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# Behavioural Rhythms during the Adaptive Phase of Introduced Milu/Père David's Deer, *Elaphurus davidianus*, in the Dongting Lake Wetland, China

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**SW, ZX and JP analyzed final data. SW and ZX wrote the article. SW, ZX and YZ conceived and designed the study. YZ and LL provided project (XLK201525) for subsidizing the study. SW, CD and LW observed the object outside.**

**Key words**
**Père David's Deer, Released, Morphological, Behavioural type, Frequency.**

## ABSTRACT

To explore the adaptation of introduced Milu (*Elaphurus davidianus*), also known as Père David's Deer, to their new environment, this study was designed to track and observe 16 Milu, which were released in the Dongting Lake wetland area from Jiangsu, from April 4 to May 12, 2016, using instantaneous scanning and recorded 486 observations. Nineteen types of behaviour were observed which were classified into nine categories: resting, feeding, moving, excreting, parental behaviour, embellishing, rutting, vigilance and social behaviour. This study analyzed the time-allotment and behavioural rhythms for resting, feeding, moving, and excreting categories. The result showed that before 9:00 and after 18:00 hours, the Milu were almost always resting. Resting behaviour was exhibited and maintained at a high frequency in other periods as well. Fluctuations in feeding frequencies was consistent, peaks occurred after 14:00 hours when the frequency was the highest. Moving behaviour was split into a non-behaviour period, fluctuation period, low-ebb period, and peak period. Vigilant behaviour was influenced by external factors, and peaks appeared suddenly after the disturbance occurred. Resting behaviour occupied most of the daytime hours during the adaptive phase, and the proportion was higher than other behavioural types except two times, resting behaviour decreased after 13:00 hours when activity behaviour increased. The adaptation of Milu was documented to a certain extent, it was found that the animal's behavioural rhythms changed during acclimation to the new habitat. It is likely that resting and feeding behaviours were influenced by temperature.

## INTRODUCTION

Milu (*Elaphurus davidianus*), also known as Père David's Deer, is endemic to China and adapted to wetlands (Asher *et al*., 2009). Historically, Milu were widely distributed in the Yangtze River basin and Yellow River basin (Hu and Jiang, 2002). Because of human factors and climate change, Milu died out in the wild in early 20th century, presently categorized as "Extinct in the wild" (Jiang and Harris, 2008). The last Milu lived in the Nanhaizi Park of China and were translocated to other countries (Zhong *et al*., 2009). The Milu were reintroduced to China from England in 1985, after continuous effort for several decades to build up population in captivity and semi-wildness for subsequent reintroduction to the wild. Deer numbers have since increased. The Milu were reintroduced in Beijing Milu Park, Jiangsu Dafeng Milu National Nature Reserve and Hubei Shishou Milu National Nature Reserve successively (Jiang and Lindsay, 2000; Jiang *et al*., 2000). Increasing human population around wildlife and biodiversity reserves, made it necessary to take action (Zhang *et al*., 2011). Because of heavy flooding in 1998, a few Milu escaped into the Dongting Lake wetland from the Hubei Shishou Milu National Nature Reserve. The population has now increased to more than one hundred, however, the increase was considered to be slow. The Dongting Lake wetland is one of the preferred habitats of the Milu, and a study on environmental factors affecting the reintroduction indicated that introducing Milu to the Donting Lake wetland was feasible (Yang *et al*., 2002). To improve the Milu population, sixteen (16) Milu were introduced from the Jiangsu Dafeng Milu National Nature

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Reserve to the Dongting Lake wetland on March 3, 2016. This was the first translocation of Milu from one facility to another in China.

Research on Milu has primarily been focused on physiology and biochemistry (Hawkey and Hart, 1985; Curlewis *et al*., 1991; Flint *et al*., 1991; Brinklow and Loudon, 1993; Yoon and Kim, 1999; Qiu *et al*., 2014), natural history (Ding *et al*., 2013), habitat and management (Xu and Ding, 1997; Hou *et al*., 2012) disease and prevention (Orr and Mackintosh, 1988; Agrimi *et al*., 1993; Ma *et al*., 2007). Meng *et al*. (2014) found that the length of the Milu's mtDNA sequence was 1199bp, and has three (3) haploid and six (6) variation points, therefore, detrimental effects in their development caused by their genetic diversity are few. Yang *et al*., (2016) suggested that because of the increase of population size, Milu may have emigrated to a new habitat. Jiang, (2000) recorded Milu behavior, including postures and movement for more than 200 behavioural types. Ding *et al*. (1989) studied the feeding behavior of Milu, 51 plant species were observed to be eaten by Milu in the Dafeng Milu National Nature Reserve.

Behavioural rhythms can be reflected in the relationship between animals and environment (Yi *et al*., 2010). To explore the response of introduced Milu to their new environment, this study recorded their behaviour and behavioural rhythms during the adaptive phase. The data were analysed to determine the characteristics of the behavioural pattern.

## METHODS

*Study area*

Dongting Lake (approximately N28°30′-N30°20′, E111°40′-E113°10′) is an international wetland located in the middle reaches of the Yangtze River region, south of Hunan Province, China (Li *et al*., 2013; Liang *et al*., 2014). The catchment area of Dongting Lake is connected with the Yangtze River and is approximately 2,625 km$^2$, being the second largest freshwater lake in China (Zhang *et al*., 2016). Because of historical extensive sediment deposition, Dongting Lake has been divided into Eastern Dongting Lake, Western Dongting Lake, and Southern Dongting Lake (Wang and Liang, 2016). The Lake is fed by four rivers (Xiang River, Zi River, Yuan River and Li River) (Liang *et al*., 2015). The water levels change seasonally and there are disparate dry and wet periods (Han *et al*., 2016). Annual mean temperature is 16.8°C and total rainfall approximately 1200–1400 mm (Cui *et al*., 2012). There are no predators on Milu in the area. The area is the important habitat for wild Milu (Yang *et al*., 2002; Xu *et al*., 2017) (Fig. 1).

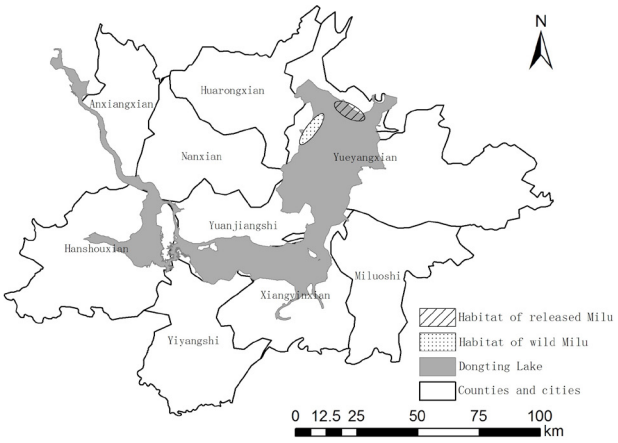


Fig. 1. Living areas of reintroduced Milu and wild Milu.

*Milu population*

Sixteen nearly 5-year-old Milu (5♂11♀) from Jiangsu Dafeng Milu National Nature Reserve were introduced to Junshan Island in the Dongting Lake wetland on March 3, 2016. The length of their head, tails, and rear legs were measured before release. The introduction site was near the wild Milu population. However, because of high water levels, the two populations had not intermixed. GPS trackers were placed on the introduced Milu to track them.

*Behaviour observations*

Following Jiang (2000) and Yang (2013), observations of typical behaviour of Milu were recorded using telescope (SWAROVSKI ATS80HD, 25-50×20-60 mm). Milu were observed from 100–200m distance. Because of thick vegetation cover, the Milu would occasionally be hidden from view. When the Milu were visible during tracking, the 5-min instantaneous scanning method was employed to record observations. The number of males and females were tracked and their behaviour recorded during daylight hours from April 13 to May 12, 2016.

*Data management*

Milu behaviour was categorised into four types, including resting, feeding, moving, and vigilance. Observations were further divided by sex. Statistics on particular behavior types by sex, and the proportion of different behavioural categories (behaviour frequency) in each time interval were recorded to determine changes in behavioural rhythms. The study calculated the proportionate behaviour in each time interval individually, summarized the number of days for each time interval, calculated their average value. Meteorological data for the study area were attained from the Chinese Atmosphere Data Network (http://date.cma.cn/), the station located

at (N29.23°, E113.05°). The differences between male and female behaviour were determined using paired *t*-test. Pearson correlation analysis was used to determine correlation coefficients.

**Table I.- Morphological parameter of released Milu.**

| Sex | Head (cm) | Tail (cm) | Rear leg (cm) |
|---|---|---|---|
| Male | 52.20±1.64 | 61.60±4.28 | 85.40±4.45 |
| Female | 41.27±1.69 | 55.09±7.91 | 72.73±4.58 |
| T value | $t=12.14<t_{0.01}$ | $t=1.71>t_{0.05}$ | $t=5.17<t_{0.01}$ |

## RESULTS

*Morphological parameters of Milu*

Morphological parameters of released Milu are shown in Table I. Average length of the head and rear legs for males was 52.20±1.64 cm and 85.40±4.45 cm, respectively, whereas they were 41.27±1.69 cm and 72.73±4.58 cm in female. Average length was significantly more in males than in females ($p<0.01$). Average tail length was more in males than in females, the difference was however not significant ($p<0.05$).

*Behaviour of Milu in adaptive phase*

Nineteen behavioural types of Milu were observed during the study, including lying, standing, tempering, eating, drinking, walking, running, jumping, defecating, lactating, cleaning, picking mud, hanging grass, smelling pudendum, sprinkling urine, bellowing, guarding, tail biting, and wrestling. Rutting behavior included picking mud, smelling pudendum, sprinkling urine, hanging grass, and bellowing. Behavioural patterns were further classified into nine categories: resting, feeding, moving, excreting, parental care, embellishing, rutting, vigilance, and social behaviour (Table II).

**Table II.- Behaviours of released** Milu **in adaptation phase.**

| Behaviour types | Behaviour | Description of behaviour | Notes |
|---|---|---|---|
| Resting | Lying | Legs are kneeling, body adjoin the land. | |
| | Standing | Legs up-right and support body, do no displacement. | |
| | Tempering | Lying in the water where submerged half of body. | |
| Feeding | Eating | Drop neck and pull plants nearby into mouth. | |
| | Drinking | Drop neck, lip stretch in water and inhale water into a mouth. | |
| Moving | Walking | Body is parallel with a floor, do displacement by legs alternately. | |
| | Running | Do displacement apace by legs alternately. | |
| | Jumping | First half of body up did, uplift the front legs, rear legs energize to do a distance. | |
| Excreting | Defecating | Uplift tail, kept anus out, then discharge some black globular granules. | Always be standing or walking |
| Parental behaviour | Lactating | Female Milu is standing, cub half squat, uplift head and move repeatedly. | |
| Embellishing | Cleaning | Use tongue or antler to touch body. | |
| Rutting | Picking mud | Male Milu rub soft mud repeatedly by using Antler then uplift head and bend the neck to sprinkle mud on backside. | |
| | Hanging grass | Male Milu rub grass repeatedly by using antler, and make the grass on the antler. | |
| | Smelling pudendum | Male Milu's nose close to female pudendum and smelling for a moment. | |
| | Sprinkling piss | When male Milu is peeing, its' penis swing left and right to sprinkle piss on its abdomen. | |
| | Bellowing | Male Milu uplift neck was slightly, close eyes, open the mouth and make a sound. | |
| Vigilance | Guarding | Face to target, neck unbend and pop-eyed, look at the target. | Always be standing |
| Social | Biting tail | One closed to another one and bite its tail for some time. | |
| | Wrestling | Two male face to each other, drop neck, touch the antlers and push forward. | |

Behaviour of male and female Milu was categorized and recorded by time interval. The female Milu exhibited only one category of behavior (resting) in 8:00 time interval representing 8:00-8:59 time interval whereas they exhibited more than three categories of behaviour during other time intervals. Dring 10:00, 13:00, and 15:00 time intervals behavior reached its peak in females when eight types were recorded. Up to six behavioural types were recorded during 11:00 and 12:00 time intervals. Behavioural types in males were the lowest in the 8:00 time interval, when only lying and standing were exhibited. The maximum number of behaviour types for males were recorded in 10:00 and 17:00 time intervals, during which nine types were exhibited. Eight behaviour types were exhibited in the 14:00 and 15:00 time intervals. Milu exhibites the lowest behaviour during 11:00 and 12:00 time intervals. Males exhibited more categories of behaviour than females before 11:00 whereas no clear pattern between males and females existed after 12:00.

The relationship of number of behavioural types with temperature in male and female behavioural types (Fig. 2) during 8:00 to 12:00 hours had correlation coefficients of 0.92 and 0.87, respectively, with temperature. Types of behaviours increased with increasing temperature before 10:00 hours. From 10:00 to 12:00 hours, the number of types declined as temperature decreased, but after 12:00, types of behaviours did not regardless of decreases in temperature. The correlation was however not significant ($p>0.05$).

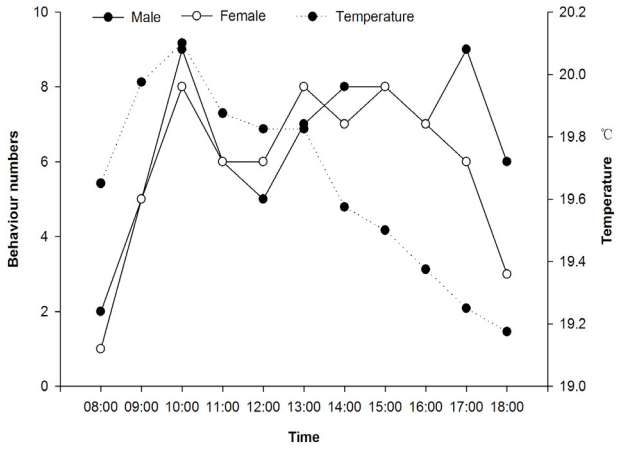


Fig. 2. The number of behavior types of male and female Milu in each time interval.

The types of behaviour between females and males were compared, the difference was not significant ($p>0.05$). Therefore, the study combined behaviour of females and males (Table III). In 8:00 time interval, only standing and lying occurred, moreover, the two behaviors occurred in all time intervals. From 9:00 time interval, activities like walking and eating increased. Maximum behavioural types including moving, resting, feeding, and rutting occurred in 10:00 time interval. In noon Behavioural types during 13:00 to 15:00 time interval were reduced only to a few. , In 17:00 time interval 10 behavioural types were recorded touching the peak in the afternoon; reduced to only 6 in 18:00 time interval in the evening.

Because of human disturbance, introduced Milu were quite vigilant during the initial adaptive period. Vigilance appeared in every time interval except in 8:00 and 18:00 time intervals. Social behaviour, such as bellowing, tail biting, and wrestling primarily occurred in the afternoon.

**Table III.- Behaviours of released Milu in each time interval.**

| Time | Behaviour | No. |
|---|---|---|
| 8:00 | Standing, lying. | 2 |
| 9:00 | Lying, guarding, eating, standing, and walking. | 5 |
| 10:00 | Eating, walking, standing, lying, lactating, cleaning, guarding, defecating, bellowing, biting tail, wrestling, running. | 12 |
| 11:00 | Walking, standing, eating, guarding, lying, cleaning, | 6 |
| 12:00 | Eating, walking, standing, lying, cleaning, defecating, guarding. | 7 |
| 13:00 | Standing, eating, walking, lying, drinking, biting tail, guarding, defecating, wrestling. | 9 |
| 14:00 | Standing, lying, guarding, eating, cleaning, walking, running, bellowing, defecating. | 9 |
| 15:00 | Walking, eating, guarding, standing, running, drinking, defecating, lying, bellowing. | 9 |
| 16:00 | Standing, eating, lying, guarding, defecating, walking, cleaning, bellowing. | 8 |
| 17:00 | Eating, standing, cleaning, walking, lying, defecating, biting tail, running, guarding, wrestling. | 10 |
| 18:00 | Eating, walking, standing, hanging grass, defecating, cleaning. | 6 |

*Behavioural rhythms of released Milu*

Excreting, parental care, embellishing, rutting, and social behaviour appeared randomly and much less frequently than others. Therefore, the study analysed the proportion of resting, feeding, moving, and vigilance of recorded samples for every 5 min period.

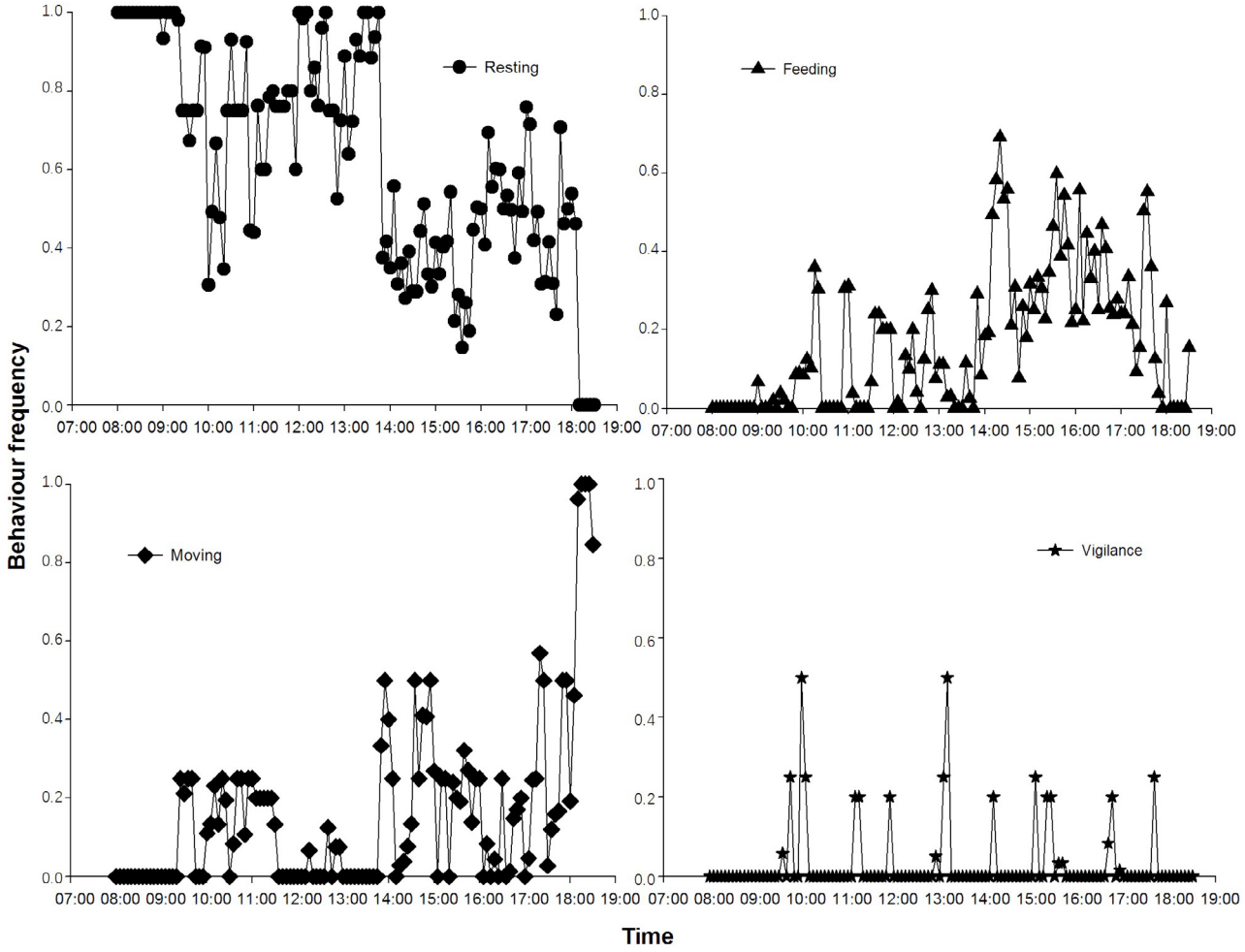


Fig. 3. Behavioral rhythm of released Milu in adaptation phase (every 5 min).

The results showed that their rhythms fluctuated and differed from each other (Fig. 3). During 8:00 hour time interval, Milu were almost always resting and they became active after 9:00 hours. Resting was the primary behaviour in every time interval, except 18:00 hour time interval, and was maintained at a high level during the adaptive phase. Fluctuation in the frequency of resting was greater before 14:00.

Feeding behaviour appeared regularly after 10:00. During 10:00 to 14:00 time intervals, variation in feeding was random and there was no feeding behaviour in some time intervals. The intervals from 14:00 to 18:00 contained the feeding peak, feeding appeared continuously, and its frequency was higher than that before 14:00.

The variation in frequency of moving could be divided into four stages. First, there was no movement before 9:00 when Milu were resting. Second, from 9:00 to 12:00 time intervals, variation in the frequency of movement was regular and maintained at a low level, the peak was 0.25 and appeared 8 times, surplus samples were almost less than 0.2. Third, moving behaviour rarely occurred during 12:00 to 14:00 time intervals. Finally, the time intervals from 14:00 to 18:00 contained the feeding peak and feeding frequency was maintained at a high level, frequency rose to 0.5 in the start, then it reduced and was maintained at about 0-0.3, extraordinarily, frequency reached 100% after 18:15 hours.

Vigilance exhibited obvious sensitivity to interference and differed from other types of behaviour. Its frequency skyrocketed when Milu were influenced by humans or environmental disturbances.

The study further analysed behavioural rhythms of released Milu for each hour (Fig. 4), and characteristics of the above four behavioural types were obvious. Although the proportion of feeding was higher than resting in 15:00 time interval and the proportion of moving was higher

than resting in 18:00 time interval, resting was the most frequent behaviour during the day with its proportion higher than that of other behavioural types. The proportion of feeding and moving was higher after 13:00 time interval. Temperature influence was related to resting and feeding, although its effect on all four behavioural types was indistinct ($p>0.05$). Before 13:00 time interval, resting was negatively correlated with temperature, and the proportion of resting declined as temperature rose from 8:00 to 10:00 hours. Conversely, the proportion of resting increased when temperature declined from 10:00 to 12:00 hours.

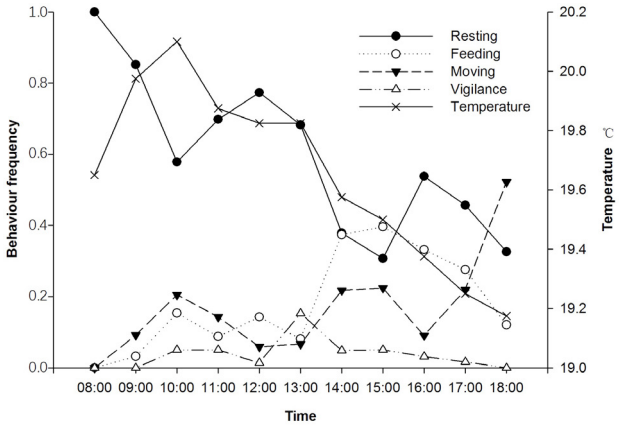


Fig. 4. Behavioral rhythm of released Milu in adaptation phase (every h).

## DISCUSSION

The recorded behaviour occurred during a time of environmental change for the Milu (Caraco, 1979). During the study period, the types of behaviour exhibited by the released Milu were similar to semi-wild Milu at that time, but the behavioural rhythms of released Milu were specific. Temperature was related to the behavioural types and rhythm of behaviour. Temperature peaked in 10:00 time interval, when types of behavior also peaked and the frequency of resting was low. Perhaps the initiation of Milu behavior relied on the rise in temperature. Types of behaviour did not decline until after 13:00 time interval. Feeding and moving behaviour increased, whereas resting was largely reduced. Temperature was lower than earlier time intervals, althoungh it was considered the appropriate temperature for Milu to feed and move during the study period. The peak of feeding of released Milu was 14:00~17:00 time interval, although the peak occurred during the morning and evening for semi-wild Milu in Hubei Shishou Milu National Nature Reserve (Yang, 2013). The difference may be because the influence of temperature was dissimilar between the two areas. Being one of the important elements of habitat, temperature affects animal habits and there is a need for more research on its influence. Compared with semi-wild Milu, rutting behaviour of released Milu occurred less often. This may have occurred because of variation in environmental elements, such as sunshine, temperature, nutrition, and humidity, impacting physiological changes in the Milu. Furthermore, and especially high level of vigilance was recorded during oestrous. Excessive disturbance could influence the breeding of Milu. The study on types of behaviour and behavioural rhythms of released Milu revealed more about influences on behavior. Milu exihibited behavioural types spontaneously to adapt to their new environment.

Frequent human activity influenced the adaptation of trans-located Milu. The perfect habitat should include abundant food, water, and shelter. However, human activity disrupted their behavior and the released Milu lived in an insecure environment during the study period. Milu occasionally change habitats passively, and most of their behaviour is in response to the environment. Adaptation of released Milu needs to be improved in future. Watch and Ward in the area where Milu were released was gradually reduced and they slowly adapted to human activity. This negatively influenced the adaptation of natural wild behavior. The administrators should provide enough living space for wildlife and maintain a balance between human and animals. During research concerned with the observation of behavior of wildlife, methods must be used that are more concealed to allow natural behavior of wildlife.

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*Statement of conflict of interest*

Authors have declared no conflict of interest.

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