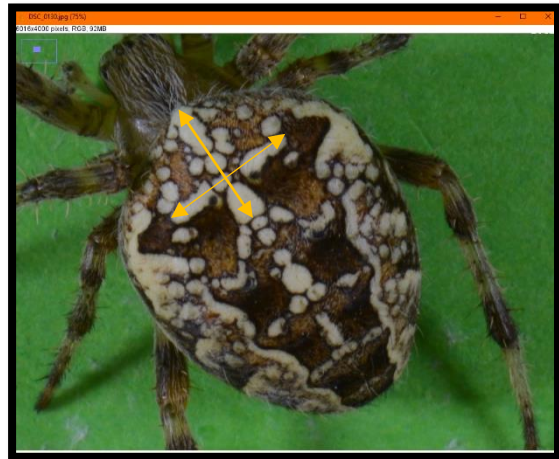


Fig 7d. Last two size measurements of the spider. The spider cross length and width are measured, after which the values area divided by the abdomen length to get a proportion.



Fig 7e. In this step the abdomen is selected using *polygon selections* after which it was analysed through *analyse > measure*. Both the area and mean gray value were measured.



Fig 7f. Second colour measurement of the abdomen. The *multi point* tool was used to select three points on the cross, on the first (dark) leaf and on the second (light) leaf. Afterwards, the colour of the 9 points was measured through *analyse > measure*.

Site selection experiment

An additional experiment was conducted to test whether the spiders will select a retreat using background matching. A subset of adult female spiders was collected (60 in total) in October around the areas Ghent and Torhout (table 1). Ghent can be considered as a big city with 568 880 residents in 2022 while Torhout is a smaller village with a population size of 20 517 (www.statbel.be). We made sure to sample enough areas to decrease dependency of our data (possibility of sampling siblings). Every spider was brought to the lab and first the same phenotypic measurements were taken as mentioned above. After this, each spider was placed in a plastic box (length-width-depth: 18cm-12cm-10cm) containing following setup (figure 8): two wooden sticks for climbing and possible web making, a dark leaf (*Rumex* sp.) in one upper corner, a pale leaf (*Carpinus betulus*) in the other upper corner and a wet piece of paper to keep the spider hydrated. We chose these plant leaves because we noticed in the field that these are typical leaves cross spiders use as a retreat and they are markedly different in colouration. The position of the leaves was randomized so that half of the boxes had the dark leaf in the upper left corner, and the other half had the dark leaf in the upper right corner. During one week, every spider's position was checked daily and given one of the following labels: Dark (D) if the spider was in or on the dark leaf, Pale (P) if the spider was in or on the pale leaf, Outside Dark (OD) if the spider was located outside but on the side of the dark leaf and Outside Pale (OP) if the spider was outside but on the side of the pale leaf. After this experiment was finished, this subset of spiders was used for the heating curves.

Table 1. Every location of spider collecting for the site selection experiment.

	Region	Latitude	Longitude	Spiders collected
Location 1	Torhout	51.090842	3.06545	5
Location 2	Torhout	51.085046	3.077741	2
Location 3	Torhout	51.089661	3.067858	3
Location 4	Torhout	51.092155	3.062834	3
Location 5	Torhout	51.088387	3.068796	2
Location 6	Torhout	51.083534	3.081335	6
Location 7	Ghent	51.0581250	3.7718397	9
Location 8	Ghent	51.0425942	3.7426720	15
Location 9	Ghent	51.0388854	3.7228250	2
Location 10	Ghent	51.0416937	3.7412135	7
Location 11	Ghent	51.0557045	3.7673057	6

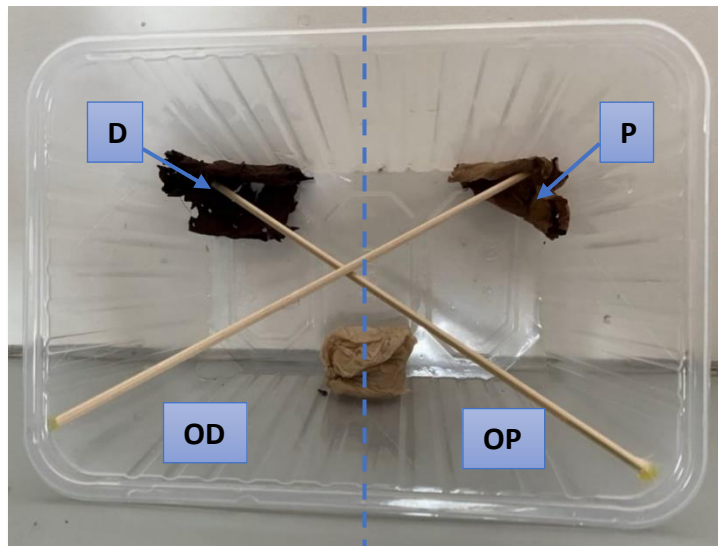


Fig 8. Photo of the setup of the site selection experiment. Every spider was placed in a plastic box containing two wooden sticks. In this case the dark leaf was placed in the upper left corner and the pale leaf in the upper right corner. Labels indicate how the position of the spider was scored: Dark (D), Outside Dark (OD), Pale (P) and Outside Pale (OP).

Heating curves

To investigate the effect of spider colour and size on heating, we performed a heating experiment. The spiders from the site selection experiment were subsequently used for this experiment, supplemented with additional spiders that were already collected during the fieldwork (76 in total, appendix table 3). Before doing the heating experiment, spider size and colour was measured based on the same method as described earlier.

First, the spiders were stored in a -80°C freezer for 15 minutes. A timing that ensures that the spiders were euthanized but were not frozen throughout to interfere with the heating. Afterwards, the spiders were placed in a breeding cabinet (aqualytic AL186) at 20°C for 30 minutes to ensure an equal body temperature of 20°C at the start of the experiment. During the experiment, the spiders were placed abdomen upwards in a petri plate filled with glass beads in groups of four (figure 9 and 10). We made sure that the spiders were placed far enough from each other, this way body temperatures were not interfering with one another. Heating was performed using a light bulb (Philips E27/ES), and the body temperatures were measured using a thermal camera which recorded the temperature changes during the experiment (FLIR T530 24° thermal camera with an accuracy of $\pm 2^{\circ}\text{C}$, range of -20°C to 1200°C and emissivity set to 0,95). The

emissivity value of 0.95 was taken because this the standard value for biological samples (Harrap et al., 2018). The petri dish was placed under the light bulb at a distance that resulted in a constant irradiance of $1000 \mu\text{W}/\text{cm}^2$ (value obtained by a Dr. Meter SM206 solar power meter, 1-3999 W/m^2 range and accuracy of $\pm 5\%$), which is typical for a sunny summer day (Rogalla et al., 2021).

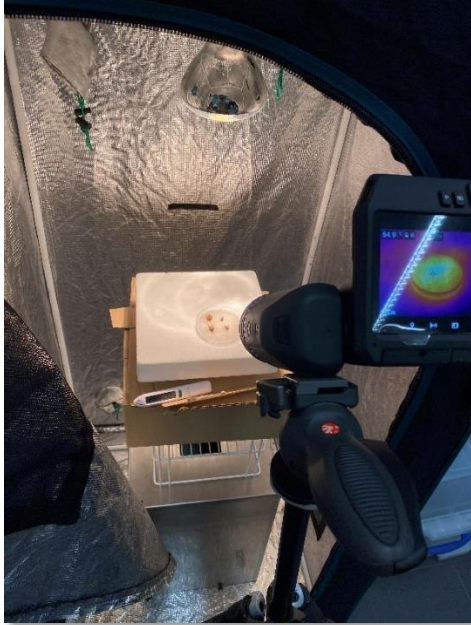


Fig 9: Photo of the experimental setup for the heating of the spiders. Four spiders are placed in a petri dish filled with glass beads and placed under a light bulb. While the spiders are heating up, the thermal camera is recording the changes in body temperature.

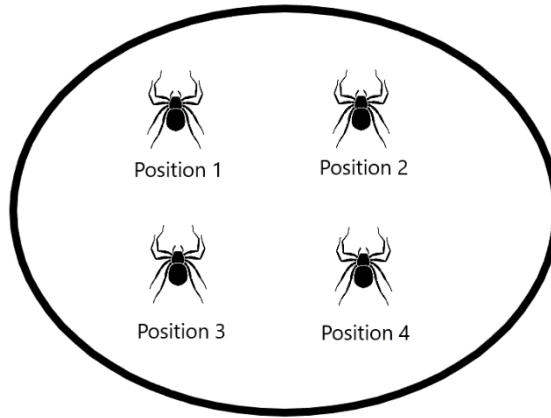


Fig 10: Graphical depiction of the positions of each spider in the petri dish. Every position was given a number from 1 to 4.

Heating was measured by turning on the light bulb for 30 minutes. Next, we extracted individual heating curves using ResearchIR (version 4.40). This was done by selecting a 3×3 box of pixels in the middle of the spider's abdomen, after which a heating curve was extracted based on the average temperature in this box. The asymptotic temperature (AT) was calculated as the mean of the last 30 temperature measurements and the heating slope (K2) as the negative derivative of the slope of the heating curve (according to Voss & Reed Hainsworth, 2001):

$$\ln \left[\frac{(T_t - T_\infty)}{(T_0 - T_\infty)} \right]$$

Where T_t is the temperature at a given time t , T_0 is the initial temperature and T_∞ is the asymptotic temperature (AT). Using this formula, we get negative slopes for each spider, after which we could find the slope of the heating curve (by taking the absolute value of the slope of the negative derivatives, resulting in a positive value). Importantly, only the temperature measurements from the first 600 timesteps were used for calculating the K2s, because after this timestep the heating curves start to deviate more leading to unreliable curves (see results).

Spider husbandry and rearing

We included a rearing experiment using the offspring of the spiders collected during the field work. Here, we reared spiderlings from urban and rural locations in different temperature and food regimes in a common garden experiment. In doing so, we could find out what the effect is of temperature and food availability on the development of these spiders and whether possible adaptations are genetic or due to phenotypic plasticity (environmentally determined).

The collected female spiders were first housed in round plastic cups (diameter of 9cm and depth of 13cm), placed upside down in a petri dish at 21°C with a 12/12 hour photo-cycle (figure 11). The cups contained a plastic straw for climbing and web making and a piece of cotton wool was placed at the bottom to ensure hydration. Spiders were misted and provided with two houseflies (*Musca domestica*) every two days. Once an egg sac was laid, the spiders were removed and euthanized by freezing, the egg sacs remained in the cup. In order to hatch, egg sacs need very high humidity (personal comm., Bram Vanthournout; Canard, 1984). For this reason, we removed the petri dishes and the cups were large cases (containing up to 10 cups) of which the bottom was filled with a few centimeters of water. This way, egg sacs were always developing under high humidity. After all egg sacs were laid, the temperature was gradually decreased (mimicking natural conditions) to stimulate egg laying (table 2). To ensure successful hatching, *A. diadematus* eggs need a cold period of at least 4 weeks (Canard, 1984). After this cold period, temperature was stepwise increased to 20°C. Once the eggs started hatching, we gave them an abundance of fruit flies (*Drosophila melanogaster*) and pollen (Eggs & Sanders, 2013) to allow growth (figure 12). After 3 weeks, the spiders big enough to be separated and they were put in plastic boxes (the same as for the site selection explained before) with two straws and a wet piece of paper (figure 13).



Fig 11: Setup of the females collected in the field. Each cup contained one female.



Fig 12: Newly hatched spiderlings from one egg sac.



Fig 13: Setup of the rearing of juvenile spiders at different temperature and food treatments.

Table 2: Stepwise increase of temperature to induce egg hatching. At 20°C, the spiders started hatching. They were kept at this temperature until they were separated for the rearing experiment.

Step	Temperature	Time
1	5°C	6 weeks
2	15°C	1 week
3	18°C	1 week
4	20°C	Until hatching and first life stages

For the rearing experiment, we used spiderlings originating from a high urbanisation degree both at landscape and local scale, and spiderlings from a low urbanisation at both scales. This would give the biggest contrast between urbanisation settings. We reared spiderlings from 18 different mothers originating from 9 subplots in high urbanisation degree and 9 from low urbanisation degree (one female per subplot) on different temperature and food regimes. The spiderlings were selected based on the total amount of offspring produced from the same egg sac, increasing our chances of having 10 spiderlings in each treatment from the same mother. From every egg sac, 10 spiderlings were placed under 20°C (rural; low temperature regime) and 10 under 24°C (urban; high temperature regime). This resulted in four treatments (40 spiderlings in total from one mother, with 10 in each treatment): High food and high temperature, high food and low temperature, low food and high temperature and low food and low temperature. Before being put in the treatment, each spider was weighed using an analytical balance (Mettler Toledo XS105 dualrange, error = 0,1 mg). This experiment lasted for four weeks, after which each surviving spider was weighed again. Weight can be seen as a proxy of how fast the spider develops.

Statistical analysis

All statistical analyses were performed in R (version 4.1.2; R Core Team, 2023).

Analysis of urbanisation effects on size, colour and microclimate variables

I analysed correlations (spearman) between size variables separately, colour variables separately and between colour and size variables together, using the `corrplot` function from the package “`corrplot`”. In addition, correlations were also made between the microclimate variables obtained in the field: `Tspider`, `Tlocal`, `Thub` and `Tretreat`.

For the analysis of the urbanisation effect, general linear mixed effects models were used (“`lme4`” and “`lmerTest`” packages; Bates et al., 2015), with spider size, colour and microclimate temperature as dependent variables and plot urbanisation, subplot urbanisation and the sampling date as fixed effects. Furthermore, we took `plotID` (P01 until P27) and `subplotID` nested within `plotID` as random intercepts. This way, we account for random plot effects (spiders from the same plot might be more similar) and random subplot effect within each plot (spiders from the same subplot might be more similar). Afterwards, effects were analysed using the `summary` function, as well as a type 3 anova (`ddf = Satterthwaite`, which corrects the degrees of freedom for dependency of data) and post-hoc analysis using Bonferroni-Holm correction (Holm, 1979) for multiple comparisons (package “`multcomp`”).

A general model looks as follows:

```
lmer(phenotypic variable ~ plot_urbanisation * subplot_urbanisation * sdate + (1|plotID) + (1|subplotID:plotID))
```

Here is “variable” any colour, size or temperature variable as dependent variable. “`Plot_urbanisation`” and “`subplot_urbanisation`” are the urbanisation levels in plots (landscape scale) and subplots (local scale) and “`sdate`” is the scaled date.

The same mixed models were used to analyse effects of the microclimate variables on the spider size and colour (in this case abdomen brightness):

```
lmer(abdomen brightness ~ Tspider + (1|plotID) + (1|subplotID:plotID))
```

Because all colour and size variables were significantly correlated with each other, I looked at separate models of only one colour or size variable as dependent variable (i.e. abdomen brightness in model above). Some of the residuals were not normally distributed, and based on the output of the “`bestNormalize`” (package “`bestNormalize`”), I decided on the particular transformation of the dependent variable. Abdomen area, abdomen length, spider length, abdomen brightness, leafdark colour, cross colour and `Tretreat` were all log transformed. Leaflight colour and `Tlocal` were `asinh` transformed. Lastly, `Tspider`, `Thub`, `Tspider - Tlocal`, `Tspider - Thub`, `Tspider - Tretreat`, `Thub - Tlocal` and `Tretreat - Tlocal` were `sqrt` transformed.

Analysis of the site selection in the lab on different days

For the analysis of site selection preference, generalized linear models were used. To be able to use these models, each position was changed to either category 0 or category 1. All positions “OP” and “P” were designated as category 1, and all positions “OD” and “D” were changed to category 0.

Afterwards, I fitted generalized linear binomial regressions for each colour variable as independent variable, and on each day separately (so from day 1 to day 7). An example of regression model would be:

```
glm(Day3 ~ abdomen_brightness, family = “binomial”)
```

Here is “Day3” all positions on day three, converted to 1s and 0s and abdomen brightness one of variables as an independent variable.

Analysis of spider heating in the lab

I analysed effects of spider size and colour on two heating variables: asymptotic temperature (AT) and heating slope (K2) of the spider. Because the colour and size variable were correlated, I made use of linear mixed effect regressions for every colour and size variable separately, against either AT or K2 as dependent variable. All assumptions were met for these models, so no transformations were necessary. A general model looks as follows:

```
lmer(AT ~ abdomen_area + (1|position))
```

Here, the position of the spider in the petri dish was added as random factor, because based on boxplots it was clear that spiders from different positions varied a lot in AT and K2 (see results).

Rearing experiment analysis

To analyse how spiderlings from different urbanisation backgrounds develop under different temperatures and food regimes, I used linear mixed effects models. Importantly, two separate datasets were analysed. One dataset contained spiders of which we did not have an initial weight, because they were still very small when separated (therefor not weighable, hereafter "dataset 1"). The second dataset contained both start and end weight, and contained spiders which were separated when they were a bit bigger. These spiders also had a period of growth under 20°C in the main lab while we were preparing for the experiment (hereafter "dataset 2"). For this reason, I analysed both datasets separately, in order to account for possible differences in development due to this earlier growth from the second data set spiders. To analyse development, I used the end weight after four weeks as dependent variable. A general model is shown below:

```
lmer(end_weight ~ urbanisation_mother * food_regime * temp_regime + (1|motherID))
```

Here is "end_weight" the weight of the spiders at the end of the experiment (in mg), "urbanisation_mother" the urbanisation degree of the mother (high or low), "food_regime" the food regime the spider was treated with (high or low), "temp_regime" the temperature regime the spider was treated with (high or low). Random intercepts were included of motherID (describes from which mother the spiderling originated) to account for dependency of spiderlings within mother (siblings might be more similar to each other).

Other packages that were used for the statistical analysis and visualisation are: "sjPlot" for visualization of the interactions, "performance" to check model fits (i.e. AIC), "lattice" for data exploration, "ggplot2" for data visualization, "ggpubr" for data exploration and visualization, "ggeffects" to find and plot predicted effects.

Results

General results and variable correlations

In this thesis, I studied spider phenotypic variation (size and colour) in response to urbanisation. I also analysed the relationship of spider microclimate variables with urbanisation. This way, I could investigate potential urban adaptation of this spider. Furthermore, I included a site selection experiment to investigate whether spiders will use background matching when choosing a retreat (hide). This way, I could potentially link colour variation with urbanisation to background matching. To verify patterns of size, colour and temperature in the field, I also performed a lab experiment to investigate the effect of spider size and colour on heating in a controlled setting. Lastly, I performed a rearing experiment to analyse effects of temperature and food on the development of spiders from different urbanisation levels. Here, I could find out how temperature, food and urbanisation background influences the development of the spiderlings, which I could then link with results from the urbanisation analysis.

An overview of all counts per plot can be found in appendix I table 2. In total, 626 spiders were collected and size and colour measurements were taken. For the measurements in the field (microclimate temperatures), 473 spiders were analysed. 241 spiders were from the red plots, 152 from the yellow plots and 233 from the green plots. Furthermore, 313 spiders were from the red subplots and 313 spiders from the green subplots. Of the 626 collected spiders, 285 spiders laid an egg sac of which 256 egg sacs hatched (90% hatching success). For the site selection experiment, 48 spiders were used in the analysis. Furthermore, 76 spiders were analysed in the heating experiment. Lastly, 467 spiderlings were reared in the common garden experiment. Of 245 spiderlings we had the start weight, and of 441 spiders we had the end weight (missing values are spiders that died during the experiment).

The correlations of the different colour and size variables of the spiders are depicted in figure 14. All correlations were positive and significant, indicating that the size variables are correlated and also the colour variables are correlated. More importantly, it shows that the colour variables are also correlated with the size variables. From this we can conclude that bigger spiders are also paler. Some correlations are less strong, but they were still significant indicating that I need to take them into account in my analysis.

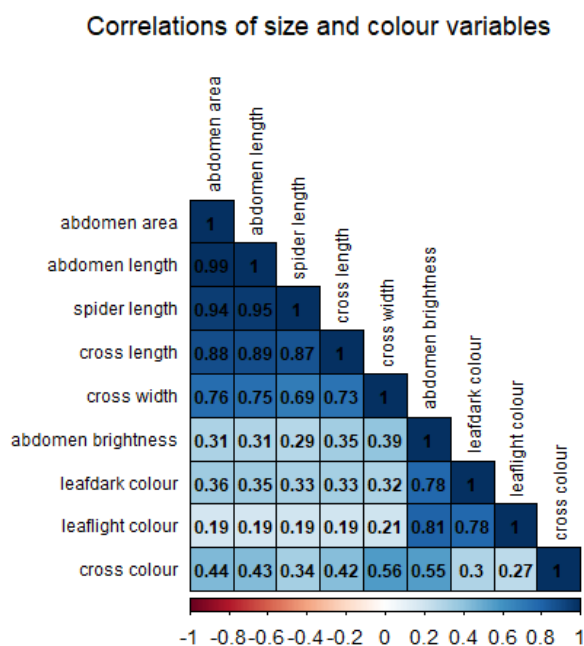


Fig 14: Correlations of colour and size variables used in the analysis. All correlations are positive and significant. Colours shown the strength of correlations (dark blue = strong positive correlation, dark red = strong negative correlation) and the values within the boxes are the correlation strengths (spearman correlations).

Following table shows effects of local (subplots) and landscape (plots) scale urbanisation and date of sampling on each analysed variable (table 3).

Table 3: Overview of all effects of urbanisation (local scale: subplots, landscape scale: plots) and date on each analysed variable. "+" means a positive effect, "-" means a negative effect and "+/-" means both positive and negative effects. Stars indicate significance of the effects (1 star = $p < 0,05$; 2 stars = $p < 0,01$; 3 stars = $p < 0,001$ and no stars means that no significant effect was found). For the colour variables, a blue box means the spiders get paler, a green box means they get darker and both colours means that they get paler or darker depending on the group. Lastly, for the interactions, "NS" means no significant interaction was found and "S" means a significant interaction was found.

length variable	plot urbanisation	subplot urbanisation	date	plot:subplot	plot:date	subplot:date	3 way interaction
abdomen area	+	-	***	NS	NS	NS	S
abdomen length	+	-	***	NS	NS	NS	S
spider length	+	-	***	NS	NS	NS	NS
cross length proportion	-	-	*	NS	NS	NS	NS
cross width proportion	+/-	-	+	NS	NS	NS	NS
colour variable							
abdomen brightness			***	NS	NS	NS	NS
leaf dark colour			***	NS	NS	NS	NS
leaf light colour				NS	NS	NS	NS
cross colour			*	NS	NS	NS	NS
temperature variable							
Tspider	+/-	*	**	NS	NS	NS	S
Thub	+	-	**	NS	NS	NS	S
Tretreat	+	*	**	NS	NS	NS	S
Tlocal	+/-	***	**	NS	NS	NS	S
Thub - Tlocal	+	+	+	NS	NS	NS	NS
Tretreat - Tlocal	+/-	+	+	NS	NS	NS	NS
Tspider - Thub	*	***	+	NS	NS	NS	NS
Tspider - Tlocal	-	**	+	NS	NS	NS	NS
Tspider - Tretreat	*	**	+	NS	NS	NS	NS

In table 4, all effects are shown of the microclimate variables on the size and colour variables.

Table 4: Overview of all effects of the microclimate variables on the size and colour variables. "+" means a positive effect, "-" means a negative effect and "+/-" means both positive and negative effects. Stars indicate significance of the effects (1 star = $p < 0,05$; 2 stars = $p < 0,01$; 3 stars = $p < 0,001$ and no stars means that no significant effect was found). For the colour variables, a blue box means the spiders get paler and a green box means they get darker. Lastly, for the interactions, "NS" means no significant interaction was found and "S" means a significant interaction was found.

size variable	Tspider	Tlocal	Thub	Tretreat	Thub - Tlocal	Tretreat - Tlocal	Tspider - Thub	Tspider - Tlocal	Tspider - Tretreat
abdomen area	***	***	***	**	+	+	***	**	***
abdomen length	***	***	***	**	+	+	***	**	***
spider length	***	**	**	*	+	+	*	*	*
cross length proportion	+	+	+	+	-	+	+	+	-
cross width proportion	+	+	+	+	-	-	+	-	-
colour variable									
abdomen brightness					*				
leafdark colour							*		*
leaflight colour					*	*	*		*
cross colour	**	**	*	*					

Effects of local and landscape scale urbanisation on spider phenotype and microclimate

Spider size

The anova outputs of all mixed models of the size variables is given in appendix IIA, table 1-5.

To investigate the effect of urbanisation on spider abdomen area, I measured this value and ran it in a mixed model that included urbanisation and sampling date as independent variables. This because spider size (and abdomen area) can be related to thermoregulation and prey availability in urban contexts. Additionally, abdomen area and length can also be seen as proxies of egg load (number of eggs) which can in turn be seen as a proxy for spider fecundity.

A significant interaction was found between plot urbanisation, subplot urbanisation and sampling date on abdomen area ($p_{\text{date}} = 7,4e-15$; $F_{\text{date}} = 105,88$; $p_{\text{subplot}} = 0,004$; $F_{\text{subplot}} = 9,61$; $p_{\text{plot}} = 0,053$; $F_{\text{plot}} = 3,26$; $p_{\text{interaction}} = 0,007$; $F_{\text{interaction}} = 5,78$). The effect of subplot urbanisation depends on plot urbanisation level and also on collection date (figure 15). The abdomen area increased over time in every plot and subplot. Moreover, it seems that for the green and yellow plots, spider abdomen area is always higher in green subplots (local scale urbanisation effect). This is different for red plots. Spiders in green subplots have smaller abdomens earlier in the season, but will reach bigger abdomen sizes at the end of season and vice versa for the spiders in the red subplots. Lastly, spiders from the red plots seem to reach highest abdomen sizes at the end of the sampling season, while green and yellow plots reach similar sizes.

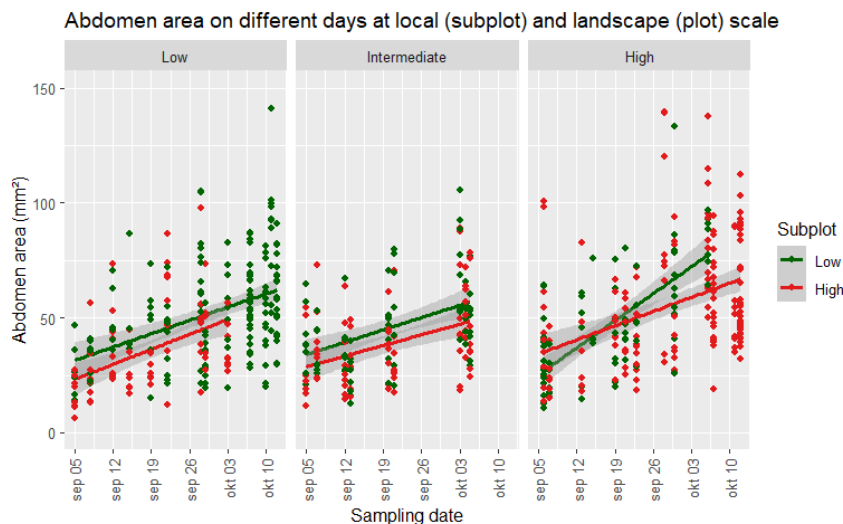


Fig 15: Three way interaction of sampling date, plot urbanisation and subplot urbanisation against abdomen area (mm²). The panels are divided into the three plot urbanisations levels (green = low, yellow = intermediate and red = high). The two differently coloured scatter plots show the two subplot urbanisation levels.

Secondly, the three-way interaction was also found to be significant when looking at abdomen length ($p_{\text{date}} = 6,7e-14$; $F_{\text{date}} = 92,2$; $p_{\text{subplot}} = 0,003$; $F_{\text{subplot}} = 10,36$; $p_{\text{plot}} = 0,033$; $F_{\text{plot}} = 3,88$; $p_{\text{interaction}} = 0,014$; $F_{\text{interaction}} = 4,85$; figure 16). So again, the effects of subplot urbanisation depends on the plot urbanisation levels and also the sampling date. In the low to moderate urbanised locations on landscape scale, and in the most urbanised areas at local scale(subplot), abdomen lengths of spiders were shorter. But, we can see that in the green plots at the end of the season the red subplots reach about the same abdomen lengths as the green subplots. The red plots reach the highest abdomen lengths of all plots, and red subplots early in the season will have higher abdomen lengths, but at the end of the season the green subplots will have the highest abdomen lengths. Lastly, we can see In general that abdomen length increases with sampling date.

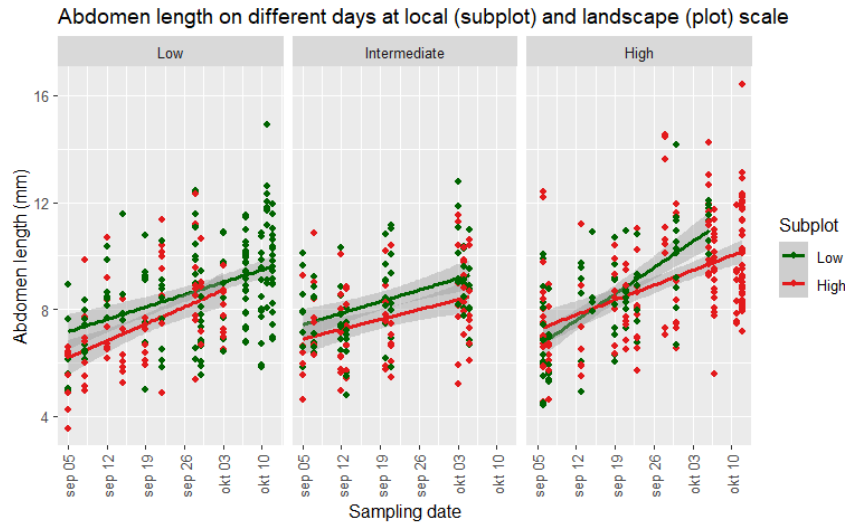


Fig 16: three way interaction of sampling date, plot urbanisation (landscape scale) and subplot urbanisation (local scale) against abdomen length (mm). The panels are divided into the three plot urbanisation levels (green = low, yellow = intermediate and red = high). Each panel depicts two subplots and their colour shows which subplot urbanisation it represents.

Urbanisation at local scale (plots) and sampling date have a significant effect on spider length ($p_{\text{plot}} = 0,01273$; $F_{\text{plot}} = 5,1377$; $p_{\text{date}} = 8,362e-10$; $F_{\text{date}} = 53,6589$; $p_{\text{subplot}} = 0,05397$; $F_{\text{subplot}} = 4,0503$; $p_{\text{interaction}} = 0,051$; $F_{\text{interaction}} = 3,2924$). Spiders from urban plots have a significantly higher spider length compared to rural plots, this effect was only significant between the red and green plots (figure 17; $p_{\text{green-red}} = 0,0226$; $Z_{\text{green-red}} = 2,672$; $p_{\text{green-yellow}} = 0,4887$; $Z_{\text{green-yellow}} = 0,692$; $p_{\text{yellow-red}} = 0,1226$; $Z_{\text{yellow-red}} = 1,871$). Additionally, spiders became longer at the end sampling period (appendix IIA, figure 1).

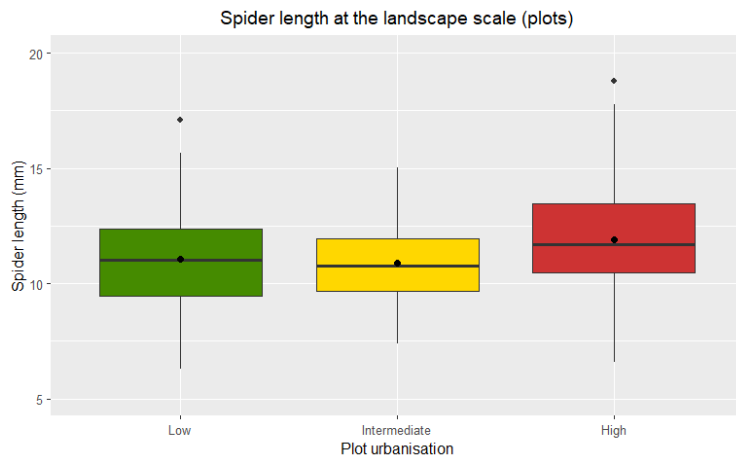


Fig 17: Spider lengths at landscape scale (plots). A significant positive effect was found of landscape scale urbanisation on spider length (but only for red plots).

Only sampling date had a significant and negative effect on the proportion cross in the abdomen ($p_{\text{date}} = 0,0003$; $F_{\text{date}} = 14,32$; $p_{\text{plot}} = 0,66$; $F_{\text{plot}} = 0,41$; $p_{\text{subplot}} = 0,86$; $F_{\text{subplot}} = 0,03$; $p_{\text{interaction}} = 0,52$; $F_{\text{interaction}} = 0,66$). So the cross length became proportionally smaller compared to the length of the abdomen towards the end of the sampling season (figure 18).

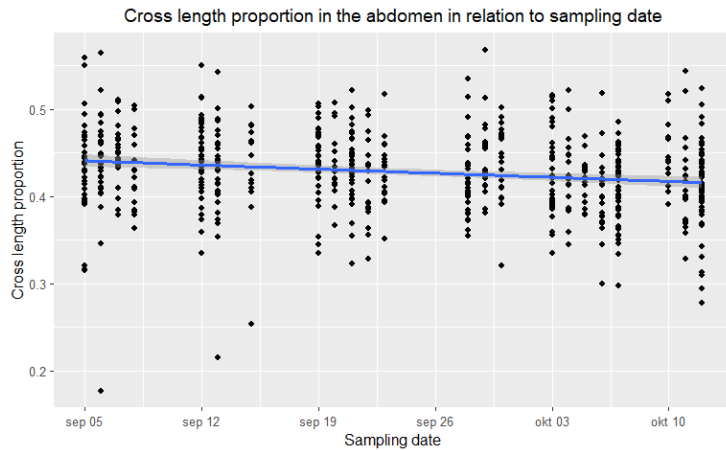


Fig 18: Proportion of the cross length in the spider's abdomen in relation to sampling date. A significant negative date effect was found ($p < 0,001$).

No significant effects were found of urbanisation or date on the proportion of the cross width in the abdomen ($p_{\text{date}} = 0,34$; $F_{\text{date}} = 0,92$; $p_{\text{plot}} = 0,72$; $F_{\text{plot}} = 0,13$; $p_{\text{subplot}} = 0,82$; $F_{\text{subplot}} = 0,20$; $p_{\text{interaction}} = 0,91$; $F_{\text{interaction}} = 0,095$).

Spider colour

The anova outputs of all mixed models of the size variables is given in appendix IIB, table 1-4.

to investigate whether garden spiders can adapt to urbanisation through colour, four colour variables were measured. For abdomen brightness, a significant positive effect was found of sampling date ($p_{\text{date}} = 0,0001$; $F_{\text{date}} = 16,81$; $p_{\text{subplot}} = 0,95$; $F_{\text{subplot}} = 0,004$; $p_{\text{plot}} = 0,97$; $F_{\text{plot}} = 0,03$; $p_{\text{interaction}} = 0,22$; $F_{\text{interaction}} = 1,58$; figure 19 and 20), indicating that later in the sampling season, spiders had paler abdomens.

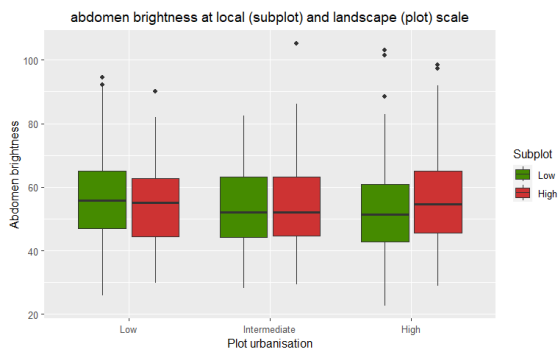


Fig 19: Boxplots of abdomen brightness on the local and landscape scale. There were no significant differences found for both local and landscape scale urbanisation.

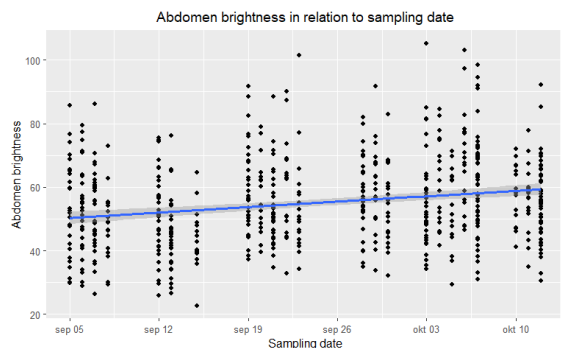


Fig 20: Abdomen brightness in relation to sampling date. A significant positive effect was found of sampling date on abdomen brightness ($p < 0,001$) meaning that abdomens became paler towards the end of the sampling period.

As a second colour measurement, the colour of the first dark leaf in the abdomen was measured. Similar to abdomen brightness, only sampling date had a significant positive effect on the leafdark colour ($p_{\text{date}} = 0,0007$; $F_{\text{date}} = 13,17$; $p_{\text{subplot}} = 0,44$; $F_{\text{subplot}} = 0,62$; $p_{\text{plot}} = 0,47$; $F_{\text{plot}} = 0,78$; $p_{\text{interaction}} = 0,44$; $F_{\text{interaction}} = 0,83$). The dark leaf became paler towards the end of the sampling period (fig 21 and 22).

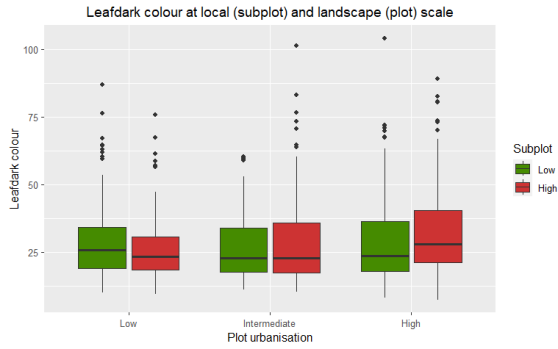


Fig 21: Boxplots of the colour of the dark leaf at local and landscape scale. No significant differences were found at local and landscape scale.

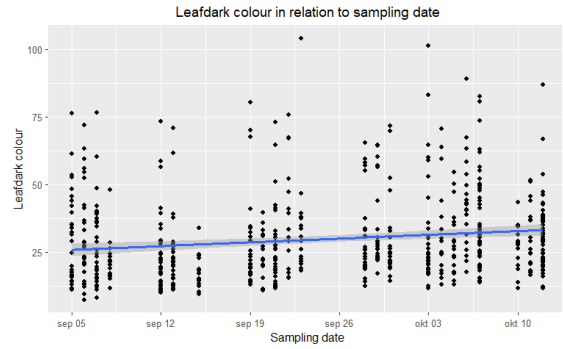


Fig 22: Figure of leafdark colour in relation to sampling date. A significant positive effect was found of sampling date on leafdark colour ($p < 0,001$). Leafdark colour was paler towards the end of the sampling period.

A third colour measurement was the colour of the first light leaf on the abdomen (leaflight colour). Unlike abdomen brightness and leafdark colour, no significant effects of urbanisation or sampling date were found on leaflight colour ($p_{\text{date}} = 0,055$; $F_{\text{date}} = 3,84$; $p_{\text{subplot}} = 0,12$; $F_{\text{subplot}} = 2,59$; $p_{\text{plot}} = 0,81$; $F_{\text{plot}} = 0,21$; $p_{\text{interaction}} = 0,52$; $F_{\text{interaction}} = 0,67$).

As a last colour measurement, I included the cross colour to test whether spiders might adapt to urbanisation (local and landscape) by “changing” their cross colour. Significant effects were found for sampling date and subplot urbanisation ($p_{\text{date}} = 9,84e-09$; $F_{\text{date}} = 41,51$; $p_{\text{subplot}} = 0,026$; $F_{\text{subplot}} = 5,34$; $p_{\text{plot}} = 0,058$; $F_{\text{plot}} = 3,068$; $p_{\text{interaction}} = 0,31$; $F_{\text{interaction}} = 1,20$). Sampling date had a positive effect on cross colour, while no significant differences were found between subplots after post-hoc analysis ($p_{\text{green-red}} = 0,23$; $Z_{\text{green-red}} = -1,21$). This means that the spider's cross became paler towards the end of the sampling period (figure 23 and 24).

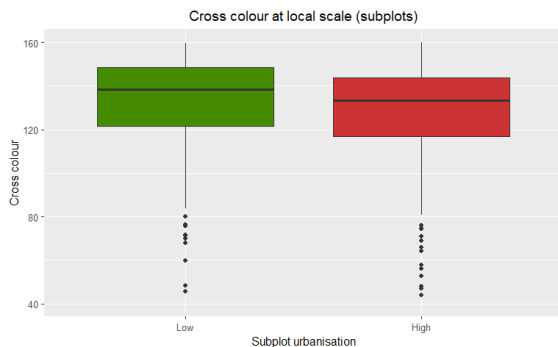


Fig 23: Boxplots of the cross colour in different subplots (local scale urbanisation). No significant difference was found between subplots.

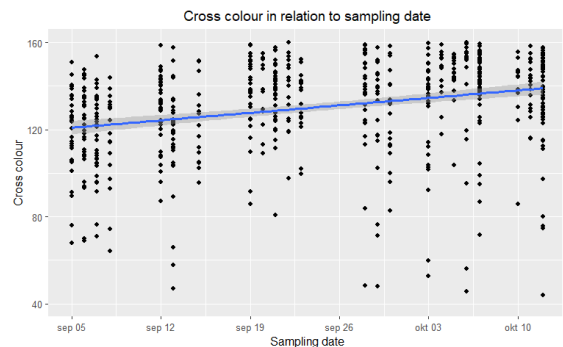


Fig 24: Figure of cross colour in relation to sampling date. A significant positive effect of sampling date was found with cross colour ($p < 0,001$). The spider's cross became paler towards the end of the sampling period.

Microclimate variables of the spider

The anova outputs of all mixed models of the size variables is given in appendix IIC, table 1-9.

After analysing urbanisation effects on the size and colour variables, I also wanted to analyse effects of local and landscape scale urbanisation on microclimatic temperature and other temperature measurements. This way I could find out how these microclimate variables change with urbanisation, and also whether spiders show microclimate selection (Tlocal – Thub) and (behavioural) thermoregulation (Tspider-Thub) and if this changes with urbanisation.

Correlations were made between Tspider and Tlocal, Tspider and Thub, Thub and Tlocal and Tretreat and Tlocal (figure 25; $\rho_{Tspider_Tlocal} = 0,93$; $\rho_{Tspider_Thub} = 0,94$; $\rho_{Thub_Tlocal} = 0,98$; $\rho_{Tretreat_Tlocal} = 0,95$; all p values < 2.2e-16). The slope of Tspider related to Thub or Tlocal is very similar as the 1:1 slope (1°C increase in Tlocal or Thub leads to 1°C increase in Tspider), but for any value of Thub or Tlocal, Tspider is a few degrees higher. This may point towards some forms of thermoregulation in this spider. In contrast, Thub and Tretreat have an almost equal slope as the 1:1 slope with Tlocal. This may explain that microclimate selection is in general less important for thermoregulation in this spider than other forms of thermoregulation, or is at least not very different from the local air temperature.

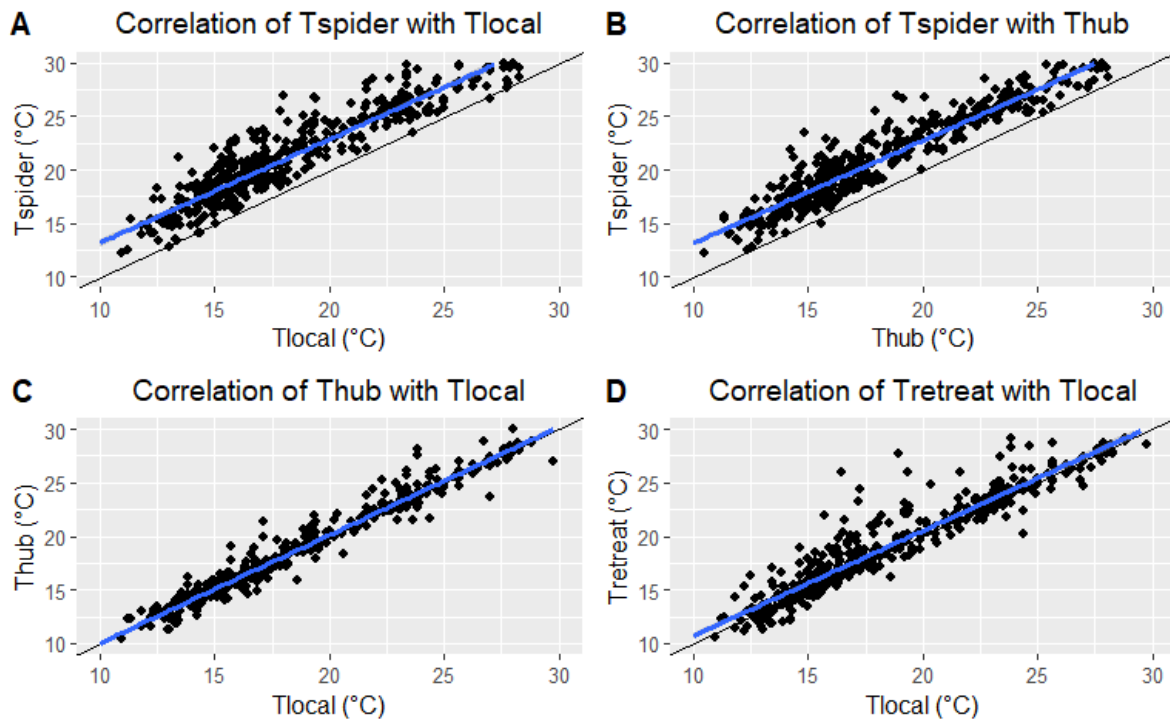


Fig 25: Correlations of the different microclimate variables. A 1:1 solid black line (1 to 1 relationship) was added to every plot, with every correlation between the actual data shown with a blue solid line. (A) Correlation of Tspider with Tlocal. (B) Correlation of Tspider with Thub. (C) Correlation of Thub with Tlocal. (D) Correlation of Tretreat with Tlocal.

The effects of local and landscape scale urbanisation and sampling date on Thub – Tlocal and Tretreat – Tlocal (microclimate selection of web hub and retreat) were not significant (Thub – Tlocal: $p_{date} = 0,09$; $F_{date} = 3,24$; $p_{subplot} = 0,67$; $F_{subplot} = 0,19$; $p_{plot} = 0,76$; $F_{plot} = 0,28$; $p_{interaction} = 0,75$; $F_{interaction} = 0,3$; figure 26; Tretreat – Tlocal: $p_{date} = 0,9$; $F_{date} = 0,017$; $p_{subplot} = 0,44$; $F_{subplot} = 0,63$; $p_{plot} = 0,54$; $F_{plot} = 0,64$; $p_{interaction} = 0,64$; $F_{interaction} = 0,45$; figure 27).

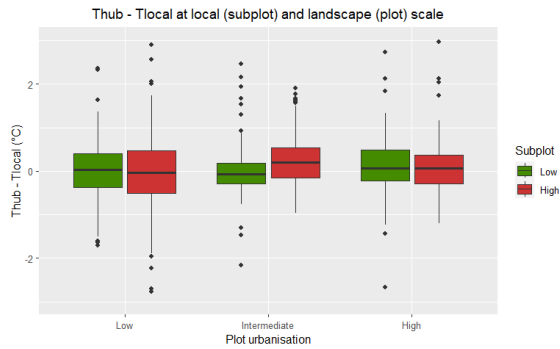


Fig 26: Boxplots of Thub – Tlocal with local (subplots) and landscape (plots) scale urbanisation. No significant differences were found between plots and subplots.

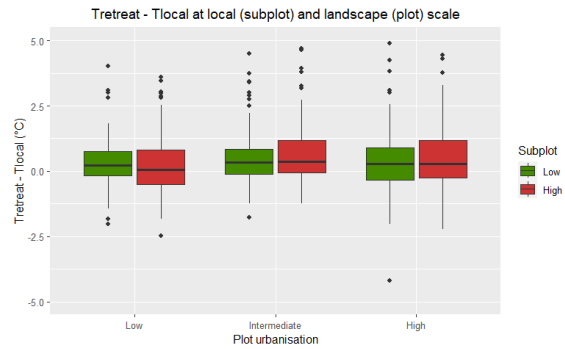


Fig 27: Boxplots of Tretreat – Tlocal with local (subplots) and landscape (plots) scale urbanisation. No significant differences were found between plots and subplots.

A significant three way interaction was found between sampling date, subplot urbanisation and plot urbanisation for Tspider ($p_{\text{date}} = 7,46e-08$; $F_{\text{date}} = 69,91$; $p_{\text{subplot}} = 0,011$; $F_{\text{subplot}} = 7,38$; $p_{\text{plot}} = 0,15$; $F_{\text{plot}} = 2,11$; $p_{\text{interaction}} = 0,029$; $F_{\text{interaction}} = 4,32$; figure 28). Tspider decreased with time in all plots and subplots. Overall it seems that Tspider does not differ that much between plots, as the values in the beginning of the season are similar between plots. In the green and yellow plots, red subplots show the highest Tspider in the beginning of the season, while in the red plots the green subplots will have higher Tspider. Towards the end of the season, this interaction changes: Green subplots will now reach higher Tspider for the green and yellow plots, while in red plots the red subplots reach higher Tspider.

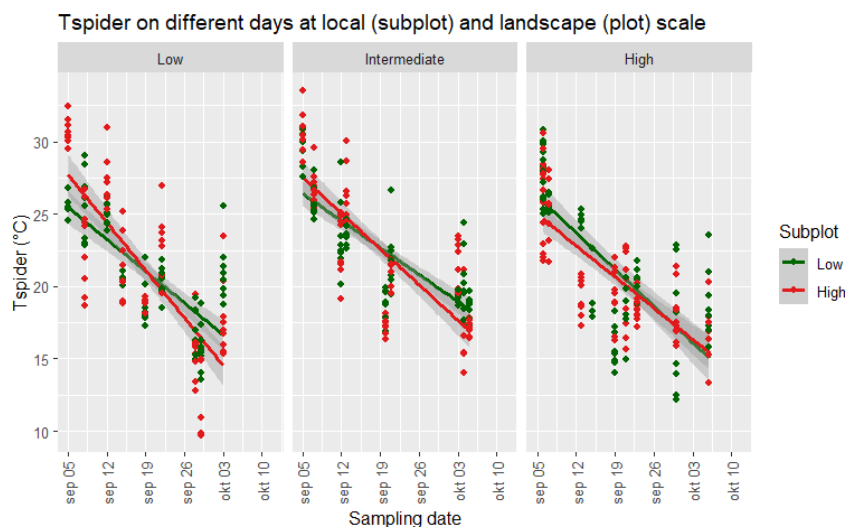


Fig 28: three way interaction of sampling date, plot urbanisation (landscape scale) and subplot urbanisation (local scale) against Tspider (°C). The panels are divided into the three plot urbanisation levels (green = low, yellow = intermediate and red = high) showing subplot urbanisation (green = low, red = high).

Similar to Tspider, a three way interaction was found between sampling date, subplot urbanisation and plot urbanisation with Tlocal ($p_{\text{date}} = 2,95e-08$; $F_{\text{date}} = 84,25$; $p_{\text{subplot}} = 0,0039$; $F_{\text{subplot}} = 9,96$; $p_{\text{plot}} = 0,02$; $F_{\text{plot}} = 4,84$; $p_{\text{interaction}} = 0,0087$; $F_{\text{interaction}} = 6,27$; figure 29). In general, Tlocal decreased with time in all plots and subplots. There is also not much difference in Tlocal between different plots, with an exception of the yellow plots, which had higher Tlocal at the end of the season compared to green and red plots. Furthermore, the red subplots were higher in Tlocal in green and yellow plots earlier in the sampling season, while green subplots had higher Tlocal towards the end of the sampling season. For the red plots, the green subplots reached higher Tlocal in the early sampling season while at the end, it was the red subplots that had higher Tlocal. The urbanisation effects were very similar for Tretreat and Thub, and can be found in the appendix (appendix IIC, figure 1 and 2).

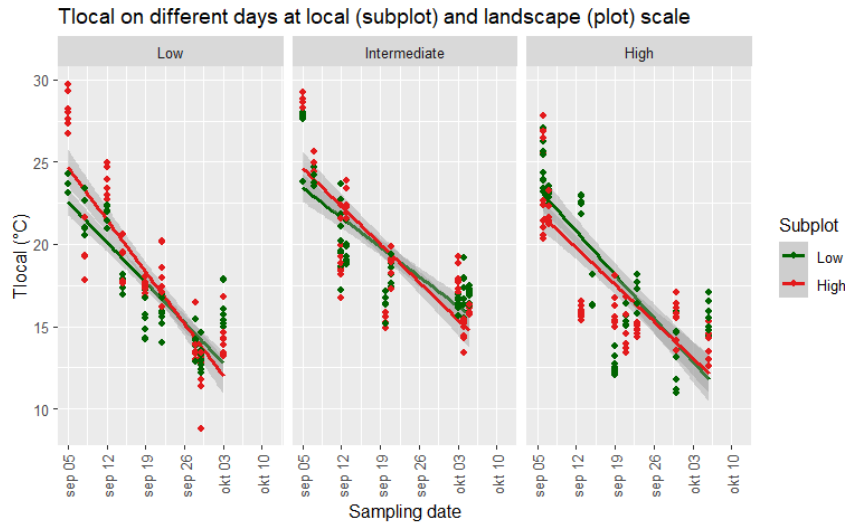


Fig 29: three way interaction of sampling date, plot urbanisation (landscape scale) and subplot urbanisation (local scale) against Tlocal (°C). The panels are divided into the three plot urbanisation levels (green = low, yellow = intermediate and red = high) showing the subplot urbanisation (green = low, red = high).

The effect of local and landscape scale urbanisation on Tspider – Thub was analysed, to find out if thermoregulation (size, colour, behaviour) changes with urbanisation. A significant effect was found of subplot urbanisation on Tspider – Thub ($p_{\text{date}} = 0,44$; $F_{\text{date}} = 0,61$; $p_{\text{subplot}} = 0,00066$; $F_{\text{subplot}} = 15,60$; $p_{\text{plot}} = 0,13$; $F_{\text{plot}} = 2,27$; $p_{\text{interaction}} = 0,20$; $F_{\text{interaction}} = 1,72$; figure 30 and 31). Tspider – Thub was significantly lower and closer to zero with local scale urbanisation ($p_{\text{green-red}} = 7,43\text{e-}05$; $Z_{\text{green-red}} = -3,96$). In red subplots the spider temperatures are closer to the temperature of the web hub than in green subplots.

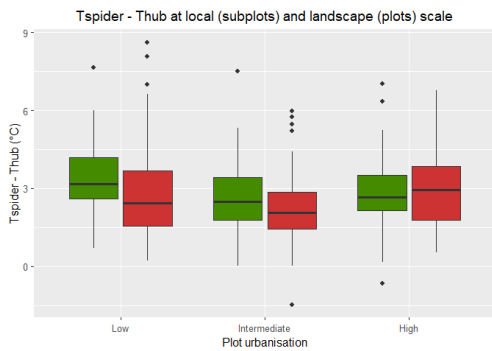


Fig 30: Boxplots of Tspider – Thub with local (subplots) and landscape (plots) scale urbanisation. Spiders had significantly lower values of Tspider – Thub in red subplots.

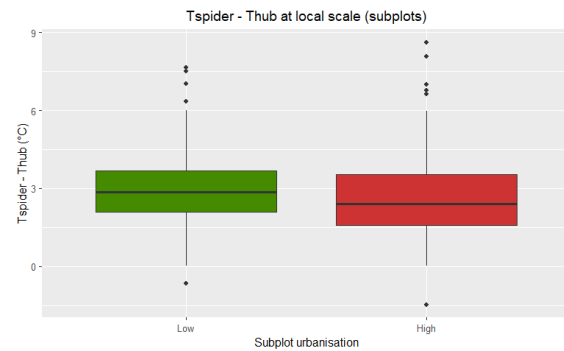


Fig 31: Boxplots of Tspider – Thub with local scale urbanisation (subplots). Spiders from red subplots had significantly lower values of Tspider – Thub compared to green subplots.

The effect of local and landscape scale urbanisation on Tspider – Tlocal was also analysed. This value shows how much the spider temperature is different from the local air temperature. I found a significant subplot urbanisation effect on Tspider – Tlocal ($p_{\text{date}} = 0,94$; $F_{\text{date}} = 0,0069$; $p_{\text{subplot}} = 0,022$; $F_{\text{subplot}} = 6,09$; $p_{\text{plot}} = 0,45$; $F_{\text{plot}} = 0,82$; $p_{\text{interaction}} = 0,54$; $F_{\text{interaction}} = 0,63$; figure 32 and 33). Tspider – Tlocal was significantly lower in red subplots compared to green subplots ($p_{\text{green-red}} = 0,0035$; $Z_{\text{green-red}} = -2,92$). In red subplots spider temperatures are closer to the local air temperature than in green subplots.

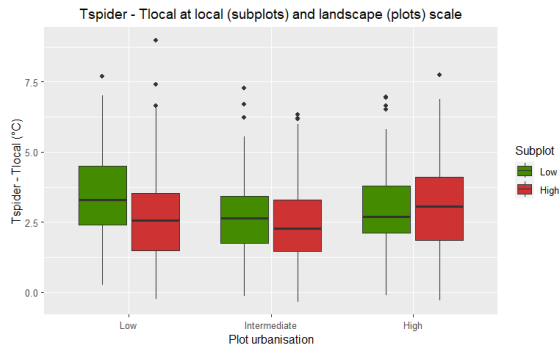


Fig 32: Boxplots of Tspider – Tlocal with local (subplots) and landscape (plots) scale urbanisation. Spiders had significantly lower values of Tspider – Tlocal in red subplots.

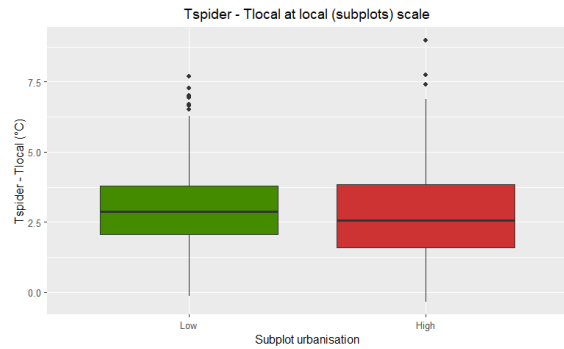


Fig 33: Boxplots of Tspider – Tlocal with local scale urbanisation (subplots). Spiders from red subplots had significantly lower values of Tspider – Tlocal compared to green subplots.

Lastly, I also analysed urbanisation effects on local and landscape scale on Tspider – Tretreat. This value explains how much the spiders temperature differs from the temperature of its retreat. Subplot urbanisation had a significant effect on Tspider – Tretreat ($p_{\text{date}} = 0,71$; $F_{\text{date}} = 0,15$; $p_{\text{subplot}} = 0,0023$; $F_{\text{subplot}} = 12,24$; $p_{\text{plot}} = 0,13$; $F_{\text{plot}} = 2,27$; $p_{\text{interaction}} = 0,43$; $F_{\text{interaction}} = 0,88$; figure 33 and 34). Tspider – Tretreat was significantly lower in red subplots compared to green subplots ($p_{\text{green-red}} = 0,00084$; $Z_{\text{green-red}} = -3,34$). In more urbanised subplots, spider temperatures were closer to the temperature of the retreat than in less urbanised subplots.

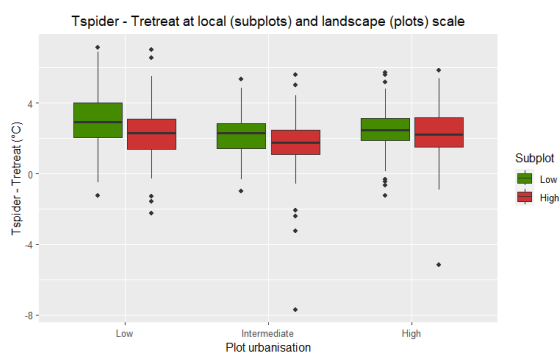


Fig 33: Boxplots of Tspider – Tretreat with local (subplots) and landscape (plots) scale urbanisation. Spiders had significantly lower values of Tspider – Tretreat in red subplots.

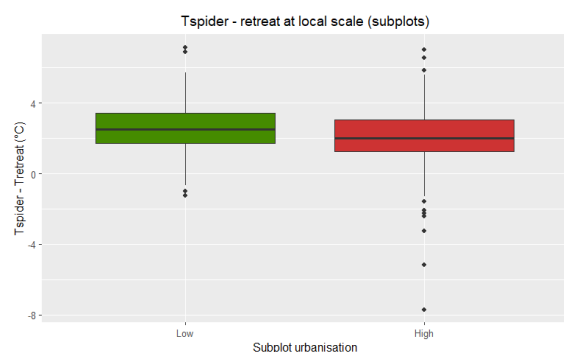


Fig 34: Boxplots of Tspider – Tretreat with local scale urbanisation (subplots). Spiders from red subplots had significantly lower values of Tspider – Tretreat compared to green subplots.

Effects of microclimate temperatures on spider size and colour

All Anova outputs of the mixed models can be find in appendix IIC table 10-18.

To investigate how spider size and colour is influenced by the microclimate variables such as Tspider, I ran a mixed model with the size and colour variables as dependent variable and the microclimate temperatures (separately) as independent variable. This way I could asses how temperature influences spider size and colour, and link this with the earlier found urbanisation effects.

Tspider, Tlocal, Tretreat, Thub, Tspider – Thub, Tspider – Tlocal and Tspider – Tretreat all had a significant negative effect on abdomen area ($p_{\text{Tspider}} = 1,28\text{e-}06$; $F_{\text{Tspider}} = 26,28$; $p_{\text{Thub}} = 0,00018$; $F_{\text{Thub}} = 15,91$; $p_{\text{Tlocal}} = 8,43\text{e-}05$; $F_{\text{Tlocal}} = 17,89$; $p_{\text{Tretreat}} = 0,0046$; $F_{\text{Tretreat}} = 8,35$; ($p_{\text{Tspider-Thub}} = 0,00081$; $F_{\text{Tspider-Thub}} = 11,38$; $p_{\text{Tspider-Tlocal}} = 0,0055$; $F_{\text{Tspider-Tlocal}} = 7,77$; $p_{\text{Tspider-Tretreat}} = 0,0009$; $F_{\text{Tspider-Tretreat}} = 11,17$; Figure 35 and 36). These effects were the same for the other size variables (abdomen length and spider length; appendix IIC figures 3,4,5 and 6). This means that spider size decreased with higher temperatures of Tspider, Tlocal,

Tretreat and Thub. Additionally, spider body temperature will also differ more from their microclimate when the spiders are smaller. Interestingly, I also found that abdomen area is significantly lower for spiders in their web hub compared to spiders that were in retreat ($p < 2.2e-16$; $F = 78.49$; $T = -8.86$; appendix IIC figure 7). This effect was also similar for abdomen length and spider length. This means that bigger spiders are more found their retreat than smaller spiders.

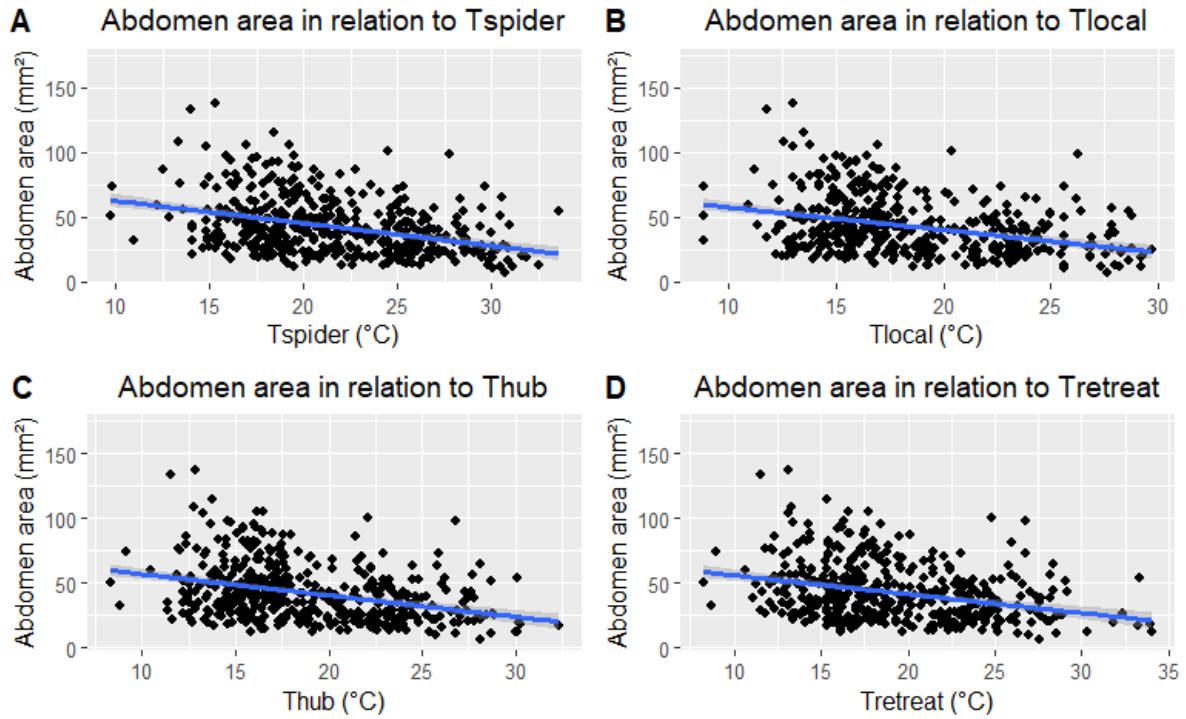


Fig 35: Abdomen area in relation to the different microclimate variables. (A) Abdomen area in relation to Tspider. (B) Abdomen area in relation to Tlocal. (C) Abdomen area in relation to Thub. (D) Abdomen area in relation to Tretreat.

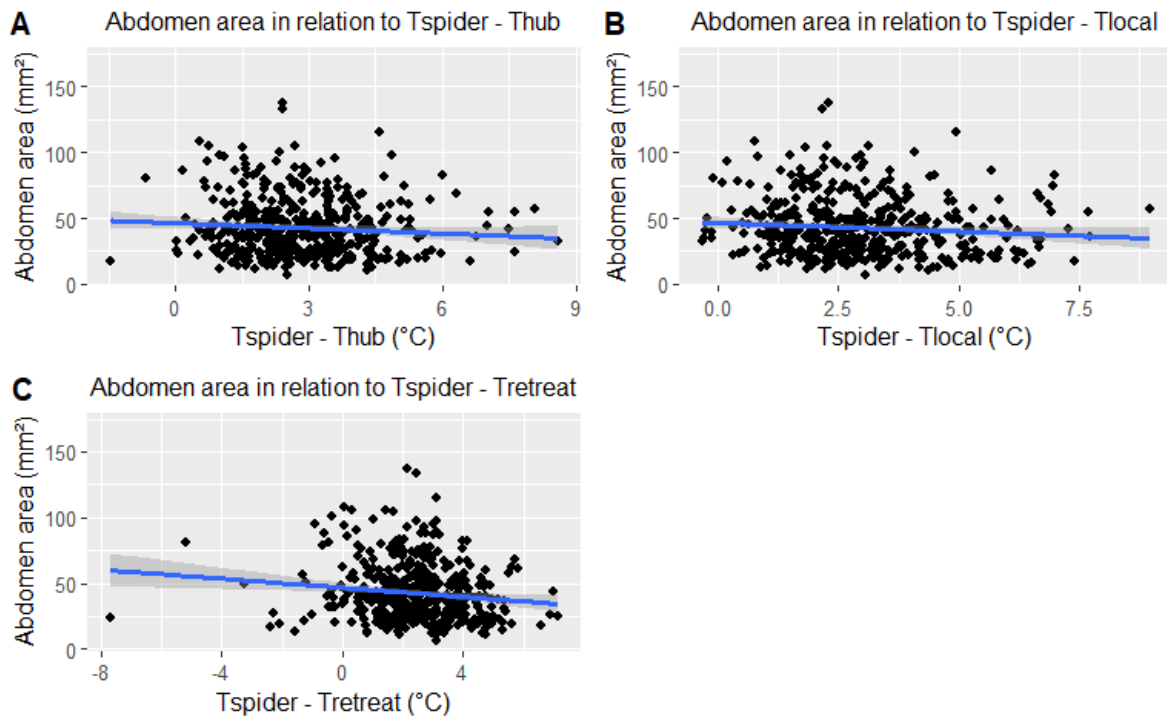


Fig 36: Abdomen area in relation to different microclimate variables. (A) Abdomen area in relation to Tspider - Thub. (B) Abdomen area in relation to Tspider - Tlocal. (C) Abdomen area in relation to Tspider - Tretreat.

Significant effects were also found of the microclimate variables on different colour variables. Tspider, Thub, Tlocal and Tretreat had significant negative effects on cross colour ($p_{\text{Tspider}} = 0,00997$; $F_{\text{Tspider}} = 7,1$; $p_{\text{Thub}} = 0,014$; $F_{\text{Thub}} = 6,65$; $p_{\text{Tlocal}} = 0,0092$; $F_{\text{Tlocal}} = 7,48$; $p_{\text{Tretreat}} = 0,003$; $F_{\text{Tretreat}} = 4,95$; figure 37). This means that with increasing temperatures, the cross colour became darker.

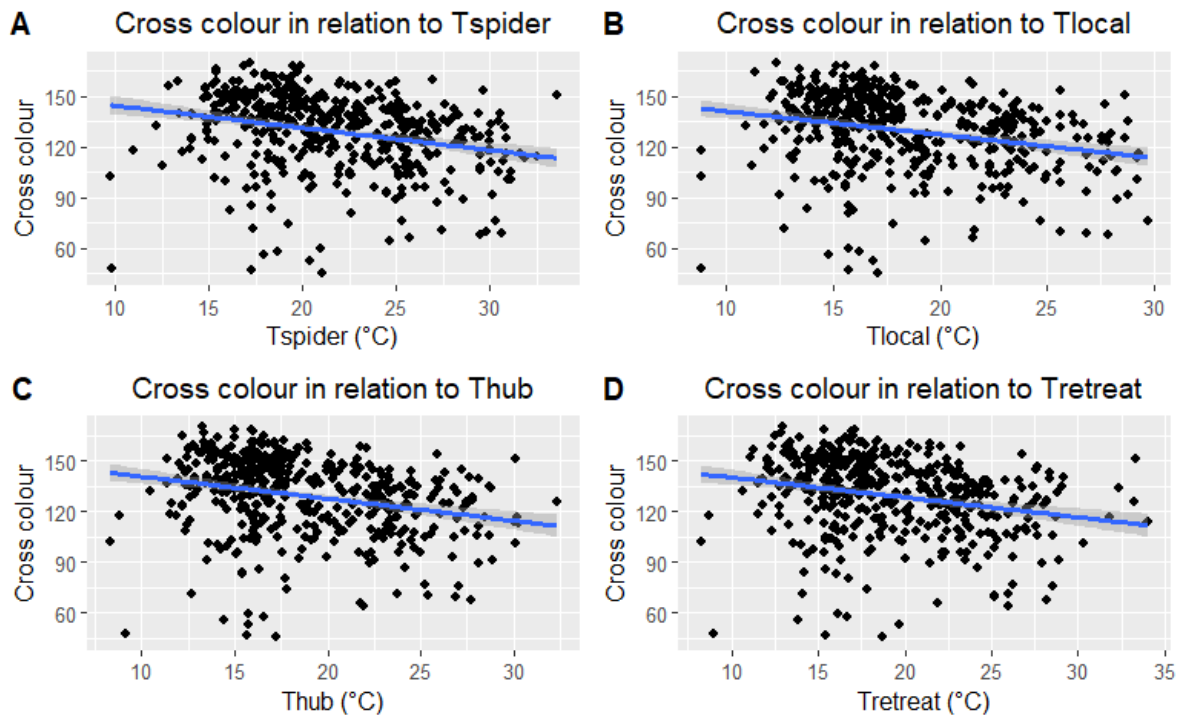


Fig 37: Cross colour in relation to the different microclimate variables. (A) Cross colour in relation to Tspider. (B) Cross colour in relation to Tlocal. (C) Cross colour in relation to Thub. (D) Cross colour in relation to Tretreat.

Thub – Tlocal had a significant positive effect on abdomen brightness ($p_{\text{Thub-Tlocal}} = 0,031$; $F_{\text{Thub-Tlocal}} = 4,71$; figure 38). This means that when the difference between the Thub and Tlocal is bigger (higher Thub), spider abdomens will be paler.

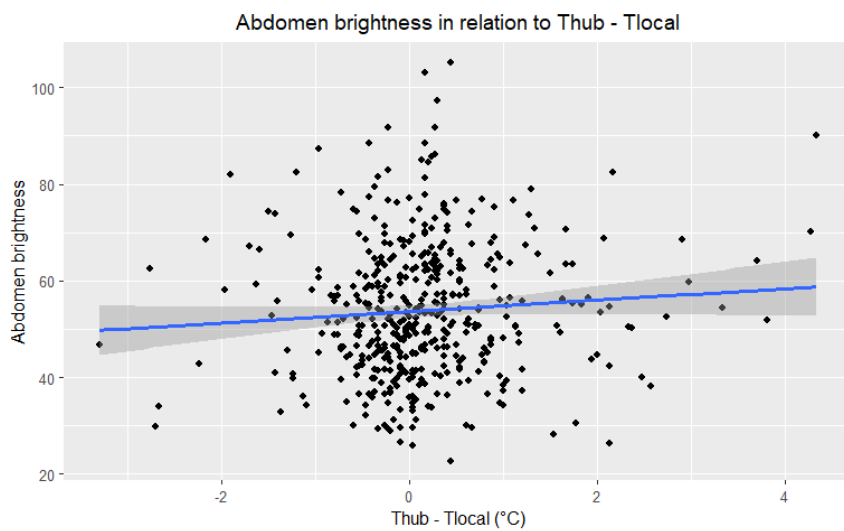


Fig 38: Abdomen brightness in relation to Thub – Tlocal. A significant positive effect was found of Thub – Tlocal local on abdomen brightness.

Both Tspider – Thub and Tspider – Tretreat had significant negative effects on leafdark color ($p_{\text{Tspider-Thub}} = 0,026$; $F_{\text{Tspider-Thub}} = 4,97$; $p_{\text{Tspider-Tretreat}} = 0,049$; $F_{\text{Tspider-Tretreat}} = 3,91$; figure 39 and 40). This means that when the difference between Tspider and Thub and Tretreat is bigger (higher Tspider), then the leafdark colour will be darker.

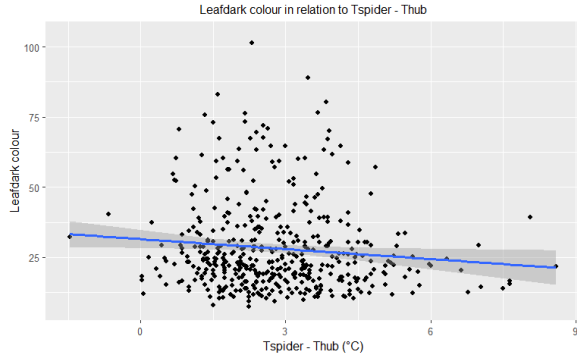


Fig 39: Leafdark colour in relation to Tspider – Thub. A significant negative effect was found of Tspider – Thub on leafdark colour.

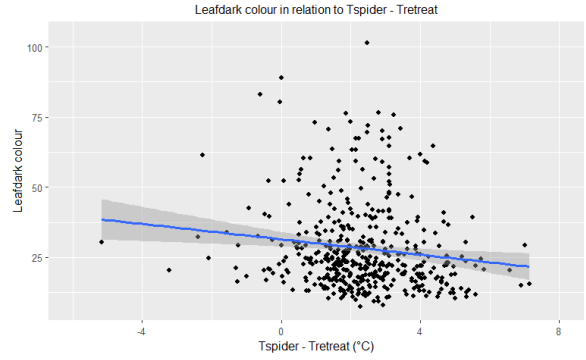


Fig 40: Leafdark colour in relation to Tspider – Tretreat. A significant negative effect was found of Tspider – Tretreat on leafdark colour.

Lastly, for leaflight colour, Thub – Tlocal and Tretreat – Tlocal had a significant positive effect on leaflight colour ($p_{\text{Thub-Tlocal}} = 0,023$; $F_{\text{Thub-Tlocal}} = 5,23$; $p_{\text{Tretreat-Tlocal}} = 0,038$; $F_{\text{Tretreat-Tlocal}} = 4,36$; figure 41) and Tspider – Thub and Tspider – Tretreat had a significant negative effect on leaflight colour ($p_{\text{Tspider-Thub}} = 0,038$; $F_{\text{Tspider-Thub}} = 4,36$; $p_{\text{Tspider-Tretreat}} = 0,011$; $F_{\text{Tspider-Tretreat}} = 6,56$). This means that when the values of Thub – Tlocal and Tretreat – Tlocal get bigger, spiders will get paler. Furthermore, when the values of Tspider – Thub and Tspider – Tretreat get bigger, spiders will get darker.

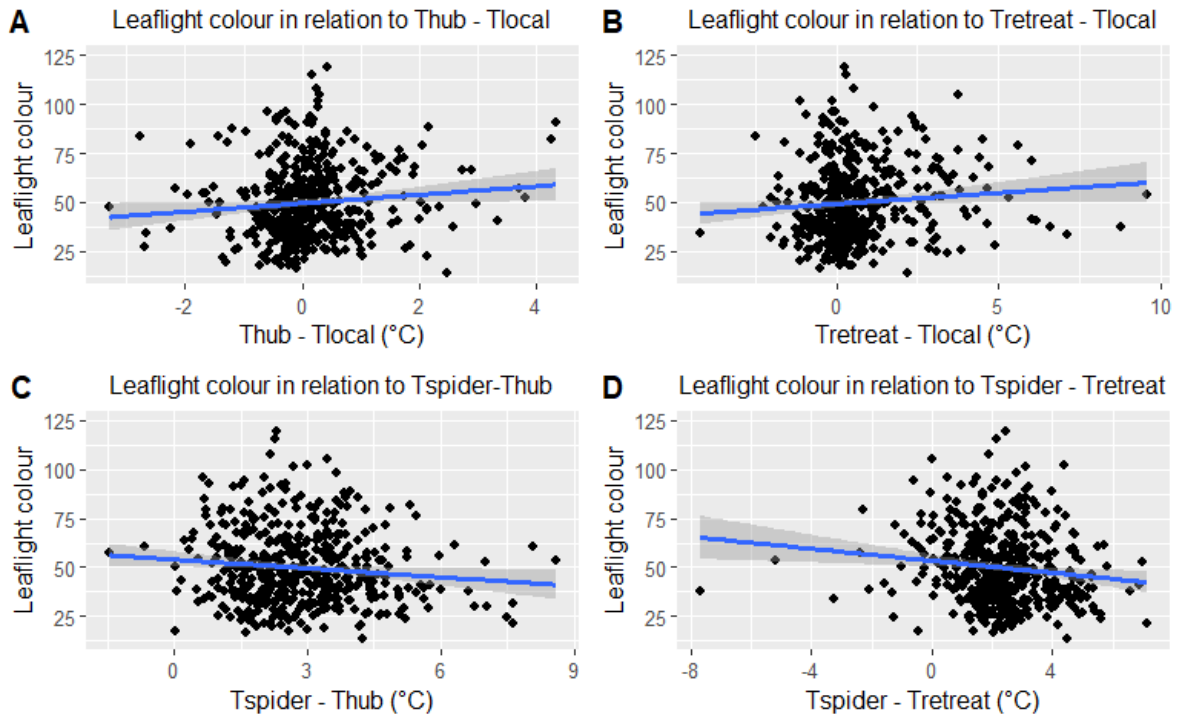


Fig 41: Leaflight colour in relation to the different microclimate variables. (A) Leaflight colour in relation to Thub – Tlocal. (B) Leaflight colour in relation to Tretreat – Tlocal. (C) Leaflight colour in relation to Tspider – Thub. (D) Leaflight colour in relation to Tspider – Tretreat.

Results of the site selection experiment

To investigate an alternative hypothesis regarding the effect of urbanisation on colour (camouflage), I analysed whether differently coloured spiders will use background matching when selection a retreat to hide. For seven days, every day was checked whether the spiders were closer to a light leaf, or a dark leaf (figure 42). From this figure it can be seen that there was quite some variation in the amount of spiders at different positions.

Only significant effects were found on day 4, which will be results shown below (for anova output: appendix III, table 1).

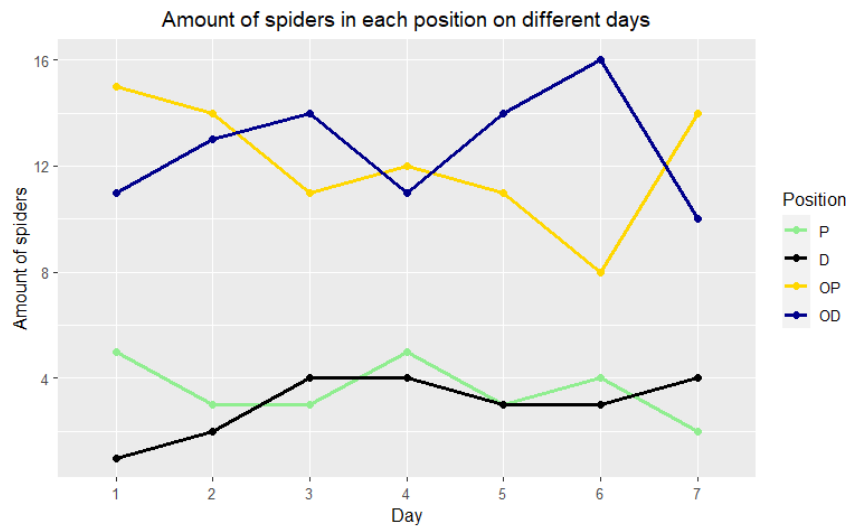


Fig 42: Positions of all spiders of different days in the site selection experiment.

On day 4, I found a significant effect of abdomen brightness on the preference of a paler background ($p_{\text{abdomen_brightness}} = 0,026$; $\chi^2 = 4,96$). Abdomen brightness had a significant positive effect on the preference of selecting a paler background (Figure 43; $p_{\text{abdomen_brightness}} = 0,038$; $Z = 2,071$).

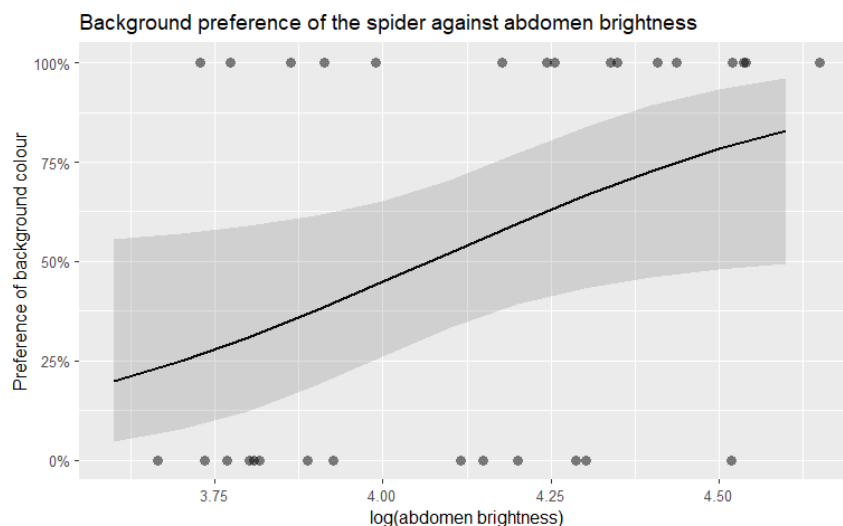


Fig 43: Preference of background of the spiders related to their abdomen brightness. A value of 0 on the x-axis means the spider was closer to the darker background, while a value of 1 means the spider is closer to a paler background. Abdomen brightness had a significant positive effect on the preference of the background colour.

Similarly, on day 4 leafdark colour also had a significant effect on the preference of a paler background ($p_{\text{leafdark_colour}} = 0,013$; $\chi^2 = 6,12$). Leafdark colour had a significant positive effect on the preference of selecting a paler background (Figure 44; $p_{\text{leafdark_colour}} = 0,035$; $Z = 2,11$).

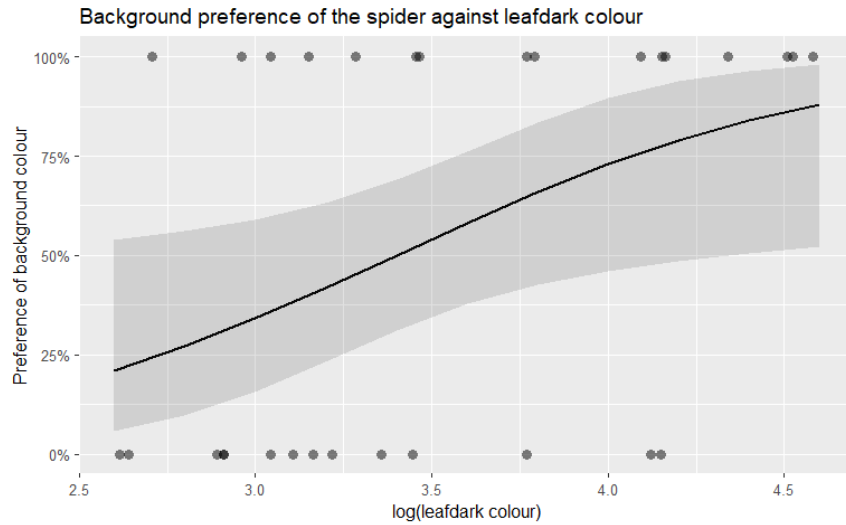


Fig 44: Preference of background of the spiders related to their leafdark colour. A value of 0 on the x-axis means the spider was closer to the darker background, while a value of 1 means the spider is closer to a paler background. Leafdark colour had a significant positive effect on the preference of the background colour.

Lastly, leaflight colour also had a significant effect on the preference of a paler background on day 4 ($p_{\text{leaflight_colour}} = 0,012$; $\chi^2 = 6,31$). Leaflight colour had a significant positive effect on the preference of selecting a paler background (Figure 45; $p_{\text{leaflight_colour}} = 0,024$; $Z = 2,26$).

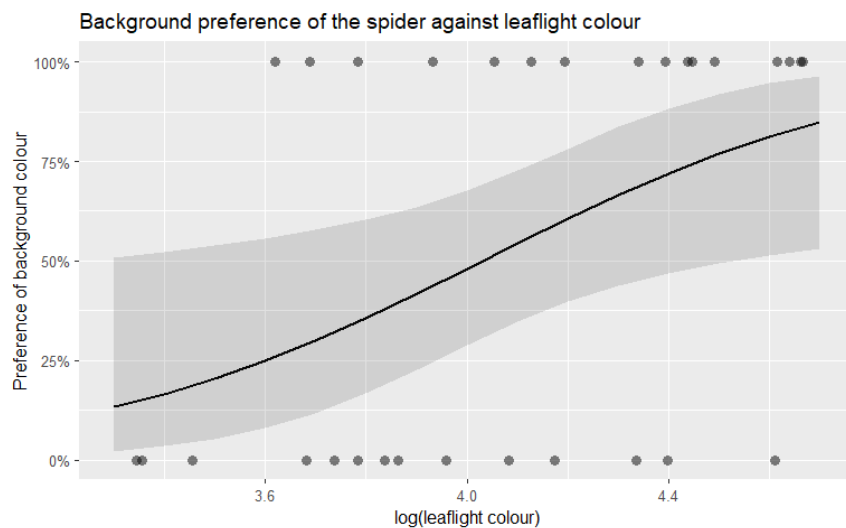


Fig 45: Preference of background of the spiders related to their leaflight colour. A value of 0 on the x-axis means the spider was closer to the darker background, while a value of 1 means the spider is closer to a paler background. Leaflight colour had a significant positive effect on the preference of the background colour.

Results of the heating experiment

General graphs and observations

To investigate how spider size and colour have an influence in the spider's thermoregulation (asymptotic temperature and slope of heating), I performed a heating experiment. In doing so, I could link possible effects of urbanisation on spider size and colour found in previous results with thermoregulation results from this experiment. Following table shows an overview of all effects of colour and size variables on spider asymptotic temperature and heating slope (table 5).

Table 5: Overview of all effects of spider size and colour on its asymptotic temperature and heating slope. "+" means a positive effect on the heating variable and "-" means a negative effect on the heating variable. Stars indicate significance of the effects (1 star = $p < 0,05$; 2 stars = $p < 0,01$; 3 stars = $p < 0,001$ and no stars means that no significant effect was found).

heating variable	abdomen area	abdomen brightness	leafdark color	leaflight color	cross color	cross length proportion	cross width proportion
AT	+	_*	-	-	-	-	+
K2	_***	_**	_**	_***	-	+	+

Following figures show an example of the heating curves of four different spiders (figure 46 and 47).

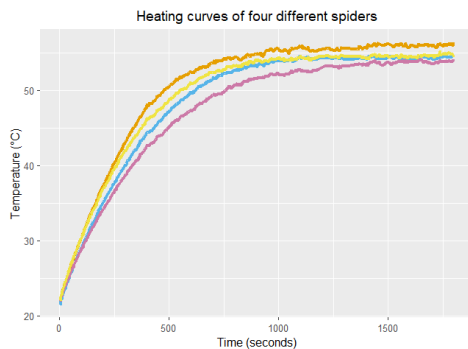


Fig 46: Heating curves of four spiders at different positions in the petri dish. Position 1 corresponds to the upper left position, position 2 to the upper right position, position 3 to the lower left position and position 4 to the lower right position.

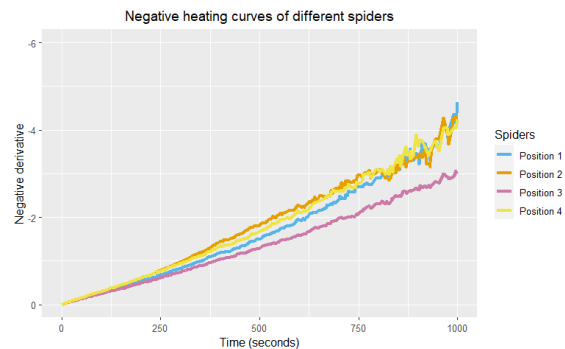


Fig 47: Negative heating curves of four spiders at different positions in the petri dish. Position 1 corresponds to the upper left position, position 2 to the upper right position, position 3 to the lower left position and position 4 to the lower right position.

Following boxplots show how AT, K2, abdomen brightness and leafdark colour differ among positions in the petri dish (figure 48). We can see that there is quite some difference in all variables among positions, but especially the AT of position 2 is higher than other positions. This could be because the light bulb has a non homogenous distribution of the solar radiation it emits. Secondly, both abdomen brightness and leafdark colour were darker on position, which could also explain why AT is higher there. The high variation in ATs and K2s among positions also explains why a mixed model with position as random effect is necessary.

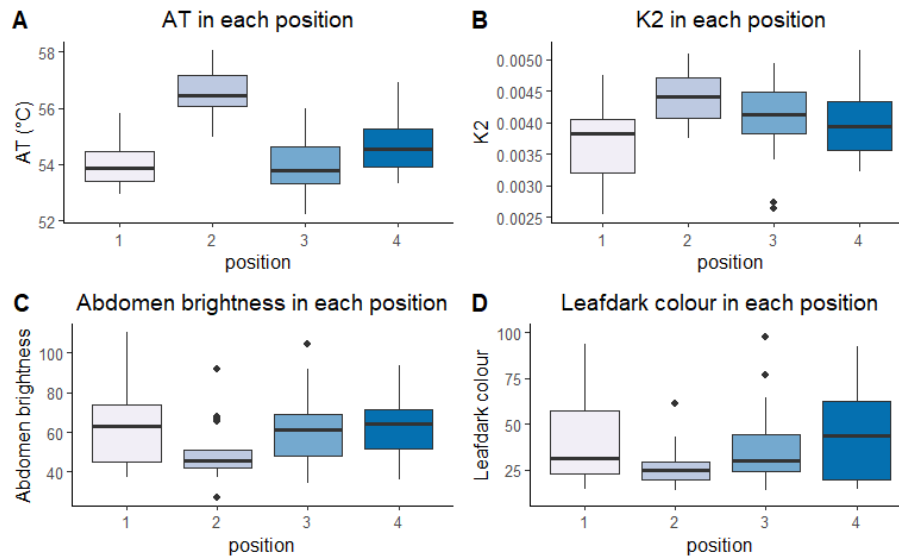


Fig 48: Boxplots showing how different variables are changing among positions in the petri dish. (A) Boxplots of asymptotic temperature (AT) with different positions in the petri dish. (B) Boxplots of heating curve slopes (K2) with different positions in the petri dish. (C) Boxplots of abdomen brightness with different positions in the petri dish. (D) Boxplots of leafdark colour with different positions in the petri dish.

Effects of size and colour variables on asymptotic temperature (AT)

None of the following variables had a significant effect on the AT: Abdomen area ($p = 0,57$; $T = 0,58$), leafdark colour ($p = 0,37$; $F = 0,81$), leaflight colour ($p = 0,39$; $F = 0,76$), cross colour ($p = 0,63$; $F = 0,23$). Only abdomen brightness had a significant effect on the asymptotic temperature of the spiders (Figure 49; $p = 0,037$; $F = 4,54$). Spiders with higher abdomen brightness (paler abdomens) have lower asymptotic temperatures ($p = 0,037$; $T = -2,13$). The anova outputs for the models with AT can be found in appendix IV, table 1.

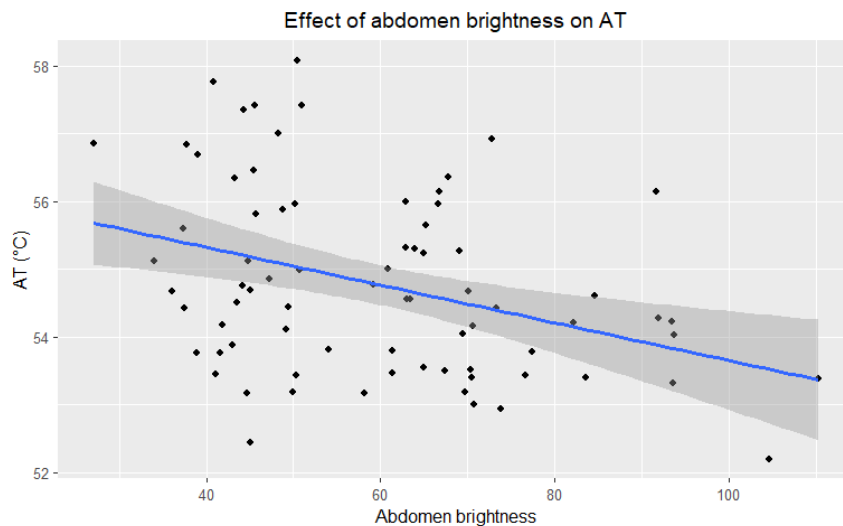


Fig 49: Figure showing the AT in relation to abdomen brightness. A significant negative effect was found of abdomen brightness against the asymptotic temperature (AT).

Effects of size and colour variables on heating slopes (K2)

I also analysed the effects of size and colour on the slope of heating of the spiders. Here, a bigger slope means faster heating of the spider, while a smaller slope means slower heating of the spider. Only cross colour had no significant effect on the K2 ($p = 0,074$; $F = 3,29$). The other variable did significantly affect the heating slope and will be explained now.

Figure 50 shows all significant effects of variables on the heating slope of the spider. The anova outputs for the models including K2 can be found in appendix IV, table 2.

Abdomen area had a significant effect on the heating slope of the spiders ($p = 1,72e-05$; $F = 21,26$). Spiders with a smaller abdomen will heat up faster than spiders with a bigger abdomen (Figure 50A; $p = 1,72e-05$; $T = -4,61$).

Abdomen brightness had a significant effect on the heating slope of the spiders ($p = 0,0025$; $F = 9,83$). Paler spiders will heat up slower than darker spiders (Figure 50 B; $p = 0,0025$; $T = -3,14$).

Leafdark colour had a significant effect on the heating slope of the spiders ($p = 0,0031$; $F = 9,34$). Spiders with paler leafdark colour will heat up slower than spiders with darker leafdark colour (Figure 50 C; $p = 0,0031$; $T = -3,06$).

Leaflight colour had a significant effect on the heating slope of the spiders ($p = 0,00087$; $F = 12,048$). Spiders with paler leafdark colour will heat up slower than spiders with darker leafdark colour (Figure 50 D; $p = 0,00087$; $T = -3,47$).

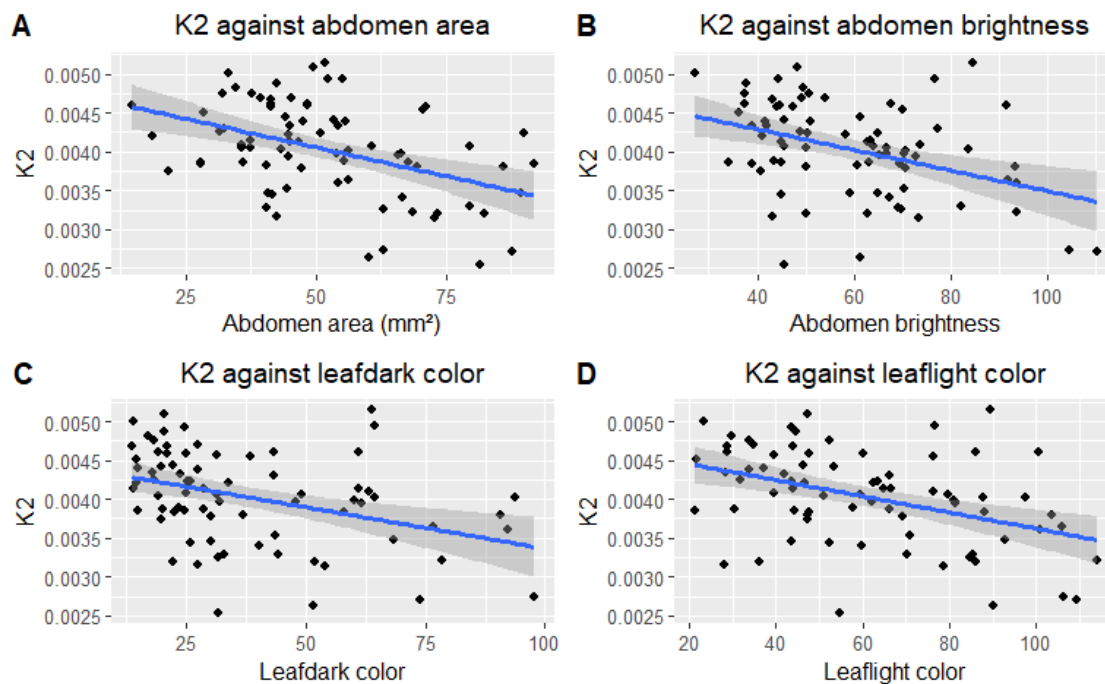


Fig 50: Plots showing the relationship between the heating slope of the spiders (K2) and different variables. (A) Plot of K2 against abdomen area. Abdomen area had a significant negative effect on K2. (B) Plot of K2 against abdomen brightness. Abdomen brightness had a significant negative effect on K2. (C) Plot of K2 against leafdark colour. Leafdark colour had a significant negative effect on K2. (D) Plot of K2 against leaflight colour. Leaflight colour had a significant negative effect on K2.

Results of the common garden experiment

A common garden experiment was conducted to test how spiderlings from mothers with different urbanisation background develop under different temperature (low/high) and food (low/high) regimes in a controlled setting. This way, I could potentially link effects of urbanisation found in the first part with results from this experiment. It also gives me a better idea of how these spiders are influenced by urbanisation during their development, since the high temperature/low food regime can be seen as an approximation of an urban setting. Lastly, it might also help me better understand how the combination of temperature and prey availability affect this spider.

Outputs of all Anovas (type 3, ddf = Satterthwaite) can be found in appendix V table 1 and 2.

The effects of urbanisation background on spiderlings in different treatments can be seen in figure 51. Urbanisation background had no significant effect on the end weight of the spiderlings in the different treatments, while food regime and temperature had a significant interacting effect on the end weight ($p_{3way\ interaction} = 0,15$; $F_{3way\ interaction} = 2,14$; $p_{food_regime:urbanisation} = 0,99$; $F_{food_regime:urbanisation} = 0,0001$; $p_{temp_regime:urbanisation} = 0,092$; $F_{temp_regime:urbanisation} = 2,87$; $p_{temp_regime:food_regime} = 0,008$; $F_{temp_regime:food_regime} = 7,19$; $p_{urbanisation} = 0,35$; $F_{urbanisation} = 0,94$; $p_{food_regime} = 6,23e-13$; $F_{food_regime} = 59,43$; $p_{temp_regime} = 0,0037$; $F_{temp_regime} = 8,603$). These effects and significance was similar in both datasets, the figures shown in this part are based on dataset 1, while results of dataset 2 are depicted in appendix V figures 1 and 2. Interacting effects of temperature and food regime will be shown in another plot below.

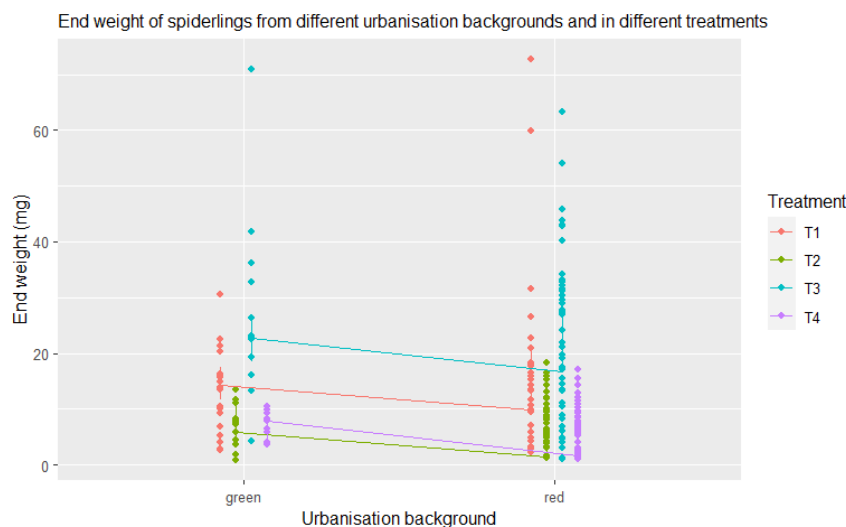


Fig 51: End weights of spiderlings from dataset 1, divided between urbanisation backgrounds and the treatment it was placed under. The lines connect predicted values of these weights. For the treatments: T1 = high food + low temperature, T2 = low food + low temperature, T3 = high food + high temperature, T4 = low food + high temperature.

Following figure (figure 52) explains better the interacting effect of temperature and food regime. Since the interaction between the two regimes is significant, the effect of food regime depends on the temperature regime and vice versa. High food has a general positive effect on spider weight, but this effect is much stronger in the high temperature treatments compared to the low temperature.

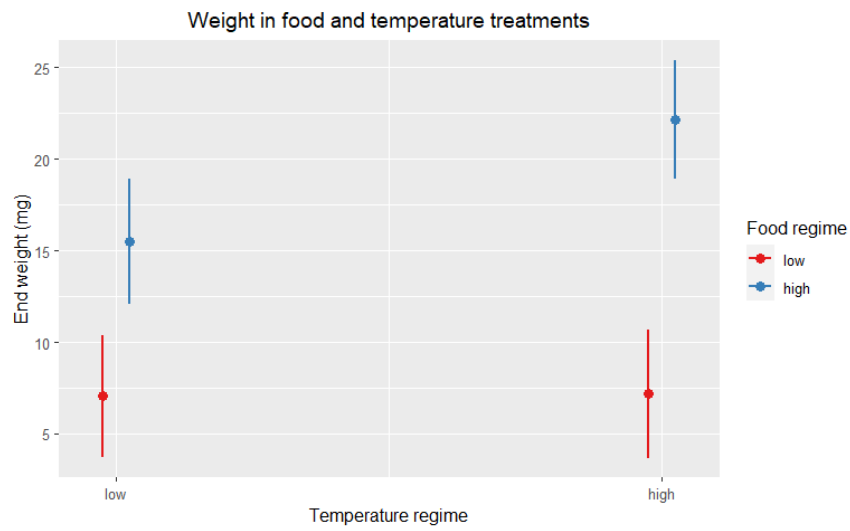


Fig 52: Figure showing the predicted values of the end weight of the spiderlings in different temperature and food regimes from dataset 1.

Discussion

In a first part of this thesis, I analysed whether spiders use their size or colour as adaptations towards urbanisation. Additionally, I also analysed microclimate variables to investigate whether spiders show microclimate selection and thermoregulation, and if these are also used as adaptations to urbanisation. Furthermore, I included a site selection experiment to investigate whether spiders will use background matching when choosing a retreat (hide). This way, I could potentially link observed colour variation with urbanisation to background matching. In order to verify possible urban adaptations in a controlled setting, I conducted a heating experiment in which the heating patterns of spiders with different size and colour were analysed. Lastly, I also wanted to link effects of temperature and prey availability on development of this spider in a controlled setup. These results could then again be linked back to the urban adaptation results from the first part.

Size as adaptation

I found strong evidence that spider size has an adaptive function in response to urbanisation. Abdomen size (area and length) decreased with increasing levels of local scale urbanisation in the field in green and yellow plots. This effect was also seen in the red plots but more towards the end of the sampling period. Abdomen size and spider length also increased with increasing levels of landscape scale urbanisation (but only in red plots; figure 15 and 16). Moreover, an increase in T_{local} is associated with a significant decrease in spider size in the field (table 4). In the heating experiment, I also confirmed that spider size influences their heating, as it was found that increasing abdomen area decreased the heating rate of the spider.

A significant interaction was found of collection date, local scale and landscape scale urbanisation for abdomen area and length (figure 15, 16 and 17). Since the interaction is significant, we cannot describe separate effects and therefore interaction effects will be described. Abdomen size decreased with increasing local scale urbanisation in the green and yellow plots, while in the red plots, this only happens towards the end of the sampling period. Local scale effects typically point towards local scale processes, such as prey availability (Dahirel et al., 2019), because spider adult size is also highly resource dependent (Mayntz et al., 2009). For example, another orb weaving spider *Nephila plumipes* reached bigger sizes in more urbanised areas of Sydney, Australia due to higher prey abundances (Lowe et al., 2014). Additionally, this result is also in line with evidence from Dahirel et al., (2019); they also found that spider size decreased with local scale urbanisation in a similar sampling design. For this particular sampling design, other results have already shown that prey numbers were similar with urbanisation (both scales), but prey biomass was shown to be lower in more urbanised areas (Dahirel et al., 2017). Furthermore, changes in size at the local scale might also indicate phenotypic plasticity, because spiders can disperse over smaller scales using ballooning (passive wind dispersion of juvenile spiders using a small silk thread, typical for a lot of araneids; Salmon & Horner, 1977) leading to full mixing of populations every year. As ballooning is expected to be a small scale process, genetic adaptation is less likely to happen (due to complete mixing). Another explanation could be the UHI effect, which leads to smaller sizes due to the temperature-size rule (Atkinson, 1994). This explanation is also supported by two other results from my thesis: Spider size decreased with temperature in the field, and decreasing abdomen area leads to a higher heating rate based on my heating experiment. Interestingly, spider size decreased both with increasing separate temperature variables (T_{spider} , T_{hub} , T_{local} and $T_{retreat}$) and also with increasing differences ($T_{spider}-T_{hub}$, $T_{spider}-T_{retreat}$ and $T_{spider}-T_{local}$). This clearly shows that in the field, smaller spiders will also reach higher temperatures. Lastly, smaller abdomen sizes also hints towards lower egg loads, which was also found by Dahirel et al., 2019.

Spider length increased with landscape scale urbanisation (in red plots), while abdomen sizes also increased the most in these plots. At larger scales, other processes might be more important, such as the UHI. While this is not completely correct (local scale UHI effects can also be found; Dahirel et al., 2017), it might be an indication of importance of UHI on larger

scales. However, smaller spiders are also expected to heat up faster (seen in heating experiment), so additional factors might play a role (such as prey availability, as explained before). Green subplots reaching the biggest sizes at the end of the sampling period could also be explained by a combination of higher temperatures (red plot) and higher food availability (green subplot). Interestingly, I also found evidence supporting this result in the common garden experiment. Here, I also found that spiders in the high food and high temperature treatment reached the highest weights. One paper suggested that body elongation in sun-exposed orb-web spiders results in better protection against heat stress, by allowing smaller areas of their body to sunlight (Ferreira-Sousa et al., 2021). This could also possibly explain spiders from my dataset also becoming longer (larger lengths) with urbanisation at landscape scale and having smaller abdomens at local scale.

All size variables increased with collection time, except for cross length proportion, which decreased. This can simply be explained by spiders reaching the end of their breeding season, which means that they will be bigger (adult size). It should also be emphasized that these graphs with time (figure 15 and 16) show different spiders, sampled at different times during the sampling period. For this reason, finding bigger spiders at the end of the season could also simply mean that the smaller spiders have already died, because they reach maturity earlier. Furthermore, cross length proportion decreased over time. This could simply mean that spiders increased in size, but the cross did not increase at a similar rate. White colour of the cross is produced by reflective prismatic guanine crystals encapsulated within vesicles located in the integument (Levy-Lior et al., 2010; Nentwig, 1987). Guanine is an excretory product of the nitrogen metabolism in spiders, and therefore the cross colouration and size can be linked to prey intake. However, Dahirel et al., (2019) did not find any effects of sampling date on spider size (cephalothorax width).

Colour as adaptation

Some evidence was found that colour is adaptive in response to urbanisation. Abdomen brightness became paler with increasing values of Thub – Tlocal. Leaflight colour also got paler with increasing Thub – Tlocal and Tretreat – Tlocal. However, both leafdark and leaflight colour got darker with increasing Tspider – Thub and Tspider – Tretreat. Lastly, cross colour also got darker with increasing Tspider, Tlocal, Thub and Tretreat. This effect was also observed in the lab: Paler spiders had lower asymptotic temperature (only abdomen brightness) and also heated up slower. Lastly, no evidence was found in the lab that spider colour is used for background matching.

Both abdomen brightness and leaflight colour became paler with increasing Thub – Tlocal or Tretreat – Tlocal. Therefore, it seems that paler spiders will select warmer hubs and retreat within their local environment. This result is also supported by my heating experiment, because there it was found that paler spiders reach lower ATs and heat up slower. This might mean that paler spiders are more advantaged in urbanised areas, as it is expected that temperatures will be higher there. Furthermore, leaflight and leafdark colour got darker with increasing Tspider – Thub and Tspider – Tretreat, which means that darker spiders will be warmer compared to the web hub and retreat. This can also be explained by darker spiders heating up faster than paler spiders. Lastly, cross colour also got darker with increasing Tspider, Tlocal, Thub and Tretreat. Again, spiders that have a darker cross will reach higher body temperatures.

Spider colour also got paler with time during the sampling period. Based on the correlations, we can say that bigger spiders are also paler, and bigger spiders also survived longer while smaller spiders die sooner. For this reason, it is expected to find bigger spiders (that are also paler) towards the end of the sampling period. Bigger spiders being paler is a phenomenon that has already been observed in previous and ongoing experiments (personal comm., Bram Vanthournout). This might also hint towards an important cost of colour, which increases when the spider is bigger. Bigger spiders were also more found in their retreat, which might be because they are less hungry and therefore less eager to stay in their web hub (more exposed, but reach prey faster; but see thesis Lukas De Jaegher).

Mixed effects were found for urbanisation effect on spider colour, since urbanisation (% built up area) had no effects, but temperature was shown to have an effect in the field. Moreover, colour also influenced their heating, as shown in the lab.

It is important to keep in mind that colour has many functions in animals, including thermoregulation and background matching, but also parasite protection, prey attraction and more (Stuart-Fox et al., 2017). Therefore, other selection pressures besides thermoregulation may play an even bigger role. This might explain why mixed effects were found, with no effect of percentage built area on spider colour. However, studies have proven that some arthropod species show different colouration in urban areas, especially related to urban melanism, which leads to darker individuals in the cities due to pollution (Grant & Wiseman, 2002; Popescu et al., 1978). One study has also partly proven that colour differences between urban and rural areas is a result of thermoregulation in common wasps (Badejo et al., 2020). Lastly, another study on thermoregulation in spiders found that white morphs of *Verrucosa arenata* were better protected against overheating, because they will heat up slower and cool down faster (Rao & Mendoza-Cuenca, 2016). Lastly, I also did not find any evidence for microclimate selection in these spiders, which could also be used as a way of thermoregulation and adaptation to urbanisation.

Microclimate selection as adaptation

Spiders show some microclimate selection, and it might also be used as adaptation for urbanisation. Paler spiders selected warmer hubs and retreats compared to darker spiders. Spider temperatures will be closer to their microclimate temperature (T_{local} , T_{hub} and $T_{retreat}$) with increasing local and landscape scale urbanisation. Therefore, proof of thermoregulation was also found in the field and it was shown to be adaptive to urbanisation. Additionally, I found proof in the field and in the lab that at least part of their thermoregulation is caused by spider size and colour. Lastly, I also found evidence for a temporal effect of the UHI.

Paler spiders selected warmer hubs and retreats compared to darker spiders. This was also supported by the heating experiment, that demonstrated that paler spiders heat up slower. One important remark is that areas with a suitable T_{local} might already be selected by the spiders, and that microclimate selection is happening on a bigger scale than the scale at which we analysed temperature. Quantifying T_{local} in areas that are close by, but lacking spiders could provide some more information on this possible mechanism. Then it would also make sense why we found very similar results for T_{local} , T_{hub} and $T_{retreat}$. Furthermore, evidence for thermoregulation was found, both in the field and in the lab. Both spider size and colour influence their temperature, as explained before. Interestingly, other research on spiders also found evidence for microclimate selection, such as sand recluses, which seem to select rocks with similar thermal conditions to hide under (Taucare-Rios et al., 2017).

I found evidence that thermoregulation (based on size and colour) is different with local scale urbanisation. $T_{spider} - T_{hub}$, $T_{spider} - T_{local}$ and $T_{spider} - T_{retreat}$ all decreased in the red subplots. This could be explained by higher temperatures in the red subplots, which makes that the spiders don't need to heat up as much to reach their optimal temperature. This is also somewhat confirmed based on the values of T_{local} , because mostly in the green and yellow plots (and early in the season), we see that red subplots indeed reach higher local temperatures than the green subplots. In addition, $T_{spider} - T_{retreat}$ and $T_{spider} - T_{retreat}$ also decreased at the landscape scale. The thermal preference of *Araneus diadematus* is between 22,5 °C and 24 °C (Napiórkowska et al., 2021). It is expected that these values are more easily reached through air temperature alone in the red subplots, where (especially in the early season) temperatures are higher. Alternatively, spiders might also use the orientation of their web for thermoregulation, as this will influence the amount of radiation to which the spider is exposed and thus its body temperature (Biere & Uetz, 1981). Besides web orientation, orientation of the spider towards the sun might also be important for thermoregulation. The golden-web spider *Nephila clavipes* responds to heat stress by pointing its abdomen in different angles towards the sun, thus leading to a system of evaporative cooling (Krakauer, 1972; Robinson & Robinson, 1974). Interestingly, these spiders are also very active during the night, which might also influence their thermoregulation and adaptations for urbanisation (thesis Nel D'haenekint).

For the separate microclimate variables (T_{spider} , T_{hub} , $T_{retreat}$ and T_{local}), a three way interaction effect was found between date, local urbanisation and landscape urbanisation. Firstly, all variables decreased with time, which is simply the result of temperatures decreasing at the end of summer. Furthermore, we can see a clear UHI effect in green and yellow

plots at local scale (based on Tspider and Tlocal; figure 28 and 29). This effect is also proven to be temporal, where the red subplots reach higher temperature values in the beginning, near the end of season this switches to green subplots being higher. For the red plots, we see a different pattern. Here, the green subplots will reach higher temperatures in the beginning, while at the end of the season both subplots reach similar temperatures. Moreover, clear urbanisation effects on temperatures were not found on the landscape scale. In general, it seems that the yellow plots reach highest temperatures, while the red and green plots have similar temperatures (appendix IIC, figure 8). Previous research with a similar hierarchical design of plots has already pointed out that local scale urbanisation shows a UHI effect, with urban subplots being on average 0,94 °C higher than rural subplots (Kaiser et al., 2016; but see also Merckx et al., 2018). My research adds an interesting temporal component to this evidence, showing that the UHI effect changes over time.

For the fieldwork, we always sampled spiders at random times of day (roughly between 9 am and 5 pm). This might have implications for the microclimate variables, as the temperature in subplot will be very different depending on the time of day. However, we tried to correct for this by calculating the temperature differences (i.e. Tspider – Tlocal, Tspider – Thub...). Additionally, it might also change whether the spiders are in retreat or not, because at the warmest hours of the day it is more expected that spiders will be in retreat. Thus, I propose for future research to either sample spiders around the same time of day, or include time of day in the mixed model as random effect. Furthermore, I only looked at temperature in thesis, but another interesting microclimatic variable could be humidity. Humidity has an effect both on the spider and its web, and we know that humidity decreases with urbanisation (Vollrath et al., 1997). Therefore, it might be interesting in the future to also include humidity in urbanisation research. We also looked at spiders that were at the end of the breeding season, but research on sub adult species might also be interesting, because earlier in the season I expect thermoregulation to be even more important (lower temperatures earlier in the season). When analysing spider colour in the lab, light intensity depending on the time of day might influence the colour calculation based on the photos. Future research should correct for this possible difference in light intensity, but only if it leads to differences in colour values. Lastly, certain characteristics that were quantified during the field work were not used in the analysis (local weather, wind, weather close to the spider), and future research should aim to include these extra variables in the analysis.

Site selection experiment

For the site selection experiment, only for day 4 effects were found of spider colour on the preference of background colour (abdomen brightness, leafdark colour and leaflight colour). Since no effects were found on the other days (with very high p values), I believe that this effect on day 4 was rather random than actual selection based on their colour. Background matching can be an important asset for spiders, especially for spiders that are ambush predators. One such spider is *Misumena vatia*, which is even known to change the colour of its full body depending on the background colour (Defrize et al., 2010; Théry, 2007). For these spiders background matching makes more sense, because their whole hunting strategy revolves around it. Interestingly, one paper suggested that *A. diadematus* are able to change the brightness of their opisthosoma, as a response to different light intensities. This might indicate background matching in this spider, but more when in their web hub and not in retreat (Blanke & Merklinger, 1983; Oxford & Gillespie, 1998). Additionally, it might also be interesting to investigate the effects of colour on prey capture rate in *Araneus diadematus*. From my study it seems that these spiders don't choose their retreat based on background matching.

Future research should try to include a more complex setup for the site selection, such as bigger containers, more background colours and also more colour variation among the different spiders.

Heating experiment

We can see that there is quite some variation in ATs and K2s between positions (due to non homogenous distribution of solar radiation from the light), which explains why position as a random factor in the mixed model is necessary for the analysis. Additionally, we can see that spider colour also differed across positions, with position 2 having darker spiders than the other positions. This may explain why the AT is way higher at position 2 compared to the other positions. I also

found that abdomen brightness has a negative effect on the AT of the spider, meaning that paler spiders will reach lower asymptotic temperatures. Yet, the biological meaning of this result might be less important, as these spiders will never reach these temperatures in nature. This can be seen from the results of field experiments, where values of T_{spider} ranged mostly between 20°C and 30 °C. Still, it is an interesting result as it shows that different abdomen colours have influence on the thermal conditions of these spiders.

Secondly, I found effects of abdomen area and abdomen colour on the slope of the spider's heating curve. Spiders with smaller abdomens will heat up faster than spiders with bigger abdomens. In addition, spider colour also plays an important role in how fast they heat up. Paler spiders will heat up slower than darker spiders. From these results, I can conclude that spider colour is important for both AT and heating rate, and size is only important for the heating rate. This is an interesting result, as it confirms patterns found in the field, such as smaller spiders reaching higher body temperatures or paler spiders selection warmer hubs and retreats.

Ideally, this experiment should be done with one spider at a time, this way the radiation from the light bulb is always the same. It might also be interesting to look at different life stages, which could explain differential importance of colour and size for thermoregulation depending on the life stage.

Common garden experiment

Because urbanisation background of the mother did not influence development in the experiment, phenotypic plasticity is expected to be an important driver of observed differences in development. Food also had a positive effect on end weight, but this way more pronounced when temperatures were also high. The effect of temperature regime thus depends on the food regime, and vice versa. If the food regime is low, then there is also no positive effect of high temperature. This is interesting, because high temperature and low food is what we expect in urbanised areas (Dahirel et al., 2019). Based on this result I can expect that spiderlings that are developing in urbanised areas (high temperature and low food) will reach lower weights. This could also explain why abdomen area and size decreased with local scale urbanisation. Furthermore, high food and high temperature is a treatment that is quite similar with the green subplots within the red plots. This might explain why in these subplots, spiders were longer based on the field analysis. Interestingly, urbanisation background of the mother had no influence in the development of the spiderlings in their respective treatments. I thus find no evidence of genetic adaptation on spider development. Since all spiders show a similar response regardless of urbanisation background, I can say that phenotypic plasticity is more important in explaining these effects than genetic adaptation.

In this experiment, we only looked at development during a period four weeks. Ideally, more variables should be included (such as size and colour) to further investigate genetic adaptations and phenotypic plasticity. We can for example compare colour and size of the mother with the spiderlings, to find out if these characteristics have a genetic basis.

Conclusion

The European garden spider shows certain adaptations in response to urbanisation at different scales. Spider abdomen area and length is smaller with local scale urbanisation. This effect is most likely due to the UHI effect or differences in prey availability between urban and rural areas. Spider abdomen size (area and length) and spider length increased with urbanisation at landscape scale (only in red plots), especially near the end of the sampling period. This is explained by both higher temperatures and higher prey availability, an effect that I also found in the common garden experiment. Lastly, spider size decreased with increasing temperature in the field, again showing possible size adaptation to urbanisation. Furthermore, spider colour can also be seen as an adaptation to urbanisation, with paler spiders selecting warmer web hubs and retreats. These results were also verified in a controlled setting in the lab. Some evidence was also found for microclimate selection, as paler spiders selected warmer web hubs and retreats. I can thus conclude that spider size and colour influence their thermoregulation. This thermoregulation also changed with urbanisation at the local scale. Spider body temperatures were closer to the air temperature in urbanised areas at the local scale. Lastly, bigger spiders were also found to be paler and more in their retreat. The former explained by higher costs of colour production and the latter explained by bigger spiders being less hungry.

Spiders do not use background matching when selection their retreat in a controlled setting. Therefore, this is not an important driver of spider colour.

In a controlled setting, it was found that spider size is important for their heating rate, and spider colour is important for both their asymptotic temperature (AT) and heating rate. These results were also confirmed in the field, and show that size and colour are important factors that might influence their thermoregulation.

Lastly, spiders show phenotypic plasticity when developing under different temperature and food regimes. Urbanisation background of the mother doesn't seem to be important in their development. In general, high food was always positive for the development.

Summary

Our world is increasingly becoming urbanised, negatively impacting natural ecosystems and causing rapid human-induced environmental change. Cities can be considered as novel environment, which have altered biotic and abiotic components both in space and time. These include higher temperatures (urban heat island), elevated noise and light pollution, but also higher habitat fragmentation and lower biodiversity. Only those species that manage to overcome these new challenges will survive and reproduce in urban areas, while others will perish. Species will undergo phenotypic changes between urban and rural environments, either through local adaptation or phenotypic plasticity, or a combination of both. One such adaptation could be through behavioural changes. In ectotherms, microclimate selection might be especially important, as they cannot regulate their own body temperature. Because of this, ectotherms might adapt to urbanisation through differential microclimate selection. Other important adaptations might be size and colour, which also strongly influence an individuals thermoregulation. Adaptations of *Araneus diadematus* to urbanisation have not been studied much. This thesis thus aims to investigate how this spider might adapt to urbanisation, and also to find proof for these adaptations with multiple experiments in a controlled setting.

In this thesis, I measured microclimatic temperatures of different spiders in a hierarchical sampling design in Flanders. This design consisted of two spatial scales, with 200 m² subplots nested in 1 km² plots. These plots and subplots also varied in their urbanisation degree. Additionally, these spiders were collected and phenotypic traits (size and colour) were also measured. I found evidence that spider size is used as an adaptation to urbanisation, both on the local scale (abdomen area and length) and on the landscape scale (spider length). I also found that smaller spiders will reach higher body temperatures. These results were also confirmed in the heating experiment. Colour was also found to be adaptive to urbanisation, as paler spiders select warmer web hubs and retreats compared to darker spiders. Darker spiders will also reach higher body temperatures, both results were again confirmed in the lab. Lastly, these results also show that spiders use microclimate selection, body size and colour as ways of thermoregulation. This thermoregulation also changed with local scale urbanisation, with urban spiders having body temperatures closer to the microclimate.

I included a site selection experiment in which I kept spiders in boxes with a pale and a dark leaf, and noted their position relative to the leaves for multiple days to analyse background matching as an alternative hypothesis. Here, I found no evidence that spiders use background matching based on the background colour and their own colour. This is evidence that background matching is not an important driver of spider colour.

Another part of this thesis consisted of a heating experiment, to find out whether spider size and colour influence their thermoregulation in a controlled setting. I heated spiders in groups of four and analysed heating patterns in terms of asymptotic temperature (mean of the last 30 temperature measurements) and slope of the heating (heating rate). Spider size had an effect on their heating rate (K2), while colour had an effect on both asymptotic temperature (AT) and K2. We find that both spider size and colour influences their heating.

In a last part of this thesis, I conducted a common garden experiment in which I reared spiderlings from different urbanisation settings in two different temperature and food regimes (high food, low food, high temperature, low temperature), and measured their weights as proxy of development. Urbanisation background did not influence development, while the interaction between temperature and food regime did. Food had a general positive effect, while this was most pronounced with higher temperatures.

Samenvatting

Onze wereld is sneller aan het urbaniseren, wat negatieve impacts heeft op natuurlijke ecosystemen en het leidt snel tot veranderingen in het milieu. Steden kunnen gezien worden als nieuwe omgevingen die veranderde abiotische en biotische componenten hebben in ruimte en tijd. Dit gaat over hogere temperaturen, verhoogde geluids- en lichtpollutie, maar ook meer habitatfragmentatie en lager biodiversiteit. Alleen de soorten die deze uitdagingen kunnen overwinnen zullen hier overleven en reproduceren, terwijl de andere zullen sterven. Soorten zijn fenotypische veranderingen ondergaan tussen urbane en rurale omgevingen, ofwel via lokale adaptatie of fenotypische plasticiteit, of een combinatie van beide. Een van deze adaptaties is verandering in gedrag. Bij ectothermen kan microklimaat selectie zelfs nog belangrijker zijn, doordat ze niet hun eigen lichaamstemperatuur kunnen regelen. Door deze reden kunnen ectothermen zich ook aanpassen aan urbanisatie via veranderingen in microklimaat selectie. Nog belangrijke adaptaties zijn grootte en kleur, die ook een invloed hebben op een individu zijn thermoregulatie. Aanpassingen van *Araneus diadematus* aan de stad zijn nog weinig onderzocht geweest. Voor deze reden wil ik in deze thesis onderzoeken hoe deze spin zich aanpast aan urbanisatie, en tracht ik bewijs te vinden van deze adaptaties via verschillende experimenten in een gecontroleerde omgeving.

In deze thesis heb ik microklimaat temperaturen gemeten van verschillende spinnen volgens een hiërarchische bemonsteringsdesign in Vlaanderen. Dit omvatte twee ruimtelijke schalen, met 200 m² "subplots" binnenin 1 km² "plots". Deze plots en subplots varieerden ook in hun urbanisatiegraad. Verder werden de spinnen ook verzameld en fenotypische kenmerken werden geanalyseerd (grootte en kleur). Ik heb bewijs gevonden grootte een adaptatie is aan urbanisatie, zowel op lokale schaal (abdomen omtrek en lengte) en op landschapsschaal (spin lengte). I vond ook dat kleinere spinnen hogere lichaamstemperaturen bereiken. Deze resultaten zijn ook bewezen in het opwarmingsexperiment. Kleur bleek ook als adaptatie gebruikt te worden voor urbanisatie, omdat lichtere spinnen warmere "web hubs" en "retreats" selecteerden dan donkere spinnen. Donkere spinnen werden ook warmer, en beide resultaten zijn opnieuw bewezen in het opwarmingsexperiment. Ten laatste tonen deze resultaten ook dat de spinnen microklimaat selectie, grootte en kleur als manieren van thermoregulatie. Deze thermoregulatie veranderde ook met urbanisatie op lokale schaal, waarbij urbane spinnen lichaamstemperaturen hebben die dicht bij hun microklimaat liggen.

Ik heb een experiment uitgevoerd dat over selectie van "retreat" ging waar spinnen werden gehouden in plasticen dozen met een donker en licht blad, hun plaatsen relatief tegenover de twee bladeren werden dagelijks opgeschreven voor meerdere dagen. Dit om camouflage als alternatieve hypothese te onderzoeken. Ik heb hier geen bewijs gevonden dat deze spinnen camouflage gebruiken op basis van de kleur van het blad en hun eigen kleur. Dit is bewijs dat camouflage geen belangrijke driver is van kleur bij deze spinnen.

Nog een onderdeel van deze thesis was een opwarmingsexperiment, om te onderzoeken of spin grootte en kleur een invloed heeft op hun thermoregulatie in een gecontroleerde omgeving. Ik heb spinnen opgewarmd in groepen van vier en heb hun opwarmingspatronen onderzocht op basis van hun asymptotische temperatuur (gemiddelde van de laatste 30 metingen) en helling van de opwarmingscurve (hoe snel ze opwarmen). Spinnengrootte had een effect op de snelheid van opwarmen (K₂), terwijl kleur een effect had op zowel de asymptotische temperatuur (AT) als op de K₂.

In een laatste onderdeel van deze thesis heb ik een "common garden experiment" uitgevoerd. Hier heb ik nakomelingen van moeders van verschillende urbanisatiegraden opgegroeid in verschillende temperatuur – en voedingsregimes (hoge of lage temperatuur en voeding), en hun gewicht gewogen dat de ontwikkeling beschrijft. Urbanisatie graad van de moeder had geen invloed op de resultaten, maar de interactie tussen voedsel en temperatuur had wel een invloed. Voedsel had een algemeen positieve invloed die het grootste was bij hoge temperatuur.

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Appendix I

General numbers and data

Table 1: Overview of the 27 plots visited for spider sampling.

PlotID	city	region	urbanisation level
P01	Ghent	Ghent	High
P02	Aalst	Ghent	High
P03	Oudenaarde	Ghent	High
P04	Bellem	Ghent	Intermediate
P05	Beervelde	Ghent	Intermediate
P06	Hillegem	Ghent	Intermediate
P07	Kalken	Ghent	Low
P08	Melsen	Ghent	Low
P09	Atembeke	Ghent	Low
P10	Antwerp	Antwerp	High
P11	Sint-Niklaas	Antwerp	High
P12	Mechelen	Antwerp	High
P13	Brasschaat	Antwerp	Intermediate
P14	Lint	Antwerp	Intermediate
P15	Ruisbroek	Antwerp	Intermediate
P16	Pulderbos	Antwerp	Low
P17	Herenthout	Antwerp	Low
P18	Bornem	Antwerp	Low
P19	Leuven	Louvain	High
P20	Brussel	Louvain	High
P21	Tienen	Louvain	High
P22	Wezemaal	Louvain	Intermediate
P23	Kortenbergh	Louvain	Intermediate
P24	Overijse	Louvain	Intermediate
P25	Houwaart	Louvain	Low
P26	Kerkom	Louvain	Low
P27	Sint-Joris-Weert	Louvain	Low

Table 2: Table of all data counts for the spider sampling, analysis and collection. The first column refers to the plot ID. The second column shows for how many spiders per plot the temperature measurements were taken. The third column shows how many spiders per plot were collected and analysed in terms of their size and colour. The last two columns depict how many egg sacs were laid and how many hatched.

PlotID	temperatures measured	Spiders collected + phenotypic measurements	egg sacs laid	egg sacs hatched
P01	16	25	16	13
P02	17	31	18	18
P03	18	27	11	10
P04	18	18	3	3
P05	16	16	4	4
P06	16	16	4	4
P07	12	12	3	3
P08	22	33	24	24
P09	15	28	14	14
P10	21	21	7	7
P11	17	28	14	13
P12	19	32	23	21
P13	17	17	5	5
P14	17	17	5	4
P15	19	19	3	2
P16	18	19	13	10
P17	19	26	16	13
P18	17	28	16	15
P19	17	28	9	5
P20	21	21	11	10
P21	18	28	13	13
P22	17	17	3	3
P23	16	16	3	2
P24	16	16	3	3
P25	17	28	11	11
P26	16	23	15	11
P27	21	36	18	15
Total	473	626	285	256

Table 3: All the spiders used in the heating experiment. 'Dark' was chosen as all spiders with an abdomen brightness under 65, and above being the light spiders.

	total	dark colour	light colour
spiders used	76	46	30

Appendix IIA

Urbanisation: size variables

Following tables show the output of each Anova (type 3, Satterthwaite method) for every mixed model of the spider size variables.

Table 1: Anova output of the mixed model which had abdomen area as dependent variable. Here is "plot" the landscape scale urbanisation, "subplot" the local scale urbanisation and "sdate" the scaled date.

variable	Sum Sq	Mean Sq	NumDF	denDF	F value	Pr(>F)
plot	1.0507	0.5253	2	28.039	3.2589	0.053389 .
subplot	1.5485	1.5485	1	31.438	9.6060	0.004069 **
sdate	17.0681	17.0681	1	59.837	105.8797	7.4e-15 ***
plot:subplot	0.1204	0.0602	2	31.437	0.3735	0.691365
plot:sdate	0.7336	0.3668	2	67.695	2.2754	0.110560
subplot:sdate	0.0055	0.0055	1	35.186	0.0341	0.854513
plot:subplot:sdate	1.8648	0.9324	2	35.367	5.7839	0.006710 **

Table 2: Anova output of the mixed model which had abdomen length as dependent variable. Here is "plot" the landscape scale urbanisation, "subplot" the local scale urbanisation and "sdate" the scaled date.

variable	Sum Sq	Mean Sq	NumDF	denDF	F value	Pr(>F)
plot	0.2768	0.1384	2	28.253	3.8796	0.032454 *
subplot	0.3694	0.3694	1	29.687	10.3566	0.003114 **
sdate	3.2889	3.2889	1	62.265	92.2034	6.697e-14 ***
plot:subplot	0.0301	0.0150	2	29.682	0.4214	0.659961
plot:sdate	0.1861	0.0930	2	71.001	2.6082	0.080715 .
subplot:sdate	0.0048	0.0048	1	33.469	0.1347	0.715870
plot:subplot:sdate	0.3457	0.1728	2	33.603	4.8453	0.014161 *

Table 3: Anova output of the mixed model which had spider length as dependent variable. Here is "plot" the landscape scale urbanisation, "subplot" the local scale urbanisation and "sdate" the scaled date.

variable	Sum Sq	Mean Sq	NumDF	denDF	F value	Pr(>F)
plot	0.21963	0.10982	2	27.432	5.1377	0.01273 *
subplot	0.08657	0.08657	1	27.715	4.0503	0.05397 .
sdate	1.14693	1.14693	1	57.953	53.6589	8.362e-10 ***
plot:subplot	0.01647	0.00824	2	27.705	0.3854	0.68379
plot:sdate	0.09321	0.04661	2	67.822	2.1804	0.12085
subplot:sdate	0.00006	0.00006	1	29.931	0.0030	0.95683
plot:subplot:sdate	0.14075	0.07037	2	29.996	3.2924	0.05097 .

Table 4: Anova output of the mixed model which had cross length proportion in the abdomen as dependent variable. Here is "plot" the landscape scale urbanisation, "subplot" the local scale urbanisation and "sdate" the scaled date.

variable	Sum Sq	Mean Sq	NumDF	denDF	F value	Pr(>F)
plot	0.004124	0.002062	2	49.839	0.4134	0.6636226
subplot	0.000168	0.000168	1	77.410	0.0336	0.8549873
sdate	0.071432	0.071432	1	86.973	14.3226	0.0002824 ***
plot:subplot	0.003776	0.001888	2	77.159	0.3785	0.6861403
plot:sdate	0.002806	0.001403	2	87.876	0.2813	0.7554942
subplot:sdate	0.002294	0.002294	1	90.804	0.4599	0.4993960
plot:subplot:sdate	0.006568	0.003284	2	91.783	0.6585	0.5200692

Table 5: Anova output of the mixed model which had cross width proportion in the abdomen as dependent variable. Here is "plot" the landscape scale urbanisation, "subplot" the local scale urbanisation and "sdate" the scaled date.

variable	Sum Sq	Mean Sq	NumDF	denDF	F value	Pr(>F)
plot	0.0021672	0.0010836	2	27.252	0.2018	0.8185
subplot	0.0006905	0.0006905	1	37.420	0.1286	0.7219
sdate	0.0049219	0.0049219	1	58.234	0.9166	0.3423
plot:subplot	0.0154722	0.0077361	2	37.459	1.4407	0.2496
plot:sdate	0.0062905	0.0031452	2	59.362	0.5857	0.5599
subplot:sdate	0.0025470	0.0025470	1	52.656	0.4743	0.4940
plot:subplot:sdate	0.0010227	0.0005113	2	53.325	0.0952	0.9093

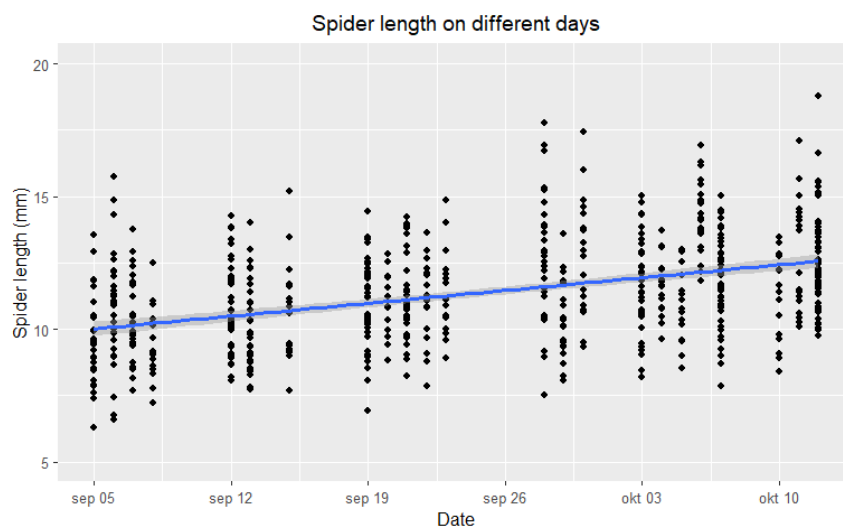


Fig 1: figure showing spider length on different days. A significant positive effect of date was found on spider length, meaning that bigger spiders were found at later dates.

Appendix IIB

Urbanisation: colour variables

Following tables show the output of each Anova (type 3, Satterthwaite method) for every mixed model of the spider colour variables.

Table 1: Anova output of the mixed model which abdomen brightness as dependent variable. Here is “plot” the landscape scale urbanisation, “subplot” the local scale urbanisation and “sdate” the scaled date.

variable	Sum Sq	Mean Sq	NumDF	denDF	F value	Pr(>F)
plot	0.00334	0.00167	2	25.121	0.0300	0.9704438
subplot	0.00024	0.00024	1	37.258	0.0043	0.9481774
sdate	0.93345	0.93345	1	54.608	16.8055	0.0001386 ***
plot:subplot	0.02285	0.01143	2	37.279	0.2057	0.8149768
plot:sdate	0.00130	0.00065	2	62.233	0.0117	0.9883762
subplot:sdate	0.00737	0.00737	1	41.293	0.1327	0.7174570
plot:subplot:sdate	0.17559	0.08779	2	41.692	1.5806	0.2179326

Table 2: Anova output of the mixed model which leafdark colour as dependent variable. Here is “plot” the landscape scale urbanisation, “subplot” the local scale urbanisation and “sdate” the scaled date.

variable	Sum Sq	Mean Sq	NumDF	denDF	F value	Pr(>F)
plot	0.31419	0.15709	2	24.126	0.7752	0.471756
subplot	0.12640	0.12640	1	34.571	0.6237	0.435046
sdate	2.66835	2.66835	1	51.356	13.1669	0.000656 ***
plot:subplot	0.02818	0.01409	2	34.564	0.0695	0.932971
plot:sdate	0.02852	0.01426	2	62.491	0.0704	0.932132
subplot:sdate	0.04539	0.04539	1	36.869	0.2240	0.638824
plot:subplot:sdate	0.33707	0.16853	2	36.936	0.8316	0.443327

Table 3: Anova output of the mixed model which leaflight colour as dependent variable. Here is “plot” the landscape scale urbanisation, “subplot” the local scale urbanisation and “sdate” the scaled date.

variable	Sum Sq	Mean Sq	NumDF	denDF	F value	Pr(>F)
plot	0.05684	0.02842	2	25.920	0.2077	0.81379
subplot	0.35447	0.35447	1	35.383	2.5905	0.11640
sdate	0.52554	0.52554	1	62.183	3.8406	0.05451 .
plot:subplot	0.09427	0.04714	2	35.364	0.3445	0.71095
plot:sdate	0.02448	0.01224	2	73.317	0.0895	0.91452
subplot:sdate	0.00575	0.00575	1	39.640	0.0420	0.83858
plot:subplot:sdate	0.18291	0.09146	2	39.743	0.6683	0.51822

Table 4: Anova output of the mixed model which cross colour as dependent variable. Here is "plot" the landscape scale urbanisation, "subplot" the local scale urbanisation and "sdate" the scaled date.

variable	Sum Sq	Mean Sq	NumDF	denDF	F value	Pr(>F)
plot	0.23906	0.11953	2	37.880	3.0682	0.05822 .
subplot	0.20785	0.20785	1	42.963	5.3351	0.02577 *
sdate	1.61699	1.61699	1	75.588	41.5058	9.838e-09 ***
plot:subplot	0.00353	0.00177	2	42.907	0.0454	0.95570
plot:sdate	0.04409	0.02204	2	76.426	0.5658	0.57024
subplot:sdate	0.00353	0.00353	1	63.770	0.0907	0.76427
plot:subplot:sdate	0.09377	0.04689	2	64.299	1.2035	0.30681

Appendix IIC

Urbanisation: microclimate variables

Following tables show the output of each Anova (type 3, Satterthwaite method) for every mixed model of the microclimate variables with urbanisation and date as independent variables.

Table 1: Anova output of the mixed model which Tspider as dependent variable. Here is "plot" the landscape scale urbanisation, "subplot" the local scale urbanisation and "sdate" the scaled date.

variable	Sum Sq	Mean Sq	NumDF	denDF	F value	Pr(>F)
plot	0.17745	0.08872	2	19.675	2.1097	0.14788
subplot	0.31035	0.31035	1	30.816	7.3795	0.01072 *
sdate	2.94027	2.94027	1	19.373	69.9138	7.456e-08 ***
plot:subplot	0.01392	0.00696	2	33.458	0.1655	0.84814
plot:sdate	0.02839	0.01420	2	19.337	0.3375	0.71765
subplot:sdate	0.08333	0.08333	1	18.678	1.9813	0.17567
plot:subplot:sdate	0.36365	0.18182	2	18.601	4.3234	0.02871 *

Table 2: Anova output of the mixed model which Thub as dependent variable. Here is "plot" the landscape scale urbanisation, "subplot" the local scale urbanisation and "sdate" the scaled date.

variable	Sum Sq	Mean Sq	NumDF	denDF	F value	Pr(>F)
plot	0.17937	0.08969	2	19.482	4.2539	0.02932 *
subplot	0.14915	0.14915	1	28.554	7.0741	0.01269 *
sdate	1.72230	1.72230	1	19.209	81.6894	2.382e-08 ***
plot:subplot	0.00425	0.00213	2	34.090	0.1008	0.90439
plot:sdate	0.03431	0.01715	2	19.168	0.8136	0.45798
subplot:sdate	0.00346	0.00346	1	17.608	0.1642	0.69019
plot:subplot:sdate	0.21155	0.10578	2	17.676	5.0170	0.01881 *

Table 3: Anova output of the mixed model which Tretreat as dependent variable. Here is "plot" the landscape scale urbanisation, "subplot" the local scale urbanisation and "sdate" the scaled date.

variable	Sum Sq	Mean Sq	NumDF	denDF	F value	Pr(>F)
plot	0.09499	0.04749	2	19.564	4.4083	0.02628 *
subplot	0.05674	0.05674	1	26.878	5.2664	0.02978 *
sdate	0.73873	0.73873	1	19.145	68.5692	9.451e-08 ***
plot:subplot	0.00611	0.00305	2	30.121	0.2835	0.75516
plot:sdate	0.02035	0.01017	2	19.095	0.9442	0.40641
subplot:sdate	0.00825	0.00825	1	15.739	0.7660	0.39461
plot:subplot:sdate	0.10281	0.05140	2	15.716	4.7713	0.02403 *

Table 4: Anova output of the mixed model which Tlocal as dependent variable. Here is "plot" the landscape scale urbanisation, "subplot" the local scale urbanisation and "sdate" the scaled date.

variable	Sum Sq	Mean Sq	NumDF	denDF	F value	Pr(>F)
plot	0.03878	0.01939	2	18.550	4.8417	0.020326 *
subplot	0.03987	0.03987	1	26.921	9.9553	0.003923 **
sdate	0.33741	0.33741	1	18.226	84.2534	2.947e-08 ***
plot:subplot	0.00324	0.00162	2	32.128	0.4051	0.670271
plot:sdate	0.01047	0.00524	2	18.177	1.3077	0.294706
subplot:sdate	0.00054	0.00054	1	17.659	0.1355	0.717126
plot:subplot:sdate	0.05021	0.02511	2	17.726	6.2693	0.008728 **

Table 5: Anova output of the mixed model which Thub - Tlocal as dependent variable. Here is "plot" the landscape scale urbanisation, "subplot" the local scale urbanisation and "sdate" the scaled date.

variable	Sum Sq	Mean Sq	NumDF	denDF	F value	Pr(>F)
plot	0.08413	0.04207	2	18.466	0.2762	0.76175
subplot	0.02827	0.02827	1	23.570	0.1856	0.67053
sdate	0.49283	0.49283	1	18.004	3.2357	0.08883 .
plot:subplot	0.26501	0.13251	2	23.751	0.8700	0.43189
plot:sdate	0.54960	0.27480	2	18.063	1.8042	0.19303
subplot:sdate	0.00354	0.00354	1	19.372	0.0232	0.88044
plot:subplot:sdate	0.09078	0.04539	2	19.406	0.2980	0.74563

Table 6: Anova output of the mixed model which Tretreat - Tlocal as dependent variable. Here is "plot" the landscape scale urbanisation, "subplot" the local scale urbanisation and "sdate" the scaled date.

variable	Sum Sq	Mean Sq	NumDF	denDF	F value	Pr(>F)
plot	0.40104	0.20052	2	18.372	0.6390	0.5392
subplot	0.19886	0.19886	1	22.138	0.6337	0.4345
sdate	0.00527	0.00527	1	18.466	0.0168	0.8983
plot:subplot	0.04687	0.02344	2	22.220	0.0747	0.9283
plot:sdate	1.27105	0.63552	2	18.461	2.0252	0.1603
subplot:sdate	0.29455	0.29455	1	20.407	0.9386	0.3440
plot:subplot:sdate	0.28515	0.14258	2	20.369	0.4543	0.6411

Table 7: Anova output of the mixed model which Tspider - Thub as dependent variable. Here is "plot" the landscape scale urbanisation, "subplot" the local scale urbanisation and "sdate" the scaled date.

variable	Sum Sq	Mean Sq	NumDF	denDF	F value	Pr(>F)
plot	0.60486	0.30243	2	21.003	2.2656	0.1285393
subplot	2.08253	2.08253	1	22.411	15.6011	0.0006626 ***
sdate	0.08132	0.08132	1	21.143	0.6092	0.4437470
plot:subplot	0.88683	0.44341	2	22.381	3.3218	0.0545431 .
plot:sdate	0.06057	0.03029	2	21.143	0.2269	0.7989241
subplot:sdate	0.50022	0.50022	1	20.041	3.7474	0.0671246 .
plot:subplot:sdate	0.46045	0.23023	2	19.838	1.7247	0.2038932

Table 8: Anova output of the mixed model which Tspider - Tlocal as dependent variable. Here is "plot" the landscape scale urbanisation, "subplot" the local scale urbanisation and "sdate" the scaled date.

variable	Sum Sq	Mean Sq	NumDF	denDF	F value	Pr(>F)
plot	0.26571	0.13286	2	21.264	0.8201	0.45383
subplot	0.98691	0.98691	1	22.767	6.0923	0.02153 *
sdate	0.00112	0.00112	1	21.336	0.0069	0.93450
plot:subplot	0.62577	0.31288	2	22.761	1.9315	0.16796
plot:sdate	0.09574	0.04787	2	21.334	0.2955	0.74715
subplot:sdate	0.22958	0.22958	1	19.700	1.4172	0.24801
plot:subplot:sdate	0.20493	0.10246	2	19.505	0.6325	0.54180

Table 9: Anova output of the mixed model which Tspider - Tretreat as dependent variable. Here is "plot" the landscape scale urbanisation, "subplot" the local scale urbanisation and "sdate" the scaled date.

variable	Sum Sq	Mean Sq	NumDF	denDF	F value	Pr(>F)
plot	0.68856	0.34428	2	21.032	2.2744	0.127577
subplot	1.85253	1.85253	1	20.019	12.2386	0.002261 **
sdate	0.02188	0.02188	1	21.467	0.1446	0.707523
plot:subplot	0.37534	0.18767	2	20.019	1.2398	0.310722
plot:sdate	0.08172	0.04086	2	21.462	0.2699	0.765986
subplot:sdate	0.46608	0.46608	1	18.619	3.0791	0.095745 .
plot:subplot:sdate	0.26557	0.13278	2	18.368	0.8772	0.432655

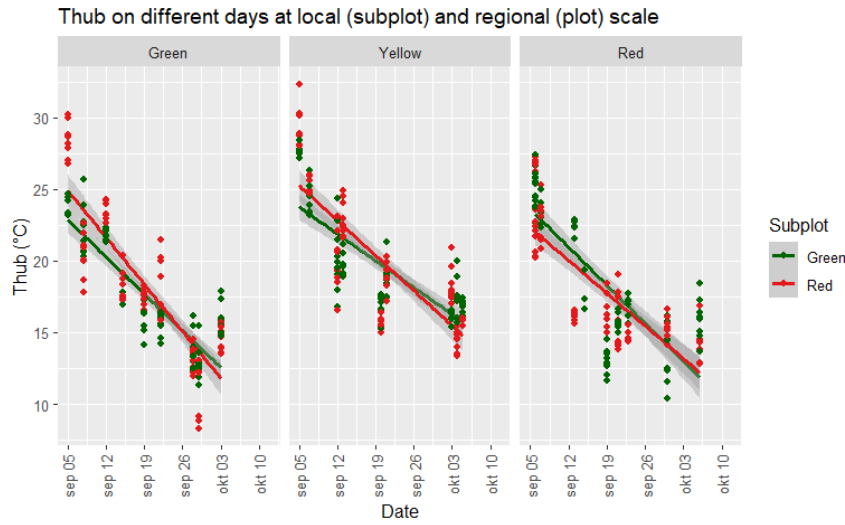


Fig 1: Three way interaction between date, subplot (local) urbanisation and plot (landscape) urbanisation against Thub. The three panel show different plots, while the red and green lines show the two different subplots.

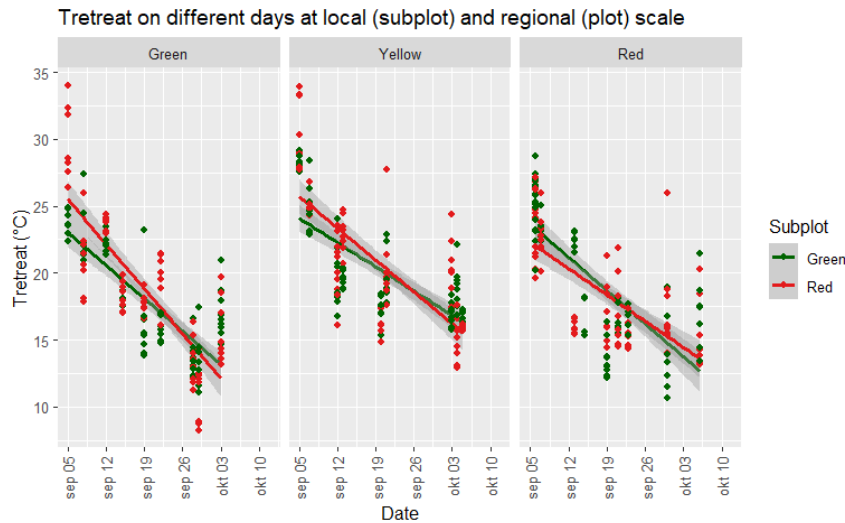


Fig 2: Three way interaction between date, subplot (local) urbanisation and plot (landscape) urbanisation against Tretreat. The three panel show different plots, while the red and green lines show the two different subplots.

Following tables show the output of each Anova (type 3, Satterthwaite method) for every mixed model where the size and colour variables are dependent variable and the microclimate variables are independent variables.

Table 10: Anova output of the mixed model with Tspider as independent variable, and each size and colour variable as dependent variable.

size variable	Sum Sq	Mean Sq	NumDF	denDF	F value	Pr(>F)
abdomen area	4.5458	4.5458	1	110.09	26.279	1.274e-06 ***
abdomen length	0.89447	0.89447	1	105.05	23.811	3.803e-06 ***
spider length	0.34601	0.34601	1	125.05	16.018	0.0001068 ***
cross length proportion	0.0038875	0.0038875	1	68.209	1.7825	0.1863
cross width proportion	0.00029827	0.00029827	1	52.191	0.0522	0.8202
colour variable						
abdomen brightness	0.17059	0.17059	1	76.179	3.0236	0.0861 .
leafdark colour	0.30932	0.30932	1	92.002	1.4754	0.2276
leaflight colour	0.026928	0.026928	1	60.889	0.1919	0.6629
cross colour	0.26885	0.26885	1	57.859	7.1006	0.009969 **

Table 11: Anova output of the mixed model with Tlocal as independent variable, and each size and colour variable as dependent variable.

size variable	Sum Sq	Mean Sq	NumDF	denDF	F value	Pr(>F)
abdomen area	3.1649	3.1649	1	57.884	17.891	8.432e-05 ***
abdomen length	0.61824	0.61824	1	56.004	16.107	0.0001795 ***
spider length	0.25158	0.25158	1	65.118	11.457	0.00121 **
cross length proportion	0.0034136	0.0034136	1	54.614	1.5652	0.2162
cross width proportion	0.0016571	0.0016571	1	37.261	0.29	0.5934
colour variable						
abdomen brightness	0.21354	0.21354	1	46.213	3.7834	0.05786 .
leafdark colour	0.11473	0.11473	1	56.454	0.5473	0.4625
leaflight colour	0.0055428	0.0055428	1	40.987	0.0395	0.8434
cross colour	0.28364	0.28364	1	40.188	7.4824	0.009228 **

Table 12: Anova output of the mixed model with Thub as independent variable, and each size and colour variable as dependent variable.

size variable	Sum Sq	Mean Sq	NumDF	denDF	F value	Pr(>F)
abdomen area	2.8225	2.8225	1	61.116	15.911	0.0001801 ***
abdomen length	0.53165	0.53165	1	59.049	13.816	0.0004502 ***
spider length	0.22391	0.22391	1	69.353	10.188	0.002125 **
cross length proportion	0.0013805	0.0013805	1	54.946	0.6317	0.4302
cross width proportion	9.1367e-05	9.1367e-05	1	38.904	0.016	0.9001
colour variable						
abdomen brightness	0.065835	0.065835	1	47.069	1.1676	0.2854
leafdark colour	0.0099384	0.0099384	1	56.705	0.0474	0.8285
leaflight colour	0.021932	0.021932	1	41.818	0.1565	0.6944
cross colour	0.25175	0.25175	1	41.183	6.6518	0.01357 *

Table 13: Anova output of the mixed model with Tretreat as independent variable, and each size and colour variable as dependent variable.

size variable	Sum Sq	Mean Sq	NumDF	denDF	F value	Pr(>F)
abdomen area	1.4897	1.4897	1	120.79	8.3448	0.004586 **
abdomen length	0.27939	0.27939	1	114.71	7.2261	0.008255 **
spider length	0.1461	0.1461	1	130.1	6.6147	0.01124 *
cross length proportion	0.0042495	0.0042495	1	69.544	1.9489	0.1671
cross width proportion	0.00029229	0.00029229	1	53.307	0.0511	0.822
colour variable						
abdomen brightness	0.015311	0.015311	1	81.794	0.2722	0.6033
leafdark colour	1.8132e-05	1.8132e-05	1	94.975	1,00E-04	0.9926
leaflight colour	0.066779	0.066779	1	61.146	0.477	0.4924
cross colour	0.18717	0.18717	1	60.666	4.9478	0.02986 *

Table 14: Anova output of the mixed model with Thub – Tlocal as independent variable, and each size and colour variable as dependent variable.

size variable	Sum Sq	Mean Sq	NumDF	denDF	F value	Pr(>F)
abdomen area	0.0053526	0.0053526	1	429.54	0.0302	0.862
abdomen length	0.0039296	0.0039296	1	431.18	0.1025	0.749
spider length	0.00049853	0.00049853	1	433.7	0.0226	0.8805
cross length proportion	0.0074072	0.0074072	1	438.61	3.4298	0.0647 .
cross width proportion	0.013297	0.013297	1	436.15	2.3258	0.128
colour variable						
abdomen brightness	0.26233	0.26233	1	441.55	4.7095	0.03053 *
leafdark colour	0.5001	0.5001	1	441.48	2.399	0.1221
leaflight colour	0.72763	0.72763	1	446.97	5.2281	0.02269 *
cross colour	0.00082049	0.00082049	1	444.05	0.0218	0.8827

Table 15: Anova output of the mixed model with Tretreat – Tlocal as independent variable, and each size and colour variable as dependent variable.

size variable	Sum Sq	Mean Sq	NumDF	denDF	F value	Pr(>F)
abdomen area	0.045653	0.045653	1	430.51	0.2582	0.6116
abdomen length	0.013613	0.013613	1	432.19	0.3555	0.5513
spider length	0.00075532	0.00075532	1	434.75	0.0343	0.8532
cross length proportion	0.00099767	0.00099767	1	437.25	0.4564	0.4997
cross width proportion	0.0020712	0.0020712	1	437.98	0.3615	0.548
colour variable						
abdomen brightness	0.20882	0.20882	1	443.2	3.735	0.05392 .
leafdark colour	0.3339	0.3339	1	442.26	1.5968	0.207
leaflight colour	0.6087	0.6087	1	446.94	4.3553	0.03746 *
cross colour	0.00060359	0.00060359	1	444.55	0.016	0.8993

Table 16: Anova output of the mixed model with Tspider – Thub as independent variable, and each size and colour variable as dependent variable.

size variable	Sum Sq	Mean Sq	NumDF	denDF	F value	Pr(>F)
abdomen area	1.9404	1.9404	1	446.55	11.378	0.000808 ***
abdomen length	0.41816	0.41816	1	447.7	11.332	0.0008274 ***
spider length	0.14047	0.14047	1	447.78	6.5413	0.01087 *
cross length proportion	0.0060026	0.0060026	1	356.38	2.7714	0.09684 .
cross width proportion	0.00042823	0.00042823	1	370.01	0.0749	0.7846
colour variable						
abdomen brightness	0.12359	0.12359	1	446.45	2.2089	0.1379
leafdark colour	1.0655	1.0655	1	445.76	5.1258	0.02405 *
leaflight colour	0.60695	0.60695	1	400.25	4.3552	0.03753 *
cross colour	0.008793	0.008793	1	440.27	0.2344	0.6285

Table 17: Anova output of the mixed model with Tspider – Tlocal as independent variable, and each size and colour variable as dependent variable.

size variable	Sum Sq	Mean Sq	NumDF	denDF	F value	Pr(>F)
abdomen area	1.344	1.344	1	443.72	7.7731	0.005531 **
abdomen length	0.27001	0.27001	1	444.83	7.1996	0.007564 **
spider length	0.096074	0.096074	1	443.98	4.4245	0.03599 *
cross length proportion	0.00037018	0.00037018	1	378.2	0.1694	0.6809
cross width proportion	0.0026145	0.0026145	1	388.76	0.4556	0.5001
colour variable						
abdomen brightness	2.9779e-05	2.9779e-05	1	447.34	5,00E-04	0.9817
leafdark colour	0.24197	0.24197	1	429.41	1.1565	0.2828
leaflight colour	0.03815	0.03815	1	419.08	0.2716	0.6025
cross colour	0.0042534	0.0042534	1	445.05	0.1131	0.7368

Table 18: Anova output of the mixed model with Tspider – Tretreat as independent variable, and each size and colour variable as dependent variable.

size variable	Sum Sq	Mean Sq	NumDF	denDF	F value	Pr(>F)
abdomen area	1.9066	1.9066	1	442.52	11.172	0.000901 ***
abdomen length	0.41218	0.41218	1	444.16	11.16	0.0009063 ***
spider length	0.11591	0.11591	1	446.21	5.3776	0.02085 *
cross length proportion	0.00014028	0.00014028	1	385.18	0.0642	0.8002
cross width proportion	1.0546e-05	1.0546e-05	1	396.08	0.0018	0.9658
colour variable						
abdomen brightness	0.21474	0.21474	1	450.45	3.8436	0.05055 .
leafdark colour	1.115	1.115	1	447.68	5.3564	0.0211 *
leaflight colour	0.91394	0.91394	1	419.93	6.5624	0.01076 *
cross colour	0.0078513	0.0078513	1	445.63	0.2092	0.6476

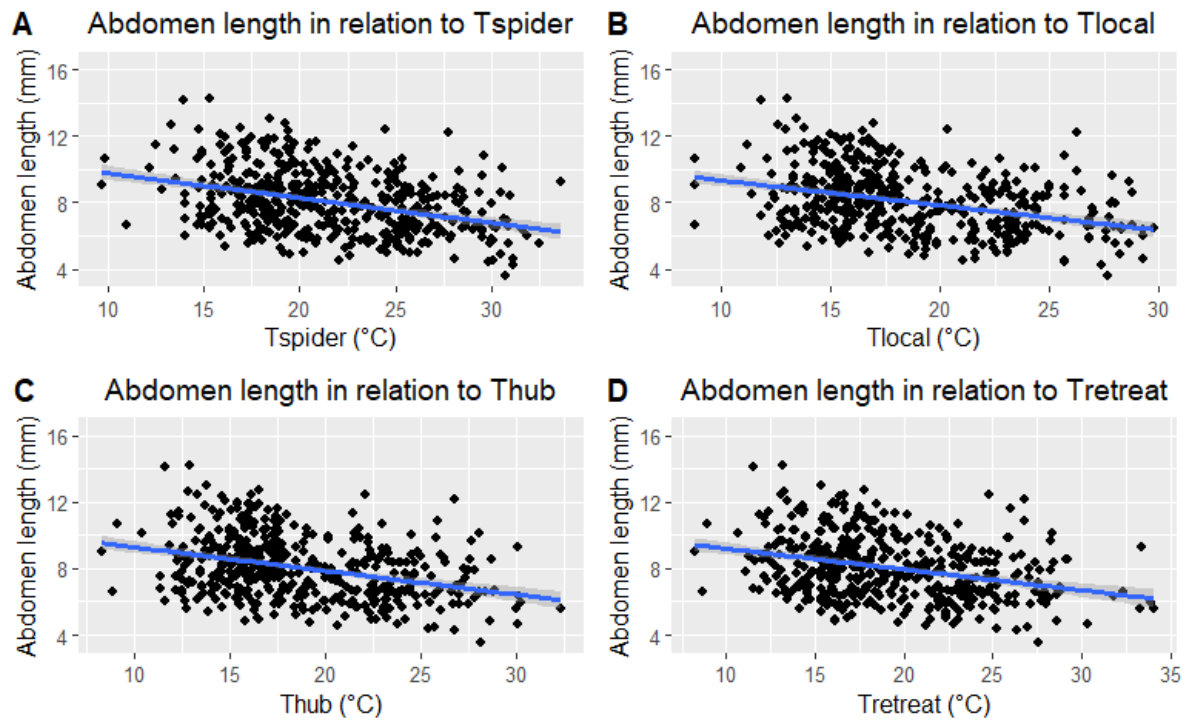


Fig 3: Abdomen length in relation to different microclimate variables. (A) Abdomen length in relation to Tspider. (B) Abdomen length in relation to Tlocal. (C) Abdomen length in relation to Thub. (D) Abdomen length in relation to Tretreat.

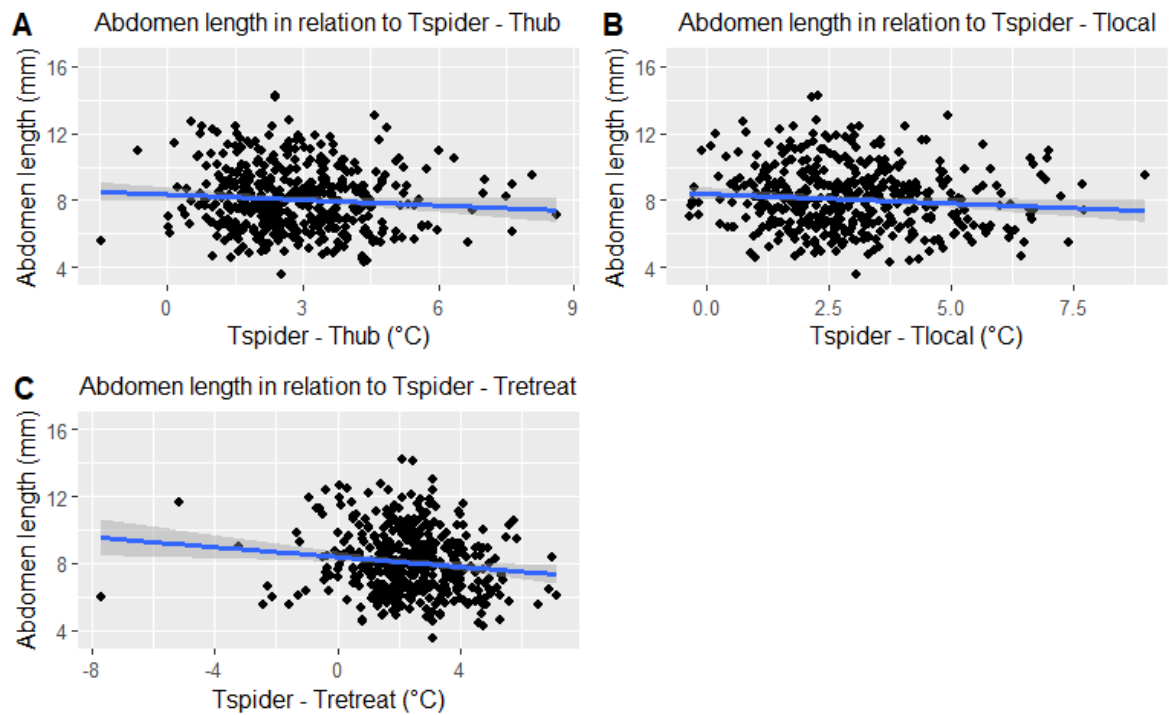


Fig 4: Abdomen length in relation to different microclimate variables. (A) Abdomen length in relation to Tspider - Thub. (B) Abdomen length in relation to Tspider - Tlocal. (C) Abdomen length in relation to Tspider - Tretreat.

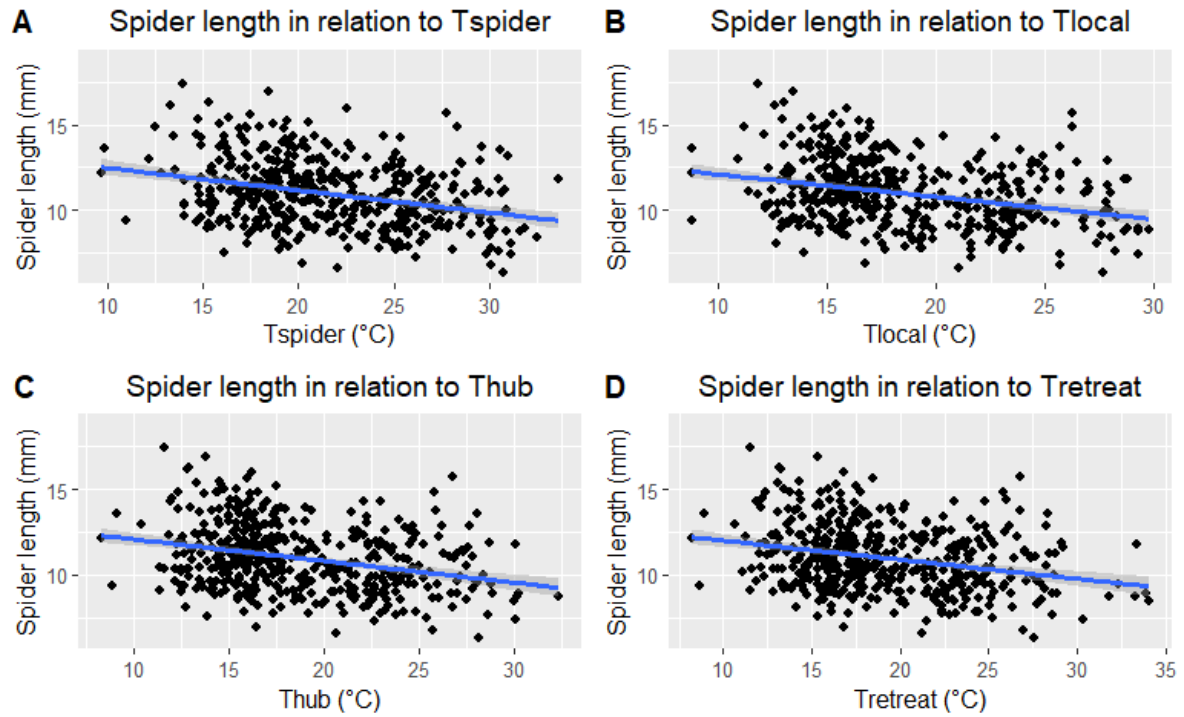


Fig 5: Spider length in relation to different microclimate variables. (A) Spider length in relation to Tspider. (B) Spider length in relation to Tlocal. (C) Spider length in relation to Thub. (D) Spider length in relation to Tretreat.

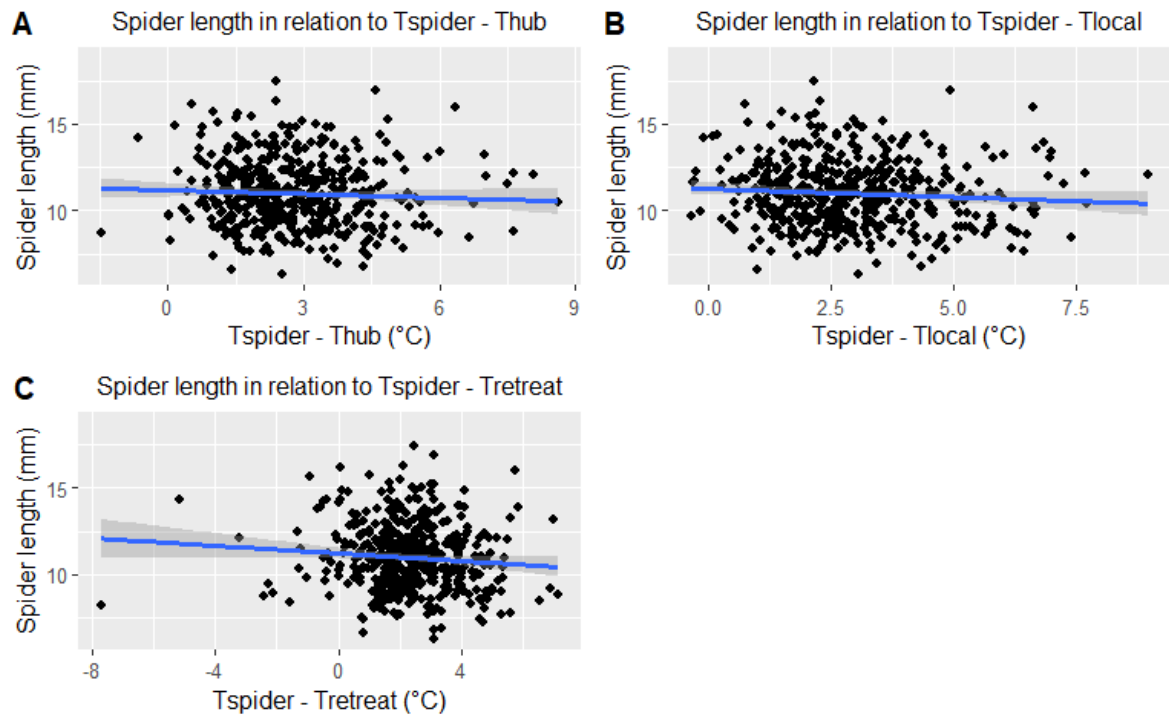


Fig 6: Spider length in relation to different microclimate variables. (A) Spider length in relation to Tspider - Thub. (B) Spider length in relation to Tspider - Tlocal. (C) Spider length in relation to Tspider - Tretreat.

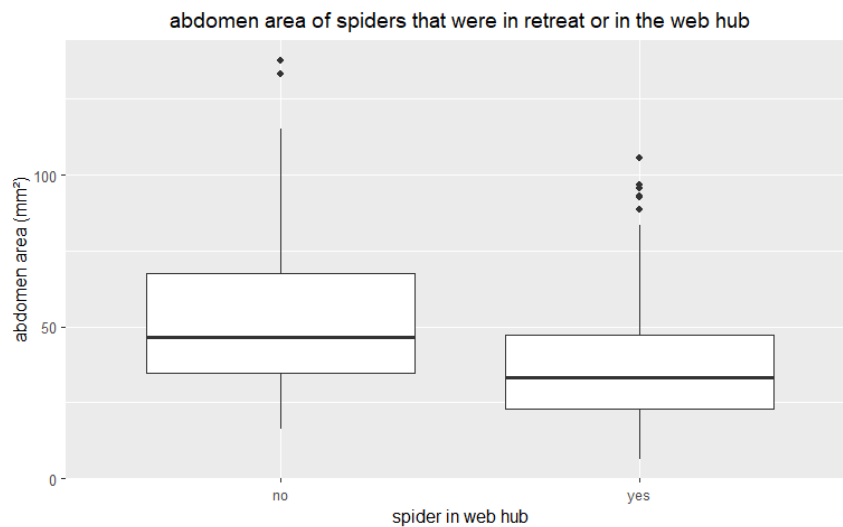


Fig 7: Abdomen area for spiders in retreat and in their hub. "yes" means spiders were in their web hub and "no" means spiders were in their retreat. This difference was found to be significant.

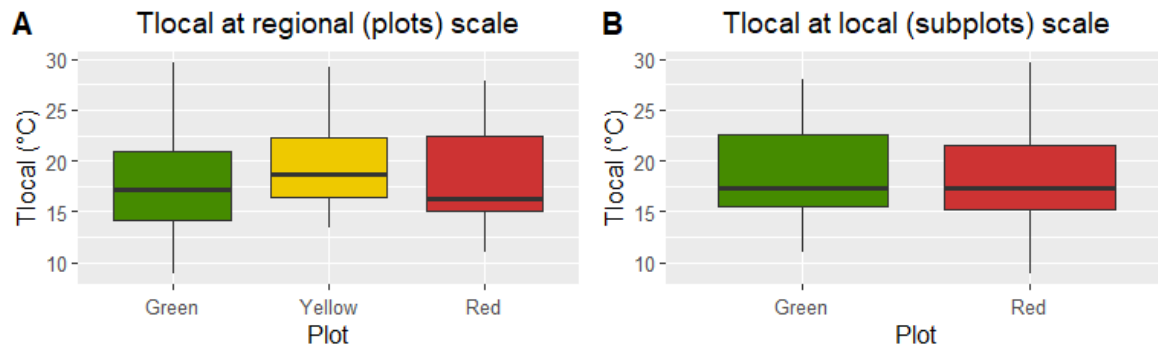


Fig 8: Tlocal at (A) landscape scale and (B) local scale. Only a significant difference was found between green and yellow plots.

Appendix III

Site selection experiment

Following tables show the output of each Anova (type 2) for every binomial regression on day 4 (only day that had significant effects).

Table 1: Output of the Anova type 2 on the binomial regression on day 4.

variable	LR Chisq	Df	Pr(>Chisq)
abdomen brightness	4.9588	1	0.02596 *
leafdark color	5.8893	1	0.01523 *
leaflight color	6.3142	1	0.01198 *
cross color	0.15382	1	0.6949

Appendix IV

Heating experiment

Following tables show the output of each Anova (type 3, Satterthwaite method) for every mixed model of the AT and K2 as dependent variable, and every size and colour variable as independent variable.

Table 1: Table showing the anova output of the mixed model with AT as dependent variable, and the colour and size variables as independent variable.

variable	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
abdomen area	0.26612	0.26612	1	71.018	0.3337	0.5653
abdomen brightness	3.4355	3.4355	1	71.481	4.5373	0.03661 *
leafdark color	0.64238	0.64238	1	71.491	0.8091	0.3714
leaflight color	0.59943	0.59943	1	71.357	0.7545	0.388
cross color	0.18854	0.18854	1	70.178	0.2327	0.631

Table 2: Table showing the anova output of the mixed model with K2 as dependent variable, and the colour and size variables as independent variable.

variable	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
abdomen area	5.0735e-06	5.0735e-06	1	71.082	21.264	1.721e-05 ***
abdomen brightness	2.7209e-06	2.7209e-06	1	73.565	9.8261	0.002472 **
leafdark color	2.6013e-06	2.6013e-06	1	73.626	9.3405	0.003122 **
leaflight color	3.2536e-06	3.2536e-06	1	73.177	12.048	0.0008733
cross color	8.9993e-07	8.9993e-07	1	70.934	3.2939	0.07377 .

Appendix V

Common garden experiment

Following tables show the output of the Anovas from the full mixed models that were used in the common garden experiment.

Table 1: Output of the anova with the end weights of the spiderlings from dataset 1 as dependent variable, and temp_regime, food_regime and mother_urbanisation as independent variables.

variable	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
temp_regime	819.5	819.5	1	203.00	8.6037	0.003741 **
food_regime	5660.2	5660.2	1	195.85	59.4279	6.234e-13 ***
urbanisation_mother	89.7	89.7	1	12.61	0.9418	0.350068
temp_regime:food_regime	684.4	684.4	1	196.18	7.1858	0.007974 **
temp_regime:urbanisation_mother	273.2	273.2	1	203.00	2.8689	0.091842 .
food_regime:urbanisation_mother	0.0	0.0	1	195.85	0.0001	0.993015
3 way interaction	203.4	203.4	1	196.18	2.1350	0.145568

Table 2: Output of the anova with the end weights of the spiderlings from dataset 2 as dependent variable, and temp_regime, food_regime and mother_urbanisation as independent variables.

variable	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
temp_regime	1695.7	1695.7	1	215.750	7.1468	0.008084 **
food_regime	8697.0	8697.0	1	214.467	36.6546	6.231e-09 ***
urbanisation_mother	9.3	9.3	1	7.576	0.0393	0.848134
temp_regime:food_regime	1288.7	1288.7	1	218.192	5.4316	0.020689 *
temp_regime:urbanisation_mother	0.4	0.4	1	215.750	0.0017	0.966808
food_regime:urbanisation_mother	2.9	2.9	1	214.467	0.0123	0.911938
3 way interaction	45.5	45.5	1	218.192	0.1917	0.661958

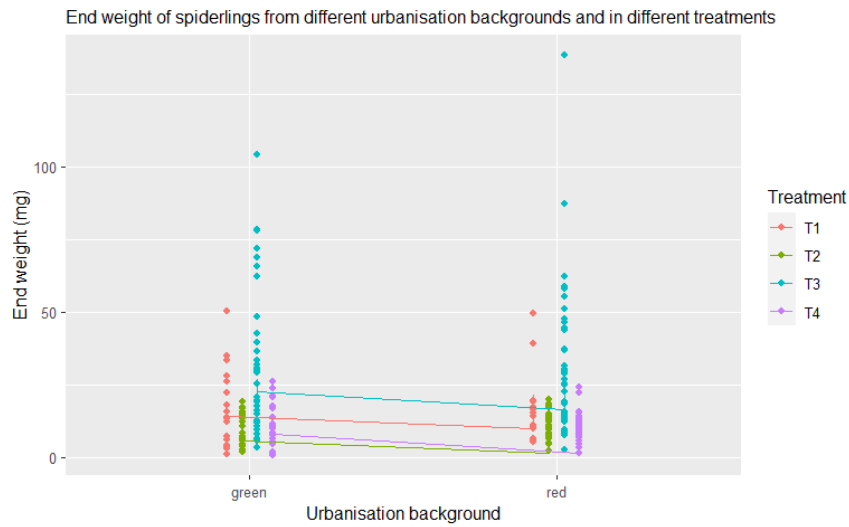


Fig 1: End weights of spiderlings from dataset 2, divided between urbanisation backgrounds and the treatment it was placed under. The lines connect predicted values of these weights based on the mixed model I ran. For the treatments: T1 = high food + low temperature, T2 = low food + low temperature, T3 = high food + high temperature, T4 = low food + high temperature.

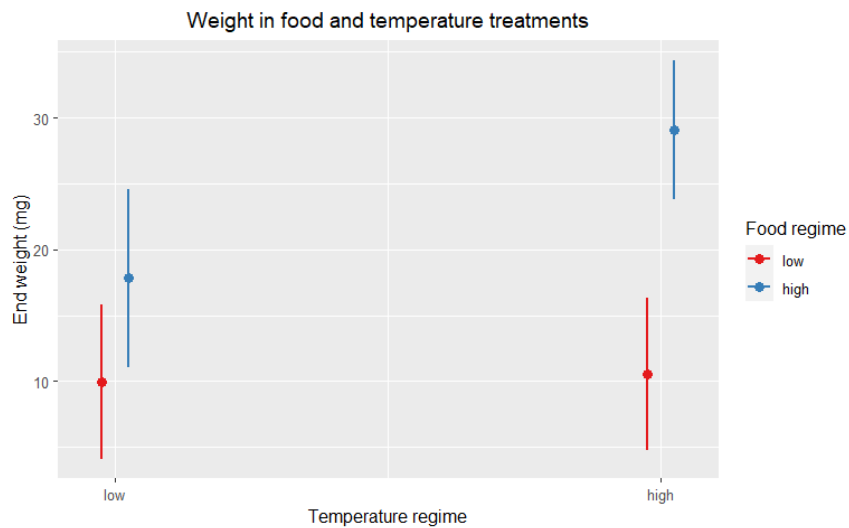


Fig 2: Figure showing the predicted values of the end weight of the spiderlings in different temperature and food regimes from dataset 2.