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Greater horseshoe bat (*Rhinolophus ferrumequinum*) ultrasound calls outside a nursery roost indicate social interaction not light sampling

Margaret M. Andrews and Peter T. Andrews

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ABSTRACT

Detailed observations of greater horseshoe bat (*Rhinolophus ferrumequinum*) excursions around the exit hole and surrounding archway, of a nursery roost in West Wales, were made with simultaneous video and ultrasound recordings. The number of flight excursions in July and October around the exit hole (0.42, 0.76, fe/hr/bat; respectively) and the archway (2.17, 0.51, fe/hr/bat; respectively) were not proportional to differences in light levels during emergence (-17.74%, -94.80 %; respectively). Flight excursions during the night in July around the exit hole (5.67 fe/hr/bat) and the archway (9.89 fe/hr/bat) exceeded those during emergence even though the difference in light level was negligible (-0.05%). Typical echolocation calls at 83-84 kHz were recorded together with social calls that had the fundamental frequency in the range 15-39 kHz and harmonics 2-7. The social call rates during the exit in July (0.11 calls/hr/bat) and October (0.14 calls/hr/bat) were not proportional to the difference in light levels and the social call rate was higher in the dark (6.97 calls/hr/bat). In July and October complex individual recognition calls and advertisement calls made during excursions outside the nursery roost demonstrated social interaction. The findings from this study demonstrate that *R. ferrumequinum* flight excursions outside a nursery roost have a social function and are not simply light sampling.

INTRODUCTION

In 1964, De Coursey & De Coursey proposed that light-sampling by greater horseshoe bats, *Rhinolophus ferrumequinum*, during the light-to-dark transition period enables them to synchronise an endogenous, non-24hr activity rhythm to the daily light cycle. Schofield (1996) observed lesser horseshoe bats, *R. hipposideros*, flight excursions outside a maternity roost at dusk and proposed that this activity was also light sampling. However, observations of adult *R. ferrumequinum* activity outside the same nursery roost in West Wales in October indicated that flights around the exit hole and surrounding archway in an old stable block were social behaviour since social calls were recorded (Andrews *et al*., 2015). The same types of calls were associated with activity inside the nursery roost (Andrews & Andrews, 2003; Andrews *et al*., 2011) and a hibernaculum in Devon (Andrews *et al*. 2006).

Since territorial advertisement and male attraction calls made outside nursery roosts are important for communication in vespertilionid species (Lundberg & Gerell, 1986; Barlow & Jones, 1997; Pfalzer & Kusch, 2003) rhinolophid communication outside a roost was investigated. The aims of this study were to monitor *R. ferrumequinum* activity outside a nursery roost with simultaneous sound recordings during July and October when light levels fell at dusk and during the night to test the hypothesis that bats were engaged in social interaction rather than just light sampling behaviour.

1 School of Biomolecular Sciences Liverpool John Moores University, Byrom Street, Liverpool L3 3AF.
2 Deceased.
3 Corresponding author: Margaret Andrews m.m.andrews@livjm.ac.uk

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MATERIALS AND METHODS

The roof space in an old stable block in West Wales (SR 977 960) is the third largest greater horseshoe bat roost in the U.K. (P. Briggs, pers. comm.). The building has an archway (1,420 cm long x 296 cm wide) for previous access to a mansion and the roost exit hole (50.5 cm long x 35.1 cm wide) is set in the archway ceiling. The average June-July exit count of adult *R. ferrumequinum* increased from 166 ± 4 SEM in 1994 to 659 ± 4 SEM in 2014 (Andrews, 2015).

Activity at the nursery roost exit hole and in the surrounding archway

The activity of *R. ferrumequinum* flying in and out of the roost, though two rows of infrared beams across the exit hole, and the time, were recorded (Andrews & Andrews, 2003).

A low light/infrared camera (Pro-642) recorded bat movements (mo) in and out of the exit hole and activity above the exit hole inside the roost and another camera (Pro-780) recorded bat flight excursions (fe) in the surrounding archway. Complete circuit flights outside around the exit hole (3-4 m) or surrounding archway (34-38 m) were counted as excursions. The cameras (Swann, Southampton, U.K.) were connected to a Medusa 960 DVR (QVIS, Hampshire, U.K.) and digital AVI files were analysed with a remote computer. Each movement (mo) through infrared beams in the exit hole was counted as a movement in or out of the roost according to the sequence in which the beams were interrupted. Movement and flight excursion rates per hour were calculated and standardised according to the number of bats.

Ultrasound measurements and analysis outside the nursery roost

During one night in July and three nights in October a modified Tranquility III time expansion bat detector (Bale, Courtpan Design Ltd. U.K.) was placed outside the nursery roost. The frequency and duration of echolocation calls and other adult and infant calls were monitored. These calls were categorised using BatSound software (Petterson, Elektronic AB, Uppsala) (Andrews & Andrews, 2003; Andrews *et al*., 2006; Andrews *et al*., 2011). Social calls had fundamental frequencies in the range 15-39 kHz and harmonics 2-7, so the average frequency was above 20 kHz, and they were classified as ultrasound social calls to distinguish them from low frequency social calls (1-10 kHz) (Andrews & Andrews, 2003). These social calls were identified according to their functions: aggressive threat calls, interactive calls, individual bat recognition and advertisement calls (Andrews & Andrews, 2003; Middleton *et al*., 2014). Modified echolocation calls, associated with flight, occurred in sequences of falling or rising frequencies lower than echolocation (Andrews *et al*. 2006). Infant ultrasound calls were similar to adult calls but there were differences in the call parameters (Andrews *et al*. 2011). The call types identified and ultrasound measurements are shown in Appendix 1.

Environmental monitoring

Light, temperature and rain were monitored by electronic equipment installed at the nursery roost site. Light intensity, measured with a CdS photoresistor, was recorded in volts and compared with light meter readings. Photocell records of 1.4 – 3.0 v were comparable with 1.0 - 600 Lux and records below 1.36 v corresponded with light meter readings of zero; thus changes in light levels were recorded as differences in voltage. Temperature was measured with a LM35DZ sensor and rain recorded with an ARG100 tipping bucket gauge (Andrews, 1996).

Social activity and ultrasound analysis

Flight activity in and out of the roost, light levels and infrared light video recordings of bat activity outside the roost were collated with analysis of ultrasound recordings to compile a simultaneous record of specific social calls with associated activity

RESULTS

Flight activity and light levels outside the nursery roost

During emergence

Rhinolophus ferrumequinum started to fly inside the roost around the exit hole 15 minutes before emergence when the external illumination was 3.0 v. In July during the exit of 650 bats the light level fell 0.47 v from 2.65 v to 2.18 v (a fall of 17.7%) (Figure 1 A, Table 1). There were few flight excursion circuits round the exit hole (0.42 fe/hr/bat) but more excursions around the archway (2.17 fe/hr/bat) (Fig. 1 C, Table 1). Flight excursions round the exit hole occurred in cycles at intervals of 6.4 ± 0.9 mins when groups of up to 10 bats flew in circuits (Figure 1 C). Movements out of the roost through the infrared beams (1.9 mo/hr/bat) were proportional to the difference in the light level (Figure 1 B, Table 1). During the exit of 258 bats in October there was a substantial fall in the light level of 2.39 v from 2.52 v to 0.13 v (94.8%) (Figure 1 E, Table 1). There were minimal flight excursions around the exit hole (0.76 fe/hr/bat) and around the archway (0.51 fe/hr/bat) (Figure 1 G, Table 1). Therefore, flight excursions around the archway were not proportional to the relatively large change in the light level. Movements out of the roost through the infrared beams (2.2 mo/hr/bat) were proportional to the light level (Figure 1 E and F, Table 1).

During the night

When 37 bats returned to the roost in July the light level fell 0.008 v from 0.148 to 0.140 v (0.05 %) (Figure 2 A). However, the largest proportion of flight excursions was around the exit hole (5.67 fe/hr/bat) and the archway (9.89 fe/hr/bat) (Table 1, Figure 2 C). There were minimal movements into (0.9 mo/hr/bat) or out of the roost (0.7 mo/hr/bat) (Figure 2 B, Table 1).

Environmental conditions

The ambient temperature during the exit in July was 20.02 ± 0.4 ^{o} C compared with 14.2 ± 0.3 ^{o} C in October and during the night in July, 19.7 ± 0.1 ^{o} C. There was no rain during the periods monitored.

Comparison of ultrasound calls in July and October

Echolocation calls

During the exit in July there were relatively few echolocation calls (1.41 calls/hr/bat) compared with the exit in October (7.74 calls/hr/bat) and infant echolocation calls were recorded in July during the exit (0.37 calls/min). During the night the echolocation call rate (60.16 calls/hr/bat) showed that this was a very active period (Table 1).

Ultrasound social calls

Social calls varied during the exits in July and October (Fig. 1. D, H) and during the night in July (Figure 2 D). Notably individual bat recognition and advertisement social calls occurred in July. Most individual recognition calls were observed during the exit in October (30.1%) but there were some during the night in July (12.2%) and few during the exit in July (9.3%). Advertisement calls were evident during the exits in July (9.3%) and October (7.2%) but also during the night in July (4.1%) (Table 2, Appendix 1). Infant modified echolocation calls, made during the exit in July (9.3%) and night (12.2%), showed that volant infant bats were present outside the roost (Figure 1 D, Fig. 2 D; Table 2, Appendix 1). The social call rate during the night in July (6.97 calls/hr/bat) far exceeded the rate during the exit in July (0.11 calls/hr/bat) and October (0.14 calls/hr/bat) when light levels changed (-0.05%, -17.7%, -94.8%; respectively) (Table 1). Therefore, social call rates were not proportional to the differences in light levels during the three periods monitored.

Figure 1. Flight activity and ultrasound social calls made by *R. ferrumequinum* during emergence from the nursery roost in West Wales. A, E: Bat exit count –✳–, light level --●--. B, F: Bat movements out of the roost –◆–, into the roost –■–. C, G: Bat excursions around the archway –✳– around the exit hole --✳--. D, H: Social calls, trill advertisement calls –●–, modified echolocation calls made by adults -.-O-.- and infants -.-□-.-.; threat calls –■–, recognition calls –◆–. Infant echolocation calls -.-X-.-.

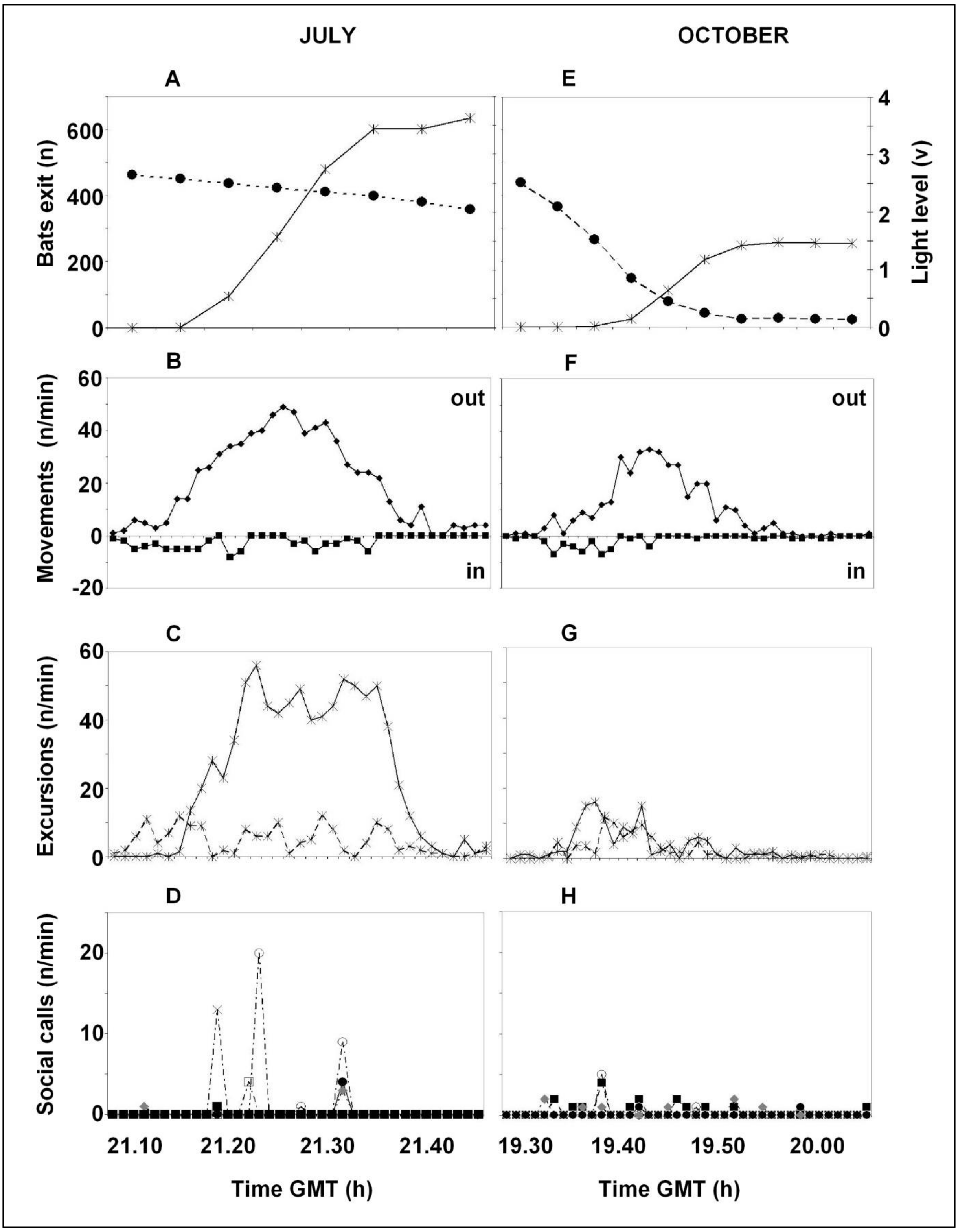

Table 1. Comparison of activity and ultrasound calls made by *R. ferrumequinum* outside a nursery roost in Pembrokeshire, West Wales, during emergence and night-time.

Period Monitored	Echolocation calls * (calls / hr / bat)	Ultrasound social calls † (calls / hr / bat)	Movements (mo / hr / bat). In ‡	Out #	Flight Excursions (fe / hr / bat) exit hole	archway ᵞ	Light level difference (%)	Bats active (max n)
Exit								
July	1.41	0.11	0.2	1.9	0.42	2.17	-17.74	650
October	7.74	0.14	0.3	2.2	0.76	0.51	-94.80	258
Night-time								
July	60.16	6.97	0.9	0.7	5.67	9.89	- 0.05	37

* Adult and infant echolocation;
† See text for details;
‡ Movements In: flights into the roost hole through infrared beams; # Movements Out: flights out of the roost through infrared beams in the exit hole;
$ Exit hole flight excursions — short flight circuits around the outside of the exit hole;
ᵞ Archway flight excursions — long flights around the whole archway outside the roost.

Figure 2. Flight activity and ultrasound social calls made by *R. ferrumequinum* during the night outside the nursery roost in West Wales. A Bats out of the roost - ✳ - and light level --●--. B Bat movements out of the roost -◆- and into the roost -■-. C Bat excursions around the archway - ✳ - and around the exit hole --✳-. D Social calls, trill advertisement calls -●-, modified echolocation calls made by adults -.-O-.- and infants -.-□-.-.; threat calls -■-, recognition calls -◆-.

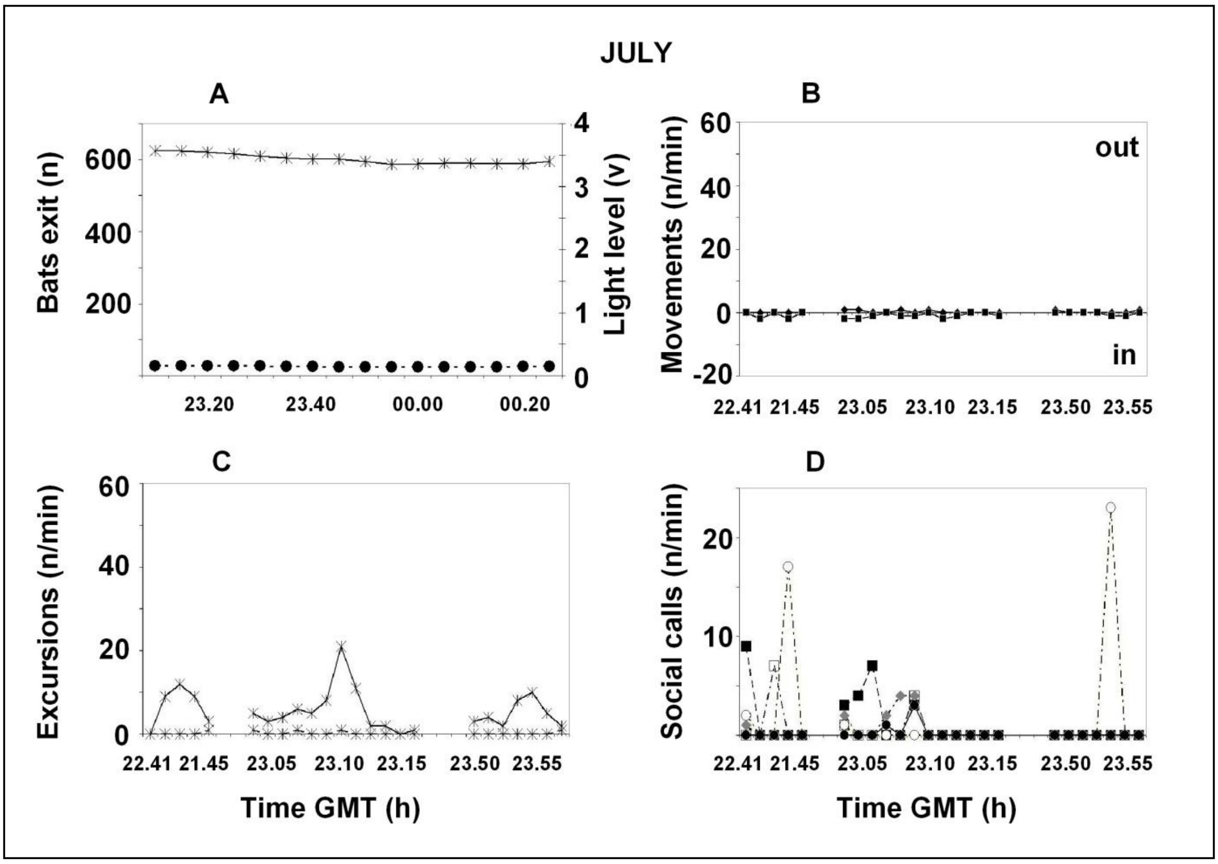

Table 2. Comparison of the proportion of ultrasound social calls made by *R. ferrumequinum* outside a nursery roost in Pembrokeshire, West Wales.

Period Monitored	Proportions of the categories of ultrasound social calls: Adult – Single component A %	Adult – Single component B %	Adult – Multiple component C %	Adult – Oscillatory trill D %	Adult – Modified echolocation E %	Infant – Modified echolocation b E %	Total – Ultrasound social calls n $
Exit							
July	2.3	0.0	9.3	9.3	69.8	9.3	43 *
October	22.9	12.0	30.1	7.2	27.7	0.0	83 †
Night-time							
July	22.1	8.1	12.2	4.1	44.9	12.2	86 ‡

Categories of social calls:
A: Threat calls; B: Interactive calls; C: Individual recognition calls; D: Advertisement oscillatory trill calls;
E: Adult modified echolocation calls: b E: Infant Modified echolocation calls.
* 650 bats in one exit sample,
† 697 bats in three exits,
‡ 37 bats in one night-time sample.
$ number of calls during video sessions

DISCUSSION

This study has shown that *R. ferrumequinum* communicate through social calls during flight excursions outside the nursery roost in July and October when the light level fell at dusk and at night. Notable social calls recorded were oscillatory trill advertisement calls, and infant modified echolocation calls. The occurrence of infant echolocation and modified echolocation calls outside the nursery roost in July showed that 21-28 day old bats were flying outside the roost (Andrews *et. al.,* 2006; Andrews *et. al.,* 2011). Some interaction was possible between the volant infants and their mothers since *R. ferrumequinum nippon* mothers have been shown to communicate with 21 day old infants (Matsumura, 1981). Oscillatory trill social calls have been categorised as advertisement calls (Andrews *et al.,* 2006) because they occurred at an established site for male *R. ferrumequinum* (Hooper & Hooper, 1956). The occurrence of advertisement calls raises questions about the presence of mature males outside a nursery roost, especially in July, since adult male *R. ferrumequinum* leave nursery roosts when the young are born and mating takes place in male territories in October (Ransome, 1991). Alternatively, advertisement calls may have a wider function in family or group recognition since *R. ferrumequinum* social interaction is based on kin-biased associations, fidelity to nursery roosts and mating sites (Rossiter *et al.*, 2002). Complex frequency modulated (FM) calls, similar to a *R. ferrumequinum* call observed by Long & Schnitzler (1975), demonstrated social interaction. Similar social calls have been identified inside a captive colony roost of *R. ferrumequinum* (Ma, *et al*., 2006) but have not been reported outside a nursery roost previously.

Social calls of the rhinolophid *R. ferrumequinum* share some general characteristics of vespertilionid social calls. Pfalzer & Kusch (2003) recorded individual recognition and advertisement calls during flight and proposed that social calls occurred independent of species and have common meanings or functions. *Myotis* spp. use social calls for individual bat recognition (Barclay *et al*., 1979; Fenton *et al.,* 1976) and pipistrelle bats use social calls to attract conspecifics (Russ *et al.,* 1998) or for acoustic recognition (Jones *et al*., 1991).Schofield (1996) proposed that during the night *R. hipposideros* flight excursions around the entrance to maternity roosts could be attributed to environmental testing for temperature but Gaisler (1963) found no relationship between temperature and *R. hipposideros* activity. However, movements out of the roost were proportional to the difference in the light level, which related to activity inside the roost prior to emergence. Temperature was not a determining factor in this study because during the emergence in July and October and at night in July the ambient temperature varied less than 0.5 º C. Also the mean temperatures ranged from 14.2 º C to 20.2 º C and *R. ferrumequinum* emerge and return to the roost if temperatures are above 8 º C (Andrews & Andrews, 2004) and insects are active (Swift *et al.* 1985).This study has shown that *R. ferrumequinum* activity outside a nursery roost in July and October is social interaction and not light sampling because:-

- the number of flight excursions was not proportional to differences in light levels,
- the social call rate was not proportional to differences in light levels during exits in July and October and was higher at night,
- specific complex FM calls and trill advertisement social calls made during excursions outside the nursery demonstrated social interaction.

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