

# Behavioural study of rams subjected to photoperiod change: sexual, social, vital and group activities monitored by video

Elena Pérez-García<sup>a,\*</sup>, Jaime Nieto<sup>a</sup>, José Alfonso Abecia<sup>b</sup>, Javier Plaza<sup>a</sup>, Carlos Palacios<sup>a</sup>

<sup>a</sup> Faculty of Agricultural and Environmental Sciences, University of Salamanca, Avenida Filiberto Villalobos, 119, Salamanca 37007, Spain

<sup>b</sup> Instituto Universitario de Investigación en Ciencias Ambientales de Aragón (IUCA), University of Zaragoza, Miguel Servet, 177, Zaragoza, Spain

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## ABSTRACT

This study examined behavioural differences in rams exposed to artificial photoperiod stimulation, within the context of the ram effect. Eleven rams were divided into two groups: six received two months of long-day artificial photoperiod (16 h light/day), followed by one month of natural light (9 h light/day, February), while five control rams remained under natural lighting. At the end of the third month, three pens were established: one with photoperiod-stimulated rams (Group L,  $n = 3$ ), one with control rams (Group C,  $n = 3$ ), both with 30 ewes, and one mixed pen with both stimulated (Group L+C,  $n = 3$ ) and control rams (Group C+L,  $n = 2$ ) with 60 ewes. Behavioural observations were conducted over 11 consecutive days. A total of 26 behaviours-classified as sexual, vital, social, and grouping- were manually recorded and analysed using BORIS software. Sexual behaviours were the most frequent across all groups. Rams in Group L exhibited higher frequencies in all behaviour categories ( $p < 0.001$ ). Vital activities had the longest durations, with Group C spending more time on these behaviours than others. In the mixed pen, both stimulated and control rams showed intermediate activity frequencies compared to their respective isolated counterparts (Group L: 36.7 % vs. L+C: 26.6 %; Group C: 17.6 % vs. C+L: 19.0 %;  $p > 0.001$ ).

In conclusion, artificial photoperiod stimulation increased the frequency of key behavioural categories, especially sexual activity. Social interactions in mixed groups modified behaviour, suggesting mutual influence. BORIS software proved valuable for detailed behavioural analysis in ethological research.

## 1. Introduction

Methods for studying animal behaviour have evolved significantly over time. Since antiquity, the most basic and widely used approach has been the direct observation of animals in their environment (López-Rull, 2014). While this method remains valuable, technological advancements have led to the development of more sophisticated tools for behavioural research. Modern techniques include the use of animal-mounted video cameras, which provide a first-person perspective of the animal's interactions (Hall et al., 2020), as well as fixed-position video systems that allow continuous and non-invasive monitoring of behavioural patterns (Southerland

\* Corresponding author.

E-mail addresses: [elenaperez955@usal.es](mailto:elenaperez955@usal.es) (E. Pérez-García), [jaimenl@usal.es](mailto:jaimenl@usal.es) (J. Nieto), [alf@unizar.es](mailto:alf@unizar.es) (J.A. Abecia), [pmjavier@usal.es](mailto:pmjavier@usal.es) (J. Plaza), [carlospalacios@usal.es](mailto:carlospalacios@usal.es) (C. Palacios).

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et al., 2022).

In the specific case of feeding behaviour, researchers have adopted innovative tools such as mobile applications capable of identifying plant species consumed by animals (Hall et al., 2021). Additionally, the analysis of acoustic data using microphones has been employed to detect and interpret chewing sounds, providing indirect yet precise insights into feeding activity (Sneddon and Mason, 2014). Hormonal profiling through blood sampling offers a physiological complement to behavioural observations, particularly in studies addressing stress or reproductive status (Aguayo-Ulloa et al., 2019).

Other technologies have also enhanced the accuracy and scope of behaviour studies. Accelerometers are now commonly used to characterize movement patterns and distinguish among different types of activity (Decandia et al., 2021). The use of automatic gates equipped with sensors allows researchers to monitor animal passage and track individual behaviours within groups (Stockman et al., 2013). In parallel, biometric devices such as breathing sensors and heart rate monitors provide real-time physiological data (Joy et al., 2022; Martí et al., 2012), while thermal imaging has proven effective for assessing body temperature and detecting changes associated with stress or metabolic shifts (Joy et al., 2022).

Conventional behavioural data collection in sheep (via rectal thermometers or physiological monitoring) can cause stress and alter natural behaviour, reducing data reliability. Therefore, non-invasive methods are increasingly recommended (Saldaña-Ríos et al., 2016). In dairy sheep, various non-invasive monitoring tools have been employed to assess both physiological and behavioural parameters. These include pedometers for activity tracking (Roelofs et al., 2010), accelerometers for detecting movement and rest patterns (Decandia et al., 2021), geographic information systems for spatial monitoring (Plaza et al., 2022a; Plaza et al., 2022b), oestrous detection devices, automated weight measurement systems, and instruments for recording milk production-related values (Odintsov Vaintrub et al., 2021). Additionally, thermographic imaging has been utilised to evaluate surface temperature changes as indicators of physiological status (Velasco-Bolaños et al., 2021), and video cameras have long been used to identify and analyse behavioural patterns (Odagiri et al., 1995).

Among these, the use of video cameras offers a particularly valuable approach for behavioural assessment, as it allows for continuous data collection within the animals' natural environment (Payne et al., 2017). Video-based systems provide several advantages, particularly with respect to animal welfare. Since animals remain in familiar surroundings, they are not subjected to novel stimuli that could influence behaviour. Furthermore, the absence of human observers eliminates potential behavioural alterations caused by human-animal interactions (Alhamada et al., 2017), ensuring that the data collected more accurately reflect the animals' natural behavioural repertoire.

Specialised software for automating behavioural video analysis has become increasingly important in animal behaviour studies. Tools such as BORIS, Interact, Etholog, and Matlab (Biasato et al., 2022); (Fernandes et al., 2021; Pokharel et al., 2018); (Vezzoli et al., 2015) are often tailored to specific species or contexts, and may require particular environmental or video quality conditions to operate effectively.

The integration of such technological tools enhances the precision and efficiency of behavioural analysis, allowing for the systematic quantification of complex behaviours over extended periods. For example, using video analysis technologies, Palacios et al. (Palacios et al., 2023) demonstrated that rams sexually stimulated by artificial photoperiod treatment exhibited greater sexual and social activity during the first 27 h following exposure to ewes, compared to non-stimulated counterparts.

This behavioural activation can be attributed to the seasonally anoestrous (discontinuous polyoestrous) reproductive pattern of sheep, which is characterised by cyclical variations in reproductive activity primarily governed by environmental cues, particularly photoperiod (Kopycińska et al., 2022). In temperate latitudes, such as Mediterranean regions, ewes undergo oestrous cycles of approximately 16–18 days, typically commencing in late summer or early autumn and ceasing by winter or early spring. This is followed by an anoestrous phase marked by the absence of ovulation and sexual receptivity (Carvajal-Serna et al., 2021). In rams, sexual behaviour generally emerges between 9 and 20 weeks of age, depending on the breed, and progressively develops through age and experience (Lynch et al., 1992).

In this context, the present study had a twofold objective. First, it aimed to evaluate the behavioural responses of rams sexually stimulated during non-breeding season through artificial light treatment, and to assess whether these stimulated males could, in turn, induce behavioural changes in a second group of non-stimulated rams through social contact. Second, the study sought to validate the use of a commercial video-based software for the detailed analysis of animal behaviour, emphasizing its utility in accurately recording and classifying various activity patterns in a naturalistic setting.

## 2. Material and methods

### 2.1. Animals and experimental design

The present study was conducted on an extensive sheep farm located in the province of Salamanca, Spain (41°01'04''N, 5°46'40''W, 800 m.a.s.l.), in the northwest of the Iberian Peninsula. All animals were handled in accordance with Spanish animal welfare regulations (RD 1201/2005), aligned with European Union Directive 2010/63 on the protection of animals used for scientific purposes (Directive 2010/63). The Ethical Committee on Animal Experimentation of the University of Salamanca reviewed and approved all procedures, issuing a favourable report under registration number 904.

A total of 11 crossbred rams (Merino × Berrichon × Castellana) of similar age (3 years) and body weight (80–100 kg) were used. Initially, all rams were kept in a shaded outdoor pen under natural photoperiod conditions. They were subsequently assigned to two experimental groups: light-stimulated rams (Group L,  $n = 6$ ) and unstimulated control rams (Group C,  $n = 5$ ). Rams in Group L underwent a sexual activation protocol via artificial photoperiod extension. This approach aimed to mimic autumnal light conditions -the

species' natural breeding season- by exposing the animals to extended daylight hours in advance. Upon return to the natural photoperiod, the rams perceive the subsequent reduction in day length as indicative of the onset of the reproductive season, thereby stimulating sexual activity. From November 1 to December 31, they were exposed to 16 h of light per day, artificially extending the natural day length of approximately 10 h in November. This was achieved using an electronic timer and artificial lighting providing an intensity > 300 lux at eye level, operating between 18:00 and 24:00. After this two-month period, the rams remained under natural photoperiod conditions during February (9 h of light/day), following the protocol established by Abecia et al. (Abecia et al., 2022). Control rams (Group C) were maintained under natural photoperiod (9 h of light/day) throughout the study.

Upon completion of the three-month treatment period, between March 1 and 15 -corresponding to the non-breeding season in this latitude-, the rams were reorganized into three experimental settings: 1) a pen (6mx7m) containing three light-stimulated rams (Group L,  $n = 3$ ) housed with 30 ewes; 2) a pen (6mx12m) with three unstimulated control rams (Group C,  $n = 3$ ), also housed with 30 ewes; and 3) a mixed pen (6mx7m) containing three rams from Group L and two from Group C, cohabiting with 60 ewes. The 120 ewes exposed to the rams were of mixed breed (Merino  $\times$  Berrichon  $\times$  Castellana), aged between 2 and 5 years, and weighed between 60 and 80 kg.

To evaluate the potential influence of social interaction between males on their reproductive behaviour, two subgroups were distinguished within the mixed pen: light-stimulated rams exposed to control males (Group L+C,  $n = 3$ ), and control rams exposed to light-stimulated males (Group C+L,  $n = 2$ ).

To facilitate continuous behavioural monitoring, each male pen was equipped with a dedicated video camera positioned at the far right corner and mounted on an elevated pole, ensuring comprehensive coverage of the entire enclosure.

## 2.2. Behavioural recording procedure and visualization

Behavioural activities were recorded using four Samsung SDC-9443BC video cameras (Samsung Digital City, Yeongtong-gu, Suwon, Seoul, South Korea). A total of 44 video segments, each lasting 30 min, were captured from 16:00 h on February 12 (designated as time 0), coinciding with the first contact between rams and ewes, until 08:00 h on February 26 (time 328). The recordings were distributed across three predefined time slots, based on seasonal daylight patterns typical for this geographical area: morning (08:30–13:30 h), afternoon (13:30–19:30 h), and evening (19:30–08:30 h).

Each video was reviewed in its entirety by a single trained observer, who was responsible for creating a detailed behavioural database. Individual rams were identified within the recordings based on unique morphological traits such as fleece coloration, head shape and size, tail, and ear morphology.

The behavioural activities recorded and categorized are shown in Table 1.

**Table 1**

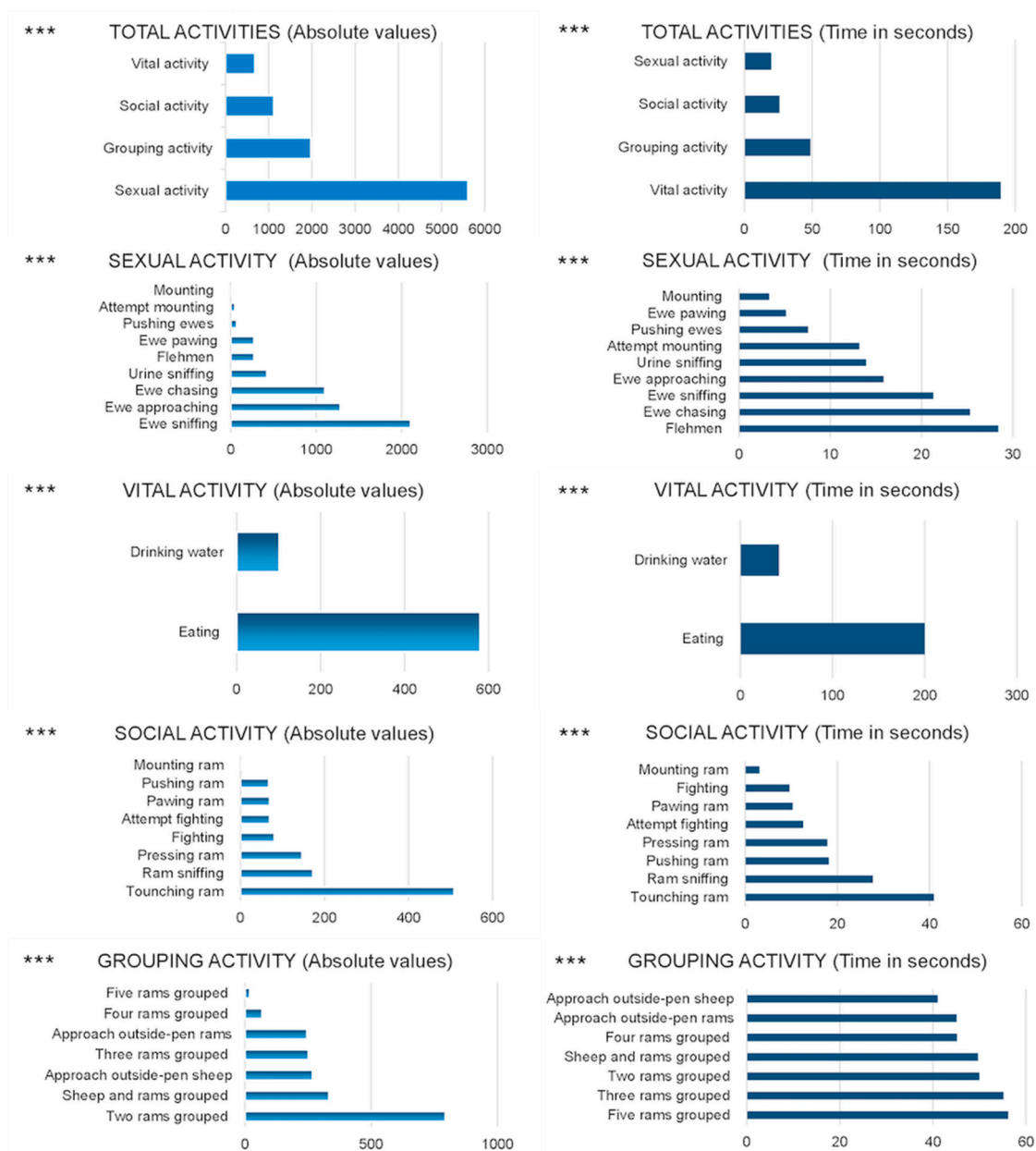
Classification of the behavioural variables recorded during the observation period. For each behavioural event, the observer recorded the exact date and time of occurrence, along with the experimental group to which the ram belonged.

Behavioural category	Behaviour
Sexual (9)	<ul style="list-style-type: none"> <li>• Ewe sniffing</li> <li>• Urine sniffing</li> <li>• Flehmen</li> <li>• Ewe chasing</li> <li>• Pushing ewes</li> <li>• Ewe pawing</li> <li>• Ewe approaching</li> <li>• Attempt mounting</li> </ul>
Vital (2)	<ul style="list-style-type: none"> <li>• Mounting</li> <li>• Drinking water</li> </ul>
Social (8)	<ul style="list-style-type: none"> <li>• Eating</li> <li>• Touching ram</li> <li>• Ram sniffing</li> <li>• Pressing ram</li> <li>• Fighting</li> <li>• Attempt fighting</li> <li>• Pawing ram</li> <li>• Pushing ram</li> </ul>
Grouping (7)	<ul style="list-style-type: none"> <li>• Mounting ram</li> <li>• Sheep and rams grouped together</li> <li>• Two rams grouped together</li> <li>• Three rams grouped together</li> <li>• Four rams grouped together</li> <li>• Five rams grouped together</li> <li>• Being near rams from another pen</li> <li>• Being near sheep from another pen</li> </ul>

### 2.3. Use of specialized video analysis software

To process the behavioural video recordings, in addition to manual observation by a single trained observer, the Behavioural Observation Research Interactive Software (BORIS), version 8.27.7 for Microsoft Windows (Friard and Gamba, 2016), was employed. A dedicated project was created within BORIS, in which the eleven rams observed in the video footage were defined as distinct subjects, and 26 behavioural variables were configured for tracking.

Each video segment was reviewed and annotated within the software by associating each recorded behavioural activity with the corresponding ram and marking its start and end times. The output was a structured dataset including the type of activity, its duration, and the individual ram that performed it.



**Fig. 1.** Total behavioural activities of rams across all treatment groups throughout the study period, expressed as absolute frequency (manual analysis) and duration in seconds (BORIS software analysis). Statistical significance: \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$  among treatment groups.

## 2.4. Statistical analysis

Statistical analyses were conducted using IBM SPSS Statistics, version 26.0 for iOS (IBM, Armonk, NY, USA). To construct a unified database, each behavioural activity observed for a given ram at a specific time was encoded into one of 26 activity categories. The dataset also included additional categorical variables: “ram,” “day,” “time slot,” and “experimental group.” The final database comprised 9396 qualitative records, each representing a single behavioural event.

Preliminary analysis with the Kolmogorov–Smirnov test indicated that neither the number of activities nor the time spent on each conformed to a normal distribution. Therefore, non-parametric statistical methods were used to assess the differences among groups.

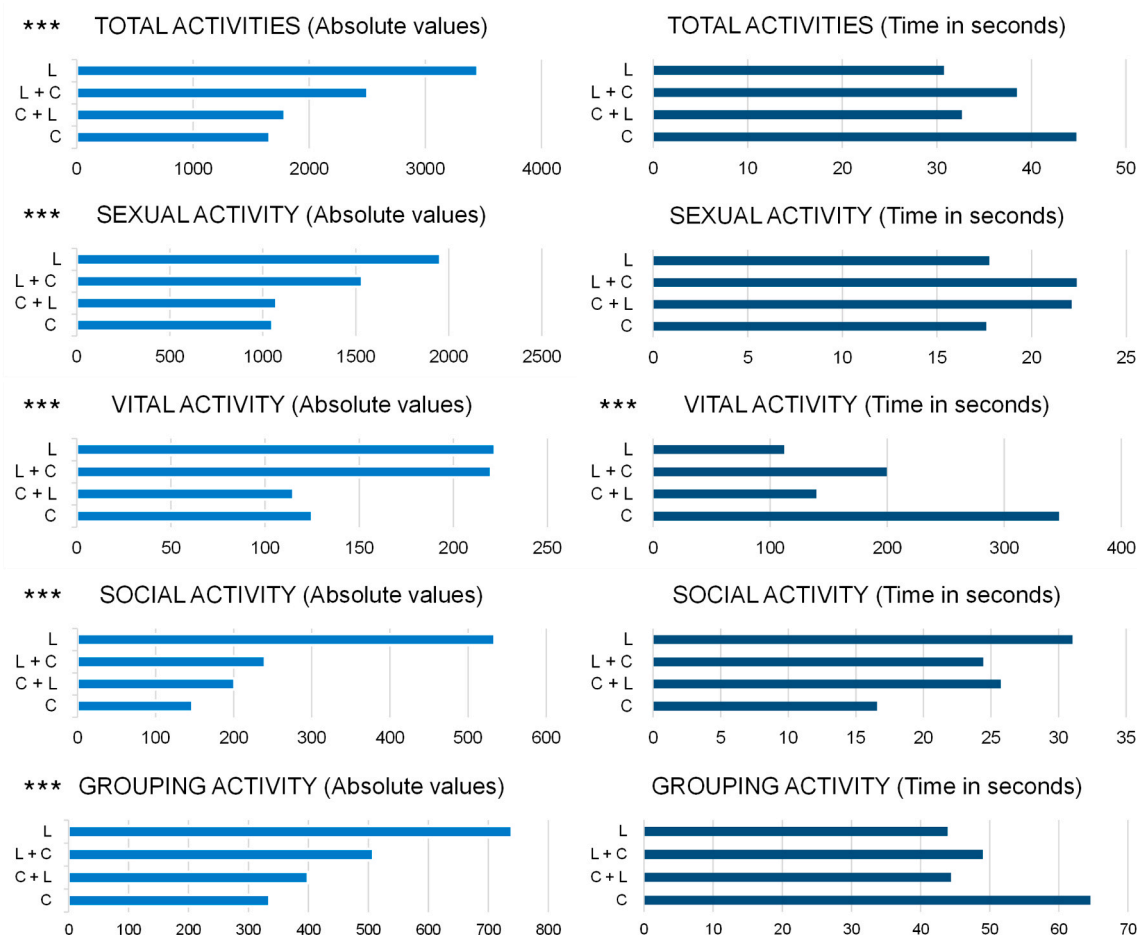
To evaluate potential relationships or independence among categorical variables, the Chi-square test ( $\chi^2$ ) was applied, assuming an equal distribution of expected frequencies and a confidence level of 95 %. The analysis included the evaluation of the influence of fixed factors such as the experimental group, recording day, and recording time slot.

Additionally, comparisons between data recorded manually and those obtained using BORIS software were performed using the Wilcoxon signed-rank test for paired samples.

## 3. Results

### 3.1. Total behavioural activities of rams

Fig. 1 summarizes the total behavioural activities recorded across all groups. Statistically differences were found between the main behavioural categories and among individual behaviours within each category ( $p < 0.0001$ ). The most frequently observed behaviours belonged to the sexual activity category, accounting for 58.6 % of the total events, with ewe sniffing being the predominant behaviour (37.5 % of all sexual activities).



**Fig. 2.** Distribution of behavioural activities by treatment group across the study period, expressed as absolute frequency (manual analysis) and duration in seconds (BORIS software analysis). L: light-stimulated rams, C: control rams, L+C: light-stimulated rams exposed to control and C+L: control rams exposed to light-stimulated males. Statistical significance: \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$  among treatment groups.

Within social activities, the most frequently recorded behaviour was touching ram (45.3 %), while eating was the most common vital activity, representing 85.0 % of this category. Among grouping behaviours, the formation of groups of two rams was most frequently observed (40.0 %).

In terms of duration, vital activities were the longest on average, with a mean duration of approximately  $189.19 \pm 24.3$  s per event. Within this category, eating accounted for the greatest time investment by the rams. Among the sexual behaviours, flehmen was the longest-lasting activity. Similarly, within the social behaviours, touching ram showed the longest average duration, consistent with its frequency of occurrence.

Grouping behaviours exhibited similar temporal patterns to their frequency distribution. However, the longest durations were associated with groups of two, three, and four rams. All behavioural durations differed between activity types ( $p < 0.0001$ ).

### 3.2. Distribution of ram activities by treatment group

Fig. 2 illustrates the distribution of behavioural activities across the different experimental groups. The light-stimulated group (L) performed the highest total number of activities (36.7 %), engaging in more behaviours than the other groups across all categories studied ( $p < 0.01$ ). However, although not significant differences were observed in time spent regarding total activities, animals from L group spent the least time on average performing these activities. Moreover, the L group tended to spend more time on social activities than the other groups, but much less time on vital activities ( $p < 0.01$ ), such as eating and drinking.

The control group (C) had the lowest total number of activities (17.6 %) ( $p < 0.01$ ), performing fewer sexual ( $p < 0.01$ ), social ( $p < 0.01$ ), and grouping behaviours ( $p < 0.01$ ) compared to the other groups. Despite the lower frequency of activities, and the lack of statistical significance, the C group seemed to dedicate more time to each individual activity, suggesting a slower pace. This group spent more time on vital activities ( $p < 0.01$ ) than the others.

The L+C group, which included both light-stimulated and control rams, exhibited a total of 26.6 % of recorded behaviours. Although their frequency of behaviours was lower than that of the L group ( $p < 0.01$ ), they spent more time on sexual activities compared to the C+L group, who shared the experimental space. The L+C group also dedicated more time to vital activities ( $p < 0.01$ ) than the L group.

Finally, the C+L group performed 19.0 % of the total activities, showing a behaviour pattern between the C and L groups. This indicates an interaction between the two types of rams, which appears to neutralize the behaviour of the individual males: stimulating the C males and reducing the activity level of the L males. The duration of activities in the C+L group, with the exception of sexual activities, was intermediate between the C and L groups ( $p < 0.001$ ) for all comparisons.

### 3.3. Activity days and treatment groups

The highest number of recorded activities occurred two days after the introduction of the rams to the ewe groups (Fig. 3), with a total of 1411 events (15.0 %), while the fewest activities were recorded on day 15, with only 302 events (3.2 %) ( $p < 0.01$ ). A progressive increase in activities was observed from the first day of the study until day 5, after which a gradual decrease in activity levels was noted, continuing through to the last day of the study.

Sexual activities reached their peak on day 5 (Fig. 3), with 875 events (15.5 %), representing the highest number of activities recorded ( $p < 0.01$ ), compared to day 13, which had the fewest sexual activities ( $p < 0.01$ ), with only 189 events (3.3 %).

Overall, statistical analysis revealed consistent differences in the number of activities recorded by day and by activity type

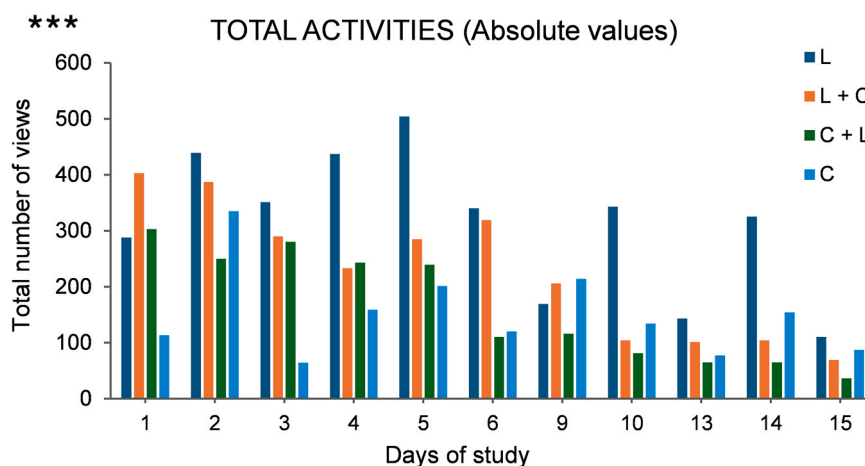


Fig. 3. Daily distribution of behavioural activities in rams by treatment group, expressed in absolute frequency. L: light-stimulated rams, C: control rams, L+C: light-stimulated rams exposed to control and C+L: control rams exposed to light-stimulated males. Statistical significance: \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$  among treatment groups.



( $p < 0.0001$ ).

The sum of activities throughout the study days expressed as a percentage (results not presented) showed differences between the treatment groups ( $p < 0.001$ ). During day 1, the highest number of activities were performed in the L+C groups (4.2 %) and the C+L group (3.2 %). On day 2, more activities were performed in group C (3.5 %). However, in group L the highest activities of all groups and all days were recorded on day 5 (5.3 %) following the introduction of the males to the sheep flock. The days with the fewest activities were day 15 for the L, L+C and C+L groups and day 3 for the C group.

### 3.4. Time slots and treatment groups

Fig. 4 illustrates the distribution of activities by time slot. The afternoon time slot accounted for the highest number of activities overall (51.1 %), with sexual activities (48.1 %), social activities (62.5 %), and grouping activities (59.4 %) predominantly occurring during this period ( $p < 0.01$ ). Conversely, vital activities were more frequent in the morning (41.8 %). The night time slot recorded the lowest number of activities (24.8 %) ( $p < 0.01$ ).

Fig. 5 displays the distribution of activities by treatment group and time slot. Across all treatment groups, the afternoon period consistently had the highest number of activities ( $p < 0.01$ ), while the evening time slot showed the lowest levels of activity ( $p < 0.01$ ). However, in the C+L group, the morning period accounted for the highest number of activities (41.4 %).

Sexual activities were primarily concentrated in the evening for the L and C+L groups ( $p < 0.01$ ). In contrast, the C+L group performed fewer sexual activities during the afternoon, while the L+C group exhibited a relatively consistent distribution of sexual activities throughout the day ( $p < 0.01$ ).

Social activities were most commonly performed in the afternoon by the L, L+C, and C groups, whereas the C+L group exhibited a preference for performing these activities in the morning ( $p < 0.01$ ).

Vital activities varied across treatment groups ( $p < 0.01$ ): the L+C group performed the most vital activities in the morning, the C+L group in the afternoon, and the L and C groups in the evening.

Grouping activities were observed most frequently in the morning for the L+C and C+L groups, and in the afternoon for the L and C groups ( $p < 0.001$ ).

## 4. Discussion

The most frequently performed activities during this study were sexual activities. This result aligns with findings by D'Occhio and Brooks (D'Occhio and Brooks, 1982), who noted that rams, as seasonal breeders, exhibit high libido during short-day periods due to increased testosterone levels. In this study, light stimulation was used to simulate autumn-like conditions, enhancing sexual behavior. These findings corroborate those of Abecia et al. (Abecia et al., 2017) and Abecia et al. (Abecia et al., 2022), which demonstrated that light treatments increase testosterone levels in treated males. In addition, Rosa and Bryant (Rosa and Bryant, 2003) emphasized that photoperiod manipulation is one of the most effective tools to control reproductive seasonality in sheep, reinforcing the observed effectiveness of artificial light stimulation.

Social and grouping activities were similarly distributed, likely due to the gregarious nature of sheep (Hauschildt and Gerken, 2015), which leads them to form cohesive flocks and interact regularly with conspecifics. Gregarious behaviours are crucial for maintaining group cohesion and reducing individual stress (Dwyer, 2008), which could also explain the regularity of social interactions observed.

Vital activities, such as eating and drinking, required the longest time per occurrence compared to other activities. This observation aligns with Berthel et al. (Berthel et al., 2023), who noted that feeding involves multiple simultaneous actions, such as chewing, swallowing, and regurgitation. Group C spent the most time on vital activities, likely because their primary focus was on these tasks. In less sexually motivated animals, energy may be preferentially allocated to essential maintenance behaviours (Forbes, 2007).

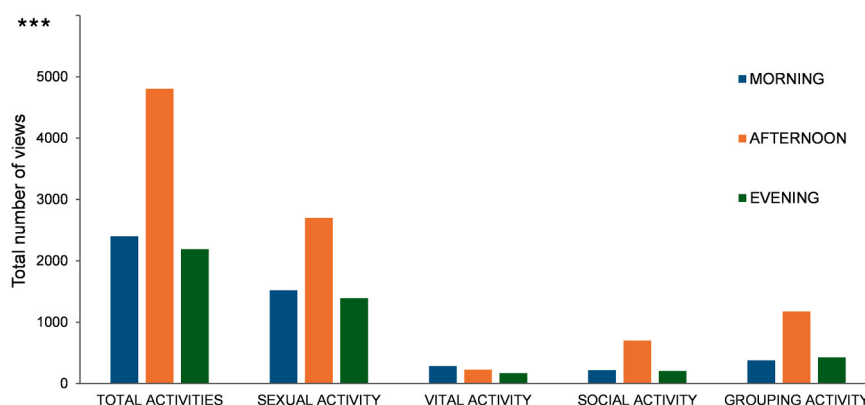
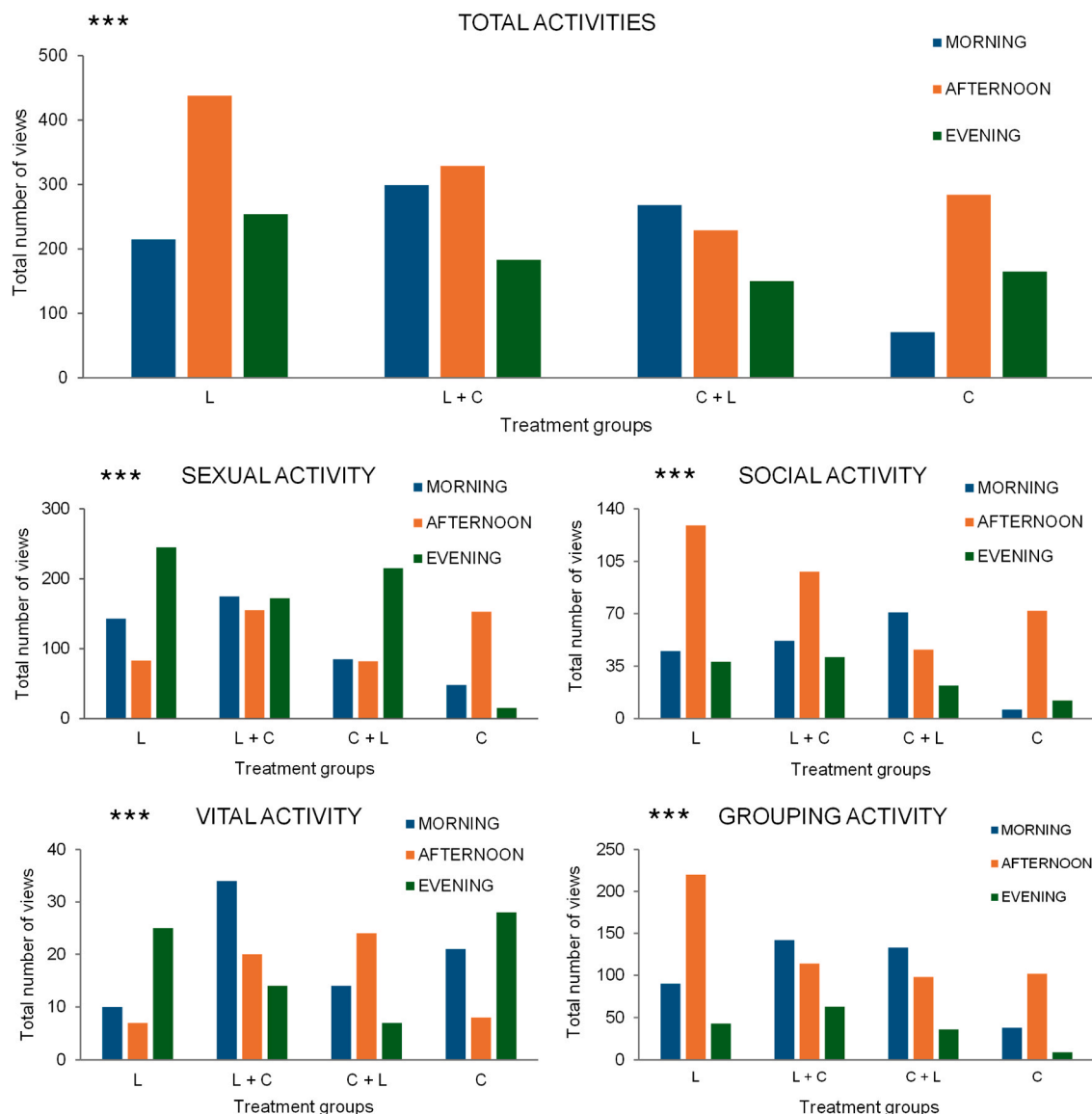


Fig. 4. Total behavioural activities across all rams during the study period, distributed by time slot and expressed in absolute frequency (manual analysis). Statistical significance: \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$  among treatment groups.



**Fig. 5.** Distribution of behavioural activities by treatment group and time slot during the study period, expressed in absolute frequency (manual analysis). L: light-stimulated rams, C: control rams, L+C: light-stimulated rams exposed to control and C+L: control rams exposed to light-stimulated males. Statistical significance: \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$  among treatment groups.

Activity frequency increased from day 1 to day 5, then gradually decreased until the end of the study. This trend corresponds with the role of luteinizing hormone, which stimulates Leydig cells in the testes to produce testosterone, thereby driving sexual behaviour (Chemineau and Delgadillo, 1993). Seasonal and physiological factors also influenced this pattern. Similar behavioural peaks in sexual activity have been reported in rams shortly after exposure to oestrous ewes (Ungerfeld et al., 2004).

The reduced activity during the evening aligns with findings by Wyse et al. (Wyse et al., 2018), who demonstrated that sheep exhibit diurnal activity patterns regulated by circadian rhythms. Additionally, melatonin synthesis at night promotes sleep and inactivity (Touitou et al., 2017). These hormonal rhythms not only affect sleep patterns but are also intricately linked to reproductive behaviour in seasonal breeders (Lincoln et al., 2006).

Light-stimulated males exhibited the highest activity levels, while the control group exhibited the least. These results are consistent with Palacios et al. (Palacios et al., 2023), who found that light-treated rams exhibited greater sexual and social activities when introduced to ewes. Mixed-group interactions indicated behavioural influences, as shown by increased activity levels in non-stimulated rams within mixed pens. Palacios et al. (Palacios et al., 2023) similarly observed enhanced sexual behaviour in non-stimulated rams when cohabiting with stimulated males during the first 24 h. This concept is further supported by the findings of



(López-Magaña et al., 2025), who concluded that sexually active bucks stimulate reproductive activity in both does and other bucks during the non-breeding season when housed together in the same group. In this context, several studies have reported associations between sexual and non-sexual behaviours in males. For example, Ungerfeld and González-Pensado, (Ungerfeld and González-Pensado, 2009) identified links between social dominance and courtship and mating behaviours in rams, under both competitive and non-competitive conditions. In line with these findings, our results indicate that sexual stimulation not only enhances sexual activity but is also associated with a general rise across all behavioural categories in stimulated rams compared with non-stimulated counterparts. Furthermore, we observed that the social contagion effect present in the mixed group influenced the entire repertoire of shared behaviours, rather than isolated behavioural categories. For instance, non-stimulated rams tended to remain inactive when not engaged in ewe-seeking behaviour, suggesting a broader pattern of behavioural inhibition. This interpretation is consistent with the findings of Lincoln and Davidson, (Lincoln and Davidson, 1977), who described the relationship between sexual and aggressive behaviour and the activity of the hypothalamic-pituitary-gonadal axis during the seasonal reproductive cycle in rams, as modulated by photoperiod.

## 5. Conclusions

In line with the dual aim of this study -to evaluate the effects of photoperiod-induced sexual stimulation in rams and to assess the utility of video analysis software for behavioural research-our results demonstrate that males stimulated through photoperiod manipulation exhibited a significantly higher frequency of behavioural activities, especially sexual behaviours, compared to unstimulated males. These findings confirm the effectiveness of artificial light treatment in activating reproductive behaviour during the non-breeding season. Given the prominence of sexual activities among the observed behaviours, future studies in this line of research should focus on further exploring the specific behavioural changes induced by this reproductive stimulation technique, particularly those related to sexual interactions and their temporal dynamics.

Furthermore, behavioural modulation was observed in the mixed group, where contact between light-stimulated and non-stimulated males resulted in increased activity in the latter. This suggests the potential existence of a social facilitation effect, whereby the presence of sexually active males can stimulate the reproductive behaviour of their non-stimulated counterparts.

The implementation of specialized software for behavioural analysis, such as BORIS, proved to be a valuable tool. Its use enabled precise and efficient annotation of large volumes of behavioural video data, significantly reducing the need for manual observation. This approach enhances the objectivity and reproducibility of behavioural studies and supports the development of non-invasive monitoring methods in animal science.

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## CRediT authorship contribution statement

**Carlos Palacios:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **José Alfonso Abecia:** Writing – review & editing, Visualization, Validation, Supervision, Data curation. **Javier Plaza:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Pérez García Elena:** Writing – review & editing, Writing – original draft, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jaime Nieto:** Writing – review & editing, Validation.

## Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Carlos Palacios reports financial support was provided by University of Salamanca. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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