Article

Does Handling for Public Talks in Zoos Affect the Behaviour of Captive Mexican Red-Kneed Spiders *Brachypelma hamorii*?

Charlotte Gresham [1,2,*], Fiona Mathews [2], Amanda Ferguson [1], Jamie Mitchell [1], Lisa Clifforde [1], Dave Clarke [1] and Lewis J. Rowden [1]

1   Zoological Society of London, London NW1 4RY, UK; amanda.ferguson@zsl.org (A.F.); jamie.mitchell@zsl.org (J.M.); lisa.clifforde@zsl.org (L.C.); dave.clarke@zsl.org (D.C.); lewis.rowden@zsl.org (L.J.R.)
2   School of Life Sciences, University of Sussex, Brighton BN1 9RH, UK; f.mathews@sussex.ac.uk
*   Correspondence: lottegresham@hotmail.co.uk

Abstract: Zoos include invertebrates in visitor interaction sessions to educate and spread conservation messages to the public. Yet, the welfare implications of these encounters on invertebrates are unstudied. Empirical studies reveal negative effects of handling on vertebrate species, thus providing reason to investigate impacts on invertebrates. Mexican red-kneed spiders *Brachypelma hamorii* are regularly handled by keepers for public talks at the Zoological Society of London, London Zoo. This study investigates whether handling affects the spiders' 24-h activity and enclosure usage. Three spiders were filmed under infrared light for 24 h following being handled, and on control (no-handling) days. The proportion of time that spiders spent under cover or exhibiting locomotion, limb-interaction, and object-interaction behaviour was recorded using instantaneous scan sampling. The spiders spent, on average, significantly more time under cover (7.8% increase) and exhibited significantly more limb-interaction behaviour (1.4% increase) on handling days. Handling for public talks therefore affects the behaviour and enclosure use of these captive *Brachypelma hamorii*. Although it is not yet possible to infer welfare implications, the presence of these behavioral responses suggests that protocols to monitor the behaviour of invertebrates following handling should be developed, and further behavioral studies are warranted to validate potential stress indices.

Keywords: handling; spiders; invertebrate; behaviour; activity; enclosure use; welfare

1. Introduction

Invertebrates are a diverse group that represent ~97% of all species and occupy every habitat on earth [1]. Invertebrate taxa are generally considered less capable than vertebrates of experiencing emotions such as stress [2], which has led to comparably less welfare research in zoos [3]. Welfare has been defined as "the state of the animal as perceived by the animal itself, with regards to its attempts to cope with its environment" [4]. Predictions of animal welfare are based on species-specific physiological and behavioral indicators of stress in the presence of a perceived threat [5]. There is a collection of evidence supporting the welfare consideration of invertebrates due to sufficient levels of sentience found in some species [6]. Studies have shown decision making, distinct personalities, and stress responses [7,8], thus providing increased justification to assess the welfare of invertebrates. The expanding number of studies finding clear behavioral responses in invertebrates offer new challenges to consider individual responses to stress [9]. Thus, further research into the welfare requirements of invertebrate species and individuals is essential for developing specific suggestions for improving welfare [6].

Many zoological collections exhibit invertebrates for educational and conservational reasons [10], and close-encounter interactions with visitors are used to spread such messages effectively [11]. Spider experts have highlighted the importance of education and

awareness as key conservation measures for arachnids; thus, large arachnid species are prime candidates for handling in zoos [12]. No known studies have evaluated the effect of handling on arachnids or any other invertebrates. However, empirical studies on vertebrates reveal behavioral and physiological stress responses to direct handling [3,13]. Evidence of the aversive effects of handling indicates a need to investigate this in invertebrate species, particularly due to the lack of research in this area.

The *Brachypelma* genus is housed across many zoos globally, at least 140 institutions [14] according to the Zoological Information Management System (ZIMS). In particular, *B. hamorii* are regularly handled by keepers at The Zoological Society of London (ZSL) London Zoo for on-site educational classes and public talks. *B. hamorii* is a New World Theraphosidae endemic to Mexico [15] which lives a solitary life in small burrows; only emerging to ambush ground-dwelling arthropods and small vertebrates which it immobilizes with venomous fangs [16]. This species is nocturnal with limited vision, but sensitive to light and dark [17]. Research on Theraphosidae show wavelength detection to be in the blue and ultraviolet range, with a primary sensitivity peak near 500 nm and a secondary peak of 270 nm [18]. *B. hamorii* is highly sensitive to vibrations, sound, touch and air currents, which are detected by pedipalps and setae covering the legs and abdomen [19]. For a defense mechanism, the species can issue a mildly venomous bite but more often flicks urticating hairs off its abdomen [17]. *B. hamorii* is currently listed as 'Vulnerable' by the International Union for Conservation of Nature (IUCN) [19]. Yet, further research is required before appropriate conservation action can be proposed.

Assessments of animal welfare in captivity often focus on observations of validated stress behaviours and comparison between wild and captive activity budgets, using ethograms to define discrete behaviours [20]. Specific ethograms for captive Theraphosids have been developed to measure anti-predatory response to predatory cues [21,22]. Other studies have looked at the behavioral repertoires and anti-predatory behaviours of *P. cambridgei, B. smithi, A. pennsylvanica, P. audax,* and *G. portentosa* (4, 8, 21). However, the 24-h behaviour budget of both wild and captive *Brachypelma* species remains unknown. Although threat perception has not been correlated with enclosure usage in captive invertebrate species, refuge use is known as an antipredator response in fish and lizards [23]. This study was able to monitor the time spiders spent under cover as a form of refuge use. Self-directed behaviours, such as scratching and overgrooming, have been linked to physiological indicators of stress in some vertebrate taxa, which can subsequently provide reliable welfare indicators [3,24]. Since stress behaviours are highly species-specific, this study identified four behaviour categories exhibited by the spiders (Table 1).

This study aimed to determine whether handling affected the 24-h enclosure usage and behaviour of *B. hamorii*. We hypothesised that there would be behaviour responses observed on handling days when compared to no-handling days. Based on the existing literature of invertebrate and vertebrate response to stress, these include increased proportions of limb-interaction and locomotion behaviour as well as proportion of time spent undercover on days following a handling event.

Table 1. Ethogram of key state behaviours exhibited by captive female *Brachypelma hamorii* at ZSL London Zoo.

Behaviour	Description
Stationary	Spider is motionless. Includes small singular, non-repetitive, movements of legs or pedipalps.
Locomotion	Directional movement on any surface. Includes climbing across or up sides of container. Includes 'scrambling' movement of front legs up container sides. Includes when spider takes 2 or more directional steps forward and then pauses.
Limb-interaction	Spider engages in purposeful and repetitive body-to-body contact. Includes rubbing legs together or onto abdomen. Includes 'grooming' behaviour where the legs or pedipalps are repeatedly drawn between the chelicerae and/or lateral pairs of legs are brushed together rapidly.
Object-interaction	Purposeful physical interaction of spider with an object or feature within its environment (e.g., flowerpot or lid). Interaction with lid involves the movement of front legs and pedipalps pressing against lid whilst stationary or shuffling sideways. Includes any manipulation of flowerpot position within enclosure.
Out of sight	Spider is out of sight. Spider is either behind the flowerpot, so out of view from the camera, or positioned far under the flowerpot where any movement cannot be determined with accuracy.

2. Materials and Methods

The project was approved by the standard ZSL research review process (project code: ZDZ127). Formal ethical approval was not needed as there was no intervention to routine husbandry.

2.1. Study Subjects

Behavioural data were collected in the B.U.G.S (Biodiversity Underpinning Global Survival) building at ZSL London Zoo (51.534000−0.151729) between December 2019 and March 2020. Three female mature adult *B. hamorii* (S1, S2, S3) were monitored due to being handled for talks at the time of the study (see Appendix A) for ZIMS numbers). All study subjects came to ZSL London Zoo on 16 January 2012 from the same captive bred source. The spiders were of similar size with an estimated birth date of 1 January 2008. During the study period, the spiders were kept in the spider nursery at an ambient temperature of 22–28 °C and humidity of 60–70%. Spiders were individually housed in transparent plastic containers with plastic mesh lids (30 cm × 25 cm × 15 cm). Half a plastic flowerpot was provided as a substitute burrow; S2 and S3 had larger flowerpots (14 cm × 13 cm × 8 cm) than S1 (10 cm × 9 cm × 6.5 cm). As a consequence of routine husbandry, the flowerpot sizes were determined by keepers and did not reflect any size or behavioural differences between spiders. Water was provided at all times in a clear circular water bowl. The bottom of the container was covered with damp coir (coconut fibre) to a depth of approximately 7 cm. Spiders were offered a locust *Schistocerca gregaria* to feed on every Thursday, with this feed presentation recorded and factored into analyses.

Daily handling was rotated between 5 keepers. The chosen spider was lifted from its enclosure by hand and moved into a clear acrylic container (11 cm × 18 cm × 6 cm), which was placed inside a larger insulated carrying container for transportation into the indoor public viewing area. The keepers were experienced in handling protocols and used a standard methodology for this. During the talk, spiders were either held on the keeper's palm, placed on the keeper's trousers whilst seated or kept in the clear acrylic container with the lid off (Appendix A). Spiders were not chosen for a talk if they had been handled the previous day or if they displayed excessive hair flicking or running behaviour when being initially handled. Handling for the talk commenced at 15:00 daily and lasted between 15–30 min (Appendix A). Once the talk had finished, spiders were placed back into the transportation container and returned to the same enclosure zone as before the talk.

2.2. Data Collection

Spiders were filmed for 24 h following handling (handling day/H) and also on days where no handling occurred (no-handling day/NH). The spiders were monitored for 24 h due to species biology; the individuals were most active in nocturnal conditions. A Go-Pro HERO4 (GoPro Inc.: San Mateo, CA, USA) camera on a 0.5 s time-lapse setting was used to record the spiders. Recordings were started immediately following the return of the spider to its enclosure, which was always between the hours of 15:00 and 15:30. The camera was positioned outside of the spider enclosure so that all enclosure zones were visible. A red LED light (REPSN 96789-R- bulbs) was positioned 30 cm away from the container and left on throughout the 24-h period; this enabled filming overnight whilst causing as little disturbance as possible to the spiders. The emitted red/orange light was within a spectral range (590–640 nm) not visible to Theraphosids [18] and did not induce any temperature change to the enclosures. Days since handling were noted for each recording (Appendix A) and spiders were not filmed on days where they were approached but not handled.

An ethogram of key state behaviours was created using existing literature from a similar species [21], based on real-time observations of handling sessions and analysis of the Go-Pro recordings (Table 1). Enclosures were split into two zones to monitor spider enclosure usage: under cover (under flowerpot) and not covered (open and side zones of the enclosure (Table 2)).

Table 2. Ethogram of key enclosure zones within captive female *Brachypelma hamorii* enclosures at ZSL London Zoo.

Zone	Description
Under flowerpot	Majority of legs and pedipalps placed underneath the flowerpot.
Open	Majority of legs and pedipalps placed on/resting over anything that is not a side or lid. This includes on the ground outside of the flowerpot or on top of the flowerpot.
Side	Majority of legs and pedipalps placed on/resting over a side of the container. Spider is vertical at 90°. Also includes when front half of body positioned at a vertical angle on side, even if only half of legs are on open surface (ground or flowerpot).

Footage of S1, S2, and S3 were reviewed by the same researcher using instantaneous scan sampling with an interval time of 15 minutes. The interval time was chosen using Lin's [25] pairwise Concordance Correlation Coefficient (CCC) test. The behaviour and enclosure zone observed for >30 s of the observation minute was recorded. Spiders were reviewed for a total number of 17 handled days and 16 non-handled days (Appendix A). Any uneaten locusts were recorded during observation sessions (Appendix A) and accounted for in statistical analysis.

2.3. Data Analyses

Microsoft Excel© 2020 was used to record the presence or absence of each behaviour and the enclosure zone occupied for 1-min, 15-min and 30-min sampling intervals. All statistical analyses were performed using RStudio [26]. Lin's [25] pairwise CCC test was performed to determine whether 15-min or 30-min sampling intervals would give similar assessments of spider behaviour to 1-min sampling interval. The epiR (v2.0.19) package [27] was used to perform pairwise CCC tests (epi.ccc) and corresponding 95% confidence intervals (CI) for each behaviour, indicating the agreement between the patterns obtained from 1-min sampling intervals compared to 15-min and 30-min.

Three behavioural categories (locomotion, limb-interaction, and object-interaction) were included in analysis based on biological relevance. Univariate analyses (chi-squared test_ lme4 [28] package) were first used to determine whether there was any significant variation in behaviour or enclosure usage between spider identity and when there was a locus present in the enclosure (locust presence). A generalised linear model (GLM) was then used to determine whether behaviour (limb-interaction, locomotion, and object-interaction)

and enclosure use differed between handling days and no-handling days. Spider identity was only included as a fixed effect in GLM analysis of locomotion, object-interaction, and time under cover to account for inter-individual differences. Locust presence was included as a fixed effect in GLM analysis of enclosure usage.

3. Results

3.1. Sampling Interval

Pairwise CCC tests showed that enclosure usage and behaviour results from 1-min sampling intervals corresponded with the results obtained from 15-min sampling intervals, since all behaviours were >80% correlated (Table 3). Thereafter, three remaining 24-h recordings of S3 were reviewed using 15-min sampling intervals. Statistical analysis was performed on results obtained from 15-min sampling intervals.

Table 3. The upper and lower 95% confidence interval (CI) results from Lin's (1989) pairwise Concordance Correlation Coefficient (CCC). The enclosure usage, locomotion, body-interaction, and limb-interaction results using 1-min sampling intervals were tested against the results using 15-min and 30-min sampling interval lengths. 27 handling and no-handling days were used in the test.

Behaviour/Zone	Pairwise CCC	95% CI
Under cover	1-min vs. 15-min	(0.84, 0.89)
	1-min vs. 30-min	(0.62, 0.86)
Locomotion	1-min vs. 15-min	(0.86, 0.97)
	1-min vs. 30-min	(0.72, 0.93)
Body-interaction	1-min vs. 15-min	(0.98, 0.99)
	1-min vs. 30-min	(0.49, 0.83)
Limb-interaction	1-min vs. 15-min	(0.84, 0.92)
	1-min vs. 30-min	(0.49, 0.83)

3.2. Enclosure Usage

Univariate analysis showed that enclosure use varied between spiders ($\chi^2 = p < 0.001$). The chi-squared test also showed a significant difference in enclosure usage when a locust was present in the enclosure ($p < 0.001$). A generalised linear model, controlling for spider identity and locust presence, showed a strong significant difference in time under cover between handling and no-handling days ($z = -7.52$, $p < 0.001$). S2 and S3 spent 0% of NH days covered (Figure 1). S1 spent more time under cover than the other spiders and had no differences in cover-usage between handling and no-handling days (50%; Figure 1).
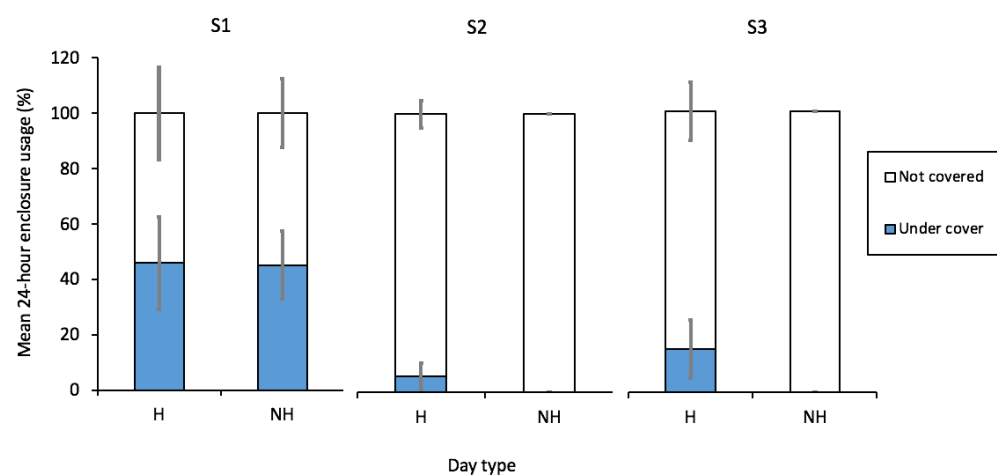

Figure 1. Mean ± SE percentage (%) of handling days (H) and no-handling days (NH) that S1 (8H, 6NH), S2 (6H, 8NH) and S3 (4H, 2NH) spent under cover and not covered.

3.3. Limb-Interaction

A chi-squared test showed no significant difference in limb-interaction between spiders (*p* = 0.14). A generalised linear model showed significantly higher mean limb-interaction on H days compared to NH days (z = −3.59, *p* < 0.001). An average 3% of 24 h was spent carrying out limb-interaction. S1 exhibited the highest mean limb-interaction on both day types and showed the largest increase in limb-interaction on handling days (~2%; Figure 2). All spiders showed a large increase in limb-interaction on handling days (Figure 2).
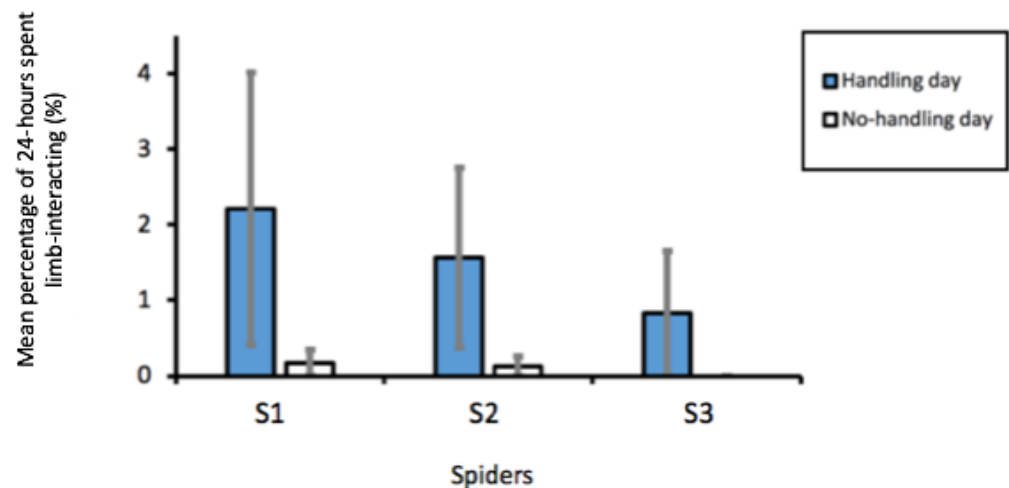

Figure 2. Mean ± SE percentage (%) of handling days (H) and no-handling days (NH) that S1 (8H, 6NH), S2 (6H, 8NH) and S3 (4H, 2NH) spent exhibiting limb-interaction behaviour.

3.4. Locomotion

A chi-squared test showed highly significant differences in locomotion between spiders (*p* < 0.001). A generalised linear fixed effects model, controlling for spider identity, showed no significant difference in locomotion between handling days and no-handling days (z = 1.67, *p* = 0.09). On average, S3 exhibited the highest locomotion level (<10%) on both day types, whereas S1 and S2 exhibited less locomotion (<2%; Figure 3). Locomotion was higher on no-handling days for all spiders (Figure 3).
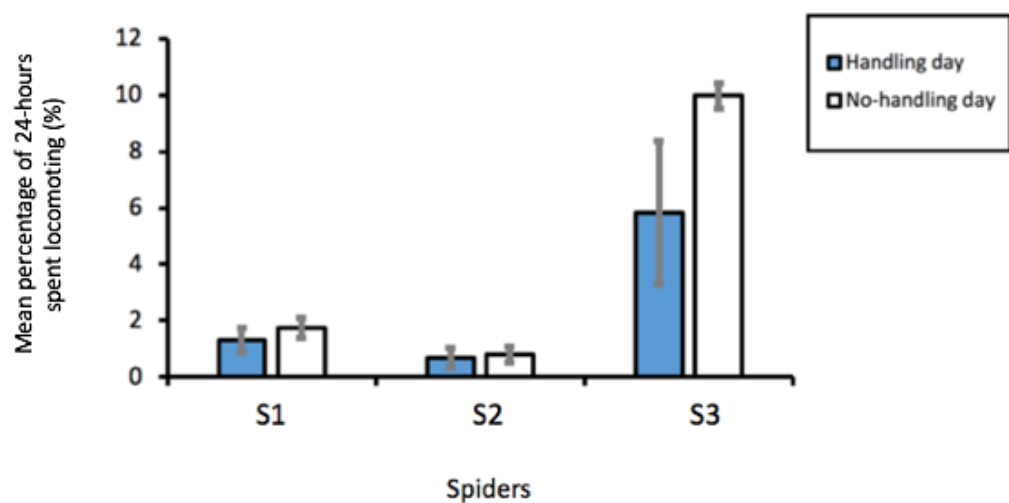

Figure 3. Mean ± SE percentage (%) of handling days (H) and no-handling days (NH) that S1 (8H, 6NH), S2 (6H, 8NH) and S3 (4H, 2NH) spent exhibiting locomotion behaviour.

3.5. Object-Interaction

A chi-squared test showed a significant difference in object-interaction between spiders (*p* = 0.01). A generalised linear model, controlling for spider identity, showed no overall

significant difference in object-interaction between handling days and no-handling days. ($z = 1.65$, $p = 0.11$). S2 showed no object-interaction behaviour whereas S3 showed the highest object-interaction behaviour (Figure 4).
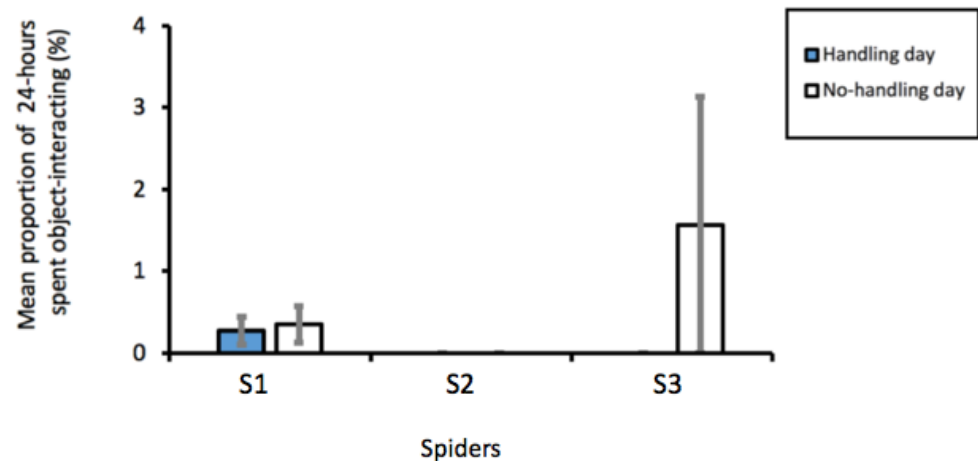

Figure 4. Mean $\pm$ SE percentage (%) of handling days (H) and no-handling days (NH) that S1 (8H, 6NH), S2 (6H, 8NH) and S3 (4H, 2NH) spent exhibiting object-interaction behaviour.

4. Discussion

A significant increase in time under cover on handling days shows that handling may have affected the spiders' refuge use. There are no enclosure usage studies on arachnids available in the literature. However, *Brachypelma smithi*, which is closely related to the study species, has been observed to retreat in response to a tactile predatory cue [8]. Refuge use has been shown to indicate perceived predation risk in vertebrates [22,29]. For example, increased time under cover has been observed in shingleback lizards *Tiliqua rugosa* as a response to handling, which subsequently indicated an aversive anti-predatory response [13]. Hence, spiders may have also viewed handling as a sublethal predation threat. Endocrinology studies and heart-rate monitoring could correlate physiological measures with behavioural observations, which could help validate refuge use as an indicator of perceived threat in arachnids [7,20]. Current technologies would need to be modified to accurately monitor invertebrate physiology.

All spiders spent less time under cover than expected (S1, 50%; S2 and S3, <20%). Particularly since wild studies indicate *Brachypelma* species to spend the majority of time concealed in burrows [19]. This suggests that the spiders may not perceive flowerpots as burrows. Studies show wild burrows to be more complex; consisting of multiple chambers with tunnels measuring three times the size of the occupying spider [30]. This examination has highlighted a possible need to provide a more physically enriching environment for captive *B. hamorii*, such as by offering a choice of burrow. Since previous arachnid studies have focused on short-term behavioural responses [8,21], enclosure enhancements could be accompanied by investigation into whether more enriching habitats induce long-term changes to activity and enclosure usage.

The significant increase in limb-interaction by spiders on handling days suggests a behavioural reaction to handling. Excessive self-directed behaviour and auto-grooming can indicate poor welfare and stress in captive vertebrate species [24,31]. Thus, increased self-directed behaviour may indicate a negative effect of handling in arachnids. Alternatively, the results may be interpreted as providing evidence that handling does not induce stress in *B. hamorii*, since increased baseline cortisol levels have been found to correlate with increased inactivity in arthropods in response to stressful situations [7]. It may be that the spiders were exhibiting self-maintenance after being handled. Furthermore, limb-interaction behaviour was recorded for a low proportion of the 24-h activity budget, meaning that spiders still spent the majority of their time on H and NH days stationary. Ul-

timately, the welfare status of *B. hamorii* cannot be confirmed, since invertebrate behaviours have not been validated as indicators of stress [20]. There is a need to identify invertebrate species-specific behaviours to be used as reliable welfare indicators. For example, web construction is suggested to be an indicator of well-being for Theraphosidae spiders in captivity [21].

It is possible that limb-interaction was an instinctive response to the environmental conditions of handling sessions, rather than the handling itself, since research has found arachnids to be highly sensitive to temperature, smell, hormones, vibrations, and even pressure level induced by weather systems [17,18]. Although data were dependent so that handling and no-handling days could be compared without compromising the validity of results. To control for environmental conditions and limit any resulting stress-response, handling sessions could be hosted behind the large glass viewing window in the spider nursery. Future studies could assess the relative importance of different talk conditions by investigating the effect of handling whilst controlling, or manipulating, specific environmental conditions such as handling surface, session length, and visitor noise level.

Our research has not provided evidence to suggest that handling affects directional movements such as walking, running, and climbing in spiders. The level of locomotion observed supports descriptions of the species being an ambush predator; which requires an animal to remain motionless for long time-periods [16,19]. The spiders may have reacted to handling by increasing actions that require less energy, such as limb-interaction. Contrasting effects of stress on different personality traits has been found in crickets [7] and cockroaches [32]. For example, sublethal predation exposure was found to significantly decrease boldness in cockroaches (measured as latency to initiate locomotion), but not activity level or aggressiveness [32]. Studies indicate differences in habitat and prey availability between wild and captive environments may alter activity budgets for *B. hamorii*. For example, Carducci and Jakob [33] found activity levels of jumping spiders *Phidippus audax* to significantly decrease when reared in less enriching, smaller cages. Although there are no published field observations of arachnid activity budgets, observations of burrows in the wild suggest captive habitats are somewhat lacking in complexity and size [30]. Although low activity level was expected, improvement to the complexity of enclosures may provide captive spiders with more opportunity to learn and practice natural behaviours.

Our research has not provided evidence to suggest that handling has an effect on object-interaction behaviour. Object-interaction was observed to mostly occur alongside directional movement and so may entail similar explanations to locomotion. Keepers selected spiders, through personal observations, that were least reactive to disturbance for handling throughout the duration of this study. Sweeney et al. [5] found less aggressive spiders *Philodromus albidus* to also be shyer than bold individuals, indicating a linked behavioural syndrome between boldness and aggressiveness. Subsequently, the study subjects may have exhibited lower levels of object interaction in comparison to the overall population of captive *B. hamorii*. To test this hypothesis, it would be useful to repeat this study with more *B. hamorii* individuals and include spiders that were never handled as controls. Furthermore, studies could assess linkage between boldness traits and object-interaction behaviour by replicating this study alongside an assessment of spider reaction to stimuli, using methods developed by this study and Bengston et al. [8].

Handling responses may have varied between *B. hamorii* individuals due to differences in past treatment of spiders. Shock-avoidance learning and habituation to non-aversive stimuli have already been demonstrated in Theraphosids [34]. In the case of this study, a high level of past handling may have reduced individuals' post-handling behavioural responses. The past handling frequency of each spider is unknown due to incomplete handling records prior to the study. However, there is a bias towards handling spiders which react best to handling. Although the best-handled spiders are already handled semi-regularly, it is advisable to ensure that selection of spiders for handling is not sporadic, to allow spiders to habituate. This proposal is supported by observations of other inver-

tebrate species, including bees [35] and cockroaches [36], which are capable of temporal learning. Future studies could assess *B. hamorii's* ability to remember environmental cues as a precursor to handling, thus providing options for habituation and potential choice over participation. Differences in behavioural traits may have also altered each individual's response to handling. Empirical studies on arthropods reveal that anti-predatory responses are affected by past experiences, such as rearing environment and previous exposure to stress [33]. Restrictive natal environments have been found to prevent the emergence of specific behavioural traits in captive *Brachypelma*, including the presence of a behavioural syndrome linking boldness with aggressiveness and exploratory behaviour [8]. However, the spiders have the same estimated birth date, came to the zoo on the same day, and were from the same captive bred source, indicating that the individuals were from the same sac and had similar rearing conditions. Nevertheless, this study has revealed the importance of considering past experiences in spider behavioural observations and the necessity for future studies to investigate a larger sample size of *B. hamorii* individuals that have known rearing conditions. Future studies observing a larger sample size could assess whether the behaviours observed in this study are explained by trait linkage, since studies have found linkages between shyness and increased reaction to threat [8]. Furthermore, a larger sample size would be useful to compare between spiders frequently handled and those which are not frequently handled, building upon this study to further evaluate the behavioural effect of handling on *B. hamorii*.

5. Conclusions

The increased proportion of limb-interaction and under-cover zone usage on handling days suggests an effect of handling on spider behaviour. Empirical studies on vertebrates indicate refuge use and self-maintenance behaviour to correlate with stress and perceived threat. Further research is required to validate these behaviours as invertebrate welfare indicators before conclusions can be made about the welfare implications of handling on captive *B. hamorii*. Thus, the measures that showed no effect in this study should not be disregarded as welfare indicators by future studies. This study demonstrates the importance of researching behavioural responses to management practices (including handling) among invertebrate species. It is also worth considering that these visitor engagement opportunities are perceived to have a positive impact on public opinion surrounding the taxa [37]. Thus, it would be useful to carry out further investigation with educational impact measured alongside animal behaviour change. It is recommended to review the protocols in place for handling, ensure in-depth monitoring of individual responses to handling, and continue to monitor the regularity of handling each spider. Finally, observations of wild counterparts are required to determine a favourable activity budget for future studies to compare wild and captive spiders. A thorough investigation of animal management methods including enrichment, enclosure size, and complexity is needed to inform best practice for any ex-situ conservation efforts.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Footage start dates, spider Zoological Information Management System (ZIMS) numbers, days since handling, locust presence, talk duration and handling surface for each handling day.

Spider	ZIMS Number	Footage Start Date	Days since Handling	Locus Present	Talk Duration	Handling Surface
S1	9411	18 December 2019	0	0	25	Palm
		14 January 2020	0	0	15	Trousers/Container
		23 January 2020	0	0	20	Palm/Container
		3 February 2020	0	0	20	Palm
		6 February 2020	0	0	20	Trousers
		7 February 2020	1	0	n/a	n/a
		8 February 2020	2	0	n/a	n/a
		12 February 2020	1	0	n/a	n/a
		13 February 2020	0	0	25	Palm
		18 February 2020	0	0	20	Palm
		2 March 2020	3	0	n/a	n/a
		4 March 2020	0	0	n/a	n/a
		4 March 2020	1	0	n/a	n/a
		11 March 2020	1	0	n/a	n/a
S2	9416	6 January 2020	0	0	30	Palm
		8 January 2020	0	0	15	Container
		15 January 2020	0	0	15	Palm/Container
		29 January 2020	1	0	n/a	n/a
		11 February 2020	2	0	n/a	n/a
		25 February 2020	2	0	n/a	n/a
		27 February 2020	0	0	15	Palm
		29 February 2020	2	0	n/a	n/a
		2 March 2020	2	0	n/a	n/a
		3 March 2020	2	0	n/a	n/a
		10 March 2020	2	0	n/a	n/a
		11 March 2020	0	0	20	Palm
		17 December 2019	2	0	n/a	n/a
		4 March 2020	0	0	35	Palm/Container

Table A1. *Cont.*

Spider	ZIMS Number	Footage Start Date	Days since Handling	Locus Present	Talk Duration	Handling Surface
S3	9413	29 February 2020	5	0	n/a	n/a
		13 January 2020	0	0	25	Container
		4 February 2020	0	0	25	Trousers
		24 February 2020	0	0	20	Container
		20 February 2020	0	1	n/a	n/a
		27 February 2020	3	1	n/a	n/a

References

1. Collen, B.; Böhm, M.; Kemp, R.; Baillie, J.E.M. *Spineless: Status and Trends of the World's Invertebrates*; Zoological Society of London: London, UK, 2012.
2. Andrews, P.L.R. Laboratory Invertebrates: Only Spineless, or Spineless and Painless? *Inst. Lab. Anim. Res.* **2011**, *52*, 121–125. [CrossRef] [PubMed]
3. Mather, J.A. Ethics and Care: For Animals, Not Just Mammals. *Animals* **2019**, *9*, 1–12. [CrossRef] [PubMed]
4. Broom, D.M. The scientific assessment of animal welfare. *Appl. Anim. Behav. Sci.* **1988**, *20*, 5–19. [CrossRef]
5. Sweeney, K.; Gadd, R.D.H.; Hess, Z.L.; McDermott, D.R.; MacDonald, L.; Cotter, P.; Armagost, F.; Chen, J.Z.; Berning, A.W.; DiRienzo, N.; et al. Assessing the effects of rearing environment, natural selection, and developmental stage on the emergence of a behavioural syndrome. *Ethology* **2013**, *119*, 436–447. [CrossRef]
6. Browning, H.; Veit, W. Improving invertebrate welfare. *Anim. Sent.* **2020**, *29*, 1–2. [CrossRef]
7. Adamo, S.A.; Baker, J.L. Conserved features of chronic stress across phyla: The effects of long-term stress on behaviour and the concentration of the neurohormone octopamine in the cricket, *Gryllus Texensis*. *Horm. Behav.* **2011**, *60*, 478–483. [CrossRef]
8. Bengston, S.E.; Pruit, J.N.; Riechert, S.E. Differences in environmental enrichment generate contrasting behavioural syndromes in a basal spider lineage. *Anim. Behav.* **2014**, *93*, 105–110. [CrossRef]
9. Mather, J.A.; Carere, C. *The Welfare of Invertebrate Animals*; Springer: Cham, Switzerland, 2019; pp. 229–245.
10. Why zoos and aquariums matter: Assessing the impact of a visit to a zoo or aquarium. Available online: https://www.informalscience.org/why-zoos-and-aquariums-matter-assessing-impact-visit-zoo-or-aquarium (accessed on 3 February 2020).
11. Sherwood, K.P.; Rallis, S.F.; Stone, J. Effects of live animals vs. preserved specimens on student learning. *Zoo. Biol.* **1989**, *8*, 99–104. [CrossRef]
12. Branco, V.V.; Cardoso, P. An expert-based assessment of global threats and conservation measures for spiders. *Glob. Ecol. Conserv.* **2020**, *24*, 1–7. [CrossRef]
13. Acaralp-Rehnberg, L. Human-animal interaction in the modern zoo: Live animal encounter programs and associated effects on animal welfare. Ph.D. Thesis, The University of Melbourne, Melbourne, Australia, 2019.
14. Species360 Zoological Information Management System (ZIMS). Available online: zims.Species360.org (accessed on 21 October 2021).
15. Mendoza, J.; Francke, O. Systematic revision of Brachypelma red-kneed tarantulas (Araneae: Theraphosidae), and the use of DNA barcodes to assist in the identification and conservation of CITES-listed species. *Invertebr. Syst.* **2017**, *31*, 157–179. [CrossRef]
16. Fukushima, C.; Mendoza, J.; West, R.; Longhorn, S.; Rivera Téllez, E.; Cooper, E.W.T.; Henriques, S.; Cardoso, P. *Brachypelma smithi*; The IUCN Red List of Threatened Species: Cambridge, UK, 2019. [CrossRef]
17. Ronald, N.B. *Keeping and Breeding Tarantulas*; Chudleigh Publishing: Ilford, UK, 1993.
18. Dahl, R.D.; Granda, A.M. Spectral sensitivities of photoreceptors in the ocelli of the tarantula *Aphonopelma chalcodes* (Araneae, Theraphosidae). *J. Arachnol.* **1989**, *17*, 195–205.
19. Fukushima, C.; Mendoza, J.; West, R.; Longhorn, S.; Rivera Téllez, E.; Cooper, E.W.T.; Henriques, S.; Cardoso, P. *Brachypelma hamorii*; The IUCN Red List of Threatened Species: Cambridge, UK, 2019. [CrossRef]
20. Wemelsfelder, F. How animals communicate quality of life: The qualitative assessment of behaviour. *Anim. Welf.* **2007**, *16*, 25–31.
21. Bennie, M.; Loaring, C.; Trim, S. Laboratory husbandry of arboreal tarantulas (Theraphosidae) and evaluation of environmental enrichment. *Anim. Technol. Welf.* **2011**, *10*, 163–169.
22. Blatchford, R.; Walker, S.; Marshal, S. A phylogeny-based comparison of tarantula spider anti-predator behaviour reveals correlation of morphology and behaviour. *Ethology* **2011**, *117*, 473–479. [CrossRef]
23. Domenici, P.; Hale, M.E. Escape responses of fish: A review of the diversity in motor control, kinematics and behaviour. *J. Exp. Biol.* **2019**, *222*, 1–15. [CrossRef]
24. Reamer, L.; Tooze, Z.; Coulson, C.; Semple, S. Correlates of self-directed and stereotypic behaviours in captive red-capped mangabeys (*Cercocebus torquatus torquatus*). *Appl. Anim. Behav. Sci.* **2010**, *124*, 68–74. [CrossRef]
25. Lin, L. A concordance correlation coefficient to evaluate reproducibility. *Biometrics* **1989**, *45*, 255–268. [CrossRef]

26. RStudio: Integrated Development for R; RStudio, PBC: Boston, MA, USA, 2020. Available online: http://www.rstudio.com/ (accessed on 3 February 2020).
27. Tools for the Analysis of Epidemiological Data, Package 'epiR'. 2021. Available online: https://cran.r-project.org/web/packages/epiR/epiR.pdf (accessed on 27 October 2021).
28. Bates, M.; Maechler, M.; Bolker, M.; Walker, S. Fitting Linear Mixed-Effects Models Using lme4. *J. Stat. Softw.* **2015**, *67*, 1–48. [CrossRef]
29. Cooper, W.E.; Perez-Mellado, V.; Baird, T.; Baird, T.A.; Caldwell, J.P.; Vitt, L.J. Effects of risk, cost, and their interaction on optimal escape by non-refuging Bonaire whiptail lizards, *Cnemidophorus murinus*. *Behav. Ecol.* **2003**, *14*, 288–293. [CrossRef]
30. Locht, A.; Yáñez, M.; Vázquez, I. Distribution and Natural History of Mexican Species of Brachypelma and Brachypelmides (Theraphosidae, Theraphosinae) with Morphological Evidence for Their Synonymy. *J. Arachnol.* **1999**, *27*, 196–200.
31. Mason, J.M. Species differences in responses to captivity: Stress, welfare and the comparative method. *Ecol. Evol.* **2010**, *25*, 713–721. [CrossRef] [PubMed]
32. McDermott, D.R.; Chips, M.J.; McGuirk, M.; Armagost, F.; DiRienzo, N.; Pruitt, J.N. Boldness is influenced by sublethal interactions with predators and is associated with successful harem infiltration in Madagascar hissing cockroaches. *Behav. Ecol. Sociobiol.* **2014**, *68*, 425–435. [CrossRef]
33. Carducci, J.P.; Jakob, E.M. Rearing environment affects behaviour of jumping spiders. *Anim. Behav.* **2000**, *59*, 39–46. [CrossRef] [PubMed]
34. Punzo, F. Reversal learning and complex maze learning in the spider *Aphonopelma hentzi* (Araneae, Theraphosidea). *Bull. Br. Arachnol. Soc.* **2002**, *12*, 153–158.
35. Ng, L.; Garcia, J.E.; Dyer, A.G. Use of temporal and color cueing in a symbolic delayed matching task by honeybees. *J. Exp. Biol.* **2020**, *223*, jeb224220. [CrossRef]
36. Arican, C.; Bulk, J.; Deisig, N.; Nawrot, M.P. Cockroaches show individuality in learning and memory during classical and operant conditioning. *Front. Physiol.* **2020**, *10*, 1539. [CrossRef]
37. Spooner, S.L.; Farnworth, M.J.; Ward, S.J.; Whitehouse-Tedd, K.M. Conservation Education: Are zoo animals effective ambassadors and is there any cost to their welfare? *J. Zool. Bot. Gard.* **2021**, *2*, 41–65. [CrossRef]