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Metz, O., Williams, J., Nielsen, R. K. and Masters, N. (2017), Retrospective study of mortality in Asiatic lions (*Panthera leo persica*) in the European breeding population between 2000 and 2014. *Zoo Biology*, 36: 66–73. doi: [10.1002/zoo.21344](https://doi.org/10.1002/zoo.21344)

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The full details of the published version of the article are as follows:

TITLE: Retrospective study of mortality in Asiatic lions (*Panthera leo persica*) in the European breeding population between 2000 and 2014

AUTHORS: Metz, O., Williams, J., Nielsen, R. K. and Masters, N.

JOURNAL TITLE: *Zoo Biology*

PUBLISHER: Wiley

PUBLICATION DATE: January 2017

DOI: 10.1002/zoo.21344

Retrospective study of mortality in Asiatic lions (*Panthera leo persica*) in the European breeding population between 2000 and 2014

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Running head: Mortality of captive Asiatic lions

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Word count: 4968 (excluding abstract, figures, tables and references)

Abstract

The intention to import Asiatic lions (*Panthera leo persica*) from Sakkarbaug Zoo to the captive population managed under the European Endangered Species Program (EEP) emphasized how little is known about the health status of the EEP population. This study was designed to characterize mortality for this population through examination of the studbook and other records on 392 Asiatic lions living in the EEP between 2000 and 2014. 270 animals died during the period with 80% of them being under one year old. The mortality rate for under one year-olds was 54%, whilst the odds of survival of cubs within a litter increased if the dam had had litters previously. Survival to reproductive age was 44%. Post mortem reports were requested and the cause of death was obtained for 133 animals. Trauma inflicted by a conspecific and lack of care were common causes of death (26% and 22% respectively), and were also responsible for most of the neonatal mortalities. Congenital defects were responsible for 9% of deaths, although the true prevalence is likely underestimated. A common necropsy protocol for all Asiatic lion collections is needed to facilitate future studies. Low cub survival and a high rate of congenital defects might indicate inbreeding depression in the EEP and points to the need for new genetic material within this population.

Key words: Asiatic lion, *Panthera leo persica*, mortality review, infant mortality, congenital defects

1 Introduction

2 The Asiatic lion (*Panthera leo persica*) is slightly smaller than the African lion and can be
3 differentiated by a prominent fold of skin that spans the length of the abdomen (O'Brien et al.
4 1987). The species is classified as endangered by the IUCN Red List and despite its historic range
5 stretching from North Africa over Europe to eastern India, the Asiatic lion now only exists as a
6 single free-living population in the Gir forest in India (Breitenmoser et al. 2008). Although this

7 population recently increased to about 500 individuals, the presence of only one free-living
8 population makes the species extremely vulnerable to catastrophic events (DeshGujarat 2015).

9 Asiatic lions went through two population bottlenecks in the past. After the first one 4,000 to
10 5,000 years ago, sport hunting and habitat encroachment reduced the population to only 20
11 individuals in the early 20th century (Driscoll et al. 2002). Today they are mainly threatened by
12 human-carnivore conflict. There are occasional incidents of attacks on humans, and livestock
13 makes up a significant part of a lion's biomass consumption (Breitenmoser et al.; Nowell and
14 Jackson 1996; Banerjee et al. 2013).

15 Captive breeding programs for Asiatic lions currently exist in India and in Europe (Zingg 2007).
16 Previous research on Asiatic lions in captivity has mainly been carried out at Sakkarbaug Zoo in
17 India, which houses the largest captive population of Asiatic lions (Nowell and Jackson 1996).
18 Sakkarbaug Zoo also takes in orphaned, sick and wounded lions from the wild, which are mated
19 with captive individuals to maintain a healthy captive population (Gujarat State Lion
20 Conservation Society 2011). Research on this population showed that reproduction starts earlier
21 in captivity than in the wild. Both males and females start reproducing at about three years of age
22 in captivity, compared to four years for females and five to eight years for males in the wild
23 (Nowell and Jackson 1996). Both genders stop reproducing at about 15 years of age (Nowell and
24 Jackson 1996). Longevity is not recorded for the free-living population and lies between 16 and
25 18 years in captivity, with 17 to 18 years for females and 16 years for males (Nowell and Jackson
26 1996). The mortality rate for lions under one year of age is estimated to be 33% in the wild and
27 36% at Sakkarbaug Zoo (Nowell and Jackson 1996). In the wild, infanticide can cause up to 60%
28 of cub mortality (Breitenmoser et al.).

29 The European breeding program started in 1990 and has been managed as a European
30 Endangered Species Program (EEP) since 1994 (Zingg 2007). From an original 12 founder
31 animals there were 121 individuals living in 42 collections in December 2014 (Nielsen 2014).
32 Both the free-living population and the population at Sakkarbaug Zoo have demonstrated high

33 counts of morphologically abnormal sperm in the past, which has been recognized as a sign of
34 inbreeding depression in African cheetahs (O'Brien et al. 1987; Wildt et al. 1987). Considering
35 this, inbreeding depression is also probably a problem within the EEP, and the population would
36 benefit from the introduction of new genetic material.

37 The Zoological Society of London (ZSL) and Gujarat Forest Department (GFD) are collaborating
38 to attempt an import of Asiatic lions from Sakkarbaug Zoo breeding facility to the EEP. As a
39 result of this collaboration, it became apparent that little is known about the health status of the
40 European population. This study aimed to improve our understanding by studying mortality
41 occurring in the European population between 2000 and 2014. Conducting a mortality review can
42 help to show trends in mortality for a captive population (Hope and Deem 2006) and may even
43 point towards management strategies that could assist in preventing some diseases (Martínez et
44 al. 2013).

45 The first objective of this study was to describe mortality occurring in the EEP, including
46 information on mortality rates and ages at death. As part of the general data obtained on mortality,
47 litter survival was investigated more closely. Generally, even if a dam starts her breeding record
48 with low litter survival she is nonetheless bred from further. Anecdotal evidence points to survival
49 improving with parity, and for other species it has been shown that parental experience has an
50 influence on the survival of the young (Daunt et al. 2007; Meijer et al. 2011). The second objective
51 was thus to look at litter survival with the hypothesis that the survival rate increases when a dam
52 becomes multiparous. The last part of the study focused on the circumstances and causes of death
53 in this population; thus the third objective was to identify the most common causes of death in
54 the population as a whole and per age group and gender.

55 **Methods**

56 Data on all Asiatic lions living in the EEP (dead and alive) between 01.01.2000 and 31.12.2014
57 were collected from the Zoological Information Management System (ZIMS). These data
58 included the date and location of birth, the date and location of death (if applicable), and the dam

59 and sire of each animal. One collection had to be excluded from the analyses as it does not use
60 ZIMS and did not supply any information for this study.

61 To allow statistical analysis, the animals were grouped into categories depending on the age at
62 death. The “neonate” category included all animals less than one month of age, which correlates
63 with the period before cubs start leaving their cubbing den. All deaths that occurred before
64 animals reached reproductive age were grouped into “juvenile” (1-36 months). All animals of
65 reproductive age (36-180 months) were classified as “adult”, while all animals that reached post
66 reproductive age were classified as “geriatric” (more than 180 months old).

67 Where applicable, the standard error of the mean was used to describe variation. A chi-square
68 analysis (R version 3.2.1 (R Core Team 2015)) was used to determine if there was a difference in
69 the number of males and females born during the study period. The mortality rate per year was
70 calculated for the study population and a Cox proportional hazard model (R version 3.2.1 (R Core
71 Team 2015), package “survival” (Therneau 2015)) was used to determine if there was a difference
72 in survival between males and females. In order to determine if cub survival increased with the
73 number of litters, a generalized linear mixed effects model was used (R version 3.2.1 (R Core
74 Team 2015), package “lme4” (Bates et al. 2015)). For this analysis, dams with two litters or more
75 were taken into account, and dams, sires and birth locations were included in the analyses as
76 random effects. It has to be noted here that the exact number of cubs was unknown for at least
77 four litters, as the litter was eaten by the dam before staff were able to see the cubs. It was assumed
78 that these litters only contained one cub.

79 Data on the circumstances of death were obtained by contacting all past and present Asiatic lion
80 holders within the EEP via the co-ordinator and collecting post mortem reports. If the original
81 reports were not in English, a translation or summary in English was requested. If this was not
82 possible the reports were translated by the first author. If the cause of death was not listed
83 specifically it was ascertained by interpretation of the post mortem report wherever possible and
84 in conjunction with the EEP Veterinary Advisor. As in other studies (Hope and Deem 2006;

85 Clancy et al. 2013), the reason for euthanasia was listed as the cause of death. Additional findings
86 at post mortem were also noted. For collections that did not respond, the cause of death was
87 obtained from ZIMS where possible. If “stillborn” was listed as the cause of death on ZIMS it
88 was not included in the analyses, as some collections do not use this term to exclusively describe
89 animals that are dead at birth.

90 Additionally, the causes of death were grouped into the following categories: “stillbirth” (dead at
91 birth), “trauma” (death due to wounds inflicted by the dam or another conspecific), “degenerative”
92 (degenerative, ‘wear and tear’ diseases), “congenital” (defects present at birth), “infectious”
93 (infectious diseases), “lack of care” (cub abandoned or not cared for properly), “neoplasia” and
94 “other” (all conditions that caused death in less than five cases). Unknown causes were grouped
95 depending on the reason for this: “no report” (not received or of insufficient quality), “carcass
96 eaten” (by dam or another conspecific) or “cause unclear” (due to carcass condition or lack of
97 findings during post mortem examination).

98 The prevalence of the different causes was calculated for the population as a whole and for each
99 gender and age group, and the leading causes of death were identified. Fisher’s exact test was
100 used to determine if the prevalence of causes of death was significantly different between male
101 and female (R version 3.2.1 (R Core Team 2015)).

102 **Results**

103 *General description:*

104 270 dead animals and 122 live animals were included in this study from records referring to a
105 total of 392 animals. Of these, 34% (132/392) were male (87 dead, 45 alive), 43% (172/392)
106 female (97 dead, 75 alive) and 22% (88/392) were of undetermined gender (86 dead, 2 alive). 340
107 animals were born during the study period and for 252 of these the sex was known. Of these 43%
108 (108/252) were male and 57% (144/252) were female, which represents a significant gender bias
109 ($p=0.02$).

110 Figure 1

111 The average age at death was 2.39 ± 0.32 years (28.79 ± 3.91 months). 80% (215/270) of the
112 animals that died during the study period did not reach one year of age (figure 1), and excluding
113 these from the calculation increases the average age at death to 11.61 ± 0.77 years (139.41 ± 9.35
114 months). The oldest animal was a female that died when she was 19 years two months and 27
115 days old. So far no animal currently alive has reached this age. The Cox proportional hazard
116 model showed that gender had no effect on survival ($p=0.19$) with the hazard ratio being 1.22
117 (95% CI: 0.91, 1.63).

118 Figure 2

119 The average yearly mortality rate during the study period was 17.5%. Over the years it fluctuated
120 between 11.5% and 23.3% as shown in figure 2. Most of the deaths fell into the category
121 “neonate”, with only about 10% of deaths in each of the other three categories (figure 3). Looking
122 at the animals that could have reached reproductive age during the study period (born no later
123 than 31.12.2011, $n=313$) showed that only 44% achieved this, and the mortality rate for animals
124 under one year of age (including animals born before 31.12.2013, $n=358$) was 54%.

125 Figure 3

126 30 dams gave birth to two litters or more during the study period and 121 litters were included in
127 the generalized linear mixed effect model. The results showed that the odds for cubs within a litter
128 to reach one year of age increase significantly with the number of litters a dam had previously
129 (OR=1.408, 95% CI: 1.19, 1.67)($p=0.00009$).

130 *Circumstances of death:*

131 For ten of the 46 collections (22%) that hold or held Asiatic lions no death occurred within the
132 study period. 50% (18/36) of the collections where deaths occurred supplied the requested data
133 on the circumstances of death. This included data on 58% (158/270) of deaths. The information
134 for whether death occurred spontaneously or due to euthanasia was available for 196 of the 270

135 deaths (73%). Of these 68% (133/196) were spontaneous and 32% (63/196) were due to
136 euthanasia. In 48 cases, the reason for euthanasia was reported and was thus noted as the cause of
137 death. In addition, the cause of death was obtained from ZIMS for seven animals. 32 reports did
138 not include a cause of death and were recorded as “cause unclear” (eight cases) or as “carcass
139 eaten” (24 cases). This resulted in a total of 133 known causes of death out of 270 deaths reported
140 (49%).

141 The quality of these reports varied, ranging from a simple statement of the cause of death to a
142 complete post mortem examination report. Furthermore, the extent of the conducted examinations
143 differed. While animals dying at an older age received a full and detailed post mortem
144 examination, cubs that died shortly after birth often received only a quick examination or none at
145 all. Excluding the 24 cases where the carcass was eaten, a full post mortem examination would
146 have been possible in 134 cases out of the 158 cases where a report was received. Information on
147 comorbid findings was only received for 31% of these (42/134) and 32% (43/134) of the reports
148 received only contained a simple statement of the cause of death.

149 **Figure 4**

150 **Table 1**

151 The causes of death per age category and in total are shown in figure 4 and table 1. The leading
152 cause of death within this study population was “trauma” (26%), closely followed by “lack of
153 care” (22%). The remaining cause categories are each responsible for 7-10% of the deaths (figure
154 4, table 2). The high incidence of “trauma” and “lack of care” in the whole study population is
155 reflected in the “neonate” category, where 36% and 35% of deaths were caused by “lack of care”
156 and “trauma” respectively. For juveniles the three most common causes of death are “trauma”
157 (26%), “congenital” (30%) and “other” (30%). Apart from “other causes” the leading cause of
158 death for adults is “degenerative” (figure 4, table 2). Only two causes of death were present in the
159 “geriatric” age group, with “degenerative” (53%) making up a slightly higher percentage than
160 “neoplasia” (47%).

161 **Figure 5**162 **Table 2**

163 The causes of death for each gender are shown in figure 5 and table 2. “Trauma” (27%) is the
164 leading cause of death for males, and for females “lack of care” (20%), closely followed by
165 “trauma” (19%), is the leading cause of death. For the individuals of unknown sex “stillbirth”
166 (23%) and “lack of care” (27%) are common causes of death while “trauma” (41%) is the leading
167 cause of death. Excluding the individuals with undetermined gender from the analyses showed
168 that there was no significant difference in the causes of death between genders ($p=0.5$).

169 *Trauma:* 34 cubs were killed through trauma. In 23 cases the individual responsible for the trauma
170 was known. In 70% (16/23) of these cases trauma was inflicted by the dam, and sires were
171 responsible for 9% (2/23). In one case trauma occurred as late as 137 days after birth, where a
172 female cub was killed by her sire.

173 *Congenital defects:* In a total of eleven cases (9%), congenital defects were the cause of death
174 during the study period. In most cases (musculo-) skeletal abnormalities were the reason for
175 euthanasia. One cub was euthanized due to multiple distal limb developmental abnormalities, and
176 three other cubs were euthanized because of severe scoliosis or other malformations of the
177 vertebral column. In two cases the congenital defects were neurological, and in one case the cause
178 of death was renal dysplasia. Two cubs of the same litter died of syringomyelia, and a cub in
179 another collection was born with a cleft palate. The most severe congenital defects were observed
180 in a cub that was born with an incomplete closure of the abdominal wall and skin, a missing tail,
181 sacrum and anus, and severe scoliosis.

182 *Infectious:* This category included five cases of pneumonia, all of which occurred in neonates.
183 There was one case of cowpox and four cases of bacterial infection, with one of these being
184 systemic. In one case an *Escherichia coli* infection resulted in acute hemorrhagic nephritis and

185 kidney failure, and in another a leg abscess caused by *Streptococcus canis* led to euthanasia. For
186 one case included in “infectious” there were no further details available.

187 *Neoplasia*: Different types of neoplasm were the cause of death during the study period, but the
188 majority were carcinomas (four cases) and haemangiosarcomas (three cases). Of the nine cases
189 overall, seven occurred in the oldest age group “geriatric”, and one occurred in the age group
190 “adult” (a 14 year old female). The youngest animal affected by neoplasia was a two year old
191 male that died of a haemangiosarcoma.

192 *Degenerative*: Renal failure was the cause of death in six of the 13 cases included in the
193 “degenerative” category. It was responsible for 10% and 13% of the deaths of adult and geriatric
194 animals respectively (three cases each). The youngest individual affected by this was a ten year
195 old female that was euthanized. In two cases death was the result of an age-related heart condition,
196 and in two cases degeneration of the central nervous system (CNS) was the cause of death. In
197 nine of the 13 cases the animal was euthanized (information unknown for one animal).

198 *Other causes*: The category “other causes” included many different causes of death: A twelve
199 year old female was killed by the male sharing her enclosure, and the male was subsequently
200 euthanized. Two animals were euthanized after sustaining injuries: an eight year old male
201 developed back paralysis, and a two month old cub suffered a fractured leg. In two cases accidents
202 resulted in death: A two year old female drowned in the wet moat of the enclosure, and a nine
203 year old female died of injuries sustained from an electrical fence. Additionally, two animals in
204 the same collection died after suffering from Vitamin A deficiency, and there were two cases of
205 herniation of the cerebellar vermis in one year old littermates. Lastly, there was one case each of
206 degenerative myelopathy, gastrointestinal ulceration and encephalitis.

207 *Additional relevant findings noted at post mortem*: Only 46 reports received included other
208 findings at post mortem. Apart from the cases described under “congenital”, there were two cases
209 of scoliosis and two cases of other musculoskeletal defects present as secondary findings. Two

210 cubs from the same litter presented with patent ductus arteriosus (PDA), but clinical significance
211 was not established and in an additional case in another collection PDA was suspected.

212 **Discussion**

213 *Mortality:*

214 Most noticeable in this study was the very young average age at death of 2.4 years, with the
215 mortality rate of animals under one year of age being 54%, and only 44% of animals reaching
216 reproductive age. A study on the free-living population in Gujarat showed that 51% of young cubs
217 survived to adult stage (Banerjee and Jhala 2012). However, it is difficult to compare these
218 numbers. Observing cubs under one month of age proved difficult in the wild, and infanticide due
219 to territorial takeovers by an adult male was a substantial cause of cub mortality (Banerjee and
220 Jhala 2012). Cubs under one month of age account for most of the mortalities in this study and
221 infanticide by an adult male was only reported in two cases.

222 For Sakkarbaug Zoo the mortality for under one year-olds is given at 36% (Nowell and Jackson
223 1996), which is comparable to the under one-year mortality rate of Amur leopards in the EEP,
224 which is given at 39% (Blomqvist 2008). If the measurements at Sakkarbaug Zoo include all
225 animals born, then mortality in the EEP is noticeably higher. A possible cause for the higher rate
226 of 54% in the EEP may be inbreeding depression. When comparing inbred individuals of certain
227 species to non-inbred individuals, juvenile mortality was shown to be higher amongst inbred
228 individuals (Ralls and Ballou 1982; Ralls and Ballou 1982; Ralls et al. 1988; Wielebnowski
229 1996). Sakkarbaug Zoo regularly breeds its captive animals with animals that have been brought
230 in from the wild for rehabilitation (Gujarat State Lion Conservation Society 2011). This way fresh
231 genetic material is constantly added to their captive population, which is currently not the case in
232 the EEP.

233 In various species parental experience also plays a role in breeding success and juvenile survival
234 (e.g. Daunt et al. 2007; Meijer et al. 2011). Amongst primiparous lionesses, infanticide is not

235 uncommon and does not mean that the lioness will repeat this behavior with her next litter (AZA
236 Lion Species Survival Plan 2012). Experience may also play a role in low cub survival rates, such
237 as provision of insufficient care or abandonment of cubs. Based on the assumption that cub
238 survival will increase with experience, many captive collections further breed from dams despite
239 previous low cub survival. This study showed that this assumption was correct for the EEP
240 population of Asiatic lions, where the odds for survival to one year of age increase with the
241 number of litters of a dam.

242 *Causes of death:*

243 The fact that “lack of care” and “trauma” are the only causes that are each responsible for over
244 20% of deaths matches the high neonatal mortality rate observed in the study population. 36% of
245 neonatal mortality is due to trauma, which is mostly inflicted by the dam. In the wild, infanticide
246 is the cause of up to 60% of cub mortality (Breitenmoser et al.). A study by Banerjee and Jhala
247 (2012) found that territorial takeovers by males that resulted in infanticide were the main cause
248 for cub mortality. The rate of infanticide in the EEP may be higher than 36%, as some of the
249 carcasses that were eaten may have been killed first, but this study points towards a lower
250 infanticide rate in the EEP compared to the wild. This is probably due to the fact that unrelated
251 males are mainly responsible for infanticide in the wild, whereas the dam is responsible in
252 captivity. It is, however, difficult to establish if there is a difference between the EEP and the free-
253 living population where trauma inflicted by the dam is concerned.

254 Unsurprisingly, degenerative diseases and neoplasia were mainly a concern for old animals. The
255 unusual case of a two year old male dying from a haemangiosarcoma will not be discussed here
256 as it is published as a case report (Vercammen et al. 2015). Renal failure caused 5% of the deaths
257 with a known cause in the whole study population and 13% of the deaths in the geriatric age class.
258 This is not unusual, as various kidney diseases are known to affect old felids, both domestic and
259 wild (Newkirk et al. 2011) and a retrospective study on wild felids in German collections found
260 renal lesions in 87% of the animals included in the study (Junginger et al. 2015).

261 Both causes of death summarized under “infectious” and “other” are a possible cause of concern,
262 but with these occurring only in a few cases they do not represent a significant problem. Cases
263 such as Vitamin A deficiency and accidents resulting in deaths are issues that need to be addressed
264 by the affected collection but do not affect the breeding population as a whole.

265 9% of the known causes of death were due to congenital defects. However, even congenital
266 defects that do not result in death can be of concern for the health and welfare of an animal. Some
267 of these defects may be hereditary in Asiatic lions. Scoliosis was present in at least six individuals
268 from the study population and is known to be hereditary in humans (Shangguan et al. 2008). PDA
269 is hereditary in some dog breeds (Buchanan and Patterson 2003) and although it has not been
270 established as the cause of death in any animal of the study population, it might play a role in
271 cubs dying of problems associated with inappropriate maternal care. In the United States, 3% of
272 humans are born with birth defects (defined as “conditions that [...] have a serious, adverse effect
273 on health, development, or functional ability” (Centers for Disease Control and Prevention)) and
274 this study points towards the percentage in Asiatic lions being higher.

275 *Limitations:*

276 The limitations of this study mainly concern the analyses of the causes of death. For only 49% of
277 the lions that died during the study period the cause of death was established. Although an
278 unknown cause of death was mainly due to lack of reporting by the collections, it would not have
279 been possible to know the cause of death for all animals. In 15% of the received reports
280 determination of the cause of death was not possible because the carcass had been eaten. Even
281 the known causes of death cannot be viewed with absolute certainty. Many collections only
282 provided a simple statement of the cause of death and it could not be verified by a post mortem
283 report. Cases recorded as “stillbirth” have to be considered with caution, as the exact definition
284 of what classifies as a stillbirth at the different collections is not clear.

285 The extent of post mortem examinations conducted differed greatly, which is reflected by the fact
286 that a lot of deceased cubs are of undetermined sex. Conditions present might have gone unnoticed

287 due to an inadequate post mortem exam. For example a thorough cardiac exam is necessary in
288 order to recognize PDA, but is only carried out in a few cases. In general, the prevalence of
289 congenital defects such as PDA and scoliosis might be underestimated. As mentioned above,
290 many cubs do not receive a full post mortem examination, so if a congenital defect was not the
291 cause of death or the reason for euthanasia it likely went unnoticed.

292 Nonetheless, a higher compliance rate would have allowed for a more detailed analysis in some
293 areas of this study. Although data analysis showed that the odds for cub survival to one year of
294 age increased with the number of litters a dam had, it was not possible to look into the reasons for
295 this. If the cause of death had been established for more cubs, it might have been possible to
296 examine whether the increasing odds for survival correlate with a decrease in incidences of “lack
297 of care” and “trauma”. Comparison between cub mortality in the wild and in the EEP was also
298 limited, as the cause of death was unknown for many cubs. Although it will never be possible to
299 establish the cause of death for each individual, a higher compliance rate could have enabled a
300 more significant comparison.

301 *Implications for the future:*

302 For many large mammals living in captive collections the management of geriatric problems is
303 becoming increasingly important. This is an issue mainly concerning the individual and not the
304 population as a whole, and research on geriatric problems in Asiatic lions has been scarce so far.
305 As in domestic cats, renal disease is a concern in older animals. Dietary adjustments play an
306 important part in managing chronic renal failure (CRF) in domestic cats. There is evidence that
307 diets specifically designed for CRF management might increase survival time (Elliott et al. 2000)
308 as well as manage the clinical signs of uremia (Rubin 1997; Elliott 2006). Protein restricted feeds
309 are used to reduce disturbances in mineral, electrolyte and vitamin balance (Rubin 1997; Elliott
310 2006), and diets for dogs and cats with CRF typically have a high fat content as this represents a
311 non-protein energy source (Elliott 2006). Research on captive cheetahs showed that the protein
312 to fat ratio was lower for whole rabbit carcass diets than for meat-only diets (Depauw et al. 2012).

313 Whole carcass feeds might thus be an option for managing renal disease in Asiatic lions, but
314 further research is needed to validate the effects in lions.

315 Further research is also needed to answer questions raised during this study. Comparing the free-
316 living, EEP and Sakkarbaug Zoo populations is currently difficult, as not all necessary
317 information is available. Knowing if there is a difference in infant mortality in the EEP compared
318 to the other two populations could show if inbreeding might be affecting the Asiatic lions in
319 Europe. Not only does the infant mortality rate itself require further investigation, but the reasons
320 for cub mortality are also difficult to compare. Especially trauma inflicted by the dam needs to be
321 explored in more detail, as it is unclear how the rate observed in the EEP compares to the other
322 populations. Furthermore, the reasons for a dam killing or abandoning her cubs need to be
323 considered. A mortality review for red foxes showed, that maternal culling of unhealthy cubs in
324 order to improve the survival chances of healthy siblings likely played a role in trauma induced
325 neonatal death (Acton et al. 2000). Knowing which cases of dams killing or abandoning their cubs
326 are due to selective culling and which are due to stress or a lack of experience might point towards
327 beneficial management actions.

328 Another important aspect raised by this study is the prevalence of congenital defects. Especially
329 those congenital defects that might be hereditary require investigation. Before being able to
330 establish heritability, the actual prevalence of birth defects, not only of those leading to death,
331 needs to be established. A first step in learning more about congenital defects would be to
332 implement a common necropsy protocol in the EEP. As mentioned, cubs often only receive a
333 superficial necropsy leading to conditions possibly going unnoticed. A common protocol could
334 include examinations needed to establish if a congenital defect known to affect the EEP is present.
335 For example, a thorough cardiac exam at each necropsy could show if PDA is a problem with a
336 significant impact or not. If it is discovered that congenital defects such as scoliosis or PDA are
337 hereditary, decisions may be taken to not breed from parents that have produced offspring with
338 these conditions in the past. The lack of a common necropsy protocol has already limited other

339 mortality studies (e.g. Maia and Gouveia 2002; Yss et al. 2012), and the implementation of such
340 a protocol may reveal additional diseases within the population not discovered during this study.
341 Both congenital defects and high infant mortality are possibly due to inbreeding depression.
342 Establishing this will however be a slow process, but the risk alone should justify the addition of
343 new genetic material to the EEP. If inbreeding depression is an issue in the EEP, this might be a
344 precautionary measure to avoid an increase in cub mortality rates and prevalence of congenital
345 defects. Artificial insemination (AI) using sperm from free-living Asiatic lions or from those at
346 Sakkarbaug Zoo would be a theoretical option, but as AI on lions has so far not resulted in live
347 offspring (Goeritz et al. 2012), this is not yet a viable option. The best option available at the
348 moment would be for ZSL and GFD to continue with their attempt to import Asiatic lions from
349 Sakkarbaug Zoo to the EEP.

350

351 **Conclusions**

- 352 1. The high neonate mortality was the most noticeable result of this study. Low cub survival rates
353 might be a sign of inbreeding depression, but more comparable research is needed to establish
354 this.
- 355 2. Investigation into litter survival in relation to parity of the dam showed that anecdotal evidence
356 of cub survival increasing with litters is supported by data collected here for Asiatic lions in the
357 EEP.
- 358 3. A common necropsy protocol for all Asiatic lions in the EEP would enable a more detailed
359 investigation into several areas, including the prevalence of congenital defects. A more detailed
360 necropsy might also reveal underlying reasons for dams abandoning or killing their cubs, which
361 are the main causes of death for young animals.

362 Overall this study pointed to some areas that require further investigation, but some issues
363 identified might be solved if new genetic material were to be added to the European population
364 of Asiatic lions.

365

366 **Acknowledgements**

367 This study was carried out in fulfilment of the Wild Animal Biology/Health MSc degree (O.M.)
368 at the Royal Veterinary College and the Zoological Society of London. A special thanks goes to
369 all Asiatic lion holders that supplied data on deaths in their collection.

370

371 **References**

372 Acton a E, Munson L, Waddell WT. 2000. Survey of necropsy results in captive red wolves (*Canis*
373 *rufus*), 1992-1996. *J. Zoo Wildl. Med.* 31:2–8.

374 AZA Lion Species Survival Plan. 2012. Lion Care Manual. Association of Zoos and Aquariums,
375 Silver Spring, MD. :143.

376 Banerjee K, Jhala Y V. 2012. Demographic parameters of endangered Asiatic lions (*Panthera leo*
377 *persica*) in Gir Forests, India. *J. Mammal.* 93:1420–1430.

378 Banerjee K, Jhala Y V., Chauhan KS, Dave C V. 2013. Living with Lions: The Economics of
379 Coexistence in the Gir Forests, India. *PLoS One* 8:1–11.

380 Bates D, Maechler M, Bolker B, Walker S. 2015. *lme4*: Linear mixed-effects models using Eigen
381 and S4_. R package version 1.1-8, <URL: <http://CRAN.R-project.org/package=lme4>>.

382 Blomqvist L. 2008. the Status of the Snow Leopard in the Eep-Program in 2007. 1992.

383 Breitenmoser U, Breitenmoser-Würsten C, von Arx M, Lanz T. Cat Specialist Group - Asiatic
384 lion *Panthera leo persica*.

- 385 Breitenmoser U, Mallon DP, Ahmad Khan J, Driscoll C. 2008. *Panthera leo* ssp. *persica*. The
386 IUCN Red List of Threatened Species. Version 2014.3. <www.iucnredlist.org>. Downloaded on
387 21 July 2015.
- 388 Buchanan JW, Patterson DF. 2003. Etiology of patent ductus arteriosus in dogs. *J. Vet. Intern.*
389 *Med.* 17:167–171.
- 390 Centers for Disease Control and Prevention. Update on Overall Prevalence of Major Birth
391 Defects--Atlanta, Georgia, 1978-2005. *MMWR Morb Mortal Wkly Rep.* 2008;57(1):1-5.
- 392 Clancy MM, Woc-Colburn M, Viner T, Sanchez C, Murray S. 2013. Retrospective analysis of
393 mortalities in elephant shrews (*Macroscelididae*) and tree shrews (*Tupaiaidae*) at the Smithsonian
394 National Zoological Park, USA. *J. Zoo Wildl. Med.* 44:302–9.
- 395 Daunt F, Wanless S, Harris MP, Money L, Monaghan P. 2007. Older and wiser: Improvements
396 in breeding success are linked to better foraging performance in European shags. *Funct. Ecol.*
397 21:561–567.
- 398 Depauw S, Hesta M, Whitehouse-Tedd K, Stagegaard J, Buyse J, Janssens GPJ. 2012. Blood
399 values of adult captive cheetahs (*Acinonyx jubatus*) fed either supplemented beef or whole rabbit
400 carcasses. *Zoo Biol.* 31:629–641.
- 401 DeshGujarat. 2015. Asiatic Lion population up from 411 to 523 in five years. Retrieved 21 July
402 2015.
- 403 Driscoll C a., Menotti-Raymond M, Nelson G, Goldstein D, O'Brien SJ. 2002. Genomic
404 microsatellites as evolutionary chronometers: A test in wild cats. *Genome Res.* 12:414–423.
- 405 Elliott D a. 2006. Nutritional Management of Chronic Renal Disease in Dogs and Cats. *Vet. Clin.*
406 *North Am. - Small Anim. Pract.* 36:1377–1384.
- 407 Elliott J, Rawlings JM, Markwell PJ, Barber PJ. 2000. Survival of cats with naturally occurring
408 chronic renal failure: effect of dietary management. *J. Small Anim. Pract.* 41:235–242.

- 409 Goeritz F, Painer J, Jewgenow K, Hermes R, Rasmussen K, Dehnhard M, Hildebrandt TB. 2012.
410 Embryo Retrieval after Hormonal Treatment to Control Ovarian Function and Non-surgical
411 Artificial Insemination in African Lions (*Panthera leo*). *Reprod. Domest. Anim.* 47:156–160.
- 412 Gujarat State Lion Conservation Society. 2011. Sakkarbaug Zoo - Breeding Programme.
- 413 Hope K, Deem SL. 2006. Retrospective Study of Morbidity and Mortality of Captive Jaguars
414 (*Panthera onca*) in North America: 1982-2002. *Zoo Biol.* 25:501–512.
- 415 Junginger J, Hansmann F, Herder V. 2015. Pathology in Captive Wild Felids at German
416 Zoological Gardens. *PLoS One* 10:1–30.
- 417 Maia OB, Gouveia a MG. 2002. Birth and mortality of maned wolves *Chrysocyon brachyurus*
418 (Illiger, 1811) in captivity. *Braz. J. Biol.* 62:25–32.
- 419 Martínez F, Manteca X, Pastor J. 2013. Retrospective study of morbidity and mortality of captive
420 Iberian lynx (*Lynx pardinus*) in the ex situ conservation programme (2004-June 2010). *J. Zoo*
421 *Wildl. Med.* 44:845–52.
- 422 Meijer T, Norén K, Angerbjörn A. 2011. The impact of maternal experience on post-weaning
423 survival in an endangered arctic fox population. *Eur. J. Wildl. Res.* 57:549–553.
- 424 Newkirk KM, Newman SJ, White L a, Rohrbach BW, Ramsay EC. 2011. Renal lesions of
425 nondomestic felids. *Vet. Pathol.* 48:698–705.
- 426 Nielsen RK. 2014. European Regional Studbook *Panthera leo persica*.
- 427 Nowell K, Jackson P. 1996. Status survey and conservation action plan: wild cats. *Biol.*
428 *Conserv.*:384.
- 429 O'Brien SJ, Joslin P, Smith III GL, Wolfe R, Schaffer N, Heath E, Ott-Joslin J, Rawal PP,
430 Bhattacharjee KK, Martenson JS. 1987. Evidence for African origins of founders of the asiatic
431 lion species survival plan. *Zoo Biol.* 6:99–116.

- 432 R Core Team. 2015. R: A language and environment for statistical computing. R Foundation for
433 Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.
- 434 Ralls K, Ballou J. 1982. Effect of inbreeding on juvenile mortality in some small mammal species.
435 Lab. Anim. 16:159–166.
- 436 Ralls K, Ballou JD, Templeton A. 1988. Estimates of Lethan Equivalents and the Cost of
437 Inbreeding in Mammals. Conserv. Biol. 2:185–192.
- 438 Rubin SI. 1997. Chronic renal failure and its management and nephrolithiasis. Vet. Clin. North
439 Am. Small Anim. Pract. 27:1331–1354.
- 440 Shangguan L, Fan X, Li M. 2008. Inheritance Involved in the Pathogenesis of Idiopathic
441 Scoliosis. Clin Orthop:104–114.
- 442 Therneau T. 2015. `_A Package for Survival Analysis in S_`. version 2.38, <URL: [http://CRAN.R-](http://CRAN.R-project.org/package=survival)
443 [project.org/package=survival](http://CRAN.R-project.org/package=survival)>.
- 444 Vercammen F, Brandt J, Brantegem L Van, Bosseler L, Ducatelle R. 2015. Haemangiosarcoma
445 in a captive Asiatic lion (*Panthera leo persica*). 5:52–55.
- 446 Wielebnowski N. 1996. Reassessing the relationship between juvenile mortality and genetic
447 monomorphism in captive cheetahs. Zoo Biology 15:353-69. Keywords: 3US/Acinonyx
448 jubatus/captive breeding/captivity/cheetah/genetics/homozygosity/ husbandry/juvenil.
- 449 Wildt DE, Bush M, Goodrowe KL, Packer C, Pusey AE, Brown JL, Joslin P, O'Brien SJ. 1987.
450 Reproductive and genetic consequences of founding isolated lion populations. Nature 329:855–
451 857.
- 452 Yss WF, Enker WC, Obert RN, Lauss CM, F VONHO. 2012. Why Do Greater One-Horned
453 Rhinoceroses (*Rhinoceros Unicornis*) Die? – an Evaluation of Necropsy Reports. Int. Conf. Dis.
454 Zoo Wild Anim.:54–61.
- 455 Zingg R. 2007. Asiatic Lion Studbooks : a short history Asiatic Lion Studbooks. XXII:4.

456 Table 1. Known causes of death per age category

	Neonate	Juvenile	Adult	Geriatric	Total
Stillbirth	12 (15%)	-	-	-	12 (9%)
Lack of care	29 (36%)	-	-	-	29 (22%)
Trauma	28 (35%)	6 (26%)	-	-	34 (26%)
Congenital	4 (5%)	7 (30%)	-	-	12 (9%)
Infectious	7 (9%)	2 (9%)	2 (14%)	-	11 (8%)
Neoplasia	-	1 (4%)	1 (7%)	7 (47%)	9 (7%)
Degenerative	-	-	5 (36%)	8 (53%)	13 (10%)
Other cause	1 (1%)	7 (30%)	6 (43%)	-	13 (10%)
Total	81	23	14	15	133

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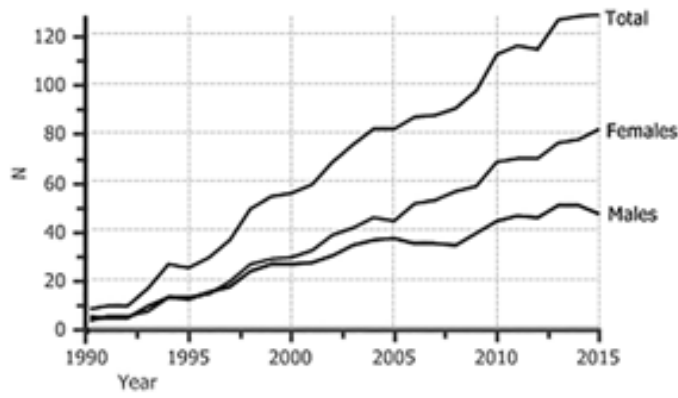
459 Table 2. Known causes of death per gender

	Male	Female	Undetermined
Stillbirth	3 (6%)	4 (7%)	5 (23%)
Lack of care	11 (21%)	12 (20%)	6 (27%)
Trauma	4 (27%)	11 (19%)	9 (41%)
Congenital	6 (12%)	4 (7%)	1 (5%)
Infectious	6 (12%)	5 (8%)	-
Neoplasia	2 (4%)	7 (12%)	-
Degenerative	7 (13%)	6 (10%)	-
Other cause	3 (6%)	10 (17%)	1 (5%)
Total	52	59	22

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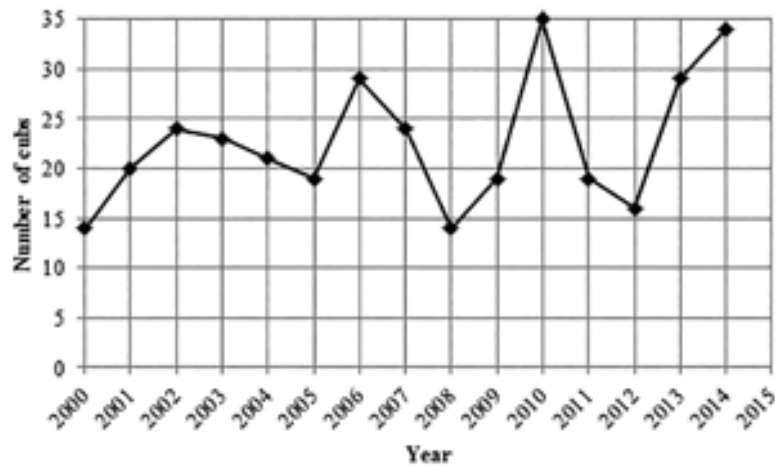
462 Figure 1. Number of animals living in the EEP population between 1990 and 2015. Figure
463 supplied by the EEP coordinator.



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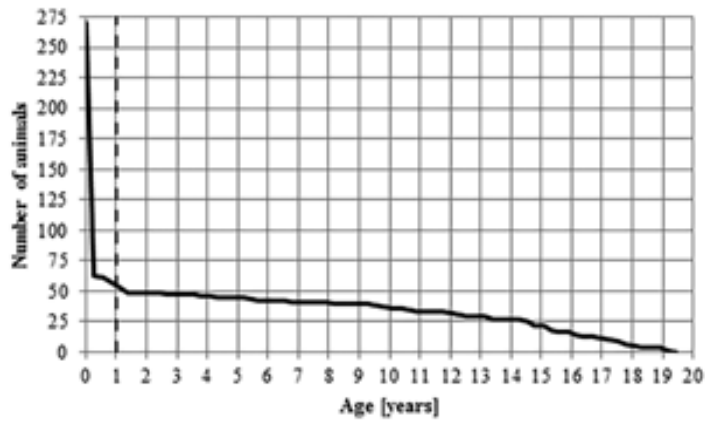
466 Figure 2. Number of cubs born per year. Graph includes all animals born during the study period
467 (n = 340).



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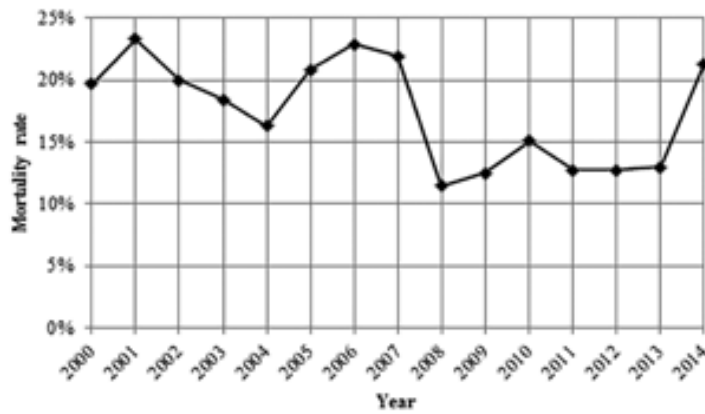
470 Figure 3. Number of animals that reached the respective age. Graph only includes animals that
471 died during the study period (n = 270).



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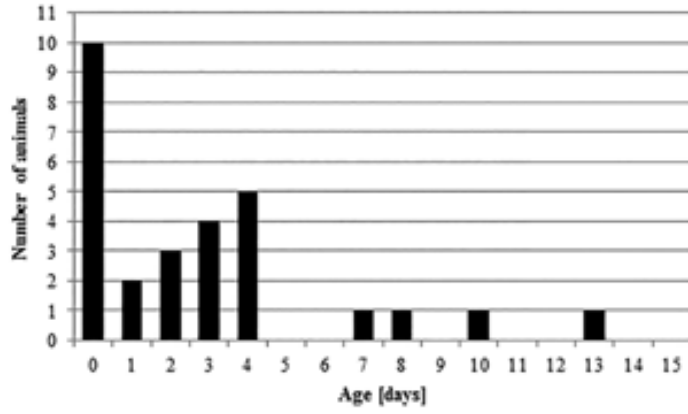
474 Figure 4. Yearly mortality rate between 2000 and 2014.



475

476

477 Figure 5. Number of animals that died due to trauma at the respective age. Only including animals
478 that were under 15 days old (n = 28).



479