Social influence on the effectiveness of virtual fencing in sheep

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38 **Abstract**

39

40 Early virtual fencing trials have effectively contained small groups of sheep within set areas of a
41 paddock when all animals were wearing manual electronic collars. With sheep farming
42 commonly involving large flocks, a potential cost-effective application of virtual fencing would
43 involve applying equipment to only a proportion of the flock. In this study, we tested the ability
44 of virtual fencing to control a small flock of sheep with differing proportions of the group
45 exposed to the virtual fence (VF). Thirty-six Merino sheep were identified as leaders, middle or
46 followers by moving them through a laneway. The sheep were then allocated to groups balanced
47 for order of movement. The groups (n = 9 per group) included applying the VF to the following
48 proportions of animals within each group: 1) 100 % (n = 9 VF) 2) 66 % (n = 6 VF; n = 3 no VF)
49 3) 33 % (n = 3 VF; n = 6 no VF) 4) 0 % (no VF; free to roam the paddock). The groups were
50 given access to their own paddock (80 x 20 m) for two consecutive days, six hours per day, with
51 the VF groups prevented from entering an exclusion zone that covered 50 % of the north side of
52 the paddock. During these hours, VF interactions, behavioural time budgets, and body
53 temperature were recorded as measures of stress, and location was tracked with GPS. Group 100
54 % VF and Control were tested on the first two days and groups 33 % VF and 66 % VF were
55 tested on the following two days. During VF implementation the 100 % VF and 66 % VF group
56 were successfully prevented from entering the exclusion zone. Having only 33 % of the flock
57 exposed to the virtual fence was not successful, with the sheep pushing forward through the VF
58 to join flock mates in the exclusion zone. For learning to respond to the audio cue, sheep in the
59 33 % group received more electrical stimuli with a 0.51 proportion for the ratio of electrical
60 stimuli to audio cue, compared to 0.22 and 0.28 for the 100 % and 66 % groups, respectively.
61 There were small differences in behavioural patterns of standing and lying on both days of
62 testing, with the 100 % VF and 66 % VF groups spending more time lying. Although stress-
63 induced hyperthermia did not occur in any of the VF groups, body temperature differed in the 33
64 % VF group. There were no differences in temperature measures between the control and 100 %
65 VF animals. This study demonstrates that for a short period, controlling two-thirds of the flock
66 was equally as effective as virtually fencing all animals, while controlling one-third of a flock
67 with a virtual fence was not effective. For the short term, it appears that implementing the VF to
68 a proportion of the flock can be an effective method of containment. Due to the limitations of
69 this study, these results warrant further testing with larger flocks and for longer periods.

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Introduction

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80   Virtual fencing is an emerging technology with the ability to contain livestock within a
81   boundary through the implementation of a warning cue (audio tone) and a negative stimulus
82   (electrical stimulus). Through associative learning animals' pair an audio warning with an
83   electrical stimulus and quickly learn to avoid the virtual fence by responding to the audio
84   warning alone (Campbell et al. 2018; Marini et al. 2018b). Virtual fencing for cattle currently
85   works on the premise that all animals require the virtual fence to be implemented, however this
86   method may not be economically feasible in the Australian sheep industry due to the large size of
87   many flocks. The efficacy of virtually fencing and controlling only a proportion of sheep in a
88   flock using collars could provide an opportunity to reduce costs.
89   Currently, there is no commercially available virtual fencing system for sheep. Virtual
90   fencing in sheep has been implemented experimentally using commercially available dog collars
91   but as a new technology, it is important to measure the impact of virtual fencing on sheep
92   welfare, particularly in situations that may affect flock interactions. Initial studies in sheep have
93   shown that they readily learn to respond to an audio cue to avoid an electrical stimulus when
94   trained individually (Marini et al. 2019; Marini et al. 2018a), a fundamental component required
95   for virtual fencing to be ethically acceptable (Lee et al. 2018). There have been few studies that
96   have evaluated group responses to the virtual fence and whether a group of sheep could be
97   trained in a paddock environment (Brunberg et al. 2017; Jouven et al. 2012). These studies found
98   mixed success in containing animals within a paddock. In a controlled grazing study using a
99   manually implemented virtual fence with a small flock of six naïve sheep, (Marini et al. 2018a)
100   numerous group interactions were observed where the flock approached the virtual fence line
101   together, but only one individual received the VF cues but all of the animals in the group reacted.
102   This suggests that there are social influences acting within a group when virtual fencing is
103   implemented. Indeed, recently, social facilitation of learning virtual fencing responses were
104   demonstrated in beef cattle (Keshavarzi et al. 2020).
105   Sheep flock movements are a continuous process of individual and group decisions that
106   influence how a group reacts and moves (Ramseyer et al. 2009a). Sheep flock movements are
107   affected by a variety of factors, including the flocks' activity, size, food availability, age, breed
108   and social relationships within the flock (Hauschildt & Gerken 2016; Pillot et al. 2010;
109   Ramseyer et al. 2009b; Taylor et al. 2011). With the strong flocking behaviour and behaviour
110   synchronisation of sheep (Hauschildt & Gerken 2015; Hauschildt & Gerken 2016), there may be
111   potential to only have a proportion of sheep controlled with the virtual fence and still gain
112   effective control (Taylor et al. 2011). However, the proportion of trained to naïve individuals can
113   impact the response of the flock, as observed by Jouven et al. (2012), where a flock with a larger
114   proportion of sheep without the virtual fence resulted in ineffective containment within the
115   virtual fence.
116   Behavioural time budgets have been used previously in cattle (Campbell et al. 2017;
117   Campbell et al. 2019) and sheep (Marini et al. 2018a) exposed to virtual fencing, as indicators of

118 impact to welfare through behavioural change. Another method of measuring impact on welfare
119 includes measures of body temperature. Stress induced hyperthermia as measured by an
120 increased body temperature has been previously shown to be an indicator of stress and anxiety
121 states in sheep (Pedernera-Romano et al. 2010; Sanger et al. 2011). These measures will be used
122 to determine the welfare of the animals in the different treatment groups. (Hauschildt & Gerken
123 2015; Hauschildt & Gerken 2016; Taylor et al. 2011).
124   In this study we tested the ability for virtual fencing to control a small flock of sheep
125 when differing proportions of the group were exposed to the virtual fence (VF). The hypotheses
126 being tested are 1) the virtual fence would be effective at containing a small flock when 66 % of
127 animals had an active virtual fence 2) the flock with 66 % of animals exposed to the virtual fence
128 would be more effective than 33 % exposed and 3) we predict sheep in the 33% group would
129 have altered behavioural time budgets and increased body temperature, but not the 66% and
130 100%, compared to control sheep. The aim of this study was to determine if virtually fencing
131 differing proportions of sheep in a flock would affect the efficacy of containment within a virtual
132 fence, as well as determine effects on sheep behaviour and body temperature as indicators of
133 animal welfare.
134
135 ## Materials & Methods
136
137 **Ethical Statement**
138 The protocol and conduct of the study were approved by the CSIRO McMaster Laboratory
139 Animal Ethics Committee under the New South Wales Animal Research Act 1985 (approval
140 ARA 17/24). The trial was conducted at Waikerie, South Australia, in the Autumn of 2018.
141 Average minimum temperature was 10.5 °C and maximum temperature was 31.5 °C, with
142 windspeeds between 22-33 kmph.
143
144 **Animals**
145 Thirty-six Merino ewes (41.6 ± 0.62 kg) were used in the study. The sheep were marked for
146 visual identification from 1 to 36 using wool paint (Dy-Mark, Australia) placed on the back and
147 either side of the barrel. Sheep were shorn the week prior to testing and required the wool around
148 their neck to be reclipped using handheld shears on the first test day. During the day sheep were
149 kept on a forage barley crop which was grown in the trial paddocks; at night they were moved to
150 holding yards and supplemented with hay (approximately 1 kg each). Water was provided ab
151 libitum in the inclusion zone of the experimental paddock and in the holding yards. Sheep were
152 kept and moved within their experimental groups throughout the trial.
153
154 **Equipment**
155 The virtual fence was implemented with Garmin dog training equipment. This included a GPS
156 track and train collar (Garmin TT15, Garmin Ltd, Olathe, KS, USA) and GPS hand-held unit
157 (Garmin Alpha 100, Garmin Ltd, Olathe, KS, USA). The collars administer an audible sound (70

158   – 80 dB, 2.7 kHz). The electrical stimulus was set to level 4 (36 mA, 20 μs with 16 pulses
159   delivered per s) as determined by previous studies (Marini et al 2018b). Each collar was paired to
160   its own hand-held unit.
161
162   The day prior to the first test day, Thermachron iButton temperature loggers (Embedded Data
163   Systems, Lawrenceburg, USA) were inserted into the vagina to measure core body temperature.
164   These were attached to an EAZI-BREED CIDR® (Zoetis, New Jersey, USA). The CIDR's were
165   leached of hormone in a phosphate-buffered saline (PBS) solution prior to use. They were
166   assembled using heat shrink tubing as previously described (Fisher et al. 2008; Lea et al. 2008).
167   Temperature was recorded at 1 min intervals for the duration of the trial. The loggers were
168   removed at the completion of testing on day two for each group.
169
170   At the beginning of each test day the sheep were fitted with HOBO® Pendant G accelerometers,
171   (Onset Computer Corporation, Pocasset, MA, USA) to measure standing and lying behaviours.
172   These were attached to the outside of the left hind leg mid-side on the cannon bone with a
173   bandage and were removed at the end of each test day. The loggers recorded x,y,x coordinates
174   that corresponded to a state of either standing/grazing, walking/running or lying, this was
175   recorded every 2 s between the hours of 9:30 a.m. to 3:30 p.m. The sheep in this study have not
176   previously worn the loggers.
177

178   **Test procedures**
179   The sheep were weighed, then identified as leaders, middle or followers prior to group
180   allocation. Group movement order in sheep has been previously identified as stable and a way to
181   categorise a sheep's position in a flock (Squires and Daws, 1975), with sheep at the back of the
182   flock having a more stable order (Doughty and Hinch, 2014). To identify group order, the sheep
183   were walked calmly down a lane way six times and visualized using a drone. Sheep that were
184   identified in the front and back of the flock four times were classed as leaders and followers
185   respectively, with eight sheep identified in each category. They were then allocated to group
186   balanced for order, it was ensured that at least one sheep from each subgroup (leader, follower
187   and middle) had the virtual fence implemented within each group i.e. in the 33 % group one
188   leader, one follower and one middle group animal had the VF implemented. The groups (n = 9
189   per group) were:
190
191   1)   100 % of sheep with active virtual fence (n = 9 virtual fence)
192   2)   66 % of sheep with active virtual fence (n = 6 virtual fence; n = 3 no virtual fence)
193   3)   33 % of sheep with active virtual fence (n = 3 virtual fence; n = 6 no virtual fence)
194   4)   0 % of sheep with active virtual fence (n = 9 no virtual fence); free to roam the paddock
195
196   Each group of sheep were tested in four separate paddocks (80 x 20 m, Figure 1) that were as
197   similar as possible. Test groups were visually isolated from each other by an opaque fence line

198   using shade cloth. The flocks were given access to a paddock for two consecutive days for six
199   hours per day. Group 100 % and control were tested on the first two days and groups 33 % and
200   66 % were tested on the following two consecutive days between 8 am and 4 pm. Sheep in
201   groups with the virtual fence were prevented from entering an exclusion zone that covered 50 %
202   of the paddock. Sheep were fitted with Garmin collars at the beginning of each test day. These
203   collars were used to implement the virtual fence and track the sheep through GPS to monitor
204   their location throughout the trial.
205

206   **Virtual fence implementation**
207   Prior to testing, a virtual fence and a warning zone (approximately 2 m apart) were discretely
208   marked on the fence lines with coloured tape to ensure the collar operators were spatially
209   consistent at delivering cues. When a sheep entered the warning zone, they were given an audio
210   cue for 2 s. If the sheep continued to walk forward or did not alter their behaviour (i.e. turn
211   around or stop) after the audio cue, an immediate electrical stimulus (< 1 s) was given. If the
212   animal ran into the exclusion zone, the audio cues and stimuli were not applied. Once the animal
213   was calm it was again given an audio cue and a stimulus if it proceeded further into the exclusion
214   zone until it either ceased forward movement or began exiting the exclusion zone.
215   The operators of the Garmin collars were positioned on a viewing platform approximately 20 m
216   outside the boundary of the test paddock. Each operator was in control of two sheep using two
217   Garmin handheld GPS units. All sheep were collared but the collars of the uncontrolled sheep
218   were not assigned to a handheld unit. Fence interactions were recorded by the operator using a
219   voice recorder and scribing. All proceedings were videotaped. audio and electrical stimuli
220   delivered were recorded during the trial and were later confirmed using video recordings. Inter-
221   observer agreement was assessed, agreement amongst the observers was 85 %.
222

223   **Statistics**
224   All data were analysed using the statistical software program R (The R Development Core Team
225   Version 3.5.1.), the *nlme* (Pinheiro et al. 2018) package was used for analysis. Data were tested
226   for normality through visual inspection of residual plots and the Shapiro-Wilks test < 0.05 was
227   considered statistically significant and $0.1 > P > 0.05$ was considered a statistical tendency. The
228   comparison of the audio cue and electrical stimulus applied to the groups was analysed using a
229   linear model, data was not normal and required a square root transformation. Analysis of the
230   cues across day within group was tested using a dependent t-test. The proportion of electrical
231   stimuli to audio cues received by sheep in the virtual fence groups (number of audio cues was the
232   number of interactions with the virtual fence) was analysed with a binomial proportions test.
233

234   Data collected from the hobo loggers were aggregated into 15 min intervals and further collated
235   and presented as the proportion of total time spent in a behaviour for each day. Behaviour was
236   analysed using a linear mixed effects model, fitting group, day and their interaction as a fixed

237 effect, individual sheep was fitted as a random effect. The effects of cohort were accounted by
238 the effect of group.
239
240 CircWave software (v 1.4, 2007 R. Hut) was used to perform a cosinor analysis in order to
241 generate a 24 h Fourier curve periodogram for the body temperature data. The transformed fitted
242 body temperature data was subsequently analysed for commonly used body temperature
243 circadian wave parameters, these were: trough, peak, acrophase (the time at which peak occurs),
244 range of oscillation, mesor (mean value of a wave), and amplitude (the difference between the
245 peak and the mesor). Groups were compared with a one-way repeated measure analysis of
246 variance (ANOVA) using a linear mixed effects model with individual sheep as a random effect.
247 Due to data collection occurring across different days, the control group could only be compared
248 with the 100 % VF group, and the 33 % VF group could only be compared to the 66 % VF group
249 for temperature data.
250
251 **GPS data**
252 GPS data from Garmin collars was collected for each trial day for each animal. Producing the
253 residency grids, was undertaken in ArcGIS software suite (v9.2; ESRI, Redlands,CA, USA)
254 using a suite of tools from the Hawth's Tools extension. The GPS data was interpolated onto a
255 30 s time step filling missing values with the previous known location, this was done for each
256 animal per day. The trial paddock was divided into a vector grid of 2 m and the individual sheep
257 data was overlaid. For each cell in the grid we summed the time spent and aggregated to produce
258 a residency map for the flock over the trial duration.
259

260 ## Results
261 **Effectiveness of the virtual fence**
262 The effectiveness of excluding sheep from the exclusion zone was successful for 100 % VF and
263 66 % VF groups but not the 33 % VF group (Figure 2). Fence interactions were recorded for all
264 groups except the control which did not have a fence implemented. Table 1 shows a summary of
265 group interactions with the virtual fence line. There were differences between the groups (100 %
266 VF, 66 % VF and 33 % VF) and the proportion of electrical stimulus to audio cues given (χ^2 (2)
267 = 13.3, P < 0.05) over the two days of testing. Proportion for the ratio of electrical stimulus to
268 audio cue were 0.22 for the 100 % VF group, 0.28 for the 66 % VF group and 0.51 for the 33 %
269 VF group, indicating that the 33 % VF group received a higher proportion of electrical stimuli.
270 Interactions with the fence increased on day 2 as seen by the increase in audio cues delivered (F_1
271 = 10.7, P < 0.05). Two of the sheep with the virtual fence implemented in the 33 % VF group
272 reached the maximum number (5) of electrical stimuli allowed to be given to an individual
273 animal within one interaction with the fence. This meant that stimuli were ceased and led to the
274 group crossing over the virtual fence and into the exclusion zone. Once in the exclusion zone the
275 animals were not able to be turned back with the technology this led to the flock residing at the
276 Northern fence line.

277
278 **Behaviours**
279 Reactions to the virtual fence cues varied between the groups (Table 2 and 3). Sheep in the 33 %
280 group were the only ones to display negative behaviours in response to the cues, such as running
281 forward in response to application of the audio cue.
282
283 For behavioural time budget, a group and day effect were seen (P < 0.05, Figure 3) for lying
284 ($F_{3,23}$ = 7.7) standing ($F_{3,23}$ = 6) and walking ($F_{3,23}$ = 12). As standing is the opposite of lying,
285 only the results of lying and walking behavior are presented. On day one there was no difference
286 in time spent lying between the control group and the other groups. A difference in lying time
287 was observed between the 100 % VF group and the 33 % VF group (t_{32} = -3.17, P = 0.003) with
288 the 33 % VF group spending 12 % less time lying. On day two, differences in lying were
289 observed between the 66 % VF (mean = 28 %) compared to the control (mean =12 %, t_{32} = 4.10,
290 P = 0.003) and 100 % VF groups (mean = 13 %, t_{32} = -3.7, P = 0.009). For walking behaviour,
291 the 66 % VF group spent significantly less time walking compared to the control and 100 %
292 group. On day one the 66 % VF group spent 2 % of their time walking, the 100 % VF group
293 spent 5 % (t_{32} = -3.50, P = 0.001). On day two the differences were observed between 66 % VF
294 (mean = 2 %) and control (mean = 5 %, t_{32} = -8.64, P < 0.05) and 100 % VF (mean = 6 %, t_{32} = -
295 10.61, P < 0.05).
296
297 **Temperature**
298 For all the body temperature parameters measured, there were no significant differences between
299 the control and 100 % VF group. The 33 % v group showed lower values for trough ($F_{(3/30)}$ =
300 4.700, P = 0.008) and acrophase ($F_{(3/30)}$ = 4.434 P = 0.011) than the 66 % VF group, and higher
301 values for range of oscillation ($F_{(3/30)}$ = 5.006, P = 0.006) and amplitude ($F_{(3/30)}$ = 6.874, P =
302 0.001) than the 66 % VF group (Table 4).
303

Discussion

305 This study looked to determine if application of virtual fencing to differing proportions of sheep
306 in a flock would affect the efficacy of containment during short term deployment. In the context
307 of the current study, it appears that there is a minimum proportion of sheep that require virtual
308 fencing to enable control of the entire group. Implementing the virtual fence on 66% of the flock
309 was highly effective at containing a group of sheep and was similar to that reported in previous
310 studies with small groups of sheep when 100 % of the animals were virtually fenced (Jouven et
311 al. 2012; Marini et al. 2018a). However, implementing the virtual fence on a smaller proportion
312 of 33% of the flock was not effective at containing the flock within the virtual boundary. Further
313 studies investigating proportions of animals controlled by the virtual fence in larger flock sizes
314 are needed to test these finding over longer periods of time.
315   We observed some instances where sheep in the 66 % VF group, that did not have the
316 virtual fence implemented entered the exclusion zone, but they were encouraged back into the

317   inclusion zone by the movements of the remaining flock. This demonstrates that having trained
318   individuals within a flock can be an effective method of controlling sheep movements. The
319   studies by Pillot et al. (2010); Taylor et al. (2011) trained sheep to respond to visual and auditory
320   cues to approach a target (food reward), when these trained sheep were in a flock with naïve
321   animals, they were successful in recruiting the naïve animals to approach the target when the cue
322   was given, thereby influencing the flocks decision and movement patterns. For the animals with
323   VF devices in the 33 % VF group, it appeared that their motivation to stay with their 'unfenced'
324   flock mates when they entered the exclusion zone, was greater than the aversiveness of the
325   electrical stimulus of the virtual fence. This was demonstrated by those sheep continuing to walk
326   forward in response to the audio cue and electrical stimulus on several occasions to join their
327   flock mates in the exclusion zone. The results seen in this study are similar to Jouven et al.
328   (2012) where, during the 30 minutes of testing, they found naïve animals crossed over to the
329   exclusion zone often but when they were in the minority they would return to the inclusion zone
330   to re-join the rest of the flock. The precise social dynamics influencing the behaviour of virtually
331   fenced sheep in groups has not yet been explored and future research in this area is needed,
332   particularly for larger flock sizes more commonly found on commercial farms.
333   These findings should be considered in relation to the limitations of the study. These
334   include the short period of the study (2 days of 8 hours per day per treatment), whereas over a
335   longer period, social influences of the uncollared sheep may draw the collared sheep into the
336   exclusion zone and reduce the effectiveness of containment of the sheep. Also, due to limitations
337   on the number of collars and people available to administer the stimuli manually and the number
338   of paddocks available only small group sizes could be tested, and treatments were not able to be
339   replicated and tested on the same day. This brings in the potential for differences in
340   environmental conditions that may have affected behaviour of the sheep in this study. With the
341   development of automated collars, longer term larger scale replicated studies will be possible, as
342   have been demonstrated in cattle (Campbell, et al. 2019).
343   The proportion of audio cue to electrical stimulus is an indicator of sheep learning to
344   respond to the audio cue and avoid receiving the electrical stimulus. The lower the ratio, the
345   better the learning shown by sheep. Within our study, the 33 % VF group were the poorest
346   learners with the highest ratio of electrical stimuli to audio cue at 0.50. This ratio was similar to
347   that seen in previous studies where sheep trained individually had a ratio of 0.48 (Marini et al.
348   2018b) and 0.51 (Marini et al. 2019). Whereas the 66 % VF and 100% VF had a similar mean
349   ratio of 0.28 and 0.22 respectively, these results were more similar to studies that have trained
350   animals in a group, where the mean application of electrical stimuli was 0.19 (Marini et al.
351   2018a). The mean number of electrical stimuli applied in the groups was also significantly
352   different with sheep in the 33 % VF group receiving more electrical stimuli over the two days.
353   This has implications for the welfare of the sheep in the 33 % VF group as animals may receive
354   high numbers of stimuli, that could negatively impact their welfare. The sheep in the 33 % group
355   also displayed some negative behaviour in response to the cues such as running forward on the
356   sound of the audio cue. Each group had an increase in audio cues and electrical stimuli applied

357 on day two, although group approaches only increased slightly. Indicating there may have been
358 more individual interactions with the fence within the group approaches on day two. More
359 approaches were expected on day two as feed availability would have declined in the grazing
360 zone and the higher feed availability in the exclusion zone may have become more attractive.
361 However even with the increased motivation to access fresh pasture, sheep in the 100 % VF and
362 66 % VF were still successfully contained by the virtual fence.

363 The use of manual training collars is labour intensive and limits the number of animals
364 that can be controlled within a group as well as the number of groups able to be tested
365 simultaneously. While, it is possible that the small number of animals used in this study may
366 have impacted the sheep behaviour and flock movements (Arnold 1977; Taylor et al. 2011),
367 sheep are able to display normal grazing behaviours in a minimum group of three (Penning et al.
368 1993). Therefore, the study size of nine animals per groups is most likely adequate in relation to
369 expression patterns of behaviour. In the current study the split of animals that did and did not
370 have the virtual fence implemented meant that there were three animals in the 33 % VF group
371 that had the virtual fence and three in the 66 % VF without, leaving the main portion of the flock
372 containing six animals. It is possible that the larger group of six had more influence over the
373 three animals. In the study by Jouven et al. (2012), they worked with flock sizes of 32 and their
374 largest split of trained animals to naïve was 50:50 leaving them with much larger groups of 16,
375 this would be a big enough group to create a sub flock, which was observed with 13 naïve ewes
376 spending time in their exclusion zone.

377 In this study, stress induced hyperthermia was not observed, and no significant
378 differences were observed in body temperature parameters for the control and 100 % virtual
379 fenced animals, however small differences were found between 33 % and 66 % virtual fence
380 animals across four of the seven parameters analysed. Temperatures circadian rhythm is
381 described using several parameters, these are the mesor, peak, trough, range of oscillation,
382 amplitude and acrophase, all of which were used in this study. These measures have been used
383 previously to determine patterns in sheep temperature (D'Alterio et al. 2012). The differences
384 observed in these measures of circadian rhythmicity indicate that there was a difference in the
385 pattern of change for body temperature across the day between the 33 % VF and 66 % VF
386 groups. The results observed in the 66% animals and between the control and the 100 % VF
387 group suggest that exposure to the virtual fence and training animals was not inherently stressful,
388 however further investigation is needed to determine whether exposing only a proportion of the
389 flock will negatively impact sheep welfare in the long term. Previous work looking at sheep's
390 initial exposure to the virtual fence electrical stimuli found that the implementation of the
391 electrical stimulus was not highly aversive (Kearton et al. 2019). Other work has highlighted the
392 importance of animals learning the audio cue in virtual fencing system in order to maintain good
393 welfare through predictability and controllability (Lee et al. 2018; Marini et al. 2019). However,
394 lack of longer-term trials to date means that the effect of repeated exposure is still to be fully
395 tested with sheep.

396   Behavioural patterns were different between the groups in this study and differed
397 between the days of testing. Due to design limitations the study was not able to be replicated and
398 the groups required testing on different days adding in additional environmental factors that may
399 have influenced patterns of behaviour. The behaviours of the sheep may also have been affected
400 by the initial adaptation period to the VF. Other studies that have looked at implementing virtual
401 fencing in sheep have not looked at the implementation of the technology over a long period of
402 time and have not been able to look into impacts on normal behaviour (Brunberg et al. 2017;
403 Brunberg et al. 2015; Jouven et al. 2012). In our previous study (Marini et al. 2018a), it was
404 found that behavioural time budgets were affected after the virtual fence was removed, through a
405 decrease in lying behaviour, but not during the fence implementation. Studies with cattle have
406 also found that the implementation of the virtual fence had limited impact on behavioural time
407 budgets in cattle (Campbell et al. 2017; Campbell et al. 2019). The differences in behaviour
408 between the two days of testing and between the groups in this study could have been due to
409 either the short period of time that the sheep spent in the paddocks (which included no
410 acclimation period). Or it could have been due to the restriction and reduction in pasture
411 availability over the time-period with animals in the 100 % and 66 % group only accessing the
412 inclusion zone. Animals in the control and 33 % VF group accessed the entire paddock giving
413 them more opportunity to walk and graze more pasture, which was reflected in the difference in
414 lying time in the 33% group.
415

Conclusions

417 This study demonstrates that social influences can affect behavioural patterns and the
418 effectiveness of virtual fencing in sheep. Only having a small portion of the flock with an active
419 virtual fence made the fence ineffective with a much higher proportion of sheep moving forward
420 and entering the exclusion zone, rather than turning in response to the audio cue. There were also
421 indications of potentially negative welfare implications for the animals that were exposed to the
422 virtual fence in the 33 % VF group due to the increase proportion in electrical stimulus received.
423 The differences seen in the behavioural patterns may have been due to sheep adapting to the
424 system, as they were only exposed for a short period of time or potential differing environmental
425 conditions due to testing on different days. Further studies using larger group sizes and
426 conducted over longer periods of time are needed to confirm these findings.
427

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433

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Figure 1

Schematic of the experimental setup, the dotted line indicates the virtual fence position.

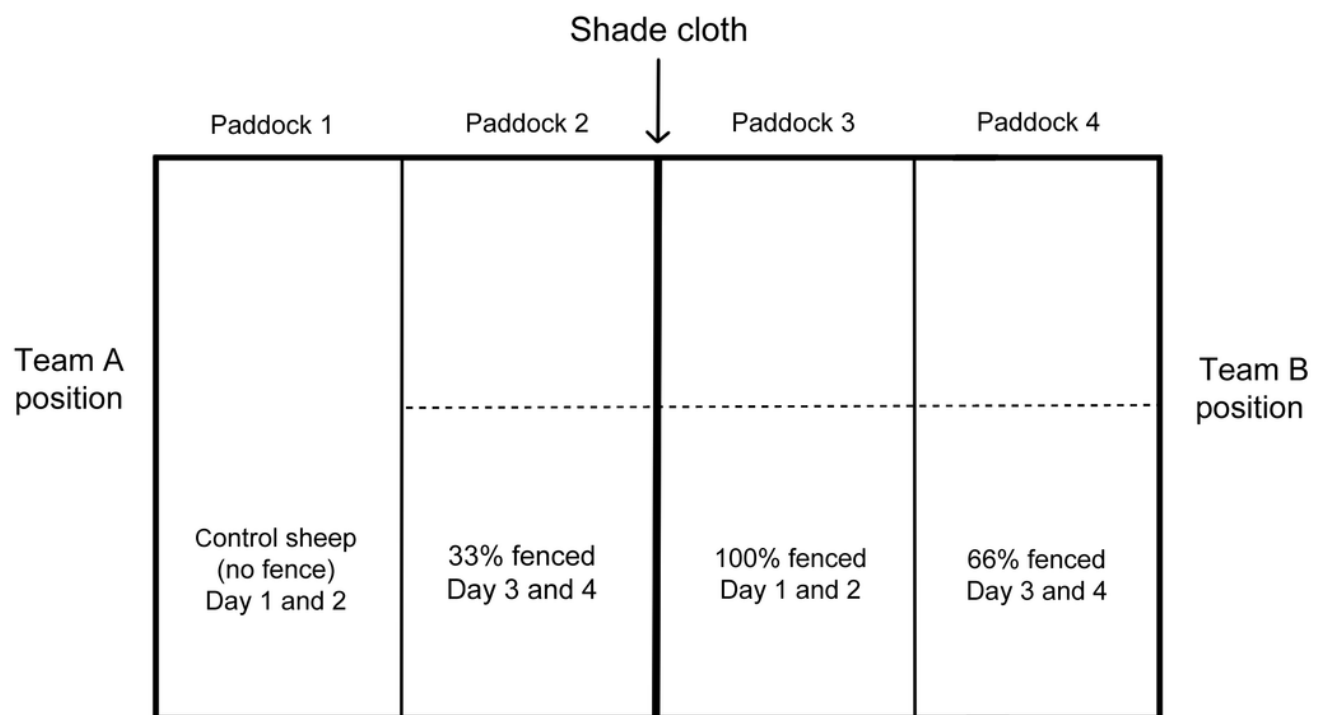



Figure 2

Residency maps for the flock in each group (n = 9 sheep each) at Waikerie showing successful exclusion from northern zone under 100% and 66% active virtual fence groups.

Each cell indicates a grid of 2 meters, colour indicates the time spent in each cell (mins). The darker the cell colour the more time spent.

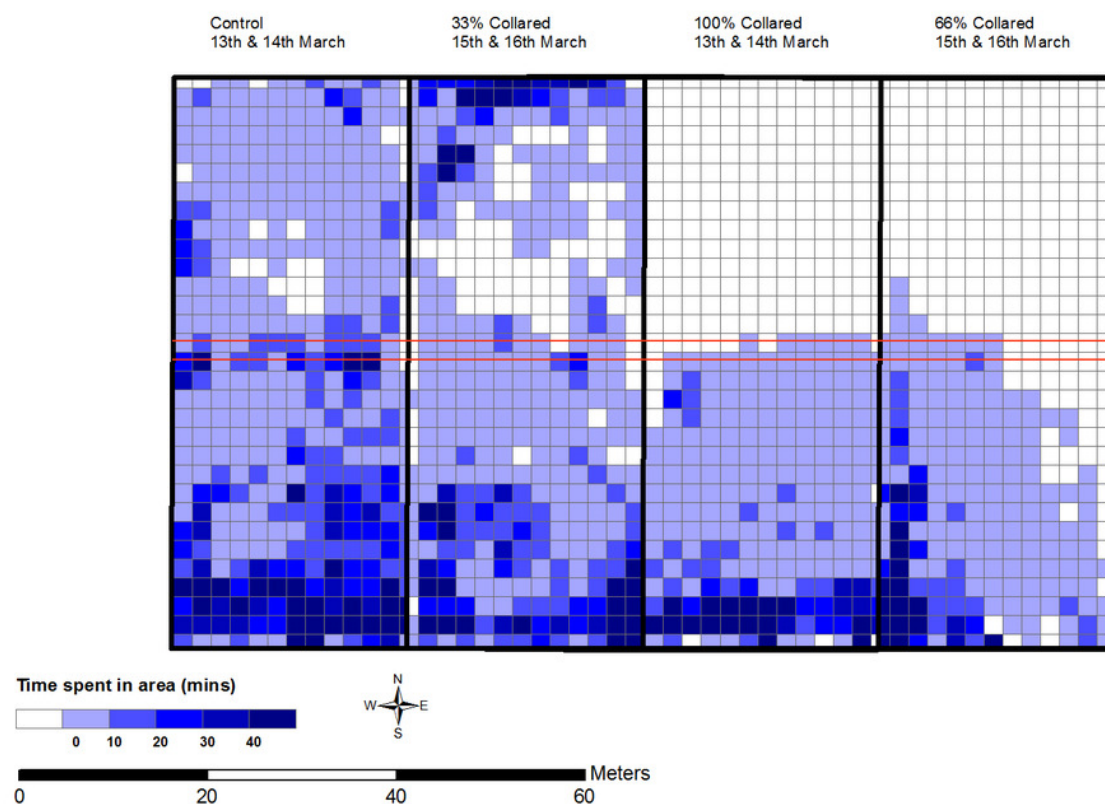
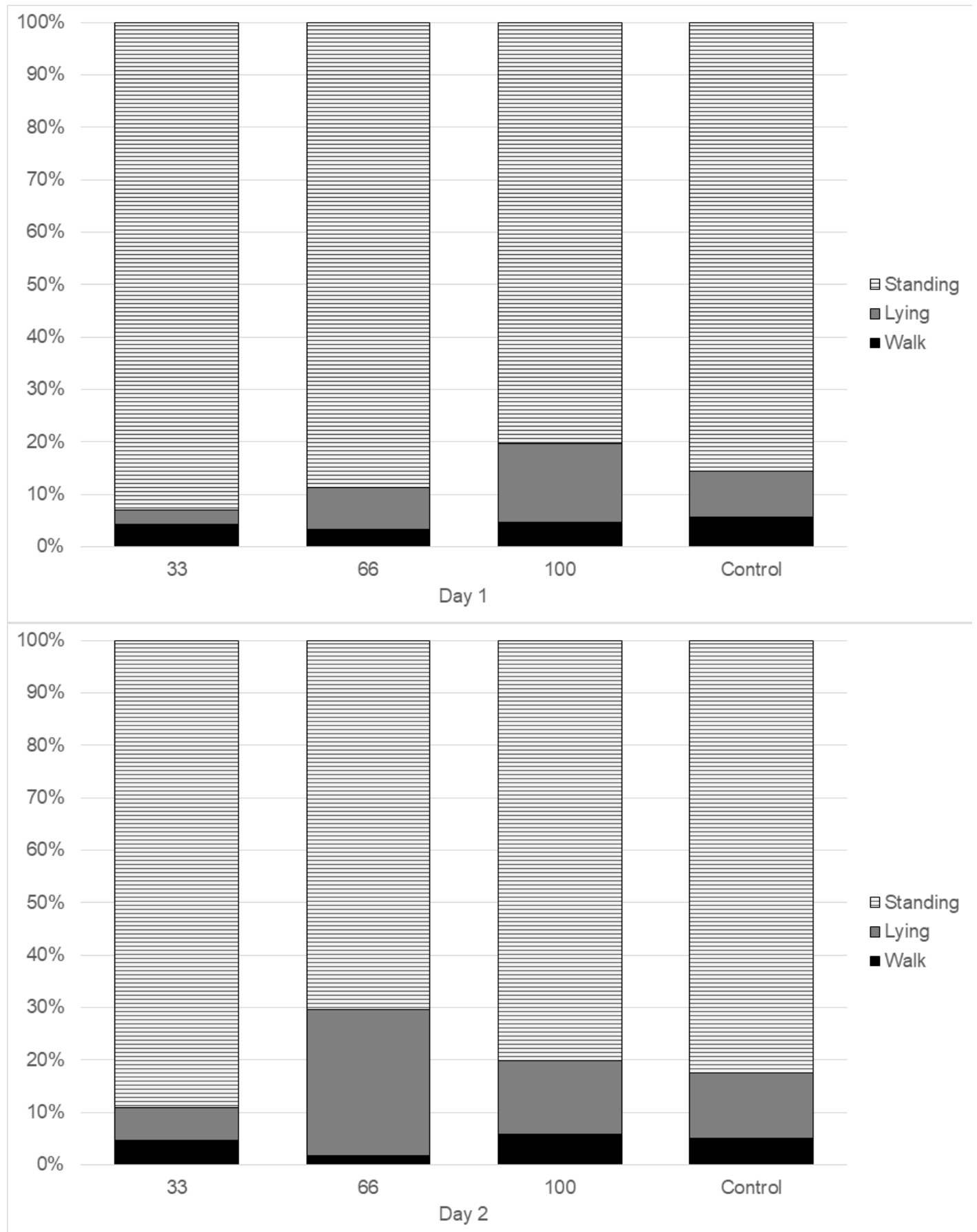



Figure 3

Proportion of time spent displaying standing, lying or walking behaviours for each group on day 1 and day 2 of the virtual fencing testing

The solid black shading is time spent walking, solid grey shading is time spent lying, horizontal lines indicate time spent standing. Time is displayed as a proportion (%) of total activity recorded for each day.

Summary of total number of group approaches and total number of audio cues and electrical stimuli given to individuals on interaction with the virtual fence. Data presented for groups that had the virtual fence implemented.

2 Table 1: Summary of total number of group approaches and total number of audio cues and electrical stimuli given to individuals on interaction
3 with the virtual fence. Data presented for groups that had the virtual fence implemented.

Group (% with fence)	Day 1			Day 2		
	Group Approaches	Audios	Elec Stim	Group Approaches	Audios	Elec Stim
33	4	14	5	9	47	26
66	3	15	5	5	25	6
100	12	17	3	12	56	13

Count of behaviours displayed in response to the audio and electrical cues from sheep in the 33%, 66% and 100% virtual fence groups over the two days the virtual fence was implemented.

Response to audio	33%	66%	100%
Grazing	17	13	35
Stop/lifted head	10	9	16
Turn	7	10	20
Walk forward	23	8	2
Run forward	4	0	0
Total interactions	61	39	73

1   Table 2: Count of behaviours displayed in response to the audio and electrical cues from sheep in the 33%, 66% and 100% virtual
2   fence groups over the two days the virtual fence was implemented.

Count of behaviours displayed in response to the audio and electrical cues from sheep in the 33%, 66% and 100% virtual fence groups over the two days the virtual fence was implemented.

1   Table 3: Count of behaviours displayed in response to the audio and electrical cues from sheep in the 33%, 66% and 100% virtual fence groups

2   over the two days the virtual fence was implemented.

Response to electrical stimulus	*33%*	*66%*	*100%*
No response	3	0	0
Turned	3	1	7
Jumped and turn	5	9	8
Run/jump forward	5	1	1
Hop forward	15	0	0
Total interactions	27	11	15

Body temperature parameters over the two days of testing for all groups

Temperature presented as mean degrees °C +- S.E.M.

1 Table 4: Body temperature parameters over the two days of testing for all groups

	Group				
	Control	**100% collared**	**33% collared**	**66% collared**	
Fitted curve parameters	**Mean ± s.e.m.**	**Mean ± s.e.m.**	**Mean ± s.e.m.**	**Mean ± s.e.m.**	
Mesor	39.0± 0.14	39.1 ± 0.12	39.0 ± 0.07	39.3 ± 0.08	n.s
Peak	39.5 ± 0.17	39.7 ± 0.13	39.9 ± 0.07	39.9 ± 0.12	n.s.
Trough	38.4 ± 0.11	38.6 ± 0.10	38.4 ± 0.11	38.8 ± 0.09	0.002
Range of oscillation	1.1 ± 0.09	1.1 ± 0.10	1.5 ± 0.08	1.1 ± 0.09	0.001
Amplitude	0.4 ± 0.04	0.6 ± 0.05	0.9 ± 0.05	0.6 ± 0.09	0.006
Acrophase	17.7 ± 0.78	16.2 ± 1.16	12.9 ± 1.17	15.9 ± 0.17	0.03
SD	0.3 ± 0.02	0.3 ± 0.03	0.3 ± 0.03	0.3 ± 0.02	n.s.