Effects of urbanization and diet on the microbiota of the western black widow (*Latrodectus hesperus*)

# Abstract

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**Potential journals**

Microbial ecology journals :

* Microbial ecology
* Animal microbiome
* Molecular ecology
* ISME journal

Conservation/urban ecology journals :

* Biological conservation?
* Urban ecosystems
* Journal of urban ecology

General ecology journals :

* Oikos?

Keywords : arthropods, microbiome, microbial communities, urban ecology

# Introduction

Human-induced environmental modifications are major drivers of predator and prey species assemblages and interactions (Faeth et al. 2005, Fischer et al. 2012, Green et al. 2022, Theodorou 2022). In urban and natural systems, predatory arthropods are essential components of local food webs and provide important ecosystem services (Schmitz 2008, Burkman and Gardiner 2014, Nyffeler and Birkhofer 2017, Korányi et al. 2021). However, urbanization is increasingly associated with a massive loss of predatory arthropods across the globe due to habitat loss, changes in habitat structure, chemical pollutants, or shifts in prey availability and quality (Vergnes et al. 2014, Seibold et al. 2019, Theodorou 2022, Vasconcelos et al. 2022). Together or individually, these effects may impair the capacity of predatory arthropods to survive in urbanized environments. Yet some predatory arthropod species can thrive and become dominant in urban and semi-urban habitats (Shochat et al. 2004, Nagy et al. 2018, Schraft et al. 2021), but the mechanisms enabling their success in these environments remain unclear.

It is now recognized that the microorganisms inhabiting arthropod hosts (i.e. microbiota) are crucial for host immune function, food digestion, nutrient acquisition, and fitness (Dillon and Dillon 2004, Zilber-Rosenberg and Rosenberg 2008, Brune and Ohkuma 2011, Engel and Moran 2013). The microbiota composition of arthropods has been shown to be majorly driven by landscape components and local habitat (Moro et al. 2011, Tiede et al. 2017, Bosmans et al. 2018, Bennett et al. 2019), as well as the host’s diet (Hammer et al. 2017, Ng et al. 2018, Kennedy et al. 2020, Dion-Phénix et al. 2021). Moreover, field and laboratory experiments show that the microbial diversity of predatory arthropods hosts is positively linked to the local biodiversity of prey (Schmid et al. 2015, Tiede et al. 2017). Together, these examples highlight how arthropod micriobiota is highly dependent on external environmental factors. Because the microbiota of arthropods may be key for their survival and reproduction in a changing world, it is thus crucial that we quantify how urbanization influences its structure (Bahrndorff et al. 2016, Speer et al. 2020). This is a particularly pressing need for predatory arthropods because of their important role in population control and ecosystem function (Hurd and Eisenberg 1990, Moran and Hurd 1997, Schmitz 2007, 2008).

While the importance of the microbiota for arthropods has been established (particularly in insects), much less is known about its structure and function in top predatory arthropods such as spiders (Kennedy et al. 2020). To fill this gap, we used the western black widow (*Latrodectus hesperus*) to test how urbanization and prey diet shape their micriobiota. Western black widows are sedentary generalist predatory spiders that inhabit both desertic and urban environments. They often nest on human-built structures, and are found at higher densities in urban habitats (0.28 individuals ) compared to desert habitats (0.006 individuals ) (Johnson et al. 2011, Trubl et al. 2012). Interestingly, urban spiders tend to be heavier than desert spiders, which is hypothesized to be related to a higher availability of prey in urban habitats (Trubl et al. 2012, Johnson et al. 2017). This may indicate potential local adaptation of black widow populations to urban environments. Western black widows use their web as a hunting tool to capture prey including beetles, crickets, cockroaches, isopods, as well as other spiders and even mammals (Schraft et al. 2021). Therefore, their webs can be used as a biomonitoring tool to infer on the pool of prey species present in the spider’s local habitat (Xu et al. 2015, Blake et al. 2016, Gregorič et al. 2022). In addition, comparing the taxonomic profiles of webs with the microbiota of spiders may help in uncovering whether they have a generally core resident, transient, or environmentally integrated microbiota (Kennedy et al. 2020).

In this study, we combine field and laboratory experiments to evaluate the impacts of urbanization and diet on microbiota communities of western black widows. First, we report on a field study, where we compared spiders collected from two desert sites (natural) and two sites located in the city of Tucson, Arizona (urban). Our objective is to characterize and compare the microbial communities of spiders and their webs according to their habitat. We hypothesize that the diversity and structure of the bacterial communities of spiders and their webs will differ between the two habitats. Because prey diversity should be lower in cities, we predict that spider and web microbial diversity will be higher for samples collected in the desert. We expect greater variation in microbial diversity in the desert compared to city sites, which should be more homogeneous. Moreover, because black widows use their webs to capture their prey, there should be an important overlap between the microbes found on the web and those on the spiders. Second, we report on a laboratory experiment where we analysed the effect of diet (i.e. crickets vs isopods) on the diversity of the western black widow’s gut microbiome, specifically. Spiders on the same diet should have similar microbiomes. A diet composed of crickets should ellicit a greater abundance and diversity since crickets are omnivores with a high protein and fruit/vegetable diet (Ng et al. 2018). A diet composed of isopods should ellicit a lower diversity of microorganisms since isopods were fed carrots exclusively.

# Materials and methods

## Study system : The western black widow spider (*Latrodectus hesperus*)

Western black widows (Latrodectus hesperus) will be used as a biological model to assess the impact of diets on the structure of their gut microbiome. L. hesperus occurs in cities and desert habitats from Mexico to western Canada. It is a polyphagous predatory species that feeds on a wide variety of prey. They are considered generalist predators. Mature females are mostly solitary and sedentary. Indeed, it is a predatory arthropod that has a predation strategy based on the creation of a 3D web that is defined by a sit and wait mode. They would tend to stay on their web for a long time and feed locally on the prey that falls on their web (Dunaj, Bettencourt, Garb, et al. ,2020). The web would also be an interesting bio indicator that could inform on the nature of prey, predators and bacteria. Indeed, the web is a rich microbial environment that could provide information about the ecosystem and available prey.

Predatory arthropods have a relatively simple gut and the diversity of their microbiome seems more likely to be affected by their diet. Black widow spiders (genus Latrodectus) are therefore a particularly suitable system to understand how microbes influence spider evolution The black widows used in the experiment come from a wild population collected and were reared and maintained in the laboratory under standardized conditions for a minimum of 3 years. The individuals were isolated and exposed to the same conditions of temperature (24°C ± 2°C), humidity (40% RH) and a day-night cycle of 12h-12h. Non-experimental individuals were fed twice a month with a live cricket.

## Methods for the field study

### Field sites

*Here, we need to describe the field conditions, temperature, precipitation, land cover, blablabla. Also provide a map of the field sites*

vicinity of and outside the city of Tucson, Arizona (USA). These specimens were distributed among 4 different sites, including 2 desert wilderness sites (Chaos Canyon (CC) and Dove Mountain (DM)) and 2 urban sites (University of Arizona Campus (UA) and a Lowe’s (LO) parking lot).

### Field data collection

During the spring of 2020, 14 black widow specimens were captured in the field sites described above. *say how many spiders where*. The web of each spider was also collected under relatively sterile conditions (latex gloves and alcohol) with a Falcon tube and a disinfected metal rod. The 14 individuals and their respective webs were identified and transported alive to be stored at -20° Celsius. The samples were then placed in a cooler with dry ice and sent by mail to the laboratory facilities at *UQAM*. The samples were placed on ice at irregular intervals, though uniformly for each sampling location, during initial storage, transportation, and final storage at UQAM facilities.

## Methods for the diet experiment

### Feeding bioassay

In a laboratory feeding experiment, we tested whether a single meal has the potential to alter the gut bacterial community of black widows. The sample size of the experiment consisted of 12 black widows. Each spider was randomly assigned to one of the following treatments: (1) no food (control, N = 4), (2) a meal consisting of *Gryllodes sigillatus* (cricket diet, N = 4) exclusively, and (3) a meal consisting of *Porcellionides pruinosus* (isopod diet, N = 4) exclusively. Crickets and isopods are within the range of potential natural prey of black widows. It has been demonstrated that isopods are present in cities (*sources?*). Isopods are nutrient-poor and contain low protein. They are however very rich in several minerals including calcium. In contrast, crickets are one of the common preys of laboratory-reared spiders. They consist of a lipid and protein-rich food that is beneficial to spiders, and they generally have a low calcium/phosphorus ratio (*source*).

Both prey were raised in a controlled and ethical farm located on the south shore of Montreal, Quebec, Canada (*which farm*). The crickets were housed in ventilated containers maintained at room temperature. They are fed regularly with an assortment of vegetables, pet food, and plants. Isopods were kept in their container in a humid culture environment at room temperature. They were fed with carrots and vegetables about twice a week.

We first transferred each individual spider into a cardboard support where they built their web for 5 days. Following this period, the black widows were assigned to one of the three feeding groups. We then provided prey weekly to maintain spiders at their satiation level. We maintained the spiders’ diets for a period of 6 weeks. In the control group, the spiders were not fed for 6 weeks.

*ne pas oublier de dire qu’on les a pesé*

## DNA extraction and illumina sequencing

The analysis of intestinal bacteria was performed on dissected digestive tracts and the lipid profile. Each spider was transferred to a plastic jar with a lid to be anesthetized by introducing CO2 for 2 to 5 minutes than by freezing in a -20°C freezer. The samples were then rinsed in three steps. I performed an initial rinse with sterile pure water for 1 minute, followed by a second rinse with 70% ethanol for 5 minutes, and then a series of three more rinses times with sterile pure water. The hindgut of each individual was dissected in 0.1 M Sodium Chloride, 0.015 M Sodium Citrate, 0.1% Diethyl Pyrocarbonate sterile solution with sterile forceps under a binocular loupe, placed in 1.5 ml microcentrifuge tubes, washed three times with sterile water. All manipulations were performed under flame and the equipment sterilized at each use. Spiders being sacrificed, then stored at -20°C until DNA was extracted. the dissection was done in the same day of their euthanasia.

An external sterilization of the cuticle was performed before the bacterial DNA extractions for each spider to avoid any contamination to the internal microbiota. The specimens were first placed in a Falcon tube containing ultrapure water and then subjected to the sonicator for 60 sec. They were then immersed in 95% ethanol and vortexed 30 sec. The same step follows with 75% ethanol. Afterwards, the spiders are placed in a sterile mortar, then crushed to extract the inner liquids with a filter pipette and added to the solution C1 of the DNeasy® PowerSoil® kit (QIAGEN), which will be used for all DNA extractions.

It is therefore the complete internal environment (fatty masses, cephalothorax, intestinal tract, ovaries, legs, etc. except for the cuticles) which is evaluated for its microbiota of spider on the field. The respective webs were first dissected and cut under the hood or Bunsen flame with knives with sterile knives and tweezers and then incorporated into solution C1 of the DNeasy® PowerSoil® kit (QIAGEN) in order to analyze their surfaces for bacteria. For spiders used in the feeding bioessay, after 6 weeks of a strict diet, 4 individuals per group were sacrificed in order to collect the digestive tract and the lipidic profile to extract the microbial DNA. The dissections were performed 72h after their last meal. Each spider was sterilized and then dissected under a binocular light microscope before used the DNeasy® PowerSoil® kit (QIAGEN). A PCR was performed with the bacterial DNA extracted with the PCR Master Mix (QIAGEN) as well as the buffer and amplifying solution from Invitrogen company.

À VALIDER Bactérie Tm = 57°C Eucaryotes Tm = 61°C Bacteria with 5uL DNA and Eucaryote 2uL DNA Polymerase : Phusion Hot Start II High-Fidelity

À ajouter : - Gène 16S et du 18S pour les eukaryotes - Quelles régions de ces gènes - Primers - Plateforme d’analyse (CERMO-FC, UQAM) - Negative controls - problèmes liés à la COVID pour les métadonnées des toiles/etc

## 16S rRNA gene sequence processing

List of things to say: - dada2 - packages and versions - reference databases - rarefaction for webs

## Statistical analyses

Process : - compare species richness between env - compare species richness between diets

* compare beta diversity using copula models for spiders
* compare beta diversity using NMDS for webs
* Compare the taxa with barplots?

# Results

# Discussion

*Our study provides a better understanding of the impacts of urbanization on the microbial ecology of L. hesperus in Arizona. It will also help determine how prey encountered by black widows shape the structure of their microbiome.*

* *Voir intro de Hammer parlant de similarités entre différentes diètes. Idem pour les problèmes méthodologiques*
* *Voir Kennedy pour effet de la diète chez des araignées et que le microbiote semble transiant + que résident*
* *Voir Hammer et al. pour des éléments de discussion sur les espèces avec peu de microorganismes*
* *Voir Bahrndorff pour implications en conservation*

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