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Assessing the reproductive status of a breeding, translocated female giant panda using data from GPS collar

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Abstract. Reintroductions and translocations have proven to be effective measures for rescuing small, isolated populations of endangered wildlife. However, the reproductive status of released wildlife is hard to obtain. To date, a giant panda named Luxin is the only translocated giant panda that has successfully bred in the wild. Using data collected from the GPS collar attached to her, we analysed her activity and home range during a breeding year and compared these values with those collected during a non-breeding (control) year. Delivery and mating days can be identified by extremely low levels of activity or even by the absence of activity. The activity of a giant panda with an infant was low, but it increased gradually after delivery. The activity rates during both the delayed implantation period and the infant-caring period were significantly lower in the breeding year than those in the control year. In the breeding year, the home ranges during the delayed implantation period and pregnancy were larger than those in the control year, while the home range during the infant-caring period was much smaller than that in the control year. Our results suggest that GPS collars embedded with activity sensors can be used to monitor the breeding status of released female giant pandas. They can provide valuable information for decision making in future release projects, providing only small disturbances to released giant pandas.

Key words: new method, activity rate, home range

Introduction

Reintroductions and translocations have proved to be effective actions in the protection of endangered species, especially for rescuing small, isolated populations (Hayward & Somers 2009). Translocations of released individuals in the wild is the key indicator of a successful release (Zhang et al. 2006). Post-release monitoring is commonly recommended by most guidelines for translocation, since it not only provides critical information about the status of released wildlife but also provides feedback to improve the process of translocations (IUCN 1987). However, the reproductive status of released wildlife is hard to obtain, even when general monitoring is employed after release.

Many methods can be used to detect pregnancy and delivery in mammalians, such as blood tests, X optical imaging technology, ultrasound imaging, or hormone levels in urine and faeces (Kähn 1992, Schwarzenberger et al. 1996, Bauman et al. 2010). However, those methods are generally difficult to apply to animals in the wild; the individuals may be elusive and difficult to observe, or they may live in large home ranges and have high activity rates (Wimsatt 1963). GPS collars embedded with activity sensors have been used to remotely monitor the behaviour of wildlife (Roberts et

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Fig. 1. The giant panda, Luxin (front), with her infant (behind).

al. 2016). They can be used to identify the reproductive status of wild animals, since the behaviour of wildlife generally changes during the breeding season (Perrigo 1987, Kleiman et al. 2013). However, this method has rarely been applied to detecting breeding in wildlife, except when Friebe et al. (2013) determined the pregnancy of a brown bear by her activity pattern using data from GPS collars.

The giant panda is a global symbol of natural protection (Wei et al. 2011). The reproductive biology of captive giant pandas has been systematically studied (Zhang & Wei 2006), while knowledge on wild giant panda reproductive biology is limited and mostly derived from casual observations (Hu et al. 1985). The hormone levels in urine and faeces have been used to identify the status of reproduction for captive giant pandas (Chaudhuri et al. 1988), but for wild ones, collecting fresh faeces from an individual during the breeding season is difficult if not impossible to obtain. He et al. (2016) identified activity status using the data from a collar with an activity sensor. However, it is still not known whether the data from GPS collars can be used to determine the reproductive status of the giant panda.

A set of photographs caught by camera traps found that Luxin, a translocated female giant panda, was caring for an infant (Fig. 1). This discovery made her the only known translocated giant panda that had successfully bred in the wild. This study, using the data from the GPS collar attached to her, analysed her behaviour patterns during breeding and infant caring and tried to identify a set of behavioural criteria to assess breeding status in female giant pandas.

Material and Methods

Study area and object

Luxin was released in the Liziping National Nature Reserve (102°10′33″-102°29′07″ E, 28°51′02″-29°08′42″ N), located in the southwest region of China. The reserve covers an area of approximately 47940 ha in the Xiaoxiangling Mountain Range, with altitudes ranging from 1330 to 4551 m (Fig. 2).

Luxin, a wild female giant panda, rescued in southwest of Qionglai Mountains in March, 2009. She was about 70 kilograms-weight and about five years old when rescued and released to Liziping National Nature Reserve on April 29, 2009. The GPS collar attached to her failed one month after releasing. She was recaptured on May 4, 2011, and then released again on May 13, 2011 with a new GPS collar, which worked for up to three years.

Data collection

We fitted a GPS 7000MU collar (Lotek Wireless Inc, Newmarket, Ontario, Canada) on Luxin's neck before releasing her into the Liziping National Nature Reserve. The GPS collar was embedded with a GPS locator and dual-axis accelerometers. Her location was recorded every three hours. The dual-axis accelerometers collected both vertical and horizontal accelerations. These acceleration values were accumulated and averaged for each direction during a recording interval of 5 minutes. This value was then converted into a unit, with a scale ranging from 0 (no activity) to 255 (highest activity).

We summed values from vertical and horizontal accelerometers at 5-min intervals to represent the activity index, which ranged from 0 to 510 (Coulombe et al. 2006, Yamazaki et al. 2008, McLellan & McLellan 2015). Using the activity index value of 32 as a threshold, Luxin's behaviours were classified as either "active" or "rest" following the methods of He et al. (2016).

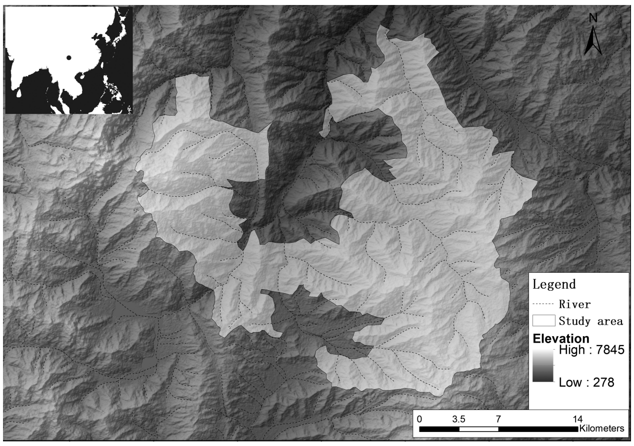

Fig. 2. Location of Liziping National Nature Reserve.

Definition of reproductive status

The breeding year calendar was used in the analysis, which starts from the beginning of a breeding season and ends before the next breeding season. Data from two years were collected: the breeding year (May 2012-May 2013) and the non-breeding year (May 2011-May 2012), which was used as a control.

The reproduction of the giant panda has five events or stages occurring in a sequence: the mating event, the period of delayed implantation, the period of pregnancy, the delivery event and the period of infant caring (Zhang & Wang 2003). The mating and delivery events were used as marking points to calibrate the breeding year, since the activities of the two behaviours are unique for the giant panda and easy to identify. Mating generally occurs between March and May, and delivery generally occurs between August and

Table 1. Time of delayed implantation, pregnancy and infant-caring in the breeding year and control year.

Period	Reproductive state	Time
Breeding year	Delayed implantation	1 May 2011-5 Jul. 2011
	Pregnancy	6 Jul. 2011-3 Sep. 2011
	Infant-caring	4 Sep. 2011-25 Nov. 2011
Control year	Delayed implantation	1 May 2012-5 Jul. 2012
	Pregnancy	6 Jul. 2012-3 Sep. 2012
	Infant-caring	4 Sep. 2012-25 Nov. 2012

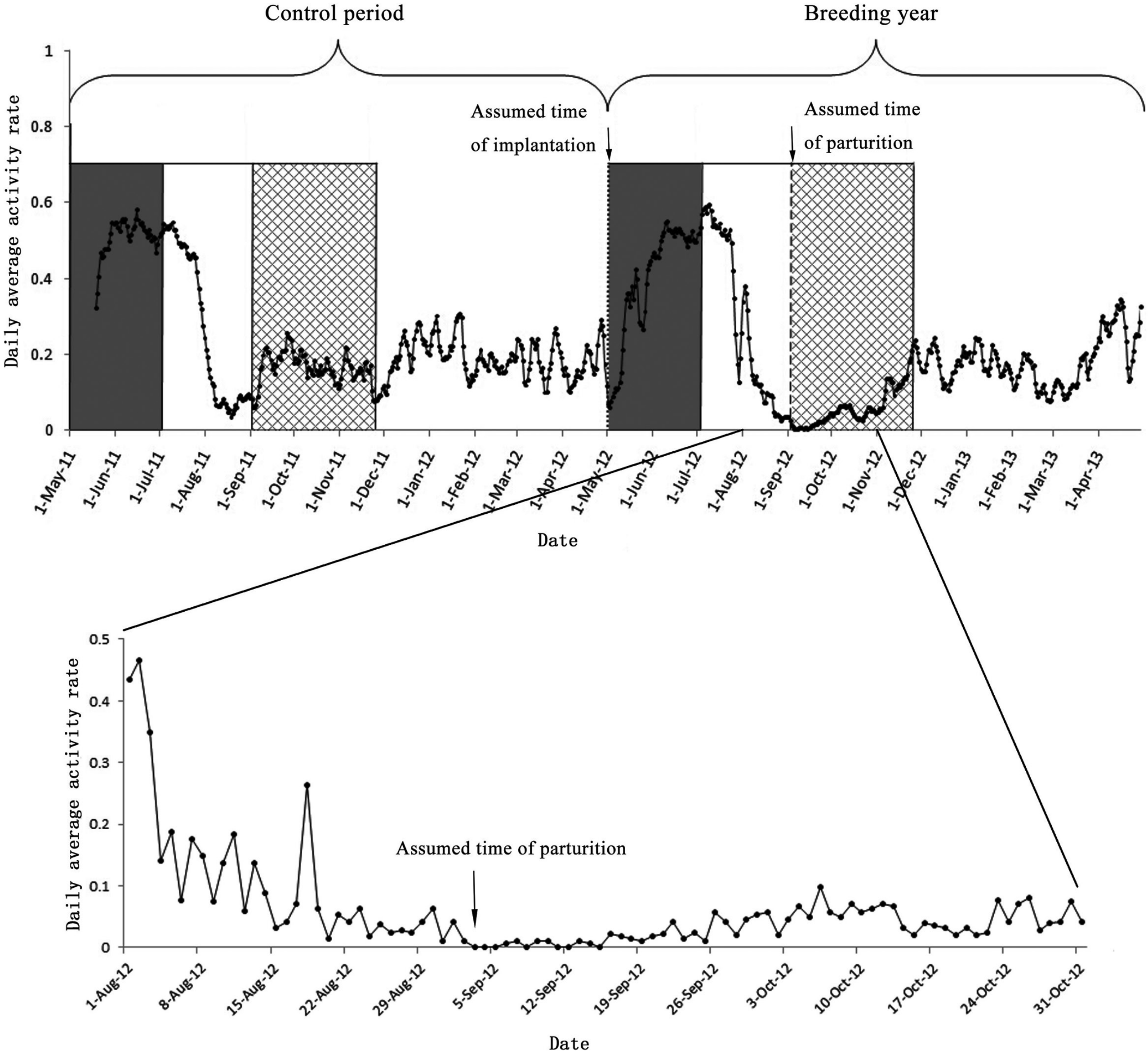

Fig. 3. Three-day moving average of activity rate for the control year (left) and the breeding year (right). The dotted line shows the time of implantation. The dashed line shows the time of parturition. The grey bar shows the defined period of delayed implantation. The white bar shows the defined period of pregnancy. The grid bar shows the defined period of infant-caring. The black line with black points shows the 3-day moving average of activity rate.

Table 2. The number of GPS sites and the area of the home range for each phase of the reproductive cycle during both the control year and breeding year.

Year	Three predefined periods	N (day)	Number of GPS sites	Kernel 95 % (ha)
Control year	Delayed Implantation	43	230	211.33
	Pregnancy	60	255	432.50
	Infant-caring	83	487	1018.98
Breeding year	Delayed Implantation	65	243	2070.09
	Pregnancy	60	47	1775.30
	Infant-caring	83	140	14.95

October. Based on the two marking points, the three other periods were identified in the breeding year. The methods to identify the five stages are outlined below.
Field observations found that female giant pandas wait in the trees without food or movement, while male giant pandas fight for mating rights (Pan et al. 2001). This can last for one or several days, and it is characterized by extremely low activity. Therefore, the event of mating was identified from the days with extremely low activity during the mating season, generally from March to May (Hu et al. 1985).
Giant pandas generally deliver their young between August and October (Hu 2001). Observations on wild and captive giant pandas show that female giant pandas stop feeding and sit for days with little movement both during delivery and during several of the following days (Zhang & Wang 2003, Zhang & Wei 2006). Therefore, the days with an activity index of 0 (or near 0) were identified as delivery days, and the first day with extremely low activity recorded between August and October is regarded as the day of the delivery event.
Delayed implantation is a special reproductive strategy of the giant panda (Zhang et al. 2009). The fertilized eggs can remain for months before implantation and pregnancy (Spady et al. 2007). The time between the mating and delivery is the period of delayed implantation and pregnancy. The exact demarcation point between delayed implantation and pregnancy is hard to determine. Spady et al. (2007) synthesized studies on delayed implantation and pregnancy of giant pandas and determined a range of 50-70 days for pregnancy. We roughly defined 60 days before delivery as the start of pregnancy and defined the days between mating and pregnancy as the period of delayed implantation.
Female giant pandas care for cubs for approximately two years (Hu et al. 1985), and their activities remain low for at least 80 days after delivery (Pan et al. 2001, Zhang & Wang 2003). The 80 days after delivery were regarded as the infant-caring period, during which the mother giant panda spends a great deal of time caring for her infant, who still lives in the den (Hu et al. 1985). The activity of the mother giant panda increases to normal levels gradually during this period. A sudden restoration of activity to normal levels generally implies the death of the infant and breeding failure (Pan et al. 2001).

Data analysis
Activity rates were calculated during the delayed implantation, pregnancy and infant-caring periods of the breeding year, and activity rates were collected again during those same times of the non-breeding year. A Mann-Whiney U-test was used to compare the activity rates between the breeding year and the non-breeding year, since the data did not follow the normal distribution.
The 95 % Kernel-based home range size during delayed implantation, pregnancy and infant caring in the breeding year and those during the corresponding times in the non-breeding year were estimated using the adehabitatHR package (Calenge 2006) in the R environment (Team 2014).

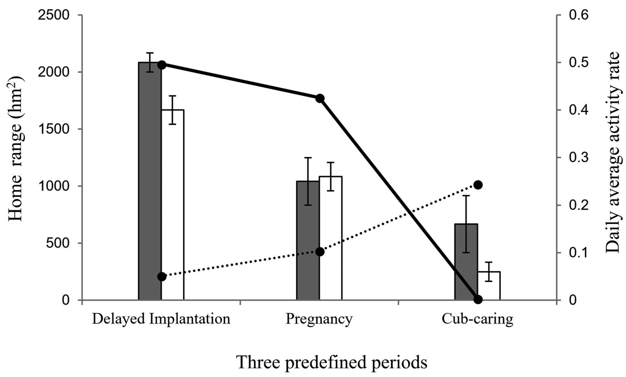

Fig. 4. Daily average activity rate (bars) and home range size (lines) of a released giant panda during delayed implantation, pregnancy, and infant caring. The grey bars represent the activity rates in the control year, and the open bars show those in the breeding year. The solid line shows the home ranges in the breeding year, and the dotted line shows those in the control year.

Results

Breeding status

Between March and May 2012, the average daily activity rates were low around 1 May, and the average daily activity rate was lowest on 1 May 2012 (Fig. 3). Therefore, we inferred that Luxin was mating on 1 May 2012, though the exact time of the mating event could be another day before or after this date (Table 1).

The delivery event occurred on 3 September 2012. Almost all activity index recorded on that day was zero, and the activity levels on the following days were low (Fig. 3).

Pregnancy period and delayed implantation period

Assuming a pregnancy period of 60 days (Tsubota et al. 1987, Zhang & Wang 2003, Zhang & Wei 2006), the pregnancy began on approximately 5 July 2012 and ended on 3 September 2012, when Luxin delivered. The days between 1 May and approximately 4 July 2012 (approximately 65 days) were regarded as the delayed implantation period. This is within the range of delayed implantation periods of the giant panda as summarized by Spady et al. (2007), i.e. 50-108 days. The infant-caring period started from the due date (4 September 2012) and lasted for 80 days, during which a gradual increase in activity was observed (Fig. 3, Table 1).

Activity rate

We collected a total of 203726 activity records from the GPS collar between 15 May 2011 and 30 April 2013 (717 days). Then we only used the data for the three periods that were defined. It contains a total of 110826 activity records data (390 days). The daily average activity rate decreased from the periods of delayed implantation to the periods of infant caring, both in the breeding year and in the control year (Fig. 4).

The daily average activity during the delayed implantation period was significantly lower in the breeding year than that in the control year (Mann-Whitney U-test, $N_1 = 43$, $N_2 = 65$, $U = 1062000$, $Z = -3.313$, $P < 0.01$). There was no significant difference in the daily average of activity rates during pregnancy between the breeding and control years (Mann-Whitney U-test, $N_1 = 60$, $N_2 = 60$, $U = 174000$, $Z = -0.297$, $P = 0.767$). During the infant-caring period, the daily average of activity rates in the breeding year was again lower than that in the control year (Mann-Whitney U-test, $N_1 = 83$, $N_2 = 83$, $U = 897000$, $Z = -8.348$, $P < 0.01$).

Home range

In 317 days, 1402 location coordinates were recorded by the GPS collar equipped on Luxin. The 95 % Kernel home range size for Luxin was 1222.01 ha in the entire breeding year and 2612.11 ha in the entire control year. In the breeding year, the home range size was large during the delayed implantation period (2070.09 ha) and then reduced to 14.95 ha during the infant-caring period. Meanwhile, in the control year, the home range was initially a small area of 211.33 ha during the delayed implantation period and then increased to 1018.98 ha during the infant-caring period (Fig. 4, Table 2).

Discussion

Luxin's successful reproduction enforced the population in the Xiaoxiangling Mountain Range, not only by adding an individual but also by increasing the genetic diversity of the local population, since she comes from the population in the Qionglai Mountain Range (Zhu et al. 2010). With the data from Luxin, our results suggested that a collar embedded with activity sensors can be used to monitor the breeding status of released female giant pandas. Previous studies have shown that the activities of giant pandas in breeding years generally differed from those in control years. Our results extend previous research by identifying the distinct characteristics of mating, delivery, and infant caring using data on activity and home range. The collar is found to be a minimal-disturbance method to detect the breeding status of released giant pandas and thus provides valuable information for decision making in future release projects. This method may also help in release projects for other wildlife.

The survival and reproduction of translocated wildlife determine the success of a translocation project at the population level (Armstrong & Seddon 2008, Campbell et al. 2015). Several milestones are key to assess the breeding status of the giant panda: mating, delivery, and infant survival. Delivery was the most definite time node of breeding and was characterized by extremely low activity or even no activity from August to October. The mating days were also easy to identify, though the exact day is hard to determine. The activity of the giant panda with an infant was low but increased gradually after delivery. It took approximately 80 days for the mother to return to normal activity levels.

The delayed implantation and pregnancy periods were harder to identify than the events of delivery or mating, since the activities during the two periods changed gradually. Lower activity rates during the period of pregnancy were recorded in the breeding year than in the control year, but it still does not serve as an identifiable indicator. It was, therefore, hard to know whether a female giant panda succeeded in mating and began pregnancy until the delivery occurred, even though the mating behaviour was identified. Adding to this confusion is the phenomenon of pseudopregnancy, which is not rare for the giant panda (Pan et al. 2001). Pseudopregnant giant pandas show behavioural patterns very similar to those of pregnant ones until they suddenly returned to normal right before the due dates (Huang et al. 1999). The possibility of pseudopregnancy for wild giant pandas cannot be ruled out, even though it was recorded only for captive ones before now.

The home range during infant caring was much smaller than that during the same period in the control year, and the activity rate was also low during this time, possibly due to infant caring. In fact, the valid GPS locations were rare and mostly concentrated near the breeding den several days before and after the delivery. Research on wild giant pandas also found that mother pandas spent more time in the breeding den during the perinatal period to prepare for delivery and care for the new-born giant panda (Hu et al. 1985, Pan et al. 2001). Meanwhile, the home ranges of Luxin during delayed implantation and pregnancy were much larger than those during the same time in the control year. This was possibly because she needed more forage to accumulate nutrients when she conceived than she did in the non-breeding year, since she will not forage as usual when she had an infant to care for. Additionally, enlarged home range sizes during pregnancy may also be due to the search for a breeding den, which can be hard to find (Zhang et al. 2011a, b).

It is noted that the power of identifying criteria for the breeding status of released female giant pandas is still weak, since these values were derived from only one breeding panda, but the behaviour patterns of Luxin match direct observations of captive and wild giant pandas (Hu 2001, Zhang & Wei 2006, Chen et al. 2015). More samples will certainly refine the criteria and enforce their power, but the number of samples needed to meet this demand cannot be attained because the translocation project of giant pandas remains at the testing stage. These results have proved, once again, the significance of long-term, post-release monitoring (IUCN 1987).

Management implications

Two criteria we known for judging whether release giant pandas was success, one criterium is that the released giant panda can survive in the wild, the other one is that the panda after release can breed and improve the genetic diversity of small populations (IUCN 1987). Our results show that using the GPS collar can be used to determine whether the giant panda is breeding and infant-caring. We recommend that continuous monitoring should be carried out after the release, especially for the giant panda which was in the sexual maturity stage. Translocation is a viable way for the protection of giant panda, based on a fact that the Luxin produce cubs and her childcare is successful. We propose to put the translocation as a basic means of giant panda protection, and continue to carry out the giant panda translocation work.

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