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Milano, January 7<sup>th</sup>, 2014

Dear Editor,

thank you very much for your answer.

I made all the changes required by the reviewer. I hope that the pictures of the enclosure are in line with the reviewer's request.

I also included all the additional information that you required in your e-mail.

I hope that this new version will be acceptable for publication.

Best regards and Happy New Year!

Silvana Mattiello

Answers to reviewer's comments:

1. Lines 84-90

I suggest moving even incomplete results of earlier studies on these two bears to section "Results" as an "unpublished data". I do not share the opinion that much more additional information would be needed.

*Au: I moved this part to the beginning of the Results section.*

2. Line 79

(Fig. 1) While suggesting a picture I meant photo rather or schematic drawing. I suggest again as explained, that authors provide a photo or schematic drawing (preferably black and white or grey scale) of the enclosure.

*Au: I replaced the map with a photo-collage with views on different parts of the enclosure (and modified the Figure caption accordingly). Sorry if I misunderstood the previous suggestion!*

1 **Effect of the change of social environment on the behavior of a captive brown bear (*Ursus***  
2 ***arctos*)**

3

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13

14

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18

19

20

21 **Abstract**

22 We observed the behavior of a captive sterilized male brown bear before and after the death of his  
23 female sibling, in order to investigate the effect of the change of social environment on his behavior  
24 and welfare. Observations were carried out by continuous recording during daylight for 6 weeks prior  
25 to hibernation when the bear was kept with the sibling, and they were repeated two years later, when  
26 the bear was alone (total observation time=108 h).

27 Feeding, moving and the total amount of time spent resting (including sleeping and alert inactive)  
28 were not affected by the change of social environment. However, when the bear was alone the  
29 percentage of time he spent alert inactive almost trebled (pair:  $17.7\pm 3.3$ , alone:  $48.5\pm 5.5$ ;  $P<0.001$ )  
30 and the time spent sleeping was less than one third (pair:  $51.1\pm 6.1$ , alone:  $14.2\pm 5.0$ ;  $P<0.001$ ) than  
31 when the female was present. The bear spent most of his sleeping time in lateral lying posture (a  
32 posture probably associated with REM sleep). The percentage of time dedicated to this posture was  
33 significantly reduced after the death of his sibling (pair:  $35.9\%\pm 7.4$ , alone:  $15.0\%\pm 5.4$ ;  $P<0.05$ ),  
34 whereas the percentage of time spent in quadrupedal posture increased (pair:  $17.1\%\pm 5.4$ , alone:  
35  $37.8\%\pm 7.8$ ;  $P<0.05$ ). One of the possible reasons for these changes may be an increased risk  
36 perception of the bear after the death of his sibling. Our results highlight the importance of social  
37 environment and of its changes, which should be carefully considered in order to maintain captive  
38 bears in good welfare conditions.

39

40 **Keywords:** brown bear, behavior, sleep, animal welfare, zoo.

41

42

43 **Introduction**

44 According to the International Species Information System (ISIS), at least 561 brown bears of  
45 various subspecies are kept in zoos around the world (as of March 22, 2013), either in group  
46 (n=79) or as individual animals (n=482). This raises some welfare issues, as captivity implies a  
47 number of environmental and management restrictions that may affect bears' welfare (Poole,  
48 1994; O'Grady, 1994).

49 One of the key issues of bear management in captivity is the social environment. Captivity can  
50 induce modifications of social behavior, for example in response to forced aggregations of  
51 individuals. Anecdotal information based on sporadic observation in zoos (Poulsen, 2011)  
52 report that these forced aggregations can sometimes result in aggressive interactions or, in some  
53 cases, they may lead to the establishment of friendly relationships that may last for a lifetime, in  
54 spite of the solitary attitude of the species. Bears are usually regarded as solitary animals, with a  
55 promiscuous mating system (Schwartz et al., 2003; Bellemain et al., 2006). This means that they  
56 can show some sort of social behavior, which varies from strict territoriality to more or less  
57 developed forms of social aggregation (Sandell, 1989). This plasticity makes the animals able to  
58 adapt to different situations. Bear social attitudes can present some variations in response to  
59 several factors, such as individual genetic factors (Stirling and Derocher, 1990) and the season  
60 or the environmental conditions in which animals are living. A more or less developed social  
61 behavior may depend, for example, from resource distribution: where resources are scarce,  
62 bears are likely to be more solitary, whereas in case of high density of high quality food (e.g. in  
63 captive environment, where food is always guaranteed) they may show a trend to aggregate with  
64 other individuals (Stirling and Derocher, 1990). Other forms of aggregation may be observed  
65 for females, which can remain with their cubs, and for littermates, which may continue to  
66 associate with one another, playing and feeding together (Novak, 1999). In brown bear this  
67 association, based on relatedness, is particularly relevant for females, whereas males tend to  
68 disperse far from their relatives (Støen et al., 2005).

69 Throughout the year, we can observe different behavioral phases, mainly related to the  
70 reproductive cycle, that may modify the social attitudes of bears, leading to a gregarious  
71 tendency (Mustoni, 2004).

72 An open issue for discussion on bears' welfare in captivity is whether males and females should  
73 be kept together all year round (Poole, 1994). This is particularly relevant when bears are  
74 sexually active and approach the reproductive season. In order to limit stress and to prevent  
75 undesired births, some zoos choose to sterilize the animals or vasectomize the males (O'Grady,  
76 1994).

77 This study was carried out in order to investigate the effect of the change of social environment  
78 on the behavior of a captive sterilized male brown bear, which spent all his life together with his  
79 sister, and suddenly remained alone after her death.

80

## 81 **Material and methods**

82 The subject of the present study is Orfeo, a male brown bear born in captivity in 1990 and kept  
83 since 2007 in an enclosure at the Osservatorio Eco-Faunistico Alpino of the Orobic Valtellinesi  
84 Park, a tourist area of natural interest, open to visitors, located in Aprica (Province of Sondrio,  
85 Northern Italy), at 1650 m a.s.l..

86 The double fenced enclosure has a surface area of approximately 10,000 m<sup>2</sup> and includes  
87 meadows, a mixed conifer wooded area, some rocks, a stream, a pond, two artificial dens and  
88 two natural dens dug by the bears (Fig. 1). Feed was delivered every morning and consisted of  
89 variable proportions of vegetables (e.g. carrots, apples, or fennels), meat or fish, and honey.

90 Until the beginning of 2011, Orfeo had been living together with his twin sister. Both animals  
91 had been sterilized, in order to prevent undesired births. The behavior of both bears was  
92 observed in 2009 during the pre-hibernation period (end of September - early November;  
93 Daldoss, 1981), in order to assess their welfare status in the captive environment.

94 Orfeo's sibling never woke up after the hibernation period of winter 2010-2011. Following her  
95 death, in autumn 2011 further observations were carried out on Orfeo alone, implementing the  
96 same observation protocol used in 2009, in order to detect possible changes of the bear's  
97 behavior in response to the modification of his social environment. Both in 2009 and in 2011,  
98 the protocol consisted of direct continuous recording observations (Martin and Bateson, 1993),  
99 conducted during daylight for 6 weeks prior to hibernation. During each week, three observation  
100 sessions (3 h each) were carried out: one in the morning (from 9:00 to 12:00), one in the  
101 afternoon (from 12:00 to 15:00) and one in the evening (from 15:00 to 18:00). The total  
102 observation time was 108 h (3 h x 3 sessions x 6 wk x 2 y).

103 Orfeo was observed from a high platform (an observation terrace normally used also by the  
104 visitors of the Osservatorio) with the aid of an 8 x 30 magnification binoculars.

105 The following behavioral categories were recorded: feeding (eating or chewing food, either  
106 delivered by the keeper or found in the fence, e.g. leaves or branches); drinking; moving  
107 (walking or hurrying, including movement while sniffing or foraging); sleeping (a state of  
108 behavioral quiescence, the body is completely relaxed, the eyes are closed and the bear is not  
109 reactive to any external stimulus); alert inactive (the bear is not moving, but his eyes are open  
110 and he is reactive to external stimuli); den preparation (including digging and raking or mowing  
111 vegetable parts to prepare for hibernation); self-grooming (including self-licking, self-  
112 scratching, scratching against trunks or rocks, bathing and scrolling) and stereotypies (pacing,  
113 circling or walking in figure-of-eight, head-tossing, swaying; Montaudouin and Le Pape, 2005).

114 All behavioral categories were mutually exclusive. Additionally, the following postures were  
115 also recorded: quadrupedal posture (standing or moving on the 4 legs); lateral lying (the bear is  
116 lying on a side, with outstretched legs and neck, occasionally with one leg partially bent);  
117 sterno-costal lying (the weight of the bear is loaded on the ventral part of the body, the fore legs  
118 are outstretched, while the hind legs are bent below the body, the head is usually upright,



119 occasionally outstretched) and sitting (the bear is sitting on his hindquarters, leaning on the  
120 distal portion of his front legs, like a dog) (Fig. 2). All postures were mutually exclusive.  
121 Orfeo was visible for most of the time, except when he was in the den or obscured by trees. The  
122 duration of each activity/posture was expressed in minutes; the percentage of time dedicated to  
123 each activity/posture out of the total visible observation time was then calculated. These  
124 variables were submitted to non-parametric analysis of variance (Mann-Whitney test; SPSS,  
125 2007), in order to test the effect of social environment (in pair *vs* alone) on the behavior of the  
126 brown bear. Results are presented as means  $\pm$  standard errors of the percentage of time  
127 dedicated to each behavioral category and to each posture. Preliminary analysis showed a very  
128 similar trend of behavioral rhythms for both years, therefore the results regarding the effect of  
129 time band (morning, afternoon, evening) will not be reported.

130

## 131 **Results**

132 The observations carried out on the pair in 2009 showed that bears exhibited a wide range of  
133 behaviors, reflecting their ethogram in the wild (with peaks of feeding activity in the morning,  
134 and peaks of resting activity in the middle of the day), and that they were free from stereotypies  
135 or other abnormal behaviors. These findings suggested that the enclosure where they lived, and  
136 the management practices to which they were submitted, were adequate to guarantee their  
137 welfare in the captive environment (unpublished data).

### 138 *Behavioral categories*

139 During the total observation period (2009 and 2011), Orfeo spent the majority of his time alert  
140 inactive (33.1%), followed by sleeping (32.7%), feeding (16.1%) and moving (9.5%). The rest  
141 of the time was dedicated to self-grooming (4.4%), den preparation (4.1%) and drinking (0.1%).  
142 No stereotypies were ever recorded. Behavioral categories with low or null occurrence were not  
143 statistically analyzed.

144 Feeding and moving time were not affected by the change of the social environment. The total  
145 amount of time spent resting (including sleeping and alert inactive) was also very similar in both  
146 periods (68.7%±4.7 in pair vs 62.7%±5.3 alone). However, the proportion of time sleeping and  
147 alert inactive was reversed: when Orfeo was alone, the time he spent alert inactive almost  
148 trebled and the time spent sleeping was less than one third than when his sibling was present  
149 (Fig. 2). These differences were statistically significant ( $P<0.001$  for both behavioral  
150 categories).

151

### 152 *Postures*

153 Orfeo spent 39.2% of his time in lateral lying posture, 27.4% of his time in quadrupedal posture,  
154 25.5% sterno-costal lying and 7.9% sitting.

155 When he was alone, Orfeo spent significantly less time in lateral lying and more time in  
156 quadrupedal posture than when he was in pair (Fig. 3;  $P<0.05$  for both postures), while the time  
157 spent sitting and in sterno-costal lying was not affected by the change of the social environment.

158 The time allocated to different behavioral categories during each posture is reported in Tab. 1.  
159 Orfeo spent most of his sleeping time in lateral lying posture. When he was sitting or in sterno-  
160 costal lying posture, he spent most of the time inactive but vigilant (alert inactive). Feeding was  
161 performed during sterno-costal lying or quadrupedal posture. Moving and den preparation were  
162 performed almost exclusively in quadrupedal posture.

163

### 164 **Discussion**

165 Orfeo spent a high overall proportion of time resting, either sleeping or alert inactive. This is in  
166 agreement with observations on wild bears, which use most of daylight time for resting, while  
167 their activity is mainly concentrated during the evening and the night (Roth, 1983). However,  
168 the resting behavior of the observed brown bear was significantly affected by the change of  
169 social environment. The absence of his sibling, which used to be vigilant by Orfeo when he was

170 resting (Cordedda, 2010), seems to have induced a reduction of the time dedicated to totally  
171 relaxed inactivity, suggesting that Orfeo was in quieter conditions when his sibling was present.  
172 The behavior that we described as sleeping (behavioral quiescence, with the body completely  
173 relaxed, the eyes closed and no reaction to external stimuli) is very similar to the behavioral  
174 phenotype of sleep described by Lima et al. (2005). However, in absence of physiological  
175 measures, we cannot state that Orfeo was really sleeping when he was lying inactive and, above  
176 all, we cannot distinguish in which phase of sleep (REM (Rapid Eyes Movement) or non REM)  
177 he was. This behavior was performed by Orfeo mainly during the lateral lying posture, that  
178 seems to be associated to REM sleep in some mammal species, such as cattle (de Wilt, 1985).  
179 The fact that most of the time that Orfeo spent in lateral lying posture was dedicated to total  
180 inactivity seems to support this statement also for bears. During sleep, and especially during the  
181 REM phase, the arousal thresholds tend to be higher than in other states, and therefore animals  
182 exposed to predation risk are likely to spend less time sleeping and less time in REM sleep, due  
183 to increased vulnerability (Lima et al., 2005). Sleep is a plastic behavior that may dynamically  
184 adapt to different environmental situations (Lima et al., 2005). Modifications of social activities,  
185 as well as of other factors, such as for example time of light exposure or food availability, can  
186 modify the sleep-wake cycle and the SWS-REM cycle (Pitrosky et al., 2003). In many  
187 gregarious species, the presence of conspecifics provides a sense of protection to other  
188 individuals, whereas isolated animals usually show higher levels of attentiveness, which are  
189 probably related to a higher sense of fear of external stimuli (Krebs and Davis, 1993). In fact,  
190 animals in risky situations prefer to form groups when they sleep (Krause and Ruxton, 2002).  
191 Although bears are usually regarded as predators, and not as prey, a solitary situation may be  
192 dangerous for them too, as some risks may occur, such as hunting, and the need for protection is  
193 important. In the specific case of bears, the cubs may decide to remain together with their  
194 brothers and sisters or to group with other young adults (Novak, 1999), in order to feel more  
195 protected. Our results seem to confirm the presence of a similar behavior also in captive brown

196 bear and highlight the importance of providing a sense of security for the animals in captivity,  
197 as recommended by Poole (1994). Therefore, in our study we can infer that the modification of  
198 the social environment can be responsible for the reduction of the time spent sleeping and of the  
199 time spent in lateral lying posture, which is strictly associated with a state of total inactivity.  
200 Sleep plays a vital role in determining balance among recovery from prior wakefulness and  
201 preparation for ensuing wakefulness. Severe sleep disruption, with alteration of the normal  
202 restorative functions linked to correct non REM-REM pattern, may alter breathing and  
203 cardiovascular functions, and may give rise to impaired emotional reactivity and impairment in  
204 cognitive functions (Zepelin and Rechtschaffen, 1974). Furthermore, studies on laboratory  
205 rodents demonstrated that REM sleep deprivation represents a stress, which can lead to  
206 hyperphagia and increased cardiovascular disease risk (Shaw et al., 1998). Therefore, we may  
207 assume that the decrease of time dedicated to the lateral lying posture, possibly related to REM  
208 sleep, is connected to a reduced welfare condition. However, in our case Orfeo still seems to be  
209 able to cope with the surrounding environment, as his general health status is good, his feeding  
210 behavior is normal and he shows no sign of stereotypies.

211 In spite of their solitary attitude, bears can show a strong attachment to other conspecifics,  
212 especially if they have been living with them for all their life, as in the case of Orfeo and his  
213 sister. Poulsen (2011) reports the case of a female polar bear in captivity that, for the first time  
214 in her life, found herself alone and then started to look for a social contact with the zookeeper:  
215 although this is an isolated case report, it supports our hypothesis that the break of a social  
216 relationship can affect bears' behavior and that a condition of isolation is not suitable for the  
217 welfare of bears that were accustomed to the presence of other conspecifics. In our case, Orfeo  
218 was probably habituated to his sister's presence and had developed a strong bond with her.  
219 After the death of his sibling, Orfeo appears in a condition of mental discomfort. This negative  
220 condition may be a further cause of the reduction of sleep, as there are growing clinical

221 evidences in humans supporting the relationship between depression and sleep disruption  
222 (Benedetti and Colombo, 2011).

223

## 224 **Conclusions**

225 Our results provide important information for captive bear management. We provide evidence  
226 that the change of social environment, and specifically the loss of a conspecific and the  
227 subsequent state of isolation and insecurity, may have short-term negative effects on the welfare  
228 of captive bears, as the change of activity patterns and the alteration of sleep may interfere with  
229 the normal restorative functions of REM sleep. We are uncertain whether these effects will last  
230 in the long-term, but preliminary observations suggest that the bear is restoring his normal  
231 behavioral patterns. Further research will be carried out in order to confirm these hypotheses.

232 Before the death of Orfeo's sibling, the pair never showed behavioral problems, suggesting that  
233 sterilized males and females can live together in captivity, provided that they have enough  
234 resources in their enclosure. The Osservatorio is presently considering the possibility of  
235 introducing a new bear mate; however, the risk of aggressive interactions between two  
236 unfamiliar adult bears in a confined enclosure is high, and the partition of the existing enclosure  
237 is under evaluation, in order to allow a period of acclimation in neighbouring enclosures, that  
238 should minimize these risks.

239 Although forcedly based only on one case report, the results of the present study provide new  
240 information that open new insights on a previously unexplored field, and highlight the  
241 importance of social environment and of its changes, that should be carefully considered in  
242 order to maintain good welfare conditions of bears kept in zoos or in other captive  
243 environments.

244

## 245 **Acknowledgements**

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247 the bear facilities for behavioral observations. Many thanks to the International Species  
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250 for the revision of the English manuscript and an anonymous reviewer for the useful comments  
251 on the first version of this manuscript.

252

253 The authors declare no conflict of interest.

254 The idea for the paper was conceived by Bernardo Pedroni and Fabia Rosi.

255 The experiment was designed by Silvana Mattiello.

256 The experiment was performed by Antonella Cordedda and Serena Brignoli.

257 The data were analyzed by Silvana Mattiello.

258 The paper was written by Silvana Mattiello, with help and inputs from all co-authors.

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260 The study met all humane standards, as no manipulation was necessary and only non invasive  
261 observations were carried out on the subjects.

262

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320



321 Table 1. Overall percentage of time allocated to different behavioral categories during each  
 322 posture. Considered behavioral categories are: alert inactive (the bear is not moving, but his  
 323 eyes are open and he is reactive to external stimuli); sleeping (a state of behavioral quiescence,  
 324 the body is completely relaxed, the eyes are closed and the bear is not reactive to any external  
 325 stimulus); feeding (eating or chewing food, either delivered by the keeper or found in the fence,  
 326 e.g. leaves or branches); moving (walking or hurrying, including movement while sniffing or  
 327 foraging); self-grooming (including self-licking, self-scratching, scratching against trunks or  
 328 rocks, bathing and scrolling); den preparation (including digging and raking or mowing  
 329 vegetable parts to prepare for hibernation); drinking.

330 Observed postures are: quadrupedal posture (standing or moving on the 4 legs); lateral lying  
 331 (the bear is lying on a side, with outstretched legs and neck, occasionally with one leg partially  
 332 bent); sterno-costal lying (the weight of the bear is loaded on the ventral part of the body, the  
 333 fore legs are outstretched, while the hind legs are bent below the body, the head is usually  
 334 upright, occasionally outstretched); sitting (the bear is sitting on his buttocks, leaning on the  
 335 distal portion of his front legs, like a dog).

336

---

	alert inactive	sleeping	feeding	moving	self- grooming	den preparation	drinking
lateral lying	22.2%	77.6%	0%	0%	0.2%	0%	0%
sterno-costal lying	75.8%	13.8%	4.6%	0%	5.4%	0.2%	0.2%
quadrupedal posture	39.8%	0.3%	7.2%	28.6%	3.3%	20.6%	0.2%
sitting	85.6%	0%	0%	0%	12.9%	0%	1.4%

---

337

338

339 **Figure legends**

340 Figure 1. Views of the enclosure. a) meadows and mixed conifer wood (the fence can be seen  
341 on the right); b) high platform from which the observations were carried out; c) artificial dens;  
342 d) natural dens dug by the bears; e) stream.

343

344 Figure 2. Percentage of time (mean  $\pm$  s.e.) devoted by Orfeo to the main behavioral categories in  
345 the presence of his sibling (pair) and alone. Asterisks over the columns indicate significant  
346 differences ( $P < 0.001$ ).

347

348 Figure 3. Percentage of time (mean  $\pm$  s.e.) spent by Orfeo in different postures in the presence of  
349 his sibling (pair) and alone. Asterisks over the columns indicate significant differences  
350 ( $P < 0.05$ ).

Figure 1  
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Figure 2  
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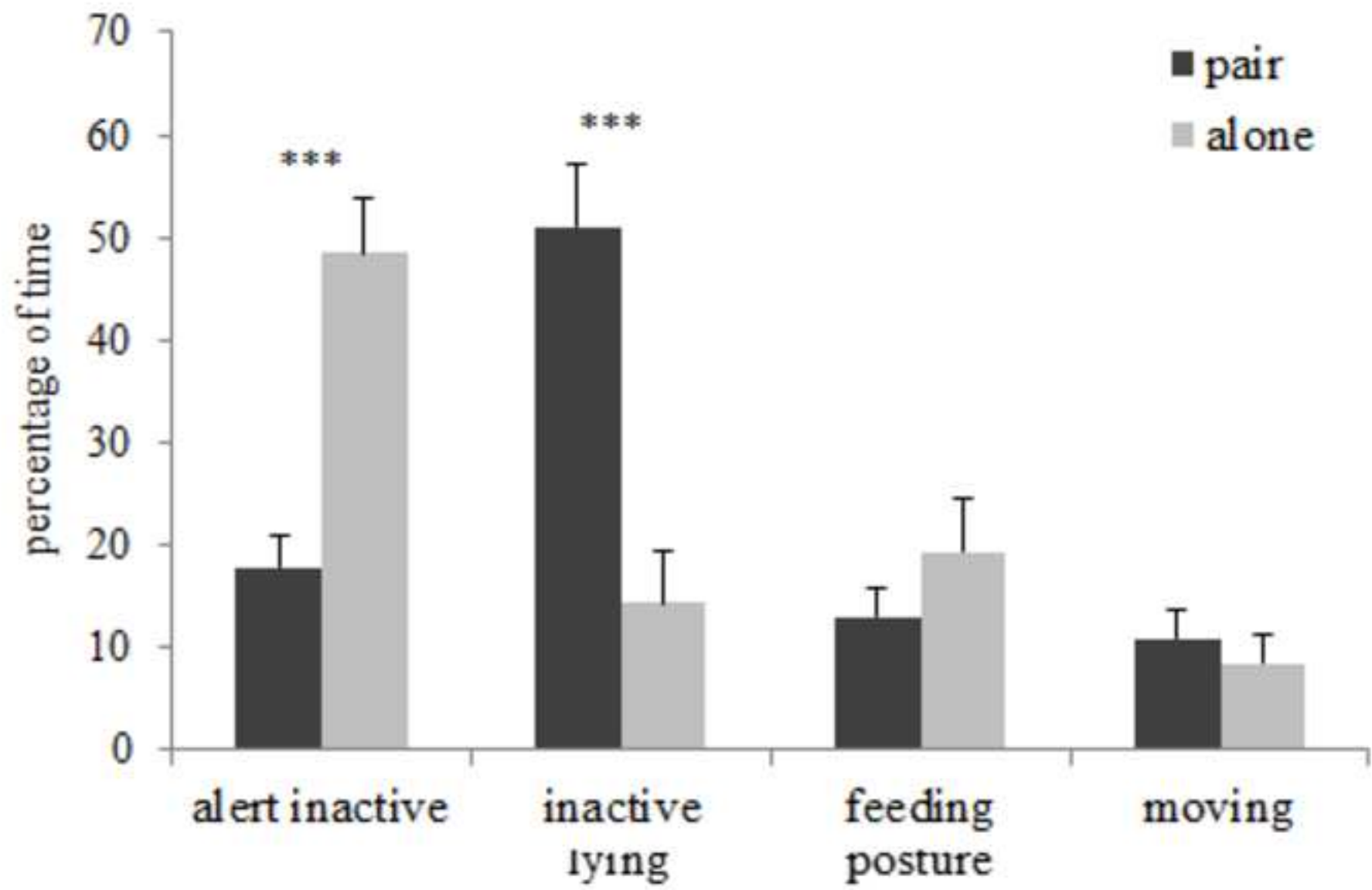


Figure 3  
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