The behaviour of sheep around natural waterways and their impact on water quality

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ABSTRACT

The impacts of extensively managed sheep on the natural environment have received little attention in comparison to beef and dairy cattle in New Zealand. In particular, there is a paucity of information on the interaction of sheep with natural waterways and their impact on water quality. Access of livestock, such as cattle, to waterways has been shown to be a cause of poor water quality due to pugging damage and excretion entering the waterway. In New Zealand, regulations require that cattle, deer, and pigs are excluded from waterways, but there are no such requirements for sheep. The aims of this thesis were to: 1) examine the behaviour of sheep on a hill country paddock, which was transected by a natural waterway during winter, spring and summer, 2) investigate whether drinking water restriction influenced the behaviour of sheep, 3) determine the impact of sheep on the water quality of the waterway across seasons and, 4) investigate the influence of slope and aspect on the spatial distribution of sheep across three seasons.

Observations of behaviour showed that sheep spent little time near the natural waterway compared to other areas within the paddock across seasons. However, study ewes showed a spatial preference for flat to low sloped areas of the paddock while utilising south and north facing slopes more than the rest of the aspect categories. Sheep preferred using culverts to cross the waterway than to try cross it via other means. In addition, the degree of interaction of sheep with the waterway was not influenced by the availability of reticulated water from a trough. Overall, ewes had minimal interaction with the waterway and therefore, had little impact on the water quality. This may have been due to the high moisture content of pasture; thus, meaning the sheep were not physiologically required to interact with the waterway to drink.

Results from this study have contributed to the knowledge of sheep behaviour around natural waterways. These results are crucial for informing future decision making related to the management of sheep in and around riparian zones, for example, potential impacts of sheep on waterways could be reduced through the strategic placement of culverts, water sources and other paddock features on areas preferred to be utilised by sheep such as lowlands areas, near crossings and those with green pasture. The results are also crucial to informing future government policy focussed on stock exclusion of sheep from waterways and suggest the current New Zealand stock exclusion policy which does not include sheep is appropriate.

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1.1 Overview

Sheep are an important part of the global agricultural economy due to the production of meat, wool, milk, and byproducts e.g., skins, tallow, blood, and renderable products (Zygoyiannis 2006). The sheep industry is essential to the New Zealand economy and New Zealand is a key player in world lamb production. While most of countries consume most of their produce and export only portion, New Zealand exports 95% of the sheep meat and 90% of the wool produced (Morris 2013; FAO 2018). By the end of 2017, New Zealand produced 362,000 tons of lamb (on a bone in basis). The major export destinations were to North Asia (37%), the European Union (36%) and North America (11%) (Beef + Lamb New Zealand 2018a). In 2019 New Zealand generated $3.1 billion per year from lamb exports (Statistics New Zealand 2019a). A record $391 million was generated from lamb exports in February in 2019 (Statistics New Zealand 2019a).

Traditionally in New Zealand sheep are managed under extensive pastoral conditions, with very few animals being housed and sheep grazing on pasture all year round (Kilgour *et al.*, 2008: Morris 2013). New Zealand is widely recognized as a world leader in pastoral agriculture (Morris 2009). Generally, pastures on which sheep are grazed are categorised into three groups based on topography and elevation, namely high, hill and flat to rolling country (Hodgson *et al.*, 2005). These topography and elevation classifications influence feed and water availability and sheep behaviour. There are three primary sources of water for extensively managed livestock species; drinking free water, water consumed from foodstuffs and metabolic water (El Shaer and Squires 2015). Feed or pasture composition can have considerable influence on the water requirements of sheep (Macfarlane *et al.*, 1958). Sheep offered copious amounts of green pasture may not need to drink, or drink less water, as the pasture can supply most of the water needs of the animal (Macfarlane *et al.*, 1958). Sheep water requirements can be entirely met through the consumption of forage when pasture contains 60 to 70% water (Macfarlane *et al.*, 1967). Stock on dry pasture however have greater water requirements in order to utilize the less digestible forage.

Water is an essential requirement for life, and its availability can have a major impact on farming productivity (Elliott 1996). It is estimated that the livestock sector uses about 8% of the available global water supply (Schlink *et al.*, 2010). A large percentage of animal's body contains water: for example, Romney ewes have a mean total body water of 68.4% of their body weight (Budtz-Olsen *et al.,* 1961). On average total body water ranges from 56% of body weight when offered dry pasture to 81% when offered lush pasture (Macfarlane *et al.,* 1967). New Zealand, however, faces challenges relating to water quality mostly due to contamination (Morgenstern and Daughney, 2012). Most of the contamination found in water ways is due to animal and human activities in agricultural, industrial, and urban areas (Foster *et al*., 2002). Animals can be a cause of poor water quality due to nutrients, sediment and pathogens deposited into waterways through urination and defecation and pugging damage to stream banks (O'Callaghan *et al*., 2019; Cournane *et al*., 2011). In 2017 it was estimated that sheep accounted for 15% of the nitrate leached from livestock systems (Statistics New Zealand 2019b). As a result, in 2019 the Government of New Zealand developed action for healthy waterways with the objective of reversing past damage and to bring New Zealand's freshwater resources, waterways and ecosystems to a healthy state within a generation. In the plan, there was a requirement that cattle, pigs and deer were excluded from the waterways more than one meter wide by 2023. There is currently no such requirement for sheep which may be due to the paucity of research on sheep behaviour and impact on waterways.

Several studies have investigated behaviours in sheep such as Champion *et al.,* (1994), Deag (1996), Dwyer and Lawrence (2005), and Al-Ramamneh *et al*., (2012), but none of them has discussed in relation to water quality and how sheep behaviour impact water quality. Therefore, the objective of this research was to study the behaviour around natural waterways and study the impact of sheep on water quality, specific areas investigated included;

- To examine the behaviour of sheep around a natural waterway during winter, spring and summer
- Investigate whether if access to a water trough influenced the behaviour of sheep around the natural waterway
- To determine the impact of sheep in a pastoral grazing system on water quality in a natural waterway
- To investigate the influence of slope and aspect on the spatial distribution of sheep across three seasons

This thesis consists of seven chapters; a general introduction, literature review and final discussion, plus four data chapters. Each research chapter (three to six) addressed a specific season with exception of chapter six which compared data across seasons i.e., winter, spring and summer. In chapters three, four and five the behaviour of sheep around a natural waterway, GPS observation of the spatial distribution of sheep and their impact on water quality were investigated during winter, spring, and summer, respectively.

In each study included in the thesis the behaviour of sheep was investigated when provided with and without access to a reticulated water trough to determine the effect on interactions of sheep with a natural waterway. It was hypothesised that sheep would exhibit limited interaction with the natural waterway and that such interactions would be unaffected by the presence of a reticulated water trough. Further, the interaction of sheep with the waterway may influence water quality due to nutrient inputs (via urine and faeces), sediment (resulting from trampling and bank erosion), and pathogen loading (from faecal matter). Together, these factors can contribute to eutrophication, increased turbidity, and alterations in stream morphology. This thesis found little evidence to reliably quantify the effects of sheep on the water quality parameters assessed. Further long-term research conducted across diverse environments is necessary to confirm these findings. The fencing of waterways to prevent sheep access is therefore unlikely to lead to significant improvements in water quality when compared with the exclusion of pigs, cattle, and deer. Further evaluation and research are required, particularly to determine the extent to which sheep contribute to stream bank erosion.

The behavior of sheep around natural waterways and how sheep impact water quality has not been presented and would be beneficial to the sheep industry in New Zealand and especially with the current action for healthy waterways in place. Therefore, this review encompasses behaviour and impact of sheep around natural waterways and due to paucity of research in sheep, some inferences are made from cattle.

2 Review of literature

2.1 Sheep industry in New Zealand

New Zealand was estimated to have 25.3 million sheep in 2022 (Statistics New Zealand 2023) with sheep numbers having steadily decreased between 1991 and 2022 by 53.6 percent (Beef + Lamb New Zealand 2022; Statistics New Zealand 2021). Despite decreasing stock numbers, total lamb production decreased only by 14% (Beef + Lamb New Zealand 2022). The decrease in sheep numbers has not affected the productivity of the industry due to several reasons, including an increase in lambing percentage (by 28%) and lamb weights (by 37%; Beef + Lamb New Zealand 2022), with little change in animal stocking rate (Morris and Kenyon 2014). In this period, there has been a dramatic increase in total lamb production (kg) per ewe in general (by 118%; Beef + Lamb New Zealand 2022).

In New Zealand, the most commonly farmed sheep breed was the Romney (Blair 2011). In the last 30 years, however, there has been a move to increase composite breeds by incorporating East Friesian, Finn and Texel genetics with the existing major breeds of Romney, Coopworth or Perendale flock to improve fertility and meat production potential (Morris 2013).

In New Zealand, meat (lamb) and wool are main products for farmers (FAOSTAT, 2014). The sheep industry in New Zealand is focused on supplying international markets, with 95% of the sheep meat and 90% of wool produced being exported (Morris 2013; FAO 2018). New Zealand exports account for nearly half of the global trade in lamb (FAO 2018) and contributes $3.1 billion per year to the economy (Statistics New Zealand 2019a).

Sheep in New Zealand are managed under extensive conditions on pasture all year round with no permanent housing (Kilgour *et al*., 2008: Morris 2013). Generally, New Zealand pastures are categorised into three groups based on climate, topography and elevation, namely high, hill and flat to rolling country (Hodgson *et al*., 2005; Beef + Lamb New Zealand 2018a; Table 2.1). In New Zealand, production systems range from intensively grazed (high stocking rate) on highly productive lowlands, through to extensive production (low stocking rate, larger paddocks) on poorer quality high country pastures (Morris, 2013). Beef + Lamb New Zealand (2018a) further categorises sheep and beef farms into eight farm classes: South Island high country, South Island hill country, North Island hard hill country, North Island hill country, North Island intensive finishing farms, South Island finishing-breeding farms, South Island intensive finishing farms, and South Island mixed cropping and finishing farms.

Pastoral grazing management systems can range from fenced enclosures/paddocks to rangeland systems where large flocks live on unfenced pastures (Kilgour *et al*., 2008). In New Zealand, sheep are managed exclusively in fenced paddocks (Morris 2013). Extensive production systems generally provide sheep with the freedom to perform their natural behavioural repertoire (Deag 1996). Extensive systems are generally described based on the number of animals per labour unit, stocking density, and degree of confinement or restriction on the movement of animals (Dwyer and Lawrence 2005; MPI, 2018). The common pasture species in New Zealand are ryegrass and white clover (Charlton and Stewart 1999).

Table 2.1: The area, pasture production, number of sheep carried average stocking units per hectare of high, hill and flat to rolling country in New Zealand

Region	Area (million ha)	Pasture production (tDM ha $^{-1}$)	Number of sheep (%)	Average stock units[1]/ha
High country	4.5	2.0	5.1	0.7
Hill country	5.0	7.0	41.0	7.5
Flat to rolling	4.5	11.0	54.0	14.0

[1] 1 stock unit = one 55 kg breeding ewe, consuming 550 kg DM per year (Parker 1998). Adapted from (Hodgson *et al*., 2005).

2.2 Water requirements of sheep

2.2.1 Sources of water

In New Zealand, sources of drinking water for sheep include water reticulated to troughs and natural waterways such as rivers, streams, creeks, drains, ponds, lakes, wetlands, and estuaries (Willms *et al*., 2002). Sheep also obtain water through the forage they consume and metabolic water from nutrient catabolism (El Shaer and Squires, 2015).

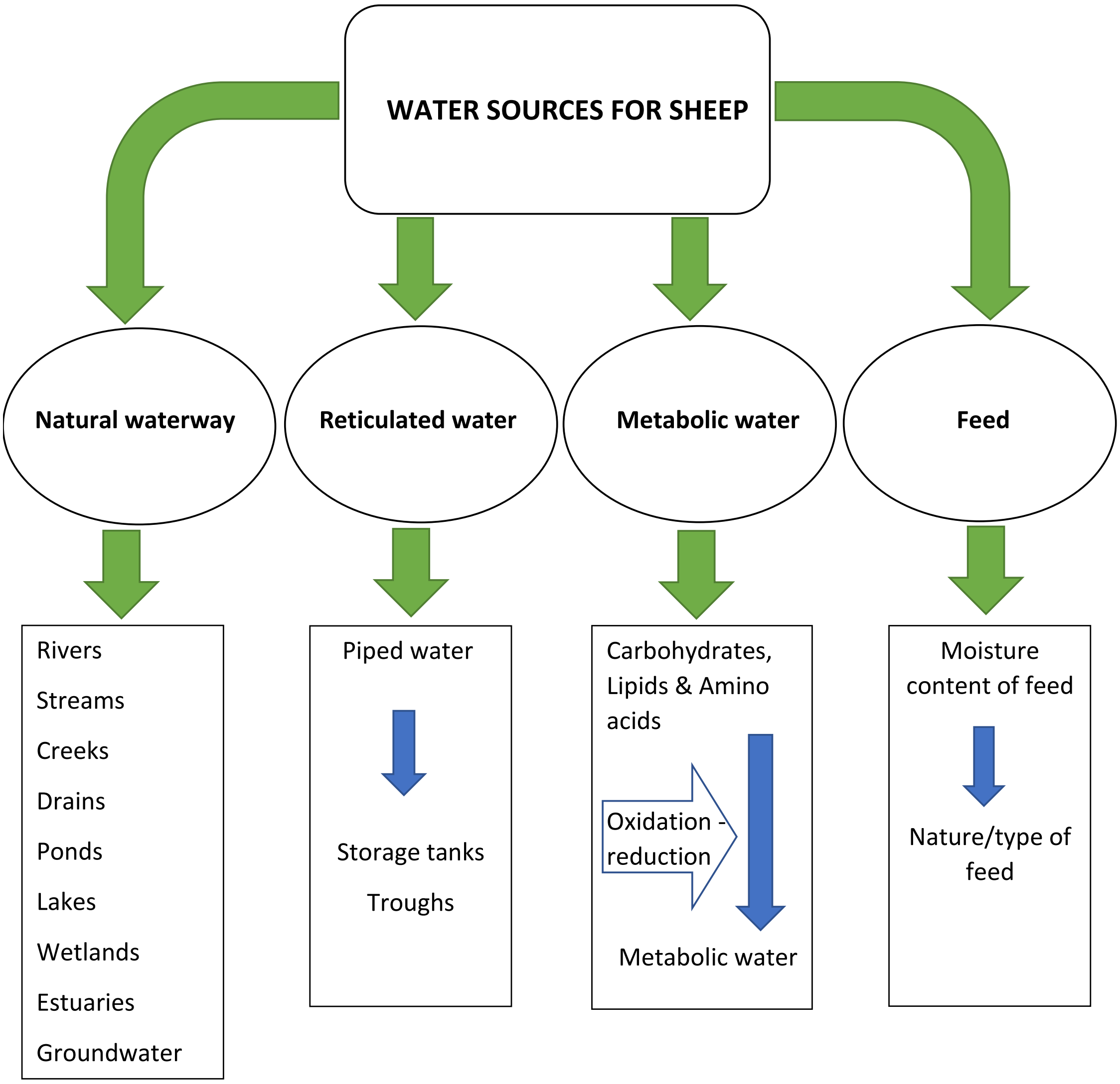


Figure 2.1:Diagram showing sources of water for sheep

2.2.1.1 Natural waterways

Natural waterways include rivers, streams, creeks, drains, ponds, lakes, wetlands, estuaries, dew, guttation and ground water (Figure 2.1: Willms *et al*., 2002). Natural waterways have traditionally been a common source of drinking water for livestock in New Zealand (Ayers and Westcot 1994). Natural sources, however, can be prone to contamination and poor water quality (Ayers and Westcot 1994). Contamination of water ways can occur through human agricultural, industrial, and urban activities (Foster *et al*., 2002).

2.2.1.2 Reticulated water

The provision of water through artificial water-points has enabled expansion of grazing lands (James *et al*., 1999). In New Zealand on large farms and on hill country areas not all paddocks have reticulated water supply which results in livestock relying on natural water sources.

2.2.1.3 Metabolic water

Metabolic water results from oxidation-reduction in the loading of carbohydrates, lipids, and amino acids (El Shaer and Squires, 2015). The overall impact though is low, because 1 kg of fat has to be oxidized to produce 1.2 L of water, and 1 kg of protein and carbohydrate only produces 0.5 L (El Shaer and Squires, 2015). Metabolic water intake for the sheep is low and reported to be only approximately 8 percent of the total intake (Aganga, 1992). It is not an efficient source of water (Atti *et al*., 2004).

2.2.1.4 Feed and forage

Forage consumed is an essential source of water for sheep in New Zealand (Markwick, 2007; Spengler *et al*., 2015). Forage and feeds differ greatly in their water content from approximately 40 for hay to greater than 80% for ryegrass pastures (Table 2.2).

High quality pasture (fresh and green) with a moisture content of greater than 80% when fed ad libitum is sufficient to meet a sheep's water requirements (Markwick 2007). Sheep grazing green pasture, therefore, may not need to drink free water or may display reduced water intake compared to sheep grazing dry pastures (Macfarlane et al., 1958). Sheep grazing feed with lower moisture contents such as hay, grain or dry pasture will need to drink more water and/or more frequently (Table 2.2; Freer and dove 2002). Water intake from forage is related to the feed moisture content with greater the moisture resulting in a lower the need to drink (Forbes 1968). Forage moisture contents vary across seasons based on stage of maturity of plants (Mendiguren et al., 2015) which can result in the amount of free water sheep require between periods of the year.

Table 2.2: Moisture content (%) of various feeds fresh and conserved feeds across various climatic conditions, seasons, and geographical locations

	Type of feed	Water content (%)	Metabolizable energy (MJKg/DM)	Season/ region/climatic condition	Reference
Fresh	Swedes	89.6	11.6	New Zealand	De Ruiter *et al*., (2007)
	Plantain	87	11.7	New Zealand	Burke (2006)
	Kale	85.8	11.5	New Zealand	De Ruiter *et al*., (2007)
	Red clover	85	11.1	New Zealand	Burke (2006)
	Chicory	86	12.5	New Zealand	Burke (2006)
	White clover	85	11.5	New Zealand	Burke (2006)
	Lotus	84	12	New Zealand	Burke (2006)
	York shire fog	84	12.7	New Zealand	Burke (2006)
	Kikuyu	83	9.8	New Zealand	Burke (2006)
	Perennial rye grass	81	10.9	New Zealand	Burke (2006)
		78.4	*	New Zealand	Chaves *et al*., (2001)
		73.2	12.6	New Zealand	De Ruiter *et al*., (2007)
	Prairie grass	81	11.2	New Zealand	Burke (2006)
	Lucerne	76	10.9	New Zealand	Burke (2006)
	Tall fescue	75	11.3	New Zealand	Burke (2006)
	Cocksfoot	73	11.1	New Zealand	Burke (2006)
	Fresh maize	64.6	10.8	New Zealand	De Ruiter *et al*. (2007)
Conserved /other	Sulla silage (1.1% condensed tannin)	77	11.5	New Zealand	Burke (2006)
	Maize silage	65.3	10.7	New Zealand	Burke *et al*., (2000)
		64.8	9.8	New Zealand	Corson *et al*., (1999)
		63.9	11.1	New Zealand	De Ruiter *et al*., (2007)
	Oat silage	60	11.1	New Zealand	Burke (2006)
	Pasture silage	59	11	New Zealand	Burke (2006)
	Lucerne silage	43	11.2	New Zealand	Burke (2006)
	Lucerne hay	42.6	9.8	New Zealand	Burke *et al*., (2000)
	Maize grain	12.9	*	New Zealand	Burke *et al*., (2000)
	Maize grain	8.7	10.8	New Zealand	De Ruiter *et al*., (2007)

*Data not provided

2.2.2 Water intake requirements

The mean total body water of an adult Romney sheep is approximately 68.4% of their total body weight (Budtz-Olsen *et al*., 1961). The water requirements of sheep are commonly described in terms of both quality and quantity (Elliott 1996). Water quantity requirements depend on factors such as the amount of moisture available in the animal's feed (Table 2.2) and that is derived from metabolic processes, the stage of production and environmental factors such as air temperature and humidity (Forbes 1968). Depending on feed type and its water content, however, total body water can range from 56% of body weight when grazing dry pasture to 81% on lush pasture (Macfarlane *et al.*, 1967).

There is a clear relationship between water intake and feed intake (Forbes 1968). Winchester and Morris (1956) and Calder *et al.*, (1964) reported that dry matter intake was inversely correlated with water consumption whereby pastures with higher moisture resulted in lower water consumed. It has been reported that in the winter and spring when the moisture content of the pasture was higher than 50 to 70% sheep did not require free water (Brown and Lynch, 1972; McFarlane and Howard, 1972).

2.2.3 Water use and intake

A sufficient supply of water is essential to avoid negative effects on animal health, performance and welfare (Murphy 1992; Meyer *et al.*, 2004). Water scarcity, often combined with heat stress, is a common challenge that animals can face, causing physiological perturbations that affect the animal's productivity (Chedid *et al.*, 2014). Maloiy and Taylor (1971) reported that sheep and goats used water amounting to approximately 8% of their body weight per day when water was available *ad libitum*, but this was reduced to 4% of body weight per day when the water intake was restricted. Table 2.3 shows that total water intake can vary depending on physiological status, location and type of feed consumed.

Table 2.3: Estimates of water requirements of sheep

Category of sheep	Type of feed	Average daily water consumption^{+} (litre/head)	Region/Climatic condition	Reference
Lambs	Commercial pelleted feed	3.8	Winter, South Africa	Schoeman and Visser (1995a)
	Dry pasture	2.2	New Zealand	Aqualinc (2004)
	Green pasture	1.1	New Zealand	Aqualinc (2004)
Weaners	*	3	Warm §	Laffan (2015)
Mature sheep	Saltbush	8.5	Warm §	Laffan (2015)
	Dry pasture	7	New Zealand	Aqualinc (2004)
	Saltbush & grassland	6.2	Semi-arid, Australia	Squires (1976)
	Kentucky blue grass + white clover + timony	4.1	Mid-temperate, Canada	Calder *et al*., (1964)
	Grassland	4	Warm §	Laffan (2015)
	Lucerne hay	3.8	Semi-arid Mediterranean zone, Greece	Hadjigeorgiou *et al*., (2000)
	Green pasture	3.5	New Zealand	Aqualinc (2004)
	Oats hay	2.9	South Africa	Van der Walt *et al*., (1999)
	Concentrate & roughage (40:60)	2.2	Lebanon	Jaber *et al*., (2004)
	Ryegrass, subterranean clover, barley grass and silver grass	1.4	Temperate, Australia	McGregor (1986)
Pregnant ewes	*	5.4	Temperate §	Schlink *et al*. (2010)
Lactating ewes	Hay + pelleted concentrate	14.4	Temperate §	Kischel *et al*., (2017)
	Dry feed	9	New Zealand	Aqualinc (2004)

*Data not provided in the respective study, § No specific country, $^{+}$ Free water

2.2.3.1 Adaptation to water restriction

During periods of water shortage animals need to reduce their water expenditure and have a range of physiological responses that can minimize water loss (Maloiy and Taylor, 1971; Laden *et al.*, 1987; Ahmed and Abdelatif, 1994; Jaber *et al.*, 2004). Withholding of water has been shown to reduce the moisture content of faeces, as well as reducing the output of urine and water loss through evaporation (Maloiy and Taylor, 1971; Laden *et al.*, 1987; Ahmed and Abdelatif, 1994; Jaber *et al.*, 2004). In sheep restriction of water intake has been reported to result in a large decrease in water loss due to reduced evaporative loss and increased rectal temperature (Maloiy and Taylor, 1971; Alamer and Al-hozab, 2004). Sheep can also reduce water loss by using a heat exchange system to cool down their brain (Strauss *et al.*, 2015). Strauss *et al.*, (2015) showed that a 50 kg sheep could save 2.6 l of water per day (~60% of daily water intake) when selective brain cooling was used for 50% of the day during heat exposure (22ºC) in South Africa.

Sheep seek shelter or shade during the day reduce heat exposure as a mechanism to reduce water loss (Cain III *et al.*, 2005). Furthermore, fleece can protect them from heat and minimize water loss (Narula *et al.*, 2010). Differences between breeds have been recorded in the efficiency of water usage (Schoeman and Visser, 1995b). Some breeds of sheep are well adapted to low water without influencing their performance for example Karagouniko and Desert Bighorn sheep (Hadjigeorgiou *et al.*, 2000; Cain III *et al.*, 2008).

A positive side effect of water restriction, however, is an improvement of the digestibility of nutrients by increasing digesta retention time, allowing more time for degradation by microbes and microbial synthesis (Osman and Fadlalla, 1974; Casamassima *et al.*, 2008; Nejad *et al.*, 2014). Many sheep can go without water for long periods for example in the tropics Merino sheep have been observed to go up to ten days without drinking water (MacFarlane 1964).

2.2.3.2 Effects of water restriction on performance

In sheep and cattle, restriction of drinking water relative to *ad libitum* intake has been reported to decrease feed intake (Mousa *et al.*, 1983; El-Nouty *et al.*, 1990; Parker *et al.*, 2003; Alamer and Al-hozab, 2004; Abdelatif *et al.*, 2010) and milk yield (El-Nouty *et al.*, 1991). Singh *et al.*, (1976), however, reported a reduction in feed intake only for the first 72 hours, thereafter there was no further reduction. These studies of the effects of water restriction differ in location, breed and type of feed utilised which may explain the differences.

Water restriction has been reported to result in decreased lamb birth weight, lamb survival and the body weight of mature sheep (El-Nouty *et al.*, 1991). The loss of body weight connected with water shortages can be attributed to reductions in feed and water intake, coupled with body water loss (Parker *et al.*, 2003). Thus, sufficient quantities of water of acceptable quality must be provided to maximize production (Murphy 1992).

2.2.4 Water turnover rate

The rate of body water turnover is an index of overall water usage of the animal in a given environment, and refers to the replacement of body water that is lost over a given period of time (Shimamoto and Komiya, 2000). Water turnover is measured as the ratio of body water that is excreted and replaced in tissues. Water turnover of an animal is controlled by several factors including; food consumption (Forbes, 1968), the type of feed consumed (Wilson 1974), and environmental conditions (Daws and Squires, 1974). Water can be gained through oxidative gain, drinking of free water or through water ingested in feed (Macfarlane *et al.*, 1958) and is lost through sweat, urine, faeces and evaporation during panting (King and Hadley, 1979).

Water turnover rates are generally measured using two methods: balance of water movement (i.e. water gain vs water loss) or by measuring the dilution of injected labelled water such as tritiated water or deuterium oxide (Morris *et al.*, 1962; Brown and Lynch, 1972; King and Hadley, 1979; Al-Ramamneh *et al.*, 2010). Use of deuterium oxide dilution technique (D_2O) or tritiated water (^3H_2O) can provide accurate water turnover rates (Morris *et al.*, 1962; Brown and Lynch, 1972; Al-Ramamneh *et al.*, 2010). Labelled water is injected either into the blood stream or into the haemolymph (King and Hadley, 1979; Al-Ramamneh *et al.*, 2010) and levels of D_2O are measured in the blood both before the administration (Al-Ramamneh *et al.*, 2010). The rate at which D_2O or ^3H_2O concentrations decrease represents the turnover rate of the water and thus allows predictions of water intake (Brown and Lynch, 1972).

2.2.4.1 Factors affecting water turnover rate

2.2.4.1.1 Breed

Water turnover rate in sheep can be influenced by breed. Desert adapted sheep breeds such as the desert Bighorn sheep (*Ovis canadensis nelsoni*) and Fat Tailed Awassi are frugal with water and therefore have low water turnover rates (Degen 1977). In general animals adapted to hot environmental conditions (33-42°C) have lower metabolic and water turnover rates (Gaughan *et al*., 2009) than those living in temperate areas. This is generally due to greater capacity to dissipate heat by panting and sweating. Water turnover rate, therefore, can also be an indication of an animal's adaptation to the environment (Degen 1977).

Water turnover rates of German Mutton Merino sheep has been reported to be between 3 and 28% greater than that at Fat Tailed Awassi when grazing native pastures in autumn (10% water), winter and spring (70-85% water), and browsing shrubs in winter (water content not reported) or grazing legumes in Summer (10% water; Degen 1977). While, Leicester wethers had greater total body water (by 6%) and water turnover rate (by 13%) than Merino wethers (originated from North Africa) when grazing *Atriplex nummularia* or *Danthonia caespitosa* pasture (Macfarlane *et al*., 1967). Therefore, as a means of potentially reducing water needs, it is important for farmers to consider suitable breeds based on their locality.

2.2.4.1.2 Feed type

Feed type can influence the water turnover rate of sheep. The water turnover rate of Merino wethers was twice as high when grazing *Atriplex nummularia* (70% water) compared with *Atriplex vesicaria* (37-43% water; Macfarlane *et al*., (1967)). Factors that contribute to differences in water turnover rates of feeds include both their water and mineral content (Wilson 1974). Salt bush (*Atriplex vesicaria*) has a high mineral content which results in sheep requiring greater water intake (Wilson 1974). In Israel when grazing lush native pastures containing 70-85% water, both Awassi and German Mutton Merino required no free daily water (Degen 1977). In New Zealand, feeds vary greatly in their moisture content (refer Table 2.3). The high-water content of feeds reduce the amount of free water an animal requires and thus is the major determinant of the water turnover among sheep (Macfarlane *et al*., 1966a).

2.2.4.1.3 Fleece length

Fleece length affects water turnover rate in sheep for example Merino wethers water turnover doubled immediately after shearing in summer when temperatures ranged between 36 and 38°C (Macfarlane *et al*., (1966b). This increase in the water turnover rate was thought to be due to an increased heat load arising from fleece removal (Macfarlane *et al*., 1966b). Fleece provides insulation from heat and solar radiation, therefore, after shearing in hot environments sheep can experience an increase in heat gain. Both skin and rectal temperatures of shorn sheep can be greater than sheep carrying wool under hot conditions (Macfarlane *et al*., 1966b). Morris *et al*., (1962) observed that there was an increase in water turnover of 37% (from 11.3 to 15.5%) when temperatures were at a maximum of 30°C in shorn sheep. When there is a greater input of heat to the body there is a corresponding increase in the respiratory rate, body temperature, extracellular fluid, and water turnover to allow for evaporative cooling (Macfarlane *et al*., 1961).

2.2.4.1.4 Environmental conditions

Environmental conditions influence both water turnover and intake (Ismail 1995; Padua *et al*., 1997). In both India and Egypt the water intake of sheep have been reported to be greater in summer compared to winter (El-Nouty *et al*., 1988; Khan and Ghosh, 1989). The increase in body water content due to higher ambient temperatures is thought to be an adaptation to cope with higher air temperatures (Yousef and Johnson, 1984). At higher temperatures there is greater water turnover with more water being required for evaporative cooling as result of respiratory evaporation (Macfarlane *et al*., 1961). Heat exposure can also affect water intake by increasing water consumption as a means of meeting the high demand of water for evaporative cooling in goats (Giger-Reverdin and Gihad, 1991). Providing shade can help sheep cope with hot conditions and reduce water turnover rate (Wilson 1974), as shade is helpful in thermoregulation. Therefore, farmers need to ensure adequate drinking water is available during summer or warmer months.

2.3 Behaviour of sheep

Animal behaviour is the way in which animals interact with each other, with other living beings, and with the environment (Barnard 2012). The behavioural repertoire of sheep includes grazing and resting (Jørgensen *et al*., 2009), social (Veissier *et al*., 1998; Fisher and Matthews, 2001) and reproductive behaviours (Lindsay 1996). The main daily behaviours include walking, resting, drinking, ruminating and grazing (Lynch *et al*., 1992; Hilario *et al*., 2017). Generally, under pastoral conditions sheep spend 8 to 11 hours per day grazing (Arnold 1982; Lynch *et al*., 1992; Betteridge *et al*., 2010a; Ekesbo and Gunnarsson, 2018), 8 to 9 hours resting (Champion *et al*., 1994), 3-8 hours ruminating (Arnold 1962; Ekesbo and Gunnarsson, 2018) and 2-3 minutes drinking (Al-Ramamneh *et al*., 2012).

2.3.1 Social behaviours

Social behaviour of sheep is characterized by the interactions among individuals, such as communication, aggression, cooperation, mating, and parental behaviour (Rubenstein and Rubenstein, 2013). Breeds can differ in their behaviour and social interaction. For example Blackface and Suffolk sheep appear to differ in their social behaviours depending on the environment with Blackface ewes being less gregarious than Suffolk ewes (Dwyer and Lawrence, 1999). Moreover, Blackface sheep preferred the upland region of the field, whereas Suffolk ewes preferred the lowland areas. In a study examining the response to being given lambs of their own or another breed, Blackface ewes preferred to associate with their own breed, whereas Suffolk ewes associated equally with their own breed and Blackface (Dwyer and Lawrence, 1999).

Leadership is an import aspect of the social behaviour of sheep which coordinates the movement of groups of animals (Syme and Syme, 1979). Leadership can be helpful to control aggression in animals and protect group members from dangers (Syme and Syme, 1979). In sheep, movement is generally initiated by older animals (Rook and Penning, 1991; Dwyer and Lawrence, 2008; Zupan *et al*., 2010).

2.3.1.1 Flocking

Sheep in flocks maintain spatial relationships and they tend to be within short distances from others (Dwyer and Lawrence, 2008). Distances between nearest neighbours 1.5 to 6.9 m have been observed among Welsh mountain, Blackface, Suffolk, and Merino breeds (Lynch *et al.*, 1992). Sheep usually move together in groups and in some cases the size of group can vary depending on what they eat. Squires (1976) found sheep grazing saltbush grazed as a single flock, but on grassland they observed small sub-flocks of 5 or 6 sheep grazing together. The sub-flocks were also observed at the watering points or grouping under the shade of trees (Squires, 1976).

2.3.2 Home range and movement

The home range is the area where animals perform their routine activities (Jewell, 1966). In the wild with no restriction, sheep show a daily pattern of the movement within their home range (Lynch *et al.*, 1992). When walking sheep normally hold their head low with their nose pointing down to the ground while ears held back (Ekesbo and Gunnarsson, 2018). Sheep exhibit synchrony of movement whereby one individual will initiate movement and the rest of the flock will follow (Hulet, 1989; Zupan *et al.*, 2010). Farmed sheep in paddocks prefer to congregate in areas that are frequently grazed which may be due to previous grazing experiences (Gibb 1991; Lynch *et al.*, 1992) (Ramos and Tennessen, 1992; Walker *et al.*, 1992; Phillips and Youssef, 2003; Simitzis *et al.*, 2008).

Changes in food and water supplies influence the nature and extent of movement in animals within and out of their home range (Leuthold 2012). Sheep usually graze as they move towards watering points (Orr 1980). Breed differences have been noted whereby breeds like Merino do not form a home range behaviour (Lynch 1974) but breeds such as Scottish Blackface, Cheviot, and Soay which show clear home range behaviour (Hunter and Milner, 1963; Squires 1975; Hewson and Wilson, 1979).

2.3.3 Grazing

Grazing is the predominant daily behaviour of sheep (Lynch *et al.*, 1992). There is scant information about the behaviour of sheep around waterways, hence much of what is presented is drawn from cattle and other animals. Some New Zealand farmers utilise grazing strategies such as rotational grazing, leader-follower grazing, and intensive winter grazing. Rotational grazing is common during the summer, while leader-follower grazing is more common when dealing with different animal groups. Intensive winter grazing is common during winter months (Beef + Lamb New Zealand 2025). In the current study, rotational grazing was used across the seasons. Sheep appear to prefer grazing in areas close to water sources in hot environments, spend most of their time near the watering points (Lynch 1974, 1977). Sheep show seasonal changes in grazing distribution (Harris and O'Connor 1980) and that better quality influence their grazing distribution (Milner and Gwynne, 1974).

Among sheep time spent grazing depends on factors such as availability of feed, day light hours and group size (Lynch *et al.*, 1992; Penning *et al.*, 1993). Sheep spend approximately eight to nine hours a day grazing, however, this can increase up to 13 hours per day if feed is limited (Lynch *et al.*, 1992). The start and cessation of grazing depends on periods when there is light in the sky for example around sunrise and sunset (Lynch *et al.*, 1992). Frequency and time spent grazing has been observed to be reduced when sheep are kept in groups of less than three animals (Penning *et al.*, 1993). Sheep tend to form groups during the morning grazing (Scott and Sutherland, 1981). Reduced grazing time is likely a result of a lack of social influence. In cattle Benham (1984) identified social facilitation for movement, but not for initiation and cessation of feeding.

When sheep graze, they tend to move head-first into the wind (Orr 1980; Andrew and Lange, 1986). This behaviour results in higher grazing pressure on parts of the paddock that are toward the prevailing wind direction (Blake 1938; Orr 1980; Andrew and Lange, 1986). Since wind direction is associated with microbial composition of water (Evans *et al.*, 2006), there can be a possibility of sheep trapping these microbes ether with their wool or feet and hence creating higher chance of impacting water quality when they access water sources. Another mechanism wind affect water quality is via induction of waves that create sediment resuspension resulting in increased concentrations of *E. coli* and nutrients (Smith *et al.*, 1999; Bachmann *et al.*, 2000). Regarding wind direction, *E. coli* counts were significantly higher when the sample collection site laid downwind of the outfall (Smith *et al.*, 1999). In hot conditions, when the shade is not available, sheep will move in search of watering points (Orr 1980). However, in winter the influence of wind has the opposite effect and graze away from wind (Griffiths 1967). Sheep appear to avoid habitat with strong winds during winter and seek shelter (Grubb and Jewell, 1966; Sibbald *et al*., 1996). Observations suggest that the presence of shade and watering sites had very little or no effect on overall grazing distribution (Harris and O'Connor 1980).

2.3.3.1 Selectivity

Sheep are selective of the plants they eat while grazing (Lynch *et al.,* 1992; Armstrong *et al.,* 1993; Edwards *et al.,* 1993; Animut *et al.,* 2005). Sheep have a narrow muzzle and can select specific parts of the plant, usually green leaf that they prefer to eat (Lynch *et al*., 1992). Their divided upper lip philtrum allows them to pick small parts of plant easily (Ekesbo and Gunnarsson, 2018). To harvest plant materials, they grasp leaves by trapping the plant material between their lower teeth and the muscular pad while the head is moved posteriorly (Ekesbo and Gunnarsson, 2018). Sheep have a muscular pad in their upper jaw instead of teeth and grind their food with a predominantly lateral movement of the jaw (Finn 1995). When offered good-quality ryegrass (green stage), sheep prefer grazing areas that do not contain pseudostem or dead plant material unless the pasture species are mixed and contain clover (Barthram 1981; Edwards *et al.,* 1993). When perennial ryegrass was sown with white clover, sheep avoided pasture that contained endophytes (Edwards *et al.,* 1993). High stocking rates used by New Zealand farmers in late summer and autumn to ensure maximum lamb production (Macdonald et al., 2008) can limit an individual sheep's ability selectively graze specific plant species (Animut *et al.,* 2005) .

2.3.4 Drinking

Sheep are adapted to survive by drinking little or no drinking water (Macfarlane *et al*., 1966a; Laffan 2015). Sheep spend approximately 0.2% of the day drinking (Al-Ramamneh *et al*., 2012). German black-head mutton sheep grazing ryegrass pastures that contained 85% moisture in temperate conditions were observed to have higher drinking frequency during periods of grazing (Al-Ramamneh *et al*., 2012). Sheep graze as they move to watering points which influences how often they walk to the watering points (Orr 1980). For example, when grazing saltbush sheep will drink water twice a day (Squires 1981). During summer, when the temperature was high (21°C-40°C), sheep walked to the watering point every day (Lynch 1974; Squires 1976) and have been observed to visit twice a day in late summer (Squires 1976). Sheep drink most frequently in the morning (8- 9 a.m.) and afternoon (3 - 7 p.m. ;Squires (1976);

Ehrlenbruch *et al*., (2010)). When sheep drink twice a day the morning drink occurred soon after sunrise (Squires 1976). Since sheep graze as they move towards the watering points, it is possible that the changes in frequency of drinking might cause overgrazing of the riparian zones. Sheep exhibit leadership behaviours therefore, when one or two animals approach a watering point, others will follow (Rook and Penning, 1991; Zupan *et al*., 2010).

2.3.5 Excretion

Sheep defecate when they are in standing position , walking or feeding (Ekesbo and Gunnarsson, 2018). There is little information on the influence of waterways on sheep excretion, however, cattle appear to defecate and urinate more in areas near water (3m close to the watering point) than areas more than 3m (White *et al*., 2001). Dairy cows were found to defecate 50 times more per metre of stream crossing than they did elsewhere on the raceway (Davies-Colley *et al*., 2004). A relationship between eating and excretion, has been reported among dairy cattle with eating activity corresponding to the period of the highest urination frequency (Betteridge *et al*., 2010a).

2.3.6 Resting

Sheep prefer to rest on higher parts of the paddock overnight, coming down to the lower parts in the morning (Taylor *et al*., 1987; Lynch *et al*., 1992). Sheep initiates lying by scraping the ground and rest with fore legs stretched (Ekesbo and Gunnarsson, 2018). Resting behaviour can be affected by time of the day, what they eat, and environmental factors such as ambient temperature and altitude (Paterson and Coleman, 1982; Dwyer 2008; Betteridge *et al*., 2010b). In England when feeding on white clover and perennial ryegrass, sheep spent 36% of their time per day lying (Champion *et al*., 1994).

Resting behaviour can be affected by environmental factors such as ambient temperature. Among desert living Bighorn sheep, those exposed to high temperatures (38-40°C) were found to rest for most of the day (Dwyer, 2008). In semi-arid conditions of India, resting time varied between Chokla, Avivastra and Rambouillet breeds in the rainy season, spring, summer but not in winter (Dhanda and Singh, 2002). When temperatures are high (35 - 44°C) most breeds spend more time standing in the summer than in spring (Dhanda and Singh, 2002; De *et al*., 2017). A study in winter in Norway reported shade and food location influenced resting behaviour more than weather (Jørgensen and Bøe, 2011). Sheep ruminate when resting, whether lying down or standing (Leme *et al*., 2013). Rams exposed to high temperature (38-44 °C) were observed to have short rumination durations (De *et al*., 2017). This may be a strategy reduce production of metabolic heat (Kadzere *et al*., 2002). Rumination while standing (De *et al*., 2017), may be a mechanism to maximize heat loss by increasing body surface area to facilitate heat dissipation (Allen *et al*., 2013).

2.3.7 Factors that affect sheep behaviour

2.3.7.1 Paddock and environmental attributes

Features in the environment can influence where sheep prefer to congregate, rest and graze (Dwyer and Lawrence, 2008). In unrestricted conditions, sheep prefer to be within 200 m of escape terrain and prefer rough, and steep slopes while avoiding areas with flat, smooth ground and with good visibility (Dwyer and Lawrence, 2008). In desert environments they prefer areas that are close to water (within 400m; Dwyer and Lawrence, 2008). These attributes are less likely to be applicable in fenced paddocks as utilised in New Zealand.

There are a number of factors that influence the distribution of sheep in their environment including availability of feed and water, visibility, shelter, altitude, topography, slope and aspect (Mysterud *et al*., 2007; Haddon 2008; Gong *et al*., 2016; Plaza 2022; Fan 2022). In different seasons of the year the importance of some of these factors increase, for example, in hot conditions the presence of shade (Mysterud *et al*., 2007).

Aspect and slope angle are two of the primary topographical factors affecting pasture growth patterns in hill country. Slope angle and aspect influence the distribution of soil moisture (Crow *et al*., 2012) and soil and air temperature, as well as the rate of photosynthesis (McAneney and Noble 1976). This is done by modifying the distribution of the incoming solar radiation and accumulated heat (McAneney and noble, 1976; Petropoulos 2014).

Generally, in the southern hemisphere, northern aspects are warmer and drier than the south facing aspects (Radcliffe and Lefever, 1981). North-facing slopes exert higher evapotranspiration rates and receive more radiation than the south-facing slopes (Radcliffe and Lefever, 1981). Suckling (1975) and Suckling (1959) observed that there were remarkable differences in seasonal pasture yield between north and south facing slopes (mainly due to temperature differences), so that shady faces were grazed more in summer and sunny faces were grazed more in winter. Additionally, changes in slope can also result in variations in organic matter, soil physical properties, pH, botanical composition and soil fertility due to animal and gravity nutrient transfer from steep slopes towards easier slopes, (Mackay *et al.*, 1999; Lambert *et al.*, 2000; López *et al.*, 2003). The greater pasture growth on easier slopes was mostly due to the greater soil water availability than on steep surfaces (Gillingham *et al.*, 1998; Bretherton 2012).

2.3.7.2 Effect of temperature

Grazing behaviour

Temperature can influence grazing behaviour with high temperatures (31-44°C) resulting in reduced feed intake and grazing time (Shinde *et al.*, 1997; De *et al.*, 2017). In very hot conditions (38-40°C), the foraging range of extensively managed sheep can be reduced to approximately 3 km (Lynch 1974). Malpura cross sheep (Malpura and Garole) under tropical conditions show reduced feed intake and slower rumination in temperature between 38-44°C (De *et al.*, 2017). Reduced feed intake may be a result of voluntary adaptive depression of metabolic rate due to reduced appetite in heat-stressed sheep (Silanikove 2000). High environmental temperatures stimulate the peripheral thermal receptor, and hence signals are sent to the hypothalamus that leads to a reduction in feed intake (Marai *et al.*, 2007). Similarly, slower rumination rates might be a mechanism to reduce metabolic heat production (Kadzere *et al.*, 2002). The temperatures observed in the pre-mentioned studies are considerably higher than those observed in New Zealand, however, there is a paucity of data on the response of sheep to temperatures between 20 to 30°C.

Excretion is also affected by temperature with defecation and urination being more frequent, in warmer months (>22°C; White *et al.*, 2001). This increase in frequency is likely due to heat stress, although a greater urination frequency is likely to require greater water intake.

An increase in temperature reduces the duration of time spent grazing and increases resting time (Shinde *et al.*, 1997). The longer resting time is likely a mechanism to protect against thermal stress (Squires 1981). Greater temperatures also result in an increase in panting, which is the primary method of evaporative heat loss for sheep (Thwaites 1985). Panting influences the respiratory rate which in turn increases the demand for water (Spengler *et al.*, 2015).

Water intake

Ambient temperature has a positive relationship with water intake (Araújo *et al.*, 2010; Petersen *et al.*, 2016). In response to elevated temperatures, sheep change their diurnal activity patterns and drinking time (Dwyer 2008). For example, in Border Leicester sheep exposed to elevated temperatures (24 °C to 45 °C) there was an increased frequency of drinking in the early morning when the overnight temperatures increased (Daws and Squires, 1974; Dwyer 2008). Greater water intake in warmer months is likely driven by more frequent defecation and urination (White *et al.*, 2001).

The amount of water the livestock drink also depends on water temperature (Markwick 2007; Huuskonen *et al.*, 2011). Typically, animals prefer to drink water that is cooler than their body temperature (Markwick 2007). In a cool environment (20°C), when water temperatures were 20°C or 30°C, sheep preferred to drink water at 20°C. In a hot environment (40°C), however, sheep preferred to drink water at 30°C (Savage *et al.*, 2008). Similarly, cattle have been shown to prefer water at a temperature near their body temperature (Szlyk *et al.*, 1989; Beede 2006).

2.3.7.3 Effect of sunlight/solar radiation

Sheep exposed to solar radiation show increased respiration rate and rectal temperatures compared with those under shade (Brosh *et al.*, 1998; Sevi *et al.*, 2001). Inactive behaviours such as lying and standing increase when exposed to solar radiation (Sevi *et al.*, 2001). Solar radiation does not appear to affect patterns of drinking or feeding (Johnson and Strack, 1992; Brosh *et al.*, 1998) but was associated with postural modification to reduce heat load (Johnson and Strack, 1992). Sheep provided with shade were observed to stand for 2 hours longer per day than sheep in the sun (Johnson and Strack, 1992). Similarly, when exposed to the sun, they laid down while stretching their heads forward in a posture that reduced reflection of solar radiation from the ground directly beneath them (Johnson and Strack, 1992).

2.3.7.4 Location of watering points

The location of water sources can influence how often sheep drink, how much they drink, and where they graze (Pond *et al.*, 2004). Squires and Wilson (1971) reported that sheep showed two daily journeys to a watering point until the food to water distance reached 4 km after which drinking frequency reduced. When distance to a water source was over 5.6 km, water intake per drinking event increased proportionally to the distance walked (Pond *et al*., 2004). A study of cattle found that when given an area of 3 acres they spent less time at a stream when water sources were nearby or off-stream access was provided (Godwin and Miner, 1996).

2.4 Effect of livestock on water quality

The quality of freshwater varies across New Zealand with some rivers and lakes reported to have pollution levels high enough to harm people and freshwater plants and animals (Ballantine & Davies Colley, 2014). Repeated public-perception surveys have reported that the majority of respondents characterised river health as "adequate" to "very poor" with fewer regarding the condition of New Zealand's rivers as "good" or "very good" (Cullen *et al.,* 2006; Hughey *et al*., 2013). National monitoring programmes have also identified that water quality and biological conditions were poor in certain New Zealand rivers, particularly in catchments influenced by urban development and agricultural activities (Ballantine & Davies Colley, 2014).

Increased agricultural intensification in the livestock farming sector between 1990 to 2020 has been linked to poorer freshwater quality (Monaghan *et al.,* 2021; Snelder *et al.,* 2021). Intensification of livestock farming is characterised by higher stocking densities and increased fertiliser application (De Haas *et al*., 2011; Capper *et al.,* 2014). Larger numbers of livestock generate greater volumes of waste, placing additional pressure on the environment (De Haas *et al.,* 2011; Capper *et al.,* 2014). Furthermore, data from 2016 to 2020 show that water quality in rivers was more degraded when more high-intensity pasture and horticultural land was present upstream (Whitehead *et al.,* 2022).

Between 2016 and 2020, approximately 45% of the country's total river length was classified as unsuitable for recreational activities such as swimming (Kuczynski *et al.,* 2024). In addition, analysis of monitored lakes between 2017 and 2022 showed that roughly 10% of sites exhibited an average infection risk for Campylobacter exceeding 3%, thereby rendering these locations unsafe for swimming (Kuczynski *et al.,* 2024). Assessment also shows higher *E. coli* and poorer water quality in pastoral farming, linking land use to microbial water quality that influences recreational safety (Larned 2016; Davies-Colley 2018). Only 6% of river length in the urban land-cover class poses low or zero toxicity risk to aquatic biota with regard to nitrate-nitrogen and ammonia. These findings highlight the ongoing need for comprehensive monitoring and effective management of freshwater resources to safeguard public health and ensure the safe use of rivers and lakes for recreational purposes.

New Zealand has made efforts to maintain water quality including farming practice changes, regulation and policy changes. These initiatives include fencing livestock out of waterways, riparian planting along streams and improved effluent management systems. Water quality is now legally prioritised over some land uses under Te Mana o te Wai - New Zealand's National Policy Statement for Freshwater Management (New Zealand Government, 2020). The policy statement requires that regional councils utilize estimates of the current state and trends of environmental indicators for several purposes. These include establishing limits on land and water use within catchments, monitoring progress toward desired environmental outcomes, and identifying the underlying causes of any observed deterioration in environmental conditions. Such assessments provide a critical evidence base to inform decision-making, ensure the sustainable management of freshwater resources, and guide interventions to achieve specified freshwater objectives. Among the measures adopted, agricultural industry groups introduced voluntary nutrient-management and stock-exclusion schemes with the aim of improving water quality and preventing further environmental degradation (Wilcock *et al.,* 2013; Swaffield 2014).

Water quality is defined in terms of the chemical, physical, and biological content of the water (Araújo *et al*., 2010). The water quality of waterways changes with season and between geographic areas, even when no pollution is present (Markwick 2007). 'Good' water quality implies that harmful substances (pollutants/contaminants) are absent from water (Markwick 2007). Elevated nitrogen (N) and phosphorus (P) in surface waters are of environmental concern as they lead to eutrophication (Chislock *et al*., 2013).

Poor water quality is an issue facing New Zealand is agriculture with high concentrations of pathogens, sediment, and nutrients such as nitrogen and phosphorous being identified in many waterways (Anon 2015). Since colonisation New Zealand has lost of 90 % of wetlands and 75 % of freshwater fish species are now classed endangered (Anon 2020). Moreover, 80-94 per cent of urban streams and pastoral waterways are not suitable for swimming at least some of the time (Anon 2020). To overcome these water quality challenges, the New Zealand government released "Action for healthy waterways" in May 2020. One of the actions implemented was to require that all waterways greater than 1m wide are fenced to exclude farmed cattle, deer, and pigs (Anon 2020). There is currently no such requirement for sheep.

The aim of these actions is to prevent further degradation of New Zealand's freshwater resources and improve water quality within five years and to have 90 % of New Zealand's waterways swimmable by 2040 (Anon 2020).

Sediment and nutrients (nitrogen and phosphorus) work together to degrade water quality and damage freshwater ecosystems (Anon 2015). Eutrophication due to elevated nitrogen and phosphorus concentrations can stimulate the growth of unwanted biological species such as algae (Chislock *et al*., 2013). Sediment decreases water clarity and degrades the habitat for other aquatic life (Ryan 1991), while pathogens such as *Escherichia coli* (*E. coli)* pose a risk to human health (Scholefield *et al*., 1993; Ledgard *et al*., 1996; Monaghan *et al*., 2005; Markwick 2007; Abell *et al*., 2011). The concentrations of contaminants (nitrogen, phosphorus, suspended sediment and *E. coli)* appear to increase with larger waterways (McDowell *et al*., 2017). One method of reducing the concentrations of these contaminants in waterways is to prevent stock access (McDowell *et al*., 2017).

Poor water quality water can reduce milk production in cattle (Umar *et al.,* 2014; Hassan *et al*., 2025) and therefore may also impact sheep milk production. High salinity in drinking water has been reported to increase milk protein and urea nitrogen but did not affect milk production in Barbarine sheep (Elgharbi, 2015). Poor water quality may result in reduced water intake which in turn can result in decrease feed intake and reduce milk production (Yirga *et al.,* 2018). In lactating ewes milk production is heavily reliant on adequate water intake (Forbes 1968). For example, the ANZG (2023) guidelines suggest that dissolved salt concentrations between 4,000 - 10,000 mg/L may cause a reluctance for sheep to drink water and could cause scouring but stock adapt without loss of production, although concentrations between 10,000 – 13,000 mg/L likely result in a decline in productivity. Though sheep are generally considered tolerant to saline drinking water, they may, under favourable conditions such as grazing on lush green pasture, tolerate Total Dissolved Solids (TDS) concentrations of up to 13,000 mg/L without significant loss of body condition or productive performance.

2.4.1 Nitrogen and phosphorus

2.4.1.1 Nitrogen

Nitrogen is among the primary limiting nutrients for pasture growth and hence is commonly applied element in fertilisers (Statistics New Zealand 2003). Over the last 50 years, but particularly in recent decades, application rates of both nitrogen and phoshorus have increased in New Zealand, (MacLeod and Moller 2006). The substantial change in nitrogen application has been driven by a shift to livestock production and a drive to increase per ha pasture production to increase animal performance (Statistics New Zealand 2019b).

Nitrogen is an essential nutrient for both plants and animals, being a vital component of amino acids, proteins, and nucleic acids (Haygarth and Jarvis 2002). Nitrogen occurs in organic, inorganic, and gaseous forms (Vymazal 2007) and is commonly added to soils by spreading fertiliser, or in the urine and dung of livestock (Figure 2.2). Livestock urine is considered the dominant source of nitrate-nitrogen leached from soil (Statistics New Zealand 2019b). Nitrate-nitrogen refers to the nitrogen present as the nitrate anion. The total amount of nitrate-nitrogen leached due to livestock in New Zealand increased from 189,000 tonnes in 1990 to 199,000 tonnes in 2017 (Statistics New Zealand 2019b). In 2017 sheep accounted for 15% of the nitrate leached from livestock (Statistics New Zealand 2019b).

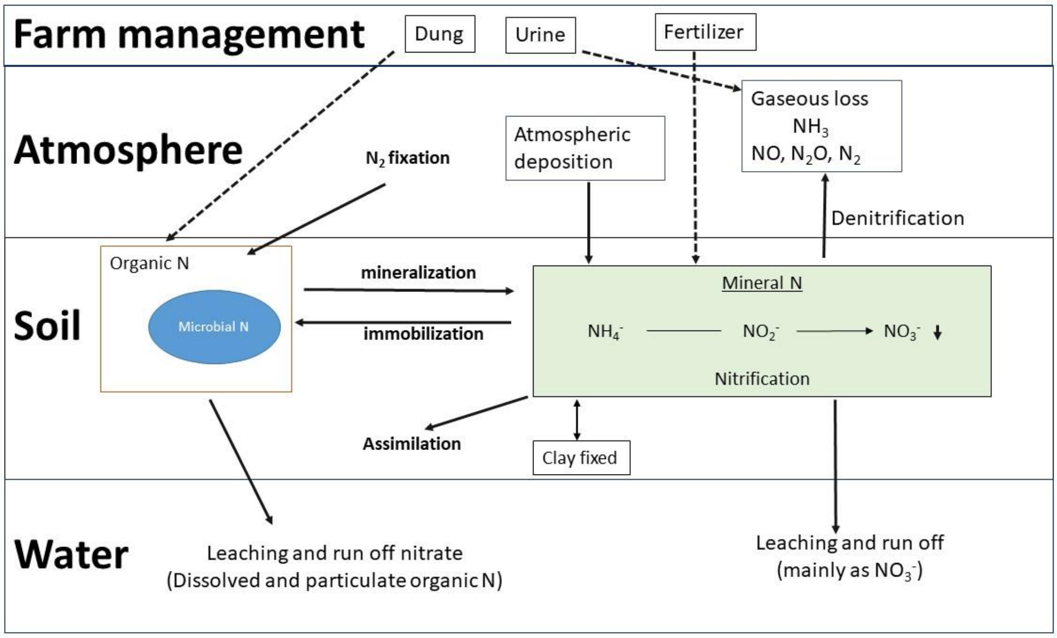


Figure 2.2: A simplified diagram showing the nitrogen cycle showing the impact of farm management on N in the atmosphere, soil and water. Modified from Haygarth and Jarvis (2002).

Ammonification can convert organic nitrogen to inorganic ammonia through microbial processes ($NH^+{}_4$; Kadlec and Wallace 2008). Inorganic ammonia is then transformed into nitrate (NO^-_3) by a bacterial process called nitrification (Kadlec and Wallace, 2008). Bacteria involved in this process include *Nitrosomonas* and *Nitrobacter*, which convert NH^+_4 to nitrite (NO^-_2). Nitrite is considered an intermediary nitrogen product, where *Nitrobacter* bacteria quickly convert NO^-_2 to NO^-_3 during the second stage of the process (McLaren and Cameron, 1996; See figure 2.2). Nitrate is the predominant form of reactive nitrogen in many waterways because it is highly soluble and readily leached from soils (Craig *et al.*, 2008). Ammonia is less prevalent in waterways as it is incorporated in organic matter and held by soil particles; however, it is highly nitrified in small streams (Craig *et al.*, 2008).

Nitrogen can enter surface waterways as surface runoff, groundwater, atmospheric deposition, municipal sewage, animal manure, soil organic nitrogen, and nitrogen fertilisers (Craig *et al.*, 2008, McIsaac 2003). Soil nitrogen inputs also include biological nitrogen fixation by legume pastures, plant residues, nitrogen fertilisers, sewage, and animal excreta (Cameron *et al.*, 2002, McIsaac 2003, Pakrou and Dillon, 2000).

2.4.1.2 Phosphorus

In New Zealand, phosphorus contamination of rivers, lakes, and streams arises from both natural sources and human activities; however, the dominant contributors are associated with land use practices, particularly agriculture, and the mobilisation of phosphorus through soil erosion. Phosphorus is one of the limiting nutrients for pasture growth and thus one of the sources is the soil from the use of fertilisers (Statistics New Zealand 2003).

Phosphorus in soil can either be immobilised in the organic form, adsorbed to the mineral soil or present in soil solution as plant available phosphorus (McLaren and Cameron, 1996). Phosphorus in water can be found in several forms such as organic, dissolved, and particulate phosphorus (Nash *et al.*, 2002). Dissolved P forms ($P < 0.45$ μm in size) are most readily available for uptake by undesirable aquatic plants and algae (Nash *et al.*, 2002). The bulk of phosphorus carried by rivers in NZ was found to be derived from particulate phosphorus sourced from gully and bank erosion (Davis *et al.*, 1998, Wallbrink *et al.*, 2003). Livestock manure and effluent contain phosphorus. During rainfall, this phosphorus can be washed into nearby waterways through runoff, where it contributes to eutrophication (Ross *et al.,* 2022). Nevertheless, catchment nutrient exports from intensively farmed land have been found to have a higher percentage of phosphorus in a soluble form, often attributable to fertiliser sources (Davis and Koop 2001).

2.4.2 Escherichia coli

Bacteria such *E. coli* in rivers and streams can originate from both livestock and human faecal contamination (Jamieson *et al*., 2004; Ministry for the Environment 2020). *E. coli* serves as an indicator organism of faecal contamination and microbiological impairment of water (Jamieson *et al*., 2004), with grazing animals often the principal source (Vinten *et al*., 2004; Collins *et al*., 2003). Increased faecal microbes pose a health risk to human health (Stephenson and Rychert, 1982), and livestock (Larsen *et al*., 1994). Most *E. coli* shed in faeces are thought to be harmless, but the occurrence of pathogenic VTEC/STEC strains has been found to be disproportionately high in rural New Zealand (Till and McBride, 2004).

2.4.2.1 Direct contamination

Dairy cattle have been found to be more likely to defecate while the herd is crossing a stream than while on a normal raceway. In the Sherry River in Tasman District, dairy cows were found to defecate 50 times more per metre of stream crossing than they did elsewhere on the raceway (Davies-Colley *et al*., 2004). In beef cattle Bagshaw (2002), reported that they spent an average of 4% of their time in or within 2 m of a stream and deposited 4% of their daily average faeces in these areas. Half of the faeces were deposited directly into water with the other half within the riparian zone which spanned 2 m on each side of the stream.

Research indicates that sheep excrete less weight of faeces than cattle (Wilcock 2006). Wilcock (2006) but that the concentrations of faecal organisms can be higher in sheep faeces than cattle. To date there is lack of data indicating sheep behaviour around waterways. Therefore, it is critical that more research is undertaken on sheep behaviour around waterways, to be able to quantify the potential impact on water quality.

2.4.2.2 Soil runoff and stream banks/riparian as sources of E. coli

E. coli can be transported to surface water from surrounding grazed paddocks via surface runoff. *E. coli* can be retained within soil particles and be transported in surface runoff with sediment (Muirhead *et al*., 2004). Unattached bacteria are neutrally buoyant in water and less likely to be filtered by vegetation than larger particles (Muirhead *et al*., 2006). Not all *E. coli* originates from recent faecal contamination, as *E. coli* can survive in the environment for longer than 1 month when soil temperatures were ≤25°C and can continue to multiply in faecal deposits (Ishii *et al.*, 2006; Moriarty *et al.*, 2011). *E. coli* can survive and regrow in the environment even after faecal waste is removed (Stephenson and Rychert, 1982; Byappanahalli *et al.*, 2003; Ishii *et al.*, 2006). Therefore, the soil can be a significant reservoir of *E. coli,* hence, previous grazing behaviour/activity is also important as it can be a significant source of *E. coli* contamination in the soil (Muirhead, 2009).

One of the main sources of *E. coli* streams is the riparian area immediately adjacent to streams. Runoff from surrounding land can elevate *E. coli* concentrations in water, however, the distance from the edge of the water affects the density of *E. coli* populations in the soil (Decamp and Warren, 2000; Byappanahalli *et al.*, 2003). These studies indicate that areas close to the stream are most likely to have a significant contribution to the *E. coli* pool in waterways.

2.4.3 Sediment

Water quality of streams, lakes, or other water bodies can be degraded by excessive amounts of dissolved or suspended sediment (SS) from surface runoff. Soil compaction due to animal trampling is one of the principal factors responsible for the degradation of the physical quality of soils under pasture and thus the formation of sediment (Da Silva *et al.*, 2003). Sediment can affect water quality by introducing contaminants such as pathogens (Jamieson *et al.*, 2004), and phosphorus (Ballantine *et al.*, 2006). In surface water, suspended sediment can be derived from natural and anthropogenic (caused by human activities) sources. Sediment transported in suspension is the most important contaminant of surface waters, as in high concentrations it can cause damage to aquatic habitat (Davies-Colley and Wilcock, 2004; Davie 2002). Suspended sediment is also important for transporting other contaminants, such as nitrogen and heavy metals (Howarth and Paerl, 2008). This is due to their physical and chemical properties, which can bind contaminants on their surfaces. For instance, phosphate is adsorbed onto sediment particles and can be transported into lakes and streams where its most significant effect is eutrophication (Howarth and Paerl, 2008).

2.4.3.1 Sources of sediment

The natural sources of sediment include atmospheric dust deposition due to wind erosion, mass movement events and erosion by water run-off (Kevin *et al.*, 2008). Other sources of sediment include erosion of channel banks and floodplains and re-suspension of channel bed sediment (Kevin *et al.*, 2008). Atmospheric sources of sediment are usually clean compared to that derived from anthropogenic sources, which may be heavily contaminated (Kevin *et al.*, 2008). The streambed acts as temporary storage for sediment (e.g., soil or atmosphere) and only releases sediment to the overlying water under certain hydrological conditions such as high flow conditions (Kevin *et al.*, 2008).

In farming, sediment sources also include soil erosion by grazing animals, machinery and tillage systems, and soil instability due to grazing animals (Kevin *et al.*, 2008). Pasture from streams and plantation forest have been reported to have a threefold higher suspended sediment and fine sediment stored in the streambed than streams in native forest catchments (Quinn *et al.*, 1997). Streambank collapse caused by animal grazing in riparian zones is also a potential source of sediment to the streams. Treading by grazing animals on stream banks, particularly cattle, by virtue of their large size and affinity for water, destabilises soils causing slumping and loss of soils directly into streams (Parkyn and Wilcock, 2004; Wilcock 2006).

2.4.3.2 Impact/effect of suspended sediment

High suspended sediment concentrations in water can damage respiratory structures of aquatic animals and reduce their ability to detect prey due to water turbidity (Davies-Colley *et al.*, 2004; Hicks *et al.*, 2004). Sediment can also limit light penetration for photosynthesis in aquatic ecosystems (Hicks *et al.*, 2004). It can also affect fish behaviour, feeding and egg laying, for example, within 1hr of measuring a rapid increase of suspended sediment, researchers reported feeding behaviour and dominance hierarchies of some fish were disrupted and alarm reactions were elicited, which may cause fish to relocate downstream to undisturbed areas (Berg and Northcote, 1985). Likewise, sediments have been associated with reducing egg survival (Erman and Ligon, 1988), and reduced feeding (Newcombe and Jensen, 1996).

Sediments also affect growth of aquatic plants (Zabarte-Maeztu *et al.*, 2020) as it settles on the streambed, filling interstitial spaces between particles, reducing the amount of habitat available to fish and invertebrates (Davies-Colley *et al.*, 2004). In New Zealand, suspensoids also have more noticeable effects even at low concentrations. When sediments settle, they can interfere with the feeding of benthos by covering the food supply of those organisms that feed on periphyton (Graham 1990). Moreover, reduction of interstitial spaces within the stream bed means less habitat and reduced exchange of oxygen and metabolites for those animals living in the bottom, or in the bottom sediment of the streams (Graham 1990).

2.4.3.3 Association between sediment and contaminants

In most cases, suspended pathogens, because of their small size and low density, will remain in suspension until they become associated with a solid substrate (Jamieson *et al*., 2005). As such, the suspended sediment provides a sink for pathogens within a system. In the absence of external sources, sediments could serve as a reservoir of high concentrations of microorganisms that could be resuspended upon changes in water turbidity, thus affecting the quality of surface water bodies negatively. Jamieson *et al*., (2005) found that enteric bacteria (*E. coli*) could survive for up to six weeks within bed sediment. Pathogens are carried on the surface of sediments; it is recognised that pathogens are associated with sediment in the aquatic environment (Jamieson *et al*., 2005). Abia *et al.,* (2017) reported that there was increased *E. coli* concentration after sediment disturbance.

2.4.4 Effects of season and flow on water quality

2.4.4.1 Nitrogen and phosphorus

The concentration of nitrogen in waterways is characterised by seasonal variation, with higher values in the winter, (Wilcock *et al*., 1999; Cooper *et al*., 1987; Wilcock *et al*., 2013; Skorbiłowicz and Ofman, 2014), and late autumn (Edwards *et al*., 2000; Yevenes and Mannaerts, 2011) than during summer (Wilcock *et al*., 1999; Arheimer and Liden, 2000; Wilcock *et al*., 2013). One reason of higher nitrate levels in winter is the higher water table resulting in water entering streams from shallow, nitrate-bearing groundwater (Wilcock *et al*., 2013). In New Zealand, nitrate concentrations are positively correlated with water flow during both winter and summer (Cooper *et al*., 1987; Rajanayaka *et al*., 2020). Nitrate accumulates in soils during drier seasons i.e., spring and summer, and is later mobilized and transported by higher rainfall, causing an increase in soil moisture, runoff, and base flow generation into streams (Yevenes and Mannaerts, 2011). A further potential explanation for lower nitrate losses during the summer period is higher temperatures and low levels of soil oxygen which are favourable for denitrification (Bremner and Shaw, 1958; Ferguson 1987). Thus, most nitrogen leaching from soil occurs during autumn and winter while most denitrification occurs during spring (Cameron *et al.*, 2002).

Levels of phosphorus in water are high in spring and autumn (Wilcock *et al.*, 1999; Rattan *et al.*, 2017), although, high concentrations can occur during storm events in winter (Wilcock *et al.*, 1999). These peak levels often coincide with spring and autumn applications of phosphate fertiliser (Wilcock *et al.*, 1999). Overall, lower levels of phosphorus in water are recorded during winter (Wilcock *et al.*, 1999). Edwards *et al.*, (2000) reported that the total phosphorus concentrations in water were generally small and only increase significantly during periods of reduced water flow in the summer / autumn periods. Phosphorus levels depend not only on temperature but also on the intensity of the runoff and peak water flow (Edwards *et al.*, 2000).

2.4.4.2 Pathogens

Water flow rates and bacteria levels show a positive correlation (Cooley *et al.*, 2007; Whitman *et al.*, 2008; Phiri *et al.*, 2020). Highest concentrations of faecal bacteria are expected in summer, when stream flows are at their lowest (Wilcock *et al.*, 1999), however, *E. coli* concentrations can increase during rainfall and under high flow conditions (Wilcock *et al.*, 1999; Giddings *et al.*, 2004; Cooley *et al.*, 2007; Whitman *et al.*, 2008; Phiri *et al.*, 2020). Therefore, the highest levels of faecal bacteria in New Zealand based on flow, are during winter followed by autumn and summer (Wilcock *et al.*, 1999). Rainfall increases streamflow, which in turn can increase bacterial concentrations both in-stream stores and wash-in and stores (Donnison *et al.*, 2006). Seasonal dynamics in *E. coli* concentrations in sediments have also been documented (Giddings *et al.*, 2004).

2.4.4.3 Sediments

Sediment in waterway begin to accumulate during spring with maximum storage and concentrations in late summer, after which, the onset of high flows during winter remobilises the stored sediment (Smith *et al.*, 1996; Ballantine *et al.*, 2006). Hence, in New Zealand suspended sediment concentrations increase with increasing flow (Collins and Rutherford, 2004) and in low flow conditions in summer (Collins and Rutherford, 2004). Winter accounts for 85% of run-off and the dominant process is the sedimentation of larger particles (Jensen *et al.*, 2006).

2.4.5 Effects of temperature on water quality

2.4.5.1 Nitrate and phosphorus

The effect of temperature on nitrogen and phosphorus concentration is less important than water flow rates. High temperatures coupled with low levels of oxygen, reduces nitrate concentrations due to denitrification (Bremner and Shaw, 1958; Ferguson, 1987). Phosphorus concentrations depend on temperature, the intensity of runoff and peak water flow (Edwards *et al.*, 2000). The rate and magnitude of phosphorus uptake by sediment was found greater at 19^{o}C compared with 26^{o}C, and when nitrogen was involved (McDowell *et al.*, 2017).

2.4.5.2 E. coli

Temperature is an essential factor affecting bacterial growth and survival in water (Faust *et al.*, 1975; Blaustein *et al.*, 2013). *E. coli* grow better and live longer in warm temperatures (25-37˚C, Hendricks, 1972; Ishii *et al.*, 2006). Padia (2011) reported that survival of *E. coli* from faecal material in creek water at 0, 10, 20, and 50˚C over the course of a week showed sustained population growth at 20˚C, but not at 50˚C. *E. coli* concentrations are not only temperature-dependent but also rainfall dependent. St Laurent and Mazumder (2014) observed that there were higher faecal coliform concentrations in water in winter than in summer which were attributed to the greater rainfall in winter months. Under controlled conditions, *E. coli* in bovine faecal slurry survived a minimum of 28 days at 4˚C, but as short as five days at 23˚C (Kudva *et al.*, 1998). This indicates that survival of *E. coli* may be improved under cooler conditions.

E. coli counts were found to be significantly higher, during the morning than the afternoon, and highest during cloudy skies (Whitman *et al.*, 2008) which may be due to exposure to sunlight (Park *et al.*, 2021). Three to six hours of sunlight exposure within the pond is sufficient to reduce the *E. coli* number*,* through inactivation (Whitman *et al.*, 2008; Park *et al.*, 2021).

But in New Zealand, solar radiation in winter appears to be too weak to result in *E. coli* inactivation, instead the cooler temperatures aid *E. coli* survival (Collins, 2004).

2.5 Precision tools and livestock management

Many behavioural and ecological studies record the movements and performance of animals. Tools to monitor animal behaviour are preferred due to the challenges of studying behaviours, such as bias due to permanent observer effects (Nanninga *et al*., 2017). Another disadvantage of human observers is that if more than one observer is involved, behavioural recordings can differ between observers (Martin *et al*., 1993; Schwarz *et al*., 2002). Also, human observers can influence the behaviours the animals display (Gordon, 1995; Crofoot *et al*., 2010; Lutz and Nevill, 2011). Costs associated with taking behavioural measures in research, have also resulted in new technologies being developed (Edwards, 2007; Sørensen *et al*., 2007). Therefore, the use of remote monitoring tools can improve efficiency and allow animals freedom to express their natural behaviours (Müller and Schrader, 2003).

Tools that have been previously used include acoustic recording tags (Johnson *et al*., 2009; Huntingford *et al*., 2012), cameras and videos (MacNulty *et al*., 2008; Moll *et al*., 2009), lasers (Fehmi and Laca, 2001), access control systems and E-Systems (Swain *et al*., 2019) and Global Positioning System (GPS) technology (Turner *et al*., 2000). E-Management system relies on the acquisition of digital data and the use of the digital data (e.g., telemetry data) in management. Methods to assist in the identification for monitoring animal behaviour include coloured collars and tags (Turner *et al*., 2000).

2.5.1 Monitoring spatial position and animal behaviour

There are two types of spatial position monitoring tools, recording of location using global positioning systems (GPS) or recording the position in a pre-defined area using lasers, cameras and video systems (Cooke *et al*., 2004). Most tools monitor spatial position by measuring the coordinates using a telemetry system. Biotelemetry systems use devices which transmit their signals to a receiver such as satellites (i.e., GPS) and devices which store their data for later retrieval (i.e., data loggers) (Cooke *et al*., 2004). Technologies such as very high-frequency (VHF) devices (Soutullo *et al*., 2007; Tomkiewicz *et al*., 2010), and ARGOS (Advanced Research and Global Observation Satellite), Doppler-based positioning (Soutullo *et al*., 2007;

McClintock *et al*., 2015) have also be used. GPS telemetry has been used to model habitat use, mechanisms of migration and for studies of the impacts of conservation (Hebblewhite and Haydon, 2010). In addition, automatic radio-tracking systems for small animals have replaced manual radio-telemetry systems (Gottwald *et al*., 2019). These systems have good accuracy, affordable and are easy to use. Recently GPS have been combined with general Packet Radio Service (GPS-GPRS) to investigate the grazing behaviour and spatial distribution of dairy cattle (Lomillos Pérez *et al*., 2018).

Other technologies have also been used to monitor spatial positions based on pre-defined coordinates including lasers (Fehmi and Laca, 2001), access control systems and E-Systems (Swain *et al*., 2019), and video systems (Moll *et al*., 2009). In addition, arrays of microphones have been used to monitor the position of free-living animals based on the sounds they produce (Mennill *et al*., 2012). This is particularly useful when studying animal ecology and behaviour, especially when integrated with GPS (Blumstein *et al*., 2011; Mennill *et al*., 2012). Other technologies include three-dimensional (3D) videography, high-speed single-camera and multi-camera videography, imaging sonar and thermal infrared imaging (Hughey *et al*., 2018).

One of the challenges of using technologies to monitor spatial position of animals is power management. Power management tools/applications can estimate the state of charge remotely, and schedule tasks that utilise less charge (Sommer *et al*., 2013). Solar powered devices can extend the duration that technologies can operate (Panckhurst *et al*., 2015; Byrne *et al*., 2017; Silva *et al*., 2017; Rahman *et al*., 2018; Hart *et al*., 2020). Alternatively, the use of smaller and lighter batteries and chips can provide a longer battery life (Vyssotski *et al*., 2006; Jurdak *et al*., 2013; Misra *et al*., 2014; Panckhurst *et al*., 2015), which improves their suitability for use in small species.

2.5.1.1 Global positioning system (GPS)

GPS units receive signals from satellites which it converts into location coordinates such as longitude and latitude (Turner *et al*., 2000). The addition of an accelerometer to a GPS unit can provide motion and head position data in addition to recording animal positions. Lightweight GPS collars with improved battery life have been used for recording the spatial location of livestock (Schlecht *et al*., 2004; Misra *et al*., 2014). Advancement in technology has led to smaller, low-voltage and low-current receivers that are powered by battery systems that can allow the tracking of small mammalian species and birds (Meyburg *et al*., 2006; Vyssotski *et al*., 2006; Grémillet *et al*., 2008).

In behavioural studies, GPS technology has been used to study the grazing behaviour of cattle (Bailey *et al*., 2004; Henkin *et al*., 2012) as well as control of grazing, i.e., virtual fencing of cattle (Campbell *et al*., 2019). GPS used in conjunction with pedometers that can count steps and measure activity levels can differentiate between standing and lying (Ungar *et al*., 2011). Accelerometers are attached to various body parts such as leg, neck, and head. Accelerometers have been used to determine the posture of sheep (i.e., grazing, standing, laying, and walking) (Marais *et al*., 2014; McLennan *et al*., 2015; Alvarenga *et al*., 2016; Radeski and Ilieski, 2017).

2.5.1.2 Visual media technology

Video footage recording is one of the standard tools used for studying animal behaviour (MacNulty *et al*., 2008). Videos can be used to study spatial movements, and activities such as feeding, standing and locomotion in both sheep (Ginelli *et al*., 2015), and cattle (Müller and Schrader, 2003). Validation studies using video observations have reported high levels of agreement between video recording and other tools such as accelerometers (Müller and Schrader, 2003). The use of videos, however, can often be time consuming and labour-intensive (Müller and Schrader, 2003). Video footage is ideal for capturing infrequent or complex behaviours such as play (Nelson and Fijn, 2013). These are often missed by observers as they can occur inconsistently. Behaviour can be monitored at the group level or individual level.

Data from video footage can provide estimates of the duration of behaviours such as walking, standing, and grazing. Video footage can also be used to validate behavioural predictions of other devices such as accelerometers e.g., ice tags leg sensors (IceTag3D™, Ice Robotics, Scotland, UK; Nielsen *et al*. (2010)). Video monitoring has been recommended for studies of the impact of grazing cattle on riparian zones to in order to improve GPS efficiency (Kaucner *et al*., 2013). Software and computer systems can be utilised to support the study of behaviours for example Observer XT (Noldus *et al*., 2000), BORIS (Friard and Gamba, 2016), CABER (Patrick, 1985) and MacSHAPA (Sanderson *et al*., 1994). Other systems include Constellations (Goldman-Segall, 1993), FERAL (Carter and Patrick, 1997), NUDIST and C Video (Richards and Richards, 1991). Behaviour, movement and location (GPS data) data can be analysed using mapping such as ArcGIS and oz Track and statistical packages such as R (Ramos *et al*., 2007; Hunter *et al*., 2013).

2.5.1.3 Other sensor technologies

There is an array of sensors to that can be used to record animal behaviour, including accelerometers, magnetometers, acoustic recorders, and pressure sensors. Sensors are useful in recording changes in activity intensity and the type of activity an animal undertakes. For example, differentiating between walking, grazing, and resting. Behaviours such as grazing, walking, lying, standing, reproduction and excretion have been studied using sensors (Turner *et al*., 2000; Ganskopp, 2001; Huntingford *et al*., 2012; Betteridge *et al*., 2013; Bishop-Hurley *et al*., 2014; Draganova *et al*., 2016; Barwick *et al*., 2018b). Sensors have enabled behavioural scientists to collect data over long periods with little impact on the behaviour of the animal being observed (Handcock *et al*., 2009; MacKay *et al*., 2012; Fogarty *et al*., 2018).

Motion sensors can record horizontal and vertical angle of the head of the animals, which can be an indicator of a particular behaviour of an animal (Schwager *et al*., 2007). Head movements can an indicator of grazing and for resting behaviours (Anderson and Bishop-Hurley, 2006). Leg movements can also be studied for predicting walking or standing status (Nielsen *et al*., 2010; Barwick *et al*., 2018b). Accelerometers use a biotelemetry system. Accelerometers are a non-invasive tool that can identify behaviours such as standing, lying or number of steps taken (McGowan *et al*., 2007; Shepard *et al*., 2008; Trénel *et al*., 2009; Nielsen *et al*., 2010; MacKay *et al*., 2012; Yoshitoshi *et al*., 2013; Diosdado *et al*., 2015; Barwick *et al*., 2018b; Lush *et al*., 2018). Accelerometers have also been used to link behavioural classification data as an early indicator of health and welfare issues (Barwick *et al*., 2017). For example, accelerometers can detect the abnormal walking pattern and other behaviour changes, and thus help predicting lameness and other health issues (Barwick *et al*., 2017; Barwick *et al*., 2018a).

A combination of spatial position tools with activity sensors can further improve the study of animal behaviour. A single tool for studying free-range animal behaviour on complex landscapes is not available; hence a combination of tools is advocated (Ungar *et al*., 2005; Anderson, 2011). When incorporated with GPS, accelerometers aid in activity classification since spatial–temporal information can be improved when the corresponding activity of the animal is known (Ungar *et al*., 2005). For example, combining GPS with accelerometers/pedometers allows the measurement of movement displayed during a given behaviour in cattle, and marine mammals (Mitani *et al.*, 2003; Aharoni *et al.*, 2009; Ungar *et al.*, 2011). GPS with urine sensors can measure where and when urination occurs (Betteridge, 2008; Betteridge *et al.*, 2010b). Further, electronic compass and telemetry coupled with videos can be used to determine where and the distribution of animals performing a given behaviour (Mitani *et al.*, 2003; MacNulty *et al.*, 2008).

2.5.2 Intrinsic errors of precision tools

2.5.2.1 Overview

A potential challenge of using GPS technology as a monitoring tool for animal behaviour is the effect of system errors in the data. GPS technology is subject to satellite clock errors, satellite position errors, receiver error, multipath errors, atmospheric errors, and selective availability errors (Hurn, 1989; Moen *et al.*, 1996; Rempel and Rodgers, 1997; Turner *et al.*, 2000; Tomkiewicz *et al.*, 2010; Li *et al.,* 2022; Cao *et al.,* 2023). Reflection of satellite signals by broad objection that lead to multipath errors (Hurn, 1989). Canopy, topography, and adjacent structures can cause errors with GPS data (Moen *et al.*, 1996; Rempel and Rodgers, 1997; Di Orio *et al.*, 2003; Hebblewhite *et al.*, 2007; Frair *et al.*, 2010). The density of forest cover has been found to increase time-to-location fix (Hurn, 1989; Moen *et al.*, 1996; Rempel and Rodgers, 1997; Tomkiewicz *et al.*, 2010) with fix rates reduced from 100% in open flat areas to 53% under dense canopy with slopes (Jiang *et al.*, 2008). There can also be position error introduced by selective availability of GPS systems (Tomkiewicz *et al.*, 2010). Selective availability is “a policy and procedure of denying non-military users full accuracy of the GPS system” (Tomkiewicz *et al.*, 2010). Selective availability errors, result in degraded accuracy of data (clock) especially for civilian users (Turner *et al.*, 2000).

Bias errors in GPS telemetry data can occur (Frair *et al.*, 2010) either in the fix rate or location precision due to device orientation (Moen *et al.*, 1996; Merrill *et al.*, 1998) and make or model of the device (Merrill *et al.*, 1998; D'Eon *et al.*, 2002; Frair *et al.*, 2004; Hebblewhite *et al.*, 2007). Vertical orientations perform better than horizontal one by increasing fix rate and location accuracy (Heard *et al.*, 2008; Jiang *et al.*, 2008).

2.5.2.2 Error correction

The accuracy of GPS receivers can be improved by the use of differential correction (DGPS). DGPS minimises the effect of position errors, atmospheric distortion and gets rid of satellite clock errors (Moen *et al.*, 1997; Rempel and Rodgers, 1997; Schlecht *et al.*, 2004; Tomkiewicz *et al.*, 2010). Errors from the base station receiver can be corrected by roving GPS receivers, hence removing the errors from location fixes (Parkinson *et al.*, 1996). Expression of errors can be in terms of circular error probable (CEP) which is the circle radius that contains percentile of points around a true location (Moen *et al.*,1997; Rempel and Rodgers 1997).

Using many evenly distributed satellites also minimises errors as the number and distribution of satellites is critical in the accuracy of a GPS receiver (Hurn, 1989). The distribution of satellites is described in terms of Position Dilution Precision (PDOP, 3-D) and Horizontal Dilution of Precision (HDOP, 2-D), compiling data from a minimum of three and four satellites, respectively. Lower dilution of precision (DOP) values indicates wider satellite spacing, with reduced spatial precision with increasing DOP values (Moen *et al.*, 1996). Triangulation errors become very small when satellites are far apart, and dilution of precision values are low. Errors become large when satellites are close together, and there is a high dilution of precision values (Hurn, 1989; Moen *et al.*, 1997; Rempel and Rodgers, 1997; Schlecht *et al.*, 2004).

When dealing with time to first fix errors (TTFF) in GPS tracking devices, receivers require 10 to 30 minutes to find GPS satellites and determine their first position. To control the first fix errors, rapid fix technologies and modern software can be used (Tomkiewicz *et al.*, 2010). The receiver can be kept in a warm start mode and hence often acquire a GPS fix of a surfacing aquatic animals in 10 seconds (Elkaim *et al.*, 2006).

A combination of spatial positions tools with sensors and data management systems can help to reduce errors and improve GPS fixes in terrestrial and aquatic animals (Hunter *et al.*, 2005; Elkaim *et al.*, 2006; Wilson *et al.*, 2007; Hunter *et al.*, 2013). Developments in sensor technology improve error reporting and ultimately improving behaviour assessment (Cooke *et al.*, 2004). Errors can also sometimes be controlled by manipulation of the device or improving the way devices are used. For example, device orientation on bears and vegetation have been shown to reduce fix rates up to 80% and location precision up to 17 m under canopy cover (Heard *et al.*, 2008; Jiang *et al.*, 2008). Fix rates declined with increasing canopy cover when the collar was on its side than when the collars were oriented upright in the open (Heard *et al.*, 2008). Jiang *et al*., (2008) noted that it was the position of the antenna that was important in closed areas.

Shorter fix intervals and reduced frequency between locations can improve fix success (Cain *et al*., 2005). Furthermore, when measuring distance travelled by an animal, selecting an optimal GPS sampling interval is useful to control GPS measurement error (McGavin *et al*., 2018).

This review focused on ovine species, however, where appropriate, due to paucity of research in sheep especially on behaviour and impact of sheep around natural waterways references from other animal species such as cattle and goats. While the behaviour of sheep in extensive production systems have been reported, their behaviour around natural waterways and impact on water quality have not been described. A greater understanding the impacts of sheep are important to understand if there are strategies needed to mitigate the effects of sheep production on the health of New Zealand waterways.

3 The behaviour of sheep around a natural waterway and impact on water quality during winter

Simple summary

The impact of extensively managed sheep on the natural environment has received little attention in comparison to beef and dairy cattle in New Zealand. There is a paucity of information on the interaction of sheep with natural waterways and the impact on water quality. The current study examined the behaviour of sheep on a hill country paddock, which was transected by a natural waterway, and assessed measures of water quality during winter. The study also investigated the effect of access to a reticulated water trough. Observations of ewe behaviour showed that they spent little time near the waterway compared to other areas of the paddock. In addition, access to the water trough had no effect on ewe time spent grazing, walking, resting, and drinking. Under the conditions of the current study, sheep had little impact on the waterway which may have been due to the high moisture content of pasture resulting in little need to interact with the waterway.

3.1 Abstract

Access of livestock, such as cattle, to waterways has been shown to be a cause of poor water quality due to pugging damage and excretion entering the water. In New Zealand, regulations require that cattle, deer, and pigs are excluded from accessing waterways, but there are no such requirements for sheep. The current study utilised 24 h video cameras, global positioning system units, and triaxial accelerometers to observe the interaction of Romney ewes ($n = 40$) with a natural waterway. Ewes were either restricted (week 1) or given access to a reticulated water trough (week 2). Proximity data showed that ewes spent more time within 3 m of the waterway when the trough was unrestricted than when restricted (14.1 ± 5.7 and 10.8 ± 5.1 min/ewe/day, respectively; $p < 0.05$). Ewes travelled shorter distances on the steeper areas of paddock than flatter areas. Similarly, ewes showed a spatial preference for the flat and low sloped areas of the paddock. Concentrations of suspended sediment and total phosphorus were higher during access to a reticulated water trough which coincided with the week with more rainy days. Phosphorus and *E. coli* concentrations in the stream water samples were above the

recommended Australian and New Zealand Environment and Conservation Council water quality guidelines, especially after rainy days, but did not appear to be directly related to sheep activity. Overall, the results suggest that during winter, ewes interacted very little with the waterway and were thus unlikely to influence the levels of nutrient and pathogens in the waterway.

3.2 Introduction

The farming of livestock on New Zealand's hill country environment can have negative effects on water quality due to the contamination of waterways with phosphorus (P), nitrogen (N), sediment, microorganisms, and faecal matter (Abell, Özkundakci, Hamilton, and Miller, 2011; Gillingham and Thorrold, 2000; Monaghan, Paton, Smith, Drewry, and Littlejohn, 2005; Scholefield *et al.*, 1993). Nitrogen, *Escherichia coli* (*E. coli*), and phosphorus contamination can originate from animal urine and faeces, while fertiliser can be a potential source of nitrogen and phosphorus (Jamieson, Joy, Lee, Kostaschuk, and Gordon, 2005; McDowell, Nash, and Robertson, 2007; McIsaac, 2003). For example, fertiliser and dung as sources of phosphorus contribute up to 40% of the total amount, and the rest is from other sources such as pasture-plants and soil components (McDowell *et al.*, 2007). Fertiliser and manure account for 57% of the total nitrogen contamination in watersheds (Bellmore *et al.*, 2018). High levels of nitrogen and phosphorus in waterways can lead to excess algal and aquatic macrophyte growth (Cooper and Thomsen, 1988; Quinn, Cooper, Davies-Colley, Rutherford, and Williamson, 1997), which may alter algal community structure and function (Thompson and Townsend, 2004). In addition, excess nitrogen and phosphorus can potentially make water unsafe for drinking for both stock and humans (Heathwaite, Johnes, and Peters, 1996; Houlbrooke, Horne, Hedley, Hanly, and Snow, 2004).

Sediment that is washed into waterways can also contain significant concentrations of phosphorus and *E. coli* (Ballantine, Walling, Collins, and Leeks, 2006; Howarth and Paerl, 2008). *E. coli*, present in animal faeces, serves as an indicator organism for faecal contamination and microbiological impairment of waterways (Jamieson *et al.*, 2005). Increased faecal microbes can pose a health risk to animals, water life, and humans (Larsen, Miner, Buckhouse, and Moore, 1994; Pal and Gupta, 1992; Stephenson and Rychert, 1982). Further, excess sediment in waterways can negatively impact the aquatic habitat by limiting penetration of light through the water, decreasing photosynthesis of aquatic ecosystems, and impacting human water use (Davies-Colley, Nagels, Smith, Young, and Phillips, 2004; Davis and Koop, 2001; Hicks, Quinn, and Trustrum, 2004). Therefore, there is a need to control the deposition of animal excreta into waterways and movement of animals, causing sediment disturbance around these areas.

In May 2020, the New Zealand government released their "Essential Freshwater" package which contained changes to the existing National Policy Statement for Freshwater Management. These polices included new regulations requiring that cattle, pigs, and deer be excluded from waterways more than 1 m wide and located on low slopes (Ministry for the Environment New Zealand, 2003). Currently, there is no such requirement for sheep. To date, the behaviour of sheep around natural waterways has received little attention, and there is a need to monitor behaviour to determine if their access to waterways should be restricted. Recently, there has been an increase in the use of digital technologies for animal behaviour studies which include the use of video cameras, tri-axial accelerometers, and global positioning systems (GPS) (Huntingford, Kadri, and Jobling, 2012; Johnson, de Soto, and Madsen, 2009; MacNulty, Plumb, and Smith, 2008; Moll *et al.*, 2009; Rahman *et al.*, 2018; Turner, Udal, Larson, and Shearer, 2000). The advantage of these digital technologies include reduced labour and time costs associated with undertaking behavioural measures, continuous observation of animals regardless of the time of the day (Edwards, Cook, Smart, and Wade, 2000; Sørensen, Rousing, Møller, Bonde, and Hegelund, 2007), and reduced bias due to observer effects (Hauser, 1993; Nanninga, Côté, Beldade, and Mills, 2017; Schwarz, Hofmann, Gutzen, Schlax, and Von der Emde, 2002).

Little information is currently available on the behaviour of sheep around natural waterways but may choose to graze close to waterway areas if the high-quality pasture is available (Oluju, 2017). On many New Zealand farms, sheep have access to free water from reticulated water supplied in troughs. Sheep can also obtain water from the pasture they consume; these pastures can contain up to 70% moisture (Brown and Lynch, 1972). It has been reported that in winter, when the dry matter content of pasture was less than 30–50 %, sheep were able to meet their water requirements from pasture alone and did not need additional free water (Brown and Lynch, 1972; McFarlane and Howard, 1972). It is therefore likely that during winter, sheep do not need to drink water. Therefore, natural water sources, such as a stream, are not required to meet their water intake needs.

The current study firstly examined the behaviour of sheep around a natural stream and secondly studied their impact on water quality in the stream. This study was conducted in the presence and absence of a reticulated water trough to examine the impact this may have on their behaviour. It was hypothesised that sheep would have little interaction with a natural waterway and that these interactions would not be influenced by the access to a reticulated water trough. It was also hypothesised that sheep would not have an impact on the water quality of the natural waterway.

3.3 Materials and methods

All the procedures in this study were carried out with the approval of the Massey University Animal Ethics Committee (MUEC 19/62). The study was conducted over a 15-day period from 16 August 2019 (D1) to 30 August 2019 (D15) at Massey University's hill country farm, Tuapaka, located approximately 15 km north-east of Palmerston North, New Zealand (40.3346° S, 175.7316° E), with study paddock located at 40.3345° S, 175.7390° E.

3.3.1 Study site

The study was conducted in a permanently fenced 1.7 ha paddock with the dimensions of 249.0 m × 249.4 m × 85.0 m × 50.2 m (Figure 3.1) that contained a discrete natural stream. The stream was classified as sixth-order based on high resolution 1 m LiDAR digital elevation data (Liu and Zhang, 2011). The stream was 233 m in length, <1 m wide, and <30cm deep in base flow conditions (Figure 3.1). The stream contained culverts at the entrance and exit of the paddock. The watershed area supplying the study paddock between the inflow and outflow was calculated by ArcGIS Pro to be 4.1 ha (Figure 3.2, watershed 2). The catchment area was grazed only by sheep throughout the year, with a pasture mass of 1263.3 ±120.7 kgDM/ha (Mean ± SD).

3.3.2 Animals and study design

The study utilised mature (3 to 5 years of age) Romney ewes (n = 40) that had been diagnosed bearing a single foetus by trans-abdominal ultrasound at approximately day 90 of gestation. Ewes had an average weight of 72.3 ±7.0 kg and body condition score of 3.0 ± 0.4 (Mean ± SD). Prior to the study, ewes were managed in an extensive pastoral system and offered 100% ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) pasture within a rotational grazing system. The ewes in the study were familiar with the paddock as it was part of their normal grazing rotation. Prior to the start of the study, the pasture mass of the paddock was measured to ensure that sufficient dry matter was present to allow ewes to remain at the study site for the entire two-week period.

During the study period, a crossover design was utilised. Ewes were grazed in the study paddock for one week when access to the trough was restricted by covering it, resulting in free water only accessible from the stream. This was followed by a second week when access to a reticulated water trough was unrestricted (trough uncovered). During the study period there were no sheep in adjacent paddocks. Ewe movement within the paddock and interaction with the waterway was monitored using GPS, triaxial accelerometers, and video surveillance footage. Water samples were collected to measure indicators of water quality. Throughout the study period, ewes were continuously stocked on a predominantly perennial ryegrass pasture (*Lolium perenne*) with masses of 1263 ±121 kgDM/ha and an average moisture content of 77 ± 3% (Mean ± SD).

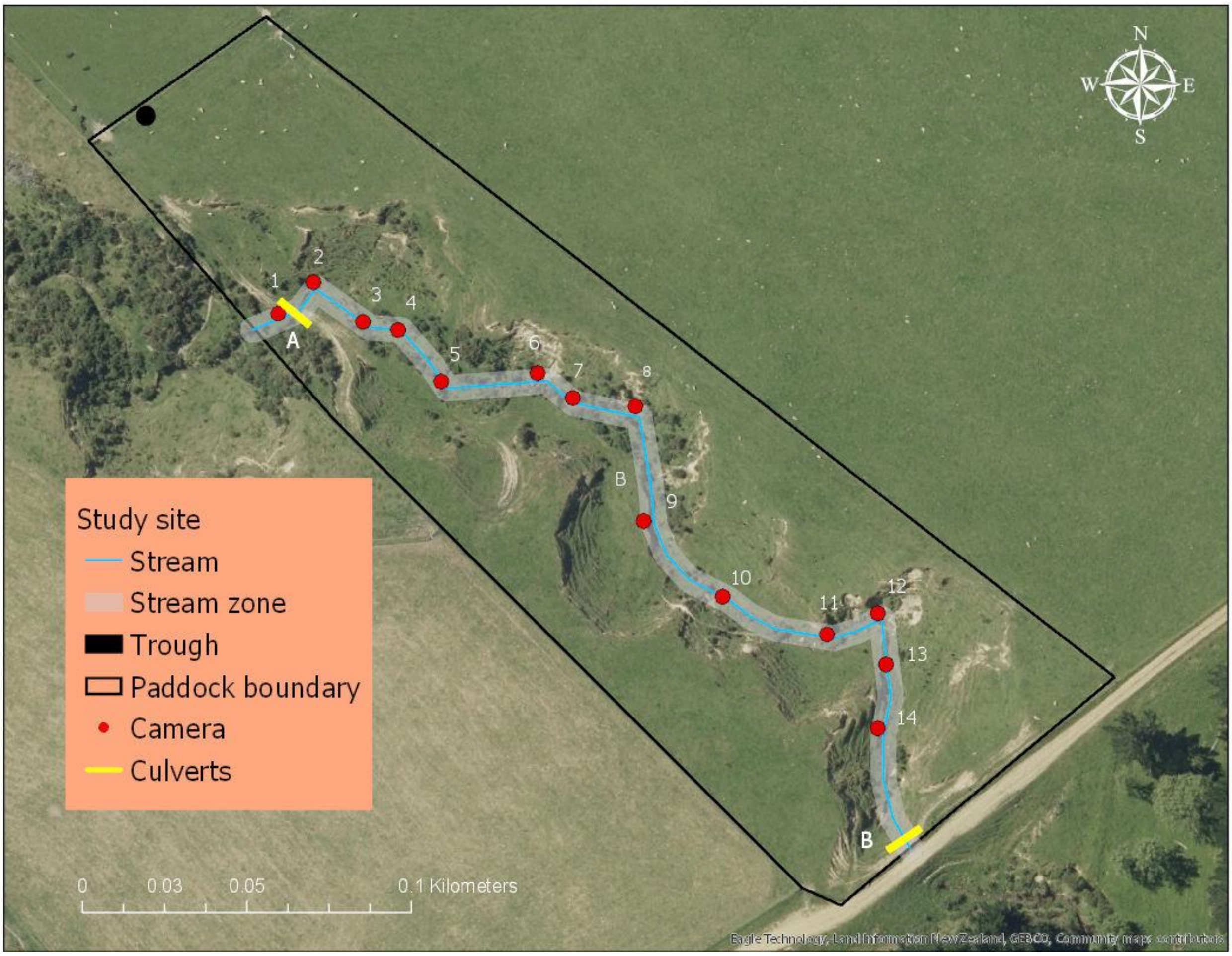


Figure 3.1: Map of the study site showing the stream (blue line), stream zone (grey shading), and the position of the trough (black dot), culverts (yellow bars (letters A & B)), and cameras (red dots).

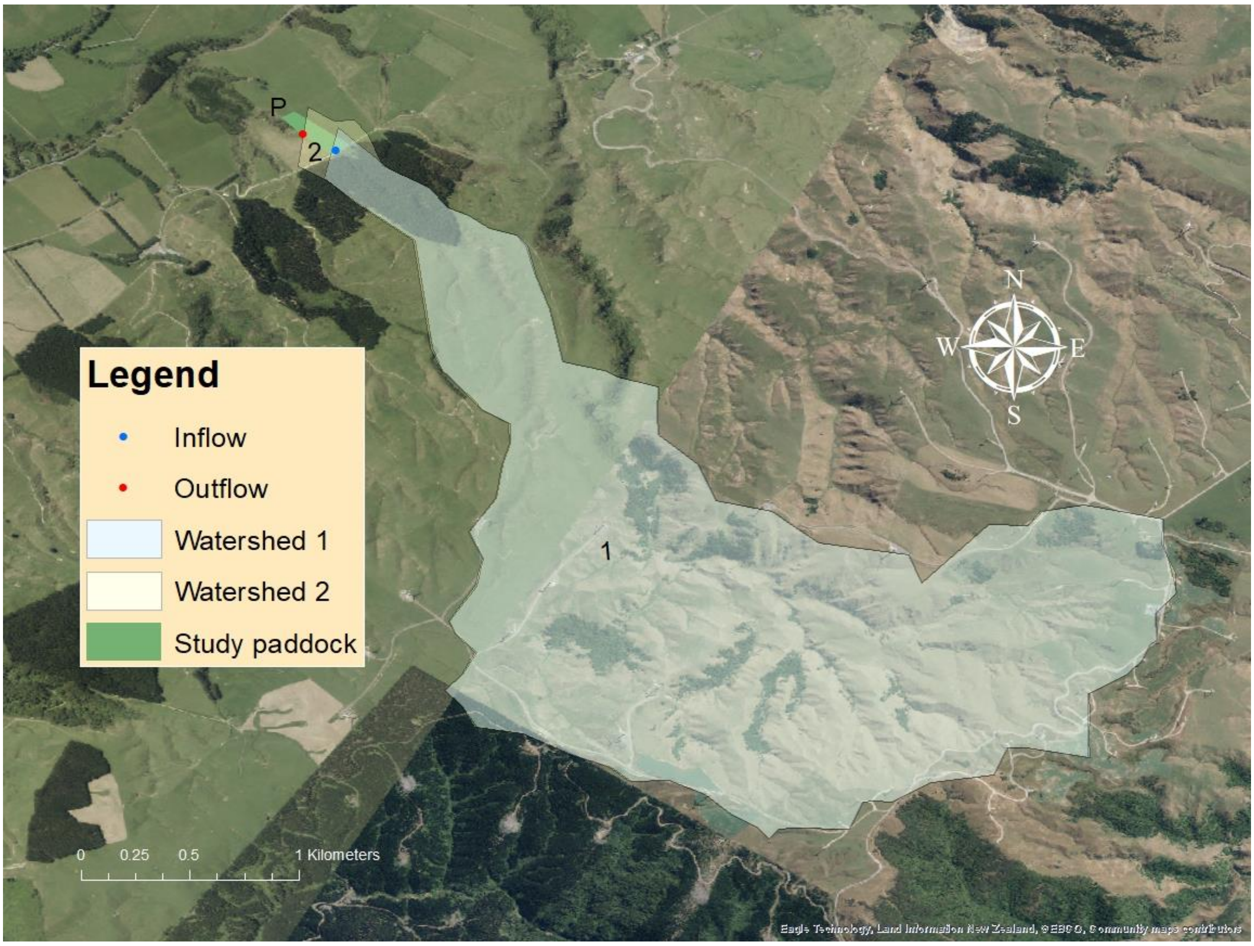


Figure 3.2: Map of watershed 1 and 2 that supplied the study paddock stream (P). Red and blue dots indicate locations of the outflow and inflow sampling sites, respectively.

3.3.2.1 *Sheep behavioural observations*

Ewe movement and behaviour around the stream was recorded using fourteen movement activated video surveillance cameras (Moultrie® model MCG-13297, Birmingham, AL, USA, n = 6; TechView® model QC8027, Kaki Bukit, Singapore, n = 4; Bushnell® model 119736, Overland Park, KS, USA, n = 4). Cameras were placed at intervals of 14 to 18 m along the length of the stream (Figure 3.1). Cameras were triggered by movement of sheep within 15 m of the unit. Footage from the cameras allowed the identification of individual sheep up to a distance of 15 m. Once triggered, cameras were programmed to record for 30 s, followed by a non-recording period of 10 s. If an animal was still moving within the range of the camera after the non-recording period, a further 30 s of footage was recorded. The cameras contained an infrared LED flash that allowed the capture of footage during hours of darkness without visible light being emitted. In order to identify each individual animal, each ewe was marked with large, coloured numbers on their both sides using stock spray (Sprayline, Donaghys, Christchurch, New Zealand; Figure 3.3) and were fitted with a plastic collar labelled with a unique number.

Figure 3.3: A photo of sheep with spray marks wearing accelerometers, GPS, and collars (two pictures above show GPS units used in the study)

Ewe behaviours were determined from video recordings using the ethogram below (Table 3.1). The behaviours included grazing, walking, stationary, drinking (further categorised by whether ewe stood on the bank or in the water), sniff water, walk in the stream, and being out of view. Sheep behaviour was coded using behaviour coding software BORIS (version 7.8.2; (Friard and Gamba, 2016)). Once the coding process was completed, a total duration for single or grouped observations was extracted.

Table 3.1: Ethogram showing the description of ewe behaviours

Behaviour	Description
Stationary	Ewe was inactive either standing or sitting. Includes sheep that were ruminating (regurgitation; re-chewing and re-swallowing) or scratching. Standing was defined as all four feet on the ground and no locomotion. Sitting was defined as at least 50% of side in contact with the ground and not being supported by all 4 feet.
Grazing	Sheep harvesting vegetation from the ground. Could be standing still or walking with muzzle close to the grass (i.e., head is below shoulders)
Walking	Moving from one point to another, did not include walking while grazing
Drink	Animal consumed water from the stream or trough for more than 5 seconds
Sniff	Moved muzzle towards water and inhaled but did not drink
Walk in stream	Sheep stepped in the stream without drinking water
Other	Sheep performed any other behaviours, such as playing or fighting
Out of view	Sheep moved out of range of the video camera

3.3.3 Global positioning system (GPS)

All study ewes (n = 40) were fitted with a collar to which a custom-built GPS unit (DataCarter Ltd., Feilding, New Zealand) weighing ~100 g was attached throughout the study period (Figure 3.3). GPS monitors were programmed to allow for continuous tracking of satellites and logging of animal position whenever ewes moved ≥5 m or every 60 s if the ewe was stationary. Each GPS unit was powered by a 3.6-volt battery (Tadiran™ lithium Inorganic battery, Kiryat Ekron, Israel) with a life under continuous GPS use of 15 to 25 days. Both the GPS and the battery were enclosed in a moulded plastic weather-proof case. GPS units recorded date and time (GMT), latitude, longitude, horizontal dilution of precision (HDOP), and the number of satellites detected. Distance was calculated between two points specified by latitude/longitude (in numeric [decimal] degrees) in excel VBA that was run with macros derived from the Vincenty inverse formula for ellipsoids (Geodetic, 1975).

3.3.4 Triaxial accelerometers

All study ewes (n = 40) were also fitted with a triaxial accelerometer with the dimensions of 4.6cm × 3.3cm × 1.5cm. That weighed 19 g (wGT3X-BT Actigraph, Pensacola, FL, USA) and was attached to the collar for the study period. The accelerometers contained Bluetooth® technology (N. semiconductor, Trondheim, Norway) and were set to be "beacons". Beacons sent signals containing their ID number at 10 s intervals to other accelerometers (receivers), indicating the proximity or location of a device. The receiver accelerometers were attached to the posts to which the video cameras were attached and recorded the proximity of any beacon devices once per minute. The distance between beacons and receivers was estimated using the received signal strength indicator value (RSSI) and transmitted power of the beacon. In the current study, three meters on either side of the stream zone corresponded to RSSI values below −56 dB (in Excel IF (A2 <= −56,1,0)). The accelerometers were initialised using proprietary software (ActiLife software, version V6.13.4, ActiGraph LLC, Pensacola, FL, USA), during which the device was identified with the sheep number or camera location, the start date, and time. The sample rate of 30 Hz was set. The accelerometers were programmed to continue to collect data until the battery was depleted.

3.3.5 Ewe measures

At D1 and D15, ewes were weighed (Tru-Test weigh scale, Auckland NZ) and body condition scored by a single experienced technician [scale 1-5; Jefferies (1961)]. On each occasion, ewes were weighed within an hour of being removed from the pasture.

3.3.6 Pasture measures

The moisture content of the pasture was determined on D1, D10, and D15. At approximately 1 pm on each day, 6 grab pasture samples of approximately 50 g were collected. Samples were randomly collected across the paddock by hand plucking in manner that simulated sheep grazing. Samples were oven-dried at 80 °C for 48 h to determine dry matter (%) in order to calculate the moisture content (%).

Moisture content Equation 1

$$Moisture\ \% = \left(\frac{Fresh\ weight\ (g) - Dry\ weight(g)}{Fresh\ weight\ (g)}\right) \times 100$$

Pasture mass was estimated on D1, D10, and D15 using a manual folding plate meter (Jenquip, Fielding, New Zealand). One hundred readings were taken across the paddock at approximately 2 m intervals, and the average mass for the paddock was recorded. Pasture mass was calculated from the equation below (White and Hodgson, 1999).

Pasture mass Equation 2

$$Pasture\ mass = (Final\ reading - Initial\ reading) \times 158 + 200$$

3.3.7 Weather data

Hourly and daily data were downloaded from Tuapaka weather station 2 (EnviroMonitor station, Davis Instruments, Davis, CA, USA) located 800m from the study site and at the same altitude. Data included rain (mm), relative humidity (%), air temperature (°C), solar radiation (MJ/mÂ²), and wind speed (m/s).

3.3.8 Water measurements

Water flow rate was measured, and samples were collected hourly for 8 h (from 0800 to 1500 hrs) on D5, D6, D12, and D13 (Table 3.2). The water flow rate of the stream was measured as water exited each of the two culverts: one at the entry (inflow) and one at the exit (outflow) of the paddock (Figure 3.1). The flow rate was manually calibrated using a 30-litre flexible bucket, stopwatch, and a graduated jug, as per the method described by Abd Ghani (Abd Ghani and Saudi, 2017). Each hour, the process was repeated three to five times and the average calculated. The equation to calculate the bucket flow rate was

Water flow rate Equation 3

$$Flow\ rate\ (L/s) = \frac{Volume\ of\ water\ in\ the\ bucket\ (L)}{Time\ to\ fill\ (s)}$$

Water samples were collected into 1 L plastic bottles from the inflow and outflow of the paddock to determine the concentration of suspended sediments (SS), total phosphorus (TP), nitrate-N, ammonium-nitrogen, and *E. coli*. Water samples were collected synchronously on the hour at both culverts. At the same intervals, additional samples to determine *E. coli* concentration were collected into 100 mL sterile microbiology bottles (Eurofins, Wellington, New Zealand) using a sterile technique to avoid sample contamination (i.e., Samples were collected using a new sterile, tightly capped container that is labelled beforehand, without touching the inside, without overfilling, avoiding disturbance of sediment, and capped immediately after collection.

All water samples were stored in a cool box with ice until they were returned to the laboratory at the conclusion of the sampling day. *E. coli* samples were then couriered to the analytical laboratory on the same day. Each 1 L sample was subsampled, with one subsample being filtered to <0.45 μm for nitrate-N. Ammonium-N analysis and the second subsample were left unfiltered for total N and P analysis. Subsamples were then frozen (−20 °C) for subsequent analysis. The remaining ~900 mL sample was refrigerated at 4 °C for subsequent SS analysis.

3.3.8.1 Nitrate-N, Ammonium-N and Total P concentrations

The nitrate-nitrogen and ammonium concentration of the water samples was determined using a colorimetric autoanalyzer (Pulse international ltd, Saskatoon, Sask. Canada) method using red azo and phenol Prussian blue dyes for nitrate and ammonium, respectively (Blakemore *et al* 1987). Quality control was assessed by analysing solutions of known concentrations of 12, 0, 0.25, 0.5, 1, 2, 4, 8, 12, 0 ppm in sequential order. Two blank (0 ppm) and a standard solution (8 ppm) were also analysed every 10 samples to monitor the accuracy of the results measured. Detection limits for both nitrate-N and ammonium using this method was 0.25mg/L.

Total P concentrations were determined by digestion and automated ascorbic acid colorimetry (APHA 4500-PH method) using a flow injection analyser (Baird, 2017). The principle is polyphosphates are converted to the orthophosphate form by a sulfuric acid digestion and organic phosphorus is converted to orthophosphate by a persulfate digestion. When the resulting solution is injected onto the manifold, the orthophosphate ion reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a complex that is reduced with ascorbic acid to form a blue complex that absorbs light at 880 nm.

3.3.8.2 Suspended sediment analysis

The concentration of SS was determined using the gravimetric analysis following the standard procedure from American Public Health Association by Baird (2017). Firstly, filter paper (Whatman 100 mm GF/C) was rinsed using reverse osmosis (RO) water and dried at 105°C for >8 hours. After drying and equilibration in a glass decanter for approximately an hour, the filter paper and water sample bottle (with lid) were weighed and weights recorded. The filter paper was then placed in vacuum funnel and the entire sample was shaken vigorously and filtered. Thereafter, the filter paper (with sediment) was dried at 105°C for >8 hours and weighed. The empty sample bottle was also weighed. Suspended sediment concentration was calculated using the equation below.

Suspended sediment Equation 4

$$mg\ total\ suspended\ sediment/L = \frac{(A - B)\ x\ 1000}{Sample\ volume\ in\ ml}$$

Where A is the weight of filter paper + dried residue in mg, and B is the weight of filter paper in mg.

3.3.8.3 E-coli analysis

E. coli concentrations were determined within 24 hours of collection by Eurofins laboratory (Wellington, NZ) using a membrane filtration procedure (Standard Method APHA 9222G; (Baird, 2017)) onto nutrient agar containing 4-methylumbelliferyl beta-D-glucuronide. Briefly, A measured volume of water is filtered through a 0.45-µm membrane filter to trap bacteria. The membrane is then placed on m-FC selective and differential medium, ensuring that no air bubbles are present. Plates are incubated at 44.5 ± 0.2 °C for 24 ± 2 hours. Fecal coliform bacteria reduce aniline blue, producing blue colonies, which are counted as fecal coliforms. Results are expressed as colony-forming units (cfu) per 100 mL of water sample, with corrections made for dilution and the volume filtered.

3.3.9 Statistical analyses

GPS data were analysed using ArcGIS mapping tools (ArcGIS Pro 2.2.4, 2018). The GPS data for each ewe was cleaned using MS Excel macros that removed data that were duplicated, improperly formatted, or that had empty fields. The distance each ewe travelled was calculated for each pair of location points using in-house macros which converted latitude and longitude to radians, computed the angle between the two points, converted the angle to nautical miles and returned a distance in kilometres. Distance travelled per sheep was analysed by time-of-day categories which included early morning (0600 to 0859), day (0900 to 1659), evening (1700 to 1959), and night (2000 to 0559).

An optimised hot spot analysis (z-score) was conducted using ArcGIS® to identify statistically significant spatial clustering of ewe GPS location fixes. A hotspot was defined as an area of higher concentration of ewe locations compared to the expected number given a random distribution of ewes. A cold spot was defined as an area that had a lower concentration of ewe locations compared to the expected number given a random distribution of ewes. The analysis of ewe interaction with waterway was based on an area defined as the 'stream zone' which represented the stream and 3 m either side.

Prior to analysis, ewe behaviour, and water quality data were checked for normality using the Kolmogorov–Smirnov and Shapiro–Wilk test, and the homogeneity of variances was studied using Levene's test and Tukey transformation (Tukey's Ladder of Powers transformation), where appropriate (Gotelli and Ellison, 2004). Behavioural data, including grazing, drinking, and walking analyses, were performed using R 3.6.0 (2019-04-26; R Core Team (2019)). The impact of water trough restriction on ewe behaviour (e.g., % grazing, drinking) was analysed using a 2-factor analysis of variance (ANOVA). The model included the fixed effects of water trough treatment and time of the day, as well as their 2-way interaction. The behavioural variables analysed were the percentage of time spent grazing and drinking. A linear regression was also used to determine if any ewe behaviours were associated with time of the day or environmental temperature.

Concentrations of *E. coli*, nitrate-N, and SS concentrations were analysed using parametric and TP using non-parametric methods in R 3.6.0. The load of nitrate-N, SS, *E. coli*, and TP in inflow and outflow water samples was calculated as the product of their concentration and stream flowrate (equation 5). One way ANOVA analyses were followed by post hoc test when significant (Tukey test $p < 0.05$); otherwise, non-parametric ANOVA models (Kruskal–Wallis) were used to assess differences between mean ranks. Spearman’s rank correlation coefficients were used to examine relationships between sheep behaviour and water treatment, as the data were not normally distributed and included ordinal measures of behaviour. Correlation coefficients and associated two-tailed p-values were reported, with statistical significance set at α=0.05.

Load Equation 5

$$Load\ (\mathrm{mg/s}) = Concentration\ (\mathrm{mg/L}) \times flowrate\ (\mathrm{L/s})$$

3.4 Results

3.4.1 Weather

Weather data were retrieved from three days prior to the start of the study (D-1 to D-3) to the completion of the study period (Figure 3.4). Rainfall occurred on 7 days of the 16-day study period, with rainfall volumes ranging from 0.2 to 27 mm/day. Maximum daily temperatures ranged from 7.2 to 13.1 °C, and the minimum daily temperature ranged from 1.7 to 8.4 °C. Relative humidity ranged from 64% to 90%.

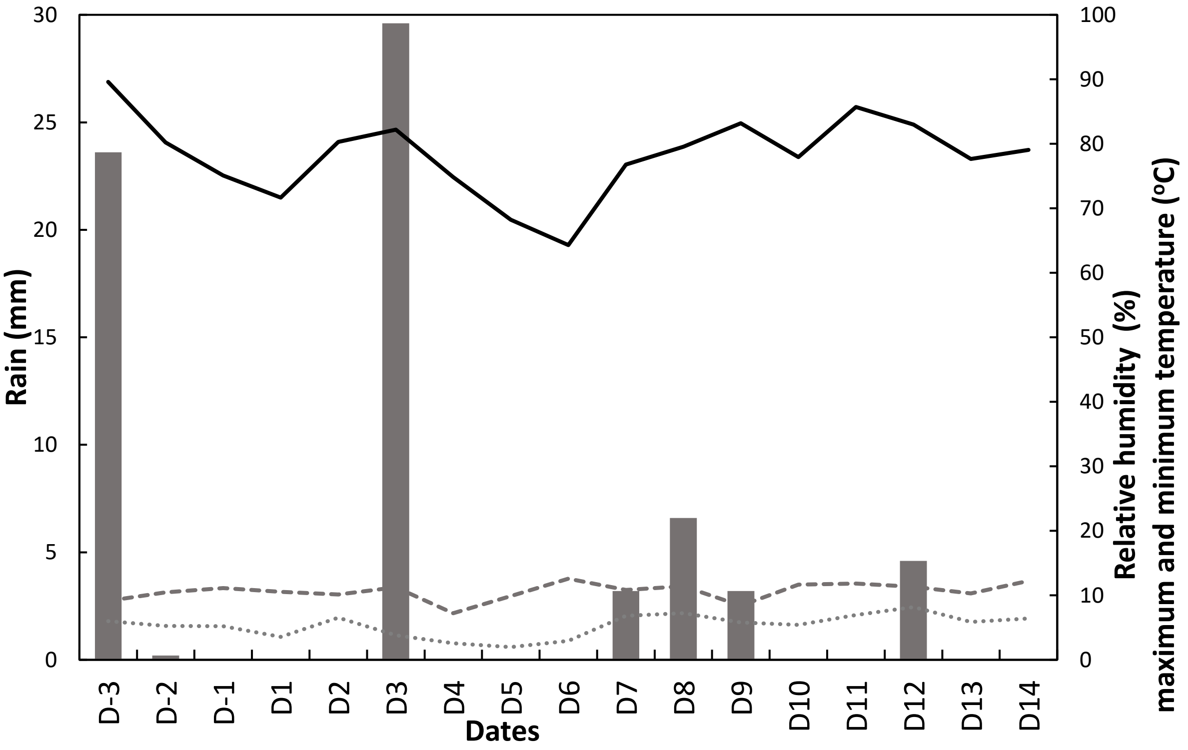
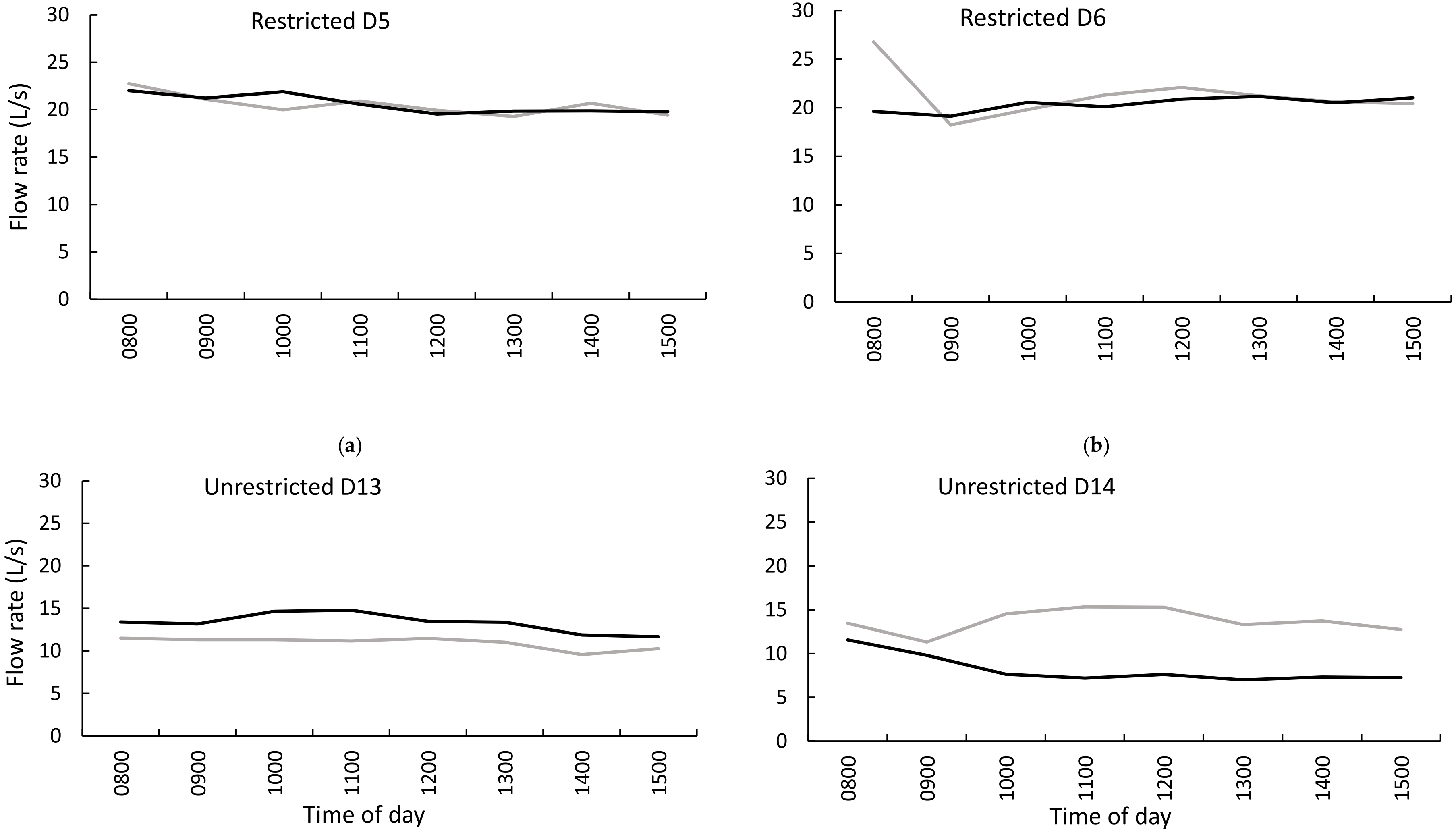


Figure 3.4: Daily mean rainfall (mm, bar), relative humidity (%, "______"), Minimum temperature (°C, "………..") and maximum temperature (°C, "--------") during the study period. D-3 to D14 indicate number of days relative to the start of the study (D1;16 August 2019)

3.4.2 Stream flowrate

Stream flowrates at the inflow and outflow monitoring sites ranged from 6.99 to 26.78 L/s but fluctuated slightly during each sampling day (Figure 3.5 a, b). The calculated duration for water to travel the length of the waterway was 15 s. Flowrate did not differ between the water trough restricted and Unrestricted periods ($p = 0.98$). Correlations between flowrate and weather parameters showed a negative correlation between flowrate and daily average temperature ($r = 0.4$ $p = 0.002$).



(**a**) (**b**)

(**c**) (**d**)

Figure 3.5: Hourly mean stream flowrate (L/s) at the inflow (grey line) and outflow (black line) monitoring sites by time of day (0800 to 1500 h) during the period of restricted access to water trough on D5 and D6 ((a,b) in upper panels) and the period of unrestricted access on D13 and D14 ((c,d) in lower panels).

3.4.3 Water quality

Water quality analyses showed that concentrations of SS and TP differed ($p < 0.05$) between the periods when access to the water trough was restricted and unrestricted; however, there was no difference ($p > 0.05$) in *E. coli* or nitrate-N (Table 3.3). Ammonium-N was below the detection limit of the analytical method throughout the study period.

Table 3.2: Timeline of the study showing the calendar date, study day, and water sample collection

Date	13-Aug	14-Aug	15-Aug	16-Aug	17-Aug	18-Aug	19-Aug	20-Aug	21-Aug	22-Aug	23-Aug	24-Aug	25-Aug	26-Aug	27-Aug	28-Aug	29-Aug
Study day	D-3	D-2	D-1	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14
Water sampling	-	-	-	-	-	-	-	√	√	-	-	-	-	-	√	√	-

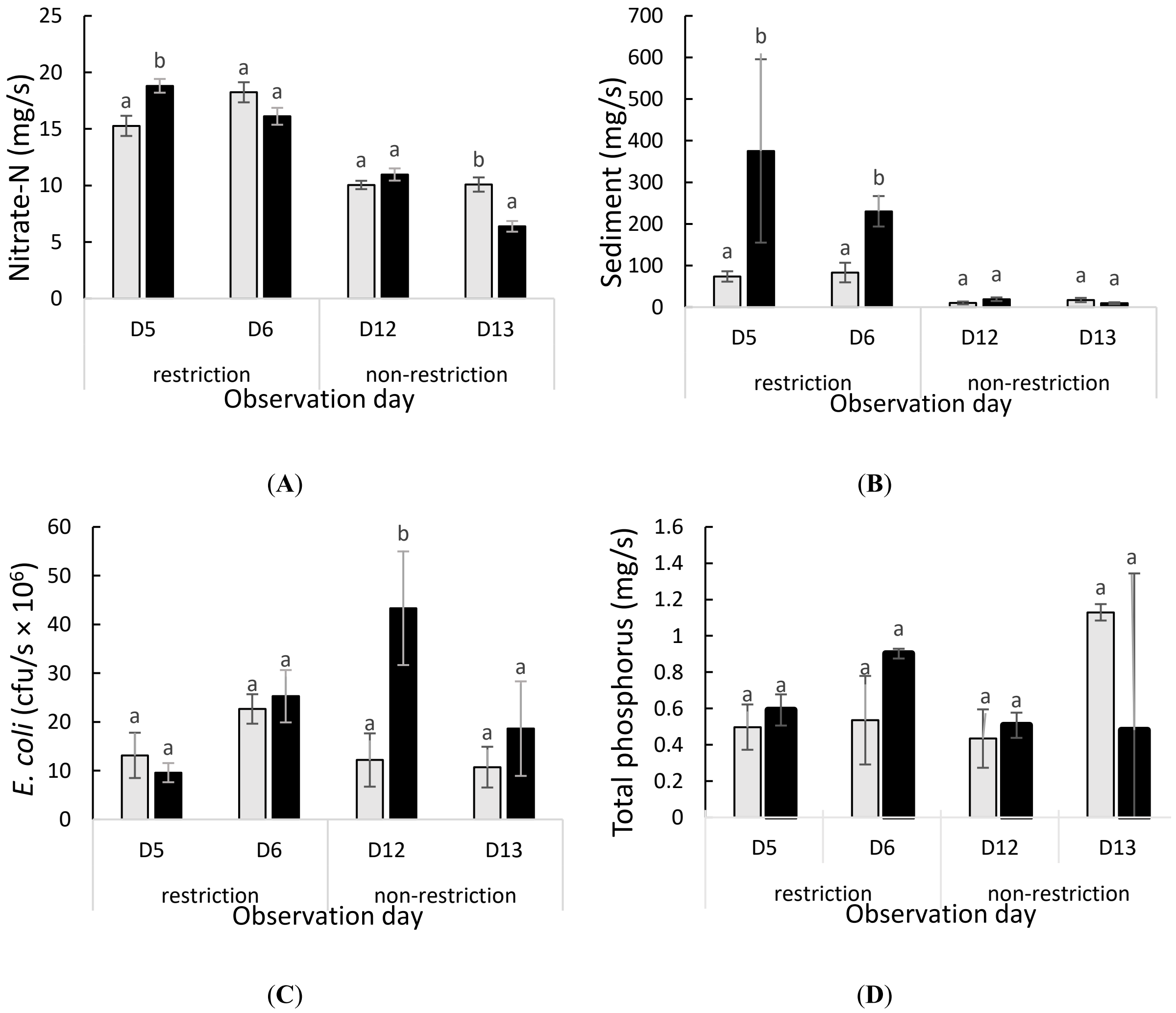
Table 3.3: Arithmetic mean (±SEM) and Tukey transformed mean (SEM) of concentration of *E. coli* (cfu/100ml), nitrate-N (mg/l), suspended sediment (mg/l) and the median (IQR) of total P (mg/l) during periods when ewes were restricted from accessing the water trough (restricted) or had access (unrestricted).

Parameter	n	Treatment	Arithmetic mean ± SEM	Transformed mean ± SEM	Median (IQR)	Treatment P-value
E. coli (cfu/100ml)	32	Restricted	84.72 ± 10.68	-0.2 ± 0.01	80 (35.5 – 98.0)	0.139
	32	Unrestricted	188.50 ± 43.20	-0.2 ± 0.01	68 (45.5 - 290.0)	
Nitrate-N (mg/l)	32	Restricted	0.71 ± 0.02	0.5 ± 0.03	0.70 (0.66 - 0.80)	0.528
	32	Unrestricted	0.66 ± 0.02	0.4 ± 0.02	0.64 (0.60 - 0.74)	
Suspended sediment (mg/l)	32	Restricted	15.08 ± 6.30	-0.8 ± 0.02	15.0 (14.0 - 18.7)	0.001
	32	Unrestricted	17.96 ± 16.70	-1.0 ± 0.02	14.60 (12.9 - 17.3)	
Total phosphorus (mg/l)	32	Restricted	0.03		0.03 (0.02-0.03)	0.018
	32	Unrestricted	0.07		0.03 (0.03-0.04)	
Ammonium (mg/l)	32	Restricted	ND			
	32	Unrestricted	ND			

ND = Concentration was below the detection limit of 0.25 mg/l.

The nitrate-N load measured at the outflow sampling site was higher than at the inflow site at D5 but lower at D13 ($p < 0.05$; Figure 3.6 A). Suspended sediment loads were higher at the outflow than inflow site on D5 and D6 during the restricted period but did not differ during the unrestricted period ($p < 0.05$; Figure 3.6 B). *E. coli* loads were higher in the outflow than inflow site at D12 ($p < 0.05$; Figure 3.6 C) but did not differ on D5, D6, or D13 ($p > 0.05$). Based on Wilcoxon test *E. coli load* showed no statistically significant difference ($p > 0.05$) between inflow and outflow during water restriction. In unrestricted period *E. coli* loads increased from inflow to outflow ($p > 0.05$). The total P load did not differ between sampling sites at any time during the study ($p > 0.05$; Figure 3.6 D).

Across the four sampling days, the load of nitrate-N, TP and SS did not vary by hour of the day ($p > 0.05$). At D13, however, *E. coli* load was higher at 10:00 am than the rest of day ($p = 0.015$).



(A)

(B)

(C)

(D)

Figure 3.6: Mean (± SEM) nitrate-N (mg/s; Panel (A)), suspended sediment (mg/s; Panel (B)), *E. coli* (cfu/s × 106; Panel (C)), and total phosphorus loads (mg/s; Panel (D)) measured in water samples collected at the inflow (grey bars) and outflow (black bars) sampling sites on study days 5 and 6 (D5 and D6; restricted access to trough) and days 12 and 13 (D12 and 13; unrestricted access to trough). Within each day, bars with different letters were significantly different ($p < 0.05$).

3.4.4 Animal density and spatial distribution

Ewe GPS locations across the study site showed that there were more fixes in the northeast than southwest areas of the paddock (Figure 3.7a panel C and D). Based on the slope profile shown in Figure 3.7a panel B, these areas coincided with the flat areas (0 – 3°) of the paddock. During the period of water trough restriction (Figure 3.7b panel C), there were fewer areas with aggregated GPS locations (focal areas) identified than when the trough was not restricted (Figure 3.7b panel D). There were, however, more ewe locations recorded near the trough when access to the trough was restricted compared to unrestricted (Figure 3.7b panel C and D).

Regardless of the period of the study, there were proportionally fewer ewe GPS locations recorded near the stream (n = 6829) than the rest of the paddock area (n = 712,400).

During the period of water trough restriction, there were two significant hot spots (statistically significant spatial clustering of locations) identified within the paddock ($p < 0.05$; Figure 3.8a panel A). When the trough was unrestricted, eight smaller areas were detected (Figure 3.8a panel B). When the stream zone was analysed independently of the rest of the paddock, six cold spots and five hot spots were identified during the unrestricted trough access period ($p < 0.05$; Figure 3.8b panel D), whereas in the restricted period, the stream zone showed only hot spot areas around each culvert (Figure 3.8b panel C).

The stream zone made up 9% of the entire paddock area. However, 0.7 and 1.0% of all ewe GPS location fixes were recorded within this area during the restricted and unrestricted water trough access periods, respectively (Table 3.4). In the stream zone, 40% of all ewe GPS location fixes during the unrestricted period were recorded in the three hot spot areas (Figure 3.8b panel D), whereas the same areas contained 24% of all GPS location fixes in the restricted period (Figure 3.8 panel C).

Table 3.4: The mean number (±SE) and percentage (%) of sheep GPS location fixes in each paddock slope class during the period when ewes had access to the water trough (Unrestricted) or were prevented from accessing the trough (Restricted).

		GPS location fix number (±SE)			
		n		%	
Slope class (degrees)	% of the study site	Unrestricted	Restricted	Unrestricted	Restricted
Flat (0-3)	18.8	121,397 ± 602^{e}	120,670 ± 201^{f}	30.0	38.4
Undulating (4-7)	14.9	95,544 ± 757^{d}	63,812 ± 118^{e}	23.6	20.3
Rolling (8-15)	17.3	62,027 ± 330^{c}	45,758 ± 93^{d}	15.3	14.6
Strong rolling (16-20)	12.1	27,583 ± 95^{b}	24,972 ± 55^{bc}	6.8	8.0
Moderately steep (21-25)	10.5	35,730 ± 460^{b}	31,619 ± 91^{c}	8.8	10.1
Steep (26-35)	21.0	58,879 ± 896^{b}	20,967 ± 57^{b}	14.6	6.7
Very steep (35-75)	5.5	3,393 ± 16^{a}	6,112 ± 86^{a}	0.8	1.9

[abcdef] Within a treatment group (column), means with different letters are significantly different ($p<0.05$).

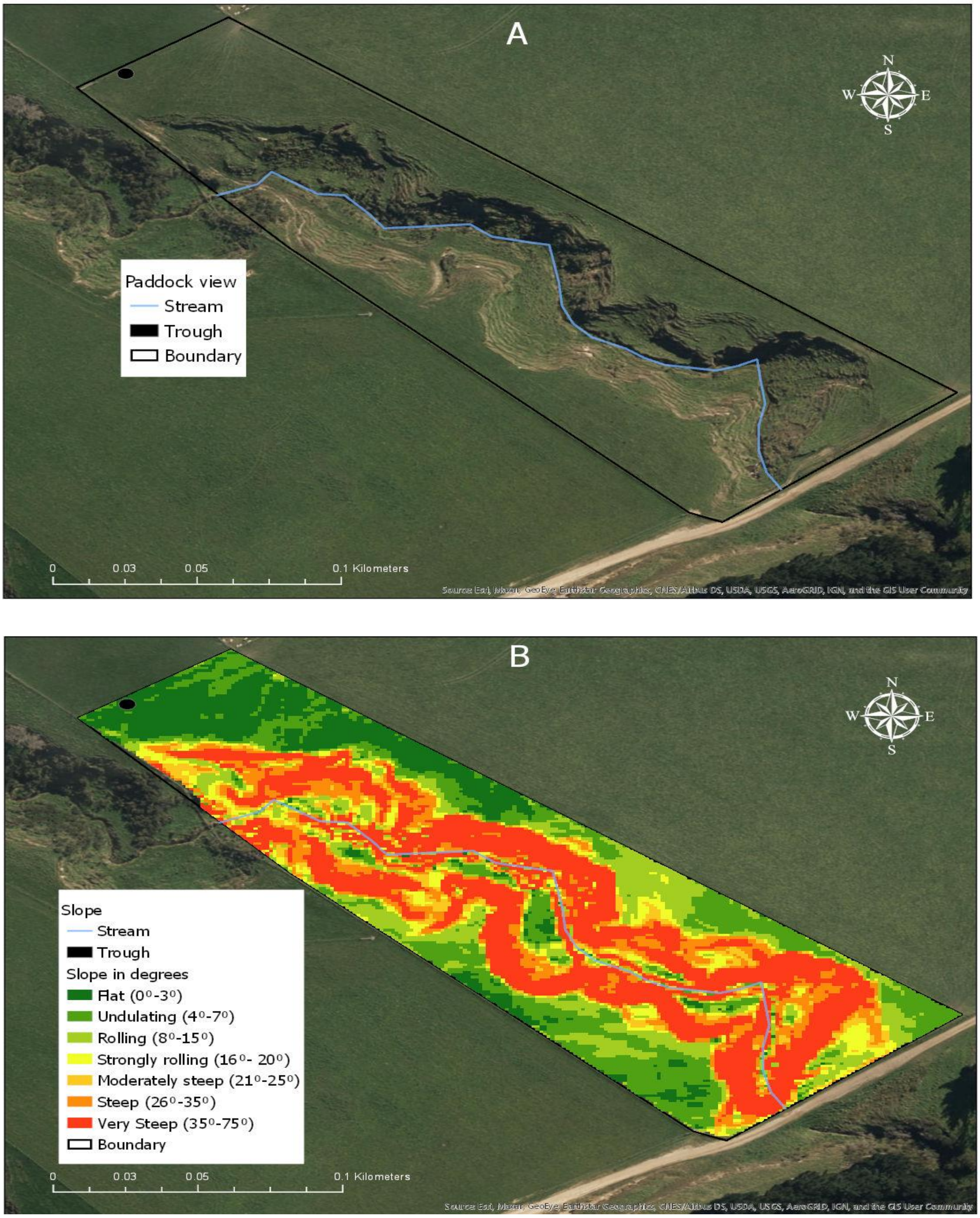


Figure 3.7a: Maps showing a satellite image of the study site (A), slope category (B) and paddock features including the stream (blue line), water trough (black circle) and paddock boundary (black line) and the slope of the terrain (green areas represent flat and red indicates very steep).

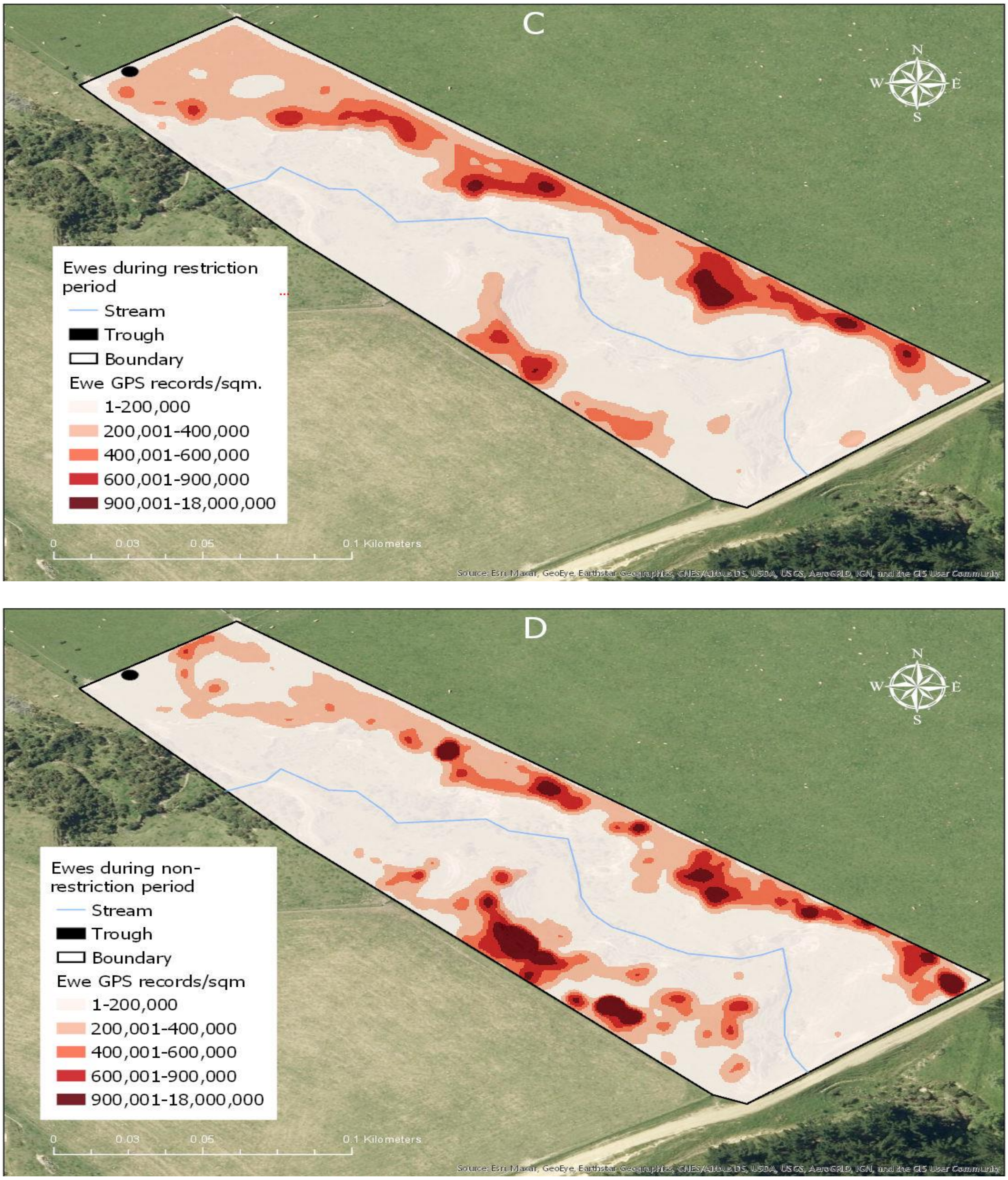


Figure 3.8b: Maps showing a satellite image of the study site and paddock features including the stream (blue line), water trough (black circle) and paddock boundary (black line) and the spatial distribution (magnitude per unit area) of sheep during the period the water-trough was restricted (C) or unrestricted (D) using kernel smoothing. Green areas represent low ewe density (1 to 200,000 GPS locations/m^2) and red areas high density of recorded locations (900,001 to 18,000,000 locations/m^2).

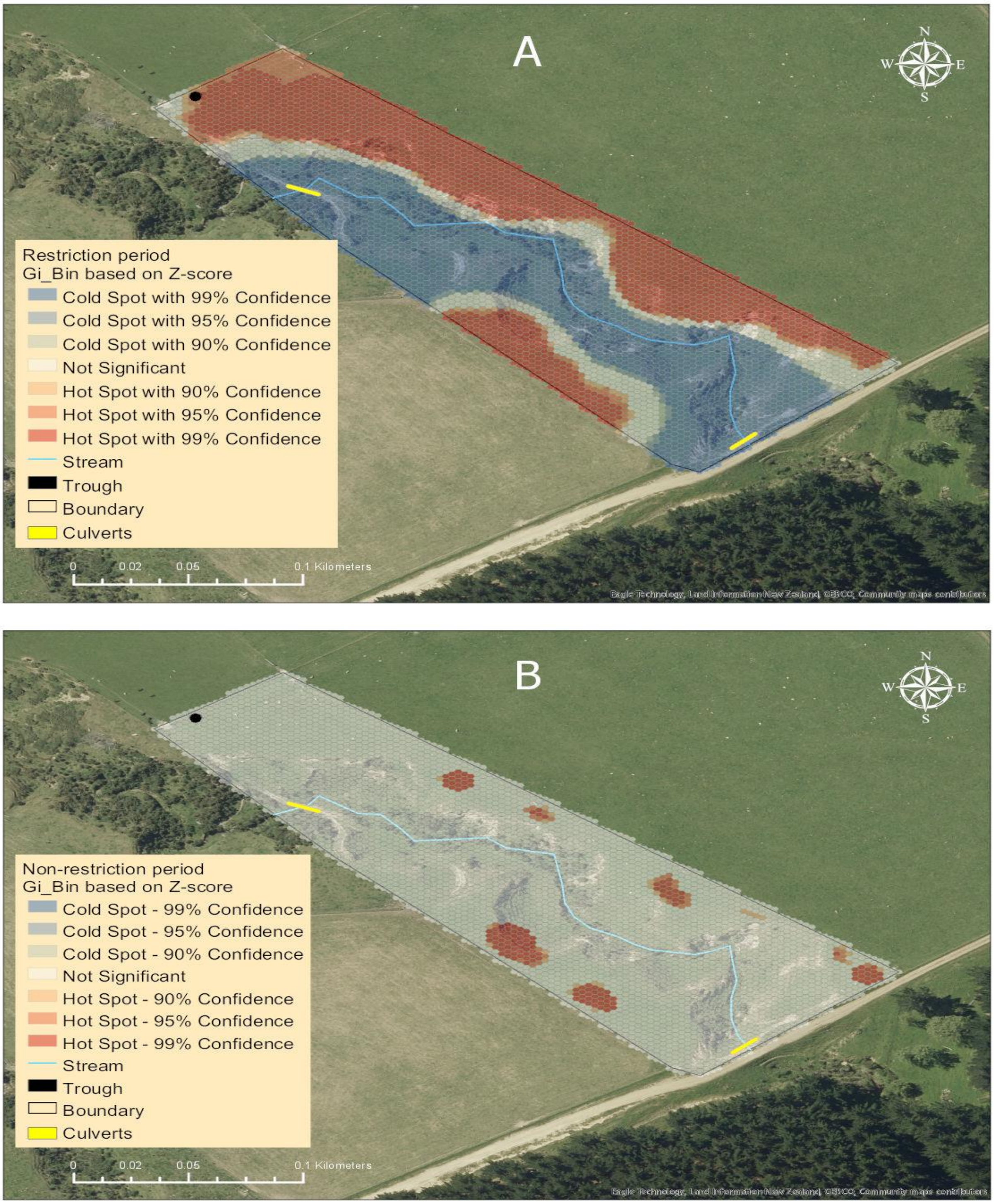


Figure 3.9a: Maps showing the spatial distribution (magnitude per unit area) of ewes within the study paddock (A,B) during the period the water trough was restricted (panel A) or unrestricted (panel B) using optimised hot spot analysis. The blue areas represent low ewe density (cold spot) and red areas high ewe density (hot spot (HS)). Hotspots indicate statistically significant ($p < 0.05$) spatial clusters of high values (larger positive z-score), cold spots indicate statistically significant ($p < 0.05$) spatial clusters of low values (smaller negative z-score), and white indicates random distribution with no spatial clustering.

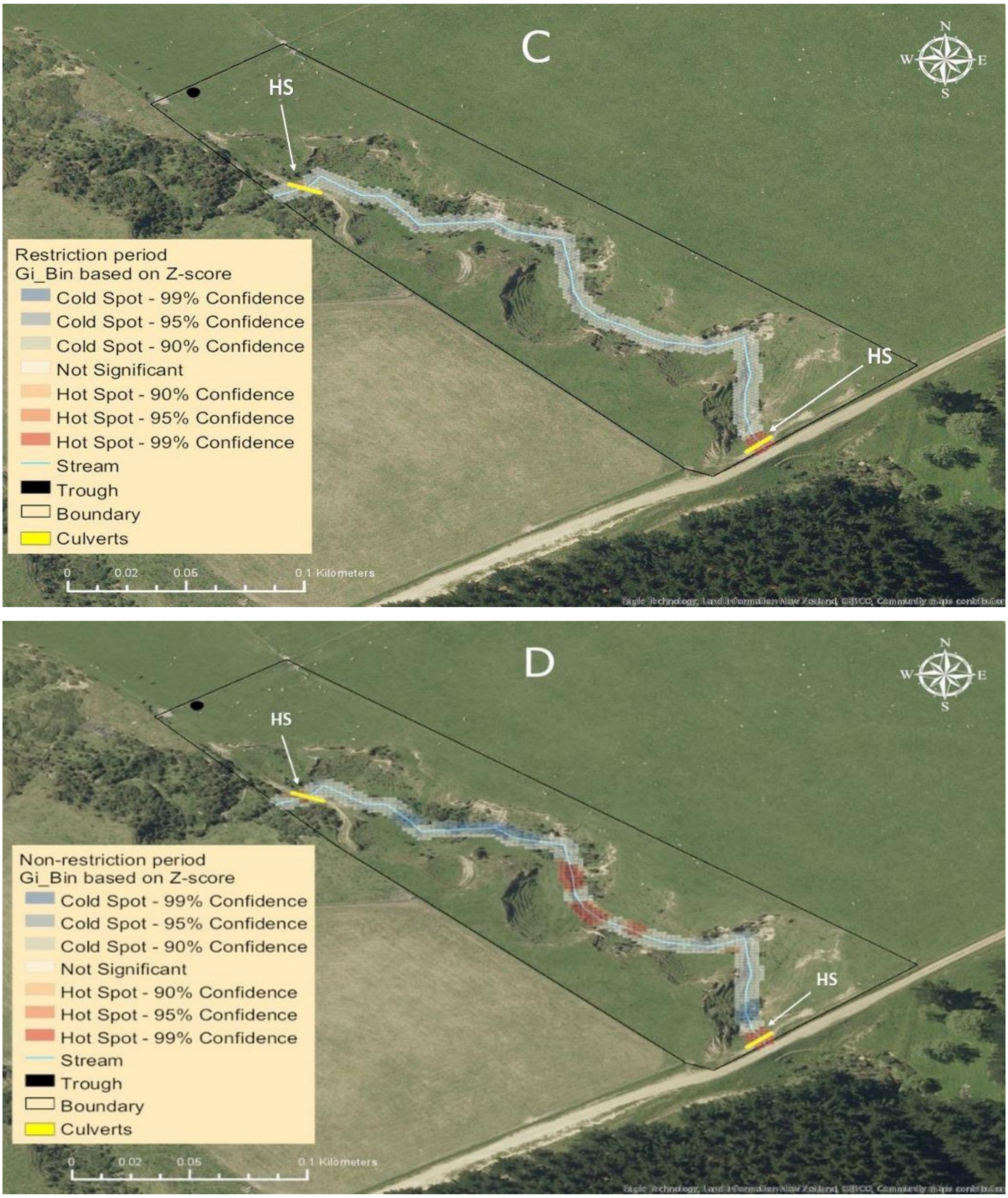


Figure 3.10b: Maps showing the spatial distribution (magnitude per unit area) of ewes within the stream zone (within 3 m of the stream) during the period the water trough was restricted (panel C) or unrestricted (panel D) using optimised hot spot analysis. The blue areas represent low ewe density (cold spot) and red areas high ewe density (hot spot (HS)). Hotspots indicate statistically significant ($p < 0.05$) spatial clusters of high values (larger positive z-score), cold spots indicate statistically significant ($p < 0.05$) spatial clusters of low values (smaller negative z-score), and white indicates random distribution with no spatial clustering.

3.4.4.1 Effect of slope

Within the paddock areas with a slope of less than 15° contained more than half of the sheep location fixes recorded during the entire study period (Table 3.4). Fewer than 2% of GPS location fixes were detected in areas with a slope of greater than 35° (very steep; Table 3.4). The area that each slope class contributed to the study site and the number of included location fixes that were recorded within each slope class in winter are shown in Table 3.5.

Table 3.5: Slope classes in the study site with the percentage of are they made up and the number of included location fixes (mean (n) with range in parentheses) and percentage of total fixes (%) recorded.

	% of	Location fixes					
Slope class	study	Winter		Spring		Summer	
(degree)	site	n	%	n	%	n	%
Flat (0-3)	18.8	2993 (2377-3610)	25.0	3364 (2964-3764)	31.9	3086 (2726-3446)	26.2
Undulating (4-7)	(14.9	3241 (2545-3937)	27.1	3595 (3196-3996)	34.1	3281 (2921-3641)	27.9
Rolling (8-15)	17.3	1992 (1542-2441)	16.6	1847 (1447-2247)	17.5	1945 (1585-2305)	16.5
Strong rolling (16-20)	12.1	957 (769-1146)	8.0	693 (293-1093)	6.6	1030 (670-1370)	8.8
Moderately steep (21-25)	10.5	772 (597-947)	6.5	493 (93-894)	4.7	868 (508-1228)	7.4
Steep (26-35)	21.0	1802 (1063-2541)	15.1	490 (90-890)	4.7	1288 (928-1648)	10.9
Very steep (36-75)	5.5	211 (137-285)	1.8	50 (-350-450)	0.5	2272 (-88-632)	2.3

3.4.5 Ewe distance travelled

The mean distance travelled per ewe per hour (m/h) varied throughout the day (Figure 3.9). Peaks were observed between 0400 and 0500, between 1400 and 1700, and between 2000 and 2100 h. Ewes travelled greater distances when the trough access was restricted (149 ± 13.8 m/h) compared to unrestricted (114 ± 8.8 m/h; $p = 0.046$; Figure 3.9). Over the entire study period, ewes travelled further ($p < 0.05$) during the night (148 ± 18.3 m/h) and in the evening (156 ± 6.8 m/h) than either in the early morning (131 ± 19.6 m/h) or day (100 ± 20.1 m/h).

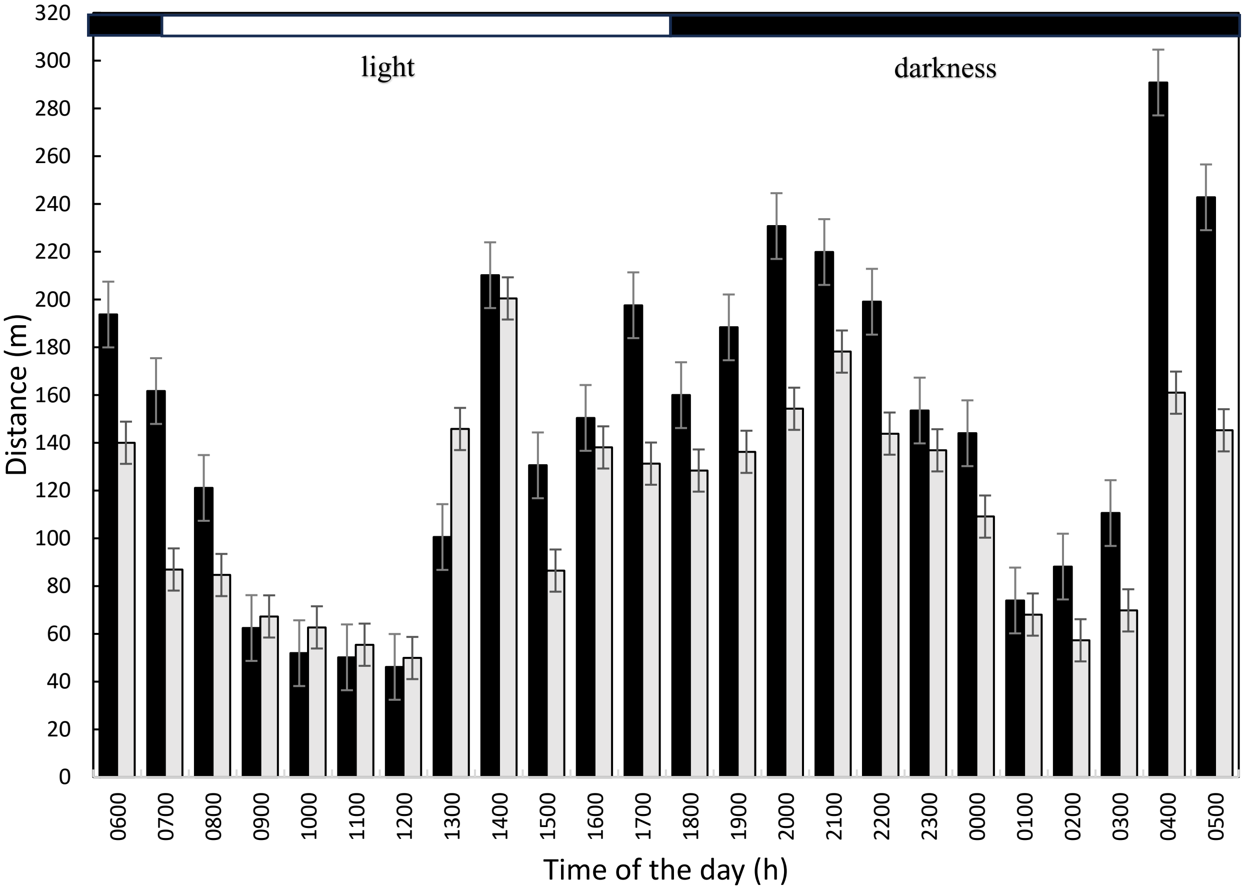


Figure 3.11: Hourly distance travelled per ewe by hour of the day (m; mean ± SEM) during the period when access to the water trough was restricted (black bars) or unrestricted (grey bars).

The mean distance ewes travelled differed across paddock slope classes ($p < 0.05$). In general, as slope increased, ewes travelled shorter distances; however, similar distances were travelled on strong rolling, moderately steep, and steep slopes in the unrestricted period (Table 3.6). During the water trough restricted period, distances travelled were similar between the flat and strongly rolling and between rolling and moderately steep areas ($p > 0.05$; Table 3.6) but differed across other slope classes.

Table 3.6: Hourly distance travelled (m) by sheep (mean ± SE) for each paddock slope class during the period when ewes had access to the water trough (Unrestricted) or were prevented from accessing the trough (Restricted).

	Distance travelled (m/h)	
Slope class (degrees)	Restricted	Unrestricted
Flat (0-3)	69.4 ± 1.36^{e}	59.6 ± 2.70^{d}
Undulating (4-7)	64.1 ± 2.12^{b}	46.4 ± 2.58^{c}
Rolling (8-15)	66.2 ± 1.73^{d}	55.9 ± 2.29^{d}
Strong rolling (16-20)	69.6 ± 1.86^{e}	62.0 ± 2.10^{e}
Moderately steep (21-25)	67.2 ± 2.04^{d}	41.9 ± 2.25^{b}
Steep (26-35)	66.0 ± 2.35^{c}	36.5 ± 2.34^{a}
Very steep (35-75)	50.7 ± 2.91^{a}	61.9 ± 3.05^{e}

[abcde] Within a treatment group (column), means with different letters are significantly different ($p<0.05$).

3.4.6 Time spent in the stream zones

Over the entire study period, proximity data showed that the time ewes spent within the stream zone (3 m either side of the stream) differed between camera locations ($p = 0.005$). Ewes spent the most time near camera 3 (19.8 ± 6.7 min/ewe/day) and camera 11 (22.2 ± 2.3 min/ewe/day; Table 3.7). When access to the trough was unrestricted, ewes spent more time ($p < 0.05$) within 3 m of any camera location (14.1 ± 5.7 min/ewe/day) than when access was restricted (10.8 ± 5.1 min/ewe/day: $p = 0.018$).

The duration that ewes were within 3 m of a camera location differed ($p < 0.05$) between time-of-day classes. Whereby, ewes were in proximity of any camera position longer in the early morning (10.8 ± 5.4 min/ewe/day) than during daylight hours (8.6 ± 4.4 min/ewe/day; $p = 0.024$). In addition, the median duration near any camera was greater in the evening (12.5 ± 7.2 min/ewe/day) than day ($p = 0.004$) and between evening and night (9.1 ± 5.4; $p = 0.001$).

A linear regression showed that daily distance travelled, average air temperature, and relative humidity accounted for 76% of the variance in the time ewes spent within 3 m of a camera. For every additional degree of temperature, ewes spent 1.2 min more within 3 m of the stream. Similarly, for every one percent increase in relative humidity, ewes spent 0.3 min more within 3 m of the cameras.

Table 3.7: The daily median and inter quartile range (IQR) in parentheses of the number of ewes detected and the duration (min) they spent within 3m of a camera per day during the period when ewes had access to the water trough (Unrestricted) or were prevented from accessing the trough (Restricted).

Camera number	Ewes detected within 3 m of each camera location			
	Restricted		Unrestricted	
	Number of ewes	Duration (min)	Number of ewes	Duration (min)
1	33 (29-34)	11.3 (10.9-22.9)	28 (27-30)	15.7 (13.7-17.3)
2	32 (25-33)	11.0 (7.7-16.3)	29 (27-30)	13.0 (8.7-15.1)
3	31 (28-32)	12.8 (6.5-18.0)	27 (25-29)	12.7 (9.1-14.2)
4	27 (19-32)	11.7 (7.6-16.3)	28 (24-30)	11.3 (9.1-12.4)
5	30 (29-33)	7.9 (4.7-10.2)	29 (28-31)	11.3 (7.8-13.6)
6	28 (16-31)	5.3 (3.6-5.9)	25 (22-29)	8.8 (6.8-10.8)
7	22 (21-24)	6.0 (4.5-7.8)	20 (18-22)	7.8 (5.2-11.2)
8	28 (16-33)	12.0 (5.2-16.6)	28 (23-29)	18.3 (14.4-19.5)
9	27 (19-28)	7.1 (5.0-13.0)	28 (24-33)	12.8 (10.7-16.5)
10	21 (18-22)	5.3 (2.9-7.3)	20 (18-27)	6.1 (4.4-7.3)
11	35 (25-36)	25.0 (17.8-29.3)	32 (29-34)	20.2 (15.2-26.1)
12	28 (18-32)	9.5 (6.2-19.1)	26 (24-29)	20.8 (16.7-26.5)
13	32 (25-32)	10.8 (7.0-16.4)	29 (23-32)	15.2 (10.4-18.5)
14	28 (17-33)	6.4 (5.8-8.5)	24 (23-29)	10.3 (9.1-13.3)

3.4.7 Behaviour in the stream zone

Video footage showed that during the entire study period when ewes were in the stream zone, they spent 68% of their time grazing ($n = 96$), 15.9% stationary ($n = 52$), 11.2% walking ($n = 53$), and 2.2% ($n = 5$) drinking from the stream. Ewes were observed to sniff water on five occasions (0.5%). On one occasion, a ewe was observed to walk in the stream (0.1%). The duration spent undertaking grazing, stationary, and walking behaviours did not differ ($p > 0.05$; Figure 3.10) between periods when the water trough was restricted or unrestricted. Video footage from both treatment periods showed that the behavioural events observed at hotspot two where there was no culvert (Figure 3.8D) included crossing the stream (12.5%), while the remaining 87.5% was grazing.

The duration ewes were observed to be stationary showed a negative relationship with solar radiation ($r = -0.8$, $p < 0.05$). For each additional one MJ/mÂ^{2} of solar radiation, there was an associated 0.48 s decrease in average time spent stationary. The average duration ewes spent undertaking all other behaviours during the entire study period was not affected ($p > 0.05$) by temperature, relative humidity, or wind speed.

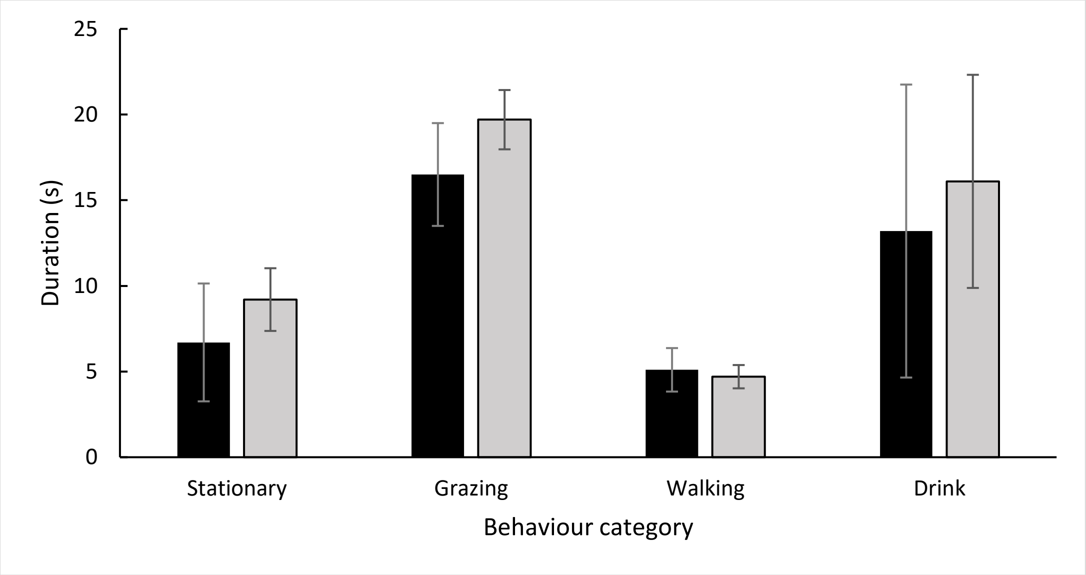


Figure 3.12: The average duration (time in seconds ± SE) of occasions (n=234) that ewes were observed to be stationary, grazing, walking, or drinking in the stream zone during periods of restricted access to water trough (black bars, n=83 occasions) or when access was not restricted (grey bars, n=151 occasions).

3.4.8 Behavioural events at the outflow culvert

During the study period, there were 185 behavioural events recorded at the outflow culvert (camera 1) which included sheep crossing the stream over the culvert (n = 145, 78.4%), crossing to graze (n = 17, 9.2%), grazing near the stream (n = 22, 11.9%), and resting (n = 1, 0.5%; Figure 3.11). All the ewes (n = 40) were observed to utilise the outflow culvert at least once during the study. Most of the ewes (n = 32) utilised the culvert more than once. Four ewes were observed to utilise the culvert ≥10 times during the study. Forty percent (n = 74) of the behavioural events were recorded during the period of unrestricted water trough access compared to 60% (n = 111) during the restricted period (p = 0.87). Of the behaviours observed, 68% were recorded during the day (0900 to 1600hrs) compared with 25% in the morning (0600 to 0800 hrs) and 7% the evening (1700 to 1900hrs); time of day only tended to affect the frequency of culvert use (p = 0.09).

Figure 3.13: Image captured by video camera number one showing ewes (ID; orange 8, blue 3, green 7 and blue 8) utilising the outflow culvert for crossing.

3.5 Discussion

The aim of the current study was to examine the behaviour of sheep in and around a natural waterway, when access to a water trough was unrestricted or restricted. The potential impacts of sheep on water quality were also assessed. It was hypothesised that the behaviour of the ewes around waterway would not differ when access to a trough was restricted or not. Likewise, it was hypothesised that the interaction of sheep with the natural waterway would not influence the level of nutrient and pathogens in the waterway.

Spatial distribution

The spatial distribution of sheep within the paddock was influenced by features such as slope and the location of culverts. Ewes showed a spatial preference for the flat to low sloped areas of the paddock, with more than 70% of GPS location fixes recorded in areas with a slope of less than 15°. This finding is in agreement with a number of studies in sheep which have reported a preference to graze slopes <30° (Sheath, 1982; Haddon, 2008; Steer, 2012). Low slopes (1–12°) have been reported to have a higher accumulation of herbage, compared to steeper slope classes (López *et al.*, 2003). Thus, the spatial preference observed in the present study may have been due to a greater pasture available in these flatter areas (Saggar *et al.*, 1990). Pasture masses were measured in the current study, although, in insufficient detail to provide information of the distribution of pasture mass across the entire paddock.

Distance travelled

The distance travelled by ewes in the current study (3157 m/day) was greater during the period when access to the water trough was restricted than when the trough was accessible. In the tropics, sheep have been observed to travel long distances, especially when feeding or searching for water (Osuji, 1974; Schlecht *et al.*, 2006). Therefore, the longer distances travelled during the restricted period of the current study may have been the result of ewes spending more time in search of water. It is acknowledged, however, that the 40m per hour difference in distance travelled between restricted and Unrestricted period was small and unlikely to be of biological significance.

Behaviour and time spent by sheep in the stream zone

Three GPS hot spot areas were identified in the study paddock where GPS location fixes were denser than the paddock average. These hotspots were located near the inflow and outflow culverts with an additional area in the middle of the stream zone (location 2; Fig. 12 D). Ewes crossing the stream using a culvert is of minimal concern in terms of water quality as there is no interaction between the animals and the water. The hot spots identified were all located in the flattest areas of the paddock based on slope map. Although the flattest area of the stream zone represented only 3.7% of the total (stream or paddock) area, it had a high density (44%) of GPS locations. At location 2, the predominant sheep behaviour observed was grazing (87.5%), which suggests that the pasture in this area was attractive.

The duration that ewes spent in the stream zone, however, was low at only 12 min/day or 0.8% of the day. Ewes were observed to drink and walk in the stream for only 0.1% of the day and only 1% of the ewe GPS locations were recorded in the stream zone. This suggests that ewes had minimal interaction with the waterway at all during the study period.

All 40 study ewes accessed the stream zone to graze but few (n=5) were observed to drink from the waterway. Oluju (2017) suggested that sheep preferred to graze around waterways to access green pasture within the stream zone. Shreffler and Hohenboken, (1980) reported that drinking activity was greatest during, or shortly after, periods of maximum eating activity. There is little published data, however, on the drinking behaviour of sheep in a temperate environment and to date none have focused on drinking from a stream. Al-Ramamneh *et al*. (2011, 2012) reported that sheep drinking behaviour was not affected by either water restriction or shearing when evaluating effect of water restriction.

In the current study, when ewes were in the stream zone, they spent 68% of their time grazing, 15.9% stationary and only 2.2% drinking. These percentages were similar to Schlecht *et al.* (2006) who reported that 60% of the day was spent grazing and 12 to 20% stationary. Al-Ramamneh *et al*., (2012) and Filipčík *et al*., (2020) reported a shorter time spent drinking than in the current study (0.1% to 0.3%). In their study Al-Ramamneh *et al*., (2012) observed penned sheep with video cameras that captured the entire area. The difference in drinking percentage between Al-Ramamneh *et al*., (2012) and the current study, therefore, was likely due footage from the current study being recorded only within the stream zone and thus, did not capture ewe behaviours in other areas of the paddock. Filipčík *et al*., (2020) used human observers to record sheep behaviours every five minutes between 7:15 a.m. and 4:15 p.m., which may have resulted in drinking events being missed.

Ewes spent 3.3 minutes more per day within 3 m of stream during the period when access to the water trough was unrestricted than when access was restricted which biologically is of little importance. When they were within the stream zone video footage showed that grazing was the dominant behaviour. Previous research suggests that ewe grazing is focused near the water-points (Graz *et al*., 2012). Sheep were observed to be within 3m of camera locations for longer periods in the early morning and evening and shorter periods during the day and night. The median duration spent within 3 m of the stream zone in the current study was greater in the early morning than during dayhours and was also greater in the evening than daylight. This pattern of time spent near the stream was in agreement with the grazing pattern reported by McGranahan *et al*., (2018) and Filipčík *et al*., (2020) where grazing behaviour was greater in the morning and evening hours.

Influence of weather

In the current study both ambient temperature and relative humidity appeared to increase the time ewes spent in the stream zone. It is possible that ewes spent less time within 3 m of the stream zone during the middle of the day as extreme temperatures have been shown to decrease the proportion of time spent feeding. Malpura cross and Polwarth ewes in climatic chamber (18°C - 45°C) showed a similar pattern (spend less time at midday) with the current study (Da Costa *et al.*, 1992; De *et al.*, 2017). The decrease in feeding activity under thermal stress may be a result of voluntary adaptive depression of metabolic rate associated with reduced appetite in heat-stressed animals (Silanikove, 2000). All the drinking events in the current study occurred when the ambient temperature was greater than 20°C which supports the impact of temperature.

Movement and drinking behaviour of sheep

Video footage in the current study showed that ewes avoided walking in the waterway and crossed the stream by jumping over it or using the culverts. This finding was similar to Askey-Doran (1999) and Dymond *et al.* (2016) who reported that sheep have reduced affinity to water and showed an aversion to standing in water. The tip of their hoof has small contact area with the ground which may allow the penetration of a film of water and possibly moss, mud and lichen (Manning, 1990). In addition, sheep have reduced blood flow to the skin of the lower legs when penetrated by or exposed to cold water (Wheeler, 1972). It is possible that this blood flow is connected with drinking behaviour as skin blood flow is adrenergically-mediated due to thermal influences which may elicit a fear reaction of sheep to water (Hales, 1982).When sheep were given access to, or were restricted from, accessing the water trough there was no effect on the proportion of time they were recorded to undertake any of the behaviours observed in the current study. This is perhaps not surprising as they had *ad libitum* access to pasture which contained 77% moisture suggesting that sheep could satisfied their water needs from pasture. This finding is in agreement with a report of desert bighorn sheep in the USA that reported the removal of water catchments did not result in changes in diet, foraging area selection, home-range size, movement rates, mortality, or productivity in ambient temperatures similar to present study (Cain III *et al.*, 2008).

Water quality

Nitrate-N load of water sampled from the stream during the current study varied between sampling sites with higher concentrations at the outflow compared to the inflow sites on study days 5 and 14 but did not differ between periods of water trough access or restriction. Cooper *et al.*, (1987) reported that in winter nitrate concentrations were positively correlated with water flow rate. Given that the flow rate of the stream did not differ between the study periods it is perhaps unsurprising that the nitrate-N load was similar. Mean nitrate-N concentrations measured in the current study (0.82 mg/l) were higher than the 0.226 mg/l reported by Cooper (1990). The difference in nitrate-N concentrations may have been due to the timing of their study which was conducted in August (winter) during a period which had no rainfall in the catchment for at least 7 days prior to water sampling. In contrast, McColl and Gibson (1979) reported a mean nitrate-N concentration of 1.8 mg/l reported during a 3-month study conducted in New Zealand in winter (June to August). It is likely in that study they recorded some of the first soil drainage events of the season which are typically high in nitrate-N (Bieroza 2019). In the current study differences in mean nitrate-N load between the inflow and outflow sampling sites at study day 5 could have been the result of outflow-N being washed through the soil due to a rain event on study day 3. It should be noted that the paddock in this study was part of a 4.1 ha catchment which was 100% pasture based whereas in images provided by McColl and Gibson (1979) showed some forested areas. Transport-limited flushing is commonly seen for nitrate in watersheds containing forest (Inamdar *et al.*, 2004; Inamdar and Mitchell, 2006). The rainfall on day 3 likely generated both subsurface and surface runoff of N which may have contributed additional N to the stream between the inflow and outflow monitoring sites.

Suspended sediment loads were found to be higher in the period ewes had unrestricted access to the water trough than when access was restricted, regardless of the water sampling site. Based on the hotspot analysis, this was the period when there was greater spatial clustering of ewes (Fig,12D). Physical disturbance of soil due to trampling has been one of the factors identified to result in the degradation of the physical quality of soils under pasture and formation of sediment (Da Silva *et al.*, 2003). On days 5 and 6 of the current study the sediment load was greater in outflow than inflow water samples which may have been the result of rainfall on day 4 generating surface runoff into the stream (approximately 5 to 24 hours prior to water sampling).

E. coli concentrations were higher on day 12 than days 5, 6 or 13. On day 12, the concentration of *E. coli* in the outflow sampling point was observed to be higher than that in the inflow. Furthermore, in unrestricted period *E. coli* loads increased between the inflow to outflow. This increase in *E. coli* may have been due to water runoff from a rainfall event on day 12. It is possible that rain washed sheep faeces into the stream thus resulting in a higher *E. coli* load at the outflow sampling point. *E. coli* also is known to attach to sediment and therefore be mobilised when sediment is lost (Muirhead, 2009). The current study also found suspended sediment to be higher in unrestricted access to the water trough than when access was restricted which could probably account for increased *E. coli* loads from inflow to outflow. Soupir *et al.* (2010) reported a high correlation between *E. coli* and soil and manure in runoff with approximately 40% of *E. coli* attached to these particles.

In the current study, *E. coli* load was positively correlated with relative humidity and negatively with solar radiation. These findings were consistent with solar radiation reducing the *E. coli* population (Sinton *et al.*, 2002; Whitman *et al.*, 2008). The New Zealand Ministry for the Environment (2003) water quality guidelines for freshwater recreation requires *E. coli* concentration to be <130 cfu/100ml. In the current study 25% of the samples collected exceeded this concentration and 3% of samples exceeded the D grade threshold of >550 cfu/100ml.

Total P load was greater when access to the trough was unrestricted than restricted. It is unclear if rainfall contributed to the high total P load recorded on study day 14 as 5mm rainfall was recorded during the two days prior to the sampling period. Previously, a study found phosphorus concentration in waterways to depend on the intensity of the runoff or during peak water flow (Edwards *et al.*, 2000). Total phosphorus in outflow water samples on D13 likely showed high variability due to the multiple forms phosphorus in water which influenced by many environmental and operational factors such as changing particulate content, flow conditions and sediment resuspension (Vianini *et al.*, 2025). The mean total P concentration of 0.05 mg/l across all samplings and treatments in the current study was similar to the 0.06 mg/l reported by Caruso (2000). The total P in the current study was greater than the guidelines of the Australia and New Zealand Environment and Conservation Council (ANZECC, 2000) which suggests that phosphorus should be less than 0.035 mg/l in upland rivers. McColl and Gibson (1979) reported concentrations were 2.18 mg/l, however, their study was conducted over three months which may explain the difference with the current one-month study as the longer the study may have had a higher the possibility of representing the whole season.

In conclusion, the current study found little evidence that sheep interacted with the natural waterway and that there was little direct impact on nitrate-N or SS. Total P and *E. coli*, however, were recorded to be above suggested thresholds on some days, however, these appeared to be unrelated to the presence of sheep in the paddock, instead appeared to be related to rain events. The degree of interaction of sheep with the waterway was not influenced by the availability of reticulated water from a trough. There was a clear indication that the study ewes showed a spatial preference for flat to low sloped areas of the paddock. Further, long-term studies are required to verify these results, especially to confirm the elevated total P and *E. coli* were due to rain events *per se* and not directly due to sheep interaction with waterways. Other periods of the year also need evaluation.

4 The behaviour and impact of sheep accessing a natural waterway on water quality in spring

Simple summary

Concern has been raised in New Zealand about the deteriorating status of water quality. Since European colonisation New Zealand has lost 90 per cent of wetlands with 75 per cent of freshwater fish now considered endangered, and 80-94 per cent of urban streams in pastoral areas being unsuitable for swimming at some times of the year. Interaction of animals with waterways is one of the major causes of the impacts to water quality, however, there is a paucity of information on the interaction of sheep with natural waterways and their impact on water quality. A study was conducted in spring to determine the behaviour of sheep on a hill country paddock and assessed measures of water quality. The study also investigated sheep behaviour and impact on the water quality of the waterway when they had access to a reticulated water trough. The expectation was that in spring sheep would likely not need to drink due to high pasture moisture contents. The spatial distribution of ewes was influenced by paddock features such as slope and culverts' location. The absence of reticulated water source had no effect on sheep interaction with the natural waterway, however, there were greater concentrations of nitrate-N and total phosphorus in water samples.

4.1 Abstract

New Zealand there are regulations to fence waterways >1m in width to restrict the access of farmed cattle, deer and pigs, however, there are no such requirements for sheep. In chapter 3 during winter there was little interaction with the waterway but the warmer temperatures in spring may result in different behaviour. In spring (November), observations of the interaction of mixed-age Romney ewes (n=40) with a natural waterway was conducted at Massey University's hill country farm Tuapaka. The animals were continuously stocked on a 1.7 ha perennial ryegrass paddock where the pasture mass was maintained above 1000 kgDM/ha. No supplementary feed was given throughout the study period. Ewe behaviour and movements were monitored continuously for two weeks using GPS devices and motion-activated trail cameras. Ewes were offered reticulated water (week 2) or were restricted from accessing the

water trough (week 1). Ewes spent more time within 3m (10.6 ± 0.6 min/ewe/day; $p<0.05$; riparian zone) of the stream during the restricted period. Ewes were also observed to travel further during the evening and early morning than during the afternoon ($P<0.05$) with shortest distance travelled during the night. During the period water trough restriction ewes spent longer grazing and drinking than during the unrestricted period ($P<0.05$). It was concluded that, during spring, absence of reticulated water source had no effect on sheep interaction with the natural waterway but there was an influence on nitrate-N and total phosphorus concentrations in water samples collected from the waterway.

4.2 Introduction

In New Zealand, the potential impacts of sheep on the environment have been given less consideration than to other forms of agriculture such as beef and dairy cattle production (Wilkins, 2002). There is lack of information on the behaviour of sheep around natural waterways and their impact on water quality. The potential for sheep to graze in riparian zones may have important implications for water quality, as the removal of plant material by grazing could decrease the filtering effect of vegetation, thus increasing sediment loss into waterways (Pearce *et al.*, 1998; McEldowney *et al.*, 2002). Further, nutrients and microorganisms present in sheep dung and urine deposited within the riparian zone are potentially at greater risk of being washed into waterways. Similarly, grazing within riparian zones could cause soil damage and stream bank erosion, which then may increase the risk of sediment being introduced to waterways.

Livestock farming in New Zealand can potentially have negative effects on water quality due to the contamination of waterways with phosphorus (P), nitrogen (N), suspended sediment (SS), microorganisms and faecal matter (Abell *et al.* 2011, Gillingham and Thorrold 2000, Ledgard *et al.* 1996, Monaghan *et al.* 2005, Scholefield *et al.* 1993). It has been predicted that the total amount of nitrate-N leached due to livestock farming in New Zealand has increased from 189,000 tonnes in 1990 to 199,000 tonnes in 2017 (Statistics New Zealand 2019b). Sheep production has been estimated to contribute 15% of the total nitrate-N leached from livestock production systems (Statistics New Zealand 2019b). Since concentrations of phosphorus, nitrogen, suspended sediment and pathogens are associated with the amount of water run off across the environment (Giddings *et al.*, 2004; Jensen *et al.*, 2006; Cooley *et al.*, 2007; Whitman *et al.*, 2008; Yevenes and Mannaerts, 2011), it might be expected that their concentration in the waterways in spring will be influenced by the amount of rain prior to or during any water sampling period.

Sheep in New Zealand can obtain water from natural waterways and through reticulated water systems. During spring livestock grazing pastures often have access to high pasture masses and a dry matter content of less than 30-50% (Brown and Lynch, 1972; McFarlane and Howard, 1972). The quality and moisture content of pasture can influence how much sheep drink (Macfarlane *et al*., 1958; Macfarlane *et al*., 1966a; Forbes, 1968) as dry matter intake of sheep is inversely related to water content (Calder *et al*. 1964). In spring, therefore, it is possible that non-lactating sheep can meet their water requirements from pasture moisture alone and do not need to drink free water. A lactating ewe, however, can produce up to 2 l of milk per day (McMillan *et al*., 2014) which increases their water requirement and intake (Johnston, 1983). Lactating ewes in spring under New Zealand conditions, therefore, may require an additional source of drinking water. This drive to drink might result in sheep consuming water from natural waterways, particularly when a reticulated water supply is not available.

In winter (chapter 3), sheep behaviour around a natural waterway showed that ewes in late pregnancy spent less time near the waterway compared to other parts of the paddock. In addition, access to a water trough had no effect on the proportion of time ewes spent grazing, walking, resting, and drinking within 15 m of the waterway. Ewes had few interactions with the waterway which was partly explained by the high moisture content of pasture during winter (77%). Restricted access to the trough resulted in the waterway being the sole water source during this period and corresponded to higher mean SS and phosphorus concentrations in the waterway, but no difference in Nitrate-N and *E. coli* concentrations were seen compared to when the trough was accessible.

The current study was designed to measure the behaviour of sheep around a natural waterway and the impact on water quality in spring. In addition, the impact of access to a reticulated water trough on sheep behaviour was investigated. It was hypothesised that the absence of reticulated water source would result in greater sheep interaction with the natural waterway, but not the amount of nutrient, sediment and pathogens in the waterway.

4.3 Materials and methods

All the procedures in this study were carried out with the approval of the Massey University Animal Ethics Committee (MUAEC 19/62). The study was conducted for two weeks from the 13th of November (D1) to the 29th of November (D17) 2019 (Table 4.1) at Massey University's Tuapaka farm, located approximately 15 km north-east of Palmerston North, New Zealand (40.3345° S, 175.7390° E). The water watershed (labelled watershed 2) that supplied the study paddock was 4.1 ha in size and fed into the outflow point (indicated by the red dot) of the study paddock (Figure 4.1). The catchment area was grazed by sheep only, with a pasture mass of 3642.4 ± 64.9 kgDM/ha (Mean ± SD).

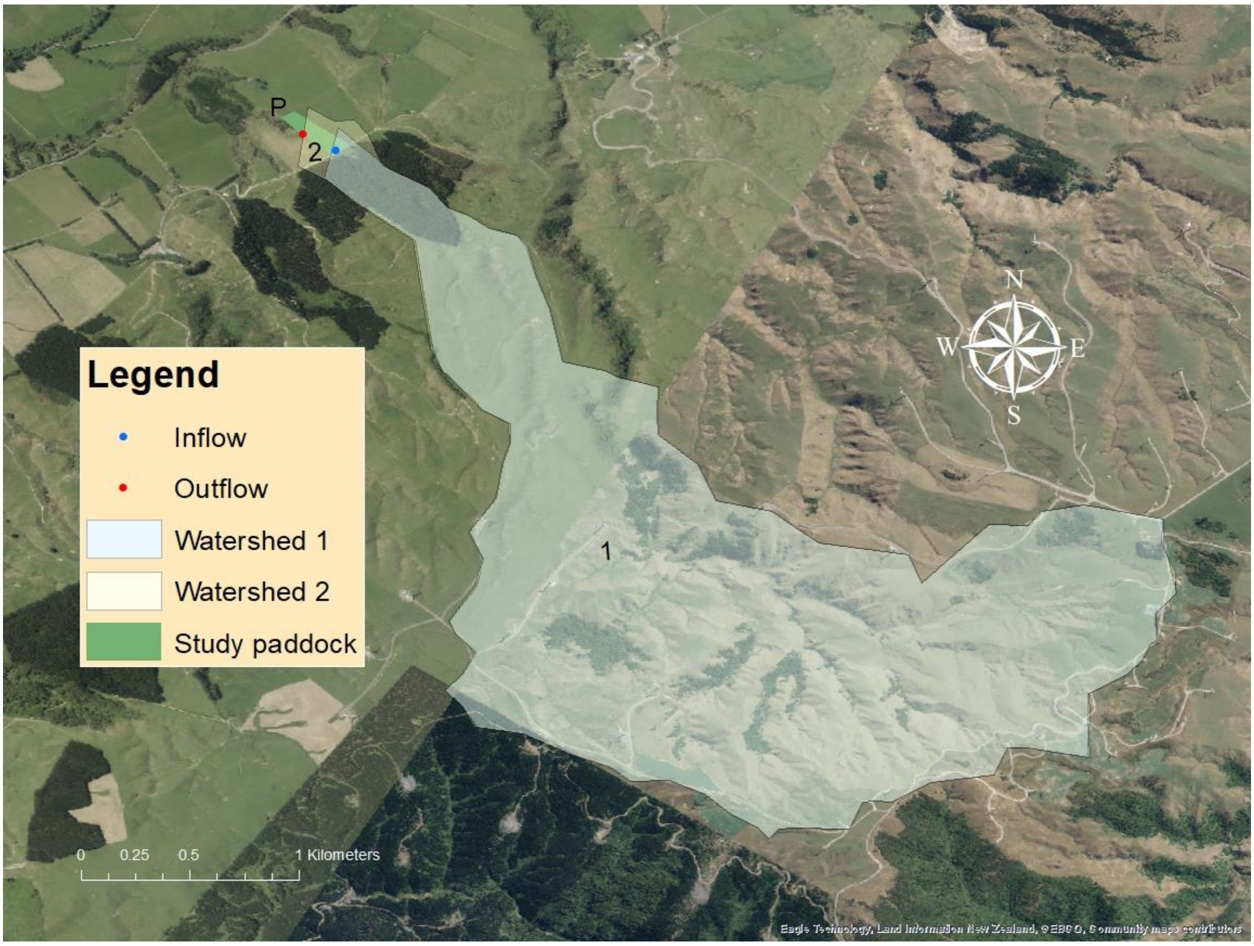


Figure 4.1:Map of watershed 1 and 2 that supplied the study paddock stream (P). Red and blue dots indicate locations of the outflow and inflow sampling sites, respectively.

4.3.1 Animals and study design

Lactating ewes (n=40) with lambs at foot were managed in a 1.7 ha paddock (Figure 4.1) that contained a discrete natural stream (the same location as chapter 3). Ewes had an average weight of 72.1 ± 8.2 kg and body condition score of 2.7 ± 0.5 (Mean ± SD). During the study period, the same ewes were used as in Chapter 3 and there were no sheep in adjacent paddocks. The stream was 233 m in length, approximately 1 m wide and 30 cm deep with an average flow rate during the study period of 4.04 l/s.

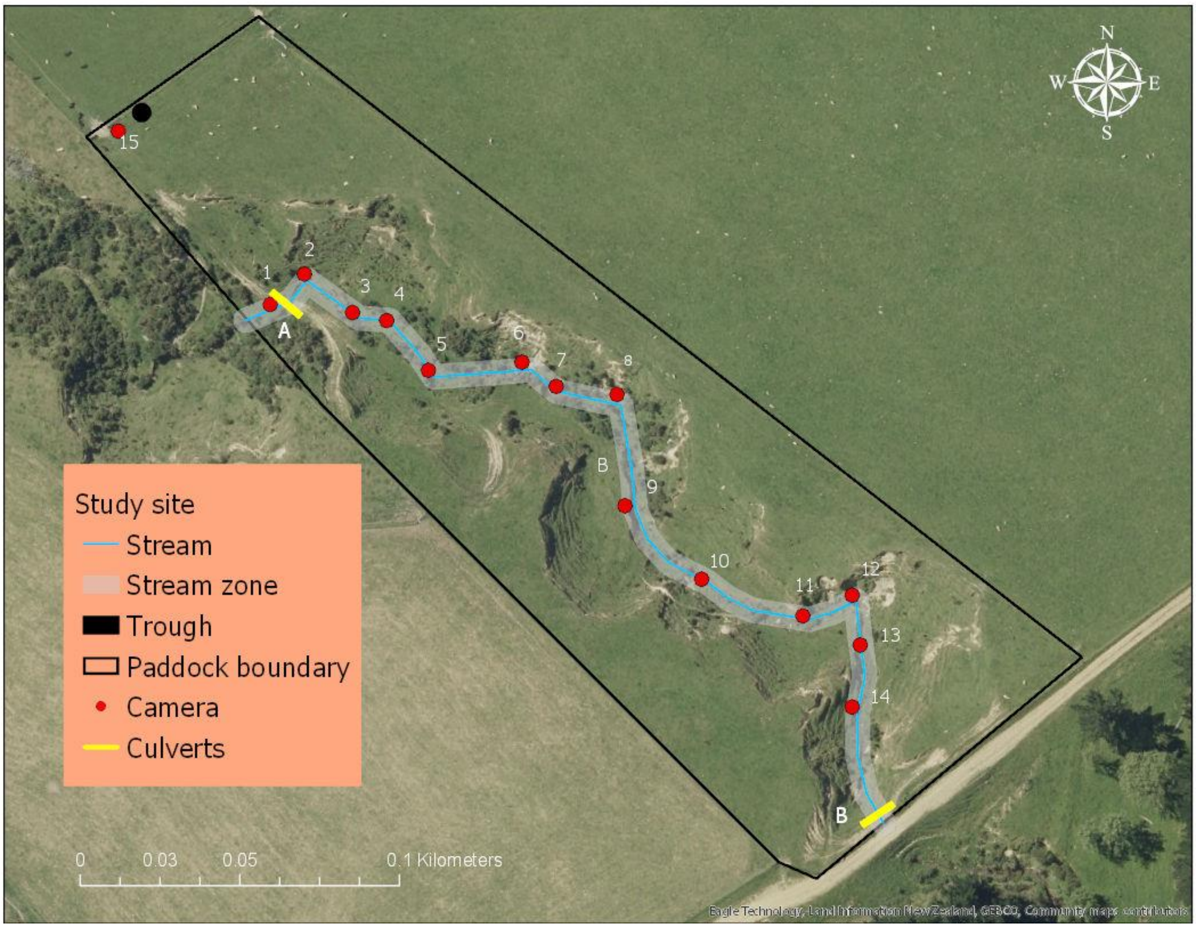


Figure 4.2: Map of the study paddock showing the paddock boundary (black line), stream (blue line), position of the trough (black dot), culverts (yellow rectangle), cameras (1-15; red dot) and the stream zone (3m; pale blue shading). A and B indicate the outflow and inflow water sampling sites, respectively.

The design of the study was as previously described in chapter 3. Briefly, a crossover study was conducted whereby ewes were grazed a single paddock for one week when offered reticulated water from a trough, resulting in the stream being the only source of free water within the paddock followed by a second week when the trough was covered. Ewe movement within the paddock and interaction with the waterway was monitored using GPS unit, accelerometers, and video surveillance footage. Water samples were collected hourly for eight hours for two days each week to determine concentrations of Nitrate-N, P, SS and *E. coli* (Table 4.1).

Table 4.1: Summary of water and pasture sample collection in spring study showing dates, study days, and the periods when ewes were restricted from drinking from the water trough (restricted) or had access (unrestricted).

Date	Study day	Treatment	Water sampling days	Pasture mass and moisture content
13-Nov	D1	Unrestricted		√
14-Nov	D2	Unrestricted		
15-Nov	D3	Unrestricted		
16-Nov	D4	Unrestricted		
17-Nov	D5	Unrestricted		
18-Nov	D6	Unrestricted		
19-Nov	D7	Unrestricted	√	
20-Nov	D8	Unrestricted	√	
21-Nov	D9	Unrestricted		
22-Nov	D10	Unrestricted		√
23-Nov	D11	Restricted		
24-Nov	D12	Restricted		
25-Nov	D13	Restricted		
26-Nov	D14	Restricted		
27-Nov	D15	Restricted	√	
28-Nov	D16	Restricted	√	√
29-Nov	D17	Restricted		

4.3.2 Ewe measures

At D1 and D17, ewes were weighed (using Tru-Test weigh scale, Auckland NZ) and body condition scored by an experienced technician [scale 1-5; Jefferies (1961)]. Lactating ewes were weighed within an hour of being removed from the pasture.

4.3.3 Pasture measures

Pasture collection and determination of dry matter yields, pasture mass and moisture content were as described in chapter 3. The moisture content of the pasture was determined using the dry oven method (Thiex and Van Erem, 1999; Wallau and Vendramini, 2019) on D1, D10 and D16 (Table 4.1). At approximately 1 pm, six grab pasture samples of approximately 50g were randomly collected across the paddock by hand plucking simulating sheep grazing. Samples were then oven-dried at 80ºC for 48 hrs to determine dry matter yields and moisture content. Pasture mass was estimated on the same study days as moisture content using a manual folding plate meter (Jenquip, New Zealand). One hundred readings were taken randomly across the paddock at approximately 2m intervals and the average mass for the paddock recorded.

4.3.4 Sheep behavioural observations

Ewe behaviour was recorded for two weeks from D1 to D17. Fifteen, motion activated video surveillance cameras (Moultrie® model MCG-13297, Birmingham, AL, USA, (n=7), TechView® model QC8027, Kaki Bukit, (n=4) and Bushnell© model 119736 Overland Park, KS, USA, (n=4)) were used to record ewe movement and behaviour around the waterway. Cameras were placed at intervals of 14 to 18m and recorded clear footage up to a distance of 15 m (riparian zone) (Figure 4.1). In addition, a camera was placed at approximately 7 m from the reticulated water trough. Camera setup and recording was as described in chapter 3. In order to identify each individual in video footage, ewes were spray marked with a coloured number on their both sides. In addition, they were fitted with a plastic collar labelled with a unique number. Behaviours were determined from video recordings were identified using the ethogram described in chapter 3. Sheep behaviour was coded using BORIS software [version 7.8.2; Friard and Gamba (2016)]. The behaviours recorded included grazing, walking, stationary, drinking, sniffing, walking in the stream and out of view.

4.3.5 GPS and accelerometers

All ewes (n=40) were fitted with a collar to which a triaxial accelerometer (Actigraph wGT3X-BT 4.6cm x 3.3cm x 1.5cm and weighing 19 grams) and GPS unit (Custom build units, DataCarter, weighing 100 g) was attached. Triaxial accelerometers and GPS units were attached for seventeen days from D1 to D17. GPS programming and specification was as described in chapter 3.

4.3.6 Weather data

Hourly and daily data were downloaded from Tuapaka weather station 2 (EnviroMonitor station, Davis Instruments, Davis, CA, USA) located 800 m away and at the same altitude as the study site. Data included rain fall (mm), relative humidity (%), air temperature (°C), solar radiation (MJ/mÂ^2) and wind speed (m/s).

4.3.7 Water measurements and samples

Stream water flow was measured at the stream inflow and outflow into and out of the paddock as described in chapter 3 (Figure 3.1). Briefly, stream flow rate was measured hourly for 8 hours (from 0800 to 1500hrs) on D7, D8, D15 and D16. Flow rate was determined manually by measuring the volume of stream flow over a set time period based on the method of Ghani and Saudi (2017).

On the same days, water grab samples were collected hourly for 8 hours from both the inflow and outflow points to determine the concentration of suspended sediments (SS), total N and P, Nitrate-N, ammonium and *E. coli.* Sampling procedures and handling were as described in chapter 3.

4.3.8 Water quality analyses

Water samples were placed in an insulated box until they were transported to the laboratory at the end of each sampling day. Samples to be analysed for nitrate-N and ammonium-N were sub-sampled and filtered to < 0.45 µm on the same day and frozen for subsequent analysis (Din, 1996). A second unfiltered sub-sample was frozen for subsequent total N and P analysis. The remaining water sample was stored at 4^{o}C for SS analysis.

4.3.8.1 Nitrate-N, Ammonium-N, Total P concentrations, Suspended sediment, and E. coli analysis

The colorimetric autoanalyzer method was used to determine nitrate-N and ammonium concentrations of filtered water samples (Blakemore *et al* 1987) using an autoanalyzer (Pulse international ltd, Saskatoon, Sask. Canada). Preparations of the autoanalyzer, standards and their sequences were as described in chapter 3.

The concentration of SS was determined using the gravimetric analysis method following the standard procedure from the American Public Health Association by Baird (2017). The procedure was as described in chapter 3. *E. coli* concentrations were determined by Eurofins laboratory (Wellington, NZ). Samples were analysed within 24 hours of collection using a membrane filtration procedure (Standard Method APHA 9222G; Baird, 2017) onto nutrient agar containing 4-methylumbelliferyl beta-D-glucuronide. *E. coli* results were reported as colony forming units (cfu) per 100mL of sample.

4.3.8.2 Loads

For each water quality parameter contaminant loads (mg/s) in the inflow and outflow samples were calculated as described in chapter 3.

4.3.9 Statistical analysis

GPS data were analysed using ArcGIS mapping tools (ArcGIS Pro 2.2.4, 2018). Distance travelled by sheep data was cleaned categorised and processed as in chapter 3. An optimized hot spot analysis (z-score) was conducted using ArcGIS to identify statistically significant spatial clustering of ewe GPS location fixes and the definition were as described in chapter 3.

Behavioural data including grazing, drinking, and walking analyses were performed using R 3.6.0 (2019-04-26; R Core Team (2019)). Prior to analysis, data (water quality data) were checked for normality using Kolmogorov–Smirnov and Shapiro-wilk test, and the homogeneity of variances using and Levene's test, and Tukey transformation (Tukey's Ladder of Powers transformation) when appropriate (Gotelli and Ellison, 2004). To determine if access to the water trough influenced the duration and percentage of time that the various ewe behaviours were observed (e.g., grazing or drinking) a 2-factor analysis of variance (ANOVA) was utilised. A linear regression was used to determine if ewe behaviours were associated with time of the day and weather parameters. To determine whether the sheep behaviour during water restriction were related to their behaviour in the non-restricted period, Spearman's rank correlation coefficients with a two-tailed level of significance ($p < 0.05$) were determined.

Nitrate- N concentrations were analysed using parametric tests while TP, SS, *E. coli* and Ammonium-N were not normally distributed and analysed using non-parametric methods. One way ANOVA analyses followed by post hoc test when significant (Tukey test $p < 0.05$), or non-parametric ANOVA models (Kruskal–Wallis) were used to assess differences between mean ranks.

4.4 Results

4.4.1 Weather

Rainfall was recorded on six of the seventeen days of the study period (Figure 4.3). It also rained on three days prior to the study period. Mean rainfall recorded per day in the study period ranged from 0 to 8.6mm (mean was 1.5mm). Daily average air temperatures ranged from 10.4 to 16.1ºC fluctuating throughout the study period with mean value of 13.2ºC. The lowest relative humidity (RH) was 73.9% and the maximum was 94.7% while the mean RH was 84.4%.

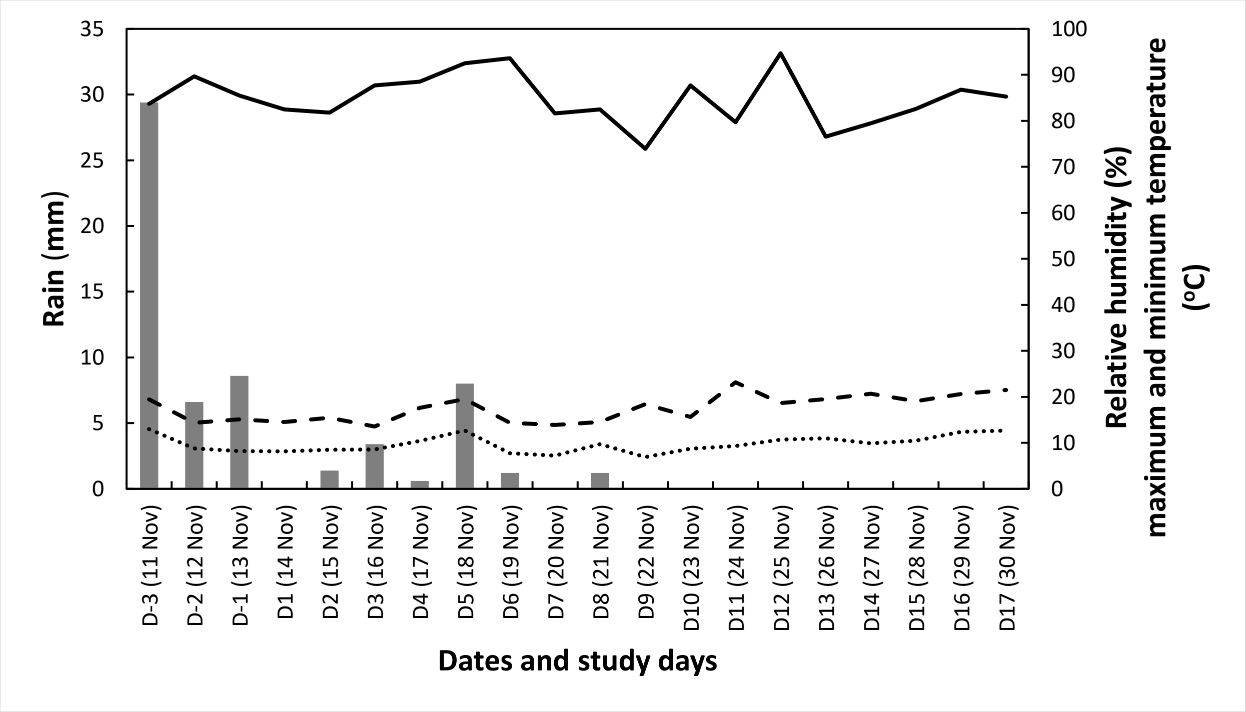


Figure 4.3: Daily mean rainfall (mm, bars), relative humidity (%, "______"), minimum daily temperature (°C, "………..") and maximum daily temperature (°C, "--------") during the study period. D-3 to D15 indicate number of days relative to the start of the study (D1;14 Nov 2019).

4.4.2 Stream flowrate

Spring stream flowrates measured at both the inflow and outflow monitoring sites fluctuated over time (D7, D8, D15 & D16) and ranged from 0.05 to 1.07 l/s (Figure 4.4). At D15 and D16 flow rate was higher at the outflow than inflow monitoring sites ($p<0.05$). It took an average of 35 sec for water to travel the length of the stream. The mean flowrate was greater in the unrestricted period (5.78 (5.10 - 6.13 l/s)) compared with the restricted period (2.24 (2.14 - 2.38l/s)) ($p<0.05$). Correlations of flowrate with environmental conditions showed that there was a positive relationship with daily average humidity ($r = 0.24$, $p<0.05$) and wind speed ($r = 0.6$, $p<0.05$) but, not rainfall ($r=0.5$, $p>0.05$). Flowrate was also negatively correlated with the concentrations of both nitrate-N concentration ($r = 0.4$, $p< 0.05$), and *E. coli* in the water samples ($r = 0.2$, $p< 0.05$).

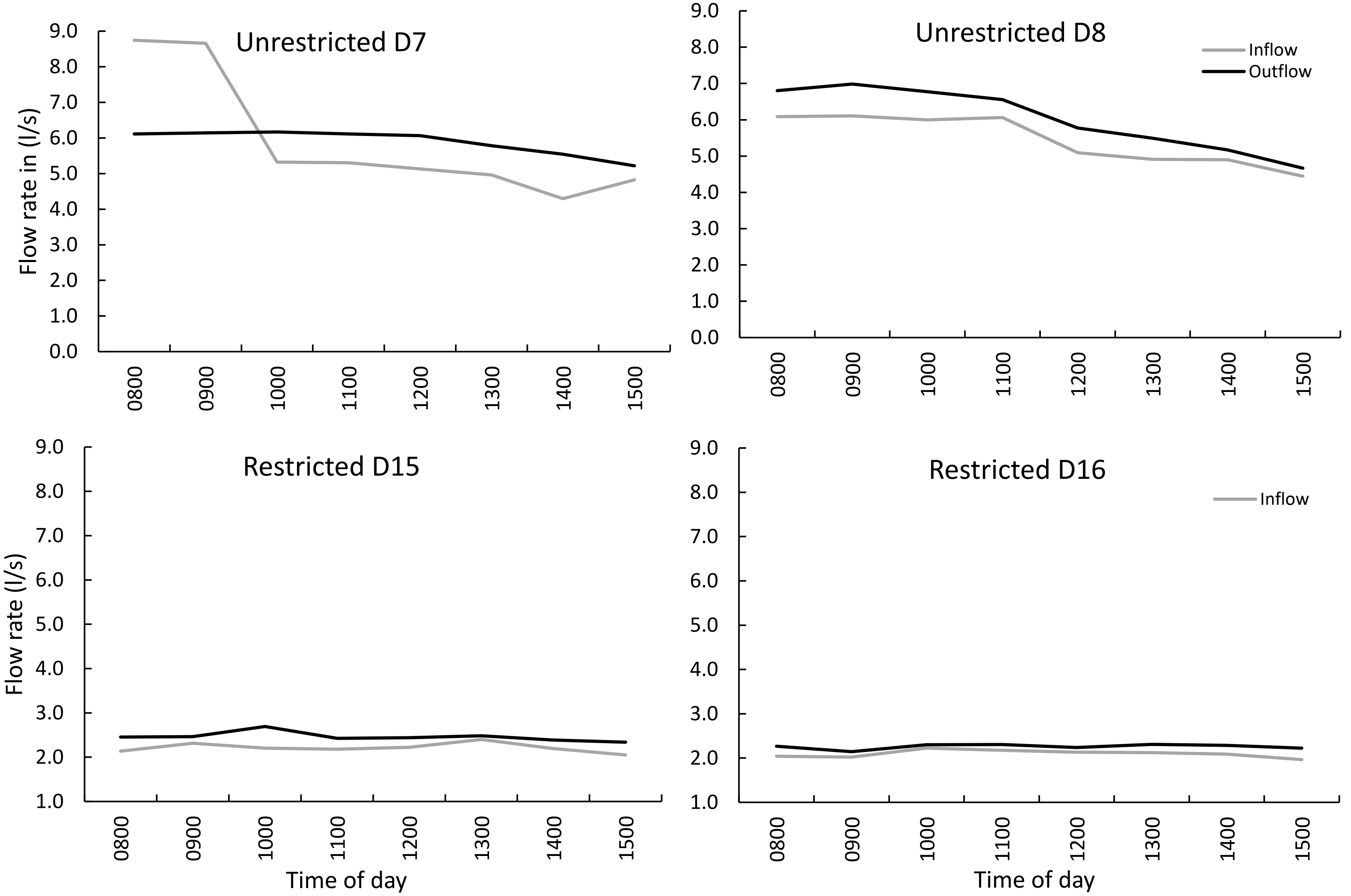

Unrestricted D7
Unrestricted D8
Restricted D15
Restricted D16
Inflow
Outflow
Inflow
Flow rate in (l/s)
Flow rate (l/s)
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0800
0900
1000
1100
1200
1300
1400
1500
Time of day
Time of day

Figure 4.4: Hourly mean stream flowrate (l/s) at the inflow (grey line) and outflow (black line) monitoring sites by time of day (0800 to 1500hr) during the period of unrestricted access to water trough on D7 and D8 (upper panels) and the period of restricted access on D15 and D16 (lower panels)

4.4.3 Water quality

Water quality analyses showed that nitrate-N and TP concentrations were greater ($p<0.05$) in water samples collected during the trough restricted period, compared to unrestricted period (Table 4.2). There was, however, no difference in *E. coli*, ammonium-N and SS concentrations between the treatment periods ($p>0.05$).

Table 4.2: Arithmetic mean (±SEM) of concentration of nitrate-nitrogen (Nitrate-N; mg/l), and the median (interquartile range) of Escherichia coli (*E. coli* cfu/100ml), total phosphorus (mg/l) and suspended sediment (mg/l) ammonium-N (mg/l) and flowrate (l/s) during periods when ewes had access to the water trough (Unrestricted) or were prevented from accessing the trough (Restricted).

Parameter	n	Treatment period	Arithmetic mean ± SEM	Median (IQR)	Treatment P-value
Nitrate-N (mg/l)	32	Restricted	0.51 ± 0.02		0.001
	32	Unrestricted	0.41 ± 0.01		
Ammonium-N (mg/l)	32	Restricted		0.13 (0.05-0.23)	0.851
	32	Unrestricted		0.10 (0.06-0.23)	
Total phosphorus (mg/l)	32	Restricted		0.04 (0.03-0.04)	0.014
	32	Unrestricted		0.04 (0.04-0.05)	
Suspended sediment (mg/l)	32	Restricted		1.74 (1.25-2.47)	0.648
	32	Unrestricted		1.99 (1.20-2.81)	
E. coli (cfu/100ml)	32	Restricted		205 (140-193)	0.185
	32	Unrestricted		215 (180-328)	
Flowrate (l/s)	32	Restricted		2.24 (2.14-2.38)	0.001
	32	Unrestricted		5.78 (5.10-6.13)	

The *E. coli* loads in water samples from the outflow site were greater than inflow on D15 and D16 ($p<0.05$), when access to the trough was restricted (Figure 4.5C). The Wilcoxon test showed no significant difference in *E. coli* concentrations between inflow and outflow during the water trough access period ($p > 0.05$). In contrast, during the water-restricted period, *E. coli* concentrations were significantly higher at the outflow compared with the inflow ($p < 0.05$). Similarly, ammonium-N and nitrate-N loads were greater at inflow than outflow sites at D15 and D16 ($p<0.05$, Figure 4.5E), respectively. SS and TP load did not differ ($p>0.05$) between sampling sites on any day during the study (Figure 4.5B & D). Nitrate-N, *E. coli*, ammonium-N, SS and TP did not vary ($p>0.05$) across the hours of the day on any study day (Appendix Figures 1 to 5).

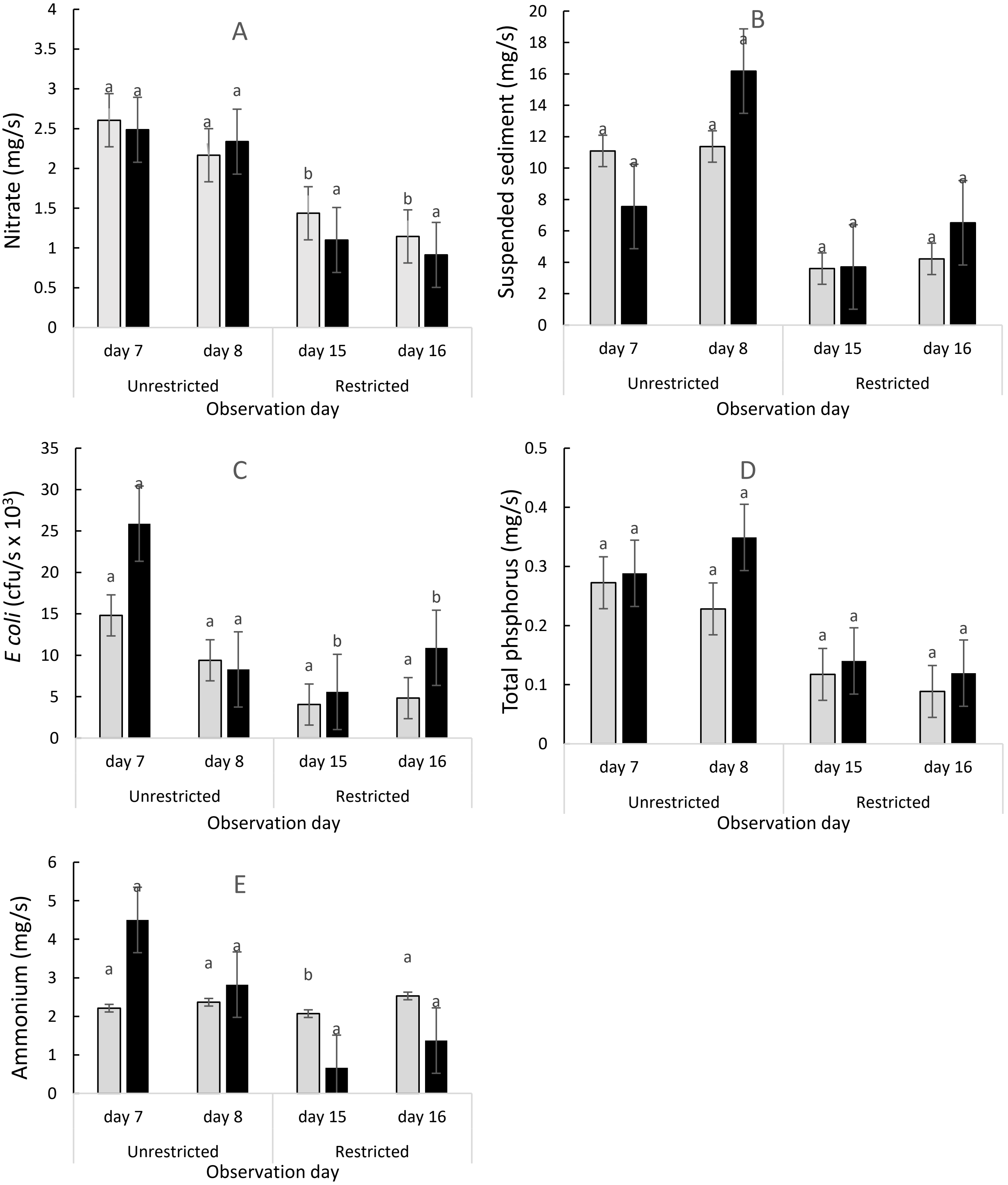


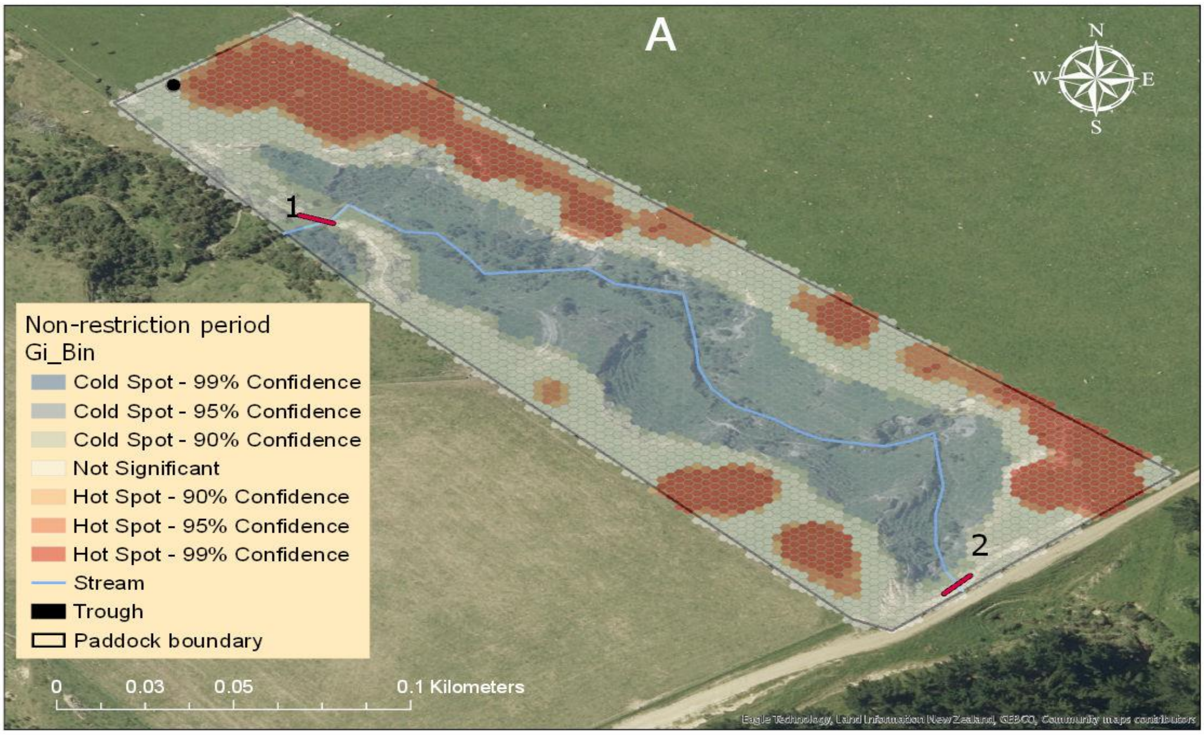
Figure 4.5: Mean (± SEM) nitrate-N (mg/s), suspended sediment (mg/s), *E. coli* (cfu/s x 10^6), total phosphorus (mg/s) and ammonium-N loads (mg/s) measured in water samples collected at the inflow (grey bars) and outflow (black bars) sampling sites on study D7 and 8 (unrestricted access to trough) and D15 and 16 (restricted access to trough). Within each day, bars with different letters were significantly different ($p<0.05$).

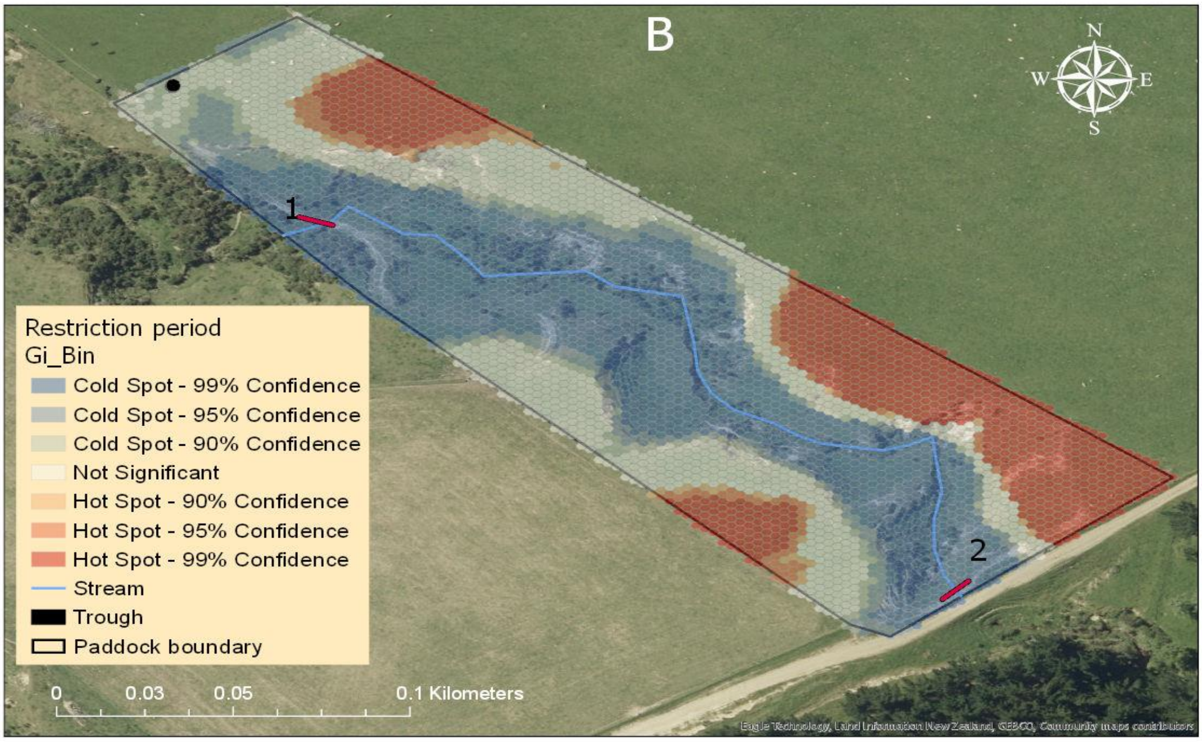
4.4.4 Animal density and spatial distribution

Optimized hot spot analysis identified statistically significant spatial clustering of ewe GPS location fixes. Six significant hot spots (high spatial clustering; $p<0.05$) were identified when ewes had unrestricted access to the water trough (Figure 4.6A), whereas there were three hot spots when the water trough was restricted (Figure 4.6B). The northern and southern areas of the paddock contained significant hotspots during the water trough restricted period (p-value < 0.05, Figure 4.6A), whereas, four focal hotspots were identified when the ewes had unrestricted access to the trough (Figure 4.6A). When the water trough was restricted, the northern hotspot was extended to be closer to the trough compared with the unrestricted period.

When the stream zone (3m either side of the stream) was analysed in isolation, two statistically significant ($p<0.05$) cold spots (areas with a low frequency of location fixes; marked CS in Figure 4.6D) were identified when access to the water trough was restricted but absent when access was not restricted. A similar pattern was observed for hot spots in the stream zones in both the water trough restricted and unrestricted periods. The significant hot spots areas were detected at locations 1 and 2 (Figure 4.6C & D).

The stream zone represented 9 % of the entire paddock area and contained 0.7 and 0.3% of all ewe GPS location fixes during restricted and unrestricted water trough periods, respectively. In the restricted period, 62% of GPS location fixes were recorded in three hot spot areas, whereas, when access was unrestricted 72% of fixes were recorded in the hot spot areas (red areas in Figure 4.6A and B). In the stream zone, during restricted water trough access, 78% of all location fixes were recorded in the hot spot areas at the culverts 1 and 2 while when access to the trough was unrestricted 72% of location fixes were recorded in the hot spot areas (red areas in Figure 4.6C and D).


A
N
W
E
S
1
2
Non-restriction period
Gi_Bin
Cold Spot - 99% Confidence
Cold Spot - 95% Confidence
Cold Spot - 90% Confidence
Not Significant
Hot Spot - 90% Confidence
Hot Spot - 95% Confidence
Hot Spot - 99% Confidence
Stream
Trough
Paddock boundary
0
0.03
0.05
0.1 Kilometers
Eagle Technology, Land Information New Zealand, GEBCO, Community maps contributors


B
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1
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Restriction period
Gi_Bin
Cold Spot - 99% Confidence
Cold Spot - 95% Confidence
Cold Spot - 90% Confidence
Not Significant
Hot Spot - 90% Confidence
Hot Spot - 95% Confidence
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Stream
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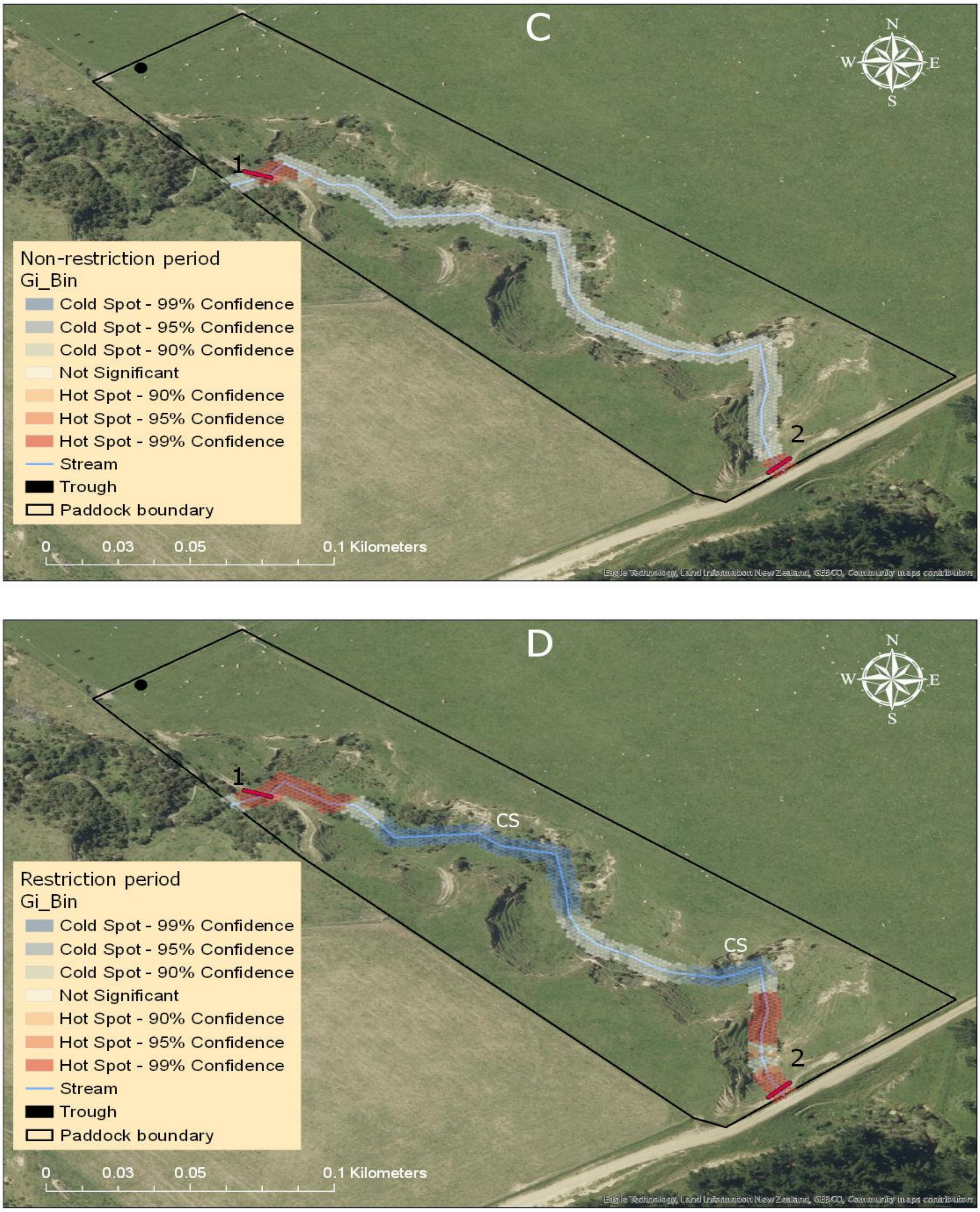

Figure 4.6: Maps showing the spatial distribution (magnitude per unit area) of ewes in the whole paddock (A & B) and stream zones (C &D; within 3m of the stream) during the period of unrestricted water-trough access (panel A & C) or water-trough restricted period (panel B & D) using optimised hot spot analysis. The blue areas represent low ewe density and red areas high ewe density. Hotspot (red areas) indicate statistically significant ($p<0.05$) spatial clusters of high values (larger positive z-score) while cold spot (CS; blue areas) indicates statistically significant ($p<0.05$) spatial clusters of low values (smaller negative z-score), and white indicates random distribution with no spatial clustering.

4.4.5 Effect of slope

During the entire study period 80% of the ewe location fixes were recorded in areas with a slope of less than 15° (Table 4.3). In the unrestricted and restricted periods, a total of 10.5% and 11.4% of location fixes were detected in areas with a slope of greater than 35°, respectively (Table 4.3). The area that each slope class contributed to the study site and the number of included location fixes that were recorded within each slope class in spring are shown in Table 3.5.

Table 4.3: The mean number (±SE) and percentage (%) of sheep GPS location fixes in each slope class (flat, undulating, rolling, strong rolling, moderately steep, steep and very steep) during the period when ewes had access to the water trough (Unrestricted) or were prevented from accessing the trough (Restricted).

		GPS location fix number (±SE)		% of GPS location fixes	
Slope class (degrees)	% of the study site	Unrestricted	Restricted	Unrestricted	Restricted
Flat (0-3°)	18.8	11229 ± 282^{c}	6233 ± 304^{b}	29.4	18.6
Undulating (4-7°)	14.9	12777 ± 382^{c}	13504 ± 355^{c}	33.5	40.3
Rolling (8-15°)	17.3	6538 ± 191^{b}	7037 ± 112^{b}	17.1	21.0
Strong rolling (16-20°)	12.1	2189 ± 173^{a}	1825 ± 144^{a}	5.7	5.4
Moderately steep (21-25°)	10.5	1435 ± 157^{a}	1093 ± 65^{a}	3.8	3.3
Steep (26-35°)	21.0	1965 ± 112^{a}	1827 ± 119^{a}	5.2	5.4
Very steep (36-75°)	5.5	2022 ± 145^{a}	2021 ± 114^{a}	5.3	6.0

Within a treatment group (column), means with different letters are significantly different ($p<0.05$).

4.4.6 Ewe distance travelled

The mean distance travelled per ewe (m/h) varied throughout the day (Figure 4.7). Peaks in distance travelled were observed at 0500, 0600, between 0900 and 1100 and between 1500 and 2000 hours. Ewes appeared to travel greater distances during the period of water trough unrestricted (74 ± 6.6 m /h) compared to the restricted period (58 ± 6.6 m/h), however, this difference was not statistically significant ($p=0.082$, Figure 4.7). Over the entire period, ewes travelled less ($p<0.05$) during the night (43 ± 6.0 m /h) than in the evening (94 ± 11.0 m /h), early morning (86 ± 11.0 m /h) or during the day (77 ± 6.7 m /h).

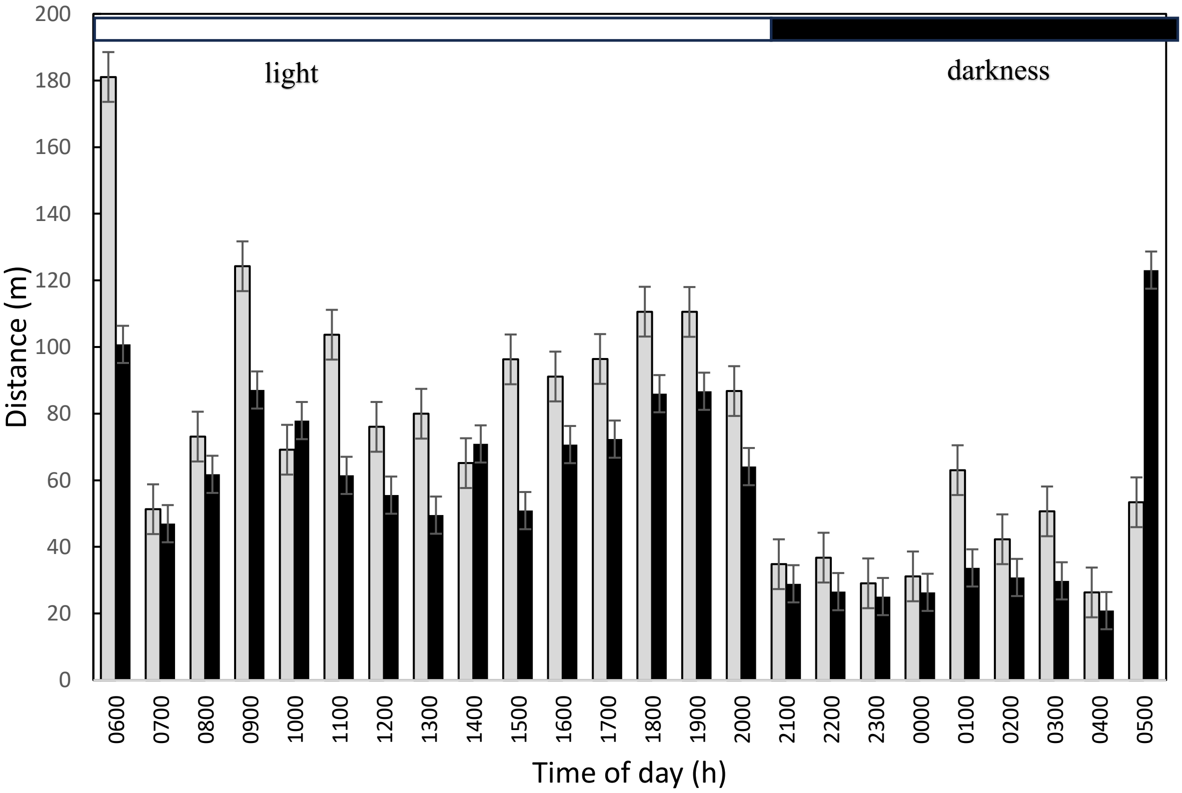

Figure 4.7: Hourly distance travelled (m) per ewe by hour of the day (mean ± SEM) during the period when access to the water trough was restricted (black bars) or unrestricted (grey bars).

In general, during the entire study period, ewe mean hourly distance travelled (m/h) reduced as slope increased (P<0.05; Table 4.4). There were, however, no differences (P>0.05) between flat and undulating slopes or between strong rolling, moderately steep and steep slopes.

Table 4.4: Mean hourly distance travelled (m) by sheep (mean ± SE) in each slope class (flat, undulating, rolling, strong rolling, moderately steep, steep and very steep) during the period when ewes had access to the water trough (Unrestricted) or were prevented from accessing the trough (Restricted).

Slope class (degrees)	Unrestricted (m/h)	Restricted (m/h)
Flat (0-3)	56.6 ± 3.90^{bc}	41.1 ± 1.90^{bc}
Undulating (4-7)	76.2 ± 7.58^{c}	50.1 ± 3.73^{c}
Rolling (8-15)	42.0 ± 5.74^{b}	27.9 ± 4.85^{b}
Strong rolling (16-20)	12.9 ± 3.20^{a}	8.9 ± 2.30^{a}
Moderately steep (21-25)	9.7 ± 2.54^{a}	7.3 ± 2.29^{a}
Steep (26-35)	16.3 ± 3.59^{a}	11.1 ± 2.88^{a}
Very steep (35-75)	40.4 ± 6.30^{b}	27.1 ± 3.37^{b}

Within a treatment group (column), means with different letters are significantly different (p<0.05).

4.4.7 Behaviour in the stream zone

Video footage showed that during the entire study period when ewes were in the stream zone, they spent 61.7% of their time grazing (n=1640), 19% stationary (n=1032), 10.2% walking (n=796) and 6.2% drinking from the stream (n=276). Ewes were also observed to sniff the water on 28 occasions (0.3%). On eight occasions ewes were observed to walk into the stream (0.07%). Ewes spent longer grazing and drinking during the water trough restricted period (p<0.05), however, the number of stationary and walking events did not differ (p>0.05) when the water trough was restricted or unrestricted (Figure 4.8).

Figure 4.8: The average duration (time in seconds ± SE) of occasions (n=1032) that ewes were observed to be stationary, grazing, walking, or drinking in the stream zone during periods of restricted access to water trough (black bars, n=390 ewes) or when access was not restricted (grey bars, n=642 ewes). Within each behavioural event, means with different letters are significantly different (p<0.05).

4.4.8 Time spent in the stream zone

Over the entire period, the time ewes spent within 3m of the stream differed by camera location ($p<0.05$). Ewes spent the most time near camera 11 (22.3 ± 1.1 min/ewe/day) and least time near camera 7 (3.2 ± 1.2 min/ewe/day, Table 4.5).

Table 4.5: The daily mean of the number of ewes (min and max in parentheses) within 3m of each camera location and the daily median and inter quartile range (IQR) in parentheses of the total duration (min) each ewe spent within 3m of each camera per day during the period when ewes had access to the water trough (Unrestricted) or were prevented from accessing the trough (Restricted).

Camera number	Ewes within 3m of each camera location			
	Restricted		Unrestricted	
	n	Total daily duration (min)	n	Total daily duration (min)
1	22 (8-30)	14.1 (12.6-22.2)	29 (8-27)	10.9 (6.9-15.6)
2	21 (10-28)	7.6 (5.1-11.7)	16 (11-20)	5.0 (4.0-7.9)
3	23 (10-29)	13.3 (10.2-15.6)	27 (9-34)	7.7 (4.9-10.7)
4	18 (8-26)	9.8 (7.1-14.0)	16 (4-24)	7.5 (4.3-11.5)
5	22 (18-22)	9.8 (8.4-15.8)	20 (5-28)	8.8 (6.8-11.3)
6	15 (4-21)	6.5 (5.3-7.1)	17 (5-25)	5.5 (4.1-7.6)
7	5 (3-6)	5.6 (2.5-7.2)	4 (3-6)	2.0 (1.8-2.4)
8	12 (4-15)	5.4 (4.0-8.1)	10 (5-20)	4.0 (2.3-5.9)
9	20 (8-27)	13.4 (7.4-17.9)	23 (16-27)	10.9 (7.8-17.0)
10	13 (3-18)	7.8 (4.6-8.7)	16 (15-19)	3.6 (3.3-4.7)
11	22 (4-30)	22.9 (13.7-26.5)	28 (22-33)	22.2 (20.3-29.8)
12	15 (3-25)	10.6 (6.9-11.3)	20 (13-25)	8.7 (4.7-11.0)
13	13 (2-22)	8.0 (6.3-12.4)	18 (11-18)	9.0 (6.1-11.14)
14	12 (3-21)	7.3 (2.7-12.1)	18 (13-22)	6.1 (3.8-7.0)

The duration that ewes were within 3m of each camera location also differed ($p<0.05$) between time-of-day classes. The median duration near any camera position was less at night (4.6 ± 0.5 min/ewe/day), than during the rest of the day ($p<0.05$; Table 4.6). Ewes spent more time ($p<0.05$) within 3m of any camera location during the restricted period (10.6 ± 0.6 min/ewe/day) than when access was unrestricted (8.3 ± 0.6 min/ewe/day: $p<0.05$).

Table 4.6: The mean total duration per day ± SEM each ewe was recorded to be within 3m of any camera location by time of day class (during the entire study

Time of the day class	n	Duration (min/ewe/day)
Night (2000 to 0559)	126	4.6 ± 0.5^{a}
Morning (0600 to 0859)	84	7.5 ± 0.6^{b}
Daylight (0900 to 1659)	70	8.1 ± 0.7^{b}
Evening (1700 to 1959)	56	8.1 ± 0.7^{b}

^{a,b,} means with different letters are significantly different ($p<0.05$).

4.5 Discussion

The aim of the current study was to examine the behaviour of sheep around a natural waterway when access to a water trough was, or was not, restricted in spring. The potential impacts of sheep on water quality were also assessed. It was hypothesised that during spring, the absence of a reticulated water source would result in greater interaction of sheep with the natural waterway due to demand of lactation but that this would not influence the loads of nutrient, sediment and pathogens in the water.

Behaviour

During the current study, video footage showed that there were more grazing and drinking events at the stream during the period of water trough restriction than when ewes were allowed access. This was supported by proximity data where ewes spent more time within 3m of each side of the stream during the period when access to the water trough was restricted than when the trough was accessible. This differed to winter where sheep spent more time within 3m of each side of the stream when access to the trough was unrestricted (Chapter 3). This was likely due greater rainfall recorded in winter (8mm) compared to spring (1.5mm). Previously, Graz *et al*., (2012) reported that the provision of water sources influenced grazing activity of sheep with most grazing focused around the water-points (Graz *et al*., 2012). Given that pasture growth rates have been reported to be 25% greater in the riparian zone than on Hill zones (Aaron *et al.,* 2013) and the high pasture moisture content of the current study, it is perhaps more likely that the ewes spent more time grazing around the waterway to access green pasture. This increase in time spent near the stream may have also then led to a greater number of drinking events.

Spatial distribution

The spatial analysis of GPS data collected during the current study showed that the ewes spent more time in the peripheral north areas of the paddock field compared to the south. This closely matched the findings of chapter 3. This spatial variation was likely due to topography of the paddock as most of the north area of the paddock (80%) had a slope less than 20 degrees. Previous research has shown that topography can affect sheep habitat use, particularly variables such as altitude, landform, slope, ruggedness, and on occasions, aspect (Mysterud *et al*., 1999; Haddon, 2008). Sheep have been shown to prefer grazing on flat to low slopes (Sheath, 1982; Haddon, 2008; Steer, 2012) which may be due a greater accumulation of herbage in these areas (López *et al*., 2003) associated with improved soil fertility (Saggar *et al*., 1990). This improved soil fertility is likely due to higher nutrient return in animal excreta resulting from greater use of the flatter areas (Saggar *et al*., 1990).

Distance travelled

The mean distance travelled per ewe (m/ewe/h) varied throughout the day with the greater distances travelled in the morning compared to the afternoon. This finding is in agreement with Bowns (1971) who reported that sheep travelled greater distances in the morning than other times of the day. In the current study, sheep travelled 1920 m/day which was less than the 5406 m/day reported by McGrannahan *et al*. (2018). This is perhaps not surprising as McGrannahan *et al*. (2018) utilised a 64 ha rangeland during summer in the United States. In comparison to winter (Chapter 3), ewes in spring moved shorter distances (1920 vs 3157 m/day). Based on previous research, the expectation was that sheep would move shorter distances in periods when there was more forage available and increased moisture content (Lalampaa *et al*., 2016). The pasture moisture content in spring was 76% which was similar to winter (77%; Chapter 3). Macfarlane *et al*. (1966) showed that when pasture contained 60 to 70% water that sheep water requirements could be met through the pasture consumed and thus, they did not need to drink free water (Macfarlane *et al*. 1966). Therefore, it is likely that in the current study ewes would be less likely to walk significant distances to find water.

Ewes in the current study were observed to travel further during the evening and early morning than during the afternoon (Figure 4.7). This finding reflects the crepuscular grazing patterns observed in sheep resulting in greater activity in the early morning and late afternoon (Arnold, 1984; McGranahan *et al.*, 2018; Filipčík *et al.*, 2020) and greater resting in the middle of the day (Venter *et al.*, 2019). In chapter 3, similar travel patterns were observed though night travel was also observed. Distance travelled has been strongly associated with time spent grazing (Loridas *et al.*, 2011). In the current study, sheep travelled shorter distances during the night than at other times. This pattern was also observed in the proximity data which showed that the mean duration near any camera was lower at night. The shorter duration spent near the cameras at night suggests that there was either less interaction near the waterway or that the ewes did not camp in the riparian areas.

Water quality

During the current study, the Nitrate-N load measured at both the inflow and outflow sites was higher during the water trough restricted period compared to the unrestricted period. During the period that the water trough was restricted there was a greater percentage of ewe GPS locations recorded in the stream zone, therefore, there may have been a greater deposition of faeces and urine in these areas. The percentage of time ewes were recorded in this area, however, was small at 0.7% and 0.3% in the restricted and unrestricted periods, respectively. In winter (Chapter 3) Nitrate-N did not differ between periods of unrestricted or restricted access to a water trough. Compared with winter (Chapter 3) spring weather conditions showed greater solar radiation (21.4 and 6.7 MJ/mÂ², respectively) and temperatures (4.8 and 13.2ºC, respectively). These greater temperatures are favourable for denitrification which can then reduce nitrate concentrations (Bremner and Shaw, 1958; Ferguson, 1987).

Nitrate-N loads in the current study were negatively correlated with flow rate ($r = 0.4$, $p < 0.05$) which is in agreement with Endut *et al.* (2009). Rain was recorded on five days during the restricted period compared to one day in the unrestricted period. Given that flowrate was increased in response to rainfall, the difference in nitrate-N load between treatments was to be expected. The mean nitrate-N concentration of 0.46mg/l measured across all sampling days in the current study was higher than the 0.01mg/l reported by (Cooper, 1990) and 0.25mg/l reported by McColl and Gibson (1979) but were within the New Zealand guidelines of less than 1 mg/L (Biggs, 2000; ANZECC, 2000). Cooper (1990) reported lower concentrations, possibly because their study was conducted over three months in spring which included a number of days without rain, whereas the current study covered a period of two weeks. The rainfall in the first week of the current study could have resulted in increased nitrate-N concentrations than was observed by Cooper (1990). Similarly, McColl and Gibson (1979) conducted their study from September to November and observed 5 days with rainfall thus resulting in less runoff to wash nitrate into the stream.

E. coli loads were higher in the outflow than inflow samples on D15 and D16 which suggests that the study paddock was the source of the bacteria. Days 15 and 16 correspond to the restricted period when there was higher interaction of sheep with the stream zone with 72% of all ewe GPS location fixes in the three stream zone hotspots which could explain higher *E. coli* loads in the outflow than inflow samples.

In the current study there were no differences in the *E. coli* loads between the restricted and unrestricted periods which was in agreement with the winter results (Chapter 3). The Ministry of Environment (2003) water quality guidelines for freshwater recreation require that the *E. coli* concentration of water ways is less than 130 most probable number (cfu)/100ml. In the current study, 92% of samples exceeded this limit. The result of the current study was similar to that of Wilcock (1999), although 4.7% of the samples in the current study still exceeded the D grade threshold of >550 cfu/100ml.

Total phosphorous loads in the current study did not differ between sampling sites but were higher during the water trough restricted period than unrestricted period. This might be expected as there were more sheep interactions with the waterway during the period of trough restriction. Interactions with waterways can be source of phosphorus as a result of streambank and riparian margin erosion and sediment loss and animal excreta (Tempero *et al.*, 2015). The concentrations of phosphorus in water samples collected in the current study (0.05 mg/l) were similar to the winter study (0.05mg/l; Chapter 3) and the 0.06mg/l reported by Caruso (2000). Higher concentrations were reported by Wilcock (1999) of 1.64mg/l and McColl and Gibson (1979) of 1.02mg/l. All four studies reported concentrations greater than the Australia and New Zealand Environment and Conservation Council guidelines of 0.035 mg/l for upland rivers (ANZECC, 2000). In the Wilcock (1999) study, stock were restricted from having direct access to the stream, however, they utilised a mixed sheep and dairy cattle farm. Cattle dairy farms often have a higher stocking rate and they bring in more supplements and use more fertiliser, so they have more nutrients cycling around the system. Being a mixed farm with cattle could probably mean more interaction with waterway and hence possibility of more faeces into the waterway. The study reported by McColl and Gibson (1979) was conducted over three months in spring with higher influence of rainfall which could explain the differences with the current study.

4.6 Conclusion

In the current study the absence of a reticulated water source in spring had no impact on the interaction of lactating ewes with the natural waterway, however, there were greater concentrations of nitrate-N and total phosphorus in water samples. In spring ewes were observed to graze and drink more frequently during the water trough restricted period than the unrestricted period. Sheep spent more time within 3m of the stream which may have been a result of greater pasture availability around the stream. The results of this spring study cannot be extrapolated to a warmer climate as pasture growth rates in summer differ greatly from spring and thus require additional study to be conducted.

5 The behaviour and impact of sheep accessing a natural waterway on water quality in summer

Simple Summary

Interaction of animals with waterways can impact water quality. The absence of reticulated water source had no effect on sheep interaction with the natural waterway in spring, however, there is a paucity of information on the interaction of sheep with natural waterways and their impact on water quality in summer. The current study, therefore, was designed to determine the behaviour of sheep on a hill country paddock during summer conditions and the impact of access to a reticulated water trough. The expectation was that during summer sheep were likely to have a greater need to drink than in other seasons due to higher dry matter content of pasture and warmer environmental conditions. Observation of behaviour showed that the spatial distribution of ewes was influenced by paddock features such as slope and culverts' location. The current study found little evidence that sheep interacted with the natural waterway and that under the current conditions of the study, sheep had little impact on the waterway.

5.1 Abstract

The behaviour of ewes in and around a natural waterway and their impact on water quality was investigated in summer. Adult ewes (n=40) were managed in a 1.7 ha paddock for one week while being offered access to a reticulated water trough followed by a second week when the trough was covered resulting in free water only being accessible from the stream. The study paddock contained a stream which was <1m wide and <30cm deep in base flow conditions with culverts at the entrance (inflow) and exit (outflow) of the paddock. A stream zone was classified as the area 3 m either side of the stream. Ewe movement within the paddock and their interaction with the waterway was monitored using GPS units, accelerometers, and video surveillance footage. The stream zone represented 9 % of the paddock area and contained 4.3 and 4.6% of the ewe GPS locations during restricted and unrestricted water trough access periods, respectively. The spatial distribution of ewes was influenced by paddock features such as slope and the location of the trough and culverts. Ewes were observed near the stream least during the night with highest activity in the daylight. The slope of the paddock influenced the distances ewes travelled with greater distances as the slope got steeper except at very steep slopes. Water quality indicators including nitrate, *Escherichia coli*, ammonium, and total

phosphorous fluctuated across the two sampling sites. Of these measures of water quality ammonium concentrations were lower and *E. coli* concentrations higher ($p<0.05$) during the water trough unrestricted period compared to the restricted period. Therefore, the current study found some evidence that sheep interacted with the natural waterway and that there was little impact on measures of water quality.

5.2 Introduction

In hot environments sheep appear to prefer to graze close to water sources and spend most of their time near watering points (Lynch, 1974, 1977). The duration that sheep spend grazing, however, depends on a number of factors including the availability of feed, hours of daylight and flock size (Lynch *et al*., 1992; Penning *et al*., 1993). Sheep have been reported to spend approximately eight to nine hours per day grazing which can increase to up to 13 hours per day if feed supply is limited (Lynch *et al*., 1992).

In New Zealand during summer, pasture can contain up to 50% moisture compared to 80% in spring (Brown and Lynch, 1972; De Ruiter *et al*., 2007; Woodward *et al*., 2013). The moisture content of pasture can directly influence how much sheep drink (Macfarlane *et al*., 1958; Macfarlane *et al*., 1966; Forbes, 1968). The dry matter intake of sheep is positively related to water consumption (Calder *et al*. 1964; Jaber *et al*., 2004). In New Zealand, therefore, during summer sheep will likely have a greater need to drink than in other seasons due to higher dry matter content of pasture and warmer environmental conditions. Sheep can access additional water from reticulated water troughs or natural sources such as streams. Thus, in summer sheep may have a greater motivation to access natural waterways to drink than in other seasons.

Farming activities in New Zealand can affect water quality due to contamination of waterways with phosphorus, sediment, nitrogen, and faecal matter (Abell *et al*. 2011, Gillingham and Thorrold 2000, Ledgard *et al*. 1996, Monaghan *et al*. 2005, Scholefield *et al*. 1993). In particular, cattle can have a significant impact on water quality of natural waterways (Cournane *et al*., 2011; O'Callaghan *et al*., 2019). Cattle crossing waterways have been reported to cause water contamination due to the deposition of urination and faeces directly into the water, and the displacement of sediment due to pugging damage (Davies-Colley *et al*., 2004). Bagshaw (2002), reported that beef cattle spent up to 6.7% of their day in or within 2 m of a stream, and deposited 4% of their faeces in these areas which then impacted on water quality. There is, however, sparse information on the behaviour of sheep around natural waterways and their impact on water quality, although small impacts on soil physical properties (e.g., soil macroporosity, bulk density, air permeability) have been reported (Platts, 1981; Drewry, 2000).

Previously, in winter (Chapter 3) and spring (Chapter 4) access to a water trough was found to have no effect on the proportion of time that ewes were observed to graze, walk, rest, or drink within 3m of stream. In spring, ewes were observed to spend more time grazing and drinking when the water trough was restricted compared with unrestricted. In both winter and spring, ewes showed minimal interaction with the stream which was partly explained by the high moisture content of pasture (77% and 76 % during winter and spring, respectively). In winter, during the period of restricted access to the trough there were higher concentrations of suspended sediment and P in the stream but no difference in N and *E. coli* compared to when the trough was accessible. In spring, nitrate-N concentrations and total phosphorus (TP) were higher between the water trough restricted periods. There is currently no data available to determine if the same trends occur during summer weather conditions.

The aim of the current study was to examine ewe behaviour and interaction with a natural waterway during summer to determine any potential impacts on water quality. In addition, the impact of providing access to a reticulated water trough on sheep behaviour was also investigated. It was hypothesised that the absence of reticulated water source would result in greater sheep interaction with the natural waterway which would negatively influence the level of nutrients and pathogens in the waterway.

5.3 Materials and methods

Procedures in this study were carried out with the approval of the Massey University Animal Ethics Committee (MUAEC 19/62). The study was conducted over two weeks from the 15th of February (D1) to the 28th of February 2020 (D14; Table 5.1) at Massey University's Tuapaka farm, located approximately 15 km north-east of Palmerston North, New Zealand (40.3345° S, 175.7390° E.). The site was the same as that used in Chapters 3 & 4 and the methodologies used were consistent, therefore, an abbreviated methodology will be outlined in the following sections.

5.3.1 Animals and study design

Adult ewes (n=40) were managed in a 1.7 ha paddock (Figure 5.1). Ewes had an average weight of 64 ± 8.8 kg and body condition score of 2.9 ± 0.6 (Mean ± SD). Briefly, a crossover study was conducted whereby ewes were grazed in a single paddock for one week while being offered reticulated water from a water trough followed by a second week when the trough was covered, resulting in the stream being the only free water access within the paddock. During the study period, the same ewes were used as in Chapter 3 and there were no sheep in adjacent paddocks. Ewe movement within the paddock and their interaction with the waterway in summer was monitored using GPS, accelerometer and video surveillance footage, and their impact on water quality was measured.

The paddock contained a discrete natural stream (Figure 5.1) which was 233m in length, <1 m wide and <30 cm deep with an average flow rate of 0.39 l/s. The water catchment (watershed 2) area supplying the study paddock calculated by ArcGIS Pro to be 4.1 ha in size (Chapter 3 Figure 3.2). The catchment area was grazed by sheep only. Throughout the study period, ewes were continuously stocked on a predominantly perennial ryegrass pasture (*Lolium perenne*) with a pasture mass of 1766 ±137 kgDM/ha (Mean ± SD) and an average moisture content of 56 % (SD=8.6%).

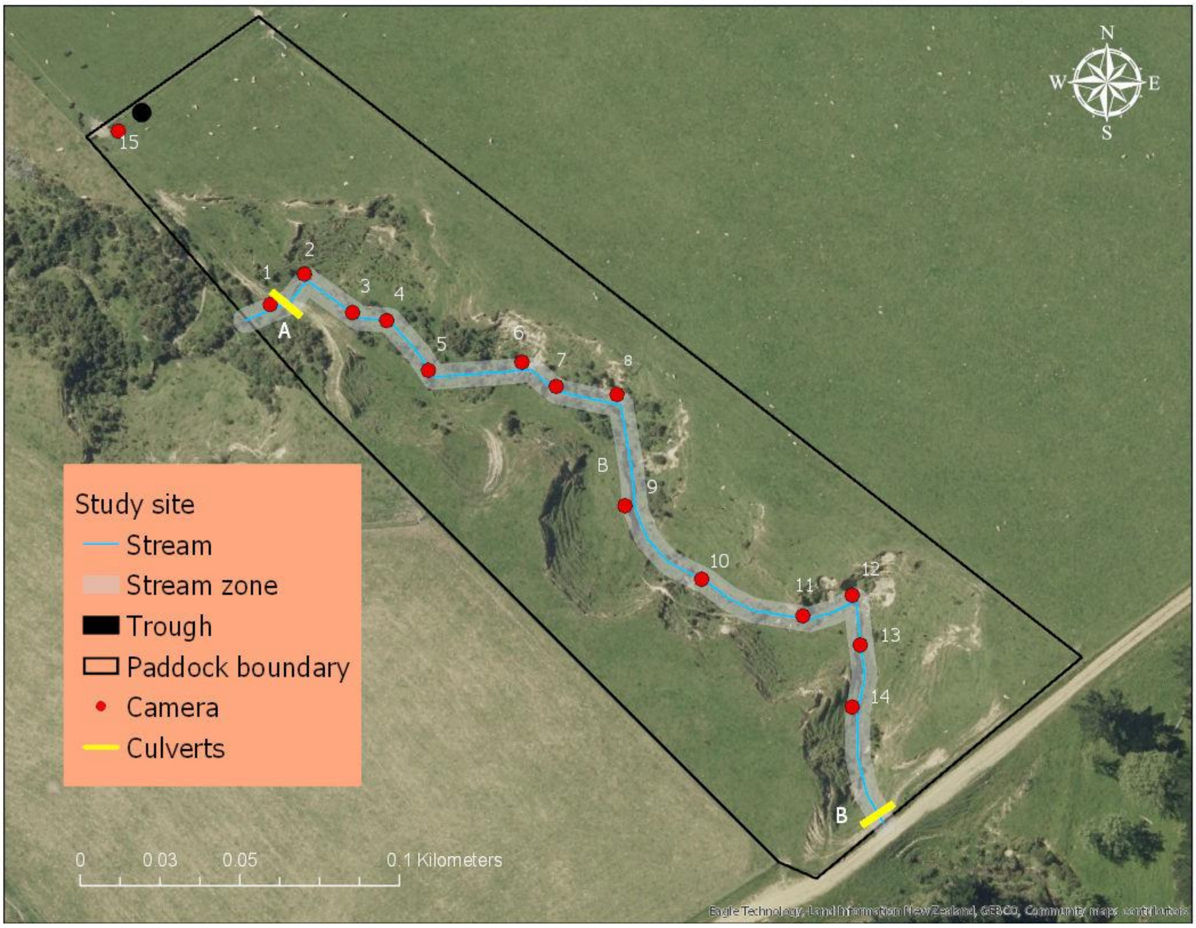

Figure 5.1: Map of the study paddock showing the paddock boundary (black line), stream (blue line), position of the trough (black dot), culverts (yellow bars (letters A & B)), cameras (red dots) and the stream zone (3m). A and B indicate the outflow and inflow water sampling sites, respectively.

5.3.1.1 Sheep behavioural observations

Ewe behaviour was recorded for two weeks from D1 to D14 (Table 5.1). Fifteen, motion activated video surveillance cameras ((Moultrie® model MCG-13297, Birmingham, AL, USA (n=7), TechView® model QC8027, Kaki Bukit, Singapore (n=4) and Bushnell © model 119736, Overland Park, KS, USA (n=4)) were used to record ewe movement and behaviour around the waterway in summer 24 hours a day. Cameras were placed at intervals of 14 to 18m along the stream and could recorded footage clearly up to a distance of 15 m (Figure 5.1). An additional camera was placed approximately 7 m from the reticulated water trough. Camera setup and recording was as described in Chapter 3. Ewes were spray marked with coloured numbers on their both sides and fitted with a plastic collar labelled with a unique number. Behaviours were coded from video recordings using the ethogram described in Chapter 3 using BORIS software [version 7.8.2; Friard and Gamba (2016)] as per Chapter 3. The behaviours recorded included grazing, walking, stationary, drinking, sniffing, walking in the stream and out of view.

Table 5.1: Summer study plan showing dates with their corresponding study days and water sampling days.

Date	15-Feb	16-Feb	17- Feb	18- Feb	19- Feb	20- Feb	21- Feb	22- Feb	23- Feb	24- Feb	25- Feb	26- Feb	27- Feb	28- Feb
Study day	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14
Water sampling days					√	√						√	√	
Pasture mass and moisture content	√							√					√	

5.3.2 GPS and accelerometers

All ewes (n=40) were fitted with a collar to which was attached a triaxial accelerometer (wGT3X-BT Actigraph, FL, USA) and a GPS unit (Custom build units, DataCarter). Triaxial accelerometers and GPS units were attached for fourteen days from D1 to D14. GPS programming and specification was as described in Chapter 3. Sheep locations were determined from data recorded from more than four satellites. Distance travelled and time spent by sheep in the paddock were calculated from GPS and accelerometer data, respectively. Time of day was classified as either early morning (0600 to 0859), day (0900 to 1659), evening (1700 to 1959) or night (2000 to 0559) as per Chapter 3.

5.3.3 Ewe measures

At D1 and D14, all ewes were weighed (Tru-Test weigh scale, Auckland NZ) and body condition scored by an experienced technician [scale 1-5; Jefferies (1961)]. All ewes were weighed within an hour of being removed from the pasture.

5.3.4 Pasture measures

Pasture sample collection and determination of dry matter yields and moisture content was as described in Chapter 3. The moisture content was determined on D1, D8 and D13 (Table 5.1). Six grab pasture samples of approximately 50g were collected by hand plucking to simulate ewe grazing. Samples were oven-dried at 80ºC for 48 hrs to determine dry matter yields and moisture content. Pasture mass was estimated on D1, D8 and D13 using a manual folding plate meter (Jenquip, NZ).

5.3.5 Weather data

Hourly and daily data were downloaded from Tuapaka weather station 2 which included rainfall (mm), relative humidity (%), air temperature (ºC), solar radiation (MJ/mÂ^2) and wind speed (m/s). The weather station was located 800m from the study site and at the same altitude.

5.3.6 Water flow rate measurements

Stream water flow was measured at the exit of two culverts located at the entry (inflow) and the exit (outflow) of the paddock as described in Chapter 3 (Figure 3.1). Stream water flow rate was measured hourly from 0800 to 1500hrs on D5, D6, D12 and D13. Flow rate was manually calibrated using a 30-litre flexible bucket, stopwatch and a graduated jug as per bucket and stopwatch method outlined in Chapter 3 using the method described by Ghani and Saudi (2017).

5.3.6.1 Water sample collection

On D5, D6, D12 and D13 (Table 5.1), water samples were collected hourly from 0800 to 1500hrs from both the inflow and outflow culverts to determine the concentration of suspended sediments, N, P, $NH^+{}_4$, and *E. coli.* Sampling procedures and handling was described in Chapter 3.

5.3.7 Water quality analyses

All water sample analyses were undertaken using the same procedures and equipment as outlined in Chapter 3. Samples to be analysed for nitrate-N and ammonium were sub-sampled and filtered for subsequent analysis (Din, 1996). A second unfiltered sub-sample was frozen for subsequent total N and P analysis. The remaining water sample was stored at 4^{o}C for suspended sediment analysis. Loads (mg/s) in inflow and outflow were calculated as in Chapter 3.

5.3.7.1 Nitrate, ammonium and total phosphorus

The colorimetric autoanalyzer method was used to determine nitrate-N and ammonium concentration of filtered water samples (Blakemore *et al.,* 1987) using an autoanalyzer (Pulse international ltd, Saskatoon, Sask. Canada). Total phosphorus concentrations were determined by Hill laboratory (Hamilton, NZ). Samples were measured using the total P digestion and the automated ascorbic acid colorimetry method (APHA 4500-PH method) and analysed via flow injection analysis (Baird, 2017). More details were described in Chapter 3.

5.3.7.2 Suspended sediment and E. coli

The concentration of suspended sediment was determined using the gravimetric analysis method following the standard procedure from American Public Health Association by Baird (2017). Briefly, sediment concentrations were calculated from the difference between the weight of the filter paper before and after filtering of water samples. Unfortunately, due to laboratory errors the concentration of sediment was not available for this study.

E. coli concentrations were determined by Eurofins laboratory (Wellington, NZ). Samples were analysed within 24 hours of collection using a membrane filtration procedure (Standard Method APHA 9222G; Baird, 2017). *E. coli* concentrations were reported as colony forming units (cfu) per 100mL of sample as described in Chapter 3.

5.3.8 Statistical analysis

GPS data were analysed using ArcGIS mapping tools (ArcGIS Pro 2.2.4, 2018). An optimized hot spot analysis (z-score) was conducted using ArcGIS to identify statistically significant spatial clustering of ewe GPS location fixes. Definition of hotspot, cold spot and stream zone are as described in chapter 3.

Distance travelled by sheep data was cleaned and processed using in-house macros in Microsoft Excel as in Chapter 3. Prior to analysis, ewe behaviour and water quality data were checked for normality using Kolmogorov–Smirnov and Shapiro-wilk test, and the homogeneity of variances using and Levene's test, and Tukey transformation (Tukey's Ladder of Powers transformation) where appropriate (Gotelli, and Ellison, 2004). Behavioural data including grazing, drinking, and walking analyses were performed using R 3.6.0 (2019-04-26; R Core Team (2019)). The impact of water trough restriction on ewe behaviour (e.g., % grazing, drinking) was analysed using a 2-factor analysis of variance (ANOVA). A linear regression was also used to determine if any ewe behaviours were associated with time of the day or environmental temperature. To assess whether the sheep behaviour during water restriction were related to their behaviour in the non-restricted period, Spearman's rank correlation coefficients with a two-tailed level of significance ($p < 0.05$).

Concentrations of *E. coli*, Nitrate-N, Ammonium and TP were analysed using parametric and TP using non-parametric methods in R 3.6.0 as shown in Chapter 3. The loads were calculated as in Chapter 3 (equation 5).

5.4 Results

5.4.1 Weather

Weather data were recorded from three days prior to the start of the study (D-1 to D-3) to the completion of the study period (D14 Figure 5.2). Rainfall was recorded on 4 days of the two-week study period, with volumes ranging from 0.6 to 13.8 mm/day. Maximum daily temperatures ranged from 17.4 to 26.6 °C, and the minimum temperature from 10 to 18.6 °C. Relative humidity ranged from 63.9% to 87.9%.

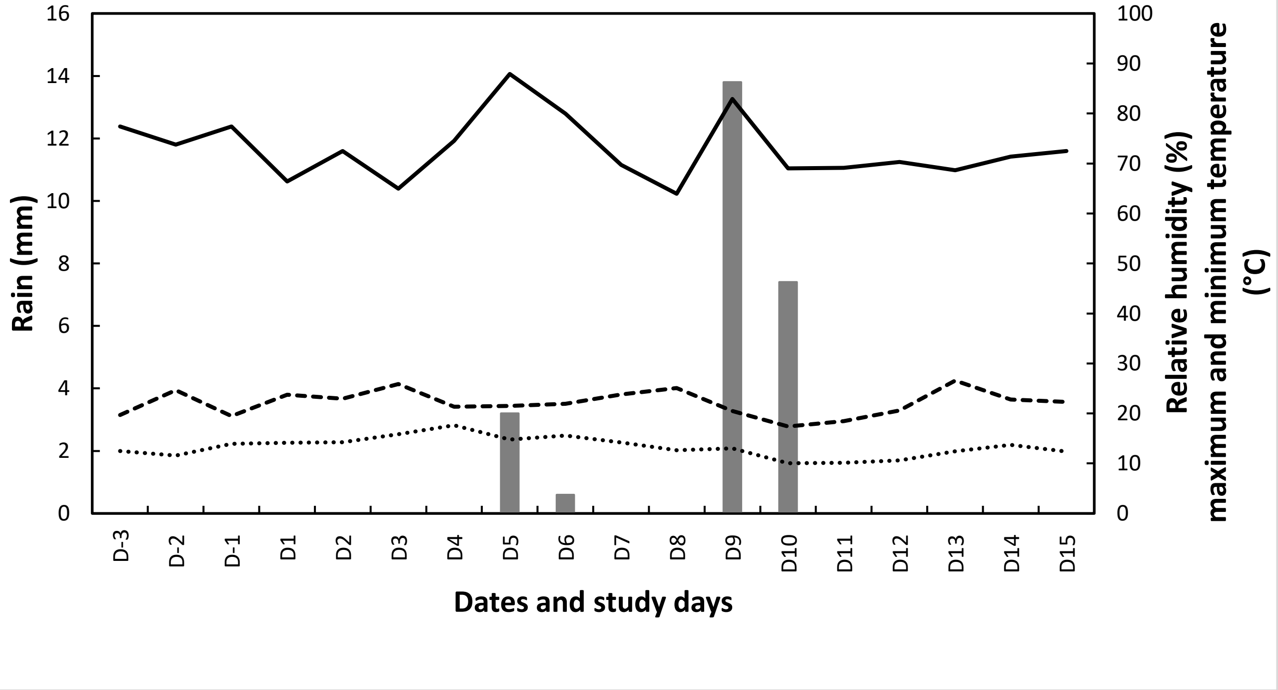

Figure 5.2: Daily mean rainfall (mm, bar), relative humidity (%,” ____”), minimum temperature (ºC,” ……”) and maximum temperature (ºC, ”------”) during the study period. D-3 to D15 indicate number of days relative to the start of the study (D1;15 Feb 2020).

5.4.2 Stream flowrate

The stream flowrate at both the inflow and outflow monitoring sites fluctuated over time (D5, D6, D12 & D13) and ranged from 0.05 to 1.07 l/s (Figure 5.3). It was calculated that it took an average of 5 min for water to flow the length of the waterway. Flowrate was higher in unrestricted water access (5.78, 5.10 - 6.13 l/s) than restricted periods (2.24, 2.14 - 2.38 l/s; $p<0.05$; Table 5.2). Flowrate was positively correlated with both rainfall ($r=0.5$, $p< 0.05$) and daily average humidity ($r = 0.1$, $p< 0.05$) and negatively correlated with wind speed ($r = 0.1$,

$p < 0.05$). At D5, D12 and D13 flow rates were greater at the inflow than outflow monitoring sites while the opposite was seen at D6 ($p<0.05$. Figure 5.3).

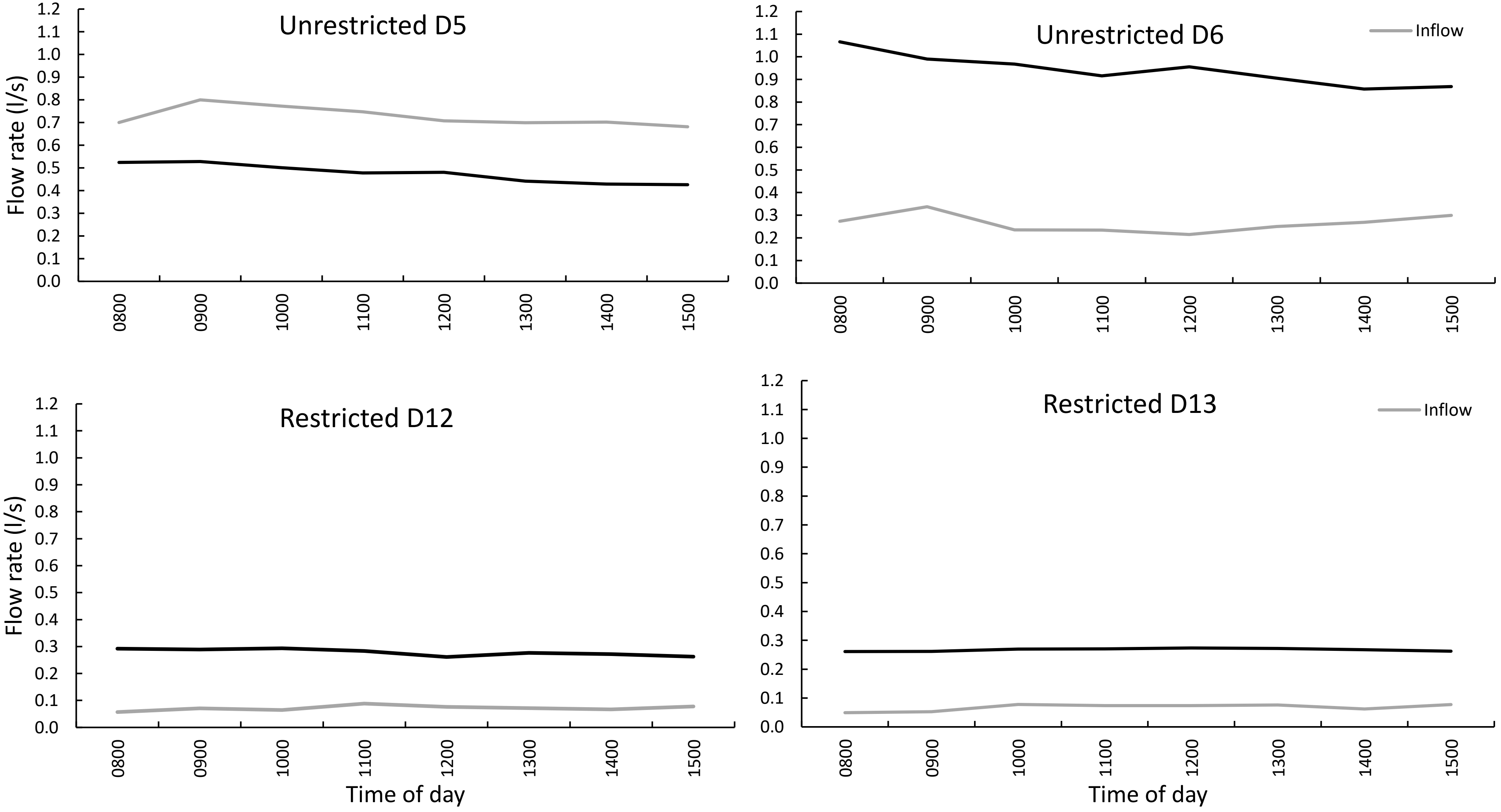

Figure 5.3: Hourly mean stream flowrate (l/s) at the inflow (grey line) and outflow (black line) monitoring sites by time of day (0800 to 1500hr) during the period of unrestricted access to water trough on D5 and D6 (upper panels) and the period of restricted access on D12 and D13 (lower panels).

5.4.3 Water quality

Water analyses showed that ammonium concentrations were higher ($p<0.05$) during the water trough restricted period compared to unrestricted period (Table 5.2). Ammonium loads were higher in the outflow than inflow on D6, D12, and D13 ($p<0.05$, Figure 5.8D)

E. coli concentrations, however, were higher ($p<0.05$) during the water trough unrestricted period compared to the water trough restricted period. The load of *E. coli* in outflow water samples were higher than inflow samples on D5, D12 and D13 ($p<0.05$, Figure 5.8B). The outflow *E. coli* load was significantly higher than the inflow load ($p \leq 0.05$) during both the water trough access period and the water-restricted period.

There was, however, no difference in Nitrate-N and TP concentrations between the treatments ($p>0.05$). Nitrate-N and TP loads were higher in inflow than outflow at D5 ($p<0.05$, Figure 5.8A and 5.8C). TP load loads were higher in the outflow than inflow on D6, D12, and D13 ($p<0.05$, Figure 5.8C)

Nitrate-N, *E. coli*, ammonium-N, and TP did not vary across the hours of the day ($p>0.05$, Figures 5.4-5.7) on any of the study days.

Table 5.2: Arithmetic mean (±SEM) of concentration of nitrate-N (mg/l), and the median (Interquartile range) of *E. coli* (cfu/100ml), total P (mg/l), suspended sediment (mg/l) ammonium-N (mg/l) and flowrate (l/s) during periods when ewes were restricted from drinking from the water trough (restricted) or had access (unrestricted)

Parameter	N	Treatment	Median (IQR)	P-value
Nitrate-N (mg/l)	32	Restricted	0.06 (0.05-0.08)	0.06
	32	Unrestricted	0.10 (0.09-0.18)	
Ammonium-N (mg/l)	32	Restricted	0.63 (0.59-0.65)	0.013
	32	Unrestricted	0.49 (0.41-0.55)	
Total phosphorus (mg/l)	32	Restricted	0.30 (0.03-0.07)	0.746
	32	Unrestricted	0.30 (0.04-0.05)	
E. coli (cfu/100ml)	32	Restricted	190 (239-802)	0.005
	32	Unrestricted	360 (382-987)	
Flowrate (l/s)	32	Restricted	0.18 (0.13-0.21)	0.001
	32	Unrestricted	0.61 (0.51-0.70)	

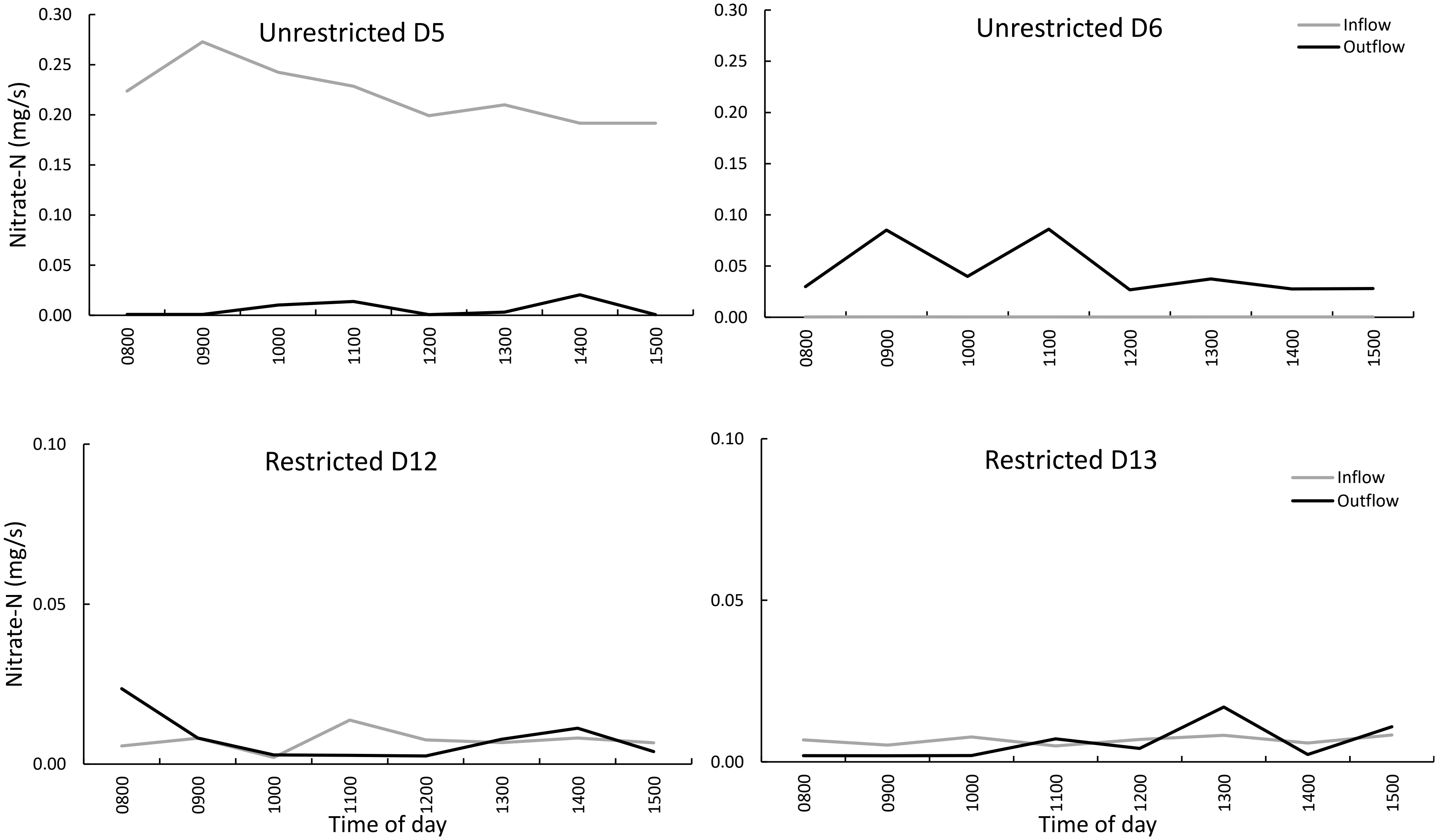

Figure 5.4: Hourly nitrate-N loads (mg/s) at the inflow (grey line) and outflow (black line) monitoring sites by time of day (0800 to 1500hrs) during the period of unrestricted access to water trough on D5 and D6 (upper panels) and the period of restricted access on D12 and D13 (lower panels).

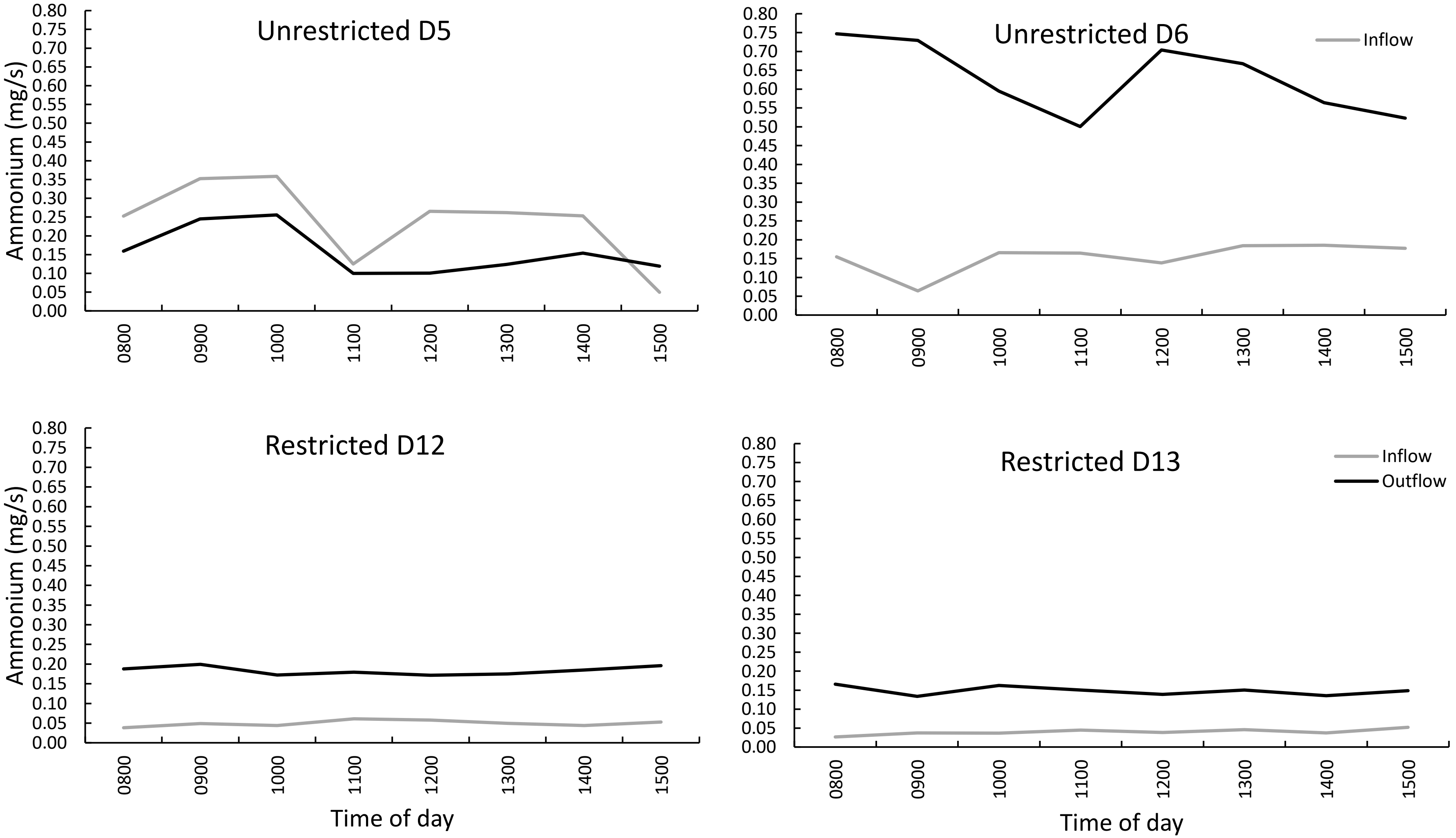

Figure 5.5: Hourly ammonium loads (cfu/s x 103) at the inflow (grey line) and outflow (black line) monitoring sites by time of day (0800 to 1500 hrs) during the period of unrestricted access to water trough on D5 and D6 (upper panels) and the period of restricted access on D12 and D13 (lower panels)

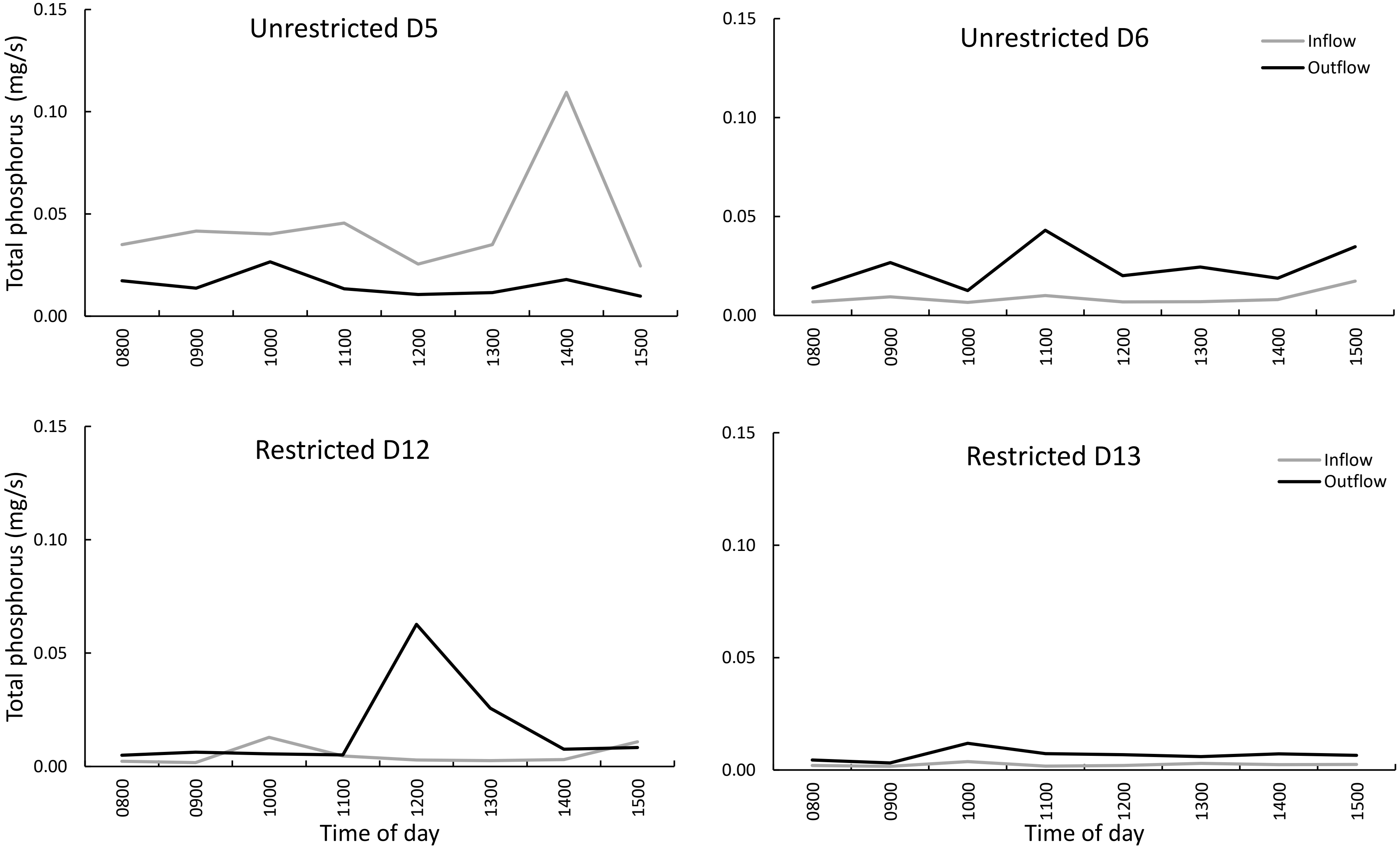

Figure 5.6: Hourly total phosphorus loads (cfu/s x 103) at the inflow (grey line) and outflow (black line) monitoring sites by time of day (0800 to 1500 hrs) during the period of unrestricted access to water trough on D5 and D6 (upper panels) and the period of restricted access on D12 and D13 (lower panels).

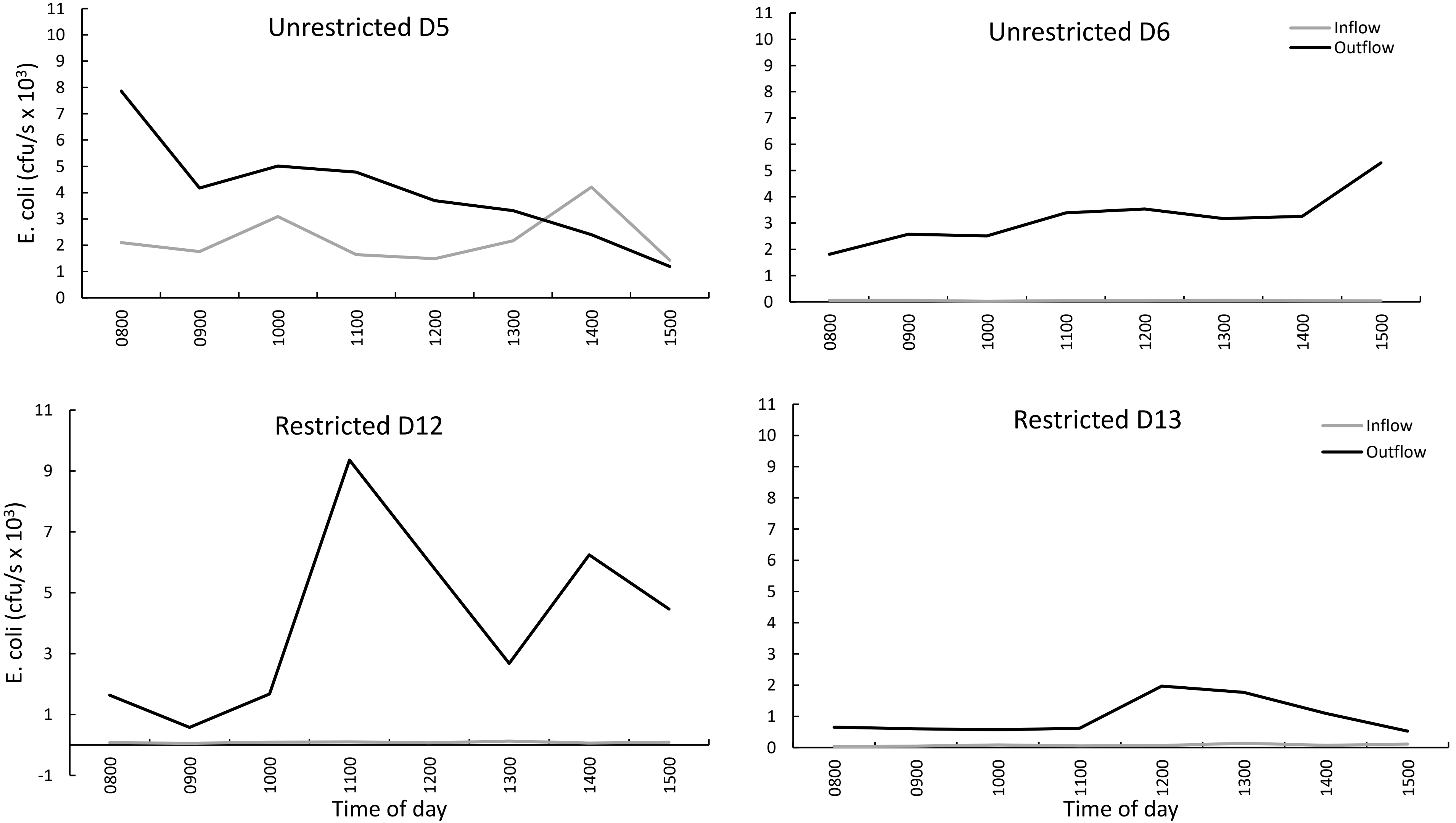

Figure 5.7: Hourly *E. coli* loads (cfu/s x 103) at the inflow (grey line) and outflow (black line) monitoring sites by time of day (0800 to 1500 hrs) during the period of unrestricted access to water trough on D5 and D6 upper panels) and the period of restricted access on D25 and D13 (lower panels).

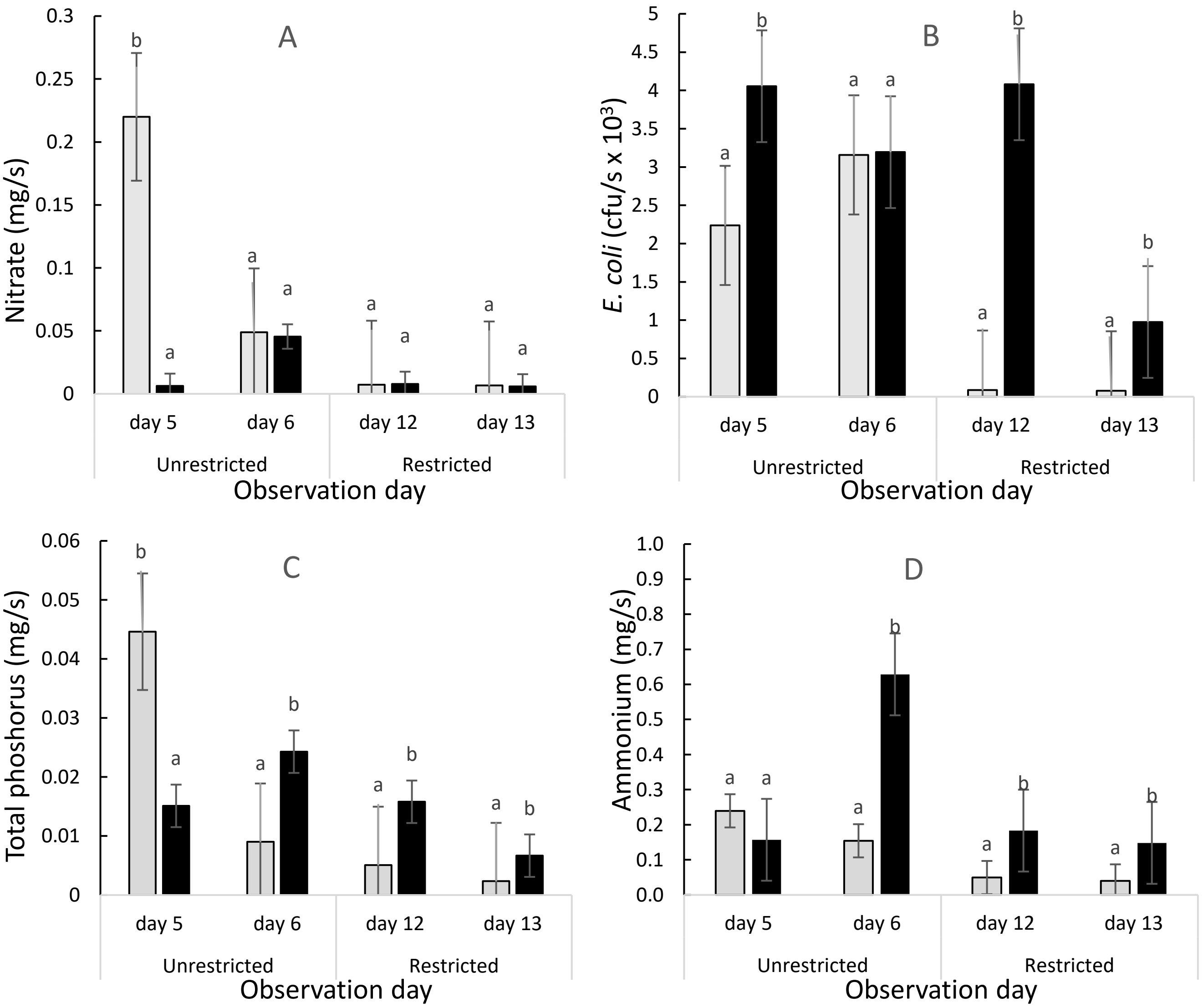

Figure 5.8: Mean (± SEM) nitrate-N (mg/s), *E. coli* (cfu/s x 10^6), total phosphorus (mg/s) and ammonium-N loads (mg/s) measured in water samples collected at the inflow (grey bars) and outflow (black bars) sampling sites on study D5 and 6 (unrestricted access to trough) and D12 and 13 (restricted access to trough). Within each day, bars with different letters were significantly different (p<0.05).

5.4.4 Animal density and spatial distribution

An optimized hot spot analysis identified statistically significant spatial clustering of ewe GPS location fixes during the period when ewes had restricted access to the water trough. During the period that access to the water trough was restricted, there was one statistically significant hot spot ($p<0.05$) was identified in the eastern area of the paddock which contained 46% of all ewe GPS fixes (Figure 5.9a panel A), whereas when the trough was unrestricted, there were two hot spots areas in the northern and eastern areas which contained 61% of location fixes (Figure 5.9a panel B). The northern hotspot detected in the unrestricted period was closer to the water trough than seen in the restricted period. Cold spots in both treatment periods were identified in the mid-portion of the paddock and along the stream zone (Figure 5.9a panels A and B).

Of the ewe GPS locations identified in the study paddock, the stream zone contained 4.3 and 4.6% during restricted and unrestricted water trough access periods, respectively. When the hotspot analysis was conducted for the stream zone in isolation, there were seven statistically significant hot spots ($p<0.05$; Figure 5.9b panel C) identified during the period of restricted water trough access which contained 40% of all the GPS fixes within the stream zone. Six significant hot spots ($p<0.05$; Figure 5.9b panel D) were identified during the unrestricted period which contained 42% of all ewe fixes. A similar pattern of cold spots identified in the stream zones in both treatment periods, although there were fewer cold spots than hot spots.

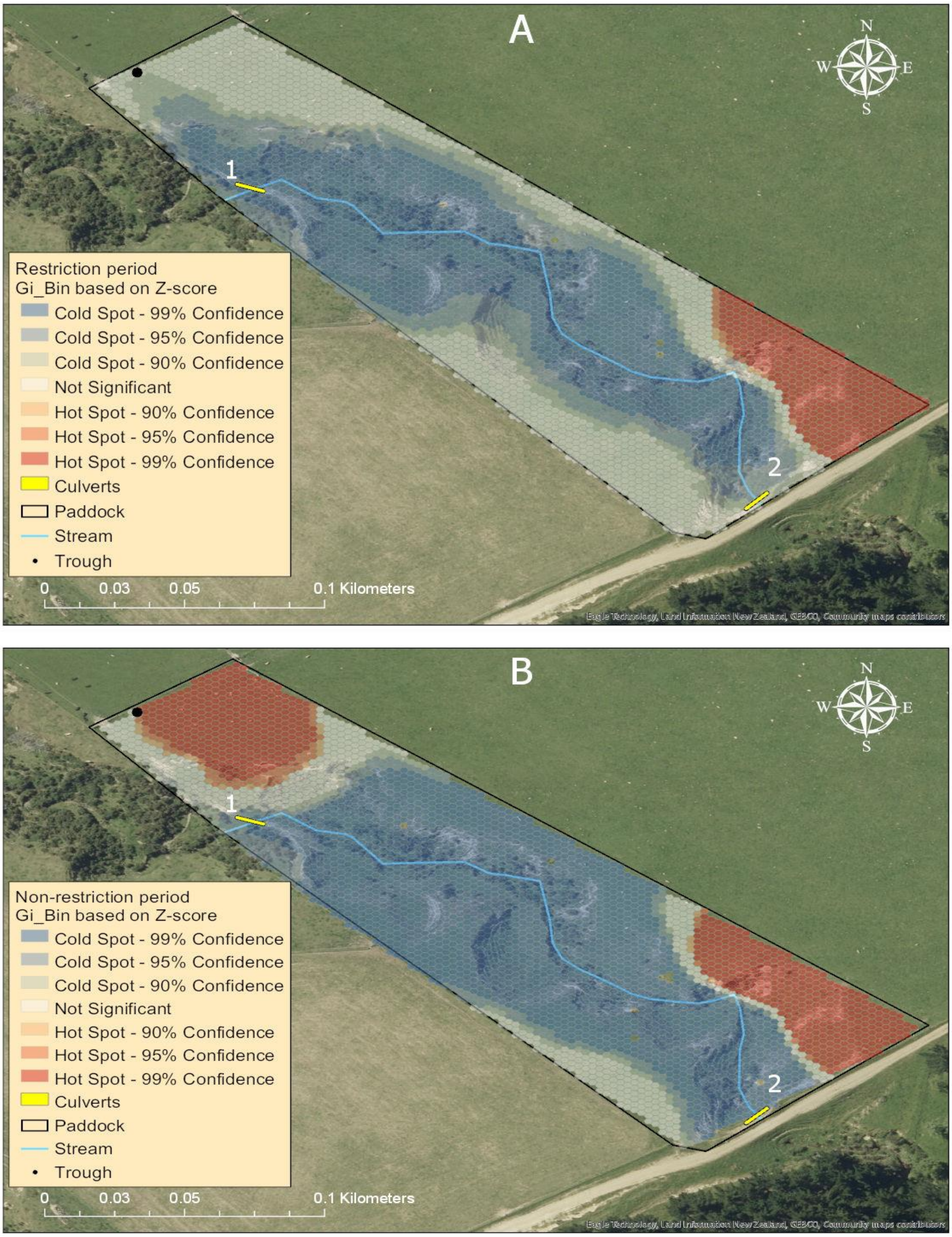

Figure 5.9a: Maps showing the spatial distribution (magnitude per unit area) of ewes in the study paddock period of restricted water-trough access (panel A) or water-trough unrestricted period (panel B) using optimised hot spot analysis. The blue areas represent low ewe density and red areas high ewe density. Hotspot (red areas) indicate statistically significant ($p<0.05$) spatial clusters of high values (larger positive z-score) while cold spot (blue areas) indicates statistically significant ($p<0.05$) spatial clusters of low values (smaller negative z-score), and white indicates random distribution with no spatial clustering.1 and 2 indicates the outflow and inflow water sampling sites respectively.

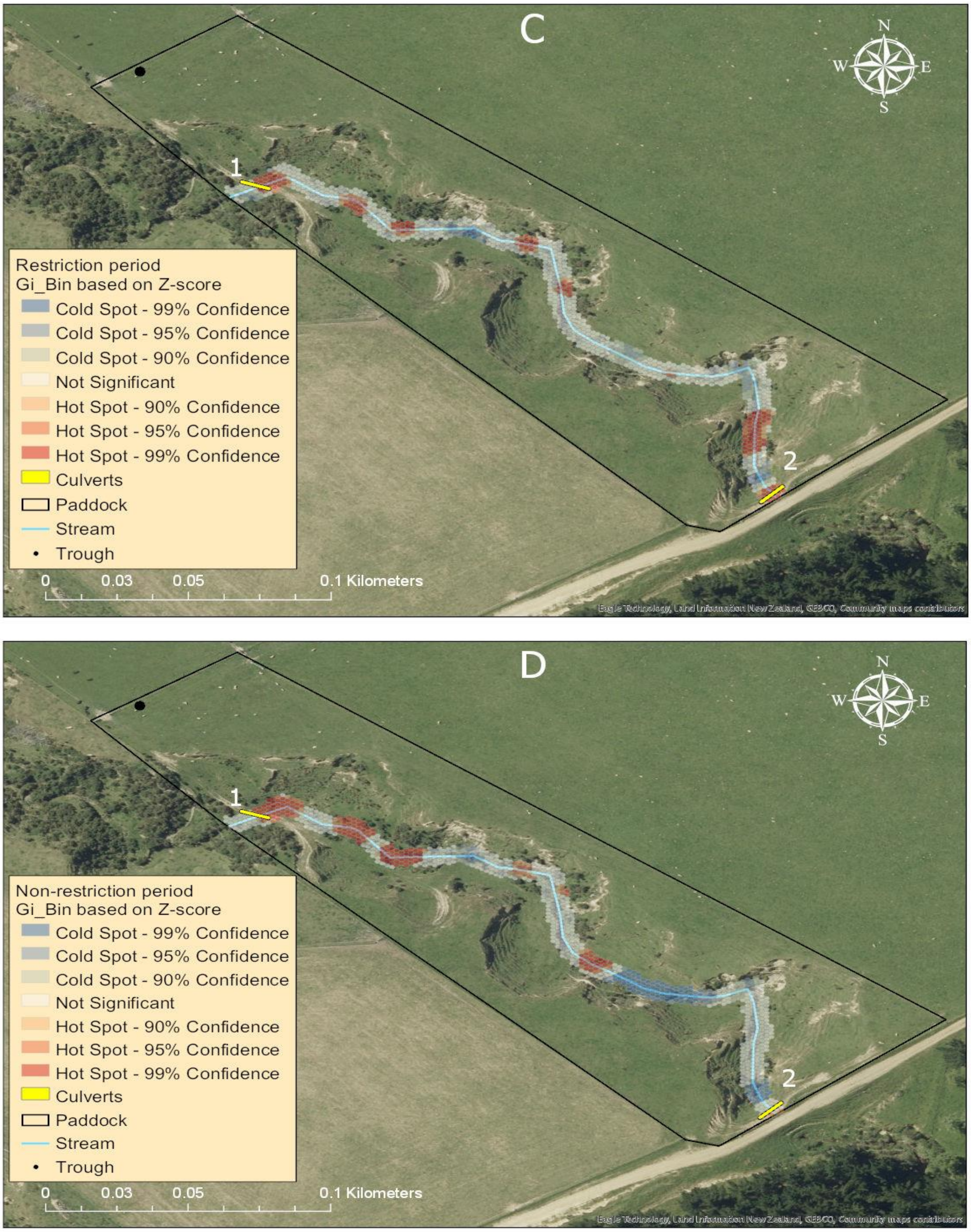

Figure 5.10: Maps showing the spatial distribution (magnitude per unit area) of ewes in the stream zone (within 3m of the stream) during the period of restricted water-trough access (panel C) or water-trough unrestricted period (panel D) using optimised hot spot analysis. The blue areas represent low ewe density and red areas high ewe density. Hotspot (red areas) indicate statistically significant ($p<0.05$) spatial clusters of high values (larger positive z-score) while cold spot (blue areas) indicates statistically significant ($p<0.05$) spatial clusters of low values (smaller negative z-score), and white indicates random distribution with no spatial clustering.1 and 2 indicates the outflow and inflow water sampling sites respectively.

5.1.1.1 Effect of slope

During the entire study period 32% of sheep locations were recorded in areas with a slope of less than 15° (Flat to rolling; Table 5.3). The majority of GPS location fixes were recorded in areas with a slope between 16° and 35° (strong rolling to steep; 65.8% in the water trough restricted period and 65.9% in the unrestricted period, Table 5.3). Few GPS fixes were detected in very steep areas with a slope greater than 35° (in both water trough restricted and unrestricted period). The area that each slope class contributed to the study site and the number of included location fixes that were recorded within each slope class in summer are shown in Table 3.5.

Table 5.3: The mean number (±SE) and percentage (%) of sheep GPS location fixes in the respective slope class during the period when ewes had access to the water trough (Unrestricted) or were prevented from accessing the trough (Restricted).

		GPS location fix number (±SE)			
		n		%	
Slope class (degrees)	% of the study site	Unrestricted	Restricted	Unrestricted	Restricted
Flat (0-3)	18.8	1438 ± 11^{ab}	1786 ± 13^{ab}	4.3	4.2
Undulating (4-7)	14.9	3295 ± 27^{abc}	4282 ± 34^{abc}	9.8	10.1
Rolling (8-15)	17.3	6121 ± 42^{bcd}	77543 ± 52^{bcd}	18.2	17.9
Strong rolling (16-20)	12.1	6545 ± 48^{cd}	8159 ± 58^{cd}	19.5	19.3
Moderately steep (21-25)	10.5	6993 ± 51^{cd}	8715 ± 62^{cd}	20.8	20.7
Steep (26-35)	21.0	8576 ± 67^{d}	10880 ± 80^{d}	25.6	25.8
Very steep (36-75)	5.5	594 ± 6^{a}	831 ± 8^{a}	1.8	2.0

Within a treatment group, means with different letters are significantly different ($p<0.05$).

5.1.2 Distance travelled

The mean distance travelled per ewe per hour (m/h) varied throughout the day (Figure 5.10). Peaks in distance travelled were observed at 0800, 1000, and between 1700 and 2000 hours. Ewes travelled negligible distances in the late evening (2100 to 2300) and early morning hours (0300 to 0500). Over the entire study period ewes travelled less ($p<0.05$) during the night (5 ± 2.7 m/h) than either evening (20 ± 2.5 m/h), the early morning (17 ± 10.3 m /h) or day (9 ± 3.7 m/h). Furthermore, ewes travelled greater distances ($p<0.05$) during the evening than during the day. No differences ($p>0.05$) were observed in the distance travelled between morning and day or morning and evening. Ewes tended to travel greater distances ($p=0.72$) in the water trough restricted period (10.4 ± 3.7m/h) compared to the unrestricted period (9.3 ± 2.4m/h; Figure 5.10).

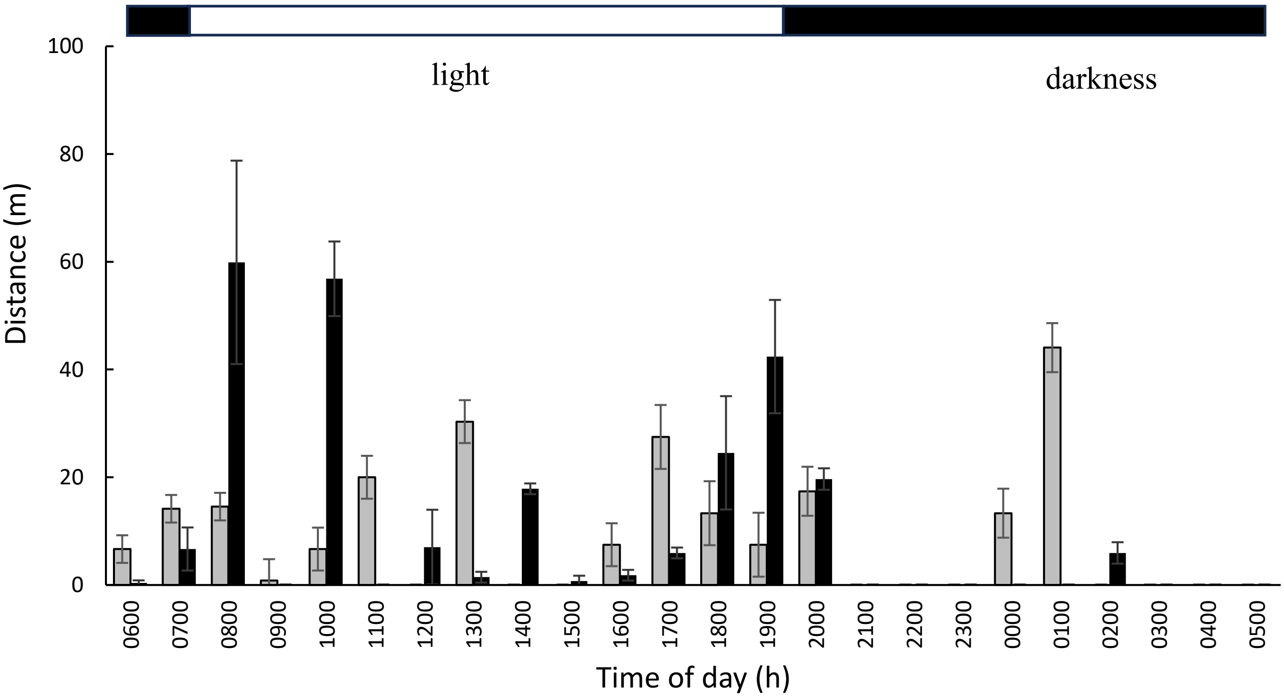

Figure 5.11: Mean hourly distance (meters) travelled per ewe per hour (mean ± SEM) during the period when access to the water trough was restricted (black bars) or Unrestricted (grey bars). Distance travelled per sheep was classified as early morning (0600 to 0859), day (0900 to 1659), evening (1700 to 1959) and night (2000 to 0559).

Paddock slope classes influenced ewe hourly distance travelled (m/h) with greater distances travelled as the slope increased up to steep slopes with a dramatic decrease in very steep slopes. The hourly distance travelled by sheep in each slope class did not differ between the treatment periods ($p>0.05$; Table 5.4).

Table 5.4: Hourly distance (meters) travelled by sheep (mean ± SE) in each slope class during the period when ewes had access to the water trough (Unrestricted) or were prevented from accessing the trough (Restricted).

Slope class (degrees)	n	Distance travelled (m/h)	
		Unrestricted	Restricted
Flat (0-3)	3086	5.7 ± 1.24^{a}	7.3 ± 1.78^{ab}
Undulating (4-7)	3281	13.2 ± 3.10^{b}	18.0 ± 4.93^{bc}
Rolling (8-15)	1945	26.3 ± 4.96^{c}	33.1 ± 7.66^{cd}
Strong rolling (16-20)	1030	26.8 ± 5.56^{c}	34.46 ± 8.37^{cd}
Moderately steep (21-25)	868	28.4 ± 5.97^{c}	36.6 ± 8.90^{d}
Steep (26-35)	1288	34.3 ± 7.90^{c}	44.5 ± 11.51^{d}
Very steep (35-90)	2272	2.5 ± 0.67^{a}	3.6 ± 1.18^{a}

Within a treatment group, means with different letters are significantly different ($p<0.05$). N represent the number of location fixes (mean) that were recorded within each slope class.

5.1.3 Time spent in the stream zones

Over the entire period, the duration that ewes were detected within 3m of each camera differed by their location ($p=0.001$). Ewes spent the most time near camera 11 (47.6 ± 6.0 min/ewe/day) and least time near camera 6 (10.0 ± 1.6 min/ewe/day; Table 5.5).

Table 5.5: The daily mean of the number of ewes (min and max in parentheses) within 3m of each camera location and the daily median and inter quartile range (IQR) in parentheses of the total duration (min) each ewe spent within 3m of each camera per day during the period when ewes had access to the water trough (Unrestricted) or were prevented from accessing the trough (Restricted).

Camera number	Ewes within 3m of each camera location			
	Restricted		Unrestricted	
	n	Daily duration (min)	n	Daily duration (min)
1	35 (33-39)	26.3 (15.7-31.0)	35 (28-38)	27.1 (16.3-31.4)
2	30 (10-35)	22.1 (9.0-27.8)	32 (16-39)	27 (13.7-31.6)
3	31 (16-37)	20.3 (14.0-24.5)	33 (18-39)	20.1 (15.1-26.0)
4	29 (5-37)	18.1 (11.4-22.08)	31 (14-39)	16.6 (14.5-23.9)
5	32 (28-35)	15.1 (7.9-19.4)	28 (13-36)	14.5 (11.9-15.7)
6	22 (1-30)	9.1 (4.6-15.3)	24 (9-33)	8.9 (5.4-13.7)
7	18 (0-27)	9.2 (8.0-32.3)	18 (6-27)	11.7 (8.0-27.1)
8	31 (22-39)	16.1 (10.9-38.2)	25 (9-36)	13.5 (7.7-25.3)
9	25 (4-35)	10.6 (7.7-14.9)	24 (10-36)	12.5 (7.0-18.5)
10	27 (13-36)	10.6 (8.0-23.7)	27 (14-35)	10.7 (3.7-16.6)
11	33 (17-39)	53.4 (31.9-58.0)	35 (18-40)	48.0 (20.0-70.8)
12	23 (4-37)	14.2 (3.6-17.5)	25 (8-38)	17.2 (4.1-27.0)
13	21 (2-34)	18.7 (6.0-24.6)	25 (7-36)	11.2 (3.0-19.1)
14	21 (4-37)	10.5 (2.1-28.4)	25 (7-36)	13.7 (3.5-14.4)

The duration that ewes were detected within 3m of each camera location differed between time-of-day ($p<0.05$; Figure 5.6) with greater durations in the daylight periods (22.7 ± 1.0 min/ewe/day) followed by evening (17.3 ± 1.4 min/ewe/day), and night (6.6 ± 1.1 min/ewe/day; Table 5.6). Time spent within 3m of any camera location did not differ ($p=0.81$) between the period the trough was restricted (13.3 ± 4.4 min/ewe/day) and unrestricted (10.3 ± 2.0 min/ewe/day).

Table 5.6: The daily duration (min/ewe/day mean ± SEM) that each ewe was recorded to be within 3m of any camera location by time of day class during the entire study

Time of the day class	n	Duration (min/ewe/day)
Night	126	6.6 ± 1.11[a]
Morning	84	11.8 ± 0.90[b]
Day	70	22.7 ± 1.02[d]
Evening	56	17.3 ± 1.38[c]

[a, b, c, d] indicate means with different letters are significantly different ($p<0.05$). Night = 2000 to 0559 hours, morning = 0600 to 0859, daylight = 0900 to 1659 and evening = 1700 to 1959.

5.1.4 Behaviour within the stream zone

Observations of video footage showed that over the 14 days of the study period when ewes were in the stream zone, they were observed to spend 41.9% grazing (n=2304 of 5496 occasions), 26.9% stationary (n=1476), 21.0% walking (n=1152) and 7.2% drinking from the stream (n=396) of the video footage recorded. Ewes were also observed to sniff the water on 72 occasions (1.3%). On twelve occasions ewes were observed to walk in the stream (0.2%). Ewes were observed to spend more time grazing and drinking during the water trough restricted than unrestricted period ($p<0.05$). The frequency of stationary and walking behaviours did not differ ($p>0.05$) when the water trough was restricted or unrestricted (Figure 5.11).

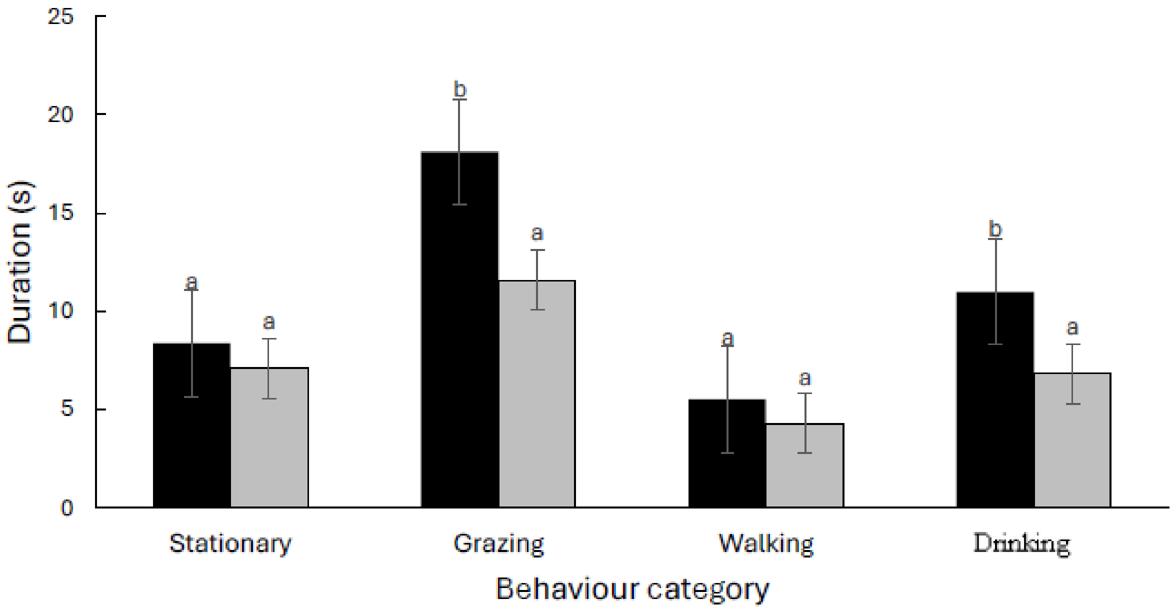

Figure 5.12: The average duration (time in seconds ± SE) of the occasions that ewes were observed to be stationary, grazing, walking, or drinking in the stream zone during periods of restricted access to water trough (black bars) or when access was not restricted (grey bars). Within each behavioural event, means with different letters are significantly different ($p<0.05$)

5.5 Discussion

The aim of the current study was to examine the behaviour of sheep around a natural waterway in summer when access to a water trough was restricted or unrestricted. The impact of ewes on water quality was also assessed. It was hypothesised that in the Manawatū region of New Zealand in summer the absence of reticulated water source would result in greater ewe interaction with the natural waterway, but this interaction would not influence the level of nutrient and pathogens in the waterway.

Spatial distribution

The spatial distribution of sheep in summer in the current study was influenced by paddock features such as slope and the location of the trough. The northern hotspot (statistically significant spatial clusters of high values for ewes) was close to the trough during unrestricted access to the water trough, indicating a greater spatial preference for this location. In warmer months (22-35°C), a greater frequency of crowding and urine patches in sheep and cattle closer to the water trough had previously been reported (White *et al.*, 2001; Graz *et al.*, 2012).

Similarly, Betteridge (2010) reported that urine patches were concentrated in areas that sheep camped, which suggests that the trough may be an area of preference in the current study.

In summer, ewes showed a preference for strong rolling to steep areas (16 to 35°) rather than the flat to rolling areas (0 to 15°) that were preferred in winter and spring. A number of studies have reported that sheep prefer to graze areas with slopes of <25° (Sheath, 1982; Haddon, 2008; Steer, 2012). It is unclear the reason for this difference between seasons as it is thought that sheep prefer flatter areas due to the greater pasture mass in these areas (Saggar *et al*., 1990). Flatter areas generally have higher soil fertility (Saggar *et al*., 1990) which retains water longer in drought conditions (Lambert *et al*., 1983).

In the current study, an increase in the slope of the paddock resulted in an increase in distance travelled per hour. This is in agreement with Loridas *et al*., (2011) who reported that in lowland pastures sheep travelled shorter distances than on mountainous pastures. It is possible that the steeper areas of the paddock had less nutritious and lower pasture mass (Aldezabal *et al.,* 1999; García-González *et al.,* 2011) thus requiring ewes in these areas to move greater distances to access enough pasture. In the current study ewes may have moved shorter distances in lowlands as a result of spending more time feeding due to greater pasture mass as a result of increased soil moisture content (Lalampaa *et al*., 2016).

Distance travelled

The pattern of activity of the ewes in the current study was similar to that reported by McGranahan *et al*., (2018) and Filipčík *et al*., (2020) with peaks in distance travelled at 0800, 1000 and between 1700 and 2000 hours and little movement during the night. (Ehrlenbruch *et al*., 2010), reported that grazing behaviour was greater in the early morning and evening than during daylight hours. This is not surprising as sheep have been observed to travel long distances when feeding or searching for water (Osuji, 1974; Schlecht *et al*., 2006).

Behaviour and time spent by sheep in the stream zone

In summer the duration ewes spent near any camera position was greater during periods of daylight than evening, and least at night. Based on previous findings it was expected that fewer ewes would be observed during the day light hours since sheep tend to be inactive or avoid grazing during the hot hours of the day (Shinde *et al*., 1997; Evangelou *et al*., 2010). This finding was contrary to McGranahan *et al*., (2018) and Filipčík *et al*., (2020) who found most of the activities in the midday and evening. In the current study maximum daily temperatures were lower than reported by McGranahan *et al*., (2018) and Filipčík *et al*., (2020) ranging from 8.6 to 29.3 °C which may explain the lack of influence of daylight periods on the grazing behaviour of sheep. The night results in the current study were in agreement with Penning *et al*. (199l) who reported little night-time grazing for sheep at pasture.

Ewes were more frequently observed to graze and drink in the stream zone during the water trough restricted period compared to unrestricted access. In the current study, ewes were observed to spend 41.9% of the time in the stream zone grazing. This finding was in agreement with Filipčík *et al*., (2020), who reported that the dominant behaviour of sheep at pasture was grazing (49.5% of their observed time). While in the stream zone ewes were observed to spend 7.2% of the time drinking from the stream which was contrary to Mateus who reported that ewes spent 0.2-0.3% drinking. In summer the free water consumption of sheep has been reported to be 40 percent higher than in winter (Markwick, 2007). Further, the water content of mountain sheep diets was reported to be insufficient to meet evaporative water losses of sheep, therefore, ewes required free water in order to maintain their water balance. This observation was supported by the current study, where during unrestricted access to the water trough there was a northern hotspot close to the trough that was not seen during the restricted period (Figure 5.9A & B). Macfarlane *et al*., (1966) demonstrated that when pasture contained 60 to 70% water, sheep water requirements could be met through the pasture consumed and thus, there was no need to drink free water (Macfarlane *et al*. 1966). In the current study pasture had a moisture content of 56% which was likely to necessitate sheep to seek sources of free water.

Water quality

Water analyses showed that ammonium concentrations were higher during the water trough restricted period, compared to unrestricted period. The mean ammonium concentration in current study was 0.56mg/l which exceeded the recommended concentration guidelines of the Australia and New Zealand Environment and Conservation Council of 0.02mg/l (ANZECC, 2000). The current study's findings support previous research showing higher ammonium concentrations and greater exceedances during the summer months in streams and rivers

(Larned, 2004; Muirhead and Meenken, 2018). Ammonium loads were higher in samples collected at the outflow than inflow sampling sites on days when flowrate was higher at the inflow than outflow sites (D6, D12, and D13). It was unlikely, however, that rainfall could explain these higher ammonium values as flowrate was found to be positively correlated with rainfall in the current study. Hence, the higher ammonium concentration observed could be due to dissimilatory nitrate reduction to ammonia where microorganisms such as *E. coli*, first reduce nitrate to nitrite and then to ammonium.

Total phosphorous loads were higher in the outflow than inflow water samples collected on D6, D12, and D13. The mean TP concentration was 0.04 mg/l which was within the proposed concentration guidelines of 0.035 mg/l for upland rivers and 0.05 mg/l for freshwater lakes (ANZECC, 2000). In general, the concentrations of TP were lower than reported in Chapters 3 and 4 (0.05 mg/l) which may be explained by the lower rainfall recorded in summer as rain has the greatest influence on the transportation of TP from pastures via surface runoff (Ahuja *et al.*, 1981; Burger *et al.*, 2007; Abell and Hamilton, 2013; Tempero *et al.*, 2015). Rainfall intensity, flowrate and pasture-plant cover influence the vulnerability of soil to physical damage, and the relative magnitude of sediment and TP transferred in surface runoff (McDowell and Wilcock, 2007). The high pasture mass and low stream flowrate recorded in the current study may also have resulted in less TP being adsorbed onto sediment particles or caused more sedimentation in waterways (Brown *et al.*, 1981; Sharpley *et al.*, 1981; McDowell *et al.*, 2006). Unfortunately, the concentration of sediment was not measured in this study, which may have helped to explain total phosphorus concentrations.

E. coli concentrations were higher during the water trough unrestricted period compared to the water trough restricted period. Outflow *E. coli* load was higher than the inflow load during both the water trough access period and the water-restricted period. In the current study 83% of all water samples contained *E. coli* concentrations that exceeded recommended 130 cfu/100 ml guidelines of Australia and New Zealand Environment and Conservation Council (ANZECC, 2000). The majority of these higher concentrations of *E. coli* were observed on D5 and D6 when rain was recorded. The *E. coli* loads in outflow water samples were higher than inflow samples on D5, D12 and D13 which was similar to the results observed in spring (Chapter 4). Higher *E. coli* loads in the outflow suggests that the source of *E. coli* was from within the paddock. The likely source of the *E. coli* was from sheep faeces that were washed into the stream by overland flow. Previously, Whitman *et al.*, (1995) reported higher *E. coli* concentrations in waterways due to rainfall events. Further high soil and faecal moisture content due to rainfall can prolong the survival and growth of *E. coli* resulting in a large land surface store (Hunter and McDonald, 1991; Moriarty 2011).

In conclusion, there was evidence that in summer sheep did interact with the natural waterway and *E. coli* loads were higher in the outflow compared to the inflow water samples suggesting that e. coli was being introduced from the paddock. Phosphates was also recorded to be above suggested thresholds on some days, which was related to rain events and presence of sheep in the paddock. The degree of interaction of sheep with the waterway was not influenced by the availability of reticulated water from a trough. GPS data indicated that the ewes showed a spatial preference for strong rolling to steep slope (between 16° and 35°) of the paddock with greater distances travelled by sheep as the slope increased, except at very steep slopes. Further long-term studies are required to verify these results, especially to confirm if the elevated ammonium and *E. coli* concentrations occur in other environmental conditions. e.g., higher ambient temperature than the current study.

6 GPS observation of spatial distribution of sheep in winter, spring, and summer

6.1 Abstract

Spatial variation in slope and aspect are key determinants of vegetation pattern and plant species distribution. Slopes of varying aspects receive different amount of solar radiation which results in differences in soil formation. The goal of the current study was to investigate the effect of paddock slope and aspect on the spatial distribution of sheep across three seasons using data collected in winter spring and summer (Chapters 3 to 5). More ewe location fixes were recorded on the south and north-facing slopes of the study compared with west and east-facing slopes across all three seasons. Up to 79% of sheep location fixes were recorded in the south and north-facing slopes which was attributed to variability in herbage growth. During winter there was a more uniform distribution of GPS locations across the study paddock, several focal hotspots were found during spring, whereas during summer, GPS locations were aggregated into one locality. Between seasons the distribution of sheep differed across categories of slope ($p<0.05$), with more sheep (69-84%) utilising flatter to gentle slopes (< 15°; Flat, undulating and rolling) than the rest of slope classes (strong rolling, moderately steep, steep, very steep). The findings from this study provide useful information to help improve management strategies including pasture and flock management.

6.2 Introduction

The primary factors that influence the distribution of sheep in their environment are feed and water availability, shelter, weather, visibility, proximity of escape terrain, altitude, topography, slope and aspect (Mysterud *et al.*, 2007; Haddon 2008; Gong *et al.*, 2016; Plaza, 2022; Fan, 2022). Season further influences the behaviour of sheep by affecting the importance of some of these factors, for example, shelter during periods of extreme weather (Mysterud *et al.*, 2007). Two key factors that affect sheep patterns of movement are terrain slope and aspect (Baum 2021). Understanding the spatial distribution of sheep across seasons can help farmers plan their management.

In New Zealand, 50% of pastoral land contains sloping terrain (Kemp and Lopez, 2016) which is categorised into low (0-12°), medium (13-25°) and high slope (>25°) (Lambert *et al.*, 1983). On NZ hill country, pasture is often grown on steep slopes (>25°) which equates to 4 million ha (80% of the total area) (Kemp and Lopez, 2016). The slope of the terrain has been reported to influence the movement and spatial distribution of sheep, where movement was found to be greater on flat areas, than on inclines (Shannon, 1975; Hitchcock and Hutson, 1979).

Aspect is defined as the compass direction in which a slope is facing (Ferraz *et al.*, 2009). Aspect influences the amount of solar radiation that an area receives and consequently affects soil temperature (Onwuka and Mang, 2018) and moisture levels, which are both important drivers of plant growth (Horvath *et al.*, 1984; Lieffers and Larkin-Lieffers, 1987; Gong *et al.*, 2008). In the northern hemisphere, south-facing slopes of mountainous rangelands receive a greater amount of radiation than north-facing slopes (Kutiel and Lavee 1999). In the southern hemisphere, soil on north-facing slopes dries out faster and is warmer than south-facing slopes due to longer exposure to sunlight (Radcliffe and Lefever 1981; Radcliffe *et al.*, 1968; Bretherton, 2012).

Spatial variation in slope and aspect are key determinants of vegetation pattern and plant species distribution (Zeng, 2014; Yang, 2020). Moreover, the distribution of pasture species is related to topography, which influences animal movement, treading, the depletion or enrichment of nutrients by animals, and soil moisture (Suckling 1954; During and Radcliffe 1962; Radcliffe 1966; Rumball and Esler 1968; Grant and Brock 1974). Sheep are selective grazers (Lynch *et al.*, 1992; Armstrong *et al.*, 1993; Edwards *et al.*, 1993; Animut *et al.*, 2005) and time spent grazing is dependent on the availability of feed (Lynch *et al.*, 1992; Penning *et al.*, 1993), which in turn affects their distribution in the paddock.

The spatial distribution of sheep is also influenced by weather variables such as temperature, rainfall and wind (Warren and Mysterud 1991; Mysterud *et al.*, 2007; Thomas *et al.*, 2008). The presence of shelter and shade can influence sheep movement patterns. Sheep exposed to higher rates of solar radiation show increased respiratory rate and rectal temperatures compared with those under shade (Brosh *et al.*, 1998; Sevi *et al.*, 2001) and are thus more likely to avoid exposed areas in hot conditions (Grubb and Jewell, 1966; Sibbald *et al.*, 1996). Inactive behaviours such as lying and standing also increase when exposed to higher rates of solar radiation (Sevi *et al.*, 2001). For example, sheep in shade were observed to stand for 2 hours longer per day than sheep without shade (Johnson and Strack, 1992). While in winter, sheep seek shelter as wind speeds increase (Munro, 1962; Scott and Sutherland 1981). Wind can greatly reduce the thermal insulation that wool provides to the animal (Cannas *et al*. 2004; Cottle and Pacheco 2017), therefore, sheep prefer areas where the aspect of the terrain provides protection from wind and topography generates different wind types (Whiteman 2000; Azizi *et al*., 2017).

The current study investigated the effect of slope and aspect on the spatial distribution of sheep across winter, spring and summer using data collected in Chapters 3 to 5. It was hypothesised that paddock topography, especially aspect, would affect the spatial distribution of sheep across seasons.

6.3 Materials and methods

All the procedures in this study were carried out with the approval of the Massey University Animal Ethics Committee (MUAEC 19/62 and 19/102). Each study was conducted for two weeks across three different seasons (winter (Chapter 3), spring (Chapter 4) and summer (Chapter 5)) at Massey University's Tuapaka farm, located approximately 15 km north-east of Palmerston North, New Zealand (40.3346° S, 175.7316° E). As each of these studies has already been described and only a brief outline has been given below.

6.3.1 Animals and study design

A series of studies were completed in winter, spring and summer using a single study site. Mixed age ewes (n=40) were managed in a 1.56 ha paddock (~149x85m) on a hill country farm (Figure 6.1A). Ewe movement within the paddock and their interaction with the waterway was monitored using global positioning system (GPS) devices. Animals were monitored between the 13th and 29th August 2019 (winter); 16th and 30th November 2019 (spring) and 15th and 28th February 2020 (summer). The paddock contained a predominantly perennial ryegrass (*Lolium perenne*) sward which included white clover (*Trifolium repens*). The pasture masses were greater than 1000kgDM/ha throughout each study (winter =1263.3 ± 120.7 kgDM/ha, spring =3642.4 ±64.9 kgDM/ha and summer = 1765.8 ± 136.8 kgDM/ha (Mean ± SD). The mean moisture content of the sward was 77 ± 3% in winter, 76 ± 4% in spring and 56 ± 8.6% (Mean ± SD) in summer.

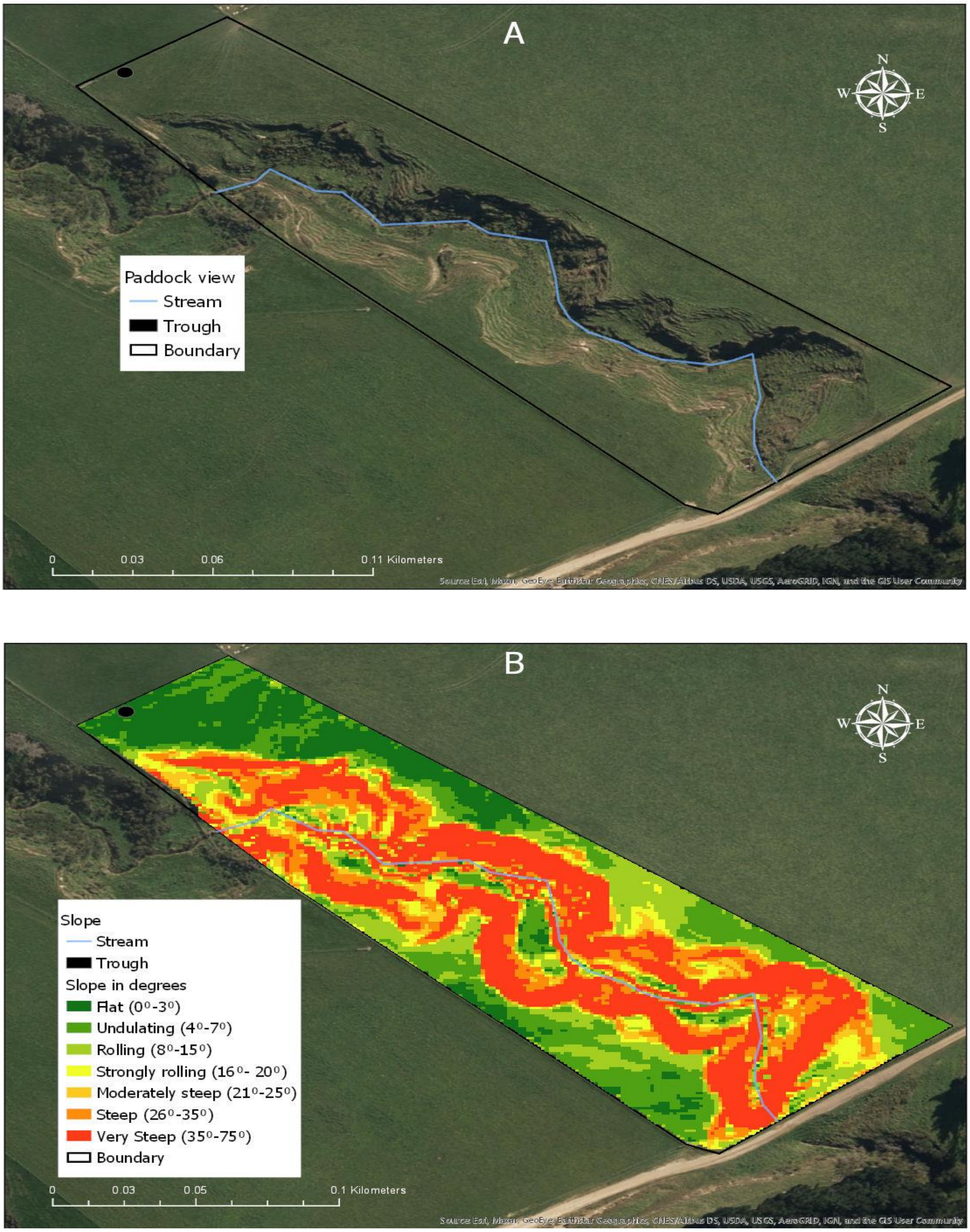

Figure 6.1: Maps showing a satellite image of the study site (panel A) and slope category including paddock features such as the stream (blue line), water trough (black circle) and paddock boundary (black line; panel B).

6.3.2 Wind data

Wind data was downloaded from New Zealand's National Climate Database (NIWA CliFlo, Pahiatua, Agno.38224 Lat -40.50652 Long 175.91586, 110 m above mean sea level) about 16 km from the study site rather than using the on-site weather station in order to have wind direction data. Hourly and daily data were downloaded which included wind speed (m/s) and wind direction (°). Wind direction was extrapolated from degrees to four intercardinal directions: North: from 292.6 to 67.5°; east: from 67.6 to 112.5°; south: from 112.6 to 247.5°; west: from 247.6 to 292.5°).

6.3.3 GPS

In each study, all ewes (n=40) were fitted with a collar to which a GPS unit (custom build units, DataCarter Ltd), was attached. GPS units were attached for two weeks in each season (winter, spring and summer). GPS units were programmed to allow for the continuous tracking of satellites and logging of animal positions whenever ewes moved ≥5m or every 60 seconds, if the ewe had not moved. GPS unit specifications were as described in Chapter 3. GPS units recorded date and time (GMT), latitude, longitude, horizontal dilution of precision (HDOP), and the number of satellites. Only location fixes based on four or more satellites were used. Analysis was further restricted to position fixes with positional dilution of precision values < 12.0 to eliminate locations with potentially large error (Lewis 2007). GIS data manipulation and analysis was done in ArcGIS Pro (Environmental Spatial Research Institute, Redwood, California, US). Advanced Spaceborne Thermal Emission and Reflection Radiometer (ASTER) files were downloaded from https://search.earthdata.nasa.gov/search/.

6.3.4 Topography data

Slope and aspect were derived from a digital elevation model (DEM) of the ASTER sensor recorded in 2010. Degrees of slope was categorised into seven classes: flat (0-3°), undulating (4-7°), rolling (8-15°), strong rolling (16-20°), moderately steep (21-25°), steep (26-35°) and very steep (35-75°; Figure 6.1B). Aspect was classified into north, south, east, and west facing slopes; the area of each aspect within the study site is given in Table 6.1.

Table 6.1: Classification of aspect showing the area (m^2) and percentage (%) of the study site represented.

Aspect of slope	Area of study site m^2 (%)
West facing	2056 (12.1)
East facing	1888 (11.1)
North facing	6269 (36.9)
South facing	6771 (39.9)

6.3.5 Statistical analysis

Statistical data analyses were performed using SPSS Statistics 20.0.0 (IBM Corp., Armonk, NY, USA), adopting a significance level of $p \leq 0.05$. Prior to analysis, data were checked for normality using Kolmogorov–Smirnov and Shapiro-wilk test, and the homogeneity of variances using Levene's test (Gotelli and Ellison, 2004).

GPS data were analysed using ArcGIS mapping tools (ArcGIS Pro 2.2.4, 2018). An optimized hot spot analysis (z-score) was used to identify statistically significant spatial clustering of ewe GPS location fixes. A hotspot was defined as an area of higher concentration of ewe locations compared to the expected number given a random distribution of ewes. A cold spot was defined as an area that had a lower concentration of ewe locations compared to the expected number given a random distribution of ewes.

Relationships between sheep site use (location fixes) and weather parameters were analysed with multiple linear regressions in SPSS version 20, using the number of ewe location fixes as dependent variables and weather parameters as predictors. These analyses were carried out separately for winter, spring, and summer.

The uneven distribution of slope within aspect would likely affect spatial distribution of the sheep as well as vegetation across seasons resulting in localised variation in microclimate and soil properties. To avoid inaccurate conclusions, the interaction of slope and aspect interactions were analysed.

Paddock use with respect to aspect and slope across the three seasons (comparing location fixes with proportion of area) was analysed using the Chi^2 test. Selection was further evaluated using Manly's selection ratios, and significance was determined using Bonferroni-adjusted confidence intervals ($\alpha = 0.05$).

6.4 Results

6.4.1 Weather

Windrose

New Zealand is situated in a zone dominated by westerly winds and south-easterly in August, with the majority arising from the westerly quarter (Ishwar & Mason, 2019). Palmerston North receives annual wind speed of 15.1 km/h. However, winter (August) generally has lower wind speeds compared to the spring and summer months (Macara, 2018). Peak wind speeds were recorded during mid-spring to mid-summer. In the current study, on the wind speed was between 0 and 3 km/h on 50% of days in spring, 49% in winter and 43.5% in summer. In November 2019, New Zealand experienced higher prevalence of north-westerly winds due to lower sea level pressure to the south and southwest of the country. This pressure resulted from the Southern Hemisphere storm tracks being displaced northwards across the entire Southern Ocean including towards New Zealand (Niwa 2019). In February 2020, the wind in Palmerston North, New Zealand, was predominantly from the west-northwest (Chappell, 2015). In the current study during winter 63.8% of wind was recorded from the east (E) and northeast (NE, ENE, E) whereas 28.9 % was from the west (W) and southwest (SSW, WSW, SW; Figure 6.2A). Similarly, in spring, westerlies (W) accounted for 17.8% % of all wind directions while wind blowing from east (E) and northeast (NE, ENE, E) accounted for 76.6% of all wind direction (figure 6.2B). In summer, westerlies (W) accounted 46% % of all wind directions while wind blowing from east (E) and northeast (NE, ENE, E) accounted for 47.8% of all wind direction (Figure 6.2C).

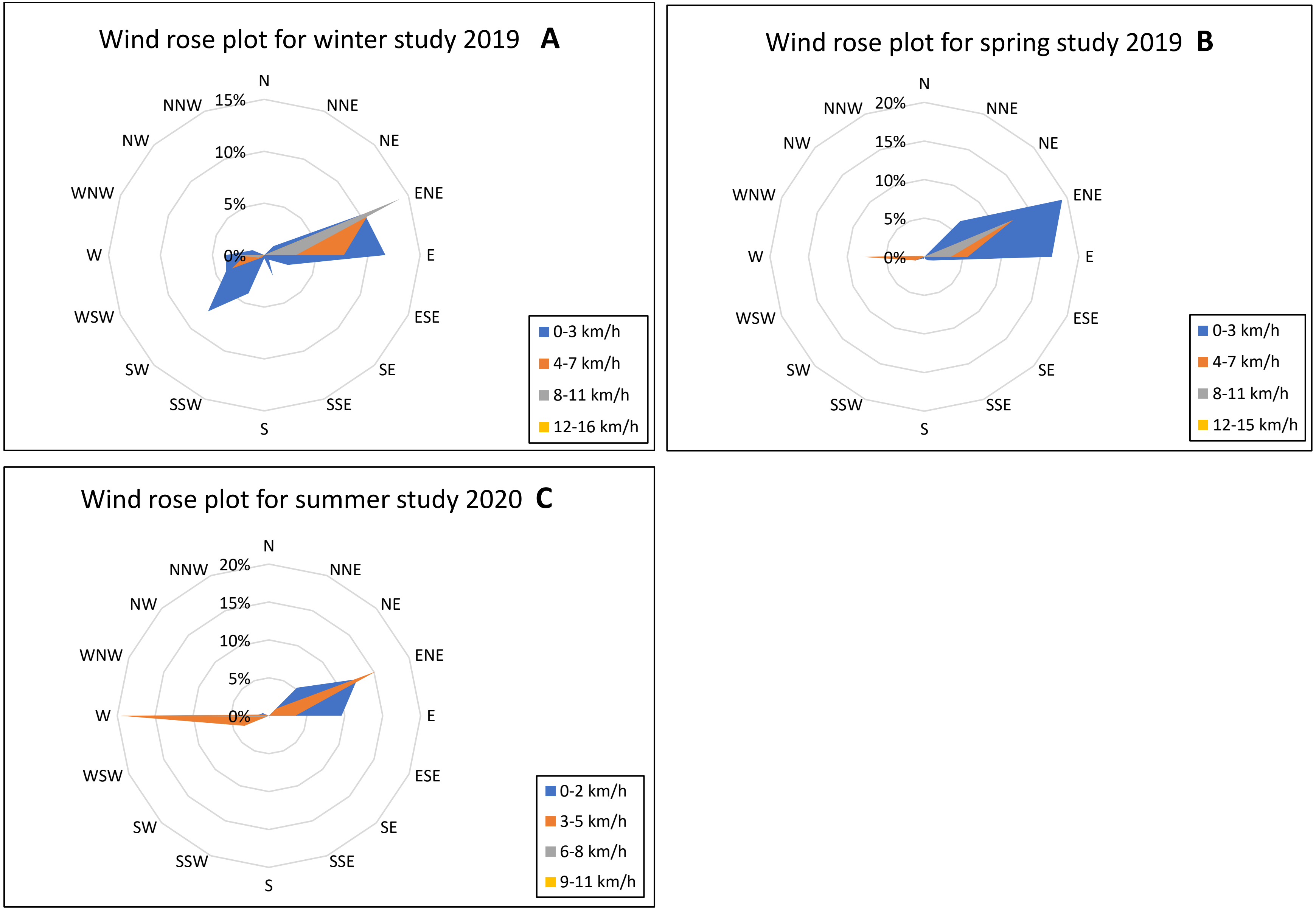

Figure 6.2: Windrose plots showing the direction and speed of wind recorded in Palmerston North in August (panel A), November (panel B) 2019 and February 2020 (panel C).

6.4.2 Slope and aspect

Within the study site, the south-and north-facing slopes each contributed approximately 40% of the area with west- and east-facing slopes contributing just over 10% each (Table 6.1). When analysed separately aspect use by sheep was similar across the three seasons. More ewe location fixes were recorded on the south and north-facing slopes (77-79%) compared with west and east-facing slopes (Table 6.2; $p<0.05$) which was proportional to their contribution to the paddock area. The location fixes were higher in the south than the north facing slopes in winter and summer ($p<0.05$) but no significant difference was observed during spring ($p>0.05$). Percentages of fixes were higher in the south than the proportion of the paddock (46% fixes vs 40% of the paddock area) and lower in the north (33% fixes vs 37% of the paddock area). The number of fixes did not differ ($p>0.05$) across seasons among the different aspect classes during the periods of water trough restricted and un-restricted (data not shown).

6.4.2.1 Aspect use

In winter the observed use of different aspects differed significantly from availability (χ^2_3 = 174.6, N = 6840, $p < 0.001$), with south- and east-facing slopes used more than was expected due to chance and north- and west-facing slopes used less than expected. Manly's selection ratios indicated preferred selection for south-facing (Wi = 1.16) and east-facing aspects (Wi = 1.13), while west-facing (Wi = 0.76) and north-facing (Wi = 0.87) slopes were avoided relative to their availability.

In spring observed use of different aspects differed significantly from their availability (χ^2_3 = 240.6, N = 6020, $p < 0.001$), indicating non-random selection of slope aspects. Manly's selection ratios showed positive selection for south-facing (Wi = 1.20) and east-facing slopes (Wi = 1.17), and avoidance of west-facing (Wi = 0.68) and north-facing slopes (Wi = 0.84) relative to their proportional availability. Bonferroni-adjusted confidence intervals ($\alpha = 0.05$) confirmed these patterns. The proportion of fixes on south (0.461-0.493) and east-facing slopes (0.119-0.141) exceeded their availability (0.399 and 0.111, respectively). In contrast, use of west (0.073-0.091) and north-facing slopes (0.296-0.326) was significantly lower than their availability (0.121 and 0.369, respectively), indicating avoidance. In spring, animals demonstrated strong selection for south-facing slopes, moderate selection for east-facing slopes, and avoidance of north- and west-facing slopes.

In summer observed us of different aspect differed significantly from their availability (χ^2_3 = 112.3, N = 6726, p < 0.001), indicating non-random selection of slope aspects. Manly's selection ratios indicated selection for east (Wi = 1.22) and south-facing slopes (Wi = 1.07), and avoidance of west-facing (Wi = 0.75) slopes. North-facing slopes were used slightly less than available (Wi = 0.94). Bonferroni-adjusted confidence intervals (α = 0.05) confirmed significant selection for east-facing (0.124-0.148) and south-facing slopes (0.413-0.443), and significant avoidance of west-facing (0.081-0.101) and north-facing slopes (0.329–0.362) relative to availability.

6.4.2.2 Slope use

Use of terrain aspects during winter differed significantly from availability (χ^2_6 = 2197.2, N = 11,968, p < 0.001), indicating strong selection patterns. Manly's selection ratios (Wi) revealed significant selection for undulating (Wi = 1.82) and flat terrain (Wi = 1.33), near-proportional use of rolling terrain (Wi = 0.96), and avoidance of strong rolling (Wi = 0.66), moderately steep (Wi = 0.61), steep (Wi = 0.71), and very steep terrain (Wi = 0.33). Bonferroni-adjusted confidence intervals (α = 0.05) confirmed these patterns: the proportion of fixes on undulating (0.260-0.282) and flat (0.239-0.261) terrain significantly exceeded availability, rolling terrain (0.158-0.174) was used in proportion to availability, and steeper terrain categories were significantly underused relative to availability. Overall, during winter, animals strongly selected lower-gradient terrain (flat and undulating) and avoided steeper slopes (Appendix 4).

In spring, use of terrain aspects differed significantly from availability (χ^2_6 = 5356, N = 10,532, p < 0.001), indicating strong habitat selection patterns. Manly's selection ratios (Wi) revealed strong selection for undulating (Wi = 2.29) and flat terrain (Wi = 1.70), neutral use of rolling terrain (Wi = 1.01), and avoidance of strong rolling (Wi = 0.55), moderately steep (Wi = 0.45), steep (Wi = 0.22), and very steep terrain (Wi = 0.09). Bonferroni-adjusted confidence intervals (α = 0.05) confirmed these patterns, with undulating (0.329-0.353) and flat (0.307–0.331) terrain significantly exceeding availability, rolling terrain (0.167-0.183) used in proportion to availability, and steeper terrain categories significantly underused relative to availability. Overall, in spring, animals strongly selected lower-gradient terrain and avoided steeper slopes.

Use of terrain aspects differed significantly from availability (χ^2_6 = 5399.88, N = 11,770, p < 0.001) during summer, indicating strong selection patterns. Manly's selection ratios (Wi) showed significant selection for undulating (Wi = 1.87) and flat terrain (Wi = 1.39), with neutral use of rolling terrain (Wi = 0.95) and avoidance of strong rolling (Wi = 0.72), moderately steep (Wi = 0.71), and steep (Wi = 0.52) terrain. Bonferroni-adjusted confidence intervals ($\alpha = 0.05$) confirmed these findings, with undulating (0.270-0.288) and flat (0.253-0.271) terrain used significantly more than expected, and strong avoidance of steeper terrain categories, except the very steep terrain (0.185-0.201). In summer, animals strongly selected lower-gradient terrain and avoided steep slopes, however strongly selected the very steep slopes.

Across the study site, the majority slopes were steep, followed by flat and then rolling (Table 6.3). The distribution of sheep differed across categories of slope ($p<0.05$), with disproportionately more sheep (69-84%) utilising slopes of <15° (Flat, undulating and rolling; Table 6.3) than the rest of slope classes (strong rolling, moderately steep, steep or very steep). Sheep largely avoid the very steepest slopes (>36°), although utilisation of steep and very steep slopes in summer and winter was greater than in spring. The flat areas (0-3°) of the paddock had a higher density of GPS locations (25%) across the seasons. During winter the distribution of GPS locations was more uniform (Figure 6.4A), than in the summer period, when GPS locations aggregated into one locality (Figure 6.4B). In spring, the distributions of GPS locations were in several focal hotspots. Shaded areas (not statistically quantified) were observed to be extensively used during summer, as shown in Figure 6.3.

Table 6.2: The area (with percentage in parentheses) that each aspect category contributed to the study site and the number of included location fixes (mean (n) with range in parentheses) and percentage of fixes (of total %) recorded within each aspect category with each season.

	Area	Location fixes					
	of study	Winter		Spring		Summer	
Aspect	site m^{2} (%)	n	%	n	%	n	%
West facing	2056 (12.1)	629 (462-796) ^{a}	9.2	494 (191-796)^{a}	8.2	611 (528-694)^{a}	9.1
East facing	1888 (11.1)	858 (688-1028) ^{b}	12.5	781 (479-1083)^{a}	13.0	913 (733-1093)^{a}	13.6
North facing	6269 (36.9)	2189 (1663-2714) ^{c}	32.0	1872 (1570-2174)^{b}	31.1	2322 (1968-2675)^{b}	34.5
South facing	6771 (39.9)	3164 (2504-3623) ^{d}	46.3	2873 (2570-3175)^{b}	47.7	2880 (2473-3287)^{c}	42.8

Within a column, different letters indicate significant differences at $p<0.05$. Winter (13th - 29th August 2019); Spring (16th -30th November 2019); Summer (15th -28th February 2020)

Table 6.3: The area (with percentage in parentheses) that each slope class contributed to the study site and the number of included location fixes (mean (n) with range in parentheses) and percentage of fixes (of total %) that were recorded within each slope class.

Slope class (degree)	Area of study site m^2 (%)	Location fixes					
		Winter		Spring		Summer	
		n	%	n	%	n	%
Flat (0-3)	2995 (18.8)	2993 (2377-3610)	25.0	3364 (2964-3764)	31.9	3086 (2726-3446)	26.2
Undulating (4-7)	2366 (14.9)	3241 (2545-3937)	27.1	3595 (3196-3996)	34.1	3281 (2921-3641)	27.9
Rolling (8-15)	2750 (17.3)	1992 (1542-2441)	16.6	1847 (1447-2247)	17.5	1945 (1585-2305)	16.5
Strong rolling (16-20)	1924 (12.1)	957 (769-1146)	8.0	693 (293-1093)	6.6	1030 (670-1370)	8.8
Moderately steep (21-25)	1677 (10.5)	772 (597-947)	6.5	493 (93-894)	4.7	868 (508-1228)	7.4
Steep (26-35)	3336 (21.0)	1802 (1063-2541)	15.1	490 (90-890)	4.7	1288 (928-1648)	10.9
Very steep (36-75)	874 (5.5)	211 (137-285)	1.8	50 (-350-450)	0.5	2272 (-88-632)	2.3

Winter (13th - 29th August 2019); Spring (16th -30th November 2019); Summer (15th -28th February 2020)

Figure 6.3: Images taken on day 3 of winter study (panel A) and day 12 of summer study (panel B), showing sheep distribution and differences in the pasture within the study paddock. Sheep grazed the flatter areas in winter and sought the aspect which provided more shade in summer.

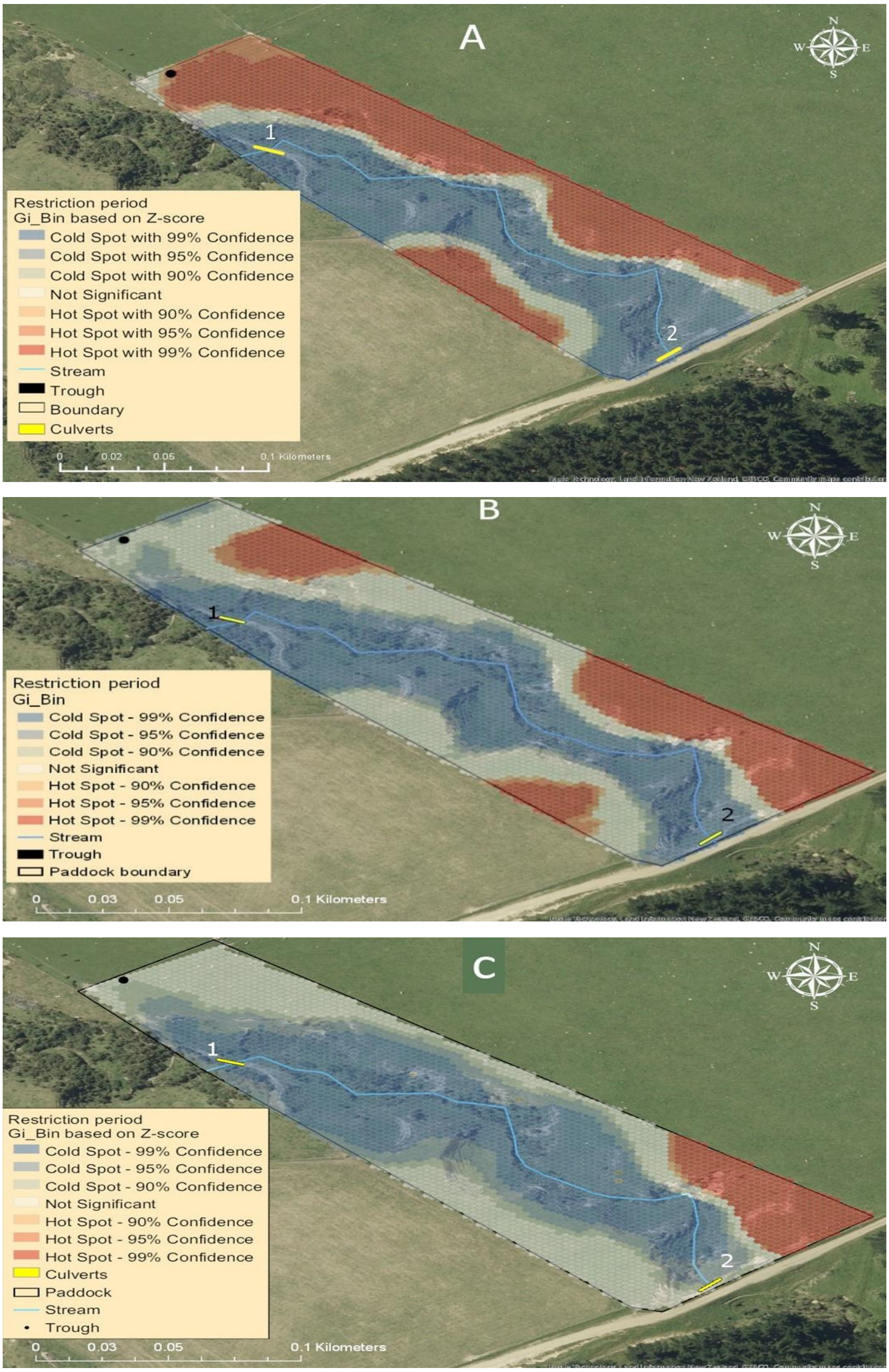

Figure 6.4: Maps of the spatial distribution (magnitude per unit area) of ewes in the study paddock during winter (panel A) spring (panel B) and summer (panel C) during the period of restricted water-trough access using optimised hot spot analysis. Yellow markings (1 and 2) represent positions of the culverts. The blue areas represent low ewe density and red areas high ewe density. Hotspot (red areas) indicate statistically significant ($p<0.05$) spatial clusters of high values (larger positive z-score) while cold spot (CS; blue areas) indicates statistically significant ($p<0.05$) spatial clusters of low values (smaller negative z-score), and white indicates random distribution with no spatial clustering.

Correlations between the number of ewe location fixes and hourly weather parameters in the south facing slopes, showed a correlation between fixes and solar radiation in spring (r^2=0.4, p=0.001) and in summer between fixes and wind speed (r^2=0.4, p=0.017). In winter, on the east facing slopes, there was a correlation between location fixes and solar radiation (r^2=0.3, p=0.006) and between fixes and average humidity (r^2=0.2, p= 0.05). In summer, there was a correlation between location fixes and wind speed on the north facing slopes (r^2=0.5, p=0.0002), also between fixes and average humidity on the north facing slopes (r^2=0.3, p=0.004). No other correlations were significant (p<0.05). A summary of weather variables (e.g., rainfall, temperature etc.) within each two-week seasonal monitoring is as shown in Table 6.4.

Table 6.4: Summary of weather variables within each two-week seasonal monitoring period showing seasonal rainfall (mm), average temperature (°C), average humidity (%), wind speed (m/s) and solar radiation (MJ/mÂ²)

Variable	Winter	Spring	Summer
Rainfall (mm)	8.0 (4.2-10)	1.5 (0-8.6)	1.5 (0-13.8)
Average temperature (°C)	4.8 (0-29.6)	13.2 (10.4-16.1)	17.2 (13.2-20.3)
Average humidity (%)	78.9 (64.3-89.6)	84.4 (73.9-94.7)	72.6 (63.9-87.9)
Wind speed (m/s)	5.2 (2.2-9.2)	7.0 (3.3-10.2)	5.0 (2.8-10.1)
Solar radiation (MJ/mÂ²)	6.7 (1.3-13.4)	21.4 (8.9-30.9)	16.5 (4.1-22.7)

Winter (13th - 29th August 2019); Spring (16th -30th November 2019); Summer (15th -28th February 2020)

6.5 Discussion

Aspect preference and herbage availability

Regardless of season, more ewe location fixes were recorded on the south and north-facing slopes of the study site compared with west and east-facing slopes. The location fixes were, however, higher with respect to the paddock area in the south than the north facing slopes in winter and summer but not during spring. Radcliffe *et al.*, (1968) also found that sheep showed a preference for northern aspects. Preference for northern and southern aspect might be due to variability in herbage growth. For example, at Whatawhata, Te Kuiti and Palmerston North, more herbage accumulated on north-facing slopes than on southerly slopes (Radcliffe *et al.*, 1968; Radcliffe 1971; Suckling 1975; Gillingham 1973, 1980). More herbage was found to accumulate on southerly and easterly aspects at Ballantrae and Wairarapa (Bircham 1976; Lambert and Roberts 1978). Therefore, sheep chose south and north-facing areas because the pastures probably had the highest nutritional quality and possibly due to the thermal comfort provided by those respective aspects. In the southern hemisphere, the northern facing slopes are warmer and drier than the south facing slopes (Radcliffe and Lefever 1981, Bretherton, 2012). High herbage accumulation may be a result of increased soil moisture levels which is always higher on south, followed by north, facing slopes than other aspects (Gillingham *et al.*, 1998). Lambert and Roberts (1976) also found that moisture loss through evapotranspiration was greater on the north facing slopes rather than the south facing slopes, possibly due to exposure to increased solar radiation on north facing slopes. Further, it is suggested that different evaporation rates were the reason for the different moisture conditions in the north facing slopes and the south facing slopes topsoil (Lambert and Roberts 1976).

More ewe location fixes were recorded on the south than north-facing slopes of the study site in all seasons (winter and summer) except spring. This is not surprising as in winter sheep might have used the south facing slope to shy away from strong winds during winter (Grubb and Jewell, 1966; Sibbald *et al.*, 1996). The average temperature in winter in the current study was 4.8°C but went as low as 0°C. In summer, however, increased use of south facing slopes may have been to take advantage of shade as in the southern hemisphere like New Zealand, the northern facing slopes are warmer and drier than the south facing slopes (Radcliffe and Lefever 1981, Bretherton, 2012). Having no difference in the number of location fixes during spring might be attributed to the moderate temperature (13°C) which would allow sheep to use any location in the paddock.

Slope preference and terrain utilisation

Sheep utilised the flat to gentle rolling land (0-15°; flat, undulating and rolling) proportionately more than their area than remainder of the slope classes. This finding was similar to previous studies that showed sheep preferred areas with slopes between 0 and 20% (Chapter 3, Chapter 4, Chapter 5, Radcliffe *et al.*, 1982, Plaza *et al.*, 2022). In the current study, sheep appeared to avoid very steep slopes (>36°), although utilisation of steep and very steep slopes in summer and winter were greater than in spring. One of the reasons that sheep may avoid very steep slopes may be that sheep prefer to move across flat terrain rather than up and down inclines

(Hitchcock and Hutson, 1979). Hitchcock and Hutson (1979) reported that sheep were more spread out and ran more slowly as the angle of incline increased.

Role of soil fertility and nutrient distribution

The spatial preference of flatter areas in the present study, may also have been due to higher soil fertility and greater masses of green pasture in these areas. Saggar *et al*., (1990) reported that fertility was highest in pasture from gentle slopes. High fertility soils have been reported to retain water longer than soils with low fertility, which then results in greater masses of green pasture in flatter areas (Lambert *et al*., 1983). Soil fertility is influenced by animal activity and gravity nutrient transfer from steep slope to easier slopes (Mackay, 1999; Lambert 2000; Lopez *et al*., 2003). However, soil fertility and nutrient transfer were not individually measured based on slope in the present study. Moreover, sheep are believed to prefer gentle slopes for grazing as steeper areas have grasses that are less nutritious and less palatable (Aldezabal *et al.,* 1999; García-González *et al.,* 2011).

Increases in summer and winter utilisation of pastures on steep slopes in the current study may also be due to ewe seeking shaded areas, as was supported by photographic observations and video analysis (Figure 6.3). In spring ewes had lambs at foot and therefore they would have maintained a close contact (distance of 10 metres of each other) throughout the study (Morgan and Arnold, 1974). It is possible, therefore, that this may have influenced the movement of the ewes in spring. The ewes preferred slopes less than 15° more frequently than steeper slopes indicating avoidance of steep terrain when forage was abundant (Lopez et al., 2003). Moreover, steep slopes increase the risk of lambs falling or becoming separated from their mothers and thus raise the chance of exposure if lambs get stranded in bad weather.

Microclimate effects

Correlations between the number of ewe location fixes and hourly weather parameters in the current study showed a negative relationship between location fixes and solar radiation in east facing slopes in winter and south facing slopes in spring. It has been shown that aspect *per se*, may be misleading and some scientists indicated that solar duration is a better predictor for sheep spatial distribution (D'Eon and Serrouya 2005). Slope and aspect govern the amount of solar radiation at the landscape level thus resulting in distinct microclimatic conditions with variation in soil properties and nutrient concentration (Agren and Andersson 2011). High levels of solar radiation, with frequent strong northerly winds can create soil moisture deficits which have been observed on the northerly aspect, especially during summer in New Zealand (Radcliffe *et al.*, 1982). This soil moisture deficit may explain the higher density of ewe GPS locations on the southerly aspect in the current study. Furthermore, during winter in NZ, radiation on south-facing slopes can be blocked by surrounding hills due to the low elevation of the sun (Tian *et al.*, 2001). Solar radiation also explains the preference of sheep to flat and low rolling areas as they may receive less solar radiation than other areas of the paddock due to greater shading (Poulos and Camp 2010). In the current study, wind speed negatively affected the spatial distribution of sheep during summer.

In conclusion, the current study found that paddock topography, slope and aspect affected the spatial distribution of sheep across winter spring and summer. These differences may be due to effects of herbage mass and growth, solar radiation, wind speed and average humidity. Sheep utilised more of the flat to gentle rolling areas (<15) than the rest of the slope categories. Moreover, more sheep were found on the south and north-facing slopes of the study site compared with west and east-facing slopes.

7 General discussion

7.1 Introduction

Several studies have investigated a range of behaviours of sheep including grazing, drinking, ruminating, idling, walking and social and reproductive interactions (Champion *et al.,* 1994; Deag, 1996; Dwyer and Lawrence, 2005; Al-Ramamneh *et al*., 2012). To date ewes' behaviour around a natural waterway has not been examined nor has their impact on water quality either in New Zealand or elsewhere. While there is clear evidence that cattle, deer, and pigs can degrade water quality when allowed to graze near waterways (MfE, 2020), little is known about the impact of sheep. Therefore, the objective of this research was to investigate the behaviour of sheep in and around natural waterways, and determine their impact on water quality.

Recently, the New Zealand Ministry for the Environment (MfE, 2020), released their "Essential Freshwater" package which contained changes to the existing national policy statement for freshwater management to maintain or improve freshwater quality. The policy required environmental agencies "account for sources of relevant contaminants" and to use collaborative processes for setting water quality targets in catchments. These polices included new regulations requiring that cattle, pigs, and deer be excluded from waterways located on low slopes that were more than 1m wide (Ministry for the Environment, 2020). The policy, however, does not require exclusion of sheep from these waterways. Until recently the behaviour of sheep around natural waterways has received little attention, but there is now a need to understand these interactions. Commonly studies of sheep behaviours include grazing and resting (Jørgensen *et al.*, 2009), social interactions (Veissier *et al.*, 1998; Fisher and Matthews, 2001) and reproductive behaviours (Lindsay, 1996). The current study employed the use of digital technologies to monitor the behaviour of sheep (grazing, resting, drinking etc.) including video cameras, tri-axial accelerometers and global positioning systems (GPS).

In Chapter six the effects of paddock topography, particularly slope and aspect on the spatial distribution of sheep across winter, spring and summer were also assessed. Aspect and slope are known to affect the microclimate of a site by altering the amount of solar energy input and wind speed due to their orientation, tilt angle, and subsequent shading effects (Oke 1978). Extremes of air temperature, rain and wind can make sheep uncomfortable and motivate them to seek shade or shelter (Munro 1962; Stafford *et al.*, 1985; Mysterud *et al.*, 2007). Inactive behaviours such as lying and standing increase when exposed to higher rates of solar radiation (Sevi *et al.*, 2001). Spatial variation in slope and aspect, therefore, are key determinants of vegetation pattern, plant species distribution and ecosystem processes (Zheng, 2014; Yang, 2020).

In summary, the aims of the studies in this thesis were to gain a clearer understanding of the behaviour of sheep around a natural waterway and determine their impact on measures of water quality. In addition, the impact of access to a reticulated water trough was investigated. The effects of slope and aspect on the spatial distribution of sheep across three seasons were also investigated. Briefly, Chapters 3, 4 & 5 examined the behaviour of sheep around a natural waterway when access to a water trough was either unrestricted or restricted. The three chapters also assessed the potential impacts of sheep on water quality. Chapter 6 further investigated the effects of slope and aspect on the spatial distribution of sheep across all three seasons (winter, spring, and summer).

7.2 Summary of main findings and conclusions

7.2.1 Animal density and spatial distribution

7.2.1.1 Slope and aspect

Within the paddock, areas with a slope of less than 15° contained more than half of the sheep location fixes in both winter (Chapter 3) and spring (Chapter 4). In summer only 32% of the sheep locations during the study period were recorded in areas with a slope of less than 15° with more than 65% in areas with a slope between 16° and 35° (strong rolling, moderately steep and steep). Across all three seasons, fewer than 6% of GPS location fixes were detected in areas with slopes greater than 35° (very steep).

These findings are in agreement with a number of studies of sheep which reported a preference for grazing slopes <30° particularly in winter and spring (Sheath, 1982; Haddon, 2008; Steer, 2012). This spatial preference may be due to higher soil fertility and result in greater pasture availability in these flatter areas compared to steeper slope classes (Saggar *et al*., 1990; López *et al*., 2003). The greater use of steep slopes by sheep in summer may have been due to the influence of higher ambient temperatures than experienced in winter or spring which resulted in sheep seeking shade and/or shelter (Munro 1962; Stafford *et al*., 1985; Mysterud *et al*., 2007).

Aspect influenced the distribution of sheep across the paddock similarly across all three seasons. South facing slopes recorded the highest number of sheep location fixes (42-48%) in all the three seasons followed by north facing slopes (32-35%). The west-facing areas were the least utilised (8-9%). The northern and southern aspects were likely preferred due to their ability to accumulate greater herbage masses than other aspects (Radcliffe *et al*., 1968; Radcliffe 1971; Suckling 1975; Bircham 1976; Lambert and Roberts 1978; Gillingham 1973, 1998). Gillingham *et al*., (1998) reported that south and north facing slopes had greater soil moisture levels across the seasons which were likely to support higher herbage accumulation (Gillingham *et al*., 1998). Conversely, Lambert and Roberts (1976), reported that soil moisture deficits occurred on northern aspects during summer in New Zealand due to higher levels of solar radiation with frequent strong northerly winds (Radcliffe *et al*., 1968).

7.2.1.2 Influence of season

Changes in weather conditions influence herbage quality and consequently the spatial distribution of animals. When studying animal density and spatial distribution, correlations between the number of ewe location fixes and hourly weather parameters on the east facing slopes showed a negative correlation with solar radiation and a positive correlation with relative humidity in winter. Average temperatures were lower in winter (4.8°C) than spring (13.2°C) and summer (17.2°C). In spring, there was a negative correlation was between location fixes and solar radiation on the south facing slopes. In summer, there was a positive correlation between location fixes and wind speed. In addition, there was a positive correlation between location fixes and wind speed on both the south facing and north facing slopes. There was also a negative correlation between location fixes and average humidity in the north facing slopes. These results suggest that the relationship between weather and location fixes was variable between seasons and showed no consistent trends.

Generally, in chapters 3, 4 and 5 sheep were observed to largely avoid the very steep slopes (>36°), however, season appeared to influence the utilisation of different slopes categories. For example, utilisation of these very steep slopes was greater in summer and winter than in spring.

The prevailing winds recorded across each season were predominantly westerlies and north easterlies, thus, it was likely that sheep preferred the south aspect slopes, especially in winter, to shelter from strong winds during winter (Grubb and Jewell, 1966; Sibbald *et al.*, 1996). In summer, however, increased use of steep slopes may have been to take advantage of shade.

7.2.2 Water quality

The flow rate of a stream directly affects the concentration of oxygen dissolved in water and the concentration of nutrients (Dou *et al.*, 2018). Flow rates in the studies included in this thesis were highest in winter followed by spring and the least during summer. In addition, the flow rate in both summer and spring was higher during the water trough unrestricted period compared to the restricted period which was primarily due to rainfall during these periods. Flow rates of streams draining dairy-grazed pastoral catchments in New Zealand have been reported to be generally higher in winter than in other seasons (Wilcock *et al.*, 1999). Concentrations of nutrients and pathogens have often been reported to be associated with flow rate for example in summer faecal bacteria were negatively correlated with flow rate (Wilcock *et al.* 1999). In this thesis the difference in nutrient and pathogen concentrations between the inflow and outflow of the paddock included nitrate-N, *E. coli*, suspended sediment, ammonium, and total phosphorus. In summer, results showed that ammonium concentrations were higher during the water trough restricted period, compared to unrestricted period. It was unlikely that rainfall would explain the higher ammonium values in summer as the flowrate was positively correlated with rainfall, resulting in a higher flowrate when access to the water trough was restricted compared to unrestricted. Higher ammonium concentrations may have been due to dissimilatory nitrate reduction to ammonia where microorganisms such as *E*. coli, first reduce nitrate to nitrite and then to ammonium coupled by favourable temperatures (10-27ºC) and humidity (64-88%) which were observed in the summer study.

Nitrate-N concentrations recorded in the studies in this thesis were highest in winter followed by spring and lowest in summer. A similar pattern was observed in previous studies in New Zealand (Hoare, 1982; Wilcock *et al*., 1999). Nitrates accumulate in soils during drier seasons i.e., summer and autumn and are leached through the soil profile following sufficient rainfall and then enter streams through groundwater in winter (Yevenes and Mannaerts, 2011). Thus, most nitrate-N leaching from soil is likely to occur during late autumn and winter (Cameron *et al*., 2002).

E. coli concentrations were highest in the summer study (Chapter 4) followed by spring (Chapter 3) and the least during winter (Chapter 2). In summer *E. coli* concentrations were higher during the water trough unrestricted period compared to the restricted period. Similar findings were reported in the United Kingdom where *E. coli* concentrations were highest in the summer months when stocking rates were increased and lowest in the winter months (Hunter *et al*., 1999, 2000). Whitman *et al*., (1995) further observed that *E. coli* concentrations increased following rainfall events in summer and concluded source was local such as sediment suspension or runoff from soils. The lowest *E. coli* values were recorded in winter and may have been due to the land stores being progressively depleted with intense and frequent rainfall (Hunter and McDonald, 1991). The Ministry for the Environment (2003) water quality guidelines for freshwater recreation recommend that the *E. coli* concentration of waterways should be less than 130 cfu/100ml. In the current study 83%, 25% and 92% of samples exceeded this concentration in summer, winter, and spring, respectively. This finding is perhaps not surprising as Joy (2015) reported that *E. coli* concentrations exceeded contact recreation standards in 62% of all New Zealand water bodies.

Suspended sediment loads varied between treatments. Suspended sediment loads were found to be higher in the period ewes had unrestricted access to the water trough than when access was restricted in winter. Suspended sediment loads did not differ between treatment in spring. Suspended sediment loads were also higher at the outflow than inflow site on some days during winter. Based on the hotspot analysis it was during the unrestricted period that there was greater spatial clustering of ewes. Treading by grazing animals on stream banks results in the degradation of the physical quality of soils under pasture and formation of sediment into the stream (Da Silva *et al.*, 2003; Parkyn and Wilcock, 2004; Wilcock, 2006). Greater sediment load in outflow than inflow water samples on some days may have been the result of rainfall on previous days generating surface runoff into the stream (approximately 5 to 24 hours prior to water sampling). Moreover, winter accounts for 85% of run-off and the dominant process is the sedimentation of larger particles (Jensen *et al.*, 2006).

7.2.3 Behaviour

Behaviour of sheep has previously been found to change according to the season (Dhanda and Singh, 2002; De *et al.*, 2017). In this thesis the proportion of time sheep spent undertaking behaviours such as grazing, walking, standing, lying, sniffing, walking in the stream and drinking was similar across all seasons. The exception was that in summer and spring sheep spent more time stationary and more time drinking than in winter. In all the seasons except summer, sheep spent more than 60% of their time grazing, which is in agreement with Filipčík *et al.*, (2020). Sheep spent more time resting in summer than in winter or spring which may have been due to increase in environmental temperature resulting in a decrease in time spent grazing (Shinde *et al.*, 1997).

In summer and spring ewes spent more time drinking from the stream than in winter. This might be due to the quality and moisture content of pasture which then influenced the motivation of sheep to drink (Macfarlane *et al.*, 1958; Macfarlane *et al.*, 1966a; Forbes, 1968). Feed dry matter intake has been reported to be associated with water intake (Calder *et al.*, 1964; Forbes, 1968; Jaber *et al.*, 2004). In the winter and spring, when the moisture content of the pasture was around 70 %, free water was not necessary for sheep (Brown and Lynch, 1972).

Video footage from the current study showed that ewes avoided walking through the waterway, instead preferring to cross the stream by jumping over it or using culverts. This behavior may be explained by several factors, including an aversion to water and thermal influences, which can trigger a fear response in sheep (Hales, 1982; Askey-Doran, 1999; Dymond et al., 2016). The observed use of culverts rather than direct water crossings indicates that crossing structure design and placement can strongly influence livestock movement patterns, highlighting the need to strategically locate culverts to support efficient paddock utilisation and improve overall grazing distribution.

The findings of this study demonstrate that sheep exhibited minimal interaction with the stream environment, and there was little evidence of any consequential effect on measured water quality parameters. These results challenge the assumption that blanket livestock exclusion policies necessarily yield uniform environmental benefits. In the context of Action for Healthy Waterways (2020), which advocates for the fencing of waterways to improve water quality, the present findings suggest that such measures may not be proportionately effective when applied to sheep. Accordingly, management strategies should consider species-management efforts may be more effectively directed toward higher-impact animals, specific behaviour and relative environmental impact to ensure that resources are allocated toward interventions likely to produce the greatest ecological benefit i.e. management efforts may be more effectively directed toward higher-impact animals.

7.2.4 Limitations

A number of water quality and paddock parameters such as water and soil temperature and soil moisture among others were not included in this study due to limited resources and time constraints. The amount of water that livestock drink has been reported to be associated with water temperature (Markwick, 2007; Huuskonen *et al*., 2011) with intake increasing as drinking water temperature increases (Ittner *et al*., 1951). Cold water has been reported to reduce respiratory rate and body temperature (Wilks *et al*., 1990). Soil temperature is crucial in the survival of *E. coli* (Ishii *et al*., 2006). Therefore, the inclusion of these parameters could provide an improved dataset as far as the behavioural and water quality study is concerned.

The Battery life of the GPS and Actigraph devices required they be retrieved to download data and replace or recharge batteries every 14 days thus limiting the duration of the study. Remote downloading of data has the potential to extend the period of similar studies. This type of system, however, requires additional infrastructure at the study site such as base stations or wireless data communication methods which were not available at the time of these studies. There is also potential for systems that utilise photovoltaic systems or power banks, however, given the relatively small size of sheep restrictions of weight and size of the devices may make this unfeasible at this time.

Each of the studies in this thesis were conducted over 2-weeks. Weather conditions including temperature and rainfall were not evenly distributed within the respective seasonal periods, therefore, extending the study periods to half of the particular season could have improved the assessment of the impact of weather conditions and provide more robust conclusions. The GPS and actigraph battery life, however, limited the duration that each study could be conducted.

The studies included in this thesis were conducted in a temperate environment which limits the extrapolation to drier areas. Repeating these studies in a dry environment could yield different results as the water quantity requirements of sheep depend on factors such as the amount of moisture available in the forage, and environmental factors such as air temperature and humidity (Forbes, 1968). Heat exposure can also affect water intake by increasing water consumption (Giger-Reverdin and Gihad, 1991), hence a dry environment could likely influence sheep to access the riparian zones for drinking and grazing.

The effect of breed was not considered in this study. Romney sheep were used, however, breeds differ in terms of behaviour, adaptability and productivity in different management systems and climatic and agro-ecological conditions. Hence, repeating the study using a different breed other than Romneys could strengthen the conclusions drawn and allow the effect of breed on animal behaviour to be explored.

An autumn study was not included due to Covid 19. Autumn in New Zealand is characterised by cooler temperature and shortened day light. Incorporating the autumn study would make a good conclusion of the sheep behaviour the whole year round in New Zealand.

The behaviours monitored by cameras in the present study were confined to the stream zone. This limitation arose due to the need to capture high-resolution still images or high-definition videos capable of recording fine details such as spray marks and collar numbers. To achieve the required image clarity, the motion detection range of the cameras was focused to a certain distance, providing optimal vision within approximately 15 metres. As a result of these constraints, the behaviours recorded and analysed in this study may not be representative of activities occurring throughout the entire paddock. The focus on the stream zone potentially excludes a range of behaviours exhibited elsewhere in the paddock environment. To obtain a more comprehensive dataset, increasing the number of cameras and improvement in terms of camera technology and including zones beyond the stream area for behavioural observation would likely yield a broader and more representative range of results.

7.2.5 Practical implications and recommendations for future research

Most studies of livestock behaviour around waterways have been conducted with cattle. The results of this thesis have provided some initial data on the behaviour and impacts of sheep, but more studies are needed. Based on these results, sheep showed minimal interactions with the stream and there was little evidence of any impact on water quality. The fencing of waterways to prevent sheep access is therefore unlikely to improve water quality compared to the exclusion of pigs, cattle, and deer. Further evaluation and research are needed particularly to determine the impacts of sheep on stream bank erosion. Trials in this thesis focused on behaviour around natural waterways and the impact of sheep on water quality. One of the of the ways sheep can impact waterways is through damage to stream banks causing erosion which was not investigated in this stud. Erosion of channel banks has been reported to be a source of sediment and phosphorus pollution which can affect water quality (Davis *et al*., 1998; Wallbrink *et al*., 2003; Kevin *et al*., 2008). It would be of interest to evaluate the extent of erosion caused by sheep on hill country farms. This will help inform future developments of the New Zealand government's stock exclusion policy.

Ewes showed a spatial preference for flat and low sloped areas of the paddock. The use of culverts to cross the waterway appeared to be driven by ewes attempting to avoid walking in water. This preference for using culverts may be useful to help managers reduce the impacts of sheep by strategic placement of culverts, water sources and other paddock features on areas preferred by sheep such as lowlands, near crossings and those with green pasture.

It would be interesting for policy makers to learn if there is an effect of stocking density on the behaviour of sheep in and around the waterway. This was not covered in this study; however, the expectation is that high sheep stocking rates would lead to concentrated grazing, trampling, and soil and streambank effects, water quality impact, altering sheep behaviour and ecosystem function.

Findings from this thesis indicate that there is currently limited empirical evidence to accurately quantify the specific impact of sheep grazing on water quality parameters studied. Longer term studies and in a range of different environments are required to verify these results, especially to confirm whether the observed impacts were due to rain events or the presence of sheep.

7.2.5.1 Recommendation for policy makers

The study suggests that the placement and design of crossing structures such as culverts in sheep grazing areas are likely to influence the impact of sheep on the water quality of natural waterways. The development and disseminate of clear guidelines for the design and placement of crossing structures such as culverts in sheep grazing areas could improve livestock safety, grazing efficiency, and environmental protection. Culverts can be strategically installed at natural stream crossings on gentle slopes frequently used by sheep, as well as away from sensitive riparian zones. Such installations may serve to reduce livestock impacts on water quality, mitigate soil erosion, and enhance both grazing efficiency and animal safety. Additionally, placing culverts at regular intervals along heavily grazed streams can prevent the overconcentration of sheep in specific areas, thereby protecting streambanks and maintaining ecosystem integrity.

In the context of Action for Healthy Waterways (2020), which advocates for the fencing of waterways to improve water quality, the present findings suggest that such measures may not be as effective for sheep. Sheep generally exert lower physical pressure on stream banks and beds because they are lighter and cause less trampling damage. They also tend to enter waterways less frequently than cattle, which are more likely to stand in streams for drinking or cooling. As a result, the direct contributions of sheep to bank erosion, sediment disturbance, and nutrient loading are comparatively smaller.

7.2.6 Overall summary and conclusions

The studies included in this thesis have led to the following conclusions:

- There was little evidence that sheep interacted with the natural waterway and had little impact on water quality parameters.
- Some water quality parameters were recorded to be above guideline concentrations on some days, however, these appeared to be unrelated to the presence of sheep in the paddock, instead to rain events.
- The degree of interaction of sheep with the waterway was not influenced by the availability of reticulated water from a trough.
- Study ewes showed a spatial preference for flat to low sloped areas of the paddock.
- Sheep utilised south and north facing slopes more than other aspects.

- Solar radiation, relative humidity and wind speed appeared to be the main weather parameters to influence the spatial distribution of sheep in the paddock across three seasons.

The current study indicates that sheep generally have little impact on waterways, although most NZ hill country farms include a mix sheep and cattle (Morris and Hickson 2016; Anon 2020). In New Zealand, it is a requirement to fence all cattle from waterways (Anon 2020), however there may be an opportunity to allow sheep to access waterways to help reduce the accumulation of forage and reduce any potential increase in weed species.

Results from this study have contributed to the knowledge of sheep behaviour around natural waterways. The results are crucial for future decision making related to management of sheep in and around riparian zones (waterways). It will help both farmers and government agencies to develop appropriate management guidelines and mitigation measures.

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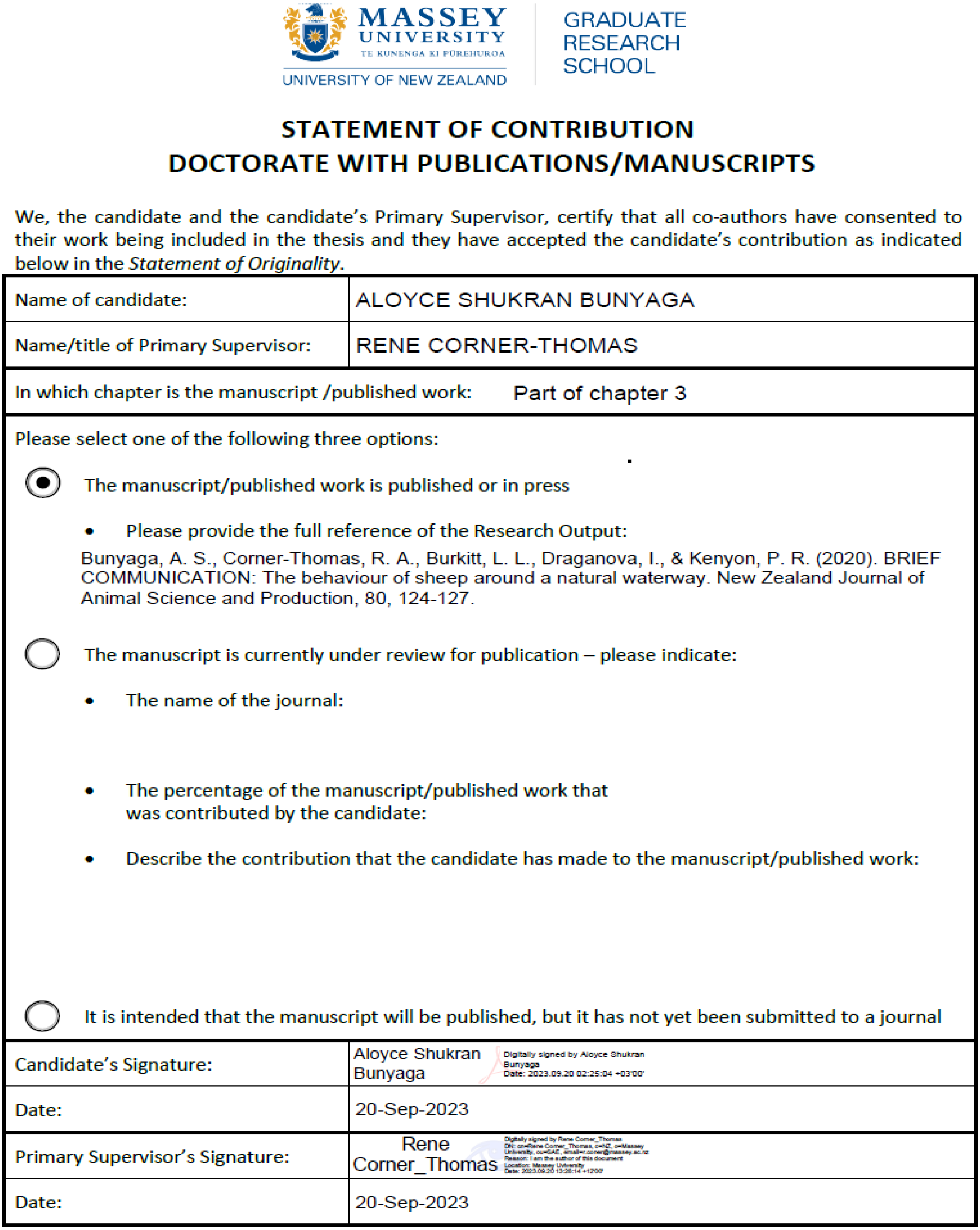
8.1 Appendices

Appendix 1



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appendix 2

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Appendix 3

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Appendix 4

Table 1. Slope selection in winter based on Manly's selection ratios (Wi) and Bonferroni-adjusted confidence intervals (α = 0.05). Availability proportions falling outside confidence intervals indicate significant selection or avoidance

Slope	Proportion Used	SE	Wi	Bonferroni CI (Use)	Availability	Interpretation
Flat	0.250	0.0040	1.33	0.239 – 0.261	0.188	Selected
Undulating	0.271	0.0041	1.82	0.260 – 0.282	0.149	Strongly selected
Rolling	0.166	0.0034	0.96	0.158 – 0.174	0.173	Neutral (overlap)
Strong rolling	0.080	0.0025	0.66	0.074 – 0.086	0.121	Avoided
Moderately steep	0.064	0.0024	0.61	0.059 – 0.069	0.105	Avoided
Steep	0.150	0.0034	0.71	142 – 0.158	0.210	Avoided
Very steep	0.018	0.0012	0.33	0.016 – 0.020	0.055	Strongly avoided