METHODOLOGY





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Abstract

Background Proximity loggers are a biotelemetry technology used to quantify animal–animal interactions noninvasively. They are small devices can be affixed to a target animal and detects and records if another device is within a certain distance, which can provide unprecedented insights into individual behavior and interactions with other animals or other aspects of the environment. Proximity loggers also can record the duration and distance between the two or more individuals detected, and have been used to study a diverse array of interactions. Studying drinking behavior in sheep is important for several critical reasons, such as improving animal welfare, enhancing productivity, managing resources efficiently, and ensuring sustainable agricultural practices. The objective of this study was to test the capacity for Bluetooth Low Energy proximity loggers to quantify drinking habits in sheep (n = 7 adult females). At all times, they had access to water from two drinking troughs. Animals wore neck collars that contained a mobile logger, and two stationary loggers were placed under the drinking troughs. To store the records downloaded from the stationary loggers, a gateway was placed close to the troughs. The mobile loggers were programmed as beacons to transmit their ID to the stationary loggers, which were programmed as receivers to scan the proximity of the mobile loggers, record the time, the IDs of the mobile loggers, and the received signal strength of the contact with the mobile loggers.

Results Ewes visited the drinking troughs 2.4 ± 0.5 times per day (rank 1.4-2.9), most frequently (42%) between 0800 and 1000 h, and 84% occurred in the daytime. Mean length of each water intake was 1.72 ± 0.30 min. The cosinor analysis of the distribution of drinking events revealed a significant (P < 0.0001) 24-h circadian rhythm in drinking events, and the acrophase (time of the highest proportion of events) occurred at 1054 h.

Conclusions Considering that studying water intake in sheep is crucial for optimizing their health, welfare, and management, the proximity loggers were demonstrated to be appropriate for documenting the drinking habits of sheep, and probably, would be suitable for studies of other behaviors that involve approaching other animals or objects.

Keywords Sheep, Proximity loggers, Drinking behavior

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Background

Proximity loggers are a biotelemetry device that can be used to quantify animal–animal interactions, noninvasively [1]. The devices can be affixed to a target animal, and can record an encounter when another device is within a certain distance, which can provide new insights into individual behavior and the animal's interactions with other animals or the environment [2].

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Proximity loggers also can record the duration and distance between the two or more individuals detected. These types of loggers have been used to study a diverse array of interactions.

In sheep, proximity loggers have been used to study pair interactions [3], the effects of feeding motivation on social cohesion among ewes [4], social interactions [5], the spatial associations of livestock guard dogs and domestic sheep [6], the effects of age, environment, and management on social contact behavior [7], and to document social relationships within an ewe-lamb group [8]. To our knowledge, however, no studies have been published that have used proximity loggers to quantify the drinking behavior of sheep. This is important for several critical reasons, such as improving animal welfare, enhancing productivity, managing resources efficiently, and ensuring sustainable agricultural practices. Moreover, it provides insights into general animal behavior, preferences, and needs, which is valuable for to develop automated watering systems, smart farming technologies, and monitoring systems that ensure optimal water availability. This understanding supports better animal husbandry practices and enhances the overall sustainability of sheep farming operations.

Studies of the drinking behavior of livestock species have been useful in predicting water and feed intake [9, 10], although monitoring those behaviors either in intensive or extensive systems is difficult. Traditionally, those behaviors have been recorded through direct observation [11–13], video recording [14–17], or a remote sensing system such as ruminal boluses [18], or RFID technologies and accelerometers [19]. Moreover, it should be considered that the type of and time of day food is supplied can influence the drinking habits of housed sheep [20], and the timing of dry-matter consumption throughout the day can influence the timing and frequency of water intake. For instance, in Churra sheep, the form of alfalfa offered (hay or pellet) influenced the daily frequency of drinking [21].

Once validated, proximity loggers can eliminate the need for visual observations. These proximity loggers, in combination with stationary sensors that are placed in fixed positions next to structures; e.g., water troughs, communicate by radio or Bluetooth signal. The use of this type of technology provide a new means of documenting animal drinking behavior. Although proximity-logging systems demand significant resources for calibration, field deployment, and operation, they facilitate fully automated, near-real-time collection of association data for entire animal populations, which has provided unprecedented spatio-temporal resolutions [22].

In view of the importance of studying water intake behavior in sheep, as a factor that can help improve animal health and welfare, and as an essential point in the design of sheep facilities, and given that such studies are complex and usually require direct observation of the animals, proximity sensors open a new door to this type of research. The objective of this study was to test the capacity of proximity loggers to quantify animal behavior; specifically, the drinking habits of sheep.

Methods

The proximity logging system

The system [2] used in this study, which was developed by IoSA, a spin-off of the Inter-University Microelectronics Centre (IMEC) and the University of Antwerp (Belgium), consists of the following four components: (1) mobile loggers, (2) stationary loggers, (3) a gateway, and (4) a mobile phone application. The mobile loggers (mass=0.9 g) were programmed as beacons that broadcast their ID to the stationary loggers. The stationary loggers were programmed as receivers that could scan the proximity of the mobile logger, as well as record the time stamp, the IDs of the mobile loggers, and the received signal strength (RSSI) of the contact with the mobile loggers. The gateway records the downloaded data from the stationary loggers and stored it on a microSD card. In addition, a mobile phone application that allowed real-time programming and monitoring was required; it also controlled the downloads from the loggers and the gateway. The system used Bluetooth Low Energy (BLE), a technology that can operate in high interference environments. The loggers and gateway were powered by rechargeable batteries.

In our system, the gateway included a GPS unit that provided an accurate time stamp, and continuously scanned for logger signals. If a logger was detected that held data that exceeded the download threshold, a connection was made between and the data were transferred to a temporary buffer. After the last data were detected, to ensure the timestamps on all loggers were synchronized, the gateway synchronized the clock on the logger with its own clock.

Animals

The seven adult not pregnant, not lactating ewes included in the study were part of a flock that contained 50 ewes, which were housed in a communal yard (6×15 m), with straw as bedding material, that did not have any artificial light, and had an uncovered area (8×7 m). Animals were fed a concentrate ration (0.50 kg of pellets, which consisted of barley 85% and soybean 15%) that was offered at 0800 h, and barley straw ad libitum. At all times, water was available from two drinking troughs (nose-paddle bowls; Suevia 370-MS¹/₂", Suevia Haiges GmbH, Germany). The two water dispensers were installed inside the building, on either side of the door that connects the enclosure with the outside yard.

Field trial

The seven ewes wore neck collars that contained a mobile logger that was housed within a plastic box $(35 \times 35 \times 10 \text{ mm})$ (Fig. 1). Stationary loggers were placed inside plastic boxes $(80 \times 80 \times 46 \text{ mm})$ that were placed under the two drinking troughs and attached to the wall (Fig. 2). The gateway was placed against the wall of the sheep house, close to the troughs (Fig. 3).

Validation

The seven mobile loggers were tested manually, without being attached to the animals. Collars were held for 2 min at 10 cm, 25 cm, or > 25 cm from the stationary loggers, under the drinking trough. The RSSI signals of those contacts were recorded, and the mean, max, and min values of the signals at the various distances were calculated to identify the signal strength associated with a drinking event.

In addition, before the start of the experiment, to confirm whether the system could distinguish between a drinking event and an animal approaching or standing near a drinking trough, 20 drinking events by the animals were observed directly in the experiment, and the number of the animal and the time recorded by the observer



Fig. 1 Mobile Bluetooth Low Energy proximity loggers (0.9 g) (**A**) used in the experiment, which were protected within a plastic box $(35 \times 35 \times 10 \text{ mm})$ (**B**) and attached to a neck collar (**C**)



Fig. 2 Stationary Bluetooth Low Energy proximity loggers used in the experiment, which were protected within a plastic box $(80 \times 80 \times 46 \text{ mm})$ that was placed under each sheep drinking trough

(Fig. 4) were compared with the corresponding values logged by the gateway.

Statistical analysis

The raw telemetry data was collected and stored into the gateway continuously from the proximity loggers attached to the ewes; a preliminary screening was performed, involving the removal of any obviously corrupted or incomplete data segments. This was done by checking for missing timestamps or logger readings outside the plausible physiological range for the species under study. In a second step, we identified erroneous data points, filtering specific physiological thresholds based on the previous manual test of the loggers. Inconsistent proximity readings, such as sudden movements without corresponding sensor data changes, or long periods of time close to the drinker through were flagged as erroneous.



Fig. 3 Gateway used in the experiment, which stored on a microSD card the records downloaded from the stationary loggers, positioned against the wall of the sheep house

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Fig. 4 Sheep drinking water from a trough while wearing a neck collar containing a mobile proximity logger. Note the short distance between the mobile and the stationary loggers

Finally, a random samples of filtered data were manually reviewed to ensure the efficacy of the automated filtering process. The final dataset, free from identified errors, was used for all subsequent statistical analyses. Only 0.4% of the total number of RSSI records were considered as part of drinking events. The rigorous filtering process ensured that the data retained for analysis was of high quality and reliable for drawing conclusions about the water intake behavior in sheep.

The mean (± SE) number of drinking events per ewe per day, their duration (min) and the distribution of events throughout the day were calculated. Pearson correlation coefficient between the number of drinking events and their duration was calculated. The hourly distribution of events was fitted to the cosine curve of a 24-h activity rhythm, which was obtained from the cosinor method at the Cosinor online platform (https://cosinor.online/app/cosinor.php) [23]. The acrophase, which is the time at which the highest activity occurs under the cosinor model, was calculated. To confirm circadian rhythmicity, an *F* test compared the cosine model with the distribution of the data. If the null hypothesis was rejected (p < 0.05), the input data fit a 24-h circadian rhythm.

Results

The mean (± SD), max, and min RSSI (dBm) at each distance tested between the mobile loggers and the stationary loggers under the drinking troughs were -44 ± 4 , -38, and -55 at 10 cm, -49 ± 3 , -46, and -55 at 25 cm, and -84 ± 4 , -70 and -92 at > 25 cm, respectively; therefore, an RSSI signal \geq -55 dBm for > 20 s and < 120 s defined a

drinking event, which was based on observations of the animals drinking.

The sheep wore the collars for ten days, but the data from the first day (adaptation to the collars) and last day (it was not a full 24-h record) of data collection were excluded; therefore, the analysis was based on data from eight days of recording (N=387,755 contacts between the mobile and the stationary loggers).

In the experiment, the actual drinking events observed had mean, max, and min RSSI of -51 ± 4 , -45, and -55dBm, respectively, which were similar to those from the calibration data recorded before the experiment. An analysis of the 387,756 records from the proximity loggers recorded indicated that, on average, ewes visited a drinking trough 2.4 ± 0.5 times per day (rank 1.4–2.9; CV 28%), most frequently (42%) between 0800 and 1000 h (Fig. 5), and 84% occurred in the daytime. Mean length of each water intake was 1.72 ± 0.30 min (rank 0.9-4.5), so that ewes spent around 4 min per day drinking water. The individual mean hourly frequency is presented in Fig. 6. The number of visits did not differ significantly between the two drinking troughs (46% vs. 54%). The correlation between the number of drinking events and their length was -0501 (P<0.05).

The cosinor analysis of the distribution of events revealed a significant (P < 0.0001) 24-h circadian rhythm in drinking frequency, and the acrophase (time of the highest proportion of drinking events) occurred at 1054 h.

Discussion

Published accounts of drinking frequencies in sheep range from 17 [14] to 0.99 times per day [13]. In our experiment, data from the proximity loggers indicated that, on average, the sheep went to a drinking trough 2.4 times per day, which is similar to the average frequencies of 2.4 and 2.8, respectively, for 'shaded' and 'non-shaded' sheep based on direct observations [12]; however, all of the water sources were in the shade in the present experiment. In the latter study [13], however, sheep were observed between 0500 and 2100 h, only. Churra sheep increased the daily frequency of drinking (5.5–7.3 times) when alfalfa hay was changed for alfalfa pellets [21], indicating that the type of food supplied can influence the drinking habits of housed sheep.

In our study, drinking frequency exhibited a distinct 24-h circadian rhythm, and was highest at about 0900 h, one hour after the concentrated feed had been offered. In pygmy goats fed ad lib and kept on a 12 h light/12 h dark cycle, 84% of the drinking events were associated with feeding bouts [24] Furthermore, cumulative food intake and cumulative water intake were positively correlated, but the correlation was weaker in



Fig. 5 Mean proportion (%) of drinking events recorded by proximity loggers, and the cosine curve of a 24-h activity rhythm of seven sheep on 8 consecutive days. Grey areas indicate night (1800–0800 h)



Fig. 6 Mean proportion (%) of drinking events recorded by proximity loggers of seven ewes under study. Grey areas indicate night (1800–0800 h)

the dark phase than it was in the light phase. It has also been reported [25] that pygmy goats that had access to food twice a day for 2 h at the beginning and near the end of the 12 h light phase drank most frequently in the last two hours before and the first hour after dark. Although the seven ewes used in the experiment presented a similar pattern of drinking behavior, a certain degree of variability was observed among individuals in the number of water intakes per sheep per day. References about this water-intake variability are scarce, although for feeding habits of domesticated ruminants, the variability is influenced by personality variations, which may explain why some individuals are unable to cope with alterations in their dietary conditions [26]. Thus, it is likely that individual drinking behavior can be affected by the welfare of individuals, and may be improved with a better understanding of their personalities. Interestingly, the higher number of water intakes per day, the lowest length of the drinking bouts, as the negative correlation between both variables indicates, confirming differences among individuals to obtain the daily water requirements (higher frequency and shorter length, or lower frequency and longest length).

Walker et al. [27] used similar BLE proximity loggers to track animals in a field environment as beacons on sheep transmitted their identity to static readers that either were at fixed locations or affixed to other sheep. In that study, the use of BLE was a less expensive and power intensive option for use in studies of sheep systems.

Conclusion

In conclusion, and considering that studying water intake in sheep is crucial for optimizing their health (it influences digestion, nutrient absorption, and overall physiological balance), and is essential for designing effective facilities, ensuring adequate access to water resources, and optimizing feeding strategies for better animal welfare and productivity, the proximity loggers were demonstrated to be appropriate for documenting the drinking habits of sheep. Probably, they would also be suitable for studies of other behaviors that involve approaching other animals or objects.

Abbreviations

- RSSI Received signal strength
- BLE Bluetooth low energy
- GPS Global positioning system
- ID Identification

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Author contributions

JAA conceptualized the study. JAA, PE, and FC collected field data. JAA, PE, and FC performed analysis and wrote the initial manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The data sets generated and analysed in the study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

The study was conducted at the experimental farm of the University of Zaragoza, Spain (41° 63 N), following procedures approved by the Ethics Committee for Animal Experiments at the University of Zaragoza. The care and use of animals were in keeping with the Spanish Policy for Animal Protection (RD 53/2013), which meets the European Union Directive 2010/63 on the protection of animals used for experimental and other scientific purposes.

Competing interests

The authors declare no competing interests.

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