Effect of grouping on behaviour of dairy heifers and cows in the transition period

Maria Soonberg, Marko Kass, Tanel Kaart, Rosie Barraclough, Marie J. Haskell and David R. Arney

Supplementary File

Material and methods

Experimental farm

The study was conducted on Edinburgh University's Langhill Farm, in Scotland in early spring before turnout. The farm comprised 228 milking cows. Cows were kept indoors through the winter. Youngstock and low yielding cows are turned out in May and return in October. High yielding cows stay indoors all year round.
Dry cows were housed in two separate groups. Far-off dry cows, in-calf cows at 10 days before expected calving date (group size n = 10 – 13), were housed in a cubicle shed, with more than one cubicle/cow and cubicles bedded with mattresses and sawdust bedding. The feed bunk during the study was 15m and the passageway width was 2.5m. At least one week before calving, the cows (n = 10 – 18) were moved to a close-up group in a strawyard, where they remained until calving. Strawyard dimensions were 11m x 19m and feed bunk length 12m.
After calving, cow and calf were moved to an individual pen. The calf was then removed at around 24h and the cow entered the main milking herd after the next morning milking.
In the main herd cows were grouped into two groups: high yielding and low yielding cows. There were four pens, three of which were open, so cows could walk freely between them (the high yielding group), and one closed pen (the low yielding group). The dimensions of this house are 24m x 84m. They had cubicles bedded with mattresses cubicles (240) and sawdust bedding was laid down three times a week and raked twice a day during milking. A DeLaval milking system was used in a parallel milking parlour with 28 places and cows were milked twice a day at 05:00 and 15:00.
Concentrate was given to each cow in the milking parlour according to their milk yield. Cows in the main herd were fed a total mixed ration, which consisted of maize, soya, barley, wheat, beet pulp, molasses, 1st cut silage and whole crop wheat. Cubicle housed cows (far-off dry) received straw and 2nd cut silage. Strawyard (close up) cows received straw, 1st cut silage, wholecrop and drycake. Water was available *ad libitum*.

Experimental design
The study was carried out according to the animal care guidelines of the ASPA (1986). It was approved by the Animal Ethics Committee of SRUC.
Twenty-nine dry Holstein cows (n=13) and heifers (n=16) were selected for this trial based on their proximity to expected calving date at the time of the study (days to calving 24 $\pm$ 21). Seventeen of these animals were observed in the far-off cubicle pen, 27 of them in the strawyard and 23 while in the main herd. Not all cows moved together from one group to another. To assess the effect of group change data were compared across five periods of three days duration (Figure 1). Two periods were in the cubicle housing and three in the strawyard.
One cow was eliminated from the study, because she was found to be not gravid. The number of experimental animals was therefore 28. Three cows were diagnosed with milk fever after calving and were not included in the main herd observation period.
Fourteen cows had IceTags and 14 cows had IceQube (IceRobotics Ltd., UK) activity monitors attached around their right hind legs to record changes in activity parameters when the

cow/heifer was introduced to a new group. Throughout the study period, activity monitors registered standing and lying times, numbers of steps and lying bouts and calculated motion index values (https://www.icerobotics.com/products/) for each cow (Kok *et al.* 2015). The same algorithms are used on both devices to calculate standing, lying steps and motion index (R. Boyce, IceRobotics pers comm), so the data from both devices is equivalent. The duration of

recorded data for each cow was different, however all animals provided activity data for at least one week before and one week after calving. Ice Tags were removed after the cow had spent two weeks in the main herd.
Aggressive behaviour was recorded to investigate changes in aggression and the consistency of individual cows' dominance behaviour after group change. Video cameras (Canon Legria) were attached on a metal pole with Manfrotto "Magic Arms" above the far-off cubicle pen and the strawyard close-up pen to record aggressive behaviour. The cubicle pen had three cameras and the strawyard pen one camera, which were directed at the feed bunk. Recording started in the morning when fresh feed was delivered, around 10:00, and lasted for one hour. Aggressors' behaviour recorded were: pushing (actor uses other parts of her body than head to attack), butting (actor uses head to attack), bulldozing (actor forcefully enters feed bunk), penetrating feeder (actor enters feed bunk between two recipients), blocking (actor physically comes between recipient and feed bunk) and threatening (actor takes a threatening posture and presents her head in the direction of recipient). Recipients' behaviours recorded were: no response, avoidance, withdraws backwards, withdraws sideways, retaliate (recipient retaliates with attack towards actor) and fight (recipient retaliates with an attack and further aggressive interactions follow) (Gibbons et al. 2009[a]).
Observations of behaviour and of nearest neighbours distances were carried out for two two-hours periods with a one hour break in the middle starting 30 mins after feed delivery. All observations were made by one observer throughout the study period. Nearest neighbour visual observations were recorded to see how group change affected animals' interactions with each other and to see if pairs stayed together, and if these changed when new cows/heifers were added to the group. The distance to the first two nearest neighbouring cows were scored in 0.5m categories up to 2.5m (i.e. <0.5, 0.5, 1, 1.5, 2, 2.5 and >2.5m). Behaviours recorded were: posture (lying or standing), idling (doing nothing), ruminating, feeding and sleeping.
Observations of the main herd were made to assess the effect of the second group change on cows' behaviours. Visual observations started on the first day that the cow entered the main herd (on average 1-2 days after calving). In cases in which the cow joined main herd 30 minutes after feed was delivered, the observations started on the following day. Cows were observed for one hour at 10-minute intervals. Data recorded were animals' location: at the feed bunk, in the passageway or elsewhere.

Statistical analysis

Several datasets were formed with different study objects for statistical analyses to reduce the bias in results caused by unbalanced raw data, because some animals spent more than one period in cubicles or in the strawyard. Three animals calved before the three days observational period ended and were observed less than the other animals; the number of cows staying together for different periods was very limited (Figure 1A).

The data were analysed using statistical software R 3.3.3 (R Foundation for Statistical Computing, Vienna, Austria), except the repeated measures analyse of variance, which was performed using SAS 9.4 procedure MIXED (SAS Institute Inc., Cary, NC, USA).

Posture, behaviour and neighbourhood before calving

A dataset was formed with one row for each animal and period combination containing the percentages of times lying, standing, ruminating, feeding and idling, the average distances to the first and to the second nearest neighbour, and the average number of aggressive actions performed and received per hour. For each animal and period, the percentage values and

average values were calculated based on 3×12 observations (except for three animals observed 2x12 because they calved earlier than expected). These distributions were close to normal and the two-way repeated measures analysis of variance was applied to test the statistical significance of the animals' age (heifers *versus* cows), housing system (loose housed system with cubicle bedding *versus* strawyard) and the age by system interaction effects. In models the potential non-zero covariance between the measures of the same animals was considered and the denominator degrees of freedom for the tests of fixed effects was adjusted by dividing the residual degrees of freedom into between-subject and within-subject portions. Model based means (*alias* least square means) were calculated and compared with the Tukey post-hoc test.

To better visualize and measure the differences or similarities between the two systems, datasets containing percentages of posture and behaviour, the average distances to the first and to the second nearest neighbour, and the number of aggressive actions performed and received per hour for each animal and system combination were formed. Correlation coefficients between the same variables registered on the same animals in the different systems was calculated to assess the concordance between two systems.

Aggressive behaviour network

When animals were present in more than one period, the consistency in aggressive behaviour was calculated from the mean number of aggressive actions performed and received for each pair of animals. Based on the data, the mean numbers of aggressive actions performed by heifers against heifers, by heifers against cows, by cows against heifers and by cows against cows were calculated and these means were compared with t-tests followed by Bonferroni correction for multiple testing.

Nearest neighbourhood score

To study the proximity network of animals, the nearest neighbour score between each pair of animals was calculated by considering both the situations where the animal j was observed as the neighbour of i and the opposite, by considering both the first and the second closest neighbours, estimating the observed distances between neighbours (closer neighbours have a larger effect) and counting the actual number of observations when two animals were in the same group. As a result, the score with values in the interval from zero (two animals were not observed being neighbours of each other at all) to one (the closeness of two animals was maximum over all pairs of animals) was achieved. More detailed description about the calculation of the nearest neighbour score is presented in the special chapter of Supplementary Material.

The nearest neighbour animal pair scores were calculated separately for each study period. If two animals were not present in the same period, their nearest neighbour score was considered missing in this period. To visually examine the similarities and differences in pairwise nearest neighbour scores, circle network diagrams (chord diagrams) were constructed, where the strength of the line connecting two animals corresponds to their nearest neighbour score value. To study the concordance of the nearest neighbours at different periods, Pearson correlation coefficients between the nearest neighbour scores for animal pairs common to the compared periods were calculated (theoretically 10 correlations in total, but as there were four pairs of periods without more than one common animal, then only six correlations were calculated).

Finally the weighted mean of these between periods' correlations with weights indicating the numbers of animals common to the pairs of periods were calculated.

Analysis of observation made in the main herd

In the post-calving period there were only six observations per cow on each day, the percentages of posture and behaviour did not conform to a normal distribution, and therefore the Wilcoxon test was used to compare the values of heifers and cows.

Analysis of data from activity monitors

The activity monitor measurements collected throughout each day were summed and divided into three time periods: measurements before calving, measurements on calving day and measurements after calving. The two-way repeated measures analysis of variance was applied to test the effect of time period, animals' age (heifers *versus* cows) and their interaction, considering the potential non-zero covariance between the measures of the same animals and adjusting the denominator degrees of freedom for the tests of fixed effects (by dividing the residual degrees of freedom into between-subject and within-subject portions). Motion index, number of steps and number of lying bouts showed right-skewed distributions and so a binary logarithm transformation was applied before analysis.

The nearest neighbourhood score

Used notations:

- n_{ij} is the number of observations, when the animals i and j were in the same group;
- $NN1_i$ and $NN2_i$ denote the first and the second closest neighbour of animal i;
- $distNN1_i$ and $distNN2_i$ denote the distances (in meters) to the first and to the second closest neighbours of animal i.

The nearest neighbourhood score between i^{th} and j^{th} animals S_{ij} was calculated as follows. At the first step the sum over all observations of animal i when it was in the same group with animal j (SUM_{nij}) was calculated, where the inverse of the distances to the closest neighbour of i being j

$$(distNN1_i|NN1_i=j)^{-1},$$

and one half of the inverse of the distances to the second closest neighbour of i being j:

$$½*(distNN2_i|NN2_i=j)^{-1},$$

were summed:

$$\mathrm{SUM}_{nij}[(distNN1_i|NN1_i=j)^{-1}+½*(distNN2_i|NN2_i=j)^{-1}].$$

After that the result was divided with the number of observations of i being in the same group with j:

$$\Sigma_{ij} = \mathrm{SUM}_{nij}[(distNN1_i|NN1_i=j)^{-1}+½*(distNN2_i|NN2_i=j)^{-1}] / n_{ij}$$

(this step is necessary to achieve comparable values also if some of the animals left groups earlier).

Similarly the sum over all observations of animal j was calculated by summing the inverse of observed distances to the first and to the second closest neighbour being i:

$$\Sigma_{ji} = \mathrm{SUM}_{nji}[(distNN1_j|NN1_j=i)^{-1} + ½*(distNN2_j|NN2_j=i)^{-1}] / n_{ji}.$$

In the last step the average of these two sums was calculated and divided with the maximum over all pairs of animals:

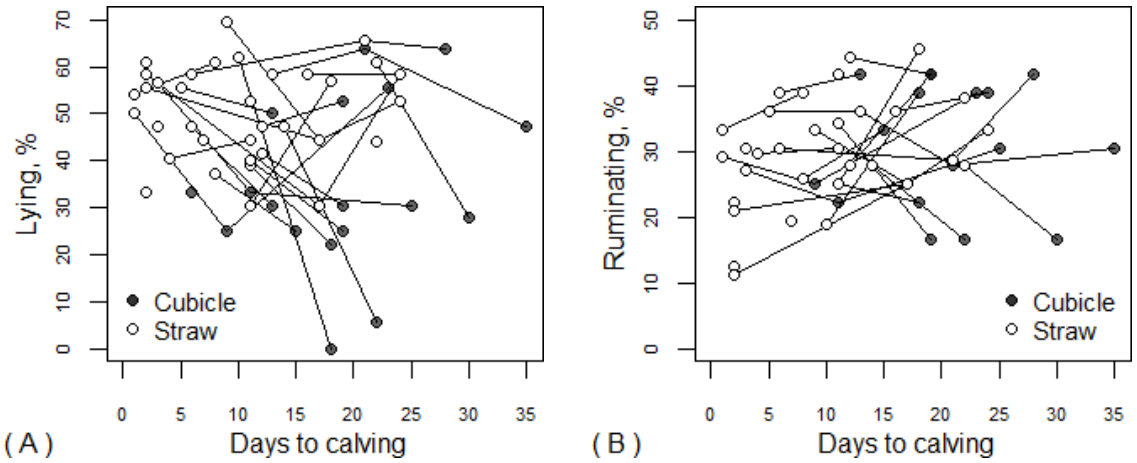
$$S_{ij} = ½(\Sigma_{ij}+\Sigma_{ji})/\max_{ij}[½(\Sigma_{ij}+\Sigma_{ji})].$$

As a result the score with values in interval from 0 to 1 was achieved. This score

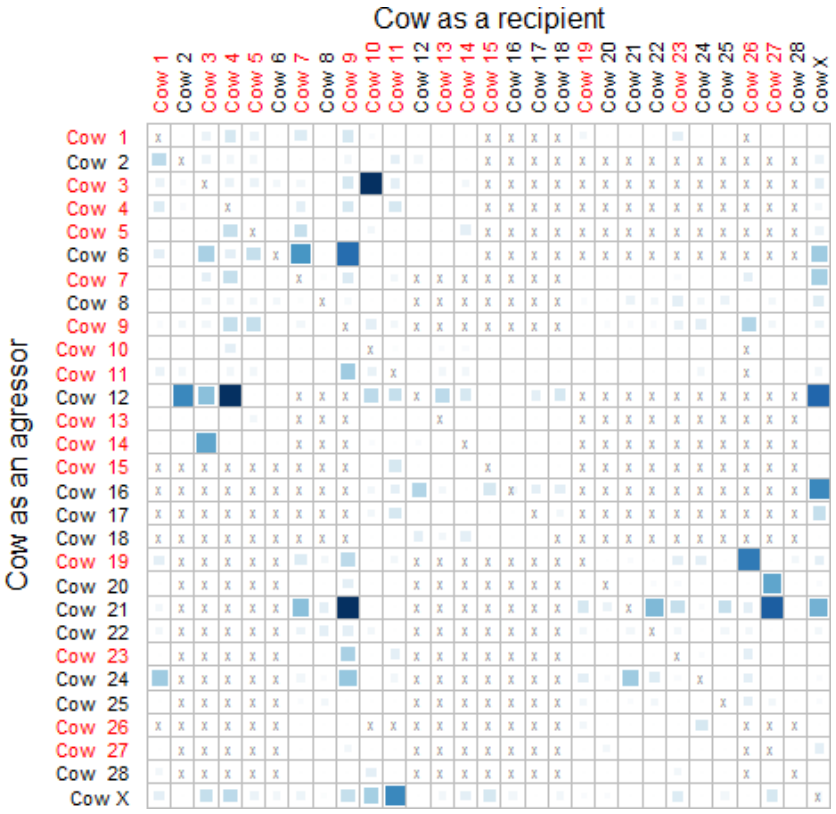
- considers both the situations where the animal j was observed as the neighbour of i and opposite,
- considers both the first and the second closest neighbours (the last with the weight ½),
- considers the observed distances (more close neighbours have a larger effect) and
- counts the actual number of observations when two animals were in the same group.

The score has value close to one in case of more close animals and value zero, if two animals were not observed being neighbours of each other at all. If two animals were not present in the same group, their nearest neighbourhood score was considered missing.

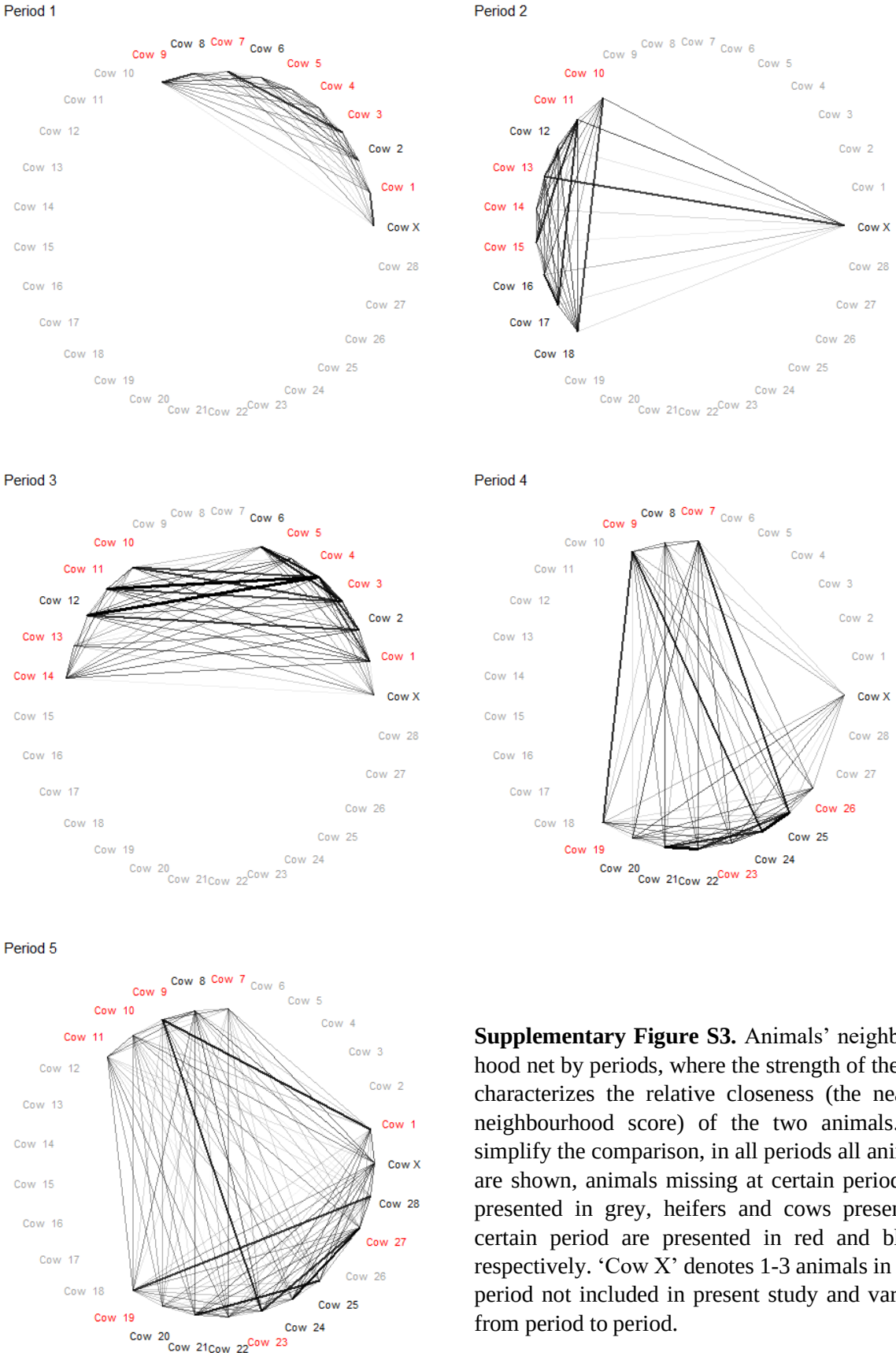
Supplementary figures and tables



Supplementary Figure S1. The percentage of (A) lying and (B) ruminating at different study periods. The days to calving on X-axis indicates the difference between the first day of the study period and the actual calving date. Observations made on the same animals are joined with lines. The animals in straw yard were more close to calving and lied more, while there were no changes in ruminating behaviour on an average. There was only weak concordance between posture and behaviour at cubicles and straw yard – animals laying and/or ruminating more in cubicles tended only slightly to behave similarly in straw yard: r=0.34 (p=0.192) and r=0.24 (p=0.366) for lying and ruminating, respectively (in figures this is expressed by non-parallel lines).



Supplementary Figure S2. The overall aggression matrix of studied animals. Colour intensity in i^{th} row and j^{th} column indicates the average number of aggression acts per hour by animal i against animal j (max=5.7), grey crosses denote animals' pairs without common periods and therefore without chance to be aggressive against each other. Heifers and cows are presented in red and black, respectively. 'Cow X' denotes 1-3 animals in each period not included in present study and varying from period to period.



Supplementary Figure S3. Animals' neighbourhood net by periods, where the strength of the line characterizes the relative closeness (the nearest neighbourhood score) of the two animals. To simplify the comparison, in all periods all animals are shown, animals missing at certain period are presented in grey, heifers and cows present at certain period are presented in red and black, respectively. 'Cow X' denotes 1-3 animals in each period not included in present study and varying from period to period.

Supplementary Table S1. Summary of behavioural characteristics seven days before and after calving and at calving day measured with Ice Tag and Ice Qube sensors on heifers and cows. The least square means (with standard errors) and factors' p-values according to the two-way repeated measures analysis of variance are presented. For better fit the right skewed characteristics motion index, number of steps and number of lying bouts are binary logarithm transformed and in their scale one unit difference corresponds to two times difference in real values.

Variable	Heifer			Cow			P-value		
	Before	Calving day	After	Before	Calving day	After	Parity	Time	Parity*Time
$\log_2$(Motion index)	11.5±0.07	12.2±0.19	12.3±0.05	11.1±0.08	12.0±0.22	11.5±0.06	<0.001	<0.001	0.010
$\log_2$(Steps, no/day)	10.2±0.08	10.7±0.22	10.9±0.06	9.0±0.09	9.9±0.24	9.6±0.07	<0.001	<0.001	0.426
$\log_2$(Lying bouts, no/day)	6.0±0.14	7.3±0.38	6.5±0.10	3.7±0.16	4.3±0.43	3.5±0.11	<0.001	<0.001	0.027
Lying, hours	13.6±0.26	11.3±0.67	8.6±0.18	14.5±0.29	9.2±0.76	9.2±0.20	0.578	<0.001	0.030