

ABSTRACT

Title of Document: **SEPARATING THE EFFECTS OF GROUP SIZE, DENSITY, AND ENCLOSURE SIZE ON MOVEMENT AND USE OF SPACE IN DOMESTIC FOWL (*Gallus gallus domesticus*)**

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This project was designed to separate the confounded effects of group size, density, and enclosure size, and to examine the role of enclosure design and its related parameters in shaping movement patterns and use of space in the domestic fowl. While previous research has suggested that group size, density, and enclosure size are highly relevant to broiler (meat-type chicken) welfare, confounding between variables makes their individual contribution difficult to distinguish. My novel treatment structure with 10, 20, and 30 birds in small (1.5 m²), medium (3.0 m²), and large (4.5 m²) enclosures enabled me to determine the impact of enclosure size while systematically controlling for group size and density. Three enclosure designs: rectangular, square, and square with partitions to maintain a constant perimeter to area ratio, were employed in order to determine the impact of enclosure parameters such as length to width and perimeter to area ratio.

Enclosure size and design were the most relevant factors for space use, which was immune to the influence of group size and density. Birds consistently had larger home ranges in larger enclosures. The design of the enclosure had a strong effect of movement, altering the response of birds to the treatment combinations. Movement appeared to be greatest in rectangular enclosures, where the largest straight-line distance is available and the perimeter to area ratio declines at a relatively slow rate with increasing enclosure size. While enclosure size played a significant role in determining nearest neighbor distances and net displacement, these parameters appeared to be limited by density. The presence of partitions designed to increase interior perimeter space appeared to reduce movement and increase inactivity. Movement patterns did not appear to be restricted by social interactions for any group size. Rather, the physical presence of group mates at even a relatively low density of 6.7 birds/ m² appeared to act as a barrier to group dispersal and movement. Group size had little effect on young domestic fowl. Overall, this project has shown that for young domestic fowl the most relevant factors to overall space use are the amount of space available and enclosure design.

SEPARATING THE EFFECTS OF GROUP SIZE, DENSITY, AND ENCLOSURE
SIZE ON MOVEMENT AND USE OF SPACE IN DOMESTIC FOWL (*Gallus
gallus domesticus*)

By

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1 **Chapter 1: Background Literature**

2 **The Effects of Group Size, Density, and Enclosure Size on** 3 **Movement and Use of Space in the Domestic Fowl**

4

5 Currently animal welfare issues are of great interest to commercial poultry
6 producers as public perception of how animals are raised and maintained has gained
7 relevance. Broiler production constitutes the largest animal industry in the United States,
8 producing more than 8 billion broilers (meat-type chickens) annually (NASS 2007),
9 therefore any improvements in management practices achieved by establishing a deep
10 understanding of the factors that affect their behavior will have major significance not
11 only in improving the quality of life of billions of birds but potentially improving their
12 performance as well.

13 One of the most reliable techniques for understanding the internal welfare state of
14 domestic fowl may be to understand their behavior (Duncan 1987; Dawkins 1999, 2003).
15 Difficulty in coping with the production environment, from physiological as well as
16 behavioral perspectives, is believed to correlate to poor welfare (for review see Broom
17 1991). Behavior can be used to assess physiological indicators of poor welfare such as
18 pain and discomfort (Dawkins 2004) as well as behavioral indicators of poor welfare
19 such as fear and frustration (Duncan 1998). Some of the most relevant features that shape
20 the behavior and use of space of animals in confined environments includes the number
21 of animals housed (group size), the amount of space available on a per animal basis
22 (stocking density), and the size and design of the enclosure. Although there is abundant
23 literature on the effects of density and group size on broiler behavior (for review see

24 Estévez 2007 and Estévez et al. 2007) how chickens move and utilize the space available
25 has been largely ignored. The way in which chickens utilize their available space is not
26 only a matter of paramount importance for the welfare of the birds, but also can have
27 important repercussion for animal production.

28 **Group Size**

29 In the domestic fowl there is a clear difference in the response of birds to small
30 (less than 20) versus large group sizes. In small groups birds form a social hierarchy or
31 pecking order (Schjelderup-Ebbe 1935; Rushen 1982), which determines access to
32 resources, including space (Banks et al. 1979; Mankovich & Banks 1982; Banks 1984;
33 Cordiner & Savory 2001). Specifically in regard to space use, dominant chickens may
34 control attractive, high value areas, such as those close to the feeders (Banks 1984;
35 reviewed in Grigor et al. 1995c). Gibson et al. (1988) suggested that dominant laying
36 hens enjoy a greater freedom of movement than subordinates, moving about the
37 enclosure freely and settling more frequently in the most attractive areas.

38 Hierarchies in chickens are established and maintained based on individual
39 recognition and the ability to remember the social status of each group mate (Douglass
40 1948; D'Eath & Keeling 2003). However the ability to recognize all group members
41 declines as group size increases, particularly beyond 30 birds (Douglass 1948). For large
42 groups McBride & Foenander (1962) hypothesized that because domestic fowl would be
43 unable to form stable hierarchies they would self-segregate and form sub-group in
44 localized areas in attempt to maintain familiarity and stabilize social relationships.
45 However this hypothesis has never been strongly supported with scientific data. Authors
46 who found incomplete space use or large differences between individuals in the amount

47 of space utilized have suggested that this serves as evidence of sub-group formation and
48 territorial behavior (McBride & Foenander 1962; Craig & Guhl 1969; Pamment et al.
49 1983; Odén et al. 2000). In order to qualify as a territory, the area would have to be set up
50 and aggressively defended from intruders or rivals (Davies & Houston 1984). In large
51 enclosures birds may not use all of the space available to them, even when there are no
52 social restrictions to movement. It is important to note that incomplete space use does not
53 necessarily reflect subgroup formation or territorial systems, but may simply be
54 indicative of individual variation or different behavioral strategies (Leone & Estévez
55 2008a).

56 Although dominance hierarchies may determine access to preferred enclosure
57 areas in small groups of domestic fowl, for the most part research has shown that group
58 size is not a factor that dictates individual space use patterns when birds are tested in
59 equally-sized enclosures. For example, Leone et al. (2007) found that the short-term
60 individual core areas of male broiler chickens did not differ between birds maintained in
61 groups of 5, 10, and 20 when given access to an equal amount of space. Similarly Lewis
62 and Hurnik (1990) found that broilers in groups of 15 to 30 ranged across the total
63 amount of space available to them, regardless of group size. These results were similar to
64 the findings of Estévez et al. (1997) who used much larger group sizes ranging from 50 to
65 200 birds and noted that birds at all group sizes had freedom to move throughout the
66 available space. This later study also suggested that as group size increases beyond 50
67 birds, broilers adopted a ‘tolerant’ social strategy categorized by few aggressive
68 interactions as access to resources based on scramble competition likely replaces
69 aggression (Estévez et al. 1997). Further evidence for a tolerant social with increasing

70 group size has been found in many other studies with laying hens (Nicol et al. 1999;
71 Carmichael et al. 1999; D'Eath & Keeling 2003; Estévez et al. 2002; Estévez et al. 2003).

72 It is likely that the switch in social behavior from aggressive hierarchies to
73 tolerance is a product of the theoretical cost of establishing a pecking order. As group
74 size increases the cost to establish a hierarchical system becomes prohibitive in terms of
75 energy invested in aggressive interactions, risk of injury, or time that could have been
76 devoted to the exploitation of resources. Additionally, in large groups individual
77 members will have little chance of encountering the same individuals repeatedly, and
78 thus recouping the initial energy required to form the hierarchy (Pagel & Dawkins 1997).
79 The physical number of individuals in an enclosure may become irrelevant once a
80 sufficiently large group size is reached. For example, Stricklin et al. (1995) demonstrated
81 with computer-simulated animals or 'animats' that 'freedom of movement' is reduced by
82 increasing group size in small groups, but that in large groups additional members did not
83 contribute to a decline in 'freedom of movement'.

84 Therefore it makes sense that many authors have found that domestic fowl in both
85 experimental and commercial settings disperse throughout their environment, using large
86 quantities of the available space (Hughes et al. 1974; Appleby et al. 1985; Appleby et al.
87 1989; Preston & Murphy 1989; Estévez et al. 1997; Leone & Estévez 2008a; Odén et al.
88 2004) and that total space use is not influenced by changes in group size (Estévez et al.
89 1997; Channing et al. 2001).

90

91 **Stocking Density**

92 Stocking density and group size are often confounded in research studies, making
93 it difficult to distinguish the individual contribution of each factor and determine if the
94 effects of increasing stocking density (via larger group sizes) are independent of the
95 effects of social hierarchies. For example, the physical barrier created by birds as they lie
96 in the path of movement of other group mates (Newberry & Hall 1990) may present more
97 of a limiting factor to movement patterns than social interactions (Estévez et al. 2005),
98 particularly at high densities. High density has been shown to restrict or reduce
99 movement in both layer and broiler strains (Andrews et al. 1997; Carmichael et al. 1999),
100 specifically decreasing the distance traveled per unit time (Lewis & Hurnik 1990; Estévez
101 et al. 1997; Febrer et al. 2006). If animal density limits movement as a result of the
102 presence of conspecifics lying in the intended pathway, then it is likely that when
103 surrounded by group-mates an individual's dispersal may be limited as well (Stricklin
104 1995).

105 In addition, the amount of 'effective free space' available, which is shaped by the
106 combination of group size, stocking density, and enclosure size, may also be a highly
107 relevant influence on movement and space use. The amount of 'effective free space'
108 available may differ substantially from the theoretical amount provided on a per animal
109 basis. Many birds, including domestic fowl, exhibit strong flocking tendencies as a result
110 of attractive forces (Clark & Mangel 1984; Febrer et al. 2006) which are expressed
111 through the maintenance of specific (close) inter-individual distances and synchronous
112 behavior. Studies have shown that in confinement groups of domestic fowl do not always
113 disperse to the full extent possible, suggesting that there an upper limit to the inter-

114 individual distances birds prefer to maintain, even when additional space is available
115 (Arnould & Faure 2004; Leone et al. 2007). This underutilization of the available space
116 can leave large areas unoccupied (Arnould & Faure 2004; Leone et al. 2007), creating
117 relative densities that differ through the enclosure (Channing et al. 2001). Therefore, even
118 at identical densities animals in larger enclosures may enjoy a greater amount of
119 ‘effective free space’ as a consequence of open areas and small relative density.

120

121 **Enclosure Size and Design**

122 The majority of investigations into the factors shaping movement and space use
123 have focused on group size and density. Far fewer studies have been conducted on the
124 effects of enclosure size and design, which are highly relevant to the movement patterns
125 of captive animals. In chickens it has been suggested that space use is ultimately
126 determined not by stocking density or group size per se but by the amount of space
127 available, or enclosure size (Newberry & Hall 1990; Estévez et al. 1997; Leone et al.,
128 2007). ‘Free range’ systems represent one common area where researchers have
129 examined the effect of enclosure size and design on space use. ‘Free range’ systems refer
130 to birds that are given access to additional outdoor space, and in both laying hens
131 (Keeling et al. 1988; Grigor et al. 1995a, c) and meat-type broilers (Estévez et al. 1997;
132 Dawkins et al. 2003) the additional space goes largely underutilized. However, even
133 when birds do not take full advantage of the available space, studies have shown that use
134 of space increases in larger enclosures (Newberry and Hall, 1990; Estévez et al. 1997)
135 regardless of differences in group size or stocking density.

136 Space use in the domestic fowl is shaped not only by the amount of space
137 available, but the quality of that space is equally important. Enclosure design, both in its
138 physical parameters (length, width, floor area, etc.) as well as elements which increase
139 environmental complexity (such as enrichment) can significantly influence movement
140 and use of space. Studies have shown that layers maintained in ‘free range’ systems make
141 greater use of outdoor areas when the quality of the space is improved by incorporating
142 various types of protective cover (Zeltner & Hirt 2003; Hegelund et al. 2005; Whay et al.
143 2007). The presence of visual cover, which increases environmental complexity and
144 perimeter (wall) space, has been found to reduce disturbances and aggression (Cornetto et
145 al. 2002), and improve bird distribution throughout the enclosure (Newberry &
146 Shackleton 1997; Cornetto & Estévez 2001). When commercial broiler breeder houses
147 were enriched with cover panels overall space use increased and males made use of a
148 wider range of areas within their environment (Leone & Estévez 2008). Similarly, when
149 broiler houses were enriched with straw bales birds used them as perches and resting
150 points, whereas and even in the control houses without enrichment birds clustered around
151 roof support poles which dotted the litter area (Kells et al. 2001).

152 Perimeter space is often the only available cover in confined environments and as
153 such is an important feature of the enclosure. Researchers have found that pigs (Wiegand
154 et al. 1994) and broilers (Cornetto & Estévez 2001b) take greater advantage of perimeter
155 space, as opposed to the more abundant central area, and use the perimeter spaces to a
156 greater extent than would be expected by chance (broilers, Newberry & Hall 1990). The
157 amount of peripheral space available is a direct consequence not only of the size of the
158 enclosure, but also its shape. The shape of the enclosure determines not only peripheral

159 space, but also a number of other potentially important parameters: such as the number of
160 corners, the distance to them, and the farthest distance an animal will have to travel to
161 reach a wall (Christman & Leone 2007). For example, when enclosures are square
162 (length to width ratio is constant) the furthest distance an animal will have to travel to
163 reach the closest wall will increase linearly with enclosure size. Conversely, in a
164 rectangular pen in which the enclosure width is held constant as floor space increases the
165 furthest distance to a wall is constant. No matter what the shape, when enclosure size
166 increases (and the total available peripheral space) the ratio of perimeter space to central
167 area (perimeter to area ratio) decreases (Stricklin et al. 1995). Even at a constant stocking
168 density the amount of peripheral space available per animal also decreases. However, in
169 rectangular (as opposed to square) enclosures this decline occurs at a slower rate.

170 Researchers who have investigated the effects enclosure shape have found
171 significant impacts on animal behavior. Aggressive interactions in group of pigs are
172 affected by the shape of the pen but most specifically by the availability of corners
173 (Wiegand et al. 1994). Post-mixing aggression was greater in circular as opposed to
174 square enclosures; however when circles were modified to include corners there were no
175 differences between enclosure types. Aggression has also been shown to decrease when
176 pigs are grouped together in rectangular as opposed to square enclosures (Barnett et al.
177 1993).

178 While group size, density, and enclosure size and design all appear to have an
179 influence on movement and use of space in domestic fowl their individual impacts cannot
180 be clearly discerned as a result of confounding between variables. Limitations to
181 movement and dispersal may be a consequence of increases in group size, density, or

182 both. While the size of enclosures, and their design, may be the factors of greatest
183 consequence for movement and space use there is surprisingly little attention paid to
184 these factors in the published literature for the domestic fowl.

185

186 **Study Purpose, Hypothesis, and Predictions**

187 The aim of this project was to separate the confounded effects of group size,
188 density, and enclosure size on movement and use of space patterns in the domestic fowl.
189 While previous research has suggested that each of these factors is highly relevant for the
190 domestic fowl the confounding between variables, which has often been ignored or
191 disregarded by authors, makes it difficult to distinguish specific contributions.
192 Furthermore, I intended to describe the relative impact of various enclosure parameters
193 such as the furthest distance from a wall, length to width ratio, and perimeter per animal
194 ratio on the domestic fowl. This information is currently lacking in the literature, but is
195 essential in the quest to understand the impact of confined environments on broiler
196 welfare. I hypothesized that each individual factor included in this work (group size,
197 density, and enclosure size and shape) would have a unique impact on movement and use
198 of space in the domestic fowl. I expected that enclosure size would most significantly
199 affect overall use of space, while group size and density would impose limits on
200 movement parameters such as total distance traveled and net displacement. The enclosure
201 design, specifically the amount of peripheral space available per animal, was expected to
202 be one of the most relevant enclosure parameters for domestic fowl, followed by the
203 farthest distance to a wall.

204

205 **Chapter 2: Separating the Effects of Group Size, Density, and**
206 **Enclosure Size**
207 **I. In Rectangular Enclosures When Length to Width Ratio**
208 **Varies as Enclosure Size Increases**
209

210

211 **Abstract**

212 To design effective captive environments that maximize animal welfare it is
213 essential to understand how confined animals move within the space available. Besides
214 the obvious effect of enclosure size, other factors such as the number of individuals in the
215 group and their density per unit of area are known to affect movement patterns. Yet
216 determining the specific contribution of each (enclosure size, group size and density) has
217 been a challenge because confounding between two or more of these factors is
218 experimentally difficult to avoid. The aim of this study was to isolate their unique effects
219 by using multiple contrasts with an efficient experimental design which included
220 combinations of groups of 10, 20, and 30 domestic fowl (*Gallus gallus domesticus*)
221 housed in 1.5 m², 3.0 m² and 4.5 m² enclosures. This treatment structure enabled me to
222 compare across increasing enclosure size both at constant group size and density. In this
223 study I demonstrate that enclosure size and density are the primary factors affecting
224 movement and use of space patterns for groups of domestic fowl. Animals in larger
225 enclosures maintained larger nearest neighbor distances, traveled greater distances, and
226 had bigger home ranges as measured by minimum convex polygons. These results
227 suggest that larger enclosures encourage more exploratory movement in groups of

228 domestic fowl. However the positive effects of large enclosures may be limited by the
229 effects of density. Contrarily, in this study group size had fewer than expected effects.

230 **Introduction**

231 Captive housing encompasses a wide variety of conditions, from zoos and
232 conservation centers, to research laboratories, and of course farms. For any captive
233 species inadequate physical and social features of the environment can be a source of
234 stress and discomfort that can lead to serious physiological, behavioral, and welfare
235 problems (Würbel 2001; Estévez et al. 2007; Morgan & Tromborg 2007). Although many
236 parameters can be controlled in captive environments, the size of the enclosure, the
237 number of animals housed in it, and density (number of animals per unit of area) are
238 some of the most salient factors that can have a major impact on how animals move and
239 use the space available to them.

240 Adequate spacing is important for the welfare of animals (Stricklin 1995) as
241 suggested by the fact that animals are willing to work for access to extra space (Faure
242 1991, 1994; Sherwin 2004, 2007). Restrictive spaces can lead to negative changes in
243 behavior (Nicol 1987; Dawkins 1988; Poon et al. 1997; Crockett et al. 2000; Bashaw et al.
244 2001) including increased stereotypies (Beattie et al. 1996; Bashaw et al. 2001), reduced
245 play (Jensen et al. 1998), and increased stress (Smith & Dobson 1990; Turner et al. 2000)
246 and anxiety (Arakawa 2005). Reduced enclosure size hinders locomotion (Crockett et al.
247 1995; Crockett et al. 2000; Estévez et al. 1997; Poon et al. 1997), group dispersal (Blanc
248 et al. 1999), and affects inter-individual distances and social spacing among animals
249 (Keeling & Duncan 1991).

250 Besides the size of the enclosure, other aspects that are not considered as often
251 such as its shape and design can also alter the behavior and space use patterns of captive
252 animals (Barnett et al. 1993; Wiegand et al. 1994; Newberry & Shackleton 1997;

253 Cornetto & Estévez 2001a, b). Changes in parameters such as peripheral (wall) space,
254 length to width ratio, or distance to walls and corners may be important features of the
255 captive environment with great biological significance for confined animals (Stricklin et
256 al. 1995; Christman & Leone 2007). For example, the addition of wall space in central
257 areas of the enclosure has been shown to improve spatial dispersion and reduce
258 disturbances in broiler chickens (Cornetto & Estévez 2001b; Cornetto et al. 2002), and
259 reduce injurious pecking and improve welfare in turkeys (Sherwin et al. 1999b).

260 The social environment is also critical to ensure the welfare of animals as
261 confining inadequate numbers in a given space can lead to social stress (Hurst et al. 1999;
262 Dronjak et al. 2004). In domestic species large group sizes have been shown to increase
263 fearfulness (Bilcik et al. 1998), reduce productivity (Gonyou & Stricklin 1998; Turner et
264 al. 2000; McLean et al. 2002), increase the chances of skin injuries (Turner et al. 2000;
265 Kjaer 2004), and reduce group stability (Takeda et al. 2000). Similar patterns have been
266 observed in captive wild animals, where inappropriate group sizes or crowding lead to
267 increased stress responses (Maestriperi et al. 1992; Boyce et al. 1998; Boal et al. 1999;
268 Dickens & Romero 2005; Raouf et al. 2006). Irrespective of enclosure size, maintaining
269 high animal densities may result in a lack of sufficient free space thereby limiting animal
270 locomotion (Estévez et al. 1997). High densities commonly used with domestic species
271 have been linked to immune suppression (Turner et al. 2000; Heckert et al. 2002),
272 increased disturbances (Cornetto et al. 2002), and reduced feeding behavior (Alanära
273 1996; Cooke et al. 2000) weight gain, feed efficiency (Horton et al. 1991; Pearce &
274 Paterson 1993; Brumm & Miller 1996; Brumm et al. 2004) and growth (Blanc & Theriez
275 1998; Bohlin et al. 2002).

276 While a great deal of research has been conducted on the impact of enclosure size,
277 group size and density in a variety of species (for review see Alanärä 1996; Sørensen et al.
278 2005; Estévez et al. 2007; Morgan & Tromborg 2007), they all involved some degree of
279 confounding between variables. For example, when testing for density effects in
280 enclosures of equal size group size has to be manipulated leading to confounding
281 between density and group size. Thus the reduction in locomotion associated with high
282 densities (Estévez et al. 1997) may be a consequence of the decline in ‘free’ enclosure
283 space, which is reduced as animal density increases (Newberry & Hall 1990), but can
284 also be associated with increased social conflict and social restriction which may occur in
285 large group sizes. Therefore, in these types of studies it is difficult to isolate the precise
286 contribution of each factor: enclosure size, group size or density, to changes in behavior
287 and movement patterns. Yet a clear understanding of the effects of enclosure size, group
288 size, and density may be critical in our efforts to improve the quality of the environment
289 for captive animals.

290 In this study I used a unique experimental design to separate the confounded
291 effects of group size, density and enclosure size on use of space and movement using the
292 domestic fowl as an animal model. I hypothesized that each characteristic of the
293 environment, defined by group size, density, and enclosure size, has a unique impact on
294 animal movement and space use.

295

296 **Methods**

297 **Facilities and Experimental Animals**

298 This project was conducted at the University of Maryland's Applied
299 Poultry Research Facility in Upper Marlboro from September through November
300 2005. A total of 540 male day-old broiler chicks were obtained from a
301 commercial hatchery. I chose to work with only a single sex in order to minimize
302 behavioral variability. Initially 12, 24, and 36 birds were placed in the
303 experimental enclosures in an effort to account for early mortalities and reach the
304 target group sizes of 10, 20, and 30 birds. At the end of 3 weeks, extra birds were
305 removed and housed in a separate enclosure. Each bird was individually tagged
306 (Leone et al. 2007) on each side of the neck using the Swiftack Poultry
307 Identification System (Heartland Animal Health Inc., Fair Play, MO). For the first
308 3 days birds were exposed to 24 hours of light, and thereafter were maintained on
309 a 14 L: 10 D program in an effort to slow growth and promote leg health.
310 Temperature and ventilation programs followed commercial practices. Feed and
311 water were provided *ad libitum* from a central tubular hopper and a line of nipple
312 drinkers located along one side of the enclosure. Three hopper sizes were
313 employed so that the proportion of enclosure space occupied by the feeder as well
314 as the amount of feeder space available per bird was constant across treatments.
315 The feeding program consisted of a standard three phase commercial diet. This
316 protocol (R-05-39) was approved by the Institutional Animal Care and Use
317 Committee at the University of Maryland.

318

319 **Experimental Design**

320 For this experiment I constructed three enclosure sizes which were 1.49
321 m² (small, 1.22 x 1.22 m), 2.96 m² (medium, 1.22 x 2.44 m), and 4.47 m² (large,
322 1.22 x 3.66 m). Each enclosure was covered with 5 cm of wood shavings.
323 Enclosure size increased in only one direction to give all enclosures the same
324 width (1.22 m) meaning that the medium-sized enclosure was twice as long as the
325 small enclosure and the large enclosure was three times as long (Appendix 6-1).
326 This arrangement enabled the floor area allowance to increase while the
327 maximum distance from the nearest wall remained constant (Table 2-1). In
328 addition to mere floor space there are a number of enclosure features which are
329 likely to have a significant impact on animal movement (Christman & Leone
330 2007) and therefore this design was developed in an attempt to maintain as much
331 consistency as possible across the enclosures. Since wall space has been
332 repeatedly shown to be a strong attraction for confined animals (Stricklin et al.
333 1979; Cornetto & Estévez 2001b; Jeanson et al. 2003), the distance to a wall may
334 heavily influence animal movement and spacing and thus I chose to control
335 variation in this parameter. The enclosure dimensions and potential parameters of
336 interest can be seen in Table 2-1 (for detailed description of calculations see
337 Christman & Leone 2007).

338 The group sizes were housed in the different enclosures to generate the
339 five experimental treatments (Table 2-1) each of them replicated five times.
340 Groups of 10 were housed all three enclosure sizes (10_S, 10_M, 10_L) while group of
341 20 and 30 were housed in the medium (20_M) and large enclosures (30_L)

342 respectively. This design enabled me to make comparisons across constant group
343 size, where density decreased with increasing enclosure size (10_S, 10_M, 10_L),
344 across a constant density, where group size increased with enclosure size(10_S, 20_M,
345 30_L), and across constant enclosure sizes where group size and density increased
346 simultaneously (10_M, 20_M and 10_L, 30_L).

347

348 **Data Collection**

349 Each enclosure was divided into a grid of 20 x 20 cm squares by placing
350 numerical and alphabetical placards along the enclosure walls. This created a
351 visual grid that allowed me to precisely record bird locations on scaled maps
352 (Cornetto & Estévez 2001b; Leone et al. 2007). For behavioral observations five
353 focal birds were randomly selected from each enclosure and were observed
354 throughout the entire experiment. Observations began at three weeks of age and
355 continued until birds were six weeks old. The birds in each enclosure were
356 observed twice per day, three days per week. The location and identity of each
357 focal individual, as well as the position of all other group members, was recorded
358 via instantaneous scan sampling *ad libitum* for a five minute period. At the
359 beginning of each observation period bird locations were recorded on a single
360 scan sheet, and once completed successive locations were recorded on additional
361 scan sheets until the 5 minutes expired. These location scans were digitized as X
362 Y coordinates with a Digitalizer (ACECAD, Taipei, Taiwan) using the
363 Chickitaizer© software (Sanchez & Estévez 1998).

364
365

Table 2-1

366
367
368

Enclosure parameters for each group size and enclosure size combination. Parameters include group size, stocking density, total floor area available, enclosure length and width, length to width ratio, total perimeter, perimeter per animal, perimeter to area ratio, farthest distance to the nearest wall and farthest distance to a corner.

Treatment	Group Size	Density (birds/m ²)	Total Area (m ²)	Length (m)	Width (m)	Length: Width Ratio	Perimeter (m)	Perimeter (m) / animal	Perimeter: Area Ratio	Distance to Wall (m)	Distance to Corner (m)
10 _L	10	2.2	4.47	3.66	1.22	3 : 1	9.76	0.98	2.2 : 1	0.61	1.93
10 _M	10	3.4	2.96	2.44	1.22	2 : 1	7.32	0.73	2.5 : 1	0.61	1.36
10 _S	10	6.7	1.49	1.22	1.22	1 : 1	4.88	0.49	3.3 : 1	0.61	0.86
20 _M	20	6.7	2.96	2.44	1.22	2 : 1	7.32	0.37	2.5 : 1	0.61	1.36
30 _L	30	6.7	4.47	3.66	1.22	3 : 1	9.76	0.33	2.2 : 1	0.61	1.93

369 From each five minute observations period I calculated a number of
370 measures to capture bird movement and space use which included: nearest
371 neighbor distances which were calculated from the locations of all group
372 members, the total distance traveled which was calculated by summing the
373 Euclidean distances between successive recorded locations for each focal animal
374 during the five minute observation period, net displacement which was calculated
375 as the Euclidean distance between the first and last observed location, and
376 movement activity which was defined as the percentage of scans where
377 movement was observed. For nearest neighbor distances I was uniquely able to
378 calculate the distances that would have been expected if birds positioned
379 themselves randomly with the environment, through the use of a simple random
380 simulation. This random simulation could not be utilized for other measures,
381 because no assumptions were made about movement patterns per se. Each
382 simulation (InsightfulCorp, S-plus 6.1, Seattle, WA) consisted of randomly
383 assigning locations to all birds according to each treatment combination. The
384 average nearest neighbor distance for the group was then calculated, and this
385 simulation was repeated 2000 times in order to generate expected nearest
386 neighbor distances. The average value from these 2000 simulations represents the
387 nearest neighbor distance that would be expected if birds randomly distributed
388 within their enclosure. Deviations were then calculated by subtracting the
389 observed nearest neighbor distances from those expected assuming randomness,
390 in order to determine to what extent the treatment influenced inter-individual
391 distance. Minimum convex polygons (Mohr 1947) were calculated using

392 ArcView GIS v8 (ESRI, Redlands, CA) with the Animal Movement Extension
393 package (Hooge & Eichenlaub 2000). A total minimum convex polygon was built
394 for each focal bird from all observed locations throughout the entire length of the
395 study. This measure provided an estimate of the total amount of space utilized by
396 each focal bird. In addition, weekly minimum convex polygons were generated
397 for each focal bird based on the observations within one weeks' time to determine
398 the effect of age on space use. A coefficient of variation was also calculated to
399 ascertain the variability in space use between individuals across age. All
400 measurements were averaged across birds within each enclosure.

401

402 **Statistical Analysis**

403 All analyses were conducted in SAS (v. 9.1, SAS Institute, Cary, NC;
404 Appendix 6-2). For all parameters except total minimum convex polygons I
405 modeled the effects of treatment, age, and their interaction. Because total
406 minimum convex polygons were calculated from all locations recorded from the
407 entire study period, the statistical model only included the treatment effect.
408 Separate mixed model ANOVAs were performed for each of the parameters
409 analyzed: nearest neighbor distances and their deviation from expected values
410 assuming random assortment, total distance traveled, net displacement, weekly
411 minimum convex polygons and their coefficient of variation, and movement
412 activity. All models included a covariance structure for repeated observations.
413 Model assumptions of normality and homogeneity of residual variances were
414 examined. In order to meet the assumptions variance components were modeled

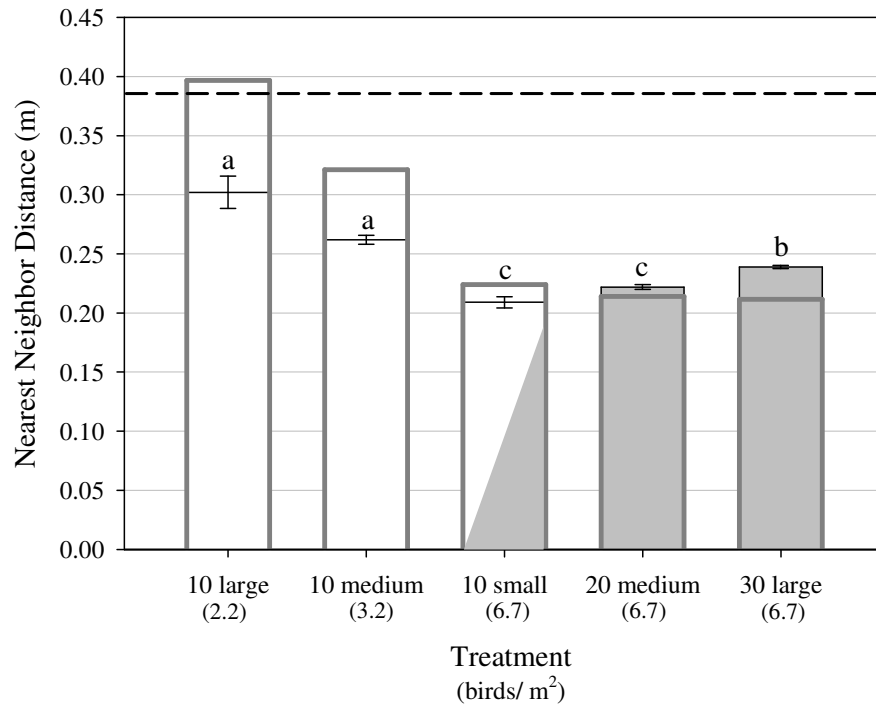
415 by treatment for nearest neighbor distances and their deviations from randomness,
416 net displacement and all minimum convex polygons.

417 The goals of this experiment were addressed with three specific *a priori*
418 contrasts, the first two detected differences between increasing enclosure sizes
419 when group size was constant (but density decreased; 10_S, 10_M, 10_L) and when
420 density was held constant (but group size increased with enclosure size; 10_S, 20_M,
421 30_L), and lastly I compared across fixed enclosure sizes (where density and group
422 size increased simultaneously; 10_M, 20_M and 10_L, 30_L). Each of these contrasts
423 maintained one factor constant, while the other two covaried; each contrast is
424 essentially an ANOVA test. In order to protect against an inflated Type I error
425 rate the contrasts and mean comparisons were only performed when the overall
426 ANOVA F-test was significant ($P < 0.05$).

427

428 **Results**

429 Nearest neighbor distance was affected by treatment ($F_{4, 6.03} = 30.80$, $P < 0.001$)
430 generally expanding with increasing enclosure size (Fig. 2-1). This increase was most
431 notable when group size remained constant with 10 birds (10_S, 10_M, 10_L; $F_{2, 11.6} = 36.61$,
432 $P < 0.001$) and to a lesser extent when density was constant (10_S, 20_M, 30_L; $F_{2, 11.1} =$
433 42.96 , $P < 0.001$). When comparing constant enclosure sizes nearest neighbor distances
434 were larger at smaller group sizes/ densities (10_M, 20_M and 10_L, 30_L; $F_{2, 4.69} = 47.15$, $P <$
435 0.001).

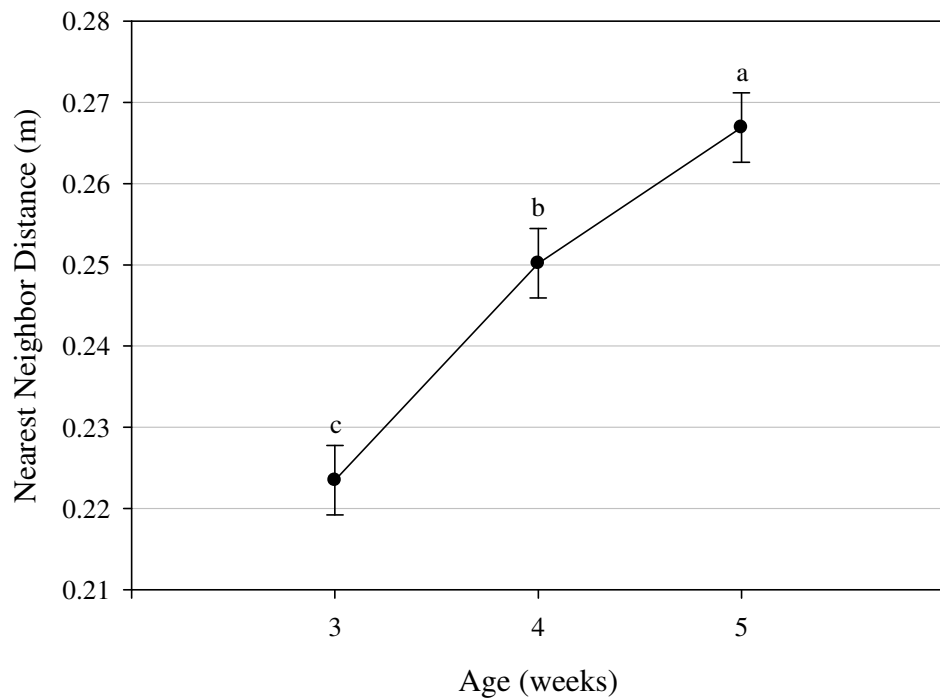


436

437 **Figure 2-1**

438 Nearest neighbor distances (least squares means \pm standard error of the mean) according
 439 to each group size (10, 20, or 30) and enclosure size (small, medium, or large) treatment,
 440 and the expected values assuming random assortment (gray outline). White fill
 441 corresponds to equal group size while grey denotes constant density, which is listed
 442 below each treatment. Means sharing any common letters are not significantly different
 443 ($P > 0.05$). All observed nearest neighbor distances differed from those expected
 444 assuming randomness ($P < 0.05$), and from the predicted uniform distribution based on a
 445 density of 6.7 birds/m² which is represented by the dotted line ($P < 0.05$).

446



447

448

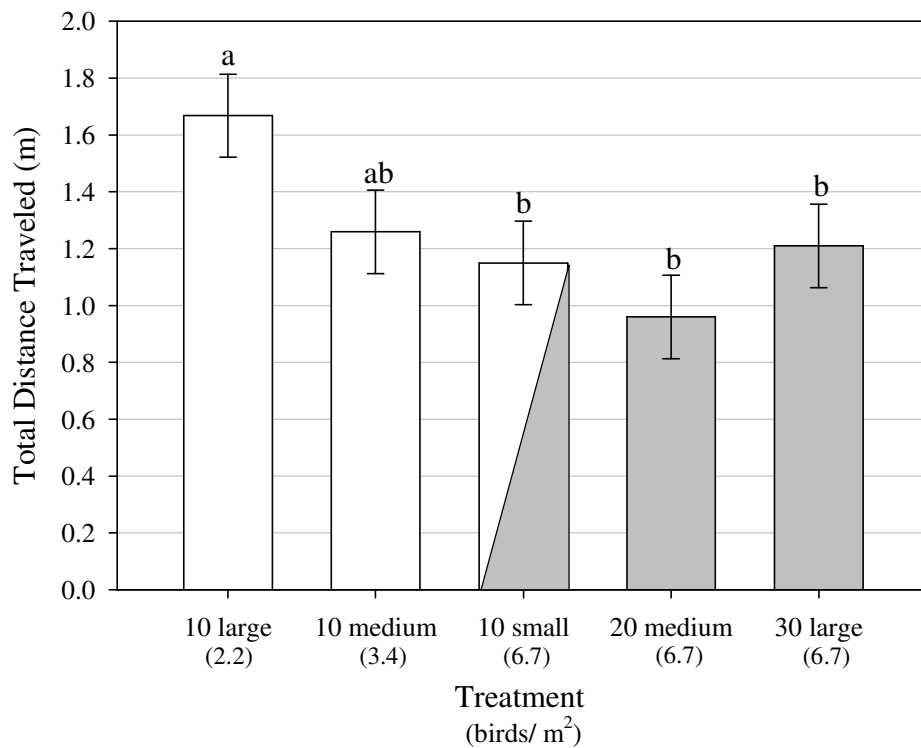
449 **Figure 2-2**

450 Effects of age on nearest neighbor distances (LSM \pm SEM). Means sharing any common

451 letters are not significantly different ($P > 0.05$).

452 In addition, group did not behave as would be expected by random assortment. There was
453 a significant deviation between the observed nearest neighbor distances and those
454 expected assuming randomness across treatment combinations ($F_{4,6.06} = 114.85$, $P <$
455 0.001 ; Fig 2-1), with all deviations differing significantly from zero ($P < 0.05$). In the
456 smaller groups of 10 birds nearest neighbor distances were smaller than would be
457 predicted by random assortment, but were farther apart than predicted in the groups of 20
458 and 30 birds. There was also a significant effect of age on the deviation of observed
459 nearest neighbor distances from the random predictions ($F_{2,13.2} = 31.67$, $P < 0.001$), but
460 there was no interaction between treatment and age. While birds were closer together
461 than randomly predicted during weeks three (deviation mean \pm standard error mean: 0.05
462 ± 0.005) and four (0.02 ± 0.004) the observed nearest neighbor distances were not
463 different than the predicted values by week five (0.007 ± 0.004 , $P = 0.14$). Nearest
464 neighbor distances were larger for older birds ($F_{2,13.2} = 31.67$, $P < 0.001$; Fig. 2-2) but
465 there was no interaction of age and treatment ($F_{8,12.4} = 1.41$, $P = 0.283$).

466 The total distance traveled was clearly affected by treatment ($F_{4,52.3} = 3.17$, $P =$
467 0.021 ; Fig. 2-3), increasing with enclosure size with constant group sizes of 10 (10_S , 10_M ,
468 10_L ; $F_{2,97.2} = 3.47$, $P = 0.035$) but not when density was maintained (10_S , 20_M , 30_L ; $F_{2,98}$
469 $= 0.79$, $P = 0.458$). Total distance traveled did not change with age ($F_{2,130} = 1.76$, $P =$
470 0.175) and there was no interaction of age and treatment ($F_{8,132} = 0.30$, $P = 0.964$).



471

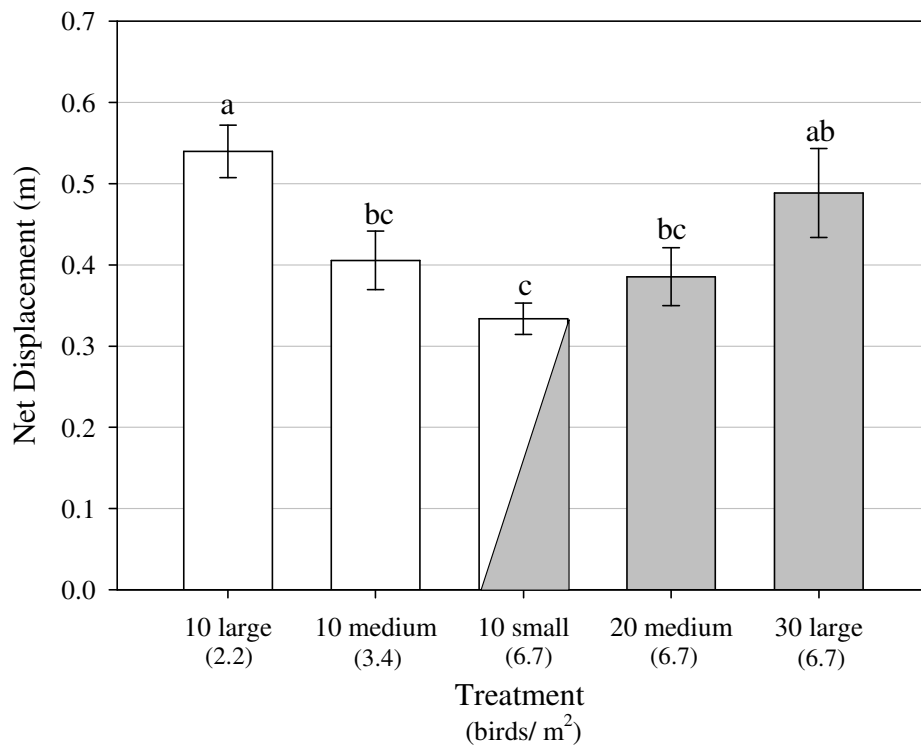
472

473 **Figure 2-3**

474 Total distance traveled during an observation period (LSM ± SEM) by each group
 475 size (10, 20, or 30) and enclosure size (small, medium, or large) treatment. White fill
 476 corresponds to equal group size while grey denotes constant density, which is listed
 477 below each treatment. Means sharing any common letters are not significantly different
 478 ($P > 0.05$).

479 Similarly, net displacement differed by treatment ($F_{4, 27.3} = 7.69, P < 0.001$; Fig.
480 2-4) but not by age ($F_{2,104} = 0.16, P = 0.856$) or their interaction ($F_{8, 76.7} = 0.32, P =$
481 0.956). Net displacement increased with enclosure size both across constant group size 10
482 ($10_S, 10_M, 10_L$; $F_{2,67.8} = 14.67, P < 0.001$) and across constant density of 6.7 birds/ m²
483 ($10_S, 20_M, 30_L$; $F_{2, 27.2} = 3.73, P = 0.037$) but were not different when comparing
484 enclosures of the same size regardless their density/ group size ($10_M, 20_M$ and $10_L, 30_L$; $F_{2,$
485 $23.2} = 0.39, P = 0.679$).

486 The total amount of space utilized, as measured by minimum convex polygons,
487 differed according to treatment ($F_{4, 13} = 76.72, P < 0.001$; Fig. 2-5) as birds used more
488 space when housed in larger enclosures regardless of density ($10_S, 10_M, 10_L$; $F_{2, 20.6} =$
489 $103.06, P < 0.001$) or group size ($10_S, 20_M, 30_L$; $F_{2, 23.6} = 98.25, P < 0.001$). There was no
490 difference in space use when a comparison was made across constant enclosure size ($10_M,$
491 20_M and $10_L, 30_L$; $F_{2, 15.2} = 1.79, P = 0.20$). There was an affect of age on minimum
492 convex polygons ($F_{2,29.7} = 7.04, P = 0.003$); as space use was greater initially and
493 decreased as the birds aged (Fig 2-6). The average amount of space used by birds during
494 each week of age different according to treatment ($F_{4,7.24} = 22.51, P < 0.001$; Fig. 2-7)
495 similar to the total minimum convex polygons, and I did not find an interaction effect
496 ($F_{8,18.4} = 1.17, P = 0.366$). The coefficient of variation for minimum convex polygons did
497 not differ by treatment ($F_{4, 7.66} = 1.09, P = 0.42$), age ($F_{2,12.7} = 0.23, P = 0.80$), or their
498 interaction ($F_{8,5.89} = 0.96, P = 0.536$).



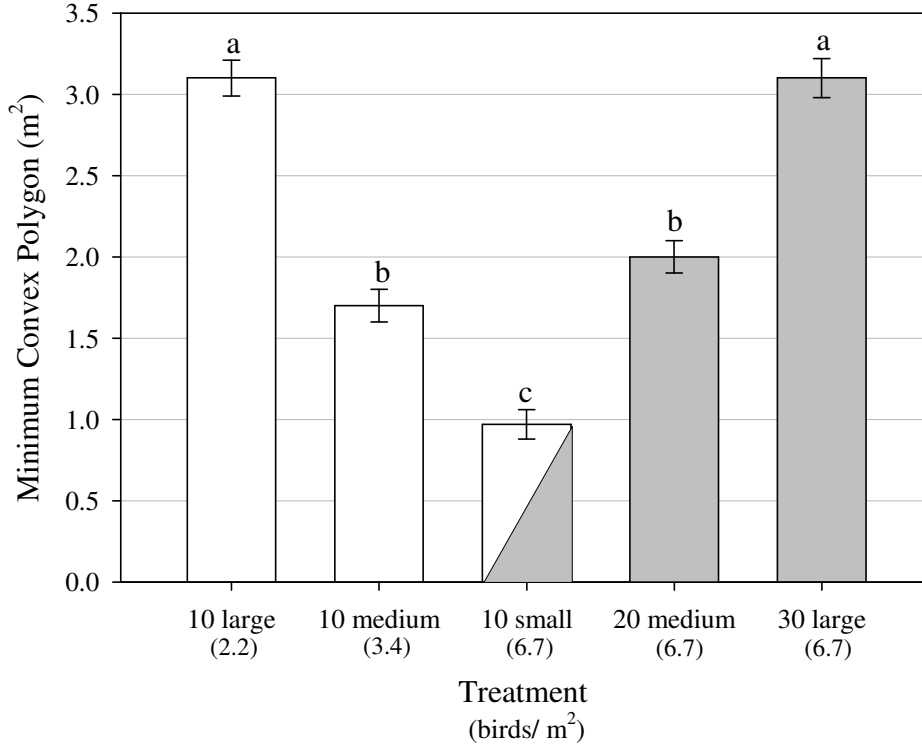
499

500

501 **Figure 2-4**

502 Net displacement, defined as the Euclidean distance between the first and last observation
 503 point, (LSM ± SEM) according to each group size (10, 20, or 30) and enclosure size
 504 (small, medium, or large) treatment. White fill corresponds to equal group size while grey
 505 denotes constant density, which is listed below each treatment. Means sharing any
 506 common letters are not significantly different ($P > 0.05$).

507

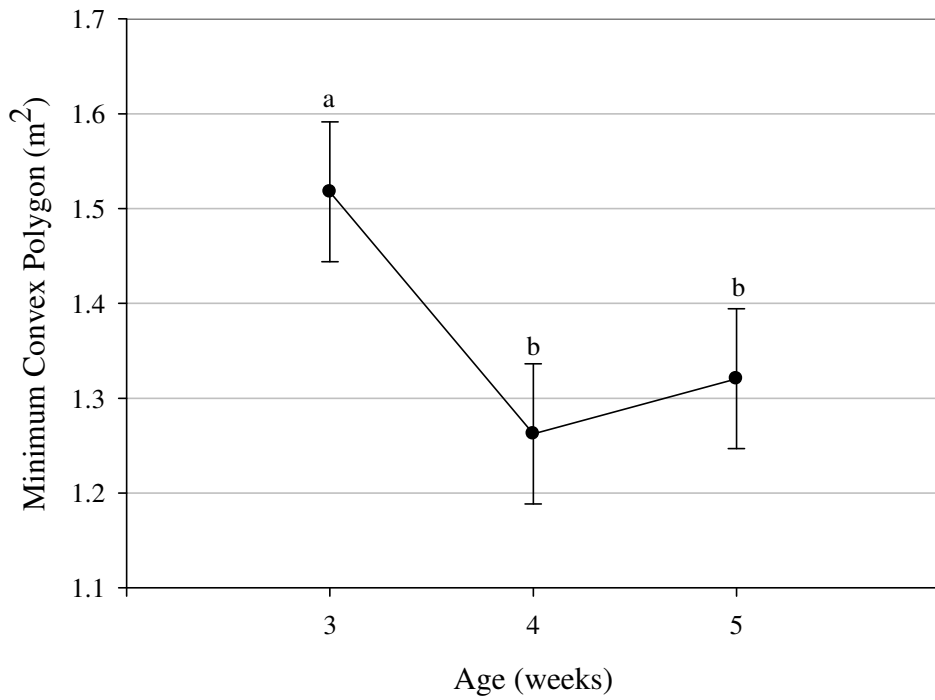


508

509

510 **Figure 2-5**

511 Total minimum convex polygon estimates of space use (LSM ± SEM), according to each
 512 group size (10, 20, or 30) and enclosure size (small, medium, or large) treatment. White
 513 fill corresponds to equal group size while grey denotes stocking density, which is listed
 514 below each treatment. Total minimum convex polygons were built from all recorded
 515 locations over the length of the study and convey the total amount of space utilized by the
 516 birds. Means sharing any common letters are not significantly different ($P > 0.05$).

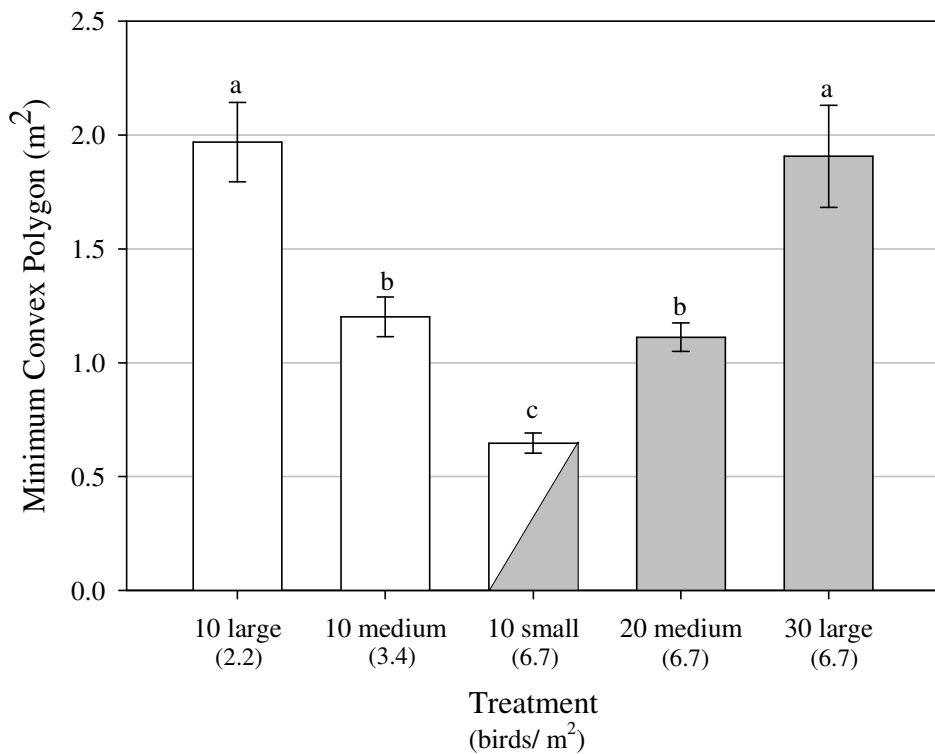


517

518 **Figure 2-6**

519 Average minimum convex polygon recorded for each week of age (LSM ± SEM). Means

520 sharing any common letters are not significantly different ($P > 0.05$).

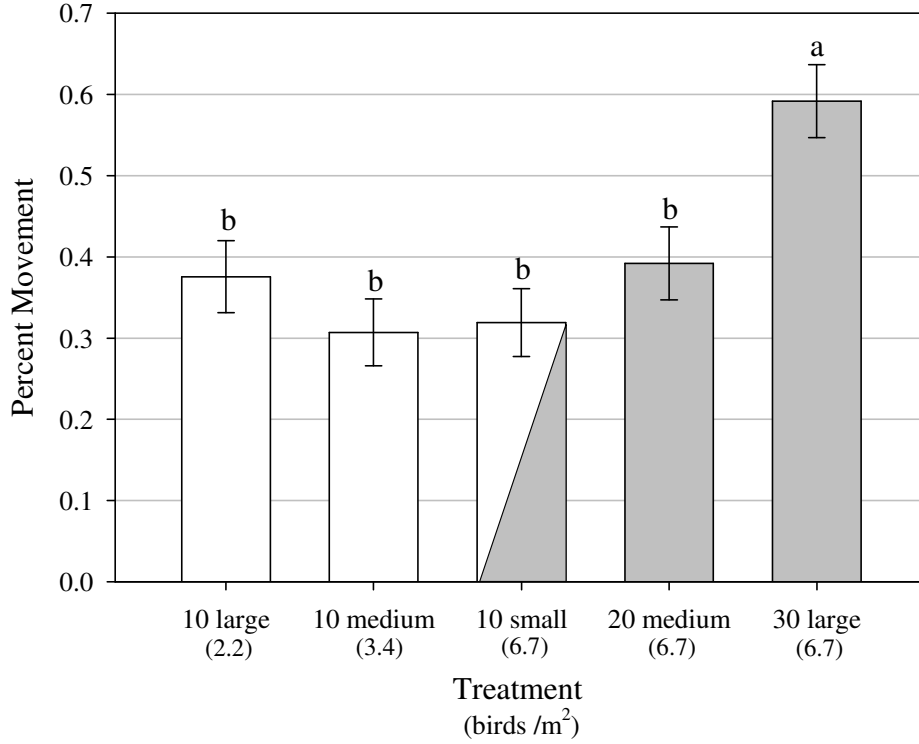


521

522 **Figure 2-7**

523 Average weekly minimum convex polygons (LSM ± SEM) according to group size (10,
 524 20, or 30) and enclosure size (small, medium, or large) treatment. Weekly minimum
 525 convex polygons convey the estimates amount of space utilized by the birds during one
 526 week's time. Means sharing any common letters are not significantly different ($P > 0.05$).

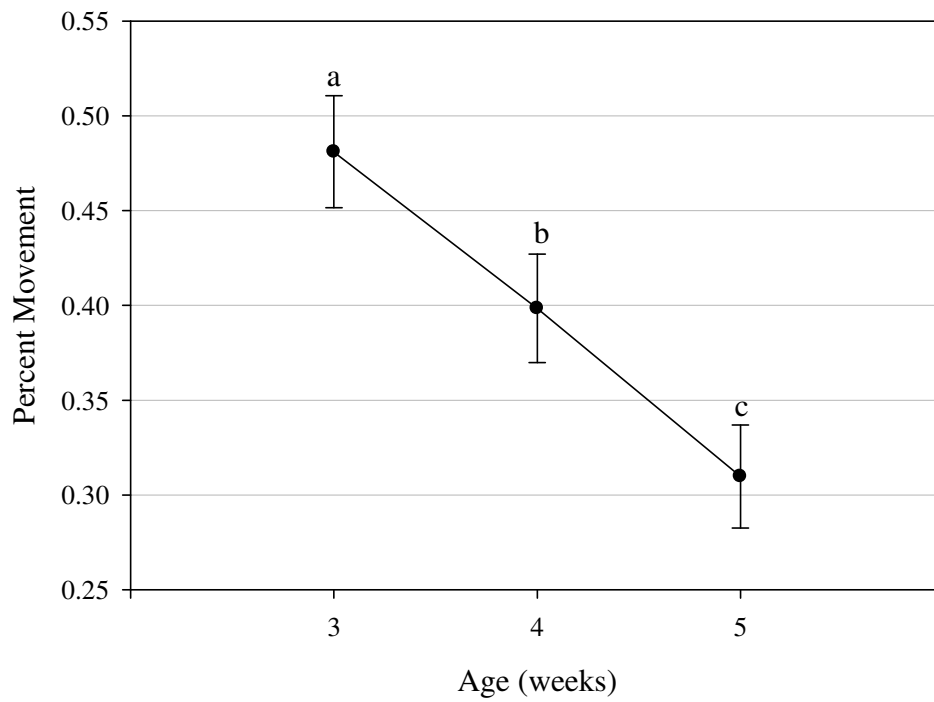
527 Whether a bird moved between scans, defined as movement activity, was
528 influenced both by treatment ($F_{4, 20} = 6.38, P = 0.002$; Fig. 2-8) and age ($F_{2, 40} = 11.04, P$
529 < 0.001 ; Fig. 2-9) but not by their interaction ($F_{8, 40} = 0.76, P = 0.64$). However the
530 treatment effect appeared to be a product of the only significant difference which
531 occurred at the largest group size of 30. This was detected in the difference between
532 enclosure/ group sizes across constant density of 6.7 birds/ m^2 ($10_S, 20_M, 30_L$; $F_{2, 36.8} =$
533 $10.22, P < 0.001$) and when movement in medium and large-sized enclosures was
534 compared ($10_M, 20_M, \text{ and } 10_L, 30_L$; $F_{2, 20} = 6.96, P = 0.005$). There was no difference in
535 movement activity when comparisons were made between enclosure sizes at constant
536 group size of 10 ($10_S, 10_M, 10_L$; $F_{2, 36.8} = 0.74, P = 0.48$).



537

538 **Figure 2-8**

539 Movement activity (LSM ± S.E.M.) according to each group size (10, 20, or 30) and
 540 enclosure size (small, medium, or large) treatment. White fill corresponds to equal group
 541 size while grey denotes constant density, which is listed below each treatment. Means
 542 sharing any common letters are not significantly different ($P > 0.05$).



543

544 **Figure 2-9**

545 Movement activity according to age (LSM \pm SEM). Means sharing any common letters

546 are not significantly different ($P > 0.05$).

547

548 **Discussion**

549 This study clearly shows that group size, density, and the size of the enclosure
550 have distinct effects on use of space in the domestic fowl. Nearest neighbor distances
551 indicate group spacing or cohesion (Clark & Evans 1954; Keeling & Duncan 1991;
552 Stahl et al. 2001; Christman & Lewis 2005) and in groups of domestic fowl they were
553 generally larger in larger enclosures, particularly when group size remained constant
554 with 10 birds per enclosure (density decreased as enclosure size increased; 10_S, 10_M,
555 10_L). These results suggest that birds adjust the distance to their nearest neighbor
556 according to the dimensions of the enclosure. Although domestic fowl, as a highly
557 social species, tend to form cohesive groups it is evident from these results that when
558 given the opportunity birds will, to a certain extent, spread out in larger enclosures.
559 However, in no treatment combination did birds take full advantage and use the entire
560 space available to them. In addition, birds did not position themselves as would be
561 expected at random; they were closer together than expected in smaller groups, but
562 slightly farther apart in the larger groups/ densities. It is not surprising that broilers do
563 not behave as would be expected by random positioning. Flocking and group
564 cohesion are anti-predatory strategies (Pulliam 1973; Clark and Mangel 1984) which
565 have been shown to exert a strong influence on the behavior of domestic fowl (Leone
566 et al. 2007), particularly in small group sizes (which likely perceive a greater
567 predatory risk as compared to larger group sizes). Domestic fowl have a strong
568 tendency to flock together in confinement, even when ample space to disperse is
569 available (Leone et al., 2007). This flocking behavior resulting in group cohesion

570 means that birds do not space themselves uniformly, or take full advantage of the
571 total amount of space available to them. Particularly in the larger group sizes birds
572 may be motivated to disperse in an attempt to minimizing the chances for resource
573 competition within a confined environment (Stahl et al. 2001; Leone & Estévez
574 2008b). Nevertheless inter-individual distances appeared to be restricted by density,
575 even in large enclosures, as suggested by the fact that nearest neighbor distances were
576 smaller and differences were not as evident when density was maintained at 6.7 birds
577 per m² across increasing enclosure size (10_S, 20_M, 30_L).

578 A second parameter that was used to measure use of space was total distance
579 traveled, which estimates the distance moved by animals when locations are collected
580 close in time (Estévez & Christman 2006) as was the case in this study. These data
581 show that similar to nearest neighbor distances, total distance traveled per observation
582 period increased with enclosure size when group size was constant (10_S, 10_M, 10_L),
583 but no differences were detected across increasing enclosure size when density was
584 controlled at 6.7 birds per m² (10_S, 20_M, 30_L). These results are in agreement with
585 results from previous studies. Keeling and Duncan (1991) found that inter-individual
586 distances among small groups of domestic fowl were larger in larger enclosures, and
587 Estévez et al. (1997) and Newberry (1999) reported that broiler chickens traveled
588 greater distances when additional space was made available to them. The constraining
589 effect of density on movement and space use has also been documented in other
590 studies (Kondo et al. 1989; Pollard & Littlejohn 1996; Estévez et al. 1997;
591 Carmichael et al. 1999; Arnould & Faure 2004).

592 The unique and novel contribution of this study is the ability to demonstrate
593 the independent effects of enclosure size and density. Nearest neighbor distances and
594 total distance traveled are primarily affected by enclosure size, with groups being
595 more dispersed and birds traveling further when more space was available. However,
596 expansions in space use can be severely constrained by density in large enclosures
597 even at relatively low densities. Despite differences in enclosure size, at a constant
598 density the total amount of ‘effective free space’ per bird is similar. It is possible that
599 this limited the birds’ potential to spread apart and attain greater inter-individual
600 distances as they otherwise would in larger enclosures. It was also quite remarkable
601 that the impact of a reduction in ‘effective free space’ was detected at the low density
602 employed in this experiment. The highest density of 6.7 birds/ m² was well below
603 commercial standards (see for example Dawkins et al. 2004; Estévez 2007; Estévez et
604 al. 2007; Leone & Estévez 2008a) and should have allowed birds sufficient ‘effective
605 free space’ to travel throughout the enclosure with little interference and negotiate
606 any individuals lying in their path of movement. These results indicate that this was
607 not the case and some degree of restriction can, and does occur in response to an
608 increase in density.

609 Although I attempted to control enclosure size, group size, and density to the
610 best of my ability, there was some inevitable confounding between enclosure size and
611 group size when comparisons were made across constant density (10_S, 20_M, 30_L). It is
612 possible that the limited expansion observed in nearest neighbor distances, and the
613 lack of differences regarding total distance traveled at constant density may have been
614 the result of limited freedom of movement related to increasing social conflict in

615 larger groups (McBride 1970; Grigor et al. 1995b) and were not related to density. If
616 group size effects were a major factor then total distance traveled and nearest
617 neighbor distances would be expected to be substantially smaller in larger groups, if
618 in fact birds limited their movements and established small subgroups (McBride &
619 Foenander 1962), or substantially larger if they were trying to avoid dominant
620 individuals (Hemelrijk 1999, 2000; Cordiner & Savory 2001). Contrarily, these
621 results appear to be more consistent with the previously described barrier effect
622 (Newberry & Hall 1990; Estevez et al. 1997; Estevez et al. 2007) which suggests that
623 limitations to movement and use of space in the domestic fowl are related to the
624 physical barriers created by the presence of other individuals in the path of movement.
625 When density is constant, as an individual moves the chance of encountering an
626 obstacle (in the form of another bird) would be similar regardless of enclosure or
627 group size, and therefore total distance traveled per observation period would be
628 expected to be similar across groups, exactly as found in this experiment. Likewise,
629 because the amount of ‘effective free space’ is similar when density remains constant
630 nearest neighbor distance was not expected to vary. However nearest neighbor
631 distance were slightly, but significantly, higher in the 30_L groups as compared with
632 10_S and 20_M. This may be interpreted as evidence that space is marginally more
633 effective for constant density in large pens, at least as far as maintaining larger
634 nearest neighbor distances but not when considering total distance traveled. In light of
635 these results both enclosure size and density appear to have a different but equally
636 important influence on movement and space use in groups of domestic fowl, as
637 measured by nearest neighbor distances and total distance traveled. In contrast the

638 effects of increasing group size appeared to be less relevant, at least under the
639 conditions of this particular study.

640 Enclosure size also appeared to be the most significant factor affecting net
641 displacement and minimum convex polygons. Net displacement measured the net
642 progress a bird made within the enclosure during a five minute observation period,
643 whereas minimum convex polygon is an estimate of the total amount of space used by
644 individuals (White & Garrott 1990). In this study both net displacement and
645 minimum convex polygon increased with enclosure size, but unlike nearest neighbor
646 distances and total distance traveled, this increase occurred irrespective of changes in
647 density or group size. Neither measure differed when comparisons were made across
648 equal enclosure sizes which suggests that birds adapted their space use patterns to the
649 size of the enclosure while density and group size had little, if any, influence on these
650 two variables.

651 Both total distance traveled and net displacement increased with enclosure
652 size for treatments 10_S, 10_M, and 10_L (constant group size). Conversely, total distance
653 traveled was similar across groups 10_S, 20_M, and 30_L (constant density), whereas net
654 displacement increased significantly with enclosure size. It is evident that if these
655 parameters differ it is because they are capturing slightly different aspects of
656 movement dynamics. For example, it is possible that birds at the smaller group sizes/
657 densities (10_S, 10_M, 10_L) were less restricted and more motivated to move and explore
658 the environment, which domestic fowl are known to do when the opportunity is
659 available (Newberry 1999; Krause et al. 2006), therefore increasing both total
660 distance travel and net displacement. Contrarily when density is higher, birds will

661 find more obstacles (other birds) in their movement path, regardless of enclosure size.
662 Although the total distance traveled may be similar for equal densities, in larger
663 enclosures birds may be able to move farther from their initial starting position, which
664 would result in greater net displacement with increasing enclosure size even at high
665 density. In other words, these results may be interpreted as evidence of more sinuous
666 movement patterns in smaller enclosures resulting in similar total distance travel but
667 less net displacement.

668 The results obtained for minimum convex polygons seem to follow from the
669 above explanation for net displacement. Birds had larger minimum convex polygons
670 in larger enclosures, irrespective of group size and density, suggesting that the overall
671 amount of space used over time is ultimately determined by enclosure size. This may
672 appear to be counterintuitive when considering the limiting effects of density for total
673 distance traveled, as birds under constant density traveled the same distance in all
674 enclosure sizes (10_S, 20_M, and 30_L). Because total minimum convex polygons were
675 constructed from all recorded locations over the duration of the experiment they
676 measured long term space use pattern, in contrast to total distance traveled which is a
677 comparatively short term measure built from five minute observation periods.
678 Similarly weekly minimum convex polygons were built from averaging all observed
679 locations over the course of each week of age. Therefore, given the increase in net
680 displacement, over time it is possible to get significant differences regarding polygon
681 size while total distance travel remains unchanged across groups 10_S, 20_M, and 30_L.

682 These results for net displacement and minimum convex polygons show that
683 birds exhibit greater overall space use with increasing enclosure size. These results

684 also provide further evidence against the idea that use of space in the domestic fowl is
685 limited by social factors and that in larger groups birds utilize a subset of available
686 space, establishing territories or home ranges (McBride & Foenander 1962; Pamment
687 et al. 1983; Odén et al. 2004). This study, together with research published for young
688 domestic fowl (Newberry & Hall 1990; Estevez et al. 1997; Leone & Estevez 2007c;
689 Leone et al. 2007), and adult broiler breeders (Appleby et al. 1985; Leone & Estevez
690 2007b) clearly suggests that movement and use of space patterns in the domestic fowl
691 are only moderately affected by the number of birds in the group. Factors such as
692 enclosure size and density seem to be far more influential for animal movement and
693 spatial distribution as clearly suggested by the results of this, and other studies
694 (Estevez et al. 1997; Newberry 1999; Sherwin 2004, 2007). I am not however
695 inferring that group size is unimportant and has no effects. Previous research has
696 shown that in equally-sized enclosures inter-individual distances are heavily affected
697 by group size (Leone et al. 2007), but enclosure size and density may be more
698 relevant factors affecting general movement and space use.

699 In this study the only parameter in which we were able to detect a clear group
700 size effect was movement activity, defined as the percentage of time a bird moved
701 between successive scans. This differed only at the largest group size for treatment
702 30_L. The increased movement activity in this group size may have been related to the
703 potential to interact with a larger number of conspecifics. When group size was
704 controlled (10_S, 10_M, 10_L) there were no differences in movement activity with
705 increasing enclosure size. Neither was there a difference between these treatments
706 and 20_M, which had the same density as 10_S. Therefore, it would not appear that the

707 increase in movement activity at 30_L was due to density or enclosure size. It is
708 interesting to note that the increase in movement activity at the 30_L treatment did not
709 translate into a subsequent increase in total distance traveled, suggesting that birds
710 were mostly shuffling or repositioning rather than truly moving through the enclosure
711 in a directed manner. Birds may have experienced a greater number of disturbances
712 while resting as a result of the larger number of individuals in the group (Cornetto et
713 al. 2002) and these disturbances may cause repositioning or jostling (Febrer et al.
714 2006). Although increased disturbances were postulated to be a result of density as
715 opposed to group size, these two factors were confounded in previous experiments.
716 These results suggest that previous findings attributed to density may also be related
717 to the number of individuals in the group and not only to density

718 I did not detect an interaction between age and treatment for any parameters
719 analyzed in this experiment, indicating that birds responded consistently as they aged.
720 However age itself had a strong influence on nearest neighbor distances and
721 movement activity. Nearest neighbor distances increased as birds grew older, most
722 likely as a consequence of their increasing physical size. Weekly minimum convex
723 polygons were largest during the third week of age, and dropped during weeks four
724 and five. This is not surprising, as there was a decline in movement activity, in
725 agreement with previous research which has shown that domestic fowl become less
726 active as they age (Cornetto & Estevez 2001; Bokkers & Koene 2003).

727 The goal of this study was to separate to the greatest degree possible the
728 unique effects of enclosure size, group size, and density on movement and use of
729 space in the domestic fowl, as ability to distinguish their effects would have the

730 potential to improve management and environmental design for captive animals. I
731 found enough evidence to suggest that movement patterns in the domestic fowl are
732 primarily determined by enclosure size, followed by density. In general birds took
733 advantage of the space available to them. Although much attention has been given to
734 the effects of group size, under these specific experimental conditions I did not find
735 evidence to suggest that group size has a fundamental impact on how domestic fowl
736 utilize space. I found only a slight effect on movement activity, which does not
737 provide any evidence for social restriction of movement related to group size. In
738 summary, when housed in enclosures of equal size domestic fowl utilized similar
739 total amounts of space as indicated by net displacement and the size of the minimum
740 convex polygons, irrespective of density or group size. On the other hand, inter-
741 individual distances and rate of movement as measured by nearest neighbor distance
742 and total distance traveled per observation period were clearly affected by density.
743 The lack of differences in these measures when density remained constant was also
744 interpreted as further evidence for the barrier effect.

745 **Chapter 3: Separating the Effects of Group Size, Density,**
746 **and Enclosure Size**
747 **II. In Square Enclosures When the Perimeter to Area Ratio**
748 **Declines Rapidly as Enclosure Size Increases**

749

750 **Abstract**

751 The goal was to determine the unique impact of enclosure size on space use
752 and movement patterns of domestic fowl, independent of group size and density.
753 Research designed to estimate the effects of group size, density, or enclosure size
754 involves inherent confounding between factors, clouding their individual. This
755 experimental design enabled me to make multiple contrasts, each holding a single
756 factor constant, in order to tease apart their specific impact. In square enclosures,
757 enclosure size increases simultaneously in two dimensions (length and width), but
758 while peripheral (wall) space increases with enclosure size, the ratio of perimeter to
759 area decreases rapidly, the implications of which are discussed. My treatments
760 consisted of a combination of three enclosure sizes: small (1.5 m²), medium (3.0 m²)
761 and large (4.5 m²) and three group sizes of 10, 20, and 30 birds. I was able to make
762 comparisons across increasing enclosure size while holding group size and density
763 constant, as well as compare the effect of increasing group size/ density at a constant
764 enclosure size. Nearest neighbor distances increased with enclosure size but were
765 constrained even by my relatively low density of 6.7 birds /m². I found no indication
766 of social restriction on space use. While I did not detect differences in the total
767 distance traveled between treatments, net displacement and minimum convex

768 polygons increased with enclosure size regardless of group size or density. These
769 results indicate that broilers adapted to their enclosures, spreading out, making greater
770 progress and utilizing a greater amount of space when it was available.
771

772 **Introduction**

773 The characteristics of confined environments can significantly alter the way
774 animals interact with one another and utilize the resources available to them (Pollard
775 & Littlejohn 1996; Nephew & Romero 2003; van Wolkenten et al. 2006; Morgan &
776 Tromborg 2007), including space (Stricklin et al. 1979; Stricklin et al. 1995; Arnould
777 & Faure 2004; Christman & Leone 2007). Currently the U.S. broiler industry
778 produces over 8 billion birds annually (NASS 2007) which represents the single
779 largest sector of domestic animals raised in confinement. The poultry production
780 environment may induce stress and discomfort in broilers as a result of high densities
781 and large group sizes (Estévez et al. 1997; Sørensen et al. 2000; McLean et al. 2002;
782 Dawkins et al. 2004), or a lack of sufficient free space (Newberry & Hall 1990).

783 While the effects of group size and density on bird performance and social
784 behavior have received much attention (for review see Estévez 2007; Estévez et al.
785 2007), few studies investigate their impact on how chickens move within and utilize
786 the available space. However, variations in bird density and group size have been
787 shown to influence movement and space use (Estévez et al. 1997; Arnould & Faure
788 2004), and some authors have suggested that group size can create social restrictions
789 to movement (McBride & Foenander 1962; Banks et al. 1979; Grigor et al. 1995b).
790 While this may be the case for relatively small group sizes where birds are able to
791 form pecking orders (Estévez et al. 2007), at larger group sizes it is more likely that
792 conspecifics merely act as a physical barrier to the movement, limiting the possibility
793 of dispersion for other birds in the group (Newberry & Hall 1990; Estévez et al. 1997).

794 On the other hand, enclosure size and configuration may have a strong
795 influence on movement and use of space patterns. Available research has shown that
796 space use increases with enclosure size in broilers (Newberry & Hall 1990; Estévez et
797 al. 1997), and that increasing environmental complexity improves bird distribution
798 both in broilers (Cornetto & Estévez 2001b) and layers (Newberry & Shackleton
799 1997). Stricklin et al. (1998) suggested that enclosure shape would have a significant
800 impact on the ‘freedom of movement’ if animals in groups, and work with pigs has
801 demonstrated that aggressive interactions are greatly influenced by enclosure shape,
802 specifically the number of corners (Barnett et al. 1993; Wiegand et al. 1994). Greater
803 space allowances lead to an increase in inter-individual distances in laying hens
804 (Keeling & Duncan 1991), sheep (Sibbald et al. 2000), and cattle (Kondo et al. 1989),
805 and encourage play behavior in calves (Jensen et al. 1998). A number of enclosure
806 parameters may influence animal behavior (Christman & Leone 2007), especially
807 peripheral (wall) space, which has been found to be highly attractive in confined
808 environments (Newberry & Hall 1990; Cornetto & Estévez 2001b). When floor space
809 increases in square enclosures, even though the total amount of peripheral space
810 increases, the perimeter to area ratio decreases rapidly (Stricklin et al. 1995).
811 Therefore it is likely that the perimeter to area ratio affects use of space in captive
812 animals.

813 Studies which examine the effects of group size, density, and enclosure size
814 involve some degree of confounding (Christman & Leone 2007), as density is a direct
815 consequence of varying either group size or enclosure size. For this reason it is
816 difficult to isolate the individual effects of each of factor. It is extremely important to

817 determine the specific impact of group size, density and enclosure size on patterns of
818 movement in chickens so that spatial requirements and enclosure design can be based
819 on sound research which details the biological needs of animals.

820 While every approach involves some level of confounding, there are a few
821 strategies that can be used to systematically address the impact of varying enclosure
822 size, group size or density (Christman & Leone 2007). The goal of this study was to
823 isolate the effects of increasing enclosure size, group size and density on use of space
824 in broiler chickens as I hypothesized that each factor would have a unique and
825 distinctive effect on movement and space use in broiler chickens. To that end I
826 employed a novel experiment design which enables me to control on factor at a time,
827 and determined significant effect through the use of multiple contrasts.

828 This is the second study in a series which investigates the effects of group
829 size, density, and enclosure size on movement and use of space in broilers chickens.
830 In the first experiment I investigated these same effects, but raised chickens in
831 rectangular enclosures which increased in floor area only in one direction (length). In
832 this second study, enclosure size increased proportionally in two directions (length
833 and width) to create square enclosures, which reduces the proportion of perimeter to
834 floor space.

835

836 **Methods**

837 **Facilities and Experimental Animals**

838 This project was conducted at the University of Maryland's Applied
839 Poultry Research Facility in Upper Marlboro from April through June 2006. A

840 total of 540 male day-old broiler chicks (Ross 703) were obtained from a
841 commercial hatchery. I chose to work with only a single sex in order to
842 minimize behavioral variability. Initially 12, 24, and 36 birds were placed in
843 the experimental enclosures in an effort to account for early mortalities and
844 reach the target group sizes of 10, 20, and 30 birds. At the end of 3 weeks,
845 extra birds were removed and housed in a separate enclosure. Each bird was
846 individually tagged (Leone et al. 2007) on each side of the neck using the
847 Swiftack Poultry Identification System (Heartland Animal Health Inc., Fair
848 Play, MO). For the first 3 days birds were exposed to 24 hours of light, and
849 thereafter were maintained on a 14 L: 10 D program in an effort to slow
850 growth and promote leg health. Temperature and ventilation programs
851 followed commercial practices. Feed and water were provided *ad libitum* from
852 a central tubular hopper and a line of nipple drinkers located along one side of
853 the enclosure. Three hopper sizes were employed so that the proportion of
854 enclosure space occupied by the feeder as well as the amount of feeder space
855 available per bird was constant across treatments. The feeding program
856 consisted of a standard three phase commercial diet. This experimental
857 protocol (R-05-39) was approved by the Institutional Animal Care and Use
858 Committee at the University of Maryland.

859

860 **Experimental Design**

861 For this experiment I constructed three enclosure sizes which provided
862 1.49 m² (small, 1.22 x 1.22 m), 2.97 m² (medium, 1.72 x 1.72 m), and 4.46 m²
863 (large, 2.11 x 2.11 m), each covered with 5 cm of wood shavings. The

864 enclosures increased in size such that the medium-sized enclosure provided
865 twice the floor area as the small, and the large provided three times the floor
866 area (Appendix 6-3). All pens were square, as the width and length of
867 available floor space increased simultaneously. The specific enclosure
868 dimensions and resulting parameters for each treatment are laid out in Table
869 3-1 (for detailed description of calculations see Christman & Leone 2007).

870 The group sizes were housed in the different enclosures to generate my
871 five experimental treatments (Table 3-1), each of them replicated five times.
872 Groups of 10 were housed in all three enclosure sizes (10_S, 10_M, 10_L) while
873 groups of 20 and 30 birds were housed in the medium (20_M) and large
874 enclosures (30_L) respectively. This design enabled me to make comparisons
875 across constant group size, while density decreased with increasing enclosure
876 size (10_S, 10_M, 10_L), across a constant density, where group size increased
877 with enclosure size (10_S, 20_M, 30_L), and finally across constant enclosure sizes
878 where group size and density increased simultaneously (10_M, 20_M and 10_L,
879 30_L).

880

881 **Data Collection**

882 Each enclosure was divided into a grid of 20 x 20 cm squares by
883 placing numerical and alphabetical placards along the enclosure walls. This
884 created a visual grid that allowed me to precisely record bird locations on
885 scaled maps of the enclosure (Cornetto & Estévez 2001b; Leone et al. 2007).
886 For behavioral observations five focal birds were randomly selected from each
887 enclosure and were observed throughout the entire experiment.

888 **Table 3-1**

889 Enclosure parameters for each treatment (noted by group size and enclosure size) including the group size, density, total floor area,
 890 enclosure walls length and width, length to width ratio, total perimeter space, perimeter per animal, perimeter to area ratio, farthest
 891 distance to a wall and farthest distance to a corner.

Treatment	Group Size	Density (birds/m ²)	Total Area (m ²)	Length (m)	Width (m)	Length: Width Ratio	Perimeter (m)	Perimeter (m) / animal	Perimeter: Area Ratio	Distance to Wall (m)	Distance to Corner (m)
10 _{Large}	10	2.2	4.47	2.11	2.11	1 : 1	8.44	0.84	1.9 : 1	1.06	1.49
10 _{Medium}	10	3.4	2.96	1.72	1.72	1 : 1	6.88	0.69	2.3 : 1	0.86	1.22
10 _{Small}	10	6.7	1.49	1.22	1.22	1 : 1	4.88	0.49	3.3 : 1	0.61	0.86
20 _{Medium}	20	6.7	2.96	1.72	1.72	1 : 1	6.88	0.34	2.3 : 1	0.86	1.49
30 _{Large}	30	6.7	4.47	2.11	2.11	1 : 1	8.44	0.28	1.9 : 1	1.06	1.22

892 Birds in each enclosure were observed twice per day, three days per week
893 from three to six weeks of age. The location and identity of each focal
894 individual, as well as the position of all other group members, was recorded
895 via instantaneous scan sampling *ad libitum* for a five minute period. At the
896 beginning of each observation period bird locations were recorded on a single
897 scan sheet, and once completed successive locations were recorded on
898 additional scan sheets until the 5 minutes expired. These location scans were
899 digitized as X Y coordinates with a Digitalizer (ACECAD, Taipei, Taiwan)
900 using the Chickitaizer© software (Sanchez & Estévez 1998).

901 From each five minute observation period I calculated a number of
902 measures that best characterize movement patterns and space use. These
903 included: nearest neighbor distances, defined as the distance between a bird
904 and its nearest group mate, which was calculated from the locations of all
905 group members (focals and non focals), total distance traveled for each focal
906 bird, defined as the sum of Euclidean distances between successive recorded
907 locations, net displacement which was calculated as the Euclidean distance
908 between the first and last observed location during the five minute observation,
909 and movement activity which was defined as the percentage of scans where
910 movement was observed. For nearest neighbor distances I was uniquely able
911 to calculate the distances which would have been expected if birds positioned
912 themselves randomly within the environment, through the use of a simple
913 random simulation. This random simulation could not be utilized for other
914 measures, because no assumptions were made about movement patterns per se.

915 Each simulation (InsightfulCorp S-plus 6.1, Seattle, WA) consisted of
916 randomly assigning locations to all birds according to each treatment
917 combination. The average nearest neighbor distance for the group was then
918 calculated, and this simulation was repeated 2000 times in order to generate
919 expected nearest neighbor distances. The average value from these 2000
920 simulations represents the nearest neighbor distance that would be expected if
921 birds randomly distributed within their enclosure. Deviations were then
922 calculated by subtracting the observed nearest neighbor distances from those
923 expected assuming randomness, in order to determine to what extent the
924 treatment influenced inter-individual distance. Minimum convex polygons
925 (Mohr 1947) were calculated using ArcView GIS v8 (ESRI, Redlands, CA)
926 with the Animal Movement Extension package (Hooge & Eichenlaub 2000).
927 One minimum convex polygon was built from all observed locations,
928 throughout the entire length of the study, which provided an estimate of the
929 total amount of space utilized by each focal bird. I also determined the percent
930 of available space that the birds utilized by dividing the minimum convex
931 polygon by the total amount of space available. Minimum convex polygons
932 were also generated for each focal bird during each week, in order to
933 understand the effect on age on space use. Additionally a coefficient of
934 variation was calculated to ascertain the variability in space use between
935 individuals across age. All measurements were averaged across birds within
936 each enclosure for statistical analysis.
937

938 **Statistical Analysis**

939 All analyses were conducted in SAS (v. 9.1, SAS Institute, Cary, NC;
940 Appendix 6-4). For all parameters calculated in this study, except minimum
941 convex polygons, I modeled the effects of treatment, age, and their interaction.
942 Because total minimum convex polygons were calculated from all locations
943 recorded over the entire study period the statistical model only included the
944 treatment effect. Separate mixed model ANOVAs were performed for each of
945 the parameters analyzed: nearest neighbor distances and their deviation from
946 random expectations, total distance traveled during an observation period, net
947 displacement, movement activity, weekly minimum convex polygons and
948 their coefficient of variation, and the percentage of enclosure space utilized.
949 All models included a covariance structure to account for repeated
950 observations. Model assumptions of normality and homogeneity of residual
951 variances were examined. In order to meet the homogeneity assumption
952 variance components were modeled by treatment for nearest neighbor
953 distances and their deviation from randomness, and both total and weekly
954 minimum convex polygons and their coefficient of variation.

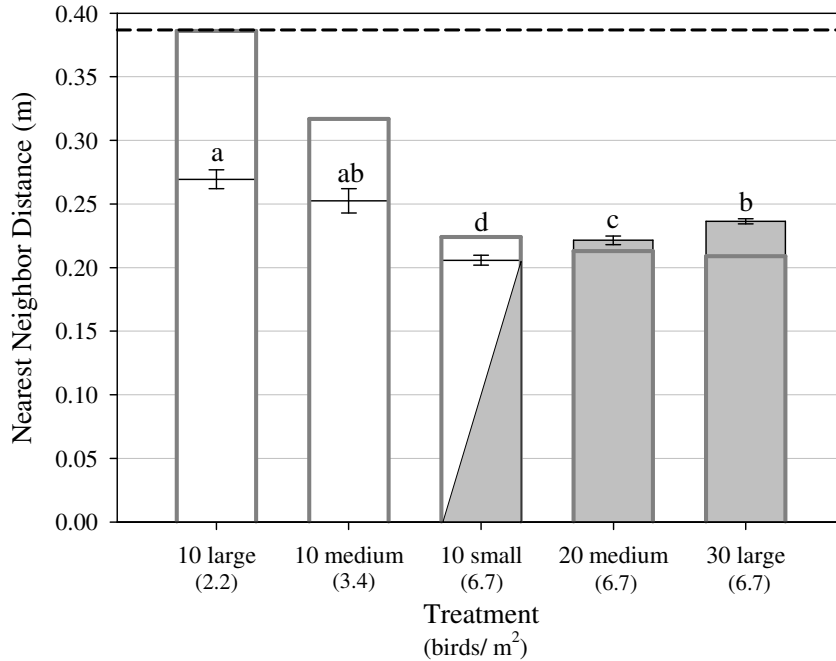
955 The goal of this experiment was addressed with three specific *a priori*
956 contrasts, I tested the effect of increasing enclosure sizes first at constant
957 group size but decreasing density (10_S, 10_M, 10_L), and second when density
958 was held constant but group size increased with enclosure size (10_S, 20_M, 30_L).
959 Lastly, I compared across equal enclosure size but density and group size
960 increased simultaneously (10_M, 20_M and 10_L, 30_L). Each of these contrasts

961 held one factor constant, while the other two covaried; each contrast is
962 essentially an ANOVA. In order to protect again an inflated Type I error rate
963 contrasts and means comparisons were only performed when the overall
964 ANOVA F-test was significant ($P < 0.05$).

965

966 **Results**

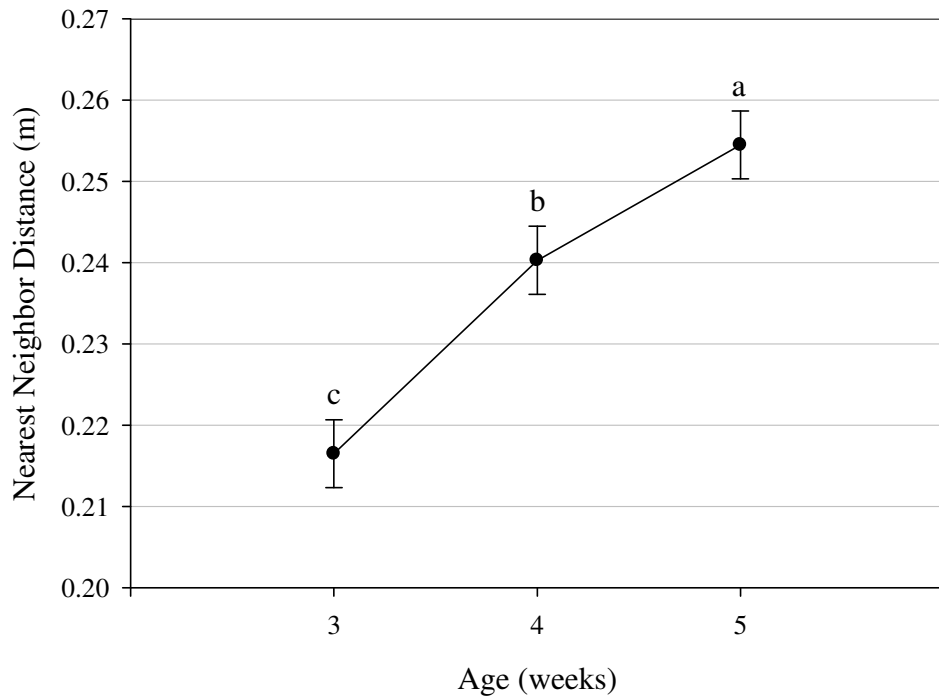
967 The degree of cohesion within the group, as measured by nearest neighbor
968 distances, was influenced both by treatment ($F_{4,9.59} = 19.42$, $P = 0.004$), and age
969 ($F_{2,26.5} = 25.19$, $P < 0.001$), but not their interaction ($F_{8,19.4} = 0.83$, $P = 0.59$). My *a*
970 *priori* contrasts revealed that nearest neighbor distances expanded with enclosure size
971 (Fig. 3-1) both at constant group size (10_S, 10_M, 10_L; $F_{2,15.2} = 32.83$, $P < 0.001$) and at
972 constant density (10_S, 20_M, 30_L; $F_{2,15.3} = 25.74$, $P < 0.001$). Comparisons between
973 enclosures of equal size revealed larger nearest neighbor distances in the smaller
974 group sizes/ densities (10_M and 20_M, 10_L and 30_L; $F_{2,7.4} = 12.12$, $P < 0.01$). Observed
975 distances deviated from those expected assuming randomness for all treatments
976 ($F_{4,6.93} = 124.12$, $P < 0.001$; Fig 3-2). In the smaller groups of 10 birds nearest
977 neighbor distances were smaller than expected, but were father apart than predicted in
978 the groups of 20 and 30 birds. There was no interaction between treatment and age on
979 the deviation of observed nearest neighbor distances from those expected assuming
980 randomness ($F_{8,5.73} = 0.59$, $P = 0.762$). Nearest neighbor distances increased with bird
981 age (Fig. 3-2). Birds were also closer together at all ages than would be expected by
982 random assortment ($F_{2,11.4} = 31.30$, $P < 0.001$).



983

984 **Figure 3-1**

985 Nearest neighbor distances (least squares means \pm standard error of the mean)
 986 according to each group size (10, 20, or 30) and enclosure size (small, medium, or
 987 large) treatment, and the expected values assuming random assortment (gray outline).
 988 White fill corresponds to equal group size while grey denotes similar densities.
 989 Means sharing any common letters are not significantly different ($P > 0.05$).
 990 Observed nearest neighbor distances differed from those expected assuming
 991 randomness ($P < 0.05$), and from the predicted uniform distribution based on a
 992 density of 6.7 birds/m² represented by the dotted line ($P < 0.05$).



993

994

995 **Figure 3-2**

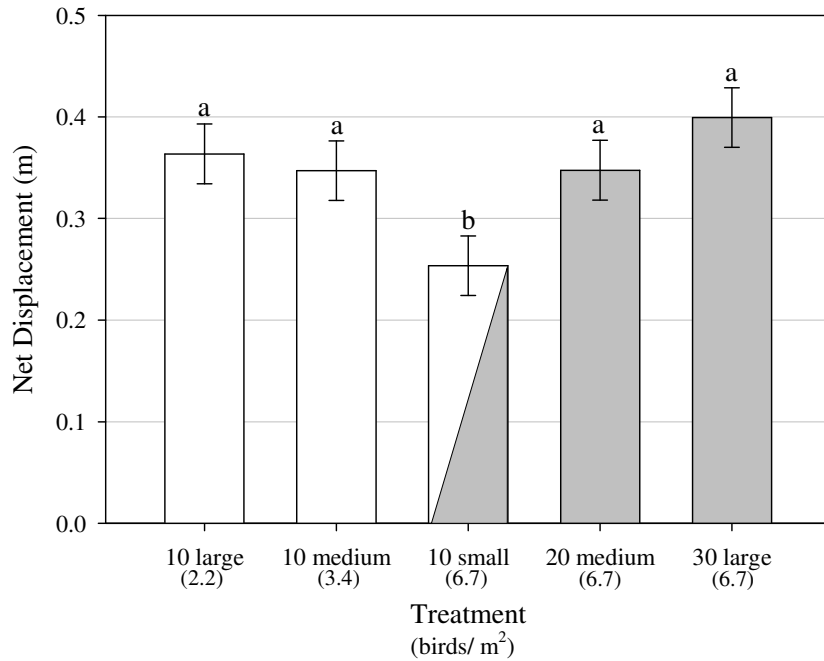
996 Nearest neighbor distances (LSM \pm SEM) were found to grow as birds aged. Means

997 sharing any common letters are not significantly different ($P > 0.05$).

998 Total distance traveled during an observation period was not affected by
999 treatment ($F_{4,23.8} = 0.68, P = 0.61$), age ($F_{2,41.2} = 2.46, P = 0.10$), or their interaction
1000 ($F_{8,41.9} = 1.31, P = 0.27$). Net displacement however was affected by treatment ($F_{4,20}$
1001 $= 3.37, P = 0.029$), but not by age ($F_{2,20} = 0.96, P = 0.39$) or their interaction ($F_{8,40} =$
1002 $1.77, P = 0.11$).

1003 Net displacement was greater as enclosure size increased from small to
1004 medium or large (Fig. 3-3) regardless of changes in group size (10_S, 20_M, 30_L; $F_{2,36.8}$
1005 $= 6.26, P = 0.004$) or density (10_S, 10_M, 10_L; $F_{2,36.8} = 4.02, P = 0.026$), but was not
1006 different when I compared equally-sized enclosures (10_M and 20_M, 10_L and 30_L; $F_{2,20}$
1007 $= 0.37, P = 0.693$).

1008 The total amount of space utilized by the birds as measured by total minimum
1009 convex polygons was affected by treatment ($F_{4,34.1} = 305.67, P < 0.001$; Fig. 3-4) and
1010 was substantially larger for birds in larger enclosures regardless group size or density.
1011 Space use increased with enclosure size both when group size (10_S, 10_M, 10_L; $F_{2,53.4} =$
1012 $300.07, P < 0.001$) and density (10_S, 20_M, 30_L; $F_{2,46.4} = 446.28, P < 0.001$) were
1013 maintained. On the contrary, there were no differences between equally-sized
1014 enclosures (10_M and 20_M, 10_L and 30_L; $F_{2,49.9} = 2.22, P = 0.12$). Similarly there was a
1015 significant effect of treatment on the average minimum convex polygon per week ($F_{4,$
1016 $7.45 = 58.47, P < 0.001$; Fig. 3-5) although there was no effect of age ($F_{2,10.4} = 1.03, P$
1017 $= 0.391$) or their interaction ($F_{8,5.47} = 0.90, P = 0.570$). The coefficient of variability
1018 in minimum convex polygons was not affected by treatment ($F_{4,20} = 1.51, P = 0.236$),
1019 age ($F_{2,19} = 1.80, P = 0.19$) or their interaction ($F_{8,24.3} = 1.91, P = 0.125$).



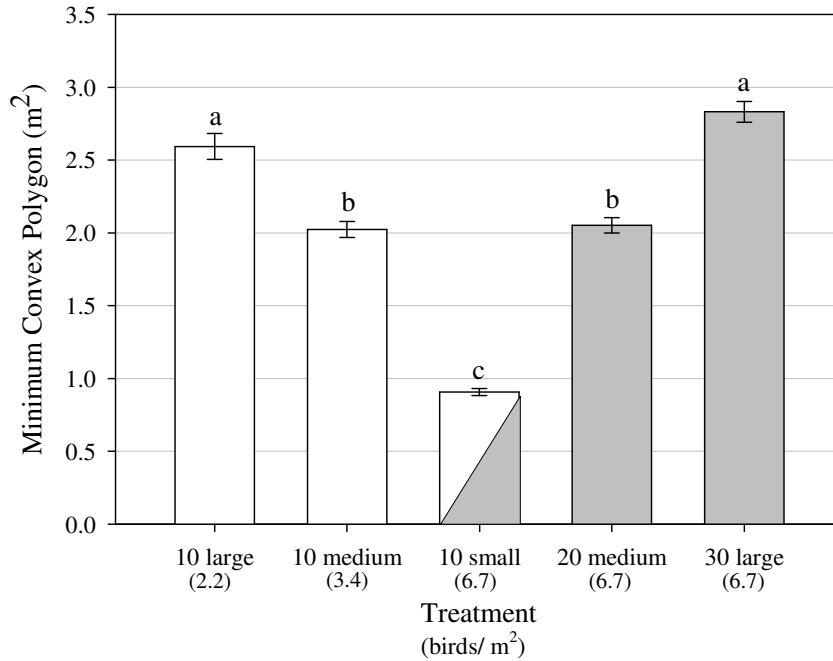
1020

1021 **Figure 3-3**

1022 Net displacement (LSM ± S.E.M.), defined as the Euclidean distance between the
 1023 first and last observation, according to each group size (10, 20, or 30) and enclosure
 1024 size (small, medium, or large) treatment. White fill corresponds to equal group size
 1025 while grey denotes similar densities. Means sharing any common letters are not
 1026 significantly different ($P > 0.05$).

1027 The proportion of total space utilized also differed between treatments ($F_{4,20} =$
1028 4.90, $P = 0.006$), varying between 60 and 70 % of the available space. The response
1029 to enclosure size was curvilinear (Fig. 3-6) regardless of density (10_S, 10_M, 10_L; $F_{2,36.8}$
1030 = 5.91, $P = 0.006$) or group size (10_S, 20_M, 30_L; $F_{2,36.8} = 3.82$, $P = 0.031$) and I found
1031 no differences in the proportion of space utilized between enclosures of equal size
1032 (10_M and 20_M, 10_L and. 30_L; $F_{2,20} = 1.77$, $P = 0.20$).

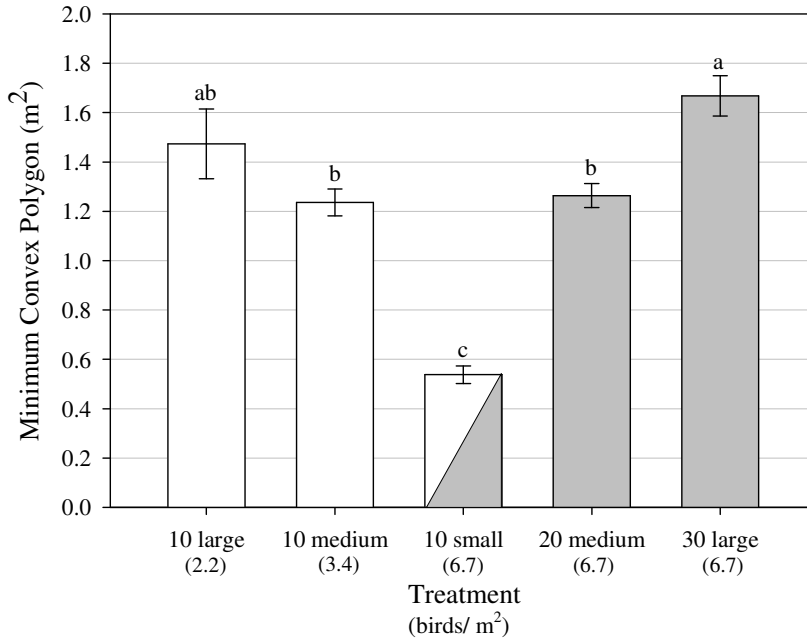
1033 Movement activity was influenced by treatment ($F_{4,20} = 5.31$, $P = 0.004$; Fig.
1034 3-7) and decreased with age ($F_{2,19} = 21.69$, $P < 0.001$; Fig. 3-8) but I did not detect
1035 any interactive effects ($F_{8,24.3} = 1.24$, $P = 0.32$). There was no effect of enclosure size
1036 on movement activity when group size was maintained (10_S, 10_M, 10_L; $F_{2,36.8} = 0.98$,
1037 $P = 0.38$) but I did find differences between enclosure sizes at constant density (10_S,
1038 20_M, 30_L; $F_{2,36.8} = 4.77$, $P = 0.014$) and when comparisons were made across constant
1039 enclosure size (10_M and 20_M, 10_L and. 30_L; $F_{2,20} = 10$, $P = 0.001$). These significant
1040 effects were most likely a result of the increase in movement at the largest group size
1041 of 30 birds (Fig. 3-8).



1042

1043 **Figure 3-4**

1044 Total minimum convex polygons (LSM ± S.E.M.) according to each group size (10,
 1045 20, or 30) and enclosure size (small, medium, or large) treatment for the entire length
 1046 of the study. White fill corresponds to equal group size while grey denotes similar
 1047 densities. Means sharing any common letters are not significantly different ($P > 0.05$).



1048

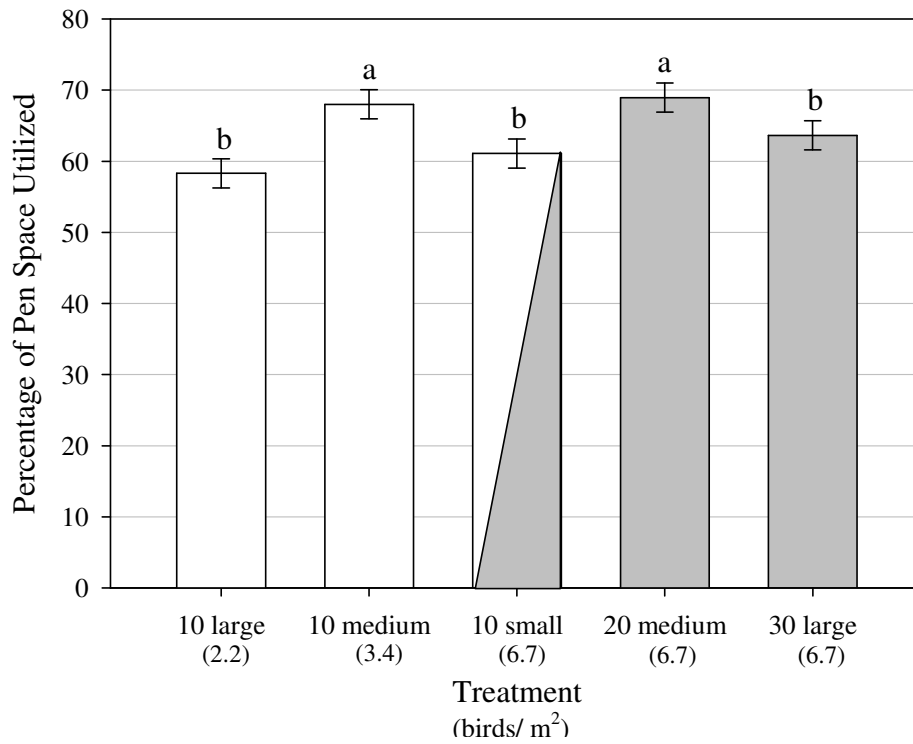
1049 **Figure 3-5**

1050 Average weekly minimum convex polygon (LSM ± SEM) according to group size

1051 (10, 20, or 30) and enclosure size (small, medium, or large) treatment. Means sharing

1052 any common letters are not significantly different ($P > 0.05$).

1053



1054

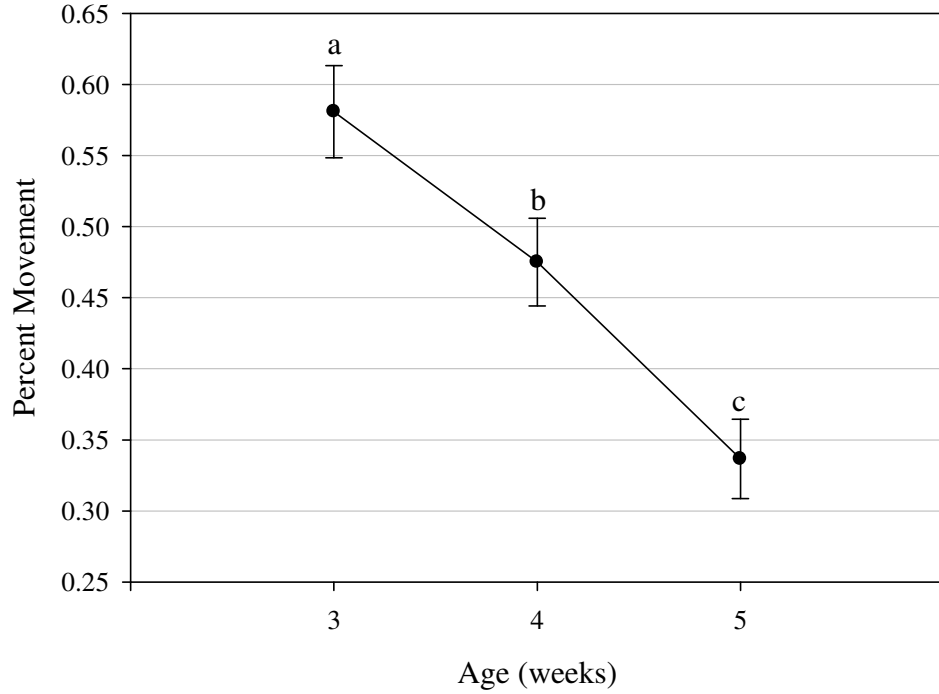
1055 **Figure 3-6**

1056 Percentage of the available enclosure space utilized (LSM ± S.E.M.) according to

1057 each group size (10, 20, or 30) and enclosure size (small, medium, or large) treatment.

1058 White fill corresponds to equal group size while grey denotes similar densities.

1059 Percentages sharing any common letters are not significantly different ($P > 0.05$).



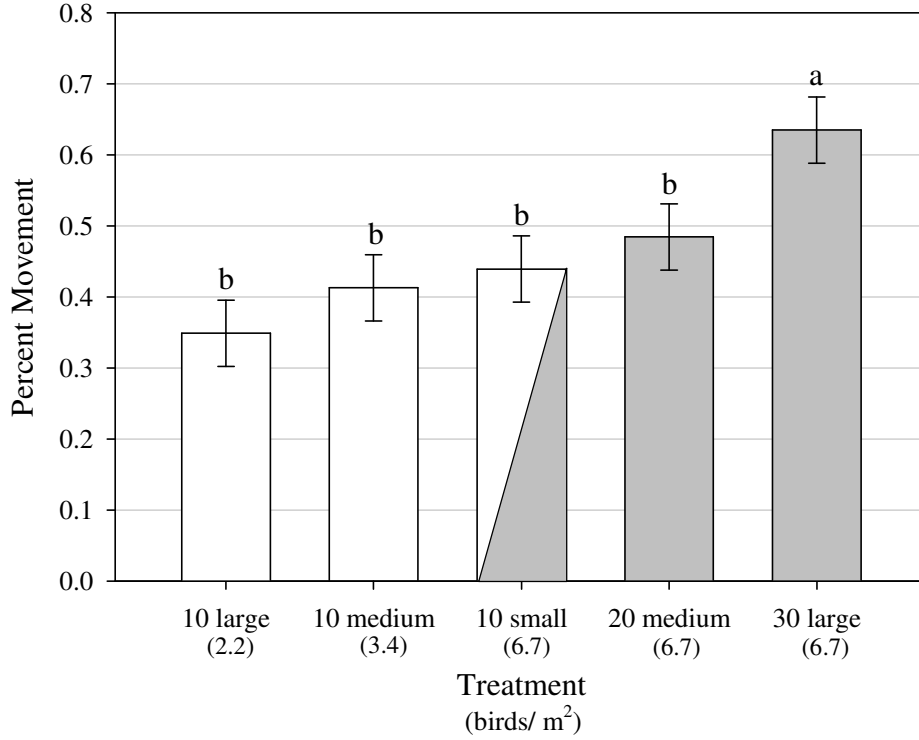
1060

1061 **Figure 3-7**

1062 Movement activity (LSM ± S.E.M.), the percentage of times birds moved between

1063 successive scans, decreased with age. Means sharing any common letters are not

1064 significantly different ($P > 0.05$).



1065

1066 **Figure 3-8**

1067 Movement activity (LSM ± S.E.M.) according to each group size (10, 20, or 30) and
 1068 enclosure size (small, medium, or large) treatment. White fill corresponds to equal
 1069 group size while grey denotes similar densities. Means sharing any common letters
 1070 are not significantly different ($P > 0.05$).

1071

1072 **Discussion**

1073 In this study, in which I carefully controlled for the effects of group size,
1074 density and enclosure size, I demonstrated that enclosure size had the strongest
1075 influence on movement and use of space in the domestic fowl. The effects of density,
1076 while significant, were not as pronounced, mostly limiting or reducing space use and
1077 movement. Surprisingly, group size had only a minor impact on space use.

1078 I found that nearest neighbor distances were affected by the treatment
1079 combinations, with birds maintaining larger distances in larger enclosures. Nearest
1080 neighbor distances expanded more substantially with increasing enclosure size when
1081 group size was maintained (10_S, 10_M, 10_L) and density decreased, as compared with
1082 increasing enclosure size at constant density (10_S, 20_M, 30_L). When not restricted by
1083 density, animals in larger enclosures had more room to spread out, resulting in larger
1084 inter-individual distances. Nearest neighbor distance was constrained by density, even
1085 though all densities in this study were well below those employed in commercial
1086 conditions (Estevez 2007). Birds however did not take full advantage of the space
1087 available to them, because the observed nearest neighbor distances were far smaller
1088 than would be expected by a uniform distribution, given the largest density of 6.7
1089 birds/ m². Similarly, birds did not behave as would be expected by random
1090 assortment. In smaller groups of 10 birds were closer together than would be expected
1091 assuming randomness, while in the larger groups of 20 and 30 birds were more
1092 dispersed than would be expected at random. Nearest neighbor distance is indicative
1093 of group cohesion and spacing (Clark & Evans 1954; Keeling & Duncan 1991; Stahl

1094 et al. 2001; Christman & Lewis 2005) and these results suggest that when given the
1095 opportunity, and when not constrained by density, birds maintained less cohesive
1096 groups with increasing enclosure size. Maintaining larger nearest neighbor distances
1097 may reduce competition for resources (Stahl et al. 2001; Leone & Estévez 2007), but
1098 more importantly it may offer thermoregulation advantages. Larger inter-individual
1099 distances may help the birds to thermoregulate, as more free space between
1100 individuals may increase air flow and heat loss, reducing the temperature at floor
1101 level. Improved thermoregulation may also explain why nearest neighbor distances
1102 increased as birds aged. Heat-production increases as birds grow, and they become
1103 more susceptible to heat stress (Xin et al. 1994). Therefore it seems reasonable that
1104 increasing inter-individual distances helps to reduce heat stress and alleviate any
1105 potential effects of increased heat production as birds age and grow.

1106 Similar to nearest neighbor distances, net displacement was also greater in
1107 larger enclosures. Net displacement essentially measures the overall progress made as
1108 a result of movement patterns (Wu et al. 2000) and under the present treatment
1109 structure was greatest in the medium and large enclosures, regardless of density or
1110 group size. It is reasonable to expect to find greater net displacement in larger
1111 enclosures as a result of birds moving more because more space is available. For
1112 example, birds made greater progress in 20_M and 30_L as compared to 10_S, even
1113 though density was identical in these three groups. At constant density the greater
1114 amount of ‘effective free space’ available in the larger enclosures may have enabled
1115 birds to make greater overall progress, whereas birds in 10_S may have been restricted
1116 by both the density and small enclosure size. There were no differences in net

1117 displacement between medium and large enclosure sizes, and taken together these
1118 results provide strong evidence that net displacement is determined most heavily by
1119 space availability.

1120 However, it is surprising that this effect of treatment on net displacement did
1121 not coincide with differences in total distance traveled, which were similar across
1122 treatments. As indicated in previous sections of this paper, total distance travel was
1123 calculated as the total distance traveled by a bird across successive locations, whereas
1124 net displacement was defined as the straight distance between the beginning and
1125 ending locations during the five minute observation period. Therefore, these results
1126 suggest that even though chickens under all treatments traveled similar distances
1127 (equal total distance traveled), birds in the larger enclosures moved farther away from
1128 the initial starting point by the end of the observation period. Changes in movement
1129 patterns according to enclosure size could explain the differences observed in
1130 response to the treatments for total distance traveled and net displacement. The
1131 amount of ‘effective free space’ would be less in smaller enclosures as birds would
1132 have a greater chance of encountering a group-mate blocking their path of movement
1133 (Newberry & Hall 1990, Estévez et al. 1997). This may have caused movements to be
1134 more sinuous or tortuous than those in the larger enclosures (and lower densities). It
1135 is also possible that birds in smaller enclosures experienced a stronger rebounding
1136 effects off the walls (as furthest distance to a wall was smaller, Table 3-1). If after
1137 each step the direction of the next move is determined in a more or less random
1138 manner, then the chances of ending up further away from the starting position will be
1139 greater in larger enclosures because there is a lower chance of encountering a wall.

1140 These differences in movement patterns may result in identical total distances
1141 traveled for birds in all treatments, but greater net displacement in larger pens.
1142 Nevertheless, I expected that total distance traveled would increase with enclosure
1143 size, especially at lower density (10_S, 10_M and 10_L), but this was clearly not the case.
1144 Broilers are characterized by a low level of activity, and generally spend upwards of
1145 80 % of their time resting (Cornetto & Estévez 2001a). Therefore it is possible that
1146 the effects of group size, density, and enclosure size were not strong enough to affect
1147 total distance traveled, at least not during the observation period employed here.

1148 In this study total minimum convex polygons were used to estimate total
1149 space use over the entire study period, and weekly minimum convex polygons were
1150 calculated to determine the average amount of space utilized in a week as birds aged.
1151 I found no effect of age on weekly minimum convex polygons, indicating that in
1152 square enclosures birds used similar amounts of space each week. Similar to the
1153 results for net displacement, both weekly and total minimum convex polygons were
1154 most heavily affected by the size of the enclosure, and appeared to be largely
1155 unaffected by density or group size. However, the effects of enclosure size on
1156 minimum convex polygons were much stronger than on net displacement, as
1157 differences were more significant between the three enclosure sizes (small, medium
1158 and large). These results suggest that given sufficient time birds in larger enclosures
1159 explored more overall space, taking advantage of the greater space availability, even
1160 at the density of 6.7 birds/ m². Whereas total distance traveled and net displacement
1161 measured short term movement patterns (over a five minute observation period),
1162 minimum convex polygons were built from all recorded locations over the entire

1163 study period and captured long term movement patterns. Thus, while I found no
1164 detectable differences in the short-term for total distance traveled, these results
1165 indicate that over time broilers utilize more space when it is offered, as in the larger
1166 the enclosures. Previous research has also shown both in broilers (Estévez et al. 1997)
1167 as well as layers (Carmichael et al. 1999) birds exhibit larger home ranges in larger
1168 enclosures. These results taken together demonstrate that broiler chickens will adapt
1169 their use of space patterns according to the amount of space available to them;
1170 spreading out, making greater progress and using a greater area. Surprisingly, these
1171 movement and use of space patterns appear to be 'immune' to the effects of group
1172 size or density, at least within the ranges employed in this experiment.

1173 While increasing enclosure size generally led to an increase in use of space, it
1174 was in the medium-sized enclosure where birds used the greatest proportion of the
1175 available space. I observed the same curvilinear response to enclosure size across
1176 constant group size (10_S, 10_M, 10_L) and constant density (10_S, 20_M, 30_L). Previous
1177 research has suggested that domestic fowl may find open space frightening, and that
1178 group size can significantly impact their willingness to explore it (Grigor et al. 1995a;
1179 Grigor et al. 1995c; Arnould & Faure 2004). Grigor et al. (1995a, b) demonstrated
1180 that even with regular exposure, laying hens did not make full use of additional
1181 outdoor space offered to them. Similarly Leone et al. (2007) found that when groups
1182 of 5, 10, and 20 broilers were housed in enclosures of equal size, the smaller groups
1183 used less overall space. It is possible that at constant group size of 10, birds may have
1184 felt more protected (less fearful) in the medium-sized enclosure than in the large
1185 enclosure, and thus explored a greater proportion of the available space. On the other

1186 hand, when density was controlled (and group size increased with enclosure size)
1187 birds in the 30_L treatment may have experienced a stronger barrier effect restricting
1188 movement than birds in the 20_M, as a result of the more numerous group mates
1189 (Newberry & Hall 1990; Estévez et al. 1997), which could explain the drop in the
1190 percentage of space utilized. It was particularly surprising that space use was
1191 relatively low in the 10_S groups, which had the smallest enclosure size (1.22 x 1.22
1192 m). Birds may have been heavily restricted in this enclosure, as I noted significantly
1193 reduced net displacements. Previous research has shown that locomotive activity is
1194 reduced in small or crowded environments (Poon et al. 1997; Boal et al. 1999;
1195 Arakawa 2005) and thus movement may have been restricted in this treatment
1196 specifically due to the relatively high density and small size of the enclosure.

1197 However, this does not explain why groups overall did not take greater
1198 advantage of the space available to them (utilization closer to 100%). Birds may have
1199 been preferentially using (and avoiding) certain areas of their enclosures, thus
1200 spontaneously limiting space use (Arnould & Faure 2004). Previous research at this
1201 facility has found that birds take greater advantage of the front of their enclosures
1202 closest to the central corridor, possibly due to better air flow at in this location closest
1203 to the ventilating fans, as opposed to the back which is closest to the exterior walls of
1204 the house (LeVan et al. 2000; Pettit-Riley & Estevez 2001; Leone unpublished data).
1205 If broilers were consistently avoiding the rear of their enclosure and more consistently
1206 using the front, then it would be logical that overall space use was less than the total
1207 amount of space available.

1208 It should be noted that in this study I found no evidence that movement or
1209 space use was restricted by social factors. McBride and Foenander, (1962) predicted
1210 that social interactions would increase with group size, and thus in large groups
1211 domestic fowl would limit their movements to avoid interactions with aggressive
1212 group mates. If this were the case, then I would have expected to find diminished
1213 space use at larger group sizes, and no differences at constant group size. To the
1214 contrary, space use (both net displacement and minimum convex polygons)
1215 consistently increased with enclosure size, regardless of group size. The only notable
1216 effect of group size in this study was on movement activity, which increased in the
1217 largest groups of 30 birds. There were no differences in movement activity between
1218 equal group sizes (10_S, 10_M, 10_L), or equal densities (10_S and 20_M), suggesting that
1219 that the greater movement activity in 30_L groups was a product of group size. Birds in
1220 these groups had the greatest potential to interact with con-specifics. Despite the
1221 increase in movement activity, I did not detect any differences between treatments in
1222 the total distance traveled. So while birds moved more often in 30_L they did not travel
1223 greater distances. It is possible that this movement activity reflected repositioning as a
1224 result of higher levels of jostling (Febrer et al. 2006) and increased disturbances
1225 (Cornetto et al. 2002), which have been detected in other studies. In both studies
1226 group size was manipulated in order to raise density, thereby confounding the two
1227 factors. Because I found a difference only at the 30_L treatment when comparing
1228 across constant density (10_S, 20_M and 30_L) I suggest that the larger group size led to
1229 an increase in bird interactions, rather than the density. Movement activity generally
1230 decreased with age, which is not surprising as broilers have been shown to become

1231 less active over time (Bizeray et al. 2000; Cornetto & Estévez 2001a; Bokkers &
1232 Koene 2002).

1233 Determining the impact of group size, density, and enclosure size is highly
1234 relevant to the design of facilities that best address the biological needs of chickens
1235 and increase efficiency in use of the available space. I found that each factor had
1236 distinct effects on movement and space use in the domestic fowl. The results of this
1237 study clearly show that enclosure size is the most critical factor affecting space use in
1238 broiler chickens, and chickens will use larger amounts of space when provided with
1239 them. The strongest effects were related to the size of the enclosure, followed by
1240 density, which appears to limit space use even at a relatively low level. Space
1241 utilization, as measured by net displacement and minimum convex polygons, was
1242 clearly affected by enclosure size irrespective of changes in group size or density.
1243 Nearest neighbor distances increased with enclosure size but were restricted by
1244 density. Group size had surprisingly minimal influence on broiler movement and
1245 space use, at least within the range employed here. Group size only appeared to
1246 increase shuffling and repositioning, as noted by greater movement activity at the
1247 largest group size of 30 birds (without a simultaneous increase in total distance
1248 traveled).

1249

1250 **Chapter 4: Separating the Effects of Group Size, Density,**
1251 **and Enclosure Size**
1252 **III. When Perimeter Space is Held Constant per Unit of**
1253 **Area**

1254

1255 **Abstract**

1256 I was interested in separating the confounded influences of group size, density,
1257 and enclosure size on movement and use of space patterns in the domestic fowl,
1258 which represent key aspects of their behavior. A number of enclosure parameters
1259 (such as the length to width and perimeter to area ratio) may influence chicken
1260 behavior, but they are often inadvertently altered when enclosure size increases. My
1261 goal was to maintain consistency across treatments both in the length to width ratio
1262 but also in the perimeter to area ratio. I housed groups of 10, 20, and 30 broilers in
1263 square enclosures of three sizes: small (1.5 m²), medium (3.0 m²) and large (4.5 m²).
1264 This experimental design enabled me to separate the influence of group size, density,
1265 and enclosure size through the use of contrasts. While group spacing increased with
1266 enclosure size, it was restricted by even the relatively low stocking density of 6.7
1267 birds/ m². The effect of treatment on movement parameters waned as birds aged, and
1268 in general movement decreased with age. Enclosure size was the most relevant factor
1269 for total space use, which was largely unaffected by group size or density.

1270

1271 **Introduction**

1272 Animal movement and space use are determined in large part by the environment, a
1273 fact which cannot be overstated for captive animals (McBride & Craig 1985; Morgan
1274 & Tromborg 2007). Three factors may be of paramount importance: group size,
1275 animal density (the theoretical amount of space available per animal), and enclosure
1276 size. For domestic fowl, enclosure size and shape has received the least amount of
1277 attention while much work has focused on the impact of group size and density (for
1278 review see Estévez 2007; Estévez et al. 2007). Initially it would appear that the
1279 effects of group size are conflicting in the published literature. However in domestic
1280 fowl, as for many other species, there are clear differences between the behavior of
1281 animals in small versus large groups.

1282 In small groups of chickens the pecking order determines access to resources
1283 (Banks 1984; Banks et al. 1979; Rushen 1982) and social factors exert a strong
1284 influence over inter-individual distances and behaviour (Mankovich & Banks 1982;
1285 Grigor et al. 1995b; Leone et al. 2007; Leone & Estévez 2007). However, in large
1286 groups birds are most likely not able to recognize all individuals (Douglass 1948) or
1287 benefit from hierarchies (Pagel & Dawkins 1997), and aggressive interaction show a
1288 marked decrease (Estévez et al. 1997; Hughes et al. 1997; D'Eath & Keeling 2003).
1289 Because they are unable to establish a stable pecking order some authors have
1290 suggested that chickens will establish small territories when housed in large groups
1291 (McBride & Foenander 1962; see also Odén et al. 2004). To the contrary, even when
1292 small groups of young broiler chickens are placed in equally-sized enclosures,
1293 individuals use similar amounts of space (Leone et al. 2007) and adults have

1294 considerable overlap in their estimated home ranges (Appleby et al. 1985; Leone &
1295 Estévez 2008a).

1296 Floor space allowance, as well as many additional features of the enclosure
1297 may influence animal movement and behaviour such as the length to width ratio,
1298 distance to a corner, and the perimeter to area ratio (Stricklin 1995; Christman &
1299 Leone 2007). Each of these parameters, inadvertently change as the size or shape of
1300 the enclosure changes. Previous work has shown that enclosure shape has a
1301 significant impact on aggression in pigs (Barnett et al. 1993; Wiegand et al. 1994) and
1302 the addition of environmental complexity which creates additional wall space reduces
1303 disturbances and aggression in domestic fowl (Cornetto et al. 2002) and turkeys
1304 (Sherwin et al. 1999a). Perimeter (wall) space is an attractive feature of the enclosure
1305 (Stricklin et al. 1979; Newberry & Hall 1990; Jeanson et al. 2003) with biological
1306 relevance for captive animals. Perimeter space may provide a sense of protective
1307 cover or enable subdominant individuals to escape aggressive group-mates in captive
1308 environments (Hemelrijk 2000). While total perimeter space increases with enclosure
1309 size, it does not rise at an equal rate with floor area, and their relationship depends on
1310 the shape and specific dimensions of the enclosure. Likewise, the amount of
1311 perimeter space available on a per animal basis decreases with increasing enclosure
1312 size even when animal density remains constant (Stricklin et al. 1995; Christman &
1313 Leone 2007). This diminishing ratio of perimeter to area may be an influential feature
1314 of the environment with a strong effect on animal movement and space use. If
1315 perimeter space is as important as floor space then merely increasing enclosure size

1316 may not be an effective means to improve animal welfare, however few publications
1317 have examined the impact of the perimeter to area ratio.

1318 Every experiment designed to elucidate the effects of group size, density or
1319 enclosure size inadvertently introduces confounding (Christman & Leone 2007). For
1320 example, in order to determine the effect of increasing group size researchers can
1321 house animals in equally-sized enclosures and allow density to increase (e.g. Cornetto
1322 & Estévez 2001b), or hold density constant and therefore house larger groups in
1323 larger enclosures (e.g. Estévez et al. 2003). In both examples the effects of group size
1324 become inexorably confounded, either with density or enclosure size, respectively.
1325 The consequence is that in these types of experiments any significant results cannot
1326 be attributed specifically to a single factor, as the observed changes may be a direct
1327 consequence of the confounded variable. Additionally, as previously indicated,
1328 altering enclosure size causes a host of parameters to differ between treatments, such
1329 as the amount of perimeter space available and the distance to the nearest wall.

1330 This experiment was designed to investigate the impact of increasing
1331 enclosure size, while maintaining a constant perimeter to area ratio, on movement and
1332 space use in domestic fowl. I employed a novel treatment design in an effort to parcel
1333 out the specific contribution of each factor as I hypothesized that they would have a
1334 unique impact on movement patterns and space use. I expected that enclosure size
1335 would have a strong effect on total space use, whereas group size and density would
1336 impact movement patterns. This is the third experiment in a series which investigates
1337 the effects of group size, density, and enclosure size and shape on movement and
1338 space use in the domestic fowl. In previous experiments broilers were housed in

1339 rectangular (first experiment) and square (second experiment) enclosures which
1340 offered the same floor space allowances as those employed here. This experiment is
1341 unique in that ‘false walls’ were added to the enclosures in order to maintain a
1342 constant perimeter to area ratio.

1343
1344

1345 **Methods**

1346 **Facilities and Experimental Animals**

1347 This project was conducted at the University of Maryland’s Applied
1348 Poultry Research Facility in Upper Marlboro from October through December
1349 2006. A total of 540 male day-old broiler chicks were obtained from a
1350 commercial hatchery. I chose to work with only a single sex in order to
1351 minimize behavioral variability. Initially 12, 24, and 36 birds were placed in
1352 the experimental enclosures in an effort to account for early mortalities and
1353 reach the target group sizes of 10, 20, and 30 birds. At the end of 3 weeks,
1354 extra birds were removed and housed in a separate enclosure. Each bird was
1355 individually tagged (Leone et al. 2007) on each side of the neck using the
1356 Swiftack Poultry Identification System (Heartland Animal Health Inc., Fair
1357 Play, MO). For the first 3 days birds were exposed to 24 hours of light, and
1358 thereafter were maintained on a 14 L: 10 D program in an effort to slow
1359 growth and promote leg health. Temperature and ventilation program
1360 followed commercial practices. Feed and water were provided *ad libitum* from
1361 a central tubular hopper and a line of nipple drinkers located along one side of
1362 the enclosure. Three hopper sizes were employed so that the proportion of

1363 enclosure space occupied by the feeder as well as the amount of feeder space
1364 available per bird was constant across treatments. The feeding program
1365 consisted of a standard three phase commercial diet. This protocol (R-05-39)
1366 was approved by the Institutional Animal Care and Use Committee at the
1367 University of Maryland.

1368

1369 **Experimental Design**

1370 For this experiment I constructed three enclosure sizes which were
1371 1.49 m² (small, 1.22 x 1.22 m), 2.96 m² (medium, 1.22 x 2.44 m), and 4.47 m²
1372 (large, 1.22 x 3.66 m). Each enclosure was covered with 5 cm of wood
1373 shavings. Enclosures were square and thus maintained a constant length to
1374 width ratio. The enclosures increased in size such that the medium-sized
1375 provided twice the floor area as the small, and the large provided three times
1376 the floor area. The specific dimensions for each enclosure and resulting
1377 parameters are laid out in Table 4-1 (for detailed description of calculations
1378 see Christman & Leone 2007). Because the amount of perimeter space per
1379 bird and the perimeter to area ratio decreases with increasing enclosure size I
1380 constructed false walls (similar in construction to Cornetto & Estévez 2001b)
1381 in order to provide birds with a constant perimeter to area ratio. These were
1382 constructed of the same white PVC piping and black plastic netting as the
1383 enclosures and were 1.2m high (same as enclosure walls) and 5 cm, 24 cm,
1384 and 46 cm wide for the small, medium, and large-sized enclosures
1385 respectively.

1386 **Table 4-1**

1387 Treatment parameters for each group size and pen size. Parameters include stocking density, total floor area, pen length and width,
 1388 length to width ratio, total perimeter space including the false walls, perimeter/ animal, perimeter to area ratio, farthest distance to a
 1389 true wall and farthest distance to a corner.

Treatment	Group Size	Density (birds/m ²)	Total Area (m ²)	Length (m)	Width (m)	Length: Width Ratio	Perimeter (m)	Perimeter (m) / animal	Perimeter: Area Ratio	Distance to True Wall (m)	Distance to Corner (m)
10 _L	10	2.2	4.47	2.11	2.11	1 : 1	17.69	1.77	4 : 1	1.06	1.49
10 _M	10	3.4	2.96	1.72	1.72	1 : 1	11.79	1.18	4 : 1	0.86	1.22
10 _S	10	6.7	1.49	1.22	1.22	1 : 1	5.90	0.59	4 : 1	0.61	0.86
20 _M	20	6.7	2.96	1.72	1.72	1 : 1	11.79	0.59	4 : 1	0.86	1.49
30 _L	30	6.7	4.47	2.11	2.11	1 : 1	17.69	0.59	4 : 1	1.06	1.22

1390

Each enclosure was outfitted with 10 false walls in the arrangement shown in Appendix 6-5, placed in a manner so as not to impede bird movements.

The group sizes were housed in the different enclosures to generate my five experimental treatments (Table 4-1), each of them replicated five times. Groups of 10 were housed in all three enclosure sizes (10_S, 10_M, 10_L), while groups of 20 and 30 were housed in the medium (20_M) and large enclosures (30_L) respectively. This design enabled me to make comparisons across constant group size, where density decreased with increasing enclosure size (10_S, 10_M, 10_L), across a constant density, where group size increased with enclosure size (10_S, 20_M, 30_L), and across constant enclosure sizes where group size and density increased simultaneously (10_M, 20_M and 10_L, 30_L).

Data Collection

Each enclosure was divided into a grid of 20 x 20 cm squares by placing numerical and alphabetical placards along the enclosure walls. This created a visual grid that allowed me to precisely record bird locations on scaled maps (Cornetto & Estévez 2001a; Leone et al. 2007). For behavioral observations five focal birds were randomly selected from each enclosure and were observed throughout the entire experiment. Observations began at three weeks of age and continued until birds were six weeks old. The birds in each enclosure were observed twice per day, three days per week. The location and identity of each focal individual, as well as the position of all other group members, was recorded via instantaneous scan sampling *ad libitum* for a five minute period. At the beginning of each observation period the location of

each focal birds was recorded in X Y coordinates using the Chickitizer© software (Sanchez & Estévez 1998) on a tablet PC (Toshiba, Irvine, CA) and represented a single scan. Once completed successive locations were recorded via additional scans until the 5 minutes expired. From the each five minute observations period I calculated a number of measures to capture bird movement and space use which included: nearest neighbor distances, defined as the average distance between each individual and its closest neighbor, which were calculated from the locations of all group members, the total distance traveled which was calculated by summing the Euclidean distances between successive recorded locations for each focal bird during the five minute observation period, net displacement which was calculated as the Euclidean distance between the first and last observed location, and movement activity which was defined as the percentage of scans where movement was observed. For nearest neighbor distances I was uniquely able to calculate the values that would have been expected if birds positioned themselves randomly within the environment, through the use of a simple random simulation. This random simulation could not be utilized for other measures because no assumptions were made about movement patterns per se. Each simulation (InsightfulCorp S-plus 6.1, Seattle, WA) consisted of randomly assigning locations to all birds according to each treatment combination. The average nearest neighbor distance for the group was then calculated, and this simulation was repeated 2000 times in order to generate expected nearest neighbor distances. The average value from these 2000

simulations represents the nearest neighbor distance that would be expected if birds randomly distributed within their enclosure. Deviations were then calculated by subtracting the observed nearest neighbor distances from those expected assuming randomness, in order to determine to what extent the treatment influenced inter-individual distance. Minimum convex polygons (Mohr 1947) were calculated using ArcView GIS v8 (ESRI, Redlands, CA) with the Animal Movement Extension package (Hooge & Eichenlaub 2000). One total minimum convex polygon was built from all observed locations, throughout the entire length of the study, which provided an estimate of the total amount of space utilized by each focal bird. I also determined the percent of available space that the birds utilized by dividing the total minimum convex polygon by the total amount of space available. Average weekly minimum convex polygons were also generated for each focal bird, in order to estimate the amount of space utilized in a week and understand the effect on age. Additionally a coefficient of variation was calculated to ascertain the variability in space use between individuals across age. All measurements were averaged across birds within each enclosure.

Statistical Analysis

All analyses were conducted in SAS (v. 9.1, SAS Institute, Cary, NC; Appendix 6-6). For all parameters except total minimum convex polygons I modeled the effects of age, treatment, and their interaction. Separate mixed model ANOVAs were performed for each of the parameters analyzed: nearest neighbor distances and their deviations from randomness, movement activity,

total distance traveled during an observation period, net displacement, minimum convex polygons by week and their coefficient of variation, and the percentage of enclosure space utilized. Because total minimum convex polygons were generated from all recorded locations over the entire study period the statistical model only included the treatment effect. All models included a covariance structure to account for repeated observations. Model assumptions of normality and homogeneity of residual variances were examined. In order to meet the assumption of homogeneity, variance components were modeled by treatment for both total and weekly minimum convex polygons, as well as their coefficient of variation.

The goals of this experiment were addressed with three specific *a priori* contrasts, the first two detected differences between increasing enclosure sizes when group size was constant (but density decreased; 10_S, 10_M, 10_L) and when density was held constant (but group size increased with enclosure size; 10_S, 20_M, 30_L), and lastly I compared across fixed enclosure sizes (where density and group size increased simultaneously; 10_M, 20_M & 10_L, 30_L). Each of these contrasts maintained one factor constant, while the other two covaried and is essentially an ANOVA test. In order to protect against an inflated Type I error rate the contrasts and mean comparisons were only performed when the overall ANOVA F-test was significant ($P < 0.05$).

Results

Nearest neighbor distances were influenced by the interaction of treatment and age ($F_{8,40} = 3.48$, $P = 0.004$; Fig 4-1), as differences between the low and high density treatments only became more exaggerated with age. Similarly, the deviation of observed values from those expected assuming randomness were also influenced by the interaction of treatment and age ($F_{8,19.8} = 3.12$, $P = 0.019$). While total distance traveled did not differ between treatments ($F_{4,16.4} = 0.84$, $P = 0.52$) and was not influenced by the interaction between treatment and age ($F_{8,33.6} = 0.67$, $P = 0.71$), there was decrease in total distance traveled as birds aged, signified by the significant effect of age ($F_{2,32.7} = 5.84$, $P = 0.007$; Fig 4-2). I also found an interaction between treatment and age for net displacement ($F_{8,40.9} = 3.53$, $P = 0.004$; Fig 4-3). Initially net displacement increased with enclosure size both across constant stocking density (10_S, 20_M, 30_L) and group size (10_S, 10_M, 10_L), however this effect of treatment waned by the fifth week. There were no differences between birds in the five treatments concerning the proportion of available pen space utilized ($F_{4,20} = 2.30$, $P = 0.09$) which averaged 68.7 ± 3.0 %, but there were treatment differences in actual total amount of space used, as measured by minimum convex polygons ($F_{4,19.2} = 116.67$, $P < 0.001$; Fig. 4-4). I found that the total minimum convex polygons built from all observed locations increased with enclosure size both when group size (10_S, 10_M, 10_L; $F_{2,39.3} = 95.63$, $P < 0.001$) and density (10_S, 20_M, 30_L; $F_{2,23.5} = 212.05$, $P < 0.001$) were maintained, but also observed differences when comparing enclosures of equal size (10_L and 30_L, 10_M and 20_M; $F_{2,30} = 5.38$, $P = 0.01$). However, the average weekly minimum convex polygons generated from the data for each week were

affected by the interaction of age and treatment ($F_{8,40} = 3.39$, $P = 0.005$; Fig. 4-5). Whereas birds in 10_S treatment used a consistent amount of space across age, minimum convex polygons declined with age in all other treatments. The coefficient of variation for weekly minimum convex polygons was not affected by treatments ($F_{4,7.52} = 0.98$, $P = 0.47$), age ($F_{2,11} = 1.94$, $P = 0.19$) or their interaction ($F_{8,5.6} = 0.46$, $P = 0.85$). I found another significant interaction between treatment and age for percent movement, defined as the percentage of time birds moved in between successive scans ($F_{8,24.3} = 2.84$, $P = 0.02$; Fig 4-6). Initially movement appeared to increase with group size, however this disappeared by the fifth week of age.

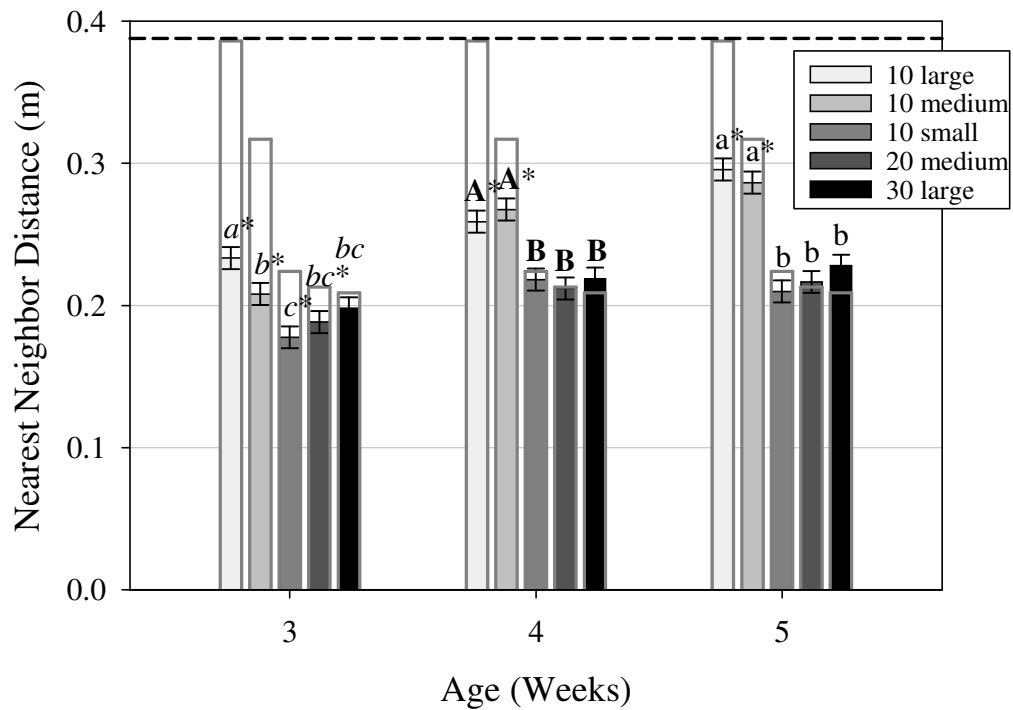


Figure 4-1

Nearest neighbor distances (least square mean \pm standard error of the mean) and their deviation from expected values assuming random assortment, according to age and group size (10, 20, or 30) and enclosure size (small, medium, or large) treatments. Means within age sharing any common letters are not significantly different ($P > 0.05$). Deviations which significantly differ from zero are denoted by an asterisk ($P > 0.05$).

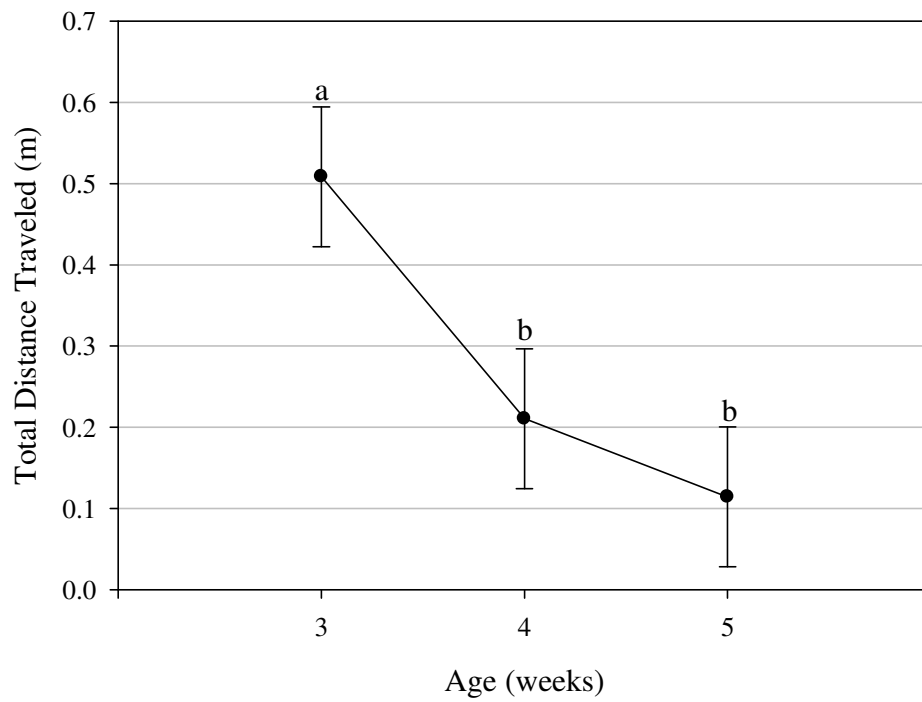


Figure 4-2

Total distance traveled (LSM \pm SEM) per week. Means sharing any common letters are not significant different ($P > 0.05$).

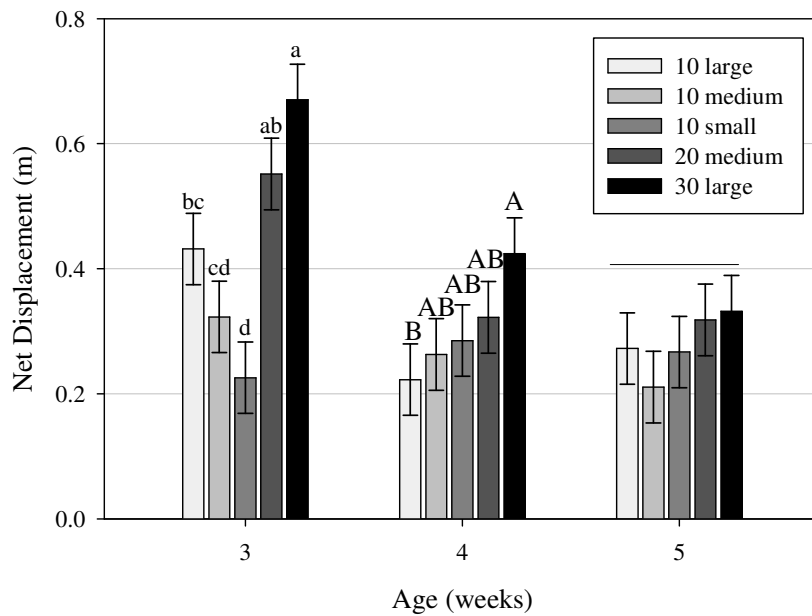


Figure 4-3

Net displacement, calculated as the distance between the first and last observed position (LSM \pm SEM), according to age and group size (10, 20, or 30) and enclosure size (small, medium, or large) treatments. Means within age sharing any common letters are not significantly different ($P > 0.05$).

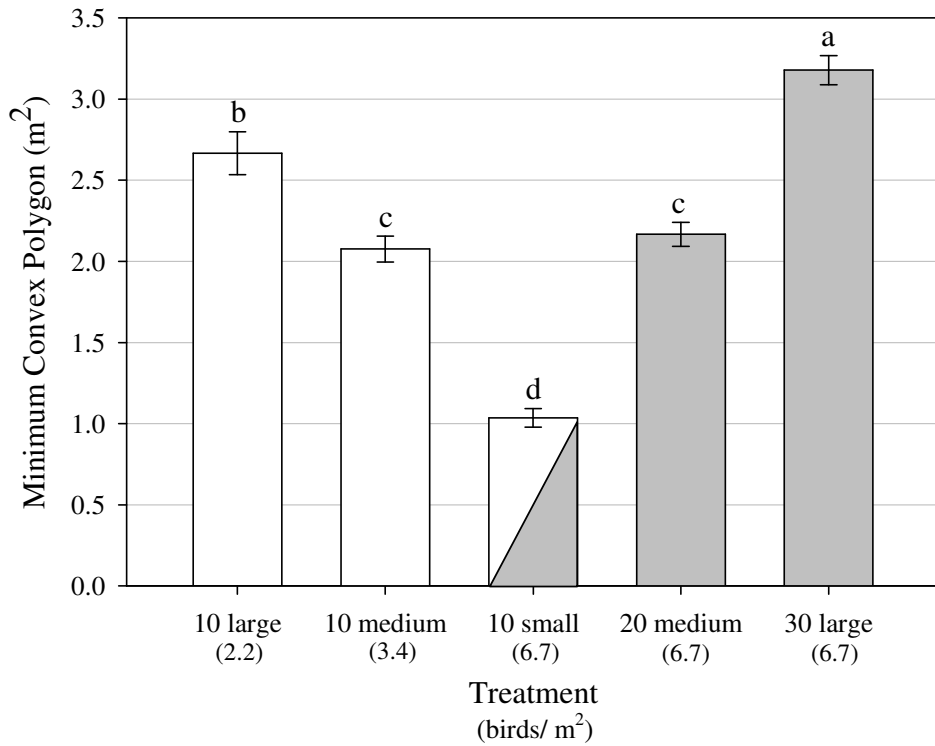


Figure 4-4

Total minimum convex polygon (LSM ± SEM) built from all locations recorded over the length of the entire study according to group size (10, 20, or 30) and enclosure size (small, medium, or large) treatments. White fill denotes constant group size whereas grey fill denotes constant density. Means sharing any common letters are not significantly different ($P > 0.05$).

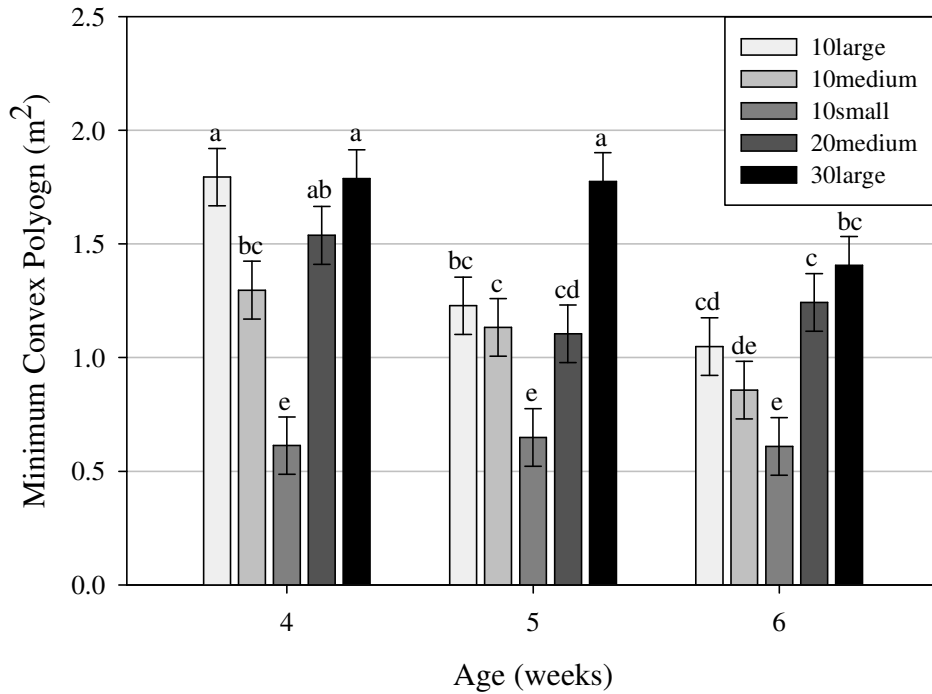


Figure 4-5

Average weekly minimum convex polygons (LSM \pm SEM), which estimate the amount of space utilized during each week of age according to group size (10, 20, or 30) and enclosure size (small, medium, or large) treatment. Means sharing any common letters are not significantly different ($P > 0.05$).

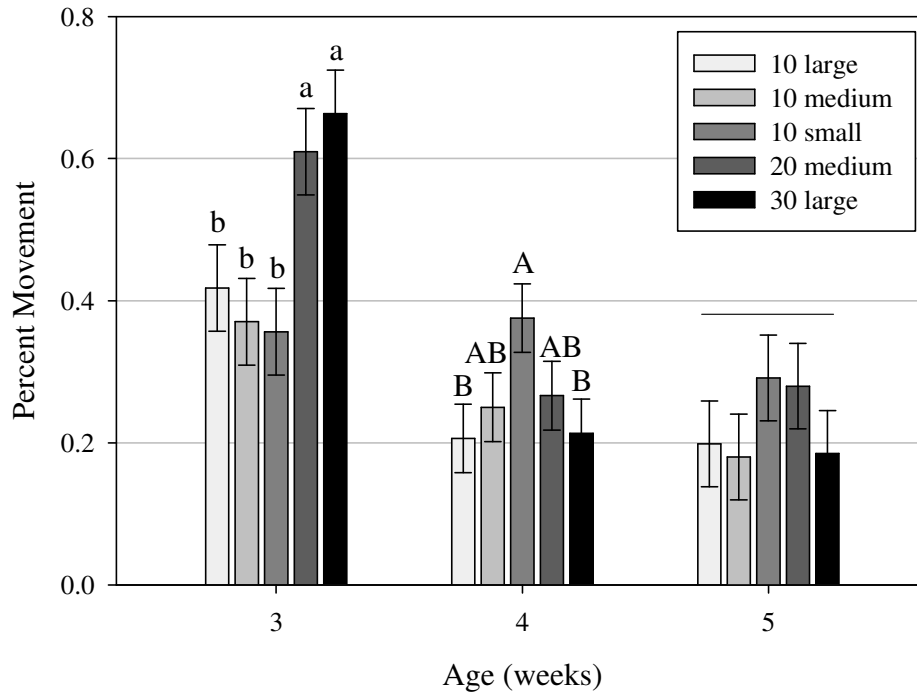


Figure 4-6

Percent movement (LSM ± SEM), defined as the percentage of time birds moved in between successive location scans, according to age and group size (10, 20, or 30) and enclosure size (small, medium, or large) treatment. Means within age sharing any common letters are not significantly different ($P > 0.05$).

Discussion

This study is the third in a series of experiments in which I tested the effects of different enclosure shapes (but identical amounts of floor area) on groups of captive domestic fowl. The overall goal of the entire project was to determine how these different designs may affect use of space in captive animals, so that recommendations can be made concerning future enclosure designs which would maximize the efficiency in use of space and promote animal movement. In this experiment I looked at use of space in enclosures that varied in size (floor area) but maintained a constant amount of perimeter space per unit area. This was accomplished by providing false walls, similar in structure to cover panels, which in essence increase the amount of perimeter space available within the interior of the pen (Cornetto & Estévez 2001b).

Consistent with results from my previous experiments I found that enclosure size, density, and group size, had distinct effects on movement patterns and use of space for domestic fowl. The results from this experiment are unique however in that I found a number of interactive effects between treatments and age, possibly as result of adding complexity to the enclosures through the use of false walls. Nearest neighbor distances were one such parameter affected by the interaction of treatment and age. Generally inter-individual distances were greater in large and medium-sized enclosures, but only under conditions of low density (10_M and 10_L). The differences between these treatments (10_M and 10_L) and the higher density groups (10_S , 20_M and 30_L) became more exaggerated as birds aged. Nearest neighbor distances in the low

density treatments appeared to increase with age, whereas when density was maintained there appeared to be no differences across the study period. Previous research has shown that density has a clear effect on movement, and that this influence increases with age. For example, Andrews et al. 1997) found that the effect of density in reducing activity in broilers was greater at four weeks of age, as opposed to two, suggesting that density has a more significant impact on birds as they grow and occupy a greater amount of space. Density has been suggested to act as a barrier to movement in broiler chickens, with its effects becoming more intense with age as less space becomes available due to the increasing size of the birds (Newberry & Hall 1990; Estévez et al. 1997). It is also likely that density acts as a barrier to group dispersion. If the physical presence of conspecifics poses as a barrier to movement, then the ability of an individual to disperse and move away from group-mates, which may be potentially surrounding it, is likely diminished at high density.

In two previous studies I found that nearest neighbor distances increased with age regardless treatment; this may be an effort by the birds to reduce the chances of competition for resources (Stahl et al. 2001; Leone & Estévez 2008b) or may be an attempt to better thermoregulate. It is possible that broilers in this study still preferred to maintain larger distances as they aged, but the presence of false walls may have exacerbated the barrier effect therefore limiting bird dispersion. However, it is clear from the deviation of observed values from those expected by a uniform distribution that even at the higher density birds still had sufficient space to maintain larger nearest neighbor distances. This underutilization of available space is supported by the fact that birds only used about 60 to 70 % of the total space available. Similar to

previous research (Leone et al. 2007) the observed nearest neighbor distances differed substantially from the expected values generated by the random simulation. Birds in the smaller groups maintained closer distances than expected, while those in larger group sizes (20_M and 30_L) were slightly larger than expected, supporting the idea that inter-individual distances in groups of domestic fowl are greatly influenced by their specific environment.

Much has been discussed about the restriction increasing group size is thought to pose for movement and use of space in the domestic fowl (McBride & Foenander 1962; Grigor et al. 1995b). The results of this experiment suggest that it is unlikely that social factors were responsible for the observed differences in nearest neighbor distances. The main evidence in support of this argument is the fact that when density was maintained (10_S, 20_M, 30_L), nearest neighbor distances remained unaffected, despite the fact that group size increased with enclosure size. If social conflict increased with group size (Al-Rawi & Craig 1975; see also Craig & Adams 1984) then I would have expected to find differences between 10_S, 20_M, 30_L. Because I did not find differences it would appear that the common factor, density, was most relevant in shaping these results. Previous authors have also supported the theory that social conflict does not increase with group size in the domestic fowl (Estévez et al. 1997; Hughes et al. 1997; Nicol et al. 1999) suggesting rather that birds are highly 'tolerant' of one another in large flocks.

Previous research has shown that increasing density not only limits inter-individual distances (Leone et al., 2007), but also reduces movement or the distance traveled per unit time (Appleby et al. 1989; Carmichael et al. 1999). However, in this

study nearest neighbor distance was the only measure that I found to be clearly affected by 'high' density. In truth, the density I maintained (6.7 birds/ m²) was well below commercial standards, which most commonly range between 15 to 20 birds/ m² (Dawkins et al. 2004; Estévez 2007). Given that even these relatively low densities impacted inter-individual distances it was again surprising that density did not have a substantial effect on total distance traveled or net displacement. What I found was a clear indication of broilers becoming increasingly inactive over time, as noted by a decline in total distance traveled, net displacement, and minimum convex polygons with age. Domestic fowl become less active as they age and grow (Bizeray et al. 2000; Cornetto & Estévez 2001a; Bokkers & Koene 2003) so it is not surprising that I found a decline in activity reflected in the parameters used to evaluate movement.

While there was a general decline in total distance traveled with age, the interaction of treatment and age for net displacement appears far more complex. Initially, at three weeks of age, net displacement was clearly higher in medium and large enclosures, especially at the higher density treatments (20_M and 30_L). By four weeks net displacement was reduced in the medium and large enclosures, and the only remaining difference was between the 10_L and 30_L treatments. By week five treatment differences had totally disappeared. The decline in net displacement for the 10_L groups was particularly surprising, given the large enclosure size and the low stocking density. The consistent perimeter to area ratio across treatments, which was achieved by adding false walls, may have initially facilitated exploration and improved bird distribution by providing the birds with a higher sense of protection, (Newberry & Shackleton 1997; Cornetto & Estévez 2001b). However, as previously

described, the presence of additional perimeter space throughout the enclosure may have created additional barriers to movement, especially later in age. The additional perimeter space may have further reduced the motivation of broilers to move around, encouraging them to spend most of their time sitting in the proximity of the devices. Previous research noted increased resting time when enclosure complexity was increased with cover panels similar in construction to the false walls (Cornetto & Estévez 2001a).

I also found an interaction between age and treatment for weekly minimum convex polygons. While birds in the smallest enclosure size/ group size treatment (10_s) appeared to use a consistent amount of space each week, for all other treatment combinations weekly minimum convex polygons decline with age. It is likely that because of the small size of the 10_s enclosure birds in this treatment consistently used a majority of the little space that was available to them, even when activity (and likely locomotion) declined with age. Even though weekly minimum convex polygons generally declined with age, there was a consistent pattern of greater space use in larger enclosures (which was the same pattern observed for total minimum convex polygons). Similarly, despite the complex relationships between density and enclosure size for short-term movement patterns in the presence of additional perimeter space, I found a clear and strong effect of enclosure size on overall space use throughout the length of the study. Similar to the results of my previous two experiments, I found that broilers took advantage of the space given to them, increasing their home ranges in larger enclosures as indicated by bigger minimum convex polygons. However, even as minimum convex polygons clearly increased

with enclosure size the persistent differences in movement between 30_L and 10_L became evident in total space use. I did not expect to find higher movement and space use in the larger group size/ density treatment (30_L as compared to 10_L). The initial increase in net displacement found in the 30_L treatment as compared to 10_L could explain why space use was ultimately greater. Given that the end of the rearing period (when treatment differenced disappeared) is the time when broilers are least active, the initial differences appear to have had a greater impact on space use simply because birds were not moving around and using as much space by the fifth week of age.

It is surprising that on average birds only accessed 68.7 % of the available enclosure space and no differences were detected between treatments in the proportion of space utilized. I would have predicted a higher percentage of space use, especially in smallest enclosure. However, birds still used a majority of space available to them, which suggests considerable overlap between individual home ranges at all treatments and therefore does not provide evidence for territorial defense (even in the small group sizes employed here). Previous research conducted at this facility has found that birds preferential utilize the front portion of their enclosures (LeVan et al. 2000; Pettit-Riley & Estevez 2001) and if they were systematically avoiding the back of the enclosure in this study I would expect to find the proportion of space use to drop below 100 %.

Movement activity, defined in this study as the percentage of time birds moved between successive scans, responded in similar fashion to the other movement parameters with the effects of treatment varying according to age. Initially movement

activity appeared to be dictated by group size; activity levels were similar when group size was constant (10_S, 10_M, 10_L) and increased with group size (20_M, 30_L). This increase could not be attributed to the higher density, because differences were found between 10_S and 20_M/ 30_L, which shared equal density. There is greater potential for interaction with a larger number of conspecifics as group size increases, but the chances of interacting with group-mates may decline over time as birds become more sedentary and less active. This could explain why I found no differences between treatments by the fifth week. Because I did not find any differences between treatments for total distance traveled the increase in movement activity may have merely represented repositioning or shuffling by the birds. Previous research has suggested that increased density leads to greater disturbances (Hall 2001; Cornetto et al. 2002) and jostling (Febrer et al. 2006). However, in these previous studies density was confounded with group size. All of these results may be reflecting an effect of group size effect on disturbances, which may lead to an increase in repositioning by birds in larger group sizes. As birds become less active with age, particularly in the presence of false walls, they may interact less frequently with one another thereby reducing the underlying differences between treatments over time.

In conclusion, despite numerous reports detailing the influence of group size and density on movement (Appleby et al. 1989; Hall 2001; Estévez et al. 2005), I found their effects to be largely transient when perimeter space increased proportionately with enclosure size as treatment differences waned by the fifth week of age for net displacement and percent movement. Larger enclosures encouraged greater space use, supporting the conclusion that birds generally range over the

majority of their environment (Hughes et al. 1974; Appleby et al. 1985; Estévez et al. 1997; Carmichael et al. 1999; Leone & Estévez 2008a) and contradicting the notion that birds set up territories and utilize a consistent subset of the available space (McBride & Foenander 1962; Craig & Guhl 1969; Pamment et al. 1983). The presence of false walls designed to maintain a constant perimeter to area ratio across increasing enclosure sizes may promote inactivity in broilers, but the results of this study show that when granted additional space chickens will take advantage of it.

Chapter 5: General Discussion

The aim of this project was to separate the individual effects of group size, density, and enclosure size on movement and use of space in domestic fowl, and to understand the relevance of enclosure shape and design. All studies which examine group size, density, and enclosure size involve some degree of confounding between factors because animal density (the theoretical amount of space available on a per animal basis) is a direct product of group size and enclosure size. Any manipulation of density will necessarily involve either changes in group size or enclosure size. For example, when attempting to quantify the effects of increasing group size researchers can house animals in enclosures of equal size, or maintain a constant density between groups. With the former, density increases with group size (and thus the two factors become confounded). Alternatively, in an effort to maintain a constant stocking density across group size groups must be housed in enclosures of increasing size, thereby confounding group size and enclosure size in this example. While I cannot completely remove confounding between group size, density, and enclosure size, in this study I intended to elucidate which factors were most relevant to broiler movement and use of space through the use of multiple contrasts. The results of these experiments are valuable in our quest to understand how the confined environment impacts poultry behavior and welfare. The uniqueness of this study is that I was able to suggest which factors; group size, density or enclosure size had the strongest and most relevant effects on the various parameters I used to measure movement and

space use. Without this information it is difficult to make concrete recommendations about which environmental factors are of greatest importance.

In this study no statistical analyses were performed when comparisons were made between variables across the different experiments. Each was performed as a separate experiment at different times of the year and with new groups of birds. Therefore many extraneous factors which could not be discretely controlled such as the variations in ambient temperature and humidity, hours of daylight, and parental stock, were confounded. For this reason, direct comparisons of the results across experiments have to be interpreted with caution. However, even without traditional analyses valuable information can be gleaned by attempting to understand the differences underlying the separate results.

In this series of experiments I found that enclosure size and design had the greatest impact in shaping movement and use of space patterns. The amount of enclosure space utilized by the birds in a given week as well as total space use, both measured by minimum convex polygons, were clearly and consistently shaped by enclosure size. Space use always increased with enclosure size in all three experiments and seemed to be invulnerable to the effects of group size and density.

The shape (square or rectangular) and design (with and without false walls) appeared to have little effect on the size of total minimum convex polygons, which were similar across experiments for enclosures of equal size. This long-term measurement of space use was built from all observed locations throughout each experiment, enabling me to create a full picture of the total amount of space broiler chickens use over time. Despite finding differences in short-term movement patterns

(total distance traveled and net displacement) across experiments, these results demonstrate that over the entire length of the study birds were utilizing a large proportion of the space provided to them, traveling throughout the enclosure and accessing multiple areas. These findings agree with previous research suggesting that use of space in broilers is mostly dictated by the amount of space available to them (Newberry & Hall 1990; Estévez et al. 1997), even at small group sizes (Leone et al. 2007). Broilers are motivated to explore (Newberry 1999) and if home ranges are not restricted by social forces (Appleby et al. 1985; Leone et al. 2007; Leone & Estévez 2008a), then over time I would expect them to travel throughout the majority of the available space in an effort to interact with and explore their environment. Even if density limited short-term movement, over time I would expect broilers to be able to compensate by directing movement paths to unexplored areas, and thus in the long term would visit the majority of enclosure spaces.

Enclosure size was also an important factor in shaping short term movement patterns such as nearest neighbor distance and net displacement. Nearest neighbor distances are often used to measure group dispersal and spacing (Clark & Evans 1954; Stricklin et al. 1979; Keeling & Duncan 1991; Christman & Lewis 2005). When provided with additional space in larger enclosures birds dispersed and maintained greater nearest neighbor distances. However, this response to enclosure size appeared to be constrained by density, as nearest neighbor distances did not increase as substantially with enclosure size when density was held constant (10_S, 20_M, 30_L). Conversely, birds in all treatments combinations and experiments did not appear to disperse to the full extent possible given the amount of space available. This is

supported by the fact that observed nearest neighbor distances were much smaller than theoretically possible if birds were to maximize their use of the available space and maintain a uniform distribution. Similar to the results of previous studies (Leone et al. 2007), birds did not space themselves randomly, but were clearly adjusting their nearest neighbor distances according to the conditions of the physical and social environment.

While the observed nearest neighbor distances clearly increased with enclosure size in all experiments, birds in smaller groups of ten (10_S , 10_M , 10_L) were closer together than would be predicted by random assortment while birds in the larger groups (20_M , 30_L) were farther apart than expected. The nearest neighbor distances maintained by groups of domestic fowl are likely shaped by both repulsive and attractive forces. The natural flocking behavior that characterizes social species (Clark & Mangel 1984) acts as an attractive force, reducing predation risk and preventing birds from moving ‘too far’ from the group (Keeling 1995). This effect is particularly relevant at smaller group sizes (Leone et al. 2007), as the theoretical predation risk is greater for smaller groups. It is therefore not surprising to find that birds in the smaller groups tended to flock together more so than would be expected by a random distribution. However, nearest neighbor distances were not only determined by group size but also by the size of the enclosure. Even for small groups nearest neighbor distances were larger when a greater amount of space was provided. While the actual distance between conspecifics was smaller at higher group size/density treatments (20_M , 30_L) birds in these groups maintained slightly larger nearest neighbor distances than would be expected at random. This may be the result of birds

trying to avoid competition in large groups as compared with the smaller group sizes. Greater dispersal may be a response to an internal motivation to maintain a certain amount of individual or 'personal space' (Mcbride et al. 1963) but also as a strategy to reduce competition for resources (Leone & Estévez 2008b), both of which would act as repulsive forces. Additionally, broilers may alter their inter-individual distances as a means of thermoregulation. This would explain why I consistently saw an increase in nearest neighbor distances with age. Early on during rearing very young chicks remain in close proximity to reduce heat loss during this sensitive period as their thermoregulatory capacity is developing. However birds grow rapidly from three to five weeks (Goliomytis et al. 2003), occupying a greater amount of space but also producing increasingly high amounts of heat as a result of their very high metabolism. Birds may be motivated to maintain greater nearest neighbor distances in larger enclosures as a strategy to maximize air flow, thereby increasing their comfort level. This would be especially important in the later weeks when birds are more susceptible to heat stress (Xin et al. 1994). Therefore, considering the interplay between attractive and repulsive forces broilers may have specific inter-individual distances they prefer to maintain, which would be determined by the balance between these two opposing forces under their specific environmental conditions. This mechanism would explain why broilers did not behave in accordance with the predictions from the random simulations or maximize inter-individual distances to their full capacity even though abundant space was available, a pattern which has also been noted in previous studies (Arnould & Faure 2004; Leone et al. 2007).

In addition to minimum convex polygons and nearest neighbor distances, net displacement was also greatly affected by enclosure size, as well as enclosure design. Net displacement gives a sense of the overall progress made by individuals as a result of their movements over an observation period. Net displacement consistently increased with enclosure size across all three experiments, similar to what I observed regarding minimum convex polygons. As enclosure size increases so does maximum distance (Stricklin et al. 1979), the area available for a bird to travel in a directional pattern. For example, in the small (square) enclosure the distance between opposing corners is 1.72 m, which represents the largest net displacement which could have been recorded for a bird in that enclosure size (maximum distance). Alternatively, in the large (square) enclosure the maximum net displacement which could have been recorded is 2.98 m.

The parameters used to measure movement and use of space in this study, nearest neighbor distances, net displacement, weekly minimum convex polygons, and movement activity were affected by changes in enclosure size as well as shape (rectangular or square) and design (with and without false walls) while total distance traveled appeared to be most heavily influenced by enclosure shape and design. Enclosure characteristics such as length to width and the perimeter to area ratio, which differed between rectangular and square enclosures, played an important role in determining broiler space use and movement patterns. When the length to width ratio is held constant, as is the case with square enclosures, the perimeter to area ratio is smaller and decreases with enclosure size at a faster rate in comparison with rectangular pens.

Perimeter (wall) space has been shown to be a highly attractive feature of the confined environment Stricklin et al. 1979, Newberry & Hall 1990; Cornetto & Estévez 2001b) and as such I expected that enclosure shape and design would heavily influence movement and space use patterns. Previous research has shown that additional perimeter space in the form of cover panels has a positive effect on the distribution and use of space of young and adult broilers Cornetto & Estévez 2001b; Leone & Estévez 2008a). Therefore it was predicted that incorporating false walls and maintaining a constant perimeter to area ratio in square pens would encourage the most movement and greatest space use, followed by rectangular enclosures, and lastly (the most commonly used) square enclosures. However this is not exactly what I found.

Enclosure design, by the means of creating additional perimeter (wall) space, produced the greatest difference in movement patterns as compared with the previous two experiments, most notably in the complex interactions that surfaced between treatment and age effects. In square enclosures with a constant perimeter to area ratio (third experiment) differences in net displacement across treatments, observed mostly during the third week of age, waned by the time birds reach five weeks. Initially net displacement increased with enclosure size, similar to the previous experiments, but unlike the others it also appeared to be affected by group size. In all three experiments movement activity was greatest at the highest group size, and in the presence of false walls it appeared that this increase in percent movement may have translated into increases in net displacement, results which were unique for this experiment. False walls may produce a propelling affect pushing birds forward and encouraging them to

move more efficiently within the enclosure when birds are young and active. This may have been further encouraged by a larger number of conspecifics with which to interact. Although I did not detect differences across treatments for total distance traveled in the presence of the false walls, net displacement increased with enclosure size and even more so at large group size, suggesting directional movement with relatively low sinuosity. Similar to net displacement, there was an interaction between age and treatment on weekly minimum convex polygons when the perimeter to area ratio was constant, which was not the case in rectangular or square enclosures.

While there was a decline in weekly minimum convex polygons with age in rectangular enclosures, surprisingly there was no such age effect in the square enclosures of experiment two. In the third experiment with square enclosures outfitted with false walls again weekly minimum convex polygons generally appeared to decline with age, except in the smallest enclosure (10_S) where space use remained constant. This decline in weekly minimum convex polygons with age is most likely a product of reduced activity. Initially, when most active, birds may have been motivated to explore their environment. In rectangular enclosures the corridor effect may have promoted movement and exploration, similar to the results for square enclosures outfitted with false walls, which likely promoted greater exploration than in square enclosures alone

I also found some differences in total distance traveled according to enclosure shape and design. While total distance traveled increased with enclosure size in rectangular enclosures at low group size/ density, I found no differences in total distance traveled according to treatment in square enclosures (with or without false

walls). When the enclosure size increased in both length and width (square enclosures) total distance traveled remained similar regardless of enclosure size, group size, or density. Although statistical analyses across experiments were not conducted, the differences that I observed in average total distance traveled in square enclosures was 0.86 ± 0.07 m (mean \pm SEM: square enclosures) and 0.28 ± 0.09 m (square enclosures with false walls), which was considerably less than the values recorded from rectangular enclosures (1.25 ± 0.11 m). Similarly, birds in large rectangular enclosures appeared to generally use more overall space, as measured by minimum convex polygons and net displacement, than when the same amount of space was provided in the form of a square enclosure. These effects may have been the result of the rectangular enclosures providing the birds the greatest possibility to travel in a constant direction within the enclosure, which perhaps reduced the chance of rebounding off the wall, and deterred sinuous movement in open spaces. To a certain extent rectangular enclosures may have a similar impact to corridors (Haddad 1999), funneling movement forward and propelling birds along the length of the walls, which is then reflected in movement patterns.

On the other hand, it is possible that the lack of a detectable treatment effect on total distance traveled (as well as lack of an age effect on weekly minimum convex polygons) in the square pens was due to the general inactivity of broilers and the reduction in total distance traveled observed (as compared to rectangular enclosures). With birds moving less in square enclosures, small differences between treatments may have become more difficult to detect during an observation period. On average broilers spend a large proportion of their time resting, up to 85 %

depending on bird age (Cornetto & Estévez 2001a; Bokkers & Koene 2003; Arnould & Faure 2004). Birds were often inactive during observation periods, especially during the fifth week, by which time many birds did not move at all. This lack of activity, coupled with the relatively short observation period, may have made it difficult to detect differences in movement patterns across treatments.

Only during the third experiment, in square pens outfitted with false walls, did I find a significant decline in total distance traveled with age. The artificial walls, designed to increase total perimeter space and maintain a constant ratio between perimeter and area, may have served as environmental enrichment. Previous research has shown that cover panels, of similar construction to the artificial walls employed here, reduce disturbances (Cornetto et al. 2002), increase resting behavior, and reduce moving in broilers (Cornetto & Estévez 2001a). Therefore, a reduction in total distance traveled with age can be explained as a direct consequence of the attractive nature of the false walls coupled with the trend for broilers to inactivity with age, which may have encouraged birds to sit and rest undisturbed. This may also explain for why total distance traveled appeared to be so much lower during the third experiment.

Nearest neighbor distances also appeared to be affected by enclosure design, more so than enclosure shape per se. In square enclosures outfitted with false walls I found a significant interaction between treatment and age, which was indicated by the fact that at the lower density treatments (10_L and 10_M) distances seemed to increase with age, which did not appear to be the case for the higher density treatments (10_S , 20_M , 30_L). As a result of the added barrier posed by group-mates birds in the large

density treatments may have found it difficult to navigate around the large number of false walls as they aged and were moving less, which would could to closer nearest neighbor distances.

Given the relatively low density that I used in these experiments (6.7 birds/ m²) I was surprised to find restrictions on movement. Nearest neighbor distances were generally limited at this higher density (10_S, 20_M, 30_L) as opposed to the lower density treatments (10_M and 10_L) as birds could have dispersed to a much greater extent than was observed in any experiment. Density has been suggested to act as a physical barrier to movement in broilers (Newberry & Hall 1990; Estévez et al. 1997; for review see Estévez et al. 2007), thus at high densities when individuals are surrounded by conspecifics, the mere physical presence those group-mates would block individuals from moving farther apart, limiting dispersal and forcing group members to maintain closer nearest neighbor distances.

An interesting result of this study is that across all three experiments I found very little evidence suggesting a large impact of group size on broiler movement and use of space. Movement activity was the only measure affected by group size; generally increasing at the largest group size of 30 birds (30_L). Density was not the causal factor because 30_L was always different from 20_M or 10_S, which all shared equal densities. Broilers in the 30_L treatment had the greatest potential to interact with group mates, and this increased interaction may have driven the rise in movement activity. Movement activity was classified as a change in a bird's location between successive scans, and given that I never observed an increase in total distance traveled at 30_L I may have inadvertently captured shuffling or repositioning behavior as

opposed to true purposeful movements. Previous research has suggested that high density leads to increased disturbances (Cornetto et al. 2002) and jostling (Febrer et al. 2006) however in both studies density was confounded with group size. When considered together, large group size, more so than density, may lead to a greater number of disturbances and therefore increased shuffling. Only during the third experiment, when birds were housed in square enclosures with false walls, did the increase in movement activity appear to translate into larger net displacements.

I was surprised that birds did not use more of the space available to them, the average proportion of available space utilized was 65.8 ± 3.0 , 62.0 ± 2.0 , 68.7 ± 3.0 % for rectangular, square and square enclosures with false walls, respectively. It is possible that birds were only taking advantage of the most attractive areas of the enclosure near the feed and water (Arnould & Faure 2004), and along the front of the enclosure. Previous research conducted at this facility indicated that birds show a consistent preference for the spaces near the front of the enclosures (LeVan et al. 2000; Pettit-Riley & Estevez 2001; Leone unpublished data). There are several reasons why the front may be an attractive area to the birds; areas in the front may receive fresh air to a greater extent than areas in the back of the enclosure. Enclosure side-walls were obscured with black plastic sheeting in order to prevent interactions between birds across adjacent enclosures, however the front of enclosures were not blocked in order to allow for proper ventilation. Therefore the front may also have been a more attractive area because it provided broilers a chance to observe birds in other enclosure across the hall or to have a better view of the activities going on in the room. This preference for the corridor-adjacent space at the front of the enclosures,

may explain why birds (even in the smallest pen) did not take greater advantage of the space available to them.

In summary, in this study I have demonstrated that the amount of enclosure space available is the most relevant factor determining use of space patterns in young domestic fowl. I found that regardless of group size, density, enclosure shape, or design, broilers had larger minimum convex polygons in larger enclosures. Nearest neighbor distances increased with enclosure size, but were constrained by 'high' density as inter-individual distances were smaller at higher density treatments. Birds consistently did not fully utilize the amount of space available to them as nearest neighbor distances were always less than what would be predicted by a uniform distribution.

The nearest neighbor distances broilers maintained were influenced by the shape (rectangular or square) and design (with or without false walls), as they appeared to be largest in rectangular enclosures, and did not increase with age when density was held constant in square enclosures outfitted with false walls. Net displacement behaved similarly, in that it generally increased with enclosure size but differences were seen according to enclosure design. However by the fifth week of age there were no differences in net displacement among treatments in the presence of false walls, which was different from the results in rectangular and square enclosures (without false walls), where the differences among treatments in enclosures of different size were consistent across age. Total distance traveled was another movement parameter highly influenced by enclosure design; it was largest in rectangular enclosures (especially when group size was constant), decreased in square

enclosures, and was smallest in the presence of false walls. The only measure which appeared to be influenced by group size was movement activity, which increased at the highest group sizes likely as a result of increased jostling and disturbances. Enclosure design also had an additional impact on movement activity; in rectangular and square enclosures movement activity was significantly higher in the 30_L treatment, however in the presence of false walls movement activity was initially higher in 20_M and 30_L, but no differences were found among treatments by the fifth week of age.

Movement and use of space patterns in the domestic fowl have important implications for both welfare and production. Inefficient space use may reduce litter quality more quickly in high-traffic or preferred areas. Poor litter quality is known to contribute to health problems, such as hoc burns and foot pad dermatitis (as reviewed in Hester 1994), which in turn are highly likely to negatively impact broiler welfare. In addition, it may be possible to alleviate some of the commonly observed leg problems in broilers by increasing movement and activity levels (as reviewed in Hester 1994). Designing environments which promote efficient space use may reduce local crowding, improve dispersal patterns, and promote movement. Therefore an understanding of the influence of key environmental factors such as group size, density, and enclosure size is highly relevant in the design of commercial broiler houses.

Conclusion

In this study I have attempted to categorize the independent effects of group size, density, and enclosure size, shape and design on movement and use of space in the domestic fowl. I demonstrated that enclosure size and design are the most relevant factors affecting movement patterns and space use in broiler chickens. In all three experiments minimum convex polygons and net displacement consistently increased in larger enclosures, suggesting that broilers will take advantage of any additional space granted to them. However, the manner in which this additional space is provided made a difference, as in this study rectangular enclosures elicited greater movement and use of space than square enclosures. Although it is hard to estimate the potential welfare benefits, the use of rectangular enclosures may be advantageous as one of the easiest ways to increase movement in broilers.

The most notable effects of density were in restricting movement, specifically in reference to group spacing as measured by nearest neighbor distances. High density likely obstructs broiler movement as it increases the number of physical barriers, in the form of conspecifics, lying in the path of movement. Group size on the other hand, had surprisingly little effects on young broilers. Despite being touted in the literature as a highly influential factor, under these conditions the experimental group size appeared to have only a marginal effect, most likely on jostling or disturbances.

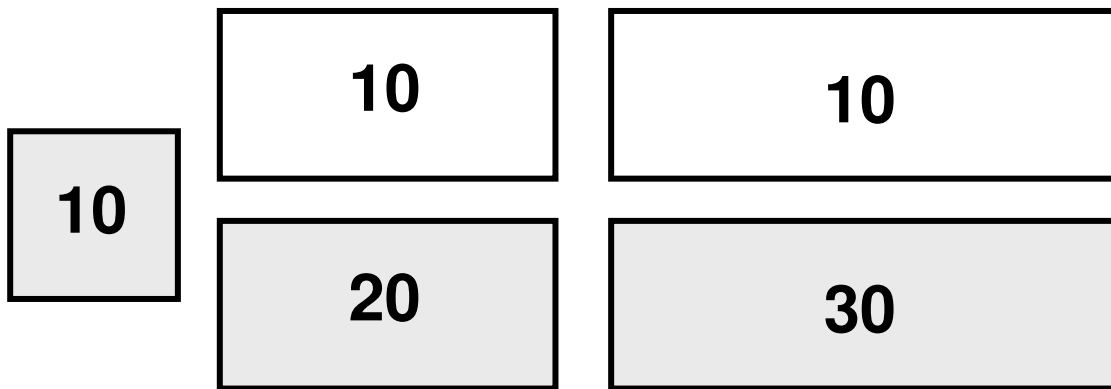
From the applied standpoint, an understanding of how the characteristics of the social and physical environment affect movement and space use is critical, as a large part of the criticism regarding intensive animal

agriculture is related to the potentially severe restrictions to movement. This is particularly relevant to broilers as any improvement in movement may help to reduce or minimize leg problems and thereby improve welfare and potentially performance.

Appendices

Appendix 6-1

Schematic for each of the group size, density, enclosure size treatments from the first experiment, when birds were housed in rectangular enclosures with equal width. Group size (10, 20, and 30) is represented in the center of each enclosure. The grey-fill enclosures have a density of 6.7 birds/ m², whereas the density for the 10_M and 10_L are 3.4 and 2.2 birds/ m² respectively.



Appendix 6-2

SAS codes for the statistical analysis of data from the first experiment using

rectangular enclosures.

```
title1 NEAREST NEIGHBOR ANALYSIS;
proc mixed data=nnweekmean covtest;
class pen treatment week;
* Testing for differences according to treatment, week, and their
interaction;
model nweekdist = treatment|week / ddfm=kr outp=resids;
repeated week /group = treatment subject=pen type=CSh r rcorr; *;
* 1 2 3 4 5; *10 10/20 10/30 20 30;
* multiple contrasts to compare treatments according to common
factor;
contrast 'SD Constant: 10 - 20 - 30 ' treatment 1 -1, treatment 1 0
-1, treatment 0 1 -1;
contrast 'GS Constant: 10 - 10/20 - 10/30 ' treatment 1 0 0 -1,
treatment 1 0 0 0 -1, treatment 0 0 0 1 -1;
contrast 'PS constant: 10/20-20 & 10/30-30' treatment 0 1 0 -1,
treatment 0 0 1 0 -1;
lsmeans treatment week / pdiff;
quit;

title1 Total Distance Traveled;
proc mixed data=sum covtest;
class trt date pen week;
model sumdist = trt week trt*week/ ddfm=kr outp=resids2;
repeated date / subject=pen type=ar(1) r rcorr;
*10 10/20 10/30 20 30;
contrast 'GS: 10 - 10/20 - 10/30' trt 1 -1, trt 1 0 -1, trt 0 1 -1;
contrast 'SD: 10 - 20 - 30 ' trt 1 0 0 -1, trt 1 0 0 0 -1, trt 0 0 0
1 -1;
contrast 'PS:10/20-20 & 10/30-30' trt 0 -1 0 1, trt 0 0 1 0 -1;
*random week(pen); *estimated at zero!;
lsmeans trt week / pdiff;
quit;

title1 Net Displacement (x1 - xn);
proc mixed data=net covtest;
class trt date pen week;
model netdist = trt week trt*week/ ddfm=kr outp=resids3;
repeated date / group=trt subject = pen type=ar(1) r rcorr;
*10 10/20 10/30 20 30;
contrast 'GS: 10 - 10/20 - 10/30' trt 1 -1, trt 1 0 -1, trt 0 1 -1;
contrast 'SD: 10 - 20 - 30 ' trt 1 0 0 -1, trt 1 0 0 0 -1, trt 0 0 0
1 -1;
contrast 'PS:10/20-20 & 10/30-30' trt 0 -1 0 1, trt 0 0 1 0 -1;
*random week(pen); *estimated at zero!;
lsmeans trt week / pdiff;
ods output lsmeans=lsm3;
ods output diffs=diff3;
```

```

quit;

title1 MCP - TOTAL AREA UTILIZED;
proc mixed data=mcpprop;
class trt pen;
model area = trt / ddfm=kr outp=resids2;
                                *10  10/20 10/30 20   30;
estimate '20 vs 10/20' trt 0 -1 0 1;
estimate '30 vs 10/30' trt 0 0 -1 0 1;
contrast 'GS: 10 - 10/20 - 10/30' trt 1 -1, trt 1 0 -1, trt 0 1 -1;
contrast 'SD: 10 - 20 - 30 ' trt 1 0 0 -1, trt 1 0 0 0 -1, trt 0 0 0
1 -1;
contrast 'PS: Med & lg' trt 0 1 0 -1, trt 0 0 -1 0 1;
random pen(trt);
lsmeans trt / pdiff;
quit;

```

```

Title1 Minimum Convex Polygon PROPORTIONS;
proc mixed data=mcpprop;
class trt pen;
model prop_real = trt / ddfm=kr outp=resids;
random pen(trt);
                                *10  10/20 10/30 20   30;
estimate '20 vs 10/20' trt 0 -1 0 1;
estimate '30 vs 10/30' trt 0 0 -1 0 1;
contrast 'GS: 10 - 10/20 - 10/30' trt 1 -1, trt 1 0 -1, trt 0 1 -1;
contrast 'SD: 10 - 20 - 30 ' trt 1 0 0 -1, trt 1 0 0 0 -1, trt 0 0 0
1 -1;
contrast 'PS: Med & lg' trt 0 1 0 -1, trt 0 0 -1 0 1;
lsmeans trt / pdiff;
quit;

```

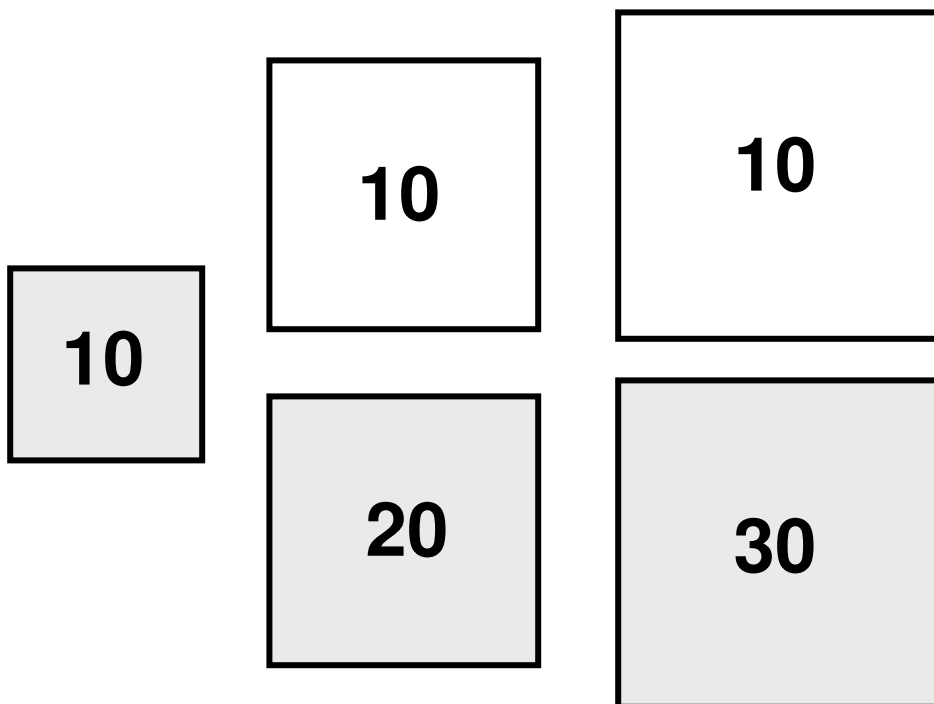
```

title1 Movement Activity;
proc mixed data=lazymeans;
class trt pen week;
model mmovep = trt week trt*week / ddfm=kr outp=mx Dresid;
repeated week / subject = pen type=cs r rcorr;
contrast 'GS Constant: 10-10/20-10/30' trt 1 -1, trt 1 0 -1, trt 0 -
1 1;
contrast 'SD Constant: 10-20-30' trt 1 0 0 -1, trt 0 0 0 1 -1, trt -
1 0 0 0 1;
contrast 'PS Constant: 10/20-20 & 10/30-30' trt 0 1 0 -1, trt 0 0 -1
0 1;
lsmeans trt week trt*week / pdiff;
quit;

```


Appendix 6-3

Schematic for each of the group size, density, enclosure size treatments from the second experiment when birds were housed in square enclosures. Group size (10, 20, and 30) is represented in the center of each enclosure. The grey-fill enclosures have a density of 6.7 birds/ m², whereas the density for the 10_M and 10_L are 3.4 and 2.2 birds/ m² respectively.



Appendix 6-4

SAS Code for statistical analysis from the second experiment, with square enclosures.

```
title1 Nearest Neighbor;
proc mixed data=nnweeks covtest;
class pen week trt;
model weekavg= trt|week / ddfm=kr outp=resids;
repeated week / subject=pen group=trt type=ar(1) r rcorr;
* lg * med * sm * ten_lg * ten_med;
contrast 'gs const' trt 0 0 1 -1, trt 0 0 0 1 -1, trt 0 0 -1 0 1;
contrast 'ps const' trt 1 0 0 -1, trt 0 -1 0 0 1;
contrast 'sd const' trt 1 -1, trt -1 0 1, trt 0 1 -1;
lsmeans trt week / pdiff;
quit;

title2 Total Distance Traveled;
proc mixed data=total_means covtest;
class trt pen week;
model mtotal = trt week trt*week/ ddfm=kr outp=resids;
repeated week / subject = pen type=ar(1) r=1,2 rcorr;
lsmeans week / pdiff;
quit;

title2 Net Displacement;
proc mixed data=net_mean covtest;
class trt pen week;
model mnet= trt|week / ddfm=kr outp=netresids;
repeated week / subject=pen type=cs r rcorr;
*10S 10M 10L 20M 30L;
contrast 'gs constant' trt 1 -1, trt -1 0 1, trt 0 1 -1;
contrast 'sd constant' trt 1 0 0 -1, trt -1 0 0 0 1, trt 0 0 0 1 -1;
contrast 'ps constant' trt 0 1 0 -1, trt 0 0 1 0 -1;
lsmeans trt / pdiff;
quit;

title2 Minimum Convex Polygon Total;
proc mixed data=mcp covtest;
class trt pen;
model meters=trt / ddfm=kr outp=resids;
random pen(trt);
lsmeans trt / pdiff;
*10lg 10md 10sm 20med 30lg;
contrast 'const. gs' trt 1 -1, trt 1 0 -1, trt 0 -1 1;
contrast 'const. sd' trt 0 0 -1 1, trt 0 0 1 0 -1, trt 0 0 0 -1 1;
contrast 'const. ps' trt 1 0 0 0 -1, trt 0 1 0 -1;
```

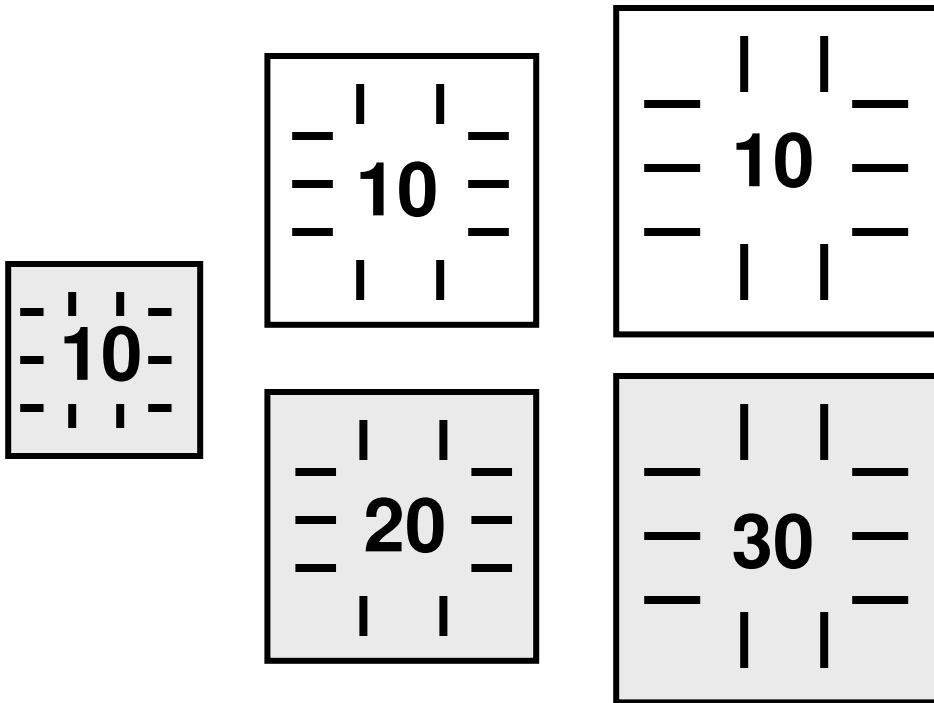
```
quit;
```

```
title2 MCP PROPORTIONS;  
proc mixed data=mcp;  
class trt pen;  
model perc=trt / ddfm=kr outp=resids;  
random pen(trt);  
lsmeans trt / pdiff;  
*10lg 10md 10sm 20med 30lg;  
contrast 'const. gs' trt 1 -1, trt 1 0 -1, trt 0 -1 1;  
contrast 'const. sd' trt 0 0 -1 1, trt 0 0 1 0 -1, trt 0 0 0 -1 1;  
contrast 'const. ps' trt 1 0 0 0 -1, trt 0 1 0 -1;  
quit;
```

```
title2 Movement Activity;  
proc mixed data=weekmeans;  
class trt pen week ;  
model prob = trt week trt*week / ddfm=kr outp=resids;  
repeated week / subject=pen type=un r rcorr;  
* 10 10m 10l 20 30;  
contrast 'gs const' trt 1 -1, trt 0 1 -1, trt -1 0 1;  
contrast 'sd const' trt 1 0 0 -1, trt 0 0 0 1 -1, trt -1 0 0 0 1;  
contrast 'ps const' trt 0 1 0 -1, trt 0 0 1 0 -1;  
lsmeans week trt / pdiff;  
quit;
```

Appendix 6-5

Schematic for each of the group size, density, enclosure size treatments from the third experiment, where false walls were added in order to maintain a constant perimeter to area ratio between enclosures. Group size (10, 20, and 30) is represented in the center of each enclosure. The grey-fill enclosures have a density of 6.7 birds/ m², whereas the density for the 10_M and 10_L are 3.4 and 2.2 birds/ m² respectively. The placement of false walls is represented by the dashed lines surrounding each group size. Figure is not to scale.



Appendix 6-6

SAS Codes for statistical analysis of data from the third experiment, when birds were housed in square enclosures with additional perimeter space.

```
title1 Nearest Neighbor distance;
proc mixed data=nnweeks covtest;
class pen week trt;
model weekavg= trt|week / ddfm=kr outp=resids;
repeated week / subject=pen type=cs r rcorr;
lsmeans trt|week / pdiff;
quit;

title2 Total Distance Traveled;
proc mixed data=mtotal covtest;
class trt pen week;
model total_dist = trt week trt*week/ ddfm=kr outp=resids;
repeated week / subject = pen type=ar(1) r rcorr;
lsmeans week / pdiff;
quit;

title2 Net Displacement;
proc mixed data=mnet covtest;
class trt pen week;
model net= trt|week / ddfm=kr outp=netresids;
repeated week / subject=pen type=ar(1) r rcorr;
lsmeans trt|week / pdiff;
quit;

title2 MCP Total;
proc mixed data=mcp covtest;
class trt pen;
model meters=trt / ddfm=kr outp=resids;
random pen(trt);
lsmeans trt / pdiff;
                                *10 10/20 10/30 20 30;
contrast 'const. gs' trt 1 -1, trt 1 0 -1, trt 0 -1 1;
contrast 'const. sd' trt 1 0 0 -1, trt -1 0 0 0 1, trt 0 0 0 -1 1;
contrast 'const. ps' trt 0 1 0 -1, trt 0 0 -1 0 1;
quit;

title2 MCP PROPORTIONS;
proc mixed data=mcp;
class trt pen;
```

```

model perc=trt / ddfm=kr outp=resids;
random pen(trt);
lsmeans trt / pdiff;
                                *10 10/20 10/30 20 30;
contrast 'const. gs' trt 1 -1, trt 1 0 -1, trt 0 -1 1;
contrast 'const. sd' trt 1 0 0 -1, trt -1 0 0 0 1, trt 0 0 0 -1 1;
contrast 'const. ps' trt 0 1 0 -1, trt 0 0 -1 0 1;
quit;

```

Appendix 6-7

Physiological results for each experiment

In all experiments the gait score measured at the end of the fifth week did not differ between treatments (1st: $F_{4,20,6} = 1.71$, $P = 0.186$; 2nd: $F_{4,20} = 0.59$, $P = 0.675$; 3rd: $F_{4,20} = 0.29$, $P = 0.883$) nor did the weight of birds measured at the end of the fifth week (2nd: $F_{3,3} = 1.17$, $P = 0.450$; 3rd: $F_{4,20} = 1.01$, $P = 0.424$).

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