Journal of
Zoological and Botanical Gardens

MDPI

Article

A Proof-of-Concept Evaluation of the Human-Assisted Soft Release Method Using Two Captive-Born Red Pandas (*A. f. styani*) During a Mock Translocation Scenario in China

Xueyang Fan, Ruiqing Lv and James Edward Ayala *

Chengdu Research Base of Giant Panda Breeding and the Conservation of Endangered Wildlife Key Laboratory of Sichuan Province, Chengdu 610016, China
* Correspondence: ayalajames@msn.com

Abstract

Translocations of endangered species are a common method of conserving wildlife; however, the release of dietary specialists pose unique challenges for conservationists. In preparation for future releases of the red panda (*Ailurus fulgens styani*), a bamboo specialist, a proof-of concept test was performed using the 'Human-Assisted Soft Release' (HASR) method previously used with giant pandas. Two red pandas (one male, one female) were trained using positive reinforcement to recall to a researcher and voluntary placement of GPS collars as well as physical examinations. Afterwards, to simulate a translocation, the individuals were 'mock-released' into a large public access red panda enclosure. Following the 'mock-release', the pandas were monitored for four weeks via GPS collars mounted with accelerometers as well as the HASR method. The protocol proved operationally successful: across eight scheduled sessions, both individuals met all four predefined criteria in every session attended (8/8 and 7/7, the latter shortened by injury), permitting recall, collar changes, body examinations and weighing inside a public enclosure without anesthesia or physical restraint, and allowing an injury to be detected from collar data and treated the following morning. Alongside this operational record, activity and space use were monitored descriptively. The odds of being active in the first week rose to 1.9 and 1.6 times each individual's own pre-release baseline (+13.7 and +8.3 percentage points; $p < 0.001$) before returning to baseline across the month, and the two individuals differed in the reshaping of their daily rhythm and in their use of space. With the limitation of two individuals and an overlap with the breeding season, the behavioral observations reported here are descriptive and hypothesis-generating rather than hypothesis-confirming and require replication. The operational findings nonetheless demonstrate that the HASR method is technically feasible with red pandas and provides a basis for the pre-release preparation of this endangered species.

Keywords: red panda; conservation translocation; reintroduction; ODBA

Check for updates

Academic Editor: Jianxu Zhang

Received: 28 June 2026
Revised: 24 August 2026
Accepted: 25 August 2026
Published: 27 August 2026

Copyright: © 2026 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

1. Introduction

Globally, the use of translocations as an in situ method to conserve wildlife has increased [1]; however, reported success rates of translocations remain mixed [2,3]. This is because for each translocation, success is defined using different metrics, amongst considerably different species and source populations (i.e., wild-born vs. captive-bred). Furthermore, there is a lack of consensus regarding the use of "soft" and "hard" release

methods, with some species showing clear benefits for one method, while others do not [4,5]. Broadly speaking though, across taxa, higher success rates are reported for the translocation of omnivorous species compared with animals that are strict carnivores or herbivores [2,6], with dietary specialists, such as the red panda (*Ailurus fulgens*), posing unique challenges for conservationists.

The red panda is a small arboreal carnivore that is a bamboo specialist, with over 90% of its diet being bamboo [7,8]. As of 2026, there has only been one formally documented translocation of captive-born red pandas into the wild. This soft release occurred in India in 2003 and used two adult *A. f. fulgens* females from the Padmaja Naidu Himalayan Zoological Park, Darjeeling, India [9]. The animals were released into the Singalila National Park, West Bengal, India, where they were monitored via GPS collars and direct observations. After five months, one female was lost as a result of predation. The remaining adult female mated and gave birth in the wild, representing the first successful integration of a captive-born red panda into a wild population. While there are reports regarding additional red panda translocations at this facility, no known peer-reviewed articles have been published documenting the results of these releases.

Currently listed as "Endangered" by the IUCN, the global red panda population is spread across five known range countries, India, Nepal, Bhutan, Myanmar, and China [10]. Conservation initiatives are in place within four of the range countries, with limited data emerging from Myanmar, partially due to ongoing conflicts [11–16]. Despite these regional conservation initiatives, empirical evaluations of pre-release training protocols remain extremely limited and the global population is still believed to be in decline with less than 10,000 individuals in the wild. Within China, a survey between 1994–1996 estimated that the population of red pandas was between 6000 and 7000 individuals across three provinces, Tibet, Yunnan, and Sichuan [14,17]. However, no current population data has been reported for the species. Comparatively, China has made considerable progress in the conservation of the more 'famous' giant panda (*Ailuropoda melanoleuca*), another bamboo specialist, resulting in the downgrade of the species from Endangered to Vulnerable. The translocations of captive-born giant pandas have played an important role in bolstering wild populations through reinforcement efforts [18]. By 2021, 12 giant pandas had been released to support the small, genetically isolated populations [19]. Encouraged by these outcomes, along with the creation of the new 27,134 km^2 Giant Panda National Park [20], which also covers core sections of the red panda habitat in Sichuan province and stronger nationwide conservation policies, China has now started developing plans to release captive-born red pandas.

In preparation for future translocations within China, a pre-release plan was formulated specifically for captive-born red pandas at the Chengdu Research Base of Giant Panda Breeding (CRBGPB) in Sichuan province. This plan was based on the CRBGPB's current giant panda translocation protocol in which a method coined the "human-assisted soft release" (HASR) was used successfully [21,22]. Using this method, as part of the pre-release preparation, captive-born giant panda cubs are trained to safely follow field staff through a completely natural pre-release environment. During this process, staff bring the young pandas to sources of water and wild growing edible bamboo, giving them the opportunity to forage and behave naturally.

In addition to learning to follow field staff, in order to ensure the young giant pandas meet their nutritional needs, they are trained for voluntary blood draws, body examinations, and weightings all without anesthesia or any type of physical restraint [21,22]. Lastly, they are trained for the voluntary placement of the GPS collars, again without any form of restraint. In terms of post-release monitoring, because of the extensive training the panda cubs receive and the bond they form with the staff, the CRBGPB field team can safely con-

tinue to approach the released giant pandas while in the wild. Again, without any physical or chemical restraint, field staff continue to change GPS collars as needed and collect blood samples and body weights as well as conduct physical examinations. As giant pandas are highly sensitive to dietary changes, this release method was specifically designed to improve the survival rate of the released animals, as it allows the staff to monitor the health of the individuals in the field even into adulthood and provide supplemental food and intervention if needed.

Based on similar needs as a bamboo specialist, to prepare red pandas for release using the HASR method, the pair were first trained using positive reinforcement for the placement of GPS collars, then received 10 months of continued training (including blood draws, body examinations, ultrasound examinations, and weightings), all without any anesthesia or restraint [23]. Following this preparation period, to safely assess the applicability of the HASR method with red pandas under a translocation scenario, a 'mock-release' was performed as a proof-of-concept test in a controlled zoo-like setting.

The design of this 'mock-release' was similar to, and inspired by, the pre-release training methods used by US zoos with the golden lion tamarin (*Leontopithecus rosalia*), a small arboreal New World monkey. As part of the tamarin reintroduction program, to provide a more complex novel environment, candidates were released into the public area of the zoo and allowed to free-range within the entire zoo grounds [24,25]. These individuals were given the freedom to explore and forage naturally throughout the zoo, experiencing different biotic and abiotic factors, such as interactions with local and exotic species (including predators), as well as zoo guests, prior to being shipped to the release area in Brazil [24,25]. This process was noted as a key component in the successful conservation of the golden lion tamarin. Regarding red pandas, this 'mock-release' entailed allowing the two GPS collared individuals access into a 1.5-hectare naturally forested enclosure that features a walkthrough trail open to guests of the facility as well as a mixed-sex group of resident conspecific red pandas. In order to assess whether this 'mock-release' presented a sufficient challenge, two a priori expectations were set: that activity levels for the mock-released individuals would increase from baseline (H1) and would then decline over the following weeks (H2). With two individuals, these expectations serve as descriptive benchmarks for whether the scenario elicited a response; they are not confirmatory tests.

To provide post-release monitoring to the red pandas during the 'mock-release' period, the animals were routinely checked in the release area using the established criteria of the HASR method. Additionally, activity data collected from the collar mounted accelerometers in the form of Overall Dynamic Body Acceleration (ODBA) was analyzed to monitor the individuals as they adjusted to the unfamiliar habitat. The use of accelerometers or similar motion sensitive devices mounted on GPS collars to analyze activity patterns post-translocation is becoming an increasingly common method of post-release monitoring in a number of species [26–28]. Lastly, GPS data from the collars were analyzed to monitor the movement and space use of the released individuals. This is the first study of its kind to test a translocation method for captive-born red pandas within the species' natural range in China and provides insights for future red panda releases.

2. Materials and Methods

2.1. Study Area

All data were collected between December 2025 and March 2026 at the Dujiangyan Field Research Center for Giant Pandas (Panda Valley) in, Dujiangyan, Sichuan China 30.9677056° N, 103.5785984° E. Panda Valley is located in the foothills of the Qionglai mountains, approximately 20 km from the Longxi-Hongkou National Nature Reserve. Longxi-Hongkou is part of both the protected core red panda and giant panda habitat in

Sichuan province. The average temperature during the study period was between 8 and 10 °C and 75% to 85% relative humidity, which is within the typical winter climatic range in this region. While Panda Valley was originally built as a giant panda reintroduction acclimation and research facility, to specifically support red panda conservation, a separate 1.5-hectare (15,000 m^2) naturally forested red panda enclosure was built and opened to the public in 2019. This area was built on the slope of a mountain and irregularly shaped, measuring approximately 168 m in width (east–west) and 153 m in height (north–south), with a total perimeter of 511 m.

Additionally, there are two separate 100 m^2 fenced in isolated breeding/ maternity enclosures with similar furnishings as the main enclosure, which is where the pre-release baseline and post-study data for this project was collected. These enclosures are attached to the large public access enclosure with a buffer zone of 44 m of forest between them and the nearest public walkway to reduce disturbance. Lastly, a separate 600 m^2 naturally forested isolated fenced area was created as a pre-release practice training yard, however, data was not collected for the pre-release baseline in this yard, as the animals only used it during brief training periods. There are four additional breeding areas that were not used for this study.

The entire 1.5-hectare area is naturally forested with native tree species such as *Magnolia officinalis*, *Phoebe zhennan*, *Ginkgo biloba*, and *Salix matsudana* as well as *Metasequoia glyptostroboides*, providing 85% tree cover along with a dense understory of local vegetation, including the bamboo species *Pleioblastus amarus*, *Chimonobambusa sichuanensis*, and *Chimonobambusa opienensis* (also known as *Qiongzhuea opienensis* and *Bashania fargesii*) all of which are consumed by red pandas. Edible bamboo comprised roughly 30% of the understory. Wooden nest boxes along with climbing structures and bridges over public walkways made from pine were installed to allow the red pandas to move freely without entering the public trail. The trail for this enclosure is open to the public daily from 8:00 to 17:30 daily. This area is patrolled regularly by staff and under strict 24 h surveillance; no instances of predation of the resident red pandas were reported at the facility. During the study period, one adult male and five adult females were living in the large enclosure. These six residents remained in the enclosure throughout the study, were not collared or otherwise manipulated, and had free access to the study animals during the 'mock-release' period.

2.2. Subjects

A five-year old adult male and four-year old adult female red panda (*A. f. styani*) were selected as release candidates from the captive population at Panda Valley. Selection was first based on age, using the red panda reintroduction in India, in which a five-year-old and six-year-old female were released, as a reference point [9]. Following this, the two individuals were selected based on their initial climbing ability, reaction to novel staff members, their overall health condition, and their genetic compatibility. Both individuals were mother-reared in separate nursery areas. The male sired a single cub in 2024 with a female outside of the study group, while the female gave birth to twins the summer of 2024 with another male not included in the study; her twins were weaned in February 2025.

In terms of personality, the male was more cautious, vigilant, and very wary of staff compared to the female, while the female was friendlier to staff, less vigilant, and bolder compared to the male. The male's average body weight during the study period was 5.9 kg and was rated as a 5 out of 10 using an internally developed body condition-scoring chart, while the female was heavier at 6.4 kg and rated as a 6 on the same scale. Both individuals were healthy and did not receive medication or treatment prior to the study. The male suffered minor injuries from fighting during the 'mock-release' period; he was

treated immediately for minor lacerations by the Panda Valley attending veterinarian and fully recovered. As the present study consists of two individuals, a male and a female, throughout the manuscript the terms 'male' and 'female' are used only as unique identifiers. Therefore, all comparisons between the two individuals are purely descriptive case observations; they are not intended to infer sex-specific or species-level patterns.

2.3. Diet

In addition to the naturally occurring bamboo in the large enclosure, all of the red pandas were also provisioned with 0.5 kg of fresh bamboo four times a day (8:00, 10:00, 13:30, 16:30) for a total of 2 kg per individual daily. This bamboo was provided in both the pre-release area as well as spread throughout the large public access enclosure. The most commonly provided bamboo species were *Bashania fargesii*, *Phyllostachys bissetii* and *Chimonobambusa opienensis* (also known as *Qiongzhuea opienensis*), while *Chimonobambusa pachystachys*, *Chimonobambusa purpurea*, *Indocalamus tessellatus*, and *Pleioblastus amarus* were seasonally provided. Additionally, 100 g of a thick dough-like mixture of corn, soybeans, rice, oats, wheat, vegetable oil, and essential minerals and vitamins was made daily at the facility and provided twice a day as a nutritional supplement to each red panda at the facility. Lastly, 300 g of fresh produce, such as apples and pumpkins, was fed per individual as part of the normal diet as well as for environmental enrichment divided across the four feeding times. Water was provided via water bowls in the pre-release enclosure and via artificial streams in the large enclosure. Throughout the entire post-release period, the study individuals were provided their full diets in both the pre-release area and public access enclosure and were given free access to all areas.

2.4. Pre-Release Experience

Both individuals had little experience in the 1.5-hectare exhibit area and exposure to the general public. The male was born in 2020 and stayed in the maternity area with his mother and twin brother until 2021. From 2021 to 2022, he was kept in an all-male sub-adult group, housed in a different location. The male was briefly released into the 1.5-hectare exhibit area in 2023; however, mid-2023, all of the animals were transferred to other enclosures due to extensive remodeling of the tourist trails. Following the renovations, the male was moved for breeding purposes into one of the mating areas where he remained until the start of this study. The female was born in 2021 and remained with her mother until 2022. From 2022 to 2023, she was kept in an all-female group in a separate location and briefly released into the large public enclosure. Again, due to the remodeling of enclosure, she was transferred to another enclosure. Late 2023, she was moved to a breeding/maternity area, where she mated, gave birth the summer of 2024, and weaned her cubs early 2025. After weaning, she was transferred, introduced, and successfully paired with the male as part of this experiment. The male was unfamiliar and unrelated to all of the six resident red pandas in the large enclosure, while the female was unrelated to all of the individuals and unfamiliar to the male and three of the females; she was previously housed as a juvenile with two of the resident females.

2.5. GPS Collars

Druid (Druid technology Co.,Ltd, Chengdu, China) Interrex B5 4G MC GPS collars were used exclusively for this investigation. The custom-made collars used the 4G cellular network and weighed 84.5 g. As the red pandas in this study weighed ~6 kg, the 84.5 g collars were 1.4% of the red panda's bodyweight, considerably lower than the 5–10% accepted range commonly used in wildlife research [29]. The GPS collars were changed on a weekly basis both to ensure ample battery power as well as to check the fit and safety of the red pandas. The collars recorded ODBA data at 1 min intervals and GNSS positioning

data each hour for 24 continuous hours over the course of the study. We used two collars per individual so that one collar would charge while the other was deployed. In order to remove the effect of the collar change process on the activity data of the red pandas, the first 15 min of data from the new collar was deleted, as was the last 15 min of data from the older collar.

2.6. Training

A significant change in behavior related to the use of GPS collars on captive red pandas was noted at Rotterdam Zoo, the Netherlands [30]. To compensate for this, a detailed plan was made to train the red pandas using only positive reinforcement to allow for the placement of the GPS collars without any form of physical or chemical restraint as part of a long-term activity study (see Fan, 2026 [23]). As only positive reinforcement training was used, both individuals could choose whether or not to participate in a training session. If an animal was uncooperative or left in the middle of a session, we discontinued that individual's training session and waited for the next session.

Training for the red pandas occurred twice daily at 10:30 and 14:00 three times a week. Initially, the red pandas were desensitized to having the collars placed around their neck, then gradually habituated to wearing the collars for extended periods. No adverse behavioral responses to positive reinforcement training were observed after habituation. It took approximately two months to fully train each panda and ensure they would cooperate reliably with the collar changes. Pieces of apple along with tactile reinforcement were used as primary reinforcers, and a training whistle was used as a secondary reinforcer. The diets of the two individuals were not altered in any way during the study period. The red pandas were continuously monitored during the habituation process to the GPS collars to ensure safety. Following habituation, the collars could be changed by a single researcher in less than one minute.

Once habituated to wearing the GPS collars, training was reduced to one session two to three times a week at 10:30 to maintain behaviors. During the three-month study period presented here, including the time the animals were in the 'mock-release' area, there were 29 training sessions in total, and both animals cooperated fully during all of these sessions. During each session the collars were checked to ensure proper fit and were changed on a weekly basis depending on battery level; following habituation, both red pandas consistently cooperated with each collar change. A small amount of fur was abraded from the dorsal surface the neck of both red pandas due to friction with collars; however, no broken or irritated skin was noted for either individual due to collar wear during the entire study period. No collar directed or abnormal behaviors were noted during this study following habituation. All data collection occurred after the animals were habituated to the collars.

2.7. Applying the HASR Method

Both red pandas were trained to follow a researcher through their 100 m^2 enclosure and recall to the researcher when given a cue. After several months of consistent recalling, the red pandas were then taken for brief trips (~30 min) into a more complex 600 m^2 naturally forested training area. Following one month of training within the 600 m^2 training area, again, once the red pandas would consistently recall, the animals were released into the 1.5-hectare public enclosure and given free access to all three locations. No activity data was analyzed or presented here while the animals were in the 600 m^2 area, as their behavior was heavily influenced by the trainer.

Post-release, the two subjects were monitored routinely as they adjusted to the 1.5-hectare habitat using the HASR method. To determine whether or not the HASR

method has the potential to be used with red pandas, the animals were checked twice a week (Monday and Friday) at 10:30 over a four-week period following the criteria listed below that was set prior to release:

(1) Once located within several meters inside the public access enclosure, the animal must return on cue to the trainer in a timely manner.
(2) While in the public access enclosure, the animal must allow the GPS collar to be checked and changed if necessary.
(3) While in the public access enclosure, the animal must allow a brief body examination and voluntary blood draw training during each session and weighings as needed.
(4) While in the public access enclosure, the animal must follow the trainer on cue and return back, on their own, to the pre-release area.

As there were eight scheduled sessions across the study period, we scored each of the four criteria independently, by the single attending trainer, based on whether or not the animal cooperated; results were purely binary, successful or unsuccessful, as no objective measure could be obtained. We considered the test successful if the animal fully complied with all four criteria during five out of eight of each of the pre-determined training sessions.

2.8. Statistical Analysis

2.8.1. Data Preparation and Analytical Unit

Raw collar output was sampled at 1 min intervals as integer Overall Dynamic Body Acceleration (ODBA) counts and converted to ODBA in units of g by dividing by 10,000. Because ODBA integrates acceleration arising from all body movement, it indexes overall movement intensity rather than locomotion per se; the "active" classification therefore reflects any non-rest movement rather than locomotion in the strict sense. Records with missing or non-positive values were excluded, and all timestamps were handled in China Standard Time (UTC + 08:00).

After exclusion of invalid minutes caused by device failure or user error, 183,906 min-level records entered the analysis (88,065 for the male; 95,841 for the female), comprising a pre-release enclosure baseline (≈32 days per individual), four one-week training phases in the large outdoor enclosure (W1–W4), and a one-week return to the pre-release enclosure. Because of these exclusions, the realized monitoring window is shorter than the nominal duration in some phases; in particular, the male's fourth training week was curtailed following an injury, leaving ≈3 days of valid recording in W4 versus ≈7 days in the other training weeks. For the spatial analyses, GPS fixes falling outside the study area were excluded prior to analysis.

Each minute was classified into one of three activity-intensity states (Section 2.8.2) and binarized into active (State 3) versus inactive (States 1–2). Minute-level records within the same hour are strongly autocorrelated; to avoid temporal pseudoreplication, all inferential models of activity used the hour as the analytical unit, with the response encoded as the pair (active minutes, inactive minutes) within each hour and a random intercept for date. This addresses temporal non-independence within an individual, not biological replication: with two animals, all models were fitted per individual, and statistical significance refers to within-individual change rather than population-level evidence. Diel-rhythm shape was modeled at the same minute-derived, hourly binned resolution (Section 2.8.5).

2.8.2. Activity-Intensity Classification by Hidden Markov Model

ODBA was classified into discrete activity-intensity states using hidden Markov models (HMMs) fitted with the momentuHMM R package [31], with a Gamma emission distribution reflecting the strictly positive, right-skewed support of accelerometer signals. Because the accelerometer (1-min) and GPS (hourly) sensors operate at different temporal

resolutions, two parallel three-state (K = 3) HMMs were fitted: a minute-scale model, used for all activity-intensity, activity-rate, and diel-rhythm analyses (the primary analyses reported below), and an hourly scale model, used to relate movement states to the hourly GPS fixes (Section 2.8.6). In both models, the three states represented biologically interpretable resting, low-intensity, and active behavior, with well-separated state-conditional means. Candidate models with one to five states were compared (Table S1); information criteria declined monotonically with the number of states, as is usual for high-frequency accelerometer data [32], so three states were retained on interpretability, recovery at both temporal scales, and agreement with the video and GPS-speed data external to model fitting (Sections 2.8.3 and 2.8.6). State-conditional parameters and transition probabilities are given in Table S2. States were decoded with the Viterbi algorithm and renumbered in ascending order of state-conditional mean ODBA (State 1 lowest, State 3 highest movement intensity); for all rate and rhythm analyses, each minute was binarized into active (State 3) versus inactive (States 1–2). The three states are defined by the fitted model, not by fixed ODBA cut-points: each was characterized by its state-conditional Gamma mean and its transition probabilities (Table S2), and each minute was assigned by Viterbi decoding of the whole series. Their behavioral content was established independently by video (Section 2.8.3).

2.8.3. Behavioral Validation of HMM Activity-Intensity States

To confirm that the decoded states corresponded to graded movement intensity, focal video observations were mapped a priori to ordinal intensity layers and cross-classified against the Viterbi-decoded HMM states. Independence was tested with a chi-square test using Monte-Carlo *p*-values (B = 10,000), and the association summarized with the bias-corrected Cramér's V (the primary statistic, since the sub-minute labels sharing one HMM state are not independent), together with Cohen's unweighted and linearly weighted κ and exact/within-one-layer agreement. Validation was carried out at the minute scale (the primary scale for the activity analyses) and, for completeness, at the hourly scale. As an independent, sensor-based check, GPS-derived movement speed was additionally compared across the hourly HMM states (Section 2.8.6).

2.8.4. Activity Response to Soft Release

Change in the overall level of activity was modeled with beta-binomial generalized linear mixed models (GLMMs) in glmmTMB [33], with the hourly (active, inactive) pair as the response, phase as a fixed effect, and a random intercept for date; the beta-binomial family accommodates the over-dispersion produced by aggregating minutes within an hour. Because the study concerned the contrast between two individuals, a separate model was fitted for each animal, giving the odds of being active in each training phase relative to that animal's own pre-release baseline (Table 1). A pooled model combining both animals was fitted for reference only (Table S3), as it averaged two distinct individual trajectories.

Table 1. Activity response by individual: active-state proportion per phase, change versus the individual's pre-release enclosure baseline, and odds of being active versus that individual's baseline (per-animal beta-binomial GLMM, date random effect; minute scale). * The male's W4 is based on approximately three days of valid recording following injury; this data should be interpreted with corresponding caution.

Individual	Phase	Active %	Δ vs. Baseline (pts)	OR [95% CI]	*p*
Male	baseline	46.4	—	—(reference)	—
	W1	60.1	+13.7	1.94 [1.48, 2.53]	<0.001
	W2	45.4	−1.0	0.99 [0.77, 1.27]	0.96
	W3	45.9	−0.5	1.02 [0.78, 1.33]	0.89
	W4 (injury *)	41.3	−5.1	1.05 [0.74, 1.47]	0.80
	return	49.7	+3.3	1.17 [0.91, 1.51]	0.23
Female	baseline	36.5	—	—(reference)	—
	W1	44.8	+8.3	1.56 [1.21, 2.02]	<0.001
	W2	41.5	+5.0	1.29 [1.01, 1.66]	0.044
	W3	37.3	+0.8	1.06 [0.83, 1.36]	0.66
	W4	37.1	+0.6	1.13 [0.88, 1.44]	0.34
	return	42.1	+5.6	1.25 [0.97, 1.59]	0.08

2.8.5. Diel Rhythm Shape

The level of activity and the shape of the 24 h activity rhythm are distinct questions. To test whether rhythm shape changed independently of overall level, a cyclic-spline generalized additive model (GAM) was fitted for each individual in mgcv [34], with a cyclic 'smooth' of hour-of-day (joined at 0 and 24 h), a per-phase difference 'smooth' (an ordered-factor interaction giving each phase's deviation in rhythm shape from baseline), and a date random effect. As mgcv does not provide a beta-binomial family, a quasi-binomial response absorbed the over-dispersion (estimated dispersion 27–29). These rhythm analyses used the minute-scale states (the primary scale); the equivalent hourly scale analysis is reported in Figure S1 and showed the same pattern.

For each per-phase difference 'smooth', the effective degrees of freedom (EDF) summarized its complexity: the smoothing penalty was selected automatically, and a 'smooth' with no support in the data was shrunk toward a flat line, giving an EDF near zero. An EDF near zero therefore indicated that a phase's diel profile did not change in shape relative to baseline, whereas a larger EDF indicated a more complex within-day reorganization. EDF and significance were distinct: a 'smooth' may use several effective degrees of freedom yet remain non-significant when its fluctuations are small relative to their uncertainty. Each difference 'smooth' was tested for departure from baseline; *p*-values were adjusted for the ten tests (two individuals × five phases) with the Benjamini–Hochberg procedure [35], and basis adequacy was confirmed with k-index diagnostics. The magnitude of any reshaping was summarized as the fitted phase-minus-baseline difference in active probability by time-of-day band.

2.8.6. Space Use

Space use during the four training weeks was characterized from the hourly GPS fixes, each carrying its hourly HMM movement state: weekly medians of step distance, movement speed, and distance to the visitor boardwalk, with weekly trends tested by Spearman rank correlations and between-individual differences by Wilcoxon rank-sum tests. Distance to the release area was summarized as daily medians to avoid pseudoreplication, tested per individual by Spearman correlation against week, and tested for between-individual divergence by a week × individual interaction (linear model on daily medians). To check

for a visitor-presence effect, distance to the boardwalk during daylight was compared between park opening hours (08:00–17:30, visitors present) and the daylight hours when the park was closed (Wilcoxon rank-sum test, per individual). GPS fixes were quality-filtered (horizontal dilution of precision ≤ 5; 94.3% retained, 1018 fixes), projected to UTM zone 48N, and screened against the enclosure polygon (fixes falling outside were removed). Weekly home-range (95%) and core (50%) areas were estimated by fixed-kernel density estimation (reference bandwidth scaled by 0.7) in adehabitatHR [36] and clipped to the enclosure boundary, and weekly between-individual home-range overlap was summarized with Bhattacharyya's affinity, the volume of intersection, and the home-range overlap index [37]. To confirm that the hourly states tracked movement, state-specific GPS speed was compared across the hourly HMM states with a linear mixed model on the log1p scale (state fixed, animal random) using lme4/lmerTest [38,39]; this validation is reported in Figure S2.

2.8.7. Software

All analyses were performed in R 4.4.0 [40]. Hidden Markov models were fitted with momentuHMM 1.5.8, beta-binomial GLMMs with glmmTMB 1.1.14, and cyclic-spline GAMs with mgcv 1.9-1; kernel-density home ranges were estimated with adehabitatHR 0.4.21 [36], and spatial data was handled with sf 1.1-0, with daylight periods assigned using suncalc 0.5.1 [41]. The supplementary GPS-speed mixed model used lme4 1.1-35.3 and lmerTest 3.1-3, and figures were produced with ggplot2 4.0.2 [42]. Effect sizes are reported alongside significance tests throughout: odds ratios with 95% confidence intervals and the absolute change in active-state percentage points for the activity models (Table 1), Cramér's V for the state validation, effective degrees of freedom for the rhythm smooths (Table 2), and Spearman's ρ for the space-use trends. The significance threshold was $\alpha = 0.05$.

Table 2. The test of whether the diel activity-rhythm shape differed from the pre-release enclosure baseline in each phase by individual (minute scale; per-individual cyclic-spline GAM difference smooths, quasi-binomial). The EDF is the effective degrees of freedom of each difference 'smooth' (≈0 = no detectable change; larger = more reorganization); *p* tests' departure from the baseline rhythm; adjusted *p* is Benjamini–Hochberg across the ten tests. The overall activity level is in Table 1. * The male's W4 is based on approximately three days of valid recording following injury; this difference smooth should be interpreted with corresponding caution.

Individual	Phase	EDF	F	*p*	*p* (BH)
Male	W1	6.38	3.44	<0.001	<0.001
	W2	6.38	4.38	<0.001	<0.001
	W3	3.95	5.18	<0.001	<0.001
	W4 (injury *)	6.04	4.55	<0.001	<0.001
	return	1.49	0.41	0.047	0.078
Female	W1	0.00	0.00	0.83	0.97
	W2	8.71	3.24	<0.001	<0.001
	W3	4.98	0.52	0.39	0.56
	W4	0.00	0.00	0.97	0.97
	return	0.00	0.00	0.90	0.97

3. Results

3.1. Activity-Intensity States and Validation

The minute-scale three-state HMM resolved well-separated resting, low-intensity, and active states along the ODBA gradient (state-conditional mean ODBA 0.010, 0.023, and 0.231 g; 5th–95th percentiles 0.008–0.012, 0.006–0.052, and 0.086–0.423 g; 38.9%, 18.1%, and 43.0% of minutes; Figure 1); the parallel hourly-scale HMM recovered the same three-state structure (Figure S1). The decoded states corresponded to graded movement intensity: at

the minute scale, the video-derived intensity layer and the HMM state were significantly associated (bias-corrected Cramér's V = 0.52; within-one-layer agreement 96.4%, exact agreement 42.2%; $p < 0.001$; n= 1461; Figure 2), with the same pattern within each individual (male V = 0.40; female V = 0.50). State-specific GPS speed provided sensor-independent confirmation of the hourly-scale states used for the spatial analyses: median speed rose monotonically across states (11.5, 18.1, 32.9 m h^{-1}), the active state moving about 2.2 times faster than the resting state ($p < 0.001$; Figure S2).

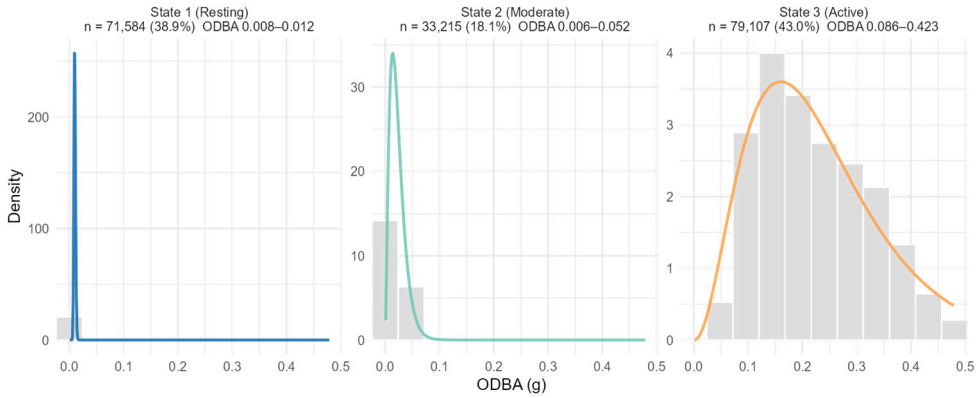


Figure 1. ODBA distribution and fitted Gamma model for each activity-intensity state (minute scale). Panel labels give the number and percentage of minutes assigned to each state and the 5th–95th percentile of ODBA within it. States were decoded from the full time series by the Viterbi algorithm rather than by fixed ODBA cut-points; the resting and moderate ranges overlap, so no single threshold separates them.

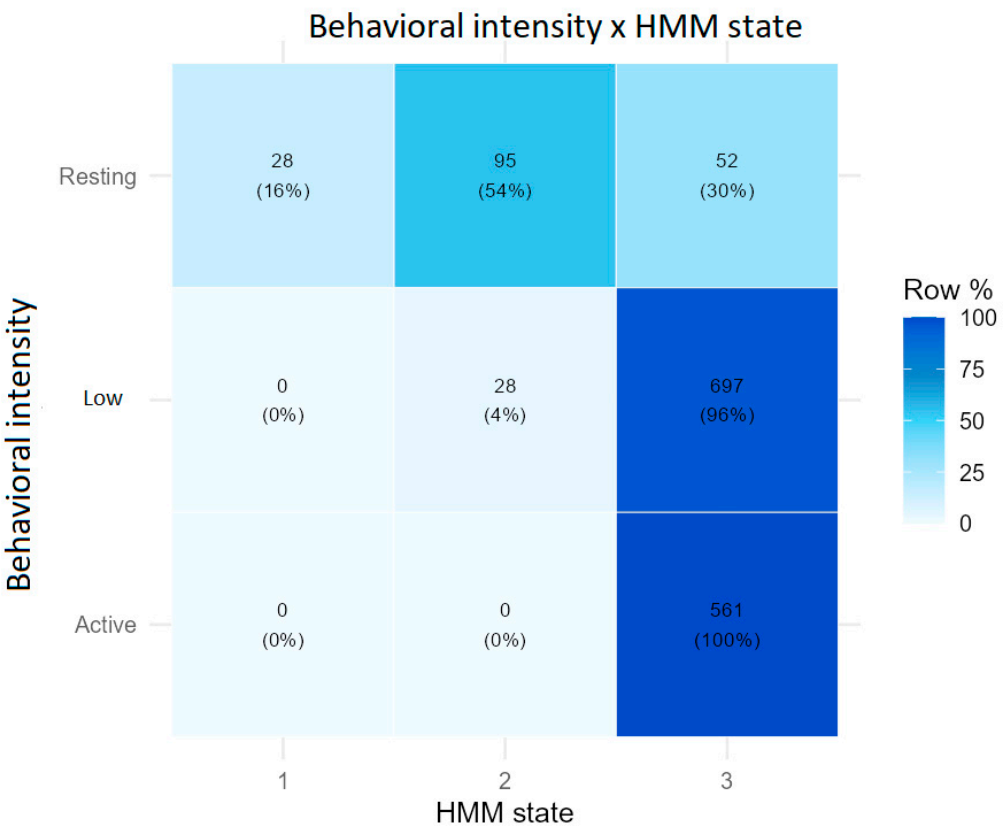


Figure 2. The validation of the HMM: the cross-classification of the a priori behavioral-intensity layers with the decoded HMM states (minute scale; bias-corrected Cramér's V = 0.52). Cell entries are counts; shading shows the within-layer percentage.

3.2. Activity Response to Soft Release, by Individual

Transfer to the large enclosure raised activity in both individuals but with different time courses (Table 1; Figure 3). In the male, the active-state proportion rose sharply in W1, from a baseline of 46.4% to 60.1% (+13.7 percentage points, +30%; OR = 1.94 versus his own baseline, $p < 0.001$); this was the only week in which his activity level differed from baseline, with W2–W4 statistically indistinguishable from baseline (45.4%, 45.9%, 41.3%; all $p \geq 0.80$) and the return phase also indistinguishable (49.7%; OR = 1.17, $p = 0.23$). In the female, the increase was smaller but more sustained: activity was elevated in both W1 (44.8%; +8.3 points; OR = 1.56, $p < 0.001$) and W2 (41.5%; OR = 1.29, $p = 0.044$) before returning to baseline in W3–W4 (37.3%, 37.1%; $p \geq 0.34$), with a marginal elevation on return (42.1%; OR = 1.25, $p = 0.08$). Thus, the male showed a larger but briefer activation confined to the first week, whereas the female showed a smaller, two-week elevation; both had returned to baseline activity levels by W3. Both individuals therefore showed elevated activity on entering the larger enclosure, with different time courses. ODBA indexes movement intensity only, so the behavioral basis of this increase cannot be determined from the collar data. In every phase without a detectable effect, the confidence interval includes 1, and the intervals are narrow enough (upper bounds ≤ 1.7) to exclude a large change rather than merely failing to detect one. The hourly scale activity-rate analysis showed the same pattern (Figure S1).

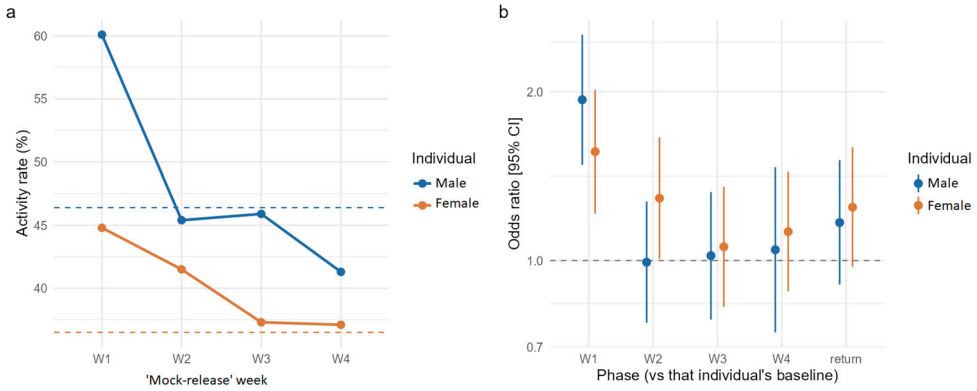


Figure 3. (**a**) Active-state proportion across 'mock-release' weeks W1–W4 for each individual; dashed lines show that individual's pre-release enclosure baseline. (**b**) The odds of being active in each phase relative to that same individual's baseline, with 95% confidence intervals (per-animal beta-binomial GLMM; values as in Table 1). The dashed line marks an odds ratio of 1, i.e., no difference from baseline; intervals crossing it indicate no detectable change.

A pooled model combining the two animals (Table S3) shows the same first-week peak (W1 OR = 1.72, $p < 0.001$) but, by averaging the two trajectories, also yields a spuriously "significant" return-phase effect (OR = 1.20, $p = 0.044$) that is not significant in either individual analyzed separately; the per-individual models are therefore the basis for inference.

3.3. Diel Rhythm Shape by Individual

Both individuals showed a strong diel rhythm, with the active state concentrated in the daylight and twilight hours and sharply reduced at night (active-state proportion—male: diurnal 68%, crepuscular 64%, nocturnal 28%; female: diurnal 53%, crepuscular 57%, nocturnal 20%; daylight periods classified relative to local sunrise and sunset, Section 2.8.5). Nocturnal activity was far lower than both diurnal and crepuscular activity in each individual (per-animal binomial GLM on the hourly analytical unit, date-clustered; both $p < 0.001$), whereas the diurnal–crepuscular difference was small (Table S4).

The shape of the 24 h activity rhythm changed independently of its overall level, and again differed between the two individuals (Table 2; Figure 4). In the male, the diel rhythm was reshaped relative to baseline in every training week (W1–W4, all Benjamini–Hochberg-adjusted $p < 0.001$; EDF 4.0–6.4), although W4 rests on approximately three days of valid recording following injury: night-time and dawn activity were elevated, while the midday peak was progressively suppressed (e.g., in W1, night +24 and dawn +20 percentage points of active probability; by W4, day −30 with night still +23 points). After the return to the pre-release enclosure, his rhythm shape was no longer distinguishable from baseline (EDF = 1.49; $p = 0.047$, adjusted $p = 0.078$), indicating that the reorganization resolved. In the female, by contrast, the rhythm shape departed from baseline only in W2 (EDF = 8.71; adjusted $p < 0.001$; mainly daytime and dusk elevation, +12 and +9 points), with W1, W3, W4, and the return all indistinguishable from baseline (adjusted $p \geq 0.56$). Her W1 increase (Section 3.2) was therefore a near-uniform lift in activity across the day rather than a change in the timing of activity. The equivalent hourly scale rhythm analysis was consistent (Figure S1).

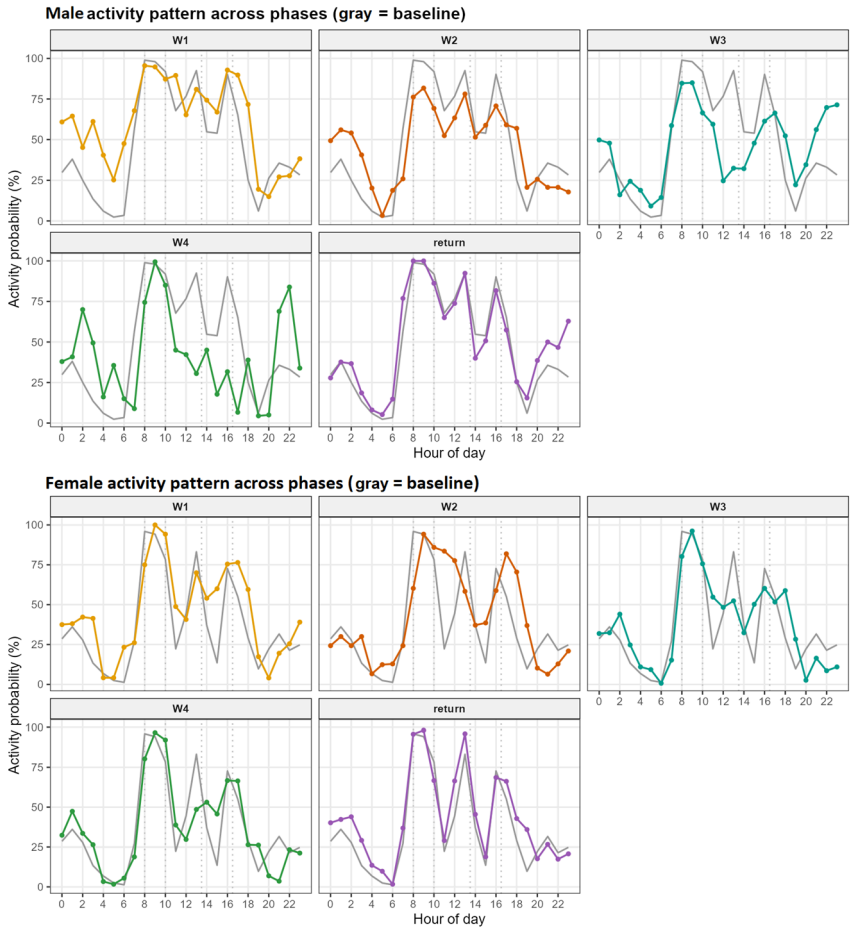


Figure 4. Diel activity rhythm across training phases for each individual (male, top; female, bottom); colored lines show each week and the return, gray lines the pre-release enclosure baseline. Difference smooths and their significance are given in Table 2; per-phase difference curves are provided in Figure S3.

The contrast between EDF and significance is illustrated by the female's W3 difference 'smooth', which used ≈5 effective degrees of freedom yet was not significant ($F = 0.52$; $p = 0.39$; adjusted $p = 0.56$). The k-index diagnostics indicated an adequate basis (k-index ≈ 0.96), so the elevated EDF reflects the 'smoother' fitting minor hour-to-hour

fluctuations that are small relative to their uncertainty rather than a genuine reorganization; this phase is read as showing no reliable change in rhythm shape.

3.4. Space Use

Space use during training also diverged between the two individuals and in opposite directions (Figure 5). Over the four weeks, the male progressively reduced his movement (step-distance Spearman $\rho = -0.18$, $p < 0.001$; speed $\rho = -0.18$, $p < 0.001$) and increased his distance from the visitor boardwalk ($\rho = +0.21$, $p < 0.001$), whereas the female slightly increased her movement (speed $\rho = +0.10$, $p = 0.03$) and decreased her distance from the boardwalk ($\rho = -0.36$, $p < 10^{-15}$). Between-individual differences in these movement and proximity metrics were significant (Wilcoxon $p < 10^{-6}$), with the male more mobile yet ranging farther from the boardwalk, while the female ranged closer to it. The two also diverged in their use of the release area itself: during the release period, the male moved progressively closer to the pre-release area while the female moved progressively farther from it (daily-median distance 82 → 22 m and 28 → 87 m, respectively; per-individual Spearman on daily medians $\rho = -0.51$, $p = 0.005$ and $\rho = +0.50$, $p = 0.003$), and this opposing divergence was highly significant (week × individual interaction on daily medians, $p < 0.001$) so that the female dispersed outward across the enclosure, while the male remained tied to the release origin. Boundary-clipped kernel home ranges quantified the same divergence (Figure 6): the female's range and especially her 50% core expanded over training (core 0.15 → 0.50 ha; 95% range 1.08 → 1.40 ha), whereas the male's remained smaller and more stable (95% range ≈ 1.3–1.45 ha) before contracting in W4 (0.77 ha, based on 31 fixes during W4, the injury week, and treated as unreliable). The two ranges overlapped broadly within the ≈1.5 ha enclosure, but their 50% cores were initially largely separate and converged over the first three weeks (core-overlap index 0.11 → 0.39; Bhattacharyya's affinity 0.76 → 0.91), with the W4 value again unreliable owing to the male's reduced sampling. Within daylight, neither individual's distance to the boardwalk differed between the public access enclosure's opening hours, in which visitors were present, and the closed hours (Wilcoxon $p \geq 0.35$). As a result, at the 2D level, we found no statistical evidence of visitor avoidance; however, vertical avoidance cannot be excluded. Furthermore, because opening hours coincide with the diurnal activity trough, a visitor-presence effect cannot be separated from the diel rhythm in this observational design.

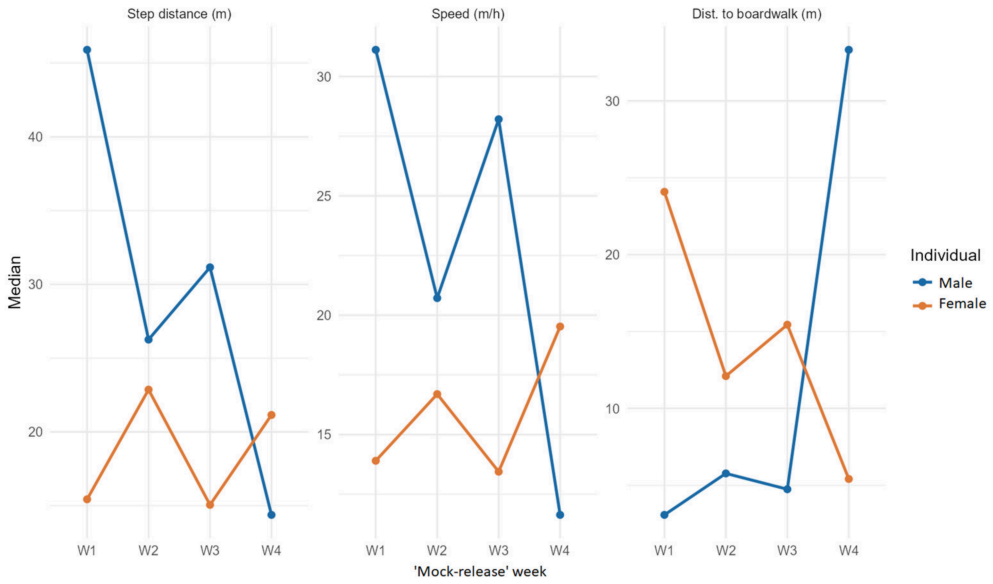


Figure 5. Weekly medians of GPS movement and proximity metrics for the two individuals.

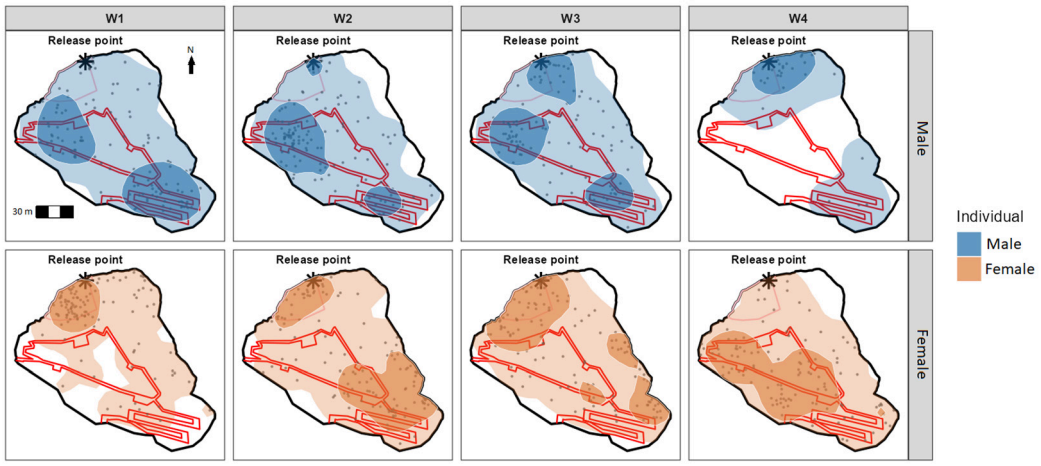


Figure 6. Weekly kernel home ranges within the enclosure across training weeks W1–W4 (male, top; female, bottom; darker = 50% core, lighter = 95% range; gray points = GPS fixes), with the study boundary (black), the visitor boardwalk (red), and the release area (∗) in the upper part of the enclosure. The female progressively shifts her range away from the release point while the male remains near it. Weekly home-range areas (Figure S5), between-individual home-range overlap (Figure S6), and the full-period home-range map (Figure S4) are in the Supplementary Material. Each map is drawn to the same scale and orientation; a north arrow is shown, and coordinates are projected to UTM zone 48N. The enclosure covers 1.5 ha (15,000 m^2), approximately 168 m east–west by 153 m north–south.

3.5. Assessment of the HASR Training

During the month-long 'mock-release', there were eight scheduled training sessions. Overall compliance with HASR protocols exceeded the predefined success threshold established before the study. The female completed all eight sessions and the male only completed seven sessions due to injury. Both animals complied 100% during each session with one exception, discussed below. Twice each week, both red pandas were located within 3–4 m in the 1.5-hectare enclosure using the track feature of the Ecotopia mobile phone application (Druid technology Co., Ltd., Chengdu, China). For each session, once located, the animals returned on cue back to the trainer in a timely manner. For each session, GPS collars were checked for fit and changed weekly as needed without incident. Twice each week, a full-body exam was performed to assess the health condition of each individual, including simulated blood collection, while in the release area without any form of physical or chemical restraint. Following examination, the red pandas were led back, regardless of distance, to the pre-release area and then let loose again. Both animals were weighed using only positive reinforcement once at the midway point during the study period. There was only one exception during the month-long period in which the red pandas did not return to the pre-release study area due to the large volume of tourists. A management decision was made not to conduct this portion of the experiment on this day because it would require the red pandas crossing through a heavily trafficked tourist path.

4. Discussion

Pre-release preparation, as well as long-term post-release monitoring, are major factors contributing to the survival of most translocated mammalian species, with dietary specialists often requiring additional attention. To meet the needs of both the pre-release preparation as well as the post-release monitoring of a bamboo specialist, the Human-Assisted Soft Release (HASR) translocation method was modified and tested with two captive-born red pandas at Panda Valley. After extensive training, the pair of red pandas were released into a large public access enclosure, as a 'mock-release' scenario.

As reflected by the ODBA data, post-release into the large public access enclosure, activity increased significantly during the first week in the novel environment for both individuals. During this period the male's activity level increased more dramatically (46.4% baseline to 60.1% week-1) than the female (36.5% baseline to 44.8% week-1) (Table 1). The high week-1 activity level for the male exceeded the reported 36.5–49.5% activity levels of wild red pandas living in Sichuan, China [43–45]. In addition to the changes in the rate of activity, notable changes occurred in the daily activity pattern shown by the individuals. Week-1, the pair showed a similar trimodal pattern, as baseline influenced by the feeding schedule (8:00, 10:00, 13:30, 16:30) in Panda Valley; however, both showed a shift to more crepuscular/nocturnal activity post-release, and this shift was more pronounced in the male than in the female. The increase in nocturnal behavior throughout the month, especially in the male, may be related to the onset of the red panda breeding season, January–March, and not solely related to the release into the novel habitat. The fact that this study coincided with the red panda breeding season is a general limitation for this study and a confound for many of the behaviors observed, as an increase in nocturnal behavior was noted for captive *A. f. fulgens* during the breeding season in Rotterdam Zoo, the Netherlands (Glatston, unpublished data). Conversely, higher rates of nocturnal activity have been reported in other captive red panda facilities outside of the breeding period [46], which may possibly be a result of the human disturbance related to the presence of zoo guests during the day and, in this case, visitors in the public access area.

By week-2, for the female and week-3 for the male, a shift from the diurnal tri-modal pattern to a more bi-modal pattern with increased crepuscular activity occurred (Figure 4). The reduction of midday activity may be a result of the larger area offsetting the disturbance of the keeper staff during the 13:30 feedings and/or a need for the animals to rest more during midday due to their increased crepuscular/nocturnal activity. Furthermore, the switch to a more crepuscular pattern may also be caused by disturbances of guests to the facility during daytime hours. Similar crepuscular activity was noted in other captive red pandas [46], while shifts to early-morning activities were also noted in red pandas living in a human-dominated landscape in Nepal [47]. Conversely, this pattern may also reflect the animals adjusting to the new area, as similar crepuscular behavior was noted for a wild sub-adult red panda that had yet to establish a territory [44]. This crepuscular pattern in the present study reduced considerably once the animals returned to the smaller pre-release area.

The female's activity patterns for week-3 and week-4 (Figure 4) did not differ detectably from baseline. However, in the case of the male, during the evening of the third day of week-4, a sharp decline in activity was detected (Figure 4). The following morning, the male was recalled using the HASR method and physically examined. Several minor injuries to his neck and ears were discovered, likely as a result of fighting with the resident male. Due to his injuries, the male study subject was led back to the pre-release area for treatment and observation. Data collection on the male was stopped at this point. Previously, two adult males were housed together yearlong in this exhibit without incident, although altercations occurred on occasion. No injuries were noted on the resident male or any of the female red pandas.

After three days of treatment and monitoring, the male fully recovered and was housed again with the study female in the isolated small pre-release area where baseline data was collected, and the post-study monitoring began. Post-study, both animals showed a higher activity rate than baseline (Figures 3 and 4). This increase in activity may again likely be related to breeding behavior and also in the case of the male, recovery from his week-4 injuries. Both individuals resumed the same diurnal tri-modal diel pattern as noted

in the baseline data with the exception of increased nocturnal activity, again possibly due to mating behavior.

In terms of area use, the two red pandas differed significantly. Once allowed free-access to the large 1.5-hectare enclosure, the male immediately dispersed from the pre-release area, with increased movement and travel distance, while the female remained close to the release site and gradually dispersed and expanded her range (Figures 5 and 6). These movement patterns coincided with the activity results: the male's week-1 activity level was higher and declined thereafter, while the female's changes were smaller (Table 1, Figure 3). The male's rhythm shape differed from baseline in every week of the study, whereas the female's differed only in week-2, the week in which her range began to expand (Table 2, Figure 4). These are concurrent observations in two individuals; the collar data cannot establish that one pattern caused the other. During week-2, as the female began to expand her range, she spent more time closer to the public walkway; in contrast, the male did the opposite and spent more time closer to the point of release the last two weeks of the 'mock-release' (Figure 6).

Regarding the behaviors displayed by the two individuals during the 'mock-release' period, ODBA indexes movement intensity, not motivation, so exploration, stress, vigilance, and territoriality cannot be determined from the collar data alone. The increase was apparent for both animals and had returned to baseline by W3 (Figure 4), consistent with the a priori expectations set out in the Introduction. With two individuals, this consistency is descriptive and does not distinguish exploration from sustained stress. Additionally, the male ranged widely and was injured fighting the resident male in W4, indicating a competitive component that cannot be distinguished from the overlap with the breeding season. With the fecal hormone data inconclusive, a stress contribution can be neither confirmed nor excluded.

One of the key factors of interest during this study was how the red pandas would respond to the guests of the facility during visiting hours. A major concern in regards of release programs that incorporate human assistance, such as the HASR, is that these methods may reduce human avoidance and therefore increase poaching risks or human–wildlife conflicts. Interestingly, neither individual's distance to the boardwalk differed during the times in which guests were present and when the facility was closed to the public (Wilcoxon $p \geq 0.35$) (Figure 5). These findings are difficult to interpret; it seems the red pandas did not avoid the visitor walkways. However, it should be noted that the walkway is along a mountain slope and is irregularly elevated several meters above ground. This walkway was specifically designed so that the red pandas may pass under the walking path unhindered. Furthermore, the entire area is heavily forested with many large trees, with added man-made bridges over the tourist path for the red pandas to use. Because of this, while the GPS locations showed the animals were near the path, they may have actually been several meters above or below the path, hiding and out of public view. Because of these confounding factors, visitor effects on the behavior of the individuals could not be isolated from temporal activity patterns. Future studies should consider the use of cameras along the path to determine to which degree the subjects interact with guests to the facility as well as experimentally determining how the presence of visitors impacts the behavior of the red pandas in this enclosure. It merits note that while human interference is a concern for future red panda releases, the planned release areas within Sichuan province are heavily monitored; all hunting is illegal, and poaching is severely punished under law.

The aim of this study was a proof-of-concept test for the use of the HASR method with captive-born red pandas in a controlled environment. There was initial concern that releasing the animals into the public access exhibit was not unfamiliar enough to the

subjects to elicit a strong response and therefore not a suitable proxy for a translocation scenario. However, given the sharp increase in activity, travel distance, and speed in both the male and female subjects during the first two weeks, it is felt that this test was adequate. In addition to the above remote monitoring, as for the use of HASR, all of the four criteria set prior to release were met. During the month-long 'mock-release', both animals complied with each of the four criteria in every session they attended (female 8/8; male 7/7, his final week shortened due to injury), with one exception on a single day when a management decision was taken not to move them across a heavily used visitor path; however, it was difficult to objectively measure compliance, in particular, recalling. The red pandas were often completely out of view, either up a tree or within a nest box. In this situation, we used the Ecotopia mobile phone application (Druid technology Co., Ltd., Chengdu, China) to locate the red pandas within 3–4 m and then called their name as a cue and waited for recall, which generally occurred within a few minutes. Because their exact location and activity at the moment of recall could not be reliably determined, recall time was not considered an accurate objective measure of compliance and therefore not assessed.

Both animals responded reliably to the HASR training throughout the study period. Any apparent differences between them in training responsiveness were not measured objectively and are not reported here. Because of the limited sample size and the overlap with the mating season, further study is needed to determine if these differences are related to sex, personality, a result of the breeding season, or any combination of the factors. A last thing of note, after abnormal activity was detected from the male's collar, he still responded well and cooperated with training. He allowed a thorough body examination in which it was determined that he had several wounds from fighting. Following examination, he then returned on his own with his trainer to the pre-release area for treatment. Similar methods of intense post-release monitoring have been applied with other species, such as in cheetahs (*Acinonyx jubatus*) [48], golden lion tamarins (*Leontopithecus rosalia*) [49], and orangutans (*Pongo pygmaeus*) [50], where the animals are checked daily to ensure they are in a healthy state, with food supplementation and veterinary assistance provided if needed. These methods have been deemed crucial for the survival of these released individuals.

Even for largely successful release programs, such as the golden lion tamarin project, that include intense post-release monitoring and support, a major limiting factor for the long-term monitoring of translocated individuals is the battery capacity of the tracking collars [51]. Typically, in a release scenario, monitoring via GPS ends once the battery is depleted, which leads to inconclusive survival rates, especially for long-lived species such as orangutans [50]. If there is a need to continue long-term monitoring after battery depletion, the animal must be recaptured, anesthetized, and fitted with a new collar, which can cause a considerable amount of stress as well as a safety risk for the animal. In the case of the golden lion tamarin and tamarins in general, because the collars are so small, batteries need to be changed every four to six months and the animals recaptured repeatedly [51,52]. A key factor that distinguishes the HASR method used with giant pandas and the red pandas presented here is that the individuals are trained for the voluntary changing of the GPS collars, so there is no recapture or restraint. This allows the researchers to monitor the animals' long term without concern for battery life and the risks associated with re-trapping individuals. Given the sensitive, elusive nature of the red panda, we believe the HASR method will reduce the stress of recapturing individuals as well as allow for longer uninterrupted monitoring periods, thus increasing the survival rate of the red pandas.

While providing useful information for the conservation translocation of red pandas, this study has several limitations. First, the study only included two individuals, a male and a female, and therefore, the results presented are limited in scope and cannot be expanded beyond the individual level. As a result, all comparisons between the two individuals

are purely descriptive case observations; they are not intended to infer sex-specific or species-level patterns. While it will be extremely challenging to simultaneously release multiple collared individuals at this facility, future studies can release pairs of unfamiliar red pandas into the large enclosure during different periods each year to expand the sample size and further test the HASR method. Secondly, as mentioned earlier, due to management issues and time constraints, this study overlapped with the red panda breeding season. As a result, it is difficult to determine exactly whether the observed behavioral changes were related directly to the 'mock-release' or to breeding activity or a combination of both factors. Given the strong increase in week-1 behaviors for both individuals that declined during the month, as stated in our original hypothesis, H1, it is most likely that these initial changes were related to the 'mock-release'; however, without hormone data or direct observations of breeding behaviors, it is difficult to make a definitive statement. We strongly recommend that future studies and release programs should avoid key life-history moments like the breeding season (January to March) and the birthing season (May to August) to reduce confounding factors. The timing of fisher (*Pekania pennanti*) releases in the USA, in relation to the breeding and birthing seasons, was determined to be a key factor in the reintroduction of this closely related species and should be taken into consideration for future investigations as well as the translocation planning for red pandas [53].

Furthermore, in an effort to monitor breeding status and stress levels during the 'mock-release', attempts were made to collect fecal samples to measure stress and hormone response in the study individuals as they transitioned into the large public access area. Despite our best efforts, it was deemed too difficult to consistently find samples in the large 1.5-hectare enclosure, as the study subjects' movements were irregular. Furthermore, it was difficult to differentiate the feces of the test subjects from the other red pandas living in this enclosure. Fecal samples, which were collected during the pre-release and post-study monitoring period, yielded inconclusive hormonal data and were therefore not reported here. To consistently monitor hormone levels in a field setting, it may be possible to train the red pandas for saliva sample collection. This method has been used in the past with giant pandas [54] and may fill the gap of non-invasive hormonal field testing with red pandas. Lastly, as this proof-of-concept test occurred in a zoo-like public red panda enclosure, the investigation of predator avoidance was beyond the scope of the present study design and warrants further study.

The HASR method has been used by the CRBGPB to successfully translocate giant pandas (*Ailuropoda melanoleuca*). This method relies heavily upon the strong bond between the release staff and the animal, which allows for intense post-release monitoring. As the giant panda is a bamboo specialist and particularly vulnerable to dietary changes, this intense monitoring is used to ensure the animals' nutritional requirements are met as they transition into the release site so as to improve the survivability of the individual. Further testing of the method is required, however; red pandas, as a bamboo specialist, will likely benefit from a similar level of intense post-release monitoring. This current study is the first known test of the HASR translocation method in red pandas and demonstrates that this method has the potential to be used for future releases of this endangered species.

5. Conclusions

This study is the first test of the Human-Assisted Soft Release method in red pandas. Its principal outcome is operational: across a month-long 'mock-release' into a 1.5-hectare public enclosure, two captive-born red pandas met all four predefined HASR criteria in every session attended, allowing recall, voluntary collar changes, body examinations and weighing without anesthesia or physical restraint, and allowing an injury to be detected remotely and treated promptly. This demonstrates that the method is technically feasi-

ble with this species and removes the recapture requirement that constrains long-term post-release monitoring in other programs. The accompanying activity and space-use findings are descriptive observations from two individuals whose study period overlapped the breeding season and included an injury; they are hypothesis-generating rather than hypothesis-confirming and require replication with larger samples outside key life-history periods before any behavioral or sex-related pattern can be inferred.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/jzbg7030035/s1, Figure S1: Hourly-scale HMM, diel-rhythm, and activity-rate results; Figure S2: GPS movement speed by HMM activity state. Figure S3: Per-phase GAM difference smooths for each individual; Figure S4: Full-period kernel home ranges within the enclosure; Figure S5: Weekly 95% and 50% kernel home-range areas (ha) across training weeks; Figure S6: Between-individual home-range overlap across training weeks; Figure S7: Distribution of GPS horizontal dilution of precision across all fixes; Table S1: Candidate hidden-Markov models with differing numbers of activity states; Table S2: State-conditional Gamma parameters and transition probabilities of the retained three-state hidden Markov models; Table S3: Pooled across-individual activity model; Table S4: Per-individual active-state proportion by daylight period.

Author Contributions: Conceptualization, J.E.A.; Methodology, J.E.A. and R.L.; Formal Analysis, X.F.; Investigation, J.E.A.; Data Curation, J.E.A.; Writing—Original Draft Preparation, J.E.A.; Writing—Review and Editing, J.E.A., X.F., and R.L.; Visualization, J.E.A. and X.F.; Supervision, J.E.A. and R.L.; Project Administration, J.E.A.; Funding Acquisition, J.E.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research study was supported by the Sichuan Science and Technology Program (2023YFWZ0010).

Institutional Review Board Statement: All experimental procedures conducted in this study were approved by the Institutional Animal Care and Use Committee of the Chengdu Research Base of Giant Panda Breeding (Protocol #2024002; approval date 31 May 2024) and adhered to the animal welfare regulations of the People's Republic of China and to the animal care guidelines of the Chinese Association of Zoological Gardens.

Data Availability Statement: Data is available upon reasonable request to the authors.

Acknowledgments: The authors would like to thank Hou Rong, Wang Hairui, Yao Ying, Wu Wei, Zhang Hao, Yi Dejiao, Gou Ling, and the red panda keeper staff and animal husbandry department of Panda Valley for their support.

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

References

1. Gaywood, M. *Conservation Translocations in a Changing Climate*; Churchill Fellowship: London, UK, 2024.
2. Morris, S.D.; Brook, B.W.; Moseby, K.E.; Johnson, C.N. Factors Affecting Success of Conservation Translocations of Terrestrial Vertebrates: A Global Systematic Review. *Glob. Ecol. Conserv.* **2021**, *28*, e01630. [CrossRef]
3. Berger-Tal, O.; Blumstein, D.T.; Swaisgood, R.R. Conservation Translocations: A Review of Common Difficulties and Promising Directions. *Anim. Conserv.* **2020**, *23*, 121–131. [CrossRef]
4. De Milliano, J.; Di Stefano, J.; Courtney, P.; Temple-Smith, P.; Coulson, G. Soft-Release versus Hard-Release for Reintroduction of an Endangered Species: An Experimental Comparison Using Eastern Barred Bandicoots (*Perameles gunnii*). *Wildl. Res.* **2016**, *43*, 1–12. [CrossRef]
5. Resende, P.S.; Viana-Junior, A.B.; Young, R.J.; Azevedo, C.S. What Is Better for Animal Conservation Translocation Programmes: Soft- or Hard-release? A Phylogenetic Meta-analytical Approach. *J. Appl. Ecol.* **2021**, *58*, 1122–1132. [CrossRef]
6. Magdalena Wolf, C.; Garland, T.; Griffith, B. Predictors of Avian and Mammalian Translocation Success: Reanalysis with Phylogenetically Independent Contrasts. *Biol. Conserv.* **1998**, *86*, 243–255. [CrossRef]
7. Zhang, Z.; Hu, J.; Yang, J.; Li, M.; Wei, F. Food Habits and Space-Use of Red Pandas *Ailurus fulgens* in the Fengtongzhai Nature Reserve, China: Food Effects and Behavioural Responses. *Acta Theriol.* **2009**, *54*, 225–234. [CrossRef]

8. Wei, F.; Thapa, A.; Hu, Y.; Zhang, Z. Red Panda Ecology. In *Red Panda: Biology and Conservation of the First Panda*; Academic Press: London, UK, 2022; ISBN 978-0-12-823753-3.
9. Jha, A. Release and Reintroduction of Captive-Bred Red Pandas into Singalila National Park, Darjeeling, India. In *Red Panda: Biology and Conservation of the First Panda*; William Andrew Elsevier Science [distributor]: Norwich, NY, USA; Oxford, UK, 2010; ISBN 978-1-4377-7813-7.
10. Glatston, A.; Wei, F.; Than, Z.; Sherpa, A. *Ailurus fulgens* (Errata Version Published in 2017). Available online: https://www.iucnredlist.org/species/714/110023718#assessment-information (accessed on 26 February 2026).
11. Shrestha, A.; Karki, S.; Koju, U.; Maraseni, T.; Gautam, A.P.; Cadman, T.; Baral, S. Stakeholder Perspectives on the Effectiveness of Governance in Red Panda Conservation Programmes in Nepal: A Comparative Analysis. *Ecosyst. People* **2022**, *18*, 547–564. [CrossRef]
12. Tajum Yomcha, A.K. Uncovering the Presence and Conservation Needs of the Elusive Red Panda (*Ailurus fulgens*) in Namdapha National Park, Arunachal Pradesh, Northeast India. *J. Wildl. Biodivers.* **2025**, *9*, 204–217. [CrossRef]
13. Letro, L.; Tandin, T.; Wangdi, S.; Wangdi, T.; Dendup, P.; Millar, J. Status, Distribution and Conservation of Red Panda *Ailurus fulgens* in Bhutan. In *Red Panda: Biology and Conservation of the First Panda*; Academic Press: London, UK, 2022; ISBN 978-0-12-823753-3.
14. Wei, F.; Thapa, A.; Hu, Y.; Zhang, Z. Red Pandas in the Wild in China. In *Red Panda: Biology and Conservation of the First Panda*; Academic Press: London, UK, 2022; ISBN 978-0-12-823753-3.
15. Lin, A.K.; Lwin, N.; Aung, S.S.; Oo, W.N.; Lum, L.Z.; Grindley, M. The conservation status of red panda in north-east Myanmar. In *Red Panda*; Academic Press: Cambridge, MA, USA, 2022; pp. 475–488.
16. Thapa, A.; Wu, R.; Hu, Y.; Nie, Y.; Singh, P.B.; Khatiwada, J.R.; Yan, L.; Gu, X.; Wei, F. Predicting the Potential Distribution of the Endangered Red Panda across Its Entire Range Using MaxEnt Modeling. *Ecol. Evol.* **2018**, *8*, 10542–10554. [CrossRef] [PubMed]
17. Wei, F.; Feng, Z.; Wang, Z.; Hu, J. Current Distribution, Status and Conservation of Wild Red Pandas *Ailurus fulgens* in China. *Biol. Conserv.* **1999**, *89*, 285–291. [CrossRef]
18. Yang, Z.; Gu, X.; Nie, Y.; Huang, F.; Huang, Y.; Dai, Q.; Hu, Y.; Yang, Y.; Zhou, X.; Zhang, H.; et al. Reintroduction of the Giant Panda into the Wild: A Good Start Suggests a Bright Future. *Biol. Conserv.* **2018**, *217*, 181–186. [CrossRef]
19. Wang, Y.; Wei, W.; Yuan, F.; Cao, D.; Zhang, Z. The Science Underlying Giant Panda Conservation Translocations. *Animals* **2023**, *13*, 3332. [CrossRef] [PubMed]
20. Huang, Q.; Fei, Y.; Yang, H.; Gu, X.; Songer, M. Giant Panda National Park, a Step towards Streamlining Protected Areas and Cohesive Conservation Management in China. *Glob. Ecol. Conserv.* **2020**, *22*, e00947. [CrossRef]
21. Bi, W.; Liu, S.; O'Connor, M.P.; Owens, J.R.; Valitutto, M.T.; Hou, R.; Qi, D.; Hayek, L.-A.C.; Wu, F.; Ma, R.; et al. Hematological and Biochemical Parameters of Giant Pandas (*Ailuropoda Melanoleuca*) in Captive and Semi-Natural Environments. *Conserv. Physiol.* **2024**, *12*, coad083. [CrossRef] [PubMed]
22. Bi, W.; Hou, R.; Owens, J.R.; Spotila, J.R.; Valitutto, M.; Yin, G.; Paladino, F.V.; Wu, F.; Qi, D.; Zhang, Z. Field Metabolic Rates of Giant Pandas Reveal Energetic Adaptations. *Sci. Rep.* **2021**, *11*, 22391. [CrossRef] [PubMed]
23. Fan, X.; Lv, R.; Ayala, J.E. Baseline Activity Patterns of Two Captive Red Pandas (*A. f. styani*) to Inform Future Conservation Translocations in Sichuan China. *Animals* **2026**, *16*, 1736. [CrossRef] [PubMed]
24. Stoinski, T.S.; Beck, B.B. Changes in Locomotor and Foraging Skills in Captive-born, Reintroduced Golden Lion Tamarins (*Leontopithecus rosalia rosalia*). *Am. J. Primatol.* **2004**, *62*, 1–13. [CrossRef] [PubMed]
25. Stoinski, T.S.; Beck, B.B.; Bloomsmith, M.A.; Maple, T.L. A Behavioral Comparison of Captive-Born, Reintroduced Golden Lion Tamarins and Their Wild-Born Offspring. *Behaviour* **2003**, *140*, 137–160. [CrossRef]
26. Cheng, Z.; Zhang, H.; Ma, J.; Feng, C.; Liu, W.; Zhong, Z.; Guo, Q.; Zhang, Q.; Zhang, P.; Zhang, S.; et al. Behavioral Shifts of Reintroduced Milu Deer *Elaphurus davidianus* in East Dongting Lake of China. *Sci. Rep.* **2025**, *15*, 34833. [CrossRef] [PubMed]
27. He, K.; Dai, Q.; Foss-Grant, A.; Gurarie, E.; Fagan, W.F.; Lewis, M.A.; Qing, J.; Huang, F.; Yang, X.; Gu, X.; et al. Movement and Activity of Reintroduced Giant Pandas. *Ursus* **2019**, *29*, 163–174. [CrossRef]
28. Shepard, E.; Wilson, R.; Quintana, F.; Gómez Laich, A.; Liebsch, N.; Albareda, D.; Halsey, L.; Gleiss, A.; Morgan, D.; Myers, A.; et al. Identification of Animal Movement Patterns Using Tri-Axial Accelerometry. *Endang. Species Res.* **2008**, *10*, 47–60. [CrossRef]
29. Sikes, R.S.; the Animal Care and Use Committee of the American Society of Mammalogists. 2016 Guidelines of the American Society of Mammalogists for the Use of Wild Mammals in Research and Education. *J. Mammal.* **2016**, *97*, 663–688. [CrossRef] [PubMed]
30. Van De Bunte, W.; Weerman, J.; Hof, A.R. Potential Effects of GPS Collars on the Behaviour of Two Red Pandas (*Ailurus fulgens*) in Rotterdam Zoo. *PLoS ONE* **2021**, *16*, e0252456. [CrossRef] [PubMed]
31. McClintock, B.T.; Michelot, T. momentuHMM: R Package for Generalized Hidden Markov Models of Animal Movement. *Methods Ecol. Evol.* **2018**, *9*, 1518–1530. [CrossRef]
32. Pohle, J.; Langrock, R.; van Beest, F.M.; Schmidt, N.M. Selecting the Number of States in Hidden Markov Models: Pragmatic Solutions Illustrated Using Animal Movement. *J. Agric. Biol. Environ. Stat.* **2017**, *22*, 270–293. [CrossRef]

33. Brooks, M.E.; Kristensen, K.; van Benthem, K.J.; Magnusson, A.; Berg, C.W.; Nielsen, A.; Skaug, H.J.; Mächler, M.; Bolker, B.M. glmmTMB Balances Speed and Flexibility Among Packages for Zero-Inflated Generalized Linear Mixed Modeling. *R J.* **2017**, *9*, 378–400. [CrossRef]
34. Wood, S.N. Generalized Additive Models. In *An Introduction with R*, 2nd ed.; Chapman and Hall/CRC: Boca Raton, FL, USA, 2017.
35. Benjamini, Y.; Hochberg, Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *J. R. Stat. Soc. Ser. B* **1995**, *57*, 289–300. [CrossRef]
36. Calenge, C. The Package "Adehabitat" for the R Software: A Tool for the Analysis of Space and Habitat Use by Animals. *Ecol. Model.* **2006**, *197*, 516–519. [CrossRef]
37. Fieberg, J.; Kochanny, C.O. Quantifying Home-Range Overlap: The Importance of the Utilization Distribution. *J. Wildl. Manag.* **2005**, *69*, 1346–1359. [CrossRef]
38. Bates, D.; Mächler, M.; Bolker, B.; Walker, S. Fitting Linear Mixed-Effects Models Using Lme4. *J. Stat. Soft.* **2015**, *67*, 1–48. [CrossRef]
39. Kuznetsova, A.; Brockhoff, P.B.; Christensen, R.H.B. lmerTest Package: Tests in Linear Mixed Effects Models. *J. Stat. Soft.* **2017**, *82*, 1–26. [CrossRef]
40. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2026; Available online: https://www.R-project.org/ (accessed on 15 May 2026).
41. Thieurmel, B.; Elmarhraoui, A. Suncalc: Compute Sun Position, Sunlight Phases, Moon Position and Lunar Phase, version 0.5.1. 2017. Available online: https://cran.r-project.org/web/packages/suncalc/index.html (accessed on 15 May 2026).
42. Wickham, H. *Ggplot2—Elegant Graphics for Data Analysis*, 2nd ed.; Springer: New York, NY, USA, 2016; ISBN 978-3-319-24275-0.
43. Reid, D.G.; Hu, J.; Huang, Y. Ecology of the Red Panda *Ailurus fulgens* in the Wolong Reserve, China. *J. Zool.* **1991**, *225*, 347–364. [CrossRef]
44. Johnson, K.G.; Schaller, G.B.; Hu, J. Comparative Behavior of Red and Giant Pandas in the Wolong Reserve, China. *J. Mammal.* **1988**, *69*, 552–564. [CrossRef]
45. Zhang, Z.; Hu, J.; Han, Z.; Wei, F. Activity Patterns of Wild Red Pandas in Fengtongzhai Nature Reserve, China. *Ital. J. Zool.* **2011**, *78*, 398–404. [CrossRef]
46. Bugler, K.A.; Ross, J.G.; Paterson, A.M. Activity Patterns of Captive Red Panda (*Ailurus fulgens*). *Animals* **2023**, *13*, 846. [CrossRef] [PubMed]
47. Bista, D.; Baxter, G.S.; Hudson, N.J.; Lama, S.T.; Weerman, J.; Murray, P.J. Movement and Dispersal of a Habitat Specialist in Human-Dominated Landscapes: A Case Study of the Red Panda. *Mov. Ecol.* **2021**, *9*, 62. [CrossRef] [PubMed]
48. Walker, E.H.; Verschueren, S.; Schmidt-Küntzel, A.; Marker, L. Recommendations for the Rehabilitation and Release of Wild-Born, Captive-Raised Cheetahs: The Importance of Pre- and Post-Release Management for Optimizing Survival. *Oryx* **2022**, *56*, 495–504. [CrossRef]
49. Kierulff, M.C.M.; Ruiz-Miranda, C.R.; De Oliveira, P.P.; Beck, B.B.; Martins, A.; Dietz, J.M.; Rambaldi, D.M.; Baker, A.J. The Golden Lion Tamarin *Leontopithecus rosalia*: A Conservation Success Story. *Int. Zoo Yearb.* **2012**, *46*, 36–45. [CrossRef]
50. Russon, A.E. Orangutan rehabilitation and reintroduction. Orangutans: Geographic variation in behavioral ecology and conservation. In *Orangutans: Geographic Variation in Behavioral Ecology and Conservation*; Oxford Academic: Oxford, UK, 2009; pp. 327–350.
51. Beck, B.B.; Kleiman, D.G.; Dietz, J.M.; Castro, I.; Carvalho, C.; Martins, A.; Rettberg-Beck, B. Losses and reproduction in reintroduced golden lion tamarins *Leontopithecus rosalia*. *Dodo J. Jersey Wildl. Preserv. Trust.* **1991**, *27*, 50–61.
52. Catenacci, L.S.; Raboy, B.E.; Oliveira, L.; de Carvalho, C.E.; Neves, L.G.; Suscke, P.; Deem, S.L.; Costa, T.; De Vleeschouwer, K.M. Golden-headed Lion Tamarins, *Leontopithecus chrysomelas* (Kühl, 1820): 27 Years of Experience in Methods for Their Capture and the Collection of Biological Material. *Primate Conserv.* **2022**, *36*, 23–35.
53. Facka, A.N.; Lewis, J.C.; Happe, P.; Jenkins, K.; Callas, R.; Powell, R.A. Timing of Translocation Influences Birth Rate and Population Dynamics in a Forest Carnivore. *Ecosphere* **2016**, *7*, e01223. [CrossRef]
54. Yan, Z.; Liu, X.; Liu, H.; Xu, H.; Liu, Y.; Li, C.; Wang, B.; Cui, S.; Jia, T.; Yang, D.; et al. Assessment of Stress Levels and Reproductive Condition in Giant Pandas: Insights from Hair, Faecal and Saliva Samples. *Conserv. Physiol.* **2024**, *12*, coae044. [CrossRef] [PubMed]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.