Chapter 5
Spider Welfare

Simona Kralj-Fišer and Matjaž Gregorič

Abstract Spiders with around 48,000 recorded species are major terrestrial predators and thus crucially important for ecosystem functioning. They are widely used as research models and for biodiversity displays and sometimes also kept as pets. Nevertheless, we are not aware of any legal ethical rules bound to spider welfare during rearing or research. To set ethical standards, we first need to detect and assess how spiders "perceive" the external world. Based on the current knowledge of spiders' sensory and nervous system, it is difficult to judge whether spiders feel pain, distress and suffering, although their behaviours like thanatosis, "bailing out", autotomy and associative avoidance learning imply so. As is now known, arthropods are not simply mini-robots as traditionally believed. Thus, spider welfare deserves more research effort, and the ethical standards for rearing or using spiders in research need to be set. Here, we describe the variety of spider physiological and behavioural characteristics and how they apply to their rearing, housing, handling and experimental use. We hope reporting these methods will help ensuring welfare and well-being of spiders in captivity.

5.1 Introduction

Spiders (order Araneae, class Arachnida) are one of the most diverse animal groups on the planet, currently with more than 48,000 described species (World Spider Catalog 2018). They rank seventh in global diversity, only surpassed by mites and ticks (Acari) among arachnids (Coddington and Levi 1991). Spiders have occupied all terrestrial ecosystems (Foelix 2011), are the most important predators in prey biomass consumption (Nyffeler and Birkhofer 2017) and thus have a crucial role in ecosystem functioning. Among their most characteristic features are hunting using venom and the production of silk, nature's toughest fibre (Foelix 2011). For millennia, spiders have been a part of human imagination, mythology and art,

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S. Kralj-Fišer · M. Gregorič (✉)
Institute of Biology, Scientific Research Centre of the Slovenian Academy of Sciences and Arts, Ljubljana, Slovenia
e-mail: MatjazGr@zrc-sazu.si

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symbolizing patience, mischief and malice. They are widely used as research models in diverse fields, for biodiversity displays, and sometimes also kept as pets. Yet, arthropods including spiders are traditionally considered mini-robots that lack flexibility (Herberstein 2011). Consequently, professional standards securing spider welfare in captivity are not clearly established.

To set such standards, we should first address issues of the ability of spiders to feel pain, distress and suffering. In the aim of comprehending the "inner world" of spiders, we describe below their sensory and notably complex nervous systems, which are needed to receive and perceive external stimuli. While spiders lack higher nerve centres and thus should only be capable of reflexive behavioural responses to dangerous stimuli (nociception), their behavioural and physiological responses imply their potential to feel pain and stress. Spiders, for example, exhibit behaviours like thanatosis, "bailing out" and autotomy when in danger. The results of several studies further imply that spiders thereby activate their stress (octopamine) system (Punzo and Punzo 2001; Jones et al. 2011). Several species are also able to modify their behaviour depending on their previous experiences and exhibit associative avoidance learning in response to previous experiences with predators.

There is thus plenty of evidence that invertebrates, including spiders, are not just instinct driven and inflexible in their behaviour; rather they show behavioural plasticity and cognitive abilities, such as attentional priming and memory (Jakob et al. 2011). This evidence should not be ignored and needs to be used while establishing the guidelines for securing their welfare. The responsibilities of researchers to take care for study animals include the experimental procedures and also providing suitable conditions at which spiders are bred or kept when not being studied. To maximize welfare, the housing of spiders should incorporate as many of the important natural living conditions as possible. Also, some research fields can hardly avoid sacrificing individuals. Many scientists use ethanol, freezing or CO_2 as a method of spider euthanasia, but it is has not been tested whether these methods indeed induce analgesia. Yet the instant death at $-60\ ^\circ C$ might be more humane than several minutes long drowning in ethanol—the method still widely used in research. In the following chapter, we aim at presenting an overview of welfare considerations when keeping and experimenting on spiders in the laboratory.

5.2 Sensory and Nervous System

The spiders' main sensory organs are eyes, lyriform organs, trichobothria and chemosensory organs (Barth 2013). The sensitivity and distribution of the sensory organs vary among taxa and largely reflect a spider's life style. For example, the visual ability is much better developed in cursorial spiders compared to web-building spiders, which mainly rely on their vibratory senses. While most web-building spiders can only detect the direction of light and motion, cursorial spiders are capable of forming images. Jumping spiders have exceptional eye sight adapted for colour vision and high spatial acuity (Blest et al. 1981).

The mechanical senses of spiders involve the specialized hairs—trichobothria and slit sensillae (slits in the exoskeleton) that detect acoustic, vibratory and tactile cues. Vibratory cues transmitted through environmental surfaces, including silk threads, are among the most important information sources for spiders. Vibrations inform them about the presence of prey, mates, parasites and predators. Furthermore, spiders commonly use vibrations as an intraspecies communication channel, which is particularly important in mate recognition and mate assessment (Uetz and Roberts 2002). Trichobothria that detect airborne vibrations cover the legs and pedipalps, slit sensillae that detect substrate-borne vibration are distributed over most of the body surface, and are most common on legs (Barth 2013). Legs and pedipalps are also covered by chemosensitive hairs enabling them to recognize conspecifics, prey and predators. Besides the sensory functions of thousands of innervated hairs covering the spider body, some serve other functions, such as adhesion to the substrate, combing out silk and cleaning (Foelix 2011).

Sensory organs are innervated. Their axons form small bundles and join into sensory nerves that conduct the sensory information to the central nervous system (CNS). The CNS of spiders is highly compacted and consists of two ganglia with exiting efferent nerves. The syncerebrum, also supraesophageal ganglion, consists of cheliceral ganglia and the brain. The brain receives optic nerves and contains visual and association centres (Foelix 2011). Despite these relatively simple and small CNS, some spiders exhibit remarkably complex behaviours. For example, some species are able to improve their prey capture technique with experience (e.g. Edwards and Jackson 1994), learn to avoid "dangerous" prey (e.g. Higgins 2007), adjust their fighting behaviour according to their previous experiences (e.g. Whitehouse 1997), etc.

Sensory hairs are extremely sensitive; touching a single hair causes a spider to escape or counterattack (Foelix 2011). Given the sensitivity and importance of their sensory organs, spiders should always be handled with care in order to prevent damaging sensory organs and overstimulating the animals. The overstimulation of mechanoreceptors can be avoided by keeping spiders in rooms with minimal vibrational stimuli, either airborne (wind, music) or substrate borne (machines causing vibrations). Similarly, routine tasks like cleaning the enclosures and feeding should be done quickly and with minimal disturbance. Also, spiders should not be handled manually, rather, we recommend using a soft brush.

5.3 The Ability to Feel Pain, Distress and Suffering

Researchers are commonly concerned about the welfare of their model animals. An animal's ability to feel pain, distress and suffering is often judged by the size and complexity of its nervous system and/or complexity of its behaviour (Mather 2011). Almost all animals with a nervous system can detect dangerous stimuli and will withdraw when stimulated. In other words, they exhibit nociception, the capacity to respond to aversive stimuli with activation of sensory and motor pathways.

Activation of the latter usually results in a reflexive behavioural response. Reflexive withdrawal may be mediated by simple sensory-motor pathways without involvement of higher processing centres. In this view, spiders, lacking higher nerve centres, should only be capable of reflexive behavioural responses to dangerous stimuli.

Thereby, it is more difficult to judge whether spiders experience pain, i.e. "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (Merskey and Bogduk 1994). While nociception occurs with or without conscious sensation, the definition of pain requires an emotional experience and thus consciousness. In vertebrates, the cognitive-emotional component of pain, or simply feeling and awareness of pain, involves higher brain structures, e.g. the limbic system, and processes. In this view, we could conclude that the spiders' nervous system precludes them to experience any kind of mental state. However, the spiders' neural architecture is not fully understood (Jakob et al. 2011). Also, arthropods in general might possess different neurobiological mechanisms for experiencing pain than vertebrates. In any way, the lack of knowledge should not be an excuse to handle spiders in a way that activates their nociceptive pathways.

Recently, additional criteria that should be fulfilled to accept potential pain experience have been proposed (Elwood 2011; Sneddon et al. 2014). Sneddon et al. (2014) stated that responses to noxious, potentially painful events should affect neurobiology, physiology and behaviour in a different manner to innocuous stimuli, and subsequent behaviour should be modified including avoidance learning and protective responses. In addition, animals should show a change in motivational state after experiencing a painful event such that future behavioural decision-making is altered and can be measured as a change in conditioned place preference, self-administration of analgesia, paying a cost to access analgesia or avoidance of painful stimuli and reduced performance in concurrent events.

Following these criteria, we will examine the below features indicating the potential for experiencing pain in spiders:

1. Nociceptors and central nervous system
2. Physiological changes to noxious stimuli
3. Protective motor reactions that might include reduced use of an affected area such as limping, rubbing, holding or autotomy
4. Avoidance learning
5. Cognitive abilities

5.3.1 Nociceptors and Central Nervous System

There is not much known about the presence of nociceptors in spiders and how nociceptive information is processed within their central nervous system. Nevertheless, spiders exhibit withdrawal or escape behaviours when exposed to noxious stimuli. They may also exhibit behaviours that may not simply be nociceptive reflexes; e.g. jumping (Suter and Gruenwald 2000), shaking web (Jackson et al. 1992; Kralj-Fišer et al. 2011) and autotomy (Eisner and Camazine 1983; Punzo 1997). Their antipredator behaviour includes thanatosis (feigning death—this behaviour is characterized by curling legs and freezing, resulting in a body posture very similar to that of a dead spider) (Bilde et al. 2006; Kralj-Fišer and Schneider 2012) and "bailing out", in which the spider drops from the web and hangs motionless from a dragline with huddled legs (Uetz et al. 2002).

5.3.2 Physiological Changes to Noxious Stimuli

Spiders detect predators by airborne vibrations stimulating their trichobothria (Foelix 2011). The increased sensitivity of trichobothria to air movements relates to increased octopamine (OA) levels (Widmer 2005). Octopamine in arthropods is considered analogous to the vertebrate norepinephrine, indicating its role in the stress system (Roeder 1999). Jones et al. (2011) experimentally elevated levels of OA and serotonin (5-HT) in the orb web spider, *Larinioides cornutus*, and observed that increased OA levels relate to decreased durations of thanatosis, while elevated 5-HT had the opposite effect (Jones et al. 2011). The elevated 5-HT likely makes the spiders more fearful and less aggressive, whereas elevated OA likely relates to increased arousal (Jones et al. 2011). The reductions of 5-HT and OA levels after agonistic encounters have been observed in the brain of male bird spiders, with subordinate males exhibiting lower levels than dominant ones (Punzo and Punzo 2001). These findings suggest that the activation of the stress system in response to aversive stimuli may be comparable to the one in vertebrates.

5.3.3 Protective Motor Reactions

Spiders regularly autotomize their legs when in dangerous situation, e.g. grasped by a predator or a conspecific during fight, in order to escape more easily (Punzo 1997; Foelix 2011). Furthermore, they self-amputate injured appendages (Kralj-Fišer et al. 2011; Kralj-Fišer and Kuntner 2012; Kuntner et al. 2014), and they "lick" or rub their wounds. Missing appendages may negatively affect development, web building, foraging success, competitive abilities and mating success in some species, whereas in several species it has no apparent costs (reviewed in Fleming et al. 2007).

Eisner and Camazine (1983) conducted an experiment that suggests a concordance between pain effects in humans and autotomy in spiders. They observed *Argiope* spiders that were stung in the leg by bees and wasps to undergo leg autotomy. They experimentally penetrated the leg-joint with a sterile pin and injected spiders with several venom components known to elicit pain in humans. Spiders employed autotomy when injected with histamine, serotonin, phospholipase and melittin, all of which induce pain in humans. Acetylcholine and bradykinin, which also induce pain in humans, did not cause the autotomy in spiders. However, injection of hyaluridase, adrenaline and dopamine, which do not induce pain in humans, also failed to be effective in causing autotomy in *Argiope*.

5.3.4 Avoidance Learning

Avoidance learning is the process by which an individual learns to avoid unpleasant situations on the basis of prior experience. There is abundant evidence for avoidance learning in spiders. For example, the wolf spider *Schizocosa avida* exhibits associative learning in response to previous experience with a predator (Punzo 1997). Individuals of the same species that have survived a scorpion attack by leg autotomy learn to avoid scorpion-scented substrates (Punzo 1997).

Several spiders are able to develop aversion to toxic and/or unpalatable prey (e.g. Edwards and Jackson 1994; Toft and Wise 1999; Skow and Jakob 2006; Hénaut et al. 2014). Furthermore, jumping spiders exhibit avoidance of visual stimuli associated with heat (Nakamura and Yamashita 2000), electric shock (Bednarski et al. 2012; Peckmezian and Taylor 2015) or vibration (Long et al. 2015). For example, jumping spiders of the species *Hasarius adansoni* were trained to associate heat with coloured papers. These spiders learned to avoid colours which were associated with high temperature, suggesting that they are not just able to learn to avoid heat but also to associate it with colour (Nakamura and Yamashita 2000). Thus, spiders are likely able to learn and take the appropriate action to avoid or reduce potential damage on the basis of prior experience with noxious stimuli; such a response is not the fixed, reflexive action of nociceptive avoidance.

5.3.5 Cognitive Abilities

There is ample of evidence indicating spiders' ability to learn. Several species are able to (adaptively) modify their behaviour in the contexts of foraging (e.g. Wilcox and Jackson 1993) and web building (e.g. Heiling and Herberstein 1999; Venner et al. 2000), intraspecific interactions (e.g. Taylor and Jackson 2003; Kasumovic et al. 2009), spatial learning and navigation (Tarsitano and Jackson 1997; Jakob et al. 2007). Some spiders even exhibit reversal learning (Liedtke and Schneider 2014). For a more exhausted literature on evidence for behavioural plasticity, learning, memory and other cognitive abilities in spiders, see reviews by Jackson and Cross (2011), Jakob et al. (2011), Nelson and Jackson (2011) and Japyassú and Laland (2017).

5.4 Keeping Spiders in Captivity

To secure the well-being of animals in captivity, we have to satisfy their general needs, e.g. ready access to fresh water; a balanced diet; appropriate housing; prevention from pain, injury or disease; and enabling an environment to express normal behaviours and ensure conditions and treatments causing no/little fear and stress. Ethical guidelines for research in vertebrates follow the “three Rs” (3Rs) principle, i.e. the replacement, reduction and refinement criteria (Russell and Burch 1959). While the latter two should be applied to spiders, the first likely does not.

Replacement generally refers to replacing animals with “non-sentient material” (Russell and Burch 1959), e.g. plants, microorganisms, animals with limited nervous and sensory systems, tissue cultures and computer models (Tannenbaum and Bennett 2015). Similarly, “lower” animals like arthropods are considered a replacement for “higher” ones, e.g. mammals, with the justification of evolutionary conservation of physiological processes allowing for application across animal groups (Horvath et al. 2013). Replacing arthropods for vertebrates might be argued for in some contexts, e.g. when experimental procedures involve trauma, and keeping a large number of experimental animals. However, arthropods and thus spiders can hardly be replaced by other animals, especially considering the gaps in our knowledge in these “lower” animal groups. These same knowledge gaps currently render replacing spiders with other invertebrates, tissue cultures and computer models impossible. The reduction and refinement principles apply to animal experiments in general and thus have to be taken into account in spider research. Reduction refers to using the minimal number of animals required to perform a study, where careful experimental and statistical planning is crucial. Refinement generally refers to finding experimental designs that maximize the scientific output while minimizing negative effects on animals, e.g. by planning for potential experimental problems, choosing the least invasive techniques and ensuring proper housing (Animal Behaviour 2018).

Until recently, the welfare of spiders and most other invertebrates in captivity has been neglected. Several invertebrate species have long been used as model organisms in research, to fulfil the “replacement” criterion in animal welfare guidelines for vertebrates (Carere et al. 2011). Do spiders and other invertebrates indeed suffer less and are they more suitable for life in captivity than vertebrates? Can we further ignore the fact that spiders fulfil several criteria that indicate their potential for experiencing pain? Instead, researchers should use the existing knowledge to establish standards for securing their welfare.

The responsibilities of researchers to take care for study animals do not only include the experimental procedures themselves but extend to providing suitable conditions at which spiders are bred or kept when not being studied. To maximize welfare, the housing of spiders should incorporate as many of the important natural living conditions as possible. While the general seasonal parameters, e.g. the day/night regime, temperature and relative humidity, can be mimicked by knowing a spider’s habitat and diurnal cycle, other maintenance parameters are important. Researchers should consider the importance of the size of enclosure, providing

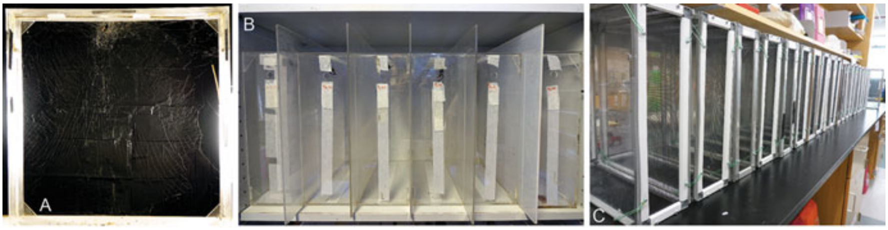

Fig. 5.1 Frame-shaped enclosures allow the construction of two-dimensional orb webs (**a**), can be made from Perspex (**b**), plastic mesh (**c**) or any other suitable material and are suitable for maximizing laboratory space (**b, c**)

biologically relevant enrichment and social interactions among individuals, as well as proper nutrition and euthanasia, all of which are discussed in the following chapters.

5.4.1 Husbandry

Spiders in general can be divided into two basic life style groups, the cursorial and web-building spiders, and within both of these several subcategories can be identified. For example, some cursorial species are completely ground dwelling, while others are arboreal, but most inhabit diverse habitats that cannot be classified into such extremes. Thus, maximizing the enclosure's floor space and climbing space or providing other enrichments is crucial. Similarly, some web-building spiders employ two-dimensional orb webs, while others employ three-dimensional webs. Thus, orb weavers can be efficiently kept in frames, usually piled like books on shelves, maximizing laboratory space and allowing the spiders to build webs (Fig. 5.1). On the other hand, three-dimensional webs demand cubic-shaped enclosures (Fig. 5.2), sometimes with supporting structures allowing web construction (Zschokke and Herberstein 2005).

While it is straightforward that larger spider species need to be housed in larger enclosures, it is sometimes difficult to identify biologically meaningful enrichment features for certain species, especially if their natural history is little known or they have not been previously kept in the laboratory. Web building spiders almost exclusively live on their webs. They either sit in the web itself or construct retreats in or at the side of the web. Thus, it is relatively easy to recognize such biologically meaningful enrichment features while collecting them in the field (e.g. providing shelters or materials for shelters). On the other hand, cursorial spiders are usually housed in simple enclosures, out of practicality often without enrichment features (e.g. easier observation, cleanliness (Jackson 1974). However, it is almost unknown how housing conditions affect cursorial spiders. There is evidence that environmental stimuli can influence behavioural syndromes, and thus laboratory-bred individuals differ from field-caught ones (Sweeney et al. 2013). This might be especially

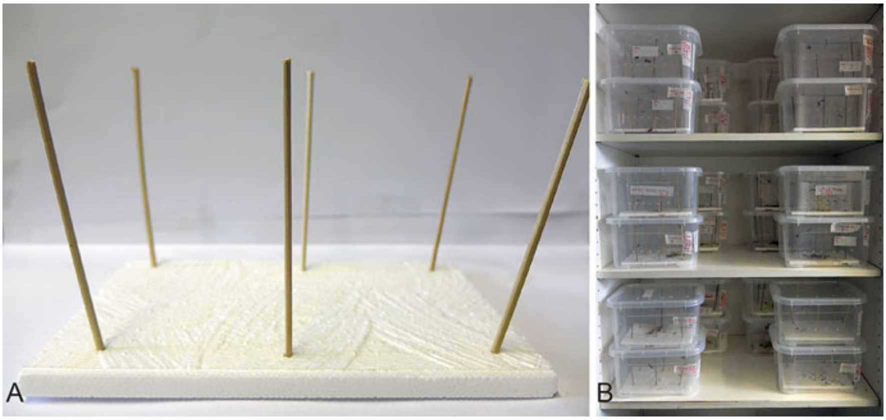

Fig. 5.2 Cubic-shaped enclosures (**b**) with supporting structures (**a**) that allow the construction of three-dimensional cobwebs

important in taxa with well-developed cognitive abilities, e.g. jumping spiders, although little is known about cognitive abilities of most other spider groups. In fact, laboratory raising jumping spiders of the species *Marpissa muscosa* in environmentally deprived, socially enriched or physically enriched enclosures consistently affects their personality traits (Liedtke et al. 2015; see Carere and Maestripieri 2013). Furthermore, recent research demonstrates neuroplasticity in jumping spiders by showing that *M. muscosa* spiders growing up solitary and in physically deprived enclosures have smaller volumes of higher order integrating brain centres compared to spiders growing up with siblings or in physically enriched enclosures (Steinhoff et al. 2018). These brain centres, composed of mushroom bodies and the arcuate body, receive visual and maybe also locomotory information and process it (Steinhoff et al. 2017). Both in vertebrates and arthropods, the variation in cognitive ability, and thus behaviour, seems to be directly influenced by brain size (Amador-Vargas et al. 2015; Benson-Amram et al. 2016; Corral-López et al. 2017; Feinerman and Traniello 2016). This highlights the need for further studies of how laboratory conditions affect the spider nervous system. Moreover, while spiders are generally considered solitary and intolerant of conspecifics, several species do live in aggregations, and some are even (sub)social (Foelix 2011).

Proper housing, be it through providing biologically meaningful enclosure enrichments or social interactions, can thus not only assure the animals' well-being but also minimizes potential factors affecting their behaviour in experiments. Furthermore, spiders are often kept permanently as laboratory model animals. Maintaining a healthy laboratory population can reduce the amount of needed wild-caught animals and allows the researchers to comply with the "reduction" and "refinement" principles of the 3Rs guiding principles for appropriate animal care. Namely, it is critical to choose correct experimental and statistical procedures, as well as planning and conducting experiments in a way to maximize the scientific benefit. Through keeping a predictable laboratory population of model taxa, one can overall reduce the number of spiders in experiments to the lowest number necessary to achieve experimental goals while ensuring the highest quality standards for the kept animals.

5.4.2 Nutrition

Nutrition importantly influences nearly all aspects of an individual's life history. Starvation and low food quality (i.e. nutrient imbalance and/or deficiency) are independent stressful conditions, and spiders can suffer from one or both (Toft 2013). The amount of food affects the rate of growing, adult size, lifespan and fecundity (Yip and Lubin 2016). Generally, smaller amounts of food increase developmental time and sometimes the number of instars, often leading to smaller adult body size, which in females is positively correlated with the number and/or biomass of produced eggs (Jakob and Dingle 1990; Vollrath 1987). Both prolonged developmental time and smaller and fewer eggs can mean heavy fitness penalties (Higgins 1995; Higgins and Goodnight 2011). Unless the experimental procedure demands otherwise, laboratory-kept spiders are generally fed ad libitum because assuring enough prey is not problematic. When providing laboratory-bred insect food, its diversity is usually lower than most natural conditions. Small instar spiders are typically fed with fruit flies or springtails, and larger spiders with house flies, mealworms and crickets. However, although a small food diversity could imply poor nutrition, it is not necessarily so. Inappropriate prey taxa can have an imbalanced nutrient composition or might even contain toxins and deterrents in too high amounts. Furthermore, an imbalanced nutrient composition and toxicity are not intrinsic to food items but properties of the consumer's physiology, i.e. the specific spider species (Toft 2013).

Food quality is best defined with its potential contribution to the fitness of the consumer. Because a certain prey taxon can be appropriate for some, but not other spider species, food quality cannot easily be determined using a chemical analysis but is best determined in fitness performance experiments. In these, the effect of single-prey diets is compared to high-quality control food and starvation (Toft 2013). Tests of multiple prey taxa have shown a continuum of prey qualities, classified into "high-quality", "intermediate-quality", "low-quality", "poor-quality" and "toxic" prey taxa. Only prey classified as high quality allows spiders on a monotypic diet a full life cycle with low mortality and successful reproduction (Toft 2013). Some prey taxa, e.g. aphids, seem to generally be of very low food quality to spiders, while others, e.g. Diptera and Collembola, seem to cover the whole spectrum of the needed nutritional composition and may be classified as high quality for most spider species (Schmidt et al. 2012; Toft and Wise 1999). A logical advice for proper spider nutrition is thus introducing known high-quality prey taxa even when keeping spiders that have not been nutritionally investigated before. Additional food enrichment can be achieved by keeping spiders on a multi-prey diet or by enriching the food of prey. For example, growth media of prey like fruit flies can be enriched with multiple or single nutrients, e.g. protein, lipids and vitamins. Similarly, adding dog food to the growth medium of prey like springtails, fruit flies, house flies and crickets proved successful (Toft 2013).

In recent years, the effects of nutrient composition on physiology and behaviour of spiders are getting better documented. For example, protein addition increases growth and the building of stabilimenta in the orb weaver *Argiope keyserlingi* (Blamires et al. 2009), while it decreases glue stickiness in the orb weaver *Nephila clavipes* and cob web *Latrodectus hesperus* (Blamires et al. 2014). Also, the protein content in food is positively correlated with growth rate in *Pardosa prativaga* (Jensen et al. 2011), but not in *Stegodyphus lineatus*, where lipids promote growth, but protein addition enhances juvenile survival (Salomon et al. 2008; Salomon et al. 2011). Unfortunately, we currently do not fully understand how nutrient demands differ among spider species and how ontogenetic stage and seasonality affect them intraspecifically. As mentioned above, starvation and nutrient imbalance represent independent stress factors (Toft 2013). In the laboratory, one can easily prevent starvation. However, well-fed spiders, i.e. such with a high body condition index (body mass regressed to body size), are not necessarily also receiving a balanced diet (Lomborg and Toft 2009; Wilder and Rypstra 2008), and there is currently no quick and easy way to assess possible nutrient imbalance. Thus, to ensure the functional definition of high-quality nutrition, laboratory-kept spiders need to be offered food that allows a full life cycle with low mortality and successful reproduction.

5.4.3 Disease Monitoring and Disposal

Like all arthropods and most invertebrates, spiders have an open circulatory system, where the haemolymph flows via a closed arterial system and an open venous system (Foelix 2011). Physical injuries are followed by haemolymph loss and expose spiders to microbial infections. Thus, the spider's immune system is composed of haemolymph coagulation and pathogen-defence that is localized in haemocytes. The immune system rapidly reacts to invasion, releasing several compounds into the haemolymph in a matter of minutes to hours, and comprises a clotting cascade to stop haemolymph loss, phagocytosis, the regulation of pathogen-destroying melanin production and the production of antimicrobial peptides (Kuhn-Nentwig and Nentwig 2013). To the best of our knowledge, the effects of diverse laboratory conditions on spider immune system appear unknown. Similarly, disease treatment is not developed, likely in large part because identification of a disease is mostly possible only in its latest stages of conspicuous symptoms or when spider behaviour changes drastically. Researchers thus mostly have to do their best to prevent disease. When choosing enclosure enrichment, it is advisable to clean, dry out or otherwise disinfect any material from nature. Prey should consist of laboratory-bred insects to prevent introducing pathogens or parasites. The enclosures should be kept as clean as possible, and especially when high humidity is necessary, air circulation needs to be ensured to minimize mould. However, the frequency of enclosure cleaning should represent a compromise between the level of required cleanliness and the amount of disturbance imposed to the spider.

Another important consideration is what happens to spiders after they die or after the end of experiments. Spiders that clearly died of disease should be disposed according to local waste handling procedures. On the other hand, it is likely safe to dispose spiders that died naturally as usual biological waste, as research on spiders usually does not include hazards occurring in other fields, e.g. dangerous chemicals (sterilants, disinfectants) and microorganisms, allergens and radiologic agents. If spiders are released after the experiments, they should be released where caught, and in the correct part of season based on their developmental stage. Released spiders should also not be in a state that will harm the natural population (e.g. because of disease or genetic modification).

5.4.4 Anaesthesia and Euthanasia

Due to a lack of research on invertebrate anaesthesia, analgesia and euthanasia, these fields are getting increasingly debated (Bennie et al. 2012; Cooper 2011) and are tightly linked to the issue of how animals perceive stress and pain (see "Spiders' ability to feel pain, distress and suffering"). Anaesthesia in spiders is usually employed to immobilize the animals, in order to facilitate tagging (e.g. for the need of individual recognition in experiments), examination (e.g. to determine the species, check for genital details) and to perform different procedures (e.g. obtain silk or venom). In terrestrial invertebrates, isoflurane (5–10%), sevoflurane, halothane (5–10%) or carbon dioxide (CO_2, 10–20%) are the suggested agents for anaesthesia (Cooper 2011). In our experience, the most common agent in spider laboratories is CO_2. While the suggested concentration of CO_2 is 10–20%, spider laboratories use a wide variety of concentration, always with fast recovery, and without mortality and visible long-term consequences. However, if anaesthetized spiders are used for potentially painful procedures, CO_2 is not advised as it is unknown to what extent, if, any at all, it induces analgesia. In such cases, using isoflurane, halothane or sevoflurane is advised, with isoflurane being the preferred in terrestrial arthropods in general (Cooper 2011) and also successfully employed in spiders (Pizzi 2006). Hypothermia is a traditional anaesthesia method in invertebrate studies but generally of limited use (Cooper 2011) and not advised in spider research. While it might be of use for some noninvasive procedures, it should not be used for invasive ones, and we generally advise against it in arachnids as many seem to not tolerate chilling well and can even die (Pizzi 2006). To avoid complications during anaesthesia, one should avoid employing it in individuals of visibly poor body conditions or otherwise unhealthy.

Some research fields and methods cannot avoid sacrificing a certain number of individuals. If spiders get euthanized, researchers have to state why and describe the method. A "good" death is an inseparable part of a "good" life for all captive animals. A variety of methods of invertebrate euthanasia are described in the literature, ranging from decapitation and injection of chemicals to freezing and immersion in ethanol, but most have not been properly studied (Cooper 2011; Lewbart 2011). Suitable methods of euthanasia need to be effective and simple to perform and cause the least distress to the animal, and they have to be compatible with given research method if performed for that reason (Bennie et al. 2012). For example, Bennie et al. (2012) suggest terrestrial arthropod euthanasia through "targeted hyperkalosis", i.e. injection of potassium chloride to depolarize the thoracic ganglia, causing rapid death. While this method has been successfully tested on a variety of terrestrial arthropods, including a scorpion, it will likely not be widely used in spiders as many spider species are too small in size, many research fields operate with too many animals for individual injections, or the field conditions would make such a method inconvenient. For spiders, Pizzi (2006) recommends immersion in 70% ethanol and warns against freezing, as the latter can compromise subsequent histological examination. In fact, the most common euthanasia method in spiders is immersion in ethanol as it is compatible with most research fields. In some fields, especially the ones relying on molecular data, freezing is necessary, be it without a medium or submerged in ethanol or buffer. Other fields (e.g. systematics and taxonomy, behavioural sciences) sometimes necessitate the euthanasia of a number of individuals to store as vouchers, without the need for subsequent experiments. In such cases, one should choose the more humane method, even if compromising tissues. For example, when euthanizing spiders to store in ethanol, it might be more humane to first anaesthetize them using CO_2, low percent ethanol or instant deep-freezing (−60 °C to −80 °C) and subsequently transfer them into ethanol for preservation. In fact, a recent study shows a two-step method of first anaesthetizing gastropods in 5% ethanol for later preservation in 70–95% ethanol, to be the most appropriate (Gilbertson and Wyatt 2016). Unfortunately, as is true for most invertebrates in general, euthanasia methods for spiders are inadequately researched and necessitate much more attention.

5.5 Conclusions

Spiders are often used as research models and for biodiversity displays while also kept as pets. Due to anthropocentric views on invertebrates, spiders are historically considered as *automata*, but the growing evidence shows that spiders are not purely instinctive but exhibit behavioural plasticity including learning. This evidence must not be further ignored, and setting the standards for securing spiders' welfare in captivity is needed, in order to comprehend (1) if/when spider feel pain and distress and (2) what we can do to avoid or minimize it.

1. Humans commonly regard spiders as animals without the capacity to experience pain, and their responses are considered as purely nociceptive reflexes. This reasoning is based on the fact that spiders lack the brain regions implicated in pain processing found in higher vertebrates. They might, however, possess

neurobiological mechanisms for experiencing pain different than vertebrates. Recent definitions of a possible pain experience include criteria such as appropriate nociceptors and a central nervous system, physiological changes to noxious stimuli, protective motor reactions that might include reduced use of an affected area (e.g. limping, rubbing, holding, autotomy), avoidance learning and cognitive abilities (Elwood 2011; Sneddon et al. 2014). We present here what criteria for potential experience of pain have been found in spiders.

In spiders, little is known about the presence of nociceptors and how nociceptive information is processed within their central nervous system. Besides exhibiting withdrawal or escape behaviours when exposed to noxious stimuli, spider avoidance includes behaviours that may not simply be nociceptive reflexes, e.g. jumping, shaking web, autotomy and thanatosis. Spiders detect predators or other potential aversive stimuli by airborne vibrations stimulating their trichobothria. Increased trichobothria stimulation relates to increased levels of octopamine, which is considered analogous to the vertebrate norepinephrine, indicating its role in the stress system in spiders. Spiders also exhibit protective motor reactions. They commonly autotomize their legs when in dangerous situation, e.g. grasped by a predator, in order to escape. Furthermore, they self-amputate injured appendages, and they “lick” or rub their wounds. An interesting experiment by Eisner and Camazine (1983) even suggests a concordance between pain effects in humans and autotomy in spiders. There is also abundant evidence for avoidance learning in spiders. Studies show that spiders are able to learn and take the appropriate action to avoid or reduce potential damage on the basis of prior experience with noxious stimuli. Such a response is not the fixed, reflexive action of nociceptive avoidance. Furthermore, there is ample evidence of the ability of spiders to learn. Several species are able to modify their behaviour in contexts of foraging and web building, intraspecific interactions, spatial learning and navigation.

2. To secure animal well-being in captivity, we have to satisfy their general needs. Thus, spiders need access to fresh water and a balanced nutrition and appropriate housing with biologically meaningful enrichments, and keeping should prevent pain, injury and disease, all of which enable an environment to express normal behaviours and ensure that conditions and treatments cause no/little fear and stress. In spider research, we need to follow the general ethical guidelines for animal care, i.e. the “3Rs principle”. However, researchers using spiders as experimental subject need to follow the general reduction and refinement guidelines, while the replacement guideline (i.e. replacing animals with tissue cultures and computer models) is currently likely not applicable.

Additionally, some research fields cannot avoid anaesthetizing and/or sacrificing spiders. In spiders, using CO_2 for anaesthesia generally seems to enable fast recovery and no visible long-term consequences, while for potentially painful procedures, isoflurane, halothane or sevoflurane is preferred. The most common euthanasia method in spiders is immersion in ethanol as it is compatible with most research fields, but when the research protocol permits it, one should choose a more humane method. For example, when euthanizing spiders to store in ethanol, it might be more humane to first anaesthetize them using CO_2, low percent ethanol or instant deep-freezing and subsequently transfer them into ethanol for preservation.

While there are well-defined ethical guidelines for using vertebrates in research, welfare in invertebrates is minimally regulated and generally neglected by researchers. To address the numerous knowledge gaps in our understanding of welfare in invertebrates, we first need to set clearly defined criteria of how to assess experiences of pain and suffering in a given invertebrate group. Such definitions will allow us to set clearly defined hypotheses that can be experimentally tested. Despite this need for research, by following the above guidelines, we can avoid at least the known sources of distress in spiders.

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