An investigation of social structure in housed dairy cows

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Abstract

The changing landscape of the UK's dairy farms poses increasing challenges to farm staff in terms of monitoring individuals behaviour within increasing herds, and more intensive conditions. Failure to detect behavioural changes may be costly, both from a welfare and financial perspective, as such alterations may indicate underlying disease or other challenges with corresponding impacts on yield and animal well-being. Social behaviour may provide a useful indicator of normal animal activity, and subsequent changes with health status, particularly if automatically monitored to reduce labour. This thesis applies a local positioning system (LPS) to collect social proximities of dairy cows, to investigate the social structure of a housed herd via social network analysis, and any relationship with traits or health.

The LPS was validated by comparing sensor reported, with human observed proximities, and accurately detected proximities at lying, feeding and in direct interactions. Use of this data to construct social networks indicated a highly connected structure, with some substructure becoming evident after filters were applied. An approaching significant effect of parity on sociality was found, but stage of lactation had no effect. Temporally, the network showed some stability but a much greater amount of variation. When divided into 'functional area' (feeding, non-feeding and milking), the non-feeding area of the shed yielded the most loosely connected network with likely most interest for further analysis due to its potential basis in choice, as opposed to forced proximity. In these functional area networks, some evidence exists for homophily (association with similar cows – based on parity and days in milk). Finally, sociality was investigated alongside health status, with evidence for a tendency for greater betweenness in lame cows than non-lame. The results suggest that sociality is a highly variable trait, and that further investigation is required to assess its suitability as a disease indicator.

Publications and conference proceedings

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Common abbreviations

AHDB – Agriculture and Horticulture Development Board

EC – Dr Edd Coddling, University of Essex

ZB – Dr Zoe Barker, Writtle College

JA – Dr Jonathan Amory, Writtle College

JVD – Dr Jorge Vasquez Diasdado

Csv file – comma separated values

SNA – social network analysis

CEP – circular error of probability

DIST – distance between the ground truth, and sensor reported position

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1.1 Introduction

Dairy cattle are kept throughout the world to produce milk for human consumption. In 2013, the UK was one of the top ten countries for milk production (AHDB, 2016). An increase in global demand for dairy products, with over 6 billion consumers of such produce (FAO, 2016), has seen a comparable increase in intensification of dairy production. A trend towards larger dairy herds has occurred, comprised predominantly of Holstein Fresian breed cattle due to their high milk yields. The total number of UK dairy farms has fallen significantly – from over 21,000 farms in 2004 to 13,355 in June 2015, whilst average herd size rose from 97-142 cows in the same time period (AHDB, 2016). This altered herd structure places new challenges on the dairy industry in terms of maintaining animal health and welfare. One result is the need to hire additional labour to maintain standards of care – creating issues in protecting human and animal safety (Barkema et al., 2015). Concerns regarding animal welfare may come into play, dependent on the speed at which herd size increases, and the stockperson to cow ratio, but there is a lack of published evidence at present regarding the effect of herd size on individual welfare (Barkema et al., 2015). These pressures within the industry highlight the value of quality health and welfare indicators in cows, particularly those which can be monitored automatically, without additional human labour. This thesis will attempt to consider one such potential indicator – namely social behaviour in dairy cattle, as reported by an automated monitoring system.

1.2 The social behaviour of cattle; an overview

1.2.1 Why be social?

Across taxa and habitats, a large number of species live in social groupings, rather than as solitary individuals. The associated advantages of group living have been well described, including reduced predation risk, improved foraging chances, mate availability, along with the cost of greater competition for resource access (Silk, 2007). These broad, group impacts of being ‘one in a crowd’ are also accompanied by potential pros and cons at the individual level – i.e. the consequences of direct relationships between two animals. These include valuable benefits such as social support – the idea that social partners may act as a buffer in challenging situations (Rault, 2012), protection of kin (and therefore genes – Stricklin, 2001), and associated negative impacts including the detrimental effect of receiving aggression (Neisen et al., 2009b) .

1.2.2 Wild and feral cattle sociality

Cattle are a social species, naturally forming small groups of females with calves and transitory males; averaging 20 members (Bouissou *et al.,* 2001). Feral and wild cattle have been studied by a few authors, namely in the UK the Chillingham cattle which form a largely unmanaged group of feral animals in a walled park. Hall (1989) reported few overt social behaviours in female feral cattle, with no prolonged aggressive interactions. Hall also noted differences in social behaviour from managed herds; namely feral cattle seldom licked one another. Other authors studying free-grazing domestic cattle on a 72ha site noted small sub-groups within the herd, with animals tending to associate with those they had been raised alongside, and were therefore familiar with (Harris et al., 2007).

1.2.3 Domestic cattle social grouping

Under farmed conditions, dairy cows are kept in large management groups comprised solely of adult females. Social dynamics are therefore likely to be altered in comparison to wild or feral cattle groups (McLennan, 2013). Given that the average herd size of dairy cows in the UK is increasing – from an average of 94 cows per herd in 2002, to 145 in 2015 (AHDB, 2016), the dairy cow's social situation will be increasingly disparate from the natural herd. The all-female grouping prevalent in dairy herds is likely to impact social interactions; although males and females perform varying types and levels of social behaviour, there is a possibility that the presence of bulls supresses aspects of a female's social repertoire (Hall, 1989). Other implications of intensified cattle management include an increase in aggression, with agonistic interactions becoming more frequent at higher stocking densities (Fregonesi et al., 2007).

1.2.4 Direct social interactions in cattle

Social behaviour in cattle has been studied by several authors, noting the affiliative and agonistic behaviours in table 1. This range of interactions shows the diversity of contact, non-contact, visual, auditory and olfactory communication displayed by cattle.

Table 1: Agonistic and non-agonistic social behaviour in cattle

Agonistic interaction	**Authors**	**Non-agonistic interaction**	**Authors**
Displacement from resource/chasing-up from lying	(von Keyserlingk et al., 2008; Proudfoot et al., 2009; Val-Laillet et al., 2009) Welfare Quality® (2009)	Allogrooming (licking) to head, neck, front or back	Val-Laillet *et al.,* (2009); (Laister et al., 2011)
Replacement at resource	(Chapinal et al., 2011)	Sniffing; inquisitive and sexual	(Boissy and Le Neindre, 1997)); (Boyle et al., 2012)
Butt	Val-Laillet *et al.,* (2009); Proudfoot *et al.,* (2009)	Social grazing; in proximity/following	(Reinhardt and Reinhardt, 1981)
Nudge	Val-Laillet *et al.,* (2009)	Rubbing heads/head play	Boyle *et al.,* (2012); Raussi *et al.,* (2005)
Push	Val-Laillet *et al.,* (2009); Proudfoot *et al.,* (2009)	Investigate/nosing	Boyle *et al.,* (2012)
Kick	Val-Laillet *et al.,* (2009)	Maintaining proximity	Bouissou *et al.,* (2001);(Gibbons et al., 2010)
Avoidance/retreat	(Gibbons et al., 2009) Neisen *et al.,* (2009)	Vocalisation to seek other cattle	Bouissou *et al.,* (2001)
Penetrate feeder/ shouldering	Gibbons *et al.,* (2009)	Mounting	Raussi *et al.,* (2005)
Block	Gibbons *et al.,* (2009)		
Threat	Gibbons *et al.,* (2009)		
Fight	(Raussi et al., 2005); Gibbons *et al.,* (2009)		
Chase	Welfare Quality® (2009)		

Visual signals such as blocks and threats are likely to be the most important method of communication for cows, with vocalisations being more generally indicative of arousal level (Keeling, 2001). Up to 90% of all 'aggressive' interactions may consist of threats from one individual, followed by submission/retreat of another, over a reasonably large distance and requiring no physical contact (Bouissou *et al.,* 2001). In the farm environment, particularly experienced by dairy cattle in more intensive housing systems, it is possible the ability to express these subtler visual signals will be limited. This could be due to reduced line-of-sight encounters (with cubicles, metalwork etc. within the shed) and a higher occurrence of recognition once at close quarters. Therefore, the agonistic behaviours described below are of important concern with regards to cattle welfare.

1.3 Agonistic social behaviour

1.3.1 The negative impacts of aggression

Aggression or agonistic interactions between animals can take the form of contact, or non-contact exchanges. In some farmed species, contact aggression can be highly injurious (e.g. lesions in pigs – Turner et al., 2006, skin and plumage damage in hens – Bilcik and Keeling, 1999), with obvious accompanying welfare issues of pain, tissue damage and risk of infection. Non-contact aggression may have less obvious negative impacts, however the concept of social 'defeat' is known to act as a stressor. This received aggression and defeat can reduce weight gain, increase physiological stress indicators (increased corticosterone levels, suppressed immune function; e.g. mice – Haller et al., 1999, pigs – Ruis et al., 2001), and even lead to reduced neurogenesis – the creation of new neurons within the brain (Lieberwirth and Wang, 2012). In cattle, agonistic responses at the feeder, even if non-contact (such as threats), may lead to displacements of lower ranking cattle, in turn thwarting their desire to feed. Such displacements may also reduce feed intake and force subordinates to feed at less preferable times (Rioja-Lang et al., 2009), as well as potentially leaving lower energy food availability due to selective eating by dominant cattle (DeVries et al., 2005). Along with the welfare consequences, social stress and experienced aggression are known to reduce productivity (Grant and Albright, 2001).

1.3.2 Dominance behaviour and resource access

Dominance is considered as a consistent, directed outcome of interactions; i.e. the same individual always wins in an encounter (Drews, 1993). In cattle, it is usually assessed by considering the outcome of displacements, or other negative interactions between dyads (e.g. Šárová et al., 2013; (Rutter et al., 1987; David Val-Laillet et al., 2008). Factors affecting position within the dominance hierarchy include age (Kabuga, 1992); older cows tend to be dominant over their younger herdmates even when the younger cattle are heavier (in 73.6% of pairings investigated in one study; Šárová et al., 2013) – as it appears that physical strength is not the main mechanism to assert dominance in these cases. Other factors associated with dominance level are weight, and whether individuals possess horns (Knierim et al., 2015). Dominance hierarchies are usually linear (David Val-Laillet et al., 2008), although evidence suggests in small groups this structure may occur by chance (Appleby, 1983). Hierarchies may also contain so-called 'circular triads' and non-linear dominance relationships, with one study finding around 41% of dyads both displaced one another (Wierenga, 1990). Dominance relationships may well be stable over time (dependent on conditions within that period) – with a high proportion of identified hierarchical relationships remaining constant up to four years later (Wierenga, 1990).

If desired resources are available to more than one individual, competition may occur. When two cows come into conflict over the resource, one may 'displace' the other from the area. Displacements in feral cattle whilst grazing and eating supplementary hay are very casual; cows approach another individual who moves away (Hall, 1989). This may be due to a well-established dominance hierarchy precluding the need for an overt interaction, or the widely distributed nature of the food. By contrast, displacements in housed cows are a more violent affair, usually requiring physical contact in the form of a push or butt (e.g. von Keyserlingk et al., 2008). Restricting available access to feed in a group of dairy cows increases displacements from food (Proudfoot et al., 2009), as well as affecting the directionality of dyads; the number of displacements between a pair becomes more reciprocal (Val-Laillet et al., 2009). Providing an additional 50cm of feeding space per cow (from 50cm – 1m) brings down aggressive interactions by 57% (DeVries et al., 2004). Age or parity may play a role in this, with unrestricted conditions seeing both multi- and primi-parous cows performing a similar number of displacements, but multiparous cows significantly more frequently displacing others in feed-restricted conditions (Val-Laillet et al., 2009). In a choice test, low ranking cows are more likely to choose eating alone at a lower value feed than to challenge a dominant animal at a higher value feeder (Rioja-Lang et al., 2009). Although cows of low, medium and high social rank have been found to spend similar amounts of time at a feeder, the time of eating is correlated with rank; subordinate cows were less likely to be seen feeding in the two hours after food delivery (Val-Laillet *et al.* 2008). This body of research suggests that older, more dominant cows engage in direct, physical displacements of subordinates, along with some decisions on the part of lower ranking cows to choose a feeding location away from more dominant herd members.

Val-Laillet *et al.* (2008) studied dominance in relation to other resources along with feed; namely a lying area and a brush. They concluded that competition was highest for feed out of the three resources, with around 88% of all displacements occurring here. A key finding of this study is the varying consistency of displacement success. A significant correlation exists between competitive success at feed and in the lying area, but this does not correlate to displacement rates at the brush. Such findings suggest that when creating dominance hierarchies, the effects of resource-specific dominance should be considered. The above evidence suggests agonistic interactions may be quite common in individual herds. Val-Laillet *et al.* (2008) recorded only 6% of possible cow pairings did not interact in displacements at the feeder over the course of three days, implying a high rate of aggressive interactions. Some agonistic interactions are more consistent than others; contact aggressive behaviours are more repeatable than non-contact interactions, and whilst individuals show similar levels of aggression across situation (e.g. competitive vs open feed-face), there can be high within-individual variation (Gibbons et al., 2009).

1.3.3 Mixing and re-grouping

The remixing of cattle is a common practice in the UK dairy industry (von Keyserlingk *et al.,* 2008), based on milk yield and stage of pregnancy (Gutmann et al., 2015). This inevitably leads to social disruption and alterations to the structure and hierarchy of the herd. The introduction of cows to an existing, stable group alters the introduced animal's behaviour; including feeding and lying, as well as significantly reducing milk production (von Keyserlingk *et al.,* 2008; Torres-Cardona et al., 2014), and some authors report as little as 4 hours lying in the 24 hours after mixing (Boyle et al., 2012). Activity levels (measured as steps) increase on the day of introduction, further indication of disruption (Torres-Cardona *et al.,* 2014). Direct social interactions are also affected, with introduced cows experiencing more displacements from feed and displacing less cows from lying than in their pre-mix group. Finally, focal cows perform less social grooming in the new group. When directly comparing stable, and traditionally managed (i.e. weekly introductions into the herd) dry cow groups, similar issues are found.

The length of time required for familiarisation of group members, and establishment of a stable dominance hierarchy, may vary. Some work suggests that pairs of introduced cattle are not completely familiar with one another by 5 days post-mixing, as there are differences in their behaviour compared to that of established pairs (Patison et al., 2010). Given the logistics of interacting with multiple other individuals, it is likely this period of familiarisation is increased in a herd setting, meaning cows may take longer to 'settle' into larger groups. Indeed, Gygax *et al.* (2009) noted variance from normal space use still present at 6 days post-mixing. Authors have suggested a range of lengths for the duration of negative social effects of mixing/re-grouping cattle. These include between 3 and 7 days in the majority of cases (Grant and Albright, 2001), or 5-15 days (Bøe and Færevik, 2003). This variance suggests the influence of study conditions – for example housing and group size, which ranged across the aforementioned studies from two cattle at pasture, to groups of 44 in cubicle sheds.

The management of a re-grouping event may affect its impact on the cattle involved. Time of day is one such management factor, with effects evident introducing young cows into an established group (Boyle *et al.,* 2012). Cows were moved into a group after morning (6-8 AM), versus evening (4-6 PM) milking. Those moved in the AM experienced more agonistic behaviour from other cows directly after re-grouping than PM cattle, and received more butts during feeding. Hunger may have played a role in the effect due to the varying 'time since feed' at both introduction points (Boyle *et al.,* 2012). The location of the mixing event may accentuate or alleviate the negative consequences. Cattle experiencing the addition of new group members in their home environment fare better than those in new environments – with the former suffering no reduced feed intake, and lower displacement rates than the latter (Schirmann et al., 2011). Space availability per cow also plays a part in the extent of aggressive behaviour, with lower stocking densities resulting in reduced displacements (and greater lying times) than higher densities (Talebi et al., 2014). The choice of composition of the mixed group may determine some aspect of the effect, with groups comprised of recently mixed multiparous cows exhibiting more aggressive behaviour than a regrouped uniparous herd (Phillips and Rind, 2001).

Another way to alleviate the effect of group introductions may be pairing or grouping the animals to be introduced. Whilst evidence suggests that adding either a single, or pair of new heifers to an existing herd of cows has little effect on the pre-existing group (in terms of space use), there is a greater effect on the new animals. Heifers introduced in either pairs or singly spend more time in 'activity' areas than control cows prior to introduction, suggesting higher levels of standing/walking and consequently reduced lying and feeding (Gygax et al., 2009). This is liable to have welfare consequences for the introduced cattle due to inability to rest and eat. However, heifers introduced in pairs achieve higher proportions of time in the lying area, perhaps allowing greater resting times and improving welfare compared to single introductions.

During the early days of introduction, heifers are more mobile than established cows; as measured by path length. This may suggest avoidance of other cattle and an inability to settle, another indicator of reduced welfare. In line with the space use results, heifers in pairs appear to reach normal movement levels sooner than single animals (Gygax *et al.,* 2009). Altered behaviour is also found amongst the existing herd members in the case of introductions to established herds, reflecting disturbance to their usual time budget. It may be relevant therefore to consider the welfare impacts on both the new and existing herd members.

All the above work indicates that mixing and re-grouping cattle is a complex social process, influenced by a broad range of factors – both individual and environmental. The benefit of such knowledge is that with careful consideration, the optimum method for group changes can be selected to minimise welfare and production effects on the cows involved.

1.3.4 Separation/isolation

Social isolation or deprivation is known to be a stressor in gregarious species such as cattle (Boissy and le Neindre, 1997), but may occur within the dairy environment – for example when cows are ill, or taken out of the herd for insemination/other management procedures. The removal of individuals from their herd results in changes to behaviour, and physiological measures. Stress behaviours displayed during the event include struggling when restrained in a crush (Boissy and le Neindre, 1997), vocalising, increased activity (locomotion; Færevik et al., 2006), frequent change in behavioural state (Munksgaard and Simonsen, 1996) and increased excretion (defecation/urination; Rushen et al., 1999). Physiologically, heart rate increases in totally isolated cattle, or those separated from the herd with a non-preferred conspecific (Rushen *et al.,* 1999; McClennan, 2013). Cows have also shown a tendency towards hypoalgesia (reduced response to pain) after isolation (Herskin et al., 2007). Cortisol (a hormone indicative of stress) also rises significantly during acute isolation in some cases (Boissy and le Neindre, 1997; Rushen *et al.,* 1999; Herskin *et al.,* 2007). Whilst some studies have noted no changes in cortisol levels after a separation event, this was suggested to be down to methodological issues (McClennan, 2013). The severe behavioural and physiological reactions of cattle to social isolation suggest that this is a major stressor, and should be avoided where possible within the dairy environment.

Several factors can be used to predict or attenuate the stress of social isolation. Cattle which engage in higher levels of social contact within the herd are more susceptible to distress when removed from the herd (Boissy and Neindre, 1997), and may therefore require more careful management. Other authors have shown the value of partial vs full isolation; namely providing one social partner if removal from the whole group is necessary (in calves; Færevik *et al.,* 2006). Individuals were markedly less vocal and moved around the pen more, as well as performing more exploratory behaviour in the presence of another calf compared to total isolation. Along with familiarity, the number of companions is relevant in diminishing fear responses to acute stressors (Takeda et al., 2003), with membership of a group of five leading to better coping than cattle in pairs.

1.4 Affiliative social behaviour

1.4.1 The value of positive interactions

A great deal of emphasis has been placed on aggressive interactions in cattle, partly due to the potential negative effects of these behaviours (Reinhardt and Reinhardt, 1981). However, the growing interest in ‘positive welfare’ indicators is highlighting the value of behaviours such as affiliative sociality in farmed animals (Abeyesinghe et al., 2013). Positive social interactions or affiliation amongst cattle are highly important, forming part of the notion of ‘social support’ in group animals. Social support is proving important in some species for improved recovery from stressors, and even a better immune system response to challenge (Rault, 2012), with more detail below.

1.4.2 Allogrooming

Allogrooming or social grooming is a measure of affiliation in adult animals (Val-Lalliet *et al.,* 2009). It may also serve as an indicator of preferred partners or “friends” in cattle; it is correlated with proximity at feed, and not all cows groom one another, possibly indicative of choice in interactions (Val-Lalliet *et al.,* 2009). There is ambiguity as to whether social grooming is a reciprocal behaviour, or potentially related to dominance. Some studies report non-existent or weak links between directionality/duration of social grooming and position in the dominance hierarchy (e.g. Val-Lalliet *et al.,* 2009), while other authors show low-ranking cows performing most allogrooming (Reinhardt et al., 1986). It is likely that study conditions such as group size and management system which affect social hierarchy influence the above findings, and whether individual herds show a dominance effect in allogrooming.

Suggestion that allogrooming can alleviate social tensions (Boissy *et al.*, 2007), improve group cohesion and strengthen bonds (Laister et al., 2011) highlights the importance of this behaviour in sociality and overall welfare. Other implications for welfare include the finding that cows receiving social licking experience had reduced heart rates regardless of the body area licking was directed to. Interestingly, performers of social licking experience the opposite effect, with raised heart rates, particularly when directing licking to the head or neck of another animal (Laister *et al.,* 2011). This potentially supports the hypothesis of subordinate cows allogrooming more dominant individuals; as an appeasement behaviour, this could be a stressful situation for the performer.

The location (within the environment) of allogrooming may vary dependent on system and other factors. In one study, most allogrooming occurred at feed, with only a quarter of this behaviour recorded in other areas (alleys/cubicles; Val-Lalliet *et al.,* 2009). Two other sets of observations noted higher levels of social licking during standing and lying respectively (Laister *et al.,* 2011). These effects indicate the importance of observing positive social interactions across all functional areas and behaviours, to avoid bias. All types of social interactions, both affiliative and agonistic, appear to occur most often between cows that are more frequently together. This makes logical sense; cows must be in proximity to have direct contact, but interestingly this suggests that bonds are not solely ‘positive’ or ‘negative’; i.e. a cow will both allogroom and displace another individual she spends time with (Val-Lalliet *et al.,* 2009).

1.4.3 Maintenance of proximity

Alongside direct measures of social interactions, the maintenance of proximity has been suggested as a proxy measure of social bonds (e.g. Gygax et al., 2010). At pasture, cows maintain grazing distances of around 4-10m from one another, and rarely move out of sight of the herd (Fraser, 2007). Proximity to another cow at feed in more intensive conditions may also shed light on relationships; for example, primiparous cow dyads are more common at feed than a primi-/multi-parous pair (Val-Lalliet *et al.,* 2009). Proximity while feeding may be important amongst pairs of different dominance ranks; particularly maintaining a greater inter-individual distance appears to have value for subordinate cows (Rioja-Lang et al., 2009), potentially to avoid aggression. When provided with a metre feeding space per cow, inter-individual distances increased by at least 60% from 50cm feeding space suggesting that when possible, proximity is reflective of choice (DeVries *et al.,* 2004).

In further support of this theory, familiar pairs of cattle maintain closer proximity during several activities than previously unfamiliar dyads, and unacquainted cattle gradually decrease their distance from one another over time as familiarisation occurs (at pasture; Patison *et al.,* 2010). Hand in hand with this “closeness” is the increase in movement towards one another in cattle undergoing familiarisation (Patison *et al.,* 2010); presumably facilitating the kinds of direct social behaviours required to become acquainted. The time a dyad have been familiar for also predicts proximity; a lengthier relationship correlates with increased time spent in proximity (across functional areas), along with reduced aggression and increased allogrooming (Raussi *et al.,* 2010; Gutmann *et al.,* 2015). Moving a pair of known preferred associates from the main herd to another area also results in shorter neighbour distances than in a random, but familiar, pairing (McClennan, 2013). A final finding suggestive of proximity reflecting affiliative relationships is that individuals which remain close during grazing or resting, also allogroom one another more frequently (Sato et al., 1991; Reinhardt and Reinhardt, 1981; Færevik *et al.,* 2007), and that networks based on affiliative behaviours match better with proximity than those formed from aggressive interactions (Boyland *et al.,* 2015).

One approach commonly used to investigate animal sociality is the 'gambit of the group' – namely the assumption that individuals spending time in locality to one another are associating. The use of such proximity (or membership of the same group) as a proxy for association has previously been applied to several ungulate species, including equids (e.g. Tong et al., 2015), goats (Godde et al., 2015), giraffes (Carter et al., 2013) and in dairy cattle (Boyland *et al.,* 2016; Gygax *et al.,* 2010). This method has several advantages over defining relationships based on direct social behaviours. The main benefit is the ease of recording proximity compared with specific interactions (Croft et al., 2008), as there is much less detail required for recording the former.

One aspect which may affect the suitability of using proximity as an indicator of association is appropriate selection of inter-animal distance. This may be based on the species being studied, or the biological question posed (Farine, 2015). When considering proximity as a proxy for direct social exchanges, the choice of distance is made somewhat simpler; by this assumption the animals must be in reach of one another in order to perform the interaction. Based on species, length of limbs/body and other factors, this distance can reasonably be calculated. In contrast, when investigating tolerance, or non-contact interaction networks, thresholds for inter-animal distance become harder to define. However, we can again draw on knowledge of the species-specific behaviours, along with environmental factors to justify selected distances (Carter *et al.,* 2013).

Previous authors investigating the sociality of dairy cattle have set criteria in the range of 1.5 – 2.5 metres (Boyland *et al.,* 2013; Neisen et al., 2009a), 4m (Patison *et al.,* 2010) or even up to 5m (partly to account for accuracy of GPS sensors – Harris *et al.,* 2007) as a between-individual distance constituting a proximity encounter. Inter-individual distances of this size may well detect direct social interactions such as grooming, but will also report animals simply standing close together. As such, the data is only suitably applied in the assessment of tolerated proximity, not as a representation of social engagement.

1.4.4 Preferential and kin relationships

The formation of structure within social groups may be, in part, due to the existence of preferred associates. Investigations of the social structure of cattle have noted repeated associations between dyads or cliques (groups) of cows, with 'attachment' relationships more common than 'avoidance' (defined as spending more/less time together than predicted by chance – Gygax *et al.,* 2010). Some evidence exists for repeated neighbours at feeding, lying and in milking order (Cooper et al., 2008) with cows seen together more frequently than would be expected at random. Zebu cattle out at pasture may have one or more individuals that they preferentially maintain proximity to, and perform social licking with – (41% of cattle had these favoured herdmates; Reinhardt and Reinhardt, 1981). Housed cattle with and without access to pasture also appear to form preferential relationships (based on proximity; Boyland *et al.,* 2015).

Several factors appear to influence the likelihood of preferential bond formation between cattle. Familiarity may be one such factor; animals which have grown up with one another (Raussi *et al.,* 2010), or spent certain times together (e.g. dry period) have stronger attachment relationships (Gygax *et al.,* 2010). Calves selectively lay with, and groomed familiar conspecifics in certain conditions (groups of 16, but not in smaller groups; Færevik *et al.,* 2007). In adult dairy cattle, both recent and long-term familiarity (e.g. entering the herd together, and growing up together respectively) predicts both the number of interactions (aggressive and affiliative combined), as well as the likelihood of maintaining proximity (Gutmann *et al.,* 2015). Longer term familiarity is better associated with these measures, suggesting that the cattle had maintained these relationships over time.

The dominance status of members of a dyad may affect preferred neighbours, with pairs of a similar position in the hierarchy preferentially eating alongside one another. Interestingly, the same factor also predicted the cattle which exchanged agonistic behaviours most often (Kabuga, 1992), reiterating a previous suggestion that relationships are more complex than simply positive or negative. The latter study indicated individual cattle showing a preference for feeding location, with the potential confounding effect that cows would choose trough position, rather than preferred neighbours. Cattle breed has been seen to affect relationships, with some breeds seeming to preferentially form bonds/associate with, and socially groom more frequently, their own kind (Stricklin, 1983; Sato, 1984; Boyland et al., 2016). Age may also play a role in the formation of cliques, with individuals of similar age tending to group together at pasture (Harris *et al.,* 2007), and cattle of a comparable lactation number (likely to be linked to age) maintaining proximity to one another (Boyland et al., 2016). This may, however, confounded by familiarity, as in the settings of the former study, cattle in the same age group had also grown up together (Harris *et al.,* 2007). Milk production (based on yield and stage of lactation) has been seen to correlate with relationships, with cows similar in this regard being more likely to associate (Boyland *et al.,* 2016).

Kinship, or relatedness, has been suggested as one mechanism driving social relationships. Under circumstances of low predation risk, the grouping of large, social herbivores is well predicted by kinship (Durand et al., 2007). Other evidence from ungulate species also exists in the literature. Female reindeer are known to associate more commonly with female herd members which are closely related to them, than random conspecifics (Djaković et al., 2012), and a similar pattern is seen in female plains zebra (Tong *et al.,* 2015). Additionally, relatedness predicts some degree of association amongst female giraffes (Carter *et al.,* 2013). In cattle, mother-calf associations are seen in herds at pasture. In one study, 71% of mothers were seen grazing most frequently with their eldest calf, which were often now adult cattle themselves, for a third consecutive year of association (Reinhardt and Reinhardt, 1981). These mothers also often preferred their sons/daughters as licking partners for several years. Other authors present similar evidence, with mothers and daughters tending to be in proximity to one another, more frequently than at random (again at pasture; Stricklin, 1983). However, these studies were based on small herds, with space to choose who they associated with, which may not always be the case in dairy cattle due to limited space and location of resources.

1.4.5 Social support

Positive social interactions and proximity are useful from a variety of evolutionary perspectives; grooming to maintain coat, predator avoidance and increased available foraging time (Koene and Ipema, 2014). Such direct effects are of immediate and obvious benefit, but other, subtler advantages may exist. As mentioned previously, social support is a concept of beneficial sociality to improve coping ability, which has been documented in both humans and animals (Rault, 2012). The presence of conspecifics in unfamiliar situations may reduce behavioural and physiological stress responses (sheep; González et al., 2013, 2013, pigs; Ruis *et al.,* 2001) – effectively providing a “stress buffer”. Alongside attenuating present stress, a stable social group provides a better platform to cope with future stressors (Fraser et al., 2013), and may have benefits to the individual regardless of whether they are currently experiencing challenge (Rault, 2012).

Early life experience is known to be important in behavioural development, including social behaviour, in animals. Raising calves in social groups rather than isolation leads to more confident individuals in a neophobia test (Costa et al., 2014), and pair-housed calves are faster to interact with an unknown conspecific than individually housed counterparts (Jensen and Larsen, 2014). Conclusions from other authors also support the idea that pair or group housing reduces reactivity to novel situations – either social or environmental (De Paula Vieira et al., 2012), and that full social contact versus limited contact increases social behaviour expression (Duve and Jensen, 2012) – suggestive of the value of companions in normal development. Another example of the value of companions for development is in the learning of grazing behaviour. Heifers which have never been out to pasture are quicker to learn to graze, and show less agitation behaviours (e.g. stamping, vocalising) in the first few hours, when grouped with experienced cows than naïve conspecifics (Costa et al., 2016).

Social support may be valuable during stressful management events such as remixing or separation from the herd. When introducing heifers into an established herd, providing a partner may alleviate some of the social stress experienced (Neisen et al., 2009b). Compared with singly introduced heifers, pairs are involved with significantly fewer aggressive encounters with cows. This may be due to another effect seen – namely the introduced pairs remain close to one another, perform behaviour synchronously and maintain greater distances to the established herd than single heifers. Other authors have provided evidence for social support in cattle during separation events. Inversely to providing a “buffer” during environmental challenge, the removal of a social group is, in itself, a stressful experience (Kikusui et al., 2006). As previously mentioned, removal of conspecifics leads to known stress reactions such as increased cortisol and heartrate, overall arousal, and ‘struggling’ behaviours (e.g. jumping, straining and kicking; Boissy and Neindre, 1997).

Provision of a companion animal during separation (McClennan, 2013), or reuniting with any familiar conspecific after a period of isolation (Boissy and Neindre, 1997) reduces these indicators, indicative of the animal returning to normal behaviour. However, whilst the presence of another individual or group may provide social support, not all groupings are ideal. Characteristics of the companions, including gender, group size and affective state, may all impact their ability to attenuate stress in a focal animal (Rault, 2012). The value of a companion animal in attenuating isolation stress may also be partially based on familiarity. Cattle can learn and distinguish between other cows (Hagen and Broom, 2003). Færevik *et al.* (2006) reported reduced stress indicators when providing a familiar, vs unfamiliar, partner to isolated calves. Adult dairy cattle may also gain more social support from a preferred animal than simply a familiar conspecific. When separated from the main body of the herd alongside one other individual, cows showed less agitation behaviour, and lower heart rates if a preferred conspecific was present, compared with a random individual pairing (McClennan, 2013).

This body of evidence highlights the significance of affiliative relationships amongst cattle; potentially allowing for more cohesive, less aggressive groups which are better prepared for environmental challenge. As stress (including social disruption) can impact milk production (e.g. von Keyserlingk et al., 2008; Torres-Cardona et al., 2014), provision for the formation of affiliative bonds in dairy cattle has value from both a production and welfare perspective.

1.4.6 Group movements and behavioural synchronicity

Animal order during group movements has been the focus of considerable study. Unified movement as a group is important for prey animals such as cattle herds, with social cohesiveness therefore being valuable (Ramseyer et al., 2009). Due to the varying motivations of individual animals at any one time (e.g. levels of hunger or thirst), members of a group must choose to pursue their own requirements, or 'yield' to the group. The benefits of group movements must therefore outweigh the disadvantages (Conradt and Roper, 2005). Questions regarding the nature of group movements have considered whether there are distinct 'leaders' and 'followers', and if specific animals always lead the group. Findings from a range of social and gregarious species seem to suggest a "distributed" leadership in group movements, rather than the existence of a single leader of a high dominance status (Petit and Bon, 2010). This has been observed in cattle; within a group of heifers at pasture, any individual within the herd could initiate a group movement – however certain individuals were more likely to be followed by a larger number of herd-mates (Ramseyer *et al.,* 2009).

However, conflicting reports do also exist, detailing good consistency of the front and back animals of a cattle herd movement (e.g. at grazing - Dumont et al., 2005). In these cases, the first leader was not always consistent. A cow who initiates a group movement at pasture may, or may not remain at the front of the herd throughout the transition (Ramseyer *et al.,* 2009, Della-Rossa et al., 2014), however there is suggestion that an individual does not need to maintain position at the front of the group to influence movements (Bode et al., 2012). Specific individuals may regularly 'lead' group movements to feed (in up to 48% of cases) but are not necessarily consistent across type of movement, for example across pasture during grazing (Dumont *et al.,* 2005). Dominant cattle have a larger impact on herd movements than subordinate individuals, and move more directly between locations. In addition, dominant cows are more cohesive with the whole herd (and direct neighbours) during these movements (Šárová et al., 2010).

Cattle order may differ between movements made by choice, and those which are forced (Reinhardt, 1983). Given the highly managed nature of the lives of dairy cattle, particularly indoor housed, their order through e.g. the parlour, may not always reflect their naturally chosen position. Walking in from pasture, queuing and then entering the milking parlour is one such ‘forced’ event. Sauter-Louis et al. (2004) observed cows walking in from pasture to milking, and noted a correlation between the order of individuals walking in, and order through the parlour. Dominance was also related to position on walking in, with more dominant animals tending to walk in the front third of the group, and cows at either end of the dominance scale being most consistent in their order. The position of cattle during milking may also be influenced by dominance, with higher ranking cows appearing to be more consistent in their choice of side within a milking parlour (Prelle et al., 2004).

Behavioural synchronicity ties in with the idea of group movements, with animals performing the same activities together. Cattle herds can be highly synchronous, and dyads of cattle appear to be more so than would be expected by chance (Šárová et al., 2007). This may be due to similar requirements of two individuals (e.g. for feed or rest) at the same time – supported by greatest concurrence of behaviour between cows of comparable body weights (therefore with potentially equal feed intake needs). The degree of synchronicity may vary across activity, for example 70% of group members expressed the same behaviour (lying or standing), 93% of the time in one study (Stoye et al., 2012), but the level of synchronicity between dyads varies by functional area of the environment (Gygax *et al.,* 2010). Conversely, Cooper *et al.* (2008) found no evidence of cows influencing one another’s time of behavioural transition, however this study only compared recently paired individuals, rather than cows within an established group. As time of day (Stoye *et al.,* 2012), and area where this concurrence of behaviour is measured both affect its expression, care should be taken in study design to account for this variance.

1.5 Recording social behaviour

The majority of studies reported above relied on manual observation of social behaviour, with matching implications for the volume and quality of data collected. The automated monitoring of behaviour, including social proximities, is discussed below, along with the benefits when compared to standard observation techniques.

1.6. Automated behavioural monitoring

1.6.1 The development and value of sensor systems

With the advent of a range of technologies, the opportunities for automated monitoring of animals has vastly increased. Prior to the existence of data loggers and sensors, the main methods of data collection for animal behaviour required direct observation by a human party, or videoing the individuals. These techniques were adequate for small groups, but with a potential need for several observers or videoing equipment – along with the time required to analyse video after collection – this proved costly in monetary and manpower terms (Gygax et al., 2007). If several observers are involved, inter-person reliability and bias may affect the data (Müller and Schrader, 2003, although individual biases are somewhat mitigated by multiple observers - Martin and Bateson, 1993), and difficulties are presented in monitoring animals during the night as illumination required for cameras and human vision may impact on cow behaviour (Meijering et al., 2004). Individual identification may be difficult in large groups, requiring the animals to be marked for observation, invoking greater cost in man-hours and equipment (Gygax *et al.,* 2007). In the study of wild animals, speed travelled by the species, visibility in its habitat and potential for human disturbance make remote monitoring a much better option than direct (Baratchi et al., 2013). Video analysis and prolonged direct observation are also impractical for commercial on-farm use, so more feasible approaches must be investigated. Data loggers and other on-cow sensors have been developed with a variety of functions to enable automatic monitoring of behaviour, health and reproductive status in both captive/domestic and wild animals.

1.6.2 Sensors for farmed animal management

1.6.2.1 Behavioural states

There are many potential applications for the automated recording of the behaviour of farmed animals, from total activity to specific behavioural states and their known relationships with health or reproductive status. Lying and standing have been successfully detected by leg-mounted mercury tilt-switches (in cattle; e.g. Champion et al., 1997). Overall activity levels can be measured via a pedometer, or a 3d accelerometer attached to the leg or neck (e.g. Müller and Schrader, 2003) of an individual animal. A global positioning system (GPS), more commonly used for assessing location, can also provide data to identify behavioural states. The application of data mining to GPS data has successfully classified grazing, resting and walking in cows at pasture (Williams et al., 2016). Other technologies, including the analysis of multiple images via an algorithm, have also been shown to detect lying cows with a sensitivity of 92% (Porto et al., 2014). A 3d accelerometer may distinguish between behavioural states with a good degree of accuracy (e.g. Vázquez Diosdado et al., 2015) when the collected data is subjected to a decision-tree algorithm. With advances in decision making algorithms, support vector machines (a machine learning method) and similar techniques, behavioural states from the simplest – e.g. lying and standing, through to grazing (Rutter et al., 1997) and lame gait (Pastell et al., 2009) may be identified through sensors reliant on a range of mechanisms.

1.6.2.2 Health and reproduction

Behavioural indicators of specific health or reproductive states may be of particular interest from a practical application viewpoint. Oestrus behaviours in dairy cattle, for example, are highly valuable, as without these signs, opportunities for insemination may be missed (incurring cost to the farmer). Leg mounted pedometers, 3D accelerometers, pressure sensors at the base of the tail and temperature-sensing implants have all been used in the detection of oestrus in cattle (for a review, see Rutten et al., 2013). Sensors have also proven useful in the detection of disease. Variance in leg-mounted accelerometer wavelets, particularly during forward movement of legs, and the phase of walking where feet hit the ground, highlighted differences between lame and sound cows (Pastell et al., 2009). Front limb movement detected by accelerometers has also successfully distinguished between lame and sound animals – with a requirement for a sensor to be attached to both legs (Chapinal et al., 2011). This is due to the change in weight distribution associated with lameness - lame cows frequently shift their weight from an injured limb to other limbs (Rushen et al., 2007). Pedometers can also be used to detect lameness. Setting this equipment to compare the daily activity of a cow (via steps per hour) to her own last ten days, and report on any changes above 5% in activity, allows for detection of lameness up to ten days before clinical signs in some cows (Mazrier et al., 2006). Beyond behaviour, other technologies incorporated in on-cow sensors have been used to highlight health concerns or fertility. Temperature detection by implanted sensors may indicate reproductive status, and the temperature or pH of the rumen as measured by in-cow loggers could be utilised in the early detection of ketosis and other disease (Rutten *et al,* 2013).

1.6.3 Automated detection of social behaviour

The social behaviour of animals may have many of the same applications as previously described for general behaviour. Sociality in animals ranges from obvious direct interactions, through the subtleties of shared space, avoidance and visual/auditory signals. From the perspective of automated monitoring, the easiest measure of sociality is the proximity between animals – with no current technologies applied to detect the minor behavioural signals (to the authors knowledge), although automated recording of feeder displacements has been applied to dairy cattle (Rutter et al., 1987). However, there is still great value in the detection of the distance between animals (see the social section of this literature review). Sensors to detect the proximity of animals work by two main mechanisms; reporting individual animal locations (to allow calculation of inter-animal distances), or detecting the presence of another sensor within a set threshold.

For the former option, local or global positioning systems can be employed, as these devices report the location of each individual within their environment. GPS has been successfully employed in wild species, or domestic animals on extensive systems (e.g wild elephants – Hacker et al., 2015) to accurately reflect social relationships. Gygax *et al.* (2007) determined that a local positioning system can be used to monitor the proximity of cows to one another in indoor housing. It was suggested by the authors that the sensors in fact provide more accurate proximity data than human reports, especially in areas where cows were active.

For the latter option, proximity sensors have been deployed effectively on a variety of species to detect the coming together of individuals, for example in birds (Levin et al., 2015), bats (Ripperger et al., 2016), and even fish (Tentelier et al., 2016). This style of sensor usually detects the presence of another node entering a radius of a set distance from itself, effectively logging a proximity encounter. Examples of this sensor type have also been utilised in on-farm monitoring – for example in dairy cattle with access to an indoor and outdoor area (Boyland et al., 2016). Both technologies allow for the production of datasets suitable for social network analysis, or other statistical techniques to investigate the existence, value and consistency of social relationships.

There is, however, an advantage to the deployment of positioning systems (either global or local), over proximity sensors, in terms of further interrogation of the dataset. Animal location derived from such systems, either in combination with activity information or as a standalone data set, can provide several outputs. Among these is the ability to gain movement data (e.g. a ‘map’ of area walked through the barn) for all animals fitted. Gygax *et al.* (2007) used a local positioning system (LPS), allowing for 300 position updates per second, with an accuracy level of around 0.5m. This permitted the monitoring of cow location through the shed, whilst stationary and during walking, by affixing a transponder and transmitter to a cow collar. Sensors based on an ultra-wide band location system (Porto *et al.,* 2014) have also been successfully used to track the location of cattle in a restricted environment, with an on-cow mean error of 0.5m. Global positioning systems (GPS) have also been employed by various researchers to collect location data about animals in outdoor systems (Nadimi et al., 2008). This can be valuable in more extensive environments, or wild animal ranges, to identify habitat use, territory size or other behaviours (e.g. distance travelled). The downfalls of GPS include high costs, both in terms of initial outlay and energy consumption (Nadimi *et al.,* 2008), and accuracy levels are typically low. Local positioning system are therefore likely to be more applicable than GPS in confined environments.

Beyond simple reports of which individuals are associating across the whole environment, positional data from an LPS may allow for assessment of the context of social bonds. Attempts by Gygax *et al.* (2007) to automatically record displacements from food using a LPS were unsuccessful; the displacements fitted within a band of characteristic behaviours, but variation was too great to allow for automatic detection. However, as a potential future avenue, advances in technology or classification algorithms may make displacements an automatically monitored behaviour. Recording the location of animal encounters permits assessment of the consistency of these relationships

1.6.4 The future of automated monitoring

Technological advances and product development have produced a wide range of equipment designed to monitor animal behaviour. However, many of these have historically been complex to use, large and therefore difficult to transport between farms (e.g. force plate sensors), high in cost or may directly affect behaviour of the animal they are applied to (Müller and Schrader, 2003). The use of on-cow sensors which do not impact usual behaviour or management practices, can remain on-farm, and provide valuable behavioural information to the farmer, is an increasingly achievable goal. This is indicated by the breadth of studies, range of existing and developing technologies, and the associated post-logging data handling techniques (e.g. SVM), and will be explored in the application of an LPS to dairy cattle in the following thesis.

1.7. Application of behavioural data – health and welfare

The ability to collect behavioural data, whether detailed states via human observation, or broader time-budgets through sensor/logger systems, is of both scientific and practical interest. An understanding of ‘normal’ animal behaviour is the basis of good husbandry, as it provides a benchmark for recognising abnormal or unusual behaviour. There is good evidence that changes in activity can be indicative of paralleled changes in health status (Weary et al., 2009). Activity alterations can be either ‘positive’ or ‘negative’ – meaning the actions may either increase or decrease when the animal is in a diseased state. This forms the notion of 'sickness behaviour' in animals.

Sickness behaviour has been defined as a group of nonspecific symptoms presented in response to an infection; in particular reduced overall activity and interest in surroundings, lowered food and water intake, and general weakness/listless behaviour (Dantzer, 2001). This "strategic" set of behaviours lead to a number of consequences which are of benefit to the recovery and survival of the individual (Hart, 1988). Animals seek to increase their body temperature, by minimising heat loss (e.g. compressing body to reduce surface area), spending time in warmer areas, shivering and piloerection. Evidence suggests that this 'fever' response (induced by the hypothalamus setting a higher body temperature requirement) provides an internal environment less suitable for some pathogens, which may not survive or reproduce as efficiently above normal body temperature (Hart, 1988).

Recognising sickness in dairy cattle is of tantamount importance for a range of welfare, financial, biosecurity and other reasons. Financially, illness can lead to cost to the dairy farmer in the price of veterinary time and drugs (Huijps et al., 2008). Cattle experiencing health challenge show a reduction in milk yield (Amory et al., 2008), and severe cases of disease may require culling of the animal, leading to loss of earnings for the farmer. Some common health issues in dairy cows, such as lameness and mastitis, are considered painful to the animal (Walker et al., 2008). An individual cow's perception of pain may vary dependent on circumstance. In situations of acute stress such as short periods of social isolation, cows have been shown to experience hypoalgesia, recorded by a reduced response to nociceptive stimuli (Herskin et al., 2004). Lame cows have lower nociceptive thresholds than their sound counterparts, and the type of foot lesion present also affects the degree to which this threshold alters (Whay et al., 1998). There is conflicting evidence on the experience of hyper/hypoalgesia by cows suffering from mastitis, with some studies finding increased responses to a pressure stimulus (Fitzpatrick et al., 1998), and others noting a decreased behavioural response to a nociceptive laser (Rasmussen et al., 2011).

The body of research suggesting the perception of pain in dairy cattle, along with the possibility of disease transmission to other members of the herd, consequences for productivity and profitability, highlight the importance of detecting illness. Early detection and treatment of the health condition may reduce the suffering (and associated cost) experienced by cattle, meaning an understanding of behavioural changes which correlate with pre-clinical disease can be highly valuable (von Keyserlingk *et al.,* 2009).

1.7.1 General behavioural indicators of disease

A number of health concerns in cattle have clearly defined behavioural indicators, explained by the underlying sickness behaviour response. Feed intake is one such indicator; although the direction of change is dependent on the health cause. Ketosis – an increase in ketone bodies in the bloodstream due to negative energy balance in early stages of lactation (Goldhawk et al., 2009) – may cause a reduction in feed ingestion. This is displayed through reduced number of visits to a feeder, less time spent at feed, and around 3kg per day reduction in dry matter intake – up to a week prior to diagnosis (Goldhawk *et al.,* 2009; Gonzalez et al., 2008). However, the inducement of ketosis artificially does not appear to cause the same effect; possibly due to other changes to physiology during the development of subclinical or clinical ketosis (Zarrin et al., 2013).

Lameness is another health condition associated with diminished feeding behaviour. Acutely lame cows have been noted to decrease feed intake significantly (Gonzalez *et al.,* 2008), have a shorter total daily feeding time (Palmer et al., 2012) and cows with lesions and a ‘lame’ locomotion score show a trend towards lower intake than control cows (Nechanitzky et al., 2016). This is logical for a number of reasons; lame cows get up from lying around 13 minutes later, and lay down again 19 minutes earlier than sound counterparts, upon delivery of feed (Yunta et al., 2012), providing less time for feed consumption. Interestingly, lame cows may eat more quickly in order to compensate for spending less time at the feeder, so in some cases, may eat as much (and under some circumstances more) as sound counterparts (Proudfoot et al., 2010; Palmer *et al.,* 2012).

Other health issues suggested to lower feed intake include metritis; metritic cows eat less in the two weeks pre-, and four weeks post-calving than healthy individuals (Huzzey et al., 2007). Cows which go on to suffer dystocia (difficult calving) also have reduced dry matter intake in the 48 hours prior to giving birth (Proudfoot *et al.,* 2009). After the artificial induction of mastitis, feeding is seen to diminish in the following 24 to 48 hours (as measured by time spent feeding - Fogsgaard et al., 2012 – and dry matter intake - Lukas et al., 2008; Yeiser et al., 2012). In contrast, one author noted increased time spent feeding on the day of induction – however with a suggestion that the cows ate more slowly, therefore not necessarily increasing feed intake overall (Siivonen et al., 2011). Infection with an abomasal parasite has also been associated with a longer average feeding bout (Szyszka et al., 2012).

Another ingestion behaviour – drinking – is affected by disease; mastitis, metritis, milk fever and ketosis have all been suggested to reduce water intake (Huzzey *et al.,* 2007; Siivonen *et al.,* 2011; Lukas *et al.,* 2008). Conversely, subacute ruminal acidosis leads to an increase in drinking – presumably due to the effect of water raising the rumen pH (Cottee et al., 2004). Rumination has also been, to a lesser extent than feeding, linked with disease. Lame cows have been noted to ruminate for significantly less time than non-lame conspecifics (Miguel-Pacheco et al., 2014), particularly during the scotoperiod (dark hours) of day 3, 4 and 6 pre-, and 0, 1 and 7 post-diagnosis and treatment of the condition (Hertem et al., 2013). During fever associated with mastitis, cows ruminate for less time than when healthy (Siivonen *et al.,* 2011; Fogsgaard *et al.,* 2012).

Lying has been cited as a behavioural indicator of several illnesses; matching as it does with the lowered activity aspect of 'sickness behaviour'. There have been conflicting reports in the literature around the change in lying behaviour with illness. Some authors have found increased lying times in cattle experiencing mobility issues, either as longer individual lying bouts (housed: Yunta *et al.,* 2012; at pasture: Navarro et al., 2013), longer lying in certain locations (out of cubicles – Galindo and Broom, 2002) or total time spent lying (at pasture; Walker *et al.,* 2008, housed; Blackie *et al.,* 2011). Specific lesions have also been suggested to increase the number of lying bouts (Navarro *et al.,* 2013). Additionally, some studies show no significant differences in total lying time between the two groups (e.g. Yunta *et al.,* 2012; Cook et al., 2007; Galindo and Broom, 2002; Navarro *et al.,* 2013), or report reduced time spent on this behaviour (Cook et al., 2004). The variety of changes seen suggest that lying time may be an indicator of lameness, however the directionality and exact effect is likely to be dependent on housing system, age, season and a myriad of other factors (Blackie et al., 2011), therefore comparing cows with their own historical time budget may be more helpful than a 'lame vs sound' comparison.

The directionality is somewhat clearer in mastitis cases. A number of authors report that individual cows experiencing mastitis lie less (e.g. Medrano-Galarza et al., 2012; Fogsgaard et al., 2015; Siivonen *et al.,* 2011), probably due to the pain associated with lying on an infected udder. However, research investigating the side of recumbency has been contradictory regarding the infected quarter and the side cows choose to lay down on. In some cases, cattle have been found to minimise lying on the infected side (Siivonen *et al.,* 2011), whereas other studies report no correlation (Cyples et al., 2012). Some other health challenges correlate with the typical expression of increased lying with 'sickness behaviour';

cattle with a high parasite load have longer lying bouts than uninfected animals (although overall lying time is not increased; Szyszka *et al.,* 2012).

Standing is another 'resting' activity which has been investigated in relation to health issues. As with lying, there is some contradictory evidence regarding the direction of change in response to challenge. Several authors report lower standing times in cows with various causes of lameness – including damaged soles and walls, infectious claw diseases and foot overgrowth – and reduced standing times with worse locomotion scores (e.g. Navarro *et al.,* 2013; Walker *et al.,* 2008; Blackie *et al.,* 2011). This appears logical given the associated pain in the feet or limbs which would likely be experienced during standing. However, other studies have reported no link between lameness and standing times (Galindo and Broom, 2002), or suggested that increased standing times, particularly in certain locations (e.g. perching in cubicles) may lead to lameness rather than serving as an indicator (Galindo *et al.*, 2000; Proudfoot *et al.,* 2010). Other work has recorded a correlation between increasing mobility score and the area of housing standing takes place – for example spending more time standing in cubicles (rather than passageways), than healthy cows (Cook *et al.,* 2007). An increasing in standing has been associated with the onset of mastitis, either in total time or length of standing bouts (Fogsgaard *et al.,* 2012; Siivonen *et al.,* 2011; Yeiser *et al.,* 2012) – potentially, as with reduced lying, due to the pain of pressure on an infected or inflamed udder.

In terms of active behaviours, a lowered amount of walking or total activity has been linked with some diseases; on average, sick cows step less than healthy counterparts (Edwards and Tozer, 2004) including in the one or two days prior to diagnosis. Lameness is one issue which has clear cause to be linked with walking or stepping due to the location of pain in the foot or limb. Several authors report reduced walking, stepping or strides per hour in lame dairy cattle (Walker et al., 2008; Reader et al., 2011), as well as changes to the nature of the gait; cows may express shorter stride lengths and travel more slowly (Blackie *et al.,* 2011). Galindo and Broom (2002) noted a significant difference in percentage of the daily time budget allocated to walking by lame and non-lame cows; 6.6 and 14% respectively. Activity as measured by neck movement also shows a similar pattern, with lame cows registering lower neck activity than sound individuals during daylight hours (Van Hertem *et al.,* 2013). Cattle in a parasitized state have also been shown to reduce their activity (again in line with the nature of 'sickness behaviour'), in some cases up to 34% lower activity in an individual with an high level of infection than a healthy counterpart (Szyszka and Kyriazakis, 2013).

In contrast to the typical expression of sickness behaviour, cows with mastitis have been observed to increase activity (measured by stepping behaviour) on the day of artificial disease induction (Siivonen *et al.,* 2011), and up to 10 days after diagnosis of naturally occurring mastitis (Fogsgaard *et al.,* 2015). This may relate to the similar finding of increased standing in mastitic cows described above (e.g. Yeiser *et al.,* 2012) where pain in the udder prevents lying. If an individual is experiencing the symptoms of illness and pain at the same time, the motivation to reduce pain may overcome the drive to perform classic 'sickness behaviours' (Fogsgaard *et al.*, 2012). Possibly for similar reasons, increased walking compared to healthy individuals has been noted in cows with a left displaced abomasum (Edwards and Tozer, 2004). The latter authors reported that a number of factors interact with the specific disease to affect general activity; namely the number of days since disease onset, and days in milk.

Behaviour during the milking event may provide some insight into the health status of individual dairy cows. Main et al. (2010) observed a relationship between lameness and milking order – with an 11.9% greater incidence of lameness in cows milked in the final third of the herd. Lame cows leave the field later than their non-lame counterparts when brought in from pasture (Walker *et al.,* 2008), and enter the parlour later. There are several possible explanations for this finding; firstly that lame cows struggle to maintain pace walking in from the field and fall behind. Another possibility is that lame cows chose to remain at the back of the herd, particularly in the crowded environment of the collecting yard, to avoid agonistic interactions with other cattle. There are also notable differences between lame and sound cows housed with an automatic milking system (AMS) where entry is voluntary; with the former visiting the AMS less often, and at specific times (namely between midnight and six am; Miguel-Pacheco *et al.,* 2014). At the other end of the milking process, lame cows return more slowly to housing after being milked, and also remain close to the housing exit (i.e. nearer the milking parlour), presumably to reduce distance walked (Juarez et al., 2003).

In terms of other diseases, cattle with a high somatic cell count, potentially indicating sub-clinical mastitis, appear to wait until the back of the herd to be milked (Rathore, 1982; Berry and McCarthy, 2012), entering the parlour at a later stage in the milking order than when they are healthy (Polikarpus et al., 2015). Another indicator of udder-based illness/injury is kicking or stepping during the milking event, with cows suffering teat lesions and ticks being more likely to perform these behaviour (Rousing et al., 2004).

Disease may also impact the expression of other, less common behaviours. Several illnesses supress behavioural expression of oestrus; representing a major problem for dairy farmers reliant on activity to schedule artificial insemination. Cows with subclinical ketosis, subclinical mastitis and lameness have lowered total activity during oestrus, including reduced mounting of other cattle (Morris et al., 2013; Rutherford et al., 2016). Self-grooming is another ‘minor’ behaviour, and is reduced in cattle experiencing mastitis (Fogsgaard *et al.,* 2012). Whilst interesting to note, it is likely that changes in these rarer behaviour will be of limited use in disease detection without extensive observation, due to the infrequency of their expression.

1.7.2 Social behavioural indicators of disease

Another typical aspect of sickness behaviour is social withdrawal or isolation. Animals are seen to avoid social interaction during illness; potentially limiting the chances of receiving a secondary infection whilst the immune system is already weakened (Proudfoot et al., 2012). Cattle with diseases including mastitis, metritis and pneumonia have been shown to isolate themselves from conspecifics within a confined area (Proudfoot et al., 2014), tying in with the classic social withdrawal aspect of 'sickness behaviour' (Hart, 1988). Cows suffering illness post-calving show a higher tendency than healthy individuals to remove themselves to a "secluded" area within a pen where there is no visual contact with other cattle, and individuals who have longer calvings may choose greater isolation than shorter parturition cows (Rørvang et al., 2017). Social isolation may be confounded by other factors, such as the temperature, comfort or other favourable aspects of the area chosen for seclusion (Proudfoot *et al.,* 2014). Additionally, aspects of the disease or illness are suggested to impact social isolation, for example (Jensen et al., 2015) noted lame cows in hospital pens did not seek distance from conspecifics, potentially as the studied conditions did not result in fever and the biological 'sickness' response.

Other authors have noted effects in direct social interactions; lame cows appear to instigate less aggressive encounters, and are licked more than sound individuals (Galindo and Broom, 2002). In the days leading up to diagnosis with mastitis, cows are seen to reduce their competitive behaviours at feed; recorded by reduced incidence of replacing healthy cows at the feeder (Sepúlveda-Varas et al., 2016). A similar pattern is true of cows suffering subclinical ketosis; these individuals perform fewer feed-face displacements (Goldhawk *et al.,* 2009).

Social rank may be affected by illness, for example metritic cows are socially subordinate in the 21 days prior to calving; measured by a significantly lower displacement index than non-metritic cattle within the same group (Patbandha et al., 2012). However, given that more displacements at the feed may reduce total possible feeding time – potentially affecting overall health – this cannot be defined as a cause or effect of illness (but may still have use as a warning sign).

When considering the use of social proximities as an indicator of illness, there are a number of potential factors to be considered. Individuals which are consistently spending time close to one another may be more susceptible to contracting contagious disease – for example (Boyland, 2015) found higher somatic cell counts (SCC) correlated with higher degree centrality, and suggested a contributing factor may be shared lying areas. The social proximities of cattle are intrinsically linked with a number of the other behaviours described above; as an example, if cows lie more with specific health conditions (e.g. Blackie *et al,* 2011), they may be more or less likely to remain close to other cows. If a sick individual chooses a lying spot in a popular area of the shed, and lies for an extended period in comparison to her usual time budget, her proximities to certain other cattle may in fact increase. Conversely, as ill cows adjust the time of their feeding (Yunta *et al,* 2012), their social encounters are also likely to change, and this may be reflected in proximities (both length of encounters and identity of the other individuals). Finally, the changes in rank or competitive behaviour described above may directly impact proximity encounters, either by altering which cows the sick individual is able to interact with (e.g. those who would normally be subordinate to herself), or by impacting on her expression of feeding/lying behaviours, with the aforementioned results.

This range of interactions between sociality, broad behaviour and proximity makes the time cows spend together a novel indicator of disease, reflecting many aspects of the cows daily life. The ability to delve into the location of encounters further expands the potential applications of this data, by assessing if, for example, cows with certain diseases leading to greater lying times experience increased/decreased proximities to other cattle. A final note is that individual differences are broadly acknowledged to be important in mammal sociality (e.g. Barocas et al., 2011), and therefore the comparison of healthy vs ill periods of individuals lives may be an essential aspect of utilising social proximities as a disease indicator.

1.8. Conclusions

An understanding of 'normal' social behaviour is a vital starting point in using this measure to assess health and welfare of individual cows – as such the broad social structure of the studied herd will be first described, with the consideration of location effects on this structure. This lends a solid basis on which to further investigate change with disease. The application of a novel LPS system, duration of the dataset and assessment of sociality across functional area combined represent a new insight into the social behaviour of housed dairy cattle.

Chapter two: General methods

2.1 Housing and animals

2.1.1 Study location; Albyns Farm

The study was conducted at Albyns Farm; a commercial dairy farm in Essex, with a herd of around 350 pedigree Holstein cattle. The management group observed were high-yielding, with an average 305-day yield of around 11,000 litres per cow. This group was monitored from July to December 2014, with a fluctuating number of cattle between 86-116. Parity ranged from one to nine. Cattle left the group periodically prior to calving, or due to severe illness, and new individuals entered the group shortly after calving. As such, the group was dynamic throughout the study period, with sections of stability (i.e. no entry/exit from the group), and slightly altered herd composition across the course of the trial. The month of October was selected for the following analysis, due to its relative stability in terms of temperature and cattle introductions (only 11 changes within the group; 101-112 cows total).

The high-yielding group were housed in a cubicle shed measuring in total 30m x 58m, of which the high yielding group had half, containing 98 useable cubicles bedded with sawdust on a geotextile mattress (see figure 1). The two main alleyways (between feed, and cubicles) were cleaned periodically throughout the day by an automatic scraper, which moved down the shed towards the slatted area. The feed face comprised a short concrete wall and a top feed-rail, interspersed with uprights at approximately 3m intervals, creating seventeen ‘bays’. The group were fed a commercial mixed ration delivered once daily and “pushed up” approximately five times daily, with the last push-up at around 9pm. Two drinking troughs were available in the cross passage between cubicles, on a slightly raised area.

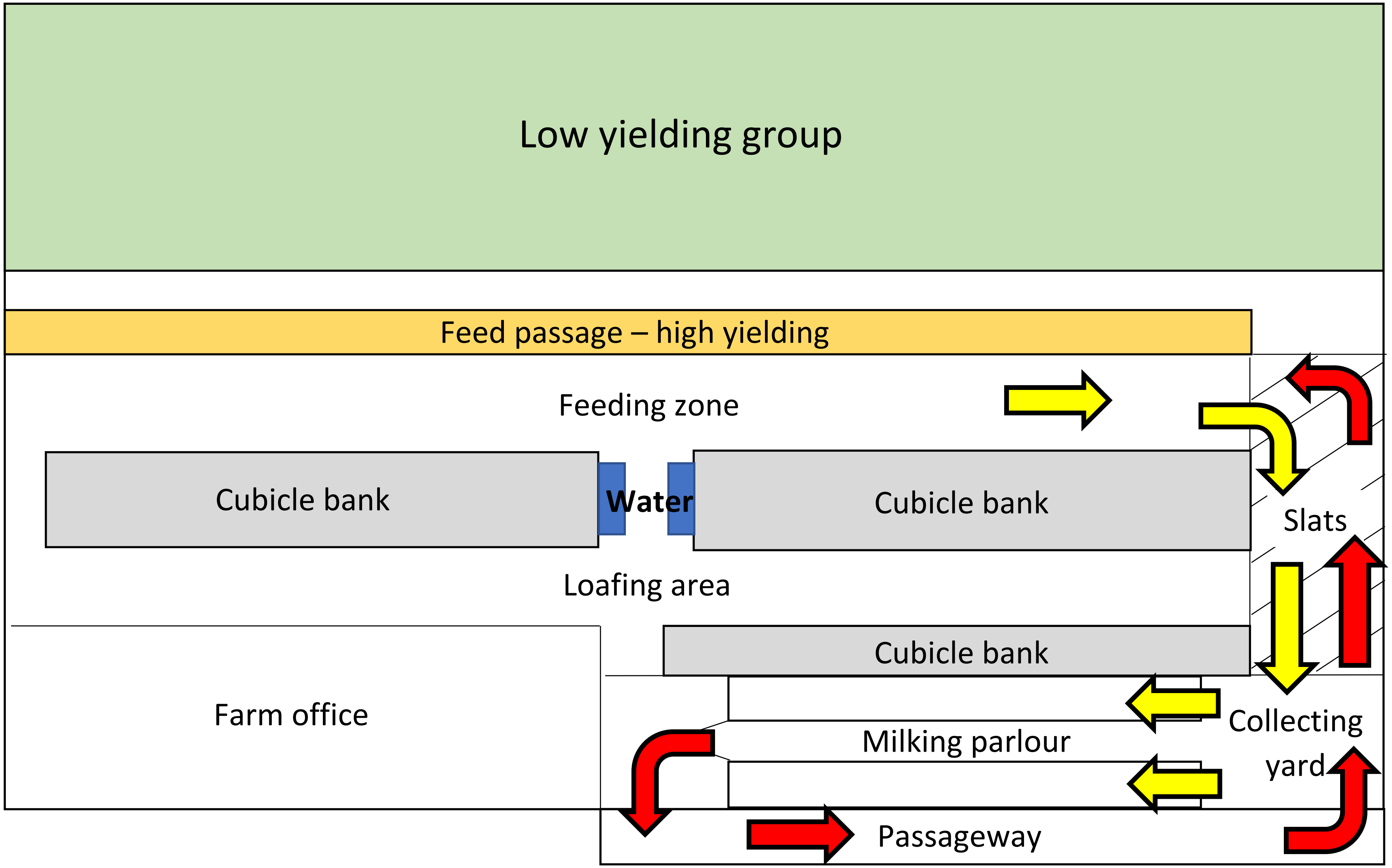


Figure 1; Layout of the cubicle shed and milking parlour with location of key resources (not to scale). Yellow arrows indicate cow movement during collection for milking, and red for return

Milking occurred three times daily, beginning at around 6am, 2pm and 10pm for the high-yielding management group. Cows were collected from the shed by a member of farm staff, and held in a slatted floor yard and the concrete-floored collecting yard prior to entering the parlour. The milking parlour was a 20:40 swingover parlour with 20 cows in each side, sides completed milking in around ten to fifteen minutes. Sides were released and, dependent on day and time of milking, could immediately return to the shed, or were pushed round into a passageway where they were filtered back into the shed in batches. The cows continued to have access to the slatted yard for a short time after milking, and occasionally for longer periods. Milk recording occurred monthly as part of the usual farm routine, to assess somatic cell count and yield of individual cattle.

2.1.2 Animal handling

All cattle in the group wore sensors for the majority of the trial; initially this involved collaring any uncollared cows, and then replacing sensors based on remaining battery life (detailed criteria below). Cows requiring collars or changes were sprayed around the rear/back legs with stock marker in the cubicle shed area in between-milking intervals. The required number of collars were prepared in advance, containing sensors and marked externally with the sensor ID. During milking, an observer in the parlour ‘pit’ recognised marked cows, and these individuals were directed into a collecting area attached to three artificial insemination pens. Finally, cows were directed into a pen, where they were collared, the cow and new sensor ID recorded, and individuals were then released back into the group via the front of the AI pens.

Cows with poor freeze brands or ear tags were, when possible, given collars with a plastic number attached to aid in identification. Collaring occurred in batches during morning, afternoon and evening milking dependent on day. Occasionally, cows were brought round from the shed into the AI pens during between-milking periods when large numbers of collar changes were required. Handling and removal of animals from the herd was carried out as quickly and calmly as possible to minimise stress to the cows involved, and limit time away from feed/lying areas.

The large majority of cattle were collared in batches at the beginning of the trial. Cows which ‘calved in’ to the group at a later date were collared as close to entry date as possible, and data collection began immediately. Overall this protocol minimised the likelihood of the collars affecting behaviour, and it was informally observed that cows quickly adjusted to them, with little attention paid to their own, or other individuals collars after an initial day.

2.2 Data collection

2.2.1 Farm event, health and cow data

Farm data were collected in the form of a weekly questionnaire with staff, detailing unusual events in the past seven days which could have affected cattle behaviour, any changes to typical management practices, cow deaths and so on. Other cow-based data collected comprised calving date, days in milk, insemination date, parity and relatedness.

Health data for all cows was collected from Uniform Agri downloads, and daily health diary records. This compiled any treatments given to cattle within the high yielding group, the health condition and length of treatment. In addition, a fortnightly mobility score was carried out by a skilled observer as cows exited the milking parlour – using the AHDB Dairy 4 point mobility score (2013) from 0 (sound) to 3 (severely lame). This score was already in use for the larger trial work on the farm. A foot trimmer visited the farm at regular intervals

(4 times during the trial), and any lesions recorded during trimming were added to the health data.

2.2.2 Social behaviour

Social behaviour was manually observed for a minimum of 45 minutes in twenty-four cattle. The cows were chosen to cover a range of parities and locomotion scores (from score 0 – 2). The observer stood either in the feed passage, or within the cubicle area, dependent on the location of the focal cow (the individual under observation). A distance was maintained to ensure no disturbance was caused to the focal cow or her near neighbours, in order to minimise impact on social interactions. Cows were continuously sampled for any incidence of social behaviour (see table 1 in chapter one for a description of recorded behaviours), and the position of the interaction within the shed was marked on a scale diagram. Finally, when at the feedface, the nearest neighbours of the focal cow was recorded, and the feeding position recorded on the scale diagram of the shed.

2.3 Automated monitoring system

2.3.1 Omnisense® Series 500 Cluster Geolocation System

A wireless sensor system – the Omnisense® Series 500 Cluster Geolocation System (hereafter referred to as OMS500) was employed for this trial. The wireless sensors (or nodes) contain a 2.4 GHz, IEE 802.15.4a transmitter module to remotely send messages to the server. The OMS500 sensors form a wireless mesh sensor-node network which allows for computation of local position based on arrival time of messages from neighbours (a local positioning system). During the trial, the system ran on a ten second ‘refresh rate’; nodes sent messages at ten second intervals. The information reported by the sensors comprised a local position (X, Y, Z format), a peak and mean reading from an internal, tri-axial accelerometer (Xtrinsic MMA8451Q 3-Axis, 14-bit/8-bit Digital Accelerometer with a sensitivity between -8g and +8g), and the sensors also contained a magnetometer (data not used for the purposes of this trial).

2.3.2 System setup and collar deployment

Fourteen network sensors were deployed strategically at known positions in the cubicle shed for maximum coverage of the area – these nodes were housed in a watertight casing and attached to an additional four 'D' battery pack for prolonged functioning. Eight additional network sensors were positioned within the collecting yard, milking parlour, and return passage to collect data during milking. Two network sensors acted as "gateways" – a sensor through which data collected from all other sensors is passed, to the server. Network sensors around the building were fixed to a wooden bracket constructed to be fitted to either Yorkshire boarding or steel I-beam. Network sensors positioned within the parlour (four sensors) were fitted to a series of ratchet straps hung down the length of the parlour above both feed troughs. The network sensors throughout the shed were all placed in the same orientation, again with the aim to maximise efficiency in receiving signals from on-cow nodes. In the parlour, four network nodes were at an alternate orientation due to the impossibility of wall-mounting, however this did not appear to adversely affect accuracy within the area in comparison with the main shed (see validation section below).

Mobile sensors of the same system (OMS500) were deployed via neck collars, onto cattle in the observed management group. The neck position gives means the head of the cow is effectively tracked, valuable for feeding behaviour (where heads are put through a feed-face), and useful for social interactions which may often be head-to-head. Small plastic

‘Really Useful®’ boxes were drilled in four places, and sewn onto webbing collars with strong twine. Sensors were placed in the boxes, surrounded with padding to prevent movement, and wrapped in a protective, waterproof layer in order to minimise risk of damage. The reported battery life of the sensors as a ‘stand-alone’ is 21 days, therefore during both trials, a varying percentage of sensors had an attached external battery back, from four to six AA alkaline batteries connected via a micro-usb cable, to extend sensor life. A voltage pre-regulator was connected between the sensor and the battery pack to ensure the voltage delivered was within the working range of the sensor. Two weights were attached to the base of the collar, to maintain sensor position. The total weight of collars and components ranged between approximately 750g and 900g, dependent on the presence or absence of battery packs attached to the sensor.

Figure 2; Location of mobile sensors in relation to cattle neck and head (contained in grey box)

The battery status of all sensors was monitored remotely (via a server interface) throughout the trial. Network sensors had the four ‘D’ battery pack replaced when they reached a low level (around 20-30% battery life remaining). Mobile sensors were removed from cows when they reached around 30% battery life (urgency assessment based on number of sensors requiring changing), and replaced with another collar and sensor combination. The unique sensor ID was recorded, along with the cow ID, at every collaring and collar change. For additional accuracy, the sensor removed from a cow was noted in order to confirm identity.

2.3.3 Validation of the OMS500 system in parlour area (pilot validation)

The original design of the project was to use the sensor system to investigate the behaviour of cattle during milking and, its relationship with disease. To this end a validation study of the sensor system within the parlour area was carried out. Nineteen stationary positions, over three repetitions, were selected to represent the range of possible cow locations during milking. The mean circular area of probability (CEP) for the whole parlour area was 1.1m – representing the distance from the mean sensor position in which 50% of positions were recorded. The mean distance (DIST) between the known ground truth, and the mean sensor-reported position was 2.5m. In addition to this stationary validation, the accuracy of on-cow sensors was assessed. Twenty cows were fitted with neck-collar mounted OMS500 sensors. Sensor data from four 24-hour periods were collected. The cows were observed at four milkings during these days, to compare observed positions with sensor data. Mean CEP values of 1.8m were reported under these conditions, with a mean 2.6m difference between the true cow location and sensor reported position. Given the level of accuracy in such a narrow area, and issues transpiring with sensors failing near the parlour, it was decided that it was not viable to use this information to create herd milking order. However the positional data from this area was still utilised in the creation of social networks, due to the smoothing methods applied as described throughout the thesis.

2.3.4 Validation of system in main shed

The accuracy and precision of the OMS500 sensors was measured throughout the cubicle shed, both on-cow and placed within the environment (from unpublished data)

Table 2; accuracy and precision of the OMS500 sensors on-cow and in fixed positions throughout the shed.

Activity	N	Mean CEP (SE)	Mean DIST(SE)
On cow –Feeding	30	2.7m (0.32)	4.4m (0.92)
On cow –Lying	14	2.7m (0.41)	5.6m (0.85)
On cow –Standing	7	1.9m (0.28)	2.8m (0.56)
Fixed position	52	1.1m (0.08)	2.7m (0.24)

2.4 Data handling

The output from the OMS500 system comprised of a comma-separated value (csv) file reporting the position, accelerometer and magnetometer reading of each sensor in the system, for every 'refresh rate' through a twenty-four hour period. This csv file was condensed to the required sensor features for reporting cow proximities (namely X and Y co-ordinates along with time, date and sensor ID), and using customised code in the programme Matlab R2015a (provided by JVD), used to produce daily matrices of cow proximity (e.g. A-B, F-Z). To classify an 'encounter', certain criteria were selected. An 'encounter' comprised two sensors coming within a four metre radius of one another, and 50% of the reported positions for the following two minutes, remaining within a two metre radius. This 50% threshold was set due to the inherent error in the reported sensor position, to allow for any momentary 'jumping' or noise in the sensor location. The full details for validation of this criteria and selection process are described in chapter three. The initial 20 minutes of each ‘sensor-day’ were removed, as the system took a short period to re-establish positions after a daily re-start at midnight.

2.5 Data analysis

The programmes UCINET 6 (Freeman, Everett and Borgatti, 2002) and Netdraw, the visualisation tool associated with UCINET were used for early stage exploration of the proximity dataset. Proximity matrices were imported into UCINET from Excel spreadsheets (xls), and saved as network files. Using the ‘network’ tools menu, centrality measures of individual nodes (including degree and betweenness) for daily networks were reported. Whole-network measures were also computed, including the density of the network, and average degree. Netdraw was used to visualise these networks, allowing thresholding of interactions by tie strength to begin visual exploration and assess if any patterns appeared. UCINET allowed for the calculation of statistical tests with node permutations, to compare groups of cattle. The programme ‘R’ (version 3.3.0, 2016) was used for further exploration of the data (as described in later chapters). More detail on the specific analysis used is detailed in the following chapters.

Chapter Three: Social sensors; validation of a local-position system for reporting proximity events in dairy cattle

3.1 Introduction

Social network analysis (SNA) has been deployed to better understand animal behaviour, and sociality, across a range of domestic and wild species (domestic: cattle – e.g. Boyland *et al.,* 2016; pigs – Durrell *et al.,* 2004; hens – Abeyesinghe *et al.,* 2013; wild: dolphins e.g. Lusseau, 2003; Tasmanian devils - Hamede et al., 2009). Captive or domestic animals are often kept in social groups, which may be disparate from the aggregations formed by the same species in the wild. Social network analysis may therefore be a useful tool to assess the degree of social structure present in these settings. Such an approach may potentially highlight social bonds, and further understanding of the roles these bonds play in a herd can inform management practices, and serve as an indicator of change within the environment, or individual (Croft *et al.,* 2008).

One approach commonly used to investigate animal sociality is the 'gambit of the group' – namely the assumption that individuals spending time in locality to one another are associating. The use of such proximity (or membership of the same group) as a proxy for association has previously been applied to several ungulate species, including equids (e.g. Tong *et al.,* 2015), goats (Godde *et al.,* 2015), giraffes (Carter *et al.,* 2013) and in dairy cattle (Boyland *et al.,* 2016; Gygax *et al.,* 2010). This method has several advantages over defining relationships based on direct social behaviours. The main benefit is the ease of recording proximity compared with specific interactions (Croft *et al.,* 2008), as there is much less detail required for recording the former. Additionally, the distance between individuals can be remotely measured – for example via a local positioning system (e.g. Neisen *et al.,* 2009a), or proximity loggers (e.g. Boyland *et al.,* 2016) – whereas subtle behaviours such as licking, grooming or even head-butts have not (to the authors knowledge) successfully been automatically recorded. When such technologies are applied, large, continuous datasets of social proximities can be produced, reducing the problems of missing data points in direct observations, and allowing for more detailed analysis – for example using the total time spent associating, rather than a binary ‘yes’ or ‘no’ to group membership (James *et al.,* 2009).

One aspect which may affect the suitability of using proximity as an indicator of association is appropriate selection of inter-animal distance. This may be based on the species being studied, or the biological question posed (Farine, 2015). When considering proximity as a proxy for direct social exchanges, the choice of distance is made somewhat simpler; by this assumption the animals must be in reach of one another in order to perform the interaction. Based on species, length of limbs/body and other factors, this distance can reasonably be calculated. In contrast, when investigating tolerance, or non-contact interaction networks, thresholds for inter-animal distance become harder to define. However, we can again draw on knowledge of the species-specific behaviours, along with environmental factors to justify selected distances (Carter *et al.,* 2015).

Previous authors investigating the sociality of dairy cattle have set criteria in the range of 1.5 – 2.5 metres as a between-individual distance constituting a proximity encounter (Boyland *et al.,* 2013; Neisen *et al.,* 2009b). Inter-individual distances of this size (and greater) as employed in the present study may well detect direct social interactions such as grooming, but will also report animals simply standing close together. As such, the data is only suitably applied in the assessment of tolerated proximity, not as a representation of social engagement.

Several technologies have been applied to monitor proximities of animals, including proximity loggers, radio-telemetry devices and local/global-positioning system collars, providing either real-time, or logged data (see Krause et al., 2013 for a recent summary). There are pros and cons associated with each technology – for example the high cost of GPS

(Nadimi *et al.,* 2008) and the short battery life of some local positioning systems (e.g. 6 days – Neisen *et al.,* 2009a). The local positioning system (Oms500®) used in the data collection for this thesis has its own positives and negatives, but aims to strike a happy balance of acceptable accuracy and reasonable battery life to allow sensor changes to fit in with management events (without external batteries reported as 21 days, with external batteries much increased). However, the system employed has not previously been utilised in the creation of social proximity data, making validation of its' performance an important step in creating reliable data for exploration of cattle sociality (Rutz *et al.,* 2015). Previous authors have highlighted the importance of validating systems for social data collection (e.g. Boyland *et al.,* 2013), with individual sensor performance and sensitivity of proximity recognition two issues to be addressed. To this end, the overall accuracy of the sensors in reporting cattle position will be assessed, along with the suitability of producing proximity matrices from the sensor data, using a variety of criteria.

3.2. Methods

3.2.1 Animals and housing

The detailed information regarding animals, housing and sensors is described in chapter two. In brief, the study was conducted between July and December 2014 on a commercial dairy farm in Essex, concentrating on the high-yielding management group, fluctuating between 86-116 individual cows. The group were housed in a cubicle shed, with 98 useable cubicles, and a commercial mixed ration fed twice daily at an open feed-face spanning the length of the barn. Milking occurred three times daily in a tandem parlour, and cattle were collected from the shed for this event by a member of the farm staff.

All cattle in the group wore OMS500 sensors for the majority of the trial; initially this involved placing neck collars with attached sensors on any uncollared cows, and then replacing sensors based on remaining battery life. Full details of the OMS500 system are described in the general methods chapter; they contain an accelerometer, magnetometer, and work as a local positioning system to triangulate individual cow location. Cattle requiring collaring/sensor changes due to diminished battery life were selected from the group during milking, and collared in the artificial insemination pens adjacent to the parlour.

3.2.2 Sensor data

The OMS500 sensors reported location (along with the aforementioned other outputs) at 10 second intervals, in the format of an X and Y co-ordinate. Over a twenty-four-hour period, the system continuously collected these reported positions, and the output at days' end (or after a manual restart of the sensor system) was a comma-separated value (csv) file. Along with a list of cow ID's, and corresponding sensor numbers, this dataset allowed for the creation of proximity matrices, as described below.

3.3.3 Data collection

3.3.3.1 Social behaviour

Social behaviour was continuously recorded by a single observer (HH), for a minimum of 45 minutes in twenty-four cattle. The cows were chosen to cover a range of parities and AHDB mobility scores (from score 0 – 2). The observer stood either in the feed passage, or within the cubicle area, dependent on the location of the focal cow. A distance was maintained to ensure no disturbance was caused to the focal cow or her near neighbours, in order to minimise impact on social interactions – this distance was based on a lack of behavioural response from cattle to observer presence but was a minimum of around four meters. Cows were continuously sampled for any incidence of social behaviour (see table 1 in chapter one for description of recorded behaviours), their rough position was also noted throughout the observation period on a map of the shed, creating a visual plot of movement and time spent in each location. Finally, when at the feedface, the nearest neighbours of the focal cow (and their rough location judged by upright posts) was recorded. In total, 758 useable interactions were produced, across 17 dates.

3.3.3.2 Position validation

On days selected from August to November, the positions of cattle in cubicles, and at the feed-face, were recorded for validation purposes. The start and end times of observations were recorded, along with cow ID, and in the cubicle observations, which bay was in use. For both the feed-face and cubicle validation, 73 unique cows and 101 individual positions were recorded, across ten and seven days respectively.

3.2.4 Data sorting

3.2.4.1 Observations of cow trajectories

Custom code in the program ‘R’ (3.3.0, 2016) for the creation of visual plots of cow trajectories was provided by EC, and adapted by HH to add delineations of shed features. Two days where there were no unplanned system stoppages, no sensor changes and no other known abnormalities (i.e. large scale farm events), were selected. On these dates, 207 sensor days-worth of data were available for observation, comprising 104 unique sensors. Using the X and Y axis positional output from the system, R3.3.0 was used to plot smoothed video trajectories of cows over a barn map. Manual observation of trajectories was carried out to assess for accuracy. Accuracy was based on several criteria:

- 'Noise' in the data: the sensor rapidly moving around in an erratic motion, clearly not representative of possible cow movement
- Performance of known movement at set times: passing through the milking parlour thrice daily, at around 6am, 2pm and 10pm
- Location of the sensor: with a small margin of error this was required to be within the area of the shed available to the management group of cows

Plots for short proportions of the day were drawn, observed for feasibility, and if necessary, shorter time periods were re-drawn, or watched as a video format. This allowed for detection of sensors getting 'stuck' – not moving for periods of time (as occurred sporadically around the parlour entrance). After plot observation, details of error, or perceived accuracy, were recorded alongside the system-reported total number of social proximities in the day (see below for classification of these proximities), for later comparison.

The start and end times of any anomalies were recorded, and a total erroneous time estimated for the sensor day. The sensor days with accuracy issues were then divided into three categories: 'short out' – where inaccuracy of sensors encompassed less than 2.5 hours, 'long out' – where inaccuracy of sensors encompassed more than 2.5 hours, and 'erratic' – where there was a lot of sensor 'noise', usually this occurred for more than 2.5 hours.

3.2.4.2 Sensor-based proximities

The 10 second interval sensor-reported positions were used to create matrices of proximity between sensors (and therefore cows). The protocol for this matrix creation was to consider rolling two minute windows of data (containing 12 sensor-reported positions). A set radius was defined around the individual sensor, within which another sensors presence would be considered a proximity event. A ‘tolerance level’ for the error, or actual movement, of reported sensor positions within and beyond the radius was also set. This comprised a percentage of reported positions required to fall within the set radius, across the two minutes (from 50% to 70%). This was varied as described in the following table. The minimum 2m radius was selected as a reasonable distance as it is similar to a cow length, and also mirrors selections by previous authors (e.g. Boyland *et al.,* 2016). The range of protocols is aimed to combat the variance in accuracy and precision of sensors on cows – the averages of which are displayed in table 2 in chapter two. Harris *et al.* (2007) employed a 5m radius, with 67% threshold, similar to the maximum distance/threshold within the present study

Table 3; nine combinations for creating proximity matrices from sensor data, by varying radius and percentage of sensor-reported positions falling within that radius (in a 2 minute smoothing window)

Combination	Radius (m)	% positions within radius
1	2	50
2	2	60
3	2	70
4	3	50
5	3	60
6	3	70
7	4	50
8	4	60
9	4	70

3.2.4.3 Proximity validation

The cubicle, feed-face and direct social observation data allowed for validation of sensor-reported proximities, based on known cow positions. A schematic of the barn was created in the programme AutoCAD, and the cow's sensor position during resting or feeding behaviour was estimated based on a mid-point within the cubicle width, or from a diagrammatic representation of the cow's position between barriers at the feedface. From the cubicle data, pairs of cattle which were reported in adjacent, or directly opposite, bays were considered as a proximity event. At the feed-face, the nearest neighbours of cattle were considered a proximity event.

Of the ten days feeding proximities were observed, six were useable after removal of poor sensor data/days with system restarts or recent sensor changes. From the original proximity events, this left 44 proximities for validation purposes, across 52 individual cows (each proximity event consisting of two cows). From the cubicle position observations, six useable days remained after applying the rejection criteria detailed above, comprising 45 unique cows, and 36 proximity events. The feeding proximities were between five and eleven minutes long, and the cubicle proximities were mostly ten-eleven minutes long, with two shorter (five and seven minutes) observations.

Of the direct social interactions, six days were selected where sensor data generally appeared accurate. Data was removed in eleven cases, from sensors where observed interactions did not match with sensor proximity, and a recent sensor change had occurred (e.g. if the observed interaction was an hour after milking, and the cow had required a collar change during that milking). In addition, seven interactions were removed where the sensor was changed shortly after the interaction due to low power, as sensors were known to reduce in reporting efficiency when running on reduced battery. This left a total of 323 observed interactions, ranging from very short encounters (e.g. pushing past in a corridor) to prolonged licking or agonistic encounters.

The time of cattle proximity, recorded as a direct social interaction, or based on two cows in proximity (as defined above) at feed or in the cubicles, was converted into “sensor time” for comparison with sensor reported times of proximity. The reported time provided from the sensor data was given as a ‘mid-point’, with an accompanying bout length time to calculate the beginning and end time of an interaction. A ±1 minute buffer around the interaction start/end time was added, due to the format of the sensor time; as there was a 2 minute minimum bout, the exact time could not be identified.

Once the two times had been compared, the interaction was marked as a match, or failing to match. This therefore represented an outcome of a ‘true positive’ classification of an interaction - where the sensors reported proximity coinciding with an observation of the same, or a ‘false negative’ – where the sensors detected no proximity event when one had been observed. Using this information, it is possible to calculate the sensitivity of the classification;

$$\text{Sensitivity} = \frac{\text{True positives}}{\text{True positives + False negatives}}$$

3.3 Results

3.3.1 Validation via observed trajectories

On the selected days, the number of proximity events per cow ranged from 146 to 646, with an average of 399 - prior to elimination of poor sensor data. Cows with less than 226 proximity events all showed accuracy issues via video observation, including sensors outside the shed, sensors “stuck” at a location and impossible trajectories to/from the milking parlour. Sensors reporting less than 300 encounters were also generally unreliable, with similar accuracy issues, and as such were rejected. The percentage of sensor days with issues, and categorised by type, are displayed in table 4 below (terms defined in 3.2.4.1 above). The division of 430 was chosen as it appeared that sensor accuracy generally improved beyond this threshold. Cows with particularly high encounter numbers did not appear to be inaccurate, suggesting more social encounters as opposed to sensor error.

Table 4: breakdown of quantity and classification of sensor inaccuracies

No. of proximity events	% and no. of erroneous sensor days	Error classification		
		Short out	*Long out*	*Erratic*
0-300	98% (40/41)	15%	40%	45%
301-430	65% (54/83)	46%	41%	13%
430+	19% (16/83)	88%	6%	6%

3.3.2 Sensitivity of sensor-reported proximities

The sensitivity was based on producing the nine combinations of radius/% of positions alterations described in table 3

Table 5: sensitivity of classification of social proximity in cubicles, at the feed-face, and in direct social interactions

Observation (%)	**2m 70%**	**2m 60%**	**2m 50%**	**3m 70%**	**3m 60%**	**3m 50%**	**4m 70%**	**4m 60%**	**4m 50%**
Cubicles	50.0	52.8	58.3	69.4	69.4	75.0	77.8	88.9	91.7
Feeding	27.3	34.1	38.6	52.3	54.5	61.4	68.2	68.2	86.4
Interactions	28.4	34.1	37.5	53.6	56.7	61.9	69.3	72.8	76.2

The sensitivity was best (using all classification methods) for the cubicle observations, and worst for the direct interactions. The 4m 50% method provided good accuracy in all scenarios.

3.4. Discussion

The results of the trajectory observations indicate that most sensors (81%) reporting over 430 proximity events in a day have a good level of sensitivity, leading to data useful for further investigation of location/proximity in cattle. Of the 19% with accuracy issues, only 2 sensors had either long periods of error, or erratic positions reported, with the rest containing only short (less than 2.5 hours) total portions of error. It should be noted that the timings are not exact due to the nature of the data (i.e. observations of a smoothed trajectory rather than position at exact timepoint) but using the custom code, sections of perceived error were ‘trimmed down’ as close as possible to the start and end time of infeasible movements.

The above results allow for the creation of a validation and rejection criteria for the rest of the dataset. All sensors reporting lower than 430 proximities a day will be manually observed (for the two following chapters) as plots to assess whether they have 2.5 hours (or greater) error. These will then be retained, or removed from the dataset if they contain less, or more error respectively. From the dataset used for this chapter, 65 out of 207 sensor days would be removed (31%). The quantity of 2.5 hours inaccurate time is considered acceptable as, at worst, only around a tenth of data will be missing, and in many observed cases the estimated total erroneous time was lower. Additionally, as the inaccurate positions are reported outside the area occupied by the collared management group, the sensor will not be reporting proximity events which did not occur (as it will not be near other sensors). Instead, it may be the case that some social proximities are missed during these periods. As a relatively small period will be lost, there should be limited bias between sensors with inaccuracies, and those reporting correctly throughout the day. As there is no current systematic way to deal with missing data/individuals in social networks, the potential impact of any difference must instead be considered in the interpretation of the results (James et al., 2009; Farine and Whitehead, 2015).

Whilst a few poor sensor days may be present in the 430+ social proximity events sensors, the risk is much lower, and these are likely to be within the occupied area of the shed – or the total number of proximity events would be lower. Due to this reduced ability of the observer to detect error, sensors reporting over 430 proximities will not be manually observed, but assumed to be accurate. The nature of the analysis to be performed on the data will incorporate several days of information, reducing the effect of any anomalies. Social networks in general are built on small, observed datasets, with many interactions passing unreported (Boyland *et al.,* 2013). Therefore the large dataset which will form the basis for the following chapters is an improvement on such small networks, which represent 'snapshots' of behaviour rather than continuous activity.

The higher sensitivity of proximity classification for cows in cubicles was likely due to the nature of the observations. Cubicle observations were mostly ten minutes or longer, with more short observations at feeding (and many short proximities in the direct social interactions). The system therefore had a longer period (in most cases) to accurately report two sensors as being in set locations, and in proximity of one another, in the cubicle area. Another likely reason is the nature of the activity carried out in the cubicle zone. Most cattle inhabiting this area were lying, or standing in the cubicle bays – sedentary activities which are unlikely to result in much sensor movement. This would again improve the chances of sensors reporting a consistent position and proximity, in comparison to the feedface, where cattle would be moving their heads up and down whilst eating, and potentially shuffling within the area whilst sorting feed. Given this knowledge, it will be important to consider that proximities in the cubicle area/during sedentary activity are more prevalent in any social networks built from the data, and that associations in other areas/activities may be under-represented. Previous authors have encountered similar issues with varying representation of interaction across functional areas of the enclosure – again finding that proximities in lying areas were well correlated between sensor, and observer reports (Gygax *et al.,* 2007).

Due to the use of a two-minute smoothing window, the 10 second refresh rate is effectively reduced to one data-point per two minutes. This can be justified via a number of means; initially by the four-metre radius, and 50% threshold providing for some movement within that two-minute window (i.e. stepping towards/away from one another). Neisen *et al.,* (2009b) investigated various sampling intervals, and their effectiveness at representatively reporting the neighbours of dairy cattle. They concluded that, given the generally slow-moving nature of the cows, and varying levels of activity across resource areas (e.g. lying areas, feed), a minimum two-minute sampling interval was recommended. The smoothing window employed in the current study is therefore likely to adequately sample any proximity events between dairy cattle. The combination of the 50% data points in the radius and 4m inter-animal distance means that some short proximities, lasting one minute, may also be captured. Additionally, cows which are just over 4m apart may also register within this classification, if their sensors both report slightly inaccurate (and closer) positions for one minute or more. Given that the analysis within this thesis will focus on compiling days of data, and investigating mainly preferred conspecifics where larger swathes of time are spent together, these small discrepancies should not pose a problem with analysis – however this issue may make this classification of proximity inapplicable in other applications.

There is conflicting evidence regarding the link between maintenance of spatial proximity and interactions. Individuals which are a short distance apart may be directly (and either non- or reciprocally) interacting, or may simply be close and not socially engaged. Equally, animals beyond this set distance apart may be interacting via non-contact means, for example vocalisations, visual or olfactory displays. Examples of these options are present in the literature. In rhesus macaques, the social connections observed through affiliative interactions (grooming) match well with the social network as formed by social proximities, and indeed the relative distances between animals during a one-hour period accurately reflect the interactions within the group (Feczko et al., 2015). However, given a lack of evidence for such an association in cattle, the maintenance of proximity will be considered as animals choosing, or tolerating the presence of their neighbour, rather than being indicative of actual interaction.

A final consideration of this dataset prior to analysis is the potential presence of 'false positive' proximities – i.e. those which are reported, but did not actually occur. Due to the nature of the data collected, it has not been possible to report the proportion of false positives made by the proximity classification. This omission is important to note, however the existence of a high number of false positives is unlikely given the validation of the data to eliminate poor accuracy. Validations of other local position systems for measuring proximity have not directly compared a known interaction with the same section of time of sensor data, but instead correlated matrices built from direct, and sensor observations (Gygax *et al.,* 2007). False positives may therefore be present and undetected in the data, but the authors considered the method acceptable (due to the good correlation of the matrices).

3.5 Conclusion

The comparison of sensor trajectories and number of reported proximity events indicates that thresholding the latter at 430 (proximity events per day) is a useful, and applicable method for eliminating erroneous sensor days. The validation of sensor reported proximities against direct observations shows that the LPS has sufficient positional accuracy, and reporting rate, to represent proximity events ranging from short interactions, to lengthier associations, with a reasonable degree of accuracy. Therefore the proximities defined from position given by the OMS500 system are suitable for taking forward to create social networks, as will be applied in the following chapters, by applying the validated 4 metre 50% threshold.

Chapter four: The social structure of a housed dairy herd

4.1 Introduction

Wild and feral cattle form small herds of females, calves and transitory males (Keeling, 2001), reaping the advantages of group living such as lower predation risk, and improved ability to forage (Silk, 2007). Domestic dairy cows tend to be kept in large management groups comprised solely of adult females, therefore social dynamics are likely to be altered in comparison to wild groups (McLennan, 2013). The size of dairy herds in the UK is increasing – from an average of 97 cows per herd in 2004, to 133 in 2014 (England – AHDB, 2016), and along with the pressures of farm management (e.g. feeding system, stocking density, group mixing) the cows social situation may be increasingly challenging. The structure of such cattle groups is of interest from a variety of perspectives, firstly in assessing the bonds and value of certain conspecifics to individual cows. This may be useful for herd management, in terms of understanding the production effects of mixing animals, or disease transmission between groups. Alongside this, social behaviour may (along with other behavioural indicators), serve as a warning sign of illness (e.g. social isolation during mastitis –Proudfoot et al., 2014), proving valuable from a management, health and welfare perspective if it allows disease to be treated promptly and reducing veterinary costs.

In order to infer changes to social structure based on disruption (e.g. illness or introduction of new cattle), the general social network of a herd must be understood. Social network analysis (SNA) is a tool which is gathering increasing interest in the field of animal science; this is a method of investigating relationships between individuals and whole groups of animals (Koene and Ipema, 2014). It has been applied across wild and domestic species to answer a range of questions, from the role of background in sociality (e.g. Levé et al., 2016) to the assessment of preferential partnerships (Abeyesinghe et al., 2013). This method has previously been employed to study cattle sociality, with authors describing the social structure of dairy cow herds with access to pasture, or housed in cubicle sheds (Gygax et al., 2009, 2010; Boyland et al., 2013). The aforementioned studies noted little ‘group level’ structure; in that there was no evidence of communities within the larger herd, and the majority of individuals associated with other herd members during the course of the study. However, both authors recorded non-random associations at the ‘individual level’ – i.e. cows preferentially spending time in proximity to specific herdmates. The existence or absence of group level structure may potentially be greatly affected by housing constraints (Tresoldi et al., 2015), for example the maximum distance individuals may maintain from one another in a confined environment, and it is therefore of interest to investigate this area on a farm-by-farm basis.

The employment of a sensor incorporating a local positioning system, along with an internal accelerometer, may provide a solution to a range of cattle-monitoring requirements. In addition to recording a daily pattern of movement and behaviour, position can be used to infer cattle proximities, and build social networks. The following study attempts to fulfil this brief, assessing the application of a novel, multi-function sensor to investigate broad and individual structure of cattle sociality within a permanent-housed dairy herd. Whilst previous authors have employed a local positioning system to social network creation (Gygax et al., 2010), these comprised six-day datasets from smaller herds (24-43 cows), some with pasture access. The present study poses the question of the benefits of a longer-term data set (a month), from sensors capable of a range of cow monitoring functions, to accurately reflect social relationships in intensively managed dairy herds.

4.2 Methods

Detailed descriptions of animals, housing and the local positioning system are presented in chapter two. They are described, in brief, below.

4.2.1 Animals and housing

The automated monitoring system described below was employed on cattle from July to December 2014 at a commercial dairy farm in Essex. A fluctuating, high-yielding management group of between 101-112 Holstein dairy cattle was observed for the course of the study, with cows moving out of the group for drying-off, and entering after calving. Animals were permanently housed in a cubicle shed with twice daily provision of a total mixed ration, and thrice daily milking. The total shed measured 30 m x 58 m, of which the high yield group had half, and contained 98 useable cubicles (geotextile mattress topped with sawdust). Cows could move freely through the shed and mix with other members of the group in the passageways and at the feed-face. Data regarding health issues, calving date, parity, lactation number, milk yield, somatic cell count and days in milk were gathered on-farm from the management diaries, the CIS system online and Uniform Agri.

4.2.2 Local positioning system

OMS500 sensors were deployed in boxes attached to webbing cow collars, encased in a damp-proof casing. The collars were equipped with attached weights to maintain sensor position on the neck and in some cases, with additional external batteries (AA) to prolong sensor active life. ‘Fixed sensors’ (in waterproof housing, with four ‘D’ batteries attached externally) were placed at strategic positions throughout the shed to maximise receipt of signal from on-cow sensors. The on-cow sensors reported animal location via a local positioning system (based on a wireless mesh network), along with a peak and mean accelerometer output for each reporting period. The sensors were set up to report every ten seconds throughout the trial, 24 hours a day, with a brief loss of data during system restart at midnight.

4.2.3 Data handling

For the purposes of this chapter, the month of October was selected for network creation. This was due to the fairly stable temperature across the month (in comparison to other trial periods), potentially important due to the effect of ambient temperature on housed cattle behaviour (Overton et al., 2002). October provided an extended period with minimal data loss across the whole sensor network (i.e. few days in which the entire system performed poorly). After elimination of these whole-system failure days, a total of 21 days were available for further analysis. Utilising the X and Y positional data of each unique sensor, relative proximities of cows were inferred; sensors within a four-metre radius of the focal sensor, for more than 50% of time points in a two-minute window were classified as a proximity event (details of the selection of this protocol are provided in chapter three). Weighted proximity matrices were constructed from this dataset; each matrix was symmetrical (i.e. undirected proximities). This process was carried out using custom code in Matlab R2015a, and the computed matrices were then transferred into Excel spreadsheets for processing.

Data were assessed as described in chapter three in order to eliminate inaccurate sensor days; in brief a trajectory of sensor movement was observed for any nodes reporting below a threshold of proximity encounters across a 24-hour period. Due to the sporadic nature of lost data points (i.e. one sensor could have several accurate days interspersed with one poor accuracy day), averages were used to maximise network size. This consisted of averaging the sum of the two-minute proximity encounters between cows, across three day periods (leading to seven matrices from 21 days), and then summing these matrices to create the final network. Upon completion of this stage, a total of 56 cows had relatively consistent data, from a minimum-maximum of 101-112 collared cows per day (and an average of 106). Of the 56 cows, most had some data loss across the seven networks (e.g. no concurrence between that individual, and a specific other within one of the seven networks), however this was never more than 10% data loss (on average less than 1%). The data loss appeared randomly spread throughout the group, and the networks were therefore considered suitable to take forward for analysis. A large proportion of studies into the social networks of animals will have incomplete data, whether through individuals present, but not included in the network, or un-observed interactions, however this data can still be put to valid use in the creation of networks, and in answering biologically relevant questions (Silk et al., 2015).

In order to assess group structure and preferential relationships, a series of thresholds were applied to the network. Previous authors have filtered animal social networks in a number of ways, for example by the number of days on which individuals were noted together (Croft et al., 2004), simple ratio and half weight index (indicators of the proportion of sampling periods in which individuals were seen together - Farine and Whitehead, 2015) or by the average number of associations between individuals (Durrell et al., 2004). For the present network, the latter seemed an appropriate measure to provide highest resolution data due to the constraints of the housing environment, where it is likely that individuals will come into proximity with one another via mechanisms other than choice, potentially daily. This is based on intensive management of the cows during events such as milking, the high stocking density of the shed and the observed gathering of cattle at key resources such as the water troughs. Associations over a certain threshold have been considered ‘preferential’ by previous authors, with a selection of twice the mean interaction rate commonly chosen (e.g. Durrell et al., 2004). As no cattle in the present network had associations of this level, a 1.5 times the mean was selected as an alternative (also used by Boyland et al., 2016).

The 56 cow network was therefore filtered within Excel and UCINET 6, to produce three matrices:

1) **Original:** the original unfiltered network
2) **Mean:** with a filter of the herd average dyadic weighted degree per day (i.e. total time spent together per day; equivalent to an average of 1 hour 3 minutes a day)
3) **1.5x mean:** a filter of 1.5x the herd average dyadic weighted degree per day (equivalent to an average of 1 hours 34 minutes a day)

4.2.4 Analysis

Analysis of social network data requires the permutation of matrices or social network measures. This is due to the lack of independence in the data; as within a matrix an individual appears in both rows and columns, and additionally the proximity of A-B, and B-A are directly related (in fact the same value; Croft et al., 2008). As such, normal statistical programmes are not suitable, instead the program ‘R’ (version 3.3.0, 2016), and the social network analysis package UCINET 6 were employed.

4.2.4.1 Network level

Network measures were calculated within Ucinet6, comprising mean degree, density and components. Degree is the number of other individuals within the network a cow has proximity encounters with, and the mean degree is the network average. Density is the proportion of 'ties' between individuals that exists, out of the total possible number of ties. A component is a connected set of individuals, linked either directly, or via other members of the network (Croft et al., 2008).

Sociograms (visual representations of the network members and ties) were created in Netdraw, where a *k*-cores analysis was also applied to the mean network – this is the division of the network into subgroups connected by a minimum of *k* members (Hanneman and Riddle, 2005). A quadratic assignment procedure (QAP) correlation was performed on the seven 'average over three day' matrices filtered by average dyadic weighted degree, with 50,000 permutations (as in Abeyesinghe et al., 2013). A QAP is a resampling method which correlates two matrices, then permutes one of the pair a given number of times, and re-correlates the matrices. The test then calculates how often the permuted results match with the observed values, and produces a p-value that indicates how likely the result is to have occurred by chance. A Bonferroni correction was applied to the results due to the multiple comparisons being performed on the same dataset.

The E-I index was also produced in UCINET 6 (the tie strengths external to the attribute group (e.g. parity, stage of lactation), minus the internal tie strength, divided by the total tie strength) from the mean network data, to assess the level of homophily or heterophily (assortment by type – either choosing similar or dissimilar group-mates respectively) within the network.

4.2.4.2 Individual level

The degree (number of cows an individual associated with) and weighted degree (the sum of proximity occurrences between dyads) of the cows in each network were calculated in

Excel. Using the mean network degrees, a one-way analysis of variance (ANOVA) with 10,000 permutations was applied to the degree by parity group (parities 1-3 were assigned to those respective groups, and parity 4-8 were grouped together), and the degree by days in milk was analysed via a node-level regression (again with 10,000 permutations) in UCINET 6. A regression was chosen for the days in milk data due to the continuous nature of the values, as opposed to the categorical nature of parity.

The weighted degree and degrees from the two filtered networks were assessed for normality, and the weighted degree and degree from the mean network were correlated (to determine the need to utilise both measures in analysis).

4.3 Results

4.3.1 Network level results

4.3.1.1 Network visualisation and descriptors

The three networks are visualised in figure 3, the sociograms show the connections between all cows which came into proximity across the 21 days (and those connections that remained once filters were applied in the latter two graphs). The edges are un-weighted and the nodes are coloured by parity group. The graph layout was drawn using ‘spring embedding’ – a method which runs random layouts until a best fit is found, where nodes of the shortest path length are closest together. These sociograms highlight the variance in individual positions within the social network (and therefore overall sociality), with some individuals having very few connections within the herd at higher filtering thresholds, and other animals holding more central, highly connected positions. For example in figure 3c, cow 2533 is poorly connected and peripheral, whereas cow 3304 has more connections and therefore sits more centrally in the network.

In the unfiltered network, weighted degree (a direct representation of time a dyad spent together, as a sum of all proximity encounters) may be useful when assessing preferences and structure. Some descriptive values for the network are displayed in table 6.

Table 6; weighted degree and time values of the unfiltered network (to the nearest minute)

Weighted degree	Time
Mean	12330.51 (411 hrs 1 mins)
Range	8313 - 16057 (277 hrs 6 mins – 535 hrs 16 mins)
Mean per ‘day’	1761.5 (58 hrs 43 mins)
Mean per dyad (total)	220.19 (7 hrs 21 mins)
Mean per dyad (per ‘day’)	31.5 (1 hr 3 mins)

A)

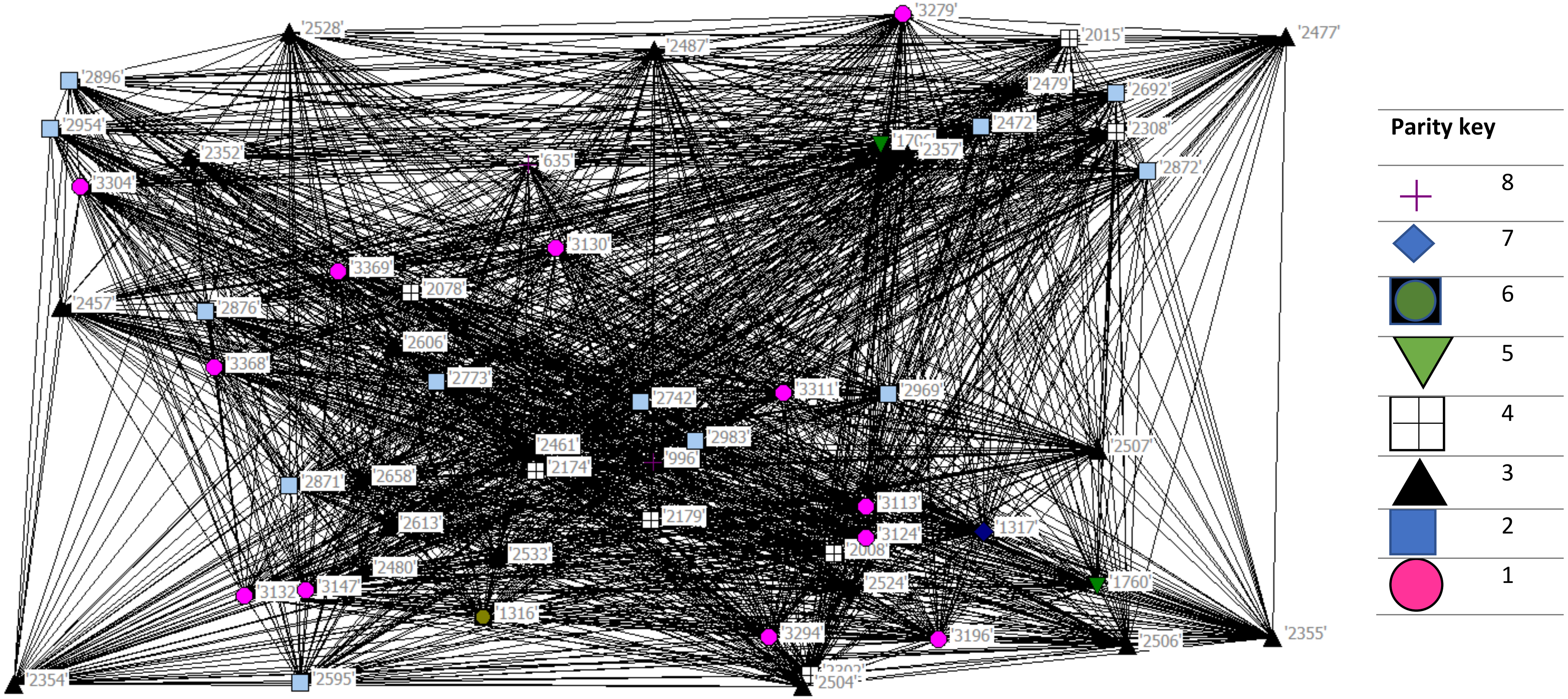
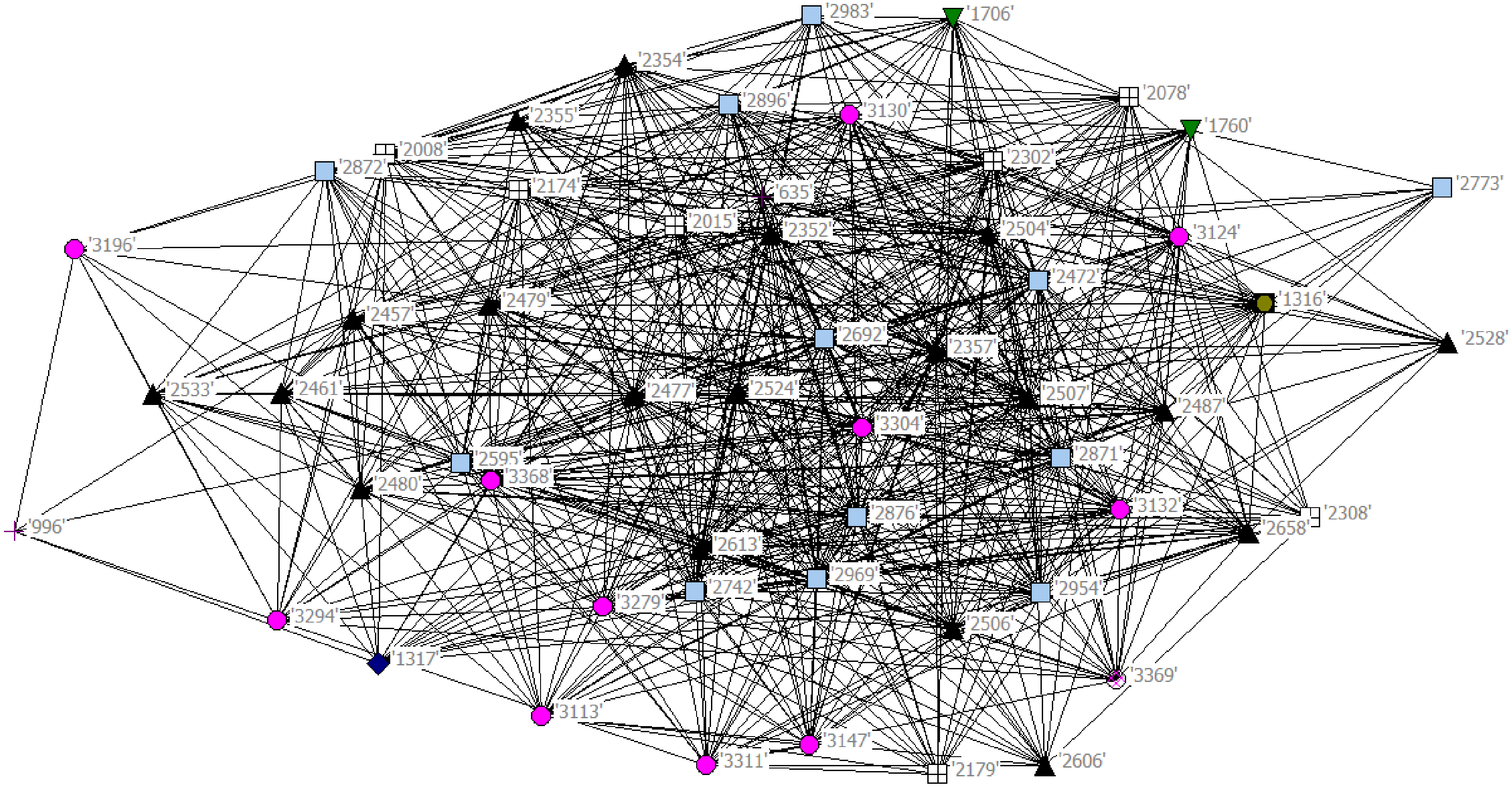


Figure 3; unweighted sociograms of the 3 networks, node colour and shape set by parity (see key). A) is the unfiltered network, b) is the mean network, and c) the 1.5x mean network (including isolates – unconnected nodes)

B)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'2078'
'2896'
'3130'
'1760'
'2355'
'2008'
'2302'
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'2479'
'2457'
'2692'
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'2528'
'2533'
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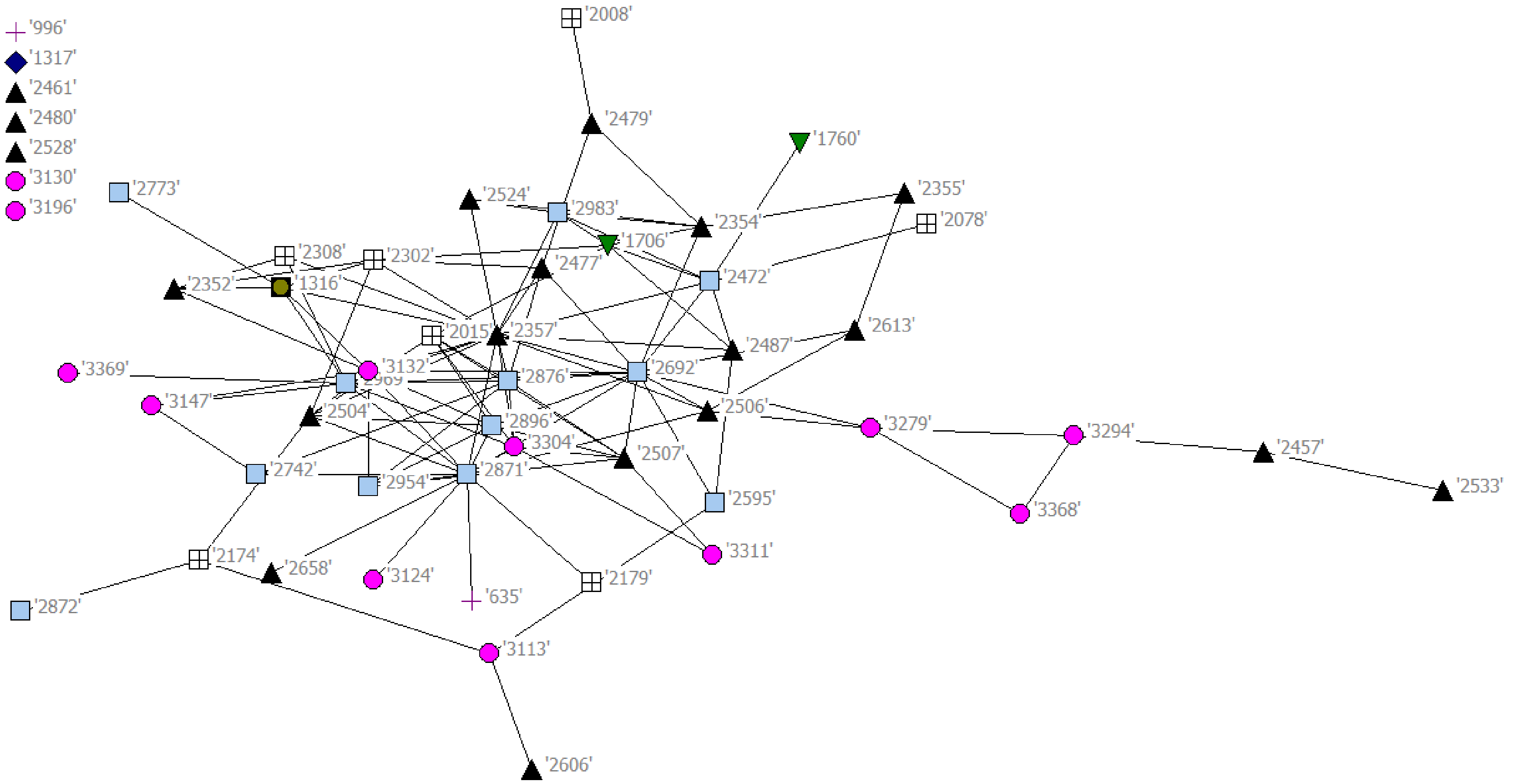
'2179'
'2606'

c)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'3311'
'2174'
'2658'
'3124'
'2179'
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4.3.1.2 Network measures and structure

All three networks consisted of one large, connected component, however in the latter two filtered networks, only 7% and 49% of the possible connections between individuals existed, as opposed to a completely saturated network when unfiltered (see table 7). The 1.5 x mean network consists of 8 components; one large connected group and 7 isolates (see figure 3c)

Table 7; network-level measures of the 3 networks

Network	Mean degree	Density	Components
Original	55	1	1
Mean	26.8	0.49	1
1.5 x mean	3.9	0.07	8

Despite the lack of apparent structure based on the existence of a single component in the first two networks, there are several '*k*-cores' (connected sub-groups) within the 1.5x mean network – a cluster of nodes connected to at least 19 other individuals within that core, and then other nodes on the periphery connected to a minimum of 5-18 other individuals within their 'cores' (see Figure 4).

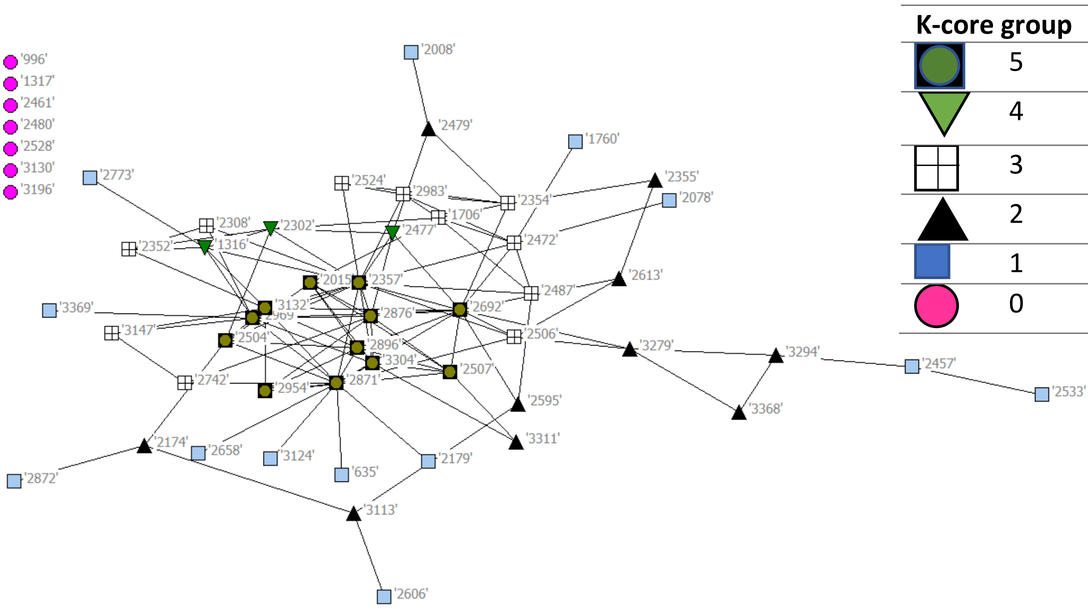


Figure 4; K-cores within the 1.5x mean network, with legend denoting maximum connections within the K-core

A final measure of network structure that was applied was the E-I index. For the mean network by parity group (1-4), the index was 0.45, with the rescaled E-I reported at -0.08 (-1 and +1 representing pefect homophily/heterophily respectively). By days in milk group (<30, 30-130, 131-230 and 230+ days – grouping based on energy requirements at stage of lactation) a similar pattern was seen – the rescaled E-I = 0.05. This re-scaling is due to the constraints of the grouping meaning that the minimum E-I index possible for the dataset is only -0.017/-0.05. The re-scaled result suggests a very slight tendency towards homophily, but it barely deviates from the 'null' assortment.

4.3.1.3 Network temporal correlations

The seven unfiltered networks (the 'average over three day' networks) containing the same 56 cows were all significantly correlated with one another (QAP with 50,000 permutations; p = <0.002 – accounting for Bonferroni correction, r values in table 8), suggesting that the networks were to some extent stable across the observed time period.

Table 8; Pearsons correlation coefficients from QAP comparisons of networks over time

Network	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1	1	0.203	0.157	0.185	0.166	0.168	0.176
2		1	0.23	0.189	0.168	0.148	0.154
3			1	0.22	0.149	0.185	0.14
4				1	0.179	0.195	0.182
5					1	0.215	0.193
6						1	0.189
7							1

4.3.2 Individual level results

The weighted degrees and degrees of the mean network were tested for normality using the Shapiro-Wilks test in ‘R’ 3.3.0, were normally distributed (p>0.05), and therefore suitable for analysis with parametric tests. The degrees from the 1.5x mean network were skewed and therefore unsuitable for parametric tests.

4.3.2.1 Correlation between social network metrics

The weighted degree from the original network was correlated (r = 0.95; see figure 5) with the degree from the mean network. As such, the degree was taken forward for analysis instead of the weighted degree, as it is representative of the former, but less susceptible to fluctuations due to data loss (i.e. it is a binary association rather than considering the quantity of time cows spent together).

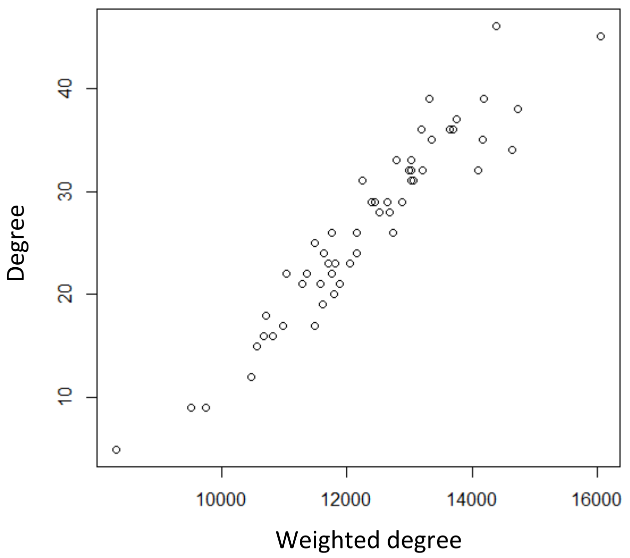


Figure 5; correlation between weighted degrees from the original network (x) and degrees from the mean network (y)

4.3.2.2 Sociality by trait – parity

Parity groups as defined previously (1-3 individually and 4-8 grouped) were compared in terms of average degree.

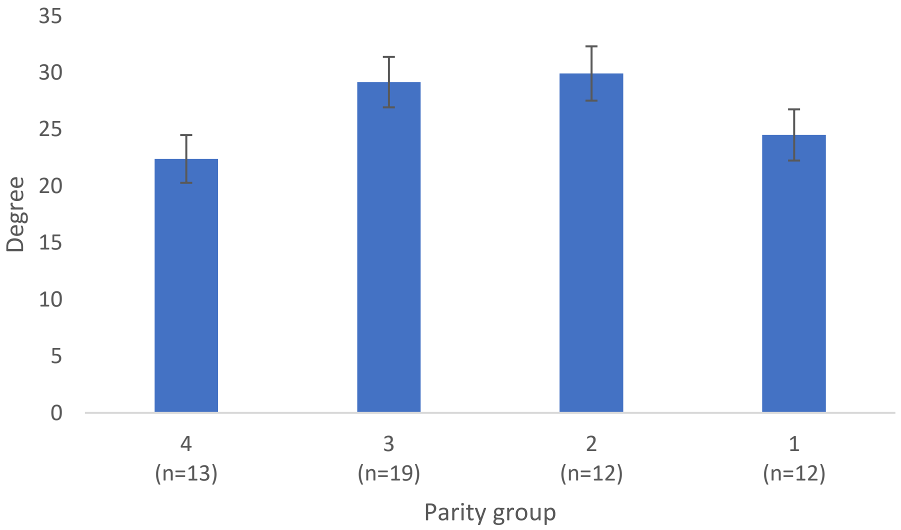


Figure 6; Degree by parity group (mean network)

The degree of cows (from the mean network) was approaching significantly different across parity groups (Figure 6 - ANOVA: $F_{(3,52)} = 2.45$, $p = 0.07$, $r^2 = 0.12$).

Table 9; weighted degree by parity group

Parity group	WD	WD as time
4	11555.45	385 hrs 11 mins
3	12611.12	420 hrs 22 mins
2	12995.39	433 hrs 11 mins
1	12028.43	400 hrs 57 mins

4.3.2.3 Sociality by trait – days in milk (DIM)

The DIM of cattle did not significantly affect their degree (r = -0.036, p = >0.05).

4.4 Discussion

The sociograms and cohesion measures indicate a highly-connected network; in fact the unfiltered network of the 56 dairy cows was completely saturated (every individual had come into proximity with each other herd member). This is perhaps unsurprising considering the environment, which provides a limited space available within the shed, further compounded by the clustering necessary to access key resources (the feed-face, water troughs and cubicles). Additionally, the proximity encounters covered the milking period, where cattle would be in enforced close proximity to other individuals, three times daily (an average 2 hours 35 minutes for non-lame cows – Barker et al., 2018). The method of defining proximity encounters also predisposes a high number of ties, as the 4m radius around each individual is a relatively large area (but necessary due to the error within the sensor position), however the applied filters will remove a large number of the weaker connections within the network.

The findings of this study are in line with previous research into the social behaviour of cattle in confined space – Gygax *et al* (2010) and Boyland *et al* (2010) both reported highly connected networks with little or no further subdivision. Given that the present study was conducted on permanent housed cattle, and some individuals within the aforementioned studies had access to pasture, it is even less likely that sub-structures would be evident in the reported network. This highlights the importance of filtering as a tool for network interrogation (Croft *et al.,* 2005); in order to reveal structure, it is necessary to apply thresholds to cattle interactions.

The sociograms of the filtered networks (figure 3) and k-cores graph (figure 4) begin to show some degree of structure; there are evidently individuals which have a greater number of connections (central within the sociograms), and cows which are have less social ties (on the periphery of the graphs). At the 1.5x mean threshold level, a number of cows have no social ties at all (figure 3c), indicating that not all cattle are equally sociable. Sociability is a highly variable trait in mammal societies (Barocas *et al.,* 2011), and fluctuates within ungulate groups (e.g. Durrell *et al.,* 2004). This is evidenced further by the wide range in individual proximity times – with the most social cattle spending around 1.9x as long in proximity to other herd members than the least social individuals. Cattle vary in the time spent performing, and receiving allogrooming (Val-Laillet et al., 2009), as well as ranging in the amount of agonistic social behaviour they perform (Gibbons et al., 2009), disposing individuals to have a unique level of social interaction. The filtered network ties are likely to be indicative of positive, rather than agonistic, connections, as cows would be tolerating one another's proximity and would be unlikely to remain close to individuals displaying aggressive behaviour (e.g. as seen by cattle moving away from aggressive conspecifics at feed - Proudfoot et al., 2009). Previous authors have linked positive cattle social networks built on observations, to proximity based networks; the two are correlated whereas proximity and aggressive networks are not (Boyland et al., 2016).

The reasoning behind connections and network structure may be valuable information if it is potentially applicable in cow management; however in the present study there was no clear indication of assortment by parity or stage of lactation (using DIM). This is in contrast to the findings of other authors who have found more frequent bonds between cows of a similar parity and milk production (linked to DIM; Boyland et al., 2016, Harris et al., 2007), but may be based on environmental differences, such as the relatively high stocking density in the present study. Despite the lack of evidence for either homo- or hetero-phily within the present network, there was an approaching significant relationship between degree and parity group (p=0.07), with parity 2 having both a higher number of associates, and a greater weighted degree (total time spent with other cattle – see table 9). The youngest and oldest cows (parity 1, or 4+) had the lowest number of associates, and interactions (see figure 6). Beef cows under two years of age have been found to be subordinate to their elders in 98% of cases (Šárová et al., 2013), therefore the lower sociality of first parity cows may be explained by avoidance of higher-ranking herd mates – as has been reported by previous authors (e.g. at feed; Rioja-Lang et al., 2009).

Whilst some studies indicate that cattle up to 16 years of age are more dominant than younger cows (Harris et al., 2007, Šárová et al., 2013), this work has been conducted on beef cattle; the production strain that dairy cattle experience may alter their social dynamics. As shown in figure 6, cows over parity 4 were less social in the present study. Dairy cows of parity 4 or higher are at increased risk of sole ulcers, and the risk of other foot issues also increases with parity (Barker et al., 2009). There is some evidence for reduced sociality and dominance associated with health conditions (e.g. Galindo and Broom, 2000, Goldhawk et al., 2009) so the higher age of these cattle and its associated disease risk, may have lowered their status within the herd. Additionally, older cattle may be less active (e.g. reduced stepping at oestrus in higher parity cows; López-Gatius et al., 2005), reducing their chance of encountering other cattle as they move around the shed. Beyond the measured factors, many other individual characteristics such as familiarity, personality and health status (Raussi et al., 2010; Wilson et al., 2013; Proudfoot et al., 2014) affect sociality of animals, which may explain why so little of the variation in the social proximities of cows in the present study was accounted for by parity and DIM.

A final aspect of the network investigated in the present study was its stability. Each 'three-day average' matrix (filtered by average weighted degree), was somewhat correlated to all other time periods (table 8). This suggests some temporal stability of the network, representative of a repeated occurrence of proximity encounters between cows over time (although there is also a high amount of variance). Additionally, each of the matrixes was most strongly correlated with the matrix either directly previous/after it (see table 8 for pearsons correlation values), indicative of a greater stability in the network across shorter time periods compared to more temporally distant matrices. This degree of stability may be indicative of some preferential partnerships (Abeyesinghe et al., 2013), or a shared preference for location causing repeated proximity encounters – these two are difficult to separate when working with proximity datasets, rather than networks built on direct social interactions (Spiegel et al., 2016). However, previous authors have strengthened the case for association by choice of partner, rather than area (Gygax et al., 2010; Boyland et al., 2016) based on direct social interactions, and assumptions of tolerance/affiliative relationships developing between cows which often meet – even if they initially associate due to area preferences. The dataset from the current study also allows for the subdivision of proximity matrixes into functional area within the shed, which will be explored in a later chapter. This may provide further evidence for or against the social/environmental preference, as networks across area can be compared.

The existence of structure within the network, and consistency of some relationships over time, has welfare implications. If certain cattle preferentially associate, there may be value in these relationships for social support (Rault, 2012), and detriment to the removal of that social partner from the herd (McLennan, 2013). Social stress has been linked to increased risk of disease (Chebel et al., 2016) and disruption of milk production (Torres-Cardona et al., 2014) therefore maintaining social structure is of benefit for cattle welfare, and profit.

It should be noted that the results presented above are effectively a ‘subsample’ of the entire herd, due to the exclusion of cattle for sensor error issues. It is therefore possible that key social relationships are not evident from the available data. However, a large proportion of studies into the social behaviour of animals are formed from incomplete datasets, either due to the low visibility of wild animal populations (e.g. cetaceans - Lusseau, 2003), human observations excluding periods of darkness, or issues with technology (e.g. Boyland et al., 2013). Approximately half the cows present in the cubicle shed (at maximum capacity) were included in the network, and methodological work by Silk et al. (2015) suggests that network measures produced from a 50% subsample of the population, correlate strongly with that from the full group. In addition, the chosen measure of the present study (degree) was resilient to change in partial networks, whereas other measures not employed in the present study (such as betweenness) did not remain representative in Silk’s study.

4.5 Conclusion

The variance in sociality seen throughout the herd suggests that individual behavioural thresholds will be highly important in the assessment of social behaviour as an indicator of disease. If general thresholds are set to determine if cows fall below an ‘average’ sociality, subtle changes in more gregarious animals may not be noticed.The broad social network of this permanent-housed dairy herd indicates that there is some aspect of choice of social partners (whether this is based on individual traits, or shared preference for areas), which will be further explored by investigating sociality across functional areas.

Chapter five: The structure and consistency of cattle relationships across functional area in a permanent housed dairy herd

5.1 Introduction

The social behaviour of farm animals is of interest from biological, welfare and financial perspectives, with the potential to reflect dominance status within a group (Šárová et al., 2016), and health or welfare state (e.g. Proudfoot et al., 2014) allowing for earlier, and more cost-effective treatment of illness. The sociality of individual cows within a herd is highly variable (e.g. Gibbons et al., 2010), which may manifest in above or below average time spent in proximity to other group members. This is reflected in the findings of the previous chapter, where there is shown to be a wide range of time spent in proximity to herd-mates by cattle throughout the environment, affecting the position and centrality of individuals within the social network of the herd.

Whilst this ‘big picture’ information regarding the social relationships within a herd is valuable, greater depth of understanding may be gained by considering the nature of sociality based on activity at the time. This is due to the myriad of factors affecting an individuals proximity to other animals – for example whilst eating cattle are known to avoid spending time close to dominant conspecifics (e.g. eating lower quality feed rather than feed alongside a dominant herd-mate - Rioja-Lang et al., 2009) as food is a valuable resource and liable to cause competition. The value of the specific area or resource to an individual may differ, leading to an altered social behaviour - for example cows which commonly displace others at feed, may not replicate this behaviour when accessing another resource (e.g. a mechanical brush, Val-Laillet et al., 2008), potentially leading to context-specific proximities.

Due to this variance, it may be beneficial to consider separate social networks by activity or area, as considering the relationships between cows across the whole day/environment may be skewed if, for example, proximity to other preferred individuals at feed is potentially hindered by the location of dominant individuals. On the other hand, if specific social relationships are highly valued, we would expect them to be consistent across activities (as their value may outweigh any cost of remaining in proximity). Some evidence exists for these consistent relationships in cattle across the environment; cattle which are preferred grazing partners in an extensive environment are also often allogrooming partners (Reinhardt and Reinhardt, 1981) – however this is based on a small herd, limited observations and may not be the case in larger groups in intensive systems. Gygax et al. (2010) compared aspects of sociality of dairy cattle across functional areas, and overall noted little consistency of relationships between activities. However there was somewhat higher consistency when considering positive, when compared to avoidance, relationships.

Regardless of the consistency of cattle proximities across area, aspects of this data may be useful for the detection of changes in behaviour by cows. If particular functional area networks are more variable or indicative of choice of partners, they may be more valuable in detecting changes in sociality. This can then be applied to consider alterations to an individuals position in the network when experiencing a health issue, change in social environment, or other welfare challenge. This chapter therefore aims to investigate the consistency and the structure of networks between functional areas, in order to better understand housed cattle sociality and it’s potential as an indicator of disease.

5.2 Methods

Detailed descriptions of animals, housing and the local positioning system are presented in chapter two. They are described, in brief, below.

5.2.1 Animals and housing

The automated monitoring system described below was employed on cattle from July to December 2014 at a commercial dairy farm in Essex. A fluctuating, high-yielding management group of 101-112 Holstein dairy cattle was observed for the course of the study, with cows moving out of the group for drying-off, and entering after calving. Animals were permanently housed in a cubicle shed with twice daily provision of a total mixed ration, and thrice daily milking. The total shed measured 30 m x 58 m, of which the high yield group had half, and contained 98 useable cubicles (geotextile mattress topped with sawdust). Cows could move freely through the shed and mix with other members of the group in the passageways and at the feed-face. Data regarding health issues, calving date, parity, lactation number, milk yield, somatic cell count and days in milk (DIM) were gathered on-farm from the management diaries, the CIS system online and Uniform Agri.

5.2.2 Local positioning system

OMS500 sensors were deployed in boxes attached to webbing cow collars, encased in a damp-proof casing. The collars were equipped with attached weights to maintain sensor position on the neck and in some cases, with additional external batteries (AA) to prolong sensor active life. ‘Fixed sensors’ (in waterproof housing, with four ‘D’ batteries attached externally) were placed at strategic positions throughout the shed to maximise receipt of signal from on-cow sensors. The on-cow sensors reported animal location via a local positioning system (based on a wireless mesh network), along with a peak and mean accelerometer output for each reporting period. The sensors were set up to report every ten seconds throughout the trial, 24 hours a day, with a brief loss of data during system restart at midnight.

5.2.3 Data handling

The same raw data as used for the previous chapter were re-sorted for the purposes of the present chapter. As previously, the X and Y positional data of each unique sensor were used to infer relative proximities of cows; sensors within a four-metre radius of the focal sensor, for more than 50% of time points in a two-minute window were classified as a proximity event (details of the selection of this protocol are provided in chapter three). Using Matlab R2015a, the proximities were divided into three matrices based on location, defined as 'functional area' matrices (see figure 1 in the general methods chapter for an outline of the shed):

- Feeding zone: the feed-face and associated alley, and half the central passage through the shed
- Non-feeding zone: The cubicles, alleys/loafing areas (other than the feeding alley) and cross-passages
- Milking zone: the slatted area, collection yard, parlour and alleyway through which cows returned from milking

This is based on the theory that location within the cubicle shed is to some extent synonymous with activity and/or level of enforced proximity (as in during the milking event) and competition (for access to feed). The computed matrices were then transferred into Excel spreadsheets for processing. Data were assessed as described in chapter three in order to eliminate inaccurate sensor days; in brief a trajectory of sensor movement was observed for any nodes reporting below a threshold of proximity encounters across a 24-hour period. Due to the sporadic nature of lost data points (i.e. one sensor could have several accurate days interspersed with one poor accuracy day), averages were used to maximise network size. 20 days were available with a total of 55 cows once this sorting had occurred, therefore seven average matrices were produced (six based on three days, one based on two days). As discussed in the previous chapter, the apparently random nature of the lost data points minimises concern around the impact of this on further analysis.

As in chapter four the below thresholds were applied in order to highlight structure within the network (based on previous authors e.g. Durrell et al., 2004, and the lack of remaining connections above 1.5x):

1) **Original:** the original unfiltered network
2) **Mean:** with a filter of the herd mean dyadic weighted degree
3) **1.5x mean:** a filter of 1.5x the herd mean dyadic weighted degree

5.2.4 Analysis

Analysis of social network data requires the permutation of matrices or social network measures. This is due to the lack of independence in the data; as within a matrix an individual appears in both rows and columns, and additionally the proximity of A-B, and B-A are directly related (in fact the same value; Croft et al., 2008). As such the social network analysis package UCINET6 was employed.

5.2.4.1 Network level

Network measures were calculated within UCINET6, comprising mean degree, density and components. Degree is the number of other individuals within the network a cow has proximity encounters with, and the mean degree is the network average. Density is the proportion of 'ties' between individuals that exists, out of the total possible number of ties. A component is a connected set of individuals, linked either directly, or via other members of the network (Croft et al., 2008).

Sociograms (visual representations of the network members and ties) were created in Netdraw, where a *K*-cores analysis was also applied to the mean networks – this is the division of the network into maximally connected subgroups (Hanneman and Riddle, 2005). A quadratic assignment procedure (QAP) correlation was performed to compare the three functional area matrices, with 50,000 permutations (as in Abeyesinghe et al., 2013). A QAP is a resampling method which correlates two matrices, then permutes one of the pair a given number of times, and re-correlates the matrices. The test then calculates how often the permuted results match with the observed values, and produces a p-value that indicates how likely the result is to have occurred by chance. A Bonferroni correction was applied to the results due to the multiple comparisons being performed on the same dataset.

To assess the assortment by type within the group, the E-I index was also produced in UCINET 6 (the tie strengths external to the attribute group, minus the internal tie strength, divided by the total tie strength) based on parity and days in milk. Additionally an ‘absolute difference’ matrix (e.g. if cow A was parity 1, and cow B parity 3, their absolute difference would be 2) for these two factors was created, and compared via a QAP correlation with 50,000 permutations to the original functional area matrices (as described by Cook, 2017). This allowed for assessment of the correlation between similarity in parity/stage of lactation and number of proximity events between cattle.

5.2.4.2 Node level

The degree was chosen as the network measure suitable for analysis as opposed to the weighted degree, this is due to the differing proportion of time cattle would spend in each of the functional areas (based on unpublished data, on average cows spent 24% of time in the feeding area, 65% in the non-feeding area and 11% in the milking area). Weighted degree is reflective of the total time individuals were in proximity, and therefore unsuitable for these datasets.

Using the average network degrees for each functional area, a one-way analysis of variance (ANOVA) with 10,000 permutations was applied to the degree by parity group (parities 1-3 were assigned to those respective groups, and parity 4-8 were grouped together), and the degree by days in milk (grouped by 0-30, 31-130, 131-230 and 231-300 days in milk based on behaviour and energy requirements at stage of lactation - Maselyne et al., 2017)

5.3 Results

5.3.1 Network level descriptors

Sociograms of the networks divided by functional area are shown in figure 7, the graphs are drawn with unweighted edges and laid out using the 'spring embedding' method (where nodes of the shortest paths between them are clustered close together). The unfiltered network sociograms are not shown due to their near complete saturation (as in chapter four), as such visualisation of these networks is not informative. At the above average filter level, some further structure becomes evident, with nodes on the periphery and more densely connected clusters of nodes at the centre of the graphs. At both filtering thresholds, the milking network has a more even distribution of connections through the network, evidenced by the lack of a clearly defined central cluster of nodes. Additionally, both the feeding and non-feeding networks at the highest filtering threshold have isolated nodes (i.e. cows which have had no connections with other individuals), whereas the milking network contains no isolates at this threshold.

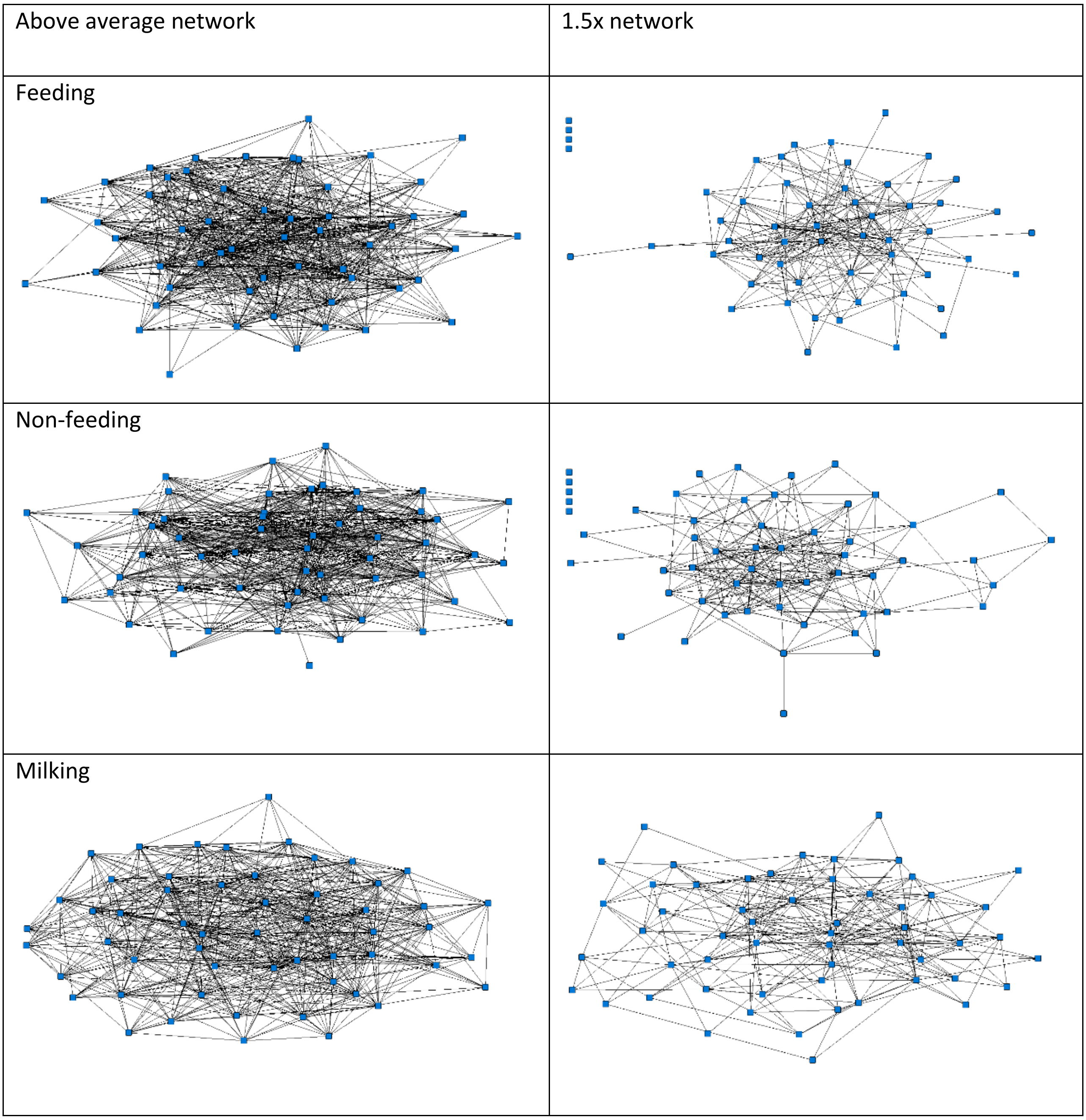


Figure 7; sociograms of the functional area networks, with two thresholds applied to number of proximity events per dyad

5.3.1.1 Network measures

When unfiltered, all three networks had a density approaching one (see table 10 below); meaning close to 100% of all possible connections existed. Unsurprisingly, at the above average threshold between 43-46% of all connections remained (as this is based on an average, logically close to half the interactions would be eliminated), and at the 1.5x average between 15-19% of all possible connections were still present. This high level of connections is mirrored in the components, with all networks having one completely connected group until the 1.5x average connections threshold is applied, and the milking network remains entirely connected (either directly or indirectly) with this filtering.

Table 10; Network-level measures of the functional area networks

Network	**Average degree**	**Density**	**Components**
Feeding unfiltered	53.96	0.99	1
Above average	24.7	0.46	1
1.5x average	9	0.16	5
Non-feeding unfiltered	54	1	1
Above average	24.5	0.45	1
1.5x average	8.3	0.15	6
Milking unfiltered	53.96	0.99	1
Above average	23.1	0.43	1
1.5x average	10.1	0.19	1

5.3.1.2 *K*-cores and cliques

The three networks at the 1.5x threshold were assessed for the number of cliques (completely connected subgroups), with a triad set as the minimum size. The milking network had the highest number of cliques with 127, the feeding network held 118 and the non-feeding network contained 101 cliques. A *k*-cores analysis was also run on these networks (shown in figure 8), indicating further variation in sociality across the areas, with the milking network containing less minimally connected clusters than the other two networks, and a larger, more densely connected central cluster in the feeding, compared to the non-feeding network.

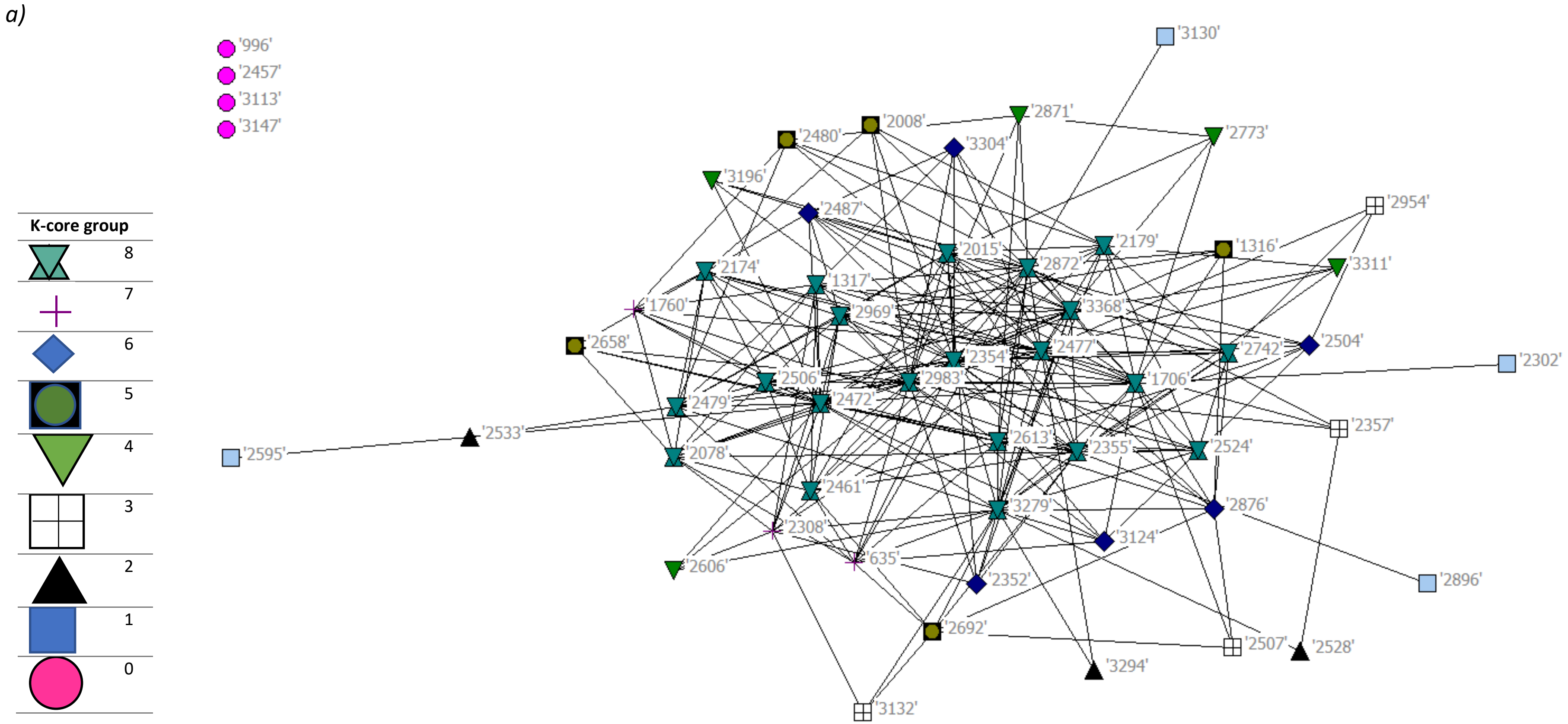
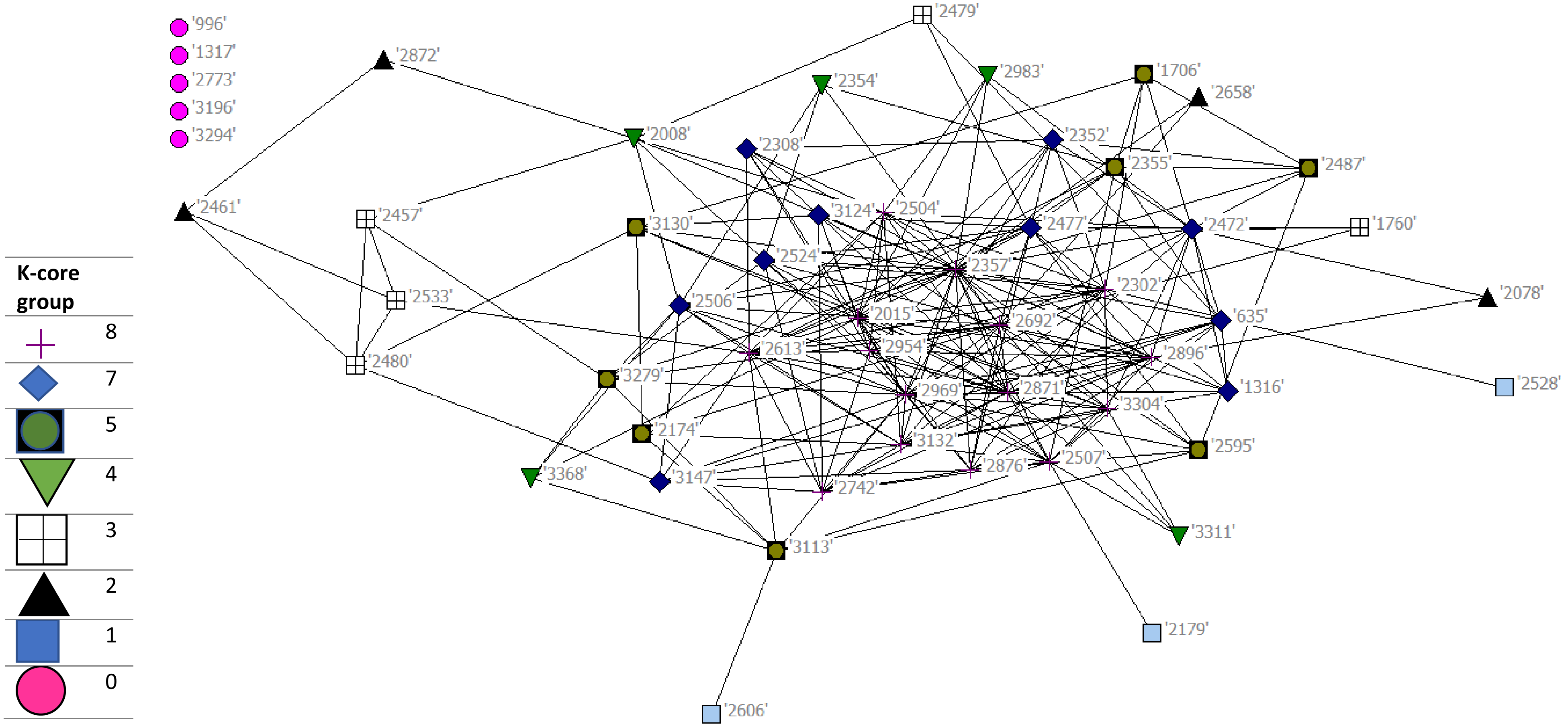


Figure 8; k-cores analysis on the three functional area networks (1.5x), nodes coloured by k-core. A) feeding network, b) non-feeding and c) milking b)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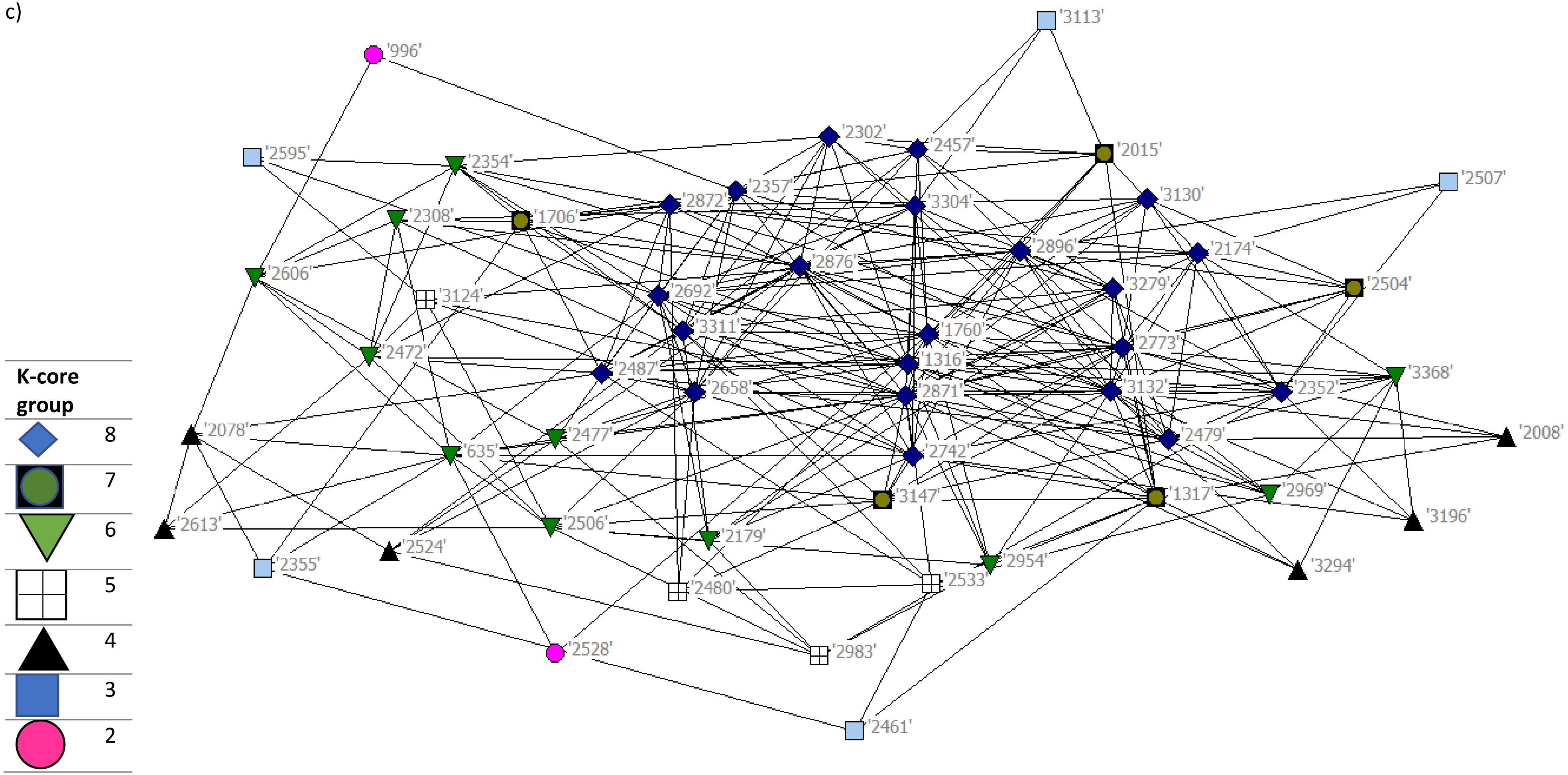
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5.3.1.3 Network assortment

To consider assortment by type, the E-I index was calculated for each of the functional area networks, by parity group and days in milk. No significant variation from the expected distribution was found – i.e. there was no evidence of non-random assortment towards heterophily or homophily by parity or stage of lactation based on this measure. However, when considering the absolute difference between parities/stage of lactation of cows, there was a significant correlation between some variables. The absolute difference in parity was significantly negatively correlated with the non-feeding functional area network (p=0.002, r=-0.21), and with the feeding network (p=0.05, r=-0.13), but uncorrelated with the milking network. Considering the absolute difference in days in milk, the non-feeding network was uncorrelated with this factor, as was the milking network (although this showed a trend towards significance; p=0.08, r=0.06) however the feeding network was significantly negatively correlated (p=0.03, r=-0.10) with the difference matrix

5.3.1.4 Network consistency across functional area

Table 11; QAP correlation between functional area proximity matrices; p value (correlation coefficient)

Functional area	**Non-feeding**	**Feeding**	**Milking**
Non-feeding	-	0.15 (0.07)	0.00 (0.18)*
Feeding	0.15 (0.07)	-	0.05 (0.09)
Milking	0.00 (0.18)*	0.05 (0.09)	-

** denotes significance after Bonferroni correction*

The networks based on proximities during milking (throughout the collecting yard and parlour), and during non-feeding periods (encompassing the cubicle and loafing areas) were significantly correlated with one another (see Table 11). This suggests that cattle which spent more time together during milking, also associated in the non-feeding areas of the shed. Before application of a Bonferroni correction, there was a significant correlation between the feeding and non-feeding networks; however this ceased to be significant once the correction was employed.

5.3.2 Node level results: degree by trait

5.3.2.1 Degree by parity

The degree of cows was compared across parity groups, indicating no significant difference in any of the functional area networks, at either the average or 1.5x threshold.

5.3.2.2 Degree by days in milk

The degree of cows was not significantly related to days in milk, in the feeding or non-feeding networks (at either threshold). In the milking network, days in milk was significantly related to degree (r^2=0.05, F=50.41, p=0.03 at the average threshold), although the r^2 indicates that this was not a strong model fit.

5.4 Discussion

The results presented above indicate that cattle are reasonably spatially inconsistent in their relationships, with different functional areas (and potentially behavioural states) yielding varying close social neighbours. The non-feeding area has the fewest proportional ‘strong ties’ (i.e. long-lasting/regular interactions) represented by lower average degree at the 1.5x mean filter. This may be due to the lower stocking density in this area of the shed compared with the milking, and potentially feeding area, but is also likely to be more representative of cattle choice in proximity partners. This is thanks to the greater space to move away from undesired partners, and given the proportion of the day spent in the non-feeding area, greater opportunity to spend time with preferred herd members or visit resources (cubicles/water troughs) at times of lower competition.

5.4.1 Network structure

The sociograms in figure 7 show the highly connected nature of each of the functional area networks, even when below average proximities are removed. The lack of a strong central cluster within the milking network and the absence of isolates compared with the presence of these structures in the other networks is perhaps unsurprising. The management of cattle during the milking event is very intense, with all herd members clustered close together within the collecting yard and milking parlour, reducing the opportunity for maintaining distance from other animals. It is interesting to note that despite some evidence of consistent milking orders in dairy cattle (e.g. Prelle et al., 2004), all cows within the milking network of the studied herd (of the subset with consistent sensor data) remained in proximity to every other member at some point across the study period. Potentially this may demonstrate a changeable milking order at the study farm (giving cows the opportunity to be close to varying individuals on different days), or possibly this is an artefact of the stocking density within the parlour and associated areas during the milking event. Given that cows are clearly coming into contact with all other herd members (across the study period) during the milking event, there may be welfare consequences. Greater stocking density increases aggression amongst cattle in a waiting area, with less crowded conditions appearing to allow for dispute resolution via avoidance, rather than agonistic interaction (Irrgang, 2012). The highly connected milking network may therefore indicate the potential for increased aggressive interactions in the milking area, with subsequent negative welfare implications for the cows.

The density of the three networks at each filtering threshold also sheds some light on the structure of the cattle proximities. When all below average connections were removed, 41-46% of possible connections within the networks remained. If there was an even spread of time spent with other cattle as in a normal distribution, we would expect the density to be close to 50% (as half the connections would fall below/above average). The lower densities of the networks suggest that there are a greater number of 'weaker' connections (i.e. those where cattle spent little time in proximity to each other), and fewer 'stronger' connections. This is particularly evident in the milking network at the above average threshold, perhaps due to the nature of the collecting yard where a large proportion of the herd were pushed into close proximity prior to the milking event.

The components of each network tell a similar story, with the milking network consisting of one component (i.e. all individuals were either directly, or indirectly linked) at all thresholds, likely for the reasons described above. The non-feeding network has six component vs the five of the feeding network; as a competitive environment (Proudfoot et al., 2009) cows were bunched together and therefore more likely to come into proximity with other individuals, potentially leading to the higher direct and indirect connections within this network. The number of triad cliques present in the networks at 1.5x threshold are likely to be indicative of prolonged or repeated interactions between the three individuals, and again the patterns in number of cliques match the above indicators to suggest a highly directly connected milking network (127 cliques) in comparison to the other functional areas.

Perhaps the network holding most interest from the perspective of demonstrating potentially genuine, choice-based social partners is the non-feeding area. This is the area of the shed where cattle have most freedom of movement, and where they spend the greatest proportion of their day (on average 65% of the time). When provided with the opportunity, cattle make decisions about which other individuals to remain close to – for example around feed (Rioja-Lang et al., 2009), when choosing which individuals to approach (Raussi et al., 2010) and evidenced by consistency of interactions over time (e.g. Gygax et al., 2010, Boyland et al., 2016 and the previous chapter). The non-feeding network contained the highest number of components, lowest cliques, lowest average degree and had the highest number of isolates in the 1.5x network. This is particularly relevant as this network is based on the greatest proportion of the cows day (65%), therefore presenting the longest period for potential interactions to occur. All these measures indicate a more subdivided network than in the other functional areas, potentially evident of the decisions of cattle regarding which individuals to interact with (within the constraints of the environment). Going forward, the non-feeding network may therefore be of greatest value in investigating changes in social behaviour, due to, for example, illness or mixing events.

5.4.2 Network assortment

The undivided network investigated in the previous chapter showed no evidence of homophily or heterophily by type using the number of ties internal/external to groups (based on parity and stage of lactation), and the same finding was evident in the application of the E-I index to the three functional area networks. However, when the data were considered more continuously rather than categorically, some significant relationships became evident. The absolute difference in parity between cows is related to the total time they are together in the feeding and non-feeding areas of the shed, with cows more similar in parity spending greater time in proximity to one another. There may be a number of factors influencing this finding; it is often the case in animal groups that individuals share more similar traits than would be expected at random (e.g. Farine, 2014). Cattle have been found to form bonds based on familiarity (Raussi et al., 2010) particularly if this is long term (Gutmann et al., 2015), so in the case of higher parity (and therefore older) animals within the group, they may have previously been in the high-yielding group together, potentially explaining increased time spent with individuals more similar in parity. Similarity in age may also be linked to association (e.g. Boyland et al., 2016), although in the present study there may be a confounding effect of familiarity (as older animals are also likely to be more familiar). The lack of relationship between association and parity in the milking network may be for many of the reasons discussed above which affect cow behaviour in this environment

– e.g. the densely stocked environment and forced movements into certain areas by farm staff.

Considering days in milk, cows of a similar stage of lactation were shown to be more likely to spend time together in the feeding network. This result may be driven by the energy requirements of dairy cattle dependent on their DIM, with changing nutritional needs as cows move through lactation (Garnsworthy, 2013). This mirrors findings by Boyland et al (2016) who also noted assortment between animals of similar milk production (based on yield and DIM). Potentially cows with a high energy requirement due to their stage of lactation may visit the feed-face at similar times (e.g. at first provision of feed) to meet their intake needs, increasing their likelihood of meeting in this area. There was also an approaching significant result when correlating stage of lactation and the milking network; interestingly this implied that cows which are less similar in terms of days in milk tended to have greater proximity encounters. Given that yield and days in milk are linked (e.g. Silvestre et al., 2009) this is a surprising result as cows of a similar stage of lactation would theoretically have similar motivation to be milked. Potentially other factors including side preference (as in Prelle et al., 2004), health and total yield which have been known to influence milking order (Rathore, 1982) may be of greater influence than days in milk, affecting milking order and therefore proximity encounters.

5.4.3 Network consistency across functional areas

The correlation results across the three functional area networks suggest that sociality is not consistent throughout all areas of the shed, and/or behaviours. The context of interactions is known to be important in affecting associations, dependent on other underlying motivations occurring at the time (Kurvers et al., 2013). As previously discussed, the feed-face is a competitive environment where choice of neighbour may fall below the motivation to feed away from a dominant individual, potentially explaining the difference between this and the non-feeding network. Gygax et al., 2010 compared networks between functional areas (subdivided into feeding, lying and activity areas), and also found little evidence for strong relationships between these, however Cooper et al. (2008) observed that a few cows within the herd associated between functional areas more than expected by chance. The latter study was based on very short observations, and out of sixty cattle, only ten consistent relationships were seen, supporting the findings of the current and previous chapter that some individuals are more consistent and highly sociable than others. Neisen et al., 2009 observed differences in synchronicity between cows, across functional areas, which may further explain the lack of consistency between these networks.

There was a significant, but weak, correlation found between the milking and non-feeding networks (see Table 11). This may be in part explained by the management of the milking event on-farm. Cows were collected from the shed for milking prior to the provision of fresh food or push-up of existing ration (dependent on time of milking), meaning that immediately before milking there would be little food available in the feeding zone. Additionally, previous research within the same herd suggests that the average length of time from last lying bout before milking was shorter than for last feeding bout – i.e. cows were more likely to have been lying than feeding prior to milking (Hodges *et al.,* 2015). Therefore cows were likely to be collected for milking alongside herd-mates they had been in proximity to within the non-feeding area of the shed.

5.4.4 Degree by trait

Whilst parity appears to be related to which specific individuals are associated with (i.e. those of a similar parity), across all three networks there was no significant difference between parity groups in terms of degree. This may be indicative of a similar average sociality regardless of parity, but a different selection of social associates for the reasons such as age and familiarity discussed earlier. In the milking network, degree was found to be related to days in milk, suggestive that cows at varying stages of lactation may spend time in proximity to more, or less individuals during the milking event. This limited finding of relationship between degree and parity/stage of lactation is similar to the results shown by other authors (Boyland et al., 2016), suggesting that choice, rather than number of, social associates may be of most value in understanding the structure of social networks and any applications for detection of health and welfare changes.

5.5 Conclusion

The findings regarding network structure across the three functional areas, and lack of correlation between some areas, highlight the impact of environmental factors on social proximities. Therefore when making assumptions about the value of, and reason for, proximities, context must be considered in concluding their biological relevance. Of the three networks, the non-feeding area may be the most promising for further analysis, as despite the extended time cows spent in this area, at the highest filtering threshold, cows in this network had the lowest average degree (and this was the area with best sensitivity of proximity classification). Finally, the highly connected networks of the feeding, and in particular the milking area may be of interest from a welfare perspective. If the reduced clustering, and lower number of extended proximity partners in the non-feeding area is indicative of cattle choice, there may be welfare impacts of enforced proximity in the feeding and milking areas, and the removal of this choice of neighbours.

Chapter six: The relationship between health and sociality in a housed dairy herd

6.1 Introduction

Social behaviour in cattle is known to be influenced by familiarity (Gutmann et al., 2015), dominance (Harris et al., 2007), milk yield (Boyland et al., 2016) and environment (Gygax et al., 2010) along with other environmental and individual characteristics. As previous chapters have shown, there is a large amount of individual variation within dairy cattle sociality not well explained by age, stage of lactation or location within the environment, warranting further investigation into the underlying mechanisms for such differences in the study herd. A further attribute which may influence social interactions in cattle is health status (e.g. Galindo and Broom, 2000). Cows suffering from a health condition, particularly one causing fever, may alter their behaviour due to the underlying response of the immune system (Hart, 1988). This manifests as increased resting time and reduced food/water intake (Dantzer, 2001) which may, for example, alter an individual's competition for resources, as their motivation to rest or eat may not coincide with other cows usual times. The association between illness and social behaviour in cattle has been the subject of a number of studies, due to the welfare and financial concerns health challenges may raise (e.g. in lost yield; Edwards and Tozer, 2004), and the potential value of behavioural indicators of disease (Proudfoot et al., 2012).

Lameness is one such common condition affecting cattle; with an average prevalence of 36.8% in dairy herds reported (Barker et al., 2010) this poses a significant issue to dairy cows and farmers. This condition alters the time budgets of cattle, most commonly considered to increase lying times (e.g. in permanent housed cows - Blackie et al., 2011). Social interactions appear to alter in cows which are lame, with affected animals showing less aggressive behaviours and receiving more allogrooming than sound cows (Galindo and Broom, 2000), however lame cows appear not to experience the social withdrawal aspect of sickness behaviour (Jensen et al., 2015). Mastitis is another problematic disease in dairy cattle, but similarly to lameness is known to affect behaviours that have the potential for disease indication. Mastitic cows reduce lying (likely due to pain in the udder) and alter feeding behaviour (Sepúlveda-Varas et al., 2016), and have been shown to be less competitive than healthy counterparts and attempt to withdraw from conspecifics (Proudfoot et al., 2014). Conversely, cattle with high somatic cell counts (indicative of sub- or clinical mastitis) may have greater social proximities under certain conditions (Boyland, 2015), highlighting the importance of environmental factors.

The above evidence for changes in social behaviour with illness suggests this may be a viable disease indicator. Alongside the direct social effects, the impacts of illness on other behaviours and thus total time budget may result in altered social proximities – for example if sick cows feed at less busy times to avoid social competition. Automated disease detection is an exciting and current topic with the potential to allow for earlier treatment of illness, in turn leading to reduced cost and improved welfare (Sepúlveda-Varas et al., 2013). The deployment of a novel sensor system to monitor cattle location, and thus proximities, may enable the discovery of any changes in sociality in diseased cattle without extensive labour and time requirements. The previous chapters have indicated that there is great variance in cattle sociality in the studied herd, and the present chapter aims to assess the relationship between common production illnesses in dairy cattle, and their social proximities .

6.2 Methods

Detailed descriptions of animals, housing and the local positioning system are presented in chapter two. They are described, in brief, below.

6.2.1 Animals and housing

The automated monitoring system described below was employed on cattle from July to December 2014 at a commercial dairy farm in Essex. A fluctuating, high-yielding management group of between 101-112 Holstein dairy cattle was observed for the course of the study, with cows moving out of the group for drying-off, and entering after calving. Animals were permanently housed in a cubicle shed with twice daily provision of a total mixed ration, and thrice daily milking. The total shed measured 30 m x 58 m, of which the high yield group had half, and contained 98 useable cubicles (geotextile mattress topped with sawdust). Cows could move freely through the shed and mix with other members of the group in the passageways and at the feed-face. Data regarding health issues, calving date, parity, lactation number, milk yield, somatic cell count and days in milk (DIM) were gathered on-farm from the management diaries, the CIS system online and Uniform Agri.

6.2.2 Local positioning system

OMS500 sensors were deployed as described in earlier chapters, on-cow and fixed sensors throughout the shed. The on-cow sensors reported animal location via a local positioning system (based on a wireless mesh network), along with a peak and mean accelerometer output for each reporting period. The sensors were set up to report every ten seconds throughout the trial, 24 hours a day, with a brief loss of data during system restart at midnight.

6.2.3 Health data

All cows were mobility scored on exiting the parlour at fortnightly intervals, using the AHDB 4-point score. For the purposes of this chapter, lame and non-lame cows were selected from the herd based on a score of 0 or 1 for non-lame, and 2 or 3 for lame. As the scoring process was potentially subjective (due to the hurried exit of cows from the parlour), cows were only selected which had two consistent consecutive scores – resulting in a final list of 26 lame and 26 non-lame individuals. The cows covered a range of parities in both groups, however there was some skew towards lower and higher parities in the non-lame/lame groups respectively (Table 12).

Table 12; parities by lame/non-lame group

Parity	n (lame)	n (non-lame)
1	3	7
2	3	6
3	10	9
4+	10	4

Somatic cell count (SCC) information was taken from the routine monthly milk collection data on the study farm. This is a routine gauge of milk quality and indicative of an immune response to infection (namely mastitis), measured as cells per ml of milk. AHDB consider SCC of under 100,000 to represent no infection, and over 200,000 as defining mastitis (AHDB, 2018), therefore for the purposes of this chapter, cows were divided into 3 groups by SCC; 0-100,000, 101-200,000 and 200,000+. This led to 73 cows with less than 100,000 SCC, 11 between 100-200,000, and 15 above 200,000.

6.2.4 Data handling

Raw, unfiltered and unvalidated data from the month of October were re-sorted for the purposes of the present chapter, with the network built from sensor data for 102 cows from the 11th-14th October (four days total). The herd was largely stable through this four day period, with only seven cows removed from the network due to partial absence. As previously, the X and Y positional data of each unique sensor were used to infer relative proximities of cows; sensors within a four-metre radius of the focal sensor, for more than 50% of time points in a two-minute window were classified as a proximity event (details of the selection of this protocol are provided in chapter three). Based on the findings of the functional area chapter, the non-feeding area was selected for creation of social networks to compare to health status (as it appears to be the area where cows display the most choice of social partners).

Unlike the earlier chapters, the raw data was un-validated in this case. This was for a number of reasons; initially as the constraints of the non-feeding location were applied, a large number of innacurate reported proximities are automatically removed (e.g. any which are reported to have occurred beyond the boundaries of the shed). Whilst the resulting data quality is likely to be poorer than in previous analysis, the use of filtered networks by above average interactions potentially removes any brief false reported proximities. Finally, the summing of four days of data may assist in smoothing out any anomalous remaining data points. This allows for the maximisation of considering sociality by health status – if the previous smaller networks on validated data were utilised, there would be much smaller sample sizes per health condition. The selection of dates was based on the timing of health data collection; milk sampling and mobility scoring both occurred close to the four days utilised (milk sampling two days previously, mobility scoring during).

The below thresholds were applied in order to highlight structure within the network (based on previous authors e.g. Durrell et al., 2004)

1) **Original:** the original unfiltered network
2) **Mean:** with a filter of the herd mean dyadic weighted degree
3) **1.5x mean:** a filter of 1.5x the herd average dyadic weighted degree

6.2.5 Analysis

The visualisation of the networks was performed in Netdraw, with spring embedding applied to the sociograms (leading to individuals with many connections between them being located close together). The social network metrics utilised for the purposes of this chapter are degree and betweenness, and were calculated in UCINET 6. Degree is simply the number of individuals a cow associates with and is therefore useful in determining broad gregariousness. Betweenness is an indicator of the number of indirect connections the cow links (i.e. the number of shortest paths between other individuals that 'pass through' the focal cow). Betweenness was more applicable in the current chapter than earlier studies within this thesis due to the more complete network available for analysis; Silk et al. (2015) noted that this measure is less representative than degree in partial networks.

The mean and 1.5x mean networks were used for the computation of degree and betweenness – due to the highly connected nature of the original network, very little variance was present in these network metrics without the application of a threshold to the network. Additionally the application of these filters potentially removed any 'false positive' proximities of short duration, due to the un-validated data used for this chapter. The lame v non lame data were not normally distributed, and as with all social network data cannot be classified as independent samples. A Wilcoxon signed rank (paired) test was therefore applied to this data, (as in Büttner et al., 2015) in *R3.3.0,* and also to the three groups of SCC data (divided as described above).

6.3 Results

6.3.1 Lame vs non-lame

A sociogram of the network (figure 9) with mobility score overlaid shows that sound cows with a mobility score 0 (pink) are more often on the periphery of the network, with individuals of worse mobility scores occupying more central positions. Twelve cows without a mobility score recorded were removed from the sociogram.

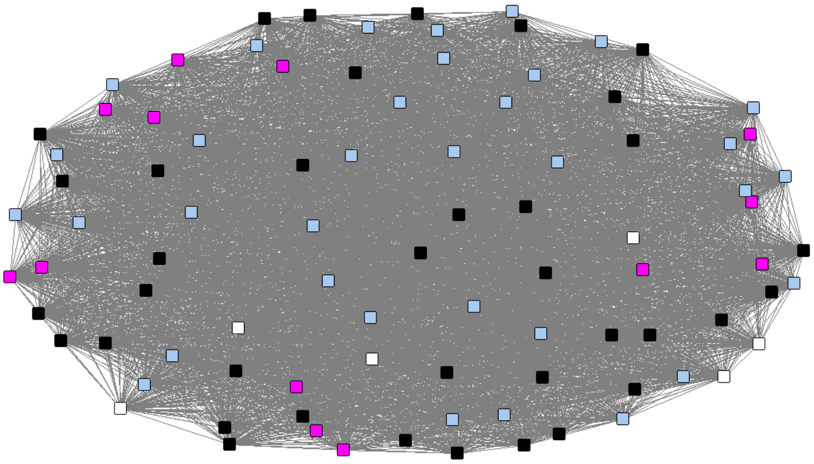

Figure 9; Sociogram of the original network with nodes coloured by mobility score. Pink = score 0, blue = 1, black = 2, white = 3

To further investigate this finding and any significance, the degree and betweenness of the two groups was compared by a Wilcoxon signed rank test for matched pairs. There was no difference between the groups in terms of degree in any network, however there was an approaching significant difference in betweenness (Z=-1.83, p=0.07) based on metrices from the mean network. Both groups contained outliers (as shown in the below box-plot), and the non-lame median betweenness was significantly lower than that of lame cows.

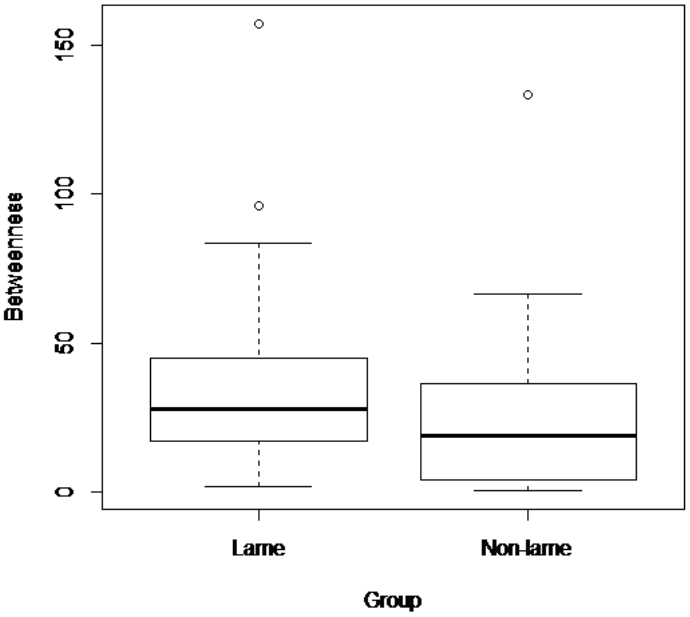

Figure 10; Box and whisker plots of betweenness in the lame and non-lame groups – mean network (boxplot presents median, first and third quartiles and whiskers represent highest/lowest values excluding outliers)

6.3.2 Somatic cell count

When the network is visualised and layout set by spring embedding, there appears to be some structure – with cows in the 101-200,000 SCC group tending to be based around the periphery of the graph, and a lesser trend in the highest SCC group (see figure 11).

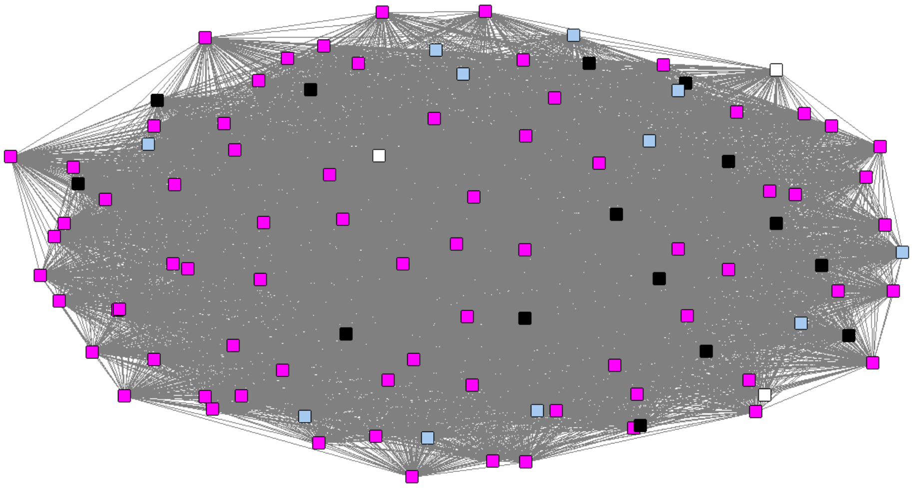

Figure 11; Sociogram of original network with cows coloured by SCC. Black = 200,000+ SCC, blue = 101-200,000, pink = <100,000, white = no SCC recorded

To investigate differences in sociality dependent on SCC, degree and betweenness were calculated. The below table shows the spread of mean network metrics across the three groups, based on the average network.

Table 13: Mean network metrices by SCC group

	<100,000 SC (n = 73)	101-200,000 SCC (n = 11)	200,000+ SCC (n = 15)	Pooled SEM
Degree	39	38.6	41.8	16.6
Betweenness	29.2	38	32.4	19.8

There was no significant difference between the three groups for either measure, at any network filter level.

6.4. Discussion

Health appears to be related to social proximities to some extent, within the studied herd. Understanding these changes in behaviour in cattle with established disease may help in identifying more subtle behavioural indicators in the early stages of illness. The difference in effect between health conditions and their relationship with sociality highlights the value of considering the specific illness, as has been indicated by earlier work noting varying social response by disease type (Jensen et al., 2015), and may be due in part to the underlying biological mechanisms occurring during each health challenge.

Lameness is a painful condition constituting a chronic stressor to affected animals (Walker et al., 2010). Cows suffering from this condition employ a suite of behavioural changes in order to adapt to and limit the pain associated with it. Reduced feeding times have been reported in lame cattle (Palmer *et al.,* 2012), and greater lying times (Blackie et al., 2011); this is logical due to the pressure of standing time (whether idling or feeding) on their feet which is likely to cause further discomfort. As such, lame cows are inclined to be within the non-feeding, rather than the feeding, area of the shed for a greater quantity of time than their non-lame counterparts. This is mirrored in unpublished data from the same herd and sensors, where lame cows have significantly increased lying during the afternoon compared with non-lame cattle.

The sociogram in figure 9 indicates that lame cows are more central within the network, which is based on the non-feeding zone of the shed. It may be that this centrality is in part due to their increased presence in this area compared to non-lame individuals. Potentially, lameness may impact the ability of these cows to compete for resources (Galindo and Broom, 2002), leading to reduced choice of cubicles – which could perhaps lead to lying in more high-traffic areas if these are less desirable to healthy cattle. The betweenness of lame cows is approaching significantly higher, potentially for the same reasons; namely that lame cows may come into contact with other cattle at varying times in the non-feeding area, including cows that have limited direct contact. In other studies, cows which are lame receive more allogrooming (Galindo and Broom, 2002) which may also explain the greater betweenness of these individuals – potentially they are groomed by cows which do not groom one another.

Lameness does not fit with the classic social withdrawal aspect of sickness behaviour (Dantzer, 2009) however this may be due to the lack of febrile response associated with the condition (Jensen et al., 2015). A cautionary note in the interpretation of these results is that the cohort of lame and non-lame cows were not matched pairs by parity, which has been suggested in chapter four to affect degree. However, when considering social network metrices in the functional area of non-feeding (chapter five), this effect did not appear significant, so may have a limited impact on the sociality of either lame/non-lame group.

When dividing cows by somatic cell count, a clear linear relationship between increasing infection and sociality does not exist. Cows in the 100-200,000 SCC region were the least social in terms of degree (i.e. had the fewest direct connections), however were the highest of the groups in betweenness – although neither was statistically significantly different than other groups. This is interesting as it may imply they have connections with many less-well connected cows (effectively acting as a hub), perhaps due to their choice of location within the shed. Mastitis is known to increase standing behaviour (Siivonen et al., 2011) and decrease feeding (Sepúlveda-Varas et al., 2016), so it would be expected that cows with a somatic cell count over 200,000 would spend proportionately more time in the non-feeding zone than those with a low SCC, possibly leading to higher degrees. This is in line with other work (Boyland, 2015b) which also noted high SCC cows to have correspondingly high degrees. It may be that within the constraints of cubicle housing, dairy cattle do not have the opportunity to socially isolate themselves as biologically motivated to do during a febrile sickness (Dantzer, 2009), with corresponding welfare implications.

As the network for this study was built from un-validated data, it is likely that some error exists in the reported proximity events. Whilst this has been minimised as described in the methods, it is worth noting due to the potential diluting effect the existence of ‘false positive’ proximities could have within the dataset. Further work on more accurate, validated datasets may therefore have the potential to define more clearly any relationships between health and sociality – however the findings of this chapter broadly reflect other examples within the literature. Another finding worthy of note is the great variance again shown within groups as defined by health, further confirming the likely multi-factorial aspects motivating sociality in individual cattle. As discussed in chapter four, the identification of changes to behaviour are likely to be more successful when comparing an individual cow’s own activity at the time of illness, to her own usual behaviour. Therefore the limited findings of this chapter utilising a broader ‘herd average’ for defining network thresholds, suggests greater promise for further work investigating an individual cows sociality over the development of a health issues.

6.5 Conclusions

These findings reflect the behaviour of cattle during a period of relative social and environmental stability (only seven cows were excluded from the network due to partial absence from the herd during the study), potentially allowing for the greatest detection of social behaviour based on individual factors. Whilst the results do not fit with those classically expected of sick cattle – namely social withdrawal – they are partially explicable by the constraints of the environment and network location. The tendency for differences in behaviour between lame and non-lame cattle suggest there may be potential for lameness detection via the automated recording of social proximities, but further work may be improved by focusing on individuals temporal changes in behaviour with disease onset, due to the high variation in sociality between cattle.

Chapter seven: General Discussion

7. Introduction

In the changing landscape of the dairy industry, growing herd sizes and a move towards more intensive housing of dairy cattle place added pressures on observing the behaviour of individual cows. Adequate monitoring of individuals is vital for assessing health, welfare and reproductive status, with negative consequences for both farmer and cow if early indicators of change are missed. Precision livestock farming is a developing field aiming to meet some of these challenges (Hostiou et al., 2017) by reducing the workload for farm staff and automatically identifying and flagging behavioural changes. Many aspects of a cows time budget offer indications about her health and welfare – from lying and standing (e.g. Blackie et al., 2011) through to social behaviours (e.g. Galindo and Broom, 2002). Sensor systems which allow for the detection of multiple behaviours and social proximities offer an exciting new avenue for potential automated monitoring of dairy cattle. Therefore the aim of this thesis was to apply a novel technology (OMS500® - a local positioning sensor system) to assess the social structure of a cubicle-housed herd of dairy cattle, and any relationship of individual sociality with health. Primarily, the work described has attempted to validate the application of a local positioning system to automatically detect the proximities of cattle, assessing the potential of such technology to be meaningfully applied in a commercial farm environment (chapter three). In chapters four and five, the data from this system is applied to the creation of social networks, across the whole shed and by functional area. Finally, chapter six furthers these findings by aiming to draw links between sociality and health status. The outcomes of these studies, their implications and suggestions for further research direction are outlined below.

7.1 The use of a local positioning system to classify social proximities

The initial validation of the OMS500 sensors for the purposes of reporting social proximity was a vital starting point for this thesis. Without adequate validation, any findings resulting from the sensor data cannot feasibly be applied to answer biological questions, as the quality and accuracy of the information will be in question (Rutz et al., 2015). The main findings from this chapter were the ability of the sensors to report location to an accuracy which allowed the detection of cattle in proximity to one another (when utilising a 4m radius and applying a 50% minimum threshold; i.e. that 50% of data points must have fallen within this radius). This was particularly evident in cows lying close together, but remained at a reasonably high level of sensitivity for cows feeding in proximity, and for direct social interactions beyond proximity (e.g. licking/butting etc). Sensors reporting location have been applied to the understanding of social behaviour and the creation of networks in animals (Handcock et al., 2009; Gygax et al., 2010) with some success. However the technology utilised for this thesis was a novel system, previously unemployed on cattle, and representing some additional benefits to the systems validated by other authors. In addition to the local positioning capability of the OMS500 sensors, they contain a magnetometer and accelerometer, lending the potential to accurately classify behaviours above and beyond social ones. The accelerometer output from these sensors can be utilised to identify feeding, lying and standing behaviour, as well as the transition between standing and lying (Vázquez Diosdado et al., 2015). The data from the magnetometer contained in the sensor were not utilised in the current study, however this could be of further use in later studies, for example to assess neck angle and infer behaviour. Therefore the future applications of such a sensor system would include the ability to integrate social and more ‘general’ behaviour for the purposes of monitoring and disease detection.

The other main finding of the sensor validation was an estimate of how much useable data is possible to retrieve from the sensor reported positions. Using the criteria established from the validation, around 31% of data would be removed (from the days assessed). This is a significant loss – however for the majority of sensors, the data loss was across short periods (less than 2.5 hours) and therefore comparable to, for example, missed interactions in observed groups. The use of appropriate social network metrices as selected for further analysis in this thesis can help combat missing data points (Silk et al., 2015), along with filtering of the networks, however the results have been interpreted with due consideration to the data quality.

7.2 Social structure within the herd, and across functional areas

The main objective of this thesis was to analyse the social structure of the studied herd, to investigate any consistencies, or evidence of relationships between individual characteristics and health. Cattle sociality has been investigated across extensive and intensive farmed conditions (e.g (Reinhardt and Reinhardt, 1981; Neisen et al., 2009b) with some evidence of social bonds and consistent relationships (e.g. Gutmann et al., 2015). There are a number of applications of social data, from ensuring important bonds area maintained for welfare and social support (McLennan, 2013), through to their potential relationship with disease (Goldhawk et al., 2009). A broad understanding of social behaviour across housing systems is therefore vital, along with ensuring that sensor-reported social networks match findings from previous observation-based studies.

The results of chapter four indicate that, before the addition of filters, the 21-day network was entirely saturated; every cow within the subset of the herd had encountered each other individual (degree of 55), making any further assessment of structure impossible. On the application of a filter of ‘above mean’ (for the whole group), and ‘1.5x mean’, some sub-structures were brought to light. Clusters of more densely connected individuals were present in each of the filtered networks, and some cattle had no encounters above the 1.5x threshold at all. This is in line with reports from other authors finding high variance in individual sociality in cattle (e.g. Val-Laillet et al., 2009). Cows in the present study did not appear to associate with others based on similarity in characteristics, however there was an approaching significant effect of parity on the number of connections cattle held within the mean network (with oldest and youngest cattle having fewer connections than those in-between).

There is conflicting evidence in the existing literature regarding ‘assortment by type’ in ungulates (e.g. preferential associations between more similar individuals), with some authors reporting this phenomena based on stage of lactation, familiarity (which may be linked to age in the present study) or reproductive state (Bouskila et al., 2015; Gutmann et al., 2015; Boyland et al., 2016), and other authors finding no assortment by production or familiarity/relatedness (Durrell et al., 2004). Therefore the findings of the current study in terms of assortment are unlikely to be universal across dairy cattle, but rather relevant to this specific herd and environment. However, the information may still be of value when assessing housed dairy herds, and also for the further interrogation of the data described below.

A final finding of chapter four was the presence of some degree of temporal stability of the network. This may imply that certain cows and/or connections are consistent through time, and further work could tease apart these more stable connections and aim to understand the motivation/factors underlying them, and any benefit for animal welfare. The existence of some temporally stable relationships reflects the work of other authors (e.g. Cooper et al., 2008; Boyland et al., 2016), but the variance within the findings of chapter four and other work re-iterates the variable nature of individual sociality and the likely effect of external factors on consistency.

When constructing networks based on the ‘gambit of the group’ – the notion that animals in proximity are associates (Croft et al., 2008) – care must be taken to ensure that this assumption has a sound basis. Within the studied herd, and indeed in almost all farm settings, there are constraints on the maximum distances that animals are able to maintain from one another. Clustering at key resources such as feed is inevitable in order to gain adequate nutrition, and during management events such as milking cows are forced into close proximity with one another. Therefore the proximity data used for chapter four was further interrogated, and subdivided into ‘functional area’ networks; for the milking area, feeding area and non-feeding area, to assess the effect of these varying sub-environments on sociality (chapter five). Each functional area matrix was assessed for structure using a number of social network metrices, with some broad findings highlighted from each area. The milking network was highly connected, with no isolates and all cows either directly or in-directly linked (at all network thresholds). The feeding area, whilst containing some isolates (at the 1.5x mean filter), produced the next most dense network, and the non-feeding network was the least connected – logical in part based on the increasing space available to cows in each area. Interestingly, only the milking and non-feeding area networks were significantly correlated, indicating that spatial neighbours are not consistent across all functional areas of the shed. Other authors report some consistency in associations across area (Cooper et al., 2008; Gygax et al., 2010) but similarly to the present study, these are limited in number/size of effect.

When assessing associations between cattle within this functional area framework, individual characteristics which appear to be unrelated to sociality at the full network level of chapter four, became evident. Cows of a similar parity spend more time together than dissimilar parity individuals (in the feeding and non-feeding area), potentially indicating a degree of assortment by parity. Stage of lactation (characterised by days in milk) may also be related to sociality in the feeding area, with again, similar cows spending greater time together than those at different stages of lactation. As described above, factors such as familiarity (which may be reflected in parity in the present study – i.e. cows which have been in the herd together longest) have been related to preference for social association by other authors (Raussi et al., 2010). Stage of lactation associations at feed are perhaps more likely driven by the comparable motivation to eat (driven by nutritional requirements) of individuals at similar days in milk (Boyland et al., 2016). Neither parity or stage of lactation appear to strongly influence total sociality (the only significant finding being a relationship between days in milk and degree in the milking network), therefore it may be that choice of close social partners varies with these factors more so than total number. The findings from this chapter indicated that the non-feeding network is likely to be most valuable in identifying choice-based proximities, which may be of assistance in detecting changes due to disease.

7.3 Relationships between sociality and disease

Chapter six aimed to compare the sociality of cattle with established health challenges to their healthy counterparts – namely lameness and high somatic cell count indicative of mastitis. A large body of evidence exists for changes of behaviour in ill cattle (e.g. Huzzey et al., 2007; Blackie et al., 2011; Siivonen et al., 2011), likely tied to the immune response to disease, or pain associated with the condition (Dantzer, 2009). Alteration to social behaviour has, to a lesser extent, been linked with disease (e.g. Sepulveda et al., 2013). There is, however, a need for greater understanding of the relationship between specific health conditions and social behaviour, to assess suitability as disease indicators.

Previous authors have compared the health status of cattle to their proximities utilising proximity loggers (Boyland et al., 2016), but chapter six has the added advantage of assessing proximities by location. The non-feeding area is likely the most suitable part of the shed for social withdrawal as part of ‘sickness behaviour’, due to the largest space availability and reduced enforced proximity compare with the milking and feeding areas. Whilst this chapter noted no significant difference in social behaviour between cows with increased/low somatic cell count, there was a tendency for lame cows to have a higher betweenness than non-lame animals. Other authors have noted lower instigation of aggression, and increased grooming of lame cattle (Galindo and Broom, 2002), potentially suggesting lame cows may come into contact with more individuals (through allogrooming, and as a result of reduced displacement of neighbours). Within the groups by health status, a high amount of variation was seen in sociality – perhaps suggesting individual responses to the health challenge rather than a blanket ‘cow response’ to sickness/lameness. This further highlights the value of individual changes, rather than application of a set threshold of alteration in sociality across the herd.

7.4 Limitations of the work

There are potential limitations of the studies due to the novel technology utilised. Chapter three highlighted the errors inherent within the sensor information, likely in part due to environmental factors such as the presence of metal structures within the shed (which interfere with signal). Such error must always be considered in interpretation of results, however as other authors have discussed, appropriate data handling and awareness of the limitations of the technology are useful tools that allow sensor-derived information to be meaningfully applied (Boyland et al., 2013; Rutz et al., 2015). Additionally, the sensors were able to report proximities on a nearly continuous basis, leading to a much larger and more complete dataset than the vast majority of observation based studies (e.g. Cooper et al., 2008).

Chapter six utilised a much larger dataset in terms of number of cows (although a shorter duration – four days) by considering all sensor data without validation. The lack of validation in this chapter poses a limitation to interpretation of the results. However, this was justifiable due to the use of the non-feeding area data only; therefore any erroneous sensor data which reports proximities occurring beyond the boundaries of the shed for example, would be removed. Additionally, all analysis was conducted on networks with an applied filter, again removing potential false positive proximities which are of short duration (e.g. if a cow passed close to a 'stuck' sensor reporting a false position).

The thesis focuses on a very specific dataset – i.e. based on one herd, at a reasonably high stocking density, in one management system. There are inevitable effects of environment on behaviour, meaning the results of this thesis are unlikely to be applicable across all housing types and herds. However, the developed principles of automated detection of social behaviour via a LPS are applicable and useful for behavioural monitoring in many situations. Additionally, many of the findings of the above work are in line with those reported by other authors, strengthening the knowledge base around social behaviour of cattle, and further supporting the use of such a sensors system in on-farm behavioural monitoring.

A final methodological limitation is the use of filtered networks, which limits available data and removes 'weak ties' between cows. Such infrequent/short interactions may have value to the animals involved or even be reliable indicators of individual state, and therefore their removal may mask important network detail (Croft et al., 2008). However in the context of this thesis where social behaviour is assessed as a practical tool for on-farm monitoring, such interactions are unlikely to be reliably used to inform changes in behaviour (as they would form such a small part of the cows time budget), but may be of value in later research, particularly if highly consistent.

7.5 Future direction and applications

The potential applications of social data from dairy cattle are many and varied; from understanding which partnerships are valued and have welfare benefit in maintaining (McLennan, 2013), to detecting disease and assessing the impact of mixing/re-grouping (Neisen et al., 2009b). As technology suitable for precision livestock farming continues to improve – in accuracy, affordability and duration of sensor/battery life, the on-farm applications of such systems will concurrently increase. Sensor systems such as the OMS500 employed for this body of work offer the ability to monitor many aspects of the cow, and farm environment; from classifying behavioural states, to identifying preferred locations and even ambient temperature. The vast datasets produced by such sensors have enormous potential for a multi-faceted approach to detecting welfare and disease, by combining the usual activity and locations, as well as sociality, of individuals to define normal behaviour, and any subsequent deviations from this.

In terms of social behaviour, there are a number of further avenues for exploration with proximity data. The results of this thesis indicate location may affect sociality, additionally time of day is known to alter certain behaviours and may be worthy of further investigation. The data produced from the OMS500 system also allows for the definition of ‘bouts’ of social proximities – the frequency and duration of these may be highly valuable for further research. For example, if cows are seen together frequently, but not for extended durations, vs longer bouts and less frequently, further detail may be gleaned about the quality and nature of these encounters. The importance of relationships between these two categories of encounters may be highly important when considering their relationship with health and welfare, as fleeting encounters are potentially less indicative of social bonds and/or tolerance than extended proximities. Some information regarding the directionality of interactions may also be obtained, if the movement of animals towards one another is detected via the local positioning system, an idea of which cow initiated the approach could be gained.

The on-board accelerometer and magnetometer within the sensors is able to detect the movement (and directionality) of the head and neck due to its neck-mounted position. The nature of some agonistic social behaviours, such as head-butting, has a classic swinging movement that may therefore have the potential to be automatically detected. As ill cows are inclined to reduce their agonistic and displacement behaviours (Sepulveda et al., 2013), as part of a broader assessment of social behaviour this may have a place as a further indicator.

The most important outcome of this thesis in terms of suggestion for further research is the need for longitudinal datasets allowing for assessment of a cow’s unique behavioural changes over time, and with disease. Given the relatively high temporal and individual variance described in chapters four to six, producing ego-networks for cows, or considering changes in their own most stable relationships are likely to have much greater scope to identify alterations to patterns of behaviour than considering total sociality.

7.6 Conclusions

A novel outcome of this thesis was the successful application of a multi-function sensor acting as part of a local positioning system (LPS) to detect proximities of cattle when validated against direct observations, and creation of social networks of the herd from this data. Whilst previous authors have utilised LPS to track cows, the networks produced were of much shorter duration, and therefore unable to consider the temporal consistency of the network. An additional novel outcome was the consideration of links between health and sociality by functional area, which although providing limited findings, may be more valuable than an overview of proximities throughout the shed in such an intensively managed setting. Some level of structure is evidenced within the studied group, based on the existence of more closely connected individuals, and the existence of isolates at high filtering thresholds. This indicates that gregariousness varies by cow, with some individuals likely to be more fundamental to the social network than others. Knowledge of key individuals/partnerships may allow more informed decisions for management and welfare of the herd – for example when considering mixing and re-grouping. The cause of the high level of variation amongst cattle is not well explained by parity, stage of lactation or health, and may well be very individual as seen in other mammals, and previous work on cattle sociality. However cow characteristics do, to some extent, appear to be linked with sociality, and within the functional area networks, some level of homophily was noted – suggesting that underlying biological mechanisms impact (to a weak extent) on social partners. The low correlation between the functional area matrices suggests that social partners may be different (and based on varying motivations) across these areas, highlighting the importance of considering sociality by location rather than throughout the shed. The evidence for some consistency of networks over time, and the approaching significant links between lameness and betweenness, offer encouragement that considering an individual cows social network may provide a method of monitoring her behavioural changes due to welfare or health status, but further research is required to investigate this.

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