Quantifying the frequency and volume of urine deposition by grazing sheep using tri-axial accelerometers

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Abstract

Urine patches deposited in pasture by grazing animals are sites of reactive nitrogen (N) loss to the environment due to high concentrations of N exceeding pasture uptake requirements. In order to upscale N losses from the urine patch, several urination parameters are required, including where, when and how often urination events occur as well as the volume and chemical composition. There are limited data available in this respect, especially for sheep. Here, we seek to address this knowledge gap by using non-invasive sensor-based technology (accelerometers) on ewes grazing *in situ*, using a Boolean algorithm to detect urination events in the accelerometer signal. We conducted an initial study with penned Welsh Mountain ewes (n = 5), with accelerometers attached to the hind, to derive urine flow rate and to determine whether urine volume could be estimated from ewe squat time. Then accelerometers attached to the hind of Welsh Mountain ewes (n = 30 at each site) were used to investigate the frequency of sheep urination events (n = 35 946) whilst grazing two extensively managed upland pastures (semi-improved and unimproved) across two seasons (spring and autumn) at each site (35 to 40 days each). Sheep urinated at a frequency of 10.2 ± 0.2 and 8.1 ± 0.3 times per day in the spring and autumn, respectively, while grazing the semi-improved pasture. Urination frequency was greater (19.0 ± 0.4 and 15.3 ± 0.3 times per day in the spring and autumn, respectively) in the unimproved pasture. Ewe squat duration could be reliably used to predict the volume of urine deposited per event and was thus used to estimate mean daily urine production volumes. Sheep urinated at a rate of 16.6 mL s^{-1} and, across the entire dataset, sheep squatted for an average of 9.62 ± 0.03 s per squatting event, producing an estimated average individual urine event volume of 159 ± 1 mL (n = 35 946 events), ranging between 17 and 745 mL (for squat durations of 1 to 45 s). The estimated mean daily urine volume was 2.15 ± 0.04 L (n = 2 669 days) across the entire dataset. The data will be useful for modelling studies estimating N losses (e.g. ammonia (NH_3) volatilisation, nitrous oxide (N_2O) emission *via* nitrification and denitrification and nitrate (NO_3^-) leaching) from urine patches.

Key words: Sensor, Pasture, Urination, Livestock, Nitrogen cycle

Implications

The study provides a large dataset on the frequency, individual urine event volume and daily urine volume production for Welsh Mountain ewes grazing *in situ*. This is expected to be useful for those wishing to model or measure (e.g. providing information to accurately simulate individual urine events in the field) N losses from sheep-grazed agroecosystems, including NH_3 volatilisation, N_2O emissions and NO_3^- leaching. Ultimately, this will improve the accuracy of N pollution estimates from sheep grazed agroecosystems.

Introduction

The urine patches of grazing animals are well recognised as hotspots of nitrogen (N) losses to the environment, due to the high N content of urine, resulting in loadings which exceed the uptake requirements of the pasture (Selbie et al., 2015). To up-scale N losses from grazing animals to the landscape level, information on the timing and season of deposition, frequency, total urinary volume, chemical composition and total urinary-N excretion are needed. Typical published datasets for sheep urination have been of limited size, assessing low numbers of replicate animals and replicates of individual urine events. Here, we seek to address this knowledge gap via the novel application of a non-invasive sensor-based technology on ewes grazing *in situ*.

Urination event data can be collected in a variety of ways, each with advantages and disadvantages. Urine can be spot-sampled from individual animals (e.g. *via* obstruction of sheep nasal and oral passages, or by stroking the side of the vulva of cattle, to stimulate the animals to urinate; Hoogendoorn et al., 2010). However, such procedures may raise animal welfare issues. Indeed, this approach to spot-sampling urine allows collection of samples for assessing urine chemical composition, but not natural frequency and volume, and cannot be considered non-invasive (Kurien et al., 2004). Urination data has also been collected from animals held in urine collection pens or metabolism crates, which allows urine events to be collected individually (e.g. Bratzler, 1951; Dick and Mules, 1953; Marsden et al., 2017, 2020). Recently, Marsden et al. (2020) analysed nearly 200 urine events from six replicate sheep in urine collection pens, assessing urine frequency, volume and chemical composition. Collecting urination datasets using these methods is thus not only challenging but it also precludes the natural behaviour of grazing animals. This highlights the need to obtain data from animals grazing *in situ*.

In contrast to the methods described above, animal-attached sensor-based logging systems can be used to determine the behaviours of free-roaming sheep, or cattle. For example, urine volume has been detected using flow-meters (Ravera et al., 2015) and thermistors (Betteridge et al., 2010a, b; Draganova et al., 2016). Here, the flow-meter weighed approximately 100 g and required an attachment to be glued to the skin around the vulva of cattle, which initially affected the animals' behaviour (Ravera et al., 2015). The thermistor-based system was housed in a silicon tube suspended below the vulva of the animal, with a data logger attached intra-vaginally. This was then coupled with a Global Navigation Satellite System (GNSS) attached to the wool of sheep or on a collar in cattle (Betteridge et al., 2010a, b). The total weight of the entire sensing unit, including batteries, was 545 g.

Accelerometers have already been used to measure grazing, ruminating, lying, walking, running and standing behaviours of sheep (Alvarenga et al., 2016; Giovanetti et al., 2017; Lush et al., 2018; Barwick et al., 2020) and, within our own programme of work, we have also detected urine volume and frequency using accelerometers (Lush et al., 2018). For the accelerometers described in Lush et al. (2018), the devices (mass 50 g) are glued to a shorn patch on the rump of the sheep. A major advantage of this approach is that animals do not need to be spot-sampled or held in crates. Additionally, the feed, water and environment found *in situ* are starting points for the urination process, all of which are modified when housing animals in crates. Sensor-based technologies can be used in combination with location tracking (e.g. using GNSS) to determine the spatial location and frequency of urination events in the field. Measuring urine N concentration in free-ranging animals is challenging but has been attempted in studies with cattle using refractive index sensors (Betteridge et al., 2013; Misselbrook et al., 2016; Shepherd et al., 2016). These sensors are fairly large, potentially affecting normal behaviour and are, anyway, not easy to implement. Therefore, although sensor-based technologies cannot provide information on urine chemical composition that is as detailed as can be collected from penned animals, this approach allows (many) monitored animals to roam and graze naturally.

In this study, we assessed the use of acceleration loggers, attached to the rump of free-roaming sheep, to understand sheep urination times, frequencies and durations using the methods of Lush et al. (2018) and Wilson et al. (2018) in two differently managed, extensively-grazed agroecosystems (semi-improved and unimproved) over two seasons (spring and autumn). We aimed to; i) ascertain the validity of tri-axial accelerometers and a Boolean algorithm analytical method for detecting urine events with a small subset of penned sheep, ii) determine if ewe squat duration is correlated with urine volume, which would allow urine volume to be estimated from tri-axial accelerometers only, and we hypothesised that iii) urine frequency and volume would differ by site and season due to differences in forage and ambient weather conditions, and iv) ewes would urinate less overnight than during the day simply due to reduced activity, as sheep often bed-down at night (Bowns, 1971; Sarout et al., 2018). Depending on the validity of the above steps, the data are projected to be of use in modelling production efficiency and N losses from grazing animals and highlight the much greater data resolution for urine frequency and volume that can be gathered from accelerometer-based technologies compared to other urine collection techniques.

Material and Methods

Initially, a urine collection trial was conducted with sensors (see below) attached to sheep contained within pens, to determine whether the systems could be utilised to predict urine frequency and volume under controlled conditions. Subsequently, four field studies were conducted over two sites (semi-improved and unimproved pastures), with two campaigns at each site (spring and autumn) using the sensors on grazing sheep to determine urination behaviour.

Logger attachment details and Boolean algorithm description

“Daily Diary” (DD) tags (Wildbyte Technologies Ltd., UK) were attached to a shaved area of the hind of Welsh Mountain ewes using a solvent-free epoxy adhesive (Fig. 1). These devices measured acceleration continuously across three orthogonal axes; X (surge), Y (sway) and Z (heave) with 12 bit resolution (range of -8 to 8 *g*) at 40 Hz, as detailed in Lush et al. (2018), to allow detection of the characteristic squatting posture exhibited by ewes during a urination event.

To quantify the time, frequency and duration of urination events in both the penned animal trial and the field-based studies (see below), we used a Boolean algorithm based on critical changes in acceleration recorded during urination (Wilson et al., 2018). This recognised that sheep urination involved the following time-linked processes: (i) the sheep stopped moving, then (ii) actively squatted, which took 0.4-0.8 s, then (iii) remained immobile for between 1 and 50 s during urination, before (iv) reversing the squatting process, again, taking 0.4-0.8 s to do so, as shown in Fig. 2. Recognition of squatting followed by standing up again was based on the differential of the static surge acceleration (smoothed over 0.5 s) exceeding 0.1 *g*/s (squatting) or being less than -0.08 *g*/s (standing up) while the smoothed (over 0.5 s) vectorial dynamic body acceleration (VeDBA – see Qasem et al., 2012 for definition) never exceeded 1 *g*. Immobility during actual urination was recognised by having a smoothed (over 0.5 s) VeDBA of less than 0.1 *g*. For a urination event to be recognised, all four processes had to occur sequentially within the defined times. Slope and topography specifically affect the static acceleration recorded by the accelerometers. The static acceleration is derived by taking a running mean of the raw acceleration over 2 s for each of the channels. The contribution of the slope to the three axes depends on animal angle (which mirrors slope angle in tags mounted on the rump) with respect to gravity. Thus, sheep facing up or down a slope have their bodies angled up or down, respectively, with respect to gravity, to an extent that is directly reflected in the static surge acceleration (which indicates body pitch angle). This is not problematic for identification of squatting during urination in sheep on flat pastures, but it cannot represent animals on slopes unless they are level. In order to correct for the slope-dependent surge axis, we specifically used the differential of the static surge acceleration as our cue for squatting because it is independent of slope (topography).

Assessing sheep urination duration as a proxy for urine volume

A urine collection study with penned barren Welsh Mountain ewes (n = 5; from the same flock as the later field campaigns) equipped with DDs was established to ascertain the validity of the urination detection algorithm (accuracy, precision and records of false positives or negatives) and to determine whether the duration of the urination squatting position could be used to estimate individual urine event volumes. Briefly, sheep with loggers were contained in urine collection pens (see Marsden et al., 2020 for details) and the start times of urination were recorded manually through direct observation with a stopwatch (although squat duration was not recorded). Feed was cut and carried to the sheep and free access to drinking water was provided. Sheep were typically housed between the hours of 10:00 and 16:00 daily for seven days. The individual urine event samples (n = 73) were collected and the volumes recorded from collection vessels installed below the slatted floor of each pen. The signals produced from the accelerometers were then analysed blind (i.e. without sharing the time of recorded urine events), using the Boolean-based algorithm described above to identify a urination event, to measure the duration of the squatting position and to determine whether there was a correlation with urine volume. In this way, known urination events were compared with the Boolean-identified events. We assessed the standard error of the estimate for the relationship between urine squatting duration with urine volume and subsequently used the duration of ewe squat times to predict the individual urine event volumes and the daily volumes of urine produced per ewe.

Field study sites and sensor deployment details

The field studies were conducted across two extensively-managed grazing areas. The first site was a semi-improved 11.5 ha upland (240-340 m asl) grassland at the Henfaes Research Centre (53°13′N, 4°0′W). The vegetation comprised a mosaic of grassland vegetation classified under the British National Vegetation Classification (NVC) scheme as U4 (*Festuca ovina* - *Agrostris capillaris* - *Galium saxatile* grassland) and MG6 (*Lolium perenne* - *Cynosurus cristatus* grassland; Rodwell, 2000). The second study site was an area of common grazing land (495 ha) on the Carneddau mountain range (322 - 943 m asl) within the Snowdonia National Park (53°22′N, 3°95′W), Wales, UK. The vegetation at the second site comprised NVC classification H12 (*Calluna vulgaris* - *Vaccinium myrtillus* heath; Elckington et al., 2001), interspersed with patches of acid grassland vegetation communities. Rainfall and air temperature were recorded at half-hourly intervals at the semi-improved site (Skye Instruments Ltd., Llandrindod Wells, UK). For the unimproved site, air temperature and rainfall data were sourced from a nearby (53°23′N, 4°0′W) COSMOS facility (Evans et al., 2016).

Barren Welsh Mountain ewes (n = 30) equipped with the DDs were deployed at the semi-improved field site in spring (12th May 2016 to 16th June 2016) and autumn (28th September 2016 to 3rd November 2016). The following year, a different set of sheep from the same flock (n = 30) were fitted with DDs and deployed at the unimproved field site in spring (31st May 2017 to 5th July 2017) and autumn (18th September 2017 to 28th October 2017). The mean ± SEM weights of the sheep at the beginning of the deployments were 33.5 ± 1.2 kg in spring and 39.7 ± 1.1 kg in autumn at the semi-improved site. In the unimproved site, the sheep weights were 41.6 ± 0.9 kg in spring and 38.0 ± 0.7 kg in autumn. The sheep were herded (on foot) up to the field sites by the shepherd after DD attachment. Data were recorded continually during the measurement campaigns, and the batteries (A cell, 3.6 Ah, 3.6V) allowed for data collection over the entire study periods (i.e. sheep were only gathered at the end of each study period). Acceleration data were downloaded from the SD cards and subsequently processed using the Boolean algorithm to record the time, frequency and duration of ewe urination events while grazing.

Field study data quality control and statistical analysis

Of the 30 DDs deployed in each season at each site, some initially failed, generally due to sheep rubbing against trees and dislodging the loggers from their rumps. Additionally, wool shedding in the spring contributed to detachment of some loggers. When the sheep had been recently sheared, the logger could be attached more securely because it was easier to shave a patch on the shorter wool of these animals. In future studies, it might be worth conducting shorter measurement campaigns and re-shaving and reattaching the logger to avoid this issue. The number of successfully recorded days of data also varied between sheep. Again, variation was caused by rubbing against trees or fences after a successful monitoring period. In addition, wool growth could result in an upward movement of the tag, increasing the length of wool binding the tag to the skin and causing noise in the accelerometer signal or resulting in the tag being re-orientated such that the surge channel (used to define squatting) stopped recording a reliable measure of true animal pitch. Given the manner by which DDs could be dislodged (see above), we expected, and saw, two basic types of failure. Data either initially failed completely or suddenly due to sheep rubbing their hindquarters against fences, or urination frequencies became irregular after a period of time due to wool growth problems. Thus, urination frequency graphs were inspected and if zero urination events were recorded on several consecutive days (> two days in a row) or when interspersed regularly throughout the data, we considered these unreliable and filtered them out.

Of the 30 loggers deployed in spring at the semi-improved site, there were nine initial failures (e.g. due to logger dislodgement not long after deployment or other sensor failures). One replicate sheep was removed due to several consecutive days of zero events interspersed throughout time, therefore indicating unreliable deployment (e.g. due to change in logger position due to rubbing). In two further replicate sheep, the data needed trimming, where loggers successfully recorded but stopped after a certain period of time, which was also due to logger dislodgement later on in the monitoring period. In autumn at the semi-improved site, there were seven initial failures and one replicate that was removed due to several consecutive zero event days and one replicate sheep data required trimming. In spring at the unimproved site, there were six initial failures and none were further removed or required trimming. In autumn at the unimproved site, there were eight initial failures and no further removal or data trimming was undertaken. The number of successful days of data recorded per sheep and days where zero events occurred are displayed in Tables S1-S4. The datasets from the successfully deployed sensors were also filtered to remove observations below 1 s of squatting duration (5 values removed), as we did not observe any squatting durations shorter than this in the penned animal study (the shortest duration directly observed was 1.9 s).

We calculated mean daily urination frequencies for each deployment and compared the spring and autumn seasons at the semi-improved site, the spring and autumn values at the unimproved site and the spring and autumn seasons between the semi-improved and unimproved sites using the Kruskal-Wallis rank sum test with pairwise comparisons *via* the Wilcoxon rank sum test in R (R Core Team, 2018). This nonparametric test was selected due to departures from normality and homogeneity of variance assumptions, precluding the use of ANOVA. The same procedures were followed for squatting duration, estimated individual urine event volume data and daily mean urine volume data. The individual event volume was estimated by using the urination rate derived from the squatting time *versus* urine volume regression, where urination rate was used as a multiplier.

Results & Discussion

Calibrating loggers to determine individual urine event volumes

In the experiment with the logger-tagged penned sheep, 73 individual urine events were analysed (Table S5). The mean ± SEM duration of squatting during urination (as recorded on the accelerometers and identified with the Boolean algorithm) was 11.9 ± 0.7 s. The Boolean algorithm successfully identified the urination events with 100% accuracy. We also did not record any false positives or negatives within this dataset and therefore concluded the algorithm should work well for the field-based deployments. Although we manually recorded the start time of the urination events occurring in the pens, we did not assess the accuracy of the start and finish times for the duration of the squatting posture. Some initial filming work was conducted for system validation for urination events under grazing conditions (see Lush et al., 2018), however, this was conducted immediately following DD deployment in the pen trials and therefore provides a poor indication of what may happen to sensing capacities of the DDs after extended deployment durations (30 days). In future work, filming the sheep (both in pens and while grazing) at several points over the deployment duration would allow for precise recording of the urination duration and may help to further validate the accuracy of our algorithm. It proved challenging to record observations of sheep urination under variable terrain conditions but we could at least ascertain that the ‘squatting’, ‘holding still’ and ‘unsquatting’ procedure was the same, irrespective of whether sheep were in a pen, a level field or in variable terrain (and variable length vegetation). Care had to be exercised when apparent urination rates in some individuals dropped after some time because this indicated an unstable tag attachment. We note, however, that all animals that manifest this, did so as a function of tag wearing time, never the reverse. For this reason, we believe that our filtered and cleaned results give representative urination metrics.

A strong linear relationship was found between the duration of ewe squatting time and the volume of urine produced (Fig. 3; $R^2 = 0.89$). Some inter-individual variation was observed e.g. sheep 4 squatted, urinated and returned to standing position quicker than others, and sheep 2 was particularly slow in squatting and returning to standing, resulting in some scatter around the best-fit line. Here, the standard error of the estimate (a measure of the uncertainty around the linear model when using it to make new predictions) was 80 mL. There could be several reasons behind inter-individual variability in the urine flow rate as recorded in this study e.g. potential ill health such as incontinence, differences in age or contrasting bladder size. In future work, we suggest individual sheep-specific calibration to allow an improvement in the estimation error for individual urine event volumes and to account for this inter-individual variability, and to account for differences in these values in different breeds or class of livestock.

Field trial weather data

Weather data for the studies involving sheep equipped with loggers at the semi-improved and unimproved sites are displayed in Figures S1 and S2. Briefly, at the semi-improved site, the mean air temperature was 12.3 °C in spring and 10.4 °C in autumn. The cumulative rainfall was 60 mm in spring and 86 mm in autumn. For the unimproved site, the mean temperature was 13.6 °C and 11.7 °C in spring and autumn, respectively. The cumulative rainfall totals at the unimproved site during the measurement campaigns were 163 mm and 211 mm in spring and autumn, respectively.

Loggers attached to free-roaming sheep: data description and summary

Across all four deployments, the number of successfully recorded urine events was n = 35 946 after accounting for failed loggers, data filtering and cleaning. Of the 30 loggers deployed in each season at the semi-improved site, data were successfully recorded for 20 sheep in the spring deployment and 22 sheep in the autumn deployment. At the unimproved site, of the 30 deployed loggers, data were successfully recorded for 24 sheep in spring and 22 sheep in autumn. The number of successful days of data recorded for each replicate sheep are displayed in Tables S1-S4, alongside the number of days where zero urination events were recorded. Zero urination events in one day are physiologically unlikely, therefore they serve as an indication of false negatives in the dataset.

Urination frequency by site and season

The mean daily urination frequencies are displayed in Figure 4 for both seasons at each study site. The overall daily mean ± SEM urine frequencies across all sites and seasons are displayed in Table 1, with significant differences displayed in columns. The Kruskal-Wallis rank sum test revealed significant differences between all sites and seasons for urine frequencies ($\chi^2 = 576$, df = 3, $p < 0.001$). The mean urine frequency was significantly lower in autumn than spring for the semi-improved and unimproved sites ($p < 0.001$ in both cases). We recorded *ca*. two less urine events animal^{-1} day^{-1} in autumn than in spring at the semi-improved site and *ca*. four less urine events animal^{-1} day^{-1} in autumn than in spring at the unimproved site. Urination frequencies were significantly higher in the unimproved site than the semi-improved site in spring ($p < 0.001$) and autumn ($p < 0.001$), being approximately double that of the semi-improved values in both cases. The identification of site and seasonal differences in urination frequencies supports the collection of site and seasonal data for up-scaling estimates of associated N losses from urine patches.

For the semi-improved site, urination frequencies can be compared to the study of Marsden et al. (2020) which was conducted with sheep grazing the same site in the same year, which were then housed in urine collection pens for periods of the day. The mean urine frequency from this study was 9.7 ± 0.7 urine events animal^{-1} day^{-1}, similar to that reported here (10.0 ± 0.2 and 8.1 ± 0.3 urine events animal^{-1} day^{-1} in spring and autumn, respectively). This suggests that containing animals in these pens may not greatly affect the observed frequency of urination events. The urination frequencies were much higher at the unimproved site than the semi-improved site in both seasons. However, the reason for this difference is currently unclear. The diversity of plants and the potential for browsing on contrasting forages was potentially greater at the unimproved site, where it is possible that secondary plant compounds such as terpenes, phenolics and alkaloids in the feed had a diuretic effect (Dearing et al., 2001). A diuretic effect has been directly observed elsewhere for plantain-fed sheep (O’Connell et al., 2016). At both sites, the sheep had access to natural water sources, which sheep may have visited to drink. There was, however, 2-3 times more rainfall at the unimproved site, providing the potential for more water ingestion from wet vegetation. There were not large differences in temperature between the sites and seasons, which had the potential to influence urine frequency. Our frequency data at the unimproved site compare well with sensor-based logging of sheep urine frequency in New Zealand hill country pasture: Betteridge et al. (2008) reported 20.6 urination events day^{-1} and Betteridge et al. (2010b) reported 17-18 events day^{-1}. These values also agree with the range of 18-20 urination events day^{-1} reported in Haynes and Williams (1993).

Urine squatting duration by site and season

Across the entire urine dataset (n = 35 946 events), the mean ± SEM squatting duration was 9.62 ± 0.03 s. The mean squatting duration split by site and season, alongside the statistical groupings based on the Wilcoxon rank sum test are displayed in Table 1. The overall Kruskal Wallis test revealed a significant effect of site and season on urination squatting duration ($\chi^2 = 237$, df = 3, $p < 0.001$). The squatting duration was significantly shorter ($p < 0.001$) in the autumn than in spring at the semi-improved site. The squatting duration was longer at the unimproved site than in the semi-improved site in both spring ($p < 0.001$) and autumn ($p < 0.001$). Squatting durations were similar ($p > 0.05$), however, when comparing between the spring and autumn at the unimproved site. Whilst different, the numerical values for squatting duration are broadly similar for this large dataset. To our knowledge, there have been no previous studies assessing the squatting duration of ewes via accelerometers in the literature.

Estimates of individual urine event volumes

Using the linear formula for the estimation of individual urination event volume derived from logger-tagged penned animals (Fig. 3), we estimated urine volumes of the free-roaming sheep based on their squatting durations. Across the entire urination event dataset, estimated individual urine event volumes ranged from 17 – 745 mL. The 25th, 50th and 75th percentiles for individual urine event volumes were 95, 125 and 177 mL, respectively. The frequency distribution of all estimated individual urine event volumes is displayed in Figure 5. This shows a similar distribution shape to that for urine volume produced by Betteridge et al. (2010a) for cattle, except that the individual urine event volumes were around ten times higher in the cattle, peaking at 1.5 L.

The mean ± SEM estimated individual urine event volume was 159 ± 1 mL across the entire urine event dataset (n = 35 946 individual urine events). This is close to the average urine volume of 150 mL for individual sheep urine events reported by Haynes and Williams (1993) and Doak (1952), which suggests that using a urine volume size of 150 mL in sheep urine patch studies is appropriate. Our data corroborate those of Haynes and Williams (1993), but are based on a far greater number of individual urine event replicates providing a much more robust data set. Importantly, the urine volume observed in another study employing pens at the same semi-improved site (Marsden et al., 2020) reported a much larger mean urine event volume of 289 ± 14 mL. This suggests that containing animals in pens may influence the volume of individual urine events but not the frequency. This may be linked to the fact that sheep have an ample supply of feed and water in pens, or that the reduced sheep movement in pens somehow causes this, or it may even be an artefact of the fight/flight response due to frequent close contact with people. The broad distribution of urine event volumes suggests that both smaller and larger urine events occur (although less frequently) up to a maximum of approximately 745 mL. It would, therefore, be useful to investigate how this range of volumes (and associated urine patch sizes) influences associated N fluxes (e.g. NH_3 volatilisation, N_2O emissions *via* nitrification and denitrification and

NO_3^- leaching). It should be noted that urine N concentrations at higher volumes are likely to be lower (Marsden et al., 2020) which may potentially result in lower N losses.

The estimated mean individual urination event volumes split by site and season can be seen in Table 1. Results of the Kruskal-Wallis test revealed a significant effect of site and season on individual urine event volumes (χ^2 = 237, df = 3, $p < 0.001$). Individual urine event volume was significantly smaller ($p < 0.001$) in autumn than in spring at the semi-improved site. When comparing between sites, the individual urine event volume was significantly larger at the unimproved site than the semi-improved site in both spring ($p < 0.001$) and autumn ($p < 0.001$). There were no significant differences ($p > 0.05$) in the estimated individual urine event volumes between DDs at the unimproved site. Although a significant seasonal difference in individual urine event volumes was recorded at the semi-improved site, the magnitude of this difference (11 mL) was minimal, and we believe unlikely to influence N losses from urine patches in any meaningful way.

Estimates of daily urine event volumes

Mean daily urination event volumes were calculated by summing the individual event volumes sheep^{-1} day^{-1} (displayed in Figure 6). Across the entire dataset, the mean daily urination event volume was 2.15 ± 0.04 L (n = 2 669 days). The mean daily urine volumes split by site and season are reported in Table 1. A significant effect of site and season was identified by the Kruskal-Wallis rank sum test for daily urine event volumes (χ^2 = 302, df = 3, $p < 0.001$). The pairwise Wilcoxon rank sum test revealed significant differences for all sites and seasons (all $p < 0.001$). The mean daily urine volume followed the trend; semi-improved site in autumn < semi-improved site in spring < unimproved site in autumn < unimproved site in spring.

The daily mean volumes ranged between 0.09 and 4.94 L across all deployments. Betteridge et al. (2010b) suggest changes in daily urine volume are linked with the animal coping with changes in ambient temperature, however, we found no relationships with daily urine variations and weather patterns. Our values for daily urine volume are in good agreement with other studies in the literature employing sheep in metabolism crates. For example, Marcilese et al. (1970) report a range of daily urine volumes between 1.65 and 3.75 L, with an average of 2.75 L. Sheep fed ryegrass or plantain in metabolism crates excreted 2.9 - 4.6 L of urine per day in O’Connell et al. (2016). Ledgard et al. (2008) reported daily urine volumes of 0.5-3.0 L per sheep per day, Doak (1952) reported a mean daily urine volume of 2.9 L per sheep per day and Marsden et al. (2020) report a mean daily volume of 2.77 L per sheep per day for animals housed in urine collection pens at the same semi-improved field site as that used here.

Assessment of diurnal variation in urination behaviour

To assess whether sheep urinated more frequently in daylight hours, the data were grouped into two periods (day and night), using the times for sunrise and sunset, and then assessed for the proportion of events within each period. In spring at the semi-improved site, 74% of the total recorded urination events occurred during daylight hours (04:49 - 21:09; ca. 67% of the total 24-hour period). This suggests that sheep do urinate overnight, although at a lower frequency than during daylight hours, presumably due to reduced grazing activity (Sarout et al., 2018). In autumn at the semi-improved site, 64% of urine events occurred during daylight hours (07:25 – 19:25; ca 50% of the total 24-hour period). Here, the lower proportion could be due to fewer daylight hours in autumn. At the unimproved site in spring, 83% of urine events were recorded during daylight hours (04:43 – 21:20; ca. 70% of the total 24-hour period). At the unimproved site in autumn, 67% of urine events occurred in daylight hours (07:16 – 18:18, ca. 46% of the total 24-hour period). Again, the lower values may be due to fewer daylight hours in the autumn compared to the spring. Upscaling individual urine event volumes from animals penned during a fraction of the day to 24 h periods to produce daily urine volume estimates should, therefore, be done with caution (as in Marsden et al., 2020).

Conclusion

In summary, this study has demonstrated the successful use of accelerometers and Boolean algorithm for the estimation of the volume and frequency of individual urination events during grazing. Sheep squat duration was correlated with individual urine event volume in penned sheep studies, with sheep urinating at a rate of 16.6 mL s^{-1}. We consider squat duration to be a good predictor of urination volume in free-ranging sheep and thus squat duration measurement (using motion sensors) is a good proxy, and multiplier, for urine volume. Furthermore, we found that urine volume and frequency differed by site and season and that ewes urinated more in daylight hours than at night. Accelerometers on free-grazing animals have several advantages over data collection from animals housed in metabolism crates. For example, they can provide larger sample sizes (number of animals, length of observation period, number of events monitored) without interfering with the animals' natural grazing behaviour, which means that they can probably provide more representative urination metrics. Unfortunately, sensor technologies do not yet allow detailed monitoring of urine chemical composition so there remains a need for urine collection and analysis e.g. in urine collection pens. Our data add to the body of literature on urination parameters which are useful for upscaling estimates of N pollution arising from urine patches to the landscape-scale. The application of accelerometer data described here is novel and represents a new and powerful technique to estimate urine volumes and frequency from grazing livestock.

Ethics approval

Use of the Daily Diary loggers was approved by Swansea University's Animal Welfare and Ethical Review Group (Reference IP-1516-5) and use of urine collection pens were approved by Bangor University's School of Natural Sciences Ethics Committee (Ethics approval code CNS2016DC01).

Data and model availability statement

At the time of publication data were in the process of being deposited to the Environmental Information Data Centre.

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Declaration of interest

None.

Acknowledgements

We thank Rob Brown, Emily Cooledge and Danielle Hunt for help with the urine collection study. We also thank Mark Hughes, Llinos Hughes and Wil Williams for technical assistance with deploying grazing animals. We also thank Snowdonia National Park, Aber & Llanfairfechan Graziers Association, National Trust & Natural Resources Wales for allowing the study sites to be used.

Financial support statement

This work was funded under the UK Natural Environment Research Council (NERC), grant award (NE/M015351/1, NE/M013847/1 and NE/M013154/1).

References

Alvarenga, F.A.P., Borges, I., Palkovič, J., Rodina, J., Oddy, V.H., Dobos, R.C., 2016. Using a three-axis accelerometer to identify and classify sheep behaviour at pasture. Applied Animal Behaviour Science 181, 91-99.

Barwick, J., Lamb, D.W., Dobos, R., Welch, M., Schneider, D., Trotter, M., 2020. Identifying sheep activity from tri-axial acceleration signals using a moving window classification model. Remote Sensing 12, 646.

Betteridge, K., Costall, D., Balladur, S., Upsdell, M., Umemura, K., 2010a. Urine distribution and grazing behaviour of female sheep and cattle grazing a steep New Zealand hill pasture. Animal Production Science 50, 624-629.

Betteridge, K., Costall, D.A., Li, F.Y., Luo, D., Ganesh, S., 2013. Why we need to know what and where cows are urinating – a urine sensor to improve nitrogen models. Proceedings of the New Zealand Grassland Association 75, 33-38.

Betteridge, K., Hoogendoorn, C., Costall, D., Carter, M., Griffiths, W., 2010b. Sensors for detecting and logging spatial distribution of urine patches of grazing female sheep and cattle. Computers and Electronics in Agriculture 73, 66-73.

Betteridge, K., Kawamura, K., Costall, D., Carter, M.L., Hoogendoorn, C., Griffiths, W., Inoue, Y., 2008. Tools to determine impact of animal behaviour on nitrate leaching and nitrous oxide emissions. In: Currie, L.D., Yates, L.J. (Eds.), Carbon and Nutrient Management in Agriculture (Occasional Report No.21). Fertiliser and Lime Research Centre, Palmerston North, New Zealand, pp.286-298.

Bowns, J.E., 1971. Sheep behavior under unherded conditions on mountain summer ranges. Journal of Range Management 24, 105-109.

Bratzler, J.W., 1951. A metabolism crate for use with sheep. Journal of Animal Science 10, 592-601.

Dearing, M.D., Mangione, A.M., Karasov, W.H., 2001. Plant secondary compounds as diuretics: an overlooked consequence. American Zoologist 41, 890-901.

Dick, A.T., Mules, M.W., 1953. Equipment for the clean collection of twenty-four-hour samples of urine and faeces for sheep. Australian Journal of Agricultural Research 5, 345-347.

Doak, B.W., 1952. Some chemical changes in the nitrogenous constituents of urine when voided on pasture. Journal of Agricultural Science 42, 162-171.

Draganova, I., Yule, I., Stevenson, M., Betteridge, K., 2016. The effects of temporal and environmental factors on the urination behaviour of dairy cows using tracking and sensor technologies. Precision Agriculture 17, 407-420.

Elkington, T., Dayton, N., Jackson, D.L., Strachan, I.M., 2001. National vegetation classification: field guide to mires and heaths. Joint Nature Conservation Committee, Peterborough

Evans, J.G., Ward, H.C., Blake, J.R., Hewitt, E.J., Morrison, M., Fry, L.A., Ball, L.C., Doughty, L.C., Libre, J.W., Hitt, O.E., Rylett, D., Ellis, R.J., Warwick, A.C., Brooks, M., Parkes, M.A., Wright, G.M.H., Singer, A.C., Boorman, D.B., Jenkins, A., 2016. Soil water content in southern England derived from a cosmic-ray soil moisture observing system – COSMOS-UK. Hydrological Processes 30, 4987-4999.

Giovanetti, V., Decandia, M., Molle, G., Acciaro, M., Mameli, M., Cabiddu, A., Cossu, R., Serra, M.G., Manca, C., Rassu, S.P.G., Dimauro, C., 2017. Automatic classification system for grazing, ruminating and resting behaviour of dairy sheep using a tri-axial accelerometer. Livestock Science 196, 42-48.

Haynes, R.J., Williams, P.H., 1993. Nutrient cycling and soil fertility in the grazed pasture ecosystem. Advances in Agronomy 49, 119-199.

Hoogendoorn, C.J., Betteridge, K., Costall, D.A., Ledgaard, S.F., 2010. Nitrogen concentration in the urine of cattle, sheep and deer grazing a common ryegrass/cocksfoot/white clover pasture. New Zealand Journal of Agricultural Research 53, 235-243.

Kurien, B.T., Everds, N.E., Scofield, R.H., 2004. Experimental animal urine collection: a review. Laboratory Animals 38, 333-361.

Ledgard, S.F., Menneer, J.C., Dexter, M.M., Kear, M.J., Lindsey, S., Peters, J.S., Pacheco, D., 2008. A novel concept to reduce nitrogen losses from grazed pastures by administering soil nitrogen process inhibitors to ruminant animals: a study with sheep. Agriculture, Ecosystems and Environment 125, 148-158.

Lush, L., Wilson, R.P., Holton, M.D., Hopkins, P., Marsden, K.A., Chadwick, D.R., King, A. J., 2018. Classification of sheep urination events using accelerometers to aid improved measurements of livestock contributions to nitrous oxide emissions. Computers and Electronics in Agriculture 150, 170-177.

Marcilese, N.A., Ammerman, C.B., Valsecchi, R.M., Dunavant, B.G., Davis, G.K., 1970. Effect of dietary molybdenum and sulfate upon urinary excretion of copper in sheep. The Journal of Nutrition 12, 1399-1405.

Marsden, K.A., Lush, L., Holmberg, J.A., Whelan, M.J., King, A.J., Wilson, R.P., Charteris, A.F., Cardenas, L.M., Jones, D.L., Chadwick, D.R., 2020. Sheep urination frequency, volume, N excretion and chemical composition: implications for subsequent agricultural N losses. Agriculture, Ecosystems and Environment 302, 107073.

Marsden, K.A., Jones, D.L., Chadwick, D.R., 2017. DMPP is ineffective at mitigating N_2O emissions from sheep urine patches in a UK grassland under summer conditions. Agriculture, Ecosystems and Environment 246, 1-11.

Misselbrook, T., Fleming, H., Camp, V., Umstatter, C., Duthie, C.A., Nicoll, L., Waterhouse, T., 2016. Automated monitoring of urination events from grazing cattle. Agriculture, Ecosystems and Environment 230, 191-198.

O'Connell, C.A., Judson, H.G., Barrell, G.K., 2016. Sustained diuretic effect of plantain when ingested by sheep. Proceedings of the New Zealand Society of Animal Production, 76, 14-17.

Quasem, L., Cardew, A., Wilson, A., Griffiths, I., Halsey, L.G., Shepard E.L.C., Gleiss, A.C., Wilson R., 2012. Tri-axial dynamic acceleration as a proxy for animal energy expenditure; should we be summing values or calculating the vector? PLoS ONE 7, e31187.

R Core Team, 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Ravera, B.L., Bryant, R.H., Cameron, K.C., Di, H.J., Edwards, G.R., Smith, N., 2015. Use of a urine meter to detect variation in urination behaviour of dairy cows on winter crops. Proceedings of the New Zealand Society of Animal Production 75, 84-88.

Rodwell, J.S., 2000. British Plant Communities, Cambridge University Press, Cambridge, UK.

Sarout, B.N.M.S., Waterhouse, A., Duthie, C-A., Poli, C.H.E.C., Haskell, M.J., Berger, A., Umstatter, C., 2018. Assessment of circadian rhythm of activity combined with random regression model as a novel approach to monitoring sheep in an extensive system. Applied Animal Behaviour Science 207, 26-38.

Selbie, D.R., Buckthought, L.E., Shepherd, M.A., 2015. The challenge of the urine patch for managing nitrogen in grazed pasture systems. Advances in Agronomy 129, 229-292.

Shepherd, M.A., Welten, B.G., Costall, D., Cosgrove, G.P., Pirie, M., Betteridge, K., 2016. Evaluation of refractive index for measuring urinary nitrogen concentration in a sensor worn by grazing female cattle. New Zealand Journal of Agricultural Research 60, 23-31.

Wilson, R.P., Holton, M.D., di Virgilio, A., Williams, H., Shepard, E.L.C., Lambertucci, S., Quintana, F., Sala, J.E., Balaji, B., Lee, E.S., Srivastava, M., Scantlebury, D.M., Duarte, C.M., 2018. Give the machine a hand: A Boolean time-based decision-tree template for rapidly finding animal behaviours in multisensory data. Methods in Ecology and Evolution 9, 2206-2215.

Table 1 Mean ± SEM of sheep urination frequency, squatting duration, individual urine event volume and daily urine volume with n for each displayed in brackets. Small letters indicate statistical groupings based on Wilcoxon rank sum test.

Site	Season	Urination frequency (urine events animal^{-1} day^{-1})	Squatting duration (s)	Individual urine event volume (mL)	Daily urine volume (L sheep^{-1} day^{-1})
Semi-improved	Spring	10.0 ± 0.2 b	9.29 ± 0.03 b	154 ± 1 b	1.55 ± 0.04 b
		(n = 590 days)	(n = 5 924 events)	(n = 5 924 events)	(n = 590 days)
	Autumn	8.1 ± 0.3 a	8.65 ± 0.08 a	143 ± 1 a	1.17 ± 0.04 a
		(n = 633 days)	(n = 5 155 events)	(n = 5 155 events)	(n = 633 days)
Unimproved	Spring	19.0 ± 0.4 d	9.93 ± 0.06 c	165 ± 1 c	3.14 ± 0.08 d
		(n = 727 days)	(n = 13 846 events)	(n = 13 846 events)	(n = 727 days)
	Autumn	15.3 ± 0.3 c	9.84 ± 0.06 c	163 ± 2 c	2.50 ± 0.06 c
		(n = 719 days)	(n = 11 021 events)	(n = 11 021 events)	(n = 719 days)

Figures

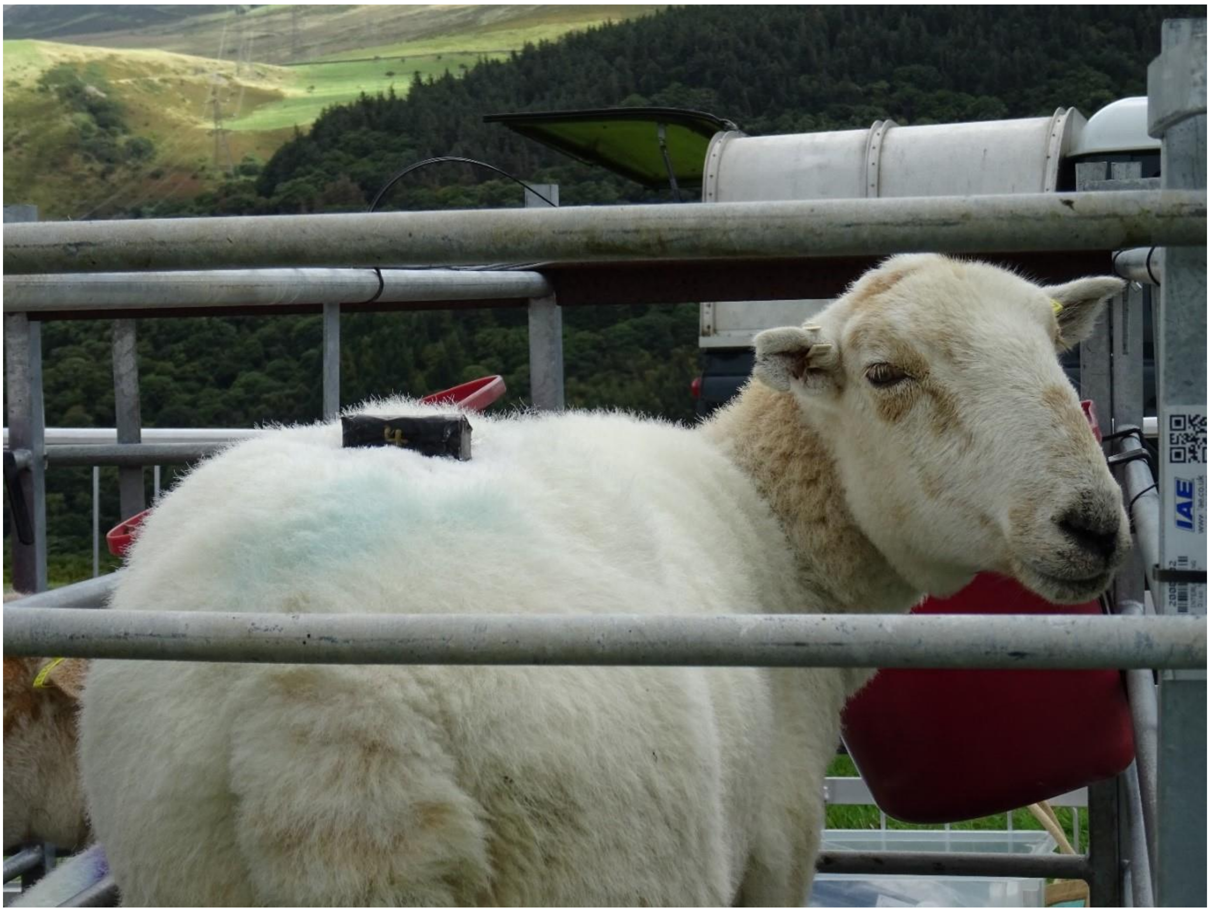

Fig. 1. Daily Diary sensor (accelerometer) glued to the hind of a Welsh Mountain ewe.

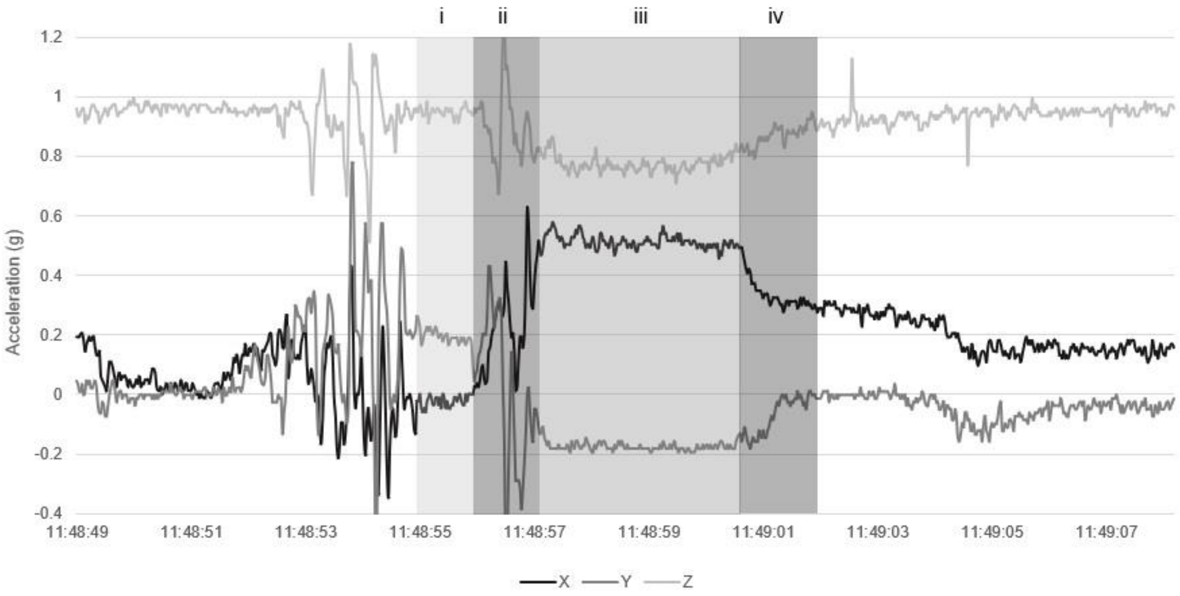

Fig. 2. Example accelerometer trace demonstrating the sheep urination time-linked processes.

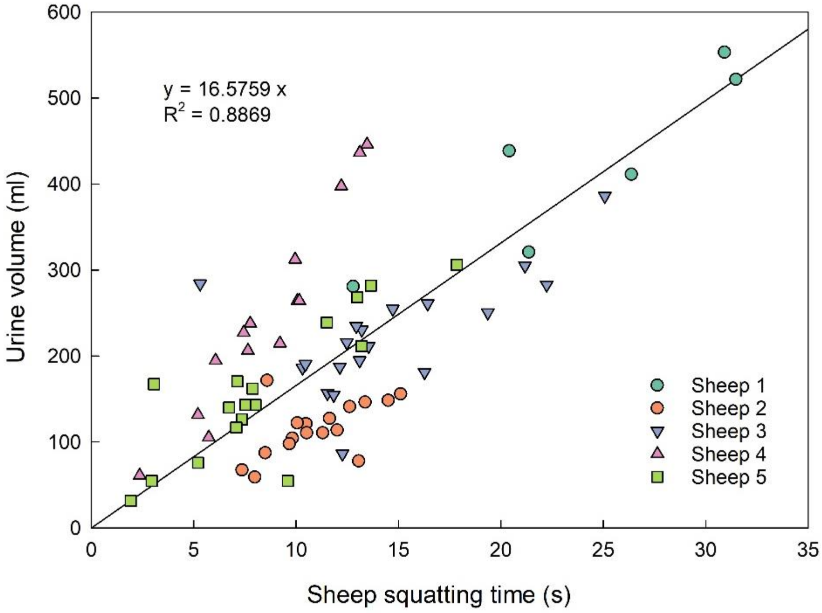

Fig. 3. Correlation between duration of ewe (n = 5) urination squatting position (measured via accelerometers attached to hind of the penned animals) and measured volume of urine produced per urination event using urine collection pens.

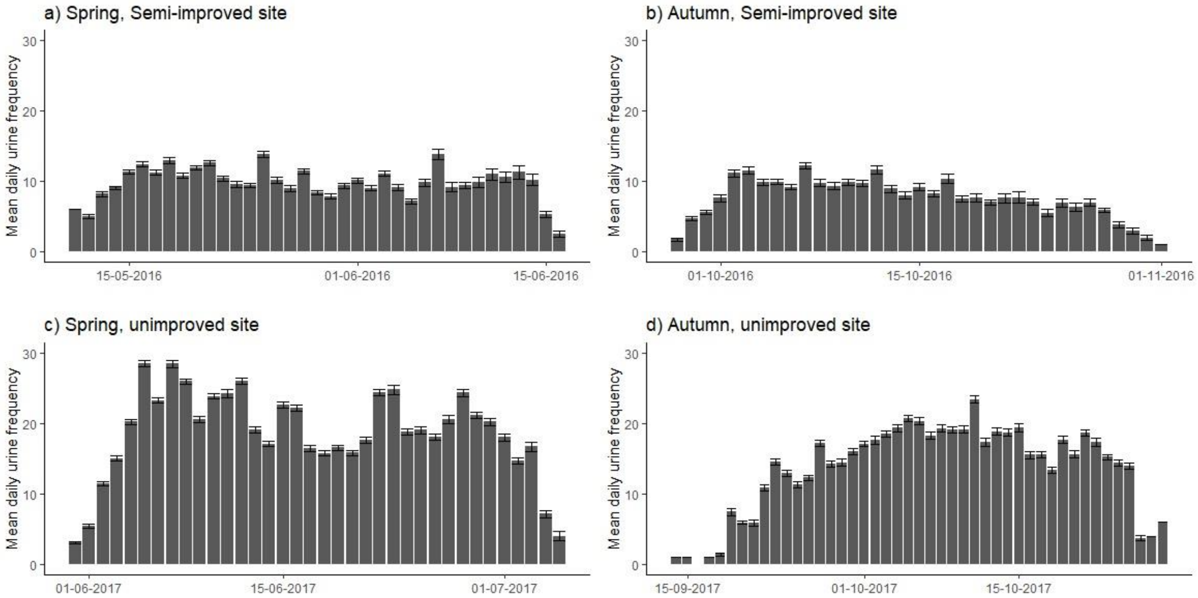

Fig. 4. Daily urination frequencies for sheep in a) spring, semi-improved pasture, b) autumn, semi-improved pasture, c) spring, unimproved pasture, and d) autumn, unimproved pasture. Bars represent daily means and error bars denote SEM. Date axes are displayed in dd/mm/yyyy.

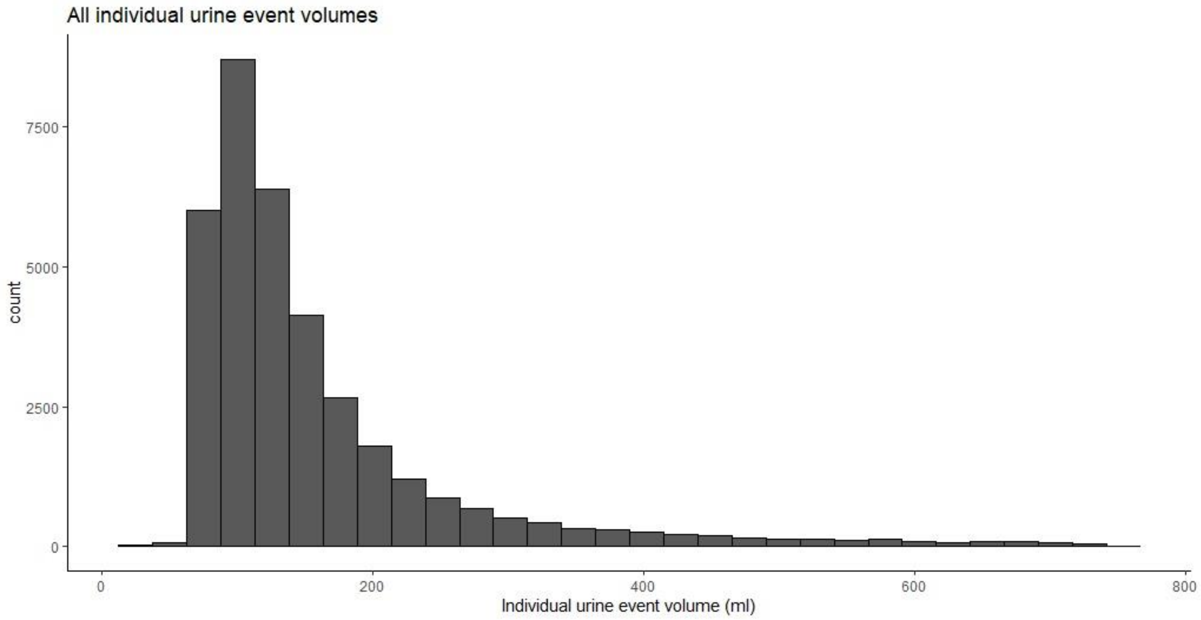

Fig. 5. Frequency distribution of individual urine event volumes across all four logger-tagged sheep deployments.

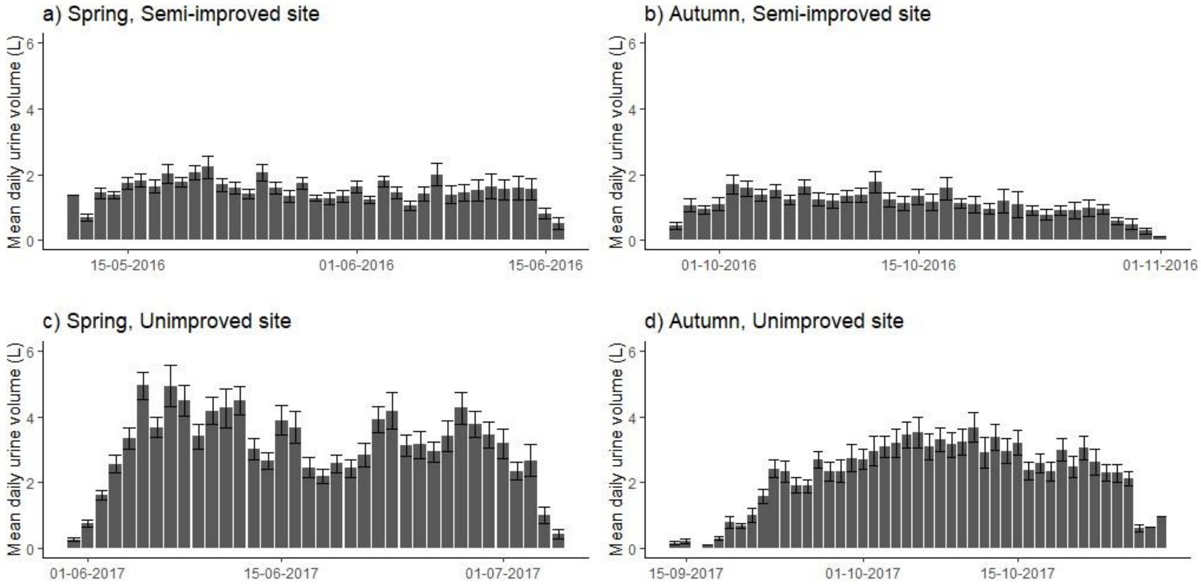

Fig. 6. Mean daily urine event volumes for sheep in a) spring, semi-improved pasture, b) autumn, semi-improved pasture, c) spring, unimproved pasture, and d) autumn, unimproved pasture. Bars denote daily mean volumes and error bars indicate SEM. Date axes are displayed in dd/mm/yyyy.