SHORT COMMUNICATION

Running Head: Food habits and prey preferences of Amur tigers

A comparison of food habits and prey preferences of Amur tiger (*Panthera tigris altaica* Temminck, 1844) at the southwest Primorskii Krai in Russia and Hunchun in China

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Abstract

A small, isolated Amur tiger population is living at the southwest Primorskii Krai in Russia and Hunchun in China region. Many of them with “dual nationality” cross the border frequently. Formulating effective conservation strategies requires a clear understanding of tiger food requirements in both Russia and China sides, while Russia side already have clear results of it. We used scat analysis combined with data on the abundance of four prey species to estimate Amur tiger diet and prey preferences in Hunchun. We examined 53 tiger samples from 2011–2016 and found that tigers preyed on 12 species (11 species in winter), four of which were domestic animals with 33.58% biomass contribution, and got the first record that Amur tiger eat lynx in this area. Tigers showed a strong preference for wild boar (Jacobs index: +0.849), which were also the most frequently consumed prey, and a strong avoidance to roe deer (Jacobs index: –0.693). On the Russian side, domestic animals (just dog) were rarely found in tiger scat, and tiger did not show strong avoidance to roe deer, but to sika deer. We also found red deer footprints during winter surveys and that tigers ate red deer on the Chinese side, while there was no record of red deer on the Russian side. Reducing or eliminating human disturbance, such as grazing, is essential to recovering tiger prey and habitat in this area and the Sino-Russia joint ungulate annual survey is indispensable for prey estimates of this small, isolated Amur tiger population.

Key words: Amur tiger, food habitat, prey preference, scat analysis, Southwest Primorie-Hunchun

INTRODUCTION

As advanced technologies like camera trapping and molecular genetic analysis have been used to monitor Amur tigers in China over the past 10 years, the whole picture of this endangered species has become clearer. The Amur tiger is now confined in two separate areas: the Sikhote-Alin Mountains of Russia connected with the Wandashan Mountains in China, with nearly 90% of the tiger population (Jiang *et al.* 2014, Miquelle *et al.* 2006, Tian *et al.* 2009), and the southwest Primorskii Krai of Russia–Hunchun of China region, with a small isolated population (Henry *et al.* 2009, Miquelle *et al.* 2006, TaroSugimoto *et al.* 2016). These two patches have long been separated by urban development and wetlands (Hebblewhite *et al.* 2011, 2014). The southern small and isolated population of tigers is at the risk of extinction from genetic, demographic, and environmental stochasticities (Henry *et al.* 2009, Sugimoto *et al.* 2014, Uphyrkina *et al.* 2002), and Russia established a single management system known as the "Land of the Leopard National Park" in 2012 in this area for more efficient conservation. Under the NEASPEC (North-East Asian Subregional Programme for Environmental Cooperation) project "Study of transboundary movements of the Amur tiger and Amur leopard using camera traps and molecular genetic analysis," the Land of the Leopard National Park and the Feline Research Center of the State Forestry Administration of China shared the camera trapping data of 2013–2015. It found out that at least 45 Amur tigers (adults only) were recorded, and 19 tigers (adults only) were registered in the territory of both countries at the southwest Primorskii Krai of Russia–Hunchun of China region during this research period (http://www.wwf.ru/resources/news/article/eng/14752). Tiger populations of such small size, especially after being isolated for 20–30 years, could drop to a threshold level below which recovery is impossible unless habitat is increased substantially within one to two generations (Kenney *et al*. 2014). The only additional potential habitat for this small population now resides on the Chinese side (Hebblewhite *et al*. 2012), because the southeast side of the southwest Primorskii Krai is the Sea of Japan. The Chinese government decided to create a national park in Jilin and Heilongjiang provinces on the border close to Land of the Leopard National Park at this area in order to improve habitat conditions with an area of 1.5 million hectares (http://programmes.putin.kremlin.ru/en/tiger/news/25404).

After obtaining a clearer picture of tiger population distribution and structure, we want to know how they survive in this area. The acquirement of food is a fundamental component for every predator’s existence, and prey selection is critical for understanding life history strategies of any carnivore (Miquelle *et al.* 1996). Kerley *et al*. (2015) and Sugimoto *et al*. (2016) reported the food habits and prey preference of Amur tiger in the southwest Primorskii Krai of Russia side in 2008–2012, 2001–2003. However, because many tigers live on the territory of both two countries, it is very valuable to know the information of Chinese side, concerning Amur tiger habitat expansion to China.

We conducted our study in Hunchun connected to the Land of the Leopard National Park and aimed to investigate the food habits and winter prey preferences of Amur tigers in this area, then compared with the results on the Russia side to complete the integrity of this small Amur tiger population’s food acquirement situation and provide a reference for habitat recovery in China.

MATERIALS AND METHODS

Study area

Hunchun municipality is an area 4938 km^2 in Jilin, northeast China, which contains Hunchun Amur Tiger National Reserve (HNR), covering 1087 km^2 (Fig. 1). It borders Russia to the east and North Korea to the southwest and is connected to the Land of the Leopard National Park in Russia. This area is a key corridor for movement of Amur tigers and Amur leopards between China, Russia and North Korea. As part of the Changbai Mountains, Hunchun is in a temperate zone and has an average rainfall of 661 mm concentrated between July and September (50% of yearly precipitation). The main forms of vegetation here are mixed broad-leaved forests and secondary Mongolian oak (*Quercus mongolica Fisch. ex Ledeb*) forest and the main animals include: the Amur tiger, Amur leopard (*Panthera pardus orientalis* Schlegel, 1857), red deer (*Cervus elaphus* Linnaeus, 1758), sika deer (*Cervus Nippon* Temminck, 1838), wild boar (*Sus scrofa* Linnaeus, 1758), roe deer (*Capreolus pygargus* Pallas, 1771) and musk deer (*Moschus moschiferus* Linnaeus, 1758).

Field methods

We collected fecal samples from 2011–2016. Sampling areas covered most of HNR and some area outside the HNR (Fig. 1). We collected scats opportunistically along roads where tigers commonly deposit scats (Karanth & Sunquist 1995, Sunquist 1981) and while snow-tracking individual tigers (Yudakov *et al.* 1988). We also collected scats near kills that were found either by snow-tracking tigers or from information given by local citizens. Scats were placed in plastic bags and stored in freezers until analyzed. Because the other sympatric big cats, Amur leopards, are living here, whose feces sometimes are similar to that of the Amur tiger, fecal samples were identified as belonging to either tigers or leopards using a fecal DNA-based method (Sugimoto *et al.* 2006).

The relative prey abundance was estimated from the snow-track survey during the winter of 2015, during which standard survey routes were traversed after sufficient snow and all fresh tracks were recorded by species (Stephens *et al.* 2006). The survey assessed ungulate abundance on the basis of track encounters and the ungulates' daily movement distance, and was conducted over 18 routes (total length is about 90 km), covering most of the tiger habitats in Hunchun.

Scat analysis

The hair of prey is relatively undamaged in carnivore scat and can thus be used to identify the prey species eaten. We recorded the presence of a species in any one scat as a single occurrence. Collected Amur tigers' scats were washed in water using a 1.5 mm sieve to separate the hair from other organic matter and remains such as hair, bones, hooves, quills and teeth of the prey consumed were also separated for species identification (Karanth & Sunquist 1995, Sunquist 1981). Among our samples, the bones, hooves, quills and teeth of prey were not found, so prey species identification depended on their hair characteristics. Separated hair was then washed in hot water to remove surface oil. Each scat sample was washed separately in acetone and dehydrated in 100% ethanol. We selected at least 10 compete hairs from each scat sample. Slides were examined at 400x using an Olympus microscope. Species identification was based on the general appearance of the hair, color, length, width, medullary structure, medullary width/hair width ratio and cuticle pattern (Moore *et al.* 1974, Mukherjee *et al.* 1994), according to the database from the laboratory of the fur herbarium of Northeast Forestry University, Harbin, China.

Sometimes, it is hard to identify scat containing cervid hair to species by using the above method (Rozhnov *et al*. 2011), so we used DNA test for the cervid species identification to test the identification results above. These hair samples were subjected to DNA extraction by TIAN amp Micro DNA Kit, following the instructions to extract DNA. Standard PCRs were performed on 10 μg of DNA extracted from scat in a 20 μL volume containing 0.6 μm of each primer, 10 μL buffer, 4 μL dNTPs, 2.5 μL ddH2O, 0.3 μL KOD FX Neo DNA polymerase (TOYOBO). Thermal cycling conditions were as follows: 94°C for 2 min then 35 cycles (94°C, 15 s/55°C, 40 s/68°C, 30 s) followed by 68°C for 20 min. Sequences from the mitochondrial DNA gene of the prey species were obtained through direct sequencing of PCR products amplified using the primers mcb398 and mcb869, others primers is L14841 and H15149 (Ficetola *et al.* 2010, Kocher *et al.* 1989).

Data analysis

We estimated the contribution of each prey species to Amur tiger diet as percent frequency occurrence and biomass contribution. We used the regression equation developed for cougars (*Puma concolor* Linnaeus, 1771) by Ackerman *et al*. (1984) to estimate biomass contribution to the diet:

$$y = 1.98 + 0.035x,$$

where y is the weight of prey consumed/scat produced and x is the live weight of the prey, to relate the number of scats containing a prey species to the prey's biomass (Ackerman 1984).

We calculated y for each species and multiplied it by the number of occurrences of the species to estimate the relative biomass of each prey type consumed. We used the estimated live weights of prey consumed by tigers in the Russian Far East (Bromley & Kucherenko 1983; Danilkin 1999; Prikhodko 2003). We then estimated percent biomass contribution (biomass of each prey type consumed/total biomass consumed × 100).

We estimated relative prey abundance from the winter track survey conducted at the study area, in which standard survey routes were traversed after sufficient snow and all fresh tracks were recorded by species in 2015 (Stephens *et al*. 2006). We used the Formozov-Malyshev-Pereleshin (FMP) formula which is considering the ungulates' daily movement distance to analysis the population density with 95% confidence intervals (Stephens et al. 2006), and then changed the density into a proportion data (relative abundance) for obtaining the Jacobs's index (Jacobs, 1974). This index ranges in values from +1 (strongly preferred) to −1 (strongly avoided). Because red deer in the tiger diet using only had one meeting in our winter samples, so we restricted our analyses of preference to three species: wild boar, sika deer, and roe deer during winter.

Considering the feces sample collected from 2011-2016 (77% samples from 2013-2015), although in the winters of 2012, 2014 there were two ungulates surveys in Hunchun with the sample plot method which is different with 2015 and Russia side, these results haven't been used here. However, the similar results of the ungulate species structure and the density order were released from these two surveys as the 2015 survey, which means the ungulate relative abundance (as the percentage form) order are stable in this area while the ungulate density may be different in each year. Therefore, for obtaining the comparable results with Russia side (same field methods), we used 2015 ungulate data to extrapolate the whole period.

The results of Amur tiger's biomass contribution, prey abundance and food preference was compared with the results of Kerley *et al.* (2015) and Sugimoto *et al.* (2016), for better understanding of the small Amur tiger population.

RESULTS

We collected 68 tiger scats in Hunchun from 2011–2016, in which 13 contained unidentifiable prey remains, 8 were multiple samples collected in the same tiger's trail or near the same killing sites. These two type samples were excluded from further analyses to reduce potential bias. Thus, 35 samples from the winter season (November to April) and 12 in the summer (May to October) were used to analysis the food habits and prey preferences of the Amur tiger in Hunchun (Hojnowski *et al.* 2012). We identified a total of 53 prey items (winter 38, summer 15) in the remaining 47 scats (Table S1). Because of limited samples in summer, seasonal relative biomass contribution was not compared.

We identified a total of 11 species in winter tiger scats in Hunchun (Table 1), among which red deer, cow, horse and sheep were not recoded on the Russia side. There were total 16 winter and summer samples containing deer's hair (Table S1). All of them were extracted DNA of hair bulbs successfully for species identification and then we found 3 mistaken identifications based on the appearance observation of hair. So in our research, the accuracy rate of species identification by the hair appearance observation was 81.25% (13/16) depend on DNA analyze results. The top three prey species with highest occurrence frequency in tiger scats were wild boar, roe deer and sika deer. Because domestic cows are much heavier than wild prey species, cow biomass contribution went into top 4 with wild boar, roe deer and sika deer, which was the other difference with the Russian side. Total biomass contribution from 4 domestic animals was 33.58%, while on the Russian side was just 0.44% and 6.3%.

There were two biggest differences between the Chinese and Russian sides in this region (Fig. 2). Firstly, Kerley *et al*. (2015) and Sugimoto *et al*. (2016) did not find red deer footprints in their snow-track surveys, but on the Chinese side we found red deer footprints in 5 survey lines. Secondly, the relative abundance order of 4 prey species in Kerley *et al*. (2015) and Sugimoto *et al*. (2016) was the same: sika deer > roe deer > wild boar (red deer = 0), but our results were: roe deer > sika deer > wild boar > red deer, which showed different ungulate population structures on the two sides.

Jacobs index values indicated that in our study Amur tigers showed a preference for wild boar (+0.849) which was similar with the Russian side, but a strong avoidance for roe deer (−0.693), while in Russia data showed a strong avoidance for sika deer (–0.698, –0.717). Besides roe deer, Amur tiger also avoided sika deer (–0.495) in proportion to their availability (Table 2).

DISCUSSION

At the southwest Primorskii Krai of Russia–Hunchun of China region, the biggest difference between the two sides was domestic animals. With fewer residents in the forest on the Russian side, only domestic dogs became Amur tigers' prey in winter, while on the Chinese side, not only domestic dogs in winter, but also cows, horses and sheep were recorded throughout the year. There are 98 villages and 4 towns in our research area (Yi *et al.* 2014). Based on a questionnaire survey, 63 pastures are within the area and 3066 cows were recorded, among which 42 pastures had an area of 355 km^2 (Li *et al.* 2016). Li *et al*. (2016) found that grazing activities mainly affected tree density and shoot numbers, which is preferred and browsed upon by ungulates. For example, grazing activities had negative effects on the number of shoots with a contribution of 30.9%. Although in winter, all most domestic animals were brought back to the villages near people, the effect on vegetation and ungulates was still present and this caused a lack of food for the tigers. Even so, the favorite food of Amur tigers is still wild boar and the majority of a tiger's diet consists of medium to large ungulates, mirroring the results of previous studies on tiger diet (Biswas & Sankar 2002, Kapfer *et al.* 2011, Karanth & Sunquist 1995, Miquelle *et al.* 2006, 2010). Therefore, with the lower ungulate density (especially wild board), Amur tigers in China side didn't avoid eating sika deer as strongly as in Russia side for their survival.

In our study, the biomass contribution of cow and horse may be overestimated, because we found that at the kill sites, tigers often eat only part of a cow or a horse, unlike wild boar, which are almost entirely eaten by the tigers. Also, tigers appear to attack calves, not usually an adult cow. Domestic animal loss to tigers may also be overestimated, because if tigers eat a domestic animal, the owner can apply for compensation after submitting the information to local government; however, local people will not report every incident of a tiger eating a wild animal. During the winter survey, we did not find cow or horse footprints in the forest, but it is not hard for tigers to find these domestic animals near villages on the Chinese side when they are on their way to find their next wild prey.

About Amur tigers' prey preferences, a strong avoidance for roe deer (−0.693) was also shown in our research, while a strong avoidance for sika deer was shown on the Russian side

(–0.698, –0.717). Prey selection analysis revealed that Amur tigers had a notable preference or avoidance for particular ungulates compared with Amur leopards: the most frequently consumed prey were sika and roe deer for leopards and wild boar for tigers (Miquelle *et al*. 1996; Sugimoto *et al*. 2016). Perhaps that is maybe why Amur leopards can coexist with Amur tigers in this region.

The other issue worthy of attention is about red deer, which are preferred in Amur tigers' diet (Zhivotchenko 1981, Miquelle *et al.* 2010b). At the southwest Primorskii Krai of Russia–Hunchun of China region, according to the results from Kerley *et al.* (2015) and Sugimoto *et al.* (2016), red deer were not eaten by Amur tiger on the Russian side, but on the Chinese side we found by hair DNA analysis that tigers did eat red deer at least once (Table S1, Fig 2a). From 2012–2013, WWF-China released 67 red deer and sika deer into the wild in Wangqing, which is in the north of Hunchun, about 50–60 km from the Sino-Russia border, to aide in the recovery of Amur tiger and leopard prey resources (http://www.wwfchina.org/pressdetail.php?id=1485). It is not known if there is a wild red deer population surviving in this region or if the released newcomers have broken into the wild network of ungulates? This needs further tracking research.

On May 24 and October 18, 2013, we collected two Amur tiger scat samples that revealed lynx hair (Table S1), earlier than when Petrunenko *et al*. (2016) announced that they found the first recorded case of a tiger killing a Eurasian lynx. Petrunenko *et al*. (2016) reported that on March 4, 2014 in Bastak Nature Reserve (48°56′37 N133°07′13 E), located in the Jewish Autonomous Region of the Russian Far East, the team found the remains of a lynx surrounded by tiger tracks. In Hunchun, we camera-trapped lynx and showed that Amur tigers, Amur leopards and lynx co-exist here. It is not clear whether tigers depress the numbers of these smaller cats, as has been suggested elsewhere (Harihar *et al.* 2011). Even less is known of the relationships between tigers and lynx. While documentation of this episode of direct competition between a tiger and lynx is compelling, there is still much to be learned about the complex interrelationships of large carnivores in this ecosystem.

About scats analysis, except for multiple samples of diet from the same individual, the majority of pseudoreplicates were for deer species (Kerley *et al.* 2015, Sugimoto *et al.* 2016). From the point of practical effect, using a compound microscope to examine the shape and thickness of hair medulla and hair cuticle patterns did not work every time for deer species identification. The simple removal of the pseudoreplicates reduced the percentage of biomass for the combined deer category, and increased the percent biomass for other prey species. We strongly recommend that DNA testing should be used in scat analysis in order to avoid pseudoreplicates. Our research is a good example, if we had not used DNA testing, we would not have found a case where a tiger ate a red deer in this area.

Our results showed that Amur tigers in Hunchun of China had different food habits and prey preferences compared with the southwest Primorskii Krai of Russia, likely due to heavy human disturbance, such as grazing, which affected local vegetation and so reduced the prey species that survive on this vegetation. Under the clear trend of the expansion of Amur tigers to the Chinese side, reducing or eliminating human disturbance is essential to the recovery of tiger prey, as well as the habitat in this region. Also, the Sino-Russia joint ungulate annual survey is also indispensable for prey estimate of this isolated small Amur tiger population.

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SUPPLEMENTARY MATERIALS

Table S1 Prey species identified in scats of Amur tigers (2011–2016)

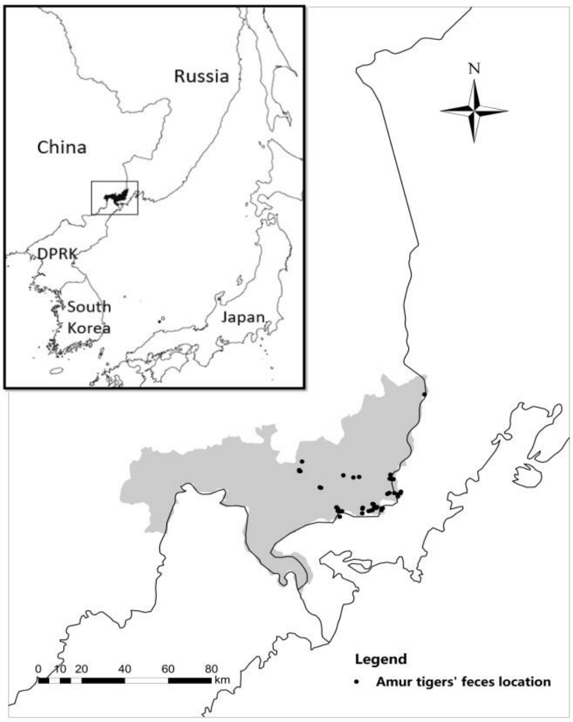

Figure 1 The study area and Amur tigers' scat sample locations.

Table 1 Percent occurrence frequency and biomass contribution of prey species to Amur tiger diet as determined by scat analysis on the Chinese side and the Russian side in winter.

Prey species	Prey weight (kg)	Occurrence frequency	Biomass contribution		
			China side (2011–2016) n = 35	Russia side † (2008–2012) n = 152	Russia side‡ (2001–2003) n = 63
Sika deer	95	11.43	9.28	25.09 (18.12–32.06)	23.86
Roe deer	37	22.86	11.45	8.31 (4.89–12.22)	41.3
Wild boar	103	42.86	36.63	58.60 (49.47–67.74)	54.0
Red deer	187	2.86	4.19	-	-
Cow	418	8.57	21.79	-	-
Domestic dog	31	5.71	2.68	0.44 (0.00–1.31)	6.30
Horse	450	2.78	7.75	-	-
Leopard cat	4	2.86	0.93	-	1.60
Bear spp.	150	2.86	3.16	3.11 (0.00–7.25)	-
European badger	6	2.86	0.96	0.34 (0.00–1.01)	6.30
Sheep	50	2.86	1.63	-	-
Red fox	5	-	-	-	3.20
Hare	1.8	-	-	-	3.20
Musk deer	-	-	-	0.37 (0.00–1.10)	-
Mustela spp.	-	-	-	0.94 (0.00–2.19)	-
Amur tiger				2.81 (0.70–5.62)	

†Cited from Kerley *et al.* (2015) Table S1, in which SW represnted the the southwest Primorskii Krai of Russia and the resuts were from 2008–2012; "n = 152" was total winter and summer scats in SW, Kerley *et al.* (2015) did not mention total winter scat number. ‡Cited from Sugimoto *et al.* (2016) showing the results from 2001–2003.

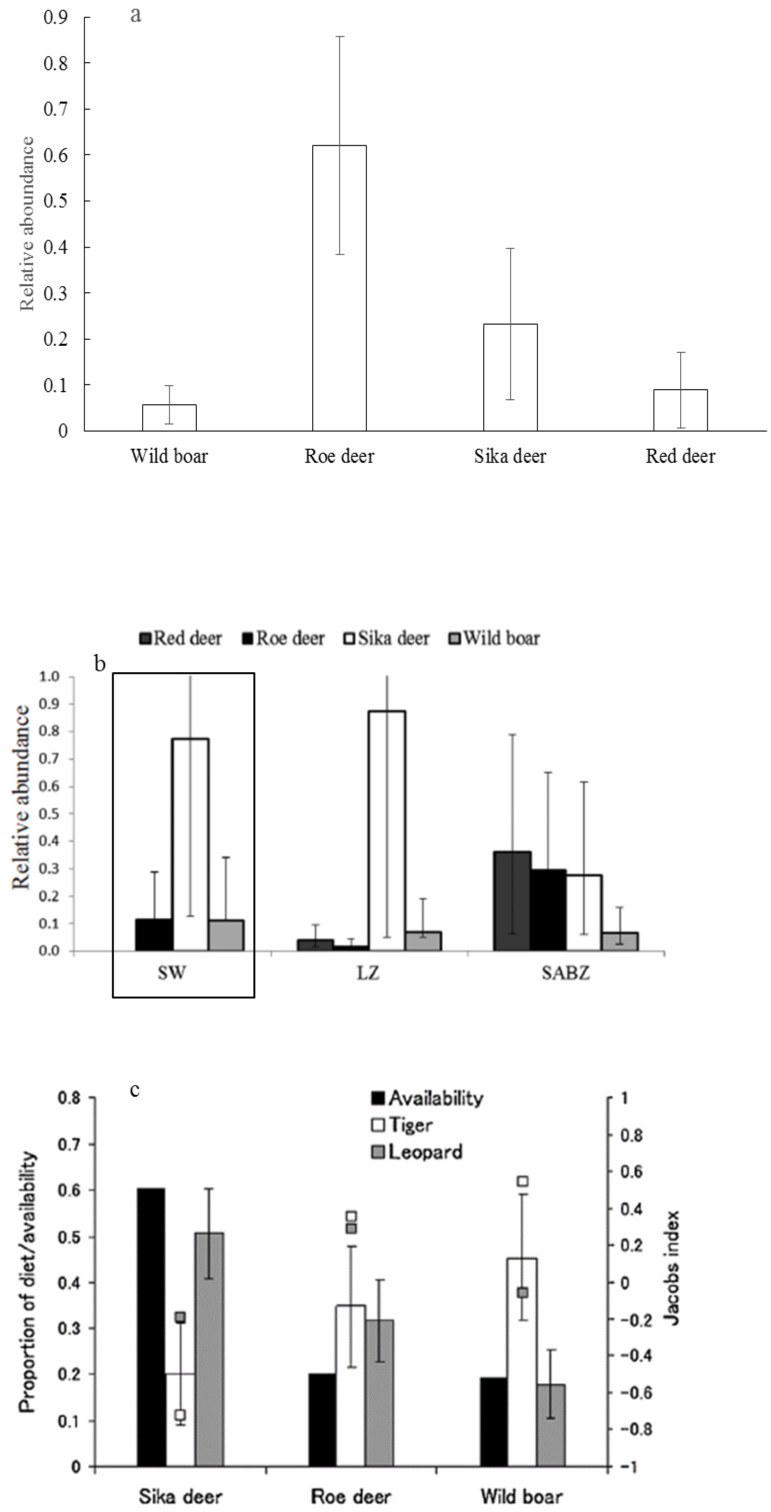

Figure 2 Relative abundance of wild boar, sika, red and roe deer as estimated by winter track counts in Hunchun: (a) shows our results from the winter survey of 2015; (b) was cited from Kerley *et al.* (2015) in which SW represnted the the southwest Primorskii Krai of Russia and the resuts were from 2008–2012; (c) was cited from Sugimoto *et al.* (2016) showing the results from 2001–2003.

Table 2 Jacobs’ index scores with 95% confidence intervals measuring tiger preference or avoidance for 4 ungulate species on the Chinese side and the Russian side

Species	Jacobs’ index		
	Chinese side 2011–2016	Russian side† 2008–2012	Russian side‡ 2001–2003
Wild boar	0.849 (0.747 to 0.957)	0.790 (0.618 to 0.962)	0.547
Red deer	-	−0.326 (−0.791 to 0.139)	-
Sika deer	−0.495 (−0.672 to 0.272)	−0.698 (−0.917 to −0.268)	–0.717
Roe deer	−0.693 (−0.906 to −0.355)	−0.368 (−1.000 to 0.275)	0.353

†Cited from Kerley *et al*. (2015), but the results were from 3 sites, not only SW, which represented the southwest Primorskii Krai of Russia; ‡cited from Sugimoto *et al*. (2016).

SUPPLEMENTARY MATERIALS

Table S1 Prey species identified in scats of Amur tigers (2011–2016)

Species	Occurrence number		
	Winter (n = 35)	Summer (n = 12)	Total items
Sika deer	4	3	7
Roe deer	8	-	8
Wild boar	15	5	20
Red deer	1	-	1
Cow	3	2	5
Domestic dog	2	-	2
Horse	1	3	4
Leopard cat	1	-	1
Bear spp.	1	-	1
European badger	1	-	1
Sheep	1	-	1
Lynx	-	2	2
Total items	38	15	53