

1 Use of abandoned structures by Przewalski's wild horses and other wildlife in the Chernobyl
2 Exclusion Zone

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13 Abstract

14 Przewalski's wild horses (*Equus ferus przewalski*) are an endangered species and current
15 conservation efforts aim to maintain genetic diversity and reintroduce wild populations. From
16 1998-2004, 36 Przewalski's horses were introduced in the Chernobyl Exclusion Zone (CEZ)
17 with no subsequent direct management, and current population size and genetic diversity are
18 unknown. We used remote cameras positioned at the entrance of abandoned structures within the
19 CEZ to elucidate visitation of these structures by Przewalski's horses and other large mammals
20 to assess the use of this method for monitoring the horse population within the CEZ. We
21 estimated frequency of structure use in winter (Nov 2016 – Feb 2017) and summer (Mar 2018 –
22 Oct 2018) periods and estimated basic group characteristics such as group type (all male vs.
23 mixed sex), group size, and number of foals. Przewalski's horses were detected 35 times at 9 of
24 10 monitored structures in winter and 149 times at all 8 monitored structures in summer. Eight
25 other mammalian species were documented using abandoned structures. Mixed sex groups of
26 horses contained 5.30 ± 0.25 adults and 1.00 ± 0.12 foals (Mean \pm S.E.). All-male groups
27 contained 1.59 ± 0.15 individuals. Our data suggest Przewalski's horses routinely use abandoned
28 structures in the CEZ with visitation patterns tending to be nocturnal in winter and crepuscular in
29 summer. Congregation of horses in these structures could have implications for future
30 monitoring of the population by providing detailed information on demographics, population
31 size, and genetic diversity which would benefit management plans for this population and
32 provide a baseline for future work.

33 Key words: camera traps, Chernobyl Exclusion Zone, population monitoring, Przewalski's wild
34 horse

35 **Introduction**

36 The Chernobyl nuclear accident in 1986 created an unprecedented radiological
37 containment issue, resulting in the creation of a 4,300 km² restricted access area spanning the
38 modern borders of Ukraine and Belarus. This area, referred to as the Chernobyl Exclusion Zone
39 (CEZ), has remained relatively untouched, allowing for natural reclamation of a primarily
40 agricultural and forest matrix. Over 190 abandoned human villages and towns are interspersed
41 throughout the landscape, consisting of thousands of abandoned structures. These structures
42 range from abandoned apartment complexes to barns and other former livestock holding
43 facilities, and represent unique habitat for the diverse wildlife that now inhabit the CEZ (Baker
44 and Chesser 2000; Deryabina et al. 2015; Webster et al. 2016; Schlichting et al. 2019). The large
45 size and restricted human access also has made the CEZ a target for the introduction of species
46 of conservation concern including European bison (*Bison bonasus*) and Przewalski's wild horses
47 (*Equus ferus przewalski*).

48 The endangered Przewalski's horse is the last remaining subspecies of wild horse (King
49 et al. 2015), whose populations declined rapidly due to hunting, habitat loss, harsh winters, and
50 competition with domestic livestock, leading to their extinction in the wild in the mid-20th
51 century (Ryder 1993; Bouman and Bouman 1994). All extant Przewalski's horses are descended
52 from a small number of captive individuals (n = 13), and captive populations are subject to a
53 comprehensive breeding program where maintenance of genetic diversity is a priority. A second
54 management priority is the reintroduction of sustainable wild populations, which has occurred at
55 five locations in Mongolia and China. In addition, from 1998-2004, 36 Przewalski's horses were
56 released into the CEZ near the town of Chernobyl as an experimental population and this
57 population gradually spread north throughout the zone (Zharkikh and Yasynetska 2008) and
58 occupied the Belarussian portion of the CEZ, the Polesie State Radiation Ecological Reserve

59 (PSRER), by 2007 (Deryabina 2015). The CEZ population was monitored 10 years post-release
60 and increased to a maximum of 65 individuals, but because of their small population size,
61 poaching, and limited gene flow, the population remains at risk (Zharkikh and Yasynetska 2008).
62 Evidence suggests that Przewalski's horses use abandoned structures in the CEZ, based on the
63 presence of feces (Klich et al. 2017), and these structures are assumed to be used as thermal
64 refugia. However, the frequency and extent to which horses (and other wildlife) use these
65 structures is currently unknown.

66 To address this knowledge gap, we conducted a pilot study to assess the use of human
67 structures by Przewalski's horses and other wildlife in the CEZ. By placing motion activated
68 cameras at the entrances of abandoned structures, we estimated frequency and diel patterns of
69 use by Przewalski's horses in winter and summer seasons. In addition, we assessed population
70 characteristics of groups visiting structures including group size, group type (mixed sex or all-
71 male groups), and number of foals in mixed sex groups during summer. Finally, we examined
72 the role of abandoned structures as refugia during summer. This information can be used to
73 improve monitoring and management strategies for the Przewalski's horse population in the
74 CEZ.

75 **Methods**

76 We deployed motion activated game cameras (A-25i, Moultrie, USA; Attack IR,
77 Cuddeback, USA; 2.6C Willfine, Suntek, China) to assess winter (n=10, November 20, 2016 to
78 February 14, 2017) and summer (n=8, March 23, 2018 to October 23, 2018) use of abandoned
79 structures by Przewalski's horses and other wildlife in the PSRER (Figure 1). Cameras were
80 placed within structures originally used for livestock husbandry (cattle, sheep, and horses) where
81 the presence of Przewalski's horses was anticipated based on observations of horse fecal

82 material. Cameras were set ~1.2 m off the ground at entrances to maximize detection of horses
83 and other large mammals and programmed to record a burst of three photos when triggered.
84 Photos were delineated into independent visits per species separated by a minimum of 6 hours.
85 Visits were used to determine presence, visits per structure, and visits per trap night in winter and
86 summer trials. In addition, we evaluated visits by horses during the winter and summer periods
87 by creating activity curves using a non-parametric kernel density approach (Ridout and Linkie
88 2009). Activity curves were created with the package “overlap” (Meredith and Ridout 2018) in
89 program R (R Development Core Team 2018).

90 Extreme cold during winter trials made cameras less reliable, meaning group composition
91 and the role of structures as refugia was estimated for summer trials only. Group size was
92 determined by recording the maximum number of individuals present in a single photo within a
93 visit. The lack of identifiable marks on Przewalski’s horses and difficulty in differentiating sub-
94 adults from adults meant that group composition could only be reliably recorded into two types:
95 mixed sex (at least one male and female identified) and all-male groups (all individuals identified
96 as male). Groups where a female could not be verified but included a foal were considered a
97 mixed sex group. Visits where group composition could not be determined were labeled as
98 unknown. The role of structures as thermal refugia was tested by comparing maximum
99 temperatures (T-test) and days with rainfall (Chi-squared test) on days with visits to days
100 without. Meteorological data were obtained within the PRSER at the Masany Research Station
101 (Figure 1).

102 **Results**

103 Przewalski’s horses were detected 35 times at 9 of 10 monitored structures (Fig. 2a and
104 b.) in winter trials and visited all 8 monitored structures 149 times in summer (Fig. 2 c. and d.).

105 Structures were visited an average of 3.89 ± 0.77 times (range 1-8) during 655 trap nights in
106 winter (0.05 visits/trap night). Summer trials totaled 1,339 trap nights (0.11 visits/trap night)
107 with an average of 18.63 ± 4.06 horse detections per structure (range 5-34). Przewalski's horses
108 used structures for extended periods of time (>5 hours), including as loafing, breeding, and
109 sleeping locations (Figure 2b), resulting in 4,668 and 7,685 photos in winter and summer,
110 respectively. Horses primarily used structures nocturnally in winter while summer visits occurred
111 throughout the day with greater use during crepuscular periods (Figure 3). The number of days
112 between visits averaged 6.27 ± 0.65 during summer. We detected eight other mid- to large-sized
113 mammals using abandoned structures in summer including brown hare (*Lepus europaeus*, $n=34$
114 detections), red deer (*Cervus elaphus*, $n=13$ detections), moose (Fig. 2e., *Alces alces*, $n=63$
115 detections), wild boar (*Sus scrofa*, $n=2$), red fox (*Vulpes vulpes*, $n=5$ detections), raccoon dogs
116 (*Nyctereutes procyonoides*, $n=9$ detections), Eurasian lynx (Fig. 2f., *Lynx lynx*, $n=9$
117 detections), and wolves (*Canis lupus*, $n=8$ detections), along with several avian and bat species.

118 Przewalski's wild horse groups averaged 3.31 ± 0.22 individuals per visit across all group
119 types during summer. Mixed sex group were detected visiting structures on 53 occasions and
120 contained $5.30 (\pm 0.25)$ adults and $1.00 (\pm 0.12)$ foals on average. Mixed groups contained 2-11
121 adults and 0-3 foals and foals were first detected May 4th. All-male groups visited monitored
122 structures on 44 occasions and were smaller than mixed sex groups, containing 1.59 ± 0.15
123 individuals on average and ranging from 1-4 adults. Groups of unknown composition averaged
124 1.73 ± 0.11 individuals. Groups of similar type and size were commonly detected on the same
125 camera for several consecutive days followed by extended periods without visits (>40 days).
126 Temperature ($p=0.81$) and days with rainfall ($p=0.91$) did not differ between days with and
127 without visits.

128 **Discussion**

129 Przewalski's horses and other wildlife appear to regularly use abandoned structures in the
130 CEZ. Visits occurred throughout both night and day, and horses were documented loafing,
131 breeding, and sleeping in structures. Successive visits tended to be temporally clustered,
132 indicating groups repeatedly use structures when in that portion of their home range, but
133 structures may go unvisited for extended periods of time. No other species documented using
134 abandoned structures appeared to stay in them for extended periods, but we likely missed
135 detections of small bodied species because cameras were set specifically to detect horses and
136 other large ungulates (>1m high). Horses were documented visiting structures more during
137 summer trials but this is likely an artifact of poor camera performance in extreme cold
138 temperatures. Alternatively, summer months with the greatest number of visits corresponded to
139 increases in blood sucking flies (families Ceratopogonidae, Simuliidae, and Tabanidae) and
140 horses could be visiting abandoned structures as a respite from these pests. Horses used
141 unmonitored barns (based on the presence of feces) as well as the barns we monitored, and the
142 lack of climatic influences based on detections could be misleading based on our small sample
143 size. Horses primarily used barns at night in winter suggesting they may serve as thermal refugia,
144 but this hypothesis should be more thoroughly tested, possibly with paired climate loggers in a
145 greater number of structures.

146 The extensive and repeated congregation of Przewalski's horses in abandoned structures
147 could have implications for future monitoring of the population in the CEZ. Przewalski's wild
148 horses in the Ukrainian portion of the CEZ also use abandoned structures (Klich et al. 2017),
149 suggesting these congregation areas represent unique focal points for which monitoring of horses
150 with remote cameras may be feasible across the CEZ. Our results provided estimates of group

151 size and usage, but monitoring a greater number of structures, using multiple cameras per
152 structure, and incorporating video surveillance could allow for more accurate estimates of
153 population structure including age/sex ratio, birth rates, herd number, herd composition, and
154 abundance. These represent critical population parameters that, in conjunction with climatic
155 variables, influence the long-term viability of reintroduced Przewalski's horse populations
156 (Slotta-Bachmayr et al 2004). Identification of key parameters for population growth and their
157 threshold values could help optimize management objectives for this population.

158 Congregation of Przewalski's horses in abandoned structures could also provide valuable
159 information about population characteristics and viability using non-invasive genetic sampling
160 (King et al. 2018). Genetic capture-recapture methods (Lukacs and Burnham 2005) could
161 provide abundance estimates as well as estimates of survival and population growth if the
162 population were repeatedly sampled. Other reintroduced Przewalski's horse populations have
163 displayed increased genetic drift and inbreeding compared to their founding population (Liu et
164 al. 2014). Although genetic diversity is currently unknown, the CEZ population has likely
165 experienced similar losses in diversity since introduction. Comparisons of genetic diversity and
166 inbreeding coefficients to reintroduced populations in China and Mongolia as well as the
167 founding population in Askania Nova Biosphere Reserve would benefit management plans for
168 this species and provide baseline population information for future work in the CEZ.

169 The future of Przewalski's horses in the wild depends on introductions that maintain
170 sustainable and genetically diverse populations (Sarrazin and Barbault 1996; Van Dierendonck
171 and Wallies de Vries 1996). Successful reintroduction of Przewalski's wild horses have
172 generally included intensive monitoring and periodic supplementation with individuals from
173 captivity (Boyd and Bandi 2002; Souris et al. 2007; Xia et al. 2014). The population in the CEZ

174 offers a unique opportunity to evaluate a reintroduction with limited direct management and
175 future investigations could identify potential management needs such as mitigating the genetic
176 consequences of founder effect and limited gene flow. In addition, the use of a range of
177 structures by a wide array of species offers an opportunity to understand the importance and
178 impact of former human settlements on wildlife populations in the CEZ and other areas
179 abandoned by people, as well as the potential importance of these structures to horses in the
180 CEZ.

181 **Acknowledgements**

182 We thank the Polesie State Radioecological Reserve staff and C. Love for field assistance
183 and discussion, as well as the Ministry of Education and Research for their support. This material
184 is based upon work supported by the Department of Energy Office of Environmental
185 Management under Award Number DE-EM0004391 to the University of Georgia Research
186 Foundation.

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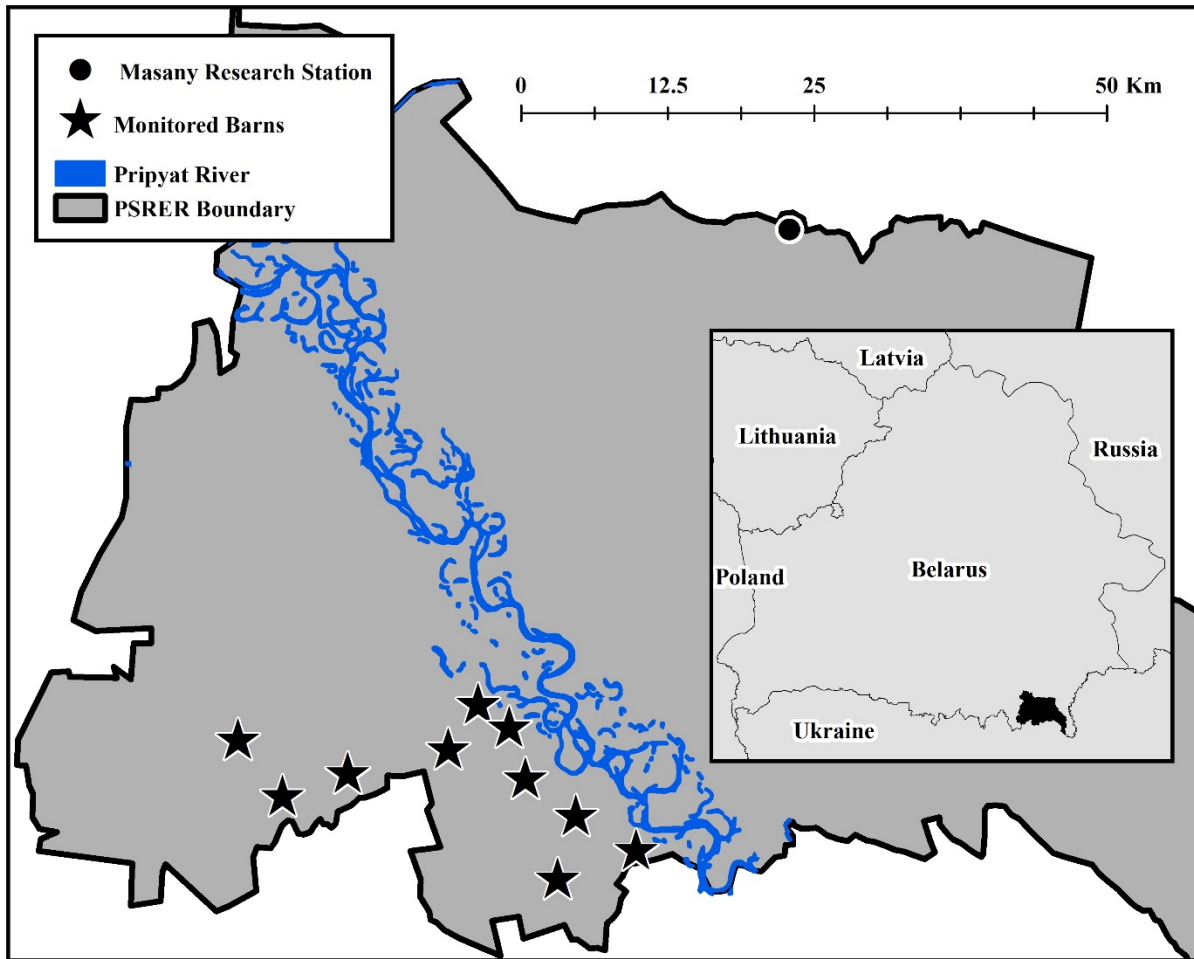
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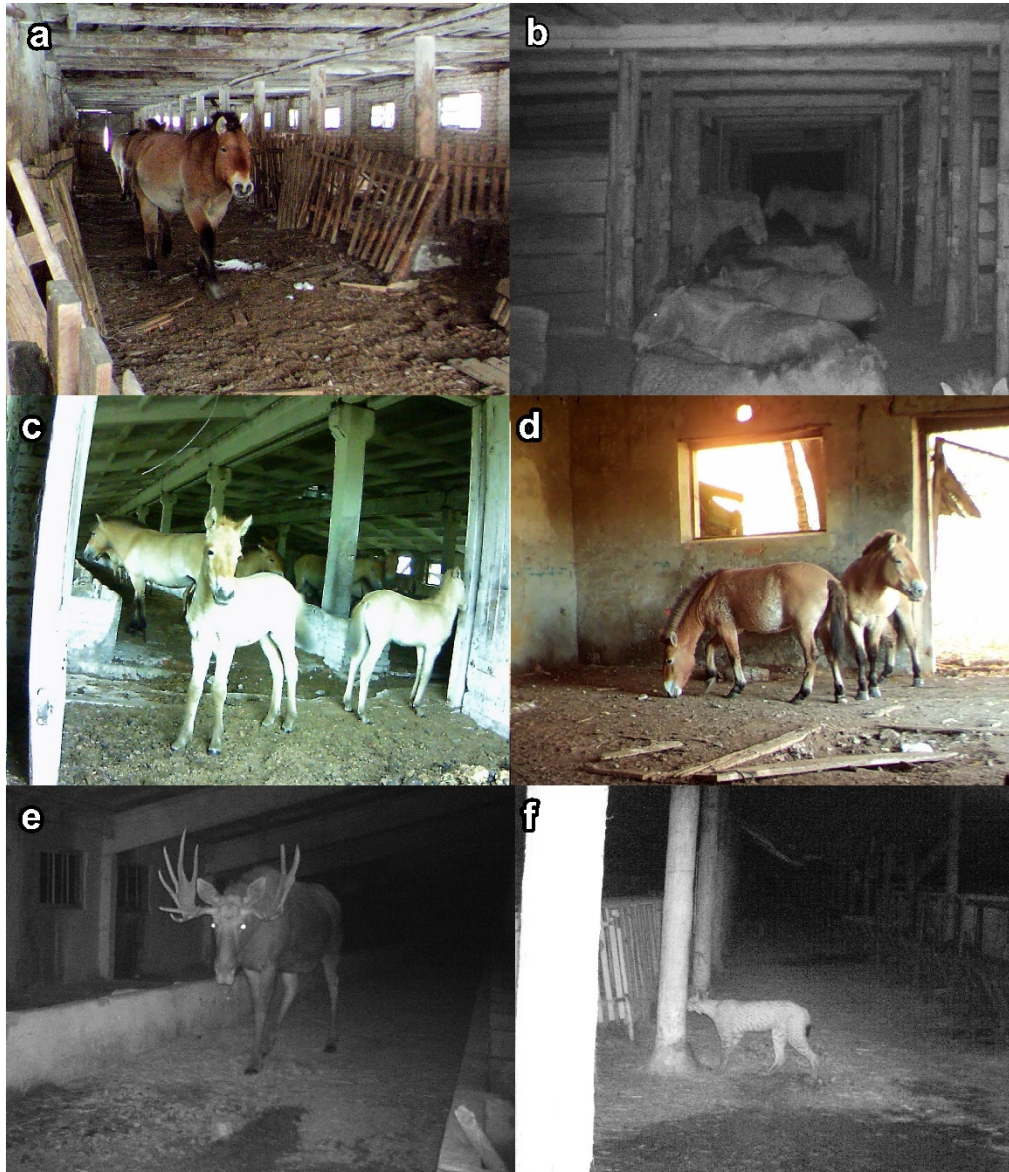
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247 **Fig. 1** Map of the Belarussian portion of the Chernobyl Exclusion Zone, the Polesie State
 248 Radiation Ecological Reserve (PSRER), indicating the location of monitored barns and the
 249 Masany Research Station. The inset displays the location of the PSRER (in black) within
 250 Belarus.

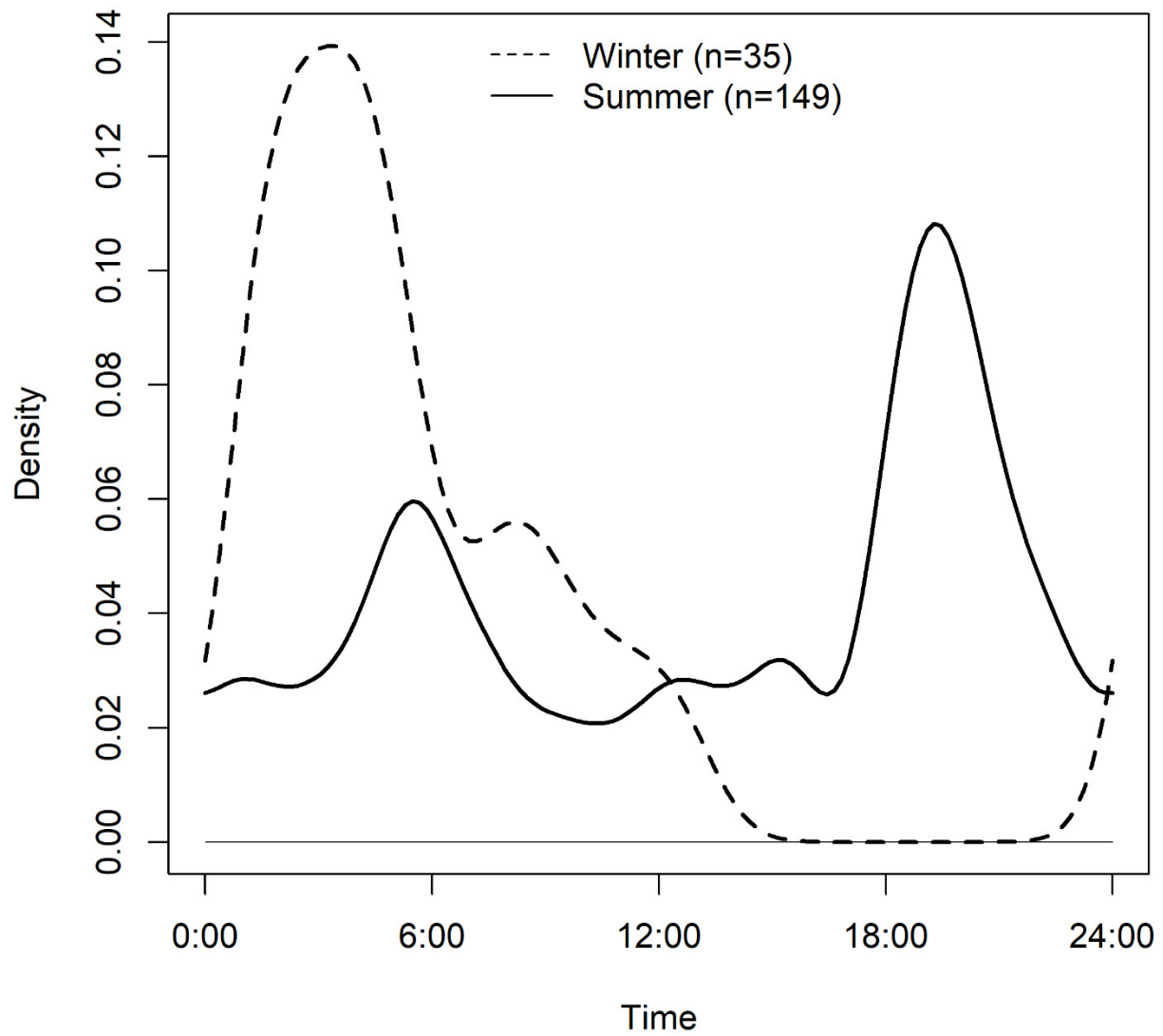
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253 **Fig. 2** Przewalski's horses (*Equus ferus przewalski*) and other wildlife occupying abandoned
254 structures in the Chernobyl Exclusion Zone. Mixed sex groups (a. – c.) and all-male groups (d.)
255 were documented occupying monitored barns previously used for livestock husbandry in winter
256 (a. and b.) and summer (c. and d.). Eight other species, including moose (*Alces alces*, e.) and
257 European lynx (*Lynx lynx*, f.) were detected utilizing abandoned structures

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260 **Fig 3.** Diel distribution of Przewalski's wild horse visits to abandoned structures in the
 261 Chernobyl Exclusion Zone in winter and summer periods. Sample sizes (n) denote the number of
 262 visits per sampling period.

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