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Impacts of man-made provisioned food on learned cub behaviours of giant pandas in pre-release reintroduction training

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Abstract. Reintroduction is a valuable tool for conserving threatened species, and pre-release training is widely considered to be crucial for successful soft-release. However, little is known about how behaviours of pre-released giant panda cubs are affected by their mothers being provided with man-made provisioned food versus mothers that naturally forage. In this study, we monitored two pairs of mother-cub giant panda pairs in Hetaoping, Wolong National Nature Reserve, China. One mother giant panda's diet was supplemented with man-made food and the other had entirely natural foraging conditions. We investigated differences in the cubs' learning ability about habitat selection, their feces abundance, and home range patterns during pre-release training and acclimatization between the two conditions. The results indicate that the cub whose mother had entirely natural food (CN) selected similar microhabitat to its mother (MN). The cub whose mother was supplemented with man-made food (CMF) differed from its mother (MMF) preferring microhabitats with higher proportions of canopy coverage and areas closer to shelter. Compared to natural feeding conditions, provisioning a giant panda mother with man-made food seemed to impact cub microhabitat selection, especially in variables describing the amount of canopy cover, proximity to water resources, and grass coverage. CMF showed no difference in preference between bamboo edge habitat and interior habitat, which differed from other pandas previously studied. Similar to her mother, the home range of CMF was significantly less than CN. Our study suggests that provisioning giant panda with man-made food may hinder behavioural development, such as microhabitat and home range selection, of giant panda cubs during the acclimatization period. We recommend lessening human intervention as much as possible for future pre-release reintroduction training. Anthropogenic interference should be limited as much as possible in pre-release reintroduction training programs and managers should strive to provide conditions similar to those found in the species' natural range in order to provide the highest probability of success for the reintroduced individual.

Key words: reintroduction, pre-release training, feces abundance, habitat selection, home range

Introduction

Reintroduction has been seen as a valuable tool for conserving many threatened species (Short et al. 1992, Kleiman 1996). Many biological and ecological factors have been shown to contribute to the success or failure of a reintroduction project, such as the provenance of released animals (wild-caught or captive-born), type of release (soft or hard), and habitat (including food) suitability (Jule et al. 2008). In particular, there are many behavioural problems unique to captive-born carnivores, which include loss of socially learned skills (e.g. hunting and mating) and high levels of stereotypies or abnormal behaviours (Soorae & Stanley Price 1997). For instance, stereotypic behaviour has been reported in captive giant pandas (Liu et al. 2005). One method to

enhance a captive-born individual's ability to adapt to wild environments is pre-release training and acclimatization (Beck et al. 1994, Kleiman 1996). Soft-releases allow for acclimation to a new location prior to release and allows animals time to gain familiarity with places to shelter and escape predation (Biggins et al. 1999). Pre-release training can assist reintroduced individuals in the development of antipredator behaviour prior to reintroductions (Alberts 2007).

The giant panda (*Ailuropoda melanoleuca*) is a threatened species (IUCN 2016), and extensive efforts have been made by conservation biologists and wildlife managers to enlarge its populations. Limited by breeding ability and habitat fragmentation, the population size of wild giant pandas has oscillated

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from the 1980s to 2014 (State Forestry Administration 2015). On the other hand, the population of captive giant pandas has increased steadily since 2000, and reached 471 by the end of 2016 (Chinese Association of Zoological Gardens 2016). With the increasing captive population, managers are now able to reintroduce some of the captive giant pandas to their historical distribution area to recover local giant panda populations, or rejuvenate isolated small populations (Zhang et al. 2006, Lei et al. 2015).

The giant panda has been a top priority for conservation action in China since the 1950s (Hu et al. 1985), but the small habitat area and low population density of giant pandas in the Xiaoxiangling mountain region are challenging conservation efforts (State Forestry Administration 2015). Although faced with difficulties typical of captive-born animals released to the wild, the captive-born giant panda Xiangxiang was first released into Wolong Nature Reserve, China in April, 2006. To enlarge the small population and enrich the genetic diversity of the isolated Xiaoxiangling population, the next attempts at reintroduction were there. The captive-born giant pandas, Taotao and Zhangxiang, were pre-release trained in Wolong Nature Reserve, China in 2012 and 2013, respectively, and subsequently released in Liziping Nature Reserve of the Xiaoxiangling mountains, China.

Although giant pandas have historically specialized as a bamboo feeder with more than 99 % of their annual diet consisting bamboo in the wild (Hu et al. 1985), previous studies have found that the diet of wild populations varies among different mountain regions (Hu et al. 1985, Pan et al. 2001, Hull et al. 2014). Spatial behaviour of giant pandas, especially for habitat selection, have also been shown to be very flexible (Hu et al. 1985, Pan et al. 2001, Hull et al. 2014, Hong et al. 2015, 2016). Flexibility in those behaviours can help giant pandas adapt to available environmental conditions (Hu et al. 1985, Zhang et al. 2009, Hull et al. 2014). Placed in enclosures for pre-release training and acclimatization, captive giant pandas experience environmental conditions that simulate the wild. Giant pandas may exhibit different behaviours, such as habitat selection, space use, and distances moved in pre-release training enclosures due to different levels of human disturbance, such as man-made food.

During the pre-release training and acclimatization phases of soft release, the effects of human disturbances on a captive born individual and its mother remain poorly understood. In this study, we monitored two pairs of mother-cub giant pandas in

Hetaoping, Wolong National Nature Reserve, China. One mother giant panda was fed man-made food and the other pair was not, and we investigated the two pairs' spatial behaviours under the presence or absence of man-made food conditions. To do this, we: 1) surveyed and compared differences between the giant pandas' learning ability about habitat selections, 2) studied the variation of feces abundance among different habitat types, and 3) tested whether different individuals exhibited different home range patterns.

Material and Methods

Study area

The pre-release training enclosure used in this study covers about 24 ha² and is located at Hetaoping, in the northeast of Wolong National Nature Reserve (Fig. 1). The elevation range is from 2100 to 2400 m. The climate is dominated by the south-east monsoon from May to October, and continental air masses in the winter. The average annual rainfall is 1800 mm (winter snowfall averages 936 mm), with an average evaporation of 874 mm. The highest temperatures occur in July with daily average temperatures of 17 °C and the lowest occur in January with daily average temperatures of -1.7 °C. The study area features broad-leaved deciduous forest with dominant species of *Juglan scathayensis*, *Rhus punjabensis* var. *sinica*, *Acer flabellatum*. Both *Fargesia robusta* and *Yushania brevipaniculata* bamboo species exist in the pre-release training enclosure, with the former covering 95 % of the study area.

Fig. 1. Study area in Wolong National Nature Reserve, China.

Study animals

To help the pre-released giant panda learn skills from its mother, we used mother with cub training methods. The male cub "Taotao" (designated CN for

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Table 1. Information of the pre-released training giant pandas. CN: cub with nature food, MN: mother with nature food, CMF: cub affected by manmade food, MMF: mother with man-made food. CCRCGP: China Conservation and Research Center for the Giant Panda.

"cub with natural food") was born in Hetaoping on 3rd August 2010, and its mother "Caocao" (designated MN for "mother with natural food") was rescued from Qionglai mountain. Managers supplemented the other mother giant panda, "Zhangka", with provisioned man-made food (e.g. bamboo shoots and vitamin enriched biscuit) thrown onto grassy areas in the enclosure. The female cub "Zhangxiang" (designated CMF for "cub with man-made food") was born in Hetaoping on 20th August 2011, and its mother "Zhangka" (designated MMF for "mother with manmade food") was born in 2000 and rescued from Qionglai mountain. MN-CN were first transferred into the acclimatization enclosure for pre-release training on 3rd May 2012. After CN was released into wild on 11th October 2012, MMF-CMF were transferred into the same acclimatization enclosure on 21st November 2012 for pre-release training. CMF was transported into another acclimatization enclosure for further prerelease training in the Liziping Nature Reserve on 6th November 2013. Detailed information for all giant pandas can be found in Table 1.

Experimental design

Field surveys were conducted on the entire enclosure from $11th$ October to $23rd$ October 2012, and $6th$ November to 29th November 2013 after pre-release training. We set transect lines at roughly 100 m equidistant from each other following the methods of Wei et al. (2000), Zhang et al. (2006) and Hong et al. (2015). Along transect lines, we established microhabitat plots $(20 \times 20 \text{ m}^2)$ centered on fecal deposits and feeding sign encountered, with the distance from each other not less than 60 m gain in elevation or not less than 100 m in horizontal distance. All microhabitat plots were set and sampled following the methods of Wei et al. (2000) and Zhang et al. (2006). Sixteen variables reflecting slope, trees, shrubs, and bamboo were measured (Table 2). During field surveys, we recorded the total number of individual feces droppings and dropping groups,

respectively, following Hong et al. (2015). We recorded the GPS coordinates of each fecal deposit and feeding sign of both the cub and the mother, and mapped the presence of bamboo edge, bamboo interior, bushwood, and grassland in the understory. We could easily distinguish the feces of the mother from the cub because the cub's feces were much smaller (means \pm standard deviation length: 7.71 ± 0.69 , width: 2.76 \pm 0.30 cm) than those of the mother's (13.28 \pm 0.85 and 5.20 ± 0.63 cm). To avoid repeated sampling, all microhabitat plots were surveyed only once during the study period, and panda feces in microhabitat plots were destroyed after the survey.

Statistical analysis

We first used one-sample Kolmogorov-Smirnov test to check if the data were normally distributed, and then conducted independent sample t-tests to compare variables between cub and mother microhabitat plots when data were normally distributed or Mann-Whitney U test when the distributional assumptions were not met. Only variables with significant difference were involved in subsequent analyses. For those predictor variables with a correlation coefficient above 0.60, the variable with a clearer biological meaning was retained for further analysis to weaken collinearity (Liang & Thomson 1994, Fabrizio et al. 2003, Sèbastien et al. 2003). Correlation coefficients were calculated through Pearson correlation analysis when data were normally distributed or Spearman correlation analysis when data were not normally distributed. Stepwise discriminant function analysis was adopted to assess the relative importance of variables contributing to the difference of microhabitat plots between the cub and mother giant panda.

To uncover the effects of provisioning man-made food on cub's learned microhabitat selection, we first calculated ratios of variables (variables in microhabitat plots of cub divided by those of its mother) and then compared them between the two pre-release feeding conditions (provisioned food versus natural foraging conditions) through independent samples t-tests (normally distributed) or Mann-Whitney U test (not normally distributed). Stepwise discriminant function analysis was used to assess the importance of variables in contributing to differences in microhabitat selection between the two feeding conditions.

To compare the spatial behaviour of giant pandas, we tracked each panda using high-resolution global positioning system (GPS) telemetry. We then calculated minimum convex polygon home ranges using the Geospatial Modelling Environment program (Beyer 2011). The relative proportion of each giant

Fig. 2. Relative proportion of each giant panda's number of feces among different habitats.

Fig. 3. Comparison of the home ranges of different giant pandas.

Fig. 4. Comparison of average feces number of different giant pandas.

panda's feces number range from different habitat types were compared through chi-square test, a method that was also used to compare the home range size among each giant panda. We also conducted independent samples t-tests to compare the number of feces per site between giant pandas (when data were normally distributed) or Mann-Whitney U test (data not normally distributed). All statistical tests were two-tailed and conducted in SPSS 17.0 (SPSS Inc., Chicago, U.S.A.). Data are presented as mean \pm SD, and the significance level of all analyses was 0.05.

Results

Differences in microhabitat selected by captive-born giant panda cubs and their mothers

There was no significant difference between variables selected by MN-CN pair through independent samples t-test or Mann-Whitney U test (Table 3). Microhabitat plots selected by CMF differed significantly from those selected by MMF in 14 variables (all except shrub dispersion and shrub DBH, Table 3), indicating MMF-CMF pair exhibited different microhabitat selection patterns. Only five variables (canopy, shrub size, shelter distance, tree dispersion and water distance) were entered into the last discriminant function. These variables made greater contributions to differentiating the microhabitat plots of CMF from those of MMF (χ^2 = 52.70, df = 5, p < 0.001; Table 4), with an overall correct prediction rate of 88.46 % (Table 5). Compared to MMF, CMF tended to select microhabitat that had higher overstory canopy, lower understory coverage, was closer to shelter, and was farther from water sources.

Difference in cub microhabitat selection learned from its mother between pairs exposed to natural vs. provisioned man-made food

Ratios of all microhabitat variables between mother and cub differed significantly in the man-made food pair, indicating the giant panda cubs exhibited different microhabitat selection patterns learned from its mother under the presence of man-made food (Table 3). Only canopy, shrub dispersion, distance from water, and grass coverage entered the last discriminant function and made greater contribution to differentiating microhabitat selection between the two types of food resources ($\chi^2 = 88.80$, df = 4, p < 0.001; Table 4), with an overall correct prediction rate of 95.16 % (Table 5).

Compared to MMF, CMF significantly selected higher overstory canopy ($p < 0.001$), farther water resources $(p < 0.001)$ and lower grass coverage $(p < 0.001)$, but

no significant difference was found in those variables between CN and MN (Table 3). Difference between ratios of shrub dispersion reflected that CMF selected areas with more shrubs compared to MMF, than CN did compared to its mother MN (Table 3).

Comparison of fecal deposition of each giant panda in different habitats

CMF was the only giant panda in this study that deposited feces in bushwood. Excluding this habitat, the relative proportion of the number of feces in the other three habitats were significantly different among the four giant pandas for bamboo edge $(\chi^2 = 24.95, d\text{f})$ $= 3, p < 0.001$), for bamboo interior ($\chi^2 = 46.39$, df = 3, $p < 0.001$), and for grass land (χ^2 = 107.68, df = 3, *p* < 0.001; Fig. 2). No significant difference existed in relative proportion of feces in the three habitat types between MN and CN. The relative number of feces of MMF in bamboo edge and interior were significantly less than that of CMF ($\chi^2 = 5.43$, df = 1, $p = 0.02$; and $\chi^2 = 22.15$, df = 1, $p < 0.001$; respectively), and there were relatively more feces in grass land than that of CMF (χ^2 = 35.84, df = 1, p < 0.001). MN more frequently appeared in bamboo edge (χ^2 = 19.56, df $= 1, p < 0.001$) with lower frequency in grass land $(\chi^2 = 42.25, df = 1, p < 0.001)$ than MMF. CN also more frequently appeared in bamboo edge habitat (χ^2) $= 4.64$, df $= 1$, $p = 0.03$), but had a lower frequency in

bamboo interior ($\chi^2 = 13.33$, df = 1, $p < 0.001$) than CMF.

Comparison of home range and trace abundance of different giant pandas

The home range among the four giant pandas was significantly different (Fig. 3; $\chi^2 = 23.41$, df = 3, *p* < 0.001). There was no difference in home range between MN and CN or MMF and CMF (Fig. 3). However, the home range of MN was larger than MMF (χ^2 = 17.86, df = 1, p < 0.001), and the home range of CN was larger than CMF $(\chi^2 = 5.43, df = 1, p$ $= 0.02$). The average number of feces among the four giant pandas was significantly different (Fig. 4; $F =$ 8.27, $df = 3$, $p < 0.001$). Even though no significant difference of average feces number existed between MN and MMF or CN and CMF, the average number of feces of the cubs in each pair was a little less than that of the mothers (Fig. 4).

Discussion

Mammals not only provide sustenance and protection to their offspring, but also teach them a variety of life skills during the rearing phase (Sun 2001). Our results clearly indicate that mother-cub pairs raised in prerelease training enclosures relying on entirely natural forage (MN-CN) exhibited similar microhabitat selection patterns, which parallels reports by Zhang

Table 3. Independent sample t-test or Mann-Whitney U test for variables among different groups.

* Data in the table are presented by mean ± SD, ^a comparisons through independent samples t-test, ^b comparisons through Mann-Whitney U test.

Table 4. Variables entered into standardized canonical discriminant function.

et al. (2013). Provisioning man-made food to MMF-CMF resulted in different microhabitat selection between the pair in almost all habitat variables. In particular, in contrast to its mother, CMF preferred

higher canopy and areas closer to shelter. Living in solitude, giant pandas naturally avoid humans (Hu et al. 1985). Our results indicate that provisioning food to mother giant pandas caused the cub to select higher

Table 5. Overall correct prediction results for different plot groups through stepwise discriminant function analysis.

security habitats. This preferential selection of higher security habitat may have been caused by the presence of the humans providing food. Our results must be interpreted with caution as we only compared two cubs and this is the first study to compare microhabitat selection within the same enclosure. Almost all previous studies on habitat utilization surveyed all giant pandas in a given area (Hull et al. 2014). Future studies should focus on investigating the similarities or differences in habitat selection behaviours between sub-adult pandas and adult individuals when they coexist in one area.

Compared to the natural feeding regime, provisioning man-made food significantly impacted giant panda cub's learned microhabitat selection behaviours, especially in regards to overstory tree coverage, water resources, and grass coverage. Giant pandas retains a short carnivorous alimentary tract even though its diet is composed almost exclusively of bamboo (Hu et al. 1985). Thus, giant pandas have very low digestive efficiency and exhibit exceptionally low daily energy expenditure during extensive foraging sessions on bamboo foods (Nie et al. 2015). Habitats of higher canopy overstory possess more nutritious bamboo (Hu et al. 1985, Hong et al. 2016), but MMF received supplemental man-made food during pre-release training that provided additional energy supplements which probably made it unnecessary for her to seek out higher quality bamboo habitat featuring more overstory trees. This behaviour was markedly different from MN, who may have needed to spend more time seeking higher quality bamboo under high canopy under the all natural feeding regime, a behavioural preference that was also passed to her cub, CN (Table 2).

Without human provisioning of food, CN and its mother MN preferred bamboo edge habitats for higher quality foods (Yu et al. 2003), and exhibited similar habitat preference (Fig. 2). In contrast, CMF spent larger periods of time in bamboo interior for shelter and bamboo resources than its mother, CN, and the mother under the natural feeding regime, MN (Fig. 2). This result may be explained by the interference caused by provisioning man-made food by the staff in grass land areas resulting in MMF spending more time on grass land consuming man-made food, subsequently spending less time teaching CMF to forage higher quality bamboo at bamboo edge habitats. In some bird species, offspring that receive low levels of care are less successful and have lower fitness (Kilner et al. 2015). We propose that CMF had less time learning skills from its mother and, therefore, CMF was found more in microhabitats with less bamboo and higher densities of vine and liana. It appears that the difference in behaviour between CMF and CN may be due to provisioning man-made food to MMF possibly resulting in less habitat selection mother-cub teaching opportunities for CMF. That provisioning mother pandas with man-made food significantly impacted cub giant panda habitat selection and foraging behaviours in the pre-release training program.

Commonly, an animal's home range may be defined as the area used to forage, mate, and foster offspring (Burt 1943). It is important to understand the spatial extent of an animal's home range, especially in comprehending a species' ecological development and in conserving adequate habitat for its survival (Connor et al. 2016). Multiple studies have found giant pandas have larger home ranges than previously thought when including data from periods longer than one year (Schaller et al. 1985, Yong et al. 2004, Hull et al. 2015). During the current study conducted over one month, home ranges of MN or MMF were obviously smaller than those of wild pandas in the Qionglai mountains ($\leq 5.14 \text{ km}^2 \pm$ 1.04, Hu et al. 1985). The home range of MMF was significantly smaller than both MN and CN's (Fig. 3). This indicates that provisioning man-made food significantly impacted MMF's movements, which subsequently impacted the size of CMF's home range. This result was also confirmed by the average number of feces of each panda, with higher average number of feces of MMF in areas provisioned with man-made food (Fig. 4).

In summary, our results indicate provisioning giant pandas with man-made food affected giant panda cub behaviours in the pre-release training program. Provisioning man-made food not only affected the mother giant panda's behaviours (i.e. MMF) but also may have hindered behavioural development of giant panda cubs during the acclimatization period, such as microhabitat selection and home range behaviours. We suggest that during pre-released training, anthropogenic interferences should be lessened and captive-born animals should be raised in the most natural conditions possible in order to develop appropriate behavioural repertoires.

Management implications

A successful reintroduction program can be impacted by many factors, such as movement capacity, sufficient protected areas, diseases, community support, etc. (Kleiman et al. 1994, Zhang et al. 2006). Keeping other conditions normal, our results clearly demonstrate that man-made foods can affect the amount of time pre-released giant panda cubs learn from its mother within the training enclosure. This subsequently affected learned behaviours, most importantly habitat selection and movements. The cub whose mother was exposed to man-made food tended to use higher security areas and lower quality bamboo habitats, and had a smaller home range. Trying to match the pre-released training environments more closely to wild habitat and minimizing anthropogenic interference may increase the chances of captive-born released success (Stamps & Swaisgood 2007). Our study results lend credence to this theory and suggests that future pre-release training must lessen human interference as much as possible during the whole acclimatization period.

The "Captive Giant Panda Release Project" was launched approximately 10 years ago, and CN and CMF were released into Liziping Nature Reserve. Perhaps resulting from its mother's exposure to man-made foods in the pre-release, CMF exhibited different microhabitat selection and foraging strategies from wild giant pandas in Liziping Nature Reserve (Lei et al. 2015). Because anthropogenic interference may significantly influence sub-adult pandas, which may persist into adulthood (Nussey et al. 2007), we suggest that future pre-released training of captive individuals should be conducted under natural conditions in order to increase the likelihood of survivorship in the wild. Though our study was conducted on giant pandas, our results may have far reaching implications for other captive-bred animals slated for reintroduction. Anthropogenic interference may significantly influence behavioural development, which could potentially adversely affect reintroduction survivorship. Pre-release training programs that prepare captive-born vertebrates for reintroduction should fully consider the potential impacts of even limited anthropogenic interference.

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