

Winter diet and food selection of the Black-necked Crane *Grus nigricollis* in Dashanbao Yunnan, China

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The Black-necked Crane *Grus nigricollis* is a globally vulnerable species, whose food is the determinant factor influencing the long-term survival of Black-necked Crane. Understanding the Black-necked Crane's dietary habits, food preferences, and the associated factors will facilitate the development of effective conservation plans for the protection of this vulnerable species. For this purpose, we used video recording to examine the dietary composition and temporal variation in food selection of Black-necked Cranes wintering in the Dashanbao National Nature Reserve, China. The composition of the birds' diets and their food selection were compared on a monthly basis. The corresponding data were analyzed using a Canonical correspondence analysis (CCA) to determine the food use and food variables, and using a Pearson correlation coefficient to determine the correlation between environmental temperatures and the availability of key food items. The results revealed that the Black-necked Crane's diet consists primarily of domestic food crops (such as grains 73.81% and potatoes 7.84%) and invertebrates 13.96%. A much smaller proportion of the diet was comprised of turnips and wild plant foods (herbaceous plants, tubers). In addition, there were monthly variations in the Black-necked Crane's food selection. Monthly food preferences were partially related to the available amount of food. The cranes mainly selected invertebrate animals in December and January, while they preferred to eat domestic crops throughout winter months, because invertebrate populations sharply declined in December and January due to the low temperature. In addition, grain consumption was negatively associated with invertebrate availability. In November, when invertebrates were most abundance, and despite a concomitant peak in grain abundance, cranes exhibited a preference for invertebrates over grains. We therefore recommend that the protection administration provide appropriate supplemental foods for cranes during icy periods.

Winter diet and food selection of the Black-necked Crane *Grus nigricollis* in Dashanbao

Yunnan, China

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Abstract

The Black-necked Crane *Grus nigricollis* is a globally vulnerable species, whose food is the determinant factor influencing the long-term survival of Black-necked Crane. Understanding the Black-necked Crane's dietary habits, food preferences, and the associated factors will facilitate the development of effective conservation plans for the protection of this vulnerable species. For this purpose, we used video recording to examine the dietary composition and temporal variation in food selection of Black-necked Cranes wintering in the Dashanbao National Nature Reserve, China. The composition of the birds' diets and their food selection were compared on a monthly basis. The corresponding data were analyzed using a Canonical correspondence analysis (CCA) to determine the food use and food variables, and using a Pearson correlation coefficient to determine the correlation between environmental temperatures and the availability of key food items. The results revealed that the Black-necked Crane's diet consists primarily of domestic food crops (such as grains 73.81% and potatoes 7.84%) and invertebrates 13.96%. A much smaller proportion of the diet was comprised of turnips and wild plant foods (herbaceous plants, tubers). In addition, there were monthly variations in the Black-necked Crane's food selection. Monthly food preferences were partially related to the available amount of food. The cranes mainly selected invertebrate animals in December and January, while they preferred to eat domestic crops throughout winter months, because invertebrate populations sharply declined in December and January due to the low temperature. In addition, grain consumption was negatively associated with invertebrate availability. In November, when invertebrates were most abundance, and despite a concomitant peak in grain abundance, cranes exhibited a preference for

invertebrates over grains. We therefore recommend that the protection administration provide appropriate supplemental foods for cranes during icy periods.

Keywords: Black-necked Crane, *Grus nigricollis*, diet composition, food numbers, food selection; Dashanbao.

Introduction

The Black-necked Crane *Grus nigricollis* is a globally vulnerable species, with the main breeding distribution in the high altitude Tibetan-Qinghai Plateau. The cranes migrate short distances to winter in the lower altitude (primarily 2000-3200 m) Yunnan-Guizhou Plateau (Harris 2013). Via telemetry and banding data, it has become clear, that the birds using the Eastern migratory route (in the following referred to as the Eastern Black-necked Cranes) breed in the north Sichuan province and the south Gansu province and mainly winter in northeast Yunnan and southwest Guizhou (Li & Li 2005; Qian et.al 2009). More than 50% of the wild populations of this species are currently suffering due to significant habitat destruction resulting from grassland degeneration (Li & Li 2012) and conventional agricultural practices that have decreased the diversity of available food types for this species in northeast Yunnan. Food is the determining factor influencing the long-term survival of Black-necked Crane (Liu Q et al. 2014a). Thus, understanding the Black-necked Crane's dietary habits, food preferences, and the associated factors will facilitate the development of effective conservation plans for the protection of this vulnerable species.

Determining the dietary composition of wild birds is essential for understanding how the animals interact with their habitats and consequently for identifying their preferred food types (Baubet et

al. 2004). Their late discovery and remote range led to a late start in research pertaining to Black-necked Crane's feeding habits (Harris & Mirande 2013). To this point, research surrounding the Black-necked Crane's diet has included quantitative studies on various types of domestic and wild plant foods (Li & Nie 1997; Bishop & Li 2001; Liu et al. 2014a) and qualitative studies on animal-based foods (Han 1995; Hu et al. 2002; Li & Li 2005; Liu et al. 2014b). Nonetheless, there remains a lack of synthetic analyses or comparative data regarding the proportions of domestic food crops, animal-matter, and wild plants consumed by the Black-necked Crane during the winter.

Until now, fecal microhistological analysis has been the only method used to identify plant material consumed by wintering Black-necked Cranes (Li & Nie 1997; Liu et al. 2014a). These studies did not mention the consumption of animal-based foods due to the need for alternative methods to collect this data (Liu et al. 2014b). Generally, fecal analysis can create a bias due to the high variability in digestibility of different food items (Redpath et al. 2001). Thus, we chose video recording as an alternative method to better understand the food selection of Black-necked Cranes. This method provided a simple, minimally invasive manner to directly observe the feeding behavior of the threatened bird species in order to estimate their dietary composition (Newton 1967; Price 1987, Yoshikawa & Osada 2015).

Previous studies suggest that variations in temperature may impact food availability (Kushlan 1978; Stapanian et al. 1999). As mentioned by Alonso et al. (1994), low temperatures may decrease grain availability for Common Cranes *Grus grus* by increasing foraging costs due to changes in soil properties. Likewise, temperature is an important correlate of insect activity,

further affecting the invertebrate-feeding birds. Higher temperatures are associated with more frequent droughts and dry soils (Martin 1985), while lower temperature cause the soil to freeze. Thus, both affect the degree of insect activity (McColloch et al. 1927; Dowdy 1937; Zhou et al. 2015) and their availability for birds. Considering this information, we considered that the temperature changes would influence the attributes of available foraging sites as well as the bird' food selection, consequently limiting available foods for birds living in cold climates. The goal of this research was to better understand factors influencing Black-necked Cranes selection of different feeding habitats during the winter. This information may facilitate the development of strategies to protect the Eastern Black-necked Crane, whose largest population winters in their most important wintering sites in the Dashanbao National Nature Reserve on the Yunnan–Guizhou Plateau (Li & Yang 2002; Qian et al. 2009). In this report, we provided a quantitative and comprehensive assessment of the cranes' wintering diet, which included domestic food crops, animal-based foods, and wild plants. We analyzed the cranes' food selection, the composition of their diet, and any correlation between environmental factors, food availability, and food selection.

Methods

Ethics statement

Our research on Black-necked Crane in Dashanbao National Nature Reserve was approved by the Chinese Wildlife Management Authority and conducted under Law of the People's Republic of China on the Protection of Wildlife (August 28, 2004).

Field Permit

The Administration of ZhaoTong Forestry Bureau approved our study on behavior observation and sampling collection in the research plot in Dashanbao National Nature Reserve (IDZTL2008163).

Study site

Dashanbao National Nature Reserve (hereafter referred to as Dashanbao Reserve, 27°18'38"N, 103°14'55"E, altitudes of 3000-3200 m), is located in southwest China (Fig. 1), and is listed as a wetland of international significance under the Ramsar Convention on Wetlands. The Dashanbao Reserve is considered an important habitat for Black-necked Cranes, as well as other wintering water birds. It is also known for its upland wetland ecosystem (Zhong & Dao 2005). The study area covers 19,200 ha and is a warm, humid plateau and monsoon climate characterized by cool, wet summers and cold, dry winters. During winter months, frequent days of sustained freezing temperatures can be expected from December to January. The mean temperature for January is -1 °C, and 12.7 °C for July. The mean annual temperature is 6.2 °C, with 123 frost-free days and 34.6 snow cover days per year. The mean annual precipitation is 1165 mm (Li & Zhong 2010). A total of c. 1,200 Black-necked Cranes winter in the Dashanbao Reserve every year, feeding on agricultural farmlands, as well as wild grasslands (Kong 2008). For the purposes of this study, supplemental feeding by humans was ignored because only c. 3 kg of corn are fed to fewer than 50 cranes every day (Kong et al. 2011a), which would have little impact on the overall dietary composition and food selection for the cranes. Farmland included fields of cereal (*Avena sativa* and *Fagopyrum tataricum*), potatoes (*Solanum tuberosum*) and turnip (*Brassica rapa* var. *rapa*). Wild grasslands were comprised of meadows with minimal water (Kong et al. 2011a) and

dominated by orchard grass (*Dactylis glomerata*), bluegrass (*Poa annua*), or *Leontopodium*,
Trifolium, *Pterospermum heterophyllum*, *Pedicularis densispica*, *Luzula multiflora*,
Hemiphragma heterophyllum etc (Kuang, et.al 2008). The cranes have been reported to forage
on *Pedicularis*, *Stellaria*, *Polygonatum* and *Veronica* (Kong et al. 2011a; Liu et al. 2014a). The
study area covered most of the foraging sites of Black-necked Cranes. Local farming uses a 3-
year rotation system, in which cereal is grown one year, followed by two years of potato or
turnip, and then back to cereal. Thus, a mosaic of patches of cereal, potato and turnip
characterizes the farmland, with each occupying about the same surface area each year.

Bird observations

Field data were collected from November 2013 to February 2015 in the Dashanbao Reserve.
Since Black-necked Cranes are highly vigilant and the landscape of the Dashanbao Reserve
consists of rolling hills and valleys, we were unable to adequately observe the flocks from our
vehicles across the main road in the Dashanbao Reserve and we had to leave the main road and
walk along smaller roads. Therefore, we selected three transect routes crossing the mountain
ridge of the reserve at two sites which housed the largest flocks of cranes according to the
reserve staff's experience and the suggestions from previous research in October 2013 (Kong et
al. 2011a) (Fig.1). The majority of cranes arrived in early November and remained feeding in
Dashanbao Reserve until in early March. The duration of our survey started in the second week
of November and lasted until the end of February in each year of observation. Severe weather
was likely to continue for a week into each survey period per year. So we spent 3 days every
week for 15 weeks per year, observing the cranes while they fed. For videotaping we chose

cranes at random from a within the total number of birds in a flock. This resulted in observations of 50-70% of all cranes in each flock. We videotaped the birds for 5 min intervals each across all transect routes. We walked transects once per day and switched direction of travel on subsequent days. We recorded each food item consumed in the feeding area. During this time, the cranes were undisturbed and at a maximum distance of 80 m from our point of observation. Thus, most sightings were between 60 and 80 m from the birds. Based on personal observation, the cranes would startle and flee their feeding site when observed from a distance of less than 60 m. A Canon PowerShot SX30 IS digital camera with a 35× optical zoom was used for all the video recordings. A total of 505 five-minute good quality videos were recorded, ensuring sufficient clarity to accurately differentiate among all the consumed food types. For this study, poor quality recordings and those lasting less than 5 minutes were discarded.

Foraging behavior

We combined feeding behavior and information about habitat type to determine food type. Food types were classified into 3 categories: (1) domestic crops (including: a. grains, b. potatoes and c. turnips); (2) animal matter (f. invertebrates, Earthworms, Coleoptera larvae); (3) wild plants (including d. herbaceous plants, e. roots or tubers). Videorecordings of foraging cranes were examined in slow motion to quantify number of pecks per each 5 minute interval. Every video was watched at least three times to confirm accurate identification of the food types consumed by the feeding crane. Depending on the types of food being eaten, and the peck frequency, four different types of feeding patterns were identified: (1) high pecking frequency and ingestion of all the target food quickly in farmland. This pattern was used primarily for aboveground food

consisting of grains (see videos 1, 2). (2) Digging up the soil to find and consume underground food, such as roots or tubers (including potato and turnip) (see videos 3). Since tubers are bulky for cranes to eat, they peck at them repeatedly, swallowing smaller pieces, until the item is completely consumed. This behavior facilitates visual identification of tuber consumption. (3) Consumption of invertebrates is also easily identifiable by a visual pattern in which the cranes peck at a plot of turf, capture their prey, and then quickly swallows it (see video 4 and video 5; picture 1). This pattern leaves an obvious disturbance of the turf that can be used for identification (see picture 2 and picture 3). (4) Lastly, the cranes used tugging (Ellis 1991), without digging up the soil, primarily for aboveground foods consisting of herbaceous plants. We distinguished this from foraging on grains via a lower pecking frequency and slower swallowing movements compared to forage on herbaceous plants (see video 6, picture 4). We recorded the numbers of pecks for each food type. In addition, for more than one food type in per 5-min recording, we recorded the pecks respectively.

Sampling area for food availability

Given the mosaic landscape of the Dashanbao Reserve, the sampling sites were selected based on two criteria: (1) The site needed to include a large section of farmland and grassland bordered by farmland with three types of crops in cultivation in the transects. (2) The site must have been selected by at least one flock of cranes for foraging across three transects. Based on these two criteria, twelve plots of farmland (2-6 ha) and twenty plots of grassland (13-43 ha) were selected using Google Earth followed by a field survey (Fig.1). The alternating proportion of land that each crop and grassland occupied was obtained via monthly sampling. The area of the sampling

site was calculated using Arcgis 9.2 (ESRI Inc.).

Availability of different food types

To investigate the availability of consumable crops, animal matter and wild plants, we examined the previously mentioned farmland and grassland sampling areas, where we recorded at least one foraging flock of cranes. We then proceeded to sample foods at intervals of 100 m along a straight line, guided by GPS localization. Due to differences of farming practices (unploughed and ploughed), we used a direct collection sampling method for cereal grains on unploughed plots and turned the soil for sampling cereal grains under ploughed lands. The latter method was used for sampling potatoes, turnip, invertebrate (e.g. Earthworm and Coleoptera larvae), herbaceous plants, as well as tubers within a depth of 10 cm. The length of a crane's bill is 12.4 cm ($n = 10$, 10.5-14.0 cm). We did not consider invertebrate sizes because the size of most invertebrate larvae and earthworm, as we assumed these to be larger than grains. The count, biomass, and the depth of food types available in each quadrat (50×50 cm, 10 cm deep) were recorded. The depths where we encountered frozen soil were recorded during the sampling. We placed 176 quadrats in grain fields, and another 222 quadrats in potato and turnip fields in 2013-2015 (sampled monthly for eight months over two years). Earthworms, Coleoptera larvae, herbaceous plants, and roots or tubers were collected from 295 quadrats in grassland. The extracted food items were stored in plastic bags and frozen until processing. After defrosting, cereals, potatoes, turnips, invertebrates, herbaceous plants, and tuber were separated, dried (60 °C, 48 h) and then weighed to determine dry biomass (0.001 g precision). We estimated the monthly availability by multiplying the monthly surface of grassland and each type of farmland by the calculated means.

Weather variables

Daily temperature values were taken from Zhonghaizi in the Dashanbao Reserve. For our analyses, we used the mean daily temperature, and the mean minimum daily temperature. We also counted the number of periods with three or more consecutive days with sustained low temperature (minimum temperature equal to or less than -10°C). These would be days when the ground would remain frozen, thus preventing the cranes from being able to dig for food.

Statistical analysis

Month trophic diversity was estimated using Shannon's diversity index: $H' = -\sum P_i \ln(P_i)$ (Pielou 1966), where P_i represents the proportion of each food type. $P_i = N_i/N$, where N_i is total number of ingestion of food type i and N is total number of all food. We calculated H' using the proportion estimate derived for each food type present in the sample. We used One-Way ANOVA to test differences between months in diversity index. Subsequently, Bonferroni techniques were applied to correct the level of significance of the index. Food selection by cranes was analyzed using the Savage selectivity index (Savage 1931, cited by Manly 1993): $W_i = O_i/\pi_i$, where O_i is the proportion of the sample of used resource units that are in category i , and π_i is the proportion of available resource units that are in category i . The proportion O_i could be calculated using the formula $O_i = u_i/u_+$, where u_i represents ingestion of a specific food and u_+ represents the total number of all food types detected. Likewise, the proportion of consumed biomass for a food type could be calculated using the formula $\pi_i = A_i/A_+$, with A_i representing the biomass of available resource units in category i , and A_+ the biomass of the total population of available resource units (Manly 1993, Avilés et al. 2002). This Savage selectivity index can

range from 0 to infinity, with 0 indicating maximum negative selection, 1 indicating no selection bias and infinity indicating maximum positive selection (Manly 1993). The statistical significance of the selection for each food type from a distribution proportional to its availability was tested using the statistic $(W_i - 1)^2 / s.e (W_i)^2$ (Manly 1993), which follows the critical value of a χ^2 distribution with one degree of freedom, with $s.e (W_i)$ being the standard error of the index $s.e (W_i)$, was calculated using the formula $\sqrt{(1 - \pi_i) / (u_+ \times \pi_i)}$ (Manly 1993). Statistical significance was obtained after applying the Bonferroni correction for the number of statistical tests (Rice 1989).

Multivariate analyses were performed with the software CANOCO (terBraak & Smilauer 1998). Preliminary detrended correspondence analysis (DCA) was applied to three food selection datasets to determine the length of the gradient. This DCA revealed that the gradient was greater than 3 standard deviation units (4.2), justifying the use of unimodal ordination techniques (terBraak & Verdonschot 1995). According to the results of our preliminary canonical correlation analysis (CCA), we eliminated collinear environmental variables with high variance inflation factors ($VIF > 20$) from further analyses. The variable with the highest significant contribution was included in the analysis (Monte Carlo permutation test $P \leq 0.05$, randomization test with 499 unrestricted permutations). Then, we recalculated the contribution and significance for each variable. Again, the variable with the highest significant contribution was included. This procedure was repeated until none of the variables had a significant contribution. The variables we included were the distributed depths of grain, the depths of potato, the depths of invertebrate, grain availability, potato availability, and invertebrate availability. Canonical correspondence

analysis (CCA) with biplot scaling on inter-species distances was used to display the relationship between food selection structure and the 6 environmental variables (main food variables). DCA analysis was performed using CANOCO version 4.5 software (terBraak & Smilauer 2002). We determined the relationship between food variables and environmental variability (the mean daily temperatures, minimum daily temperatures, and number of days with frozen soil) via applying the Pearson correlation coefficient in SPSS 20. We used the Kruskal–Wallis nonparametric test to explore monthly differences in available biomass of four foods. If the monthly differences were statistically significant, we used Nemenyi by programming in SPSS 20. Statistical significance was obtained after applying the Bonferroni correction.

Results

Diet composition and monthly variation

Domestic crops (grains and potatoes) and animal matter (invertebrates) collectively comprised the majority of the Black-necked Crane's diet, followed by turnips and wild plants (herbaceous plants, tubers) (Table.1). When we pooled yearly data, domestic crops and animal matter accounted for 95.62% in total food items, of which grains accounted for 73.81%, potatoes 7.84% and animal matter 13.96% respectively. In November (both years combined), the proportion of grains consumed was the lowest compared to other months. From December to February, the proportion of grains consumed was increased more than twofold of the amount of grain consumed in November. In contrast, the highest consumption of potato and invertebrates occurred in November, followed by January (for potato) and February (invertebrate), while the lowest of both was December. Turnips and herbaceous plants comprised a minimal proportion of

the diet during the entire winter. The diversity of the diet (H') was higher in November than in other months (November and December: $P = 0.006$; November and January: $P = 0.05$; November and February: $P = 0.028$) (Fig. 2).

Food Selection

Wild plant food accounted for the largest proportion of food available in the Black-necked Crane's environment (Table 2). When we pooled yearly data, herbaceous plants and tubers accounted for 89.75%, with 43.25% for herbaceous plants and 46.50% for tubers. Domestic crops (grain 1.17%, potato 1.64%, and turnip 2.94%) and animal matter (invertebrates 4.48%) accounted for a much lower proportion of total food available. Kruskal–Wallis test indicated significant effects on grain and invertebrate available biomass in all four months (grain : $H = 16.402$, $P = 0.001$; invertebrate: $H = 13.081$, $P = 0.004$), whereas we did not find significant effects on other types of food ($P > 0.05$). The available biomass of grains in November and December was higher than in the other two months (Table 2, Nov and Jan: $H = 7.53$, $P = 0.006$; Nov and Feb: $H = 13.60$, $P = 0.000$; Dec and Feb: $H = 6.46$, $P = 0.010$). Invertebrate available biomass was higher in November and February than in the other two months (Table 2, Nov and Dec: $H = 7.55$, $P = 0.006$, Feb and Dec: $H = 8.38$, $P = 0.004$, via Nemenyi test).

In comparing the six types (3 categories) of foods available to the foods selected, the Savage index showed that the cranes preferred grain, invertebrates, and potatoes in November (Table 3). Grains were preferred through the wintering period, invertebrates were the second most preferred food type in November and February, and cranes preferred potatoes in November and January.

Environmental factors compared to food selection

287 The eigenvalues for the first two axes in Fig.3 were 0.223 and 0.007, respectively. The food use-
 288 environmental correlations for the first two axes were 0.986 and 0.714 respectively. The first two
 289 axis of the CCA explained 96.6 % of the total variance in food selection data and food variables,
 290 of which 93.8% was contributed by the first axis, and 2.88% by the second axis. All CCA results
 291 are presented in Fig 3. Invertebrate availability (0.77), potato availability (0.65) and grain
 292 availability (0.53) were positively associated with the first axis, while the distributed depths of
 293 invertebrate (-0.78), the depths of potato (-0.68) and the depths of grain (-0.49) were negatively
 294 associated with the first axis. CCA axis 1 and 2 separated the food use into groups for grain use,
 295 potato use and invertebrate use. The first group for invertebrate use was positively associated
 296 with invertebrate availability, and was negatively associated with the depths of invertebrate. The
 297 second group for potato use was negatively associated with the depths of invertebrate. The third
 298 group for grain use was positively associated with the depths of invertebrate, followed by the
 299 depths of potato and the depths of grain, while these were negatively associated with invertebrate
 300 availability. As invertebrate availability seasonally decreased, cranes increased their grain
 301 consumption. The depth distribution of invertebrate was significantly negatively correlated with
 302 mean temperature and mean of minimum temperature, and was positively correlated with the
 303 number of days during which the ground was frozen (Table 4). Further analysis revealed that the
 304 available numbers of two levels of distributed depths for invertebrates were positively correlated
 305 with the temperature values, that is 0–1 cm, and 1.1–2 cm, and were negatively correlated with
 306 the number of days with frozen ground (Table 4). The mean depth of the frozen ground was 4.93
 307 cm in December (n = 10, 2.6–6.9 cm) and 3.12 cm (n = 5, 2.9–3.5 cm) in January (see picture 5).

Discussion

Diet composition

The variation in diet of the Black-necked Cranes was systematically studied for the first time using video recording. The results revealed that the wintering diet of the Black-necked Crane in the Dashanbao Reserve mainly consisted of domestic crops (e.g. grains and potatoes), and invertebrate animals. Turnips and wild plants foods (such as herbaceous plants, and tubers) accounted for a much lower proportion of their diet. These results are similar to those of a previous report in which fecal analysis was used to study the crop and wild plant consumption of a subpopulation of Black-necked Cranes wintering at the Yarlung Zangpo Valley National Natural Reserve. However, the report on the cranes in the Yarlung Zangpo Valley National Natural Reserve did not calculate the proportion of animal-based food (Bishop & Li 2001). It is important to note that initial estimates approximated that 13.96% of the Dashanbao Black-necked Crane's diet would consist of invertebrates. In comparison, animal matter comprises less than 10% of the diet for Common Cranes in the Holm Oak Dehesas (Avil  t et al. 2002), and 2–3% of the diet for various crane species in different regions of the world (Irene 1980; Reinecke & Krapu 1986). Certain crane species feed primarily on animal matter while wintering in certain sites. These include, the Lesser Sandhill Crane (*G. canadensis canadensis*) (Davis & Vohs 1993), Whooping Crane (*G. americana*) (Pugesek et al. 2013), and Red-crowned Crane (*G. japonensis*) (Li et al. 2014). Demoiselle Cranes (*Anthropoides virgo*) (Sarwar et al. 2013), Florida Sandhill Cranes (*G. c. pratensis*) (Rucker 1992) and Common Cranes show similar preferences for invertebrates (Avil  s et al. 2002).

Current research on the proportion of animal-based foods in the diet of Black-necked Cranes has solely focused on describing species tendencies (Han 1995; Hu et al. 2002; Li & Li 2005; Liu et al. 2014b). Thus, there is a need for additional quantitative investigations into the Black-necked Cranes feeding habits, including invertebrate consumption. Likewise, more data is needed to study the feeding habits of Black-necked Cranes over a greater distribution of locations. This would greatly enhance our understanding of the dietary habits of this species.

Previous studies using fecal analysis to assess the proportion of the mentioned food categories in the Black-necked Crane's diet have produced inconsistent results in comparison to our study.

These studies largely reported a wild plant diet (leaves, roots and tubers), while failing to mention the inclusion of domestic crops or invertebrates in the diet of cranes in the Dashanbao Reserve (Liu et al. 2014a). This inconsistency has two possible explanations: the method to analyze the data and the sampling procedures. First, different methods were used to analyze the diet. With fecal analysis, wild plant fiber may therefore have been easier to detect in feces than the potato and grain fibers or invertebrate larvae residues, despite the latter two making up a larger proportion of the diet. Liu et al. (2014a) mentioned potato cuticles failed to detect in the fecal sample of a crane that due to the digestibility of the food type. With video observation, we were able to directly estimate the frequency on which a particular food type was fed on, without concern for variations in digestibility. While, video observation enables the detection of even highly digestible food, it is often more difficult to identify the specific food types that are seen consumed in the video. Thus, it requires more careful observation and detection of feeding patterns to identify food items. This may also be seen as an advantage, as it can provide us with

more complete foraging information, including actual foraging behavior. We are thus able to successfully estimate the digestible compositions of a birds' diet (Robinson & Holmes 1982; Rundle 1982). Second, our results infer that the sampling time may have greater impact on identifying food types which change with monthly variations. For example, as a climate-restricted food, invertebrates are difficult for Black-necked Cranes to find in December and January (Table. 2) (see below discussion). Fecal analysis of Black-necked Crane's diet in the previous study did not mention sampling time in Dashanbao Reserve (Liu et al. 2014a). So we speculate that whether the arbitrary or discontinuous sampling times caused the bias of fecal analysis. However, video observation was based on monthly sampling in this study.

Monthly variation and diet selection

In November, a high proportion of the Black-necked Crane's diet consisted of domestic crops (principally grain) and invertebrate organisms (Table 1). The phenomena may be because the availability of those food types was the highest during the time of early migration (November) as compared to the other three months (Table 2), or during the warmer weather. The birds require a balanced diet, including a variety of nutrients from different food types. In November when both grains and invertebrates were most available, invertebrates were consumed more than they were consumed at any other time. In contrast, grains were consumed less than in other months. This suggests that the cranes likely prefer invertebrates over grains, potentially because invertebrate organisms provide a greater source of protein and calcium than available in grains. These nutrients are essential for their migration fitness and overall survival. Cranes consumed only a minimal quantity of wild plants despite their larger proportion of available biomass as compared

to that of domestic crops and animal matter (Table 2). It is possible that cranes prefer domestic crops or animal matter over wild plants because (1) herbaceous plants may have lower caloric content than grains or animal matter ; (2) there is insufficient density of vegetation suitable for the cranes to forage on (*Pedicularis*, *Stellaria*, *Polygonatum* and *Veronica*) (Kong et al. 2011a; Liu et al. 2014a).

Environmental factors compared to food selection

Based on the results of our CCA, the grain use and invertebrate use present two different patterns. Grain use was positively correlated with invertebrate depth and negatively correlated with invertebrate availability. However, invertebrate use shows the opposite pattern. Falling temperatures and freezing soils reduced the availability of invertebrates and increased the depth of invertebrates, especially for December and January and (Table 4). Therefore, cranes primarily fed on grains during December and January and fed on invertebrate animals in December and January. Potato use was negatively associated with the depths of invertebrates. With increased depth distribution of invertebrates during icy conditions or low temperature periods, cranes consumed underground food potatoes increasing the cost of digging to obtain food resource.

Management implications

Our results support previous reports that Black-necked Cranes generally prefer farmlands, and avoid grasslands (Kong et al. 2011a), likely due to the availability of domestic crops and invertebrates to feed on, as well as other habitat features. We agree with Kong's views (2011b) that higher quantities and densities of food as well as looser soil structure in farmlands facilitate food collection by the cranes. During icy conditions (December or January), the invertebrate

392 shortage is exacerbated. We recommend that the protection administration should supplement
 393 additional foods for cranes during icy periods, and restore grassland foraging habitat. This would
 394 support the cranes' need for dietary diversity and would benefit the farmers by reducing
 395 economic losses resulting from the cranes feeding on newly planted crop seeds during their late
 396 spring migration (in March). To further ease the conflict between cranes and local farmers, it is
 397 advisable to cultivate crops in a certain area that may be left unharvested for the cranes to eat.
 398 Furthermore, it is necessary to maintain adequate traditional croplands to sustain this vulnerable
 399 species, as many of these conventional cultivations (grains, potatoes and turnips) have been
 400 replaced by more economic crops (*Lepidium meyenii* Walp) in the Dashanbao Reserve.

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 403 National Nature Reserve for their valuable support in the field.

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Supplementary material

Raw data

Raw data 1-available food biomass

Raw data 2-distributed depth of three food types

Raw data 3-Temperature

Raw data 4-video data

Photographs

Picture 1 shows a Black-necked Crane foraging a Coleoptera larva on grassland.

Picture 2 and **Picture 3** show feeding traces of Black-necked Cranes on grassland

Picture 4 shows a Black-necked Crane foraging herbaceous plants on grassland.

Picture 5 shows the frozen ground of the grassland in winter.

Videos

Video 1 shows two adult Black-necked Cranes with a juvenile walked along a corn grain, frequently pecking grains and ingesting them quickly. The video was taken on 12:46 on December 14th, 2014.

Video 2 shows a flock of Black-necked Cranes feeding on grains on 10:34 on December 20th, 2014. Ten individual Black-necked Cranes were chosen from this foraging flock before we moved on the next flock.

541

542 **Video 3** shows two f Black-necked Cranes foraging in a potato field on 15:23 on February 24th,
543 2014. At 00:00 the crane toward the right of the video starts to feed on a potato tuber, and then it
544 moves on to pecking up an invertebrate. At 00:00 the other crane towards on the left of the video
545 is searching for food by thrusting an open bill into the substrate. At 01:39 this crane starts to feed
546 on a potato tuber for the rest of the sequence, swallowing two large pieces and many small pieces.

547

548

549 **Video 4** shows a juvenile Black-necked Crane, catching an earthworm and pecking it quickly on
550 16:37 on February 25th 2014.

551

552 **Video 5** shows five Black-necked Cranes feeding on invertebrates in grassland on 12:20 on
553 November 20th, 2014. We selected an adult crane with a juvenile to film the feeding behavior.
554 The adult crane touches the soil surface with a thrusting movement of its bill. Subsequent
555 pecking at a plot of turf, results in it catching an invertebrate and quickly swallowing it. The
556 adult crane may also pass the invertebrate to its offspring.

557

558 **Video 6** shows a Black-necked Crane foraging herbaceous plants in grassland on 16:02 on
559 November 29th, 2013. A crane tugs the herbaceous plants with a quick rotation of its bill towards
560 the right and left.

561

Figures and Tables

Figure 1 Map of the Dashanbao National Nature Reserve, showing the location of our study areas. The red dot at the upper right designates the location of the Reserve in a map of China. Circles indicate sites where we recorded video material of cranes. The pentagram indicates the sites for our food availability sampling. Black lines designate the transect lines 1, yellow line designate the transect lines 2, red line designate the transect lines 3. The blue rectangle designates Dahaizi reservoir, the yellow rectangle designates Tiaodunhe reservoir, and the white rectangle designates the Dashanbao Reserve.

Figure 2 Monthly Shannon index of diversity (H') for the diet of the Black-necked Crane *G. nigricollis* wintering in the Dashanbao National Nature Reserve, China.

Figure 3 Results projection of the plane of the Canonical Correspondence Analysis (CCA) that we performed. The food uses (invertebrate use, potato use and grain use) are the constraining variables and the food variables are the dependent variables. Dependent variables are represented by arrows and their abbreviation: Ia = Invertebrate availability; Pa = Potato availability; Ga = Gain availability; Id = Invertebrate depth; Gd = Grain depth; Pd= Potato depth. The right axis refers to Axis 1 and the left axe refers to Axis 2.

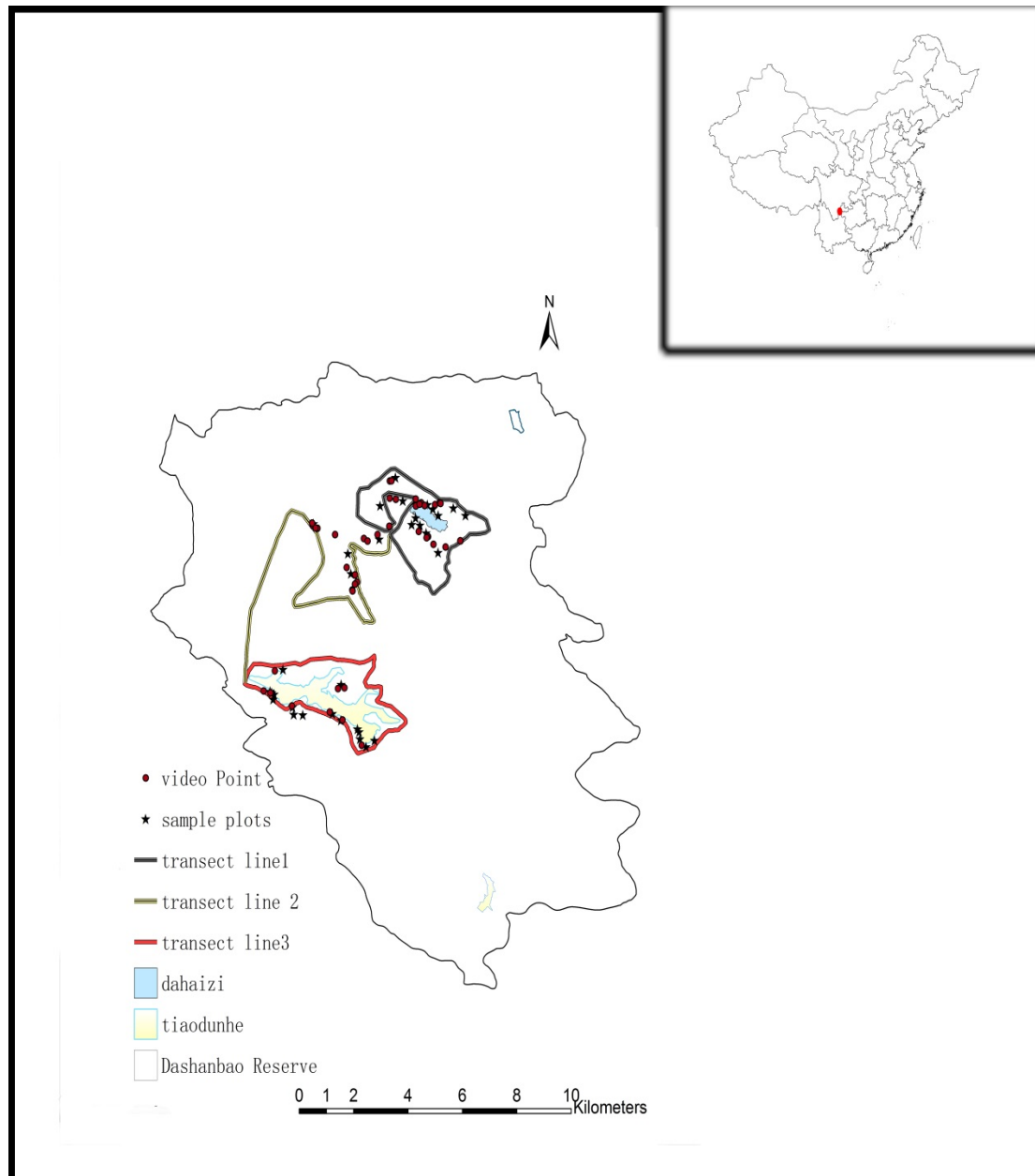
Table 1 Monthly, yearly and whole percentage of food items in dietary composition of the Black-necked Crane *G. nigricollis* wintering in the Dashanbao National Nature Reserve, China.

Table 2 Monthly availability of biomass of all food in the Dashanbao National Nature Reserve, China.

Table 3. Depicted is food selection of Black-necked Cranes *G. nigricollis* for the six most available food types in the Dashanbao National Nature Reserve China in relation to month and year. Shown is the number of videos, the frequency of pecking, food availability (π_i), food use (O_i), the Savage selectivity index (W_i) for each food group, standard error of the index (s.e), and the statistical significance (P) for our results. Significance is reached at $P < 0.006$, after applying the Bonferroni correction. NS: $P > 0.05$; +: positive selection, -: negative selection.

Table 4 Pearson correlations between the environmental variables and invertebrate food variables for Black-necked cranes *G. nigricollis* in the Dashanbao National Nature Reserve China.* Correlation is significant at the 0.05 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed).

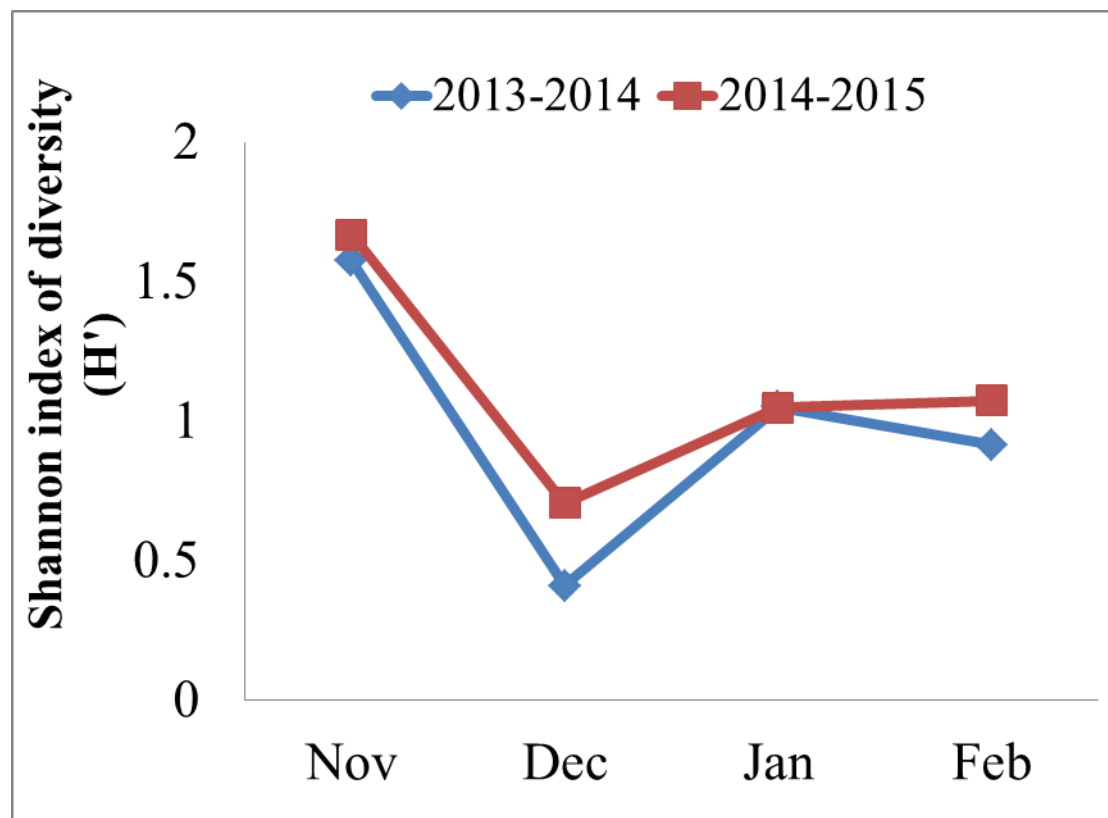
599 **Figure 1** Map of the Dashanbao National Nature Reserve, showing the location of our study
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 605 rectangle designates the Dashanbao Reserve.



606

607 **Figure 2** Monthly Shannon index of diversity (H') for the diet of the Black-necked Crane *G.*

608 *nigricollis* wintering in the Dashanbao National Nature Reserve, China.



610 **Figure 3** Results projection of the plane of the Canonical Correspondence Analysis (CCA) that
 611 we performed. The food uses (invertebrate use, potato use and grain use) are the constraining
 612 variables and the food variables are the dependent variables. Dependent variables are represented
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 614 Gain availability; Id = Invertebrate depth; Gd = Grain depth; Pd= Potato depth. The right axis
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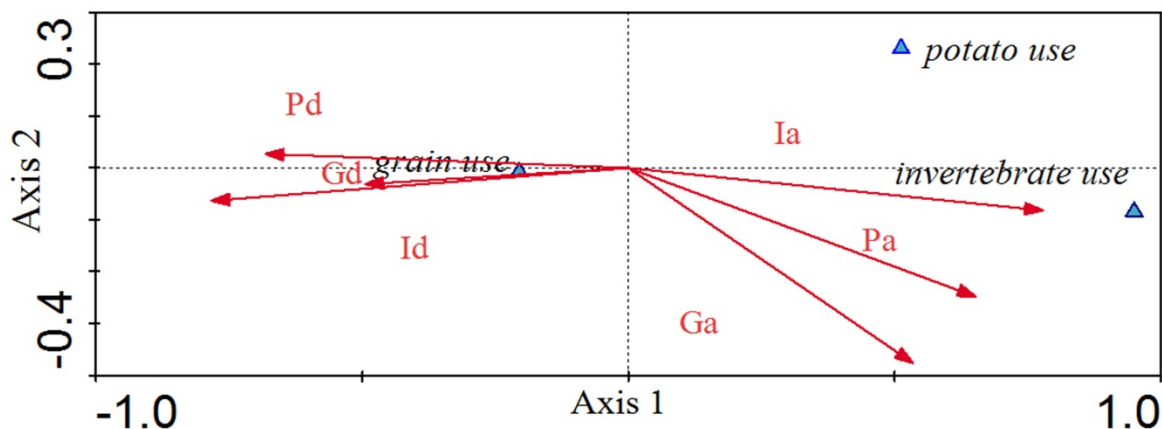


Table 1 Monthly, yearly and whole percentage of food items in dietary composition of the Black-necked Crane *G. nigricollis* wintering in the Dashanbao National Nature Reserve, China.

		sample	pecks frequency	Grain (%)	Potato (%)	Turnip (%)	Invertebrate (%)	Herbaceous plant (%)	Tuber (%)
2013-2014	Nov	46	1180	39.73	20.29	0.01	39.05	0.93	0.00
	Dec	47	1608	95.24	1.88	0.00	1.63	1.00	0.25
	Jan	70	1808	81.42	5.81	0.11	1.83	5.97	4.87
	Feb	50	1212	82.51	4.95	0.00	10.56	1.49	0.50
	Total year	213	5808	74.72	8.23	0.03	13.27	2.35	1.40
2014-2015	Nov	105	1342	49.55	11.18	0.07	33.83	1.86	3.50
	Dec	53	1861	88.55	4.30	0.00	1.61	4.89	0.64
	Jan	66	1495	80.27	9.03	0.07	2.34	7.16	1.14
	Feb	68	1502	73.24	5.33	0.00	20.84	0.47	0.13
	Total year	292	6200	72.90	7.46	0.04	14.66	3.59	1.35
Two year combined		505	12008	73.81	7.84	0.03	13.96	2.97	1.38

620 **Table 2** Monthly availability of biomass of all food in the Dashanbao National Nature Reserve, China.

		2013- 2014					2014- 2015					Two year combined
		Nov	Dec	Jan	Feb	Total year	Nov	Dec	Jan	Feb	Total year	
Grains	mean food biomass(g/0.25m ²)	0.79	0.73	0.35	0.18	0.52	2.45	0.99	0.49	0.43	1.07	0.81
	sample N	21	23	20	20	84	23	19	20	30	92	176
	Percentage of food availability (%)	0.45	0.54	0.38	0.26	0.41	5.08	1.17	0.56	0.93	1.94	1.17
Potatoes	mean food biomass(g/0.25m ²)	2.16	1.22	0.62	0.77	1.11	1.38	1.18	0.82	0.82	1.02	1.05
	sample N	12	14	18	15	59	26	19	34	30	109	168
	Percentage of food availability (%)	1.07	1.16	1.08	1.56	1.22	2.51	1.81	1.50	2.47	2.07	1.64
Turnip	mean food biomass(g/0.25m ²)	12.50	16.01	7.15	5.99	11.94	4.21	5.74	5.78	3.38	4.44	7.63
	sample N	8	8	4	3	23	8	8	3	12	31	54
	Percentage of food availability (%)	2.37	5.31	3.32	2.02	3.25	2.91	3.05	2.81	1.70	2.62	2.94
Invertebrate	mean food biomass(g/0.25m ²)	1.03	0.39	0.20	0.81	0.59	0.62	0.29	0.49	0.60	0.49	0.51
	sample N	10	12	12	13	47	35	41	41	31	148	195
	Percentage of food availability (%)	3.77	2.54	1.74	5.65	3.43	8.23	3.05	4.56	6.30	5.54	4.48

Herbaceous plant	mean food biomass(g/0.25m ²)	7.60	3.79	1.36	3.18	4.12	2.38	2.38	2.55	2.47	2.45	2.99
	sample N	25	22	20	26	93	49	40	51	62	202	295
	Percentage of food availability (%)	43.53	45.05	50.73	41.4	45.18	47.11	45.41	50.42	48.45	47.85	46.51
Tubers	mean food biomass(g/0.25m ²)	7.88	6.95	8.17	7.01	7.46	1.81	4.34	4.33	3.85	3.47	4.73
	sample N	11	19	19	18	67	41	29	28	47	145	212
	Percentage of food availability (%)	48.82	45.4	42.71	49.1	46.51	34.17	45.50	40.14	40.15	39.99	43.25

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Table 3 Depicted is food selection of Black-necked Cranes *G. nigricollis* for the six most available food types in the Dashanbao National Nature Reserve China in relation to month and year. Shown is the number of videos, the frequency of pecking, food availability (π_i), food use (O_i), the Savage selectivity index (W_i) for each food group, standard error of the index (s.e), and the statistical significance (P) for our results. Significance is reached at $P < 0.006$, after applying the Bonferroni correction. **NS**: $P > 0.05$; +: positive selection, -: negative selection.

			Sample	Pecks frequency	O_i	π_i	W_i	s.e	P	selection
2013-2014	Nov	grain	13	470	0.4	0.04	10.34	0.15	*	+
		potato	3	240	0.2	0.06	3.37	0.11	*	+
		turnip	0	0	0	0.23	0	0.05	*	-
		invertebrate	26	462	0.39	0.02	16.32	0.19	*	+
		herbaceous plants	4	8	0.01	0.44	0.02	0.03	*	-
		tuber	0	0	0	0.2	0	0.06	*	-
	Dec	grain	26	1520	0.95	0.04	21.49	0.12	*	+
		potato	4	30	0.02	0.04	0.42	0.12	*	-
		turnip	0	0	0	0.34	0	0.04	*	-
		invertebrate	7	26	0.02	0.01	1.32	0.22	NS	+
		herbaceous plants	7	21	0.01	0.22	0.06	0.05	*	-
		tuber	3	11	0.01	0.35	0.02	0.03	*	-
	Jan	grain	22	1472	0.81	0.03	26.71	0.13	*	+

2014-2015	Feb	potato	13	105	0.06	0.05	1.21	0.1	NS	+
		turnip	2	2	0	0.12	0.01	0.06	*	-
		invertebrate	12	33	0.02	0.01	1.77	0.23	*	+
		herbaceous plants	6	108	0.06	0.11	0.53	0.07	*	-
		tuber	16	88	0.05	0.67	0.07	0.02	*	-
		grain	22	1000	0.83	0.01	57.09	0.24	*	+
	Nov	potato	5	60	0.05	0.05	1.08	0.13	NS	+
		turnip	0	0	0	0.07	0	0.1	*	-
		invertebrate	14	128	0.11	0.04	2.54	0.14	*	+
		herbaceous plants	5	18	0.01	0.33	0.05	0.04	*	-
		tuber	4	6	0	0.5	0.01	0.03	*	-
		grain	18	665	0.5	0.17	2.97	0.06	*	+
	Dec	potato	18	150	0.11	0.11	1.05	0.08	NS	+
		turnip	6	1	0	0.1	0.01	0.08	*	-
		invertebrate	41	454	0.34	0.06	5.32	0.1	*	+
		herbaceous plants	14	25	0.02	0.34	0.05	0.04	*	-
		tuber	8	47	0.04	0.22	0.16	0.05	*	-
		grain	20	1648	0.89	0.06	15.07	0.09	*	+
		potato	10	80	0.04	0.07	0.62	0.08	*	-
		turnip	0	0	0	0.14	0	0.06	*	-
		invertebrate	8	30	0.02	0.04	0.43	0.12	*	-
		herbaceous plants	11	91	0.05	0.3	0.16	0.04	*	-
		tuber	4	12	0.01	0.39	0.02	0.03	*	-

Jan	grain	22	1200	0.8	0.03	26.79	0.15	*	+
	potato	19	135	0.09	0.09	1.06	0.08	NS	+
	turnip	3	1	0	0.05	0.01	0.11	*	-
	invertebrate	10	35	0.02	0.06	0.38	0.1	*	-
	herbaceous								
	plants	10	107	0.07	0.4	0.18	0.03	*	-
	tuber	2	17	0.01	0.37	0.03	0.03	*	-
Feb	grain	26	1100	0.73	0.03	24.41	0.15	*	+
	potato	7	80	0.05	0.06	0.93	0.1	NS	-
	turnip	0	0	0	0.09	0	0.08	*	-
	invertebrate	28	313	0.21	0.04	4.79	0.12	*	+
	herbaceous								
	plants	5	7	0	0.36	0.01	0.03	*	-
	tuber	2	2	0	0.42	0	0.03	*	-

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629

630 **Table 4** Pearson correlations between the environmental variables and invertebrate food variables for Black-necked cranes *G.*
 631 *nigricollis* in the Dashanbao National Nature Reserve China.* Correlation is significant at the 0.05 level (2-tailed). ** Correlation is
 632 significant at the 0.01 level (2-tailed).

		invertebrate depth	invertebrate numbers in 0-1 cm deep	invertebrate numbers in 1.1-2 cm deep
Mean temperature	Correlation coefficients	-0.721*	0.740*	0.690
	P-value	0.043	0.036	0.058
Minmum temperature	Correlation coefficients	-0.730*	0.843**	0.775*
	P-value	0.040	0.009	0.024
the number of days with frozen ground	Correlation coefficients	0.779*	-0.842**	-0.797*
	P-value	0.023	0.009	0.018

633