

THE CITY WEB: HOW THE PREY AVAILABILITY AND QUALITY AFFECTS SPIDER FORAGING BEHAVIOUR



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1 INTRODUCTION

1.1 IMPACT OF URBANIZATION

In 2018 an estimated 55.3 % of the global population lived in urban settlements. By 2050 It's expected that 1 out of 3 people will live in an urbanized city with at least 500 000 inhabitants (United Nations Population Division 2018, 2015), meanwhile the built-up area will increase threefold (Angel et al. 2011). Urbanization changes our environment and is considered one of the most important processes defining biologic patterns and processes that maintain life (McKinney 2008). This process incurs change by altering the eco-evolutionary processes by not only directly and indirectly impacting the actors, but the stage as well (Hutchinson 1967, Alberti 2015, Parris 2016). This gave rise to an increase in research trying to uncover the unknown underlying patterns and processes associated with urbanization, yet many aspects of its impact remains misunderstood. Nevertheless, humans have always had an impact on ecosystems, however the speed, intensity and scale at which urbanization currently occurs is unprecedented. Not only that but the impact will only further increase in the future (Ellis et al. 2013, Ellis 2015, Bai et al. 2017). Moreover urbanization contributes to global change and is considered one of the most dramatic forms of human-induced rapid environmental change (HIREC) (Parris 2016, Sih et al. 2013).

The impact of HIREC is capable of altering entire ecosystems to the point at which prevailing historical conditions are transformed into ecosystems with novel biotic and abiotic characteristics. The aforementioned differ substantially in structure and/or function Urban areas are a good example of such novel ecosystem induced by HIREC, with urbanization as largest contributor (Evers et al. 2018, Hobbs et al. 2009, Theodorou 2022). Cities are characterized by replacement and fragmentation of vegetation by buildings and roads, which decreases connectivity. Furthermore high heat retaining materials such as concrete and asphalt are used, creating impervious surfaces, which disrupts evapotranspiration. Additionally, increased heat retention contributed to the introduction of man-made composite materials (i.e. concrete and asphalt) combined with disrupted evapotranspiration contribute significantly to the urban heat island effect. Resulting in higher temperatures in urban environments compared to their rural counterparts (Liu et al. 2016, McDonald et al. 2008, Bai et al. 2017, Dahirel et al. 2018). Urban environments are highly altered by the presence of large human activity. The impact of human presence is often characterized by intense and frequent disturbance of habitats likely accompanied by increased chemical, sound and light pollution. (Dahirel et al. 2018, Parris 2016, Theodorou 2022).

Altered food availability is an important determining component of urban communities. A paper published by Seress et al. in 2020 conducted on *Parus major* theorized that urban-dwelling animals' reproductive performance is limited by the reduced availability of high-quality food sources food. Other studies conducted on birds (Jones et al. 2012, De Satgé et al 2019) observed similar results of decreased reproductive success associated with lowered abundance of high-quality food as an important contributing factor in urban environments. Conversely anthropogenic food sources are abundant and can be a large portion of the animals' diet. However these food sources are of much lower nutritional quality. Furthermore, numerous studies have found a link between anthropogenic food sources and adverse effects on the physiology, increase risk-prone behaviour and reproductive success of an animal. The combined impact of reduced natural food availability and increased anthropogenic food availability have been well studied birds and a general pattern of lower reproductive success in urban environments is observed, although its extent remains

unclear (Seress et al. 2020, Jones et al. 2012, De Satgé et al. 2019, Theodorou 2022). Contrary to mammals and birds, orb-weaver spiders are fully dependent on insect abundance due to their inability to feed on anthropogenic food sources (Penick et al. 2015).

1.2 ADAPTATION TO URBANIZATION

HIREC (which includes urbanization) is accompanied by the exertion of new selective pressures on species. This alteration of processes and patterns and modification of the pre-existing environment in environments subjected to urbanization acts as a filter on biodiversity (Seto et al. 2012). Biological adaptations are the species' response to these new selective pressures. Adaptations can take place at morphological, physiological, behavioural and molecular level to increase fitness in altered environments. Adaptation are often accompanied by trade-offs, usually manifested as lowered fitness in other environments. A maladaptation results in an ecological trap, causing further reduction of survival rate. Biological adaptation is seen as the driving force of evolution (Wong & Candolin 2014, Zhang & Luo 2014, Sih et al. 2013). Even though decreased survival rates coinciding with lowered fitness are commonly observed caused by maladaptation and/or inability to adapt. Nevertheless well-adapted species are able to thrive in new conditions (Trimmer et al. 2019, Theodorou 2022). This difference in biological responses is contributed to the interaction a plethora of dependencies. Where both species- and spatial dependence are regarding as 2 major contributing giving shape to certain outcome of selection. The outcome of urbanization is among other species-, place- and scale dependent, which can result in variation in biological responses. Deeming urbanization a complex multifaceted research topic, where the impact can easily be missed, when not considering these factors (Theodorou 2022, Bai et al. 2017, Alberti 2015).

For animals behavioural change is considered the first step towards adaptation to rapid environmental changes. Behavioural plasticity plays a crucial role in mitigating the initial impact of HIREC. (Wong & Candolin, 2015, Dahirel et al. 2018, Sih et al. 2013). A number of studies have documented behavioural changes in response to HIREC, although the potential cost and constraints regarding the changes remain poorly understood. It is possible that behaviour is linked to metabolic and physiological processes that directly impact fitness (Dahirel et al 2017, Bonte et al. 2011). A review published in 2020 by Ritzel & Gallo on the effect of urbanization on behaviour of mammals concluded that mammals clearly respond to urban environment by altering behaviour. These behavioural changes include harm avoidance or decreased flight initiation distance in urban birds and this behaviour can develop within seconds to hours. This in contrast to acclimation which develops gradually over days and weeks. Increased boldness and neophilia may indicate more permanent developmental response which can potentially lead to evolutionary change. Similar results for boldness were observed for lizards present in urban environments (Baxter-Gilbert et al. 2019). For spiders (which includes this research' species) a link between presence of artificial light and local increase in abundance of predatory species and their corresponding prey has been observed (Davies et al. 2013) by building webs near artificial light and so altering their foraging behaviour and their corresponding foraging success (Willmott et al. 2019, Heiling 1999). Another study conducted in 2009 by Mayntz, Toft & Vollrath showed that when spiders experience lowered prey biomass, changes in web building behaviour are observed. This response consists of increasing the capture area of the web, which leads to increased foraging efficiency and compensates for the low abundance of prey. Additionally, alterations in web-building behaviour in European Garden spiders in relation to urbanization have been observed. With the reduction

in web mesh at local scale being a central component (Dahirel et al. 2018). Nonetheless, the effect of urbanization on foraging behaviour in urban-dwelling spider species remains under sampled.

Alternatively adaptations to new environments can be achieved by an alteration of the phenotype. A well-documented example is the change of colour polymorph in *Biston betularia* due to the onset of the industrial revolution in the United Kingdom. Soot pollution on birch trees acted as a new selective pressure which induced a shift from the light to dark polymorph (Cook & Saccheri 2012). Other studies have shown a decrease in body size in arthropods in relation to urbanization, more specifically by the heat-island effect (Merckx et al. 2018, Dahirel et al. 2018, Dahirel et al. 2017). The associated increase in temperature results in increased metabolic activity and costs. Resulting in decreased body size as trade-off. Body size is intrinsically linked to metabolic rate and life-history traits, deeming it a crucial determinant of food webs and community dynamics. Greatly affecting current and future ecological interactions. Where future communities' body size will further decrease in response to global warming (Merckx et al. 2018, Dahirel et al. 2018).

1.3 PREDATORY BEHAVIOUR OF ARANEAE

A wide range of predatory behaviour is found within the Order Araneae. Some of the main strategies involve: actively pursuing, cautiously stalking and ambushing, using a web and even prey attraction and aggressive mimicry. Spiders have fangs which are used to insert venom. This incapacitates the prey and liquifies the internal structures enabling its consumption (Cohen 1995). The use of venom grants advantages, but is costly, spiders will assess this trade-off and respond accordingly (Uetz 1989, Uetz 1992, Cooper et al. 2015).

Orb-weaver spiders (Araneidae) such as *A. diadematus* are passive hunters specialized in the utilisation of an orb web to catch prey (Lee & Thomas, 2002). The typical orb web consists of a flat wheel of stiff radial threads, which connect in a central point (= hub; Zschokke 1999). The radial threads are overlaid by a spiral of elastic and sticky threads suspended freely in vegetation. When the spider is not present in the hub, it takes shelter in a retreat (a built hiding place in the foliage around the web). The direction of illumination combined with gravity provides a general compass direction. This gives them the required local and global cues to construct their web (Vollrath & Selden 2007, Vollrath 1992, Dahirel et al, 2017). The web of most araneid spiders is asymmetric, where the lower part is larger (Mayer 1952, Witt & Reed 1965). The webs are recycled and rebuilt daily, allowing spiders to match currently/recently experienced environmental conditions (Witt & Reed 1964). As a consequence of passive hunting, an individual's growth rate is dependent on the abundance (encounter rate), quality (nutritional value) and size of prey. (Li 2005, Wilder et al 2011). Additionally, decreased quality, quantity and size of prey can result in body size reduction in orb-weaver spiders (Higgins & Goodnight 2011). Vibrational cues are received as result of prey caught in the web, thus these cues are dependent on the prey's movement. Therefore, web plucking, an alternative method to receive information on both prey and condition of the web. Which is done by actively pulling the web, receiving information in vibrational waves that reflected off prey. As such circumventing the dependence on the prey's input. This behaviour is only expressed, when necessary. This behaviour is not specific for Araneidae and can also be part of courtship behaviour. (Mortimer et al. 2019, Robinson & Mirick 1971)

1.4 EUROPEAN GARDEN SPIDER

Araneus diadematus Clerck 1757 is a orb-weaver spider of the Araneidae, commonly found in the Holarctic realm (Lee & Thomas 2002; Nentwig, Blick, Gloor, Hänggi & Kropf 2016). They are among the most common species found in both rural and urban environments, deeming it a perfect species to conduct research on the effect of urbanization. Confirmed by results on changes in web-building behaviour in urban populations (Dahirel et al. 2017, Dahirel et al. 2018). Spiders are ectothermic organisms, therefore the prevailing environment (e.g. weather, temperature) impacts an ectotherm's metabolic activity and consequently change its behaviour accordingly (Kearney et al. 2009). On the dorsal side of the abdomen, a leaf-like structure with a white cross is present. This is one of the important characteristics to determine the species, which makes it easy to recognize. Variation in cross size and colour is present (Oxford & Gillespie 1998). The orb-webs are commonly found in bushes and herbaceous vegetation (Lee & Thomas 2002). The spider can be found in the centre of the web during both day and night (D'haenekint 2021). It is a polyphagous species where the largest component of its diet consists of Diptera, aphids and hymenopterans (Bonte & Nyffeler 2020, Ludy 2007). Female individuals mature in late summer and can survive until late fall. Around this time they will lay egg sacs once they have mated and will eventually die (Foelix, 1982). The spiderlings will hatch in early spring. At first they remain clumped together but eventually will disperse by ballooning. Ballooning is a passive method of dispersing by using a silk strand as airborne sail. Thus, eliminating ability of habitat choice due to lack of control of movement while airborne. Long distance dispersal events achieved by ballooning are considered rare, therefore limiting gene flow at large scale. (Dahirel et al. 2018, Bonte et al. 2011, Edelaar & Bolnick 2012) The spiderlings mature in mid to late summer (Lee & Thomas 2002, Bonte et al. 2011). The spiders have an annual or biennial life cycle, where the latter mostly occurs in northerly regions (Johannesen & Toft 2002).

1.5 OBJECTIVES

Previous studies on *A. diadematus* mainly focussed on sexual behaviour (Roggenbuck et al. 2011, Fischer et al. 2021) and web building behaviour (Zschokke 2011, Hesselberg et al. 2004, Dahirel et al. 2018), but never on spiders' foraging behaviour in relation to urbanization. The research analyses the difference in behaviour to a prey stimulus between rural and urban population. It is expected that urban individuals will exhibit more voracious behaviour as result of urbanization-induced decrease in quality, quantity and size of prey. Voracious behaviour is characterized by faster reaction times. Consequently, assuming that urban individuals are more probable of expressing voracious behaviour. Furthermore, spider body size and web size are examined. Which are both expected to decrease with increasing urbanization degree. Lastly, data on spider development is obtained from common-garden experiment simulating urban and rural conditions using a combination of temperature and food regimes. This will be combined with information on the degree of urbanization the mother was exposed to. Enabling this research to determine the contribution of phenotypic plasticity and/or genetic effects to the adaptive potential in response to urbanization. The acquired results will serve as reference for other orb-weaver species. Behavioural adaptations to urbanization are understudied in spiders and more generally Arthropods. Although recent studies conducted on *A. diadematus* have proven altered web-building behaviour along with size reduction regarding urbanization (Dahirel et al. 2017, Dahirel et al. 2018). Yet, with the prospect of further increased urbanization, the demand for information on biological responses among all urban-dwelling taxa is required to insure further conservation of biodiversity. Flanders is regarded among the most urbanized and densely populated regions in Europe, acting as an ideal frame of reference in regards the impact of urbanization on biodiversity (United Nations Population Division 2018). Moreover, the framework in which the process of urbanization functions has yet to be fully understood. This research contributes to insights on behavioural change as response to urbanization. Understanding these complex eco-evolutionary dynamics of urbanization will grant the possibility for future sustainable strategies required for conservation of biodiversity (Palkovacs & Post 2009).

1.6 HYPOTHESES

Previous studies have shown the impact of urbanization-induced heat-island effect on body size of many Arthropod species. Furthermore, conclusive evidence indicating a trifold decrease in insect quality, quantity and size in urbanized environments altering the functional response. Hence urban environments exert new selective pressures on spiders, which lack capability to exploit anthropogenic food sources. Leading to this research' proposed change in behaviour as response to the effect of urbanization on the prey population. It is expected that the effects of urbanization on prey populations result in increased the voracity and risk proneness due to malnourishment experienced by urban populations. Previous studies have shown adaptative capability of *A. diadematus* in web-building behaviour regarding urbanization (Dahirel et al. 2017, Dahirel et al. 2018, Bonte et al. 2008). However, this research focusses on changes in foraging behaviour. Specifically, the increase of voracious behaviour in urban populations. Observed as decreased time until attacking prey stimulus. This will be tested on regional and local scale . Where, in alignment with other studies (Dahirel et al 2018), it is assumed that local scale urbanization has greater contribution in shaping behavioural changes compared to regional scale. As the effects of urbanization should be more pronounced at local scales (Dahirel et al. 2018, Dahirel et al. 2017, Merckx et al. 2018)

Alternatively the low abundance of large prey items could resulted in urban individuals to be inexperienced in handling them. Hence, a possibility of observing more risk-prone behaviour as a consequence of lower quantity of high-risk prey interactions.

The second part focusses on differences in development between populations exposed to varying degrees of urbanization. Urban spiders are expected to be more accustomed to urban environments such as higher temperatures and lower prey availability and are expected to experience a better growth compared to rural individuals raised in common garden setting. This setting consists of predetermined conditions, which is assumed for simulating key aspects of the environmental pressure (under which temperature and diet) exerted by urbanization. Furthermore, we hypothesize that the body size of urban individuals will be smaller than rural individuals as a result of urbanization induced heat island effect. Again expecting greater contribution to size reduction at local scale urbanization.

- **It is expected that individuals of *A. diadematus* present in urban environments will react quicker to prey stimuli (i.e. vibrations) than individuals in rural conditions due to decreased quantity, quality and size of prey as result of urbanization.**
- **It is expected that individuals originating from urban areas have decreased body size (i.e. length) in comparison to their rural counterparts due to the urban-heat island effect.**
- **It is expected that spiderlings from urban mothers have enhanced development in urban conditions (high temperature and low food regime). Conversely spiderlings from rural mothers have enhanced development to rural conditions (moderate temperature and high food regime).**
- **It is expected that phenotypic plasticity contributes more to adaptation in developmental traits compared to maternal genetic effects.**

2 METHODOLOGY

2.1 FIELD WORK

2.1.1 SAMPLING

The sampling design was defined according to the Spatial and environmental determinants of Eco-Evolutionary Dynamics (SPEEDY) project. The strength of this design is the hierarchical structure that allows to study urbanization over multiple sites across Flanders. Urbanization is determined by calculating the percentage of build-up cover (BUC) (Merckx et al. 2008, Dahirel et al. 2017, Piano et al. 2017). Urbanization is thus defined by the proxy BUC calculated at different scales. Thus the SPEEDY set-up implements 2 spatial scales: large (3x3 km) and small (200x200 m). The smaller spatial scale is nested within the larger. The BUC values result in 3 categories: low (green; < 3% BUC), intermediate (yellow; 5 – 10 % BUC) and high (red; >10% BUC). The categories are nested within the spatial scales. In total 27 different plots were chosen across Flanders, specifically East-Flanders, Antwerp and Flemish Brabant. Each plot consist of 2 sampling areas. Resulting in total of 54 sampling sites. Each region contains 9 sites with different urbanization levels and spatial scales. Figure 1 visualizes the sampling design according to SPEEDY.

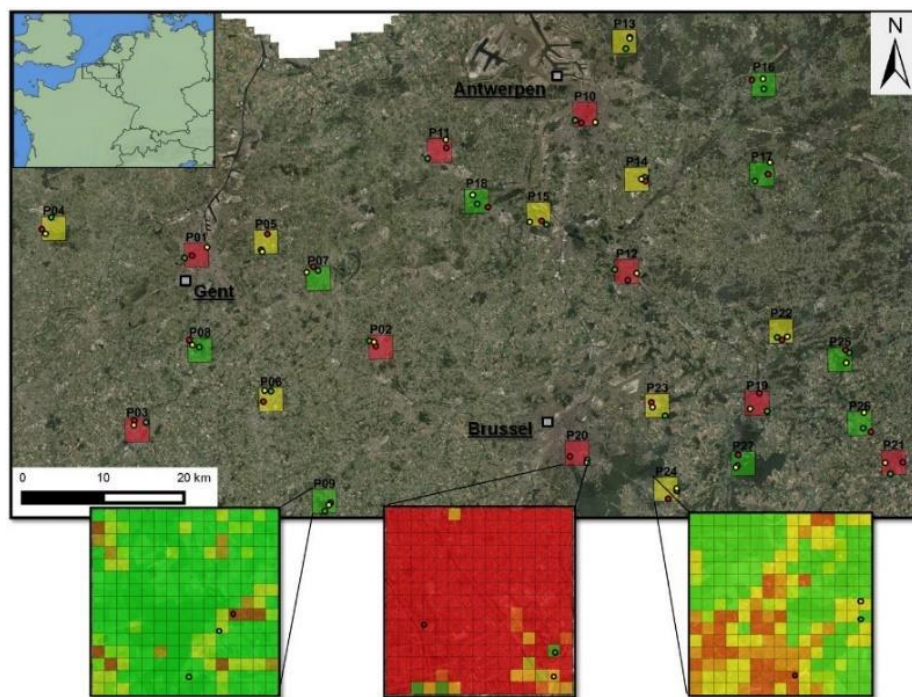


Figure 1| Map of the study area (Northern Belgium) depicting the locations of the sample plots (27) with corresponding degree of urbanization (green; low, yellow; intermediate, red; high) at regional scale (3x3km). Subplots are nested within each plot. The lower part of the figure depicts the degree of urbanization at local scale (200x200m). For each plot, 2 subplots (green; low and red; high) were sampled. Resulting in a total of 54 sites sampled. Urbanization was quantified as percentage built-up cover. (Piano et al. 2017)

2.1.2 DATA COLLECTION

During a period of roughly one month (05/09/2022 - 06/10/2022) 54 different sites (Appendix 1) were visited. Every subsequent day of sampling the locations alternated by region and degree of urbanization. By doing this, we approached a random sampling strategy. The objective for each site was collecting a minimum of 8 spiders, where preferably 5 spiders were present in their web hub and the other 3 in their retreat. At some sites additional spiders were collected for rearing. Conversely, at some sites the preferable amount was not found due to a lack of individuals or less representation in either web hub or retreat. On location we started searching for individuals. When a spider was found, reaction time to prey stimulus was sampled. Followed by temperature measurements of the abdomen, web hub and local environment.

Firstly, the reaction time to prey stimuli was measured. This was only collected if the individual was present in the web hub. To measure behavioural response of the spider to prey stimuli, an electric toothbrush was used to mimic vibration by flying insects caught in the web. Therefore, we attached a tie-wrap on the toothbrush using tape (Figure 2). The area below the web hub is bigger, as a consequence of the asymmetric architecture of orb webs. Therefore, the prey stimulus was introduced centrally in the lower half of the web (Figure 3). The behaviour was filmed using a go-pro (GoPro hero 10). A chronometer was used to determine how long it took until the spider attacked the stimulus. Timing the reaction was stopped when 120 seconds was reached. This ensured quicker detection of the stimulus and doesn't require reorientation of the spider (Rhisiart & Vollrath 1994). Consequently increasing prey-handling efficiency and indirectly reliability of the observations (Landolf and Barth 1996). The vertical length of that part of the web was measured.



Figure 2| Electric toothbrush with tie-wrap to simulate prey stimulus

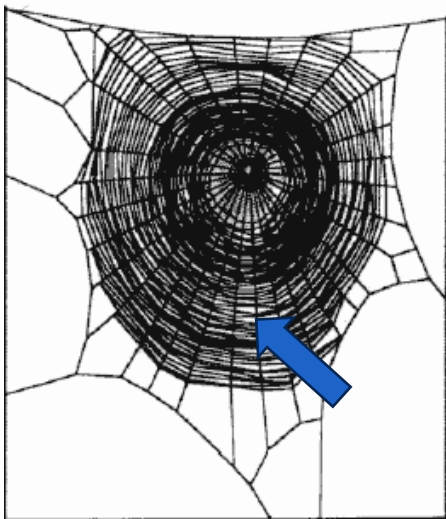


Figure 3| Spider located in web hub. The arrow indicates the point where the prey stimulus is introduced. (Zschokke 1996)

Furthermore, temperatures were measured with a Thermocouple type T (Omega, T-type 36 gauge thermocouple; 5SC-TT-TI-36-204 2M; accuracy = $\pm 1^\circ\text{C}$), which accurately measured temperature of small areas (e.g. abdomen of an individual). During measurements tweezers were utilized to avoid influence of body temperature (Figure 4), which could have led to incorrect data. The spider was captured and fixated in a plastic tube. The lid of the tube was modified with mesh. This was done to ensure a reliable measurement was carried out without inference of the spider's movement (Figure 5). Three repeated measurements of spider surface temperature in the leaf structure (darker part of the abdomen), web hub and local temperatures were performed to reduce variation between the measurements. In my thesis we will only use the local and abdomen temperatures. The other temperatures were collected to acquire information about microclimate selection as a way to reduced heat stress in urban areas (results are presented in the master thesis of Thomas Roels). Spiders were labelled and collected for rearing and breeding purposes. At the lab each individual was photographed. Using these photos spider size was later measurement by means of the proxy body length in the computer program ImageJ (Schneider et al. 2012). All data was collected in an excel file.



Figure 4| Tweezers were used to reduce interference of body temperature



Figure 5| Body surface temperature measured in the leaf structure. Spider was fixated against mesh.

When the initial field work was completed, some locations were revisited and extra individuals collected without measuring temperature and prey capture behaviour. This was done to ensure that enough females were available for the breeding experiment. This was due to the fact that, sampling at the beginning of September could result in unmated females despite being adult. Only the sites (n=15) with strongest contrast in urbanization degree were visited, i.e. most urbanized and rural sites at local and regional scale.

2.1.3 VIDEO ANALYSIS

During video assessment, behaviour was divided in 3 categories using time intervals (Table 1). *Immediate* reaction was assigned when an individual attacked the stimulus within 3 seconds. The *flee* category was used for spiders that fled or exceeded 120 seconds. Everything in between the aforementioned categories is regarded as *assess*. The time measurements acquired in the field with a chronometer were verified by watching the video and if necessary amended to the most correct time estimation. To ensure reliable categorisation, a trial period took place. In which random videos were chosen and categorized. This was done on multiple dates. Afterwards a comparison indicated no big discrepancies. Therefore, further analysis of remaining videos was justified. All the data was added to an Excel file. Later, a second analysis of the videos was done, because the accuracy of time was not desirable. The second analysis, Filmora (Wondershare, Version 12.0.9), a computer program was used. This enabled slowing down and editing the videos. During first video analysis, there were more than 3 categories. This was reduced to 3. This was done to ensure that the observations were grouped better (i.e. previous categories were added under a broader definition). In total, 280 videos were categorised consisting mostly of immediate reaction (Table 1, second column). Cropped videos consisting of only the reaction of a certain individual were saved. This was done in case videos needed a further reanalysis.

Categories	Amount	Time interval
immediate	195	0-3 sec
assess	40	3-120 sec
flee	45	> 120 sec or flight
Total	280	

Table 1| Overview of categories. The second column (*Amount*) represents how many times a certain behaviour is observed. The categories were divided according to time intervals. They consist of immediate (within 3s), assess (between 3 and 120 sec) and flee (flight response or over 120 s).

2.2 SPIDER HUSBANDRY AND REARING

Adult *A. diadematus* females were collected during sampling in the field during the period where it was most probable that the females had already mated, but not yet laid their egg sacs. The females were housed in separate enclosures in the lab. An enclosure consisted of a petri dish and a plastic cup. Three moist cotton-wool circles were placed inside the petri dish. This ensured proper humidity. A hole at the top of the plastic cup allowed feeding and watering. This hole was closed off with cotton-wool. Watering was done with spray bottles filled with tap water. The amount of moisture in the cotton-wool was used as indicator. To aid the spiders with web building, the inside of the cups were scratched with a boxcutter and wooden sticks were added, to grant anchoring points. The latter were later replaced by plastic straws due to the presence of mould on the wooden sticks as a result of the moist environment. Recently collected individual were fed houseflies (*Musca domestica*). After the spiders got accustomed to the lab environment, a more regular feeding schedule was incorporated (3x a week). The spiders were kept in the lab at a constant 21 °C until all individuals were collected. After which temperature was gradually reduced (to mimic natural conditions) to stimulate egg laying. To ensure successful hatching *A. diadematus* eggs need a cold period of at least 4 weeks (Canard, 1984). Afterwards temperatures were increased in a stepwise manner to 20 °C according to regime shown in Table 2.

6 weeks at 5°C
1 week at 15°C
1 week at 18°C
20°C until hatching and conditions during first life stages

Table 2| overview of temperature regime required for egg hatching. Where for a number of weeks a particular temperature was imposed to ensure successful hatching.

Starting at the end of November the first females started laying their egg sacs. Females typically die within some days after egg laying. The presence of a possible egg sac was checked daily. The eggs sacs remained in the enclosure after the female was removed. The egg sacs need a high humidity, hence the cotton was drenched in water. Females that laid eggs were collected in Eppendorf tubes in the freezer at -20°C, for possible later analysis.

From February 14 to February 28, the eggs hatched. Initially the spiderlings were fed with pollen due to their inability to capture the much larger prey (Figure 6). When the spiderlings reached larger sizes they were fed with fruit flies (*Drosophila melanogaster*). Initially, the spiderlings were given an excess of fruit flies. Followed by a feeding regime of 2 x 3 flies. Ensuring enough nutrition to aid in their growth. At predetermined dates, spiderlings that weren't needed for further experiments were killed off, put in 2ml Eppendorf tubes with 100% ethanol and put in the freezer at -20°C. Spiderlings that were selected for further rearing, had 10 individuals collected in 2ml 100% ethanol filled Eppendorf tubes as a means of reference for the initial size. After a few weeks, selected spiderlings were put in a



Figure 6| Recently hatched spiderlings in a plastic cup enclosure.

separate enclosures to prevent cannibalism. For each clutch, around 50 individuals were rehoused. This was done for 18 females, amounting in around 1000 spiderlings. The enclosures consisted of in rectangular cup with plastic straws. A hole in the lid was also present and closed off with cotton-wool. When all spiderlings of interest were housed, the common-garden experiment started. Urbanization is simulated by the combination of temperature and food regimes. Urbanized environments are characterized by low food regime and high temperature. Conversely, high food regime associated with low temperatures are considered rural conditions. Furthermore, two additional combinations were assigned. A treatment with high temperature regime and low food regime. Following that a treatment with low temperature regime along with high food regime. The different treatment are shown in table 3. Treatments were uniformly allocated to spiderlings from each mother.

	Feeding regime	Feeding regime
Temperature	High x High	High x Low
Temperature	Low x High	Low x Low

Table 3 | overview different food and temperature regime regarding common-garden experiment. Feeding regimes could weekly be high (12x (cfr. unlimited amount of fruit flies)) or low (4 fruit flies / week). Temperature regimes could be by high (24°C) or low (20°C).

To evaluate growth between the juvenile spiders from different locations, 2 measurements of weight at different dates were carried out. Individuals were selected after 4 weeks of exposure to a treatment. A considerable amount of individuals' weight was too low during the first measurement resulting in an absence of start weight in the data. However, at such small sizes the effect of the treatment is not pronounced evident by a lack of variation between individuals. To fixate the spiderlings, they were placed inside of 2 weight boats and placed on an analytical scale (Figure 7). Data on weight was obtained for 246 individuals and subsequently added to excel.

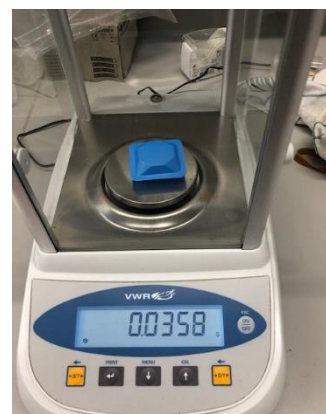


Figure 7| Weight boats fixating a spiderling enabled measuring with a precision scale

2.3 STATISTICAL ANALYSIS

Data analysis was conducted using R (R core Team, 2023). Firstly, data exploration was carried out, to get an indication of possible trends present in the data. It helps with visualization of observed behaviour across the different degrees of urbanization (local and regional). Pearson correlation tests between the variables were conducted and visualized in a heatmap. The sampling dates were scales enabling the inclusion of the temporal effect. Violin plots (R package ggplot2) were used to visually evaluate possible trends between a certain variable and the two scales of urbanization, where local scale is nested within the regional scale. This was done accordingly for spider length, spider web length, reaction time, and velocity. Velocity is calculated by dividing reaction time by web length. When analysis reaction time and effect only data from individuals that reacted within 3 seconds was used. For analysis on reaction time and velocity The effect of urbanization was analysed with linear mixed models (R package lme4, Bates et al. 2015). Information on local urbanization and regional urbanization is included in all models as fixed effects. Some models required the inclusion of an interaction term and/or sampling dates. The random factor for these models consisted of the id's of the individuals, which were nested within the plot id's. Accounting for the variability in the data, which can't be assigned to the independent variables. Starting with the most complex model, which was gradually reduced to acquire the best fit. Additionally, behaviour was divided into 3 categories, however we did not use a multinomial statistical analysis. Instead binomial proportions (immediate reaction = 1, other behaviour = 0) were analysed using a generalized linear mixed model assuming binomial distribution with logit link function (R package lme4). An overview of data on categories is located in Appendix 2.

3 RESULTS

3.1 GENERAL RESULTS

The large amount of data in combination with multiple variables give grounds for including general graphs. Ensuring a better understanding of the collected data during field and lab sampling, before covering specific results in relation the proposed hypotheses.

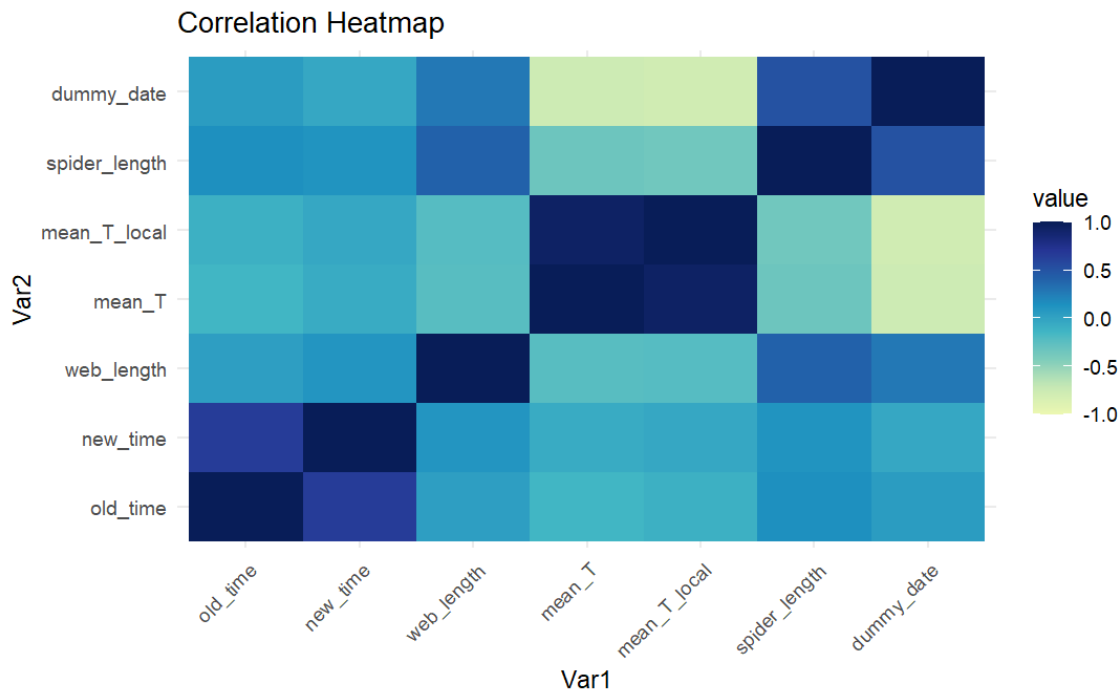


Figure 8 | heatmap composed of Pearson correlation values. A colour gradient visualizes the direction of the correlation with +1 (positive correlation), -1 (negative correlation) and 0 (no correlation). Data for dummy_date (sampling dates), spider_length, mean_T_local (mean local temperature), mean_T (mean spider surface temperature), web_length, new_time (time measurements from second video analysis), old_time (time measurements from first video analysis) is included.

Data independency was checked by using a heat map composed of Pearson correlation values (Figure 8). A colour gradient is used to visualize the direction of the correlation (-1; negative correlation, 0; no correlation, +1: positive correlation). A supplementary table containing all Pearson correlation values can be found in Appendix 3.1. A strong negative correlation (-0.78) is present for sampling date and the local temperature. This indicates that temperature decreased in relation with later sampling dates. This is explained by the time sampling took place. We started at the beginning of September the beginning of October. Which includes the seasonal change from Summer to Autumn. Moreover, an additional strong negative correlation (-0.76) is present for spider surface temperature and sampling data. Explained by the dependency on surrounding temperatures for thermoregulation in ectothermic organisms. This dependency is also emphasised by the strong positive correlation (0.94) between spider surface temperature (mean_T) and local temperature. A moderate positive correlation (0.66) between the time measurements acquired from the first and second video analysis is observed. Therefore, data from both analysis have similar relationships. Thus, it can be concluded that no major discrepancies between data are present. An additional moderate positive correlation (0.51) is present for spider length and sampling data (Appendix 3.2). It appears that size increased

in relation to sampling date. This could be accounted to the temporal effect linked to sampling during developmental period of subadult spiders. However, the temporal effect will be incorporated into statistical model.

The effect of temperature on reaction times is visually evaluated by a scatterplot (Appendix 3.3). The graph appears to show a random distribution. Temperature does not influence reaction time.

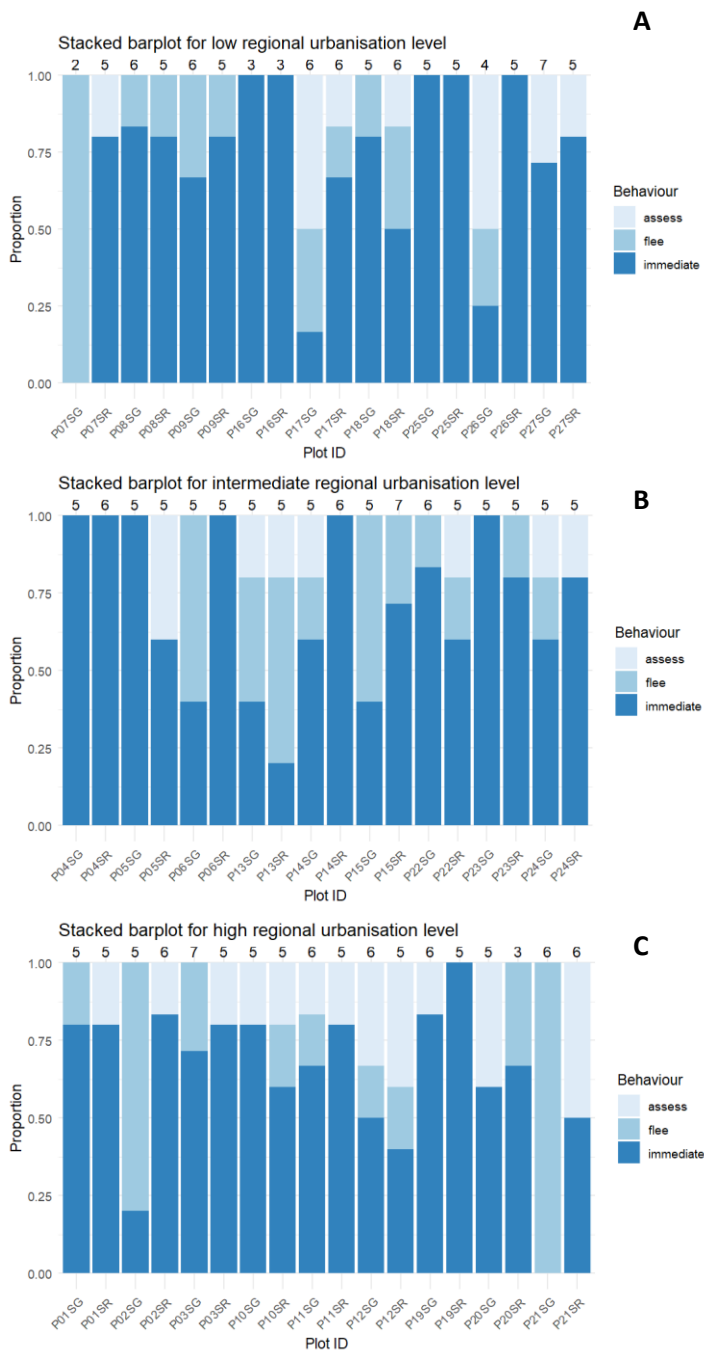


Figure 9 | Bar plots with proportions of categories for each degree of urbanization at regional scale (9.A; low, 9.B; intermediate, 9.C; high). Local degree urbanization is indicated by the final character of Plot ID (G; low, R; high)

Bar plots for each degree of regional urbanization (Figure 9.A; low, Figure 9.B; intermediate, Figure 9.B; high) show the proportions of the behaviour for each sampling plot. Local degree of urbanization is indicated by the final character of Plot ID (G; low, R; high). The number of categories for each subplot is displayed above the bar plots. Almost all subplots contain data on at least 5 individuals which is in accordance with the objective. However, certain sites have lower observations. To evaluate the impact of these low observations on statistical testing, concerned plot were eliminated and models were evaluated. No significant effect was found, which support the inclusion of the data.

3.2 URBANIZATION

The main focus of this research is on behavioural change as adaptation to decreased quality, quantity and size of prey items caused by the urban heat island effect. Urbanization-induced alterations in prey dynamics lead to a fitness decrease in predators. For that reason, increased voracity is expected as mitigation to the adverse consequence of urbanization. The impact of the urban heat island effect on web and spider size will be analysed. Additionally, adaptations to urbanization can already be present during development. Therefore expecting urbanized individuals may experience increased development compared to rural individuals exposed to urban conditions. Statistical analysis will enable to evaluate the contribution of both temperature and food regime. Moreover, a conclusion will be made on the attribution of phenotypic plasticity and maternal genetic effects to adaptive ability in relation to urbanization. Furthermore, results on probability of exhibiting voracious behaviour will be covered. It is relevant to highlight that important factors will be missed during sampling, this is inherent to ecological research' complexity. However, assessment and therefore disentanglement of sampled variables is crucial, to acquire the most reliable results.

3.2.1 SPIDER LENGTH

The statistical analyses on reaction time included 627 observations from 55 different locations. Violin plots are used to visualize possible trends. A linear mixed model was used to statistically evaluate the effect of urbanization at regional and local scale on spider length.

violin plots with urbanization and spider length

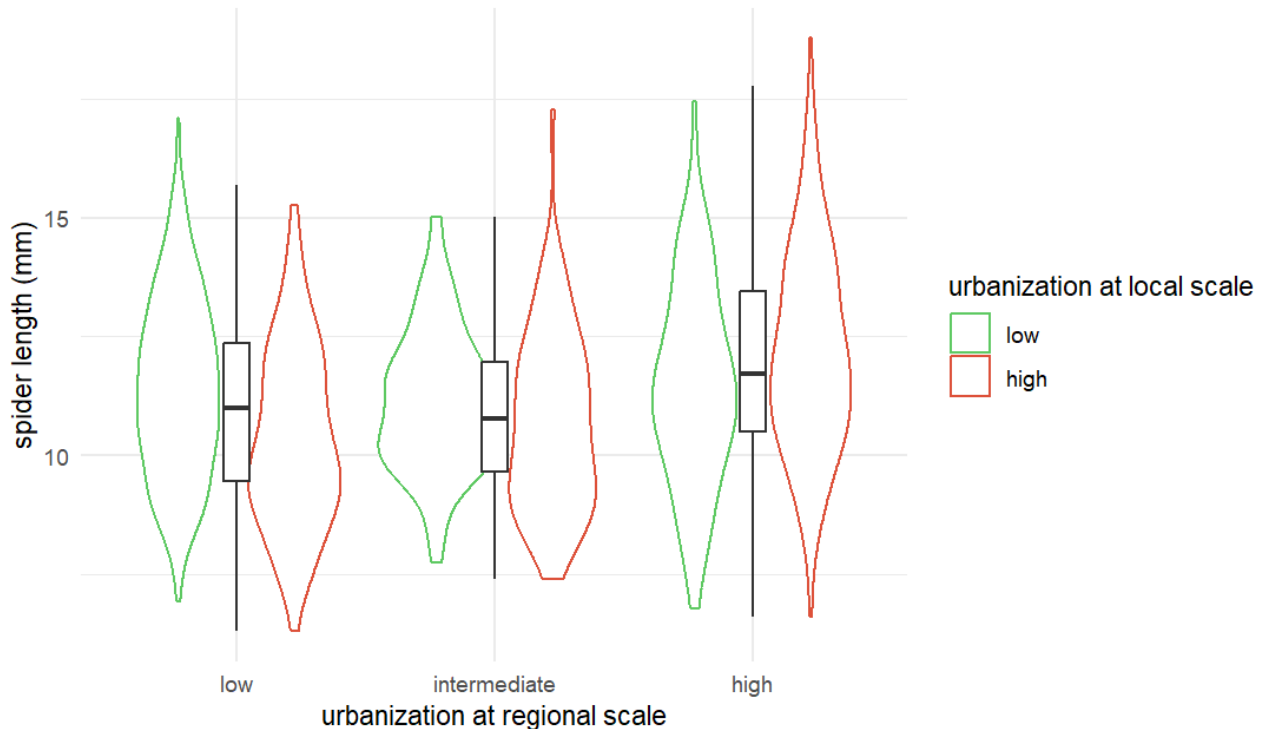


Figure 10 | Violin plots with spider length (in mm) and degree of urbanization at regional (3km x 3km) and local scale (200m x 200m).

The violin plots on spider length and regional and local degree of urbanization (=DOU) indicate clear trends (Figure 10). An indication on size difference is present between local scales irrespective of regional scale ($p = 0.0158$). However, the direction varies on regional DOU. Urban populations at low and intermediate DOU have smaller body sizes compared to rural populations. Urban populations are characterized by size reduction for low and intermediate DOU at regional scale. Conversely, the combined effect of high local and regional DOU causes a body size increase ($p = 0.02776$).

```
lmer(spider_length ~ plot * subplot + sdate + (1|plot_id:subplot_id), data = final_length)
```

The model with the inclusion of an interaction term between regional DOU, local DOU and sampling date is statistically supported. The additional fixed effects contributed sufficiently to explain observed variability within the data. Additional information on model selection is found in Appendix 3.4.

Fixed effects	Estimate	Std.Error	Df	t-value	Pr(> t)
(Intercept)	11.4908	0.2864	42.9746	40.127	< 2e-16
plotintermediate	-0.3199	0.427	53.5396	-0.749	0.457
plothigh	0.345	0.4364	53.3355	0.791	0.43266
subplothigh	-1.1186	0.4512	64.4732	-2.479	0.0158
sdate	0.4906	0.1847	572.2089	2.656	0.00813
plotintermediate:subplothigh	0.8565	0.6459	64.841	1.326	0.18953
plothigh:subplothigh	1.4021	0.6216	59.8079	2.256	0.02776
plotintermediate:sdate	-0.3607	0.3306	192.9694	-1.091	0.2767
plothigh:sdate	0.5311	0.3537	117.2945	1.502	0.13586
subplothigh:sdate	-0.5633	0.3137	529.0264	-1.796	0.07309
plotintermediate:subplothigh:sdate	1.0259	0.5452	138.6208	1.882	0.06196
plothigh:subplothigh:sdate	0.2991	0.4625	215.3857	0.647	0.51858

Table 4 | Output from linear mixed model of effect of urbanization on spider length. Fixed effects included are regional (low, intermediate, high), local (high, low) degree of urbanization and *sdate* (scaled sampling dates). In addition to interaction effects for each possible combination of variables. The intercept equals low regional DOU and acts as reference for the effect of urbanization..

Output generated by the model shows significant p-values for 2 factors (Table 4). Firstly, *subplothigh*, which represent differences in spider length by urbanization within the same plot (cfr. local scale DOU). The p-value is 0.0158, providing statistical backing. It indicates the presence of spider length differences between local urbanization degrees. Further elaboration about the direction of this effect is possible by comparing corresponding output with the intercept. The estimate (-1.1186) indicates that on average individuals sampled in high local urbanization are 1.12 centimetres smaller compared to individuals originating from low local urbanization exposure. Furthermore, the model confirms a second effect allocated to the interaction between *subplothigh* and *plothigh* (local and regional DOU respectively). Indicated by a p-value of 0.02776. The interaction represents the combined effect of urbanization on spider length at both local and regional scale. The magnitude of the effect will vary according to the joint impact of urbanization at both scales. The direction of this effect, indicated by the value 1.4021, is positive. As such average spider length will be greatest at high DOU at both scales. Resulting in an average size increase in urban population of 1.4 cm compared to rural populations. However, obtaining a clear picture of the combined effect becomes intricate in reference to results observed within local scales. Where an average size reduction in function of increased levels of urbanization is expected. Figure 11 allows visual interpretation of this interaction. A difference in body size is present between all local scales. The direction is negative at both low and intermediate regional scale. Favouring smaller body size at high local urbanization. However, the inverse relationship is observed at the highest exposure of urbanization due to the effect of the interaction between the scales. Thus resulting in an average size increase of individuals originating from urban environments.

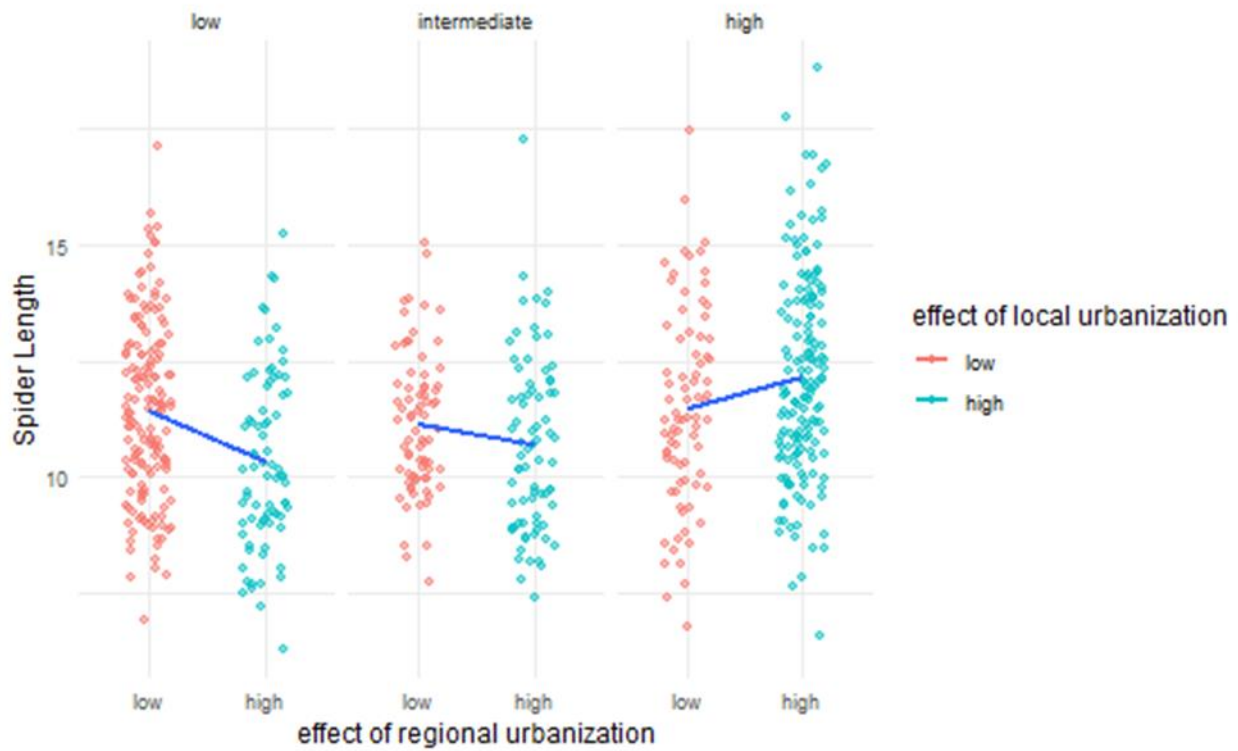


Figure 11 | Violin plots with spider length (in mm) and degree of urbanization at regional (3km x 3km) and local scale (200m x 2000m). Trend lines are added to visually represent the significant interaction effect of local and regional high DOU.

3.2.2 WEB LENGTH

The statistical analyses on reaction time included 258 observations from 54 different locations. Violin plots are used to visualize possible trends. A linear mixed model was used to statistically evaluate the effect of urbanization at regional and local scale on web length.

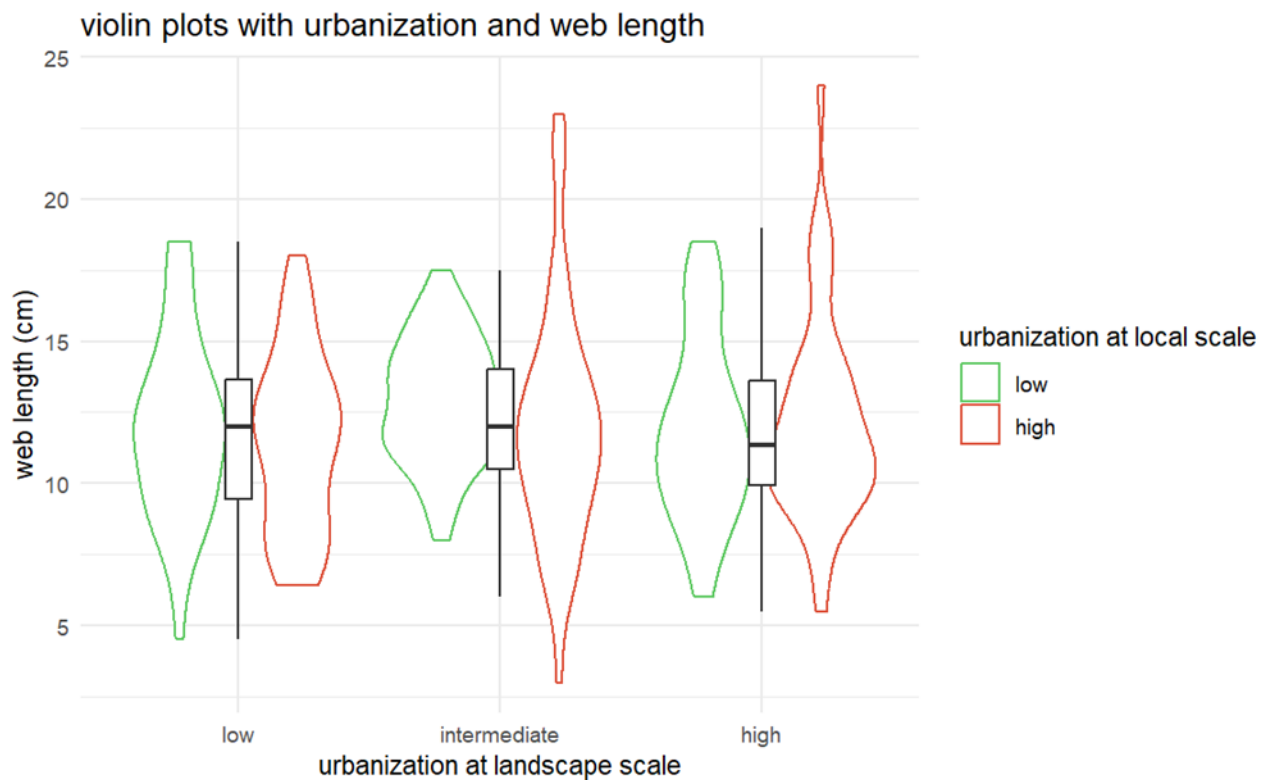


Figure 12 | Violin plots with web length (in cm) and degree of urbanization at regional (3x3km) and local scale (200x200m).

The violin plots on web length and regional and local degree of urbanization (=DOU) indicate no clear trends (Figure 12). Further evidenced by absence of significant p-values (table 5)

```
lmer(web_lengte_onderaan ~ plot + subplot + (1|plot_id:subplot_id), data = final_data)
```

The best fit was acquired after excluding additional fixed effects comprising sampling data and interaction terms. These did not contribute sufficiently to explain variability at the cost of a considerable amount of statistical power. Additional information on model selection is found in Appendix 3.5. The output of the model did not result in significant p-values (Table 5). No significant effect at both regional and local scale on web length is present.

Fixed effects	Estimate	Std.Error	Df	t-value	Pr(> t)
(Intercept)	11.7779	0.5465	47.1234	21.55	<2e-16
plotintermediate	0.8752	0.6576	46.1057	1.331	0.19
plothigh	0.4501	0.6619	46.8134	0.68	0.5
subplothigh	-0.3861	0.5375	46.3498	-0.718	0.476

Table 5 | Output from linear mixed model of effect of urbanization on web length. Fixed effects included are regional (low, intermediate, high) and local (high, low) degree of urbanization. The intercept equals low regional DOU and acts as reference for the effect of urbanization.

3.2.3 REACTION TIME

The statistical analyses on reaction time included 196 observations from 54 different locations. Violin plots are used to visualize possible trends. A linear mixed model was used to statistically evaluate the effect of urbanization at regional and local scale on reaction time.

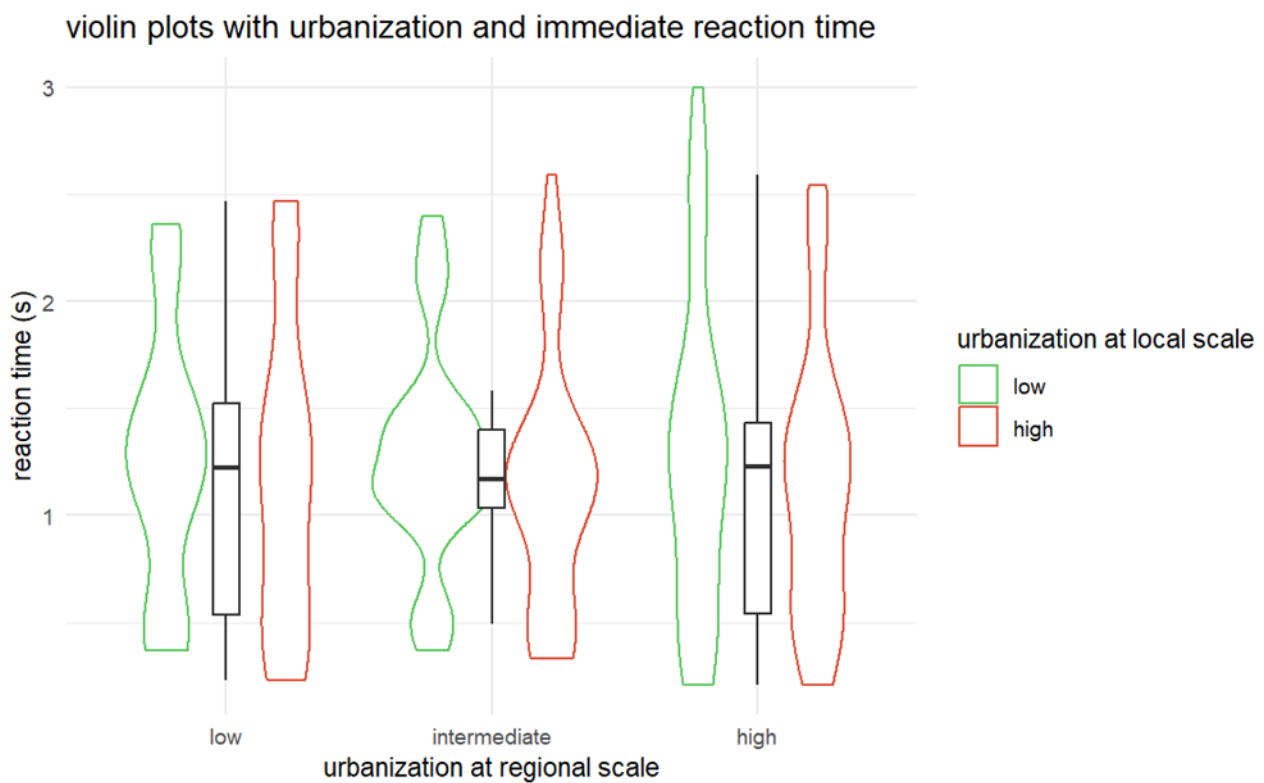


Figure 13 | Violin plots with reaction time (in seconds) and degree of urbanization at regional (3x3km) and local scale (200x200m).

The violin plots on reaction time and regional and local degree of urbanization display similar distributions (Figure 13). Consequently, no trends are present, which is statistically supported (Table 6).

```
lmer(time ~ plot + subplot + (1|plot_id:subplot_id), data = data_immediate)
```

The best fit was acquired after excluding additional fixed effects comprising sampling data and interaction terms. These did not contribute sufficiently to explain variability at the cost of a considerable amount of statistical power. Additional information on model selection is found in Appendix 3.6. The output of the model did not result in significant p-values (Table 6).

Fixed effects	Estimate	Std.Error	df	t-value	Pr(> t)
(Intercept)	1.282025	0.112786	47.52408	11.367	3.73e-15
plotintermediate	-0.0114	0.131192	46.00444	-0.087	0.931
plothigh	-0.00427	0.135009	48.57475	-0.032	0.975
subplothigh	-0.10741	0.108688	47.58644	-0.988	0.328

Table 6 | Output from linear mixed model of effect of urbanization on reaction time. Fixed effects included are regional (low, intermediate, high) and local (high, low) degree of urbanization. The intercept equals low regional DOU and acts as reference for the effect of urbanization.

3.2.4 VELOCITY

The statistical analyses on reaction time included 196 observations from 54 different locations. Violin plots are used to visualize possible trends. A linear mixed model was used to statistically evaluate the effect of urbanization at regional and local scale on velocity.

No significant p-values were acquired for the effect of urbanization on reaction time. However, other variables can be used to evaluate further behavioural adaptations. Velocity is calculated by dividing the measurement of web length by the reaction time. This gives an idea of what distance is covered in a certain time interval. Implementing this variable grants the benefit that both web size and reaction time are accounted for in a single variable.

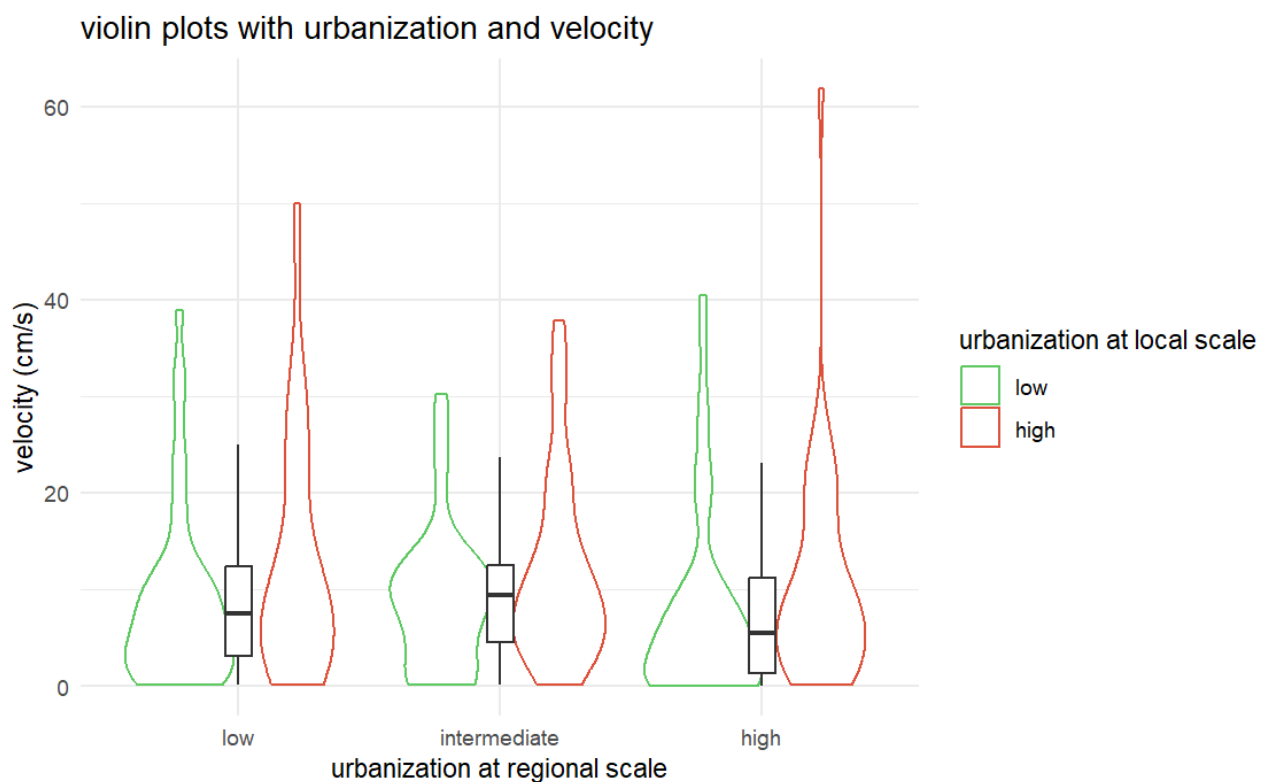


Figure 14 | Violin plots with velocity (in cm/s) and degree of urbanization at regional (3x3km) and local scale (200x200m). A positive trend is present at local scale.

The violin plots on velocity and regional and local degree of urbanization show a positive trend at local DOU (Figure 14). Velocity increases as result of high exposure of local urbanization. Output form linear model supports this (Table 7).

```
lmer(velocity ~ plot + subplot + (1|plot_id:subplot_id), data = data_immediate)
```

The best fit was acquired after excluding additional fixed effects comprising sampling data and interaction terms. These did not contribute sufficiently to explain variability at the cost of a considerable amount of statistical power. Additional information on model selection is found in Appendix 3.7. The output of the model resulted in a significant p-value (0.0497) at local scale DOU. There is a difference in velocity between individuals originating from populations subjected to contrasting levels of urbanization. Where increased urbanization drives average velocity of individuals in a positive direction. Urban populations are 3.2 cm/s faster than rural populations.

Fixed effects	Estimate	Std.Error	DF	t-value	Pr(> t)
(Intercept)	8.90062	1.60785	49.20589	5.536	1.19e-06
plotintermediate	-0.08191	1.94547	48.20687	-0.042	0.9666
plothigh	-1.44784	1.95768	48.86882	-0.74	0.4631
subplothigh	3.19909	1.58935	48.27897	2.013	0.0497

Table 7 | Output from linear mixed model of effect of urbanization on velocity. Fixed effects included are regional (low, intermediate, high) and local (high, low) degree of urbanization. The intercept equals low regional DOU and acts as reference for the effect of urbanization.

3.2.5 BEHAVIOUR CATEGORIES

280 observations from 54 different locations were included in the analysis on reaction time. Dummy variables were assigned (*immediate* reaction; 1, other behaviour: 0). This enabled analysis with a generalized linear mixed model assuming binomial distribution. Consequently, analysing the probability of exhibiting immediate behaviour in relation to urbanization at regional and local scale.

```
glmer(reaction_dummy ~ plot + subplot + (1|plot_id:subplot_id), data = final_data,
family = binomial(link="logit"))
```

The best fit was acquired after excluding additional fixed effects comprising sampling data and interaction terms. These did not contribute sufficiently to explain variability at the cost of a considerable amount of statistical power. Additional information on model selection is found in Appendix 3.8. The output of the model resulted in significant p-values (table 7). The output shows significant evidence for the effect of high urbanization at local scale (p-value = 0.04630). Therefore, the odds of exhibiting immediate behaviour in response to prey stimulus are 68.59% higher for individuals from urban populations compared to individuals not subjected to high urbanization. On top of that cementing the role of local urbanization in shaping behavioural changes. No further conclusions can be made.

Fixed effects	Estimate	Std.Error	df	Pr(> t)
(Intercept)	0.9665	0.3425	2.822	0.00478
plotintermediate	0.2933	0.4342	0.675	0.49942
plothigh	-0.4323	0.4075	-1.061	0.28866
subplothigh	0.6859	0.3442	1.993	0.04630

Table 8 | Output from generalized mixed model of effect of urbanization on probability of exhibiting *immediate behaviour*. Fixed effects included are regional (low, intermediate, high) and local (high, low) degree of urbanization. The intercept equals low regional DOU and acts as reference for the effect of urbanization.

3.3 COMMON GARDEN EXPIREMENT

A common garden experiment was conducted to analyse the effect of urban conditions on development. Observations are acquired on 245 spiderlings originating from 10 mothers. The mothers were collected from sites with the strongest contrast in urbanization degree (i.e. most urbanized and rural sites at local and regional scale). Combinations of food and temperature regime were uniformly allocated to spiderlings from a certain mother. This enables evaluation on the contribution of maternal effects and heritability. Maternal effects are analysed regarding maternal urbanization exposure. Heritability is assessed according to variation observed within related offspring. Subsequently, conclusion are made regarding the influence of phenotypic plasticity. Additionally, the contribution of temperature and food regime on spiderling development is unravelled. The difference start and end date is regarded as a proxy for development. Random effect are assigned in regards to spiderlings originating from the same mother.

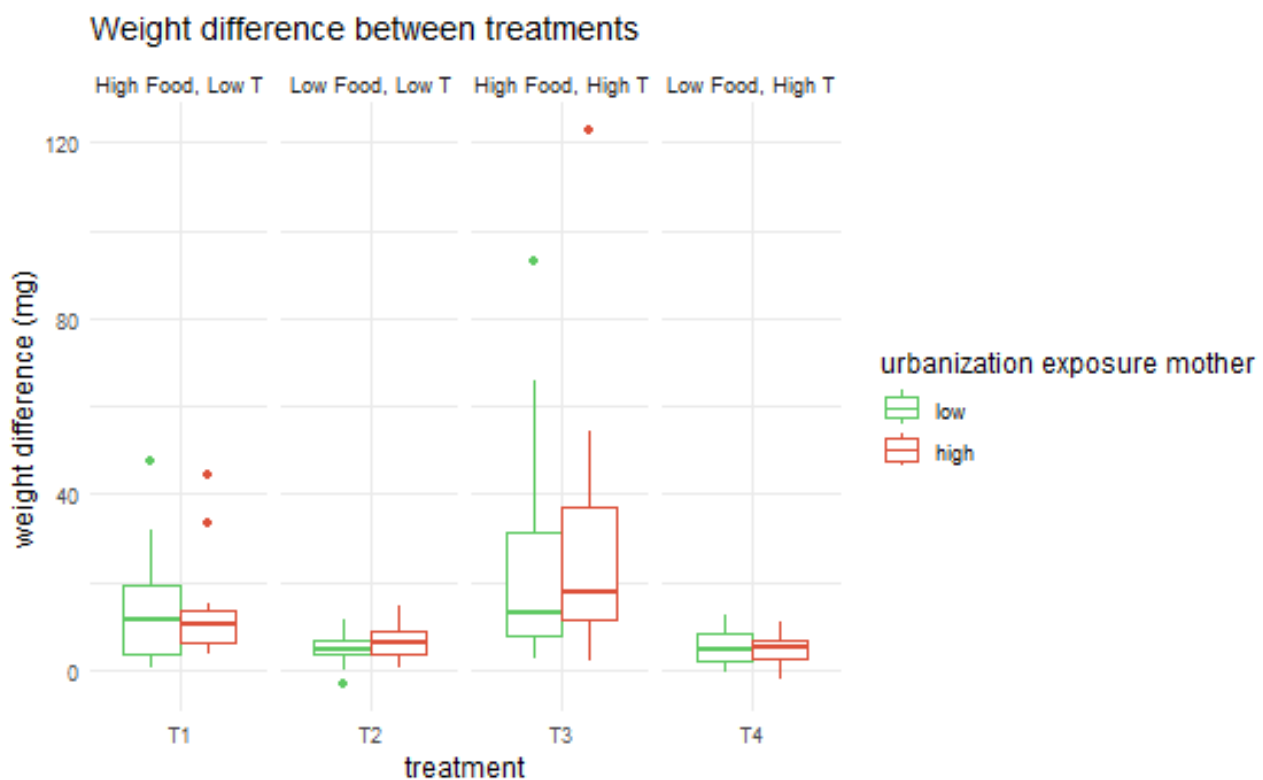


Figure 15 | Box plots containing data on weight difference between treatments with regards to the urbanization context of mother.

Box plots shown in Figure 15 give a general overview of effect on treatments. It is clear that treatment 3 (i.e. T3; high food and high temperature) result in largest effect on development (0.01071). Food regime contribute more to spiderling development compared to temperature regimes (0.00469). Furthermore, boxplot colours indicate the urbanization context of the mother. However, no evidence (visually and statistically) for the effect on development is present.

```
lmer(difference ~ food_regime * temp_regime + (1|motherID), data = data_immediate)
```

The best fit was acquired after excluding an additional fixed effect comprising maternal urbanization exposure. This did not contribute sufficiently to explain variability at the cost of a considerable amount of statistical power. Additional information on model selection is found in Appendix 3.9.

Fixed effects	Estimate	Std.Error	Df	t-value	Pr(> t)
(Intercept)	5.8906	2.256	33.4671	2.611	0.01341
temp_regimehigh	-0.7508	2.6064	215.8263	-0.288	0.77359
food_regimehigh	7.7717	3.0197	223.8632	2.574	0.01071
temp_regimehigh:food_regimehigh	11.0522	3.8691	223.9155	2.856	0.00469

Table 9 | Output from linear mixed model of effect of food and temperature regimes on weight difference. Fixed effects included are regional (low, intermediate, high) and local (high, low) degree of urbanization. The intercept represent weight difference when both food and temperature regimes are at reference levels.

The inclusion of data on the urbanization context of the mother lacked statistical backing. This is visually shown by response plots in Figure 16, the effect of treatment conditions on development is not influenced by the urbanization context of the mother.

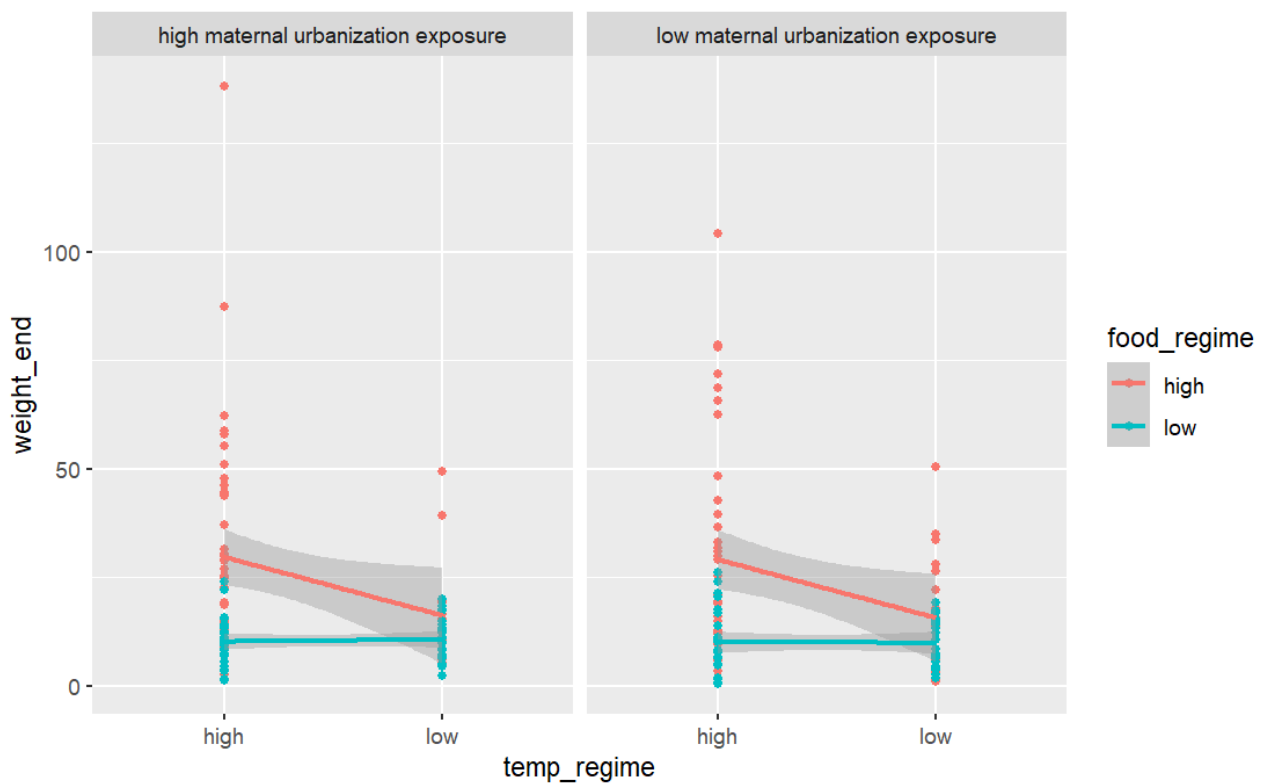


Figure 16 | Response plots containing the effect of food and temperature regimes on spiderling development in relation to the urbanization context of the mother. Similar trends are observed irrespective of maternal urbanization exposure.

Additionally, further analysis on the possible influence of the mother on development was conducted. This was done by interpreting the variation in weight among siblings. This was done by separately plotting boxplots of the effect of treatments (Figure 17). Visually similar results are observed compared to Figure 15. A linear model without the random effect was used to determine significance of the mother's influence on weight difference. Inclusion of the random effect is statistically validated after comparing the models (Appendix 3.10). Thus, variation among siblings cannot be explained as contribution of maternal genetic effects. Rather random variation is present in the data, however can't be explained by the fixed effects as such needs to be accounted for. No evidence on effect of maternal genetic effect is found. This results in the assumption that phenotypic plasticity is the main contributing factor in determining adaptative capability to urbanization.

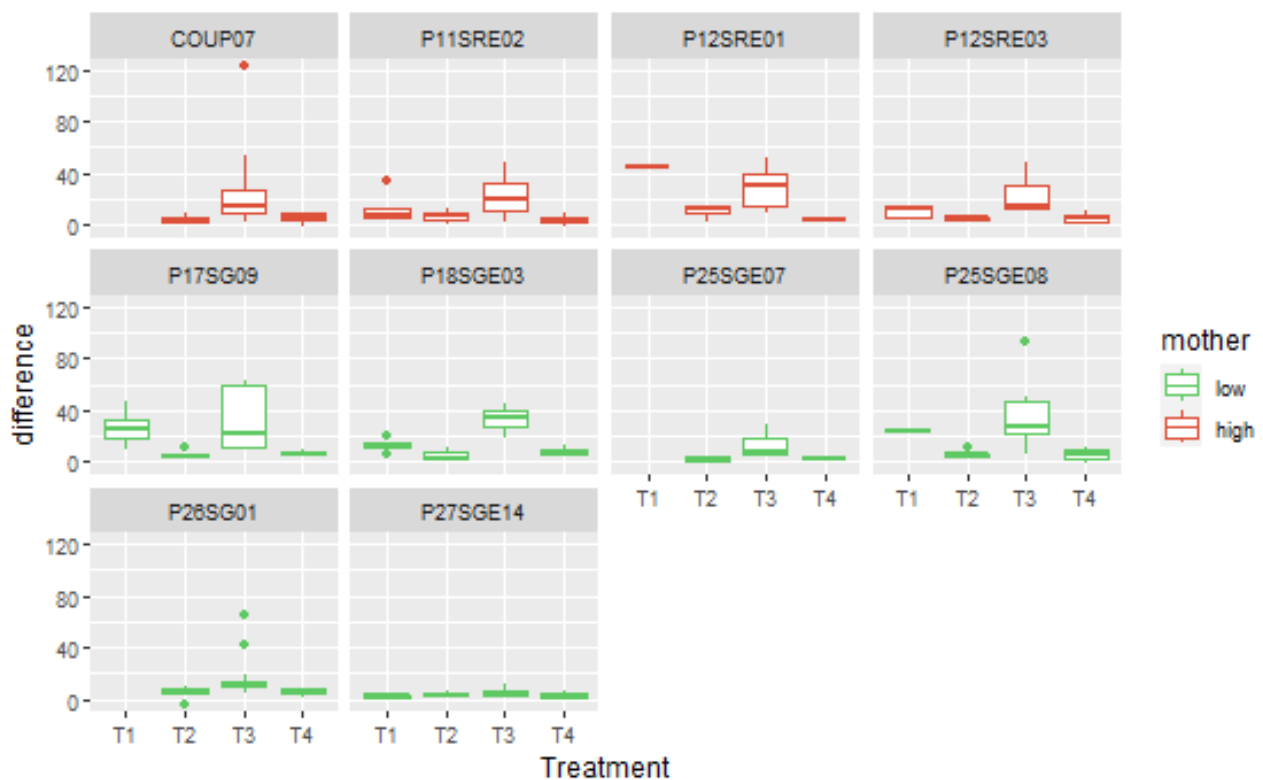


Figure 17 | Box plots subdivided for siblings containing data on weight differences between treatments with regards to the urbanization context mother.

4 DISCUSSION

The goal of this research was to determine a possible biological response in European garden spiders to varying degrees of urbanization at two spatial scales. Urbanization is a main contributor to the current global biodiversity crisis by inducing among other things biotic homogenization (McKinney 2005). Consequently, decreasing Arthropod diversity, abundance, quality and size evidenced by numerous research (under which; Piano et al. 2017, Svenningsen et al. 2022, Merckx et al. 2018, McIntyre 2000). The main focus was on behavioural change as adaptation to urbanization. Based on the is a general consensus on the importance of behavioural adaptation as response to new selection pressures. Possible alterations in behaviour can occur in; web-building micro-habitat selection, foraging behaviour, et cetera... This research focussed on foraging behaviour. This resulted in evidence of altered behaviour in response to urbanization. Where an increase in average velocity is observed in individuals exposed to high local urbanization. Furthermore, it can be concluded that individuals exposed to high local urbanization have increased probability of exhibiting risk-prone and voracious behaviour. The combined results further cement the importance of the effect of urbanization on predator-prey dynamics as main driver of behaviour.

4.1 FORAGING BEHAVIOUR

This research found evidence for behavioural changes as mitigatory adaptations to the impact of urbanization. Evidence was found for differences in velocity between population exposed to varying degrees of urbanization. In conjunction with proof of increased probabilities of observing voracious behaviour in individuals exposed to local urbanization. Similar distributions of reaction times are observed at both spatial scales of urbanization. Consequently, no statistical proof on the effect of urbanization on reaction time was obtained. When we corrected the recorded time for web size, we could calculate the spider's velocity during the reaction. Which reflected the distance covered in certain time interval. This was used as an indication to evaluate voracity and risk-proneness. As was hypothesised an effect of local scale urbanization on velocity is detected. Velocities observed in urbanized individuals are greater on average. This regarded as an adaptation to the fitness reduction caused by the disrupted prey dynamics, where greater velocity could signify increased levels of voracity. Subsequently, significant results are acquired for the behaviour categories. An increased probability of exhibiting immediate behaviour at high local scale urbanization is observed. Hence confirming urbanization as important driving of behaviour.

The acquired results highlight the importance of the scale dependency of urbanization. Observations sampled at local scale resulted in more pronounced effects. Which can be attributed to the accumulation of the environmental changes caused by urbanization at smaller scales (Dahirel et al. 2017). Complemented by the sedentary lifestyle resulting in increased sensitivity to local environmental pressures (Dahirel et al. 2017, Foelix 2010). Furthermore, previous research also showed an increased contribution of urban heat islands effect at local scale in the sampled region (Dahirel et al. 2017, Merckx et al. 2018). Which is assumed to be a major driving factor of alterations in prey dynamics. Where results from previous studies (Dahirel et al. 2017, Dahirel et al. 2018, Merckx et al. 2018) validate this assumption. Implying a more locally pronounced impact on spiders in regards to decreased quality and quantity of prey. Consequently, explaining the absence of the effect at regional scale in our data without rejecting urbanization's scale dependency. Furthermore, A shift towards more voracious behaviour was expected as result of malnourishment. Increased levels of voracious behaviour are associated with decrease in risk-averse behaviour. Reflected in a higher proportions of

immediate reaction exhibiting by urban populations. An indication of such behavioural change in relation with urbanization was already observed in male individuals of Western black widow spiders. These individuals displayed increased levels of voracity during development in a common-garden setting (Johnson et al. 2019). Evidence backing aforementioned indication is provided by this study. Following that providing further evidence of the importance of behavioural adaptation as mitigation to HIREC. Urbanization-driven alterations in behaviour have been well documented in mammals (reviewed in Ritzel & Gallo 2020) and birds (Jones et al. 2012, De Satgé et al. 2019). In turn, a general consensus exists on urbanization's contribution to increasing risk-prone - and explorative behaviour. However, the limited amount of research on arthropods indicate no general trends. Research on a species of jumping spider demonstrated the effect of environmental conditions during development as a factor shaping behaviour (Liedtke et al. 2015). Spiders reared in enriched environments exhibited increased level of exploration. Conversely, poor environmental conditions (cfr. urban environments) resulted in a decreased explorative behaviour. Additionally, urbanization did not contribute in shaping explorative behaviour in three rove beetle species. However, it is hypothesized that mobility differences among species could result in the absence of significant effect of urbanization-driven alterations in behaviour. Where rove beetles, which are highly mobile can escape the persistent impact of urbanization by micro-habitat selection (Magura et al. 2022). Conversely, the European garden spider is a sit-and-wait predator with limited dispersal capacity. Consequently unable to mitigate urban selective pressures by relocating. Potentially explaining why urbanization-driven alterations in its behaviour are observed. Finally, observations on four species of ground beetles (Schuett et al. 2018) and a grasshopper species (Waterschoot et al. 2023) imply increased bold behaviour as response to urbanization. Ultimately, results on *A. diadematus* concerning urbanization-driven alterations in foraging behaviour are in line with the proposed hypotheses. It can be concluded for *A. diadematus* that urbanization induced increased voracious behaviour. However, both presence and direction of behavioural adaptations in arthropods varies greatly among species. Yet, evidence provided in this research aids in unravelling the extent of urbanization's consequences on arthropods' behaviour.

4.2 SPIDER SIZE REDUCTION

The urban heat island effect associated with urbanization resulting in a body size reduction in Arthropods has gained traction in recent years. Providing insights on an important impact of urbanization shaping urban populations. This effect has been observed in both aquatic and terrestrial urban communities (Merckx et al. 2018). Reduction in size in arthropods is the consequence of the temperature-size rule, which exerts selective pressure. Size reduction is theorized to be an adaptation to temperature-induced increased metabolic activity and the associated costs (Merckx et al. 2018, Atkinson 1994). Additionally, studies on *A. diadematus* have shown differences in body sizes between urban and rural population. More specifically an average size decrease in urban populations, which is conform to expectations. An effect of urbanization on body size is not observed at regional scale. The results of this study mostly are consistent with the expectations based off previous research. A local effect on body size of reduction urban population was observed. As well, a significant effect assigned to the interactive effect was detected. This interaction contributing significantly when urbanization degree was high at both scales. However, a body size increase was observed in urban populations opposed to a decrease. The positive effect of increased temperature on spider growth could be a possible explanation for this effect (Löwe et al. 2014). Additionally, increased prey abundance at small scales in urban areas can result in bigger body size (Löwe et al. 2014, Miyashita 1992) as increased prey availability often results in large increases in growth (Wilder et al. 2011, Toft 2013). A possible contributing

factor is the presence of artificial light can alter fitness by increasing prey at that location irrespective of local prey abundance (Eisenbeis et al. 2009, Löwe et al. 2014, Heiling 1999). Furthermore, waste from humans could increase the abundance and nutritional value of prey at micro-scale (McIntyre 2000). Alternatively, environmental variation at micro-scale in urban areas can be important factor in shaping the observed biological response. Where micro-habitat selection could play a role in mitigating impact of high urbanization exposure.

4.3 DEVELOPMENT

A common garden experiment was carried out to evaluate the response in development to urban conditions. Four treatments with different food and temperate regimes (both high and low) were allocated among related spiderlings. Numerous studies on the effects on growth and body size have shown the importance of high capture rates especially of high nutritional prey (cfr. Toft 2013, Löwe et al. 2014, Wilder et al. 2011, Wilder et al 2019). Further evidenced by results observed in this research. Food regime has the greatest contribution in the response on development. High feeding regime resulted in the biggest weight differences, consequently growth rate. Therefore, highlighting the impact on spider size and development caused by the urban heat island effect on prey dynamics. This impact is expected to reduce spider body size and limit development (Higgins & Goodnight 2011, Dahirel et al. 2017, Mayntz et al. 2009). Additionally, the impact associated with urbanization is amplified by *A. diadematus*' inability to feed on anthropogenic food sources (Penick et al. 2015). However, decreased prey abundance can be mitigated by mixed diets that supplement nutritional values. Implying a dependence on prey species composition at micro-scale (Toft 2005). Furthermore, even the impact related to low abundance of poor quality prey can be partially counteracted by mixed diets consisting of prey with different nutritional values (Toft 2005, Toft 2013). Despite this it is important to mention that low and intermediate quality diets enable substantial growth and size. But this is only temporary as development will stop before maturation resulting in a large decrease fitness (Toft & Wise 1999, Oelbermann & Scheu 2002). Moreover, the probability encountering a particular type of prey (e.g. toxic, low quality, high quality) varies heavily, thus having further important consequences on spider fitness (Toft 2013). As such habitat selection could be beneficial or detrimental. Nonetheless, selection for optimal sites is crucial for increasing foraging success (Jakob et al. 2001, Napiórkowska et al. 2021). Additionally, encounter rates depend on micro-habitat prey abundance resulting in additional difficulty in determining the scope of the effect regarding the pressure exerted by altered prey dynamics on individual spiders development (Li 2005, Wilder et al. 2011). The effect of prey species composition as mitigating factor emphasises importance of conserving biodiversity. Especially since urbanization is regarded as major cause of biotic homogenization (McKinney 2005).

Following this, results on the combined effect of food - and temperature regime prompted the biggest weight differences. This indicates the beneficial impact of temperature on development as result of increased metabolic activity (Johnson et al. 2019, Löwe et al. 2014). However, beneficial effects are only present below a critical temperature threshold. Surpassing this threshold could result in slowed development, reduced mass and increased mortality rates (Kingsolver et al. 2013, Johnson et al. 2019). Furthermore, it is possible that implemented temperature regimes don't give an actual representation of the effect experienced by the urban heat island effect. Where microclimates in urban areas could be characterized by either higher or lower temperatures. Temperature also acts as a cue in micro-habitat selection in *A. diadematus* (Napiórkowska et al. 2021), further cementing the relation between temperature and nutrition. On top of that microclimate is able to buffer or amplify environmental conditions (Woods et al. 2015). Additionally, temperatures were kept

constant during the experiment, however evidence suggest greater impact by variation in temperature rather than a high mean temperature (Dowd et al 2015, Johnson et al. 2019).

Results from the common garden experiment combined with data on maternal exposure to urbanization (high or low resp.) enabled analysis on the contribution of maternal effects on adaptive ability to urbanization was evaluated. No proof of maternal effects influencing weight was obtained, thus providing evidence that urbanization exposure of mother does not contribute to difference in weight in the offspring. Similar responses in development as result of simulated urban conditions are observed in offspring originating from mothers exposed to both degrees of urbanization. Furthermore, as a means of evaluating the heritability of adaptive ability, random effects were analysed. This was done to determine whether weight differences in related offspring could be attributed to the mother's genetic contribution. Similar to maternal effects, the effect of heritability resulting in a non-significant contribution. However, it is of key importance to mention that although this method gives an indication on heritability. A caveat in this analysis is the absence of equal observations for each mother, which can lead to skewed results (Appendix 3.11). This condition was not met due to high mortality rate in infant spiderlings. Nonetheless, an indication on the importance of phenotypic plasticity as driving factor for adaptation is given. However, further research is required to quantify contributing factors aiding adaptive ability to urbanization in developmental traits.

5 CONCLUSION

The selective pressures exerted as a result of HIREC (especially urbanization) are major drivers of both behavioural and morphological change in *A. diadematus*. The acquired results granted additional insights in uncovering the consequences of urbanization. It can be concluded that urbanization is a major contributor in increasing both velocity and probability of voracious behaviour. Subsequently, increased temperatures exert selective pressure leading in of body size differences at local DOU. This result complements previous research. Furthermore, the combined effect of high degrees of urbanization at both scales lead to an increase in size in urban populations. However, the underlying mechanism remains unclear. Although, micro-habitat variation, increased prey abundance and temperate-induced increased growth in urban areas are suggested as possible explanations. Which, could be explored in further research. Additionally, this research used a constant prey stimulus, however information on prey characterized by irregular vibrational cues could be of interest. Developmental responses proved more difficult to disentangle due to complexity of ecological research. However, the beneficial effect on development in relation with prey abundance has been shown. Furthermore, combination of higher prey abundance along with temperatures resulted in biggest size differences. This emphasizes the positive outcome of temperature-induced increase of metabolic activity on development. Finally, we evaluated the possible contribution of maternal effects to adaptation in offspring. After which, an indication on the importance of phenotypical plasticity in adaptative ability as response to urbanization is present, although no concrete conclusion will be drawn. This would require additional research. As for behaviour, the complexity of ecological research hinders the full comprehension on underlying mechanisms. Nevertheless, possible contributing variables are discussed.

The relevancy of research in relation to HIREC will only further increase based on future predictions. Therefore, clear understanding of both selection pressures and biological responses is required to establish a framework for conservation. Conservation at local scale will prove fruitful regarding the combined results of research on urbanization inducing small scale importance. Furthermore, spiders have an important role in regulating populations of prey items. Deeming them crucial in maintaining ecological balance, thus conserving biodiversity.

6 SUMMARY

Urbanization is considered as one of the most dramatic forms of human-induced rapid environmental change, besides it is expected to further increase. Urbanization is characterized by novel biotic and abiotic conditions. Therefore exerting new selective pressures without historical analogues. Mitigation of detrimental impact is achieved by adaptation. Adaptations can take place at morphological, physiological, behavioural and molecular level as a means to increase fitness in altered environments. Urbanization is associated with the pollution, fragmentation, artificial light and altered food availability. Additionally, the urban heat island effect, resulting from disrupted evapotranspiration and increased heat retention lead to higher temperatures in urban areas. Despite knowledge about the impact of urbanization, the process is a complex and operates at multiple spatial scales.

Behavioural change is considered to be the first step towards adaptation to rapid environmental change. This is evidenced by numerous studies on mammals and birds. However, little information is known about the biological response in Arthropods. However, altered prey dynamics in insects under which decrease in abundance, quality and size in relation to urbanization have been observed. Consequently, indirectly impacting the food web. Leading to the expectation that urban population experience more malnutrition and consequently will exhibit increased voracious behaviour. Therefore, the goal of this research was uncovering behavioural adaptations as result of urbanization-induced prey dynamics. *A. diadematus* is a orb-weaver spider, common in rural and urban areas.

54 sites were sampled according to the SPEEDY-design, where urbanization degree is calculated by percentage of built-up cover. Information on behaviour was obtained by time measurement and division of categories. Additionally, web - and spider length are analysed. Finally, sampled individuals were collected for breeding purposes. A common garden experiment with the offspring was used to evaluate development in relation to urbanization. The offspring was exposed to different treatments simulated urban conditions by food and temperature regimes. Moreover, 2 spatial scales of the degree of urbanization are incorporating into the analysis (local and regional scale, resp.)

Results on spider size showed the local impact of urbanization on spider size. Low and intermediate regional DOU caused a size reduction in urban population. Furthermore, the combined effect of high DOU at both spatial scales resulted in a size increase in urban population. This emphasizes the complexity of the underlying processes of urbanization. No conclusion on web size could be made.

Significant results on spider velocity, which is calculated as the division of reaction time by web length were obtained. This evidence support the hypothesis that urban populations react faster compared to rural population. Further proof of our hypothesis is acquired by analysis of the probability of exhibiting an immediate reaction. Where urban-dwelling individuals were more likely to exhibit such behaviour.

The common garden experiment proved the importance of prey abundance on development. Furthermore, strongest effect on weight was observed when both temperature and food regime were high. This implies the importance of temperature in development as result of increased metabolic activity. However, the effect is beneficial below a critical threshold temperature. Lastly, indication on the importance of phenotypic plasticity were observed. However, conclusions were not drawn.

7 SAMENVATTING

Urbanisatie wordt beschouwd als een van de meest dramatische vormen van door de mens veroorzaakte milieuveranderingen en zal naar verwachting nog verder toenemen. De omvang van urbanisatie verloopt aan een ongekend snel tempo. Geurbaniseerde gebieden worden gekenmerkt door nieuwe biotische en abiotische factoren. Deze nieuwe omstandigheden oefenen onbekende nieuwe selectiedrukken uit. Biologische adaptaties zijn de reactie van organismen op deze selectiedruk, hierdoor kunnen ze de negatieve impact verzwakken. Deze adaptaties kunnen plaatsvinden op morfologisch, fysiologisch, gedragsmatig en moleculair niveau om de fitness te verhogen in gewijzigde milieus. Urbanisatie wordt gekenmerkt door vervuiling, fragmentatie, kunstmatig licht en een veranderde beschikbaarheid van voedsel. Verder gaan verstedelijkte gebieden gepaard met een verstoorde evapotranspiratie en verhoogde warmteretentie. Dit resulteert in een temperatuurverhoging ten opzichte van het platteland. Dit wordt het hitte-eilandeffect genoemd. Ondanks de kennis over de impact van verstedelijking, blijft het een complex proces dat zich op meerdere ruimtelijke schalen afspeelt.

Verandering in het gedrag worden beschouwd als de eerste stap richting adaptatie aan milieuverandering. Dit blijkt uit talloze onderzoeken naar zoogdieren en vogels. Echter, is er maar weinig geweten over de impact van urbanisatie op het gedrag van geleedpotigen. Alhoewel, onderzoek heeft uitgewezen dat urbanisatie een veranderde prooidynamiek bij insecten teweegbrengt. Kortom, neemt de abundantie, kwaliteit en grootte van insecten af in verstedelijkte gebieden. Hierdoor is er een indirect invloed op het voedsel web. Dit leidt tot de verwachting dat insect predatoren ondervoed raken en bijgevolg meer vraatzuchtig gedrag zullen vertonen. Daarom was het doel van dit onderzoek het blootleggen van gedragsaanpassingen als gevolg van door verstedelijking verstoorde prooidynamiek. Waar de kruisspin een centrale rol speelt. De kruisspin is een wielwebspin die veelvuldig voorkomt in zowel landelijke en stedelijke gebieden.

54 locaties werden onderzocht volgens het SPEEDY-ontwerp, waarbij de urbanisatiegraad werd berekend aan de hand van het percentage bebouwde oppervlakte. Informatie over gedrag werd verkregen door tijdmetingen en het indelen van gedrag in categorieën. Daarnaast werden web- en spinlengte geanalyseerd. Tot slot werden bemonsterde individuen verzameld voor kweekdoeleinden. Een “common garden” experiment met de nakomelingen werd gebruikt om de ontwikkeling in functie van verstedelijking na te gaan. De nakomelingen werden blootgesteld aan verschillende behandelingen die urbanisatie simuleerden door middel van voedsel- en temperatuurregimes. Bovendien werden 2 ruimtelijke schalen van de mate van verstedelijking meegenomen in de analyse (respectievelijk lokale en regionale schaal).

De invloed van urbanisatie op spinlengte werd tweemaal aangetoond. Ten eerste, lage en gemiddelde regionale urbanisatiegraden veroorzaakten een lengteafname in verstedelijkte gebieden. Vervolgens induceerde het gecombineerde effect van hoge urbanisatiegraad op beide ruimtelijke schalen, een toename in lengte in verstedelijkte gebieden. Dit benadrukt de complexiteit van de onderliggende verstedelijkingsprocessen. Er kon geen conclusie worden getrokken over de web grootte.

Vervolgens is de invloed van urbanisatie op snelheid van spinnen aangetoond. Snelheid wordt bekomen door het delen van reactietijd en web lengte. Dit bewijs ondersteunt de hypothese dat stedelijke populaties sneller reageren in vergelijking met landelijke populaties. Dit werd verder gestaafd door resultaten omtrent de

waarschijnlijkheid om onmiddellijk te reageren. Verstedelijkte spinnen vertonen een grote kans om direct op een prooi te reageren.

Het kweekexperiment toonde een positieve effect op spinontwikkeling als gevolg van de aanwezigheid van een grote aantal prooi. Bovendien werd het sterkste effect op het gewicht waargenomen wanneer zowel de temperatuur als het voedselregime hoog waren. Dit impliceert het belang van temperatuur voor de ontwikkeling als gevolg van verhoogde metabolische activiteit. Het effect is echter gunstig zolang een kritische temperatuur niet overschreden wordt. Tot slot werden er aanwijzingen gevonden voor het belang van fenotypische plasticiteit. Er werden echter geen conclusies getrokken.

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10 APPENDIX

10.1 Overview of sampling sites

PlotID	city	region	regional DOU plot (1)	local DOU subplot (2)
P01	Gent	Gent	high	high and low
P02	Aalst	Gent	high	high and low
P03	Oudenaarde	Gent	high	high and low
P04	Bellem	Gent	intermediate	high and low
P05	Beervelde	Gent	intermediate	high and low
P06	Hillegem	Gent	intermediate	high and low
P07	Kalken	Gent	low	high and low
P08	Melsen	Gent	low	high and low
P09	Atembeke	Gent	low	high and low
P10	Antwerpen	Antwerpen	high	high and low
P11	Sint-Niklaas	Antwerpen	high	high and low
P12	Mechelen	Antwerpen	high	high and low
P13	Brasschaat	Antwerpen	intermediate	high and low
P14	Lint	Antwerpen	intermediate	high and low
P15	Ruisbroek	Antwerpen	intermediate	high and low
P16	Pulderbos	Antwerpen	low	high and low
P17	Herenthout	Antwerpen	low	high and low
P18	Bornem	Antwerpen	low	high and low
P19	Leuven	Leuven	high	high and low
P20	Brussel	Leuven	high	high and low
P21	Tienen	Leuven	high	high and low
P22	Wezemaal	Leuven	intermediate	high and low
P23	Kortenbergh	Leuven	intermediate	high and low
P24	Overijse	Leuven	intermediate	high and low
P25	Houwaart	Leuven	low	high and low
P26	Kerkom	Leuven	low	high and low
P27	Sint-Joris-Weert	Leuven	low	high and low

10.2 Overview of data on proportions

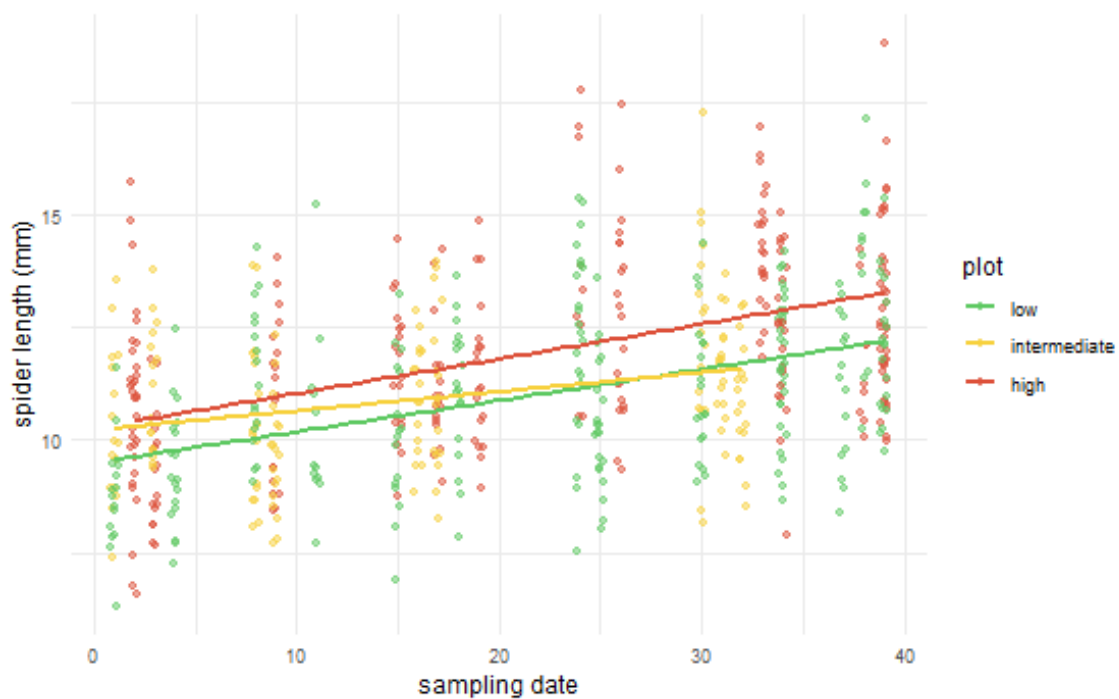
plot_id	subplot_id	immediate	assess	Flee	Total	Prop_immediate	Prop_other	Plot	subplot
P01SG	P01	4	0	1	5	0.8	0.2	high	low
P01SR	P01	4	1	0	5	0.8	0.2	high	high
P02SG	P02	1	0	4	5	0.2	0.8	high	low
P02SR	P02	5	1	0	6	0.83333333	0.1666667	high	high
P03SG	P03	5	0	2	7	0.71428571	0.2857143	high	low
P03SR	P03	4	1	0	5	0.8	0.2	high	high
P04SG	P04	5	0	0	5	1	0	intermediate	low
P04SR	P04	6	0	0	6	1	0	intermediate	high
P05SG	P05	5	0	0	5	1	0	intermediate	low
P05SR	P05	3	2	0	5	0.6	0.4	intermediate	high
P06SG	P06	2	0	3	5	0.4	0.6	intermediate	low
P06SR	P06	5	0	0	5	1	0	intermediate	high
P07SG	P07	0	0	2	2	0	1	low	low
P07SR	P07	4	1	0	5	0.8	0.2	low	high
P08SG	P08	5	0	1	6	0.83333333	0.1666667	low	low
P08SR	P08	4	0	1	5	0.8	0.2	low	high
P09SG	P09	4	0	2	6	0.66666667	0.33333333	low	low
P09SR	P09	4	0	1	5	0.8	0.2	low	high
P10SG	P10	4	1	0	5	0.8	0.2	high	low
P10SR	P10	3	1	1	5	0.6	0.4	high	high
P11SG	P11	4	1	1	6	0.66666667	0.33333333	high	low
P11SR	P11	4	1	0	5	0.8	0.2	high	high
P12SG	P12	3	2	1	6	0.5	0.5	high	low
P12SR	P12	2	2	1	5	0.4	0.6	high	high
P13SG	P13	2	1	2	5	0.4	0.6	intermediate	low
P13SR	P13	1	1	3	5	0.6	0.4	intermediate	high
P14SG	P14	3	1	1	5	0.6	0.4	intermediate	low
P14SR	P14	6	0	0	6	1	0	intermediate	high
P15SG	P15	2	0	3	5	0.4	0.6	intermediate	low
P15SR	P15	5	0	2	7	0.71428571	0.2857143	intermediate	high
P16SG	P16	3	0	0	3	1	0	low	low
P16SR	P16	3	0	0	3	1	0	low	high
P17SG	P17	1	3	2	6	0.16666667	0.83333333	low	low
P17SR	P17	4	1	1	6	0.66666667	0.33333333	low	high
P18SG	P18	4	0	1	5	0.8	0.2	low	low
P18SR	P18	3	1	2	6	0.5	0.5	low	high
P19SG	P19	5	1	0	6	0.83333333	0.1666667	high	low
P19SR	P19	5	0	0	5	1	0	high	high
P20SG	P20	3	2	0	5	0.6	0.4	high	low
P20SR	P20	2	0	1	3	0.66666667	0.33333333	high	high
P21SG	P21	0	0	6	6	0	1	high	low
P21SR	P21	3	3	0	6	0.5	0.5	high	high
P22SG	P22	5	0	1	6	0.6	0.4	intermediate	low
P22SR	P22	3	1	1	5	0.6	0.4	intermediate	high
P23SG	P23	5	0	0	5	1	0	intermediate	low
P23SR	P23	4	0	1	5	0.8	0.2	intermediate	high
P24SG	P24	3	1	1	5	0.6	0.4	intermediate	low
P24SR	P24	4	1	0	5	0.8	0.2	intermediate	high
P25SG	P25	5	0	0	5	1	0	low	low
P25SR	P25	5	0	0	5	1	0	low	high
P26SG	P26	1	2	1	4	0.25	0.75	low	low
P26SR	P26	5	0	0	5	1	0	low	high
P27SG	P27	5	2	0	7	0.71428571	0.2857143	low	low
P27SR	P27	4	1	0	5	0.8	0.2	low	high

10.3 Additional results

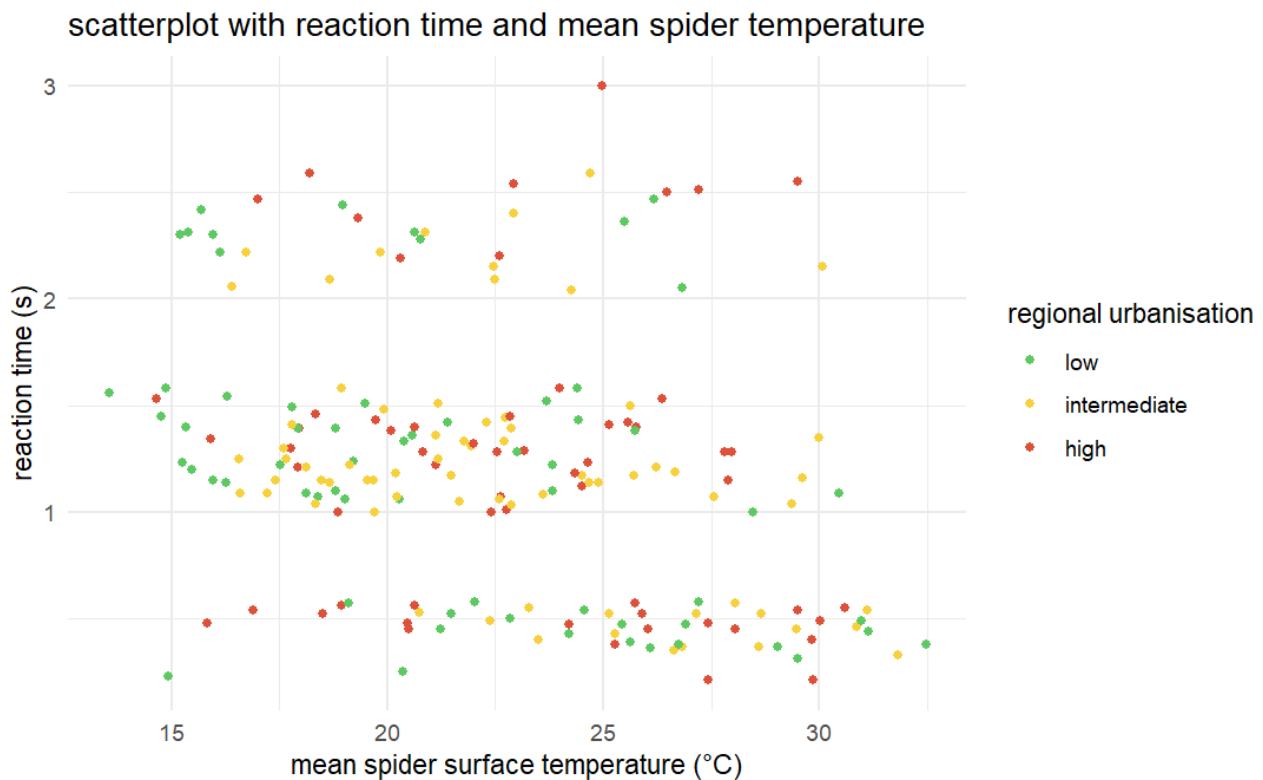
10.3.1 Correlation matrix

	old_time	new_time	web_length	mean_T	mean_T_local	spider_length	dummy_date
old_time	1	0.66	0.04	-0.14	-0.1	0.15	0.06
new_time	0.66	1	0.11	-0.06	-0.03	0.12	-0.03
web_length	0.04	0.11	1	-0.23	-0.22	0.41	0.28
mean_T	-0.14	-0.06	-0.23	1	0.94	-0.33	-0.76
mean_T_local	-0.1	-0.03	-0.22	0.94	1	-0.35	-0.78
spider_length	0.15	0.12	0.41	-0.33	-0.35	1	0.51
dummy_date	0.06	-0.03	0.28	-0.76	-0.78	0.51	1

10.3.2 Visual representation of temporal effect



10.3.3 Scatterplot mean spider surface temperature and reaction time



10.3.4 Goodness of fit spider length

```
## Models:
## length.1: spider_length ~ plot + subplot + (1 | plot_id:subplot_id)
## length.null: spider_length ~ plot + subplot + sdate + (1 | plot_id:subplot_id)
##      npar  AIC   BIC logLik deviance Chisq Df Pr(>Chisq)
## length.1    6 2561.9 2588.5 -1275.0  2549.9
## length.null  7 2537.0 2568.1 -1261.5  2523.0 26.881  1 2.164e-07 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
## Data: final_length
## Models:
## length.1: spider_length ~ plot + subplot + (1 | plot_id:subplot_id)
## length.2: spider_length ~ plot * subplot + sdate + (1 | plot_id:subplot_id)
##      npar  AIC   BIC logLik deviance Chisq Df Pr(>Chisq)
## length.1    6 2561.9 2588.5 -1275.0  2549.9
## length.2    9 2530.9 2570.8 -1256.4  2512.9 37.028  3 4.538e-08 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
## Data: final_length
## Models:
```

```
## length.2: spider_length ~ plot * subplot + sdate + (1 | plot_id:subplot_id)
## length.3: spider_length ~ plot * subplot * sdate + (1 | plot_id:subplot_id)
##      npar  AIC   BIC logLik deviance Chisq Df Pr(>Chisq)
## length.2   9 2530.9 2570.8 -1256.4  2512.9
## length.3  14 2528.4 2590.6 -1250.2  2500.4 12.478  5  0.02879 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

10.3.5 Goodness of fit web length

```
## Data: final_data
## Models:
## web.null: web_lengte_onderaan ~ plot + subplot + (1 | plot_id:subplot_id)
## web.1: web_lengte_onderaan ~ plot * subplot + (1 | plot_id:subplot_id)
##      npar  AIC   BIC logLik deviance Chisq Df Pr(>Chisq)
## web.null   6 1326.4 1347.6 -657.20  1314.4
## web.1      8 1329.6 1357.9 -656.78  1313.6 0.8273  2  0.6612
```

10.3.6 Goodness of fit reaction time

```
## Data: data_immediate
## Models:
## time.null: time ~ plot + subplot + (1 | plot_id:subplot_id)
## time.1: time ~ plot * subplot + (1 | plot_id:subplot_id)
##      npar  AIC   BIC logLik deviance Chisq Df Pr(>Chisq)
## time.null   6 384.24 403.91 -186.12  372.24
## time.1      8 388.18 414.41 -186.09  372.18 0.0547  2  0.973
```

10.3.7 Goodness of fit velocity

```
## Data: snelheid
## Models:
## velocity.null: velocity ~ plot + subplot + (1 | plot_id:subplot_id)
## velocity.1: velocity ~ plot * subplot + (1 | plot_id:subplot_id)
##      npar  AIC   BIC logLik deviance Chisq Df Pr(>Chisq)
## velocity.null   6 2037.1 2058.7 -1012.5  2025.1
## velocity.1      8 2041.0 2069.9 -1012.5  2025.0 0.0914  2  0.9553
```

10.3.8 Goodness of proportions

```
## Data: final_data
## Models:
## binomial.null: reaction_dummy ~ plot + subplot + (1 | plot_id:subplot_id)
## binomial.lme: reaction_dummy ~ plot + subplot + plot:subplot + (1 | plot_id:subplot_id)
##      npar  AIC   BIC logLik deviance Chisq Df Pr(>Chisq)
## binomial.null   5 340.18 358.36 -165.09  330.18
## binomial.lme    7 343.98 369.42 -164.99  329.98 0.207  2  0.9017
```

10.3.9 Goodness of fit treatments

```
## Data: weight_dif
## Models:
## model: difference ~ temp_regime * food_regime + (1 | motherID)
## model.null: difference ~ temp_regime * food_regime + mother + (1 | motherID)
##      npar  AIC   BIC logLik deviance Chisq Df Pr(>Chisq)
## model      6 1850.5 1871.1 -919.25  1838.5
## model.null  7 1852.4 1876.4 -919.19  1838.4 0.1167 1    0.7326
```

10.3.10 Output non-mix vs mixed

```
## Data: both_weights
## Models:
## model: weight_end ~ temp_regime * food_regime
## model_random: weight_end ~ temp_regime * food_regime + (1 | motherID)
##      npar  AIC   BIC logLik deviance Chisq Df Pr(>Chisq)
## model      5 1926.3 1943.5 -958.15  1916.3
## model_random  6 1914.8 1935.4 -951.41  1902.8 13.492 1 0.0002396 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

10.3.11 Observations for each mother

motherID	observations
COUP07	39
P11SRE02	50
P12SRE01	17
P12SRE03	22
P17SG09	20
P18SGE03	17
P25SGE07	9
P25SGE08	19
P26SG01	26
P27SGE14	26