Seasonal differences of the Milu's home range at the early rewilding stage in Dongting Lake area, China

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ABSTRACT

The study of home range utilization of rewilded animals is important for informed reintroduction management. Sixteen Milu adult individuals (5♂11♀) were reintroduced from Jiangsu Dafeng Milu National Nature Reserve to Hunan East Dongting Lake National Nature Reserve on February 28, 2016, out of which 11 Milu individuals (1♂10♀) were wearing GPS satellite tracking collars. Subsequently, with the aid of GPS collar technology, combined with on-ground tracking observations, we tracked the reintroduced Milu for one year from March 2016 to February 2017. We used the dynamic Brownian Bridge Movement Model to estimate the individual home range of the 10 rewilded Milu (1♂9♀, 1 female individual was eliminated because its collar fell off) and seasonal home range of 5 rewilded female Milu (all tracked for up to one year). 95% level represented the home range, and 50% level represented the core areas. Temporal variation in normalized difference vegetation index were used to quantify changes in food availability. We also quantified resource use of rewilded Milu by calculating the selection ratio for all habitats within their core areas. The results showed that: (1) a total of 52,960 coordinate fixes were collected; (2) during the early stage of rewilding, the average home range size of the rewilded Milu was 17.62 ± 3.79 km^2 and the average core areas size was 0.77 ± 0.10 km^2; (3) the annual average home range size of the female deer was 26.08 ± 5.21 km^2 and the annual average core areas size was 1.01 ± 0.14 km^2 at the early stage of rewilding; (4) during the early stage of rewilding, the home range and core areas of the rewilded Milu were significantly affected by season, and the difference between summer and winter was significant (home range: $p = 0.003$; core areas: $p = 0.008$); (5) home range and core areas of the rewilded female deer in the Dongting Lake area in different seasons showed significant negative correlation with NDVI (home range: $p = 0.000$; core areas: $p = 0.003$); (6) Most rewilded female Milu showed a high preference for farmland in all seasons except winter, when they focused on using lake and beach. The home range of the rewilded Milu in the Dongting Lake area at the early stage of rewilding experienced significantly seasonal changes. Our study reveals seasonal differences in the home ranges of rewilded Milu and the resource use strategies of individual Milu in response to seasonal changes. At last, we put forward the following management recommendations: (1) to establish habitat islands; (2) to implement community co-management; (3) to reduce human disturbance; (4) to strengthen population monitoring for formulating species conservation plans.

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1. Introduction

Home range can be used to measure the demand for resources by wildlife and provide important information for managing wildlife populations and their habitats (Ofstad et al., 2016). The earliest concept of home range referred to the area where animals moved to feed, to mate, and to raise their young (Burt, 1943), and later it referred to the extent of the area where a particular animal had a probability of occurrence (e.g., 95% probability of occurrence) within a certain time frame (Kernohan et al., 2001). In recent years, with the wide application of technologies such as satellite positioning and radio telemetry, home range research has gradually become one of the burning issues in conservation biology of rewilding animals, such as giant panda (*Ailuropoda melanoleuca*), Przewalski's wild horse (*Equus ferus*), Burmese python (*Python bivittatus*), Swift foxes (*Vulpes velox*), Lions (*Panthera leo*), Tiger (*Panthera tigris*), etc. (Duan et al., 2015; Sarkar et al., 2016; Yiu et al., 2017; Wang et al., 2019; Zhu et al., 2019; Butler et al., 2020). Rewilded animals often show a strong desire to explore unknown or new habitats when they had relatively indistinct cognition of the area. Their spatial utilization patterns changed accordingly as they became familiar with environmental resources (He et al., 2019; Yang et al., 2018). Space using by animals is determined by the interaction between movement and the environment and is influenced by a combination of habitat selection, biotic interactions and intrinsic factors (Viana et al., 2018). These processes ultimately determine the extent and size of home range. Conducting home range studies on rewilded animals not only helped to understand the factors influencing individual spatial exploring behavior after their release to the wild, providing the basis for the effective management of rewilded populations, but also helped to grasp the health status of the released individuals promptly, thus improving the chance of success of species rewilding projects (Armstrong and Seddon, 2008; Berger-Tal and Saltz, 2014).

Milu (*Elaphurus davidianus,* Milne Edwards 1866), native to the swampy areas of the middle and lower reaches of the Yangtze and Yellow rivers in China, became extinct from the wild in the early 20th century (Jiang et al., 2000; Yang et al., 2016). Since the reintroduction of Milu in China in 1985, the population had increased to over 8000 individuals, including over 2420 living in five populations in the wild (Zhang et al., 2021). Despite the remarkable progress in the reintroduction of Milu population development and research, the reestablishing of wild Milu populations still faced multiple challenges, including the lack of genetic diversity, few field distribution sites, and the contradiction between population size and environmental carrying capacity (Bai et al., 2021; Cheng et al., 2021; Zeng et al., 2006). Till now, the soft release of Milu has been carried out in Jiangsu Dafeng Milu National Nature Reserve (JDMNNR), Hunan East Dongting Lake National Nature Reserve (HEDLNNR), Jiangxi Poyang Lake National Nature Reserve (JPLNNR), and Beijing Milu Park (BMP) (Hu and Jiang, 2002; Ding et al., 2017; Wang et al., 2017; Xia et al., 2021).

Fig. 1. The habitats of the rewilded Milu in the Dongting Lake area of Hunan Province, China, (a)and (b) were at Junshan Dike farmland area; (c) was at Junshan Houhu wetland.

Dongting Lake area was one of the original habitats of the Milu; however, the flooding of the Yangtze River in 1998 caused some Milu individuals to escape from the fences of Hubei Shishou Milu National Nature Reserve (HSMNNR). Eventually, a natural wild Milu population was formed in the Dongting Lake area, of which the East Dongting Lake subpopulation was mainly distributed in the Zhuzi River wetland (ZRW) and Hongqi Lake wetland (HLW) (Yang et al., 2016). On February 28, 2016, in order to reduce inbreeding and promote population rejuvenation of natural wild Milu in the Dongting Lake area, 16 adult Milu (5♂11♀) were reintroduced from
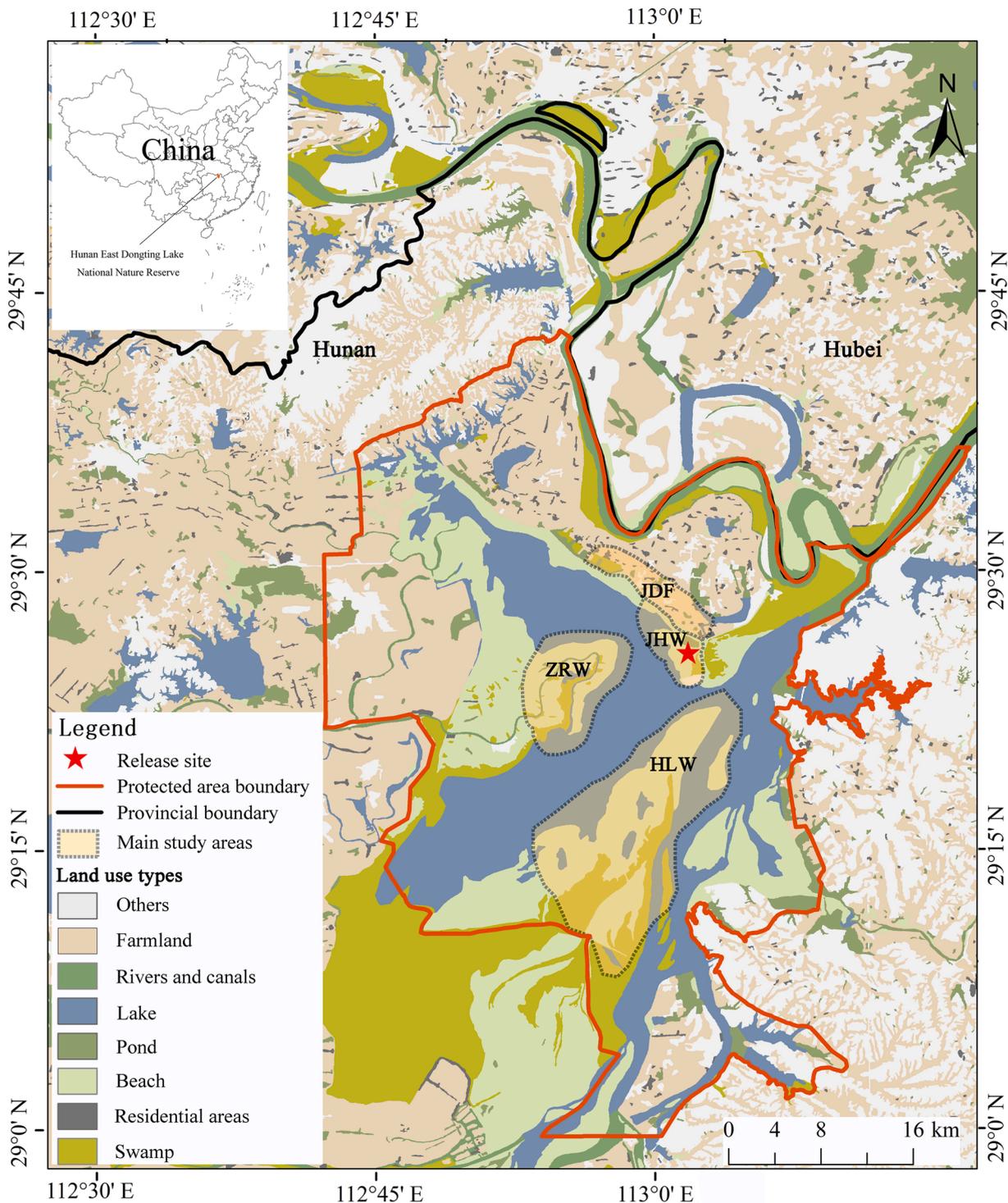

Fig. 2. The location and land use types of HEDLNNR and main study areas.

JDMNNR to HEDLNNR, led by the former State Forestry Administration and Hunan Provincial People's Government, of which 11 Milu (1♂10♀) were fitted with GPS satellite tracking collars. That release was the first wild release of Milu carried out off-site in China (Fig. 1). Their ability to optimize movement and their space utilization decisions directly affect the reintroduction success (Yiu et al., 2017). Home range links animal ranging behavior and habitat selection, and changes in their extent and size reflect the changing ecological needs of animals over time (Viana et al., 2018). For instance, seasonal variation in home range size is often associated with seasonal changes in food resources and habitat characteristics (Xu et al., 2009; Hasapes and Comer, 2016; Plotz et al., 2016). In habitats with high food availability, animals foraged a small area (Smith and Schaefer, 2002; Xu et al., 2009). Much evidence suggests that ungulates can effectively adjust their habitat use and resource selection in response to spatial and temporal variation in food resources (Carvalho et al., 2008; Ding et al., 2017; Henderson et al., 2018; Viana et al., 2018; Makhado et al., 2020). In this study, we hypothesized that home range of rewilded Milu has seasonal variations and that its ranging behavior and resource use are relevant to food abundance and availability. We investigated the home ranges of the above 11 rewilded Milu using the tracking data from the GPS collars and the on-ground tracking method, aiming to understand the changing behavior and home range size of the Milu at the beginning of rewilding, and to explore the influence of seasonal changes on their home ranges.

2. Material and methods

2.1. Study area

Hunan East Dongting Lake National Nature Reserve (112°43′59.5″−113°13′13.4″ E, 29°0′0″−29°37′45.7″ N) is located in Yueyang City, Hunan Province, south of the Jing River in the middle reaches of the Yangtze River, with an area of about 1900 km². HEDLNNR has a subtropical monsoon type climate with mild, humid and distinct seasons. The annual mean temperature is 16.8 °C; the annual mean rainfall is 1300 mm, and the annual mean relative humidity is 80%. The Dongting Lake is connected to the Yangtze River and is a buffer for the Yangtze River floods, and the water level in the lake area has a seasonal fluctuation pattern (Zhou et al., 2020). Seasonal water level fluctuations caused changes in habitat structure and resource distribution in Dongting Lake area, thereby affecting the wildlife activities in the lake area (Huang et al., 2019; Xu et al., 2017; Yang et al., 2016). The dominant plants in the HEDLNNR are *Phalaris arundinacea*, *Carex* spp., *Polygonum hydropiper*, *Artemisia selengensis*, *Phragmites australis*, *Miscanthus lutarioriparius*, etc. (Hou et al., 2016); the dominant small mammal species are *Microtus fortis*, *Erinaceus amurensis*, *Mustela sibirica*, *Crocidura attenuata*, and there are no natural terrestrial predators of Milu in the area.

This study area was divided into the Junshan Houhu wetland (JHW), Junshan Dike farmland area (JDF), ZRW, and HLW (Fig. 2). Among them, JHW was the field release site for Milu. The plants in the area were dominated by *Carex marsh*; thus Milu had sufficient food and shelters. The Junshan Houhu Management Station in the area facilitated the monitoring of the released deer. The ZRW is a reed farm, and the HLW has large reed swamps; the eastern part of the JDF is mostly planted with *Brassica campestris* and *Zea mays*, and the western part is mainly planted with *Oryza sativa*, etc., which provided sufficient food sources for the rewilded Milu (Li et al., 2018). Human activities in the Dongting Lake area during the study period included asparagus harvesting in spring, reed harvesting in winter (Song et al., 2015; Yang et al., 2016).

2.2. Data collection

The Milu individuals were monitored by GPS satellite tracking and on-ground observation from March 3, 2016 to February 28, 2017. The GPS satellite tracking collars (model HQAN, 800–1000 g, 12 months battery life) were manufactured in Hunan Global Messenger Technology Co., Ltd., China. Position fixes were downloaded using User Manual v2.0 of the global faith satellite tracking data service platform. GPS coordinates were recorded at scheduled intervals of an hour per fix. With the continuous use of the equipment, the time interval gradually increased to 1 fix / 6 h to prolong the life of diminishing batteries. The seasons were divided according to the local climatic conditions: spring from March to May, summer from June to August, autumn from September to November, and winter from December to February (Wang et al., 2018).

The study was performed in accordance with the recommendations of the Institution of Animal Care and the Ethics Committee of Central South University of Forestry and Technology (approval number: CSUFT-201628). Permission for field work was obtained from the former State Forestry Administration (permit number: 174).

2.3. Home range estimators

The choice of home range estimators significantly affects management and conservation actions, as different methods lead to vastly different interpretations of movement patterns, habitat selection, as well as home range requirements (Cohen et al., 2018; Powell and Mitchell, 2012; Silva et al., 2018; Wilson et al., 2020). Common methods used to study the home range included the Minimum Convex Polygon (MCP), Kernel Density Estimation (KDE), Brownian Bridge Movement Model (BBMM), dynamic Brownian Bridge Movement Model (dBBMM) and Time Local Convex Hull (T-LoCoH) (Silva et al., 2018; Peris et al., 2020; Wilson et al., 2020). dBBMM is a model that is hardly limited by loci autocorrelation and sample size (Kranstauber et al., 2012; Silva et al., 2018; Wang et al., 2020, 2021). Although all of the above methods identify some general spatial-temporal patterns, dBBMM is more efficient at detecting movement corridors and accurately representing long-term shelters sites (Silva et al., 2018). During the monitoring period, the time interval for positioning gradually increased, resulting in different numbers of loci collected in different seasons. dBBMM took into account the animal movement speed and measurement loci error, and integrated the time interval between active loci into the model (Byrne et al.,

2014; Cohen et al., 2018; Wilson et al., 2020). For the dBBMM parameters, we chose to use a constant window and margin size equal to 11 and 3 respectively, a location error of 5 and a raster of 100 (Kranstauber et al., 2012; Byrne et al., 2014). We kept values for the window and margin size constant instead of altering them to account for changes in GPS sampling frequency because we failed to see any measurable effects of changing these values when we began our analyses.

2.4. Data analyses

All loci data were summarized and sorted in Excel 2010, and values in the results section are expressed as Mean $\pm$ Standard Error (SE). dBBMM was employed to compute the Home Range (HR) and Core Areas (CA), where the HR and CA were expressed in 95% and 50% levels, respectively (Peris et al., 2020; Silva et al., 2018; Wilson et al., 2020). A dBBMM was constructed with the help of the move package (Kranstauber et al., 2019) in R 4.0.4 software (R Core Development Team, 2020), and the generated shp files were imported into ArcGIS 10.6 to map the HR of individuals in different seasons and to calculate the area of HR and CA. The data of F9 female Milu was not involved in home range estimation because its collar fell off. Secondly, the female and male HR differences were briefly compared because only one male Milu was tracked (Liu et al., 2020). The Kolmogorov-Smirnov (K-S) test was used to test the normality of the HR of the females, and the one-sample t-test was used to compare the mean values with the HR of the male if the distribution was normal. Finally, only the annual HR of the 5 Milu hinds with complete loci data for a whole year were calculated, and the differences in HR areas between seasons were examined. If the data conformed to both normal distribution ($p > 0.05$) and chi-square ($p > 0.05$), One-way Analysis of Variance (ANOVA) (SPSS ver.26) was used to analyze the differences; otherwise, non-parametric tests were used. The overall differences in HR size between seasons were first tested using the Kruskal-Wallis (K-W) test, and significant differences were tested using the Mann-Whitney U test for two-way comparison of HR area between seasons (Zar, 2019).

To account for productivity of HR, we obtained 30 m resolution Normalized Difference Vegetation Index (NDVI) data at 16 day for four seasons during the study period. Thus, temporal variation in NDVI values quantify changes in food availability for herbivores (Lehmann et al., 2020). Moreover, correlation coefficients between HR and NDVI within HR in different seasons were calculated. Pearson Analysis was used if the data conformed to a normal distribution; otherwise, Spearman Analysis was used (SPSS ver.26). The *p*-value threshold as 0.05 to determine the significant variables (Zar, 2019).

The land-use remote sensing monitoring data were downloaded from Chinese Academy of Sciences Resource and Environment Science and Data Center (https://www.resdc.cn). A secondary classification system was used in the assessment, and the main classifications in this study were farmland, lake, pond, beach, residential areas, swamp and others. We quantified resource use of rewilded Milu by calculating the selection ratio $w_{ij} = u_i/a_j$ for each habitat within the CA following Manly et al. (2002), where u_i is the proportion of used habitat and a_j is the proportion of available habitat. The ratio is 1 if an individual uses an habitat in relation to their availability, between 1 and 0 if they use it proportionally less than its availability, and > 1 if they use it proportionally more its availability.

3. Results

3.1. Summary of telemetry data

A total of 52,960 coordinate fixes for the 11 rewilded Milu were obtained (Table 1). During the monitoring period, the period of tracking for each Milu varied due to individual death or collar malfunction. Overall, each rewilded Milu was tracked for an average of 249 ± 33 d. In spring, a total of 11 rewilded Milu GPS fixes were obtained, mainly concentrated in JHW, with a small number distributed in the JDF (Fig. 3). After entering summer, the female Milu F9 had no locality information due to collar falling off, while females F4, F7 and F8 and the remaining 6 females (F1, F2, F3, F5, F6 and F10) were active in the western and eastern parts of the JDF respectively, and were occasionally seen in the residential areas, while M1 male spread from the western part of the JDF to Chiba Town, Jianli City, Hubei Province (Fig. 4). By autumn and winter, only 5 rewilded females (F2, F4, F5, F8, F10) had continuous GPS fixes, which showed southward migration, with F4 and F8 migrating to the ZRW, F5 and F10 moving to the HLW, and F2 returning to the JHW again (Fig. 3).

Table 1
Location information and home range of the Milu at the early rewilding stage in Dongting Lake area.

| Individuals	Sex	Monitoring period	Valid fixes	Home range (km^2)	Core areas (km^2)
F1	♀	2016.3.3 - 2016.8.14	4596	8.86	0.65
F2	♀	2016.3.3 - 2017.2.28	5178	10.56	0.68
F3	♀	2016.3.3 - 2016.8.31	4441	9.07	0.57
F4	♀	2016.3.3 - 2017.2.28	5994	20.40	0.77
F5	♀	2016.3.3 - 2017.2.28	5046	39.13	1.30
F6	♀	2016.3.3 - 2016.7.20	3763	8.14	0.45
F7	♀	2016.3.3 - 2016.8.30	4346	5.71	0.42
F8	♀	2016.3.3 - 2017.2.28	4820	24.38	0.90
F9	♀	2016.3.3 - 2016.5.26	3083	–	–
F10	♀	2016.3.3 - 2017.2.28	6491	35.92	1.37
M1	♂	2016.3.3 - 2016.8.31	5202	13.99	0.57
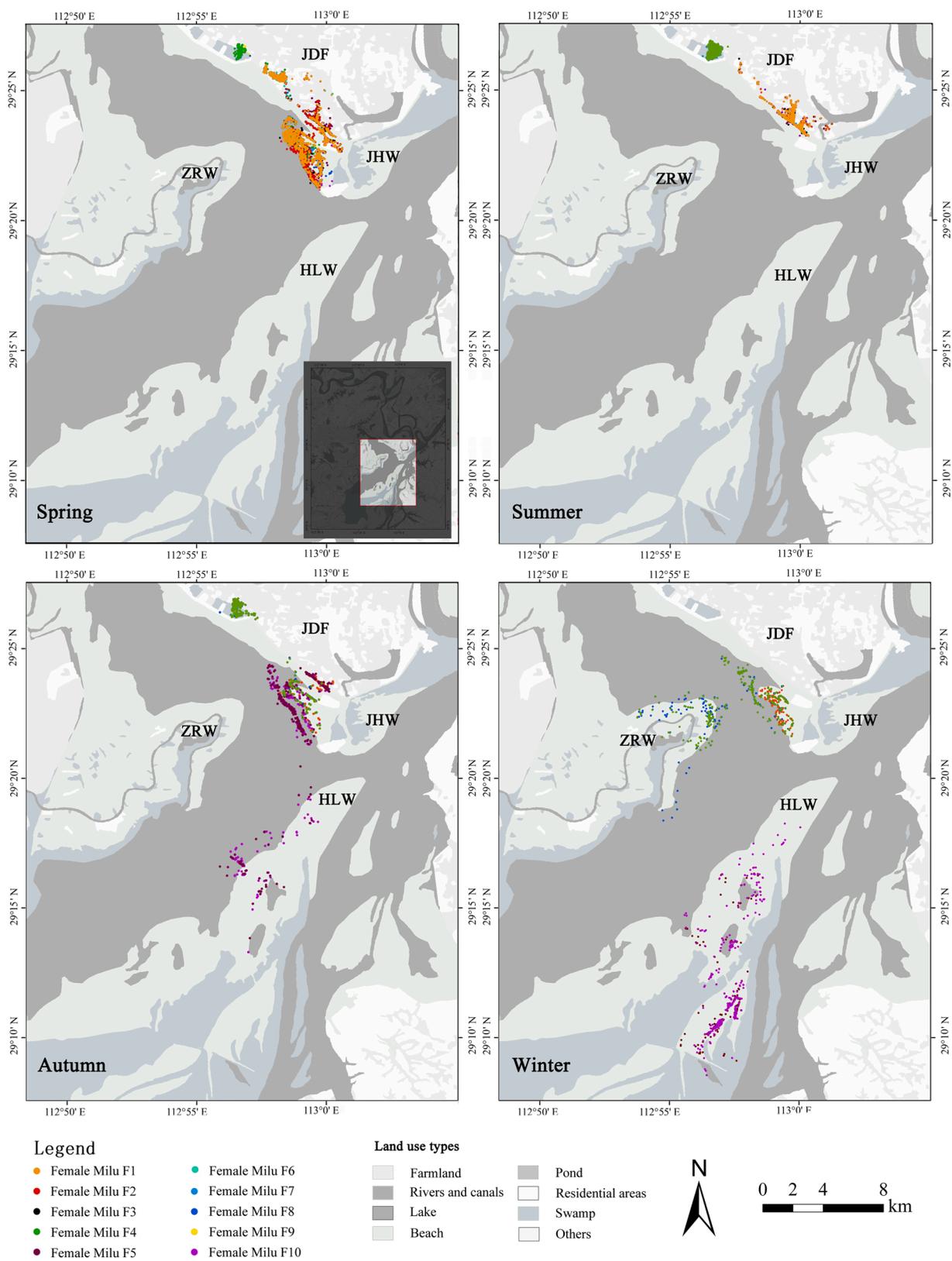
Fig. 3. Coordinate fixes of the rewilded female Milu in Dongting Lake area in different seasons.

3.2. Size of home ranges and core areas

The mean HR size of rewilded Milu was estimated to be 17.62 ± 3.79 km^2 (n = 10), and the mean CA size was estimated to be 0.77 ± 0.10 km^2 (Table 1). Amongst them, the mean HR size of the females was estimated to be 18.02 ± 4.22 km^2 (n = 9), and the mean CA size of the females was estimated to be 0.79 ± 0.11 km^2 (Table 1). The results of the one-sample t-test showed that there were no significant differences between the mean HR and the mean CA of female Milu and the male M1 (1♂9♀) ($p = 0.367$). In addition, the average annual HR of rewilded female Milu was 26.08 ± 5.21 km^2), and the average annual CA was 1.01 ± 0.14 km^2 (Table 1).

3.3. Home range and core areas of the rewilded Milu in different seasons and its relationship with NDVI

For both HR and CA of the rewilded female Milu in different seasons, reached the largest in winter, decreased in autumn and spring, and decreased to the smallest in summer (Fig. 5). The data were not normally distributed, nor were the variances homogeneous, so non-parametric analyses were used to test the differences (Zar, 2019). The K-W test showed that the HR area of rewilding Milu differed significantly between seasons ($p = 0.015$) and the CA size differed further significantly ($p = 0.005$). Further pairwise comparisons showed that the differences between summer and winter were highly significant (HR: $p = 0.003$; CA: $p = 0.008$). Furthermore, the results of the one-sample t-test showed that there were no significant difference among HR of females and the male in spring (1♂10♀) ($p = 0.230$), while the HR was significantly larger in males in summer (1♂9♀) ($p = 0.000$) (Fig. 5).

The HR size and NDVI within HR in different seasons did not conform to the normal distribution (Table 2). Spearman Correlation Analysis showed that the HR and CA of the rewilded female Milu in different seasons were significantly negatively correlated with NDVI (HR: $p = 0.000$; CA: $p = 0.003$).

3.4. Within-home range resource selection

The resource use strategies of rewilded female Milu showed more significant seasonal and individual differences (Fig. 6). In spring, some individuals showed a higher preference for farmland, others chose lake and swamp. In summer, all individuals showed avoidance of lake, pond and others, while farmland, beach, residential areas were relatively more utilized habitats. In autumn, all Milu showed
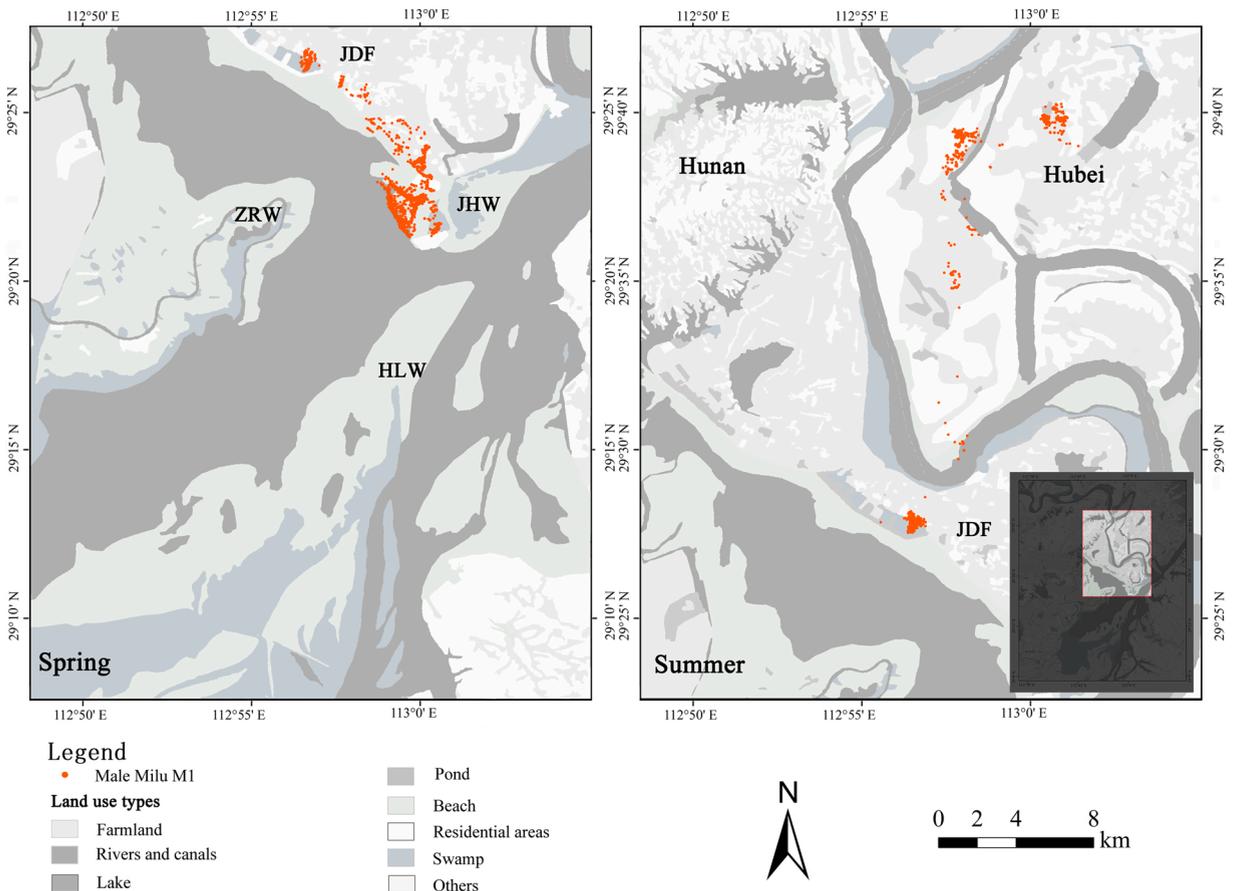

Fig. 4. Coordinate fixes of the rewilded male Milu in Dongting Lake area in different seasons.
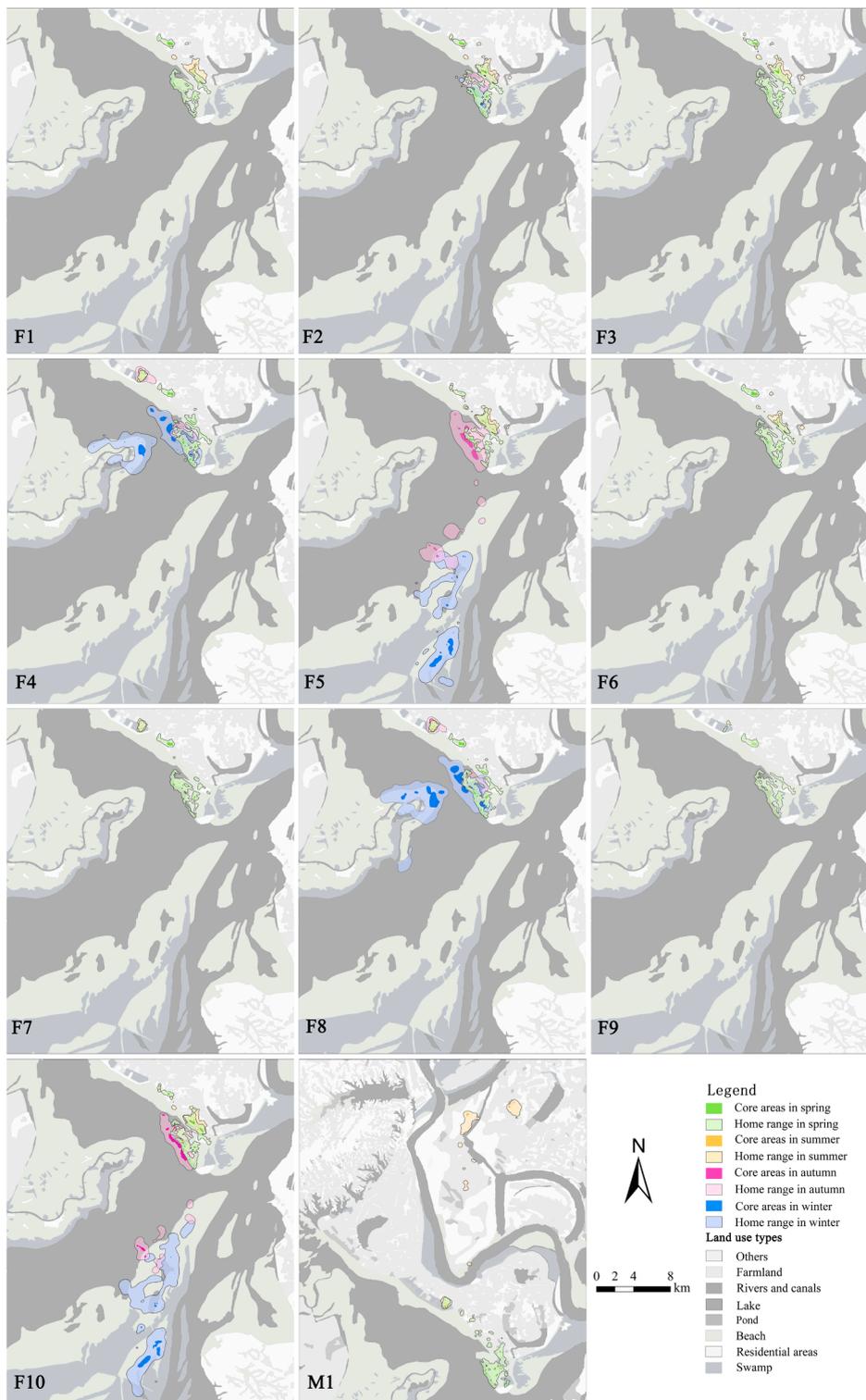
Fig. 5. Home range and core areas of the rewilded Milu in Dongting Lake area in different seasons.

Table 2

The home range sizes of the rewilded female Milu and NDVI in different seasons.

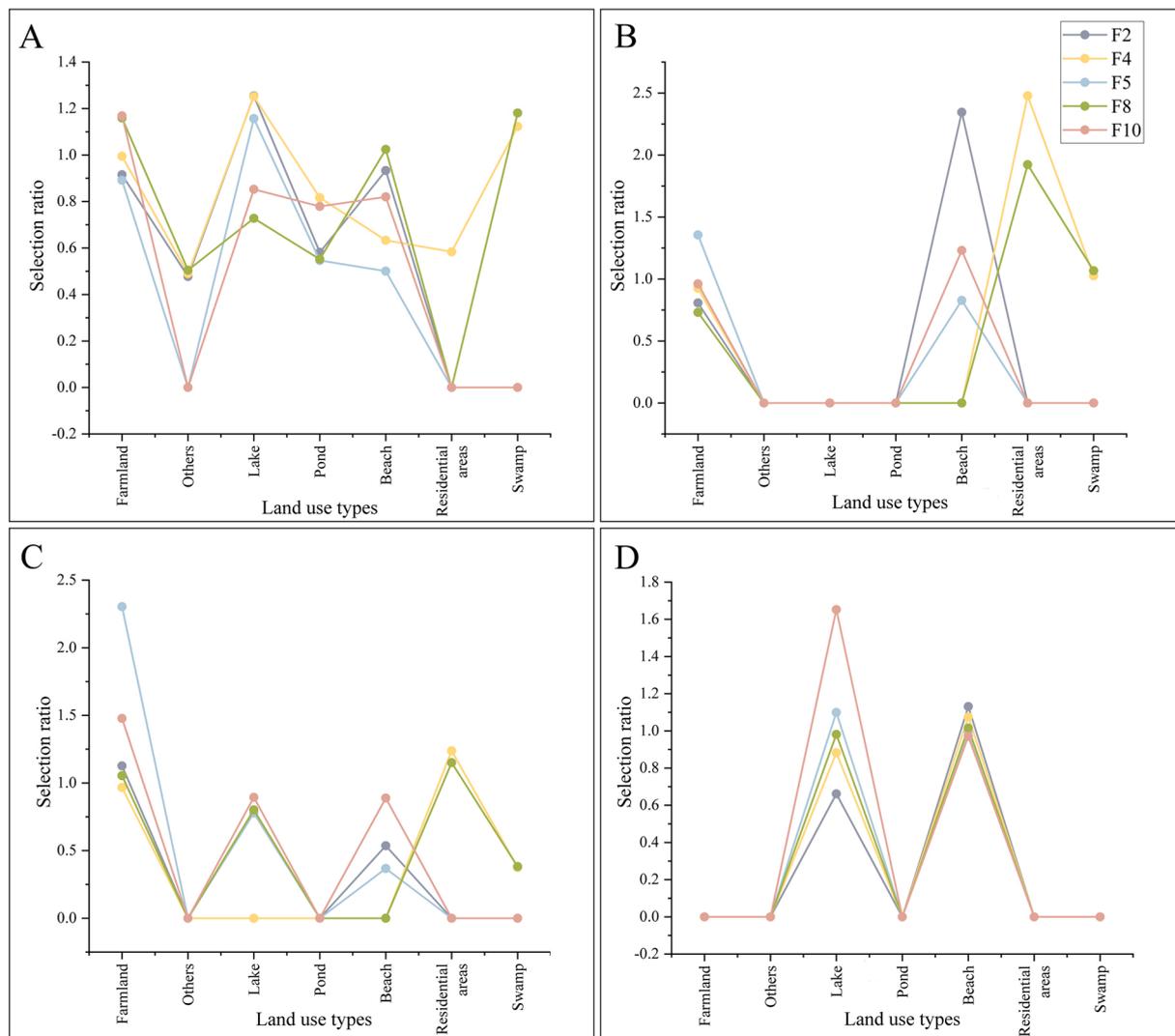| Seasons	Sample size	Mean home-range size (km^2)	Mean NDVI within home range	Mean core-areas size (km^2)	Mean NDVI within core areas
Spring	5	7.84 ± 0.53(5.77–8.61)	0.24 ± 0.01	0.40 ± 0.06(0.14–0.48)	0.25 ± 0.01
Summer	5	2.32 ± 0.59(0.90–3.47)	0.40 ± 0.02	0.20 ± 0.03(0.11–0.30)	0.46 ± 0.01
Autumn	5	12.66 ± 3.66(6.64–24.22)	0.27 ± 0.01	1.01 ± 0.48(0.20–2.41)	0.25 ± 0.02
Winter	5	24.75 ± 5.05(5.15–33.12)	0.13 ± 0.01	2.56 ± 0.90(0.77–5.71)	0.14 ± 0.01

Fig. 6. Manly's selection ratios calculated for all habitats for rewilded female Milu in different seasons, A-spring; B-summer; C-autumn; D-winter.

more utilization of farmland. Finally, each Milu only showed focused use of lake and beach in winter.

4. Discussion

The formation of home range is a dynamic process. Both the habitat and intrinsic factors of animals might change through time and cause home range size to vary (Hasapes et al., 2016; Viana et al., 2018; Butler et al., 2020). Our results are consistent with our prediction that home range of rewilded Milu has seasonal differences. Similar to the rewilded female Milu in JDMNNR and most ungulates, the activities of the rewilded female Milu in Dongting Lake area were also significantly affected by seasonal changes, and most of them showed that the HR size in winter was larger than that in summer (Ding et al., 2017; Makhado et al., 2020; Ofstad et al., 2016). In general, home range and core areas of Milu increased with decreasing NDVI values among seasons. Due to the shortage of food in

winter, wild animals usually expanded their feeding range to ensure their energy supply (Volampeno et al., 2011). Dongting Lake area is in a dry period in winter and a large number of aquatic plants wither (Zhou et al., 2020). Moreover, although all rewilded female Milu utilized the same habitat within the CA (Fig. 6), geographically they were clearly divided into three groups (Fig. 3). The dispersal of Milu is mainly influenced by population density (Yang et al., 2016), coupled with the harvesting of reeds by local residents in winter, which causes the expansion of the winter home range (Seigle-Ferrand et al., 2021; Song et al., 2015). Secondly, summer was the season with the highest temperature in Dongting Lake area throughout the year, and the activity area of Milu was limited by water (Ding et al., 2017; Xu et al., 2017). Nevertheless, all individuals showed avoidance of lake, pond and others in summer (Fig. 6). We observed that the rising water level in the lake area led to the inundation of large areas of JHW、HLW、ZRW. The rewilded Milu in Dongting Lake area fed on carex, reeds, and other continental vegetation, and water level fluctuations directly affected the size and spatial distribution of vegetation (Yang et al., 2002; Hu et al., 2018; Zhou et al., 2020). At the same time, this season coincided with the rut of the Milu; the opposite sexes of Milu would show different feeding and breeding strategies during the rut season. Previous studies illustrated that reproductive status was the main factor explaining differences in home ranges among female ungulates (Lehmann et al., 2020; van Beest et al., 2011). Females would reduce their range as they nurture offspring, while males would disperse due to habitat quality or mate availability (Carvalho et al., 2008; Makhado et al., 2020; Song et al., 2015). The summer range of the rewilded females was limited to the JDF, while the M1 stag left the herd in June 2016 (Fig. 5). A rewilded male without a collar was observed mixed in the herd in the field, and it is speculated that M1 chose to move out after failing to compete with other stags for mates (Cheng et al., 2020; Song et al., 2015; Yuan et al., 2019).

Seasonal differences in resource use within an individual home range may reflect changes in habitat requirements (Henderson et al., 2018; Nugent, 1994; Xu et al., 2009; Xue et al., 2020). Core areas are the areas of wild animals in the home range that contain most hidden places and foods (Nielsen and Stroud-Settles, 2018; Samuel et al., 1985). In this study, individual differences in resource use within the CA can be observed for each season (Fig. 6), and it can effectively avoid competition for resources. By comparison, fewer habitat types were intensively used in summer and winter, possibly affected by flooding and shelter shortages, respectively (Xia et al., 2021; Xu et al., 2017; Zhang et al., 2020).

5. Conclusions and suggestions

The ultimate objective of Milu rewilding was to maintain viable rewilded populations (Bai et al., 2021). Rewilded Milu are able to respond to dynamic changes in resource abundance and availability over time, similarly to other ungulate species when subject to limited resources (He et al., 2016; Lehmann et al., 2020; Seigle-Ferrand et al., 2021; Viana et al., 2018). In spite of this, the survival of its population remains threatened by seasonal severe weather. Given this, the following conservation management recommendations are made: (1) to establish habitat islands for the released Milu. Habitat islands should be established in areas with relatively high terrain and adjacent to water sources (e.g., ZRW and JHW) to provide sufficient food and shelter for Milu during the flood season and winter, (2) implement community co-management, hire local residents to take care of the habitat island, and set up co-management committees to encourage participatory conservation, (3) reduce human activities (e.g., reed harvesting, etc.) in the activity areas of Milu to leave enough space for the rewilded Milu population to survive, and (4) strengthen the population monitoring of rewilded Milu. We should extend the monitoring period to grasp the inter-annual activity rhythm of rewilded deer, and collect data to develop a long-term species conservation plan.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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