

Does nest size matter to laying hens?

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Abstract

Laying hens in loose housing systems have access to group-nests which provide space for several hens at a time to lay their eggs. They are thus rather large and the trend in the industry is to further increase the size of these nests. Though practicality is important for the producer, group-nests should also cater to the egg-laying behaviour of hens to promote good welfare. One of the factors playing a role in the attractiveness of a nest is the amount of enclosure: hens prefer more enclosure when having a choice between different nest types. The aim of this study was to investigate if hens prefer smaller group-nests to lay their eggs given that they may seem more enclosed than larger nests.

The relative preference of groups of laying hens for two nest sizes—0.43 m² vs. 0.86 m²—was tested in a free-access choice test. The experiment was conducted in two consecutive trials with 100 hens each. They were housed from 18 to 36 weeks of age in five groups of 20 animals and had access to two commercial group-nests differing in internal size only. We counted eggs daily as a measure of nest preference. At 28 and 36 weeks of age, videos were taken of the pens and inside the nests on one day during the first 5 hours of lights-on. The nest videos were used to record the number of hens per nest and their behaviour

27 with a 10 min scan sampling interval. The pen videos were observed continuously to count the total number
28 of nest visits per nest and to calculate the duration of nest visits of five focal hens per pen.

29 We found a relative preference for the small nest as more eggs, fewer nest visits per egg and longer nest
30 visit durations were recorded for that nest. In addition, more hens—including more sitting hens—were in the
31 small nests during the main egg-laying period, while the number of standing hens did not differ. These
32 observations indicate that even though both nests may have been explored to a similar extent, the hens
33 preferred the small nest for egg-laying.

34 **Keywords**

35 Laying hens, nest size, group-nests, nest choice, preference test, animal welfare

36

1. Introduction

Humans feel safer in spaces perceived as having more enclosure, which is the degree to which spaces are visually defined by surrounding surfaces (Alkhresheh, 2007; Stamps, 2005). And small spaces give a greater feeling of enclosure compared with large spaces (Alkhresheh, 2007). Similarly, in laying hens, a smaller nest may provide a greater sense of protection than a larger one given that the main purpose of a nest is to provide the hens with an isolated and safe place to lay their eggs (Duncan, 1978). Hens are also more motivated to gain access to enclosed nest sites compared with open nest sites (Appleby and McRae, 1986; Zupan et al., 2008). However, the current trend in the industry is to increase the size of group-nests (for example through removal of side walls) as these are cheaper to build (E. Fröhlich, personal communication).

Commercial rollaway group-nests used in free-run housing systems range in floor surface area from approximately 0.5 to 1.8 m², with a relatively constant depth of 0.5 to 0.6 m and a width of up to 3 m. Legal requirements exist for group-nests in a few countries but they only pertain to the maximum number of hens allowed per m² of nest surface area: 100 hens per m² in Switzerland (Animal Welfare Ordinance, 2008) and 120 hens per m² in the EU and New Zealand (CEC, 1999; NAWAC, 2012). In Switzerland, commercial farm animal housing systems or equipment, including nests for laying hens, must be approved by the Federal Veterinary Office before they can be sold to producers (Wechsler, 2005). Therefore, various nest properties have recently been examined experimentally (Buchwalder and Fröhlich, 2011; Kruschwitz et al., 2008; Stämpfli et al., 2011; Stämpfli et al., 2012). Buchwalder and Fröhlich (2011) used preference tests to compare commercial group-nests with simple wooden rollaway group-nests (with only a thin plastic mat on the nest floor) and found smaller nests often preferred by the hens for egg-laying. Similarly, Holcman et al. (2007) reported that broiler breeder hens laid more eggs in smaller individual nests than larger group-nests. In captive-reared partridges given a choice between three nest types, a preference was shown for nests providing the least amount of internal space and resembling natural conditions the most (Robles et al., 2001). However, the results from the previous three studies are confounded as many characteristics differed between the nest types; it is unclear whether nest size affected the choice of the hens. The relationship between nest size and nest use, predation rate and reproductive characteristics has been investigated in studies of wild birds (ex: Lambrechts et al., 2011; Soler et al., 1998; Weidinger, 2004). But it

65 is difficult to draw relevant conclusions from these studies for domestic laying hens as they are held in
66 artificial conditions, are provided with formed nests and do not reproduce.

67 Our aim was to test the hypothesis that hens prefer smaller over larger group-nests as a site to lay their
68 eggs. Commercial group-nests were used and hens were tested in groups to mimic commercial housing
69 systems. Thus, groups of hens were given a free choice between two identical group-nests that differed in
70 size only. We expected that hens would lay more eggs, show fewer nest visits per egg, spend more time,
71 and sit more in the smaller nests given that such effects are characteristic for preferred nests (Kruschwitz et
72 al. 2008; Struelens et al., 2008).

73 **2. Materials and methods**

74 *2.1. Animals and housing*

75 The relative preference for nest size was assessed in two consecutive trials, each with a different batch of a
76 commercial strain of laying hens (Lohmann Selected Leghorns) in the winter of 2011/2012 and in the spring
77 of 2012. For each trial, non-beak trimmed day-old chicks were purchased from a commercial hatchery.

78 They were reared in a pen (18 m²) until 9 weeks of age at which time they were split into two groups of 120
79 animals (2 pens of 18 m²) with unrestricted access to water, commercial feed, perches and sawdust
80 bedding. At 18 weeks of age, 100 hens were randomly chosen from the 240 animals, moved to the
81 experimental barn and assigned to five pens in groups of 20.

82 The experimental pens were of identical size (3 x 3 x 2 m, length x width x height) and arranged in two rows
83 (Fig.1a). The hens had access to sawdust bedding, three perches (0.3 m apart horizontally; at 0.6, 1.3 and
84 1.6 m high), *ad libitum* commercial layer mash feed from a round feeder and water from 8 nipple drinkers.

85 There were visual barriers up to a height of 1.6 m between the pens. Two group-nests differing in internal
86 size only were placed opposite each other on either side of the door in each pen (Fig. 1a). Their position
87 was counterbalanced across pen and trial. The hens had access to both nests at all times.

88 The group-nests were of a rollaway type commercially available in Switzerland. The large nest was the
89 unmodified version with internal dimensions of 0.60 x 1.44 m and the floor of the small nest was half of this
90 size with internal dimensions of 0.60 x 0.72 m (Fig. 1a). The small nest was modified by adding two internal
91 walls and closing off the front edges of the nest. The walls of both nests were made up of plywood which

92 was painted black. Both nests looked identical from the outside and were closed on three sides with a roof,
93 two red curtains in the front (0.60 x 0.45 m, width x height) with an entry of 0.25 m in the middle and a
94 platform to access the nest made up of a metal grid (0.30 x 1.44 m, width x length). They had a floor
95 covered in brown AstroTurf® and divided in two with both parts slanting towards the middle (Fig. 1b). The
96 front floor was higher than the rear to allow eggs to roll onto the egg collection belt. The light intensity on
97 the floor in the rear of the nest was 0.7 ± 0.1 lux in the large nest and 0.6 ± 0.1 lux in the small nest in both
98 trials.

99 From the first day of age until the end of the experiment, artificial light was used to prevent seasonal effects
100 of natural daylight on egg-laying behaviour. The photoperiod followed standard commercial practice. At 18
101 weeks of age, the hens had 10 hours of light from 6:30 to 16:30 h with a 15 min twilight phase at the
102 beginning and end of the day. Light exposure was then gradually increased by 30 min each week until 15
103 hours of light was reached in week 28 of age (1:30 to 16:30 h); the photoperiod then remained constant
104 until the end of the study. In the experimental barn the average light intensity at bird height on the pen
105 floors was 7.8 ± 1.0 lux and temperature was maintained at 17.7 ± 4.3 °C in trial 1 and 18.9 ± 3.2 °C in trial
106 2. The hens were kept in the experimental barn until 37 weeks of age and were then sold to local farmers.

107 2.2. *Data collection*

108 The number of eggs laid per nest was our primary outcome variable used to assess nest preference, based
109 on previous studies also having used egg number as the main criterion for nest attractiveness in choice
110 tests (Buchwalder and Fröhlich, 2011; Cooper and Appleby, 1996; Duncan and Kite, 1989). Nest and floor
111 eggs were collected and recorded once daily between 9:00 and 13:00 h from 18 until 36 weeks of age
112 given that by this age most hens have gone through several egg-laying sequences (Icken et al., 2008). We
113 also recorded behavioural data, which were our secondary outcome variables, to gain additional
114 information on nest preference. To observe the hens during the egg-laying phase, two infra-red digital video
115 cameras (Conrad, BP258IR) were mounted in each nest. Additionally, a digital video camera (Samsung,
116 SCC-C4305P) was installed above each pen providing a complete outside view of the nests. Videos of the
117 pens and inside the nests were taken for a 5 hour period after the lights were turned on once during the
118 28th week of age and once during the 36th week of age. This time frame was chosen as most hens lay

119 their eggs within the first 5 hours of the day (Lentfer et al., 2011; Riber, 2010) and verified during both trials:
120 more than 95 % of eggs were laid by the time the lights had been on for 5 hours.

121 Videos from inside the nests were analyzed with a 10 min scan sampling method using the behaviours
122 described in Table 1. The pen footage was observed continuously during both 5 h periods and all nest visits
123 were recorded to calculate the number of nest visits per egg. In addition, five hens per pen were randomly
124 chosen and marked with a blue animal marker spray (Raidex GmbH) on their back at 23 weeks of age for
125 individual identification. These focal hens were observed continuously to calculate individual nest visit
126 durations (time of nest exit - time of nest entry) (Table 1). To speculate on which nest the focal hens laid
127 their eggs in, the two longest nest visits per day were extracted from the data set. If both were in the same
128 nest or if the longest visit was 50 % greater in duration than the other, we assumed that this visit was the
129 one during which the egg was laid. We were however unable to confirm this as it was very difficult to see
130 the hens laying their eggs due to crowding on the rear nest floors.

131 All behavioural data were viewed and analyzed using the behavioural observation software package
132 INTERACT (Mangold International GmbH, 2011, Version 9, Arnstorf, Germany). Blinding the researcher to
133 treatment was impossible when collecting eggs and analyzing videos inside nests but the videos taken from
134 above the pen could be analyzed blindly since the nests looked identical from the outside.

135 The Cantonal Veterinary Office approved this experiment (Bern, Switzerland, Approval BE110/11) and we
136 followed the ethical guidelines of the International Society of Applied Ethology.

137 2.3. *Statistical analysis*

138 All statistical analyses were performed with R (version 3.0.1) and R Studio (version 0.97.551). *P*-values
139 below 0.05 were considered significant for all analyses and the function lme in the R package nlme
140 (Pinheiro et al., 2013) was used to fit linear mixed effects models. The assumptions of normally distributed
141 errors and homogeneity of variance were examined graphically with the use of the Normal plot (residual
142 quantiles versus quantiles of a normal distribution) and the Tukey-Anscombe plot (residuals versus
143 estimates). To satisfy these assumptions, data on the number of nest visits per egg and the number of nest
144 visits for focal hens were square-root transformed; data on mean number of standing hens per nest and on
145 mean nest visit duration were log-transformed. Results shown are untransformed means.

146 The proportion of eggs in the small nest was compared with 50 % in the model since the distribution of
147 eggs in both nests was not independent. Week of age was included in the model as a fixed effect. Data
148 reported for eggs are mean proportion of eggs per nest per day, averaged over week. To investigate the
149 role of nest size on the mean number of nest visits per egg per day, nest size, week of age and their
150 interactions were specified in the model as fixed effects. For the mean number of hens in the nests per
151 scan averaged over hour (for standing, sitting and total hens), nest size, week of age, hour and all two-way
152 interactions were included in the model as fixed effects. Finally, for the mean duration of nest visits per
153 focal hen per day, nest size, week of age, hour and all two-way interactions were in the model as fixed-
154 effects. In all models trial and pen were included as random effects (as well as hen for the focal animal
155 data).

156 The full models were reduced using the function stepAIC in the R package MASS (Venables and Ripley,
157 2002) that performs stepwise backward model selection using Akaike's information criterion. When there
158 was a statistically significant interaction, the models were run separately for each hour and the interaction
159 terms were removed.

160 **3. Results**

161 3.1. *Egg numbers and nest size*

162 The hens started laying eggs during their 19th week of age and as expected reached 50 % production by 21
163 weeks of age. We collected a total of 10'002 eggs from the nests from the beginning of egg-laying at 19
164 weeks of age until the end of the experiment at 36 weeks of age (6'157 eggs from the small nests and
165 3'845 eggs from the large nests). The proportion of eggs laid on the floor was 3.3 % in trial 1 and 4.1 % in
166 trial 2. During early egg-laying from 19 until 20 weeks of age, we found no evidence of nest preference
167 (proportion of eggs to total eggs in nests: 0.37 ± 0.06 in the small nest vs. 0.31 ± 0.04 in the large nest, $F_{1,9}$
168 = 1.85, $P = 0.21$). Between 21 and 36 of age, the hens laid a greater proportion of eggs in the small nests
169 compared with the large nests and we found no evidence that the age of the hens in that period influenced
170 this egg-laying pattern (Fig. 2a).

171 3.2. *Behaviour of all hens and nest size*

172 More nest visits per egg occurred in the large nest than in the small nest and at 36 weeks of age than at 28
173 weeks of age (Fig. 2b).

174 For the video observations inside the nests, we pooled the data for the number of hens on the front and
175 rear floors as few hens were observed sitting on the front floor of the nests. Out of the total number of hens
176 sitting per scan, only 4.66 ± 0.93 % of hens sat on the front floor of the small nest and only 5.50 ± 0.98 %
177 sat on the front floor of the large nest. The total number of hens per scan ranged from 0 to 9 hens in the
178 small nest and from 0 to 7 hens in the large nest (sitting hens + standing hens). We found an interaction
179 between nest size and hour ($F_{4,176} = 3.15$, $P = 0.02$, Fig. 3a), indicating that nest size affected the total
180 number of hens in nests, though the number depended on the hour: there were more hens in the small nest
181 during hour 3 and 4 after lights-on than in the large nest. We also found an interaction between week of
182 age and hour ($F_{4,176} = 3.15$, $P = 0.02$, Fig. 3b) with more hens in the nests during the first 2 hours of lights-
183 on during week 36 of age compared with week 28 of age.

184 For the number of sitting hens in the nests, we found an interaction between nest size and hour ($F_{4,181} =$
185 2.55 , $P = 0.04$, Fig. 3c), with more hens sitting in the small nest during hour 3 and 4 after lights-on than in
186 the large nest, but we found no evidence that week of age affected the number of sitting hens ($F_{1,181} = 0.34$,
187 *ns*, Fig. 3d).

188 There was no evidence that the mean number of hens standing in the small nest (0.93 ± 0.55) differed from
189 the mean number of standing hens in the large nest (0.95 ± 0.72 , $F_{1,179} = 0.25$, *ns*, Fig. 3c). But there was
190 an interaction between week of age and hour ($F_{4,179} = 3.37$, $P = 0.01$), with more hens standing in the nests
191 during the first 2 hours of lights-on when they were 36 weeks of age compared with 28 weeks of age (Fig.
192 3f).

193 3.3. *Behaviour of focal hens and nest size*

194 Of the 50 focal hens, we excluded two from the analysis (one died during the experiment and the other did
195 not enter the nests on the days of observation). Most focal hens visited both nests on both days of
196 observation (77.1 % of focal hens) while 16.7 % visited two nests on only one day and 6.2 % visited one
197 nest only on both days. Hens visited the small nest an average of 16.11 ± 2.23 and the large nest an

198 average of 13.29 ± 1.72 times per day (neither nest size nor week of age affected these numbers: $F_{1,124} =$
199 0.59 , *ns* and $F_{1,124} = 0.34$, *ns*, respectively)

200 Focal hens showed a longer mean nest visit duration in the small nest (8.31 ± 1.65 min) compared with the
201 large nest (3.69 ± 0.79 min, $F_{1,129} = 7.49$, $P < 0.05$). For the longest visit durations, data from nine hens was
202 excluded (similar maximum nest visit duration in large and small nest). We found that at 28 weeks of age,
203 37.8 % of focal hens had their longest nest visit in the large nest and 62.2 % had their longest nest visit in
204 the small nest. At 36 weeks of age, 37.2 % of hens had their longest nest visit in the large nest while for
205 62.8 % of hens it was in the small nest. Of these hens, 77.1 % had their longest nest visit in the same nest
206 on both days. We found no evidence that the longest nest visit duration was affected by nest size (small
207 nest: 31.23 ± 3.69 min, large nest: 23.23 ± 2.97 min, $F_{1,38} = 0.32$, *ns*).

208 **4. Discussion**

209 We report here that hens show a relative preference for smaller group-nests. The increased proportion of
210 eggs in the small nest points to a preference for that nest as egg-laying is the final purpose of nest-seeking
211 and nesting behaviour. In addition, behavioural data from two days during the peak egg-laying period
212 reinforces this conclusion. The increased number of nest visits per egg in the large nest implies that it was
213 the less attractive nest as the hens required more nest visits to lay one egg than in the small nest. A high
214 number of nest visits per egg has also previously been associated with less preferred group-nests
215 (Buchwalder and Fröhlich, 2011). We also found more hens overall, more sitting hens and longer nest visit
216 durations in the small nest. Although the increased numbers of sitting hens only occurred during hour 3 and
217 4 after lights-on, this is the time during which most hens lay their eggs (Lentfer et al., 2011). The similar
218 number of standing hens in both nests and the focal hen data suggest that hens explored both nests prior
219 to egg-laying. Other studies found that preferred nest sites resulted in less locomotion, fewer nest visits,
220 longer nest visit durations and more sitting behaviour (Buchwalder and Fröhlich, 2011; Cooper and
221 Appleby, 1995; Freire et al., 1996; Kruschwitz et al., 2008). Kruschwitz et al. (2008) reported that laying
222 hens performed less exploratory behaviour prior to choosing nests with a greater degree of cover and more
223 nesting behaviour in such nests than in more open nest sites. Similarly, nest boxes in furnished cages with
224 plastic curtains received fewer nest visits per egg and resulted in longer nests visits than nests with open
225 fronts (Struelens et al., 2005).

226 The focal hen data demonstrates that although hens spent more time overall in the small nest, the longest
227 nest visit duration did not differ between nests which supports our prediction that this was the nest visit in
228 which eggs were laid. These results agree with Stämpfli et al. (2011) who reported that nest visits in which
229 hens laid an egg lasted between 10 and 90 minutes. Most focal hens were consistent in their nest choice
230 even though a majority of them did visit both nests each day. However, we were unable to assign all
231 individual hens to their eggs and to infer whether or not all hens were exclusive in their choice of nest.

232 The Oxford Dictionary defines size as “the relative extent of something; a thing’s overall dimensions”. We
233 also use the term “nest size” in a relatively broad sense to define the space available in a nest which
234 inherently included differences in floor surface area, wall surface area and curtain surface area between the
235 small and large nest. Since the nests only differed in size, we imply that the hens preferred the small nest
236 due to this characteristic. But there could be explanations for this nest choice other than size. Social factors
237 may have influenced this preference as hens were tested in groups to mimic commercial conditions.
238 Rietveld-Piepers et al. (1985) reported that dominant hens come into lay before subordinate hens, thus the
239 nest choice of dominant hens may influence the choice of the other hens. Furthermore, familiarity of nest
240 position, rather than preference alone, may have affected nest choice once the hens were older and
241 accustomed to egg-laying (Duncan and Kite, 1989). Both of these explanations are however unlikely as 77
242 % of focal hens visited both nests on both days of observation, so we assume that most hens made an
243 active nest choice throughout the experiment. Nests may also be entered for purposes other than egg-
244 laying behaviour such as hiding from other hens, but since we used egg number as the primary criterion to
245 assess preference and recorded behaviour only during the main egg-laying period of the day this should
246 not affect our conclusions.

247 Even though the hens laid more eggs in the small nest, some also laid eggs in the large nest. At least three
248 possible reasons exist for choosing this nest. First, as Kruschwitz et al. (2008) suggested, laying hens may
249 have different needs when it comes to an appropriate nest site; some hens may simply have preferred the
250 large nests for egg-laying due to its size or due to the lower hen density. In fact, three focal hens only
251 entered the large nest on both days of observations. Second, we cannot exclude the possibility that they
252 chose the large nest due to lack of space in the small nest as not all 20 hens could fit in the small nest,
253 especially since all of the hens tried to fit on the rear floor. We counted a maximum of nine hens in the
254 small nest, which was then full, whereas the large nest contained a maximum of seven hens only. Thirdly,

255 social factors also likely played a role in the selection of the large nest. Freire et al. (1998) showed that
256 subordinate hens were more active prior to egg-laying and were displaced from the nest site more often
257 than dominant hens. In our experiment some hens may have been displaced from the small nest and had
258 to use the large nest, however we were unable to assess dominance status in this experiment as the hens
259 were not all individually identified. But the large nest was probably not unattractive enough to disregard it
260 as a nest or to delay the timing of oviposition which laying hens are to some extent able to control (Reynard
261 and Savory, 1999).

262 Older hens entered the nests earlier in the day than when they were younger, which is consistent with the
263 work of Riber (2010), and suggests that once the hens were more experienced they were faster in choosing
264 a nest. And yet, there were more nest visits per egg when the hens were older. This unexpected result and
265 the relatively high number of nest visits per egg compared with other studies is difficult to explain (for
266 example, less than 15 visits per egg in Stämpfli et al., 2011 and 2-17 nest visits per egg in Buchwalder and
267 Fröhlich, 2011). However, one of the nests tested by Buchwalder and Fröhlich (2011) was of the same type
268 as the one used in this study and a similar high number of nest visits per egg was reported (40.29 ± 11.38
269 nest visits per egg compared with 34.86 ± 3.67 nest visits per egg in our experiment). Thus, the nest type
270 itself may be responsible for the hens performing such a high number of nest visits. This is supported by
271 our observations: the hens predominantly used the rear floor of the nest for sitting while the front floor was
272 used for standing or for sitting only when the rear floor was occupied. Such a split floor nest design may be
273 unattractive to laying hens.

274 **5. Conclusion**

275 From this study, we conclude that nest size does matter to laying hens, at least in a small group setting.
276 The hens showed a relative preference for the small group-nest—even though it was half the size of the
277 large nest—as demonstrated by the greater proportion of eggs. The hens may have found the small nests
278 to offer more protection and enclosure than the large nest. Therefore, when designing attractive group-
279 nests their size should be taken into account.

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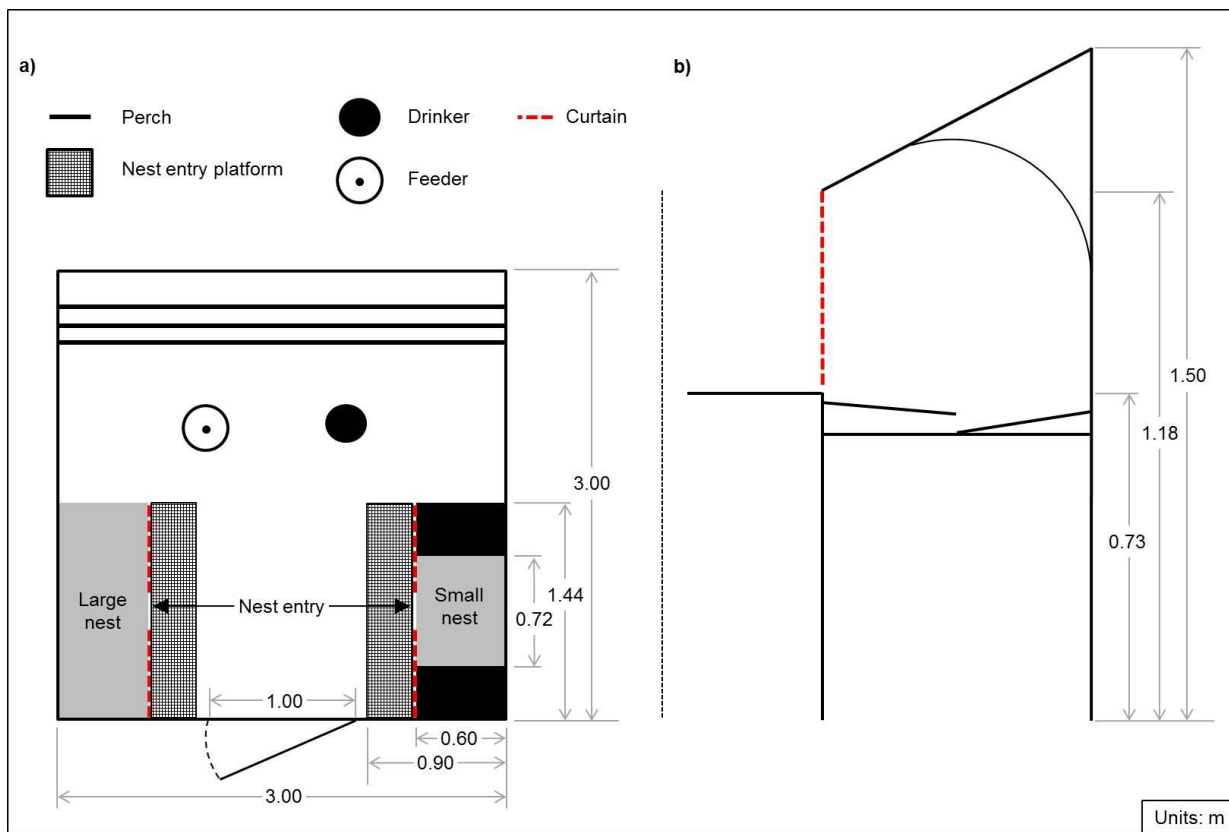
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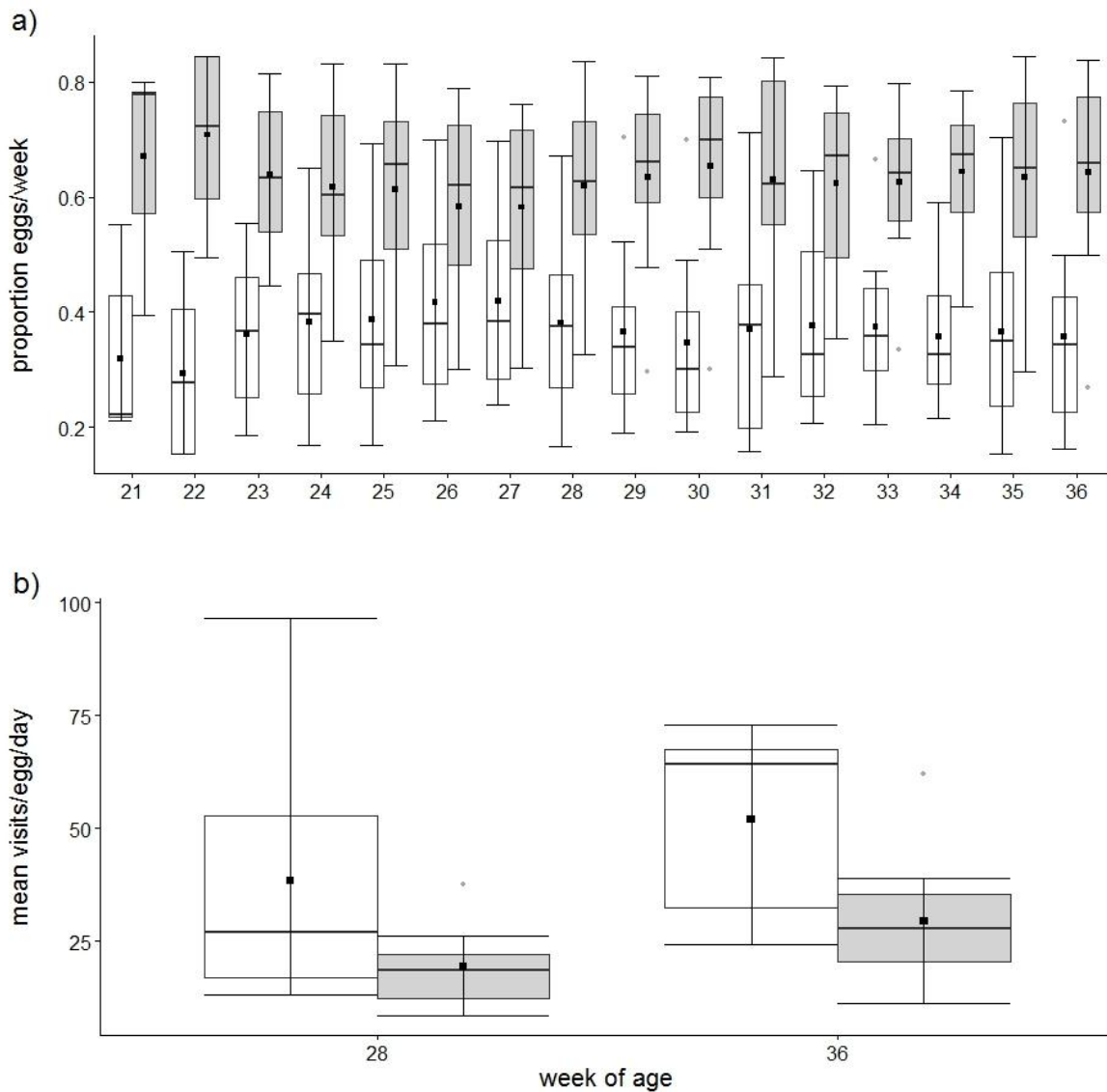
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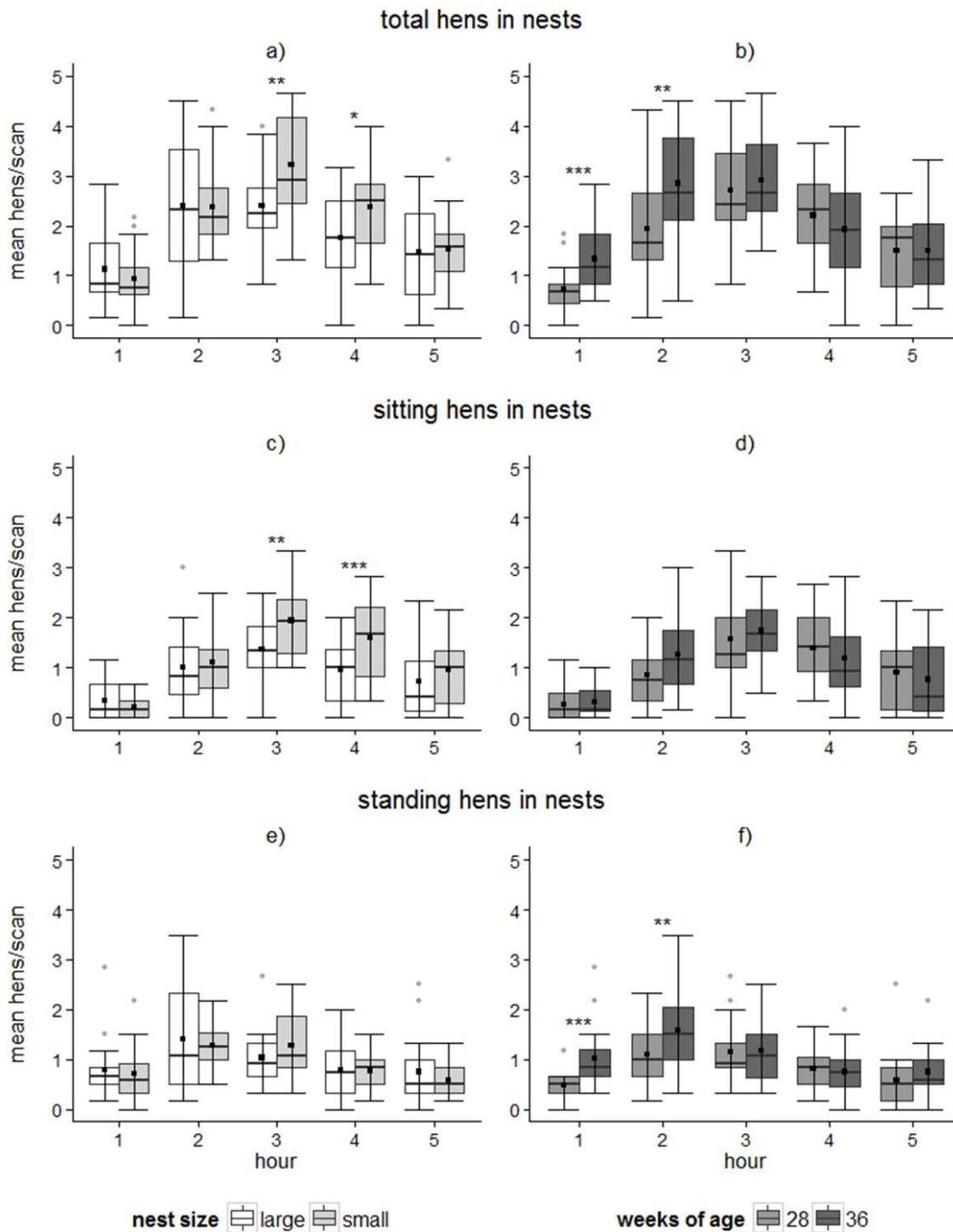
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356 **Fig 1.** (a) Top view of an experimental pen for a group of 20 hens, (b) cross section of nest, slopes of 10 %
 357 for front floor and 15 % for rear floor.

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 360 **Fig 2.** (a) Mean proportion of eggs per nest from week 21 to 36 of age (nest size: $F_{1,149} = 11.54$, $P < 0.001$,
 361 week of age: $F_{1,149} = 0.34$, *ns*), (b) mean number of nest visits per egg per nest for one day during week 28
 362 of age and one day during week 36 of age (for the first 5 hours of lights-on, nest size: $F_{1,24} = 11.18$, $P =$
 363 0.003, week of age: $F_{1,24} = 4.67$, $P = 0.044$). Boxplots: boxes represent 1st and 3rd quartile, the thick lines
 364 are the medians, the squares represent means, whiskers extend to most extreme data points (within 1.5 x
 365 interquartile range) and grey dots represent outliers.



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Fig 3. Mean number of hens in nests per hour after lights-on and nest (a, c, e) and week (b, d, f). Stars represent significant differences between nest sizes or weeks of age: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Boxplots: boxes represent 1st and 3rd quartile, the thick lines are the medians, the squares represent means, whiskers extend to most extreme data points (within 1.5 x interquartile range) and grey dots represent outliers.

