ANALYSING THE MOVEMENT AND BEHAVIOUR OF HOUSED DAIRY COWS

Kareemah Chopra

A thesis submitted for the degree of PhD Biostatistics

Department of Mathematics and School of Life Sciences
University of Essex
Submitted for examination in January 2023

I. ABSTRACT

Cows in modern dairy systems are at risk of comprised health and welfare, and monitoring changes in behaviour can help identify early-warning signs. This thesis uses a local positioning system to detect changes in group-level behaviour. The proximity interaction network structure and consistency of a herd housed in a closed barn on a commercial farm in Essex is explored. Next, the network structure, alongside group-level space-use patterns, on the commercial farm in Essex are compared to those of a second dairy cow herd housed in an open barn (RVC Research farm). In the subsequent chapters, the relationship between barn temperature and bunching behaviour, a potentially maladaptive response to warmer than average temperatures, was investigated in both herds, through various bunching metrics: range size, inter-cow distance and nearest neighbour distance. The herd on the commercial farm in Essex was highly connected and temporally unstable, with inter-individual variation in interactions in the non-feeding zone, and social differentiation across functional zones. No social assortment by parity, days in milk or lameness state was detected. The herd on the RVC Research farm were less connected than the herd on the commercial farm in Essex. Inter-individual variation in proximity interactions was found in the feeding zone of the RVC Research farm, alongside social differentiation across functional zones. Cows showed preferences for specific areas of the non-feeding zones, more so on the commercial farm in Essex than on the RVC Research farm. Cows increased their bunching behaviour ≥ 20°C in terms of all bunching metrics on the commercial farm in Essex. This pattern was observed for nearest neighbour distance on the RVC Research farm ≥ 15.91°C. This thesis demonstrates the use of precision livestock farming to monitor changes in group-level behaviour to improve the health and welfare of livestock.

II. PUBLICATIONS

Chopra, K., Hodges, H. R., Barker, Z. E., Vázquez Diosdado, J. A., Amory, J. R., Cameron, T. C., Croft, D.P., Bell, N.J., Codling, E. A. (2020). Proximity interactions in a permanently housed dairy herd: Network structure, consistency, and individual differences. Frontiers in veterinary science, 1040.

III. CONFERENCE AND SEMINAR PRESENTATIONS

Animal Behaviour Live: Annual Online Conference. August 2020, virtual. *Investigating the social network of dairy cows* (poster).

ASAB Winter Meeting. December 2020, virtual. * (talk).

National Centre for Statistical Ecology Meeting. June 2021, virtual. *Social networks of cows* (talk).

Essex Data Science Seminar Series. November 2021, virtual. *The behaviour of dairy cows* (talk).

University of Essex: Life Sciences Graduate Forum, September 2021, virtual. * (talk).

University of Reading. March 2022, virtual. *The Social Behaviour of Cows* (guest talk).

UFAW Advancing Animal Welfare Science, Edinburgh, UK. June 2022. * (poster).

ECBB: All of life is social. Groningen, the Netherlands. July 2022. * (poster).

* Proximity Interactions in a Permanently Housed Dairy Herd: Network Structure, Consistency, and Individual Differences

IV. ACKNOWLEDGEMENTS

A huge thank you to the University of Essex, for enabling me to carry out my research through an interdisciplinary faculty studentship and for helping me attend invaluable conferences through Proficio.

I am profoundly grateful to my supervisors Professor Edward Codling and Dr Thomas Cameron for all their guidance and patience throughout my PhD. You have taught me so much- I couldn’t have asked for better supervisors. My sincere thanks to my collaborators, Dr Zoe Barker, Dr Jonathan Amory, Dr Jorge Vazquez Diosdado, Professor Darren Croft, and Dr Nick Bell, all of whom I enjoyed working with. I appreciate the invaluable input you provided. Thank you to Dr Holly Hodges whose work provided me with a basis for my own. I am also grateful to the farm staff who helped facilitate my studies. Thank you to my colleagues who welcomed me across both departments. I am thankful for the time and invaluable feedback from my examiners, Dr Simon Turner, and Dr Delaram Jarchi.

My appreciation extends to my family and friends for their continued moral support throughout my studies, particularly my mother, sister, and grandma. Finally, I would like to thank my partner for always listening to me deliberate over my work.

V. LIST OF FIGURES

Chapter 2.

Chapter 3.

Chapter 4.

Chapter 5.

Chapter 6.

VI. LIST OF TABLES

Chapter 2.

Chapter 3.

Chapter 4.

Chapter 5.

Chapter 6.

VII. CONTENTS

1. CHAPTER 1: GENERAL INTRODUCTION

1.1. Introduction

Dairy products are an important global commodity, with consumption predicted to further increase over the next decade, despite the growing interest in plant-based alternatives, largely due to rising consumption in developing countries (1). To meet this demand, world milk production alone is expected to continue growing by 1.6% per annum over the next decade (1). Stocking densities have increased, and cows are genetically selected for milk quantity (2).

Although modern farming systems are efficient, dairy cows are susceptible to decreased health and welfare, with high-yielding cows particularly prone to production diseases (3). However, consumer and legislation demand for improved health and welfare of managed animals is increasing, and its importance is being recognised in the farming industry (2,4,5). Compromised health and welfare has in turn been shown to decrease overall productivity, so indicators are vital to maintain productivity and demonstrate the high welfare linked to products to gain premium prices from consumers (6). In this introductory chapter, I will outline prominent health and welfare concerns in the dairy industry. Next, I will provide an overview of the individual and social behaviour of cattle, then link this to indicators used to identify health and welfare concerns. The latter are reported through studies using traditional in-person observations, of which I highlight difficulties with, and more advanced methods using automated monitoring systems. I then identify knowledge gaps in the identification of prominent health and welfare concerns before setting out my research objectives to address these gaps.

1.2. Health and Welfare

This section defines animal health and welfare and addresses some of the main issues associated with disease, environmental factors, and grouping in the context of housed herds, and how they are typically diagnosed on-site. I focus on four of the most prevalent issues in the dairy industry, namely lameness, mastitis, heat stress and ketosis, which significantly impact the welfare of dairy livestock and the sustainability of the dairy industry. Mastitis is the most common production disease affecting dairy herds worldwide, with an estimated incidence rate of clinical mastitis in England and Wales ranging from 47% to 65% (7). Lameness, with an average farm prevalence of 32% across England and Wales, is the second most common production disease (8,9). Heat stress is already a significant issue impacting production, and climate change is expected to rapidly increase its prevalence and severity (10). Finally, ketosis is the most common metabolic disease in high-performance dairy cows during the transition period (11,12), with an average prevalence estimated across ten European countries of 22% (13). These issues are not extensive and nor are they mutually exclusive i.e., animals can suffer from co-occurrences of these conditions simultaneously. I later demonstrate how these complications can alternatively be identified through changes in behaviour, with a focus on social behaviour, in Sections 1.3 and 1.4.

1.2.1. Defining animal health and welfare

Animal health refers to an individual’s overall state of physical, mental, and social fitness, with better health involving the prevention and treatment of illness, as well as positive mental health (14). Welfare instead refers to well-being in any respect, incorporating the physical and mental state of an individual (15). An animal’s health is often considered a principal component of animal welfare, although measures of the former only more recently consider the latter (14). Indicators of welfare are varied, including hunger and thirst, physical and thermal discomfort, pain, injury and disease, fear and stress and the ability to express normal behaviour (as outlined in The Five Freedoms) (16). It must be considered that negative experiences are vital for survival, so management should aim to reduce rather than eliminate these experiences (17). Moreover, the Five Freedoms focus primarily on preventing negative experiences rather than providing opportunities for positive ones, which are also important (17). By taking into account the three main concepts of animal welfare- that animals can lead natural lives through development and use of their natural adaptations and capabilities, be free from prolonged and intense fear, pain and negative states, and experience normal pleasures while functioning well biologically- we can further improve animal health and welfare (18). While these concepts are not mutually exclusive, different individuals and groups tend to place varying degrees of emphasis on each. Assurance schemes are often used for monitoring animal health and welfare, involving on-farm assessment, advice and implementation (19).

1.2.2. Lameness

Lameness, a defect causing abnormalities in an animal's gait due to injury or disease to the leg or foot, is a prominent global issue (20). It is considered the third greatest health concern in dairy cow herds, following reduced fertility and mastitis, and is one of the primary reasons for culling young sows (21,22). The extremity of the condition varies, from uneven strides to an inability to bear weight on the affected limb (23). Affected cows produce less milk, with an estimated mean loss of 360kg per cow's lactation cycle, although the extent to which appears to be wound or lesion-dependent (24–27). Reproduction is also impacted; cows experience increased calving to conception intervals and a hindered ability to express oestrus and ovulate, and lame sows produce a smaller litter size than non-lame sows, and show delays in post-weaning oestrus (28–30).

Measuring the welfare impacts of lameness is difficult, as we are unable to grasp the internal experience of individuals. Nevertheless, it is well established that it is a painful condition; the use of analgesics, medications used to treat pain, demonstrates that lame individuals have lower pain thresholds, the extent to which is lesion dependent (31–38). Physiological measures also reflect the stressful nature of the condition, with individuals experiencing increases in heart rate variability and blood cortisol when lame (39,40). Particularly in cases of severe lameness, additional discomfort and stress arises from corrective trimming, which may be partly triggered by separation from the herd for this procedure (40,41). Although the impacts of handling and restraint are hard to distinguish from the procedure itself, discomfort and stress caused in the process clearly impact individual welfare.

Detecting an animal's gait is primarily used to detect lameness. Typically, an individual is assigned a locomotion score indicating the presence or absence of specific behaviours and postures, for instance an arched back, during routine procedures such as herding or hoof trimming (42–45). Although useful, observer scoring is subjective, prone to error and abnormal behaviours may not be immediately obvious; studies find farmers underestimate the severity of several pathologies through locomotion scoring (46–49). The uses and disadvantages of locomotion scoring, and alternatives for diagnosing lameness, are discussed further in Section 1.4.1.1.

1.2.3. Heat stress

As with lameness, heat stress, the failure to dissipate body heat to maintain homeostasis, hinders the reproductive success of mammals through an accumulation of effects, of particular concern in farm animals due to economic loss (50). Dairy cow conception rates are reduced in warmer months, particularly in high-yielding cows, further highlighting the need to address this in the livestock industry (51–53). Under high temperatures, individuals increase their respiration rate to facilitate evaporative cooling, while also elevating their pulse rate to enhance blood flow to the skin's surface, promoting heat loss (54,55). Both respiration rate and pulse rate are indicators of stress (54,55). An individual's mental state and their ability to live a natural life may therefore be breached when animals lose the ability to maintain a comfortable body temperature.

Heat stress is frequently detected using manual vaginal data loggers, but these can only be used for a limited number of consecutive days to maintain vaginal health, and data are not provided in real-time or require downloading (56,57). Other techniques for detecting heat stress include recording rumen temperature, but this is impacted by heat from fermentation and cooling through water intake (58). Although current strategies to target heat stress are useful e.g., the installation of cooling techniques, shade, ventilation, fans and sprinklers, these do not consider the individual or current weather conditions (59–61). There is a call for continuous monitoring and detection of heat stress at the individual level, particularly pressing given climate change and the high demand for milk production, with higher stocking rates used to accommodate for this which could heighten the impacts.

1.2.4. Mastitis

Mastitis, inflammation of the mammary gland or udder, is estimated to be the most prevalent disease in the dairy industry (62). Profit is lost through mortality, decreased milk production and quality, contamination of milk, and treatment costs (63–66). There is conflicting evidence of whether animals with mastitis suffer hyperalgesia, abnormally heightened sensitivity to pain, as studies find both increased and decreased thresholds to nociceptive pressure and temperature

(67–70). Nonetheless, other indicators such as changes in facial expression in sheep suggest mastitis is painful (71).

Currently, mastitis is typically detected through somatic cell counts and culture-based methods, due to the low cost and ease of data collection (72–74). These techniques help identify the disease but lack sensitivity in determining the extent of the infection, so there is a need for more sensitive methods (72). Farm management routines such as milking and unhygienic environments can give rise to the condition, and the uptake of automated milking systems is reducing inspection time required to identify mastitic cows, further accentuating the need for more sophisticated, automated identification (75–77). Typically, animals are treated through antibiotics, but there is a demand for alternatives due to growing concerns of antibiotic resistance, and quicker identification could help with this (78).

1.2.5. Ketosis

Ketosis is a common metabolic disease characterised by high concentrations of ketone bodies in the bloodstream, urine, and milk due to a negative energy balance (79). Transition dairy cows, individuals undergoing physiological changes before and after calving to support milk production, often develop the disorder (80). Cows with ketosis experience decreased milk yield and impaired reproduction, plus increased susceptibility to lameness and mastitis, all of which are costly for farmers (81). Current diagnosis techniques focus on individuals which are already sick and involve identifying ketone bodies in bodily fluids e.g., milk, blood and urine (79,82). These methods have low in sensitivity and specificity; blood samples are the ‘gold standard’ as they provide a relatively accurate measure of ketone bodies, but this is invasive and time-consuming (79,82).

1.2.6. Other conditions

Other common dairy diseases include complications during and after calving. Dystocia, a difficult birth resulting from prolonged calving or severe assisted extraction of a calf at birth, is considered extremely painful and increases the risk of calf mortality (83–87). Scoring systems are used but these are subjective, and can vary so there is a need for standardisation to better understand the prevalence and extent of dystocia on farms (86–90). Another birthing complication is a retained placenta that increases the risk of metritis, inflammation of the uterus, leading to reduced milk yield and reproductive problems (91–95). Metritis is typically identified through clinical examination of the uterus and discharge, but veterinary examination is relatively infrequent on most farms (96,97). Although antibiotics can be used to treat the condition, they increase selective pressure for the emergence of resistant bacteria (98–101). After calving, animals may also develop hypocalcaemia (milk fever), defined as low plasma calcium, which has been associated with impaired reproduction and milk production (102–105). Definitive diagnosis requires an expensive blood test, and this typically requires sample processing. However, development of on-site diagnostic tests and procedures for cows are underway, including the use of portable blood analysers that detect ion concentrations in the blood through a small sensor (106).

Another prominent issue impacting farm animals is the left displacement of the abomasum, whereby this stomach compartment is filled with gas and then trapped by the rumen to the left side of the abdominal cavity; in dairy cows, this mainly affects high yielding lactating individuals postpartum (107–109). The condition is traditionally diagnosed by detecting gas present in the abomasum through a high-pitched noise produced by simultaneous auscultation (listening) and percussion (tapping on a surface to determine underlying structures) (110). Treatments involve attempting to move the abomasum back into position non-surgically or surgically but the former has variable success rates and the latter is invasive; detecting early warning signs is crucial for profitability and welfare (110,111).

1.3. An overview of the behaviours of dairy cows

Behaviour can be defined as ‘the internally coordinated responses (actions or inactions) of whole living organisms (individuals or groups) to internal and/or external stimuli, excluding responses more easily understood as developmental changes’ (112). Here, an overview of both individual and social/ group behaviours is provided, the latter divided into affiliative (positive) and agonistic (negative). Differences between housed dairy cow behaviours and those of their wild counterparts are highlighted.

1.3.1. Individual behaviour

The main individual behaviours of cattle can be categorised into: walking, grazing/feeding, ruminating and resting, and the latter three can be further divided into lying and standing (113,114). Grazing is the most observed behaviour in pasture-based systems, enabling cattle to obtain enough nutrition for maintenance and production, whereas this is not needed in housed settings where cows are typically fed regularly (115,116). Instead, housed dairy cows spend most of their time lying rather than feeding, although the latter is still a frequent behaviour (114). Less observed behaviours include drinking, grooming, head shaking, scratching and urinating, which may be equally important; an increase or decrease in non-essential behaviours, which may be dependent on the behaviour, might indicate a stressed state (113).

To detect abnormalities which are indicative of welfare concerns, cataloguing individual-specific baseline behaviours is essential. Individual time budgets can be affected by the specific housing system and management, so it is crucial to monitor deviations from known norms. Shifts in time budgets may be effective adaptations to a specific environment e.g., restricting access to food increases the time housed cows spend eating, in attempt to obtain a similar amount of feed (117). Alternatively, changes could have negative consequences. Inadequate access to space can inhibit essential behaviours, as shown by a reduction in lying time of up to one hour per day for cows housed at 1.5 cows per stall, compared to those housed at one cow per stall (118–122). A reduction in lying time should be monitored as it increases the risk of lameness in cows (123). However, lying time is also weather-dependent; housed cows spend more time lying in winter than in hotter seasons, to reduce heat loss (114). Behavioural time budgets may not only be environmentally dependent but attribute-dependent; higher yielding cows spend more time eating and less time lying than lower yielding cows (124). Moreover, a single trait is not fully representative of an individual’s state and so it is useful to consider repeatable groups of traits stable across time and contexts- or personality (125).

To test the presence of personality, individual differences in behaviours must show temporal and contextual consistency (126). Indeed, individual cows show temporal consistency in lying duration and overall activity, with more active individuals showing higher daily variation in their behavioural activity (127,128). This could be linked to sociality; individuals show repeatability in behaviours and saliva cortisol in response to a social isolation test over time (129). Exploration and anxiety could contribute as well as sociality, in terms of underlying personality structures which may be important concerning animal welfare (129). These studies suggest both individual differences and temporal consistency- aspects of personality (126).

Furthermore, cows show intra-individual consistency in inactivity and inhibition behaviours across contexts, to various fear-eliciting situations: novel environments and objects, unfamiliar areas and surprise effects (130). Responses to fear are age-dependent; older individuals show a lower cortisol response when separated from their herd, potentially due to habituation to husbandry situations (129). Despite artificial selection of farm animals, distinct behavioural consistencies are present which could help us understand the ability of individuals to cope with husbandry conditions and predict stress responses, relevant from the perspectives of both animal welfare and production (130,131).

1.3.2. Grouping

Cattle are naturally gregarious, forming dominance-based hierarchies, in groups generally consisting of 20 individuals including females, calves, and transitory males (132). Group living provides numerous benefits, including reduced predation risk, facilitated learning for resource exploitation and mating opportunities (133,134). The advantages of group living tend to outweigh the costs e.g., increased competition for resources, and so it has evolved independently in a range of species (133). Wild Bovidae groups self-regulate, with group size and density subject to change based on current environmental conditions, predation risk and resource availability e.g., bison select foraging sites with wheat sedge less with increasing risk of wolf predation, and wildebeest favour grouping in open habitats under high predation risk (133,135–137).

Although on most commercial farms cows are housed in groups, the herd social structure of dairy cows differs significantly from their wild counterparts; they are usually housed in exclusively female groups for efficiency, reducing time and labour, and to prevent uncontrolled breeding (135,138). Typically, non-lactating cows are separated from the milking herd and the latter are separated by lactation stage and age, and calves are separated from dams shortly after birth (139,140). This differs from the mixed-sex herds found in the wild, where males protect the herd from predators (138). Dairy cows are also subject to regrouping events following changing status, exposing cows to new individuals often without considering social stress (141–143). Furthermore, domestic cows typically have access to restricted space allowance in the form of indoor housing or fenced grazing paddocks (135,139,140,142,144). High stocking densities increase competition for resources, hence aggression and social stress, which will be further explored in Section 1.3.2.1 (145). Ultimately, grouping of dairy herds is not led by animal choice but through farmer decisions, therefore the social structures of cattle in a domestic setting may not align with their evolutionary adaptations, so understanding the structure and dynamics of these networks may provide insights to optimise management (146,147).

1.3.3. Social behaviour

Social behaviours can be broadly categorised into agonistic and affiliative. Agonistic interactions are negative and include displacement, kicking, pushing and avoidance (148). Affiliative behaviour is positive, and includes allogrooming (social licking), sniffing and rubbing (149–151). Monitoring an individual’s negative and positive social interactions over time could help predict responses to management practices (152). The importance of considering the individual in social interactions will be discussed in more depth in the following sections of this review (Section 1.3.2- 1.3.3).

1.3.3.1. Agonistic

Agonistic behaviours may involve direct contact, such as a butt, nudge, push or kick, and the aftermath of such interactions may be conspicuous via physical inspection of individuals e.g., bruising, cuts and scratches, bite marks, limping or lameness (148,153). Non-contact agonistic behaviours, such as threatening and displacements, may not have immediate observable effects but can result in stress. Responses can manifest as increased heart rate, raised cortisol levels, vocalisations, and stereotypic behaviour, which are indicative of an animal's psychological state (154). Studies have shown that housed cows exhibit more agonistic behaviours than their wild counterparts, which suggests that they may experience greater levels of stress and compromised welfare (120,148,155). Agonistic interactions heighten after regrouping events and with increasing stocking density, of particular concern giving increasing herd sizes (120,148,156,157).

High stocking densities lessen the chances of subordinates being able to avoid dominants e.g., whilst feeding, increasing social stress (135,158). It is therefore clearly important to consider the dominance hierarchy in herd groups, including how cows are ranked e.g., by age and weight, in relation to management (148,159). Dairy cow hierarchies have been assessed considering displacements, at feed barriers and cubicles, and such interactions do not always involve physical contact (144,159). Non-contact displacements of lower ranking cows may be significant, resulting in excessive stress and reduced feed intake and lying duration, shifting individual time budgets to become less favourable (158,160). Dominance hierarchies are largely stable but can change over time, according to resources and environmental conditions, particularly with frequent regrouping (135,152).

1.3.3.2. Affiliative

Opportunities for positive interactions are essential for maintaining a good standard of welfare in managed herds (161). Allogrooming can serve not only as a hygienic practice, but also to promote social bonding, and can be particularly useful to monitor after the introduction of a new cow into a group (148,149,162,163). Allogrooming reduces social tension, and recent arrivals into a group are found to groom more herd members (162).

Social support is further reflected in the responses of beef cattle to stressors, whereby individuals are more likely to seek social contact in isolation (164). Maintenance of closeness serves to provide social support and promote social bonding (165–167). Animals maintain closer proximity to more familiar individuals, observed across taxa from fish to primates (168–171). The importance of closeness and familiarity is further demonstrated in studies showing behavioural stress responses of cows during social isolation, which are less extreme in the presence of a companion, particularly when individuals are familiar (129,141). The provision of sufficient space is therefore crucial to not only allow subordinates to avoid dominant individuals, but to also allow social bonds to form, of notable concern given increasing herd sizes (135). Indeed, poultry show increased tonic immobility with increasing group size, indicating higher levels of fear, and pigs housed in large groups show increased aggression; the stress imposed by the social environment may increase the risk of any individual sow becoming a biter (145,172,173). However, this is only above a given group size e.g., pigs and calves housed in pairs are more stressed than when housed in groups of four to eight, respectively (174,175). This number will differ between livestock and according to conditions. Maintenance of proximity is widely used to measure social interactions over time. It is used as a proxy for both affiliative contact and non-contact social interactions but does not indicate which animal in a dyad, if any, drives the spatial relationship, or whether relationships may appear where none exist, such as when two individuals share the same diurnal pattern or micro-climatic preferences. Nonetheless, maintenance of proximity has been found to be positively correlated with affiliative social interactions between cows (176) which is why it is adopted in this thesis to measure such interactions (Chapters 3 and 4) (165–167).

1.4. Recording behaviour to improve health and welfare

As established in Section 1.2, dairy cows in modern farming systems are at risk for compromised health and welfare. Behavioural indicators are vital to maintain and improve the welfare of livestock, so stress and pain can be alleviated.

1.4.1. Observational studies of behaviour

Earlier observational studies demonstrate the use of behavioural indicators to assess the health and welfare of dairy cows. This section aims to provide a brief overview of these studies, whilst discussing their limitations in monitoring behaviour.

1.4.1.1. Gait analysis

As highlighted in Section 1.2.1.1, locomotion scoring is widely used to diagnose mobility issues, particularly lameness, providing convenient, immediate on-site assessment without the need for technical equipment (177). Main approaches are numerical rating scores (NRS) which use discrete units, and visual analogue scales (VAS) which measure on a less restrictive and continuous scale (178–180). Handling VAS data is more difficult though, and inter-observer agreement in cow lameness scores is lower than when using a NRS (178–180). Therefore, NRS is more commonly used (178–180). Nonetheless, both scoring methods are inherently subjective; farmers are found to identify equivalent to, or less than, one in four hoof injuries or incidences of disease (46,181). Furthermore, inter-observer reliability in diagnosing lameness through both scoring methods is low e.g., only as high as 60% in horses (*n* = 20) scored between sixteen observers (182). Observer training and limiting the number of scoring categories improves repeatability but the latter could limit important information (183,184). Moreover, inter-observer repeatability increases with the severity of lameness, so animals with slight gait abnormalities are at greater risk of being classified as non-lame, as found with sheep and horses (180,185). With growing herd sizes to meet rising demand for dairy products, scoring is particularly prone to error.

1.4.1.2. Individual time budgets and space-use

Individuals may adjust the time they spend standing when in pain e.g., lame broilers are shown to decrease the time they spend standing compared to their non-lame counterparts (186). Additionally, individuals may alter the time they spend lying when lame, as demonstrated by lame cows that spent more time lying than non-lame cows (187). Shifts in resting times when ill may be dependent on the comfort of stalls; lame cows spend more time lying when housed with sand bedding than with rubber crumb-filled mattresses (188). A reduction in lying could therefore signal lameness, and the use of comfortable bedding could aid recovery (187–189). Similarly, cows with dystocia change between standing and lying positions more frequently, indicating discomfort (190). In contrast, cows undergoing heat stress limit the time they spend lying (189,191,192). Instead, cows spend more time standing under heat stress, to increase exposed body surface area for heat loss, but this may also maladaptively hinder blood circulation in the udders, so monitoring a change in standing behaviour could reduce discomfort from not only heat stress but also from further complications (191).

Shifts in behaviours other than lying and standing are observed in unhealthy individuals; cows with lameness and dystocia spend less time feeding, and this is also seen prior to the development of displacement of the abomasum and in cows undergoing heat stress (188,190,193,194), although patterns are inconsistent across herd groups (187,195–197). Limiting food intake under heat stress serves to decrease metabolic rate to reduce heat load (198–202). Under heat stress, cows also increase their water intake to cool down, as temperatures increase past a threshold (203). Similarly, goats are shown to spend less time eating and more time drinking under heat stress (204). The time dedicated to non-essential behaviours also decrease under heat stress, as shown with grooming in mastitic dairy cows (205), and with preening in lame broilers (186). Overall, a change in behavioural time budgets can indicate discomfort, and prompt detection of shifts can help address potentially associated health concerns.

An alteration in individual space-use could further help identify health complications; non-lame cows are found to spend more time in a cool alley of a study barn compared to lame cows (189). Under hot conditions, cows seek areas of shade with the aim to reduce body temperature (206), but the effectiveness of this likely depends on factors such as stocking density and shade availability. It is therefore useful to flag changes in livestock movement and understand where these may indicate discomfort to improve welfare.

1.4.1.3. Social behaviour

Observations show cows are displaced more often from feeding areas at high stocking densities, particularly subordinates (207). Lame cows are at higher risk of being displaced; the encounter itself and an inability to access equal feed is likely to induce stress (207). Cows may also avoid aggressive interactions when ill, likely associated with areas holding resources such as food, as found with lame cows and matristic cows prior to calving (208,209). The order individuals enter the milking parlour can shed light on the health status of cows too, with observations showing lame cows position towards the rear of the herd upon entering, suggesting a lack of ability to compete for relief from milking (187). Moreover, the likelihood of developing health conditions might in itself be dependent on social rank, with observations showing low-ranking cows are more likely to develop soft tissue lesions and lameness (210).

A modification in social behaviour could help identify heat stress; aggressive interactions between cows increase with temperature (211,212). This is observed in shaded areas, unsurprising given this resource becomes more important to relieve discomfort so cattle are more likely to compete for it (213). Installing more shaded areas can help, and is shown to reduce respiration rate, a sign of stress, but the amount of shade required is herd-specific and should consider housing conditions and group size (211).

1.4.1.4. Limitations

In-person observations and video recordings, alongside the measurement of daily feed intake, have shed light on behavioural changes in gait, time budgets and space-use when livestock are ill. There is, however, a need for more objective measures to detect illness. Observing and measuring cow footprints show promise in objectively detecting lameness but multiple trackway measurements are required due to the high inter-individual variability in tracks (214,215). Manual measurements are limited by time and resources. It is also difficult to identify conditions whilst maintaining a suitable distance, to reduce the potential behavioural influence of observers, a concern as animals are known to mask vulnerable behaviours (216).

Given time is pressing for farmers, especially with increasing herd sizes, there is a call for automated detection of illnesses such as lameness and heat stress.

1.4.2. U*sing technology for automated detection of illness*

The recent adoption of Precision Livestock Farming (PLF), where farm management is aided through continuous automated real-time monitoring of animals or the environment (217–219) can help overcome limitations of traditional techniques. In this section, an overview of studies using various technologies to identify changes in livestock behaviour, indicative of the health and welfare issues covered in Section 1.2, are outlined. Limitations in these approaches are then identified, as well as gaps in our knowledge.

1.4.2.1. Gait analysis

The main technologies used to detect lameness through gait are force plates, pressure mats, imaging, and motion sensing. Changes in weight distribution can be measured using ground reaction forces via force plates; lame cows place more weight on healthy limbs and adopt longer stances compared to non-lame cows (220–222), and a deviation in front-to-hind leg weight distribution may be indicative of lameness in sows (223). The use of force plates is validated by the finding that the weight lame cows apply to injured legs increases after administration of local anaesthetic (224). Assessing aspects of gait through pressure mats, which measure vertical forces such as the speed and asymmetry of gait, can also be used to assess health, found to successfully identify lameness in cows up to a sensitivity of up to 90% (225). The use of pressure mats for gait analysis is further demonstrated in research comparing flooring types used by pigs, revealing major improvements in pressure relief through the use of rubber mats compared to bare sensors (226). Furthermore, a study has showed that by utilising pressure mats and imaging techniques to measure range of motion and release angle of front hooves, there was an observed improvement in gait score in 83% of cows (227). However, the study also identified that 7% of the recordings were unusable due to difficulties in synchronising the pressure mat and the camera system (227). Furthermore, space may be an issue for pressure and force mat systems, although force mat length and sensor resolution can be compromised to a degree without influencing lameness detection (228).

Other image analysis techniques have been used to detect lameness through posture, and such systems can capture at high frequencies; for example, a system monitoring a cow's back posture shows promise, although this alone may not be an accurate indicator (55). A more comprehensive technique has been proposed, whereby the body movement pattern (BMP) analyses back posture in combination with head position using a side-view camera; 76% of lame cows were correctly classified (229). Furthermore, lameness can be detected through overlap in a cow's consecutive steps, and comparing manually labelled hoof locations to those located using imaging analysis, to a mean correlation coefficient of 95% (230).

The technologies explored above require animals to keep their legs in certain positions, or continuously move through specific measurement zones (222,225,227,229). The latter constraint has resulted in 12% of total cow gait recordings being unusable (227). Furthermore, imaging and weight analyses can be costly and rely on assessment with only a few steps per individual, and the technologies are prone to disruption from their surroundings. In imaging analysis, this is often due to changing shadows and dynamic backgrounds, which decreases detection accuracy (227,231,232). Interference can be minimised during set-up and through pre-processing but is impossible to eradicate completely (227). Top-view 3D cameras could help overcome these difficulties, outputting similar accuracies to side-view 2D cameras (233).

Alternatively, motion sensing for gait analysis can help overcome imaging interference issues (234). Lame cows show less symmetry in the acceleration of their hind legs than non-lame cows (235). Variance in acceleration between front and hind legs has also been found to indicate lameness in cows (236). However, it is important to consider inter-individual variation in gait and posture (237). A specific posture may indicate a healthy or a lame individual depending on the individual. A baseline for normal behaviour must be catalogued for each individual. This has been addressed previously, with lameness detected in cows using a supporting computer vision system with an accuracy of 96% (231). Furthermore, using accelerometers attached to cows' hind legs, deviations from their normal walking patterns have been detected with an accuracy of 91% (234). This shows promising potential for identifying abnormalities that could be indicative of lameness (234).

Overall, the technologies discussed in this section show promise in detecting lameness through changes in individual gait. Still, it must be considered that factors such as weather, milk load and lactation stage may influence gait, so this needs further exploration (220,225,238).

1.4.2.2. Individual time budgets and space-use

Monitoring individual behavioural budgets over time has the potential to indicate illness. To do this, it is vital to catalogue baseline behaviours, and this has been achieved for animals using accelerometers, including cows and sows (239,240). For instance, accelerometery data has been analysed using algorithms to detect cow behaviours, including lying, standing, and feeding, as well as transitions between standing and lying, with high sensitivities ranging from 77% to 99% (239). Changes in quantified baseline behaviours can then detect illness; standing bouts and walking speed are lower in lame cows compared to non-lame cows, and a model incorporating both can predict lameness to a sensitivity of 90% (241). Cows are also found to decrease their daily feeding duration when lame compared to when they are non-lame, found with the use of local positioning system (LPS) data in combination with accelerometery data, and more recently with accelerometery data alone (242,243). Similarly, cows with metritis reduce their feeding behaviour and spend less time at the feed alley during the transition period, and cows with dystocia reduce their feeding and drinking behaviour 24 hours before calving (208,244,245). Sows with neurocysticercosis, a parasitic disease of the nervous system, also reduce the time they spend at feeding troughs (246). Time spent feeding may not indicate consumption though; the use of electronic feeding systems shows that cows with subclinical hypercalcaemia visit feed and water troughs less often following calving but also increase their dry matter intake, perhaps to reduce the chance of competition and discomfort (247).

When lame, housed dairy cows decrease the time they dedicate to standing and increase the time they spend lying (243,248). Changes in activity levels may depend on the lesion causing lameness, impacting the pain caused by standing (248,249). Similarly, cows with subclinical hypocalcaemia spend less time standing after calving and cows with dystocia transition between standing and lying more frequently, both indicating discomfort (244,247). As explained in Section 1.4.1., accelerometery recordings reveal cows instead decrease their lying duration under heat stress during the day, measured using Temperature Humidity Index (THI) as a proxy (243). Monitoring other behaviours can also be useful; walking time decreases with increasing temperature as shown with accelerometers to a sensitivity of ≥ 80% (243,250). Furthermore, cows with severe metritis change their rumination and activity patterns, but the algorithm used to detect this needs developing for less severe cases, as the overall sensitivity for detecting all cases of metritis is 59% (251).

Monitoring space-use can also help locate ill individuals; using LPS, housed cows have been shown to reduce their full range size when lame, and spend more time in resting areas compared to non-lame cows (252). The use of LPS is thus valuable to monitor housed cows. LPS errors may result from inference of metal structures, but the technology has been validated with dairy cows with mean errors typically around 2-3m, and 0.5m mean errors may be achievable (15,25–28). LPS can be successfully set up indoors whereas GPS (Global Positioning System) has similar success in detecting behavioural changes indicative of lameness in pasture-based systems (116,253–256).

Spatiotemporal positioning of individuals can also be monitored using proximity sensors, detecting the presence of an individual within a given distance from a feature or another individual. Proximity sensors have been used to detect 'brushing events', defined as when cows were within one metre underneath a rotating grooming brush for at least ten seconds (257,257). Lame cows are detected to use brushes located away from the feed bunk less than those installed next to it, to a validity of 75% (257,257). Similarly, cows equipped with proximity sensors detecting presence at a feeder reveal a change in short-term feeding behaviour during the onset of chronic lameness (258). As mentioned in Section 1.4.1.1., inter-individual differences in behaviours can occur due to a variety of factors, such as parity and days in milk, so it is crucial to consider potential changes in behaviour at an individual level (252).

1.4.2.3. Social behaviour

Cows are less likely to approach humans when ill compared to when healthy (259,260), and both cows and calves have been observed to alter their positioning in a herd when ill (261–263). Lame cows may alter their time budgets due to a reduced ability to gain preferable positions and resources, and to reduce competition when their immune system is more vulnerable, suggested through evidence that lame individuals spend less time feeding and are less aggressive than non-lame cows (209,242,252). Individuals may also isolate themselves when they are ill, as shown with matristic cows and sows with neurocysticercosis; this may serve to reduce competition and limit chances of secondary infection (246,264). Interestingly, lame cows appear to be licked by conspecifics more than non-lame cows; perhaps initiated by lame cows for comfort (209).

Livestock such as cattle appear to show inter-individual variation in sociality; some individuals are more influential than others over the social structure, perhaps due to personality or dominance (167,265). Furthermore, relationships between individuals may differ according to the context. Housed cattle are shown to avoid interactions with dominant conspecifics whilst feeding, to reduce competition (158). This pattern may be altered if the resource is deemed more valuable, perhaps when food is limited (266). It is therefore useful to monitor social behaviour over time and across contexts, to consider deviations from normal behaviour indicative of illness. For example, competition between cows at an electronic water bin increases during times of heat stress, and less successful cows avoid the drinker at the hottest, most competitive times (267). Although social behaviour plays a crucial role in animal welfare, current technologies for monitoring animal behaviour mostly rely on location and movement patterns, which are not specifically designed to track social interactions. This limits our ability to understand and address social issues within animal groups.

1.5. Application of Precision Livestock Farming

As discussed in the previous sections of this thesis, monitoring potential changes in behaviour can help farmers identify early-warning signs of compromised health and welfare to refine management techniques. Collaboration between researchers and the industry has led to advancements in objective animal welfare assessment, and there is potential to further integrate automated monitoring into welfare protocols. This requires easy to install, low-cost systems, with user-interfaces accessible to farmers for data interpretation with minimal training. An example of a technology which is currently used in the dairy industry for managing cow health and welfare, is the accelerometer system by IceRobotics introduced in 2018 which allows farmers to detect lameness in cows using a traffic light colour system (268). In this system, the severity of lameness is determined based on the degree of deviation from a normal gait, and green indicates normal gait, amber indicates mild deviation from normal, and red indicates severe deviation from normal (268). This allows farmers to quickly and easily identify cows that may be experiencing lameness and take appropriate action (268). Another example of a system adopted in the dairy industry is CattleEye, a tool which uses visual imagery to identify body condition score to detect lameness (269) Although advancements in detecting compromised health and welfare issues through PLF in recent years have contributed to faster identification, there is demand for more accurate systems detecting concerns promptly, for the adoption of such technologies by farmers (270).

1.6. Gaps in our knowledge and thesis aims

While automated PLF techniques have been successful in detecting patterns of individual behaviour, these have not been widely applied to monitor changes in social behaviour that could be associated with illness, such as lameness and heat stress. LPS is used throughout this thesis to better understand group-level behaviours, with a view to detect health and welfare issues. Firstly, I aim to monitor potential changes in the proximity interaction network structure and consistency of a dairy cow herd housed in a closed barn, on a commercial farm in Essex (Chapter 3), and I compare the network structure to that of a second dairy cow herd housed in an open barn (RVC Research farm) (Chapter 4). In Chapter 4, I also compare the space-use patterns and time budgets of these two herds and explore reasons behind differences. In Chapters 5 and 6, I aim to understand the relationship between temperature and bunching behaviour in both dairy cow herds, a potentially maladaptive behaviour, with a view to improve management regimes.

2. CHAPTER 2: GENERAL METHODOLOGY

In this thesis, data recorded from a local positioning system (LPS) will be analysed to 1) explore the social network structure of two dairy cow herds, the first housed in a closed barn on a commercial farm in Essex and the second housed in an open barn on a farm referred to as the RVC Research farm (Chapters 3 and 4), 2) compare the space-use patterns and time budgets of these two herds (Chapter 4) and 3) analyse the relationship between temperature and the bunching behaviour of both herds, a potentially maladaptive response (Chapters 5 and 6).

2.1. Animals and housing

Two groups of high-yielding dairy cattle were monitored continuously for this study, both in southeast England. The first group were Holstein-Friesian cows, monitored from 1st August 2014 to 30th November 2014 (n = 127, a minimum of 86 and a maximum of 111 cows present in each month; feed space = 4.80m^2 to 6.20m^2 per cow, lying space = 3.53m^2 to 4.55m^2 per cow; overall area = 2898.00m^2 (63.00m x 46.00m), feeding zone = 391.28m^2 (58.40m x 6.70m), non-feeding zone = 553.13m^2 (left and right cubicle area = 52.60m x 9.70m = 510.22m^2 excluding two unusable cubicles, each 2.71m^2 (1.18m x 2.30m), = 504.80m^2; lower cubicle area = (27.75m x 2.42m = 67.16m^2 excluding seven unusable cubicles, each 2.69m^2, = 48.33m^2; space between neighbouring cubicles: left and right cubicle blocks = 3.59m; right and lower cubicle blocks = 2.40m). The cows were housed in a free-stall barn, with 98 cubicles available, and central passageways allowing free movement to areas including the feeding passage. Due to the presence of cubicles and barriers, this barn is considered to have a ‘closed’ barn layout. The group were milked thrice daily (at approximately 5am, 1pm and 9pm). This first group are henceforth referred to as the herd on the commercial farm in Essex. The second group were approximately 90% Holstein-Friesian and approximately 10% Brown Swiss, monitored from 17th October 2015 to 7th December 2015 (n = 55, a minimum of 45 cows present each month; stocking density of feed space = 4.04m^2 to 4.93m^2 per cow, lying space = 7.28m^2 to 8.90m^2 per cow; overall area = 2469.60m^2 (58.8m x 42m), feeding zone = 222.05m^2 (40.3m x 5.51m), non-feeding zone = 400.58m^2 (40.30m x 9.94m)). The cows were housed in an ‘open barn’ (no cubicles) and milked twice daily (at approximately 8am and 5pm). This second group are henceforth referred to as the RVC Research farm herd. Both study groups were fed a total mixed ration during the first milking and feed was provided several times daily. Layouts of the two barns are shown in Figure 1A and 1B for the commercial farm in Essex and the RVC Research farm, respectively.

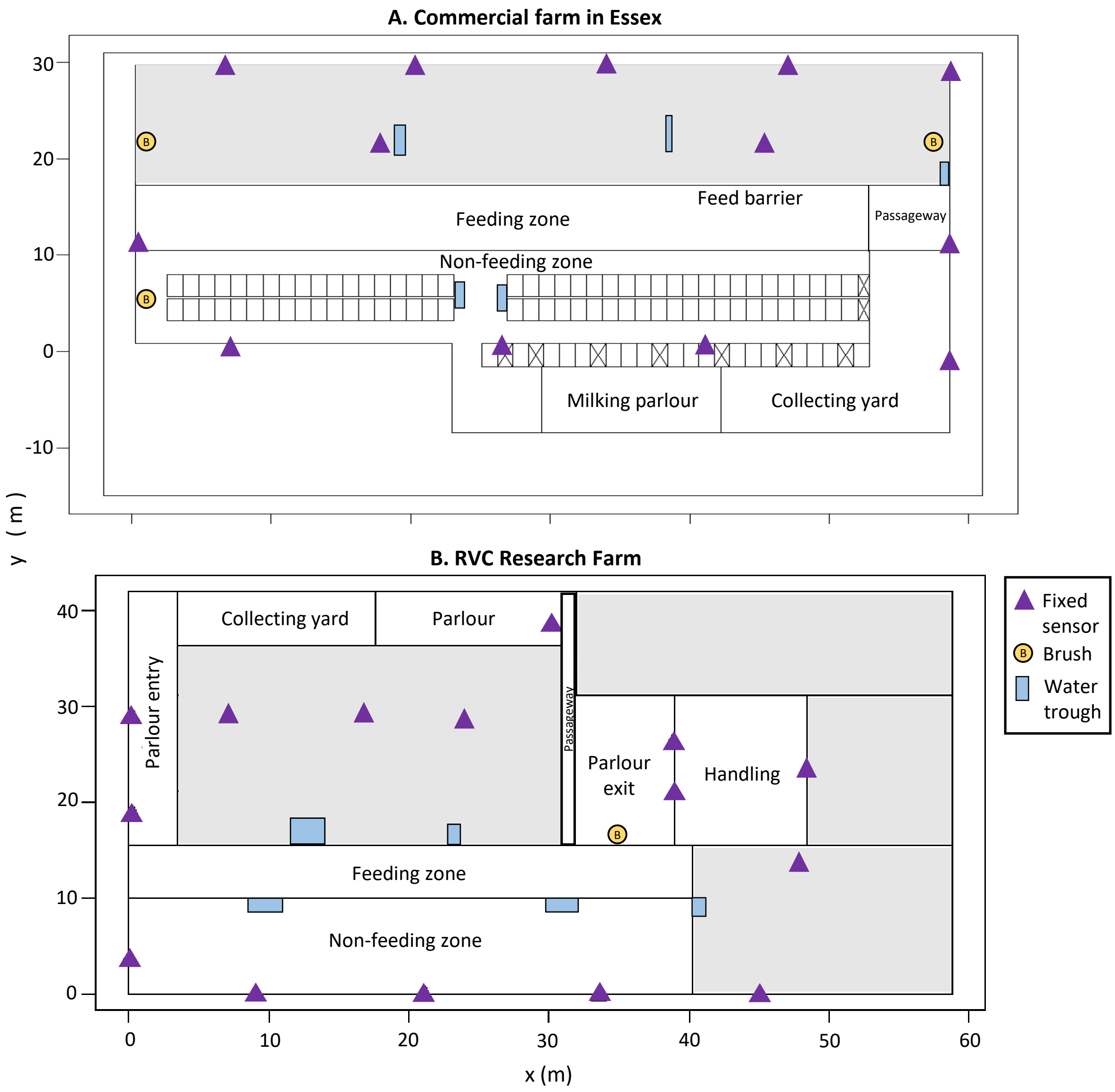

Figure 1. Barn layouts for (A) the commercial farm in Essex and (B) the RVC Research farm. Overall areas are 63m x 46m for the commercial farm in Essex and 58.8m x 42m for the RVC Research farm. Fixed Omnisense 500 sensors ('Oms500'; part of the local positioning system) are represented with purple triangles, brushes are indicated with yellow circles labelled 'B' and water troughs are represented as blue rectangles. For the commercial farm in Essex, cubicles are shown with small rectangles- referred to in this research as a 'closed barn' layout, whereas the RVC Research farm does not contain cubicles and is referred to as having an 'open barn' layout. Note that the location of the brush in the parlour exit of the RVC Research farm is approximate but could only be accessed during milking times. The areas shown in grey were

not used by the study groups. On the commercial farm in Essex, the grey area above the feeding zone housed a different high-stocked dairy cow herd during the study period. On the RVC Research farm, the grey area between the collecting yard/parlour and feeding zone housed a low-yield dairy cow herd, and the area shown in grey below the handling area where x = ~ > 40m housed young stock during the study period.

2.2. Local positioning System

Cows were deployed with mobile Oms500 devices (Omnisense Ltd, Cambridge, UK) which encompass both local positioning and accelerometer sensors. Orientation was fixed using a weighted neck collar. The sensors formed a localised wireless network, using triangulated radio signal communication to automatically calculate the relative local position of each cow throughout the study duration, at a temporal resolution of 0.1Hz. Strategically positioned sensors across the barn fixed the absolute spatial location of each sensor and optimised the sensor network performance by increasing coverage, improving signal strength, reducing interference, and enhancing triangulation. This specific sensor system was previously evaluated on the commercial farm in Essex reporting a 50% circular error of probability (CEP) measurement of 1.07m for a static sensor and 1.90m for a sensor deployed on a standing cow (i.e., 50% of all measurements lay within 1.07m of the mean location of static sensors and within 1.90m of the mean location of cow mounted sensors) (242). Mean distance errors of 2.66m (static sensors) and 2.80m (sensors on standing cows) are reported in the same study (242).

2.3. Pre-processing and Cleaning of Positional Data

All data processing and analysis were conducted in R using Windows 3.6.3 64 bit, with RStudio.

2.3.1. Data selection

For the commercial farm in Essex, within the study period, 15 days (04/08/2014, 07/09/2014, 13/09/2014, 16/09/2014, 17/09/2014, 22/09/2014, 24/09/2014, 09/10/2014, 27/10/2014, 31/10/2014, 06/11/2014 and 22/11/2014 to 30/11/2014) were excluded from the analysis due to the system malfunctioning. Within the remaining days of the study period (days (*d*) = 99; August = 30*d*, September = 22*d*, October = 28*d*, November = 19*d*), the number of cows in the barn on a given day across the study period (not including removed days) varied from $n = 86$ to 111. A total of 88,576,716 location data points were collected from these cows (Fig 2A).

For the RVC Research farm, six days within the study period (20/10/2015, 27/10/2015, 13/11/2015, 19/11/2015, 29/11/2015 and 30/11/2015) were excluded due to the system malfunctioning. Within the remaining (*d* = 46) days of the study period the number of cows in the barn on a given day varied from $n = 22$ to 50. From these cows, 22,743,650 location data points were collected (Fig 2B).

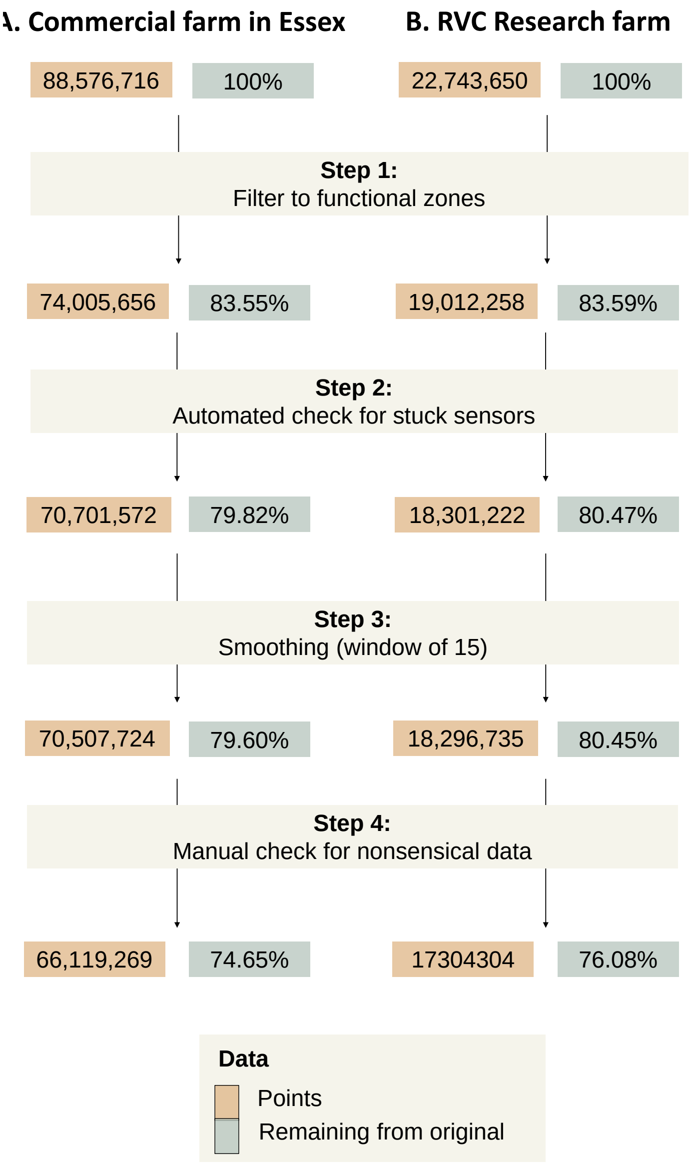

Figure 2. Overview of the data cleaning and processing steps for (A) the commercial farm in Essex and (B) the RVC Research farm. Step 1: filtering data points to those detected within the functional zones; step 2: automated check for stuck sensors (x and/or y coordinate(s) exactly the same for two or more consecutive time points); step 3: smoothing (simple moving average with a window of 15 time points); step 4: manual check for nonsensical data (cows seemingly restricted to an unrealistically small area for a full day).

2.3.2. Data cleaning and processing

Data cleaning and processing consisted of four steps, resulting in a total data removal of 25.35% for the commercial farm in Essex and 23.92% for the RVC Research farm (see flow charts in Fig 2).

2.3.2.1. Step 1: Filtering to functional zones

Data recorded outside of the defined functional zones, the feeding and non-feeding zones, including a 3m buffer region to allow for minor positional errors, were excluded from analysis. This included (correct) location data from the milking parlours and collecting yards when cows were constrained during milking periods, as well as erroneous data from non-milking periods where locations were (incorrectly) recorded more than 3m outside the barn area.

Of the total data points collected, the data located outside of the barn areas, and therefore excluded during this first step, were 16.45% (14,7571,060 data points) for the commercial farm in Essex (Step 1, Fig 2A) and 16.41% (3,731,392 data points) for the RVC Research farm (Step 1, Fig 2B).

2.3.2.2. Step 2: Automated removal of 'stuck' sensor data

An automated algorithm, created for the purposes of this study, was used to detect data where the sensor was located at exactly the same *x* and/or *y* coordinate(s) for two or more consecutive time points i.e., indicating an issue with the LPS (the commercial farm in Essex: 3.73% of data (3,304,084 data points); the RVC Research farm: 3.12% of data (711,036 data points); Step 2 in Fig 2A and Fig 2B respectively). This is the case when the system occasionally lost signal, and then its last known location is repeatedly reported. We can be certain that a functioning sensor would not report this, based on the typical known sensor error and noise, as well as the trajectories of the cows; minor variations in point location would be present. Figure 3 shows an illustrative example of working and sensors stuck at both *x* and *y* coordinates (A-B and C-D, respectively).

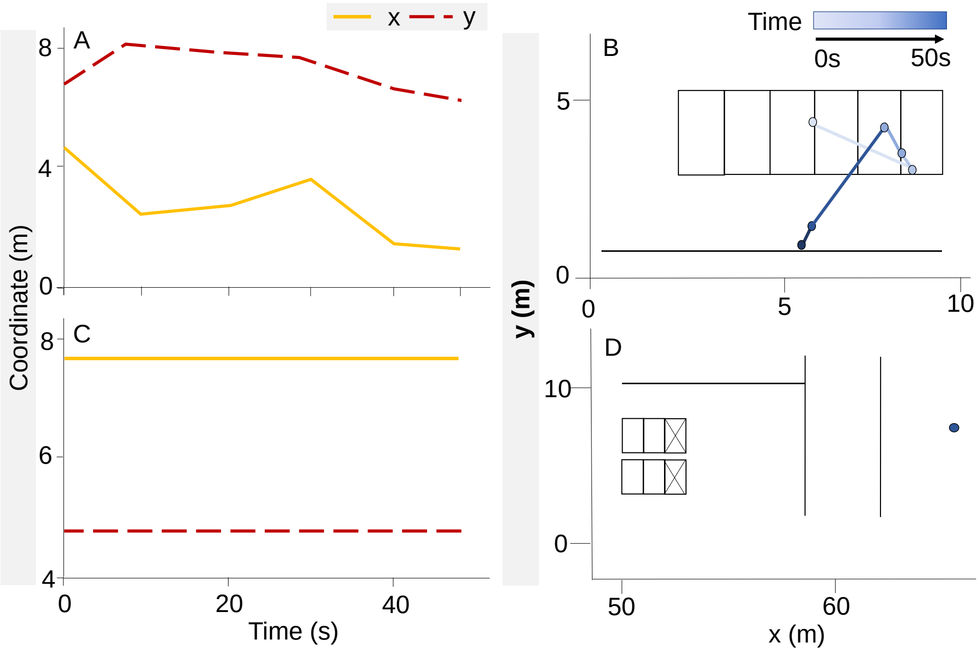

Figure 3. Examples of (A-B) sensical and (C-D) nonsensical cow trajectories recorded by a local positioning sensor on the commercial farm in Essex. (A) Coordinates of sensor ID 0002f1 (cow ID 2352) on 08/10/2014 from 05:39:53 (time = 0*s*) to 05:40:43 (time = 50*s*); (B) the same coordinates plotted as a trajectory in a zoomed-in region of the barn (refer to Fig 1). (C, D) Coordinates and trajectory (single location point) of sensor ID 0000ee (cow ID 3233) at the same time, illustrating how this sensor was apparently 'stuck' and was recording incorrect location data.

2.3.2.3. Step 3: Smoothing

A simple moving average (SMA, see Equation S1 below) was applied to the remaining data, with a two-sided window of 15 data points to smooth location outliers and to reduce instantaneous noise:

$$SMA_{15} = \frac{(x,y)_{t-7} + \ (x,y)_{t-1} + \cdots \ (x,y)_{t} + \cdots (x,y)_{t+1} \ + \ (x,y)_{t+7}}{15} \quad (\mathbf{S1})$$

where (x, y) = position and t = time.

To apply the SMA with this window size, it is necessary to remove seven data points from the start and end of the location time series for each sensor per day. In total this led to 0.22% of the data being removed for the commercial farm in Essex, and 0.04% for the RVC Research farm (Step 3 in Fig 2A and Fig 2B respectively).

Figure 4A shows an illustrative example of the raw trajectories of three cows (cow IDs = 2478, 2352 and 88), on the commercial farm in Essex, over a 5-minute period on 1/10/2014. Prior to smoothing, cow 1317 was detected to have interacted with cow 88 during this time, using a proximity threshold of 3m (Fig 4A), whereas after smoothing the cow was not detected to have interacted with any other cows during this time (Fig 4B). Cows 2352 and 88 were detected to have interacted both before and after smoothing (Fig 4A-B).

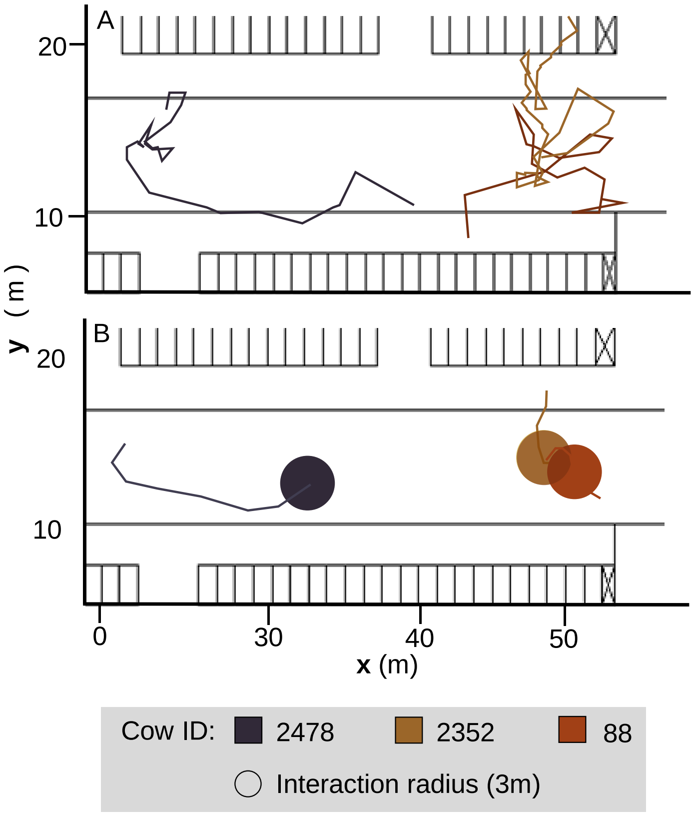

Figure 4. (A) Raw and (B) smoothed trajectory data for three cows, demonstrating how smoothing the data may have impacted the classification of an interaction, using the protocol of 3m (and 60s). The data were smoothed using a simple moving average with a window size of 15 data points. Trajectories are coloured by cow identity: 2478 = purple, 2352 = yellow and 88 = orange, on 01/10/2014 between 15:18:30 to 15:23:30 on the commercial farm in Essex. The radius required to define an interaction (3m) is shown for each cow in (B). Cow 2352 and 88 were classed as interacting, whereas cow 4 was not shown to have interacted with any other cows.

2.3.2.4. Step 4: Further nonsensical data

Periods where the trajectory of a cow was restricted to a small area for an extended time duration (but not exactly the same coordinates as in Step 2) were detected using a semi-automated algorithm created for the purposes of this study (Step 4 in Fig 2). These cases were then checked manually to determine whether the trajectory was biologically plausible, or if any farm interventions had taken place for that cow during the given time period; implausible data were removed. This resulted in the removal of 4.95% of data for the commercial farm in Essex, and 4.37% of data for the RVC Research farm (Step 4 in Fig 2A and Fig 2B respectively).

Figure 5 shows an example, which was in fact the only instance found of this type in October on the commercial farm in Essex, with sensor ID 000284 on day 05/10/2014. Visualising the trajectory of the cow deployed with this sensor (cow ID = 2585) on this day implies the cow only briefly entered the non-feeding zone, when in fact it must have spent most of the day there and was not subject to any farm management actions this day (Fig 5). Upon closer inspection, only 1101 data points were recorded for this cow in the non-feeding zone on this day, and a total of 2187 data points throughout the day, whereas the average points collected across all cows in the non-feeding zone alone was 6273 in October (*n* = 91). The data for cow ID 2585 on this day were therefore removed from the analysis (Fig 5).

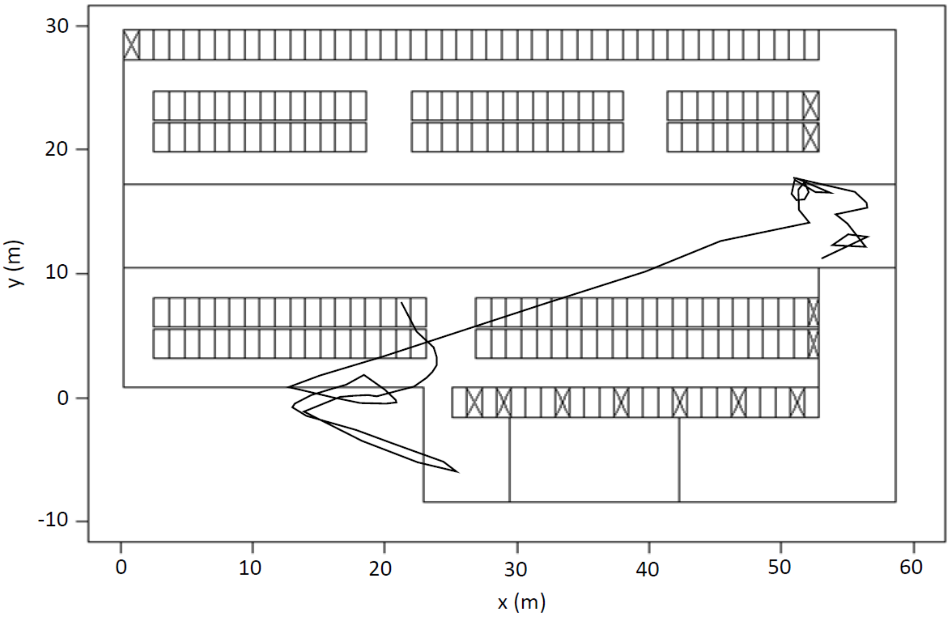

Figure 5. Trajectory plot showing data removed during step 4 of data cleaning for the commercial farm in Essex, removal of nonsensical data based on visualisation of unrealistic movement. Sensor ID 000284 (cow ID 2585) on 05/10/2014 was only shown to move within a very small area throughout the entire day.

2.4. Social network analysis

2.4.1. Protocol for determining proximity interactions

Using the smoothed and cleaned positional data, an interaction was defined between dyads (each pair of cows) using a protocol based on sustained proximity (radial metric distance) over a specified time period and was hence non-directed (if cow A is close to cow B, then B is close to A, and so on).

Positional data within the barn were filtered by coordinate into functional zones: the ‘feeding zone’ (defined as the feeding passage and nearest passageway; in the range of $10.5\text{m} \leq y \leq 17.2\text{m}$ for the commercial farm in Essex and $0\text{m} \leq y \leq 9.94\text{m}$ for the RVC Research farm in Figure 1), the ‘non-feeding zone’ (cubicles and passageways; $1.62\text{m} \leq y \leq 10.5\text{m}$, $-1.6\text{m} \leq x \leq 58.6\text{m}$ for the commercial farm in Essex and $0\text{m} \leq y \geq 9.94\text{m}$ for the RVC Research farm in Figure 1) and the ‘full barn’ (the combined feeding and non-feeding areas); a buffer of 3m was used around each zone. The proximity protocol defining an interaction described above was subsequently applied to the data for every given dyad located in each functional zone, outputting the total number of interactions over the course of each day. A non-directed matrix, weighted by the frequency of proximity interactions, for every given day (the commercial farm in Essex: $d = 99$, the RVC Research farm: $d = 46$) and functional zone was produced, holding the number of interactions recorded for every possible dyad (e.g., if the number of cows of a given day was 92, the matrix would have dimensions of 92 x 92). The matrices were therefore symmetrical, with ‘NA’ inputted along the diagonals of each.

Below it is explained how and why a ‘strict’ protocol was selected for identifying proximity interactions.

2.4.1.1. Validation

To validate the algorithm for identifying and classifying proximity interactions, an observer (HH) recorded (*n* = 35) verified instances of cows lying in adjacent cubicles within a localized area of the commercial farm in Essex barn (non-feeding zone) on five different dates during the main study period: 08/08/2014, 22/08/2014, 23/09/2014, 21/10/2014 and 18/11/2014. Each of these verified proximity interactions lasted on average ten minutes. A one-minute buffer was added around the start and end times of each observation (in case of any minor time-sync issues between observer and sensor clocks) before comparing them to proximity interactions detected from the sensor-collected location data using the automated algorithm.

The algorithm uses a 'strict' protocol to identify proximity interactions based on pairs of individual cows being within a specified radius (spatial threshold, *r*) for <u>all</u> time points within a sustained time period (duration, *t*). To test the sensitivity of the algorithm to the (arbitrary) choice of parameters, the classification performance was considered over a range of spatial thresholds (*r* = 1-5m) and time durations (*t* = 20-160s; 2-16 time points at 0.1Hz). Each of the verified observed proximity interactions were either marked as a true positive (TP), where the algorithm reported a proximity interaction coinciding with an observation, or a false negative (FN) where the algorithm did not detect a proximity interaction when one had been observed. The sensitivity, or true positive rate (TPR), was then defined as TPR = TP / (TP + FN). Note that the nature of the observation process (identifying those cows lying in adjacent cubicles such that proximity interactions could be verified) meant that observations where cows were not interacting were not recorded; hence it was not possible to estimate the true negative (TN) or false positive (FP) rates, or the associated specificity (true negative rate, TNR).

Table 1 provides the sensitivity results for the proximity identification algorithm based on the 'strict' protocol described above. As might be expected, using a higher spatial threshold, *r*, and a lower time duration, *t*, increased the sensitivity, up to 0.97 (5m and 20-100s), while using a very small spatial threshold (1m) and a longer time duration (160s) reduced sensitivity to as low as 0.03. Notably, according to Table 1, the sensitivity of detecting proximity interactions using a 3m threshold does not change significantly between a 60s and an 80s duration (both are at 83%). However, using an 80s duration may result in missing some social interactions that lasted between 60s and 80s. This could potentially lead to an underestimation of the frequency of interactions between cows, some of which may be of particular importance.

The stringency of the algorithm can be reduced by identifying a proximity interaction when only a certain percentage of points within the time period, *t*, are within the specified spatial threshold, *r*. For example, classifying an interaction as when sensors are within a 3m proximity for at least 50% of the time points within an 80s window (eight time points) increased the sensitivity to 0.89 (from 0.83 with the strict protocol) (Table 1). However, reducing the stringency of the algorithm would have almost certainly increased the false positive rate, while offering only minimal increases in sensitivity, based on the validation results in Table 1.

Table 1. Sensitivity of proximity interaction algorithm based on verified observations on the commercial farm in Essex across five days in August-November 2014 (*n* = 35, average duration of ten minutes), using different combinations of spatial thresholds (*r* = 1-5m) and time durations (*t* = 20-160s). The sensitivity is calculated using the equation: true positives/ (true positives + false negatives)). In all these cases, a ‘strict’ identification protocol was used: the distance between cows must be less than the spatial threshold, *r*, for all points throughout the time period, *t*, in order for a proximity interaction to be identified. The protocol based on a spatial threshold of *r* = 3m and time duration of *t* = 60s (indicated in bold) was chosen for use in the algorithm based on acceptable sensitivity (true positive rate = 0.83), expected lower rate of false positives, and to fit with practical considerations such as the size of an individual cow, the sensor location error, and the typical minimum duration of important social interactions.

		Time (s)							
		20	40	**60**	80	100	120	140	160
Radius (m)	1	0.46	0.31	0.29	0.17	0.14	0.09	0.06	0.03
	2	0.71	0.66	0.63	0.63	0.49	0.46	0.37	0.23
	3	0.86	0.86	**0.83**	0.83	0.74	0.71	0.69	0.53
	4	0.91	0.91	0.89	0.86	0.86	0.80	0.77	0.74
	5	0.97	0.97	0.97	0.97	0.97	0.91	0.86	0.86

2.4.1.2. Parameter choice

Ideally, the chosen parameters for the algorithm would have maximized both the sensitivity and specificity. However, as it was not possible to estimate specificity, parameters were selected based on several considerations. Basic logic dictates that using a larger spatial threshold or reducing the time duration would have increased false positives, where cows were a larger distance apart or only briefly passing each other are classified as interacting. Conversely, Table 1 illustrates how using a very small spatial threshold, or too long a time duration, significantly reduced the sensitivity of the algorithm. In addition, there are practical considerations to take into account: the location sensors have previously been reported to have a mean error distance of 2.66m in this barn environment (242,252); the typical body length of a dairy cow is 2.3-2.6m; the main interest was in sustained proximity interactions that were not simply incidental ‘passing’ interactions. Hence, to balance the validated sensitivity, expectations about how specificity would relate to parameter choice, and the practical considerations mentioned, a protocol was adopted based on a spatial threshold of $r = 3$m and a time duration of $t = 60$s. Longer time durations were counted as more than one proximity interaction i.e., 60*s* = one interaction, 80*s* = two interactions, 100*s* = three interactions and so forth.

The choice of time duration compares to other recent studies on dairy cow social interactions that use thresholds ranging from 2s (271) to 10 minutes (272), and is similar to a study where a minimum of 6 time points within a window of 12 consecutive time points [0.1 Hz] was used (alongside a spatial threshold of two metres) (273). Previous studies have also used similar spatial thresholds to detect cattle interactions, ranging from $r = 1.5$ to 4m (176,271–273).

To further test the logic behind the choice of parameters, the algorithm was run over a range of spatial thresholds and time durations with the full herd (all dyad parings) for individual days, within the study period on the commercial farm in Essex. Fig 6A illustrates how using a short time duration dramatically increased the number of proximity interactions (92664 interactions at 20s, compared to 58499 interactions at 60s, or 37628 interactions at 160s). Fig 6B illustrates how increasing the spatial threshold resulted in an increased number of proximity interactions (214,813 interactions at 5m, compared to 76,330 interactions at 3m, and 5,393 interactions at 1m).

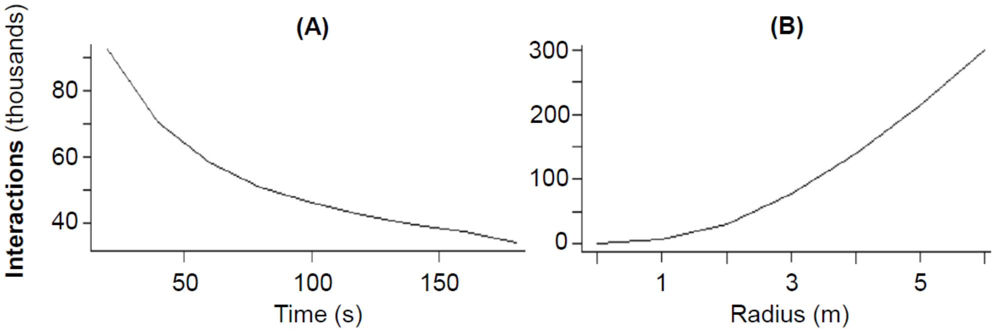

Figure 6. Interactions identified between all cow dyads (*n* = 4186) on the commercial farm in Essex on an example day, 01/10/2014, for different time durations and spatial threshold radii (B; *r* = 0-6m). Where *t* = 20s-180s, this corresponds to 2-18 time points recorded at 0.1Hz, and results are shown in increments of 20s or two time points. Increments of one metre are used for the spatial threshold radii. Note that results are very similar for other days.

2.4.1.3. Additional results for different spatial thresholds

To fully explore the effect of the spatial threshold radius, *r*, and time duration threshold, *t*, additional full network analyses were run, for the data collected on the commercial farm in Essex, using one, two, four and five metres for a fixed time duration of $t = 60$s, and using 40, 80 and 100s for a fixed radius of $r = 3$m. Qualitatively similar results were obtained, demonstrating that the overall results and conclusions are robust to this parameter choice (see Chapter 4, Appendix 1, Tables A1 to A7).

2.5. Network visualisation

The interaction matrices for each day and each farm, for the full barn, and each functional zone, were converted into network graphs, using the package 'igraph' (274) in R (274–276), where nodes represent individuals, and edges represent interactions between dyads, with increasing weight (more interactions) indicated by increasing width of the edges. The Fruchterman-Reingold layout algorithm was used to determine the node positions; connected nodes are pulled towards each other and unconnected vertices are repelled.

2.6. Social Network Analysis

The edge density, the proportion of direct ties in a network relative to the total ties possible, was calculated for the full barn and functional zones (feeding and non-feeding zone) on each farm. Cows periodically entered and left the feeding zones, so edge density was expected to be lower in these zones, in comparison to the non-feeding zones.

Permutations are used to test the normality of observed network data and are essentially a form of null model (277,278). A widely used method to account for the non-independence of dyads in SNA is by using a node-level permutation (277,278). Node identities are randomised, and the original test statistic is compared against permuted test statistics. Here node-level permutations are implemented to test the hypotheses by randomizing the identity of cows:

Equation 1. $$p\text{-value} = \frac{\Sigma\left(t_p \geq t_o\right)}{q} \quad (\mathbf{1})$$

, where t_p and t_o are the permutated and original test statistics respectively, and q is the number of permutations (in this case, 10,000).

The test statistic comparing a given measure, i.e., differences in daily interactions between lameness states etc., was calculated for each permutation (t_p). If the proportion of permuted test statistics equal to or more than the original test statistic (t_o), was ≥ 5% ($p \geq 0.05$) (see Equation 1), then the null hypothesis was accepted i.e., there was no significant difference in the measure between the groups. A Bonferroni correction was applied to the p-value to account for multiple comparisons on the same dataset. As computing an exact p-value is not possible with a finite number of permutations, if the p-value was calculated to be zero a biased estimator was applied: one was added to both the numerator and denominator of Equation 1, following the suggestion in (279).

2.6.1. Social differentiation

As the residual data on daily interactions were found to be not normally distributed (Shapiro-Wilk normality test; the commercial farm in Essex: W = 1.00, 1.00, 0.98, $p = 0.04$, < 0.01 and < 0.001, for the full barn, feeding zone and non-feeding zone, respectively and the RVC Research farm: W = 0.99, 0.96, 0.94, $p < 0.001$, < 0.001 and $p < 0.001$, for the full barn, feeding zone and non-feeding zone, respectively), a Kruskal-Wallis Rank Sum test was conducted to assess whether there is a significant difference in the median daily interactions individuals had, with 10,000 node-level permutations to account for non-independence of dyads.

The interactions between each dyad may be uniformly distributed across an interaction matrix for a given day, or specific dyads may interact more or less than other dyads. The structure of a network can be assessed by comparing the number of observed interactions between every given dyad with the number of expected interactions between every dyad. To assess whether associations between individuals were more heterogeneous than we would expect given a null hypothesis that all dyads associate uniformly, the following statistic for social differentiation (*S*) was calculated (see Equation 2) based on (280), Appendix 9.4, and following (176):

Equation 2. $$\boldsymbol{S = \frac{\sum_i^n \sum_j^n (O_{ij} - E_{ij})^2}{n(n-1)}} \quad \textbf{(2)}$$

, where *O* and *E* are the observed and expected number of interactions, respectively, *i* and *j* are two given individuals, and *n* is the number of individuals.

As shown in Equation 2, the difference between the observed number of interactions and the expected number of interactions was summed for each dyad, and then divided by the total number of dyads, for each day (e.g., if the number of individuals was 92, $n = 4186$ [= ((92 x 91) /2)]).

2.7. Bunching metrics

There is no formal definition of 'bunching' behaviour in the context of PLF, although a range of different metrics are available to measure spatial aggregation, spatial clustering, or social proximity from positional data. Most previous papers have used observations or basic nearest neighbour aggregation indices to measure bunching, the latter of which would provide a distorted score if all cows are in pairs (281–284). Here, a range of bunching metrics are used to obtain a better understanding of this behaviour. Hence, three complementary bunching metrics are considered, each measured on an hourly basis across the full herd: range size (core and full), mean inter-cow distance (ICD), and mean nearest-neighbour distance (NND).

2.7.1. Range size

Range was calculated by overlaying a virtual grid (1.5m by 1.5m) over the barn maps. For each herd, at each timestep, location data were used to assign each individual within the herd to a given cell (or rejected if it lay outside the main barn region, see step one of pre-processing in Section 2.4.2.1). The number of individuals assigned to each cell across the full herd and over a full hour (360-time steps at 0.1 Hz) was counted giving a final hourly total for each virtual cell within each barn. Following the standard methodology outlined in (75), the areas where the cows spent most time were identified by selecting the highest density cells that cumulatively add to either 50% or 95% of the total observed density. These high-density cells were then used to create an hourly utility distribution for each farm, showing the relative amount of space used by the herd over time. The utility distribution distinguishes between the 'core range' (50%) and 'full range' (95%) of the herd's spatial usage. The number of unique cells included in each hourly core range and full range distribution are then defined as the range size (CR and FR respectively, measured in units of 1.5m x 1.5m cells).

2.7.2. Inter-cow distance

At a given time-step, the herd mean inter-cow distance is the mean distance between each of the possible dyad pairs, $(n^2 - n)/2$, across a herd with *n* individuals. An hourly value (defined as ICD, measured in metres) is then calculated as the arithmetic mean of all (360) values recorded over that hour. The equation for this is given below (Equation 3).

Equation 3. $$ICD = \frac{1}{360}\sum_{t=1}^{360}\left(\frac{2}{n^2-n}\sum_{(i,j)\in n}\sqrt{(x_i(t) - x_j(t))^2 + (y_i(t) - y_j(t))^2}\right),$$

where (*i* ,*j*) is a given dyad pair and $i \neq j$, and *n* is the number of individuals.

2.7.3. Nearest neighbour distance

At a given time-step, the nearest neighbour distance for a given individual is simply the distance to its closest neighbour in the herd (i.e., the smallest inter-cow distance when considering all dyad pairs involving that individual). A mean value can then be determined across the full herd on an hourly basis (defined as NND, measured in metres). The equation for this is given below (Equation 4).

Equation 4. $$NND = \frac{1}{360}\sum_{t=1}^{360}\left(\frac{1}{n}\sum_{i\in n} min_{j\neq i}\sqrt{(x_i(t) - x_j(t))^2 + (y_i(t) - y_j(t))^2}\right), \quad (2)$$

where *j* indexes all possible dyad pairs for *i* across the herd, and *n* is the number of individuals.

2.7.4. Justification for the use of various bunching metrics

Although the three bunching metrics defined above measure different aspects of space-use and social proximity, they will all decrease as spatial clustering ('bunching') increases. Range size is entirely independent to ICD and NND, but as it is aggregated at an hourly level from the outset, it will not capture short-time variations in proximity between individuals. Nevertheless, a smaller (core or full) range size indicates that the herd as a whole are using less of the barn and are sharing a smaller space. NND and ICD are loosely dependent but capture different aspects of herd bunching. ICD measures distances across the full herd and hence can be skewed by individuals that are far apart or in separate subgroups (e.g., a herd split into two distinct and tightly bunched subgroups could still have a high ICD value, even though they would be considered to be bunching). Meanwhile, NND only accounts for the distance between animals nearest each other and hence may not capture bunching between multiple individuals (e.g., a herd split into spatially separated dyad pairs could have a very low NND, but would have high ICD, and would not usually be considered as herd-level bunching). By measuring the above three metrics, it is aimed to accurately capture a wide range of herd dynamics that may be indicative of bunching behaviour.

3. CHAPTER 3: PROXIMITY NETWORK STRUCTURE OF A HOUSED DAIRY HERD

The work in this chapter has been published in a journal publication: (285)**.**

3.1. Introduction

The herd social structure of cows on most commercial dairy farms differs significantly from their wild counterparts (138). Dairy cows are typically kept in exclusively female groups, separated by age and reproductive status, with access to a more restricted space allowance in the form of either indoor housing or fenced grazing paddocks and may be subject to frequent regrouping events (139,140,142,144). Understanding the structure and dynamics of housed dairy cattle networks may provide insights on preferential interactions and aid in optimising their management (146,147).

The social structure of animal groups, including how associations and interactions between individuals change over time, can be assessed using social network analysis (SNA) (165). The approach is well established; SNA is used across multiple disciplines including sociology (286), computer science (287) and transport (287,288), and has been developed to study animal social networks, particularly over the last decade (289,290). The simplest interaction networks can be developed by assuming interactions occur when two individuals are within a given proximity, usually based on metric distance, for a specified time duration (132,150,251,258). More advanced methods such as analysis based on topological distances, which take into account an individual’s neighbours and their neighbours (291), as well as more complex analyses of interactions considering social dominance are also possible (292).

SNA has been used to explore interactions in dairy cattle, revealing highly clustered herds (166,176,273). Cows appear to associate non-randomly based on attributes; studies show that cows tend to associate more frequently with other cows at similar lactation stages (166,176). Dairy cattle exhibit inter-individual variation in sociality, which may be influenced by dominance or personality traits, which are established as consistent from calf to adulthood (except during puberty) (293). Housed cattle are known to avoid interactions with dominant conspecifics whilst feeding to reduce competition (19), and the social positioning of individuals may also be altered where a resource is deemed more valuable (266). Individual attributes are thought to be important in disease transmission (147), as cows participate in contact behaviours based on age and sex. Dairy cows may groom conspecifics based on familiarity and dominance (162), although affiliative and agonistic interaction networks may not be correlated (294).

Precision Livestock Farming (PLF) techniques provide opportunities to support rapid identification of lameness and other production diseases. Sick cows are less likely to approach humans (259,260), and both cows and calves have been observed to alter their positioning in a herd when ill (261–263). Evidence suggests cows with ketosis and mastitis displace conspecifics less frequently (295–297). Lame cows may alter their time budgets with lame individuals spending less time feeding than their healthy counterparts (242,252). Lame cows also appear to be licked by conspecifics more than non-lame cows (209). Despite this existing evidence, to my knowledge automated PLF techniques have not been applied to monitor changes in social behaviour in cattle that could be associated with disease.

In this study, the structure and consistency of the proximity interaction network of the herd housed on a commercial farm in Essex is investigated, using positional data collected from an automated local positioning system (LPS). The level of inter-individual variation in proximity interactions is determined across different functional zones of the barn (feeding, non-feeding) and it is assessed how these interactions vary during the month-long study period. The influence of health status (specifically lameness), parity, and days in milk (DIM), on the sociality and interactions of individuals within the herd are also considered.

3.2. Methodology

3.2.1. Animals and housing

A Holstein-Friesian high-yielding dairy cow herd was monitored continuously in a closed free-stall barn on a commercial farm in Essex, from 1st October 2014 to 31st October 2014. The study group consisted of 92 cows that were continuously present in the barn throughout the study duration. These cows formed part of a larger group (*n* = 100 to 111 on any given day in the month). Refer to Chapter 2 (Section 2.4) for further details on the herd and their management.

3.2.2. Local positioning system and data pre-processing

Each cow was equipped with a combined local positioning sensor and accelerometer, Omnisense 500 (298), mounted on a weighted neck collar. Refer to Chapter 2, Section 2.5 for further information on the sensor system.

Data selection and pre-processing was conducted as outlined in Chapter 2, Section 2.4. For the main analysis, fixed interval periods when most cows were in the milking parlour or collecting yard were excluded (05:00-07:59, 12:00-14:59, 20:00-22:59), since their behaviour during these time periods was constrained by farm staff. The sensor system reset at midnight each day and hence times between 23:00 and 00:59 were also excluded.

3.2.3. Protocol for proximity interactions

The protocol for determining proximity interactions, defined as when dyads within 3m for a minimum of 60s, with longer time durations counting as more than one interaction, is outlined in detail in Chapter 2, Section 2.4.

While this parameter choice is consistent with previous studies e.g., (176,273), a range of other parameter values for r and t are also considered, as well as less stringent protocols (where only a certain percentage of points within the specified time period need to be within the radius for an interaction to be identified). Using observed data of ($n = 35$) known proximity interactions it was possible to validate the algorithm and determine the sensitivity (true positive rate) of this protocol (0.83); it was not possible to estimate the specificity using this observed data, but the r and t parameters were chosen to reduce the expected false positive rate, as well as taking into account practical and biological considerations, including the sensor mean error distance and the typical size of a dairy cow (see Chapter 2, Section 2.5). Also, qualitatively similar results were obtained when using t = 40, 80, 100s (for r = 3m) and r = 1, 2, 4 and 5m (for t = 60s), and hence the conclusions should be robust to this selected parameter values of r = 3m and t = 60s (see Appendix 1).

3.2.4. Social network analysis

An explanation of how daily networks were visualised, for the full barn, the feeding zone (FZ) and the non-feeding zone (NFZ), is provided in Chapter 2 (Section 2.5.2).

Edge density, the proportion of direct ties in a network relative to total ties possible, was calculated for the full barn and each of the functional zones (as outlined in Chapter 2, Section 2.6). Permutations (*q* = 10,000) were used to account for the non-independence of dyads, and details of this are provided in Chapter 2, Section 2.6.

3.2.4.1. Social differentiation

Inter-individual variation in median daily interactions (the median number of proximity interactions an individual had per day) across the study duration was assessed through a Kruskal-Wallis Rank Sum test, using 10,000 node-level permutations to account for the non-independence of dyads. Social differentiation was calculated daily for each functional zone (FZ and NFZ), by finding the difference between the observed number of interactions and the expected number of interactions, summing this for each dyad, and then dividing this by the total number of dyads (refer to Chapter 2.5.4 for details).

3.2.4.2. Temporal variation in sociality

A Kruskal-Wallis Rank Sum test was conducted to assess whether there was a significant difference in median daily interactions between days, for each functional zone, with 10,000 permutations. Pearson's correlation was used to test if temporal variations in daily interactions were correlated across time in each functional zone, and then with mean daily temperature.

To assess whether the network structure was stable or varied over time, seven interaction matrices were created, each holding the average number of interactions between dyads (n = 4186) over four consecutive days. Each consecutive network was compared by conducting a Mantel Test (165,299). The 'mantel' function was used, from the 'vegan' package in R (300). The distribution of proximity interaction data within the daily network matrices were not normally distributed, shown through one-sample Kolmogorov-Smirnov tests. To investigate the degree of association between consecutive averaged matrices, a Mantel statistic Z was therefore calculated using a Spearman's Rank Sum test, with 10,000 permutations and Bonferroni correction to account for multiple comparisons. A similar analysis using shorter- and longer-day partitions was also completed, and results were found to be qualitatively similar (Table 2).

3.2.4.3. Impact of lameness status, parity, and days in milk on sociality

3.2.4.3.1. Lameness state

Cows were assigned a mobility score fortnightly as they exited the parlour (30/9/2014, 13/10/2014, and 27/10/2014) using the AHDB mobility score (301). A mobility score of zero to three was assigned, where zero is good mobility, one is imperfect mobility, two is impaired mobility and three is severely impaired mobility. If a score was not recorded, 'NS' was noted. All cows in each session were scored by ZB who had scored approximately 70,000 cows over ten years before this study. The average observer reliability, score blind zero to three by ZB using AHDB dairy standardized videos (301), was 80% for two sessions (19/05/2013 (*n* = 10) and 18/02/2015 (*n* = 10)).

For this study, cows with scores two or three were considered as clinically lame (L) and cows with scores zero or one were considered non-lame (NL). Cows scored as non-lame for two successive scoring sessions (NL-NL-L or L-NL-NL) were classed as ‘dominant not lame’ (DNL), and cows scored as lame for most sessions (L-L-NL or NL-L-L) were classed as ‘dominant lame’ (DL). Cows that apparently changed status twice within the study (NL-L-NL or L-NL-L), or those with missing data (no recordings in one or more of the sessions, typically due to the individual not being sighted) were not included in the lameness classification, see Table 1.

Table 1. Classified lameness status of (*n* = 92) dairy cows within the study.

Lameness status	**Frequency (number of cows)**
Non-lame (NL)	15
Lame (L)	12
Dominant non-lame (DNL)	11
Dominant lame (DL)	10
Multiple change of status	14
No / missing data	30
Total	92

The behaviour of cows in the L and DL groups were compared, as was the behaviour of the L and DL groups (using the chosen protocol to define an interaction, as when cows were within a 3m radius for at least 60s [recorded at 0.1Hz]) (as defined in Chapter 2, Section 2.4) to determine whether the groups could be reasonably combined.

In the feeding zone, there were significant differences in mean daily interactions (the mean number of proximity interactions an individual had per day), between DNL and NL cows (Wilcox test statistic [hereafter W] = 89, after 10000 permutations, $p < 0.001$), and between DL and L cows (W = 69, after 10,000 permutations $p < 0.001$), see Figure 1A. In the non-feeding zone, there were no significant differences in mean daily interactions, between DNL and NL cows (W = 89, after 10,000 permutations, $p = 0.03$) or between DL and L cows (W = 51.5, after 10000 permutations, $p = 0.73$), see Figure 1B.

In the feeding zone, there were no significant differences in the node-level measures mean degree (the average number of connections each cow in the network has) or mean clustering coefficient (a measure of the extent to which cows in the network tend to cluster) between NL cows and DNL cows (respectively W = 101.5, $p = 0.20$; W = 111, $p = 0.10$; 10,000 permutations; Figure 1C and 1E), or between L and DL cows (respectively W = 59, $p = 0.56$; W = 61, $p = 0.50$; 10,000 permutations; Figure 1C and 1E). In the non-feeding zone, there were no significant differences in mean degree or clustering coefficient between NL and DNL cows (respectively W = 70.5, $p = 0.77$; W = 69, p = 0.79; 10,000 permutations; Figure 1D and 1F), or between L and DL cows (respectively W = 62, $p = 0.46$; W = 68, $p = 0.32$; 10,000 permutations; Figure 1D and 1F).

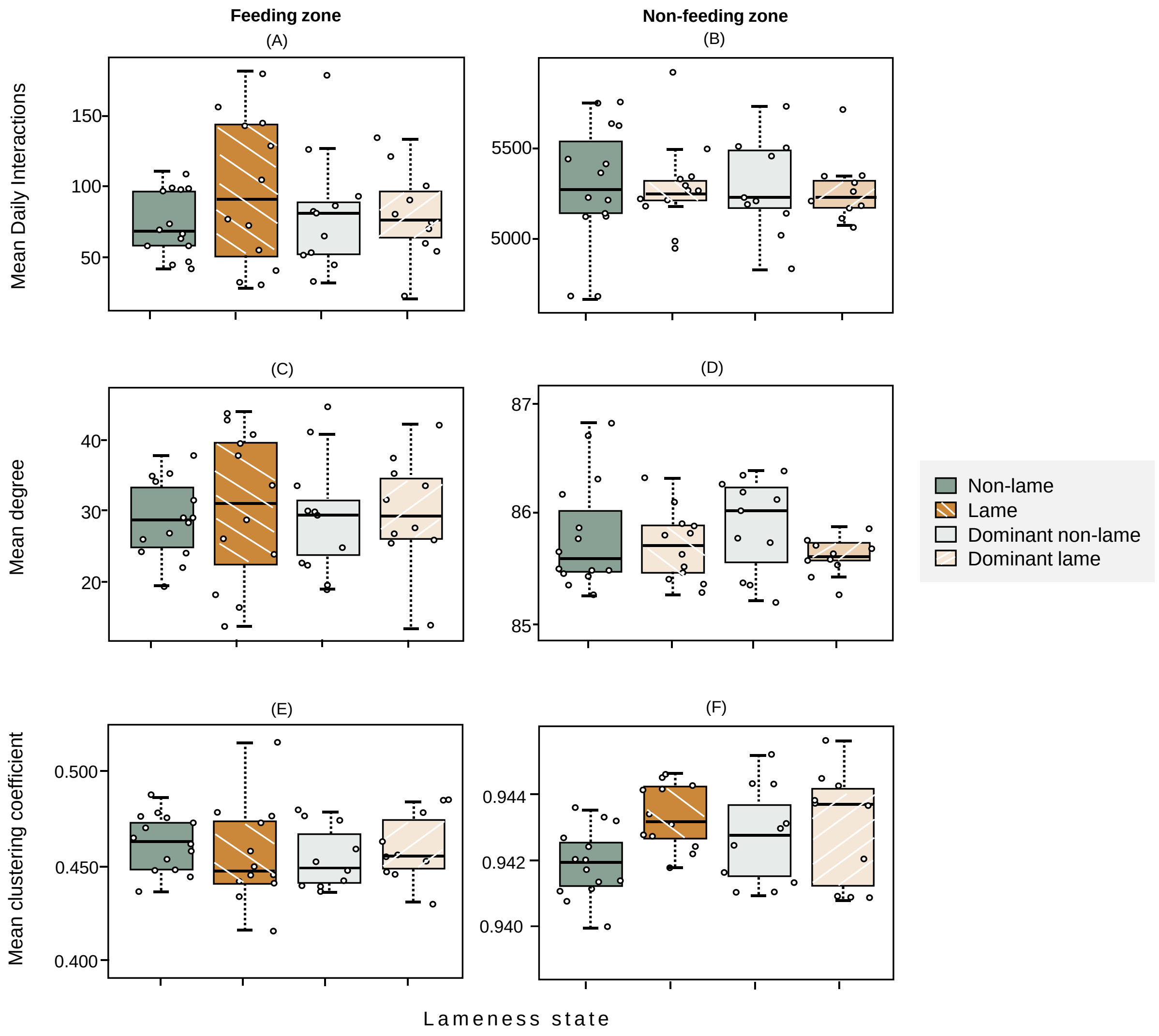

Figure 1. (A) Mean daily interactions and (B-C) mean node-level measures (degree and clustering coefficient), compared between lameness states in each functional zone on the commercial farm in Essex. Lameness status is defined as either: non-lame (NL), lame (L), dominant non-lame (DNL), and dominant lame (DL) cows (n = 48). Plots corresponding to the feeding zone are (A, C, E) and for the non-feeding are (B, D, F).

As the results are qualitatively similar if the groups are not combined and the four separate classifications for lameness are kept, for the purposes of the main analysis presented in this chapter, ‘lame’ and ‘dominant lame’ cows are combined into a single group (‘lame’), and similarly ‘non-lame’ and ‘dominant non-lame’ cows are combined into a single group (‘non-lame’). In total, 48 of the 92 cows within the study group were classified as either ‘lame’ (22 cows) or ‘non-lame’ (26 cows) using this approach and were included in the part of the analysis focusing on lameness differences.

The mean daily interactions between non-lame ($n = 26$) and lame ($n = 22$) cows were compared using a two-tailed Wilcoxon test, with 10,000 permutations. Node degree (the number of immediate neighbours each node in the network has) was also compared between non-lame and lame cows. As a cumulative measure, node degree is less prone to sampling error, such as temporal loss of signal of the sensor system, than other measures such as betweenness (the number of shortest paths that pass through a given node), which can change dramatically with removed or missing data (302), so mean node degree was compared between non-lame and lame cows. Local clustering coefficient, which measures the degree of clustering of nodes in a graph by calculating the ratio of a node’s connections with its neighbours to the maximum possible number of such connections (303), was calculated between non-lame and lame cows. The mean node-level measures, calculated for each individual over the full study period, were compared between lameness states using two-tailed Wilcoxon tests with 10,000 permutations (Shapiro-Wilk normality test, $p < 0.01$).

A matrix was created, showing the absolute differences in lameness between all dyads ($n = 1128$), as in (273) (e.g. if cow A was lame, a score of 1 was assigned, and cow B was not lame, a score of 0 was assigned, and their absolute difference would be 1). The absolute difference matrix was compared to the original interaction matrix for every given day, using a Mantel test again with Spearman's Rank Correlation Coefficient (as described in Section 2.6.3). Bonferroni correction was applied to account for multiple comparisons (*n* = 28).

3.2.4.3.2. Parity and days in milk

To assess whether parity and days in milk (DIM) affected social assortment, a matrix was created, showing the absolute differences in parity between all dyads (*n* = 4186), as in (273) (e.g. if cow A had a parity of 1, and cow B had a parity of 3, their absolute difference would be 2). An absolute difference matrix for days in milk (DIM) was also created. The absolute difference matrix for a given attribute was compared to the original matrix for every given day, using a Mantel test (as described in Section 2.6.3).

3.3. Results

3.3.1. Basic network measures and visualization

Figure 2 compares visualisations of the original and mean node degree filtered networks (nodes with a degree less than or equal to the mean node degree were excluded) for the full barn, the FZ and the NFZ. The full barn network was more connected than the NFZ network (0.02 difference in edge density) and the FZ network (0.63 difference in edge density; Figure 2; Table 3). This is expected since the full barn network contains more interactions i.e., those occurring both in the FZ and NFZ. A key notable difference is that the NFZ network was more connected than the FZ network (0.31 difference in edge density) (Figure 2; Table 3).

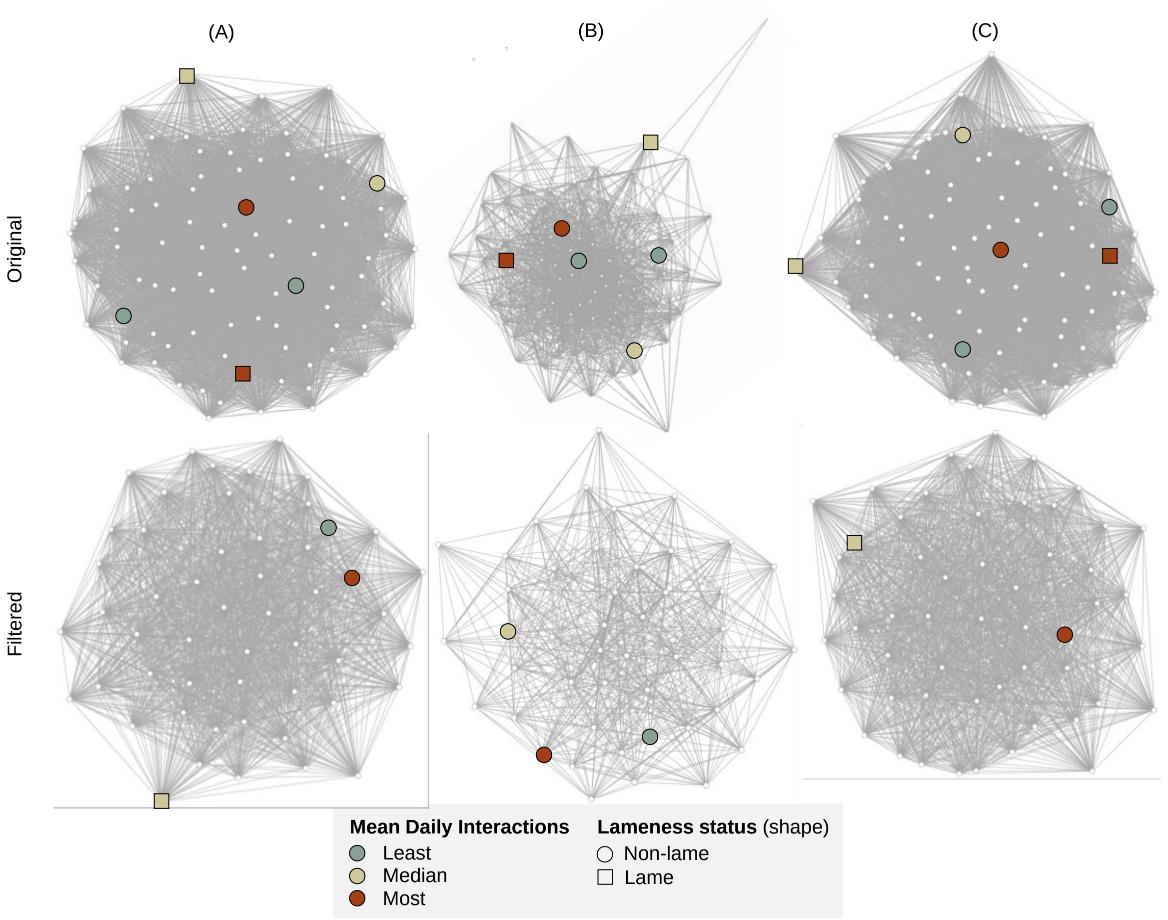

Figure 2. Undirected original and filtered (by mean degree) networks on a randomly chosen day, 01/10/2014, in (A) the full barn, (B) feeding zone, and (C) non-feeding zone of the commercial farm in Essex, showing mean daily interactions between cows (*n* = 92 in original networks). Thicker edges indicate a higher number of daily interactions. The Fruchterman-Reingold layout algorithm was used to determine the node positions; unconnected vertices are repelled. The highlighted illustrative subset of cows corresponds respectively to the least (blue, cow ID = 3124 and 3317), median (green, cow ID = 3132 and 635), and most (red, cow ID = 2273 and 2266) mean daily interactions, with squared nodes representing lame cows. A clearer network structure is shown after filtering (where nodes with a degree less than or equal to the mean node degree were excluded), with a more uniform distribution of interactions in the main barn and the non-feeding zone in comparison to the feeding zone. Created in RStudio using the ‘vegan’ package (275,276,300).

The full barn and NFZ networks remained as one component, whereas in the FZ network one to three individuals isolated from the main component on each day (Table 3).

3.3.2. Inter-individual variation

Throughout the following analysis and presentation of results, a subset of individuals at the middle and extreme ends of the data are highlighted to aid interpretation and to illustrate the extent of the observed data: two cows with the lowest mean daily interactions over the full study period (cow ID = 3324 and 3317 with mean daily interactions of 1955 and 1956, respectively), two cows with mean daily interactions closest to the median (cow ID = 2602 and 3132, with mean daily interactions of 2084 and 2085, respectively), and two cows with the highest mean daily interactions (cow ID = 635 and 3361, with mean daily interactions of 2266 and 2273, respectively); across the full herd the mean daily interactions were 2093 (median = 2085, standard deviation = 76.63).

There was significant inter-individual variation in daily interactions in the NFZ (Kruskal-Wallis chi-squared [hereafter K-W] = 19.21, df = 91, after 10,000 permutations, $p < 0.01$), but not in the full barn (K-W = 26.53, df = 91, after 10,000 permutations, $p < 0.001$) or the FZ (K-W = 851.71, df = 91, after 10,000 permutations, $p = 1$).

Figure 3 illustrates the lack of inter-individual variation in daily interactions in the full barn and the FZ, and the greater inter-individual variation in daily interactions in the NFZ for the highlighted subset of individuals.

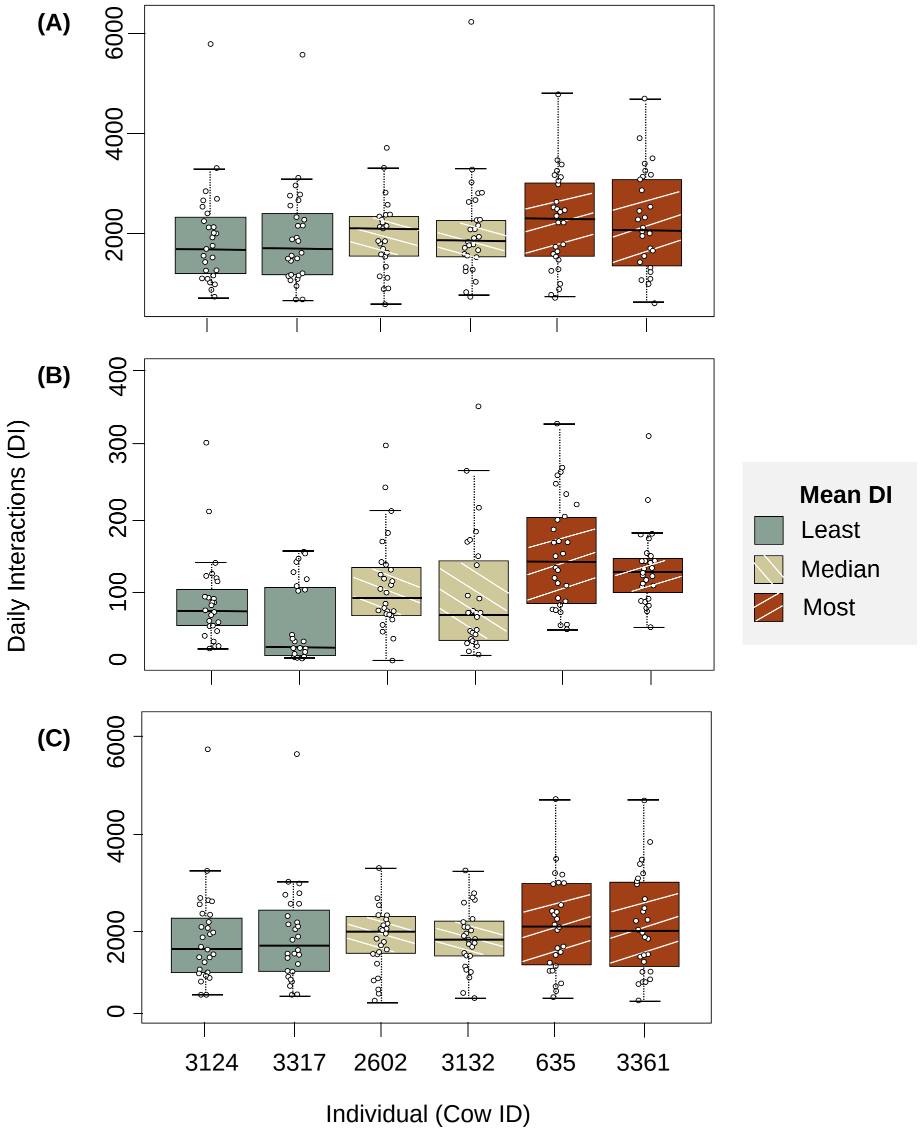

Figure 3. Daily interactions in (A) the full barn, (B) feeding zone, and (C) non-feeding zone of the commercial farm in Essex, for a highlighted illustrative subset of individuals. Individuals selected are two cows with the least mean daily interactions (blue, cow ID = 3124 and 3317), two cows with mean daily interactions closest to the median value (green, cow ID = 2602 and 3132) and two cows with the highest mean daily interactions (red, cow ID = 635 and 3361).

Social differentiation was observed across the full barn (> 92.96 % of dyads, $p < 0.01$ across days), the FZ (100 % of dyads, $p < 0.01$ across days), and the NFZ (92.96 % of dyads, $p < 0.01$ across days), see Table 1.

3.3.3. Temporal variation in sociality

There was no significant difference in median daily interactions between days in the full barn (K-W chi-squared = 2252.30, df = 27, after 10,000 permutations, $p = 1$; Table 11), FZ (K-W = 61.00, df = 27, after 10,000 permutations, $p = 1$; Table 1), nor in the NFZ (K-W = 2268.9, df = 27, respectively after 10,000 permutations, $p = 1$; Table 11).

Figure 4 highlights the temporal instability in both the functional zone networks. Although there were no clear trends over time, where there were changes these are seen to be highly correlated across all individuals in the NFZ ($n = 92$; Pearson's coefficient [hereafter ρ] = 0.55, $p < 0.01$) (Fig 4). Conversely, individual interactions in the FZ showed much more random variation than in the NFZ ($\rho = 0.02$, $n = 92$, $p < 0.90$), as demonstrated with the highlighted subset of individuals (Fig 4). There was a weak but non-significant relationship between mean temperature and mean daily interactions across days in both the FZ ($\rho = -0.09$, df = 26, $p = 0.66$; Table 1; Fig 4) and NFZ ($\rho = 0.04$, df = 26, $p = 0.82$; Table 1; Figure 9).

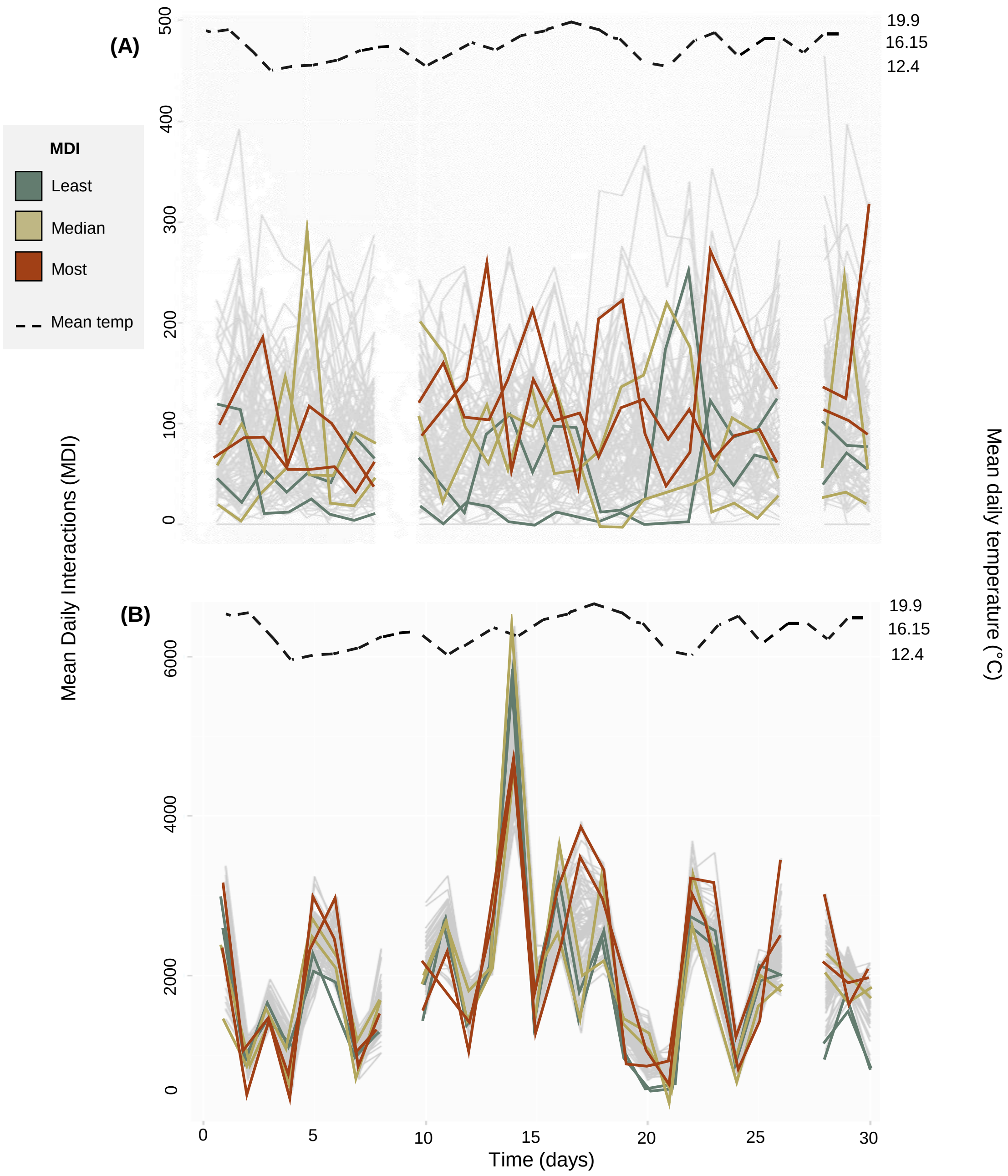

Figure 4. Mean daily interactions across time (01/10/2014 to 31/10/2014 with days excluded from the study omitted) in (A) feeding zone, and (B) non-feeding zone of the commercial farm in Essex. An illustrative subset of individuals is highlighted: two cows with the least mean daily interactions (blue, cow ID = 3124 and 3317), two cows with mean daily interactions closest to the median value (green, cow ID = 2602 and 3132) and two cows with the highest mean daily interactions (red, cow ID = 635 and 3361). Data for each individual cow are indicated with a grey line. Mean daily temperature is shown with the dashed black line.

In the FZ, there were significant weak positive correlations between all the four-day block averaged- consecutive networks (*n* = 7, comparisons = 6) (range of Spearman's coefficient [hereafter R_s] across days = 0.20 to 0.31, after 10,000 permutations and Bonferroni correction, $p < 0.001$ for all comparisons; Table 3; Fig 5). In the full barn there were also weak correlations between the four-day averaged consecutive networks (range of R_s = 0.03 to 0.23, after 10,000 permutations and Bonferroni correction, $p \leq 0.001$ for three comparisons (day blocks 1-2, 2-3, 5-6); range of R_s = -0.04 to -0.001, after 10,000 permutations and Bonferroni correction, $p > 0.23$ for three comparisons (day blocks 3-4, 4-5, 6-7) (Table 3; Fig 5). In the NFZ, there were inconsistent (significant or non-significant) weak correlations between consecutive networks (range of R_s = 0.05 to 0.24, after 10,000 permutations and Bonferroni correction, $p < 0.01$ for four comparisons (day blocks 1-2, 2-3, 5-6, 6-7); range of R_s = -0.04 to 0.01, after 10,000 permutations and Bonferroni correction, $p = 1$ for two comparisons (day blocks 3-4, 4-5); Table 3; Fig 5).

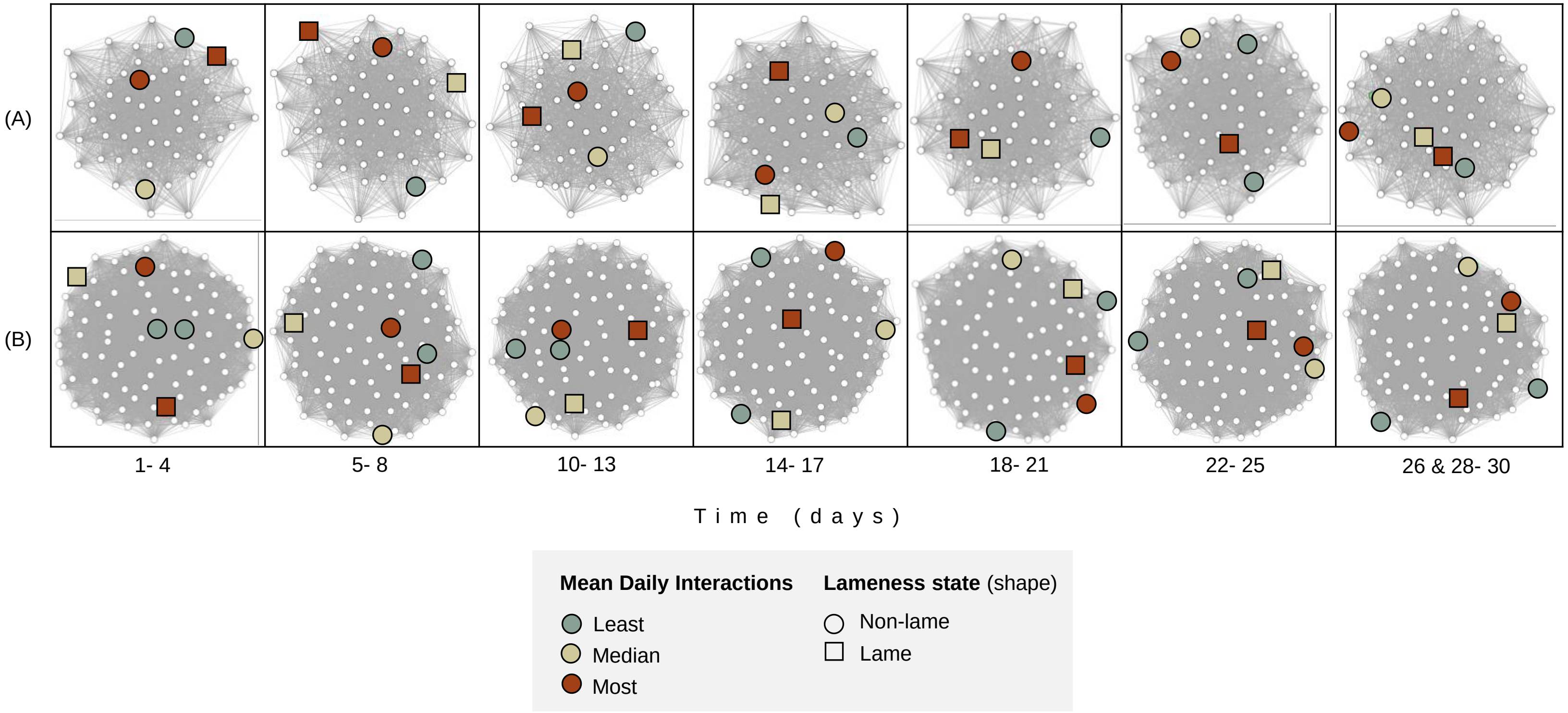

Figure 5. Interaction networks, filtered by mean node degree, over time for (A) feeding zone, and (B) non-feeding zone of the commercial farm in Essex. An illustrative subset of individuals is shown: two cows with the least mean daily interactions (blue, cow ID = 3124 and 3317), two cows with mean daily interactions closest to the median (green, cow ID = 2602 and 3132) and two cows with the highest mean daily interactions (red, cow ID = 635 and 3361). It should be noted that some individuals may not appear in the FZ networks due to their interactions not exceeding the mean node degree (as nodes with a degree less than or equal to the mean node degree were excluded). The Fruchterman-Reingold layout

algorithm determined the node positions; unconnected vertices are repelled. Created in RStudio using the ‘vegan’ package (275,276,300). Days 9 and 27 were excluded due to nonsensical recordings (See Chapter 2, Section 2.4).

For completeness, each of the original daily networks (*n* = 28) are compared, as well as two-, seven-, and 14-day blocks. The results were qualitatively similar, showing weak correlations between all consecutive networks (Table 2).

Table 2. Results from temporal variation analysis of the interaction network by segmenting and averaging the networks (feeding and non-feeding zone), derived from data recorded on the commercial farm in Essex, into one, two, seven and 14 day-blocks. A proximity interaction was defined where cows (*n* = 92) were within a three-metre radius for more than 60 seconds. To calculate the *p*-value, 10,000 permutations were used, and *p*-values are after Bonferroni correction; significant results ($p < 0.05$) are in bold.

	Non-parametric statistical test and reported *p*-value		
Segmented components (days)	**Feeding zone**	**Non-feeding zone**	**Summary**
28 (1 day)	Range of R_s = 0.09 to 0.18 **($p \leq 0.01$)** for 25 comparisons; range of R_s = 0.02 to 0.04 ($p = 1$) for two comparisons (days 21-22, 26-27)	Range of R_s = -0.30 to 0.37 **($p \leq 0.05$)** for seven comparisons (days 3-4, 7-8, 13-14, 17-18, 18-19, 25-26, 27-28); range of R_s = -0.28 to 0.03 ($p \geq 0.42$) for 20 comparisons	
14 (2 days)	Range of R_s = 0.10 to 0.26 **($p \leq 0.01$)**	Range of R_s = 0.06 to 0.29 (**$p < 0.01$** for six comparisons (day blocks 2-3, 6-7, 10-11, 11-12, 12-13, 13-14);	

		range of R_s = -0.22 to 0.19 ($p = 1$) for seven comparisons	Weak correlation between all consecutive networks.
7 (4 days)	Range of R_s = 0.20 to 0.31 **($p < 0.001$)**	Range of R_s = 0.05 to 0.24 (**$p < 0.01$**) for four comparisons (day blocks 1-2, 2-3, 5-6, 6-7); range of R_s = -0.04 to 0.01 ($p = 1$) for two comparisons (day blocks 3-4, 4-5)	
4 (7 days)	Range of R_s = 0.14 to 0.41 (**$p < 0.01$**)	R_s = 0.28 **($p < 0.001$)** for one comparison (day block 3-4); R_s = 0.004 ($p = 1$) for one comparison (day block 1-2); R_s = -0.16 ($p = 1$) for one comparison (day block 2-3)	
2 (14 days)	R_s = 0.41 (**$p < 0.001$**)	R_s = -0.07 ($p = 1$)	

3.3.4. Impact of health status, parity, and days in milk on sociality

3.3.4.1. Lameness

Lame cows (*n* = 22) did not have significantly more mean daily interactions than non-lame cows (*n* = 26) in the FZ (Wilcoxon test statistic [hereafter W] = 342, *p* = 0.86 after 10,000 permutations; Table 3; Figure 6) nor in the NFZ (W = 276, *p* = 0.40 after 10,000 permutations; Table 3; Figure 6).

In the FZ, lame cows did not show a significantly different mean clustering coefficient or degree than non-lame cows (W = 284 and 321.5 respectively, after 10,000 permutations, *p* = 0.53 and 0.25, respectively; Table 3; Figure 6). Similarly, in the NFZ, mean clustering coefficient or degree did not differ between the lameness states (W = 398 and 241.5, after 10,000 permutations, *p* = 0.99 and 0.17 respectively; Table 3; Figure 6).

There was no significant social assortment by lameness in the FZ (range of across days R_s = -0.06 to 0.04), nor the NFZ (range of across days R_s = -0.07 to 0.06) where, after Bonferroni Correction and 10,000 permutations, $p > 0.80$ in all cases for all days (*n* = 28; Table 3). In other words, cows with the same lameness state did not associate more than cows of different lameness states.

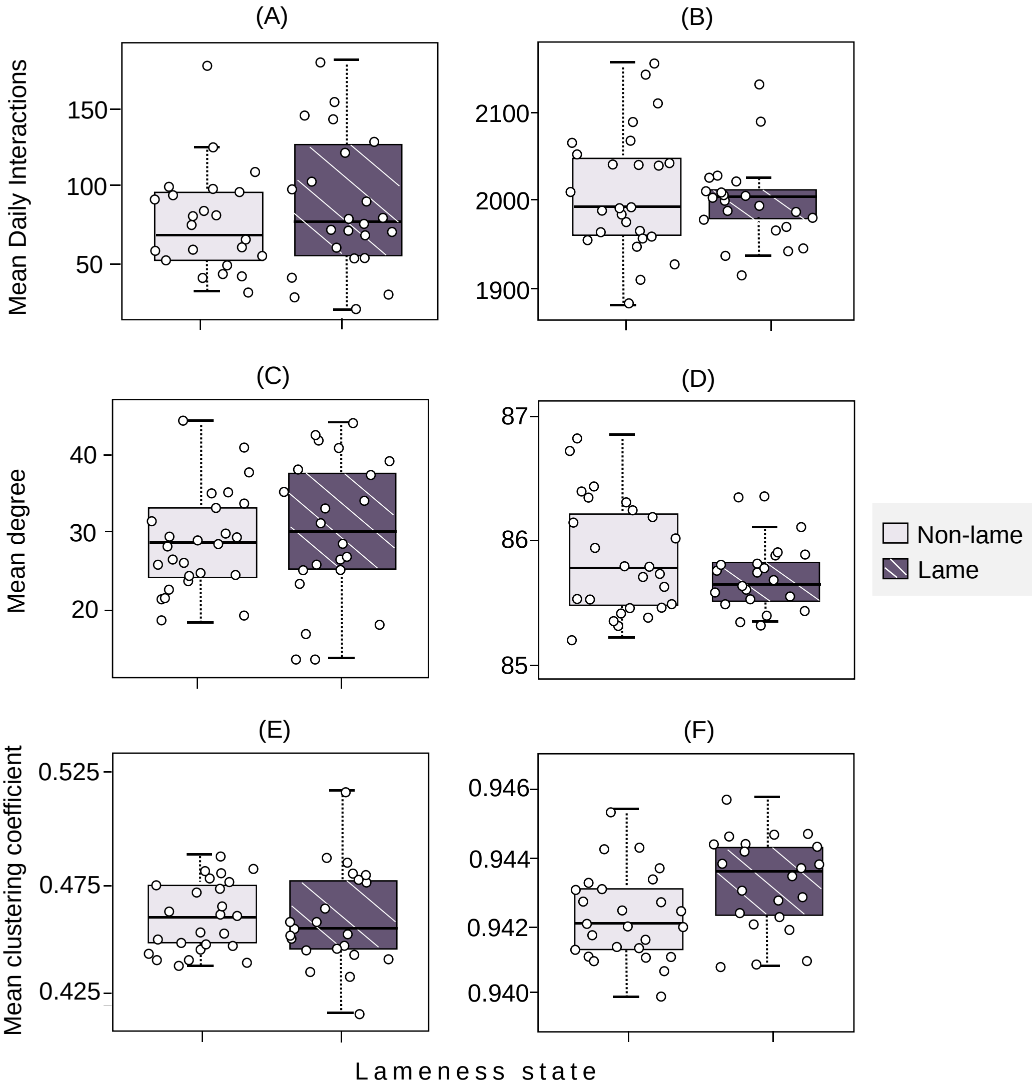

Figure 6. (A, B) Comparison of Mean Daily Interactions and node-level measures (C, D) degree and (E, F) clustering coefficient, between lameness states in the (A, C, E) feeding zone and (B, D, F) non-feeding zone of the commercial farm in Essex. Cows were classified as either non-lame (NL) (*n* = 26) or lame (L) (*n* = 22). The horizontal line in each boxplot represents the median value. The mean [standard deviation] values for NL and L cows are given respectively by: (A) 74 [35] and 90 [44]; (B) 2015 [70] and 2006 [45]; (C) 27.92 [8.53] and 30.14 [9.37]; (D) 85.85 [0.45] and 85.68 [0.26]; (E) 0.44 [0.09] and 0.46 [0.02]; (F) 0.94 [0.001] and 0.94 [0.001]. Each individual cow is indicated with a small circle.

3.3.4.2. Parity and days in milk

There was no significant social assortment by parity in the FZ (range of across days R_s = -0.05 to 0.03, after 10,000 permutations and Bonferroni Correction, $p = 1$ for all days; Table 3) or in the NFZ network (range of R_s across days = -0.02 to 0.03, after 10,000 permutations and Bonferroni Correction, $p = 1$ for all days; Table 3).

There was also no significant social assortment by DIM in the FZ (range of across days R_s = -0.03 to 0.04, after 10,000 permutations and Bonferroni Correction $p = 1$ for all days) or the NFZ (range of R_s across days = -0.3 to 0.03, after 10,000 permutations and Bonferroni Correction, $p \geq 0.44$ for all days; Table 3).

The results for social assortment by lameness, parity and DIM in the full barn network were similar to those of the NFZ (results in Table 3).

Table 3. Overview of results, basic network measures, inter-individual variation, temporal variation in sociality and social assortment by lameness, parity, and days in milk, for the full barn (FB) and each functional zone of the commercial barn in Essex. A spatial threshold radius of $r = 3$m and time duration of $t = 60$s was used define an interaction. The functional zones shown are the feeding zone (FZ) and non-feeding zone (NFZ). (Median) daily interactions are denoted with (M)DI. Significant results ($p < 0.05$) are in bold.

	Measure	**Test value** (p-value)			**Summary**
		Full barn	**Feeding zone**	**Non-feeding zone**	
Basic network measures	Mean edge density ($d = 28$)	0.96	0.33	0.94	The networks are highly dense, more so the NFZ than the FZ.
	Components (by day) ($d = 28$)	1	2-6	1	The networks typically consist of one component.
Inter-individual variation	Inter-individual differences in median DI ($n = 92$)	K-W = 26.53 **($p < 0.001$)**	K-W = 851.71 ($p = 1$)	K-W = 19.21 **($p < 0.001$)**	**Inter-individual variation in DI in the NFZ** but not in the FZ or the FB.
	Social differentiation (SD) ($n = 92$)	SD between ≤ 92.96 % of dyads **($p < 0.01$)**	SD between 100 % of dyads **($p < 0.01$)**	SD between 92.96 % of dyads **($p < 0.01$)**	**Social differentiation present in all networks.**
	Difference in median DI between days ($n = 92$, $d = 28$)	K-W = 2252.30 ($p = 1$)	K-W = 61.00 ($p = 1$)	K-W = 2268.9 ($p = 1$)	No difference in DI between days in all networks.
	Relationship between MDI and days ($n = 92$, $d = 28$)	Pearson correlation, $\rho = 0.03$ ($p = 0.88$)	Pearson correlation, $\rho = 0.55$ **($p < 0.01$)**	Pearson correlation, $\rho = 0.02$ ($p = 0.90$)	MDI correlated over time in the feeding zone but not in the non-feeding zone.

Temporal variation in sociality	Relationship between MDI and temperature ($n = 92$, $d = 28$)	Pearson correlation, $\rho = 0.04$ ($p = 0.83$)	Pearson correlation, $\rho = -0.09$ ($p = 0.66$)	Pearson correlation, $\rho = 0.04$ ($p = 0.82$)	Weak non-significant correlation between MDI and temperature in both functional zones.
	Relationship between four-day block consecutive networks (six networks, $n = 92$ per network)	Mantel test, range of $R_s = 0.03$ to 0.23 **($p \leq 0.001$)** for three comparisons (day blocks 1-2, 2-3, 5-6); range of $R_s = -0.04$ to -0.001 ($p > 0.23$) for three comparisons (day blocks 3-4, 4-5, 6-7)	Mantel test, range of $R_s = 0.20$ to 0.31 **($p < 0.001$)**	Mantel test, range of $R_s = 0.05$ to 0.24 **($p < 0.01$)** for four comparisons (day blocks 1-2, 2-3, 5-6, 6-7); range of $R_s = -0.04$ to 0.01 ($p = 1$) for two comparisons (day blocks 3-4, 4-5)	Weak or no correlation between all consecutive networks.
Individual characteristics	Difference in mean DI between non-lame ($n = 26$) and lame cows ($n = 22$)	Wilcoxon test, W = 297 ($p = 0.56$)	Wilcoxon test, W = 342 ($p = 0.86$)	Wilcoxon test, W = 276 ($p = 0.40$)	No difference in DI between non-lame and lame cows in both functional zones.
	Difference in mean clustering coefficient between non-lame ($n = 26$) and lame cows ($n = 22$)	Wilcoxon test, W = 392 ($p = 0.98$)	Wilcoxon test, W = 284 ($p = 0.53$)	Wilcoxon test, W = 398 ($p = 0.99$)	No difference in clustering coefficient between non-lame and lame cows in either functional zone.
	Difference in mean node degree between non-lame ($n = 26$) and lame cows ($n = 22$)	Wilcoxon test, W = 304.5 ($p = 0.63$)	Wilcoxon test, W = 321.5 ($p = 0.25$)	Wilcoxon test, W = 241.5 ($p = 0.17$)	No difference in node degree between non-lame and lame cows in either functional zone.

	Social assortment by lameness status by day, *n* = 48)	Mantel test, $R_s = 0.11$ **($p < 0.01$)** for day 16; range of R_s = -0.07 to 0.05 (p = 1) for remaining 27 days	Mantel test, range of R_s = -0.06 to 0.04 (p = 1 for all days)	Mantel test, range of R_s = -0.07 to 0.06 ($p > 0.80$)	Cows did not socially assort according to their lameness status, parity, or DIM in either functional zone.
	Social assortment by parity (by day, *n* = 92)	Mantel test, range of R_s = -0.02 to 0.03 (p = 1 for all days)	Mantel test, range of R_s = -0.05 to 0.03 (p = 1 for all days)	Mantel test, range of R_s = -0.02 to 0.03 (p = 1 for all days)	
	Social assortment by DIM (by day, *n* = 92)	Mantel test, range of R_s = -0.03 to 0.03 (p = 0.80 for all days)	Mantel test, range of R_s = -0.03 to 0.04 (p = 1 for all days)	Mantel test, range of R_s = -0.03 to 0.03 ($p > 0.44$ for all days)	

3.4. Discussion

Within this chapter, the interaction network of the dairy herd housed on a commercial farm in Essex was found to be highly connected with significant social differentiation. Interactions between cows were more heterogenous than expected by chance, but the network structure was temporally unstable. There was no evidence of preferential social assortment, showing cows did not associate more than expected by chance according to lameness state, parity, or days in milk (DIM).

Visualisation of the full barn interaction network illustrates that the herd was highly connected, as confirmed by the mean edge density (96%). This indicates that each cow was likely to have had interactions with most other cows in the herd each day. It is not clear from this study whether these cows actively seek out and connect with their conspecifics, perhaps to maintain social structure in the group, or whether this high connectivity is a function of the building layout and high stocking density. It must be acknowledged that, due to building works on the farm, the stocking rates were high during the study period (feed space = 0.48m per cow, lying space = 0.72 cubicles per cow). This may have reduced the ability of the cows to actively choose with whom to be in close proximity with. In agreement with this study, high connectivity was also reported for cows housed in loose straw yards with concrete loafing areas with moderate to high stocking rates of 9.50m^2 per cow to 7.66m^2 per cow from sensor derived proximity measurements (166,176). Lower edge densities have been reported in a grazing system, in (147), but in their study an interaction was based on the occurrence of specific behaviours considered to increase the risk of disease transmission rather than social proximity. Lower edge density and a sparse structure was also reported for cows housed in cubicles at a moderate stocking rate (1.03 cubicles per cow), but the group in their study only comprised of 36 cows and interactions were only recorded during two 15-minute time slots per day, therefore not capturing changes in location and near neighbours throughout the day (304). Further investigations of dairy cows in a range of housing types and stocking rates are needed to determine if cows are naturally highly connected or whether aspects of the commercial dairy result in cows spending time in proximity to a greater number of conspecifics.

Analysis on the interaction networks revealed significant inter-individual variation in daily interactions in the non-feeding zone (NFZ), but not across the feeding zone (FZ) or when considering the full barn. The FZ is likely to be a more dynamic location than the loafing and resting areas. Feed bouts are shorter than lying bouts and cows will begin and end their eating bouts at different times, leading to a greater turnaround of contacts at the feed face than other areas of the barn. It is possible however, that cows have greater control over the individual interactions they have in the non-feeding zone and therefore a greater degree of individuality is observed. Researchers have demonstrated that inter-individual variation in sociality is an individual trait in dairy cows (272) influenced by dominance status and personality traits. This may affect an individual's ability to gain resources, such as cubicles, impacting their proximity interactions in the non-feeding zone (162,293), as also speculated by (176), although it is not possible to distinguish between these potential factors in this study.

The structure of the interaction network was weakly correlated over time, and individuals periodically isolated from the main network component of the feeding zone. These individuals were not the same each day, and they were not of the same lameness status, suggesting their isolation was due to them choosing not to feed at the same time, or being unable to compete due to the lack of space. The overall herd was subject to changes throughout the study period, with the addition and removal of cows outside of the study group (*n* = 92, whole herd = 100 to 111 cows on a given day), which could have affected the social structure of the herd. In a previous study, while introductions of new cows to a stable group did not affect the sociality of individual cows, it did weaken the overall social network (272). Researchers similarly reported weak to moderate correlations in structure between consecutive one-week networks in a highly connected network also subject to changing group composition (176). Further analysis on the temporal stability of dairy cow networks whilst removing specific individuals could aid management.

There were no significant correlations between daily interactions and temperature in this study. However, the study period was selected based on there being a relatively stable temperature throughout with temperature low enough not to induce heat stress. Cows have been shown to modify their collective behaviour, in terms of clustering for example, or individual behaviours in extreme heat conditions, or show long-term signs of heat stress due to high stocking densities (191,305–307). Therefore, environmental temperature and even individual cow temperature should be considered when monitoring the herd social structure over longer study periods. Furthermore, the social network may have been more dynamic than initially envisioned due to factors not accounted for, such as farm management actions or treatment interventions (308).

Considering the known social withdrawal response of unhealthy cows (309), it might be predicted that lame cows would be less willing to compete for preferred food or access to cubicles, but no differences in the sociality or positioning were found between lame and non-lame cows. At a particularly high stocking rate in intensive cubicle housing, there may have been little opportunity for the 22 ‘lame’ cows identified in this study to self-isolate. Lame cows have been shown to modify their space-use in this barn, but this was with access to an additional loafing area at the end of the cubicle shed which would make social distancing easier than in this study (252). Furthermore, lame cows are found to receive approximately twice as much allogrooming as cows that are non-lame (310), and this explanation would also support the finding in this study of no individual-level social assortment by lameness state i.e. cows of the same lameness state did not associate more or less than expected (310). When interpreting the result above it should be considered that use of a visual locomotion score is not without the potential for classification errors, especially when scoring large groups of cows at the parlour exit as was the case in this study. It has been reported that mild claw lesions are not always accompanied with a corresponding increase in locomotion score, indicating that locomotion scoring even by trained observers may not be sensitive enough to detect all lameness cases (311). Indeed in a previous study a predictive statistical model correctly classified two cows that were incorrectly classified by observer locomotion scoring (252). Cows with dominant lameness status were also discretely grouped as either 'non-lame' or 'lame' during analysis (312,313), and these cows may have behaved differently during various time periods of the study. Nonetheless, this study demonstrates a potential way to assess the influence of health status on social interactions within a typical herd. Quantitative measures of individual social interactions and network position may be useful indicators to use within automated monitoring approaches in PLF.

Social differentiation was present in both functional zones (Table 1); some dyads interacted more than others, as similarly shown in (166,314). A number of previous studies have indicated social differentiation can occur with age, as cows of a similar age would have had greater opportunity to develop social ties with one another (314,315), particularly if they calved at similar times. In addition, stronger bonds may form between calves born at similar times, who remain together throughout rearing before joining the milking herd; cows have been shown to invest more time and energy into relationships with herd members sharing long-term experiences (168). This study does not find that cows differentiate by parity, a proxy for age. While parity may give an indication as to a cow's experience in the herd and may contribute to her personality traits, this measure is probably too coarse to identify cows with historical associations, such as shared calf cohorts, which has been suggested to result in stronger bonds. In this study a recent shared transition period, as indicated by similar DIM, was not sufficient to result in differentiation on this basis. This is in line with previous findings, where recent familiarity with cows had no effect on lying down behaviours of cows transitioning to the herd but early familiarity led to greater synchrony of lying behaviours (316). Greater detail of the cohorts of cows kept from birth through to the milking herd, unmeasured in this study, may explain the social differentiation observed. It is possible that the high temporal variation of the network structure, and insufficient space within the barn may have impeded the ability to identify these structures. Alternatively, non-random associations may have been the result of cows of similar dominance rank positioning closely, with subordinates displaced from favourable feeding positions by dominant cows (266), particularly as feed space was limited to < 0.60 m/cow. Interactions may be more likely to develop between cows with similar energy requirements and motivation, and hence similar activity time budgets. For example, cows that spend more time eating may spend a lot of time near the feed face and hence position closely to similar cows (166,314,315). Stage of lactation affects the time an individual allocates to feeding, given that energy requirements vary with milk yield; for instance, dry matter intake is typically highest during mid-lactation (317).

When interpreting the results, it is important to consider potential limitations of the relatively novel technology and SNA techniques used in this study (308,318). Although the proximity used to define an interaction was also tested for other radii and time durations, and qualitatively similar results were obtained, any interactions detected were limited by the accuracy of the LPS system (2.66m mean error for a static sensor). Additionally, a fundamental problem with this type of automated approach to identify proximity interactions is that we are unable to distinguish between which proximity interactions were true social interactions (e.g. allogrooming) and which were non-deliberate or non-social proximity events (e.g. due to the positioning of neighbouring cows at the feed face (140,308) or in cubicles (140). The results are likely to contain both genuine sustained social interactions, as well as proximity events which were not directly social. Distinguishing between genuine social interactions and indirect or non-social proximity interactions is an open research question that requires further investigation. The chosen proximity identification protocol was tested and validated using observational data and was found to have a sensitivity of 83% (r = 3m and t = 60s), but a direct estimate of the rate of false positives and hence the specificity is not possible. Using a time duration of 60s is likely to reduce the rate of false positives (compared to using a shorter time duration) but will also potentially exclude genuine social interactions of short duration. Multiple shorter interactions may be as socially relevant as longer sustained interactions. The analysis was based on a comparison of daily-level network statistics and comparison of these over time or between individuals with different lameness status, parity, and DIM. It is quite plausible that, although the daily level behaviour may be similar across the network, there could be significant individual variability in social interactions on a finer timescale (e.g., hourly, or less), particularly around key events such as feeding and milking, and this variability in social behaviour may be linked to social status or health. A further limitation is that, although most cows that were present in the herd throughout the study period (n = 92) were included, there were cows that entered and left the group throughout this period, and hence some potential interactions involving these cows would not have been recorded. The effect of missing individuals on the conclusions drawn from a social network analysis are not well understood and this remains an open research question (308,318). Despite the drawbacks to using proximity to detect potential social interactions, this approach based on using a local positioning system is useful for quickly accumulating the large datasets needed for SNA in an automated way (308).

3.5. Conclusions

Automated spatial positioning technology was used to monitor a large herd group of permanently housed dairy cows on a commercial farm in Essex at high temporal resolution for a full month. Proximity interactions were identified by sustained periods of closeness between dyads. The proximity interaction network structure of the herd was highly connected, with significant differentiation in interactions between dyads, and high temporal variability. Lameness, parity, and days in milk were not found to directly influence social interactions or network position. This chapter demonstrates how automated sensor technology could be used to monitor the social structure of a large commercially relevant group of livestock, and how individual differences in social interactions and network measures could be used to potentially identify health differences between animals. Future work should aim to better distinguish social interactions from indirect non-social interactions and consider how interactions within a larger group may differ in different housing environments and at different stocking densities.

The main results of this chapter are outlined below:

- The structure and consistency of a proximity interaction network of a dairy herd, housed on a commercial farm in Essex, was assessed using local positioning system data over a month-long duration.
- Cow dyads were considered to have interacted if they were continuously within three meters for ≥ 60s, and from the resulting proximity interaction network, social differentiation, temporal stability, and social assortment were derived.
- The network was highly connected but temporally unstable, with social differentiation and inter-individual variation in daily proximity interactions in the non-feeding zone.
- Cows were not found to assort by lameness, parity, or days in milk.

3.6. Appendix 1. Results from different spatial thresholds and radii

To fully explore the effect of the spatial threshold radius, *r*, additional full network analyses were run using one, two, four and five meters for a fixed time duration of *t* = 60s. In all cases, qualitatively similar results were obtained, including significant social differentiation, high temporal variation in the network structure, and no social assortment by lameness, parity, or DIM (Tables A1 to A4), demonstrating that the overall results and conclusions are robust to this parameter choice.

To fully explore the effect of the time duration threshold, *t*, additional full network analyses were run using 40, 80 and 100s for a fixed radius of *r* = 3m. In all cases, qualitatively similar results were obtained, including significant social differentiation, high temporal variation in the network structure, and no social assortment by lameness, parity, or DIM (Tables A5 to A7), demonstrating that the overall results and conclusions are robust to this parameter choice.

Table A1. Overview of results using a spatial threshold radius of *r* = 1m and time duration of *t* = 60s to define an interaction on the commercial farm in Essex for the full barn and the functional zones: feeding zone and non-feeding zone. Basic network measures (original and filtered by mean degree), inter-individual variation, temporal variation in sociality, lameness status, and parity and days in milk, where (M)DI = (median) daily interactions, are shown Significant results ($p < 0.05$) are in bold.

	Measure	**Test value** (*p*-value)			**Summary**
		Full barn	**Feeding zone**	**Non-feeding zone**	
Basic network measures	Mean edge density ($d = 28$)	0.46	0.04	0.43	The networks are highly dense, more so the NFZ than the FZ.
	Components (by day) ($d = 28$)	1	6-19	1	The full barn and the NFZ consisted of one component, but the FZ consisted of numerous.
Inter-individual variation	Inter-individual differences in median DI ($n = 92$)	K-W = 36.06 ($p = 1$)	K-W = 701.83 ($p = 1$)	K-W = 31.24 **($p < 0.001$)**	**Inter-individual variation in DI in the NFZ** but not in the full barn or the FZ.
	Social differentiation (SD) ($n = 92$)	SD between >100% of dyads **($p < 0.01$)**	SD between 100% of dyads **($p < 0.01$)**	SD between 100% of dyads **($p < 0.01$)**	**Social differentiation present in all networks.**
Temporal variation in sociality	Difference in median DI between days ($n = 92$, d = 28)	K-W =2154.40 ($p = 1$)	K-W = 43.65 ($p = 0.98$)	K-W = 2162.90 ($p = 1$)	No difference in DI between days in all networks.
	Relationship between MDI and days ($n = 92$, $d = 28$)	Pearson correlation, $\rho = -0.01$ ($p = 0.97$)	Pearson correlation, $\rho = 0.28$ ($p = 0.15$)	Pearson correlation, $\rho = -0.01$ ($p = 0.96$)	MDI weakly correlated over time in both functional zones.
	Relationship between MDI and	Pearson correlation, $\rho = -0.04$ ($p = 0.84$)	Pearson correlation, $\rho = -0.23$ ($p = 0.24$)	Pearson correlation, $\rho = -0.04$ ($p = 0.85$)	Weak negative correlation between MDI and temperature in both functional zones.

	temperature (n = 92, d = 28)				
	Relationship between four-day block consecutive networks (six networks, n = 92 per network)	Mantel test, range of R_s = 0.05to 0.34 **(p< 0.01)** for five comparisons (day blocks 1-2, 2-3, 3-4, 4-5, 5-6); R_s = -0.14 (p = 1) for one comparison (day block 6-7)	Mantel test, range of R_s = 0.05 to 0.14 **(p ≤ 0.01)** for five comparisons (1-2, 2-3, 3-4; 4-5, 6-7); $R_{s\,=}$ = 0.02 (p = 0.54) for one comparison (day block 5-6)	Mantel test, range of R_s = 0.05 to 0.35 **(p < 0.01)** for five comparisons (day blocks 1-2, 2-3, 3-4, 4-5, 5-6); R_s= -0.15 (p = 1) for one comparison (day block 6-7)	Weak correlation between all consecutive networks.
Individual characteristics	Difference in mean DI between non-lame (n = 26) and lame cows (n = 22)	Wilcoxon test, W = 255 (p = 0.25)	Wilcoxon test, W = 317 (p = 0.72)	Wilcoxon test, W = 237 (p = 0.15)	No difference in DI between non-lame and lame cows in both functional zones.
	Difference in mean clustering coefficient between non-lame (n = 26) and lame cows (n = 22)	Wilcoxon test, W = 419 (p = 1)	Wilcoxon test, W = 262.50 (p = 0.30)	Wilcoxon test, W = 417 (p = 1)	No difference in clustering coefficient between non-lame and lame cows in either functional zone.
	Difference in mean node degree between non-lame (n = 26) and lame cows (n = 22)	Wilcoxon test, W = 261.50 (p = 0.29)	Wilcoxon test, W = 305.50 (p = 0.64)	Wilcoxon test, W = 210 (p = 0.05)	No difference in node degree between non-lame and lame cows in either functional zone.

	Social assortment by lameness status by day, *n* = 48)	Mantel test, range of R_s= -0.07 to 0.07 ($p > 0.34$ for all days)	Mantel test, range of R_s = -0.06 to0.06 ($p > 0.70$ for all days)	Mantel test, range of R_s = -0.07 to 0.07 ($p \geq 0.35$)	Cows did not socially assort according to their lameness status, parity, or DIM in either functional zone.
	Social assortment by parity (by day, *n* = 92)	Mantel test, range of R_s = -0.03 to 0.02 ($p = 1$ for all days)	Mantel test, range of R_s = -0.04 to 0.02 ($p = 1$ for all days)	Mantel test, range of R_s= -0.02 to 0.03 ($p = 1$ for all days)	
	Social assortment by DIM (by day, n = 92)	Mantel test, range of R_s = -0.02 to 0.02 ($p = 1$ for all days)	Mantel test, range of R_s = -0.04 to 0.03 ($p = 1$ for all days)	Mantel test, range of R_s = -0.02 to 0.03 ($p = 1$ for all days)	

Table A2. Overview of results using a spatial threshold radius of *r* = 2m and time duration of *t* = 60s to define an interaction on the commercial farm in Essex for the full barn and the functional zones: feeding zone and non-feeding zone. Basic network measures (original and filtered by mean degree), inter-individual variation, temporal variation in sociality, lameness status, and parity and days in milk, where (M)DI = (median) daily interactions, are shown Significant results ($p < 0.05$) are in bold.

	Measure	**Test value** (*p*-value)			**Summary**
		Full barn	**Feeding zone**	**Non-feeding zone**	
Basic network measures	Mean edge density ($d = 28$)	0.84	0.19	0.80	The networks are highly dense, more so the NFZ than the FZ.
	Components (by day) ($d = 28$)	1	1-4	1	The networks typically consist of one component.
Inter-individual variation	Inter-individual differences in median DI ($n = 92$)	K-W = 29.22 **($p < 0.001$)**	K-W = 928.39 ($p = 1$)	K-W = 21.57 **($p < 0.001$)**	**Inter-individual variation in DI in the NFZ** but not in the FZ.
	Social differentiation (SD) ($n = 92$)	SD between > 96.43% of dyads **($p < 0.01$)**	SD between 100% of dyads **($p < 0.01$)**	SD between 96.46% of dyads **($p < 0.01$)**	**Social differentiation present in all networks.**
Temporal variation in sociality	Difference in median DI between days ($n = 92$, d = 28)	K-W = 2282.10 ($p = 1$)	K-W = 49.47 ($p = 0.99$)	K-W = 2303.00 ($p = 1$)	No difference in DI between days in all networks.
	Relationship between MDI and days (**n** = 92, $d = 28$)	Pearson correlation, $\rho = -0.03$ ($p = 0.87$)	Pearson correlation, $\rho = 0.32$ ($p = 0.09$)	Pearson correlation, $\rho = -0.04$ ($p = 0.86$)	MDI weakly correlated over time in both functional zones.
	Relationship between MDI and temperature ($n = 92$, $d = 28$)	Pearson correlation, $\rho = -0.04$ ($p = 0.83$)	Pearson correlation, $\rho = -0.19$ ($p = 0.34$)	Pearson correlation, $\rho = -0.04$ ($p = 0.83$)	Weak negative correlation between MDI and temperature in both functional zones.

	Relationship between four-day block consecutive networks (six networks, $n = 92$ per network)	Mantel test, range of $R_s = 0.04$to 0.26 **($p \leq 0.01$)** for five comparisons (day blocks 2-3, 3-4, 4-5, 5-6, 6-7); $R_s = -0.10$ ($p = 1$) for one comparison (day block 1-2)	Mantel test, range of $R_s = 0.10$to 0.25 **($p < 0.001$)**	Mantel test, range of $R_s = 0.05$ to 0.28 ($p \leq 0.10$) for five comparisons (day blocks 2-3, 3-4, 4-5, 5-6, 6-7); $R_s = -0.10$ ($p= 1$) for one comparison (day block 1-2)	Weak correlation between all consecutive networks.
Individual characteristics	Difference in mean DI between non-lame ($n = 26$) and lame cows ($n = 22$)	Wilcoxon test, W = 272.00 ($p = 0.37$)	Wilcoxon test, W = 311.50 ($p = 0.68$)	Wilcoxon test, W = 236.50 ($p = 0.14$)	No difference in DI between non-lame and lame cows in both functional zones.
	Difference in mean clustering coefficient between non-lame ($n = 26$) and lame cows ($n = 22$)	Wilcoxon test, W = 397.00 ($p = 0.99$)	Wilcoxon test, W = 290.00 ($p = 0.51$)	Wilcoxon test, W = 412.00 ($p = 0.99$)	No difference in clustering coefficient between non-lame and lame cows in either functional zone.
	Difference in mean node degree between non-lame ($n = 26$) and lame cows ($n = 22$)	Wilcoxon test, W = 306.50 ($p = 0.65$)	Wilcoxon test, W = 299 ($p = 0.59$)	Wilcoxon test, W = 250.50 ($p = 0.22$)	No difference in node degree between non-lame and lame cows in either functional zone.
	Social assortment by lameness status by day, $n = 48$)	Mantel test, $R_s = -0.06$ to 0.09 ($p \geq 0.06$) for all days	Mantel test, range of $R_s = -0.04$ to 0.06 ($p \geq 0.46$ for all days)	Mantel test, range of $R_s = -0.07$ to 0.07 ($p > 0.38$)	

	Social assortment by parity (by day, n = 92)	Mantel test, range of R_s = -0.03 to 0.02 (p = 1 for all days)	Mantel test, range of R_s = -0.05 to 0.04 (p =1 for all days)	Mantel test, range of R_s = -0.02 to 0.03 (p = 1 for all days)	Cows did not socially assort according to their lameness status, parity, or DIM in either functional zone.
	Social assortment by DIM (by day, n = 92)	Mantel test, range of R_s = -0.03 to 0.02 (p = 1 all days)	Mantel test, range of R_s = -0.04 to 0.02 (p = 1 for all days)	Mantel test, range of R_s = -0.03 to 0.03 ($p \geq 0.87$ for all days)	

Table A3. Overview of results using a spatial threshold radius of $r = 4$m and time duration of $t = 60$s to define an interaction on the commercial farm in Essex for the full barn and the functional zones: feeding zone and non-feeding zone. Basic network measures (original and filtered by mean degree), inter-individual variation, temporal variation in sociality, lameness status, and parity and days in milk, where (M)DI = (median) daily interactions, are shown Significant results ($p < 0.05$) are in bold.

	Measure	**Test value** (p-value)			**Summary**
		Full barn	**Feeding zone**	**Non-feeding zone**	
Basic network measures	Mean edge density ($d = 28$)	0.99	0.49	0.99	The networks are highly dense, more so the NFZ than the FZ.
	Components (by day) ($d = 28$)	1	1-3	1	The networks typically consist of one component.
Inter-individual variation	Inter-individual differences in median DI ($n = 92$)	K-W = 18.49 (p =1)	K-W = 1001.70 ($p = 1$)	K-W = 9.76 **($p < 0.001$)**	**Inter-individual variation in DI in the NFZ** but not in the NFZ.
	Social differentiation (SD) ($n = 92$)	SD between ≥ 78.76 % of dyads **($p < 0.01$)**	SD between ≥ 99.95 % of dyads **($p < 0.01$)**	SD between ≥78.76 % of dyads **($p < 0.01$)**	**Social differentiation present in all networks.**
Temporal variation in sociality	Difference in median DI between days ($n = 92$, $d = 28$)	K-W =2353.70 ($p = 1$)	K-W = 63.41 ($p = 1$)	K-W = 2376.40 ($p = 1$)	No difference in DI between days in all networks.
	Relationship between MDI and days ($n = 92$, $d = 28$)	Pearson correlation, $\rho = -0.05$ ($p = 0.81$)	Pearson correlation, $\rho = 0.34$ ($p = 0.07$)	Pearson correlation, $\rho = -0.11$ ($p = 0.28$)	MDI weakly correlated over time in both functional zones.
	Relationship between MDI and temperature ($n = 92$, $d = 28$)	Pearson correlation, $\rho = -0.11$ ($p = 0.59$)	Pearson correlation, $\rho = -0.10$ ($p = 0.62$)	Pearson correlation, $\rho = -0.55$ ($p = 0.79$)	Weak negative correlation between MDI and temperature in both functional zones.

	Relationship between four-day block consecutive networks (six networks, n = 92 per network)	Mantel test, range of R_s = 0.09to 0.25 **($p < 0.001$)** for four comparisons (day blocks 3-4, 4-5, 5-6, 6-7); range of R_s = -0.26 to 0.03 ($p \geq 0.09$) for two comparisons (day blocks 1-2, 2-3)	Mantel test, range of R_s = 0.21 to 0.44 (**$p < 0.001$**)	Mantel test, range of R_s = 0.07 to 0.25 **($p < 0.001$)** for four comparisons (day blocks 3-4, 4-5, 5-6, 6-7); range of R_s = -0.34 to -0.003 (p= 1) for two comparisons (day blocks 1-2, 2-3)	Weak correlation between all consecutive networks.
Individual characteristics	Difference in mean DI between non-lame (n = 26) and lame cows (n = 22)	Wilcoxon test, W = 286.00 (p = 0.49)	Wilcoxon test, W = 315.50 (p = 0.71)	Wilcoxon test, W = 271.00 (p = 0.35)	No difference in DI between non-lame and lame cows in both functional zones.
	Difference in mean clustering coefficient between non-lame (n = 26) and lame cows (n = 22)	Wilcoxon test, W = 325.00 (p = 0.79)	Wilcoxon test, W = 301.00 (p = 0.61)	Wilcoxon test, W = 374.00 (p = 0.96)	No difference in clustering coefficient between non-lame and lame cows in either functional zone.
	Difference in mean node degree between non-lame (n = 26) and lame cows (n = 22)	Wilcoxon test, W = 321.50 (p = 0.75)	Wilcoxon test, W = 295.50 (p = 0.56)	Wilcoxon test, W = 245.50 (p = 0.18)	No difference in node degree between non-lame and lame cows in either functional zone.
	Social assortment by lameness status by day, n = 48)	Mantel test, R_s = 0.10 **($p = 0.01$)** for day 26;	Mantel test, range of R_s = -0.07 to 0.06	Mantel test, range of R_s = -0.07 to 0.07 ($p \geq 0.39$)	

		range of R_s= -0.06 to 0.06 ($p \geq 0.89$) for 27 days	($p \geq 0.37$ for all days)		Cows did not socially assort according to their lameness status, parity, or DIM in either functional zone.
	Social assortment by parity (by day, n = 92)	Mantel test, range of R_s = -0.02 to 0.03 ($p = 1$ for all days)	Mantel test, range of R_s = -0.04 to 0.03 ($p = 1$ for all days)	Mantel test, range of R_s = -0.02 to 0.03 ($p \geq 0.84$ for all days)	
	Social assortment by DIM (by day, n = 92)	Mantel test, range of R_s = -0.03 to 0.03 ($p = 1$ for all days)	Mantel test, range of R_s = -0.04 to 0.03 ($p = 1$ for all days)	Mantel test, range of R_s = 0.02 to 0.04 ($p \geq 0.45$ for all days)	

Table A4. Overview of results using a spatial threshold radius of r = 5m and time duration of t = 60s to define an interaction on the commercial farm in Essex for the full barn and the functional zones: feeding zone and non-feeding zone. Basic network measures (original and filtered by mean degree), inter-individual variation, temporal variation in sociality, lameness status, and parity and days in milk, where (M)DI = (median) daily interactions, are shown Significant results ($p < 0.05$) are in bold.

	Measure	**Test value** (p-value)			**Summary**
		Full barn	**Feeding zone**	**Non-feeding zone**	
Basic network measures	Mean edge density (d = 28)	1	0.59	1.00	The networks are highly dense, more so the NFZ than the FZ.
	Components (by day) (d = 28)	1	1-3	1	The networks typically consist of one component.
Inter-individual variation	Inter-individual differences in median DI (n = 92)	K-W = 21.78 (p =1)	K-W = 1018.60 (p = 1)	K-W = 11.98 **($p < 0.001$)**	**Inter-individual variation in DI in the NFZ** but not in the NFZ.
	Social differentiation (SD) (n = 92)	SD between ≥ 70.91 % of dyads **($p < 0.01$)**	SD between ≥ 99.95 % of dyads **($p < 0.01$)**	SD between ≥ 70.60 % of dyads **($p < 0.01$)**	**Social differentiation present in all networks.**
Temporal variation in sociality	Difference in median DI between days (n = 92, d = 28)	K-W =2370.70 (p = 1)	K-W = 65.58 (p = 1)	K-W = 2394.40 (p = 1)	No difference in DI between days in all networks.
	Relationship between MDI and days (n = 92, d = 28)	Pearson correlation, ρ = -0.06 (p = 0.76)	Pearson correlation, ρ = 0.33 (p = 0.08)	Pearson correlation, ρ = -0.06 (p = 0.74)	MDI weakly correlated over time in both functional zones.
	Relationship between MDI and	Pearson correlation, ρ = -0.15 (p = 0.44)	Pearson correlation, ρ = -0.07 (p = 0.73)	Pearson correlation, ρ = -0.15 (p = 0.44)	Weak negative correlation between MDI and temperature in both functional zones.

	temperature (n = 92, d = 28)				
	Relationship between four-day block consecutive networks (six networks, n = 92 per network)	Mantel test, range of R_s = 0.04 to 0.24 **($p \leq 0.02$)** for five comparisons (day blocks 2-3, 3-4, 4-5, 5-6, 6-7); R_s = -0.26 (p = 1) for one comparison (day block 1-2)	Mantel test, range of R_s = 0.23 to 0.50 (**$p < 0.001$**)	Mantel test, range of R_s = 0.19 to 0.25 **($p < 0.001$)** for four comparisons; (day blocks 2-3, 4-5, 5-6, 5-7); range of R_s = -0.33 to 0.01 (p = 1) for two comparisons (day blocks 1-2, 3-4)	Weak correlation between all consecutive networks.
Individual characteristics	Difference in mean DI between non-lame (n = 26) and lame cows (n = 22)	Wilcoxon test, W = 292.00 (p = 0.52)	Wilcoxon test, W = 312.50 (p = 0.69)	Wilcoxon test, W = 277 (p = 0.40)	No difference in DI between non-lame and lame cows in both functional zones.
	Difference in mean clustering coefficient between non-lame (n = 26) and lame cows (n = 22)	Wilcoxon test, W = 266.00 (p = 0.33)	Wilcoxon test, W = 297 (p = 0.56)	Wilcoxon test, W = 343.00 (p = 0.87)	No difference in clustering coefficient between non-lame and lame cows in either functional zone.
	Difference in mean node degree between non-lame (n = 26) and lame cows (n = 22)	Wilcoxon test, W = 337.50 (p = 0.85)	Wilcoxon test, W = 303.50 (p = 0.62)	Wilcoxon test, W = 267.50 (p = 0.34)	No difference in node degree between non-lame and lame cows in either functional zone.

	Social assortment by lameness status by day, *n* = 48)	Mantel test, $R_s = 0.10$ **(*p*= 0.02)** for day 26; range of R_s= -0.06 to 0.06 ($p \geq 0.58$) for 27 days	Mantel test, range of R_s = -0.06 to 0.06 ($p \geq 0.48$ for all days)	Mantel test, range of R_s = -0.06 to 0.08 ($p \geq 0.17$)	Cows did not socially assort according to their lameness status, parity, or DIM in either functional zone.
	Social assortment by parity (by day, *n* = 92)	Mantel test, range of R_s = -0.02 to 0.03 (p =1 for all days)	Mantel test, range of R_s = -0.05 to 0.04 (p = 1 for all days)	Mantel test, range of R_s = -0.01 to 0.03 (p = 1 for all days)	
	Social assortment by DIM (by day, *n* = 92)	Mantel test, range of R_s = -0.03 to 0.03 (p = 1 for all days)	Mantel test, range of R_s = -0.04 to 0.03 (p = 1 for all days)	Mantel test, range of R_s= -0.03 to 0.03 ($p \geq 0.59$ for all days)	

Table A5. Overview of results using a spatial threshold radius of r = 3m and time duration of t = 40s to define an interaction on the commercial farm in Essex for the full barn and the functional zones: feeding zone and non-feeding zone. Basic network measures (original and filtered by mean degree), inter-individual variation, temporal variation in sociality, lameness status, and parity and days in milk, where (M)DI = (median) daily interactions, are shown Significant results ($p < 0.05$) are in bold.

	Measure	**Test value** (p-value)			**Summary**
		Full barn	**Feeding zone**	**Non-feeding zone**	
Basic network measures	Mean edge density (d = 28)	0.99	0.52	0.98	The networks are highly dense, more so the NFZ than the FZ.
	Components (by day) (d = 28)	1	1-3	1	The networks typically consist of one component.
Inter-individual variation	Inter-individual differences in median DI (n = 92)	K-W = 20.90 **($p < 0.001$)**	K-W = 1001.30 ($p = 1$)	K-W = 12.94 **($p < 0.001$)**	**Inter-individual variation in DI in the NFZ** but not in the NFZ.
	Social differentiation (SD) (n = 92)	SD between ≥ 82.30 % of dyads **($p < 0.01$)**	SD between 100 % of dyads **($p < 0.01$)**	SD between ≥ 83.30 % of dyads **($p < 0.01$)**	**Social differentiation present in all networks.**
Temporal variation in sociality	Difference in median DI between days (n = 92, d = 28)	K-W =2340.10 ($p = 1$)	K-W = 57.27 ($p = 1$)	K-W = 2358.10 ($p = 1$)	No difference in DI between days in all networks.
	Relationship between MDI and days (n = 92, d = 28)	Pearson correlation, $\rho = -0.06$ ($p = 0.74$)	Pearson correlation, $\rho = 0.27$ ($p = 0.16$)	Pearson correlation, $\rho = -0.07$ ($p =0.73$)	MDI weakly correlated over time in both functional zones.
	Relationship between MDI and temperature (n = 92, d = 28)	Pearson correlation, $\rho = -0.06$ ($p = 0.76$)	Pearson correlation, $\rho = -0.13$ ($p = 0.52$)	Pearson correlation, $\rho = -0.06$ ($p = 0.77$)	Weak negative correlation between MDI and temperature in both functional zones.

	Relationship between four-day block consecutive networks (six networks, $n = 92$ per network)	Mantel test, range of $R_s = 0.05$ to 0.29 **($p < 0.01$)** for five comparisons (day blocks 2-3, 3-4, 4-5, 5-6, 6-7); $R_s = -0.25$ ($p = 1$ for one comparison (day block 1-2)	Mantel test, range of $R_s = 0.18$ to 0.41 (**$p < 0.001$**)	Mantel test, range of $R_s = -0.28$ to 0.29 **($p < 0.001$)** for five comparisons (day blocks 2-3, 3-4, 4-5, 5-6, 6-7); $R_s = -0.28$ ($p = 1$) for one comparison (day block 1-2)	Weak correlation between all consecutive networks.
Individual characteristics	Difference in mean DI between non-lame ($n = 26$) and lame cows ($n = 22$)	Wilcoxon test, W = 280.00 ($p = 0.42$)	Wilcoxon test, W = 305.50 ($p = 0.63$)	Wilcoxon test, W = 236.00 ($p = 0.14$)	No difference in DI between non-lame and lame cows in both functional zones.
	Difference in mean clustering coefficient between non-lame ($n = 26$) and lame cows ($n = 22$)	Wilcoxon test, W = 338.00 ($p = 0.85$)	Wilcoxon test, W = 270.00 ($p = 0.35$)	Wilcoxon test, W = 375.00 ($p = 0.96$)	No difference in clustering coefficient between non-lame and lame cows in either functional zone.
	Difference in mean node degree between non-lame ($n = 26$) and lame cows ($n = 22$)	Wilcoxon test, W = 331.50 ($p = 0.81$)	Wilcoxon test, W = 293.00 ($p = 0.54$)	Wilcoxon test, W = 251.00 ($p = 0.22$)	No difference in node degree between non-lame and lame cows in either functional zone.
	Social assortment by lameness status by day, $n = 48$)	Mantel test, $R_s = 0.09$ **($p = 0.03$)** for day 26; range of $R_s = -0.07$ to 0.06	Mantel test, range of $R_s = -0.06$ to 0.05 ($p \geq 0.76$ for all days)	Mantel test, range of $R_s = -0.07$ to 0.07 ($p \geq 0.24$)	

		($p \geq 0.84$) for 27 days			Cows did not socially assort according to their lameness status, parity, or DIM in either functional zone.
	Social assortment by parity (by day, $n = 92$)	Mantel test, range of $R_s = -0.02$ to 0.02 ($p = 1$ for all days)	Mantel test, range of $R_s = -0.05$ to 0.02 ($p = 1$ for all days)	Mantel test, range of $R_s = -0.02$ to 0.03 ($p \geq 0.97$ for all days)	
	Social assortment by DIM (by day, $n = 92$)	Mantel test, range of $R_s = -0.02$ to 0.03 ($p \geq 0.74$ for all days)	Mantel test, range of $R_s = -0.05$ to 0.02 ($p = 1$ for all days)	Mantel test, range of $R_s = -0.02$ to 0.03 ($p \geq 0.42$ for all days)	

Table A6. Overview of results using a spatial threshold radius of r = 3m and time duration of t = 80s to define an interaction on the commercial farm in Essex for the full barn and the functional zones: feeding zone and non-feeding zone. Basic network measures (original and filtered by mean degree), inter-individual variation, temporal variation in sociality, lameness status, and parity and days in milk, where (M)DI = (median) daily interactions, are shown Significant results ($p < 0.05$) are in bold.

	Measure	**Test value** (p-value)			**Summary**
		Full barn	**Feeding zone**	**Non-feeding zone**	
Basic network measures	Mean edge density (d = 28)	0.93	0.23	0.91	The networks are highly dense, more so the NFZ than the FZ.
	Components (by day) (d = 28)	1	1-4	1	The networks typically consist of one component.
Inter-individual variation	Inter-individual differences in median DI (n = 92)	K-W = 18.92 **($p < 0.001$)**	K-W = 953.09 (p =1)	K-W = 13.94 **($p < 0.001$)**	**Inter-individual variation in DI in the NFZ** but not in the FZ.
	Social differentiation (SD) (n = 92)	SD between > 100 % of dyads **($p < 0.01$)**	SD between 99.98 % of dyads **($p < 0.01$)**	SD between 82.30 % of dyads **($p < 0.01$)**	**Social differentiation present in all networks.**
	Difference in median DI between days (n = 92, d = 28)	K-W = 2327.80 (p = 1)	K-W = 57.29 (p = 1)	K-W = 2338.4 (p = 1)	No difference in DI between days in all networks.
	Relationship between MDI and days (n = 92, d = 28)	Pearson correlation, ρ = -0.05 (p = 0.79)	Pearson correlation, ρ = 0.28 (p = 0.15)	Pearson correlation, ρ = -0.06 (p = 0.80)	MDI not correlated over time in either functional zone.

Temporal variation in sociality	Relationship between MDI and temperature ($n = 92$, $d = 28$)	Pearson correlation, $\rho = -0.04$ ($p = 0.86$)	Pearson correlation, $\rho = -0.17$ ($p = 0.39$)	Pearson correlation, $\rho = -0.04$ ($p = 0.86$)	Weak correlation between MDI and temperature in both functional zones.
	Relationship between four-day block consecutive networks (six networks, $n = 92$ per network)	Mantel test, range of $R_s = 0.03$ to 0.31 **($p \leq 0.001$)** for four comparisons (day blocks 3-4, 4-5, 5-6, 6-7); range of $R_s = -0.25$ to 0.03 ($p \geq 0.18$) for two comparisons (day blocks 1-2, 2-3)	Mantel test, range of $R_s = 0.12$ to 0.30 **($p < 0.001$)**	Mantel test, range of $R_s = -0.08$ to 0.31 **($p < 0.001$)** for four comparisons (day blocks 3-4, 4-5, 5-6, 6-7); range of $R_s = -0.28$ to 0.02 ($p \geq 0.58$) for two comparisons (day blocks 1-2, 2-3)	Weak correlation between all consecutive networks.
Individual characteristics	Difference in mean DI between non-lame (n = 26) and lame cows (n = 22)	Wilcoxon test, W = 274.50 ($p = 0.39$)	Wilcoxon test, W = 317 ($p = 0.73$)	Wilcoxon test, W = 235.00 ($p = 0.14$)	No difference in DI between non-lame and lame cows in both functional zones.
	Difference in mean clustering coefficient between non-lame (n = 26) and lame cows (n = 22)	Wilcoxon test, W = 429.00 ($p = 1$)	Wilcoxon test, W = 284.00 ($p = 0.46$)	Wilcoxon test, W = 441 ($p = 1$)	No difference in clustering coefficient between non-lame and lame cows in either functional zone.
	Difference in mean node degree between	Wilcoxon test, W = 308.00 ($p = 0.66$)	Wilcoxon test, W = 267.00 ($p = 0.33$)	Wilcoxon test, W = 274.50 ($p = 0.39$)	No difference in node degree between non-lame and lame cows in either functional zone.

	non-lame (n = 26) and lame cows (n = 22)				
	Social assortment by lameness status by day, n = 48)	Mantel test, range of R_s= -0.07 to 0.08 ($p \geq 0.14$)	Mantel tests, range of R_s = -0.04 to 0.08 ($p \geq 0.07$ for all days)	Mantel test, range of R_s= -0.07 to 0.07 ($p \geq 0.32$)	Cows did not socially assort according to their lameness status, parity, or DIM in either functional zone.
	Social assortment by parity (by day, n = 92)	Mantel test, range of R_s = -0.02 to 0.03 (p = 1 for all days)	Mantel test, range of R_s = -0.04 to 0.04 (p = 1 for all days)	Mantel test, range of R_s = -0.02 to 0.03 (p =1 for all days)	
	Social assortment by DIM (by day, n = 92)	Mantel test, range of R_s = -0.02 to 0.03 (p = 0.78 for all days)	Mantel test, range of R_s = -0.04 to 0.03 (p = 1 for all days)	Mantel test, range of R_s = -0.02 to 0.03 ($p \geq 0.41$ for all days)	

Table A7. Overview of results using a spatial threshold radius of r = 3m and time duration of t = 100s to define an interaction on the commercial farm in Essex for the full barn and the functional zones: feeding zone and non-feeding zone. Basic network measures (original and filtered by mean degree), inter-individual variation, temporal variation in sociality, lameness status, and parity and days in milk, where (M)DI = (median) daily interactions, are shown Significant results ($p < 0.05$) are in bold.

	Measure	**Test value** (p-value)			**Summary**
		Full barn	**Feeding zone**	**Non-feeding zone**	
Basic network measures	Mean edge density ($d = 28$)	0.89	0.15	0.87	The networks are highly dense, more so the NFZ than the FZ.
	Components (by day) ($d = 28$)	1	1-5	1	The networks typically consist of one component.
Inter-individual variation	Inter-individual differences in median DI ($n = 92$)	K-W = 16.79 **($p < 0.001$)**	K-W = 937.39 ($p = 1$)	K-W = 13.95 **($p < 0.001$)**	**Inter-individual variation in DI in the NFZ** but not in the NFZ.
	Social differentiation (SD) ($n = 92$)	SD between ≥ 85.63 % of dyads **($p < 0.01$)**	SD between 100 % of dyads **($p < 0.01$)**	SD between 99.98 % of dyads **($p < 0.01$)**	**Social differentiation present in all networks.**
	Difference in median DI between days ($n = 92$, $d = 28$)	K-W =2327.10 ($p = 1$)	K-W = 50.69 ($p = 1$)	K-W = 2332.50 ($p = 1$)	No difference in DI between days in all networks.
	Relationship between MDI and days ($n = 92$, $d = 28$)	Pearson correlation, $\rho = -0.05$ ($p = 0.79$)	Pearson correlation, $\rho = 0.28$ ($p = 0.14$)	Pearson correlation, $\rho = -0.05$ ($p = 0.79$)	MDI weakly correlated over time in both functional zones.

Temporal variation in sociality	Relationship between MDI and temperature (n = 92, d = 28)	Pearson correlation, ρ = -0.03 (p = 0.88)	Pearson correlation, ρ = -0.16 (p = 0.42)	Pearson correlation, ρ = -0.03 (p = 0.88)	Weak negative correlation between MDI and temperature in both functional zones.
	Relationship between four-day block consecutive networks (six networks, n = 92 per network)	Mantel test, range of R_s = 0.06 to 0.31 **(p ≤ 0.001)** for five comparisons (day block 2-3, 3-4, 4-5, 5-6, 6-7); R_s = -0.24(p = 1) for one comparison (day block 1-2)	Mantel test, range of R_s = 0.12 to 0.28 (**p ≤ 0.001** for all days)	Mantel test, range of R_s= 0.07 to 0.30 **(p < 0.001)** for five comparisons (day blocks 2-3, 3-4, 4-5, 5-6, 6-7); R_s = -0.25 (p = 1) for one comparison (day block 1-2)	Weak correlation between all consecutive networks.
Individual characteristics	Difference in mean DI between non-lame (n = 26) and lame cows (n = 22)	Wilcoxon test, W = 263.50 (p = 0.30)	Wilcoxon test, W = 315.00 (p = 0.71)	Wilcoxon test, W = 236.00 (p = 0.14)	No difference in DI between non-lame and lame cows in both functional zones.
	Difference in mean clustering coefficient between non-lame (n = 26) and lame cows (n = 22)	Wilcoxon test, W = 420.00 (p = 1)	Wilcoxon test, W = 236.00 (p = 0.15)	Wilcoxon test, W = 436.00 (p = 1)	No difference in clustering coefficient between non-lame and lame cows in either functional zone.
	Difference in mean node degree between non-lame (n = 26) and lame cows (n = 22)	Wilcoxon test, W = 317.50 (p = 0.73)	Wilcoxon test, W = 315.00 (p = 0.71)	Wilcoxon test, W = 309.00 (p = 0.66)	No difference in node degree between non-lame and lame cows in either functional zone.

	Social assortment by lameness status by day, $n = 48$)	Mantel test, range of $R_s = -0.06$ to 0.08 ($p \geq 0.17$ for all days)	Mantel test, range of $R_s = -0.04$ to 0.06 ($p \geq 0.60$ for all days)	Mantel test, range of $R_s = -0.07$ to 0.07 ($p \geq 0.32$)	Cows did not socially assort according to their lameness status, parity, or DIM in either functional zone.
	Social assortment by parity (by day, $n = 92$)	Mantel test, range of $R_s = -0.02$ to 0.03 ($p = 1$ for all days)	Mantel test, range of $R_s = -0.03$ to 0.05 ($p \geq 0.96$ for all days)	Mantel test, range of $R_s = -0.02$ to 0.03 ($p = 1$ for all days)	
	Social assortment by DIM (by day, $n = 92$)	Mantel test, range of $R_s = -0.02$ to 0.03 ($p = 1$ for all days)	Mantel test, range of $R_s = -0.03$ to 0.03 ($p = 1$ for all days)	Mantel test, range of $R_s = -0.02$ to 0.04 ($p \geq 0.35$ for all days)	

3.7. Appendix 2: Additional results for different temporal segmentation of study period

In the main results, the temporal stability of the proximity interaction networks (feeding and non-feeding zone) are assessed by segmenting the 28-day study period into seven averaged four-day consecutive blocks, showing weak or non-significant, between networks in both functional zones. For completeness, each of the original daily networks (*n* = 28), as well as two-, seven-, and 14-day blocks are also compared. The results are qualitatively similar, showing weak correlations between all consecutive networks (Table A1).

Table A1. Temporal variation of the interaction network (interaction defined when cows (n = 92) were within a three-metre radius for more than 60 seconds), by segmenting and averaging the networks (feeding and non-feeding zone) into one, two, seven and 14 day-blocks. To calculate the p-value, 10,000 permutations were used, and p-values are after Bonferroni correction; significant results (p < 0.05) are in bold.

	Test value (p-value)		
Segmented components (days)	**Feeding zone**	**Non-feeding zone**	**Summary**
28 (1 day)	Range of R_s = 0.09 to 0.18 **($p \le 0.01$)** for 25 comparisons; range of R_s = 0.02 to 0.04 (p =1) for two comparisons (days 21-22, 26-27)	Range of R_s= -0.30 to 0.37 **($p \le 0.05$)** for seven comparisons (days 3-4, 7-8, 13-14, 17-18, 18-19, 25-26, 27-28); range of R_s = -0.28 to 0.03 ($p \ge$ 0.42) for20 comparisons	Weak correlation between all consecutive networks.
14 (2 days)	Range of R_s = 0.10 to 0.26 (**$p \le 0.01$**)	Range of R_s = 0.06 to 0.29 (**p < 0.01** for six comparisons (day blocks 2-3, 6-7, 10-11, 11-12, 12-13, 13-14); range of R_s = -0.22 to 0.19 (p = 1) for seven comparisons	
7 (4 days)	Range of R_s= 0.20 to 0.31 **(p < 0.001)**	Mantel test, range of R_s = 0.05 to 0.24 (**p < 0.01**) for four comparisons (day blocks 1-2, 2-3, 5-6, 6-7); range of R_s= -0.04 to 0.01 (p = 1) for two comparisons (day blocks 3-4, 4-5)	
4 (7 days)	Range of R_s= 0.14 to 0.41 (**p < 0.01**)	R_s = 0.28 **(p < 0.001)** for one comparison (day block 3-4); R_s = 0.004 (p = 1) for one comparison (day block 1-2); R_s = -0.16 (p = 1) for one comparison (day block 2-3)	
2 (14 days)	R_s = 0.41 (**p < 0.001**)	R_s= -0.07 (p = 1)	

4. CHAPTER 4: ANALYSING THE SOCIAL NETWORK STRUCTURE OF A DAIRY HERD GROUP SUBJECT TO FREQUENT REGROUPING

4.1. Introduction

Animal health and welfare varies amongst housed livestock herds for multiple reasons, partly due to their housing and management (319–321). Housing systems e.g., the physical infrastructure, size and shape of buildings, stocking density, group size, and management practices e.g., milking times, regrouping events, are typically chosen based on available funds and the farmer's perceived needs of the herd. These factors have the potential to impact interactions within the herd, which is important for dairy cows given they have social preferences, as demonstrated in Chapter 3 of this thesis, and considering social bonds provide support under stressful conditions and help with recovery from diseases (132,176,272,322).

High stocking densities are common in the dairy industry, which may inhibit the ability of cows to interact with preferred conspecifics, potentially explaining why assortment by attributes such as parity is found in some herds but not others; the latter is the case in Chapter 3 (176,273,315). This may be dependent on the area of the barn, if there are varying space allowances, as in Chapter 3. With limited space, aggressive encounters may increase, such as dominant cows displacing subordinates more often for resources, particularly when these are limited and without the presence of barriers (207,323–326). Moreover, subordinates may be unable to avoid dominants, leading to an increase in social stress of the former (135,158). It may that subordinate behaviour is temporary and related to illness, which would be useful to identify.

It is well established that removing a cow from a herd is stressful for the individual, but regrouping can also impact the remaining herd's interactions (144,327–329). After introducing a new individual into a herd, cows within the group have been found to reduce allogrooming, and overall agonistic interactions in the group increase e.g., displacements from feeding areas (144,157). Individual factors can influence social interactions; younger and smaller individuals may be more vulnerable to aggression and social exclusion, particularly following regrouping. Calves, in particular, may be less physically competitive and less dominant in social interactions compared to larger and older individuals, making them more susceptible to negative outcomes (330). To reduce agonistic behaviours among adults, consistent group composition is likely to be important, as it allows individuals to become familiar with each other and establish a clear dominance hierarchy. However, with younger animals like calves, a clear dominance hierarchy may not establish, making their social interactions less predictable and more aggressive (132,168,331,332).

Although social network analysis (SNA) has been recognised for use with farm animals, it is yet to be applied widely for management purposes, despite its use in understanding animal interactions, networks, and behaviour in productivity and disease outcomes (147,333–336). Understanding factors impacting group dynamics and space-use could help refine management regimes to optimise animal welfare whilst maximising productivity. A first step is finding whether there are differences between groups under various management systems.

In this chapter, SNA is used to analyse and compare the social network structure and space-use patterns of two dairy cow herds, the first housed on a commercial farm in Essex and a second referred to as the ‘RVC Research farm’, using local positioning data (see Chapter 2 for details). These groups were subject to different management practices in terms of their housing, group sizes, stocking density and frequency of regrouping, all of which may affect the social network structure and space-use. So, the main aim of this chapter is to determine whether social networks derived from local positioning data can capture differences in behaviour between two dairy herds, most likely attributed to their housing in two different animal productions systems.

4.2. Methods

4.2.1. Animals and housing

As detailed in the General Methodology (Chapter 2, Section 2.4), two dairy cow herds were monitored for this study: the first on a commercial farm in Essex (monitored from 1st August 2014 to 30th November 2014), and the second is referred to as the RVC Research farm (monitored from 17th October 2015 to 7th December 2015). The main design and area differences in the production systems focussed on are that the cows on the commercial farm in Essex were housed in a barn with cubicles, henceforth referred to as a 'closed barn', at a high stocking density (n = 127; minimum of 86 unique cows each month and a maximum of 111 unique cows per month; feed space = 4.80m^2 to 6.20 m^2 per cow, lying space = 3.53m^2 to 4.55m^2 per cow; overall area = 2898m^2 (63m x 46m), feeding zone (FZ) = 391.28m^2 (58.40m x 6.7m), non-feeding zone (NFZ) = 553.13m^2 (left and right cubicle area = 52.60m x 9.7m = 510.22m^2 excluding two unusable cubicles, each 2.71m^2 (1.18m x 2.30m), = 504.80m^2; lower cubicle area = (27.75m x 2.42m = 67.16m^2 excluding seven unusable cubicles, each 2.69m^2, = 48.33m^2; space between neighbouring cubicles: left and right cubicle blocks = 3.59m; right and lower cubicle blocks = 2.4m), whereas the herd on the RVC Research farm were housed in a barn without cubicles, henceforth referred to as an 'open barn', at a moderate stocking density (n = 55, a minimum of 45 cows each month; stocking density of feed space = 4.04m^2 to 4.93m^2 per cow, lying space = 7.28m^2 to 8.90m^2 per cow; overall area = 2469.60m^2 (58.8m x 42m), feeding zone (FZ) = 222.05m^2 (40.3m x 5.51m), non-feeding zone (NFZ) = 400.58m^2 (40.30m x 9.94m)). For further details on the herds and visualisations of the barn layouts, please refer to Chapter 2, Section 2.4.

4.2.2. Local positioning system

Each cow was equipped with a combined local positioning sensor and accelerometer, Omnisense 500 (298), mounted on a weighted neck collar. Further information on the sensor system is provided in Chapter 2, Section 2.5.

Data selection and pre-processing were conducted as outlined in Chapter 2, Section 2.4. For the main analysis, hours when most cows were in the milking parlour or collecting yard were excluded (for the commercial farm in Essex: 05:00 to 07:59, 12:00 to14:59 and 20:00 to 22:59 and for the RVC Research farm: 05:00 to 07:59 and 14:00 to 16:59), as well as periods during which the sensor reset (approximately 23:00 to 00:59 on both farms), since their behaviour during these time periods was constrained by farm staff.

4.2.3. Protocol for determining proximity interactions

The protocol for determining proximity interactions was defined as when dyads were within 3m for a minimum of 60s, with longer time durations counting as more than one interaction, as outlined in detail in Chapter 2, Section 2.4. While these parameter values are consistent with previous studies e.g., (176,273), a range of other values for r and t are also considered, as well as less stringent protocols (where only a certain percentage of points within the specified time period need to be within the specified radius for an interaction to be identified). Using observed data of (n = 35) known proximity interactions it was possible to validate the algorithm and determine the sensitivity (true positive rate) of this protocol (0.83); it was not possible to estimate the specificity using this observed data, but the r and t parameters were chosen to reduce the expected false positive rate, as well as taking into account practical and biological considerations, including the sensor mean error distance and the typical size of a dairy cow (see Chapter 2, Section 2.4.1.). Also, qualitatively similar results were obtained for the commercial farm in Essex herd when using $t = 40, 80, 100$ s (for $r = 3$m) and $r = 1, 2, 4$ and 5m (for $t = 60$ s), and hence the conclusions should be robust to this parameter choice (see Chapter 3, Appendix 1).

4.2.4. Social network analysis

An explanation of how daily networks were visualised, for the full barn, the feeding zone (FZ) and the non-feeding zone (NFZ), is provided in Chapter 2 (Section 2.5.2).

Edge density, the proportion of direct ties in a network relative to total ties possible, was calculated for the full barn and each of the functional zones (as outlined in Chapter 2, Section 2.6). Permutations ($q = 10{,}000$) were used to account for the non-independence of dyads, and details of this are provided in Chapter 2, Section 2.6.

Direct comparison between the barns using graph measures such as edge density and social differentiation without bias is not possible, given these are influenced by the number of nodes and edges (165,278,337,338), and considering the herd sizes are different (maximum $n = 127$ and $n = 55$ for the commercial farm in Essex and the RVC Research farm, respectively). A network with more nodes leads to more possible edges, but a smaller network (fewer nodes) is likely to be more connected i.e., a higher edge density. Attempts have been made to compare social networks across group sizes with some success through the triadic census method, which counts the frequency of different types of triads (groups of three nodes) and their connections (165,337). These methods are limited, however, with triadic census explained by simple node-based measures, and so there is ongoing attempt to compare networks which is beyond the scope of the work in this chapter (165,337). This chapter therefore does not set out to draw direct quantitative comparisons between the social network structure and space-use of the herd groups, but broad patterns within each group are detected and compared.

Although direct comparisons cannot be drawn between the farms, a crude comparison of edge density is used to understand the extent to which each network is connected, for the full barns and each of the functional zones (NFZ and FZ). Moreover, if the open, lower stocked barn of the RVC Research farm is found to be less connected than the closed, higher stocked barn network of the commercial farm in Essex, this would support a hypothesis of the RVC Research farm having reduced connectivity compared to the commercial farm in Essex. This is given a network with less nodes is more, not less, likely to be highly connected compared to a network with more nodes.

Inter-individual variation in median daily interactions (the mean number of proximity interactions an individual had per day) across each of the study durations was assessed through a Kruskal-Wallis Rank Sum test, using 10,000 node-level permutations to account for the non-independence of dyads. Social differentiation was calculated daily for each functional zone (FZ and NFZ), by finding the difference between the observed number of interactions and the expected number of interactions, summing this for each dyad, and then dividing this by the total number of dyads (refer to Chapter 2.5.4 for details). Again, the numerical outputs for these measures of social differentiation are not directly compared; instead, broad patterns are compared as an initial step to understand potential differences.

4.2.5. Space-use patterns

As outlined in Chapter 2 (Section 2.6.1), space-use heatmaps were created by overlaying virtual grids (1.5m by 1.5m) over the barn layouts and counting the number of points lying in each cell. The highest density cells cumulatively adding to 50% or 95% of the total observed density were defined as the core and full range size. Patterns in space-use and activity patterns were then investigated e.g., clear gaps in specific areas of the barn, using core and full range size, as well as range size ratio (core range divided by full range). Comparisons were drawn between the findings from each herd.

To demonstrate the efficacy of the range size metrics, time-dependent effects were explored. The data were split into periods between milking times: interval one (I1; the commercial farm in Essex: 01:00 to 04:00; the RVC Research farm: 08:00 to 13:00), interval two (I2; the commercial farm in Essex: 08:00 to 11:00; the RVC Research farm: 19:00 to 22:00 and 01:00 to 04:00) and interval three (I3; the commercial farm in Essex: 15:00-19:00). Range size ratios were calculated for each interval and compared between the two herds.

Finally, the space-use metrics were confirmed as sensible by comparing core range and full range sizes calculated for hours during and between milking periods. The range sizes were expected to decrease during milking periods, as areas outside of the functional zones were not taken into account to calculate range size (and hence range size ratio) i.e., only a small number of cows were expected to remain in the functional zones during milking times which would reduce the calculated values.

4.3. Results

4.3.1. Basic network measures and visualization

Figure 1 compares visualisations of the original networks, for the full barn and each functional zone on the RVC Research farm (similar to Fig 2 in Chapter 3, Section 3.3.1 for the commercial farm in Essex).

As expected, on both farms, the full barns are more connected than the non-feeding zones (NFZ) (0.02 and 0.16 difference in edge density for the commercial farm in Essex and the RVC Research farm respectively) and feeding zones (FZ) (0.63 and 0.09 difference in edge density for the commercial farm in Essex and the RVC Research farm respectively) (Fig 1; Table 1). On the commercial farm in Essex, the FZ is less connected than the NFZ zone (0.61 difference in edge density) whereas on the RVC Research farm the FZ is more connected than the NFZ (0.07 difference in edge density) (Fig 1; Table 1).

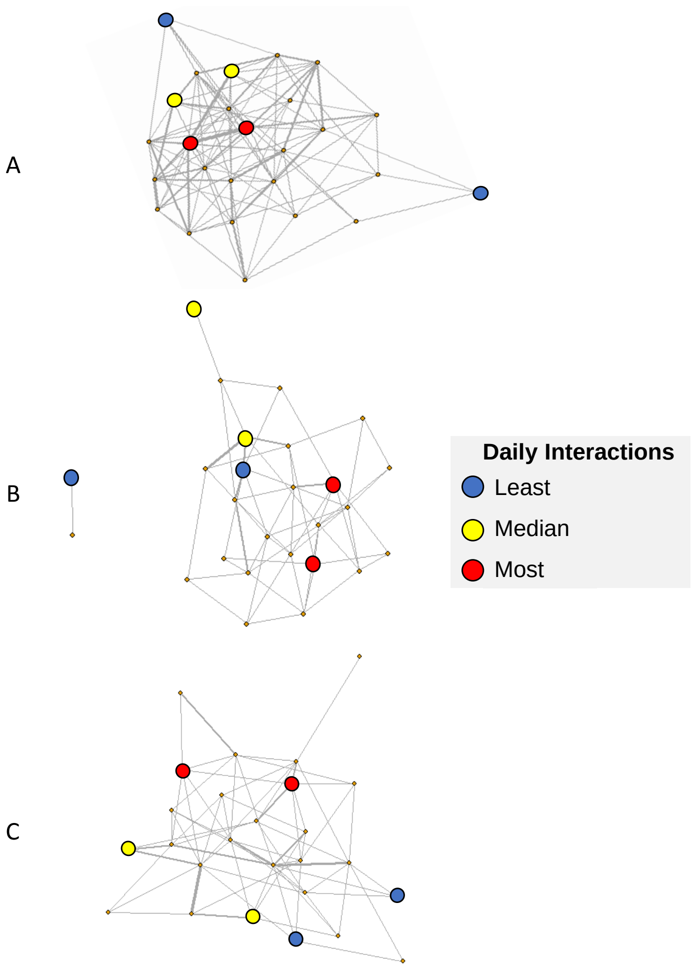

Figure 1. Undirected networks on a randomly chosen day, 17/10/2015, in (A) the full barn, (B) feeding zone, and (C) non-feeding zone of the RVC Research farm herd, showing interactions between individual cows. Thicker edges indicate a higher number of daily interactions. The Fruchterman-Reingold layout algorithm was used to determine the node positions; unconnected vertices are repelled. A subset of individuals at the middle and extreme ends of the dataset for this example day are highlighted to illustrate the extent of the observed data: two cows with the lowest daily interactions (DI) during the example day (cow ID = 765 and 530 both with DI of 3 and 5, respectively), two cows with DI closest to the median (cow ID = 785 and 652 both with DI of 11 and 10 respectively), and two cows with the most DI (cow ID = 576 and 702 with DI of 19 and 18, respectively). Across the full herd, the mean DI were 15 (median = 14, standard deviation = 2.64). The graphs were created in RStudio using the ‘vegan’ package (68,69,75).

4.3.2. Social differentiation

There is evidence of significant inter-individual variation in daily interactions in the full barn on the RVC Research farm (Table 1; Kruskal-Wallis chi-squared [hereafter K-W] = 69.29, df = 53, after 10,000 permutations $p = 0.001$) and in the FZ (Table 1; K=W = 109.36, df = 53, after 10,000 permutations, $p < 0.001$), but not in the NFZ (Table 1; K-W = 69.29, df = 53, after 10,000 permutations, $p = 0.06$).

Social differentiation is present across the full barn on the RVC Research farm (Table 1; ≥ 99.98% of dyads, $p < 0.01$ across days), the FZ (Table 1; 100% of dyads, $p < 0.01$ across days), and the NFZ (Table 1; 100% of dyads, $p < 0.01$ across days) on the RVC Research farm.

Table 1. Overview of the social network analysis results for the herd on the commercial farm in Essex and the herd on the RVC Research farm, for the full barns, the feeding zones (FZ) and the non-feeding zones (NFZ): basic network measures and inter-individual variation. A spatial threshold radius of 3m and time duration of $t = 60$s was used to define an interaction, where (M)DI = (median) daily interactions. Significant results ($p < 0.05$) are in bold.

		Test results with *p*-values			**Summary**
Metric	**Farm**	**Full barn**	**Feeding zone**	**Non-feeding zone**	
Mean edge density	Commercial farm in Essex ($d = 28$)	0.96	0.33	0.94	**Highly dense networks**, more so the NFZ than the FZ.
	RVC Research farm ($d = 47$)	0.28	0.19	0.12	**Weakly connected networks**, more so the NFZ than the FZ.
Inter-individual differences in median DI	Commercial farm in Essex	K-W = 26.53 ($p < 0.001$)	K-W = 851.71 **($p = 1$)**	K-W = 19.21 ($p < 0.001$)	**Inter-individual variation in DI the NFZ** but not the FZ **on the commercial farm in Essex** whereas **inter-individual variation in the FZ** but not the NFZ **on the RVC Research farm.**
	RVC Research farm	K-W = 149.10 **($p < 0.001$)**	K-W = 109.36 **($p < 0.001$)**	K-W = 69.29 ($p = 0.06$)	
Social differentiation (SD)	Commercial farm in Essex	SD between > 92.96 % of dyads **($p < 0.01$)**	SD between 100% of dyads **($p < 0.01$)**	SD between 92.96% of dyads **($p < 0.01$)**	Strong social differentiation present in all networks.
	RVC Research farm	SD between ≥ 99.98% **($p < 0.01$)**	SD between 100% of dyads **($p < 0.01$)**		

4.3.3. Space-use comparison between farms

4.3.3.1. General space-use

There are clear gaps between cubicles in the NFZ ($y \leq 10.5$) on the commercial farm in Essex where passageways are present, across the study duration (Fig 2). For example, there is a space between the set of left and right cubicles at approximately $x = 20$, and these grid cells are not incorporated into the highest density cells comprising the core range, whereas the areas corresponding to cubicles are (Fig 2). Despite a decline in use from left to right, the core range on the RVC Research farm remains unsegmented across the y-axis in Figure 2, with no noticeable gaps in the NFZ ($y \leq 10$; boundary rounded to nearest 0.5).

The mean range size ratio on the commercial farm in Essex is higher than on the RVC Research farm (mean = 0.279, SD = 0.011 and mean = 0.268, SD = 0.006 respectively; $t = 3.37$, $df = 17.82$, $p = 0.003$, excluding milking hours) (Fig 2; Table 2). The higher range size ratio on the commercial farm in Essex suggests a more equitable use of the space i.e., less use of specific areas, compared to on the RVC Research farm. This is further supported when comparing the time that individuals spend in the passages of the NFZ (16.67%) to the cubicles of the NFZ (83.33%) on the commercial farm in Essex, which can interpretated using visualisations provided in Figure 2, although this does not consider sensor error or that the cubicles covered a greater area in this zone in comparison to passageways (Table 2).

4.3.3.2. Time-dependent space-use

On the commercial farm in Essex, as expected, the cows appear to be more active during the day (I2: 08:00 to 11:00 and I3: 15:00 to 19:00) particularly during the morning (I2), compared to during the night (I1: 01:00-04:00; Table 2; Fig 3). This is also the case on the RVC Research farm, with cows more active during I1 (08:00 to 13:00) than I2 (19:00 to 22:00 and 01:00 to 04:00) (Table 2; Fig 3). Cows appear to be spending more time in fewer cells during the night (mean hourly range size ratios on the commercial farm in Essex during I1 = 0.262 to 0.274 and the RVC Research farm during I2 = 0.263 to 0.279, compared to during the day when they are more likely to be active (range size ratio on the commercial farm in Essex during I2 = 0.276 to 0.287 and I3 = 0.274 to 0.296, and on the RVC Research farm during I1 = 0.256 to 0.268 (Table 2; Fig 3).

Supporting this, on both farms, cows show greater use of the FZ during the day, indicated through the higher mean hourly y coordinate during the day (the commercial farm in Essex: I2 = 6.26 to 7.98, I3 = 5.38 to 6.30, compared to I1 = 4.46 to 5.38; the RVC Research farm: I1 = 8.49 to 9.39 compared to I2 = 4.81 to 6.63) (Table 2; Fig 4). This is further shown in the raw data (the commercial farm in Essex: 64.67% and 71.14% of $y \leq 10.5$ (most likely in NFZ) during I2 and I3 respectively whereas 84.48% of $y \leq 10.5$ during I1; the RVC Research farm: 54.79% of $y \leq 10$ (most likely in NFZ) during I1 whereas 79.56% of $y \leq 10$ during I2) (Table 2).

Table 2. Summary of descriptive statistics showing the time that herds on the commercial farm in Essex and on the RVC Research farm spent in the feeding zone (FZ) and non-feeding zone (NFZ) during different time intervals, divided as hours between milking, across the study period. The feeding zone is defined as where y ≤ 10.5 and y ≤ 10 for the commercial farm in Essex and the RVC Research farm respectively, and the non-feeding zone as where y ≥ 10.5 and y ≥ 10 for the commercial farm in Essex and the RVC Research farm respectively. Time intervals shown are interval one (I1; the commercial farm in Essex: 01:00 to 04:00; the RVC Research farm: 08:00 to 13:00), interval two (I2; the commercial farm in Essex: 08:00-11:00; the RVC Research farm: 19:00 to 22:00 and 01:00 to 04:00) and interval three (I3; the commercial farm in Essex: 15:00 to 19:00). The mean hourly range size ratio is also shown for each time interval, defined as the core range (50%) divided by the full range (95%); this is given as a range from the lowest mean hourly size ratio e.g., 0.262 at 04:00 for the commercial farm in Essex, I1 to the highest mean hourly range size ratio e.g., 0.274 at 01:00 for the commercial farm in Essex, I1.

Time interval	Farm	Time	Range of mean hourly y (m)	Time in NFZ (relative to time in NFZ or FZ; %)	Range of mean hourly range size ratios (CR/FR)	Summary
I1	Commercial farm in Essex	01:00 to 04:00	4.46 to 5.38	84.48	0.262 to 0.274	More time spent in the FZ during the day
I2		08:00 to 11:00	6.26 to 7.98	64.67	0.276 to 0.287	
I3		15:00 to 19:00	5.38 to 6.30	71.14	0.274 to 0.296	
I1	RVC Research farm	08:00 to 13:00	8.49 to 9.39	54.79	0.256 to 0.268	
I2		19:00 to 22:00 & 01:00 to 04:00	4.81 to 6.63	79.56	0.263 to 0.279	

4.3.3.3. Preferences for specific areas of the barns

More specifically, visualisation of the heatmaps over time suggests that cows on the commercial farm in Essex prefer to position in the right-hand cubicles over the left-hand and lower cubicles in the NFZ, and the left over the lower (Fig 2). This is supported with basic summing of the data; cows spent 51.71% in the right cubicles ($26.90m \leq x \geq 52.85m$ and $0.80m \leq y \geq 10.50m$), 29.93% in the left cubicles ($2.55m \leq x \geq 23.10m$ and $0.80m \leq y \geq 10.50m$), and 18.36% in the lower cubicles ($25.10m \leq x \geq 52.85m$ and $-1.62m \leq y \geq 0.80m$) in the NFZ, although this doesn't consider sensor error (Table 3; Fig 2). Preferences for certain areas in the NFZ are shown to a lesser extent in the RVC Research farm, with cows dedicating 41.10% of their time to the left of the NFZ ($x \leq 13.43m$), 33.01% to the middle ($13.43m \leq x \geq 26.87m$) and least to the right at 25.89% ($x \geq 26.87m$) (Table 3; Fig 2).

Table 3. Descriptive statistics showing the time (as %) herds on the commercial farm in Essex and on the RVC Research farm spent in areas of the non-feeding zone. Areas are defined on the commercial farm in Essex as: left = $2.55 \leq x \leq 23.10$ and $0.80 \leq y \leq 10.50$; lower = $25.10 \leq x \leq 52.85$ and $-1.62 \leq y \leq 0.80$ and right = $26.90 \leq x \leq 52.85$ and $0.80 \leq y \leq 10.50$, and on the RVC Research farm as: left = $x \leq 13.43$; middle = $13.43 \leq x \leq 26.87$; right = $x \geq 26.87$).

Farm	**Cubicles/area**	**Coordinates (m)**	**Time in area (%)**	**Summary**
Commercial farm in Essex	Left	$2.55 \leq x \leq 23.10$ and $0.80 \leq y \leq 10.50$	29.93	Cows show preferences for specific areas of the NFZ on both farms, especially on the commercial farm in Essex.
	Lower	$25.10 \leq x \leq 52.85$ and $-1.62 \leq y \leq 0.80$	18.36	
	Right	$26.90 \leq x \leq 52.85$ and $0.80 \leq y \leq 10.50$	51.71	
RVC Research farm	Left	$x \leq 13.43$	41.10	
	Middle	$13.43 \leq x \leq 26.87$	33.01	
	Right	$x \geq 26.87$	25.89	

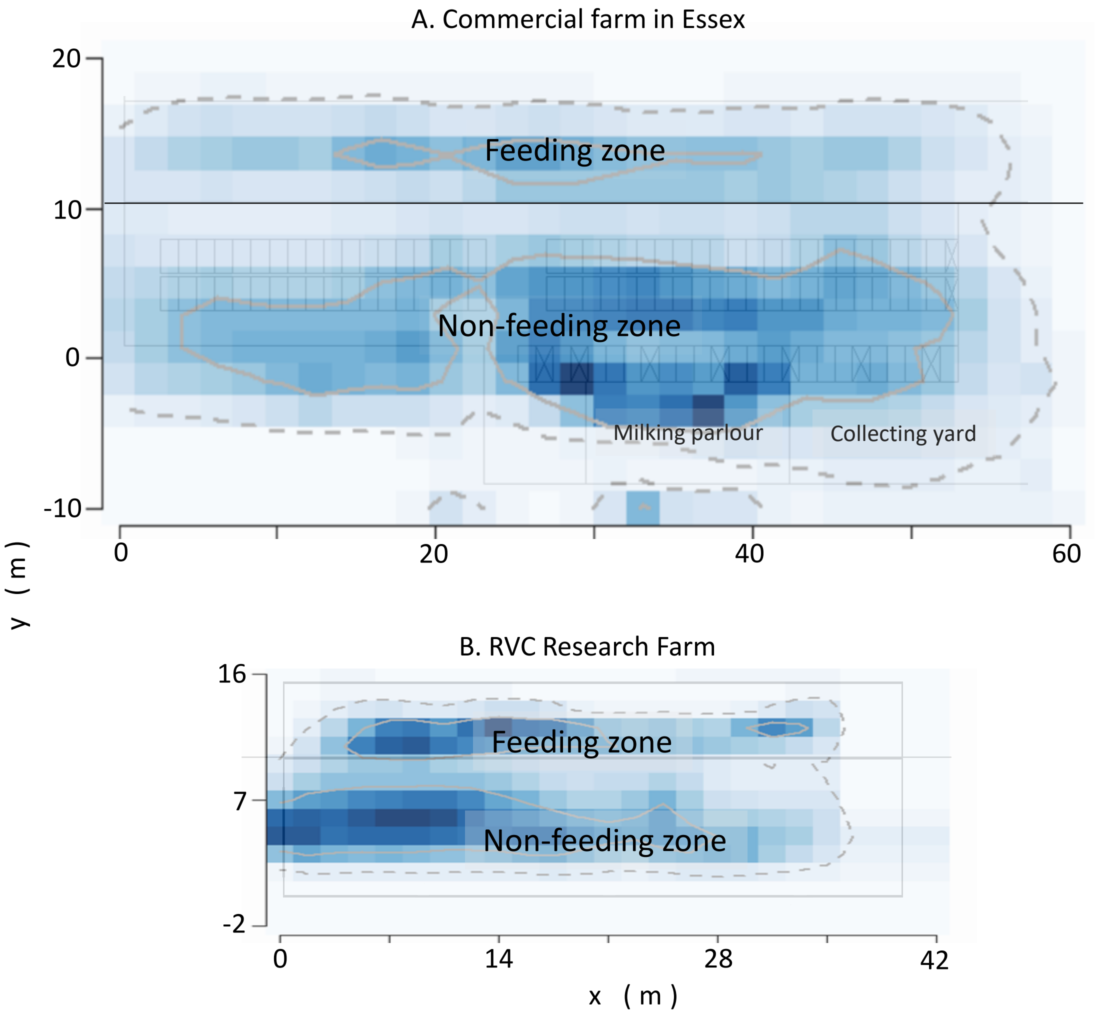

Figure 2. Example heatmaps showing herd-level space-use on the (A) commercial farm in Essex and (B) the RVC Research farm over the full study durations in the non-feeding zone and the feeding zone. The non-feeding zone is defined as where $y \leq 10.5$ for the commercial farm in Essex and $y \leq 10$ for the RVC Research farm, and the feeding zone is defined as where $y \geq 10.5$ for both farms. The lighter shades of blue indicate areas of low density or space-use, whereas the darker shades of blue indicate areas of high density. A black line is marked to show the division between the zones.

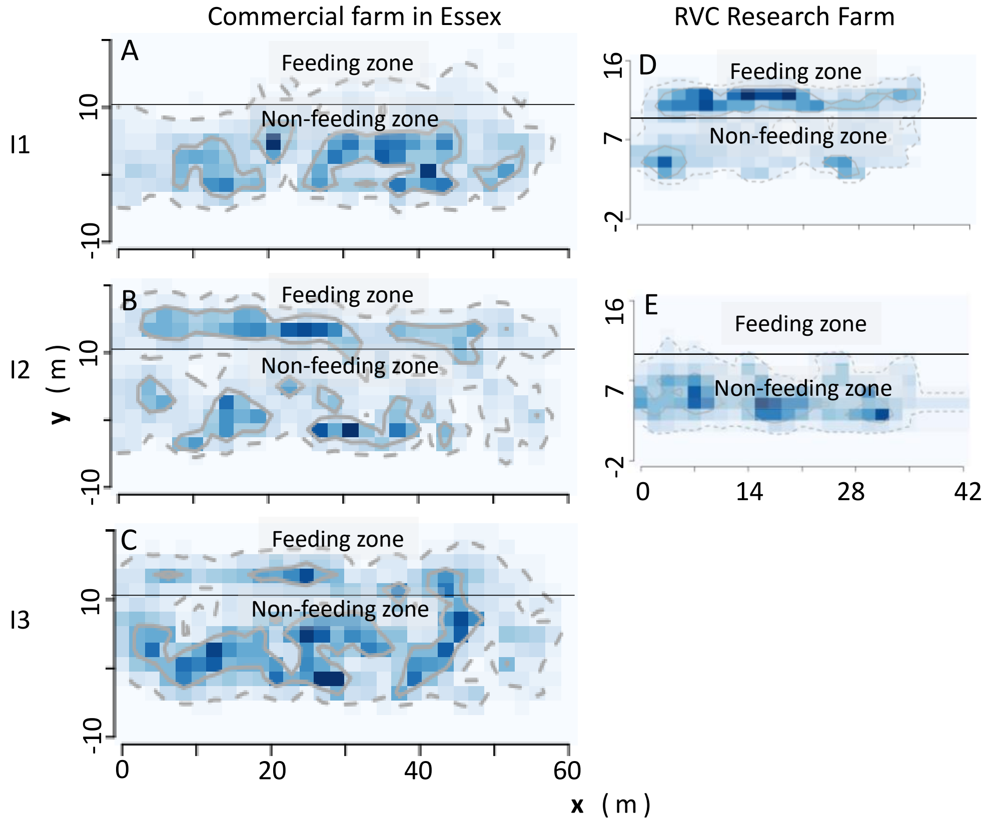

Figure 3. Example heatmaps comparing herd-level space-use (core range and full range) between the non-feeding zones (NFZ) and the feeding zones (FZ) at different time intervals, on the commercial farm in Essex (A-C) and the RVC Research farm (D-E). The solid grey lines represent the core range (50%) while the dashed grey lines represent the full range (95%). The NFZ is defined as where y ≤ 10.5 for the commercial farm in Essex and y ≤ 10 for the RVC Research farm, and the FZ is defined as where y ≥ 10.5 for both farms. Hours are divided into periods between milking as interval one (I1; the commercial farm in Essex: 01:00-04:00; the RVC Research farm: 08:00 to 13:00), interval two (I2; the commercial farm in Essex: 08:00-11:00; the RVC Research farm: 19:00 to 22:00 and 01:00 to 04:00) and interval three (I3; the commercial farm in Essex: 15:00-19:00). Examples show 01/10/2014 at 04:00 (I1), 08:00 (I2) and 18:00 (I3) for the commercial farm in Essex, and 01/11/2015 at 12:00 (I1) and 03:00 (I2) for the RVC Research farm. The lighter shades of blue indicate areas of low density or space-use, whereas the darker shades of blue indicate areas of high density. Black

lines mark the divisions between the zones. For these example hours, the usage of the FZ on the commercial farm in Essex is shown to be lower during I1 (range size ratio (core range divided by full range) = 0.283) compared to I2 (range size ratio = 0.277) and I3 (range size ratio = 0.302). The usage of the FZ on the RVC Research farm is shown to be lower during I2 (range size ratio = 0.267) compared to I1 (range size ratio = 0.290).

4.3.3.4. Metric confirmation: space-use differences during and between milking periods

The range sizes on both farms fluctuate between milking (on the commercial farm in Essex: 05:00 to 07:00 and 12:00 to 14:00; the RVC Research farm: 05:00 to 07:00 and 14:00 to 16:00) and periods between milking, with mean CR as low as 50.28 and 23.76 on the commercial farm in Essex and the RVC Research farm respectively during the milking, and highest CR at 55.22 and 40.12 respectively between milking on the commercial farm in Essex and the RVC Research farm respectively (Fig 4). A similar pattern is observed for FR on the RVC Research farm, with mean FR as low as 101.53 during milking and as high as 148.31 between milking (Fig 4). On the commercial farm in Essex, the pattern for FR occasionally deviates from that of CR during the last hour of milking due to a few cows moving back into the FZ and NFZ, which is considered in the FR but not the CR e.g., from 06:00 (mid-milking) to 07:00 (last hour of milking), the mean CR drops from 23.86 to 23.76 whereas the mean FR increases from 101.53 to 118.65 (Fig 4).

The range size ratio decreases during milking on both farms, e.g., mean range size ratio during milking = 0.210 to 0.294 and 0.200 to 0.268 on the commercial farm in Essex and on the RVC Research farm respectively whereas mean range size ratio = 0.262 to 0.295 and 0.256 to 0.278

between milking on the commercial farm in Essex and on the RVC Research farm respectively, which is expected given cows are spending most of their time in fewer cells (of the FZ and NFZ) during milking (Fig 4).

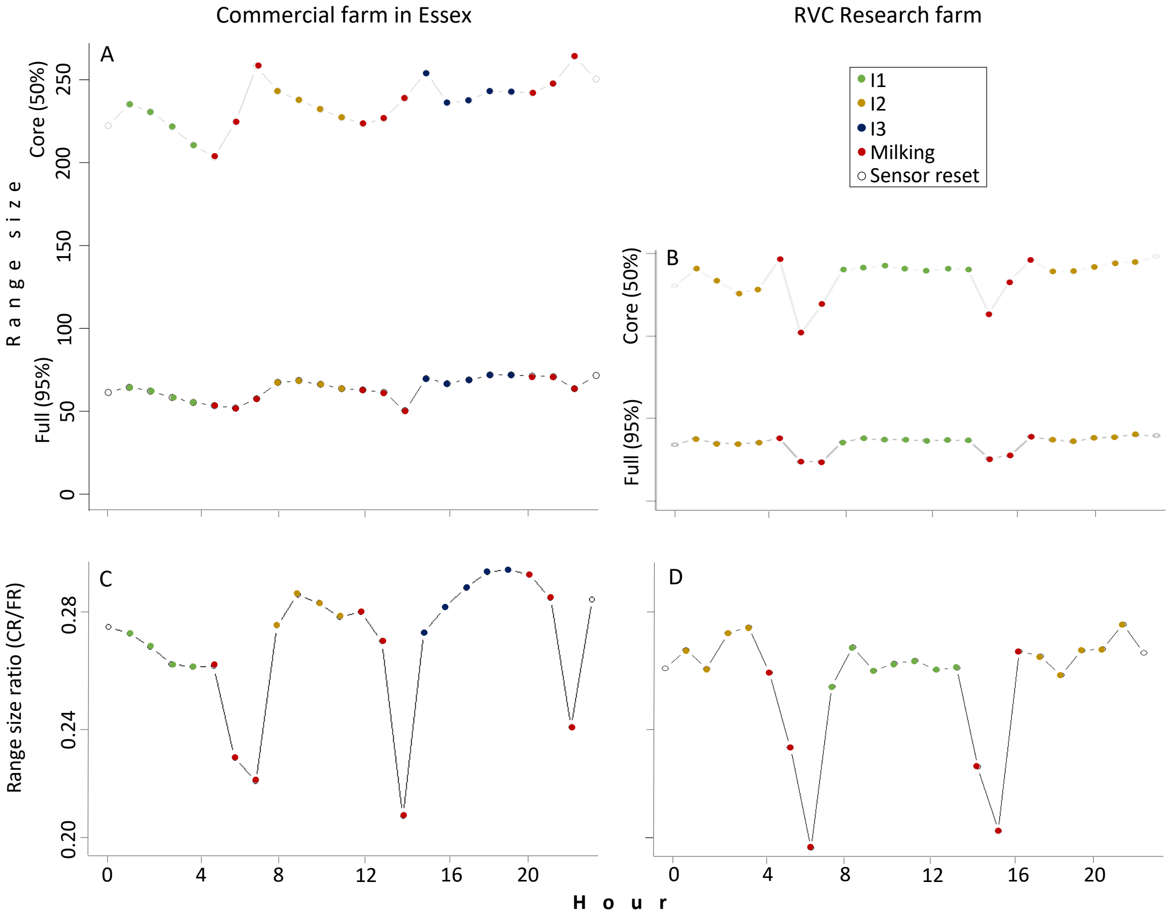

Figure 4. (A-B) Mean core range (50%, CR) and full range (95%, FR) and (C-D) range size ratio (CR/FR) per hour, across the study durations for the commercial farm in Essex and the RVC research farm. Hours divided into periods between milking as interval one (I1; commercial farm in Essex: 01:00 to 04:00; RVC research farm: 08:00 to 13:00), interval two (I2; commercial farm in Essex: 08:00 to 11:00; RVC research farm: 19:00 to 22:00 and 01:00 to 04:00) and interval three (I3; commercial farm in Essex: 15:00 to 19:00).

4.4. Discussion

This section reveals moderately to highly connected networks on the commercial farm in Essex in comparison to weakly connected networks on the RVC Research farm. The NFZ is more strongly connected than the FZ of the RVC Research farm, as found on the commercial farm in Essex. On the commercial farm in Essex, significant inter-individual variation was found in the NFZ but not in the FZ whereas on the RVC Research farm significant inter-individual variation was found in the FZ but not in the NFZ. Daily social differentiation was found in both functional zones across the farms. Exploration into space-use patterns show both the herd on the commercial farm in Essex and the herd on the RVC Research farm use a greater area of the barn during the day compared to the night. Interestingly, cows appear to show preferences for certain areas in the NFZ on both farms, especially on the commercial farm.

Individuals with the least daily proximity interactions were clearly shown to be on the periphery of, or disconnected from, the weakly connected networks on the RVC Research farm. This herd group was subject to frequent changes in group composition, with an average of 12 individuals being added or removed daily. These frequent changes could have weakened social ties within the herd. In contrast, the group composition on the commercial farm in Essex was more stable over time, with an average of one individual being added or removed daily, and the network was found to be highly connected. In addition, the herd on the RVC Research farm saw a complete turnover of individuals during the study period (where n = 22 to 52 daily), whereas the 91 cows studied on the commercial farm in Essex were present throughout the study period (where n = 100 to 110 daily). Therefore, the cows studied on the commercial farm in Essex may have had stronger connections due to their greater familiarity with each other. The introduction of new individuals into a dairy herd can weaken the network, due to a lack of interactions between new and resident cows, and weakened links between resident cows (272).

Although networks may strengthen over time following a regrouping event, as dominance hierarchies are thought to be established after a couple of weeks, this may not be possible with frequent regrouping (132,157,272). The differences in network connectivity may also be a result of the differences in space allowances. Stocking density in the NFZ, where cows spent most of their time, was lower on the RVC Research farm (stocking density of lying space = 7.28m^2 to 8.90m^2 per cow*)* than on the commercial farm in Essex (lying space = 3.53m^2 to 4.55m^2 per cow), and the former were housed in an open barn whereas the latter were housed in a closed barn. The greater space allowance on the RVC Research farm could have enabled individuals to choose whom to distance from to a greater degree, unlike on the commercial farm in Essex where individuals may have been forced to maintain less differentiation in closeness.

Despite the high connectivity of the commercial farm in Essex network, on both farms highly social individuals continued to be highly social over time, as found in previous studies on cattle (249,251). The stronger individual differences in sociality in the FZ, compared to the NFZ, on the RVC Research farm may have been due to a high demand for preferable feeding spots, with subordinate cows not gaining optimal areas. Differences in individual sociality may have been masked in the NFZ on the RVC Research farm due to a lower demand for specific areas given the lack of cubicles and greater space allowance allowing distancing. However, on the commercial farm in Essex there may have been a high demand for preferable cubicles in the NFZ, revealing individual differences in proximity interactions. Patterns in the FZ may have been masked on the commercial farm in Essex due a greater turnover of individuals at the feed face, with cows starting and ending eating at various times due to limited space.

Not only are individual differences in proximity interactions in the FZ revealed, but preferences for interactions between specific dyads across zones are shown. This is despite the daily changes in group composition both herds were subject to throughout the study durations, particularly on the RVC Research farm. This could be as cows removed from the herd were often added back later, and social bonds may have remained despite temporary separation, given long-term familiarity has a stronger impact on social relationships than recently shared experiences (168). However, as found on the commercial farm in Essex with the lack of temporal stability across averaged four-day networks (Chapter 3), herds housed at high stocking rates, may form associations, and maintain preferences over a short time i.e., daily but not in the longer term, which could not be assessed on the RVC Research farm due to a high number of daily changes in group composition. Still, cows with similar energy requirements or routines may position together throughout the day, and while these may change over time, this could result in the appearance of associations where they don’t exist.

Investigation of the potential impact of familiarity on social preferences was considered in this chapter, by assessing social differentiation against the number of changes to the group over time, but this was not possible due to little fluctuation in the high daily social differentiation values. Familiarity has indeed been found to impact the social relations of cows, through proxies for familiarity such as parity (176), although this is not always found to be the case e.g., Chapter 3, which may be attributed to these proxies being too coarse to model familiarity. Considering which cows were kept together from birth through to joining their milking herd, which was unmeasured in this study, may explain the daily social differentiation observed. Direct measures of familiarity i.e., time spent together, shows that individuals across taxa, from fish to primates, favour interactions with familiar individuals over those with unfamiliar individuals (166,339–343). Directly after regrouping, and to some extent a week later, familiar cows are preferred over unfamiliar ones as grooming partners and feeding neighbours (339). Assessing whether familiar individuals are favoured throughout frequent regrouping e.g., daily as in this study, could help us further understand the impact of removing individuals from a herd on the social network.

Both herds displayed preferences for space-use, as well as proximity interactions. The study group on the commercial farm in Essex were housed in a closed barn, which appears to have led to cows spend more time in specific areas. In contrast, the open barn on the RVC Research farm resulted in the cows being less likely to occupy specific areas. Visualisation of space-use suggest that the herd on the commercial farm in Essex spent more time in the cubicles than the passageways. Although the mean range size ratio differed significantly between the farms, suggesting that the herd on the commercial farm in Essex used a more restricted area of the barn, this difference is small (mean difference of 0.011) and may not be biologically meaningful. The preference for the left of the NFZ on the RVC Research farm, as well as the preference for the right-hand cubicles on the commercial farm in Essex, could partly explain this. Preferences for specific areas of barns has also been found in other studies (197,344). For each farm, the preferred areas were closer to the passageways to the collecting yards. Positioning in these areas would have given cows a greater chance of relieving a high milk yield sooner than cows positioned elsewhere. Indeed, studies show that cows at the beginning of the milking order have a higher milk yield (345,346). Evidence for higher parity cows positioning in areas closer to milking areas has been found previously, supporting this theory (252,347). Other factors are likely to contribute as well; older, more experienced cows with a high milk yield may be more likely to gain a position in preferred cubicles as opposed to a younger cow with a high milk yield (252). To further this, the most dominant cow is first in line to be milked, and ill cows enter later than when they are healthy, further highlighting advantages of positioning close to the collecting yard (348,349). The demand for specific areas may also change over time. If the number of cubicles closer to feeding areas is restricted, the demand for those closer to a feed alley may increase, especially prior to scheduled feeding times (197,347). Alternatively, the physical space restrictions may have restricted rest in certain locations of the NFZ, more so on the commercial farm in Essex due to the fixed cubicles and higher stocking density.

The finding that cows were more active during the day than during the night is well known but demonstrates the efficacy of the LPS and confirms the use of the range size metrics used; cattle are diurnal e.g., they consume the majority of their feed intake between dawn and dusk, assuming housing conditions allow this as the study farms did (350). Moreover, comparison of the range size metrics between and during milking times revealed patterns which were expected. Specifically, a lower core range size was calculated during milking periods, sensical given most cows were positioned in areas associated with milking. The range size ratio was found to be lower during than between milking periods, also expected given a few cows remained in the functional zones during milking periods.

Space-use patterns between the two barns could be further explored, investigating where individuals position in relation to each other, and whether this is based on their attributes. It has been found that lame cows have a higher site-fidelity in a feeding area, perhaps due to a lack of motivation and a lack of ability to choose high-traffic, preferable feeding positions (209,351). Perhaps this is clear in closed barns (with cubicles) such as on the commercial farm in Essex but not in open barns (without cubicles) such as the RVC Research farm; an understanding of this would help identify cows with lameness specific to housing environments. It would be of interest to investigate if other differences in housing e.g., bedding, as well as management e.g., milking system impact the space-use preferences of herds. For example, individuals may show preferences for specific areas prior to scheduled feeding times, but perhaps only if access is quicker from specific areas of a barn, and unhealthy individuals may not gain these. This would have implications for disease spread, as well as health and social stress, and other aspects of welfare.

4.5. Conclusions

Although direct quantitative comparisons of network measures between these barns of different sizes were not possible, this work provides evidence for differences in the network structure and space-use of two dairy herds. Specifically, this chapter suggests that a greater space allowance, in terms of barn layout and stocking density, and a high turnover in group composition may weaken social ties. These results provide a key first step to understanding how to optimise the housing and management of dairy herd groups.

The main results of this chapter are outlined below:

- Two dairy herd groups, one on a commercial farm in Essex and the second on an RVC Research farm, were tracked continuously using a local positioning system over one and three months respectively. Proximity interaction networks were compared in terms of structure, and space-use patterns were detected and compared between the herds using range size metrics.
- The networks derived from the herd housed on the commercial farm in Essex were more connected than those housed on the RVC Research farm. The non-feeding zone network was particularly connected on both farms, compared to the feeding zone network.

- On the commercial farm in Essex there was significant inter-individual variation in the non-feeding zone whereas on the RVC Research farm there was significant inter-individual variation in the feeding zone, and daily social differentiation was present on both farms in both functional zones.
- Both herds used a greater area of the barn during the day and preferences for areas were clearer on the commercial farm in Essex than on the RVC Research farm.
- The use of the range size metrics was confirmed by comparing space-use patterns between and during milking periods.

5. CHAPTER 5: ANALYSIS OF LOCAL POSITIONING DATA REVEALS BUNCHING IN HOUSED DAIRY COWS IS ASSOCIATED WITH HIGHER TEMPERATURES.

5.1. Introduction

Maintaining close proximity to other individuals can serve to thermoregulate in cold conditions (351–353). For instance, piglets spend over 60% of their time bunching to reduce heat loss (354). In contrast, cattle have been observed to instead bunch in normal conditions or when their environment is warmer than average ambient temperatures, through observational studies (281,283,284,355).

Cattle may bunch in shade to decrease heat load; the body temperature of cattle at pasture can be reduced through the use of tree shading (356,357). Shaded structures are favoured by housed dairy cows during warmer periods of the day, and cows at pasture favour shade with increasing sunlight (346,357,358). Strong preference for shade is demonstrated further by dairy cows choosing shade in a test arena over lying after half a day of lying deprivation (359). Cattle may bunch with the intention to take advantage of shade or to create shade by exposing less body surface area to direct sunlight. With housed dairy cows, the former is more likely than the latter, given sunlight is likely to be blocked by roofing.

At high temperatures, cattle may congregate at water troughs since drinking will help them cool down, or ventilated areas to avoid fly burdens. Moreover, bunching in itself may reduce fly burdens; cattle position their tails on the outside of groups and kick and swish their tails to protect themselves against stable flies (282,356,360–363). Increased fly burdens could trigger defensive aggregation, a known response of both invertebrates and vertebrates to a predatory threat (364,365). Bunching may effectively result in an encounter-dilution effect whereby the number of flies per cow in the group decreases, as shown with tsetse flies (366), but cows may also lose weight by reducing the time they spend on other activities (363). If significant bunching occurs, the herd’s body heat may contribute to an overall rise in ambient temperature, which would then raise internal body temperature in a feedback cycle (55,211).

In the context of dairy cattle, heat stress is defined as the sum of factors external to an animal that displace their body temperature from a set point, mainly caused by the microclimate within their localised environment including temperature, humidity, solar radiation and wind speed (367). Behavioural responses of dairy cattle to rising temperatures include a reduction in lying time and increase in standing time (368–370). Standing serves to increase body surface area exposed for heat loss, but also raises the risk of lameness and can reduce sleep, which can weaken the immune system. Indication of heat stress is also apparent through other behavioural responses including reduced feed intake, thought to serve to reduce metabolic heat production, and an increase in drinking behaviour to cool individuals down (200,372). Physiological changes to heat stress have also been observed including: lower milk yield, increased respiration rate, increased heart rate and hindered reproduction (203,373–385).

Previous research has mostly used ambient temperature (barn or local weather station recordings) and body temperature, and the Temperature-Humidity Index (THI) as indicators of heat stress (Table 1) (199,200,200,202,372,373,376,379,380,385–391). If air movement is low and solar radiation is effectively blocked, THI considers both the effects of temperature and humidity associated with thermal stress. It is relatively simple to measure on farms with large herds and is considered suitable for forecasting the thermal environment indoors (392). THI was initially developed to gauge human heat discomfort (393) but numerous modifications have been created for use on cattle (386,394,395). Temperature and THI are commonly used to assess physiological changes, with alterations typically detected above thresholds of THI = 65 to 72 and temperatures ranging from 19°C to 26°C (Table 1). Above THI = 60 and particularly above 19°C, behavioural changes have been found. THI appears to be most widely used to measure various metrics associated with production, and it has been found that losses occur when the THI reaches between 60 and 73 (Table 1). Thresholds therefore vary (THI = 65 to 72 and 19°C to 26°C) (Table 1).

Table 1. Thermal thresholds of production loss: milk yield and conception rate, physiological changes: rectal temperature, respiration rate and heart rate and behavioural changes: feed intake, drinking rate and lying rate, of cattle. Where the relationship with temperature (200,200,202,372,373,376,385–391,396) or THI (368–370) was not recorded, this is estimated based on a humidity of 75%, to enable crude comparisons.

Category	Measure	THI	Temperature
Production loss	Milk yield	Decrease at threshold of 60 to 72 (200,202,376,387–391,396).	Approximately 16°C to 23°C at a relative humidity of 75%.
	Conception rate	Decrease at threshold of 72 to 73 (373,386).	Approximately 23°C to 24°C at a relative humidity of 75%.
Physiological changes	Rectal temperature	Increase at threshold of 65 to 69 (379,380).	Increase at threshold of 20°C to 26°C (381,382).
	Respiration rate	Increase at threshold of 65 to 69 (379,380).	Increase at threshold of 19°C to 25.5°C (381,383,384).
	Heart rate	Increase at threshold of 72 (385).	Approximately 23°C to 24°C at a relative humidity of 75%.
Behavioural changes	Bunching	Increase at approximately THI = 67 at a relative humidity of 75%.	Increase above 19°C to 20°C (281,355), or higher ≤ 30°C than > 30°C (282).
	Feed intake	Decrease at threshold of 68 (200).	Approximately 21°C at a relative humidity of 75%.
	Drinking rate	Increase above 60 (372).	Approximately 21°C at a relative humidity of 75%.
	Lying rate	Decrease at approximately THI = 65 at a relative humidity of 75%.	For every 1°C increase in mean hourly ambient temperature from 19°C and from 21°C, respectively in (368,369), decrease of approximately 21 to 22 minutes. Another study reported that at lower temperatures ranging from -15°C to 19°C, reduction in lying by 1.5 minutes for every degree increase (370).

In both temperate and subtropical regions, prior studies have shown baseline evidence for bunching at high temperatures above a particular threshold (281,283,355), however, the study durations and frequency of behavioural recordings have been infrequent and not utilised continuous monitoring via sensors. In temperate regions, it was discovered that bunching in dairy herds increases when the air temperature surpasses a threshold of 19-20°C (281,355). These two studies used either video recordings at 15-minute intervals one day per week for thirteen weeks (281), or photographs at 15-minute intervals for three months to measure the distribution of cows in each of two or three divisions within a barn (355).

A previous study observed a dairy herd in a humid subtropical climate, categorising individuals into one of four nearest neighbour groups, with recordings taken every 20 minutes for ten days, then one day per week for six weeks (283). Although an increase in bunching was observed with higher THI values (using an ambient temperature measure), a specific temperature threshold at which a qualitative change in behaviour occurred was not reported (283). This may be attributed to the high temperatures recorded throughout the study period; the weekly least mean squares of daily ambient temperature ranged from 21°C to 28°C (283). In contrast, a previous study reported a higher incidence of cows at ambient temperatures of 30°C or lower compared to temperatures above 30°C (282). However, it is important to note that the study did not explore the correlation between temperature and bunching, and used a simple binary method to categorise bunching (either present or absent) (282).

This study investigates the association between higher temperatures and bunching behaviour in a dairy herd housed on a commercial farm in Essex. Bunching behaviour is defined using three metrics based on local positioning data (range size, inter-cow distance and nearest neighbour distance) from continuous recordings made over four months in 2014.

5.2. Methodology

5.2.1. Animals and housing

A Holstein-Friesian high-yielding dairy cow herd was monitored continuously in a closed free-stall barn on a commercial farm in Essex, from 1st August 2014 to 30th November 2014 (*n* = 127 cows, minimum and maximum unique cows present per day during the study period: *n* = 86 days and *n* = 111 days respectively). Refer to Chapter 2, Section 2.4 for further details on the herd and their management.

5.2.2. Local positioning system and data pre-processing

Each cow was equipped with a combined local positioning sensor, temperature sensor and accelerometer, Omnisense 500 (298), mounted on a weighted neck collar. Refer to Chapter 2, Section 2.5 for further information on the sensor system.

Data selection and pre-processing were conducted as outlined in Chapter 2, Section 2.4. For the main analysis, hours when most cows were in the milking parlour or collecting yard were excluded (05:00-07:59, 12:00-14:59, 20:00-22:59), since their behaviour during these time periods was constrained by farm staff. The sensor system reset at midnight each day and hence times between 23:00 and 00:59 were also excluded.

5.2.3. Temperature data

Barn temperature (BT12) was recorded every eight seconds via Oms500 sensors (BT2; *n* = 22) and hourly via wall-mounted fixed ‘Tinytag’ sensors (BT1; *n* = 4) (Fig 1A) (298,397). The BT1 sensors were placed at approximately equal distances down the length (x axis) of the barn at a height of approximately 2.5-3m, alongside four BT2 sensors for comparison (Fig 1A). A mean hourly measurement was calculated (across all sensors, $n = 26$) to give a measure of the ambient barn temperature over this period. Other measures of temperature, derived from cow-equipped sensors and a local weather station, were considered but barn temperature readings were used for the bunching analysis as these are the most reliable, independent measures of temperature (Appendix 1).

Humidity was also considered but results from this analysis are not included in the main body of this chapter, as the readings were derived from a local weather station rather than from within the barn, and patterns were shown to be mainly dependent on temperature alone (Appendix 2).

Examples of heatmaps for hours of extreme bunching, alongside corresponding cow positions at a given time, visually demonstrate the variations present across the study duration (Fig 1B to 1D). Fig 1B-C shows an hour during which the herd spent most time in the right cubicles, with some time spent in the middle and right of the feeding zone (FZ), corresponding to a low range size (CR = 46, FR = 166). In contrast, Fig 1C-D shows an hour where the cows were spread out across the non-feeding zone (NFZ) and the FZ, resulting in a higher range size (CR = 88, FR = 268).

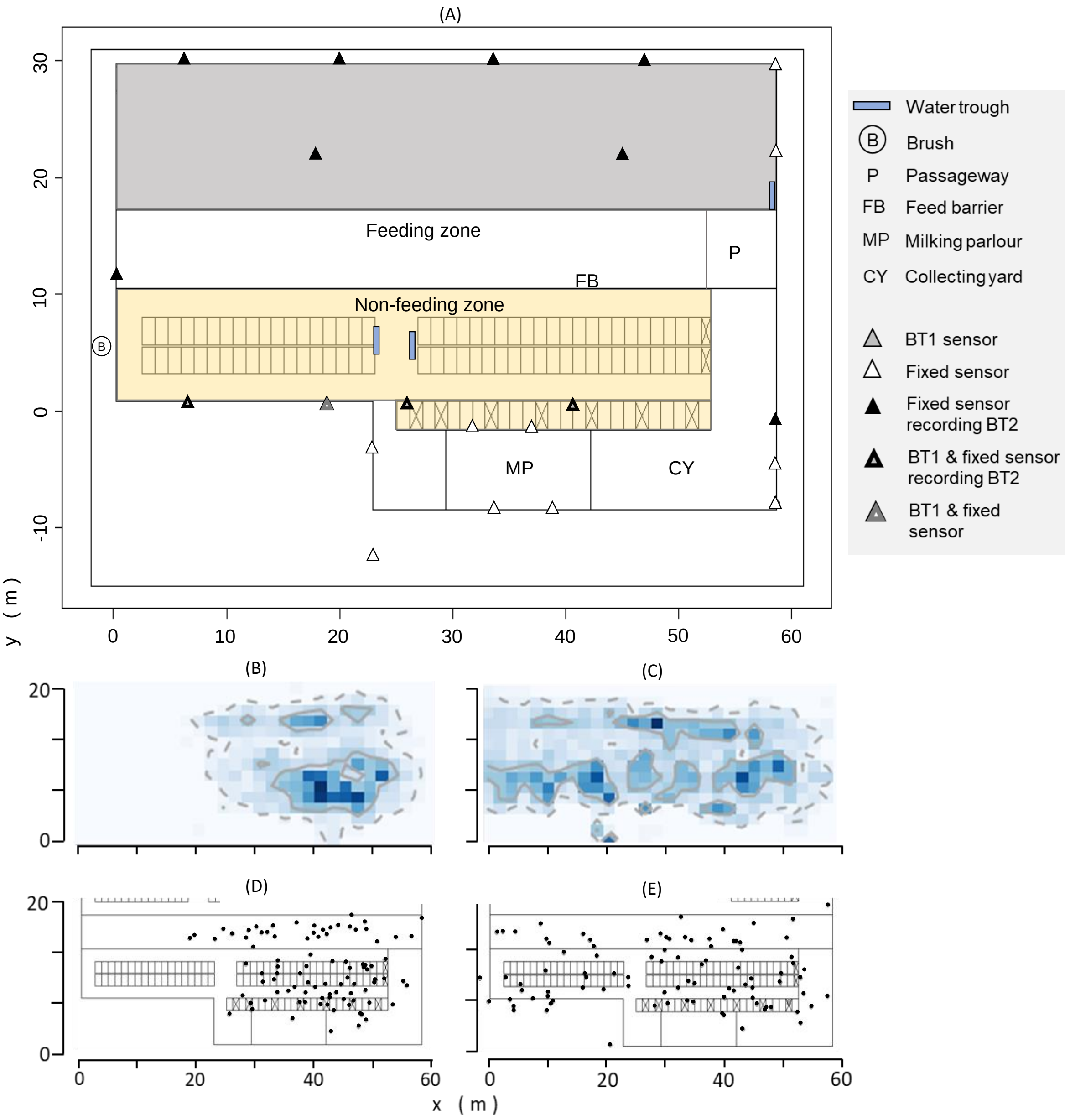

Figure 1. (A) Barn layout of the commercial farm in Essex showing the positions of sensors recording barn temperature, both Oms-500 sensors (*n* = 11, BT2; black triangles) and Tinytag sensors (*n* = 4, grey triangles, BT1), as well as (B-E) examples of high and low bunching. The Oms-500 sensors (*n* = 22) were also used to form a triangled network to fix absolute spatial position (white triangles in A)**.** The area shown in grey (upper barn area) was not used by this group of cows during the study period. The barn is divided into the feeding zone and the non-feeding zone where cubicles were located. The milking parlour (MP), collecting yard (CY), feed barrier (FB) and passageway (P) are also shown. The area shown in

grey above the feeding zone was not used by the study group but housed a high-stocked dairy cow herd during the study period. The space-use heatmaps show examples of high bunching where core range (CR, 50%) = 46, full range (FR, 95%) = 166, and ICD and NND = 12.78m and 2.66m respectively (B: 18/09/2014 at 11:00-12:00) and low bunching where CR = 88, FR = 268, and ICD and NND = 20.87m and 3.61m respectively (C: 12/10/2014 at 18:00-19:00). Examples of point location data for all individuals in the herd are shown for specific time points within each hour (D: 18/09/2014 at 11:01:32 and E: 12/10/2014 at 18:00:02).

BT12 peaked during the afternoon, with drops during the night, as expected (Fig 2A). The coolest hourly median BT12 was recorded in November (11.89°C) and the hottest in August (18.20°C), with median for September and October as 18.01°C and 15.16°C respectively. There was a significant difference in mean temperature between months (Fig 2A; K-W chi-squared = 1043.40, $p < 2.2e\text{-}16$). The mean hourly BT12 reaches above 20°C during the afternoon in August and September, but not during October and November (Fig 2). More specifically, Fig 2B shows hourly barn temperature over time for the entire study period; most of the values which lie above 20°C occur in August and September (93.30%, $n = 334$ of 358 hours).

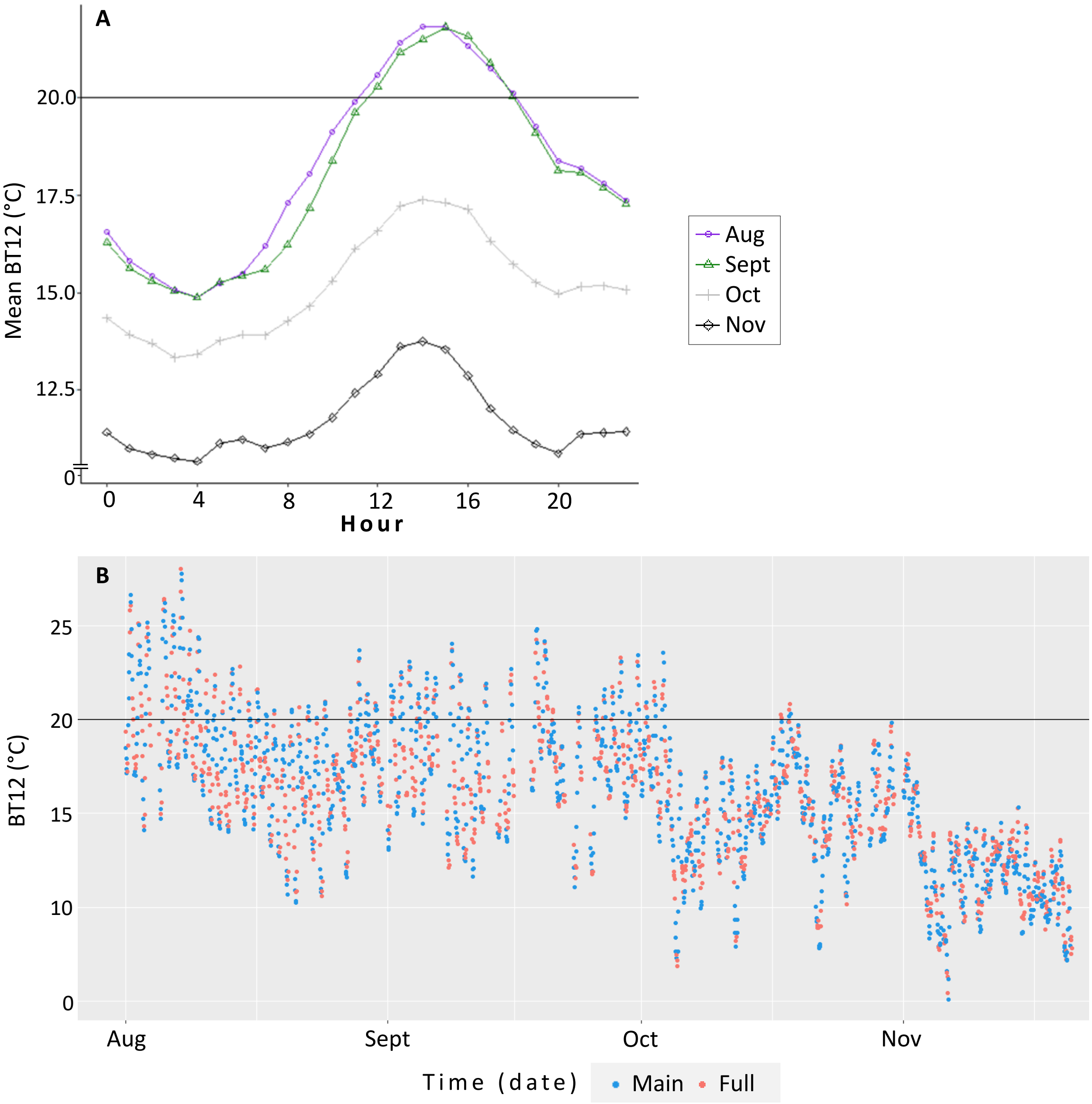

Figure 2. (A) Mean 24-hourly barn temperature (BT12 in °C) averaged over all days per month, and (B) mean hourly barn temperature (BT12 in °C) recorded during the full study period on the commercial farm in Essex (August to November 2014). In A, months shown are August = purple circles, September = green triangles, October = grey crosses, November = black diamonds (milking times are included). In B, the blue dots data points included in the analysis ("main"). The remaining data points, in red, were removed from the analysis due to milking (05:00-07:59, 12:00-14:59, 20:00-22:59), or because they are when the system reset (approximately 23:00 to 00:59) ("full"). Of the 2905 data points, *n* = 358 are above 20°C, with 334 of these occurring in August and September.

5.2.4. Bunching metrics

Given there are no formal definition of ‘bunching’ behaviour in the context of PLF, three bunching metrics are used: range size (core and full), mean inter-cow distance (ICD), and mean nearest-neighbour distance (NND). These metrics measure different aspects of space-use and social proximity, which may be indicative of bunching behaviour. Please refer to Section 2.4.12 (Chapter 2) for further details on why and how these metrics were calculated.

5.2.5. Segmentation and breakpoints

A segmented relationship describes changes in a trend at specific values, with a point of change referred to as a breakpoint (BP) (398). To explore whether bunching behaviour is affected by temperature, and whether this is above a certain threshold, the following approach is adopted, considering findings from previous studies (281,355).

5.2.6. Breakpoints

Based on previous studies on dairy cow behaviour (Table 1) (368,369), it is hypothesised that changes in bunching behaviour may occur at 20°C. To test this hypothesis, eight linear regression models were created, two per bunching metric (CR, FR, ICD and NND), one for data < 20°C and the second for data ≥ 20°C. For this, the lm() function in base R was used (e.g., CR ~ BT12). For each model, the dependent variable was the bunching metric, and the independent variable was BT12. Chow tests were performed to test the significance of the hypothesised BP of 20°C, for all bunching metrics. For this, the function ‘sctest’ from the R package ‘strucchange’ was used (398,399), inputting ‘bunching metric ~ temperature’ and specifying the BP.

For completeness, the R package ‘segmented’ was used to identify the locations of forced breakpoints (399). For each bunching metric, the original linear model (for all BT12 values) was inputted into the ‘segmented()’ function e.g., segmented(CR ~ BT12), specifying one to two breakpoint(s) in BT12. The identified BP was used to create two new linear regression models e.g., 1) CR ~ BT12 where BT12 < BP and 2) CR ~ BT12 where BT12 ≥ BP, and the results were analysed for each of the eight models. Chow tests were performed to test the significance of these breakpoints.

For all linear models, default R settings were used, which excludes observations containing NA values and uses QR factorisation, an extensively used method for solving least squares problems. Model assumptions were considered before drawing conclusions: heteroskedasticity through residual vs fitted plots and NVC tests, and residual normality through QQ-plots and the Shapiro-Wilks test (see Appendix 2).

5.2.7. Time of day

To explore whether time of day effects bunching behaviour, the data were split into time intervals between milking periods: one (I1: 1am to 4am), two (I2: 8am to 11am) and three (I3: 3pm to 7pm). The data corresponding to each of the three intervals were then used to create linear regression models e.g., CR ~ BT12 for data recorded during I1 and BT12 ≥ 20°C, and the results are analysed.

5.3. Results

5.3.1. Bunching metrics: time

The bunching metrics vary over time, and drop during milking periods, where cows are located within the restricted space of the milking parlour, except for full range as some cows remained in the main barn at a given time during milking times (Fig 3). More specifically, Fig 4 shows the hourly bunching metrics over the entire study period, demonstrating variation in the bunching metrics over time.

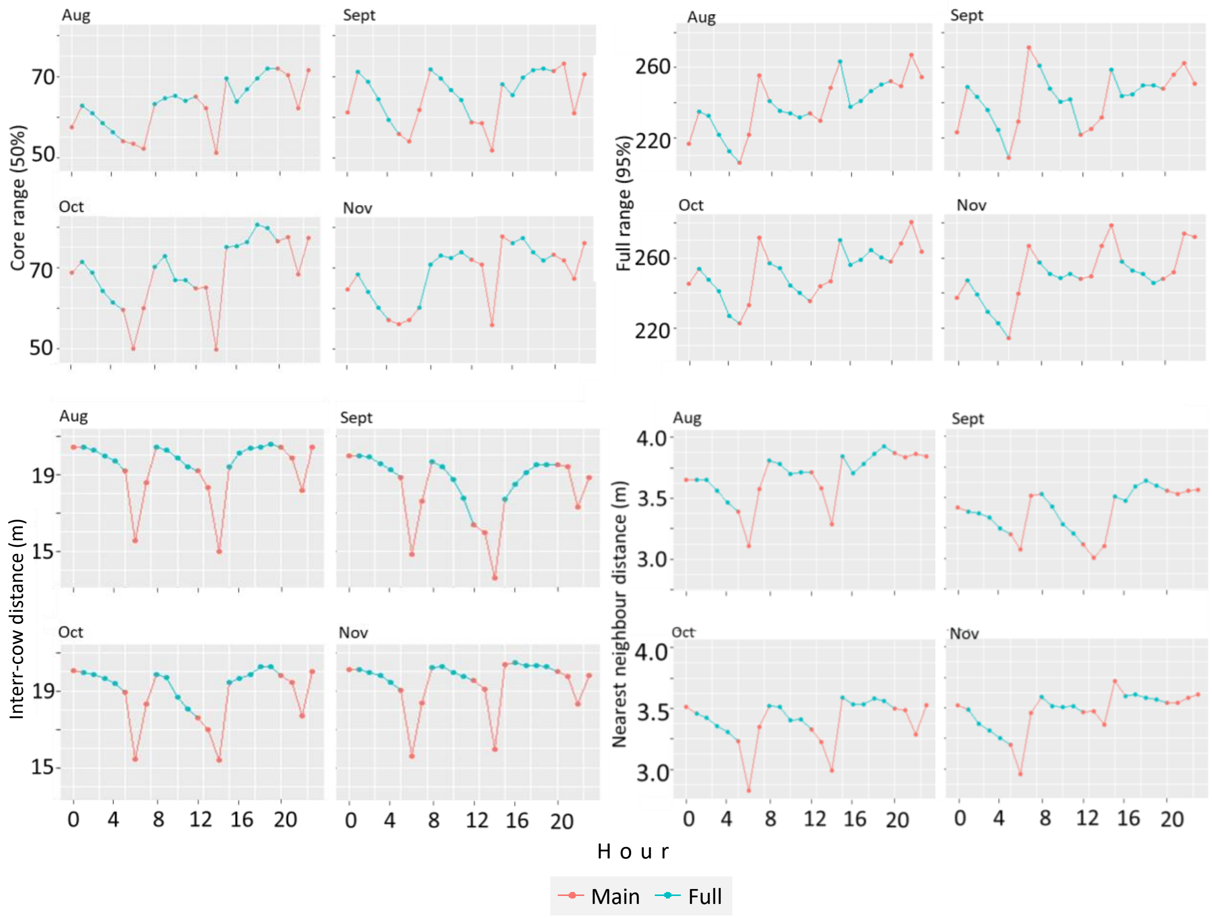

Figure 3. Mean hourly bunching metric: core range (50%), full range (95%), inter-cow distance (m) and nearest neighbour (m) per month on the commercial farm in Essex. Covered months are August, September, October, and November 2014. Hours are divided into those in the main barn (blue; “main”) and those in the collected yard and milking parlour (05:00-07:59, 12:00-14:59, 20:00-22:59) or when the sensor rest at midnight (approximately 23:00 to 00:59) (red; “full”).

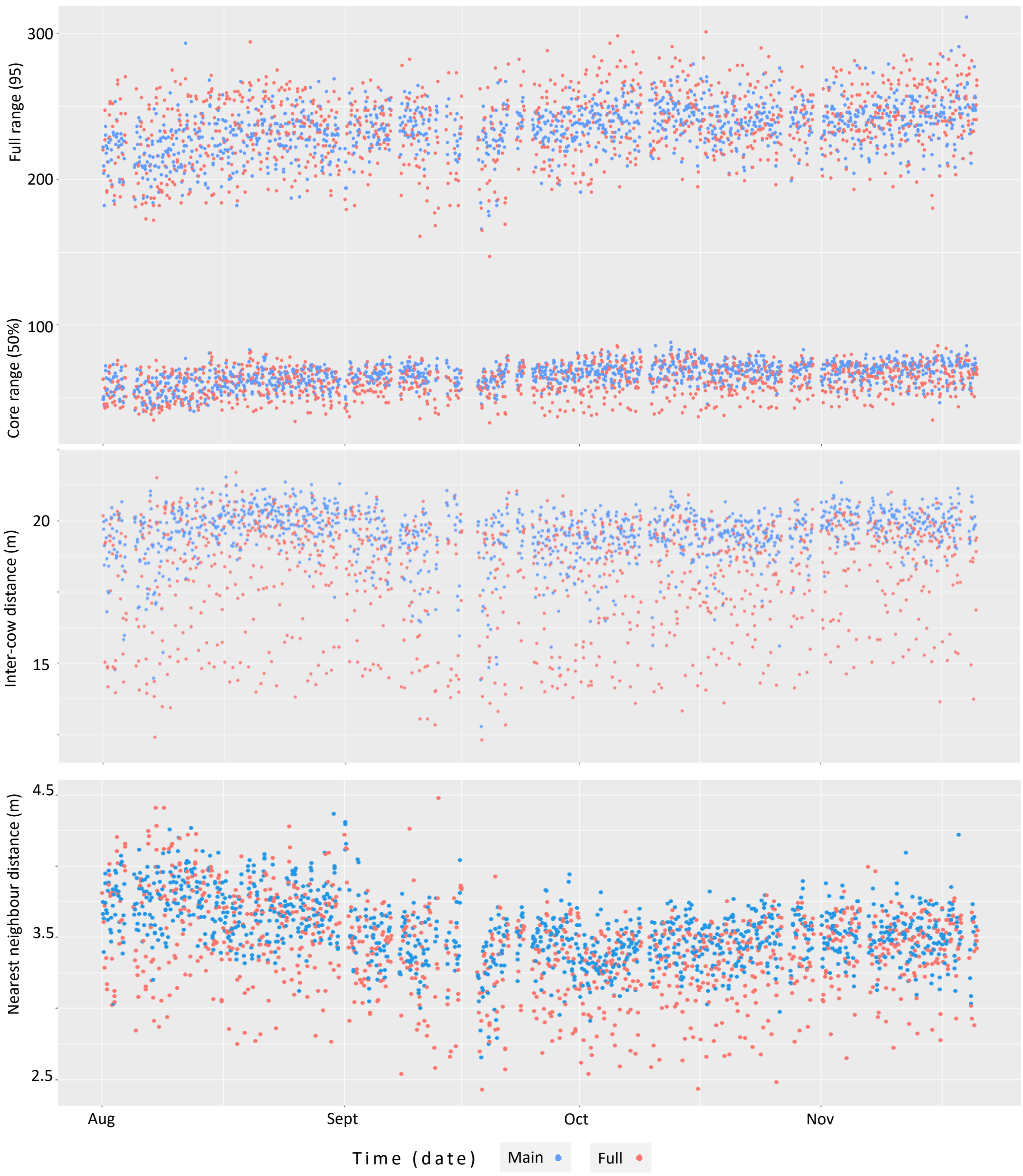

Figure 4. Bunching metrics: core range (50%), full range (95%), inter-cow distance (m) and nearest neighbour (m) against time per month, from the data recorded on the commercial farm of Essex. Months are August, September, October, and November 2014. The blue dots data points included in the analysis ("main"). The remaining data points, in red, were removed from the analysis due to milking (05:00-07:59, 12:00-14:59, 20:00-22:59) or because they are when the system reset (approximately 23:00 to 00:59) (red; "full").

5.3.2. Segmented relationship: bunching metrics ~ temperature

Above or equal to 20°C, a clear negative trend is shown between all four bunching metrics and BT12, and these are significant for CR, FR, and ICD (Table 2; Fig 5). The trends for the range sizes are the strongest, followed by ICD, and the weakest (and non-significant) trend is for NND (Table 2; Fig 5). Below 20°C, the relationships between BT12 and the range sizes and ICD are also negative but to a lesser extent e.g., for CR, e = -0.15 and $p = 0.06$ where BT12 < 20°C whereas e = -2.08 and $p = 1.26e-13$ where BT12 ≥ 20°C (Table 2; Fig 5). The direction of the relationship between NND and BT12 changes from positive to negative either side of the breakpoint (Table 2; Fig 5). This broadly supports the prediction that higher temperatures are associated with increasing bunching, as well as the existence of a temperature threshold in this relationship throughout the entire study period.

For August, for a given BT12 value, the range size values are generally lower and the NND values are generally higher in comparison to September (Fig 5). For example, where BT12 is 20.1°C (rounded to nearest one decimal point), CR = 60 to 65 and NND = 3.57m to 3.80m for August ($n = 2$) whereas CR = 65 to 78 and NND = 3.32m to 3.68m for September ($n = 4$). The pattern is the opposite for ICD and NND (Fig 5). This can be explained by the number of cows present in the barn, consistently lower in August (n = 85 to 86) compared to in September (n = 92 to 96). Moreover, there are negative trends between BT12 and the bunching metrics ≥ 20°C when considering August and September separately (Table 2; Fig 5; CR: e = -1.81, SE = 0.30, t-value = -6.03, $p = 2.88e-8$ and e = -1.35, SE = 0.49, t-value = -2.79, $p = 0.007$; FR: e = -2.48, SE = 0.81, t-value = -3.05, $p = 0.003$ and e = -0.66, SE = 1.11, t-value = -0.60, $p = 0.55$; ICD: e = -0.38, SE = 0.05, t-value = -7.44, $p = 3.65e-11$ and e = -0.31, SE = 0.09, t-value = -3.27, $p = 0.002$ and NND: e = -0.02, SE = 0.01, t-value = -2.33, $p = 0.02$ and e = -0.06, SE = 0.02, t-value = -4.00, $p = 0.0002$, for August and September respectively).

Table 2. Outputs from the linear regression model assessing the relationship between barn temperature (BT12) and bunching, on the commercial farm in Essex, below and above the breakpoint of BT12 = 20°C (*n* = 170), for all the months (overall) and for August and September alone (O, A and S, respectively), excluding outliers (*n* = 5). Outputs from Chow tests, which test the significance of the breakpoint, are also shown. Each test is conducted for each bunching metric (CR = core range, FR = full range, ICD = inter-cow distance, NND = nearest neighbour distance). Significant *p*-values are in bold. Periods during which cows were in the milking parlour or collecting yard were removed (05:00-07:59, 12:00-14:59, 20:00-22:59), as well as periods during which the sensor reset (23:00 to 00:59).

Bunching metric	**Chow-test**	**< 20**°C	**Selected data**	**≥ 20**°C
CR	F = 2.23, p = 0.11	e = -0.15, SE = 0.08, t-value = -1.88, *p* = 0.06	O	e = -2.08, SE = 0.26, t-value = -8.00, **_p_ = 1.26e-13**
			A	e = -1.81, SE = 0.30, t-value = -6.03, **_p_ = 2.88e-8**
			S	e = -1.35, SE = 0.49, t-value = -2.79, **_p_ = 0.007**
FR	F = 1.59, p = 0.20	e = -0.35, SE = 0.16, t-value = -2.19, **_p_ = 0.03**	O	e = -2.65, SE = 0.63, t-value = -4.24, **_p_ = 3.51e-5**
			A	e = -2.48, SE = 0.81, t-value = -3.05, **_p_ = 0.003**
			S	e = -0.66, SE = 1.11, t-value = -0.60, *p* = 0.55
ICD	F = 0.91, p = 0.40	e = -0.03, SE = 0.01, t-value = -3.95, **_p_ = 8.46e-5**	O	e = -0.33, SE = 0.04, t-value = -7.58, **_p_ = 1.52e-12**
			A	e = -0.38, SE = 0.05, t-value = -7.44, **_p_ = 3.65e-11**
			S	e = -0.31, SE = 0.09, t-value = -3.27, **_p_ = 0.002**
NND	F = 3.17, p = 0.04	e = 0.02, SE = 0.002, t-value = 9.40, **_p_ < 2e-16**	O	e = -0.01, SE = 0.01, t-value = -1.31, *p* = 0.19
			A	e = -0.02, SE = 0.01, t-value = -2.33, **_p_ = 0.02**
			S	e = -0.06, SE = 0.02, t-value = -4.00, **_p_ = 0.0002**

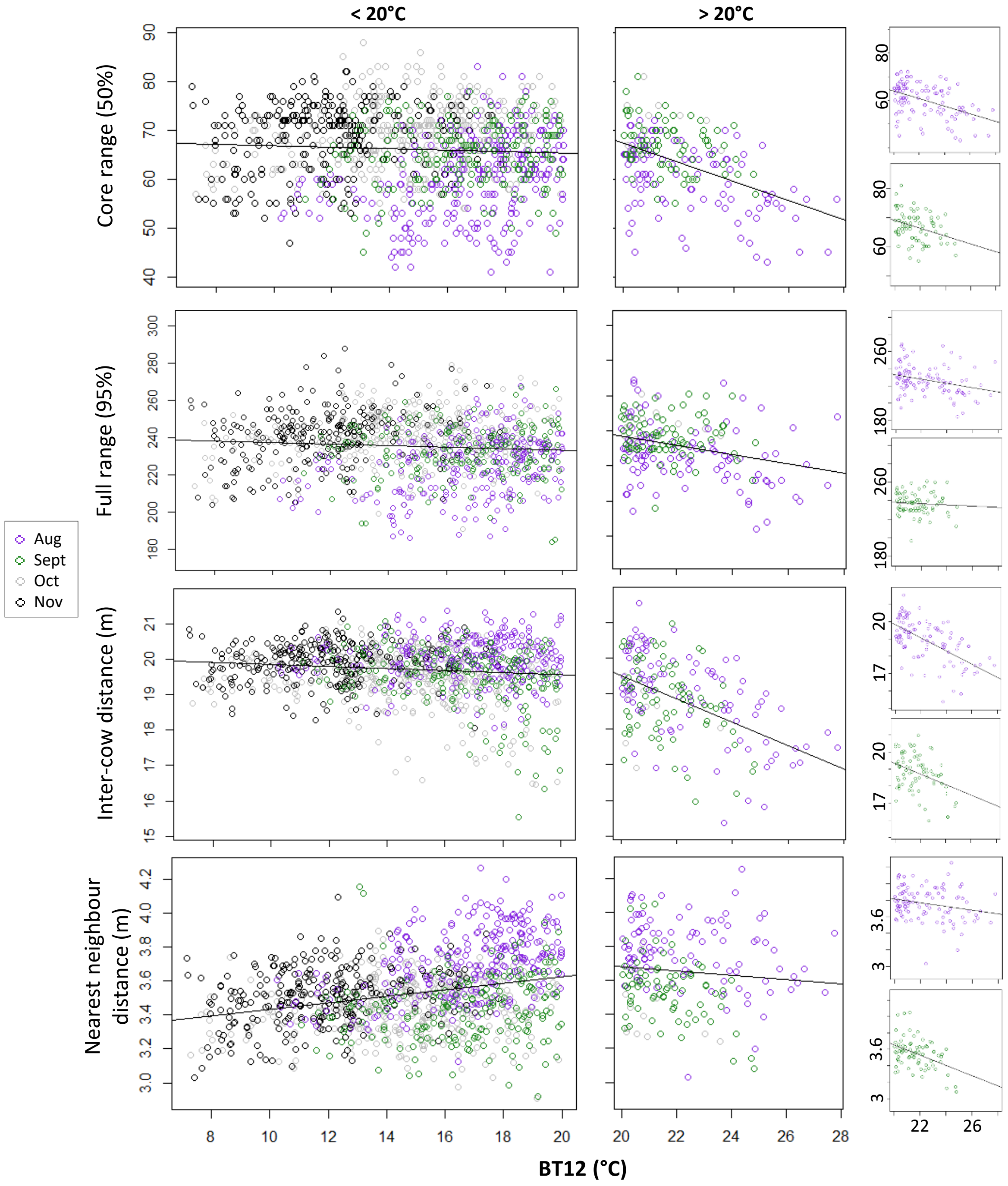

Figure 5. Linear regression plot outputs assessing the relationship between bunching and barn temperature (BT12) on the commercial farm in Essex, below and above (or equal to) the breakpoint of 20°C (≥ 20°C: *n* = 170). Plots are shown for each bunching metric (core range (50%), full range (95%), inter-cow distance (m) and nearest neighbour distance (m)), excluding outliers (*n* = 5), based on model assumptions, as shown in Appendix 1. A single point represents an average per hour and points are coloured by month: August = purple,

September = green, October = grey and November = black. Insets showing the patterns for August and September alone are also shown. Periods during which cows were in the milking parlour or collecting yard were removed (05:00-07:59, 12:00-14:59, 20:00-22:59), as well as periods during which the sensor reset (23:00 to 00:59).

For completeness, the analysis in this section was conducted using THI at a breakpoint of 65, roughly equivalent to 20°C at a relative humidity of 60% (the mean hourly value over the study duration). Humidity readings from two local weather stations were considered, and the results are qualitatively similar; there are significantly negative patterns between THI and CR, FR, and ICD, and a positive but negligible and non-significant relationship with NND (Appendix 3).

5.3.3. Cross-checking the breakpoint

Forcing a data-driven algorithm to find the location of breakpoints finds values close to 20°C for all the bunching metrics, except for FR which is not unexpected given outliers e.g., if a small number of cows remained in the FZ whereas the rest were bunching in the NFZ, this would skew the metric (Table 3). The (significant) negative trends observed using all four bunching metrics remain consistent above these forced breakpoints (Table 3).

Table 3. Data-driven barn temperature (BT12) breakpoints (BP) and corresponding results assessing the linear relationship between barn temperature (BT12) and each bunching metric: CR = core range, FR = full range, ICD = inter-cow distance, NND = nearest neighbour distance, on the commercial farm in Essex. Significant *p*-values are in bold. Outliers have been excluded, based on linear model assumptions (*n* = 5). Periods during which cows were in the milking parlour or collecting yard were removed (05:00-07:59, 12:00-14:59, 20:00-22:59), as well as periods during which the sensor reset (23:00 to 00:59).

Bunching metric	**BP (°C)**	**Chow-test**	**< BP**	**≥ BP**
CR	23.02	F = 2.07, *p* = 0.13	e = -0.15, SE = 0.08, t-value = -1.88, *p* = 0.06	e = -2.08, SE = 0.26, t-value = -8.00, ***p* = 1.26e-13**
FR	12.18	F = 0.63, *p* = 0.54	e = 1.60, SE = 0.78, t-value = 2.06, ***p* = 0.04**	e = -0.82, SE = 0.16, t-value = -5.19, ***p* = 2.58e-7**
ICD	19.69	F = 1.38, *p* = 0.25	e = -0.03, SE = 0.01, t-value = -3.56, ***p* = 0.0004**	e = -0.35, SE = 0.04, t-value = -7.86, ***p* = 1.87e-13**
NND	20.77	F = 4.14, ***p* = 0.02**	e = 0.02, SE = 0.002, t-value = 11.00, ***p* < 2e-16**	e = -0.01, SE = 0.01, t-value = -0.50, *p* = 0.62

5.3.4. Time of day

Time of day is divided into intervals between milking times; three intervals: one (I1: 1am to 4am- red; no values where BT12 ≥ 20°C), two (I2: 8am to 11am- blue; *n* = 33) and three (I3: 3pm to 7pm- yellow; *n* = 142).

Focussing on data above 20°C, none of the data points are in I1 and most data points lie in I3 as opposed to I2 (*n* = 156 and 39, respectively), which is likely due to the barn reaching higher temperatures during the morning and afternoon in comparison to the night/early morning. Above the breakpoint, the relationship is negative during interval two (Fig 6; CR: e = -1.49, SE = 0.87, t-value = -1.71, *p* = 0.10, FR: e = -3.41, SE = 2.20, t-value = -1.55, *p* = 0.13, ICD: e = -0.42, SE = 0.19, t-value = -2.16, *p* = 0.04, NND: e = -0.05, SE = 0.03, t-value = -1.53, *p* = 0.14; *n* = 35) and interval three (Fig 6; CR: e = -2.30, SE = 0.27, t-value = -8.61, p = 7.93e-15, FR: e = -3.28, SE = 0.66, t-value = -4.93, *p* = 2.08e-6, ICD: e = -0.35, SE = 0.04, t-value = -8.20, *p* = 8.85e-14, NND: e = -0.01, SE = 0.01, t-value = -0.66, *p* = 0.51; *n* = 155).

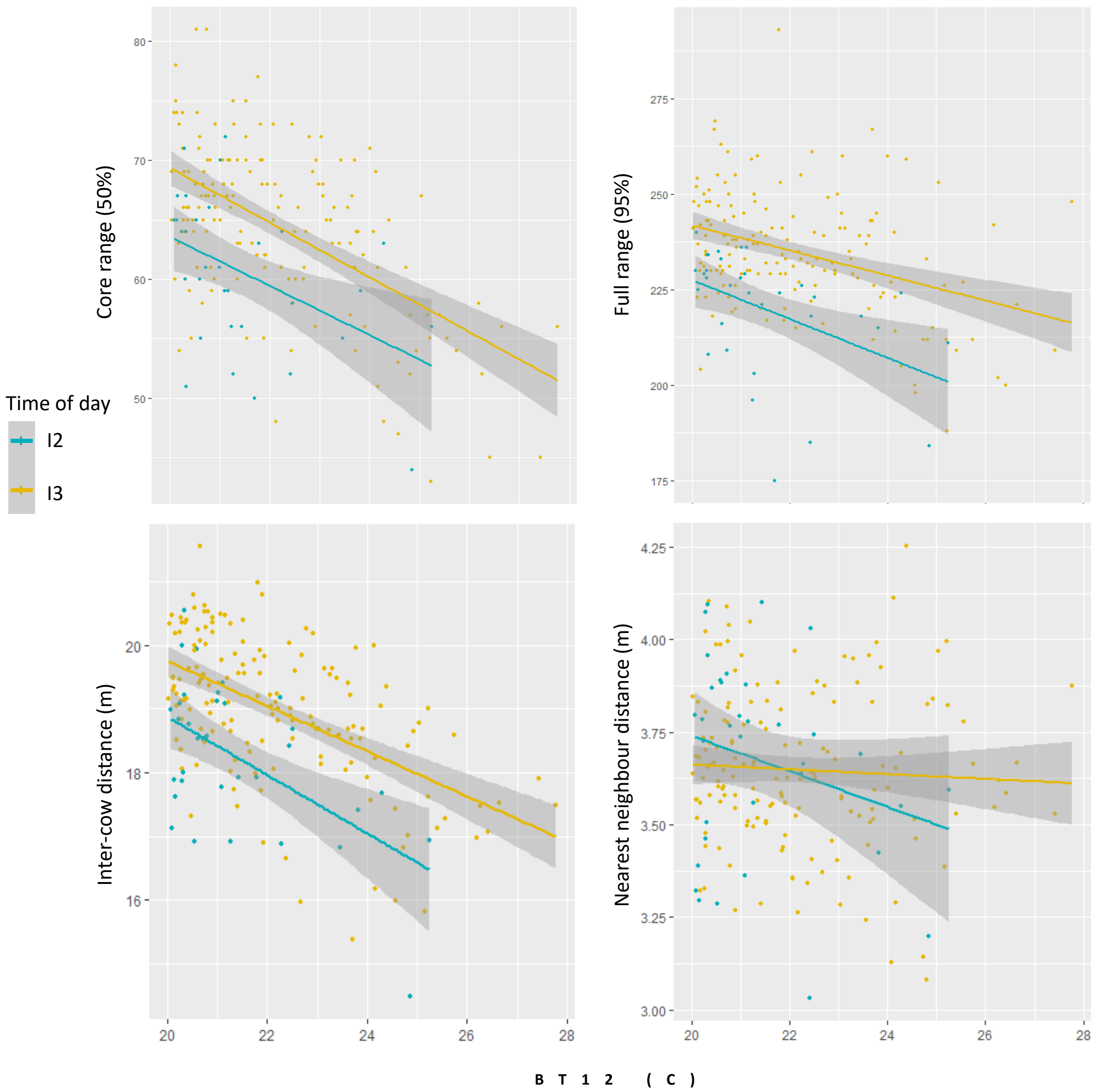

Figure 6. Barn temperature (BT12) against the bunching metrics: core range, full range, inter-cow distance and nearest neighbour distance, showing data from the commercial farm in Essex, where BT12 ≥ the breakpoint of 20°C and according to time of day. Time of day is inputted as a categorical variable, divided into intervals between milking times; three intervals: interval one (I1; 1am to 4am- red), interval two (I2; 8am to 11am- blue) and interval three (I3;3pm to 7pm- yellow). Note that there are no values in I1 for this dataset where BT12 ≥ 20°C. Periods during which cows were in the milking parlour or collecting yard were removed (05:00-07:59, 12:00-14:59, 20:00-22:59), as well as periods during which the sensor reset (23:00 to 00:59).

5.4. Discussion

Above or equal to 20°C, the herd increased their bunching behaviour, measured in terms of reduced: full and core range size, inter-cow distance (ICD) and nearest neighbour distance (NND) with increasing barn temperature, and this pattern is found to be significant for the range sizes and inter-cow distance. These trends persist during the afternoon and evening (8am to 11am and 3pm to 7pm), but not during the night/early morning (1am to 4am) because temperatures did not reach 20°C.

The main finding suggests that cows increase their bunching behaviour at warmer than average ambient temperatures. This supports previous observational studies investigating the relationship between bunching of dairy cows and temperature, by counting the number of cows in each of three to four areas of a barn at 15-minute intervals; cows have been found to crowd above but not below an ambient temperature of 19°C to 20°C (281,355). The findings from this chapter provide further support and a more detailed understanding of how cows may bunch at high temperatures, given the data are derived from higher frequency recordings and numerous measures of bunching are used, which consider various aspects in space-use and social proximity. Specifically, the results indicate that the herd uses less space as a whole at high barn temperatures (core range), and not only dyads (NND) position closer together at higher temperatures, but multiple individuals follow the same pattern (ICD).

The increase in bunching, using all measures, at warmer than average ambient temperatures is found to be present during the morning and afternoon but not during the night/early morning, as temperatures did not reach above the 20°C breakpoint during the latter periods. Similar findings have been found previously, where cows were found to crowd more during the afternoon, where temperatures were consistently recorded above 22°C than during the evening/early morning, where temperatures did not exceed 11°C (281,355). These alterations to the spatial positioning of the herd during specific periods of the day are likely to disrupt normal behaviours such as feeding, as stocking rates at the feed face are increased. Such changes have the potential to disproportionately affect cows with heath issues such as lameness who have been reported to moderate their feeding times to avoid competition at the feed face (242,400). The lack of bunching in the evening and overnight indicates that these periods are important for cows to undertake activities which may have been avoided during the day. The occurrence of increased tropical nights in the UK (401) and in other temperature zones may diminish the ability of cows to adapt to changing climatic conditions and to catch up on important behaviours e.g., feeding.

Cows may alter their spatial positioning during the day due to a preference for specific areas of the barn at high temperatures. Possible factors on the commercial farm in Essex which might result in the presence of microclimates, namely temperature, humidity and air flow within the barn follow: 1) mechanical ventilation was provided by fans in the left and the middle of the barn 2) water troughs were located in the centre cross passage and 3) sunlight was radiating through the wooden cladding on the right side of the shed, whereas no was sunlight on the left side of the shed due to the position of the parlour. Although the overall barn temperature did not vary significantly, cows may have detected variations in the microclimate of the barn due to these factors. It is not clear to what extent each of these factors affect the bunching behaviour, but it can be hypothesised that cows are either drawn to preferable microclimates or driven away from adverse microclimates when temperatures increase above 20°C. Further work to investigate temporal and spatial changes of the barn environment is needed to elucidate the drivers for bunching behaviours to improve housing designs.

Humidity readings from a local weather station were also used, given many studies use THI as well as ambient temperature to measure behavioural changes in dairy cattle, and major changes in THI were found to be driven by temperature (see Appendix 3). The relationships between THI and the bunching metrics were also qualitatively similar to those for temperature alone, with cows bunching to a greater degree with increasing THI beyond a threshold of 65. Regional or local humidity is unlikely to reflect the fine scale microclimate changes experienced by cows in different areas of the barn at different times. The breakpoint is based on previous studies on dairy cows and logical reasoning (243,367,384,402), and is checked by imposing at least one breakpoint using an algorithm. This is outputted as close to 20°C for three of the four bunching metrics, and the negative trend above these thresholds remain. It must also be considered that environmental conditions interact complexly so other measures may also contribute to heat stress, such as sunlight, humidity, wind speed and ventilation (358,384,403,404).

Due to the high metabolic demands of dairy cows, their bodies radiate significant heat into their local environment to maintain a stable core body temperature (405). Bunching of dairy cattle, whether due to management practices such as collection for milking or because of behavioural choices, has the potential to create hot and humid conditions capable of inducing heat stress. This has implications for welfare, with cows under heat stress showing signs of distress, such as an increase in respiration rate, excessive salivation and panting (346,379). Dairy milk yield, milk fat and milk protein decrease under heat stress, and production losses reduce profit (202,377,378,388,396). This is particularly pressing given heat stress is predicted to become a greater challenge in the dairy industry as demand increases (2,4,5), and due to the increase in tropical nights, including in regions where cattle have experienced little pressure and time to adapt to heat stress (401). Further study is required to better understand the relationship between bunching and temperature, considering appropriate heat stress thresholds at which to alert farmers.

5.5. Conclusions

This study provides evidence for an increase in bunching behaviour, measured using various spatial and social proximity measures, at higher than average ambient temperatures in a large housed dairy herd. This behaviour may be a maladaptive response and so the relationship between bunching and temperature needs further exploration, particularly given global temperatures are predicted to increase.

The main results of this chapter are outlined below:

- A dairy herd group housed on a commercial farm in Essex were monitored using a local positioning system, to understand the relationship between bunching behaviour and temperature.
- Bunching was measured using a range of metrics to capture various degrees of spatial positioning and social proximity: range size, inter-cow distance and nearest neighbour distance.
- The herd increased their bunching behaviour in terms of all four bunching metrics at barn temperatures exceeding or equal to 20°C.
- Data-driven breakpoints were outputted as close to 20°C, and the corresponding results are qualitatively similar.

5.6. Appendix 1. Temperature

The hourly barn temperature measures, BT1 and BT2, each a mean of independent sensors, are highly correlated throughout the study duration (Fig A1; all hours included: correlation = 0.90). Both barn temperature measures are correlated to sensor temperature (ST) (Fig A1 A-B; excluding milking times and sensor reset: BT1 = 0.91, BT2 = 0.95). A buffering effect is reflected in the differences in temperature ranges between ST and barn temperature (Fig A1; excluding milking hours and midnight reset; ST = 12.40°C to 24.96°C, BT1 = 7.40°C to 28.72°C, BT2 = 6.99°C to 27.60°C). Local temperature (LT) is correlated to ST, BT1 and BT2 (Fig A1 D-F; excluding milking times; correlations = 0.77, 0.79 and 0.80, respectively). The weaker relation between barn temperature and LT therefore confirms this as the most unreliable temperature measure. BT1 and BT2 are reliable, independent measures of temperature, so were combined as BT12 for the subsequent analysis.

Although BT1 and BT2 are well correlated overall, Fig A1A shows outliers in the BT1 measures (September 18th to September 29th), also evident when comparing BT1 to ST in Fig A2. The correlation is higher at 0.98 without these days, as shown in Fig A2. These outliers are likely due to a temporary malfunctioning of the BT1 sensors, potentially due to battery life. For these days, BT1 (*n* = 4) is used alone, rather than BT12. For readability, all data are referred to as BT12 henceforth.

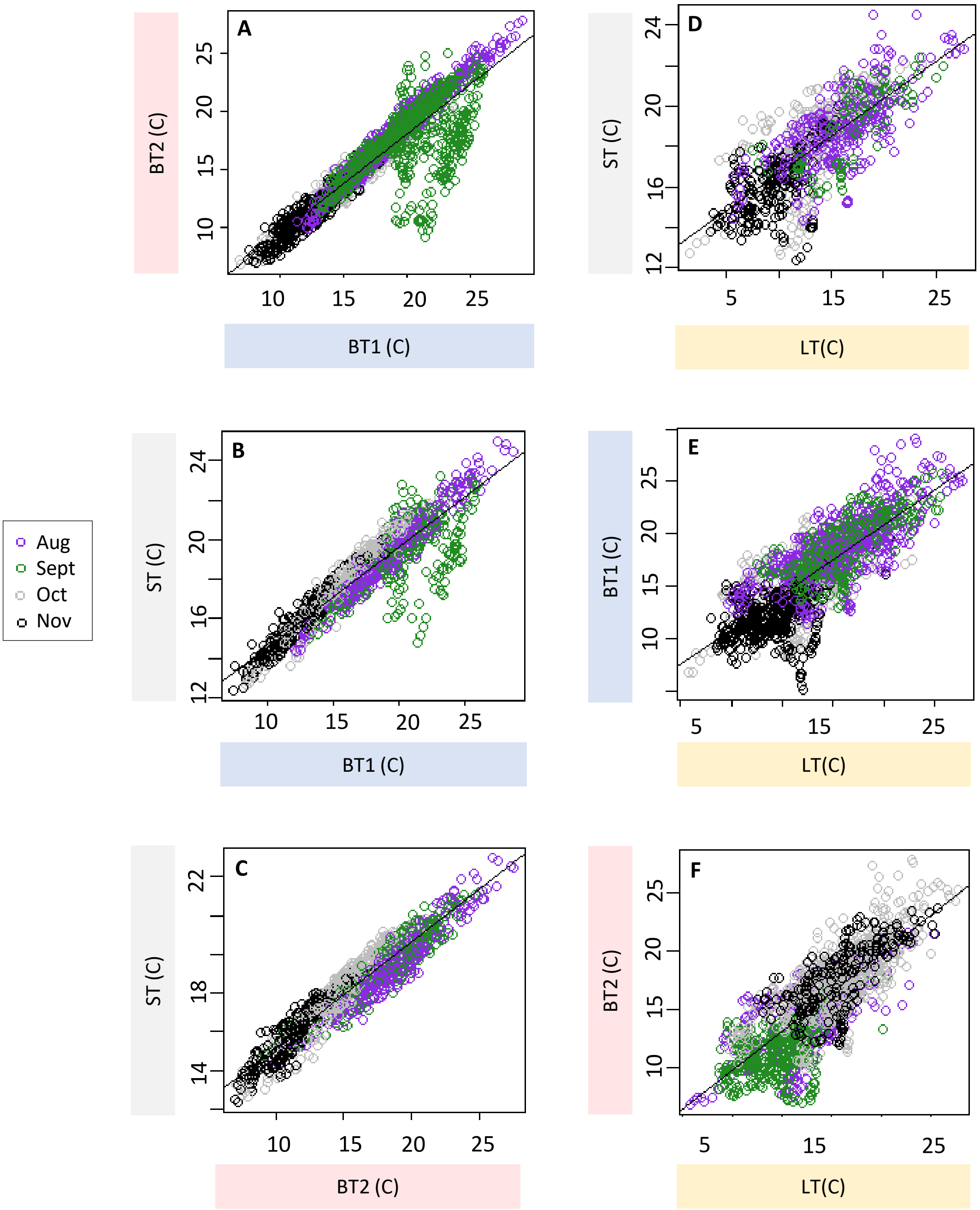

Figure A1. Relation between temperature measures: barn temperature 1 (BT1), barn temperature 2 (BT2), sensor temperature (ST) and local temperature (LT) (°C) on the commercial farm in Essex. (A) = BT1 and BT2, (B) = BT1 and ST, (C) = ST and BT2, (d) = LT and ST, (e) = LT and BT1 and (f) = LT and BT2. Months are colour coded: August = purple, September = green, October = grey and November = black. (A), (B) and (F) contain all hours, except sensor reset, whereas (B) to (D) exclude milking hours and sensor reset.

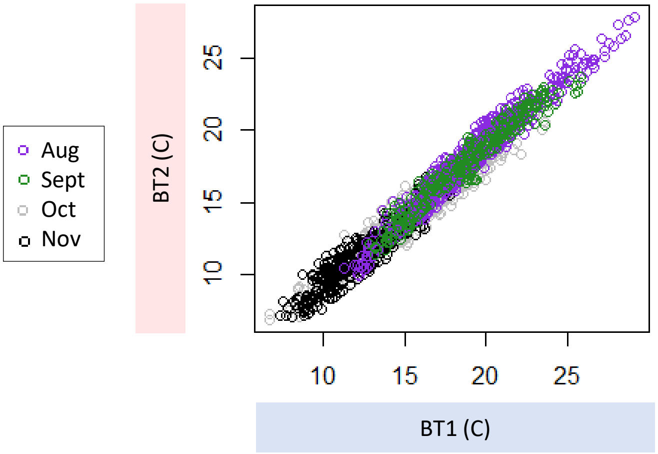

Figure A2. Relation between temperature measures: barn temperature 1 (BT1), barn temperature 2 (BT2), on the commercial farm in Essex, with outliers in September excluded. Outliers were recorded from September 18th to September 29th. Months are colour coded: August = purple, September = green, October = grey and November = black. Plot contains all hours otherwise, except sensor reset.

5.7. Appendix 2. Model assumptions.

The linear model assumptions, heteroscedasticity, and residual normality were tested where BT12 is below and equal to or above the breakpoint of 20°C, and the results show that assumptions are met for all the bunching metrics ≥ 20°C when excluding outliers (*n* = 5), apart from for residual normality for ICD (Table A1; Fig A1). Below 20°C, both model assumptions are not met for ICD, nor is the heteroscedasticity assumption for NND (Table A1; Fig A1). However, given the large sample size, particularly below the breakpoint (< 20°C: *n* = 1067, ≥ 20°C: *n* = 170), extreme values skewing the data from residual normality and homoscedasticity are not unexpected, so linear regression was used. Furthermore, comparing Table A2 to Table 2 in the main text, the conclusions drawn from the linear model outputs do not change drastically before excluding these outliers (*n* = 5).

Table A1. Outputs from the linear model assessing the relationship between bunching and barn temperature (BT12) on the commercial farm in Essex, below and above (or equal to) the breakpoint of 20°C (*n* = 195). Assumptions of heteroscedasticity and residual normality were tested using NVC and Shapiro-Wilks, respectively. Each test result is shown for each bunching metric (CR = core range, FR = full range, ICD = inter-cow distance, NND = nearest neighbour distance), including all the data (Y) and excluding outliers (N) (*n* = 5). Significant *p*-values are in bold.

		Heteroscedasticity		**Residual normality**	
Bunching metric	**Outliers exc.** (*n* = 5)	**< 20°C**	**≥ 20°C**	**< 20°C**	**≥ 20°C**
CR	N	Chi-sq = 0.01, *p* = 0.91	Chi-sq = 2.31, *p* = 0.13	W = 0.98, ***p* = 0.004**	W = 0.99, *p* = 0.22
	Y	Chi-sq = 0.39, *p* = 0.53	Chi-sq = 0.22, *p* = 0.64	W = 0.99, ***p* = 1.41e-8**	W = 0.99, *p* = 0.21
FR	N	Chi-sq = 0.85, *p* = 0.36	Chi-sq = 0.38, *p* = 0.54	W = 0.99, ***p* = 2.69e-8**	W = 0.96, ***p* = 5.67e-5**
	Y	Chi-sq = 0.19, *p* = 0.66	Chi-sq = 5.04, ***p* = 0.02**	W = 0.99, ***p* = 6.09e-7**	W = 0.99, *p* = 0.10
ICD	N	Chi-sq = 141.36, ***p* < 2.2e-16**	Chi-sq = 0.89, *p* = 0.35	W = 0.91, ***p* < 2.2e-16**	W = 0.91, ***p* = 1.14e-9**
	Y	Chi-sq = 93.97, ***p* < 2.2e-16**	Chi-sq = 0.84, *p* = 0.36	W = 0.94, ***p* < 2.2e-16**	W = 0.97, ***p* = 0.002**
NND	N	Chi-sq = 38.57, ***p* = 5.27e-10**	Chi-sq = 0.15, *p* = 0.70	W = 0.99, ***p* = 0.0007**	W = 0.97, ***p* = 0.0008**
	Y	Chi-sq = 36.78, ***p* = 1.32e-9**	Chi-sq = 2.47, *p* = 0.12	W = 1.00, *p* = 0.40	W = 1.00, *p* = 0.82

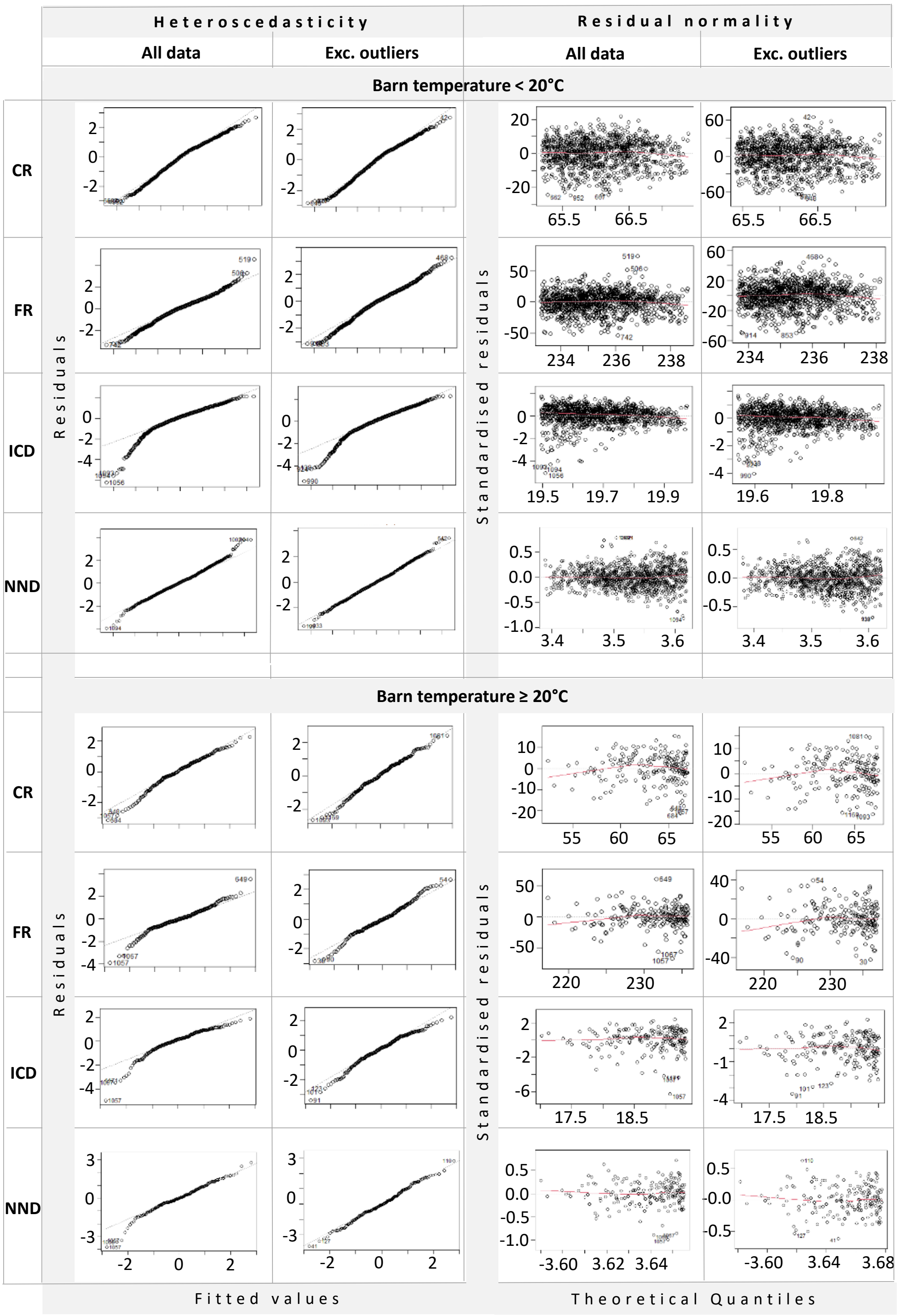

Figure A1. Visualisations of regression results and model assumptions assessing the relationship between bunching and barn temperature (BT12) on the commercial farm in Essex, below and above (or equal to) the breakpoint of BT12 = 20°C: heteroscedasticity, and residual normality. For each assumption, plots are shown for each bunching metric (CR = core range, FR = full range, ICD = inter-cow distance, NND = nearest neighbour distance).

Table A2. Outputs from the linear model assessing the relationship between barn temperature (BT12) and bunching, on the commercial farm in Essex, below and above (or equal to) the breakpoint of BT12 = 20°C (*n* = 195), including outliers (*n* = 5). Outputs from Chow tests, which test the significance of the breakpoint, are also shown. Each test result is shown for each bunching metric (CR = core range, FR = full range, ICD = inter-cow distance, NND = nearest neighbour distance). Significant *p*-values are in bold. Periods during which cows were in the milking parlour or collecting yard were removed (05:00-07:59, 12:00-14:59, 20:00-22:59), as well as periods during which the sensor reset (23:00 to 00:59).

Bunching metric	**Chow-test**	**< 20°C**	**≥ 20°C**
CR	F = 3.63, ***p* = 0.03**	e = -0.17, SE = 0.08, t-value = -2.12, ***p* = 0.03**	e = -1.89, SE = 0.28, t-value = -6.64, ***p* = 3.09e-10**
FR	F = 1.50, *p* = 0.22	e = -0.42, SE = 0.16, t-value = -2.57, ***p* = 0.01**	e = -2.39, SE = 0.73, t-value = -3.27, ***p* = 0.001**
ICD	F = 2.03, *p* = 0.13	e = -0.04, SE = 0.01, t-value = -4.63, ***p* = 4.1e-6**	e = -0.30, SE = 0.05, t-value = -5.66, ***p* = 5.52e-8**
NND	F = 8.21, ***p* = 0.0003**	e = 0.02, SE = 0.002, t-value = 8.60, ***p* < 2e-16**	e = -0.01, SE = 0.01, t-value = -0.78, *p* = 0.44

5.8. Appendix 3. THI analysis.

For completeness, the main analysis was conducted using THI at a breakpoint of 65, roughly equivalent to 20°C at a relative humidity of 60% (the mean hourly value over the study duration).

Humidity was not recorded within the barn. Although readings from a local weather station are not a direct measure of the indoor environment, and so micro-variation will not be captured correctly and corresponding results would rely on a few assumptions, macro-level changes in humidity should be. Hourly relative humidity readings were therefore obtained from local weather stations, Stanford Rivers and Harold Hill (406,407), approximately 3.5 miles and 7 miles from the study location respectively, and the Temperature Humidity Index (THI) is calculated using the equation from (408) (Equation A1):

Equation A1. $$1.8 \times T - (1 - RH)(T - 14.3) + 32,$$

where T = sensor temperature (°C), and RH = relative humidity.

Fig A1 shows the frequency of relative humidity recordings from the two local weather stations.

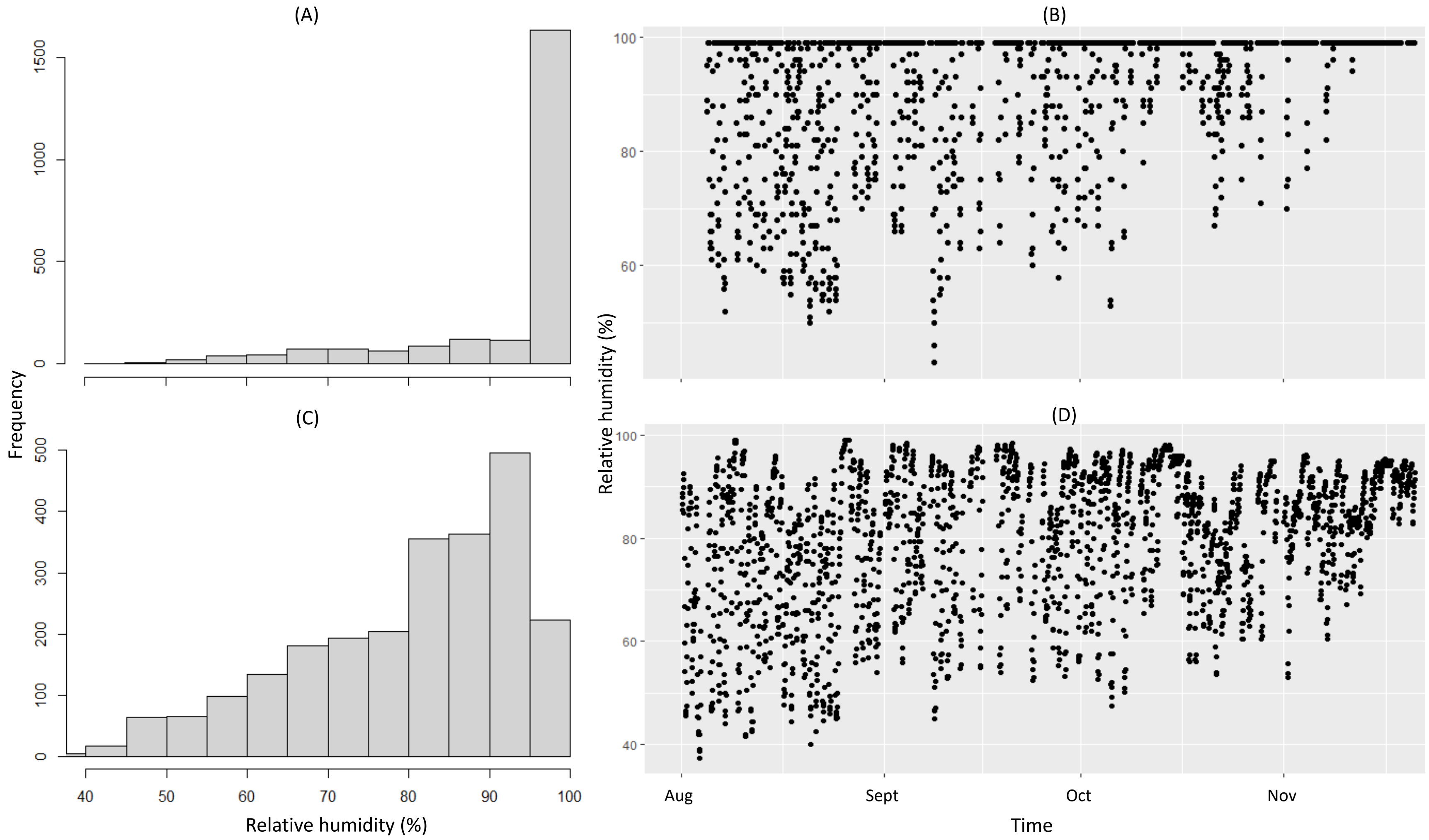

Figure A1. Plots showing relative frequency recordings from two weather stations: (A and B) Stanford Rivers and (C and D) Harold Hill near the commercial farm in Essex. For each weather station, the distribution of relative humidity (%) recordings are shown as a histogram (A and C) and the recordings are shown across the study duration (B and D). Stanford Rivers was located approximately 3.5 miles from study site and Harold Hill was located approximately seven miles from study site. Humidity readings were not available for a few days during the study period: August 1st to 3rd and September 1st, 2nd, 8th, 9th (9 of 13 hours) 11th, 12th (5 hours), 15th).

Figure A2 shows the humidity recordings from both weather stations over the study duration, and the results follow a similar pattern over time. Given humidity was often recorded at ~100% for consecutive recordings at Stanford Rivers, which indicates an issue with the sensor system, the following analysis was conducted using the data from Harold Hill (Fig A2).

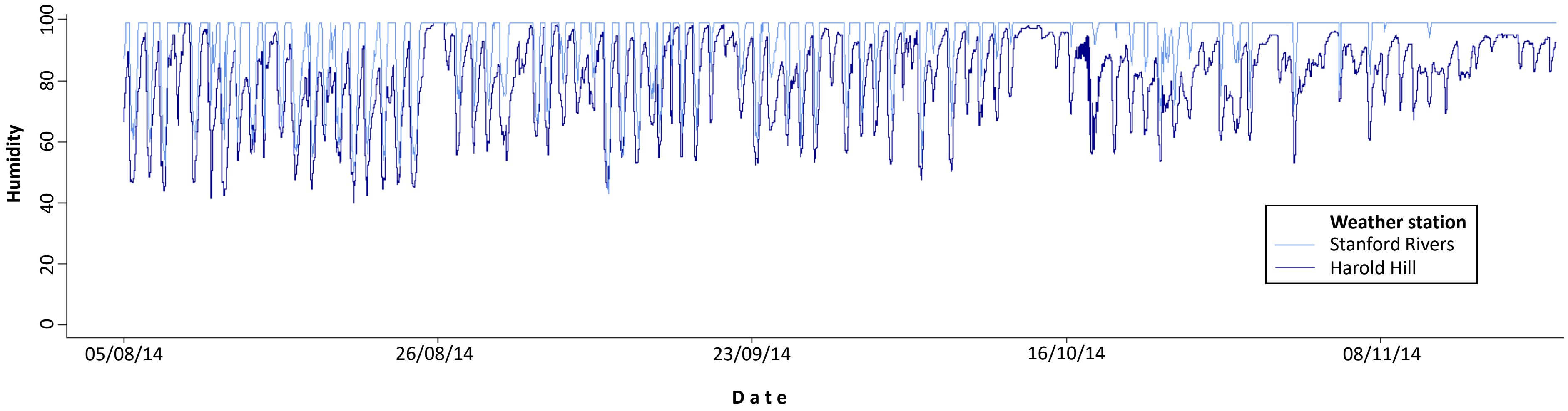

Figure A2. Hourly relative humidity, derived from two local weather stations, Stanford Rivers (light blue) and Harold Hill (dark blue) near the commercial farm in Essex. Values are shown per hour across the study duration. Stanford Rivers was located approximately 3.5 miles from study site and Harold Hill was located approximately seven miles from study site.

5.8.1. Harold Hill THI analysis

Fig A3 shows that THI using humidity from Harold Hill peaked during the afternoon. The mean hourly THI reaches above the breakpoint of THI = 65 during the afternoon in August and September, but not during October and November (Fig A3). Fig A4 shows THI per hour across time, showing that most of the values above the breakpoint of THI = 65 lie in August and September (~88.52%, *n* = 455 of 514 hours).

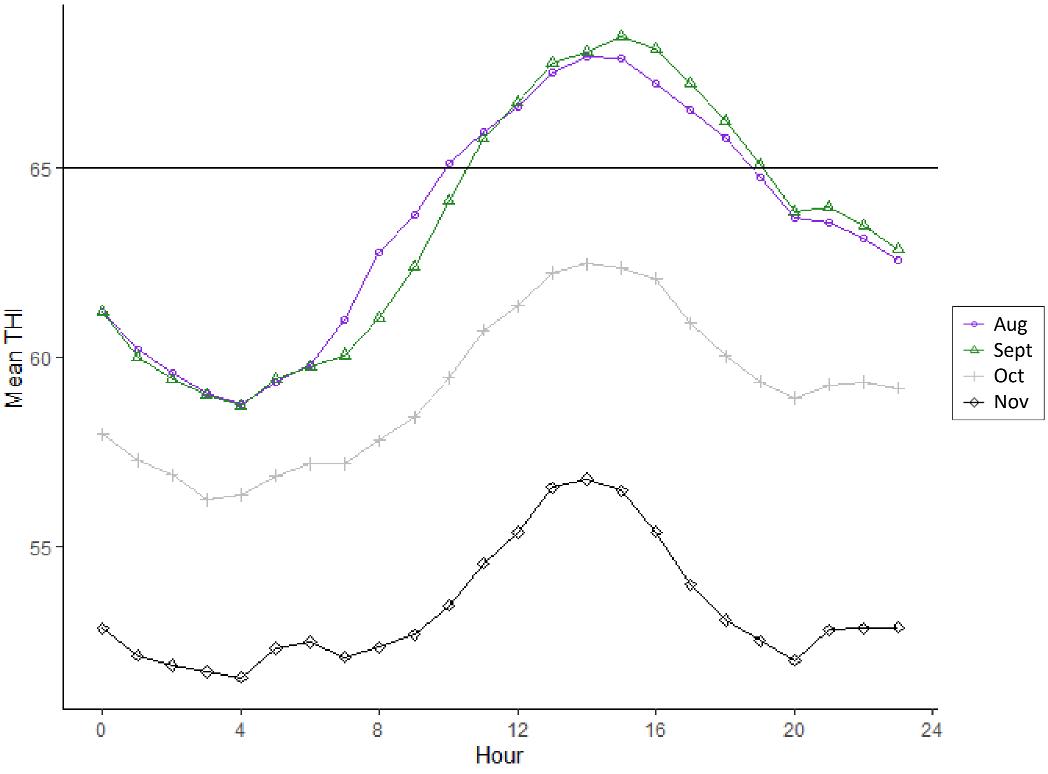

Figure A3. Mean hourly Temperature Humidity Index (THI) across the study duration, using barn temperature (BT12) and relative humidity derived from Harold Hill, a weather station near the commercial farm in Essex. Months are in 2014, and are August = red, September = light blue, October = dark blue, November = green. The breakpoint of THI = 65 is marked with a black line. Note that milking times are included.

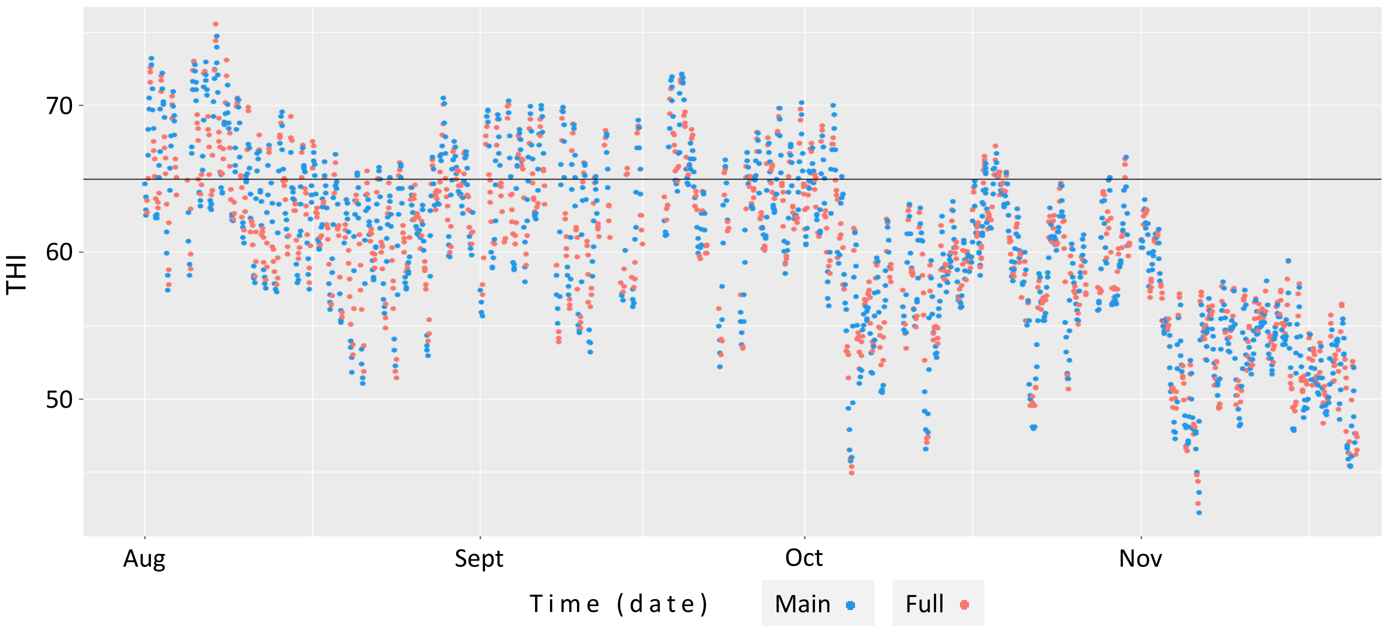

Figure A4. Hourly Temperature Humidity Index (THI) across time, using barn temperature (BT12) and relative humidity derived from a local weather station (Harold Hill) near the commercial farm in Essex. The times shown are from August 2014 to November 2014. The breakpoint of THI = 65 is marked with a black line (*n* = 451 of 1125 hours above THI = 65). The blue dots data points included in the analysis ("main"). The remaining data points, in red, were removed from the analysis due to milking or because they are when the system reset ("full").

The breakpoint of THI = 65 is significant for all the metrics except ICD, considering all the data, although this also becomes non-significant when excluding outliers for FR and NND (*n* = 5) to satisfy model assumptions (Table A1-A2; Fig A5). The relation is (significantly) negative above this breakpoint, before and after excluding the outliers (*n* = 5), except for with NND after excluding outliers (Table A1-A2; Fig A5). It is not unexpected that the negative pattern does not hold for NND i.e., the positive relationship with THI is negligible and non-significant (e = 0.001, *p* = 0.51; Table A2), as this is similar to the non-significant weak relationship found between NND and BT12 (e = -0.01, *p* = 0.19; main paper- Table 2).

Table A1. Outputs from a linear regression model assessing the relationship between the Temperature Humidity Index (THI) and bunching metrics, on the commercial farm in Essex, below and above the breakpoint of THI = 65 (*n* = 889 and 226, respectively), using humidity readings from Harold Hill weather station, with outliers included (*n* = 5). Chow test outputs testing the significance of the breakpoint are also shown. Each test is conducted for each bunching metric (CR = core range, FR = full range, ICD = inter-cow distance, NND = nearest neighbour distance). Significant *p*-values are in bold.

Bunching metric	**Chow-test**	**THI < 65** *[Shapiro-Wilks; NVC]*	**THI ≥ 65** *[Shapiro-Wilks; NVC]*
CR	F = 12.87, ***p* = 2.94e-6**	e = -0.13, SE = 0.06, t-value = -2.38, ***p* = 0.02** *[W = 0.98,* ***p = 5.54e-9;*** *Chi-sq = 0.97, p = 0.33]*	e = -1.44, SE = 0.18, t-value = -7.86, ***p* = 8.12e-14** *[W = 0.99,* ***p = 0.006;*** *Chi-sq = 0.68, p = 0.41]*
FR	F = 8.56, ***p* = 0.0002**	e = -0.20, SE = 0.11, t-value = -1.85, *p* = 0.06 *[W = 0.99,* ***p = 1.61e-7;*** *Chi-sq = 0.48, p = 0.49]*	e = -1.77, SE = 0.47, t-value = -3.81, ***p* = 0.0002** *[W = 0.98,* ***p = 0.0001;*** *Chi-sq = 5.44, p = 0.02]*
ICD	F = 1.98, *p* = 0.14	e = -0.01, SE = 0.005, t-value = -2.78, ***p* = 0.005** *[W = 0.94,* ***p < 2.2e-16;*** *Chi-sq = 61.71,* ***p = 3.99e-15****]*	e = -0.24, SE = 0.03, t-value = -6.97, ***p* = 2.28e-11** *[W = 0.91,* ***p = 6.84e-12;*** *Chi-sq = 0.73, p = 0.39]*
NND	F = 7.94, ***p* = 0.0004**	e = 0.01, SE = 0.001, t-value = 8.19, ***p* = 7.88e-16** *[W = 0.99,* ***p = 0.0003;*** *Chi-sq = 24.46, p =****7.59e-7****]*	e = -0.002, SE = 0.007, t-value = -0.23, *p* = 0.82 *[W = 0.98,* ***p = 0.0001;*** *Chi-sq = 0.96, p = 0.33]*

Table A2. Outputs from a linear regression model assessing the relationship between the Temperature Humidity Index (THI) and bunching metrics, on the commercial farm in Essex, below and above the breakpoint of THI = 65 (*n* = 884 and 221, respectively), using humidity readings from Harold Hill weather station, with outliers excluded (*n* = 5). Chow test outputs testing the significance of the breakpoint are also shown. Each test is conducted for each bunching metric (CR = core range, FR = full range, ICD = inter-cow distance, NND = nearest neighbour distance), based on model assumption testing. Humidity readings were derived from a local weather station, Harold Hill. Significant *p*-values are in bold.

Bunching metric	**Chow-test**	**THI < 65** *[Shapiro-Wilks; NVC]*	**THI > 65** *[Shapiro-Wilks; NVC]*
CR	F = 5.84, ***p* = 0.003**	e = -0.11, SE = 0.05, t-value = -2.07, ***p* = 0.04** *[W = 0.98,* ***p = 5.54e-9;*** *Chi-sq = 0.97, p = 0.33]*	e = -1.52, SE = 0.17, t-value = -8.76, *p* < 2.e-16 *[W = 0.99, p = 0.06;* *Chi-sq = 2.60, p = 0.11]*
FR	F = 1.84, *p* = 0.16	e = -0.20, SE = 0.11, t-value = -1.85, *p* = 0.06 *[W = 0.99,* ***p = 1.61e-7;*** *Chi-sq = 0.48, p = 0.49]*	e = -0.19, SE = 0.04, t-value = -4.98, ***p* = 7.21e-7** *[W = 0.99,* ***p = 0.06;*** *Chi-sq = 5.78, p = 0.02]*
ICD	F = 1.87, *p* = 0.15	e = -0.01, SE = 0.005, t-value = -2.45, ***p* = 0.01** *[W = 0.94,* ***p < 2.2e-16;*** *Chi-sq = 61.71,* ***p = 3.99e-15****]*	e = -0.25, SE = 0.03, t-value = -7.98, ***p* = 3.84e-14** *[W = 0.95,* ***p = 2.27e-8;*** *Chi-sq = 1.49, p = 0.22]*
NND	F = 0.14, *p* = 0.87	e = 0.01, SE = 0.001, t-value = 8.53, ***p* < 2e-16** *[W = 0.99,* ***p = 0.0003;*** *Chi-sq = 24.46,* ***p = 7.59e-7****]*	e = 0.001, SE = 0.01, t-value = 0.14, *p* = 0.89 *[W = 1.00, p = 0.51;* *Chi-sq = 0.07, p = 0.79]*

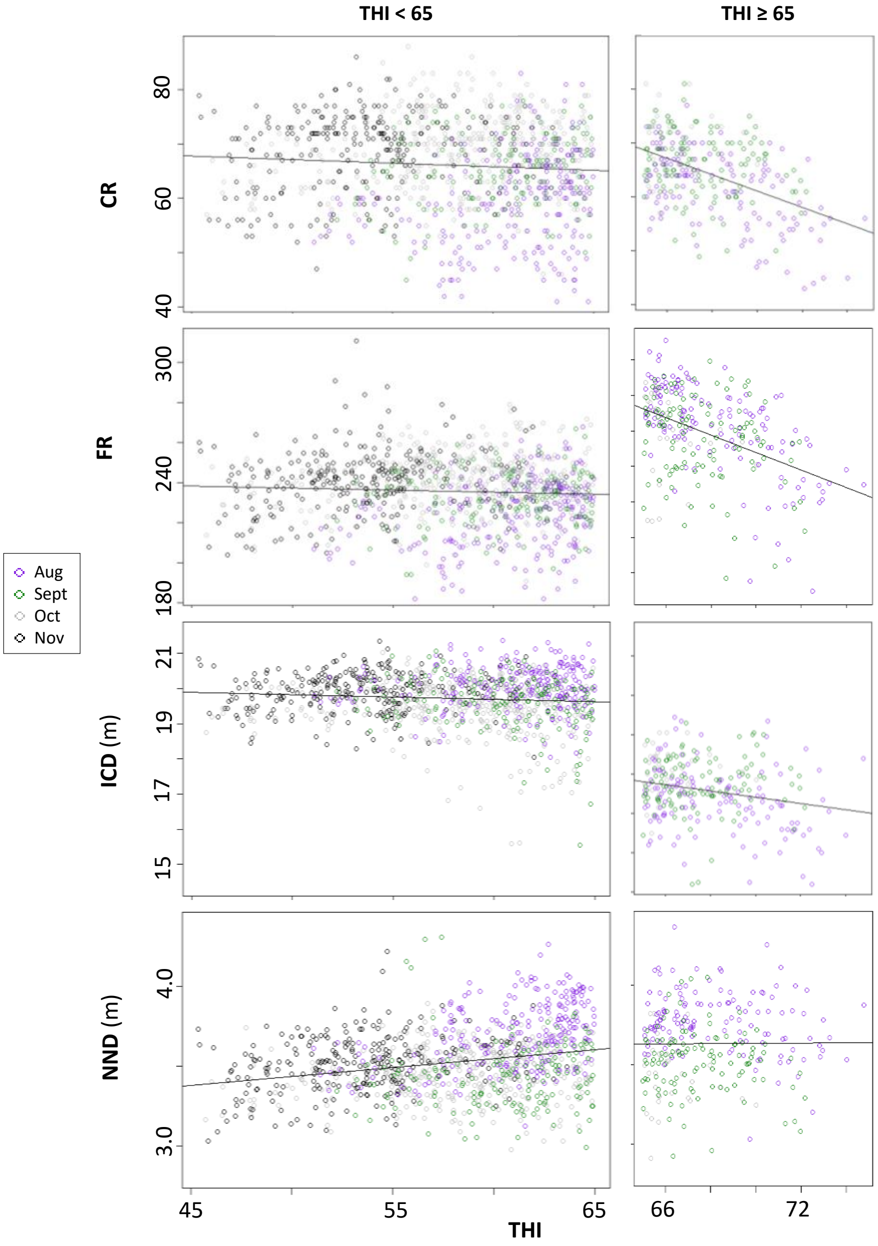

Figure A5. Visualisation of linear model outputs, assessing the relationship between the Temperature Humidity Index (THI) and bunching metrics, on the commercial farm in Essex, below and above the breakpoint of THI = 65 (*n* = 884 where THI < 65 and 221 where THI ≥ 65), with humidity derived from Harold Hill weather station. Plots are shown for each bunching metric (CR = core range, FR = full range, ICD = inter-cow distance, NND = nearest neighbour distance), excluding outliers (*n* = 5), based on model assumption testing. A single point represents an average per hour and points are coloured by month: August = purple, September = green, October = grey and November = black.

Forced breakpoints are outputted as approximately THI = 54 to 68, and the relations above these breakpoints remain negative, and are significant for all metrics except for NND (Table A3).

Table A3. Data-driven barn temperature (BT12) breakpoints (BP) and corresponding results assessing the linear relationship between the Temperature Humidity Index (THI) and each bunching metric: CR = core range, FR = full range, ICD = inter-cow distance, NND = nearest neighbour distance, on the commercial barn in Essex, using humidity readings from Harold Hill weather station. THI breakpoints were found using a data-driven approach. Significant *p*-values are in bold.

Bunching metric	**BP**	**Chow-test**	**< BP**	**> BP**
CR	68.27	F = 5.20, ***p* = 0.01**	e = -0.07, SE = 0.04, t-value = -1.68, *p* = 0.09	e = -2.36, SE = 0.47, t-value = -5.02, ***p* = 2.29e-6**
FR	54.39	F = 2.34, *p* = 0.10	e = 0.90, SE = 0.43, t-value = 2.11, ***p* = 0.04**	e = -0.58, SE = 0.11, t-value = -5.22, ***p* = 2.19e-7**
ICD	65.52	F = 2.43, *p* = 0.09	e = -0.02, SE = 0.005, t-value = -3.45, ***p* = 0.001**	e = -0.27, SE = 0.03, t-value = -7.92, ***p* = 8.5e-14**
NND	68.47	F = 1.05, *p* = 0.35	e = 0.01, SE = 0.001, t-value = 10.23, ***p* < 2e-16**	e = -0.01, SE = 0.02, t-value = -0.80, *p* = 0.42

6. CHAPTER 6: EFFECTS OF TEMPERATURE ON A DAIRY HERD HOUSED IN AN OPEN BARN

6.1. Introduction

In Chapter 5, evidence of a dairy cow herd housed in a commercial barn with cubicles (defined as a closed barn) increasing their bunching behaviour in response to increasing temperature is provided. Bunching may occur around shaded areas, water or places with better ventilation, to reduce heat load, or the behaviour may serve to distribute fly burdens (49,282,356,362,363,409). Nonetheless, close proximity to other animals means that bunching may maladaptively increase internal body temperature, and so an investigation into whether there are relationships between temperature and bunching (or other behaviours) in different herd groups and barn environments is vital (55,211).

The temperature at which deviations from normal physiological states, production, and behaviour are visible proxies for heat stress, varies between studies. For instance, reports show respiration rate (RR) increases above threshold temperatures (expected as metabolic costs are known to scale with temperature in mammals (410), but this pattern is observed from as low as 15°C or temperatures as high as 26°C depending on the study group (381,383,384). Although temperature alone is used as a proxy for heat stress, any behavioural changes in cattle could also be attributed to a significant impact of relative humidity (e.g., Temperature-Humidity Index (THI)), often used to assess heat stress, which was not reported in some earlier studies (381,382). However, comparisons between THI thresholds, which consider humidity, still show differences, with measured RR increasing above environmental THI as low as 64.7 or THI as high as 70 depending on the study group (379,411). Furthermore, with every 1°C increase in mean hourly ambient temperature, lying down rate is shown to decrease by 21 minutes from 19°C or 22 minutes from 21°C (368,369). The temperature threshold at which changes can be detected therefore does not only appear to be dependent on the measured variable, but also on the study group itself.

Cow behaviour may also be linked with temperature outside of the context of heat stress. For example, bunching occurs at low temperatures to share warmth (352,353,404). Other behaviours may also change depending on mid-range temperatures, such as drinking; for each degree Celsius increase in ambient temperature, water intake is found to rise (409,412–414), although this is not the case across all herds, highlighting the need to investigate changes in behaviour across herd groups (415,416).

Differences in temperature and THI thresholds at which changes in behavioural, physiological, and other metrics are found may be dependent on housing type. As temperature or THI increases, cows are more likely to undergo heat stress if barns are designed without consideration for shade, building materials, and ventilation (417), but this remains an assumption that has rarely been tested. The overall layout of a barn can contribute to the effectiveness of thermoregulation, with open spaces providing more opportunity to distance from conspecifics to cool down. A cow’s ability to use shaded areas to thermoregulate may in turn be compromised in housing systems with less space, leading to maladaptive behaviours, such as bunching at high temperatures. Housing may also affect behaviour irrespective of temperature changes. The physical layout may preclude or allow certain behaviours which other barn layouts do or do not allow. Negative impacts are reported in tie stall housing, including: increased time spent standing, increased lying interruptions and more collisions with housing equipment, compared to loose housing (418–422). The slope and width of stalls may further impact lying behaviour, with evidence of steeper slopes reducing lying duration, and narrower stall partitions increasing lying duration (423,424). Bar biting and leaning against equipment is also shown to be more prevalent in a tie stall system compared to a loose system (425). It therefore appears that natural behaviour is suppressed to a greater extent in areas with less space. In line with this, the impact of a high heat load is likely to be heightened at higher stocking densities (417), or in constrained spaces. Given the investment in the design of barns, financial decisions should be informed by animal welfare and production. Comparing between barn designs can help inform this debate so it is useful to investigate whether animal comfort, including thermoregulation, is optimised in specific housing.

This chapter aims to further the investigation of bunching behaviour with temperature, which was conducted in Chapter 5 using data collected from a herd housed on a commercial farm in Essex. This is carried out by investigating whether a second herd group, referred to as the herd on the RVC Research farm, also bunch in response to increasing temperature. Cows on the RVC Research farm, also in southeast England, were housed with a greater space allowance (lower stocking density and open barn layout i.e., no cubicles) than the herd on the commercial farm in Essex (higher stocking density and closed barn layout i.e., with cubicles). On the RVC Research farm, mean hourly barn temperature (BT2) was only recorded ≥ 20°C on two occasions, due to the location of the sensors in areas of the barn other than the non-feeding and feeding zones. Therefore, the hypothesis from Chapter 5 (bunching increases with barn temperature above ≥ 20°C), based on heat stress onset, is not appropriate. Instead, a purely data-driven approach is adopted in this chapter to investigate possible behavioural links between bunching and temperature.

6.2. Methods

6.2.1. Animals and housing

As detailed in the General Methodology (Chapter 2), a dairy cow herd (approximately 90% Holstein-Friesian and approximately 10% Brown Swiss) was continuously monitored on a farm referred to as the RVC Research farm, where cows were housed in an open barn (no cubicles) at a moderate stocking density (17th October 2015 to 7th December 2015; $n = 55$, a minimum of 45 cows each month; stocking density of feed space = 4.04m^2 to 4.93m^2 per cow, lying space = 7.28m^2 to 8.90m^2 per cow) (Figure 1). For further details on the herd and their management, please refer to Chapter 2, Section 2.4.

6.2.2. Local positioning system and data pre-processing

Each cow was equipped with a combined local positioning sensor and accelerometer, Omnisense 500 (298), mounted on a weighted neck collar. Refer to Chapter 2, Section 2.5 for further information on the sensor system.

Data selection and pre-processing was conducted as outlined in Chapter 2, Section 2.4. For the main analysis, hours when most cows were in the milking parlour or collecting yard were excluded (05:00 to 07:59 and 14:00 to 16:59), since their behaviour during these time periods was constrained by farm staff. The sensor system reset at midnight each day and hence times between 23:00 and 00:59 were also excluded.

6.2.3. Temperature

Barn temperature was recorded every ten seconds via fixed Oms500 sensors (BT2; *n* = 3) (298) which were placed in the parlour, parlour exit and handling area (referred to as 'fixed sensors recording BT2' in Fig 1). It was planned to derive barn temperature recordings from the remaining Om500 sensors (*n* = 12; see 'fixed sensors' in Fig 1), but upon review of the data, it was found that these sensors were not set to record temperature correctly. Although readings from inside the feeding and non-feeding zones would have provided a better direct indication of the temperatures the herd were experiencing, the measurements derived from the three BT2 sensors are nevertheless still a good indicator of the ambient barn temperature and are more accurate compared to other possible sources of temperature data (e.g., from local weather stations). For the purposes of analysis, the average hourly measure across BT2 sensors (*n* = 3) was calculated (as for BT12 in Chapter 5).

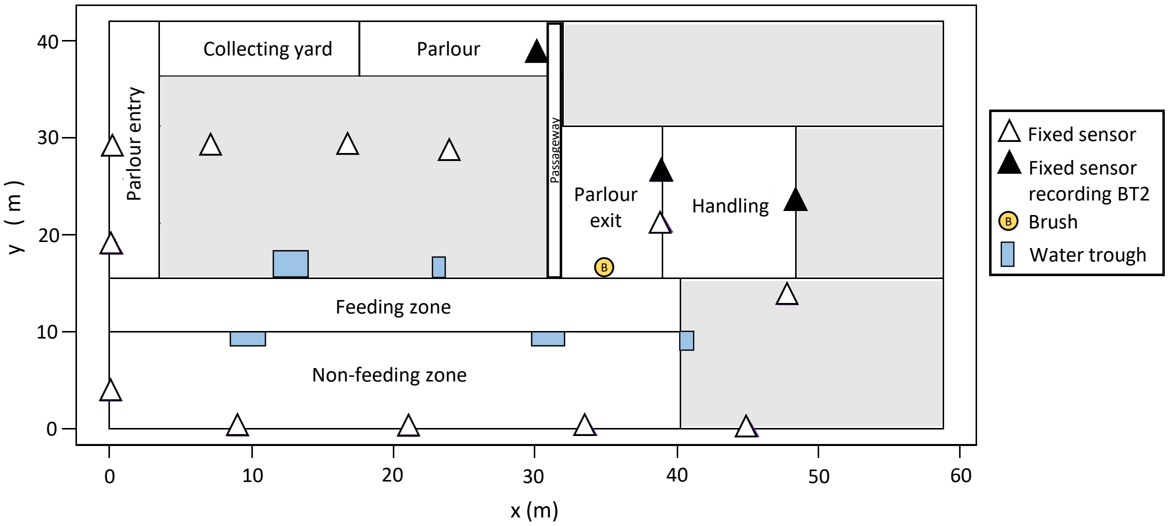

Figure 1. Barn layout of the RVC Research farm showing the positions of the fixed sensors (Oms-500) used to form a triangulated network to fix the absolute spatial position and record barn temperature. Fixed sensors are shown with white triangles, and those that recorded barn temperature (BT2; *n* = 3) are shown with black triangles. The barn is divided into the feeding zone (FZ) and the non-feeding zone (NFZ). The milking parlour (MP), collecting yard (CY), feed barrier (FB) and passageway (P) are also shown. The grey areas were not used by the study group. However, during the study period, a low-yield dairy cow herd was housed in the grey area between the collecting yard/parlour and feeding zone. Furthermore, during the study period, a group of young stock were also housed in the grey area below the handling area, where x = ~ > 40m.

To explore time-dependent effects, the data were split into roughly two-week periods: division one (16/10/2015 to 28/10/2015), division two (29/10/2015 to 10/11/2015), division three (11/11/2015 to 23/11/2015) and division four (24/11/2015 to 08/12/2015).

Although division four of the study duration (24/11/2015 to 08/12/2015) is on average a cooler period compared to the remaining time periods (16/10/2015 to 23/11/2015) in the UK, in 2015 the temperatures for this division were the warmest on record across the UK to date according to numerous weather stations (426). BT2 peaked during the afternoon with troughs during the night, as expected (Fig 2). The coolest median temperature (BT2) was recorded in division one (16/10/2015 to 28/10/2015) at 13.76°C and the hottest in division two (29/10/2015 to 10/11/2015) at 17.50°C), with divisions three (11/11/2015 to 23/11/2015) and four (24/11/2015 to 08/12/2015) as 14.78°C and 15.15°C respectively, and a significant difference in median temperature between divisions (Fig 1; K-W chi-squared = 308.65, $p < 2.2e\text{-}16$). The mean hourly BT2 is consistently higher in division two, always above 15°C, compared to in the remaining divisions where BT2 only reaches above 15°C during the afternoon.

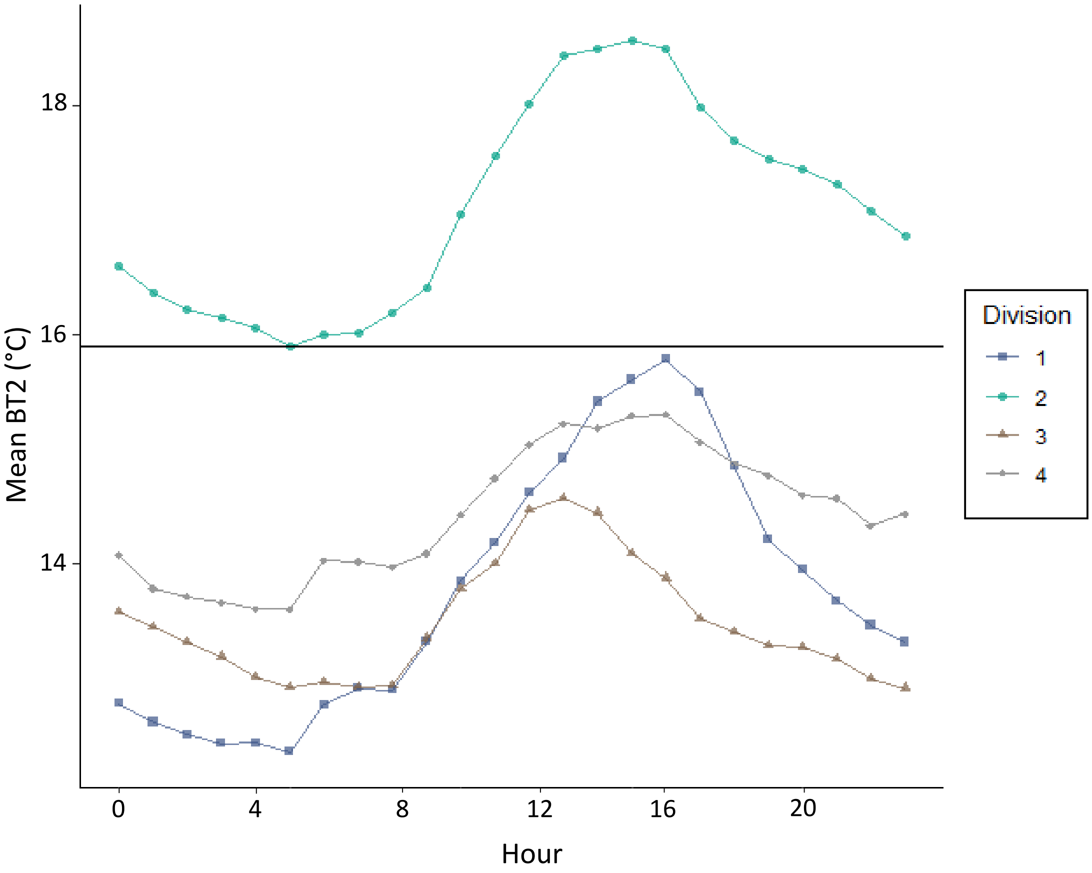

Figure 2. Mean barn temperature (BT2 in °C) per hour for each of four 13-day divisions of the study duration on the RVC Research farm. Divisions are defined as: division one (16/10/2015 to 28/10/2015) = blue squares, division two (29/10/2015 to 10/11/2015) = green circles, division three (11/11/2015 to 23/11/2015) = brown triangles, division four (24/11/2015 to 08/12/2015) = grey diamonds). A breakpoint of 15.91°C is marked with a black line. Note that data from milking periods are included.

Figure 3 shows BT2 per hour across time for the entire study period; the highest values lie in division two, where mean hourly BT2 reaches up to 20.50°C.

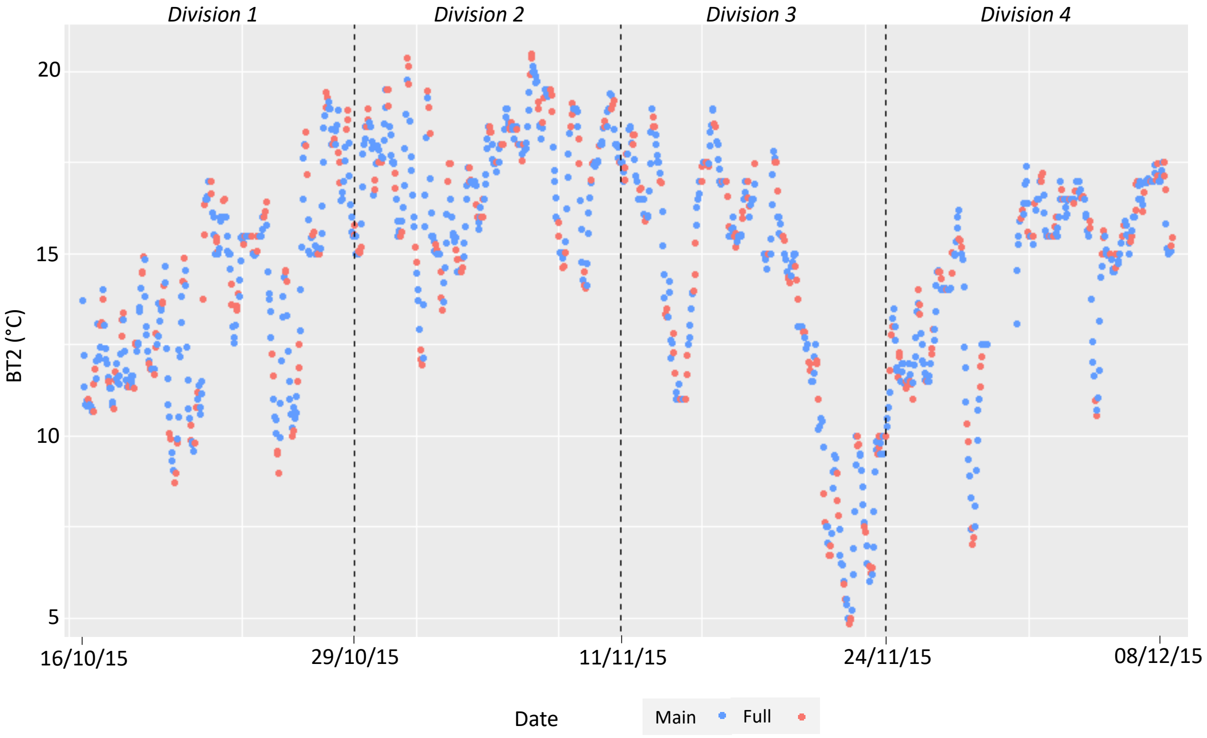

Figure 3. Temperature (BT2 in °C) per hour across time for the entire study duration on the RVC Research farm. Divisions marking 13-day blocks are marked with black vertical lines: division one (16/10/2015 to 28/10/2015), division two (29/10/2015 to 10/11/2015), division three (11/11/2015 to 23/11/2015) and division four (24/11/2015 to 08/12/2015)). The blue dots data points included in the analysis ("main"). The remaining data points, in red, were removed from the analysis due to milking (05:00 to 07:59 and 14:00 to 16:59) or when the system reset (approximately 23:00 to 00:59) ("full").

6.2.4. Bunching metrics

Given there are no formal definition of 'bunching' behaviour in the context of precision livestock farming (PLF), four bunching metrics are used: core range (CR), full range (FR), mean inter-cow distance (ICD), and mean nearest-neighbour distance (NND). These metrics measure different aspects of space-use and social proximity, which may be indicative of bunching behaviour. Please refer to Section 2.4.12 (Chapter 2) for further details on why and how these metrics were calculated.

6.2.5. Segmentation and breakpoints

6.2.5.1. Breakpoints

To identify the locations of forced breakpoints, the R package 'segmented' was used (399). For each bunching metric, the original linear model (for all BT2 values) was inputted into the 'segmented()' function e.g., segmented(CR ~ BT2), specifying one to two breakpoint(s) in BT2. The identified BP was used to create two new linear regression models e.g., 1) CR ~ BT2 where BT2 < BP and 2) CR ~ BT2 where BT2 ≥ BP, and the results were analysed for each of the eight models. After identifying the location of these breakpoints, Chow tests were performed to test their significance. Based on these results and the BT2 distribution, a breakpoint of 15.91°C was adopted for the final linear regression model.

For all linear models, default R settings were used, which excludes observations containing NA values and uses QR factorisation, an extensively used method for solving least squares problems. Model assumptions were considered before drawing conclusions: heteroskedasticity through residual vs fitted plots and NVC tests, and residual normality through QQ-plots and the Shapiro-Wilks test (Appendix 1).

6.2.5.2. Time of day

To explore whether time of day effects bunching behaviour, the data were split into time intervals between milking periods: one (I1: 8am to 1pm- blue; n = 130) and two (I2: 5pm to 4am- yellow; n = 315). The data corresponding to each of the two intervals were then used to create linear regression models e.g., CR ~ BT2 for data recorded during I1 and BT2 ≥ 15.91°C, and the results were analysed.

6.3. Results

6.3.1. Bunching metrics: time

Visualising patterns in bunching metrics shows variation across time (Fig 4-5). There are sharp declines in the bunching metrics during milking periods, where cows were located within the restricted space of the milking parlour, except for full range as several cows remained in the main barn at a given time during milking periods (Fig 4-5).

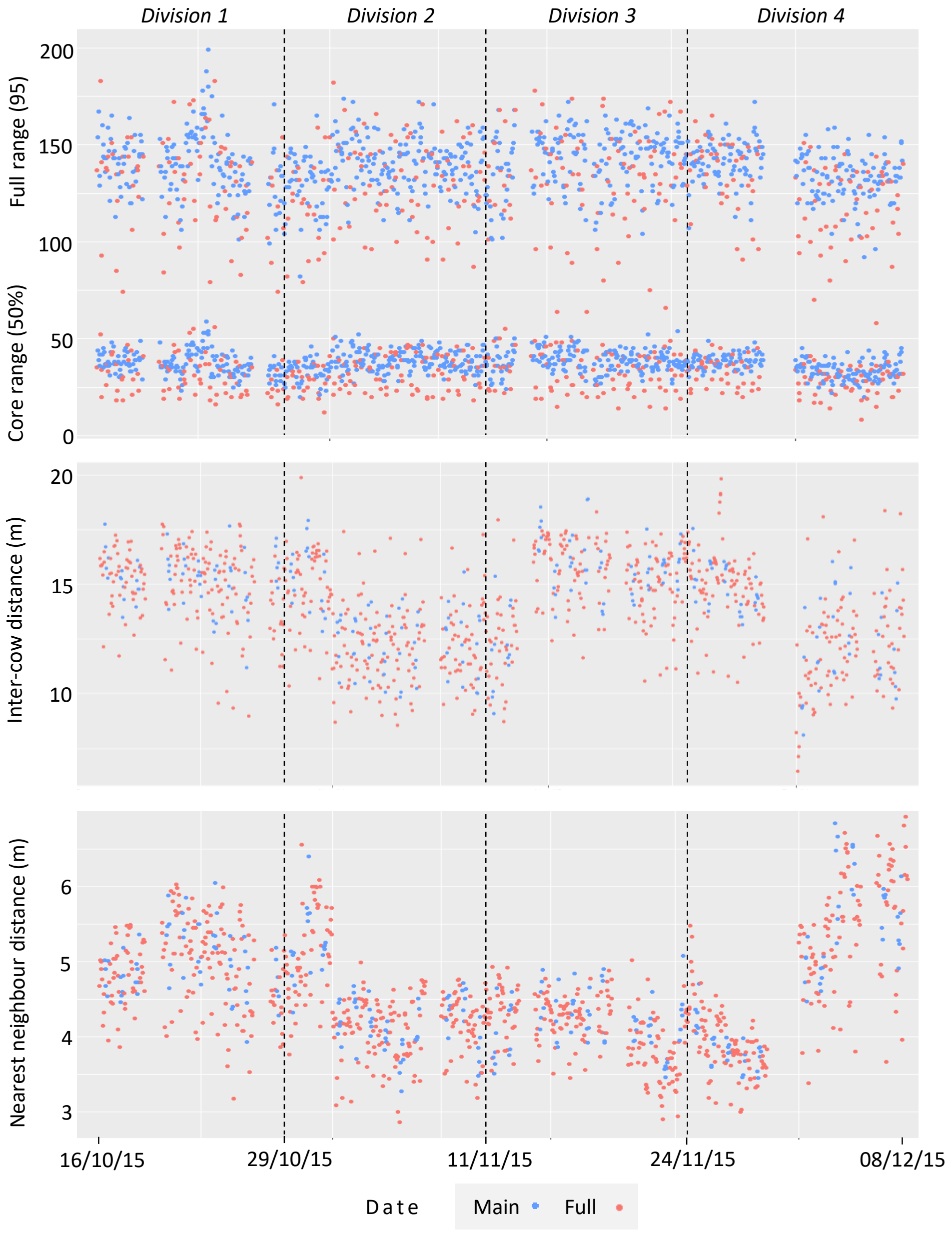

Figure 4. Bunching metrics: hourly core range (50%), full range (95%), inter-cow distance (m) and nearest neighbour (m) against time on the full RVC Research farm. Divisions marking 13-day blocks are marked with black vertical lines: division one (16/10/2015 to 28/10/2015), division two (29/10/2015 to 10/11/2015), division three (11/11/2015 to 23/11/2015) and division four (24/11/2015 to 08/12/2015)). Data points are colour-coded according to hours cows spent in the main barn ('main'; blue) and in the milking yard/collecting parlour (05:00 to 07:59 and 14:00 to 16:59) or when the sensor system reset at midnight (approximately 23:00 to 00:59) ('full', red).

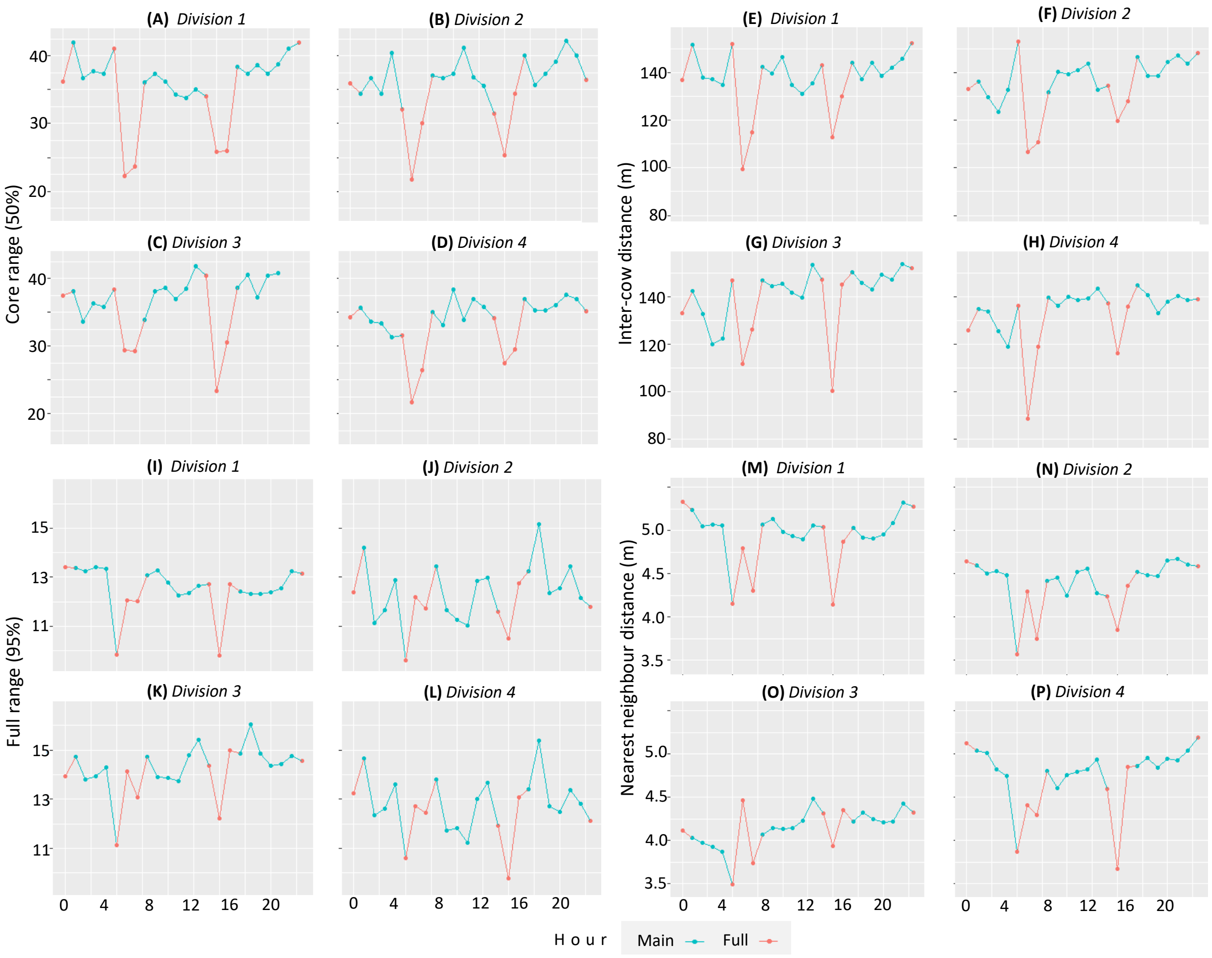

Figure 5. Mean hourly bunching metric: core range (50%; CR), full range (95%; FR), inter-cow distance (m; ICD) and nearest neighbour (m, NND), for the full herd on the RVC Research farm, by divisions of 13-day blocks. Divisions are defined as: division one (16/10/2015 to 28/10/2015; A, E, I and M for CR, FR, ICD and NND, respectively), division two (29/10/2015 to 10/11/2015; B, F, J and N for CR, FR, ICD and NND,, respectively), division three (11/11/2015 to 23/11/2015; C, G, K and O for CR, FR, ICD and NND, respectively) and division four (24/11/2015 to 08/12/2015; D, H, L and P for CR, FR, ICD and NND, respectively)). Hours are divided into those spent in the main barn ('main'; blue; 08:00 to 13:59, 17:00 to 22:59 and 01:00 to 04:59) and those spent in the collected yard and milking parlour (05:00 to 07:59 and 14:00 to 16:59) or when the sensor rest at midnight approximately 23:00 to 00:59) ('full'; red).

6.3.2. Breakpoints for bunching metrics ~ barn temperature

Forcing an algorithm to find the location of breakpoints outputs values at 18.90°C for core range (CR), 13.00°C for full range (FR), 9.77°C for inter-cow distance (ICD) and 15.91°C for nearest neighbour distance (NND), and the breakpoints for ICD and NND are significant (Table 1). Negative trends, significant or close to significant, hold above these breakpoints for all the bunching metrics (Table 1). However, the minority of the data lies where BT2 ≥ 18.90°C (4.18%), and most of the data lies where BT2 ≥ 13.00°C (72.50%) or BT2 ≥ 9.77°C (92.90%) so interpreting the model outputs based on this skewed distribution either side of this breakpoint would not be sensible. A breakpoint of 15.91°C is therefore adopted for the subsequent analysis, where the BT2 is significant for NND, and where approximately half (42.90%) of the data lies above or equal to this threshold.

Table 1. Results from analysis investigating the linear relationship between barn temperature (BT2) and each bunching metrics (CR = core range, FR = full range, ICD = inter-cow distance, NND = nearest neighbour distance), on the RVC Research farm, using a data-driven approach to identify breakpoints in BT2. Significant *p*-values are in bold. The breakpoints were estimated regardless of their statistical significance. After identifying the locations of these breakpoints, Chow tests were conducted to evaluate their significance. Periods during which cows were in the milking parlour or collecting yard were removed (05:00 to 07:59 and 14:00 to 16:59), as well as periods during which the sensor reset (approximately 23:00 to 00:59).

Bunching metric	**BP (°C)**	**Chow-test**	**< BP**	**≥ BP**
CR	18.90	F = 1.72, *p* = 0.18	e = -0.18, SE = 0.07, t-value = -2.47, ***p* = 0.01**	e = 4.27, SE = 2.53, t-value = 2.53, *p* = 0.10
FR	13.00	F = 1.52, *p* = 0.22	e = 0.22, SE = 0.49, t-value = 0.46, *p* = 0.65	e = -1.21, SE = 0.41, t-value = -2.99, ***p* = 0.003**
ICD	9.77	F = 3.19, ***p* = 0.04**	e = -0.28, SE = 0.07, t-value = -4.15, ***p* = 3.92e-5**	e = -0.29, SE = 0.03, t-value = -8.94, ***p* < 2e-16**
NND	15.91	F = 3.36, ***p* = 0.04**	e = 0.03, SE = 0.11, t-value = 0.28, *p* = 0.78	e = -0.23, SE = 0.04, t-value = -5.92, ***p* = 8.43e-9**

Breakpoints are compared between divisions: division one (16/10/2015 to 28/10/2015), division two (29/10/2015 to 10/11/2015), division three (11/11/2015 to 23/11/2015) and division four (24/11/2015 to 08/12/2015) for completeness, to test whether the breakpoint causes the change in bunching behaviour, or whether this is due to the time period of the study. Results show that most breakpoints lie between 12.95°C (lower quantile) and 15.79°C (upper quantile), with the median breakpoint as 15.45°C, close to the previously found breakpoint of 15.91°C (Table 3). In a few cases, the breakpoint is found on the lower or upper end of the BT2 distribution e.g., only 3.80% of the full data lie where BT2 < 7.88°C and only 1.17% of the full dataset lie where BT2 ≥ 19.50°C, so these breakpoints are not sensible (Table 3). Moreover, there is no clear pattern between breakpoints and the divisions, so the following analysis is conducted using the full dataset and a breakpoint of 15.91°C (Table 3).

Table 2. Linear regression model outputs above or equal to data-driven barn temperature (BT2) breakpoints, for each 13-day division of the study conducted on the RVC Research farm. Divisions are defined as: division one (16/10/2015 to 28/10/2015), division two (29/10/2015 to 10/11/2015), division three (11/11/2015 to 23/11/2015) and division four (24/11/2015 to 08/12/2015). The breakpoints were estimated regardless of their statistical significance. After identifying the locations of these breakpoints, Chow tests were conducted to evaluate their significance. Each test is conducted for each bunching metric (CR = core range, FR = full range, ICD = inter-cow distance, NND = nearest neighbour distance). Significant *p*-values are in bold. Periods during which cows were in the milking parlour or collecting yard were removed (05:00 to 07:59 and 14:00 to 16:59), as well as periods during which the sensor system reset (approximately 23:00 to 00:59).

Bunching metric	**Division**	**BP (°C)**	**Chow-test**	**≥ BP °C**
CR	1	15.00	F = 0.66, *p* = 0.52	e = -2.86, SE = 0.83, t-value = -3.46, ***p* = 0.001**
	2	19.50	F = 8.06, ***p* = 0.0004**	e = 15.18, SE = 7.37, t-value = 2.06, *p* = 0.07
	3	15.72	F = 5.59, ***p* = 0.04**	e = -0.90, SE = 0.91, t-value = -0.99, *p* = 0.33
	4	9.04	F = 1.96, *p* = 0.14	e = -0.73, SE = 0.18, t-value = -4.06, ***p* = 7.24e-5**
FR	1	15.39	F = 2.96, *p* = 0.05	e = -9.80, SE = 2.27, t-value = -4.31, ***p* = 7.55e-5**
	2	19.50	F = 6.54, ***p* = 0.002**	e = 41.63, SE = 21.36, t-value = 1.95, *p* = 0.08
	3	15.50	F = 6.28, ***p* = 0.002**	e = -4.65, SE = 1.88, t-value = -2.48, ***p* = 0.02**
	4	7.88	F = 0.86, *p* = 0.43	e = -1.40, SE = 0.40, t-value = -3.46, ***p* = 0.001**
ICD	1	9.99	F = 1.95, *p* = 0.15	e = -0.13, SE = 0.04, t-value = -3.10, ***p* = 0.02**
	2	17.49	F = 11.49, ***p* = 1.86e-5**	e = -0.23, SE = 0.31, t-value = -0.74, *p* = 0.46

	3	14.24	F = 17.36, ***p* = 1.25e-7**	e = -0.72, SE = 0.18, t-value = -4.04, ***p* = 0.0001**
	4	11.50	F = 1.08, *p* = 0.34	e = -0.54, SE = 0.09, t-value = -5.98, ***p* = 1.24e-8**
NND	1	16.00	F = 1.61, *p* = 0.20	e = -0.28, SE = 0.12, t-value = -2.26, ***p* = 0.03**
	2	15.65	F = 3.39, ***p* = 0.04**	e = -0.07, SE = 0.04, t-value = -1.81, *p* = 0.07
	3	15.72	F = 6.14, ***p* = 0.003**	e = -0.07, SE = 0.04, t-value = -1.55, *p* = 0.13
	4	13.43	F = 2.54, *p* = 0.08	e = 0.39, SE = 0.07, t-value = 5.31, ***p* = 4.74e-7**

For NND, there is a clear qualitative change around the breakpoint of 15.91°C; the non-significant positive increase in NND with increasing temperature below the BP becomes a significant negative decrease above or equal to the breakpoint (Table 6; Fig 7). There is also a qualitative change below and above (or equal to) the breakpoint for FR, but these trends are non-significant (Table 5; Fig 7). Either side of the breakpoint, there is a change in the gradient for CR, but the behaviour is qualitatively the same, and there is no change for ICD (Table 5; Fig 7).

Table 3. Outputs from linear regression assessing the relationship between bunching and barn temperature (BT2), on the RVC Research farm, below and above a breakpoint of BT12 = 15.91°C (*n* = 320 ≥ 15.91°C), excluding outliers (*n* = 5). Chow test outputs testing the significance of the breakpoint are also shown. Each test is conducted for each bunching metric (CR = core range, FR = full range, ICD = inter-cow distance, NND = nearest neighbour distance). Outliers are removed based on model assumption testing, as shown in Appendix 1. Significant *p*-values are in bold. Periods during which cows were in the milking parlour or collecting yard were removed (05:00 to 07:59 and 14:00 to 16:59), as well as periods during which the sensor system reset (approximately 23:00 to 00:59).

Bunching metric	**Chow-test**	**< 15.91**°C	≥ **15.91**°C
CR	F = 2.57, *p* = 0.08	e = 0.0002, SE = 0.11, t-value = 0.002, *p* = 1.00	e = 0.74, SE = 0.31, t-value = 2.39, ***p* = 0.02**
FR	F = 0.61, *p* = 0.55	e = -0.08, SE = 0.26, t-value = -0.32, *p* = 0.75	e = 0.49, SE = 0.76, t-value = 0.64, *p* = 0.52
ICD	F = 1.80, *p* = 0.17	e = -0.13, SE = 0.04, t-value = -4.19, ***p* = 3.39e-5**	e = -0.20, SE = 0.12, t-value = -1.63, *p* = 0.11
NND	F = 2.51, *p* = 0.08	e = 0.12, SE = 0.01, t-value = 9.59, ***p* < 2.2e-16**	e = -0.22, SE = 0.04, t-value = -6.09, ***p* = 3.39e-9**

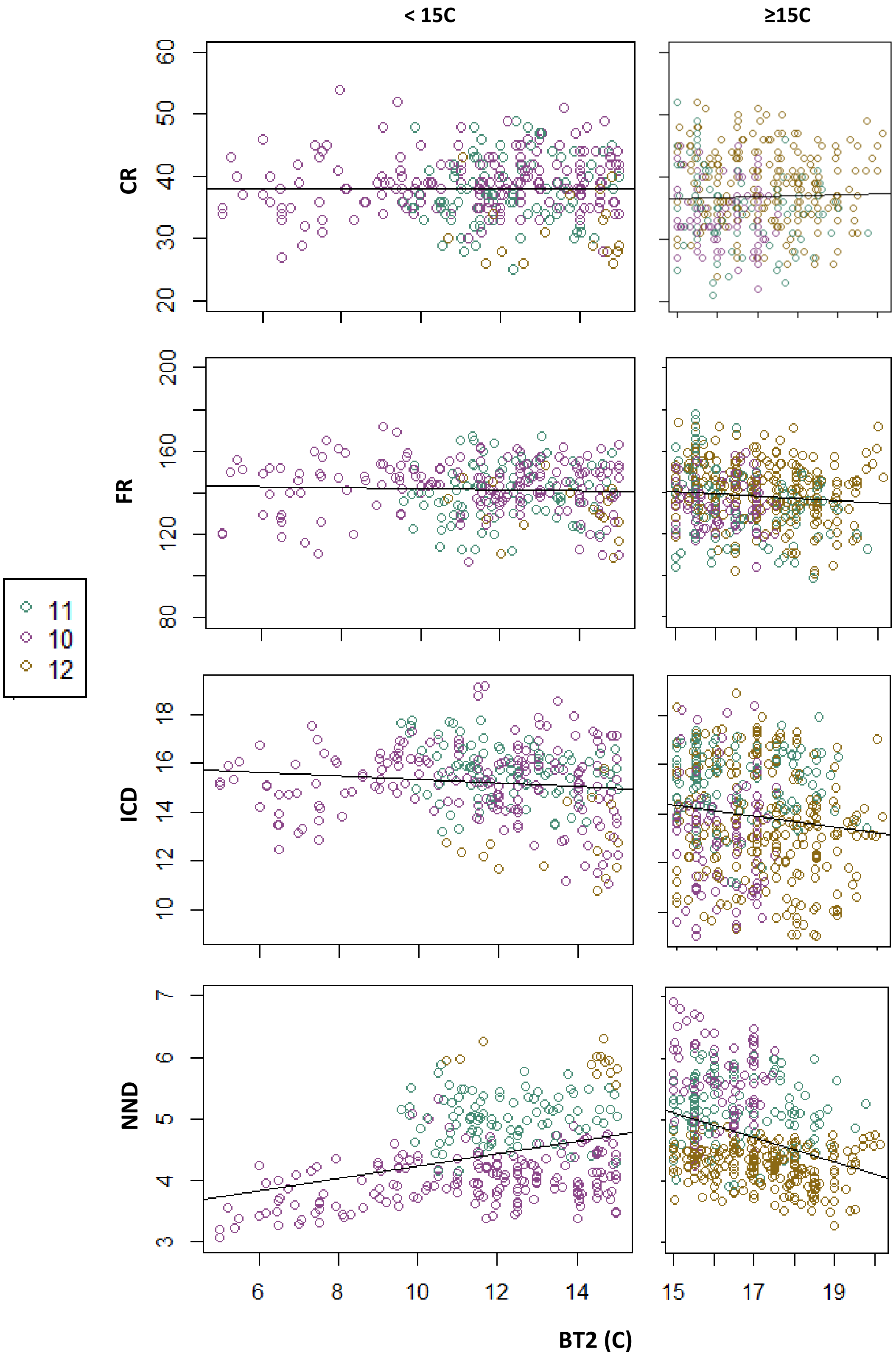

Figure 6. Linear regression plot outputs assessing the relationship between bunching and barn temperature (BT2) on the RVC Research Farm, below and above (or equal to) 15.91°C (≥ 15.91°C: *n* = 315). Results are shown for each bunching metric (CR = core range,

FR = full range, ICD = inter-cow distance, NND = nearest neighbour distance). Outliers are excluded (*n* = *5*), based on model assumption testing. A single point represents an average per hour and points are coloured by 13-day divisions: division one (16/10/2015 to 28/10/2015; green), division two (29/10/2015 to 10/11/2015; purple), division three (11/11/2015 to 23/11/2015; yellow) and division four (24/11/2015 to 08/12/2015; grey)). Periods during which cows were in the milking parlour or collecting yard were removed (05:00 to 07:59 and 14:00 to 16:59), as well as periods during which the sensor reset (approximately 23:00 to 00:59).

Considering the divisions separately, the negative trend between BT2 and the bunching metrics ≥ 15.91°C is shown in most cases, except for with range size for division two and with three of the four metrics in division four where the relationships are positive but non-significant, so there is no clear impact of division on the relationship (Table 6).

Table 4. Outputs from linear model assessing the relationship between bunching and barn temperature (BT2), on the RVC Research farm, above or equal to a breakpoint of 15.91°C (*n* = 746 in total, with *n* = 315 ≥ 15.91°C), for each 13-day division, with outliers excluded (*n* = 5). Divisions are defined as: division one (16/10/2015 to 28/10/2015), division two (29/10/2015 to 10/11/2015), division three (11/11/2015 to 23/11/2015) and division four (24/11/2015 to 08/12/2015)). Outliers are excluded, based on model assumption testing, as shown in Appendix 1. Significant *p*-values are in bold. Periods during which cows were in the milking parlour or collecting yard were removed (05:00 to 07:59 and 14:00 to 16:59), as well as periods during which the sensor system reset (approximately 23:00 to 00:59).

	≥ 15.91°C			
Bunching metric	**Division 1**	**Division 2**	**Division 3**	**Division 4**
CR	e = -0.50, SE = 0.87, t-value = -0.57, *p* = 0.57	e = 0.23, SE = 0.43, t-value = 0.54, *p* = 0.59	e = -1.51, SE = 0.93, t-value = -0.55, *p* = 0.59	e = 0.46, SE = 1.50, t-value = 0.31, *p* = 0.76
FR	e = -7.23, SE = 2.87, t-value = -2.52, ***p* = 0.02**	e = 0.32, SE = 1.02, t-value = 0.31, *p* = 0.76	e = -1.03, SE = 2.46, t-value = -0.42, *p* = 0.68	e = 1.75, SE = 3.68, t-value = 0.48, *p* = 0.64
ICD	e = -0.22, SE = 0.33, t-value = -0.67, *p* = 0.51	e = -0.10, SE = 0.17, t-value = 0.60, *p* = 0.55	e = -0.54, SE = 0.33, t-value = -1.60, *p* = 0.11	e = -0.17, SE = 0.67, t-value = -0.25, *p* = 0.80
NND	e = -0.30, SE = 0.12, t-value = -2.46, ***p* = 0.02**	e = -0.08, SE = 0.05, t-value = -1.61, *p* = 0.11	e = -0.07, SE = 0.05, t-value = -1.43, *p* = 0.16	e = 0.34, SE = 0.19, t-value = 1.82, *p* = 0.07

6.3.3. Time of day

Time of day is divided into intervals between milking times: interval one (I1: 8am to 1pm- blue; $n = 130$) and interval two (I2: 5pm to 4am- yellow; $n = 315$).

Above or equal to 15.91°C, the relationship between BT2 and NND remains significantly negative during both time intervals (Fig 8; I1: e = -0.21, SE = 0.05, t-value = -4.11, $p = 7.04e-5$ and I2: e = -0.22, SE = 0.05, t-value = -4.28, $p = 3.07e-5$). The case is similar for ICD; the relationship remains negative across time intervals (Fig 8; I1: e = -0.15, SE = 0.17, t-value = -0.87, $p = 0.38$ and I2: e = -0.17, SE = 0.17, t-value = -0.99, $p = 0.33$). This suggests that the cows were bunching across time intervals in terms of NND and ICD.

The positive relationship between BT2 and CR is stronger during I2 than during I1 (Fig 8; I1: CR: e = 0.35, SE = 0.45, t-value = -0.77, $p = 0.44$ and I2: CR: e = 1.36, SE = 0.41, t-value = 3.31, $p = 0.001$), and the negative relationship between BT2 and FR during I1 becomes positive during I2 (Fig 8; I1: FR: e = -2.18, SE = 1.09, t-value = -2.00, $p = 0.048$ and I2: FR: e = 3.11, SE = 1.04, t-value = 2.99, $p = 0.003$).

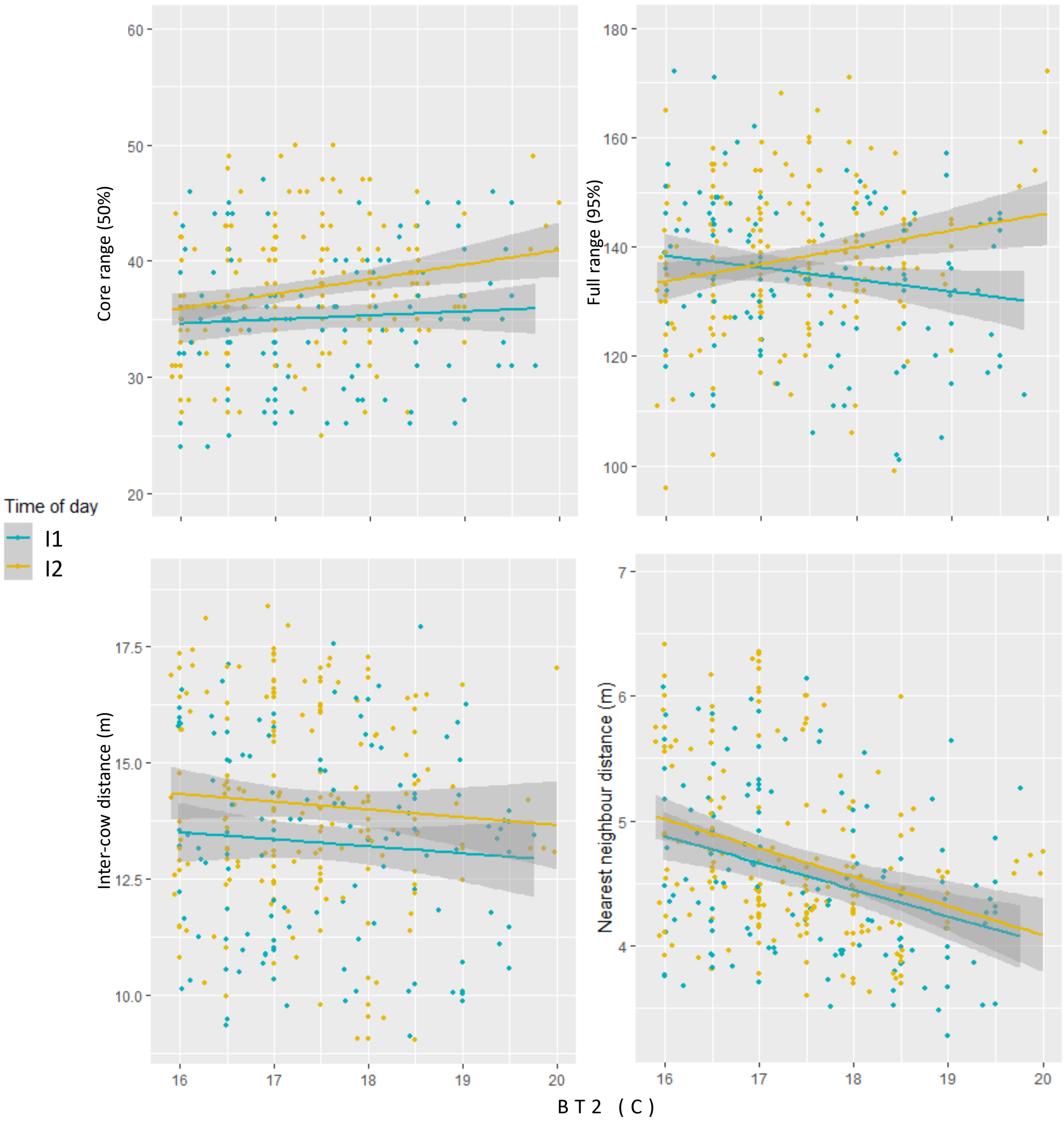

Figure 7. Barn temperature (BT2) against the bunching metrics: core range, full range, inter-cow distance and nearest neighbour distance, showing data collected from the RVC Research farm, where BT2 ≥ the breakpoint of 15.91°C, according to time of day. Time of day is inputted as a categorical variable, divided into intervals between milking times; three intervals: interval one (I1: 8am to 1pm- blue; *n* = 169) and interval two (I2: 5pm to 4am- yellow; *n* = 227). Periods during which cows were in the milking parlour or collecting yard were removed (05:00 to 07:59 and 14:00 to 16:59), as well as periods during which the sensor reset (approximately 23:00 to 00:59).

6.4. Discussion

In this chapter, the relationship between barn temperature (BT2) and four measures of bunching behaviour, core range (CR), full range (FR), inter-cow distance (ICD) and nearest neighbour distance (NND) were examined using data collected from a herd housed in an open barn system (no cubicles) on an RVC Research farm; as per a study of cows housed in a closed barn system (with cubicles), on a commercial farm in Essex, in Chapter 5. The herd significantly increased their bunching response in terms of NND with increasing barn temperature above or equal to, but not below, 15.91°C. On both sides of this threshold, the relationships between temperature and the remaining bunching metrics were found to be either negative (ICD) or qualitatively dissimilar to the trends for NND but non-significant (CR and FR). No clear pattern was detected between 13-day block divisions and the relationships between BT2 and the bunching metrics. The trends for NND and ICD were found to hold across times of the day but are inconsistent for the range sizes. This study provides evidence of cows responding to increasing temperature by bunching with other herd members, using various measures of bunching, an important finding as it could demonstrate that cows maladaptively respond to an increased heat load.

The patterns between the bunching metrics and BT2 $\geq$ 15.91°C on the RVC Research farm are similar to those found on the commercial farm in Essex $\geq$ 20°C, in that cows are shown to bunch more above these thresholds in terms of NND. This trend holds for range size and ICD on the commercial farm in Essex but not on the RVC Research farm; although ICD is found to decline with increasing BT2, this is not only above a breakpoint. The cows bunched in terms of NND on the RVC Research farm $\geq$ 15.91°C across times of the day, as on the commercial farm in Essex $\geq$ 20°C. The greater space allowance on the RVC Research farm, compared to on the commercial farm in Essex, may have meant cows were more able to find areas to cool down whilst less active during the evening and night, making it less likely they would bunch in terms of range size, whether this is driven by seeking shade, social support, or other reasons.

Differences in patterns of bunching between farms could also be explained by numerous factors including a herd's ability to cope with heat. Cows may become acclimatised to local environmental conditions such that one herd may consider a temperature warm whereas cows in warmer climates may not. Therefore, some herds may be less likely to respond, e.g., behaviourally, to high temperatures. The degree to which a cow is acclimated to a local temperature is likely dependent on rearing history and breed. Some cattle breeds are more heat tolerant than others, and tolerance could be tested in relation to behavioural responses such as bunching (427). Cows on the commercial farm in Essex were only Holstein-Friesian whereas 10% of cows on the RVC Research farm were Brown Swiss, and the remaining 90% were Holstein-Friesian, which may have contributed differences in overall heat tolerance. Investigation into whether differences in temperature thresholds vary between groups would be useful, to help optimise management.

Other individual attributes may contribute as well; cows in early lactation have been found to have a higher rectal temperature compared to cows in late lactation, suggesting that the former are more susceptible to heat stress, although this is not consistent across studies (346,379). However, this inconsistency may be attributed to how cows were grouped by lactation; a different number of categories were used in these studies, but this needs further investigation (346,379). Other attributes such as parity may contribute; a pattern between parity and rectal temperature has been observed, although this was non-significant (346,379).

An individual cow's stance could also impact heat tolerance, as found with physiological changes including respiration rate (RR) (379,380). RR thresholds appear to be lower for lying cows, at THI = 60 to 66, compared to standing cows where RR thresholds are reported at THI = 68 to 70 (379,380). Standing exposes a greater skin surface area compared to lying, allowing greater heat loss, which could explain these differences. Cows on the RVC Research farm may have been more likely to find a comfortable space to lie down during the evening and night, given the space allowance was greater than on the commercial farm in Essex in terms of stocking density and the open barn layout, potentially lowering the extent of the bunching response in terms of range size and ICD due to a greater tolerance of high temperatures.

The strength of bunching at higher temperatures, alongside the breakpoint, could change if temperature on the RVC Research farm was more frequently recorded above 20°C, or heat stress may occur between 15.91°C and 20°C. As it was not possible to determine the difference in ambient temperature between those recorded in the gateways and parlour, by the sensors, and within the feeding and non-feeding zones where the herd were located, it is possible that the relationship between temperature and bunching was masked for range size and ICD using this data. Alternatively, temperature may not be the (main) cause of the bunching behaviour. Time of day did not appear to contribute, as there was no significant difference in the relationship between temperature and NND between periods where temperatures reached above the threshold, on both the commercial farm in Essex with 20°C and the RVC Research farm with 15.91°C. Instead, management may contribute to the behaviour e.g., cows may bunch before milking in preparation to move to the collecting yard to relieve milk yield, or before scheduled feeding times to gain resources promptly.

Understanding the prevalence of bunching behaviour at high temperatures across farms is crucial, as is understanding the temperature at which this starts occurring in case this behaviour is maladaptive. Even if temperature is not the main driver behind bunching, if this is the case across farms, cows under high temperatures are prone to increased heat load therefore heat stress. This needs to be addressed for health, welfare, and productivity (55). Another important step would be to investigate the reasons behind bunching behaviour, e.g., shade, water, fly burden, stress, so that mitigation strategies can be implemented.

6.5. Conclusions

This chapter provides further support for cows bunching with increasing temperature above a threshold, as found on the commercial farm in Essex in Chapter 4. It is demonstrated that a second dairy cow herd housed on a RVC Research farm bunch in terms of NND with increasing barn temperature above or equal to a threshold of 15.91°C. This behaviour needs further exploration across herd groups, as it may be maladaptive, in which case it could potentially be prevented through investigating its causes.

The main results of this chapter are outlined below:

- The relationship between bunching behaviour and barn temperature was assessed on a second farm, an RVC Research farm, using a local positioning system and temperature sensors recording in the barn over three months.
- Bunching behaviour was defined as in Chapter 5, as range size, inter-cow distance (ICD) and nearest neighbour distance (NND).
- The herd increased their bunching behaviour in terms of NND with increasing barn temperature above or equal to 15.91°C.

- No clear patterns in the relationships between barn temperature and the bunching metrics were detected based on 13-day block divisions.
- The increased bunching response in terms of NND was found to be present across times of the day, and to a similar degree.

6.6. Appendix 1. Model Assumptions

The linear model assumptions, heteroscedasticity, and residual normality were tested where BT2 is below and above (or equal to) the breakpoint of 15.91°C, and the results show that assumptions are met for all the bunching metrics ≥ 15.91°C when excluding outliers (*n* = 5), apart from for heteroscedasticity for ICD and residual normality for ICD and NND (Table A1, Fig A1). However, given the large sample size (< 15.9°C: *n* = 426, ≥ 15.91°C: *n* = 320), extreme values skewing the data from residual normality and homoscedasticity are not unexpected, so linear regression was carried forward.

Table A1. Linear model assumptions for the model assessing bunching against barn temperature (BT2), on the RVC Research farm, below and above (or equal to) a breakpoint of 15.91°C (total *n* = 320): heteroscedasticity, and residual normality. Assumptions were tested using NVC and Shapiro-Wilks, respectively. Each test result is shown for each bunching metric (CR = core range, FR = full range, ICD = inter-cow distance, NND = nearest neighbour distance), including all the data and excluding outliers (*n* = 5). Significant *p*-values are in bold.

		Heteroscedasticity		**Residual normality**	
Bunching metric	**Outliers exc.** (n = 5)	**< 15.91°C**	**≥ 15.91°C**	**< 15.91°C**	**≥ 15.91°C**
CR	N	Chi-sq = 11.01, **p = 0.001**	Chi-sq = 0.46, p = 0.50	W = 1.00, p = 0.25	W = 1,00, p = 0.66
	Y	Chi-sq = 9.11, **p = 0.003**	Chi-sq = 0.18, p = 0.67	W = 0.99, p = 0.14	W = 0.99, p = 0.07
FR	N	Chi-sq = 3.21, p = 0.07	Chi-sq = 0.31, p = 0.58	W = 0.99, **p = 0.008**	W = 0.99, p = 0.05
	Y	Chi-sq = 0.89, p = 0.35	Chi-sq = 0.67, p = 0.41	W = 0.99, **p = 0.008**	W = 0.99, p = 0.39
ICD	N	Chi-sq = 25.29, **p = 4.92e-7**	Chi-sq = 2.06, p = 0.15	W = 0.97, **p = 3.73e-7**	W = 0.99, **p = 0.005**
	Y	Chi-sq = 13.29, **p = 0.0003**	Chi-sq = 0.59, p = 0.44	W = 0.98, **p = 0.0001**	W = 0.98, **p = 0.0003**
NND	N	Chi-sq = 11.46, **p = 0.001**	Chi-sq = 6.03, **p = 0.01**	W = 0.97, **p = 2.77e-8**	W = 0.96, **p = 6.64e-8**
	Y	Chi-sq = 9.83, **p = 0.002**	Chi-sq = 6.54, p = 0.01	W = 0.97, **p = 2.74e-7**	W = 0.96, **p = 1.94e-7**

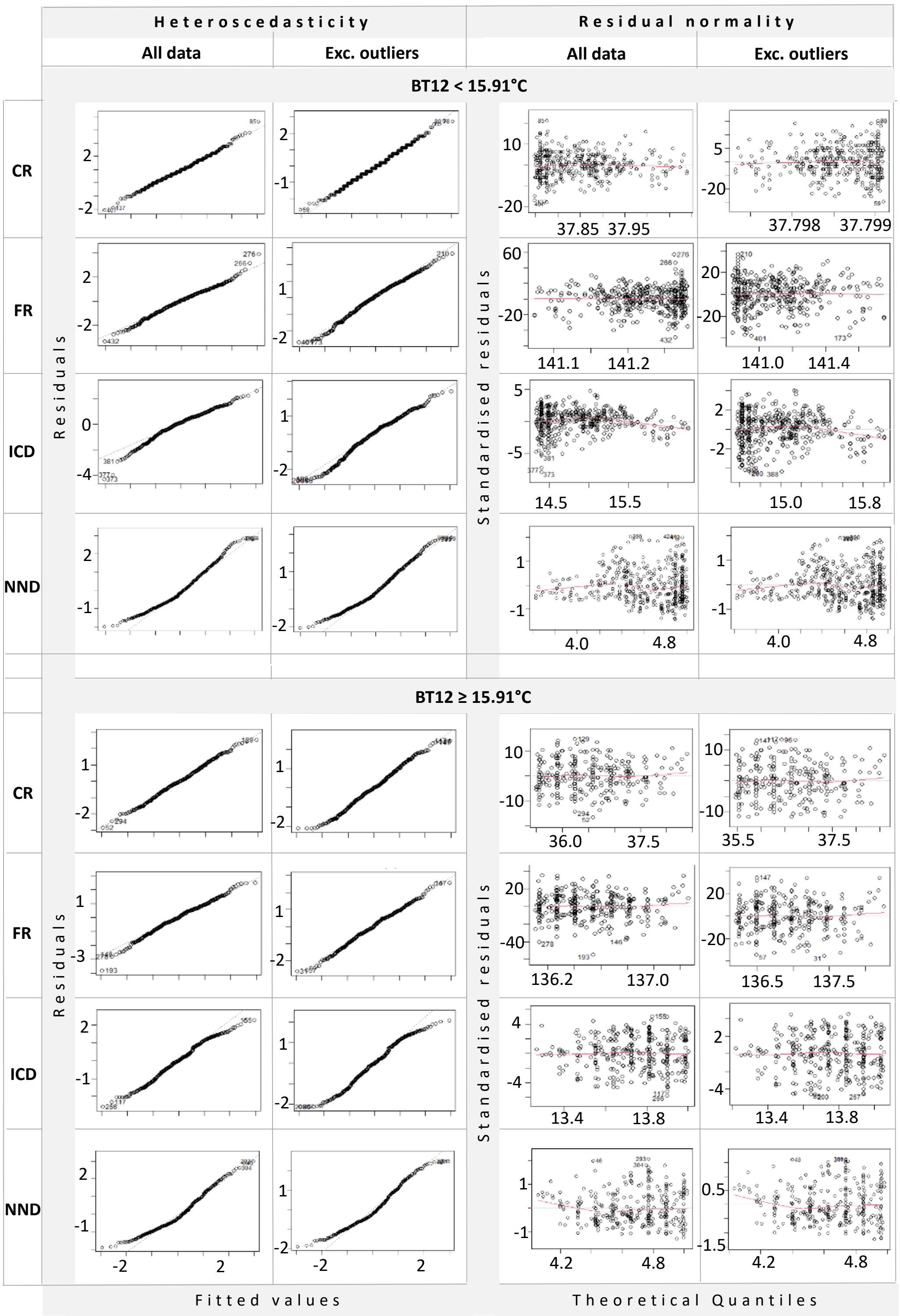

Figure A1. Plots viewing assumptions of the model assessing the relationship between bunching and barn temperature (BT2) on the RVC Research farm, below and above (or equal to) the breakpoint of BT2 = 15.91°C: heteroscedasticity, and residual normality. For each assumption, plots are shown for each bunching metric (CR = core range, FR = full range, ICD = inter-cow distance, NND = nearest neighbour distance).

Furthermore, comparing Table A2 to Table 4 in the main text, the conclusions drawn from the linear model outputs do not change drastically before excluding these outliers (*n* = 5). The only qualitative difference is in the direction of the relationship between BT2 and each of the range sizes < 15.91°C, before and after the removal of the outliers but all these relationships below the breakpoint are non-significant (Table A2 and Table 4).

Table A2. Outputs from linear regression assessing the relationship between bunching and barn temperature (BT2), on the RVC Research farm, below and above (or equal to) a breakpoint of 15.91°C (*n* = 320), with outliers included (*n* = 5). Chow test outputs testing the significance of the breakpoint are also shown. Each test is conducted for each bunching metric (CR = core range, FR = full range, ICD = inter-cow distance, NND = nearest neighbour distance). Significant *p*-values are in bold.

Bunching metric	**Chow-test**	**< 15.91°C**	**≥ 15.91°C**
CR	F = 1.07, *p* = 0.34	e = -0.03, SE = 0.11, t-value = -0.29, *p* = 0.78	e = 0.69, SE = 0.32, t-value = 2.12, ***p* = 0.04**
FR	F = 1.98, *p* = 0.14	e = 0.02, SE = 0.28, t-value = 0.07, *p* = 0.95	e = 0.27, SE = 0.80, t-value = 0.34, *p* = 0.73
ICD	F = 5.77, ***p* = 0.003**	e = -0.18, SE = 0.03, t-value = -5.12, ***p* = 4.57e-7**	e = -0.17, SE = 0.12, t-value = -1.40, *p* = 0.16
NND	F = 3.36, ***p* = 0.04**	e = 0.13, SE = 0.01, t-value = 9.50, ***p* < 2.2e-16**	e = -0.23, SE = 0.04, t-value = -5.92, ***p* = 8.43e-9**

7. CHAPTER 7: GENERAL DISCUSSION

Increasing demand for dairy commodities has incentivised the housing of livestock at high stocking densities with unstable group compositions (2). Understanding the impact of modern systems is important to improve dairy cow health and welfare as well as productivity, including social requirements and housing conditions. Monitoring individual cows can be difficult in such systems, and early indicators are often missed, but precision livestock farming shows promise, allowing the rapid identification of behavioural changes indicative of health and welfare issues to prompt treatment (167,241–243,250,252,267). Automated monitoring through changes in social and group-level behaviour will aid the development of such systems. This thesis therefore aimed to investigate the social networks of two dairy cow groups through a local positioning system (LPS), with a view to identify health and welfare complications through changes in both the network structure and consistency of herds (Chapters 3 and 4). Next, the relationship between temperature and the bunching behaviour of dairy cows was explored, a potential maladaptive behaviour which could induce heat stress, with the use of the same LPS as well as barn temperature readings (Chapters 5 and 6). In this discussion, the main findings from this thesis are highlighted, alongside limitations of this work, before suggesting future research questions.

7.1. Accuracy and reliability of data collected through the LPS

The LPS used for this work was validified to a 50% circular error of probability measurement of 1.07m to 1.90m and a mean distance error of 2.66m to 2.80m, dependent on whether the sensor was on a static or standing cow (242).

The system reset at approximately midnight, and data recorded during this period was removed as planned. Positions located more than 3m outside of the functional zones of interest were removed and unexpected errors required further pre-processing, including sensors occasionally ‘freezing’ at the exact same or similar coordinates. Partly attributed to the metal structure of the housing, noise was reduced through smoothing of the data, and a few instances of unrealistic trajectories were removed. The total data loss from the original datasets were below 30% for both farms, minimal considering data removed included correct location data in the milking parlour and other areas outside of the functional zones of interest. This pre-processing was carried out through a combination of automated and manual checking, the latter of which is unpractical on site for farmers, although only less than 26% of the original data were removed manually for both datasets.

7.2. Proximity interaction network of two herd groups

The cows housed on the commercial farm in Essex were found to have a highly connected network, whereas the cows on the RVC Research farm had a weakly connected network. Differences were expected, given the herds were of different sizes and subject to different management; varying levels of connectivity in different cow herds have previously been reported (166,176). The herd on the RVC Research farm were subject to more frequent changes in group composition, with an average of 12 individuals being added or removed daily, compared to on the commercial farm in Essex where only one individual was added or removed daily on average. Furthermore, the herd on the RVC Research farm saw a complete turnover of individuals during the study period (where n = 22 to 52 daily), while 91 individuals remained in the herd on the commercial farm in Essex throughout the entire study period (where n = 100 to 110 daily). The daily changes in group composition on the RVC Research farm may have hindered the formation of strong social bonds, given the dominance hierarchy is thought to take a fortnight to establish (132,157,272). It is important to note that the analysis on the network structure conducted for the herd housed on the commercial farm in Essex only included the cows (*n* = 92) that were always present throughout the study period, allowing for analysis of temporal variation over time. However, on the RVC Research farm, the network structure analysis included all cows, including those that were added or removed from the herd during the study period. Therefore, it is reasonable to expect that the cows studied on the commercial farm in Essex had stronger connections as they were more familiar with each other, even with an average of one individual being added or removed daily, compared to the cows on the RVC Research farm. However, it remains unclear whether this is the case. Moreover, the differences in the network connectivity between the groups could also be attributed to the lower stocking density and the open barn layout (no cubicles) on the RVC Research farm, in comparison to the closed barn (with cubicles) on the commercial farm in Essex. The more restricted space allowance on the commercial farm in Essex likely gave individuals less opportunity to choose whom to position closely to, compared to on the RVC Research farm. Moreover, connectivity was zone-dependent; higher connectivity was revealed in the non-feeding zones (NFZ) compared to the feeding zones (FZ). Higher connectivity has been found in lying areas compared to standing/activity areas, which supports these findings (166,428)

Despite the differences in connectivity of the two networks, significant inter-individual variation was shown on the commercial farm in Essex in the NFZ, suggesting that highly social individuals remain highly social over time, irrespective of group composition. These findings agree with previous research, finding variation in individual sociality in cattle (148,176,272). Potential differences may have been masked in the FZ due to the high turnover of individuals and the high stocking density. Significant inter-individual variation was shown in the FZ on the RVC Research farm, which may have been due to the lower stocking density, whereas in the NFZ patterns may have been masked due to less demand for preferable areas i.e., cubicles and a greater opportunity to distance from each other.

On both farms, there was significant social differentiation in both functional zones as found in other herd groups (166,176). Social differentiation was apparent in both zones despite the turnover of individuals (particularly on the RVC Research farm), perhaps due to individuals maintaining long-term familiarity (168). This does not appear to be the case in all unstable groups however, as found in a herd with individuals sourced from different farms without a rearing history; although observations over a time period longer than a week could have revealed greater preferences (429). Moreover, a lack of temporal stability over time was shown on the closed barn (with cubicles) on the commercial farm in Essex, which could also be the case on the open barn (no cubicles) on the RVC Research farm. Alternatively, the network on the RVC Research farm could have changed less frequently due to a greater opportunity for cows to choose whom to maintain closeness to, although this was beyond the scope of this thesis. The weak temporal stability of the commercial farm in Essex network suggests cows change their social bonds frequently, although these may be maintained over a shorter time, and restricted by the space allowance. The former suggestion is reflected in the differing results from a previous study, finding evidence for temporal stability of a cow network across weeks, of 17% to 57% (176). The level of temporal stability is likely to be herd-dependent, based on the frequency of regrouping, group size and composition and differences in individual sociality.

The potential impact of individual attributes on social preferences was assessed on the commercial farm in Essex. Social assortment could not be assessed on the RVC Research farm due to frequent changes in group composition. While cows were not found to assort by lameness, parity, or days in milk on the commercial farm, research has found significant assortment based on parity or milk production (linked to days in milk) in other herds (176,315). A study on a farm with a stocking density of 9.5m^2 per cow (assuming the average group size of *n* = 110) found cows assorted by milk production (176), whereas cows on the commercial farm in Essex were housed at a stocking density of 3.8m^2 per cow (feed space) and 5.3m^2 per cow (lying space) (assuming the average group size of *n* = 104 during the analysed month). Limited space allowance may therefore mask social preferences, as on the commercial farm in Essex, but research on this is lacking. Furthermore, there is currently no research on social preferences of cattle at pasture, where social preferences would be expected to be revealed given the opportunity for unrestricted space. Non-random associations may also be influenced by other attributes such as personality and dominance, which requires further investigation (169,264,266).

7.3. Space-use preferences

Cows spread across more cells during the day compared to the night, more so on the RVC Research farm than on the commercial farm in Essex, as expected due to the latter barn holding cubicles. This was further demonstrated through visualisations of space-use, showing clear gaps between cubicles in the commercial farm in Essex and a fragmented core range whereas no obvious gaps in the space-use on the RVC Research farm. Interestingly, cows on both farms show preferences for certain areas in the NFZ, and this is more so the case on the commercial farm in Essex than on the RVC Research farm, perhaps as specific cubicles were considered favourable. Likewise, preference for specific areas of a barn is demonstrated in other studies (197,344). The preferred cubicles on both farms were closer to the collecting yards, which would have allowed cows in these areas to relieve a high milk yield sooner than cows positioned elsewhere. Evidence for higher parity cows positioning in areas closer to milking areas has been found previously, supporting this theory (252,347). Other factors are expected to contribute as well; older, more experienced cows with high milk yields may be more likely to gain positions in preferred cubicles as opposed to younger cows with high milk yields (252). The demand for specific areas may also change over time. If the number of cubicles closer to a feed face is restricted, the demand for these may increase, especially before scheduled feeding times (197,347).

7.4. Relationship between barn temperature and bunching behaviour

Cows on the commercial farm in Essex significantly increased their bunching behaviour above a barn temperature threshold of 20°C, in terms of range size, inter-cow distance (ICD) and nearest neighbour distance (NND). Observational studies counting individuals present in a few areas of a barn every quarter of an hour also find that cows crowd above an ambient temperature of 19°C to 20°C, supporting the findings from this thesis (281,355). Barn temperatures on the RVC Research farm did not reach above 20°C on more than two occasions, but a significant negative relationship between temperature and nearest neighbour distance was found above (or equal to) 15.91°C.

The effect of warmer than average ambient temperatures on bunching behaviour varied according to the time of day. Specifically, while an increase in bunching with increasing temperature was observed during the day, this was not present during the night/early morning as temperatures did not reach high values, and this finding is consistent with previous research (281,355).

7.5. Limitations of this work

Along with the issue of occasional nonsensical data recording, another drawback of this work is that the accuracy of the LPS system limits the range of interactions that can be detected. However, the protocol for determining proximity interactions was validated to a sensitivity of 83% and the analysis was conducted using various time and radii parameters, and similar results were obtained. Error must still be considered whilst interpreting outputs from automated data collection to allow meaningful application. The chosen protocol aimed to reduce false positives but would have excluded any important genuine shorter social interactions. Moreover, it was unable to be determined whether detected interactions were directly social or non-deliberate/non-social, and the results probably contain both. Particularly with space-constraints of housing, individuals are forced in proximity to a degree, especially to gain resources, as indicated through the inconsistency in proximity interactions across functional zones. The impact of the changing group composition on the conclusions is also unknown, more so on the RVC Research farm than the commercial farm in Essex, and a direct comparison between the social networks structures on the farms was not possible due to the different group sizes. Furthermore, the visual mobility locomotion scores used in Chapter 3 were prone to classification errors, especially due to the large group size. Parity and days in milk may have also been too coarse to identify cows with historical associations or shared transition periods, thought to strengthen bonds.

Previous studies used humidity, as well as ambient temperature, to measure the responses of dairy cows to changing weather. However, temperature has been used alone to measure responses (368–370,381,382), and when humidity is considered from a local weather station in the results, qualitatively similar results are obtained and changes in bunching are found to be primarily driven by temperature.

7.6. Future directions

7.6.1. Further research

To be used for health and welfare improvement, detecting individual baseline behaviours is essential. From this, deviations in individual and group-level/social behaviour can be detected over time to allow fast identification of abnormal behaviours indicative of illness or reduced welfare status.

To expand the social network analysis of this thesis, detecting the nature of proximity interactions would be useful. For example, if a cow continually approaches another, it can be implied that the cow is initiating an interaction, whereas if the individual stops this behaviour, this may indicate a health or welfare problem. This was attempted using LPS data from a smaller beef herd, but a lack of observational data meant the classifications of outcomes from cow-cow approaches could not be validated. Similar work has been conducted, determining the direction of dairy cow interactions through video recordings (294). Identifying this automatically through an ‘approach and leave’ technique could enable easier detection of welfare status in cows consistently failing to respond to interactions in an expected manner, on-site for farmers. Additionally, automatic detection of affiliative and agonistic interactions, as achieved through video recordings (294), could help improve group management. For instance, the maximum stocking density before the frequency of agonistic interactions reach above an a priori threshold, e.g., more than in self-regulated wild groups, could be determined to maintain good welfare. This has been attempted using an indoor positioning system and standardised residuals (SR), assuming that interactions are positive when SR reaches above a threshold and the remainder are negative/avoidance interactions (430,431). Although the technique shows promise, it requires comparison against different association metrics and the method needs to be replicated for evaluation (430).

As the sensor was combined with an accelerometer, social behaviours could be catalogued and combined with local positioning recordings to better understand the nature of interactions and determine whether these are reciprocated or asymmetric. An accelerometer has been used in combination with a LPS to determine individual behaviour and space-use patterns linked to lameness (252), and this could be extended to understand individual behaviours in relation to social interactions, which could potentially reveal abnormal interactions indicative of conditions such as lameness. Magnetometers could help us better understand the directionality of interactions, as well as determining ‘approach and leave’ interactions. For example, agonistic interactions such as head butting in combination with a cow approaching would provide evidence for unsocial rather than social behaviour. The use of accelerometers to catalogue social behaviour has indeed been shown; locomotor play has been estimated to an accuracy of 79% at 1Hz, in dairy calves, a figure which could be improved at a higher recording frequency (432). Combining accelerometery data with LPS data could therefore help us better understand the frequency, dynamics, and types of interactions between cows.

We could explore how the removal of an individual from a network may alter social interactions. Shifts in a network following removals are likely, given the value of and preference for social interactions in cow herds (166,176), and this is demonstrated with our current knowledge of reduced feeding behaviour and hindered immunological response of the remaining herd (329). Social bonds are expected to weaken with the removal of herd mates and are likely to be individual-specific; the removal of a key member connecting associates may have drastic impacts on the welfare of the remaining herd, as well as the individual subject to removal, and thus could be avoided. Removal of specific individuals may be necessary, however, if medical treatment is required. The impact of this could be better understood using less crude mobility data, i.e., assessment more than once every fortnight for all individuals as in Chapter 3. If assortment is then found between cows of similar lameness states, which may have been masked in this thesis, lame cows could be removed in pairs or small groups for treatment, to remove drastic impacts to their welfare. In addition, if differences in sociality between lame and non-lame cows are revealed, this would further highlight the need to promptly detect lameness and enable us to do this more effectively.

To directly compare networks, they must hold a similar number of nodes and edges, whether this is to monitor a group over time (as in Chapter 3), or compare groups (165,433). Even the removal of a single uncommon node between networks could result in the loss of vital information. Basic network measures like clustering coefficient are not appropriate for analysing groups subject to a high turnover of individuals or for comparing networks of vastly different sizes (308). Researchers have proposed other methods, such as the association of strength, but these are in their infancy (165,434). Given the difficulty in finding two herds that are exactly the same size, it would be extremely useful to develop a method that allows for meaningful comparisons between networks of different sizes. Such a method, developed with input from mathematicians and psychologists who use similar techniques, would enable us to better disentangle differences in network formations to better inform the management of livestock groups. For example, this approach could be used to investigate the impact of stocking density or barn layouts on the revelation of social preferences within livestock groups.

As well as comparing social networks between herds, space-use patterns between herds could be further explored, investigating where individuals position, and whether there are patterns based on their attributes. Lame cows are found to have a higher site-fidelity in a feeding area, perhaps due to a lack of motivation and ability to choose high-traffic, preferable feeding positions (209,252). Perhaps this is clear in barns with cubicles, such as the barn on the commercial farm in Essex, but not in open barns such as the barn on the RVC Research farm; an understanding of this would help identify cows with lameness whilst considering housing. Likewise, it would be of interest to investigate if other differences in housing and management, e.g., bedding, milking system, and stocking density, impact the space-use of herds. Individuals may favour specific areas prior to scheduled feeding times, but perhaps only if access is quicker from specific areas of a barn, and unhealthy individuals may not gain these. This would have implications for disease spread, as well as health and social stress, and other aspects of welfare.

Bunching at higher than average ambient temperatures also has implications for disease spread e.g., tuberculosis, especially indoors and at high humidity, as this makes pathogens more contagious, in turn impacting the health and welfare of cattle (435,436). In terms of the temperature and bunching relationship, a follow-up study could incorporate barn humidity readings, to better understand its impact. The extent of heat stress is determined by other variables, including ventilation, wind speed and solar radiation, so understanding the impact of these mixed effects on bunching behaviour would further aid mitigation strategies, although the latter may be irrelevant in housed herds. Testing whether patterns hold above similar thresholds in the summer months on the RVC Research farm, and on other farms, would be useful, as would investigating the cause behind bunching at warmer than average ambient temperatures. This could be achieved through mapping out shaded areas, water troughs and recording fly counts, and considering a herd’s space-use patterns in relation to these points of interest.

7.6.2. Implementation of PLF

The potential benefits of PLF are vast: increased production, reduced environmental impact, as well as improved animal health and welfare (437). It is still largely in the early-adoption phase and is still progressing; but end-user demands are predicted to increase (280).

The main issues with farmers not implementing PLF are: the expertise required to analyse and understand data collected, trust in the technology, poor maintenance service, the initial investment and the overall benefit of the technology (438–441). Learning the use of a new technology increases a farmer's workload, with large quantities of data making it difficult to select key information for decision-making, especially if support post-purchase is not sufficiently provided (441,442). The lack of trust in automated systems by farmers is emphasised in a study showing dairy farmers only respond to 3% of mastitis warnings generated by milking robots (443). PLF uptake by farmers is dependent on socio-demographics, financial resources, herd size and experience of and trust in technology, the latter often based on age (440,444–446).

The stress and time in handling automated systems may lessen once farmers familiarise themselves with the tools, and can prioritise alerts, potentially lowering overall stress, as has been reported in a case study of farmers using an automated oestrus system over time (447–449). Farmers should be provided with enough support to enable uncomplicated and quick management and interpretation of data (444). Once farmers are familiarised with a given technology, trust can be solidified through transparent communication between scientists and farmers, aiming for continual improvement of systems to lessen false alarms and improve farmer satisfaction, alongside farmers and providers (450). This is particularly important given the shortage of research on the quantitative effects of PLF adoption on farms (450). Companies providing sensor systems must continually support farmers to enable this (441).

Investment decisions should be thorough, with profitability evaluated, and accessibility to sensor systems could be expanded by offsetting initial costs through government funded schemes. Measuring the overall benefit of systems is difficult, given financial circumstances are not the only contributing factor; the wellbeing of the farmer should be considered too. The overall benefit to farmers could be amplified by detecting multiple conditions with one sensor, to lower financial cost and workload, or by combining data from multiple sources to increase precision e.g., production and physiology as well as behaviour (451,452). Benefits must outweigh the costs, so it is crucial to work with farmers to understand these and improve the cost to benefit ratio.

Rather than aiming to replace humans, technologies ought to support decision-making, by identifying complications before farmer intervention; the farmer's knowledge remains critical and should not be lost through dependency on technologies (453). The bond between farmer and livestock should not be diminished through automated systems, as animals deprived of this may become more fearful of humans which could reduce their wellbeing, and farmers may lose motivation in their profession (454). Instead, the time saved using systems should aim to provide opportunities for farmers to form stronger bonds through activities such as more frequent visits (455).

Besides considering the benefit to farmers, other parties should be carefully considered and involved (450). The public view of farmers may detach consumers who view automated systems negatively, so research on perceptions is imperative. There is also a need to create standards for sharing sensor-generated data, as regulation for data ownership and sharing is currently limited (451,456). Data could be shared so parties other than farmers also benefit, such as veterinarians and slaughterhouse operators, as well as researchers to continually improve sensor systems, but this requires legitimisation (456).

7.7. Conclusions

This thesis demonstrates the use of a LPS to monitor the social network structure of two large dairy cow herds. Secondly, this thesis provides evidence for a relationship between bunching behaviour and warmer than average ambient temperatures in both the studied cow herds, using LPS and barn temperature recordings. The results show promise for the expanded use of PLF to improve animal health and welfare within the growing dairy industry, particularly relevant due to the ongoing increases in group sizes, selective grouping, and milk production per cow (457). Caution must be taken when implementing PLF, ensuring farmers can successfully use the technology and interpret outputs, using results to aid but not to finalise decision-making. This thesis also sheds light on potential issues associated with increasing intensity in the dairy industry, such as higher stocking densities restricting space allowance for preferred interactions, and potentially maladaptive bunching behaviour resulting from management regimes. It is therefore vital to understand responses of livestock to management regimes and the environment at the individual-level, to maintain and improve the health and welfare of groups across the growing industry. To do this, effective communication amongst farmers, product companies, equipment manufacturers, suppliers, scientists, vets, and the government is required (457).

8. REFERENCES

1. OECD. OECD-FAO Agricultural Outlook 2020-2029 [Internet]. Paris: Organisation for Economic Co-operation and Development; 2020 [cited 2022 Apr 26]. Available from: https://www.oecd-ilibrary.org/agriculture-and-food/oecd-fao-agricultural-outlook-2020-2029_1112c23b-en

2. Miglior F, Fleming A, Malchiodi F, Brito LF, Martin P, Baes CF. A 100-Year Review: Identification and genetic selection of economically important traits in dairy cattle. J Dairy Sci. 2017 Dec 1;100(12):10251–71.

3. Oltenacu PA, Broom DM. The impact of genetic selection for increased milk yield on the welfare of dairy cows. Anim Welf. 2010;19(Supplement):39–49.

4. Cembalo L, Caracciolo F, Lombardi A, Del Giudice T, Grunert KG, Cicia G. Determinants of Individual Attitudes Toward Animal Welfare-Friendly Food Products. J Agric Environ Ethics. 2016 Apr 1;29(2):237–54.

5. Bayvel A, Diesch T, Cross N. Animal welfare: a complex international public policy issue: economic, policy, societal, cultural and other drivers and constraints. A 20-year international perspective. Anim Welf. 2012 May 17;21(1):11–8.

6. Gonçalves JL, Kamphuis C, Martins CMMR, Barreiro JR, Tomazi T, Gameiro AH, et al. Bovine subclinical mastitis reduces milk yield and economic return. Livest Sci. 2018 Apr 1;210:25–32.

7. Bradley AJ, Leach KA, Breen JE, Green LE, Green MJ. Survey of the incidence and aetiology of mastitis on dairy farms in England and Wales. Vet Rec. 2007;160(8):253–8.

8. Esslemont RJ, Kossaibati MA. Incidence of production diseases and other health problems in a group of dairy herds in England. Vet Rec. 1996;139(20):486–90.

9. Griffiths BE, Grove White D, Oikonomou G. A Cross-Sectional Study Into the Prevalence of Dairy Cattle Lameness and Associated Herd-Level Risk Factors in England and Wales. Front Vet Sci [Internet]. 2018 [cited 2020 Sep 30];5. Available from: https://www.frontiersin.org/articles/10.3389/fvets.2018.00065/full

10. Polsky L, von Keyserlingk MAG. Invited review: Effects of heat stress on dairy cattle welfare. J Dairy Sci. 2017 Nov 1;100(11):8645–57.

11. Guliński P. Ketone bodies – causes and effects of their increased presence in cows' body fluids: A review. Vet World. 2021 Jun;14(6):1492–503.

12. Lei MAC, Simões J. Invited Review: Ketosis Diagnosis and Monitoring in High-Producing Dairy Cows. Dairy. 2021 Jun;2(2):303–25.

13. Suthar VS, Canelas-Raposo J, Deniz A, Heuwieser W. Prevalence of subclinical ketosis and relationships with postpartum diseases in European dairy cows. J Dairy Sci. 2013 May 1;96(5):2925–38.

14. Nicks B, Vandenheede M. Animal health and welfare: equivalent or complementary? Rev Sci Tech. 2014 Apr;33(1):97–101.

15. Fraser D. Science, Values and Animal Welfare: Exploring the 'Inextricable Connection'. Anim Welf. 1995 May 1;4(2):103–17.

16. United Kingdom. Farm Animal Welfare Council (FAWC) D of E Food and Rural Affairs. Second Report on Priorities for Research and Development in Farm Animal Welfare. London; 1993.

17. Mellor DJ. Updating Animal Welfare Thinking: Moving beyond the "Five Freedoms" towards "A Life Worth Living". Anim Open Access J MDPI. 2016 Mar 14;6(3):21.

18. Fraser D, Weary DM, Pajor EA, Milligan BN. A Scientific Conception of Animal Welfare that Reflects Ethical Concerns. Anim Welf. 1997 Aug;6(3):187–205.

19. Main D, Mullan S. Economic, education, encouragement and enforcement influences within farm assurance schemes. Anim Welf. 2012 May 17;21(1):107–11.

20. Gaynor JS, Muir WW. Handbook of Veterinary Pain Management. Elsevier Health Sciences; 2008. 661 p.

21. Leach KA, Whay HR, Maggs CM, Barker ZE, Paul ES, Bell AK, et al. Working towards a reduction in cattle lameness: 1. Understanding barriers to lameness control on dairy farms. Res Vet Sci. 2010 Oct 1;89(2):311–7.

22. Anil SS, Anil L, Deen J. Effect of lameness on sow longevity. J Am Vet Med Assoc. 2009 Sep 15;235(6):734–8.

23. Blowey RW. Cattle Lameness and Hoofcare: An Illustrated Guide (3rd Edition). 5m Books Ltd; 2020. 271 p.

24. Green LE, Hedges VJ, Schukken YH, Blowey RW, Packington AJ. The Impact of Clinical Lameness on the Milk Yield of Dairy Cows. J Dairy Sci. 2002 Sep 1;85(9):2250–6.

25. Amory JR, Barker ZE, Wright JL, Mason SA, Blowey RW, Green LE. Associations between sole ulcer, white line disease and digital dermatitis and the milk yield of 1824 dairy cows on 30 dairy cow farms in England and Wales from February 2003–November 2004. Prev Vet Med. 2008 Mar 17;83(3):381–91.

26. Mitev J, Gergovska Z, Miteva T, Penev T. Influence of lameness on daily milk yield, lactation curve and body condition score during lactation in Black-and White cows. Bulg J Agric Sci. 2011 Jan;17(5):704–11.

27. Juarez ST, Robinson PH, DePeters EJ, Price EO. Impact of lameness on behavior and productivity of lactating Holstein cows. Appl Anim Behav Sci. 2003 Aug 27;83(1):1–14.

28. Morris MJ, Kaneko K, Walker SL, Jones DN, Routly JE, Smith RF, et al. Influence of lameness on follicular growth, ovulation, reproductive hormone concentrations and estrus behavior in dairy cows. Theriogenology. 2011 Sep 1;76(4):658–68.

29. Hernandez J, Shearer JK, Webb DW. Effect of lameness on the calving-to-conception interval in dairy cows. J Am Vet Med Assoc. 2001 May 15;218(10):1611–4.

30. Iida R, Piñeiro C, Koketsu Y. Removal of sows in Spanish breeding herds due to lameness: Incidence, related factors and reproductive performance of removed sows. Prev Vet Med. 2020 Jun 1;179:105002.

31. Ley SJ, Waterman AE, Livingston A. A field study of the effect of lameness on mechanical nociceptive thresholds in sheep. Vet Rec. 1995 Jul 1;137(4):85–7.

32. Whay HR, Waterman AE, Webster AJ, O’Brien JK. The influence of lesion type on the duration of hyperalgesia associated with hindlimb lameness in dairy cattle. Vet J Lond Engl 1997. 1998 Jul;156(1):23–9.

33. Dolan S, Kelly JG, Monteiro AM, Nolan AM. Up-regulation of metabotropic glutamate receptor subtypes 3 and 5 in spinal cord in a clinical model of persistent inflammation and hyperalgesia. Pain. 2003 Dec 1;106(3):501–12.

34. Tadich N, Tejeda C, Bastias S, Rosenfeld C, Green LE. Nociceptive threshold, blood constituents and physiological values in 213 cows with locomotion scores ranging from normal to severely lame. Vet J Lond Engl 1997. 2013 Aug;197(2):401–5.

35. Conte S, Bergeron R, Gonyou H, Brown J, Rioja-Lang FC, Connor ML, et al. Use of an analgesic to identify pain-related indicators of lameness in sows. Livest Sci. 2015 Oct 1;180:203–8.

36. Passos LT, Cruz EA da, Fischer V, Porciuncula GC da, Werncke D, Dalto AGC, et al. Dairy cows change locomotion score and sensitivity to pain with trimming and infectious or non-infectious lesions. Trop Anim Health Prod. 2017 Apr 1;49(4):851–6.

37. Dyson S, Berger JM, Ellis AD, Mullard J. Behavioral observations and comparisons of nonlame horses and lame horses before and after resolution of lameness by diagnostic analgesia. J Vet Behav. 2018 Jul 1;26:64–70.

38. Thomas AD, Orsel K, Cortés JA, Pajor EA. Objective determination and quantification of pain and inflammation associated with digital dermatitis in feedlot cattle. Appl Anim Behav Sci. 2022 Aug 1;253:105684.

39. Kovács L, Kézér FL, Jurkovich V, Kulcsár-Huszenicza M, Tőzsér J. Heart Rate Variability as an Indicator of Chronic Stress Caused by Lameness in Dairy Cows. PLOS ONE. 2015 Aug 13;10(8):e0134792.

40. Janßen S, Wunderlich C, Heppelmann M, Palme R, Starke A, Kehler W, et al. Short communication: Pilot study on hormonal, metabolic, and behavioral stress response to treatment of claw horn lesions in acutely lame dairy cows. J Dairy Sci. 2016 Sep 1;99(9):7481–8.

41. Reinemann D, Rasmussen M, Wiltbank M, Sheffield L, LeMire S. Dairy Cow Response to Electrical Environment Final Report Part I. Comparison of Behavioral to Physiological Responses and Part II. Comparison of Treatments Applied during Milking. 1999 Jun 30;

42. Wells SJ, Trent AM, Marsh WE, Robinson RA. Prevalence and severity of lameness in lactating dairy cows in a sample of Minnesota and Wisconsin herds. J Am Vet Med Assoc. 1993 Jan 1;202(1):78–82.

43. Clarkson MJ, Downham DY, Faull WB, Hughes JW, Manson FJ, Merritt JB, et al. Incidence and prevalence of lameness in dairy cattle. Vet Rec. 1996;138(23):563–7.

44. Sprecher DJ, Hostetler DE, Kaneene JB. A lameness scoring system that uses posture and gait to predict dairy cattle reproductive performance. Theriogenology. 1997 Apr 15;47(6):1179–87.

45. Nalon E, Conte S, Maes D, Tuyttens FAM, Devillers N. Assessment of lameness and claw lesions in sows. Livest Sci. 2013 Sep 1;156(1):10–23.

46. Whay HR, Main DCJ, Green LE, Webster AJF. Assessment of the welfare of dairy caftle using animal-based measurements: direct observations and investigation of farm records. Vet Rec Lond. 2003 Aug 16;153(7):197.

47. Flower FC, Weary DM. Effect of Hoof Pathologies on Subjective Assessments of Dairy Cow Gait. J Dairy Sci. 2006 Jan 1;89(1):139–46.

48. Espejo LA, Endres MI, Salfer JA. Prevalence of Lameness in High-Producing Holstein Cows Housed in Freestall Barns in Minnesota. J Dairy Sci. 2006 Aug 1;89(8):3052–8.

49. Fabian J, Laven RA, Whay HR. The prevalence of lameness on New Zealand dairy farms: A comparison of farmer estimate and locomotion scoring. Vet J. 2014 Jul 1;201(1):31–8.

50. Hansen PJ. Effects of heat stress on mammalian reproduction. Philos Trans R Soc B Biol Sci. 2009 Nov 27;364(1534):3341–50.

51. López-Gatius F. Is fertility declining in dairy cattle?: A retrospective study in northeastern Spain. Theriogenology. 2003 Jun 1;60(1):89–99.

52. Badinga L, Collier RJ, Thatcher WW, Wilcox CJ. Effects of Climatic and Management Factors on Conception Rate of Dairy Cattle in Subtropical Environment1. J Dairy Sci. 1985 Jan 1;68(1):78–85.

53. al-Katanani YM, Webb DW, Hansen PJ. Factors affecting seasonal variation in 90-day nonreturn rate to first service in lactating Holstein cows in a hot climate. J Dairy Sci. 1999 Dec;82(12):2611–6.

54. Gaughan JB, Holt SM, Hahn GL, Mader TL, Eigenberg R. Respiration rate - is it a good measure of heat stress in cattle? Asian-Australas J Anim Sci. 2000 Jan;13(Suppl.):329–32.

55. Poursaberi A, Bahr C, Pluk A, Van Nuffel A, Berckmans D. Real-time automatic lameness detection based on back posture extraction in dairy cattle: Shape analysis of cow with image processing techniques. Comput Electron Agric. 2010 Oct 1;74(1):110–9.

56. Burdick NC, Carroll JA, Dailey JW, Randel RD, Falkenberg SM, Schmidt TB. Development of a self-contained, indwelling vaginal temperature probe for use in cattle research. J Therm Biol. 2012 Jul 1;37(4):339–43.

57. Sakatani M, Takahashi M, Takenouchi N. The efficiency of vaginal temperature measurement for detection of estrus in Japanese Black cows. J Reprod Dev. Apr Estimating US dairy clinical disease costs with a stochastic simulation model2016;62(2):2015–95.

58. Bewley JM, Grott MW, Einstein ME, Schutz MM. Impact of Intake Water Temperatures on Reticular Temperatures of Lactating Dairy Cows. J Dairy Sci. 2008;91(10):3880–7.

59. Tresoldi G, Schütz KE, Tucker CB. Cooling cows with sprinklers: Timing strategy affects physiological responses to heat load. J Dairy Sci. 2018 Dec 1;101(12):11237–46.

60. Tresoldi G, Schütz KE, Tucker CB. Cooling cows with sprinklers: Effects of soaker flow rate and timing on behavioral and physiological responses to heat load and production. J Dairy Sci. 2019 Jan 1;102(1):528–38.

61. Dikmen S, Larson CC, De Vries A, Hansen PJ. Effectiveness of tunnel ventilation as dairy cow housing in hot climates: rectal temperatures during heat stress and seasonal variation in milk yield. Trop Anim Health Prod. 2020 Sep 1;52(5):2687–93.

62. Ruegg PL. A 100-Year Review: Mastitis detection, management, and prevention. J Dairy Sci. 2017 Dec 1;100(12):10381–97.

63. Puerto MA, Shepley E, Cue RI, Warner D, Dubuc J, Vasseur E. The hidden cost of disease: I. Impact of the first incidence of mastitis on production and economic indicators of primiparous dairy cows. J Dairy Sci. 2021 Jul 1;104(7):7932–43.

64. Liang D, Arnold LM, Stowe CJ, Harmon RJ, Bewley JM. Estimating US dairy clinical disease costs with a stochastic simulation model. J Dairy Sci. 2017 Feb;100(2):1472–86.

65. Le Maréchal C, Thiéry R, Vautor E, Le Loir Y. Mastitis impact on technological properties of milk and quality of milk products—a review. Dairy Sci Technol. 2011 May 1;91(3):247–82.

66. Aghamohammadi M, Haine D, Kelton DF, Barkema HW, Hogeveen H, Keefe GP, et al. Herd-Level Mastitis-Associated Costs on Canadian Dairy Farms. Front Vet Sci. 2018 May 14;5:100.

67. Fitzpatrick J, Young F, Eckersall D, Logue D, Knight C, Nolan A. Recognising and controlling pain and inflammation in mastitis. Proc Br Mastit Conf. 1998 Oct 7;36–44.

68. Rasmussen DB, Fogsgaard K, Røntved CM, Klaas IC, Herskin MS. Changes in thermal nociceptive responses in dairy cows following experimentally induced Escherichia coli mastitis. Acta Vet Scand. 2011 May 18;53:32.

69. Dolan S, Field LC, Nolan AM. The role of nitric oxide and prostaglandin signaling pathways in spinal nociceptive processing in chronic inflammation. Pain. 2000 Jun 1;86(3):311–20.

70. Peters MDP, Silveira IDB, Fischer V. Impact of subclinical and clinical mastitis on sensitivity to pain of dairy cows. Animal. 2015 Dec 1;9(12):2024–8.

71. McLennan KM, Rebelo CJB, Corke MJ, Holmes MA, Leach MC, Constantino-Casas F. Development of a facial expression scale using footrot and mastitis as models of pain in sheep. Appl Anim Behav Sci. 2016 Mar 1;176:19–26.

72. Viguier C, Arora S, Gilmartin N, Welbeck K, O’Kennedy R. Mastitis detection: current trends and future perspectives. Trends Biotechnol. 2009 Aug 1;27(8):486–93.

73. Adkins PRF, Middleton JR. Methods for Diagnosing Mastitis. Vet Clin North Am Food Anim Pract. 2018 Nov 1;34(3):479–91.

74. Menzies PI, Ramanoon SZ. Mastitis of Sheep and Goats. Vet Clin North Am Food Anim Pract. 2001 Jul 1;17(2):333–58.

75. Hovinen M, Pyörälä S. Invited review: Udder health of dairy cows in automatic milking. J Dairy Sci. 2011 Feb 1;94(2):547–62.

76. Mollenhorst H, Rijkaart LJ, Hogeveen H. Mastitis alert preferences of farmers milking with automatic milking systems. J Dairy Sci. 2012 May 1;95(5):2523–30.

77. Hogeveen H, Kamphuis C, Steeneveld W, Mollenhorst H. Sensors and Clinical Mastitis—The Quest for the Perfect Alert. Sensors. 2010 Sep;10(9):7991–8009.

78. Gomes F, Henriques M. Control of Bovine Mastitis: Old and Recent Therapeutic Approaches. Curr Microbiol. 2016 Apr 1;72(4):377–82.

79. Zhang G, Ametaj BN. Ketosis an Old Story Under a New Approach. Dairy. 2020 Jun;1(1):42–60.

80. Ingvartsen KL. Feeding- and management-related diseases in the transition cow: Physiological adaptations around calving and strategies to reduce feeding-related diseases. Anim Feed Sci Technol. 2006 Mar 9;126(3):175–213.

81. Suthar VS, Canelas-Raposo J, Deniz A, Heuwieser W. Prevalence of subclinical ketosis and relationships with postpartum diseases in European dairy cows. J Dairy Sci. 2013 May 1;96(5):2925–38.

82. Binev R, Marutsova V, Radev V. Clinical and haematological studies on subclinical lactational ketosis in dairy goats. Agric Sci Technol Bulg. 2014;6(4):427–30.

83. Mee JF. Managing the dairy cow at calving time. Vet Clin Food Anim Pract. 2004 Nov 1;20(3):521–46.

84. Schuenemann GM, Nieto I, Bas S, Galvão KN, Workman J. Assessment of calving progress and reference times for obstetric intervention during dystocia in Holstein dairy cows. J Dairy Sci. 2011 Nov;94(11):5494–501.

85. Lombard JE, Garry FB, Tomlinson SM, Garber LP. Impacts of Dystocia on Health and Survival of Dairy Calves. J Dairy Sci. 2007 Apr 1;90(4):1751–60.

86. Mee JF. Prevalence and risk factors for dystocia in dairy cattle: A review. Vet J. 2008 Apr 1;176(1):93–101.

87. Jacobson C, Bruce M, Kenyon PR, Lockwood A, Miller D, Refshauge G, et al. A review of dystocia in sheep. Small Rumin Res. 2020 Nov 1;192:106209.

88. Mee JF. Managing the dairy cow at calving time. Vet Clin North Am Food Anim Pract. 2004 Nov;20(3):521–46.

89. McClintock SEJ. A genetic evaluation of dystocia in Australian Holstein-Friesian cattle – ScienceOpen [Internet] [Thesis]. University of Melbourne; 2004 [cited 2023 Jan 4]. Available from: https://www.scienceopen.com/document?vid=4c4c1440-c59b-4b10-9e40-5b7e0822984b

90. Bar-Anan R, Heiman M, Ron M, Weller JI. Comparison of proven sires from five Holstein-Friesian strains in high-yield Israeli dairy herds. Livest Prod Sci. 1987 Jan 1;17:305–22.

91. Drillich M, Beetz O, Pfützner A, Sabin M, Sabin HJ, Kutzer P, et al. Evaluation of a Systemic Antibiotic Treatment of Toxic Puerperal Metritis in Dairy Cows. J Dairy Sci. 2001 Sep 1;84(9):2010–7.

92. C. Bartlett P, Kirk JH, Wilke MA, Kaneene JB, Mather EC. Metritis complex in Michigan Holstein-Friesian cattle: incidence, descriptive epidemiology and estimated economic impact. Prev Vet Med. 1986 Oct 1;4(3):235–48.

93. Günay A, Günay Ü, Orman A. Effects of retained placenta on the fertility in treated dairy cows. 2011 Feb;126–31.

94. Gilbert RO, Shin ST, Guard CL, Erb HN, Frajblat M. Prevalence of endometritis and its effects on reproductive performance of dairy cows. Theriogenology. 2005 Dec;64(9):1879–88.

95. Björkman S, Oliviero C, Kauffold J, Soede NM, Peltoniemi OAT. Prolonged parturition and impaired placenta expulsion increase the risk of postpartum metritis and delay uterine involution in sows. Theriogenology. 2018 Jan 15;106:87–92.

96. Sheldon IM, Lewis GS, LeBlanc S, Gilbert RO. Defining postpartum uterine disease in cattle. Theriogenology. 2006 May 1;65(8):1516–30.

97. Sheldon IM, Cronin J, Goetze L, Donofrio G, Schuberth HJ. Defining Postpartum Uterine Disease and the Mechanisms of Infection and Immunity in the Female Reproductive Tract in Cattle1. Biol Reprod. 2009 Dec 1;81(6):1025–32.

98. Fishman N. Antimicrobial stewardship. Am J Infect Control. 2006 Jun 1;34(5, Supplement):S55–63.

99. Malinowski E, Lassa H, Markiewicz H, Kaptur M, Nadolny M, Niewitecki W, et al. Sensitivity to antibiotics of Arcanobacterium pyogenes and Escherichia coli from the uteri of cows with metritis/endometritis. Vet J. 2011 Feb 1;187(2):234–8.

100. Ozawa T, Kiku Y, Mizuno M, Inumaru S, Kushibiki S, Shingu H, et al. Effect of intramammary infusion of rbGM-CSF on SCC and expression of polymorphonuclear neutrophil adhesion molecules in subclinical mastitis cows. Vet Res Commun. 2012 Mar 1;36(1):21–7.

101. Pol M, Ruegg PL. Treatment practices and quantification of antimicrobial drug usage in conventional and organic dairy farms in Wisconsin. J Dairy Sci. 2007 Jan 1;90(1):249–61.

102. Kossaibati MA, Esslemont RJ. The costs of production diseases in dairy herds in England. Vet J Lond Engl. 1997 Jul 1;154(1):41–51.

103. Wilson DJ, González RN, Hertl J, Schulte HF, Bennett GJ, Schukken YH, et al. Effect of Clinical Mastitis on the Lactation Curve: A Mixed Model Estimation Using Daily Milk Weights. J Dairy Sci. 2004 Jul 1;87(7):2073–84.

104. Chapinal N, LeBlanc SJ, Carson ME, Leslie KE, Godden S, Capel M, et al. Herd-level association of serum metabolites in the transition period with disease, milk production, and early lactation reproductive performance. J Dairy Sci. 2012 Oct 1;95(10):5676–82.

105. Ribeiro ES, Lima FS, Greco LF, Bisinotto RS, Monteiro APA, Favoreto M, et al. Prevalence of periparturient diseases and effects on fertility of seasonally calving grazing dairy cows supplemented with concentrates. J Dairy Sci. 2013 Sep 1;96(9):5682–97.

106. Fu Y, Colazo MG, De Buck J. Development of a blood calcium test for hypocalcemia diagnosis in dairy cows. Res Vet Sci. 2022 Oct 1;147:60–7.

107. Raizman EA, Santos JEP. The Effect of Left Displacement of Abomasum Corrected by Toggle-Pin Suture on Lactation, Reproduction, and Health of Holstein Dairy Cows. J Dairy Sci. 2002 May 1;85(5):1157–64.

108. Sexton MF, Buckley W, Ryan E. A study of 54 cases of left displacement of the abomasum: February to July 2005. Ir Vet J. 2007 Oct 1;60(10):605.

109. Shakespeare A. Left displaced abomasum in sheep. Vet Rec. 1997 Nov 15;141(20):527–8.

110. Li XW, Xu QS, Zhang RH, Yang W, Li Y, Zhang YM, et al. Ultrasonographic findings in cows with left displacement of abomasum, before and after reposition surgery. BMC Vet Res. 2018 Feb 1;14(1):44.

111. Niederberger MD, Hirsbrunner G, Steiner A, Brechbühl M, Meylan M. In vitro effects of bethanechol on abomasal and duodenal smooth muscle preparations from dairy cows with left displacement of the abomasum and from healthy dairy cows. Vet J. 2010 Apr 1;184(1):88–94.

112. Levitis DA, Lidicker WZ, Freund G. Behavioural biologists do not agree on what constitutes behaviour. Anim Behav. 2009 Jul 1;78(1):103–10.

113. Riaboff L, Poggi S, Madouasse A, Couvreur S, Aubin S, Bédère N, et al. Development of a methodological framework for a robust prediction of the main behaviours of dairy cows using a combination of machine learning algorithms on accelerometer data. Comput Electron Agric. 2020 Feb;169:105179.

114. Uzal S, Ugurlu N. The time budget of dairy cows as affected by season and housing system. J Int Environ Appl Amp Sci. 2010;5(4):638–47.

115. Andriamandroso A, Bindelle J, Lebeau F. Cattle grazing dynamics under contrasted pasture characteristics at temporal and spatial scales [Internet] [Thesis]. Université de

Liège, Liège, Belgique; 2017 [cited 2023 Jan 5]. Available from: https://orbi.uliege.be/handle/2268/209464

116. Riaboff L, Relun A, Petiot CE, Feuilloy M, Couvreur S, Madouasse A. Identification of discriminating behavioural and movement variables in lameness scores of dairy cows at pasture from accelerometer and GPS sensors using a Partial Least Squares Discriminant Analysis. Prev Vet Med. 2021 Aug 1;193:105383.

117. Munksgaard L, Jensen MB, Pedersen LJ, Hansen SW, Matthews L. Quantifying behavioural priorities—effects of time constraints on behaviour of dairy cows, Bos taurus. Appl Anim Behav Sci. 2005 Jul 1;92(1):3–14.

118. Winckler C, Tucker CB, Weary DM. Effects of under- and overstocking freestalls on dairy cattle behaviour. Appl Anim Behav Sci. 2015 Sep 1;170:14–9.

119. Falk AC, Weary DM, Winckler C, von Keyserlingk MAG. Preference for pasture versus freestall housing by dairy cattle when stall availability indoors is reduced. J Dairy Sci. 2012 Nov 1;95(11):6409–15.

120. Fregonesi JA, Tucker CB, Weary DM. Overstocking Reduces Lying Time in Dairy Cows. J Dairy Sci. 2007 Jul 1;90(7):3349–54.

121. Hill CT, Krawczel PD, Dann HM, Ballard CS, Hovey RC, Falls WA, et al. Effect of stocking density on the short-term behavioural responses of dairy cows. Appl Anim Behav Sci. 2009 Mar 1;117(3):144–9.

122. Krawczel PD, Mooney CS, Dann HM, Carter MP, Butzler RE, Ballard CS, et al. Effect of alternative models for increasing stocking density on the short-term behavior and hygiene of Holstein dairy cows. J Dairy Sci. 2012 May 1;95(5):2467–75.

123. Leonard FC, O'Connell JM, O'Farrell KJ. Effect of overcrowding on claw health in first-calved friesian heifers. Br Vet J. 1996 Jul 1;152(4):459–72.

124. Løvendahl P, Munksgaard L. An investigation into genetic and phenotypic variation in time budgets and yield of dairy cows. J Dairy Sci. 2016 Jan 1;99(1):408–17.

125. Carere C, Maestripieri D. Animal Personalities: Behavior, Physiology, and Evolution. University of Chicago Press; 2013. 518 p.

126. Kaiser MI, Müller C. What is an animal personality? Biol Philos. 2021 Jan 10;36(1):1.

127. Schrader L. Consistency of individual behavioural characteristics of dairy cows in their home pen. Appl Anim Behav Sci. 2002 Jun 28;77(4):255–66.

128. Müller R, Schrader L. Individual Consistency of Dairy Cows' Activity in Their Home Pen. J Dairy Sci. 2005 Jan 1;88(1):171–5.

129. Schrader L, Müller R. Behavioural consistency during social separation and personality in dairy cows. Behaviour. 2005 Jan 1;142(9–10):1289–306.

130. Boissy A, Bouissou MF. Assessment of individual differences in behavioural reactions of heifers exposed to various fear-eliciting situations. Appl Anim Behav Sci. 1995 Dec 1;46(1):17–31.

131. Manteca X, Deag JM. Individual Differences in Temperament of Domestic Animals: A Review of Methodology. Anim Welf. 1993 Aug 1;2(3):247–68.

132. Bouissou M, Boissy A, Le Neindre P, Veissier I. Social Behavior of Cattle. CABI; 2001. 22 p.

133. Krause J, Krause P of FB and EJ, Ruxton GD, Ruxton G, Ruxton IG. Living in Groups. OUP Oxford; 2002. 228 p.

134. Beilharz RG, Zeeb K. Social dominance in dairy cattle. Appl Anim Ethol. 1982 Jan 1;8(1):79–97.

135. Estevez I, Andersen IL, Nævdal E. Group size, density and social dynamics in farm animals. Appl Anim Behav Sci. 2007 Mar 20;103(3):185–204.

136. Fortin D, Fortin ME. Group-size-dependent association between food profitability, predation risk and distribution of free-ranging bison. Anim Behav. 2009 Oct 1;78(4):887–92.

137. Thaker M, Vanak AT, Owen CR, Ogden MB, Slotow R. Group dynamics of zebra and wildebeest in a woodland savanna: effects of predation risk and habitat density. PloS One. 2010 Sep 20;5(9):e12758.

138. Keeling L, Gonyou HW. Social behaviour in farm animals. CABI; 2001. 406 p.

139. Hasegawa N, Nishiwaki A, Sugawara K, Ito I. The effects of social exchange between two groups of lactating primiparous heifers on milk production, dominance order, behavior and adrenocortical response. Appl Anim Behav Sci. 1997 Jan 1;51(1):15–27.

140. Phillips CJC, Rind MI. The Effects on Production and Behavior of Mixing Uniparous and Multiparous Cows. J Dairy Sci. 2001 Nov 1;84(11):2424–9.

141. Færevik G, Jensen MB, Bøe KE. Dairy calves social preferences and the significance of a companion animal during separation from the group. Appl Anim Behav Sci. 2006 Sep 1;99(3):205–21.

142. Schirmann K, Chapinal N, Weary DM, Heuwieser W, von Keyserlingk MAG. Short-term effects of regrouping on behavior of prepartum dairy cows. J Dairy Sci. 2011 May 1;94(5):2312–9.

143. McLennan KM. Social bonds in dairy cattle: the effect of dynamic group systems on welfare and productivity [phd]. University of Northampton; 2013.

144. von Keyserlingk MAG, Olenick D, Weary DM. Acute Behavioral Effects of Regrouping Dairy Cows. J Dairy Sci. 2008 Mar 1;91(3):1011–6.

145. Rodenburg TB, Koene P. The impact of group size on damaging behaviours, aggression, fear and stress in farm animals. Appl Anim Behav Sci. 2007;103(3–4):205.

146. Wey T, Blumstein DT, Shen W, Jordán F. Social network analysis of animal behaviour: A promising tool for the study of sociality. Anim Behav. 2008;75(2):333–44.

147. de Freslon I, Martínez-López B, Belkhiria J, Strappini A, Monti G. Use of social network analysis to improve the understanding of social behaviour in dairy cattle and its impact on disease transmission. Appl Anim Behav Sci. 2019 Apr 1;213:47–54.

148. Val-Laillet D, Guesdon V, von Keyserlingk MAG, de Passillé AM, Rushen J. Allogrooming in cattle: Relationships between social preferences, feeding displacements and social dominance. Appl Anim Behav Sci. 2009 Jan 31;116(2):141–9.

149. Laister S, Stockinger B, Regner AM, Zenger K, Knierim U, Winckler C. Social licking in dairy cattle—Effects on heart rate in performers and receivers. Appl Anim Behav Sci. 2011 Mar 1;130(3):81–90.

150. Boyle AR, Ferris CP, O'Connell NE. Are there benefits in introducing dairy heifers to the main dairy herd in the evening rather than the morning? J Dairy Sci. 2012 Jul 1;95(7):3650–61.

151. Gibbons JM, Lawrence AB, Haskell MJ. Measuring sociability in dairy cows. Appl Anim Behav Sci. 2010 Jan 31;122(2):84–91.

152. Wierenga HK. Social dominance in dairy cattle and the influences of housing and management. Appl Anim Behav Sci. 1990 Sep 1;27(3):201–29.

153. Proudfoot KL, Veira DM, Weary DM, von Keyserlingk MAG. Competition at the feed bunk changes the feeding, standing, and social behavior of transition dairy cows. J Dairy Sci. 2009 Jul 1;92(7):3116–23.

154. Kumar B, Manuja A, Aich P. Stress and its impact on farm animals. Front Biosci-Elite. 2012 Jan 1;4(5):1759–67.

155. Hall SJG. Chillingham cattle: social and maintenance behaviour in an ungulate that breeds all year round. Anim Behav. 1989 Aug 1;38(2):215–25.

156. A. Talebi, M.A.G. von Keyserlingk, E. Telezhenko, D.M. Weary. Reduced stocking density mitigates the negative effects of regrouping in dairy cattle. J Dairy Sci. 2014;97(3):1358–63.

157. Raussi S, Boissy A, Delval E, Pradel P, Kaihilahti J, Veissier I. Does repeated regrouping alter the social behaviour of heifers? Appl Anim Behav Sci. 2005 Sep 1;93(1):1–12.

158. Rioja-Lang FC, Roberts DJ, Healy SD, Lawrence AB, Haskell MJ. Dairy cows trade-off feed quality with proximity to a dominant individual in Y-maze choice tests. Appl Anim Behav Sci. 2009 Mar 1;117(3):159–64.

159. Radka Šárová, Marek Špinka, Ilona Stěhulová, Francisco Ceacero, Marie Šimečková, Radim Kotrba. Pay respect to the elders: age, more than body mass, determines dominance in female beef cattle. Anim Behav. 2013 Dec 1;86(6):1315–23.

160. DeVries TJ, von Keyserlingk M a. G, Beauchemin KA. Frequency of feed delivery affects the behavior of lactating dairy cows. J Dairy Sci. 2005 Oct;88(10):3553–62.

161. Mellor D, Beausoleil N. Extending the ‘Five Domains’ model for animal welfare assessment to incorporate positive welfare states. Anim Welf. 2015 Aug 1;24(3):241–53.

162. de Freslon I, Peralta JM, Strappini AC, Monti G. Understanding Allogrooming Through a Dynamic Social Network Approach: An Example in a Group of Dairy Cows. Front Vet Sci [Internet]. 2020 Aug 4 [cited 2020 Sep 9];7. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7417353/

163. Sato S, Sako S, Maeda A. Social licking patterns in cattle (Bos taurus): influence of environmental and social factors. Appl Anim Behav Sci. 1991 Oct 1;32(1):3–12.

164. Ishiwata T, Kilgour R, Uetake K, Eguchi Y, Tanaka T. Choice of attractive conditions by beef cattle in a Y-maze just after release from restraint. J Anim Sci. 2007;85(4):1080–5.

165. Croft DP, James R, Krause J. Exploring Animal Social Networks. Princeton University Press; 2008.

166. Gygax L, Neisen G, Wechsler B. Socio-Spatial Relationships in Dairy Cows. Ethology. 2010 Jan 1;116:10–23.

167. Boyland NK. The influence of social networks on welfare and productivity in dairy cattle [Internet]. University of Exeter; 2015 [cited 2020 Jul 11]. Available from: https://ore.exeter.ac.uk/repository/handle/10871/19360

168. Gutmann AK, Špinka M, Winckler C. Long-term familiarity creates preferred social partners in dairy cows. Appl Anim Behav Sci. 2015 Aug 1;169:1–8.

169. Raussi S, Niskanen S, Siivonen J, Hänninen L, Hepola H, Jauhiainen L, et al. The formation of preferential relationships at early age in cattle. Behav Processes. 2010 Jul;3(84):726–31.

170. Magurran AE, Seghers BH, Shaw PW, Carvalho GR. Schooling preferences for familiar fish in the guppy, Poecilia reticulata. J Fish Biol. 1994 Sep;45(3):401–6.

171. Wikberg EC, Ting N, Sicotte P. Familiarity is more important than phenotypic similarity in shaping social relationships in a facultative female dispersed primate, Colobus vellerosus. Behav Processes. 2014 Jul 1;106:27–35.

172. Bilčík B, Keeling LJ, Newberry RC. Effect of group size on tonic immobility in laying hens. Behav Processes. 1998 Apr 1;43(1):53–9.

173. Rizvi S, Nicol CJ, Green LE. Risk factors for vulva biting in breeding sows in south-west England. Vet Rec. 1998;143(24):654–8.

174. Barnett JL, Hemsworth PH, Winfield CG, Hansen C. Effects of social environment on welfare status and sexual behaviour of female pigs. I. Effects of group size. Appl Anim Behav Sci. 1986 Nov 1;16(3):249–57.

175. Takeda K ichi, Sato S, Sugawara K. Familiarity and group size affect emotional stress in Japanese Black heifers. Appl Anim Behav Sci. 2003 Jun 3;82(1):1–11.

176. Boyland NK, Mlynski DT, James R, Brent LJN, Croft DP. The social network structure of a dynamic group of dairy cows: from individual to group level patterns. Appl Anim Behav Sci. 2016 Jan;174(1):1–10.

177. Hewetson M, Christley RM, Hunt ID, Voute LC. Investigations of the reliability of observational gait analysis for the assessment of lameness in horses. Vet Rec. 2006 Jun 24;158(25):852–7.

178. Engel B, Bruin G, Andre G, Buist W. Assessment of observer performance in a subjective scoring system: visual classification of the gait of cows. J Agric Sci. 2003 May;140(3):317–33.

179. Flower FC, Weary DM. Gait assessment in dairy cattle. Animal. 2009 Jan 1;3(1):87–95.

180. Welsh EM, Gettinby G, Nolan AM. Comparison of a visual analogue scale and a numerical rating scale for assessment of lameness, using sheep as a model. Am J Vet Res. 1993 Jun;54(6):976–83.

181. Espejo LA, Endres MI, Salfer JA. Prevalence of Lameness in High-Producing Holstein Cows Housed in Freestall Barns in Minnesota. J Dairy Sci. 2006 Aug 1;89(8):3052–8.

182. Hewetson M, Christley RM, Hunt ID, Voute LC. Investigations of the reliability of observational gait analysis for the assessment of lameness in horses. Vet Rec. 2006;158(25):852–8.

183. Hjermstad MJ, Fayers PM, Haugen DF, Caraceni A, Hanks GW, Loge JH, et al. Studies comparing Numerical Rating Scales, Verbal Rating Scales, and Visual Analogue Scales for assessment of pain intensity in adults: a systematic literature review. J Pain Symptom Manage. 2011 Jun;41(6):1073–93.

184. D’Eath R. Repeated locomotion scoring of a sow herd to measure lameness: consistency over time, the effect of sow characteristics and inter-observer reliability. Anim Welf. 2012 May 1;21(2):219–31.

185. Menzies-Gow NJ, Stevens KB, Sepulveda MF, Jarvis N, Marr CM. Repeatability and reproducibility of the Obel grading system for equine laminitis. Vet Rec. 2010 Jul 10;167(2):52–5.

186. Weeks CA, Danbury TD, Davies HC, Hunt P, Kestin SC. The behaviour of broiler chickens and its modification by lameness. Appl Anim Behav Sci. 2000 Mar 22;67(1):111–25.

187. Walker SL, Smith RF, Routly JE, Jones DN, Morris MJ, Dobson H. Lameness, Activity Time-Budgets, and Estrus Expression in Dairy Cattle. J Dairy Sci. 2008 Dec 1;91(12):4552–9.

188. Gomez A, Cook NB. Time budgets of lactating dairy cattle in commercial freestall herds. J Dairy Sci. 2010 Dec 1;93(12):5772–81.

189. Cook NB, Mentink RL, Bennett TB, Burgi K. The Effect of Heat Stress and Lameness on Time Budgets of Lactating Dairy Cows. J Dairy Sci. 2007 Apr 1;90(4):1674–82.

190. Metz J, Metz JHM. Behavioural phenomena related to normal and difficult deliveries in dairy cows. Neth J Agric Sci. 1987 May 1;35(2):87–101.

191. Herbut P, Angrecka S. Full article: Relationship between THI level and dairy cows’ behaviour during summer period. Ital J Anim Sci. 2018;17(1):226–33.

192. Overton MW, Sischo WM, Temple GD, Moore DA. Using Time-Lapse Video Photography to Assess Dairy Cattle Lying Behavior in a Free-Stall Barn. J Dairy Sci. 2002 Sep 1;85(9):2407–13.

193. Almeida PE, Weber PSD, Burton JL, Zanella AJ. Depressed DHEA and increased sickness response behaviors in lame dairy cows with inflammatory foot lesions. Domest Anim Endocrinol. 2008 Jan 1;34(1):89–99.

194. Van Winden SCL, Jorritsma R, Müller KE, Noordhuizen JPTM. Feed Intake, Milk Yield, and Metabolic Parameters Prior to Left Displaced Abomasum in Dairy Cows. J Dairy Sci. 2003 Apr 1;86(4):1465–71.

195. Singh SS, Ward WR, Lautenbach K, Hughes JW, Murray RD. Behaviour of first lactation and adult dairy cows while housed and at pasture and its relationship with sole lesions. Vet Rec. 1993 Nov 1;133(19):469–74.

196. Hassall SA, Ward WR, Murray RD. Effects of lameness on the behaviour of cows during the summer. Vet Rec. 1993 Jun 1;132(23):578–80.

197. Cook NB, Bennett TB, Nordlund KV. Effect of Free Stall Surface on Daily Activity Patterns in Dairy Cows with Relevance to Lameness Prevalence. J Dairy Sci. 2004 Sep 1;87(9):2912–22.

198. Rhoads RP, Baumgard LH, Suagee JK. 2011 and 2012 Early Careers Achievement Awards: metabolic priorities during heat stress with an emphasis on skeletal muscle. J Anim Sci. 2013 Jun;91(6):2492–503.

199. Hill DL, Wall E. Weather influences feed intake and feed efficiency in a temperate climate. J Dairy Sci. 2017 Mar 1;100(3):2240–57.

200. Bouraoui R, Lahmar M, Majdoub A, Djemali M, Belyea R. The relationship of temperature-humidity index with milk production of dairy cows in a Mediterranean climate. Anim Res. 2002 Nov 1;51(6):479–91.

201. West JW, Mullinix BG, Bernard JK. Effects of Hot, Humid Weather on Milk Temperature, Dry Matter Intake, and Milk Yield of Lactating Dairy Cows. J Dairy Sci. 2003 Jan 1;86(1):232–42.

202. Gorniak T, Meyer U, Südekum KH, Dänicke S. Impact of mild heat stress on dry matter intake, milk yield and milk composition in mid-lactation Holstein dairy cows in a temperate climate. Arch Anim Nutr. 2014 Sep 3;68(5):358–69.

203. West JW. Effects of Heat-Stress on Production in Dairy Cattle. J Dairy Sci. 2003 Jun 1;86(6):2131–44.

204. Salama AAK, Hamzaoui S, Albanell E, Such X, Caja G. Metabolic and behavior responses of lactating goats under heat stress. Small Rumin Res. 2021 Oct 1;203:106496.

205. Mandel R, Whay HR, Nicol CJ, Klement E. The effect of food location, heat load, and intrusive medical procedures on brushing activity in dairy cows. J Dairy Sci. 2013 Oct 1;96(10):6506–13.

206. Tucker C, Schütz K. Behavioral responses to heat stress: dairy cows tell the story. In Tepme, Arizona; 2009. p. 3–04.

207. Huzzey JM, DeVries TJ, Valois P, von Keyserlingk MAG. Stocking Density and Feed Barrier Design Affect the Feeding and Social Behavior of Dairy Cattle. J Dairy Sci. 2006 Jan 1;89(1):126–33.

208. Huzzey JM, Veira DM, Weary DM, von Keyserlingk MAG. Prepartum Behavior and Dry Matter Intake Identify Dairy Cows at Risk for Metritis. J Dairy Sci. 2007 Jul 1;90(7):3220–33.

209. Galindo F, Broom DM. The Effects of Lameness on Social and Individual Behavior of Dairy Cows. J Appl Anim Welf Sci. 2002 Jul 1;5(3):193–201.

210. Galindo F, Broom DM, Jackson PGG. A note on possible link between behaviour and the occurrence of lameness in dairy cows. Appl Anim Behav Sci. 2000 Apr 19;67(4):335–41.

211. Schütz KE, Rogers AR, Poulouin YA, Cox NR, Tucker CB. The amount of shade influences the behavior and physiology of dairy cattle. J Dairy Sci. 2010 Jan;93(1):125–33.

212. Vizzotto EF, Fischer V, Neto AT, Abreu AS, Stumpf MT, Werncke D, et al. Access to shade changes behavioral and physiological attributes of dairy cows during the hot season in the subtropics. animal. 2015 Sep;9(9):1559–66.

213. Schütz KE, Cox NR, Matthews LR. How important is shade to dairy cattle? Choice between shade or lying following different levels of lying deprivation. Appl Anim Behav Sci. 2008 Dec 1;114(3):307–18.

214. Telezhenko E. Measurement of spatial gait parameters from footprints of dairy cows. Animal. 2009 Dec;3(12):1746–53.

215. Telezhenko E. Measurements of trackways as a method for assessing locomotion in dairy cows [Internet]. Vol. 2005:2. Skara; 2005 [cited 2022 May 17]. Available from: https://pub.epsilon.slu.se/926/

216. Reader JD, Green MJ, Kaler J, Mason SA, Green LE. Effect of mobility score on milk yield and activity in dairy cattle. J Dairy Sci. 2011 Oct 1;94(10):5045–52.

217. Duncan S, Stewart TI, Oliver M, Mavoa S, MacRae D, Badland HM, et al. Portable Global Positioning System Receivers: Static Validity and Environmental Conditions. Am J Prev Med. 2013 Feb 1;44(2):e19–29.

218. Gygax L, Neisen G, Bollhalder H. Accuracy and validation of a radar-based automatic local position measurement system for tracking dairy cows in free-stall barns. Comput Electron Agric. 2007 Mar 1;56(1):23–33.

219. Tullo E, Fontana I, Gottardo D, Sloth KH, Guarino M. Technical note: Validation of a commercial system for the continuous and automated monitoring of dairy cow activity. J Dairy Sci. 2016 Sep 1;99(9):7489–94.

220. Neveux S, Weary DM, Rushen J, Keyserlingk MAG von, Passillé AM de. Hoof Discomfort Changes How Dairy Cattle Distribute Their Body Weight. J Dairy Sci. 2006 Jul 1;89(7):2503–9.

221. Scott GB. Changes in limb loading with lameness for a number of friesian cattle. Br Vet J. 1989 Jan 1;145(1):28–38.

222. Pastell M, Kujala M, Aisla AM, Hautala M, Poikalainen V, Praks J, et al. Detecting cow's lameness using force sensors. Comput Electron Agric. 2008 Nov 1;64(1):34–8.

223. Sun G, Fitzgerald RF, Stalder KJ, Karriker LA, Johnson AK, Hoff SJ. Development of an Embedded Microcomputer-based Force Plate System for Measuring Sow Weight Distribution and Detection of Lameness. Appl Eng Agric. 2011 Jan;27(3):475–82.

224. Rushen J, Pombourcq E, de Passille AM. Validation of two measures of lameness in dairy cows. Appl Anim Behav Sci. 2007;106(1–3):173–7.

225. Maertens W, Vangeyte J, Baert J, Jantuan A, Mertens KC, De Campeneere S, et al. Development of a real time cow gait tracking and analysing tool to assess lameness using a pressure sensitive walkway: The GAITWISE system. Biosyst Eng. 2011 Sep 1;110(1):29–39.

226. Carvalho VC de, de Alencar Nääs I, Mollo Neto M, Souza SRL de. Measurement of pig claw pressure distribution. Biosyst Eng. 2009 Jul 1;103(3):357–63.

227. Pluk A, Bahr C, Poursaberi A, Maertens W, Nuffel A van, Berckmans D. Automatic measurement of touch and release angles of the fetlock joint for lameness detection in dairy cattle using vision techniques. J Dairy Sci. 2012 Apr 1;95(4):1738–48.

228. Van De Gucht T, Saeys W, Van Weyenberg S, Lauwers L, Mertens K, Vandaele L, et al. Automatically measured variables related to tenderness of hoof placement and weight distribution are valuable indicators for lameness in dairy cows. Appl Anim Behav Sci. 2017 Apr 1;189:13–22.

229. Viazzi S, Bahr C, Schlageter-Tello A, Van Hertem T, Romanini CEB, Pluk A, et al. Analysis of individual classification of lameness using automatic measurement of back posture in dairy cattle. J Dairy Sci. 2013 Jan 1;96(1):257–66.

230. Song X, Leroy T, Vranken E, Maertens W, Sonck B, Berckmans D. Automatic detection of lameness in dairy cattle—Vision-based trackway analysis in cow’s locomotion. Comput Electron Agric. 2008 Nov 1;64(1):39–44.

231. Kang X, Zhang XD, Liu G. Accurate detection of lameness in dairy cattle with computer vision: A new and individualized detection strategy based on the analysis of the supporting phase. 2020 Nov 1;103(11):10628–38.

232. Van Hertem T, Alchanatis V, Antler A, Maltz E, Halachmi I, Schlageter-Tello A, et al. Comparison of segmentation algorithms for cow contour extraction from natural barn background in side view images. Comput Electron Agric. 2013 Feb 1;91:65–74.

233. Van Hertem T, Viazzi S, Steensels M, Maltz E, Antler A, Alchanatis V, et al. Automatic lameness detection based on consecutive 3D-video recordings. Biosyst Eng. 2014 Mar 1;119:108–16.

234. Haladjian J, Hodaie Z, Nüske S, Brügge B. Gait Anomaly Detection in Dairy Cattle. In: Proceedings of the Fourth International Conference on Animal-Computer Interaction. 2017. p. 1–8.

235. Pastell M, Tiusanen J, Hakojärvi M, Hänninen L. A wireless accelerometer system with wavelet analysis for assessing lameness in cattle. Biosyst Eng. 2009 Dec 1;104(4):545–51.

236. Chapinal N, de Passille AM, Pastell M, Hänninen L, Munksgaard L, Rushen J. Measurement of acceleration while walking as an automated method for gait assessment in dairy cattle. J Dairy Sci. 2011;94(6):2895–901.

237. Alsaaod M, Römer C, Kleinmanns J, Hendriksen K, Rose-Meierhöfer S, Plümer L, et al. Electronic detection of lameness in dairy cows through measuring pedometric activity and lying behavior. Appl Anim Behav Sci. 2012 Dec 31;142(3):134–41.

238. Flower FC, Sanderson DJ, Weary DM. Effects of Milking on Dairy Cow Gait. J Dairy Sci. 2006 Jun 1;89(6):2084–9.

239. Vázquez Diosdado JA, Barker ZE, Hodges HR, Amory JR, Croft DP, Bell NJ, et al. Classification of behaviour in housed dairy cows using an accelerometer-based activity monitoring system. Anim Biotelemetry. 2015 Jun 10;3(1):15.

240. Ringgenberg N, Bergeron R, Devillers N. Validation of accelerometers to automatically record sow postures and stepping behaviour. Appl Anim Behav Sci. 2010 Dec 1;128(1):37–44.

241. Beer G, Alsaaod M, Starke A, Schuepbach-Regula G, Müller H, Kohler P, et al. Use of Extended Characteristics of Locomotion and Feeding Behavior for Automated Identification of Lame Dairy Cows. PLOS ONE. 2016 May 17;11(5):e0155796.

242. Barker ZE, Vázquez Diosdado JA, Codling EA, Bell NJ, Hodges HR, Croft DP, et al. Use of novel sensors combining local positioning and acceleration to measure feeding behavior differences associated with lameness in dairy cattle. J Dairy Sci. 2018 Jul 1;101(7):6310–21.

243. Hut PR, Scheurwater J, Nielen M, van den Broek J, Hostens MM. Heat stress in a temperate climate leads to adapted sensor-based behavioral patterns of dairy cows. J Dairy Sci. 2022 Aug 1;105(8):6909–22.

244. Proudfoot KL, Huzzey JM, von Keyserlingk MAG. The effect of dystocia on the dry matter intake and behavior of Holstein cows. J Dairy Sci. 2009 Oct 1;92(10):4937–44.

245. Urton G, von Keyserlingk MAG, Weary DM. Feeding Behavior Identifies Dairy Cows at Risk for Metritis. J Dairy Sci. 2005 Aug 1;88(8):2843–9.

246. Trevisan C, Johansen MV, Mkupasi EM, Ngowi HA, Forkman B. Disease behaviours of sows naturally infected with Taenia solium in Tanzania. Vet Parasitol. 2017 Feb 15;235:69–74.

247. Jawor PE, Huzzey JM, LeBlanc SJ, von Keyserlingk MAG. Associations of subclinical hypocalcemia at calving with milk yield, and feeding, drinking, and standing behaviors around parturition in Holstein cows. J Dairy Sci. 2012 Mar 1;95(3):1240–8.

248. Navarro G, Green LE, Tadich N. Effect of lameness and lesion specific causes of lameness on time budgets of dairy cows at pasture and when housed. Vet J. 2013 Sep 1;197(3):788–93.

249. Chapinal N, de Passillé AM, Weary DM, von Keyserlingk MAG, Rushen J. Using gait score, walking speed, and lying behavior to detect hoof lesions in dairy cows. J Dairy Sci. 2009 Sep 1;92(9):4365–74.

250. Borchers MR, Gavigan S, Harbers A, Bewley J. An evaluation of a novel device for measuring eating, rumination, and inactive behaviors in lactating Holstein dairy cattle. Animal. 2021 Jan 1;15(1):100008.

251. Stangaferro ML, Wijma R, Caixeta LS, Al-Abri MA, Giordano JO. Use of rumination and activity monitoring for the identification of dairy cows with health disorders: Part III. Metritis. J Dairy Sci. 2016 Sep 1;99(9):7422–33.

252. Vázquez Diosdado JA, Barker ZE, Hodges HR, Amory JR, Croft DP, Bell NJ, et al. Space-use patterns highlight behavioural differences linked to lameness, parity, and days in milk in barn-housed dairy cows. PLoS ONE [Internet]. 2018 Dec 19 [cited 2020 Jul 15];13(12). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6300209/

253. Hebblewhite M, Haydon DT. Distinguishing technology from biology: a critical review of the use of GPS telemetry data in ecology. Philos Trans R Soc B Biol Sci. 2010 Jul 27;365(1550):2303–12.

254. Haskell MJ, Rennie LJ, Bowell VA, Bell MJ, Lawrence AB. Housing System, Milk Production, and Zero-Grazing Effects on Lameness and Leg Injury in Dairy Cows. J Dairy Sci. 2006 Nov 1;89(11):4259–66.

255. Williams ML, Mac Parthaláin N, Brewer P, James WPJ, Rose MT. A novel behavioral model of the pasture-based dairy cow from GPS data using data mining and machine learning techniques. J Dairy Sci. 2016 Mar 1;99(3):2063–75.

256. Homburger H, Schneider MK, Hilfiker S, Lüscher A. Inferring Behavioral States of Grazing Livestock from High-Frequency Position Data Alone. PLOS ONE. 2014 Dec 4;9(12):e114522.

257. Mandel R, Nicol CJ, Whay HR, Klement E. Short communication: Detection and monitoring of metritis in dairy cows using an automated grooming device. J Dairy Sci. 2017 Jul 1;100(7):5724–8.

258. González LA, Tolkamp BJ, Coffey MP, Ferret A, Kyriazakis I. Changes in Feeding Behavior as Possible Indicators for the Automatic Monitoring of Health Disorders in Dairy Cows. J Dairy Sci. 2008 Mar 1;91(3):1017–28.

259. Cramer MC, Stanton AL. Associations between health status and the probability of approaching a novel object or stationary human in preweaned group-housed dairy calves. J Dairy Sci. 2015 Oct 1;98(10):7298–308.

260. Cramer MC, Ollivett TL, Stanton AL. Associations of behavior-based measurements and clinical disease in preweaned, group-housed dairy calves. J Dairy Sci. 2016 Sep 1;99(9):7434–43.

261. Fogsgaard KK, Røntved CM, Sørensen P, Herskin MS. Sickness behavior in dairy cows during Escherichia coli mastitis. J Dairy Sci. 2012 Feb 1;95(2):630–8.

262. Dittrich I, Gertz M, Krieter J. Alterations in sick dairy cows' daily behavioural patterns. Heliyon. 2019 Nov;5(11):e02902.

263. Belaid MA, Rodríguez-Prado M, Rodríguez-Prado DV, Chevaux E, Calsamiglia S. Using behavior as an early predictor of sickness in veal calves. J Dairy Sci. 2020 Feb 1;103(2):1874–83.

264. Proudfoot KL, Jensen MB, Weary DM, von Keyserlingk MAG. Dairy cows seek isolation at calving and when ill. J Dairy Sci. 2014 May 1;97(5):2731–9.

265. Nawroth C, Prentice PM, McElligott AG. Individual personality differences in goats predict their performance in visual learning and non-associative cognitive tasks. Behav Processes. 2017 Jan 1;134:43–53.

266. Val-Laillet D, Rushen J, Keyserlingk M. The concept of social dominance and the social distribution of feeding-related displacements between cows. Appl Anim Behav Sci. 2007 Aug 19;111:158–72.

267. McDonald PV, von Keyserlingk MAG, Weary DM. Hot weather increases competition between dairy cows at the drinker. J Dairy Sci. 2020 Apr 1;103(4):3447–58.

268. Icerobotics. Lameness Alerts | IceRobotics | CowAlert [Internet]. [cited 2022 Jul 28]. Available from: https://www.icerobotics.com/cowalert/lameness-alerts/

269. CattleEye. CattleEye | Autonomous Livestock Monitoring [Internet]. CattleEye. [cited 2023 Mar 27]. Available from: https://cattleeye.com/

270. Silva SR, Araujo JP, Guedes C, Silva F, Almeida M, Cerqueira JL. Precision Technologies to Address Dairy Cattle Welfare: Focus on Lameness, Mastitis and Body Condition. Animals. 2021 Aug;11(8):2253.

271. Patison KP, Swain DL, Bishop-Hurley GJ, Robins G, Pattison P, Reid DJ. Changes in temporal and spatial associations between pairs of cattle during the process of familiarisation. Appl Anim Behav Sci. 2010 Dec 1;128(1):10–7.

272. Rocha LEC, Terenius O, Veissier I, Meunier B, Nielsen PP. Persistence of sociality in group dynamics of dairy cattle. Appl Anim Behav Sci. 2020 Feb 1;223:104921.

273. Hodges HR. An investigation of social structure in housed dairy cows [Internet] [phd]. University of Essex; 2018 [cited 2020 Jul 11]. Available from: http://repository.essex.ac.uk/23324/

274. Csardi G, Nepusz T. igraph: Network Analysis and Visualization [Internet]. 2022 [cited 2023 Jan 5]. Available from: https://CRAN.R-project.org/package=igraph

275. R Core Team. R: A language and environment for statistical computing. [Internet]. Vienna, Austria: R Foundation for Statistical Computing; 2022. Available from: https://www.R-project.org/

276. RStudio Team. RStudio: Integrated Development for R. [Internet]. RStudio; 2020 [cited 2020 Jul 11]. Available from: https://rstudio.com/products/rstudio/

277. Farine D. A guide to null models for animal social network analysis. Methods Ecol Evol. 2017;8(10):1309–20.

278. Farine DR, Whitehead H. Constructing, conducting and interpreting animal social network analysis. J Anim Ecol. 2015;84(5).

279. Phipson B, Smyth GK. Permutation p-values should never be zero: calculating exact p-values when permutations are randomly drawn. Stat Appl Genet Mol Biol [Internet]. 2010 Jan 31 [cited 2020 Jul 11];9(1). Available from: http://arxiv.org/abs/1603.05766

280. Whitehead H. Analyzing Animal Societies: Quantitative Methods for Vertebrate Social Analysis. Chicago: University of Chicago Press; 2008. 350 p.

281. Erbez M, Bøe KE, Falta D, Chládek G. Crowding of dairy cows in a cubicle barn during the hot summer months. Arch Anim Breed. 2012 Oct 10;55(4):325–31.

282. Ashmawy WRE, Williams DR, Gerry AC, Champagne JD, Lehenbauer TW, Aly SS. Risk factors affecting dairy cattle protective grouping behavior, commonly known as bunching, against Stomoxys calcitrans (L.) on California dairies. PLOS ONE. 2019 Nov 7;14(11):1–22.

283. Lefcourt AM, Schmidtmann ET. Body Temperature of Dry Cows on Pasture: Environmental and Behavioral Effects. J Dairy Sci. 1989 Nov 1;72(11):3040–9.

284. Mader TL, Holt SM, Hahn GL, Davis MS, Spiers DE. Feeding strategies for managing heat load in feedlot cattle. J Anim Sci. 2002 Sep;80(9):2373–82.

285. Chopra K, Hodges HR, Barker ZE, Vázquez Diosdado JA, Amory JR, Cameron TC, et al. Proximity Interactions in a Permanently Housed Dairy Herd: Network Structure, Consistency, and Individual Differences. Front Vet Sci [Internet]. 2020 [cited 2021 May 24];7. Available from: https://www.frontiersin.org/articles/10.3389/fvets.2020.583715/full

286. Kim J, Hastak M. Social network analysis: Characteristics of online social networks after a disaster. Int J Inf Manag. 2018 Feb 1;38(1):86–96.

287. Otte E, Rousseau R. Social Network Analysis: A Powerful Strategy, also for the Information Sciences. J Inf Sci. 2002 Dec 1;28:441–53.

288. CHOI J, Barnett G, CHON BS. Comparing World City Networks: a network analysis of Internet backbone and air transport intercity linkages. Glob Netw. 2006 Feb 21;6:81–99.

289. Makagon MM, McCowan B, Mench JA. How can social network analysis contribute to social behavior research in applied ethology? Appl Anim Behav Sci. 2012 May 1;138(3–4).

290. Croft D, Darden S, Wey T. Current directions in animal social networks. Curr Opin Behav Sci. 2016 Dec 1;12:52–8.

291. Ballerini M, Cabibbo N, Candelier R, Cavagna A, Cisbani E, Giardina I, et al. Interaction ruling animal collective behavior depends on topological rather than metric distance: Evidence from a field study. Proc Natl Acad Sci. 2008 Jan 29;105(4):1232–7.

292. Nagy M, Vásárhelyi G, Pettit B, Roberts-Mariani I, Vicsek T, Biro D. Context-dependent hierarchies in pigeons Máté Nagy. Proc Natl Acad Sci U S A. 2013 Jul 22;110(32):13049–54.

293. Neave HW, Costa JHC, Weary DM, von Keyserlingk MAG. Long-term consistency of personality traits of cattle. R Soc Open Sci. 2020 Feb 1;7:191849.

294. Foris B, Zebunke M, Langbein J, Melzer N. Comprehensive analysis of affiliative and agonistic social networks in lactating dairy cattle groups. Appl Anim Behav Sci. 2019 Jan 1;210:60–7.

295. Goldhawk C, Chapinal N, Veira DM, Weary DM, von Keyserlingk MAG. Prepartum feeding behavior is an early indicator of subclinical ketosis. J Dairy Sci. 2009 Oct 1;92(10):4971–7.

296. Patbandha TK, Mohanty TK, Layek SS, Kumaresan A, Behera K. Application of pre-partum feeding and social behaviour in predicting risk of developing metritis in crossbred cows. Appl Anim Behav Sci. 2012 Jun 1;139(1):10–7.

297. Sepúlveda-Varas P, Proudfoot KL, Weary DM, von Keyserlingk MAG. Changes in behaviour of dairy cows with clinical mastitis. Appl Anim Behav Sci. 2016 Feb 1;175:8–13.

298. Omnisense. Technology Overview [Internet]. [cited 2023 Jan 5]. Available from: http://www.omnisense.co.uk/technology.html

299. Mantel N. The detection of disease clustering and a generalized regression approach. Cancer Res. 1967;

300. Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, et al. vegan: Community Ecology Package [Internet]. 2019. Available from: https://CRAN.R-project.org/package=vegan

301. AHDB. Mobility scoring: how to score your cows [Internet]. [cited 2023 Jan 5]. Available from: https://ahdb.org.uk/knowledge-library/mobility-scoring-how-to-score-your-cows

302. Krause J, Croft DP, James R. Social network theory in the behavioural sciences: potential applications. Behav Ecol Sociobiol. 2007 Nov 1;62(1):15–27.

303. Watts DJ, Strogatz SH. Collective dynamics of ‘small-world’ networks. Nature. 1998 Jun;393(6684):440–2.

304. Salau J, Lamp O, Krieter J. Dairy cows’ contact networks derived from videos of eight cameras. Biosyst Eng. 2019 Dec 1;188:106–13.

305. Allen JD, Hall LW, Collier RJ, Smith JF. Effect of core body temperature, time of day, and climate conditions on behavioral patterns of lactating dairy cows experiencing mild to moderate heat stress. J Dairy Sci. 2015 Jan 1;98(1):118–27.

306. Shahriar MdS, Smith D, Rahman A, Freeman M, Hills J, Rawnsley R, et al. Detecting heat events in dairy cows using accelerometers and unsupervised learning. Comput Electron Agric. 2016 Oct 1;128:20–6.

307. Shahriar MdS, Smith D, Rahman A, Henry D, Bishop-Hurley G, Rawnsley R, et al. Heat event detection in dairy cows with collar sensors: An unsupervised machine learning approach. In: 2015 IEEE SENSORS. 2015. p. 1–4.

308. James R, Croft DP, Krause J. Potential banana skins in animal social network analysis. Behav Ecol Sociobiol. 2009 May 1;63(7):989–97.

309. Dantzer R. Cytokine-Induced Sickness Behavior: Where Do We Stand? Brain Behav Immun. 2001 Mar 1;15(1):7–24.

310. Galindo F, Broom DM. The relationships between social behaviour of dairy cows and the occurrence of lameness in three herds. Res Vet Sci. 2000 Aug 1;69(1):75–9.

311. Tadich N, Flor E, Green L. Associations between hoof lesions and locomotion score in 1098 unsound dairy cows. Vet J. 2010 Apr 1;184(1):60–5.

312. Archer SC, Green MJ, Huxley JN. Association between milk yield and serial locomotion score assessments in UK dairy cows. J Dairy Sci. 2010 Sep 1;93(9):4045–53.

313. Groenevelt M, Main DCJ, Tisdall D, Knowles TG, Bell NJ. Measuring the response to therapeutic foot trimming in dairy cows with fortnightly lameness scoring. Vet J. 2014 Sep 1;201(3):283–8.

314. Boyland NK, James R, Mlynski DT, Madden JR, Croft DP. Spatial proximity loggers for recording animal social networks: consequences of inter-logger variation in performance. Behav Ecol Sociobiol. 2013 Nov 1;67(11):1877–90.

315. Harris NR, Johnson DE, McDougald NK, George MR. Social Associations and Dominance of Individuals in Small Herds of Cattle. Rangel Ecol Manag. 2007 Jul 1;60(4):339–49.

316. Gutmann AK, Špinka M, Winckler C. Do familiar group mates facilitate integration into the milking group after calving in dairy cows? Appl Anim Behav Sci. 2020 Aug 1;229:105033.

317. Managing Cow Lactation Cycles [Internet]. The Cattle Site. [cited 2020 Jul 14]. Available from: http://www.thecattlesite.com/articles/4248/managing-cow-lactation-cycles/

318. Croft DP, Madden JR, Franks DW, James R. Hypothesis testing in animal social networks. Trends Ecol Evol. 2011 Oct 1;26(10):502–7.

319. de Vries M, Bokkers EAM, van Reenen CG, Engel B, van Schaik G, Dijkstra T, et al. Housing and management factors associated with indicators of dairy cattle welfare. Prev Vet Med. 2015 Jan 1;118(1):80–92.

320. Millet S, Moons CP, Van Oeckel MJ, Janssens GP. Welfare, performance and meat quality of fattening pigs in alternative housing and management systems: a review. J Sci Food Agric. 2005 Feb 8;85(5):709–19.

321. Sevi A, Casamassima D, Pulina G, Pazzona A. Factors of welfare reduction in dairy sheep and goats. Ital J Anim Sci. 2009 Jan 1;8(sup1):81–101.

322. Rault JL. Friends with benefits: Social support and its relevance for farm animal welfare. Appl Anim Behav Sci. 2012 Jan 15;136(1):1–14.

323. DeVries TJ, von Keyserlingk MAG, Weary DM. Effect of Feeding Space on the Inter-Cow Distance, Aggression, and Feeding Behavior of Free-Stall Housed Lactating Dairy Cows. J Dairy Sci. 2004 May 1;87(5):1432–8.

324. Krawczel PD, Klaiber LB, Butzler RE, Klaiber LM, Dann HM, Mooney CS, et al. Short-term increases in stocking density affect the lying and social behavior, but not the productivity, of lactating Holstein dairy cows. J Dairy Sci. 2012 Aug 1;95(8):4298–308.

325. Proudfoot KL, Veira DM, Weary DM, von Keyserlingk MAG. Competition at the feed bunk changes the feeding, standing, and social behavior of transition dairy cows. Journal of Dairy Science. 2009;92(7):3116–23.

326. Collings LKM, Weary DM, Chapinal N, von Keyserlingk MAG. Temporal feed restriction and overstocking increase competition for feed by dairy cattle. J Dairy Sci. 2011 Nov 1;94(11):5480–6.

327. Herskin MS, Munksgaard L, Andersen JB. Effects of social isolation and restraint on adrenocortical responses and hypoalgesia in loose-housed dairy cows1. J Anim Sci. 2007 Jan 1;85(1):240–7.

328. Rushen J, Boissy A, Terlouw EMC, de Passillé AMB. Opioid peptides and behavioral and physiological responses of dairy cows to social isolation in unfamiliar surroundings. J Anim Sci. 1999 Nov 1;77(11):2918–24.

329. Walker JK, Arney DR, Waran NK, Handel IG, Phillips CJC. The effect of conspecific removal on behavioral and physiological responses of dairy cattle. J Dairy Sci. 2015 Dec 1;98(12):8610–22.

330. Veissier I, Boissy A, Passille AM de, Rushen J, Reenen CG van, Roussel S, et al. Calves' responses to repeated social regrouping and relocation. J Anim Sci. 2001;79:2580–93.

331. Proudfoot KL, Weary DM, LeBlanc SJ, Mamedova LK, von Keyserlingk MAG. Exposure to an unpredictable and competitive social environment affects behavior and health of transition dairy cows. J Dairy Sci. 2018 Oct 1;101(10):9309–20.

332. Proudfoot K, Habing G. Social stress as a cause of diseases in farm animals: Current knowledge and future directions. Vet J. 2015 Oct 1;206(1):15–21.

333. Bolt SL, Boyland NK, Mlynski DT, James R, Croft DP. Pair Housing of Dairy Calves and Age at Pairing: Effects on Weaning Stress, Health, Production and Social Networks. PLOS ONE. 2017 Jan 4;12(1):e0166926.

334. Dubé C, RIBBLE CS, Kelton D, McNab B. Inroduction to network analysis and its implications for animal disease modelling. Rev Sci Tech Int Off Epizoot. 2011 Aug 1;30:425–36.

335. Veit C, Foister S, Valros A, Munsterhjelm C, Sandercock DA, Janczak AM, et al. The use of social network analysis to describe the effect of immune activation on group dynamics in pigs. Animal. 2021 Sep 1;15(9):100332.

336. Liddell C, Morgan ER, Bull K, Ioannou CC. Response to resources and parasites depends on health status in extensively grazed sheep. Proc R Soc B Biol Sci. 2020 Feb 12;287(1920):20192905.

337. Faust K. Comparing social networks: Size, density, and local structure. Adv Methodol Stat. 2006 Jul 1;3(2):185–216.

338. Wijk BCM van, Stam CJ, Daffertshofer A. Comparing Brain Networks of Different Size and Connectivity Density Using Graph Theory. PLOS ONE. 2010 Oct 28;5(10):e13701.

339. Foris B, Haas HG, Langbein J, Melzer N. Familiarity influences social networks in dairy cows after regrouping. J Dairy Sci. 2021 Mar 1;104(3):3485–94.

340. Edenbrow M, Croft DP. Kin and familiarity influence association preferences and aggression in the mangrove killifish Kryptolebias marmoratus. J Fish Biol. 2012 Mar;80(3):503–18.

341. Wikberg EC, Ting N, Sicotte P. Familiarity is more important than phenotypic similarity in shaping social relationships in a facultative female dispersed primate, Colobus vellerosus. Behav Processes. 2014 Jul 1;106:27–35.

342. Gomes ACR, Beltrão P, Boogert NJ, Cardoso GC. Familiarity, dominance, sex and season shape common waxbill social networks. Behav Ecol. 2022 May 1;33(3):526–40.

343. Atton N, Galef BJ, Hoppitt W, Webster MM, Laland KN. Familiarity affects social network structure and discovery of prey patch locations in foraging stickleback shoals. Proc R Soc B Biol Sci. 2014 Aug 22;281(1789):20140579.

344. Natzke RP, Bray DR, Everett RW. Cow Preference for Free Stall Surface Material. J Dairy Sci. 1982 Jan 1;65(1):146–53.

345. Rathore AK. Order of cow entry at milking and its relationships with milk yield and consistency of the order. Appl Anim Ethol. 1982 Jan 1;8(1):45–52.

346. Osei-Amponsah R, Dunshea FR, Leury BJ, Cheng L, Cullen B, Joy A, et al. Heat Stress Impacts on Lactating Cows Grazing Australian Summer Pastures on an Automatic Robotic Dairy. Animals. 2020 May 1;10(5):E869.

347. Churakov M, Silvera AM, Gussmann M, Nielsen PP. Parity and days in milk affect cubicle occupancy in dairy cows. Appl Anim Behav Sci. 2021 Nov 1;244:105494.

348. Soffié M, Thinès G, De Marneffe G. Relation between milking order and dominance value in a group of dairy cows. Appl Anim Ethol. 1976 Aug 1;2(3):271–6.

349. Polikarpus A, Kaart T, Mootse H, De Rosa G, Arney D. Influences of various factors on cows' entrance order into the milking parlour. Appl Anim Behav Sci. 2015 May 1;166:20–4.

350. Ray DE, Roubicek CB. Behavior of feedlot cattle during two seasons. J Anim Sci. 1971 Jul 1;33(1):72–6.

351. Andersen IL, Bøe KE, Hove K. Behavioural and physiolgical thermoregulation in groups of pregnant sows housed in a kennel system at low temperatures. Can J Anim Sci. 2000 Mar;80(1):1–8.

352. Bøe K. Thermoregulatory behaviour of sheep housed in insulated and uninsulated buildings. Appl Anim Behav Sci. 1990 Sep 1;27(3):243–52.

353. Ozella L, Langford J, Gauvin L, Price E, Cattuto C, Croft DP. The effect of age, environment and management on social contact patterns in sheep. Appl Anim Behav Sci. 2020 Apr 1;225:104964.

354. Conrad L, Aubé L, Heuchan E, Conte S, Bergeron R, Devillers N. Effects of farrowing hut design on maternal and thermoregulatory behaviour in outdoor housed sows and piglets. Appl Anim Behav Sci. 2022 Jun 1;251:105616.

355. Javorová J, Velecká M, Falta D, Večeřa M, Andrýsek J, Studený S, et al. The Effect of Air Temperature and Time of Day on Distribution of Czech Fleckvieh Cows Within the Barn. Acta Univ Agric Silvic Mendel Brun. 2014 Feb 1;62(1):117–24.

356. Giro A, Pezzopane JRM, Barioni Junior W, Pedroso A de F, Lemes AP, Botta D, et al. Behavior and body surface temperature of beef cattle in integrated crop-livestock systems with or without tree shading. Sci Total Environ. 2019 Sep 20;684:587–96.

357. Kendall PE, Nielsen PP, Webster JR, Verkerk GA, Littlejohn RP, Matthews LR. The effects of providing shade to lactating dairy cows in a temperate climate. Livest Sci. 2006 Aug 1;103(1):148–57.

358. Tucker CB, Rogers AR, Schütz KE. Effect of solar radiation on dairy cattle behaviour, use of shade and body temperature in a pasture-based system. Appl Anim Behav Sci. 2008 Feb 1;109(2):141–54.

359. Schütz KE, Cox NR, Matthews LR. How important is shade to dairy cattle? Choice between shade or lying following different levels of lying deprivation. Appl Anim Behav Sci. 2008 Dec 1;114(3):307–18.

360. Schmidtmann ET, Valla ME. Face-fly pest intensity, fly-avoidance behavior (bunching) and grazing time in Holstein heifers. Appl Anim Ethol. 1982 Jun 1;8(5):429–38.

361. Schmidtmann ET, Berkebile DR. Relationship Between Numbers of Face Flies (Diptera: Muscidae) and Nearest-neighbor Distance Among Holstein Cows. J Econ Entomol. 1985 Feb 1;78(1):102–5.

362. Gerry AC, Peterson NG, Mullens BA. Predicting and Controlling Stable Flies on California Dairies. 2007.

363. Wieman GA, Campbell JB, Deshazer JA, Berry IL. Effects of Stable Flies (Diptera: Muscidae) and Heat Stress on Weight Gain and Feed Efficiency of Feeder Cattle. J Econ Entomol. 1992 Oct 1;85(5):1835–42.

364. Foster WA, Treherne JE. Evidence for the dilution effect in the selfish herd from fish predation on a marine insect. Nature. 1981 Oct;293(5832):466–7.

365. Bowen MT, Kevin RC, May M, Staples LG, Hunt GE, McGregor IS. Defensive Aggregation (Huddling) in Rattus Norvegicus toward Predator Odor: Individual Differences, Social Buffering Effects and Neural Correlates. PLOS ONE. 2013 Jul 29;8(7):e68483.

366. Torr SJ, Prior A, Wilson PJ, Schofield S. Is there safety in numbers? The effect of cattle herding on biting risk from tsetse flies. Med Vet Entomol. 2007;21(4):301–11.

367. Dikmen S, Hansen PJ. Is the temperature-humidity index the best indicator of heat stress in lactating dairy cows in a subtropical environment? J Dairy Sci. 2009 Jan 1;92(1):109–16.

368. Chen JM, Schütz KE, Tucker CB. Cooling cows efficiently with water spray: Behavioral, physiological, and production responses to sprinklers at the feed bunk. J Dairy Sci. 2016 Jun 1;99(6):4607–18.

369. Tresoldi G, Schütz KE, Tucker CB. Cooling cows with sprinklers: Effects of soaker flow rate and timing on behavioral and physiological responses to heat load and production. J Dairy Sci. 2019 Jan 1;102(1):528–38.

370. King MTM, Pajor EA, LeBlanc SJ, DeVries TJ. Associations of herd-level housing, management, and lameness prevalence with productivity and cow behavior in herds with automated milking systems. J Dairy Sci. 2016 Nov;99(11):9069–79.

371. Grandin T. Evaluation of the welfare of cattle housed in outdoor feedlot pens. Vet Anim Sci. 2016 Dec 1;1–2:23–8.

372. Tsai YC, Hsu JT, Ding ST, Rustia DJ, Lin TT. Assessment of dairy cow heat stress by monitoring drinking behaviour using an embedded imaging system. Biosyst Eng. 2020 Apr 1;199.

373. Schüller LK, Burfeind O, Heuwieser W. Impact of heat stress on conception rate of dairy cows in the moderate climate considering different temperature–humidity index

thresholds, periods relative to breeding, and heat load indices. Theriogenology. 2014 May 1;81(8):1050–7.

374. Sammad A, Umer S, Shi R, Zhu H, Zhao X, Wang Y. Dairy cow reproduction under the influence of heat stress. J Anim Physiol Anim Nutr. 2020;104(4):978–86.

375. Brouček J, Novák P, Vokřálová J, Šoch M, Kišac P, Uhrinčať M. Effect of high temperature on milk production of cows from free-stall housing with natural ventilation. Slovak J Anim Sci. 2009 Dec 31;42(4):167–73.

376. Tao S, Orellana Rivas RM, Marins TN, Chen YC, Gao J, Bernard JK. Impact of heat stress on lactational performance of dairy cows. Theriogenology. 2020 Jul 1;150:437–44.

377. Ominski KH, Kennedy AD, Wittenberg KM, Moshtaghi Nia SA. Physiological and Production Responses to Feeding Schedule in Lactating Dairy Cows Exposed to Short-Term, Moderate Heat Stress. J Dairy Sci. 2002 Apr 1;85(4):730–7.

378. Wildridge AM, Thomson PC, Garcia SC, John AJ, Jongman EC, Clark CEF, et al. Short communication: The effect of temperature-humidity index on milk yield and milking frequency of dairy cows in pasture-based automatic milking systems. J Dairy Sci. 2018 May 1;101(5):4479–82.

379. Yan G, Liu K, Hao Z, Shi Z, Li H. The effects of cow-related factors on rectal temperature, respiration rate, and temperature-humidity index thresholds for lactating cows exposed to heat stress. J Therm Biol. 2021 Aug 1;100:103041.

380. Pinto S, Hoffmann G, Ammon C, Amon T. Critical THI thresholds based on the physiological parameters of lactating dairy cows. J Therm Biol. 2020 Feb 1;88:102523.

381. Zhou M, Aarnink AJA, Huynh TTT, van Dixhoorn IDE, Groot Koerkamp PWG. Effects of increasing air temperature on physiological and productive responses of dairy cows at different relative humidity and air velocity levels. J Dairy Sci. 2022 Feb 1;105(2):1701–16.

382. Li G, Chen S, Chen J, Peng D, Gu X. Predicting rectal temperature and respiration rate responses in lactating dairy cows exposed to heat stress. J Dairy Sci. 2020 Jun;103(6):5466–84.

383. Hahn G, Parkhurst A, Gaughan J. Cattle Respiration Rate as a function of Ambient Temperature. ASAE Pap NMC97. 1997;121.

384. Berman A, Folman Y, Kaim M, Mamen M, Herz Z, Wolfenson D, et al. Upper Critical Temperatures and Forced Ventilation Effects for High-Yielding Dairy Cows in a Subtropical Climate. J Dairy Sci. 1985 Jun 1;68(6):1488–95.

385. Dalcin VC, Fischer V, Daltro D dos S, Alfonzo EPM, Stumpf MT, Kolling GJ, et al. Physiological parameters for thermal stress in dairy cattle. Rev Bras Zootec. 2016 Aug;45:458–65.

386. Morton JM, Tranter WP, Mayer DG, Jonsson NN. Effects of Environmental Heat on Conception Rates in Lactating Dairy Cows: Critical Periods of Exposure. J Dairy Sci. 2007 May 1;90(5):2271–8.

387. Linvill DE, Pardue FE. Heat stress and milk production in the South Carolina coastal plains. J Dairy Sci. 1992 Sep;75(9):2598–604.

388. Gantner V, Mijić P, Kuterovac K, Solić D, Gantner R. Temperature-humidity index values and their significance on the daily production of dairy cattle. Mljekarstvo Časopis Za Unaprjeđenje Proizv Prerade Mlijeka. 2011 Mar 15;61(1):56–63.

389. Zimbelman R, Rhoads R, Rhoads M, Duff G, Baumgard L, Collier R. A Re-evaluation of the Impact of Temperature Humidity Index (THI) and Black Globe Humidity Index (BGHI) on Milk Production in High Producing Dairy Cows. Proc 24th Annu Southwest Nutr Manag Conf. 2009 Jan 1;

390. Gauly M, Bollwein H, Breves G, Brügemann K, Dänicke S, Daş G, et al. Future consequences and challenges for dairy cow production systems arising from climate change in Central Europe – a review. Animal. 2013 Jan 1;7(5):843–59.

391. Ekine-Dzivenu CC, Mrode R, Oyieng E, Komwihangilo D, Lyatuu E, Msuta G, et al. Evaluating the impact of heat stress as measured by temperature-humidity index (THI) on test-day milk yield of small holder dairy cattle in a sub-Sahara African climate. Livest Sci. 2020 Dec 1;242:104314.

392. Li S, Gebremedhin KG, Lee C, Collier R. Evaluation of Thermal Stress Indices for Cattle. In Reno, Nevada: American Society of Agricultural and Biological Engineers; 2009. p. 1.

393. Thom EC. The Discomfort Index. Weatherwise. 1959 Apr 1;12(2):57–61.

394. Mader TL, Davis MS, Brown-Brandl T. Environmental factors influencing heat stress in feedlot cattle1,2. J Anim Sci. 2006 Apr 1;84(3):712–9.

395. Berry IL. Dairy shelter design based on milk production decline as affected by temperature and humidity. Trans ASAE. 1964;7(3):329–31.

396. Hill DL, Wall E. Dairy cattle in a temperate climate: the effects of weather on milk yield and composition depend on management. Animal. 2015 Jan 1;9(1):138–49.

397. Data Logger | Gemini Data Loggers UK manufactures of Tinytag [Internet]. geminidataloggers.com. [cited 2022 Jul 26]. Available from: https://www.geminidataloggers.com/

398. Muggeo VMR. Estimating regression models with unknown break-points. Stat Med. 2003 Oct 15;22(19):3055–71.

399. Muggeo VMR. segmented: An R package to Fit Regression Models with Broken-Line Relationships. R NEWS. 2008;8/1:20–5.

400. Blackie N, Amory J, Bleach E, Scaife J. The effect of lameness on lying behaviour of zero grazed Holstein dairy cattle. Appl Anim Behav Sci. 2011 Nov 15;134(3):85–91.

401. Hanlon HM, Bernie D, Carigi G, Lowe JA. Future changes to high impact weather in the UK. Clim Change. 2021 Jun 30;166(3):50.

402. Nonaka I, Takusari N, Tajima K, Suzuki T, Higuchi K, Kurihara M. Effects of high environmental temperatures on physiological and nutritional status of prepubertal Holstein heifers. Livest Sci. 2008 Jan 1;113(1):14–23.

403. Bun C, Watanabe Y, Uenoyama Y, Inoue N, Ieda N, Matsuda F, et al. Evaluation of heat stress response in crossbred dairy cows under tropical climate by analysis of heart rate variability. J Vet Med Sci. 2018 Feb 2;80(1):181–5.

404. Anderson SD, Bradford BJ, Harner JP, Tucker CB, Choi CY, Allen JD, et al. Effects of adjustable and stationary fans with misters on core body temperature and lying behavior of lactating dairy cows in a semiarid climate. J Dairy Sci. 2013 Jul;96(7):4738–50.

405. Spiers DE. How cows dissipate heat. In 2003. p. 77.

406. Weather Underground. Stanford Rivers, FORECAST FOR STANFORD RIVERS, GB [Internet]. 2014 [cited 2022 Jul 11]. Available from: https://www.wunderground.com/dashboard/pws/ISTANFOR2/table/2014-10-6/2014-10-6/daily

407. Weather Underground. Harold Hill, FORECAST FOR ROMFORD, GB [Internet]. 2014 [cited 2022 Sep 22]. Available from: https://www.wunderground.com/dashboard/pws/IROMFORD2

408. Kibler HH (Hudson H). Environmental physiology and shelter engineering with special reference to domestic animals. LXVII, Thermal effects of various temperature-humidity combinations on Holstein cattle as measured by eight physiological responses. MIZZOU. 1964;1–40.

409. Meyer U, Everinghoff M, Gädeken D, Flachowsky G. Investigations on the water intake of lactating dairy cows. Livest Prod Sci. 2004 Nov 1;90(2):117–21.

410. Clarke A. Principles of Thermal Ecology: Temperature, Energy and Life. Oxford University Press; 2017. 497 p.

411. Atkins IK, Cook NB, Mondaca MR, Choi CY. Continuous Respiration Rate Measurement of Heat-Stressed Dairy Cows and Relation to Environment, Body Temperature, and Lying Time. Trans ASABE. 2018;61(5):1475–85.

412. Stockdale CR, King KR. A note on some of the factors that affect the water consumption of lactating dairy cows at pasture. Anim Sci. 1983 Apr;36(2):303–6.

413. Murphy MR, Davis CL, McCoy GC. Factors Affecting Water Consumption by Holstein Cows in Early Lactation1. J Dairy Sci. 1983 Jan 1;66(1):35–8.

414. Economides S. Factors affecting water consumption of dry pregant and lactating cows in a mediterranean environment. 1998 Oct;(190):11.

415. Castle ME, Thomas TP. The water intake of British Friesian cows on rations containing various forages. Anim Sci. 1975 Apr;20(2):181–9.

416. Little W, Shaw SR. A note on the individuality of the intake of drinking water by dairy cows. Anim Sci. 1978 Apr;26(2):225–7.

417. Becker CA, Stone AE. Graduate Student Literature Review: Heat abatement strategies used to reduce negative effects of heat stress in dairy cows. J Dairy Sci. 2020 Oct 1;103(10):9667–75.

418. Jensen MB. Adaptation to tethering in yearling dairy heifers assessed by the use of lying down behaviour. Appl Anim Behav Sci. 1999 Feb 15;62(2):115–23.

419. Haley DB, Rushen J, Passillé AM de. Behavioural indicators of cow comfort: activity and resting behaviour of dairy cows in two types of housing. Can J Anim Sci. 2000 Jun;80(2):257–63.

420. Popescu S, Borda C, Diugan EA, Niculae M, Stefan R, Sandru CD. The Effect of the Housing System on the Welfare Quality of Dairy Cows. Ital J Anim Sci. 2014 Jan 1;13(1):2940.

421. Shepley E, Obinu G, Bruneau T, Vasseur E. Housing tiestall dairy cows in deep-bedded pens during an 8-week dry period: Effects on lying time, lying postures, and rising and lying-down behaviors. J Dairy Sci. 2019 Jul 1;102(7):6508–17.

422. Beaver A, Weary DM, von Keyserlingk MAG. Invited review: The welfare of dairy cattle housed in tiestalls compared to less-restrictive housing types: A systematic review. J Dairy Sci. 2021 Sep 1;104(9):9383–417.

423. Wilson AM, Wright TC, Cant JP, Osborne VR. Development of a novel stall design for dairy cattle: Part I. The effect of an increased slope on lying behavior, rumination, cleanliness, and preference. Animal. 2022 Jan 1;16(1):100427.

424. Tucker CB, Weary DM, Fraser D. Free-Stall Dimensions: Effects on Preference and Stall Usage. J Dairy Sci. 2004 May 1;87(5):1208–16.

425. Crohn CC. Behaviour of dairy cows kept in extensive (loose housing/pasture) or intensive (tie stall) environments: III. Grooming, exploration and abnormal behaviour. Appl Anim Behav Sci. 1994;42:73–86.

426. Burt S, Kendon M. December 2015 – an exceptionally mild month in the United Kingdom. Weather. 2016 Dec 4;71(12):314–20.

427. Toledo IM, Dahl GE, De Vries A. Dairy cattle management and housing for warm environments. Livest Sci. 2022 Jan 1;255:104802.

428. Meckbach C, Elsholz S, Siede C, Traulsen I. An Information-Theoretic Approach to Detect the Associations of GPS-Tracked Heifers in Pasture. Sensors. 2021 Jan;21(22):7585.

429. Fielding HR, Silk MJ, McKinley TJ, Delahay RJ, Wilson-Aggarwal JK, Gauvin L, et al. Spatial and temporal variation in proximity networks of commercial dairy cattle in Great Britain. Prev Vet Med. 2021 Sep 1;194:105443.

430. Koene P, Ipema B. Social Networks and Welfare in Future Animal Management. Animals. 2014 Mar;4(1):93–118.

431. Fagen RM, Mankovich NJ. Two-act transitions, partitioned contingency tables, and the ‘significant cells’ problem. Anim Behav. 1980 Nov 1;28(4):1017–23.

432. Größbacher V, Bučková K, Lawrence AB, Špinka M, Winckler C. Discriminating spontaneous locomotor play of dairy calves using accelerometers. J Dairy Sci. 2020 Feb 1;103(2):1866–73.

433. Sundaresan S, Fischhoff I, Dushoff J, Rubenstein D. Network metrics reveal differences in social organization between two fission-fusion species, Grevy’s zebra and onager. Oecologia. 2007 Mar 1;151:140–9.

434. Whitehead H, Bejder L, Ottensmeyer CA. Testing association patterns: issues arising and extensions. Anim Behav. 2005 May 1;69(5):e1–6.

435. O’Reilly LM, Daborn CJ. The epidemiology of Mycobacterium bovis infections in animals and man: A review. Tuber Lung Dis. 1995 Aug 1;76:1–46.

436. Xiong Y, Meng QS, Gao J, Tang XF, Zhang HF. Effects of relative humidity on animal health and welfare. J Integr Agric. 2017 Aug 1;16(8):1653–8.

437. Banhazi TM, Lehr H, Black JL, Crabtree H, Schofield P, Tscharke M, et al. Precision Livestock Farming: An international review of scientific and commercial aspects. Int J Agric Biol Eng. 2012 Sep 22;5(3):1–9.

438. Rojo-Gimeno C, van der Voort M, Niemi JK, Lauwers L, Kristensen AR, Wauters E. Assessment of the value of information of precision livestock farming: A conceptual framework. NJAS - Wagening J Life Sci. 2019 Dec 1;90–91:100311.

439. Borchers MR, Bewley JM. An assessment of producer precision dairy farming technology use, prepurchase considerations, and usefulness. J Dairy Sci. 2015 Jun 1;98(6):4198–205.

440. Bianchi MC, Bava L, Sandrucci A, Tangorra FM, Tamburini A, Gislon G, et al. Diffusion of precision livestock farming technologies in dairy cattle farms. animal. 2022 Nov 1;16(11):100650.

441. Hartung J, Banhazi T, Vranken E, Guarino M. European farmers' experiences with precision livestock farming systems. Anim Front. 2017 Jan 1;7(1):38–44.

442. Schewe RL, Stuart D. Diversity in agricultural technology adoption: How are automatic milking systems used and to what end? Agric Hum Values. 2015 Jun 1;32(2):199–213.

443. Hogeveen H, Buma KJ, Jorritsma R. Use and interpretation of mastitis alerts by farmers. In: Proceedings of the 6th European Conference on Precision Livestock Farming [Internet]. Leuven, Belgium; 2013. p. 313–9. Available from: https://research.wur.nl/en/publications/use-and-interpretation-of-mastitis-alerts-by-farmers

444. Abeni F, Petrera F, Galli A. A Survey of Italian Dairy Farmers' Propensity for Precision Livestock Farming Tools. Animals. 2019 May;9(5):202.

445. Pindado E, Sánchez M, Verstegen JAAM, Lans T. Searching for the entrepreneurs among new entrants in European Agriculture: the role of human and social capital. Land Use Policy. 2018 Sep 1;77:19–30.

446. Carillo F, Abeni F. An Estimate of the Effects from Precision Livestock Farming on a Productivity Index at Farm Level. Some Evidences from a Dairy Farms' Sample of Lombardy. Animals. 2020 Oct;10(10):1781.

447. Désiré C, Hostiou N. L'élevage de précision : quels changements dans l'organisation du travail et la gestion de données en élevage ? In: 4es Rencontres Nationales sur le Travail en Elevage [Internet]. 2015. Available from: https://hal.inrae.fr/hal-02740598

448. Allain C, Chanvallon A, Courties R, Billon D, Bareille NN. Technical, economic and sociological impacts of an automated estrus detection system for dairy cows. In: Precision Dairy Farming [Internet]. Leeuwarden, Netherlands: Wageningen Academic Publishers; 2016 [cited 2022 Nov 9]. p. 460-p. (Precision Dairy Farming). Available from: https://hal.inrae.fr/hal-02743241

449. Eastwood CR, Chapman DF, Paine MS. Networks of practice for co-construction of agricultural decision support systems: Case studies of precision dairy farms in Australia. Agric Syst. 2012;108(C):10–8.

450. Lovarelli D, Bacenetti J, Guarino M. A review on dairy cattle farming: Is precision livestock farming the compromise for an environmental, economic and social sustainable production? J Clean Prod. 2020 Jul 20;262:121409.

451. Halachmi I, Guarino M, Bewley J, Pastell M. Smart animal agriculture: application of real-time sensors to improve animal well-being and production. Annu Rev Anim Biosci. 2019 Feb 15;7:403–25.

452. Hofstra G, Roelofs J, Rutter SM, van Erp-van der Kooij E, de Vlieg J. Mapping Welfare: Location Determining Techniques and Their Potential for Managing Cattle Welfare—A Review. Dairy. 2022 Dec;3(4):776–88.

453. Berckmans D. Precision livestock farming technologies for welfare management in intensive livestock systems. Rev Sci Tech Int Off Epizoot. 2014 Apr;33(1):189–96.

454. Boivin X, Bensoussan S, L’hotellier N, Bignon L, Brives H, Brule A, et al. Hommes et animaux d’élevage au travail : vers une approche pluridisciplinaire des pratiques relationnelles. INRAE Prod Anim. 2012 Jun 1;25(2):159–68.

455. Lagneaux S, Servais O. De la traite robotisée au raid d’avatars. Incorporation et virtualisation. Parcours Anthropol. 2014 Sep 23;(9):73–101.

456. Isabelle A. The legitimacy of precision livestock farming [Internet] [First cycle, G2E]. [Uppsala]: Swedish University of Agricultural Sciences; 2021. Available from: https://stud.epsilon.slu.se/16970/

457. Britt JH, Cushman RA, Dechow CD, Dobson H, Humblot P, Hutjens MF, et al. Invited review: Learning from the future—A vision for dairy farms and cows in 2067. J Dairy Sci. 2018 May 1;101(5):3722–41.