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1 Effect of immobilizations on female moose (*Alces alces*) activity and space  
2 use

3

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20

21 Effect of immobilizations on female moose (*Alces alces*) activity and space use

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23 **Abstract**

24 Studies of free-ranging wildlife often involve animal capture and fitting of tracking devices.  
25 Capturing wildlife may result in behavioral alterations. Thus, there is a need to evaluate the effects  
26 of capture on study animals to identify potential biases influencing the research. We assessed the  
27 short-term response of 15 GPS/GSM-collared adult female moose (*Alces alces* L., 1758) and  
28 immobilization and handling by comparing moose rates of movement and net square displacement  
29 before and after re-capture. Moose were more active up to seven hours and increased their spatial  
30 displacement for 4.5 days after re-capture compared to movement patterns before re-capture.  
31 Opposing to our predictions, moose did not reduced their rates of movement after their initial  
32 displacement following capture and recovery, i.e., moose did not show any indication for a  
33 residual effect. We recommend using individuals as their own controls in analyses of capture  
34 impacts to account adequately for individual behavioral differences. We recommend omitting data  
35 of at least the first five days following capture for analyses of moose movement and distribution.

36

37 *Keywords:* rates of movement, net square displacement, capture, handling, chemical  
38 immobilization, free-ranging ungulates.

39

## 40 Introduction

41 Studying the behavior of free-ranging wildlife often involves capture, handling, and equipping  
42 individuals with a tracking device, especially in wide-ranging wildlife that utilize remote areas or  
43 occur in forested habitats. For many species, researchers use mixtures of anesthetic drugs and  
44 tranquilizers to facilitate handling of free-ranging animals and to reduce stress (Arnemo et al.  
45 2006; Kreeger and Arnemo 2007). Irrespective of methodology, capture is a potentially stressful  
46 event for free-ranging wildlife (Kock et al. 1987; Haulton et al. 2001, Fahlman et al. 2008).  
47 Capture procedure impacts not only on animals' physiological and physical parameters, but may  
48 also result in behavioral alterations following capture and handling, which calls for an assessment  
49 of the potential short-term and long-term disturbances effects on study animals and research  
50 results (Laurenson and Caro 1997; Côté et al. 1998; Cattet et al. 2008; Morellet et al. 2009).

51 To evaluate the effect of capture with chemical immobilization on behavior of free-ranging  
52 wildlife, researchers must characterize normal behavior for those individuals. Because detailed  
53 monitoring of an animal before it is equipped with a tracking device is impossible, researchers  
54 must rely on data associated with an initial capture and subsequent re-captures over time (i.e.,  
55 animal as its own control). We studied the short-term impact of capture, handling, and chemical  
56 immobilization on rates of movement and net square displacement of 15 free-ranging GPS-marked  
57 female moose during a 120 hour period *before* their re-capture and *after* their re-capture. We used  
58 net square displacement to reflect moose' spatial displacement between moose' capture location  
59 and moose' locations at different time stamps after capture and recovery (Calenge et al. 2009).

60 Based on our literature search, we predict capture and handling to alter moose rates of  
61 movement and spatial displacement. First, we predict that moose leave the capture area

62 immediately after recovery, reflected as instantaneous increased rates of movement and net square  
63 displacement. Secondly, we predict the capture and handling to affect moose rates of movement  
64 even after initial recovery and movement away from the re-capture location, i.e., animals show a  
65 residual effect reflected as delayed decreased movement. We therefore predict moose would be  
66 less active and move less after their initial displacement when compared to their rates of  
67 movement and spatial displacement before recapture. Thirdly, we expect specific characteristics  
68 associated with each chemical immobilization and capture (induction time, immobilization time,  
69 handling time, dosage, body temperature, and presence of conspecifics) would be correlated with  
70 changes in moose rates of movement and displacement. Thus, we predict that individuals receiving  
71 a higher dose of immobilizing drugs, being exposed to longer induction, immobilization and  
72 handling time, or exhibiting higher body temperatures, have a relatively greater change in their  
73 rates of movement and displacement when compared to those with lower dosages, shorter  
74 induction, immobilization and handling times, or lower body temperatures.

75

## 76 Material and methods

### 77 Study area

78 Female moose were captured in the low alpine mountain region of Northern Scandinavia in the  
79 regions of Västerbotten, Sweden and in Nordland, Norway. The whole region (65° 47' N 15° 19'  
80 E, WGS84) is characterized by boreal and mountainous forest that is dominated by Scots pine  
81 (*Pinus sylvestris* L.), Norway spruce (*Picea abies* L.), birch (*Betula pubescens* Ehrh.), and willow  
82 (*Salix* spp.), and areas that are above the tree line. Mean temperatures in January and July are -13°

83 C and 13° C, respectively. Snow cover lasts from the beginning of October until late May, and the  
84 vegetation growing season is about 110-130 days (Raab and Vedin 1995).

85

## 86 Moose immobilization

87 We immobilized 15 free-ranging female moose (mean year of birth  $1999 \pm 2.4$  SD, range 1995-  
88 2003, determinable for 12 / 15 females; mean bodyweight  $323 \text{ kg} \pm 17$  SD, available for 2 / 15  
89 females) from a helicopter. We used a dart gun (DAN-INJECT ApS , Børkop, Danmark) for all  
90 immobilizations. *Large Animal Immobilon* (2.25 mg etorphine per ml (2.45 mg per ml as etorphine  
91 hydrochloride) and 7.38 mg acepromazine per ml (10 mg per ml as acepromazine maleat),  
92 Novartis Animal Health UK Ltd, Surrey, UK; Arnemo *et al.* 2006) was used for 11 captures. The  
93 mean dosage per capture was  $6.5 \text{ mg etorphine} \pm 2.5$  SD ( $0.020 \text{ mg kg}^{-1}$  bodyweight). *M99* (9 mg  
94 etorphine per ml (9.8 mg per ml as etorphine hydrochloride; Novartis Animal Health, Basel,  
95 Switzerland) was used in 4 captures and mean dosage per capture was  $5.1 \text{ mg etorphine} \pm 1.7$  SD  
96 ( $0.016 \text{ mg kg}^{-1}$  bodyweight). Together with etorphine, we injected xylazine (*Rompun*, KVP  
97 Pharma and Veterinär Produkte GmbH, Germany). For 11 captures mean dosage per capture was  
98  $145.5 \text{ mg xylazine} \pm 56.8$  SD ( $0.45 \text{ mg kg}^{-1}$  bodyweight) and for 4 captures mean dosage per  
99 capture was  $137.5 \text{ mg xylazine} \pm 75.0$  SD ( $0.43 \text{ mg kg}^{-1}$  bodyweight; Arnemo *et al.* 2006). We  
100 reversed immobilization by intravenous administration of the antagonists atipamezole (*Antisedan*,  
101 as 5 mg per ml atipamezole hydrochloride; Orion Pharma, Espoo, Finland) and diprenorphine  
102 (*Large Animal Revivon* ( $n = 11$ ), as 2.45 mg diprenorphine hydrochloride per ml; Novartis Animal  
103 Health UK Ltd, Surrey, UK; *Diprenorphine* ( $n = 4$ ), as 12 mg diprenorphine hydrochloride per ml;  
104 Novartis Animal Health, Basel, Switzerland; Kreeger and Arnemo 2007). The mean dosage per  
105 capture ( $n = 11$ ) was  $9.1 \text{ mg atipamezole} \pm 2.8$  SD ( $0.028 \text{ mg kg}^{-1}$  bodyweight) and  $8.5 \text{ mg}$

106 diprenorphine  $\pm$  4.8 SD (0.026 mg kg<sup>-1</sup> bodyweight). In 4 captures we used *Diprenorphine* and  
107 mean dosage per capture was 15 mg  $\pm$  6.0 SD (0.046 mg kg<sup>-1</sup> bodyweight) and 6.9 mg atipamezole  
108  $\pm$  3.8 SD (0.021 mg kg<sup>-1</sup> bodyweight). We equipped each moose with a neck collar tracking device  
109 that included a Global Positioning System (GPS) receiver, Global System for Mobile  
110 communication (GSM) modem, and a traditional VHF-beacon (Vectronic Aerospace GmbH,  
111 Berlin, Germany). We received a location for each moose every hour. We immobilized moose  
112 between early November and early December in 2005 and 2007. The total handling time (from  
113 close-up approach by the helicopter until reversed and standing) was 33 min  $\pm$  8 SD per capture.  
114 On average, we used 1.2 darts  $\pm$  0.4 SD per capture. No capture related injuries or mortalities  
115 occurred.

116

## 117 Data analysis

### 118 *Rates of Movement*

119 To determine relative response in rates of movement towards chemical immobilization and  
120 handling, we calculated Euclidean distance [m] between consecutive locations, and compared the  
121 estimated “speed” [m h<sup>-1</sup>] each individual travelled 120 hours *before* re-capture and as well as  
122 *after* re-capture using the Wilcoxon Signed Rank test. For our reference data (hereafter referred as  
123 ‘*before* re-capture’) we sampled each individual location data starting at the very same hour of the  
124 day as the re-capture started minus 168 hours. Of this data we used the first 120 hours for our  
125 reference material. Thus, we ignored location data 48 hours directly prior the re-capture event,  
126 leaving a gap of two days as a conservative estimate to avoid dilution by any potential

127 disturbances related to re-capture event. We used each individual as its own control and controlled  
128 for moose circadian rhythm.

129

### 130 *Net square displacement*

131 Using R package *adehabitat* (version 1.8.3) we calculated the net square displacement [m]  
132 (hereafter referred as ‘displacement’; Dettki and Ericsson 2008; Calenge et al. 2009) of each  
133 moose post-capture by comparing its location at re-capture and its locations during a 120 hour  
134 period *after* recovery. As before we obtained animal’s relative response by comparing  
135 displacement *after* re-capture with displacement during *before* re-capture as given by the reference  
136 data using the Wilcoxon Signed Rank test. Thus, the origin position is the first location of the  
137 reference data.

138

### 139 *Impact of immobilization procedure*

140 We addressed the effect of specific characteristics of each immobilization and capture on the  
141 relative change in moose rates of movement and displacement during the first 24 hours following  
142 recovery. To avoid dilution from the initial displacement, we excluded the first seven hours  
143 immediately after recovery as indicated by the changes in rates of movement (Figure 1). Thus, we  
144 averaged the change in moose rates of movement and displacement using data between the 8<sup>th</sup> and  
145 24<sup>th</sup> hour *after* re-capture. We compared each animal’s rate of movement and displacement after  
146 recovery as related to 1) time until lateral recumbency (*MinDown*, i.e. induction time), 2) duration  
147 of immobilization (*MinImmo*), 3) total handling time (*MinHandling*), 4) dosage (ethorphine [mg]  
148 (*Emg*) and xylazine [mg] (*Rmg*); given as a principal component (*pc1*) due to their high correlation  
149  $r = 0.94$ ), 5) moose’ rectal body temperature (*Temp*), and 6) whether the moose was with other



150 moose aside from their own offspring (*Company*). Four of the fifteen females were in company of  
151 other moose. The majority of females (14/15) were accompanied by offspring, and thus we could  
152 not evaluate the effect of being barren or not. *Pc1* combined 97% of the variance, received strong  
153 positive loadings (0.7) from both *Emg* and *Rmg*, and had an eigenvalue of 1.9. To avoid an over-  
154 parameterization of the model, we evaluated the impact of the different explanatory variables one  
155 at a time using a linear model.

156  
157 We used the software open-source program R 2.10.1 for all statistics and set  $p < 0.05$  (R  
158 Development Core Team, 2009). Values are given with standard errors if not otherwise indicated.  
159 Data was heavily right skewed, and thus we used log-, or cube root transformation to access  
160 normality.

161

## 162 Results

163 On average, it took  $4 \text{ min} \pm 3 \text{ SD}$  until the first dart was injected after helicopter approach and  $13$   
164  $\text{min} \pm 5 \text{ SD}$  from dart injection until moose were laterally recumbent (i.e. the induction time).  
165 Moose were immobilized for about  $21 \text{ min} \pm 9 \text{ SD}$ . Reversal of immobilization (i.e. time from  
166 administration of the antagonists until standing), took  $1 \text{ min} \pm 0.4 \text{ SD}$ .

167

### 168 *Rates of movement and displacement*

169 Moose were more active up to seven hours after capture and recovery, with the largest increase  
170 during the first two hours (Wilcoxon Sign Rank test: 1<sup>st</sup> hour:  $s = 33$ ,  $p = 0.001$ ; 2<sup>nd</sup> hour:  $s = 22$ ,  $p$

171 = 0.008; 3<sup>rd</sup> hour:  $s = 25$ ,  $p = 0.052$ ; 4<sup>th</sup> hour:  $s = 21$ ,  $p = 0.1$ ; 5<sup>th</sup> hour:  $s = 29$ ,  $p = 0.02$ , 6<sup>th</sup> hour:  $s$   
172 = 40,  $p = 0.02$ ; 7<sup>th</sup> hour:  $s = 20$ ,  $p = 0.048$ ; Figure 1; Appendix 1). Moose showed greater  
173 displacement up to 4.5 days following immobilization compared to their displacement *before* re-  
174 capture (Figure 2; Appendix 2).

175

#### 176 *Response in relation to immobilization parameters*

177 Moose that had a longer induction time increased their rates of movement and their spatial  
178 displacement more compared to moose with a shorter induction time (Table 1). However, in both  
179 cases the effect is driven by an outlier, i.e. one female that changed her behavior significantly and  
180 for which it took long time until the immobilization drugs showed effect (Table 1; movement rate  
181 = 1003 [m hr<sup>-1</sup>]; displacement = 10619 m; induction time = 22 minutes).

182

## 183 Discussion

184 As we predicted, moose were more active the very first hours after their capture and recovery than  
185 during the period before capture, and animals moved away from the area of their capture.  
186 However, while rates of movement were increased only a few hours after capture, moose had  
187 greater spatial displacement up to 4.5 days after capture. Although their movements immediately  
188 after capture suggested flight behavior, the longer-lasting spatial displacement suggests that moose  
189 moved from the area of capture to a greater extent than just an initial displacement. Alternatively,  
190 the observed larger values of displacement could reflect that capture may trigger an onset of  
191 migration since captured female moose in this study belonged to a migratory moose population

192 that was still in their summer range at the time of capture. Unfortunately, literature is limited  
193 regarding capture effects on migration timing in migratory species. Still, some studies suggest that  
194 capture disturbance trigger movement to other areas as Ramsay and Stirling (1986) documented  
195 den abandonment and area shift in female polar bears (*Ursus maritimus* Phipps, 1774), and  
196 Morellet et al. (2009) describe refuge behavior in roe deer (*Capreolus capreolus* L., 1758)  
197 following capture.

198 In contrast to our prediction, moose showed no residual effect following capture in form of  
199 lowered activity with respect to their movement rates or displacement following initial recovery  
200 and movement away from the capture location. This may imply that the immobilization and  
201 capture procedure itself, as used in this study, did not considerably affect moose movement. Cattet  
202 et al. (2008) found that both grizzly (*Ursus arctos* L., 1758) and American black bears (*Ursus*  
203 *americanus* Pallas, 1780) lower their movement rates for several weeks following capture.  
204 Although, Støen et al. (2010), documented that species belonging to different guilds show different  
205 strategies to cope with human-induced disturbances, e.g. European brown bear (*Ursus arctos*)  
206 lowered their movement activity following research-related close-up approaches by helicopters,  
207 while moose increased their movement activity. Alternatively, one hour intervals may give a  
208 resolution too coarse to pick up decreased movement patterns related to immobilization in moose.  
209 Individuals may take several smaller resting breaks yet they are still moving, possibly diluting the  
210 effects within the one hour intervals.

211 Our results agree with findings by Morellet et al. (2009) suggesting an altered spatial behavior  
212 in roe deer although they document an effect present up to ten days. We judge our methodological  
213 approach, comparing individual behavior directly before and after re-capture using individuals as

214 their own control and accounting for circadian rhythms, to provide an appropriate evaluation of  
215 movement patterns of individuals after immobilization and capture. In contrast, the risk for  
216 misdirected conclusions may increase when studying individuals' response following capture  
217 where neither access to reference data for a given individual nor analysis accounts for individuals'  
218 circadian or seasonal rhythm is present. Furthermore, conclusions have to be carefully made when  
219 animals have been followed over longer distance or transported and thereby are displaced from  
220 their original home range. Especially in territorial species, or species with distinct home ranges, a  
221 larger spatial displacement to the periphery of individuals' area of residence due to capture  
222 procedure may result in misleading conclusions on the impact of capture on animals' movement  
223 behavior.

224 Longer inductions times may be found in high-strung individuals as increased stress levels may  
225 delay the chemical immobilization to take effect (Kreeger and Arnemo 2007). In turn, high-strung  
226 individuals may be more predisposed to increased movement behavior both before capture and  
227 following recovery, which should be addressed in future studies by comparing stress hormone  
228 levels with observed movement behavior. Of all immobilization parameters evaluated, our data  
229 indicated only a relationship between moose rates of movement and spatial displacement with  
230 induction time. Yet, this relationship was driven by an outlier, which in combination of the  
231 variation in the behavioral response indicates a need of a larger sample size to properly address  
232 that question. Effects of chemical immobilization are complex and differ among species (Kreeger  
233 and Arnemo 2007), and most likely also differ among individuals, complicating impact detection  
234 in small sample sizes. Side-effects differ with drug combinations (Kreeger and Arnemo 2007). Our  
235 study focused on chemical immobilization using a combination of etorphine-xylazine and reversed

236 the effect with antagonists atipamezole and diprenorphine; this should be kept in mind when  
237 evaluating the specific side-effects.

238 In summary, our results suggest a momentary effect of capture on moose movement rates, i.e.,  
239 only the first few hours after recovery, but an impact on moose displacement that lasts for some  
240 days. Thus, we recommend omitting location data at least the first five days following capture  
241 when addressing behavioral movement analyses. Our results further evoke the value of using  
242 individuals as their own control to account for individual differences when evaluating capture  
243 impacts. Our results suggest that larger sample sizes are needed to evaluate the influence of  
244 immobilization and capture parameters due to variation in behavioral response. In particular, we  
245 recommend long-term wildlife research projects to specifically address the affect of multiple  
246 capture procedures, as a process to evaluate the capture protocol. Such analyses should take  
247 advantage of information given by multiple captures of the same study animals as repeated  
248 measures enable to control for differences among individuals.

249

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256 Northern Sweden in Umeå (Dnr A124-05 2005-11-15) and was carried out in accordance with the

257 Swedish laws concerning animal research ethics. All personnel were certified according to the  
258 standards by the Swedish Animal Welfare Agency and the Swedish Board of Agriculture.

259

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298



299 Tables

300 Table 1 Female adult moose *Alces alces* change in movement rates and displacement in relation to  
 301 capture characteristics, Northern Scandinavia. Values represent mean  $\pm$  SE. Significant results as  
 302 indicated by the linear model in bold.

| Explanatory variables |                                      | Response variables                 |                             |
|-----------------------|--------------------------------------|------------------------------------|-----------------------------|
|                       |                                      | Rates of movement                  | Displacement                |
| Mean $\pm$ SE         |                                      | 192 [m hr <sup>-1</sup> ] $\pm$ 77 | 2857 [m] $\pm$ 716          |
| Dosage <i>pc1</i>     | -0.06 $\pm$ 0.4                      | df=11, F=1.8, p=0.2                | df=12, F=0.1, p=0.7         |
| <b>MinDown</b>        | <b>13 [min] <math>\pm</math> 1.3</b> | <b>df=11, F=5.9, p=0.03</b>        | <b>df=12, F=5.3, p=0.04</b> |
| MinImmo               | 21 [min] $\pm$ 2.6                   | df=11, F=0.9, p=0.4                | df=12, F=0.1, p=0.7         |
| MinHandling           | 36 [min] $\pm$ 2.3                   | df=11, F=0.09, p=0.8               | df=12, F=0.9, p=0.4         |
| Temperature           | 39.3 [°C] $\pm$ 0.2                  | df=9, F=0.3, p=0.6                 | df=9, F=0.4, p=0.5          |
| Company               | 4/15                                 | df=11, F=4.1, p=0.07               | df=12, F=0.1, p=0.7         |

303 *Pc1*: principal component of ethorphine [mg] and xylazine [mg], MinDown: induction time,  
 304 MinImmo: duration of immobilization, MinHandling: total handling time, Temperature: rectal  
 305 body temperature, Company: other moose aside from female's offspring

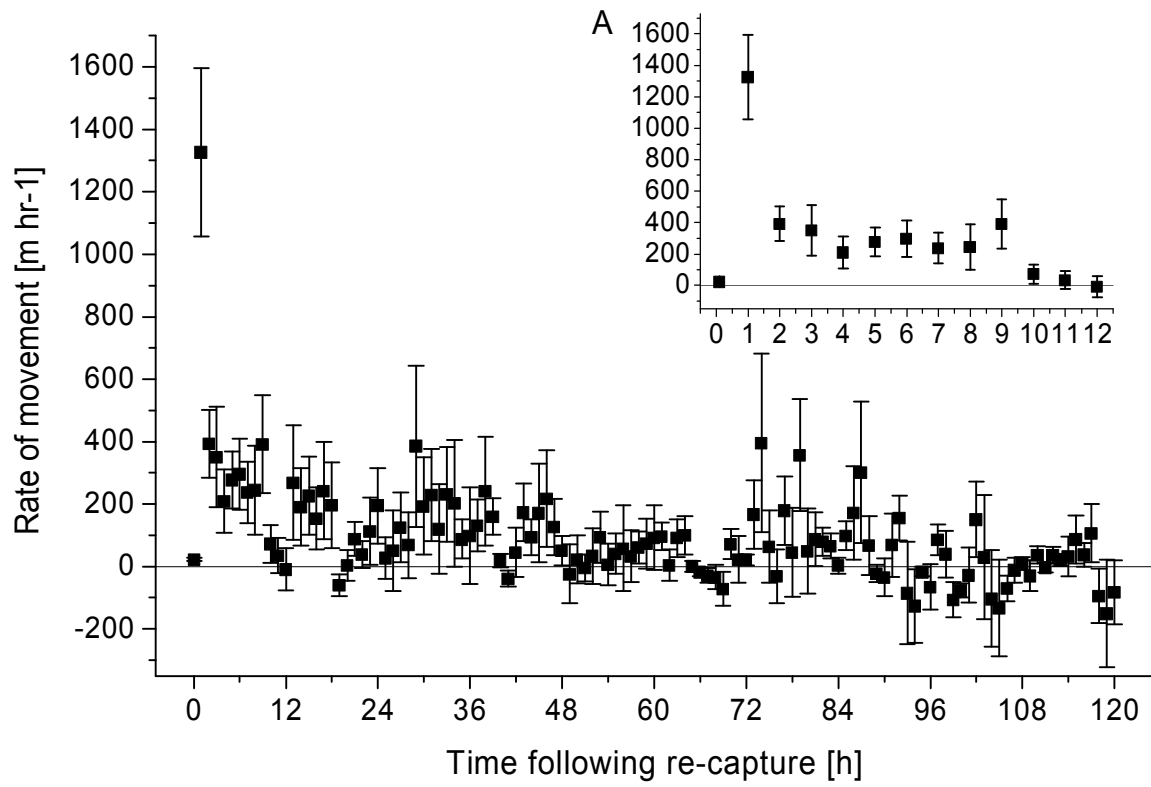
306 **Figures**

307 **Fig. 1** Change in adult female moose, *Alces alces*, movement rates [ $\text{m hr}^{-1}$ ] *after* re-capture; zero  
308 line indicates no difference in movement rates *before* and *after* re-capture. (A) shows the first 12  
309 hours. Northern Scandinavia.

310

311 **Fig. 2** Change in adult female moose, *Alces alces*, net square displacement [m] *after* re-capture;  
312 zero line indicates no difference in displacement *before* and *after* re-capture. Northern  
313 Scandinavia.

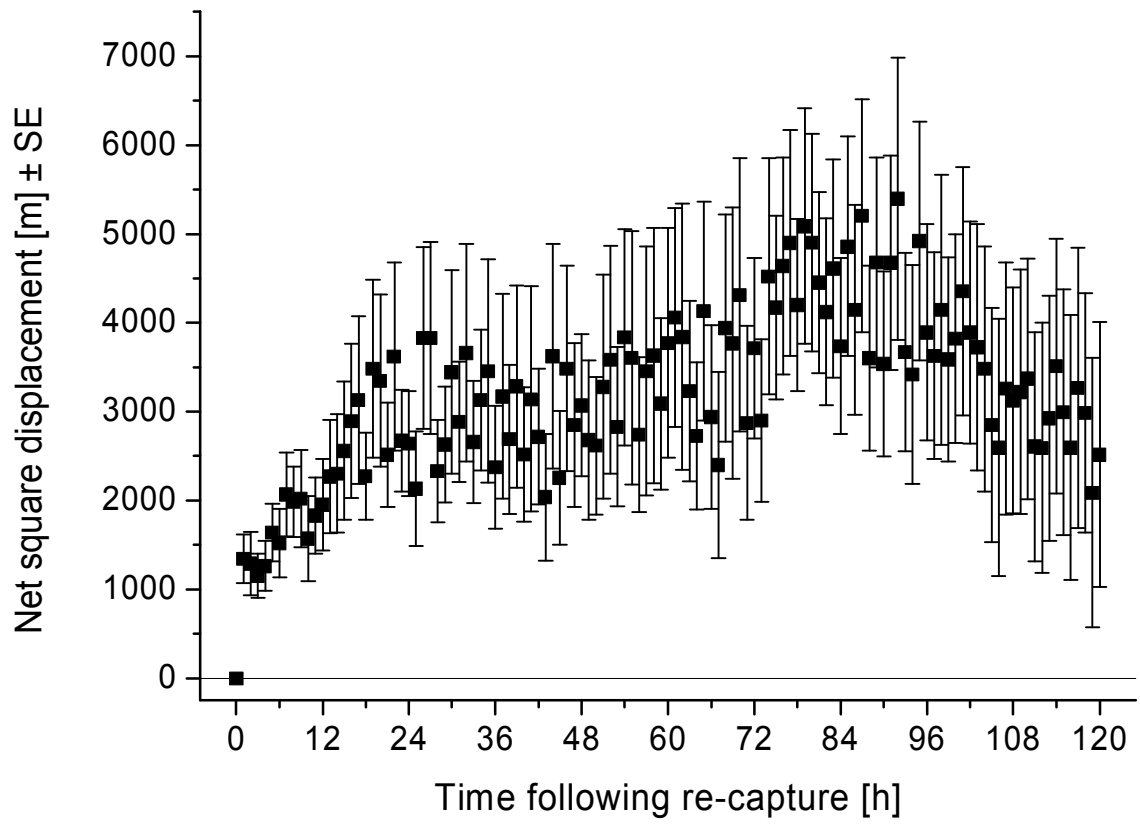
314



315

316 Fig.1

317



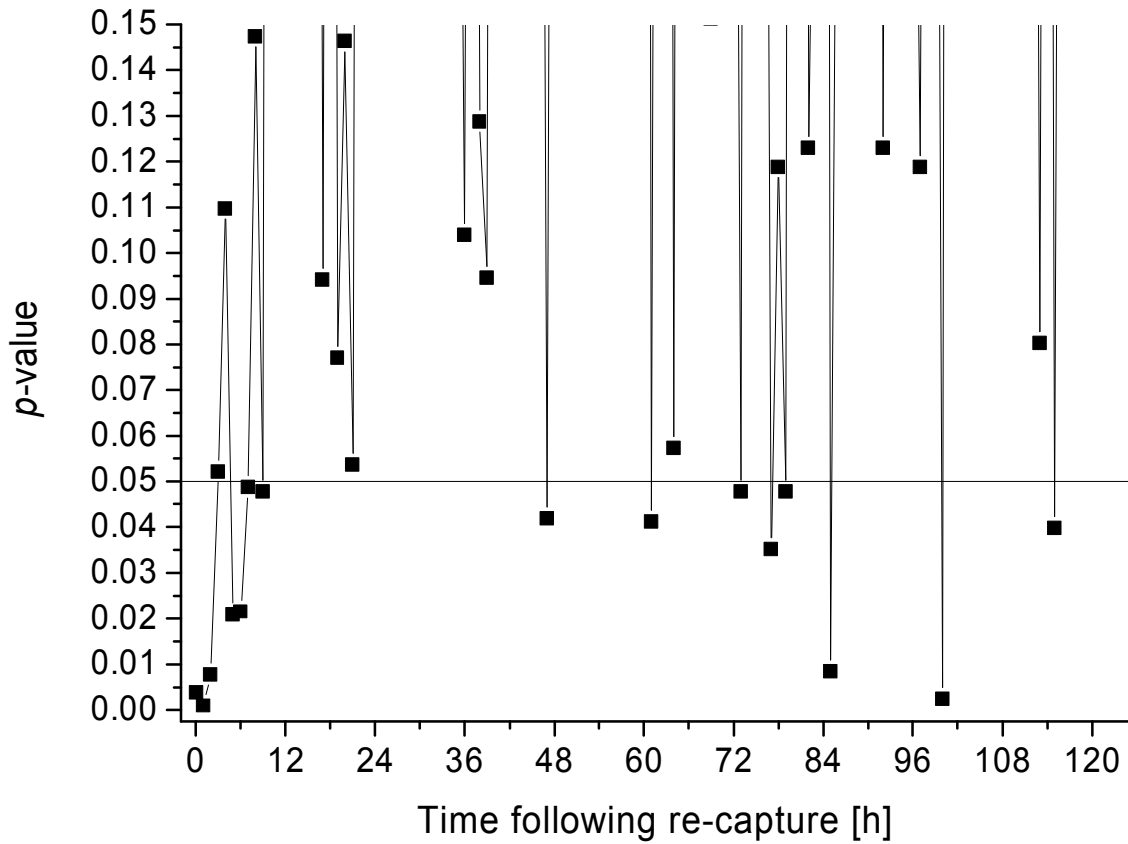
318

319 Fig. 2

320

321 **Appendix 1**

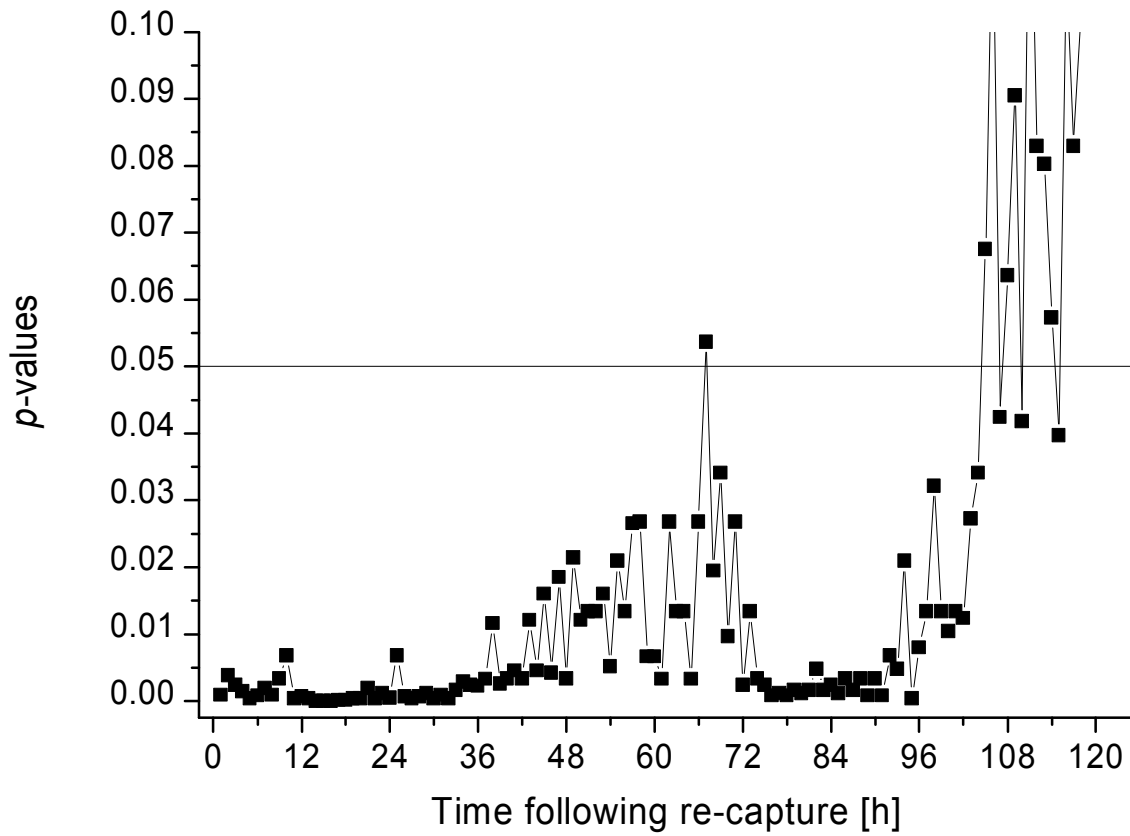
322 Probability of difference in adult female moose, *Alces alces*, movement rates [m hr<sup>-1</sup>] *before* and  
323 *after* re-capture (Wilcoxon Signed Rank test). Black line indicates p = 0.05.



324

325 **Appendix 2**

326 Probability of difference in adult female moose, *Alces alces*, net square displacement [m] *before*  
327 and *after* re-capture (Wilcoxon Signed Rank test). Black line indicates  $p = 0.05$ .



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