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Dew-worms in white nights: high latitude light constrains earthworm (*Lumbricus terrestris*) behaviour at the soil surface

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Abstract

Soil is an effective barrier to light penetration that limits the direct influence of light on belowground organisms. Variation in aboveground light conditions, however, is important to soil-dwelling animals that are periodically active on the soil surface. A prime example is the earthworm *Lumbricus terrestris* L. (the dew-worm), an ecosystem engineer that emerges nocturnally on the soil surface. In the summer, the northernmost populations of *L. terrestris* are exposed to a time interval with no daily dark period. During a two-week period preceding the summer solstice, we studied the constraints that boreal night illumination imposes on *L. terrestris* surface activity by comparing their behaviour under ambient light with artificially-induced darkness. Looking for evidence of geographical divergence in light response, we compared the behaviour of native *L. terrestris* (Jokioinen, S-W Finland; 60°48'N) with two markedly more southern populations, from Preston (Lancashire, UK; 53°47'N) and Coshocton (Ohio, USA; 40°22'N) where the nights have a period of darkness throughout the year (total latitudinal range ca. 2 300 km). Under ambient light conditions, *L. terrestris* emergence on the soil surface was diminished by half compared with the darkened treatment and it peaked at the darkest period of the night. Also mating rate decreased considerably under ambient light. The native dew-worms were generally the most active under ambient light. They emerged earlier in the evening and ceased their activity later in the morning than dew-worms from the two more southerly populations. The differences in behaviour were, however, significant mainly between native and UK dew-worms. In the darkened treatment, the behaviour of the

three earthworm origins did not differ. Under the experimental conditions light condition was the dominant environmental factor controlling surface activity, but elevated night-time air temperature and humidity also encouraged dew-worm emergence without discernible differences among geographical origins. Our results show, that in boreal summer, the high level of night illumination strongly limits soil-surface activity of dew-worms. Considering the important regulatory role of *L. terrestris* in many ecosystem processes, this can have significant corollaries in dew-worm impacts on the environment. Although evidence for geographical differentiation in behaviour was obtained, the results point to phenotypic flexibility in *L. terrestris* light response.

Key-words: adaptation, geographical variation, illumination, light environment, phenotypic flexibility, sensory ecology

Highlights:

- We studied how the light during boreal summer nights limits dew-worm activity on the soil surface.
- Compared with a darkened treatment, soil surface activity was halved in ambient light.
- Mating frequency was consequently also reduced.
- Response to light differed, although not consistently, in dew-worms from different latitudes.

1. Introduction

Light is a great provider and pacemaker of life and adaptation to variation in the light environment is a central quest for all organisms (Gross, 2002; Bradshaw and Holzapfel, 2007). Soil is an efficient light barrier and, based on plant models, it has been asserted that ecologically and physiologically significant amounts of light rarely penetrate deeper than 4-5 mm into soil (Tester and Morris, 1987). The direct impact of light on the development of soil microbial community characteristics may even be limited to the surface 1 mm of soil (Jeffery et al., 2009). Therefore, direct impacts of light on belowground dwelling invertebrates, in general is likely to be small. Exceptions would be species residing permanently in the uppermost part of the soil or in habitats where the soil is too thin to allow escape from light (Wallwork, 1970).

Among soil invertebrates, there are also deep burrowing species that regularly visit the soil surface and become directly subjected to prevailing light conditions. A prime example is the anecic earthworm *Lumbricus terrestris* L. (the dew-worm), which as an adult lives in a deep (often > 1 m; e.g. Nuutinen and Butt, 2003), vertical burrow that opens at the soil surface below a midden, a mixture of collected organic and inorganic material and surface castings. The original authority for this species recognized the habit of *L. terrestris* to emerge nocturnally onto the soil surface to copulate (Linné, 1767) and Darwin (1881) realised that such emergence was important to foraging and midden construction.

Dew-worms display a diurnal vertical activity pattern, where individuals are nearest to the soil surface close to midnight and in the deepest position at noon (Joyner and Harmon, 1961). *L. terrestris* prefer moist and cool conditions and their emergence is known to be correlated with high air humidity, affected by air temperature and elapsed time from preceding rainfall events (Macdonald, 1983). Dew formation most certainly favours emergence but - remarkably - it seems that the importance of dew on *L. terrestris* activity has not been systematically investigated. While the nocturnal activity pattern relates to the prevalence of suitable moisture and temperature, avoidance of harmful daylight UV-radiation and the risks imposed by day-active predators are also likely to have affected the evolution of this behaviour (Edwards and Bohlen, 1996). The diurnal activity patterns of *L. terrestris* can also have an endogenous physiological component as the rhythms can persist under continuous darkness (Laverack, 1963).

L. terrestris senses light with photoreceptor cells that are mainly found in the first few anterior segments of the body, with greatest densities at the prostomium (Hess, 1925). Photoreceptors are found in other body regions too, chiefly within the posterior segments that are exposed to light when a dew-worm casts on the soil surface. The negative reaction of *L. terrestris* to light is well documented, as is its positive reaction to very weak illumination (Hess, 1924). Under artificial laboratory illumination and altered day-night cycles, *L. terrestris* strictly avoids light and adjusts its surface activity to the dark period (e.g. Butt et al., 2003; Field and Michiels, 2006). To our knowledge, the importance of light conditions on dew-worm emergence and mating activity has not been investigated under natural daily variation of illumination.

The geographical distribution of *L. terrestris* covers a wide latitudinal range and, consequently, a great variation in day-length and illumination conditions. In the Northern Hemisphere, it reaches beyond the Arctic Circle (66.56°N; e.g. Nieminen et al., 2011) where populations in summer are exposed to the polar day. Populations in the more southern parts of the boreal zone live in conditions where there is no dark period over a number of midsummer weeks, with twilight conditions prevailing throughout the night. The Global Biodiversity Information Facility (GBIF, 2013) records of *L. terrestris* distribution suggest that in the Northern Hemisphere the southern range extends to the northern parts of the Iberian Peninsula in Europe (41°N) and in North America still further south (37°N). A distribution map of Reynolds (1995) notes *L. terrestris* as far south as 33°N in North-America. In these southern latitudes the night always has a dark period which even at its minimum lasts for several hours.

Since soil-surface activity is critical for feeding, reproduction, and dispersal of *L. terrestris* (Mather and Christensen, 1988; Nuutinen and Butt, 1997; Butt et al., 2003) and thereby for the many ecological processes that this species regulates (Lee, 1985; Edwards and Bohlen, 1996; Borken et al., 2000; Shipitalo and Le Bayon, 2004; Milcu et al., 2006; Griffith et al., 2013), we investigated the constraints that a northern light environment imposes on dew-worm activity at the soil-surface. We hypothesized that boreal mid-summer white nights severely limit the time available for *L. terrestris* soil-surface activity, with temperature and humidity also playing an important, but secondary role. At the same time, we considered that populations residing in widely different environments could diverge in their behaviour (Foster and Endler, 1999; Hut et al., 2013). We predicted that a population from a northern area exposed to high illumination levels in midsummer, would show a higher level of activity under these conditions compared with more southerly populations with no previous exposure to white nights. Alternatively, phenotypic flexibility (*sensu* Piersma and van Gils, 2011) could lead to a similar response of populations, irrespective of their latitudinal origin.

2. Material and Methods

2.1. Experimental animals

In mid-May 2009, we collected *L. terrestris* from three deciduous forests, selected to represent a wide latitudinal gradient with clear differences in day-length conditions during the Northern Hemisphere midsummer. The sites were, from north to south: Jokioinen (Finland (“FI”); 60°48’N, 23°28’E), Preston (Lancashire, United Kingdom (“UK”), 53°47’N, 2°41’W) and Coshocton (Ohio, USA (“US”), 40°22’N, 81°48’W). The set of locations represents a total latitudinal range of ca. 2 300 km. The US site was chosen to represent the most southern conditions as we were not familiar with equally southern continental European populations of *L. terrestris* and the experimental animals could be readily obtained based on previous research collaborations. The differences among locations in length of darkness during midsummer 2009 are depicted in Fig. 1. In Preston, even the shortest midsummer night has 5 hrs 7 min of darkness and in Coshocton this is 7 hrs 51 min. In Jokioinen, there is a period of no darkness in midsummer, with civil twilight conditions prevailing at midnight. By definition, civil twilight begins in the morning (before sunrise) and ends in the evening (after sunset) when the center of the Sun is geometrically 6° below the horizon (e.g. Forsythe et al., 1995). Under favourable weather conditions, illumination is then sufficient for terrestrial objects to be clearly distinguished and according to a traditional, although questionable definition, artificial light is not needed to perform outdoor activities (Leibowitz and Owens, 1991).

UK and US dew-worms were shipped to Jokioinen by express delivery lasting from 2 to 4 days. Prior to introduction into the experimental units, dew-worms from each origin were stored in groups of 6-10 individuals in 10 l buckets filled with sieved (4 mm), heat-treated and remoistened, earthworm-free soil. Chopped barley (*Hordeum vulgare* L.) straw was provided on the soil surface as food. Storage was at +15°C and in continuous darkness, lasting for 4-11 days depending on batch collection or arrival date.

2.2 Experimental set-up

As experimental units, we used 0.15 m inner diameter, 0.45 m tall PVC-tubes that were sealed with a watertight cap at the base and filled with soil. The soil was field-collected top soil (fine sand; 15% clay, 29% silt and 56% sand; pH (H₂O 1:2,5) 6.1, C content 2.1%) that was passed through a 4-mm sieve, mixed thoroughly, and kept for 5 days at +70 - 80°C to eradicate any remaining earthworms and their cocoons. It was then re-moistened to 20% gravimetric water content in small batches and mixed into a single batch. In total, twenty tubes were filled with soil close to the upper margin by a standard tapping procedure to ensure a uniform soil bulk density throughout each column.

Adult, healthy and individually weighed *L. terrestris* from a particular geographical origin were randomly selected and introduced into six tubes, so that altogether there were 3 (origins) x 6 (replicates) = 18 experimental tubes, each containing three earthworms. Transparent, 0.1 m tall, plastic collars were mounted on top of the tubes to deter earthworm escape. Two tubes were kept without earthworms for monitoring experimental conditions. After earthworm addition, but prior to the experiment, all tubes were kept for one week at +15°C in a 12:12 hr artificial light cycle, with lights on at 23:00 hrs and off at 11:00. This was to equally distort

possible emergence patterns originating from the different longitudinal origins of the populations.

During the experiment, the tubes were kept in a temperature-controlled water bath (a modified, rectangular chest freezer) to stabilize the soil temperature at +13°C (Fig. 2). The bath was divided into two sides: ambient illumination (AMB) and artificially induced nighttime darkness (DARK). Two aquarium pumps were installed to circulate the water for equal temperature distribution in the bath. Three tubes with earthworms from each geographical origin were placed randomly at opposite sides of the bath, so that there were two 3x3 groups of tubes. The two tubes without earthworms were placed on the AMB side between the treatments and monitored for soil water content using a pair of 0.15 m long Time Domain Reflectometry rods (TDR Model no. 6050X1 Trase System, Soilmoisture Equipment Corp., Santa Barbara, CA) and temperature at 0.1 m depth using a permanently installed mercury thermometer.

The experiment was conducted outdoors in MTT's Jokioinen campus in south west Finland (60°48'N, 23°28'E), in a glass-roofed shelter to protect the tubes and monitoring equipment from rain. The shelter had metal meshwork walls that permitted the passage of air and light. The light conditions in the shelter corresponded closely to those under the open sky and there was no artificial lighting in the vicinity of the shelter that affected illumination of the tubes. The water bath was positioned so that the longer side was oriented E-W. The DARK treatment was achieved by covering the tubes with a three-ply thickness of brown garden cloth from 21:30 to 03:30 each night (all times relating to the experiment are expressed in standard time). The cloth was draped in a tent-like fashion over a ridge at a height of 1.5 m above the tube surfaces. To the human eye, this induced complete darkness under the cloth. The AMB treatment remained constantly under ambient light.

The illumination level above the tubes was measured at the end of the behavioural recording in both treatments with a standard hand-held lux meter (LMT pocket-lux, Lichtmesstechnik, Berlin; measuring accuracy at low illumination 0.001 lux) before un-covering the DARK treatment. The Lux-scale was used to provide a comprehensible reference for a human observer. At the same time, air temperature and humidity above the tubes was measured with a hand-held meter (HMI41, Vaisala, Helsinki, Finland). To quantify the shading effect of the glass roof and meshwork walls on the light conditions, we measured the illumination level under open sky on two sides of the shelter on the six final mornings of the experiment at ca. 03:40 hrs. Soil temperature in the control tube was determined immediately before and after the recording sessions while soil moisture was monitored twice (9 and 16 June) with TDR-measurement in the respective control tube.

During the experiment the earthworms were fed horse manure that could be regarded as an equally unfamiliar food source for all three earthworm origins. At the start of the experiment, ten grams of oven-dried and remoistened manure was spread evenly on the soil surface. The same amount of manure was added after 10 days to all tubes, to replenish the consumed / buried feed. Immediately before each recording session, all tubes were equally moistened by spraying tap water on the soil surface. At the end of the experiment, the tubes were emptied, worms were retrieved by hand-sorting the soil and their general condition and fresh-weight recorded. As a recent investigation revealed notable cryptic diversity in *L. terrestris* (James et al., 2010), three individuals of each geographical origin were genetically analysed at the Canadian Centre for DNA Barcoding (CCDB) after the experiment. Results confirmed that all

specimens were *L. terrestris* (David Porco, CCDB Guelph, Ontario, Canada, personal communication).

2.3 Recording of behaviour

The surface activity of the dew-worms was recorded with two night-vision web-cams (StarCam mini+, Micro-Star International, New Taipei City, Taiwan), one installed 1.5 m above each of AMB and DARK treatments (Fig. 2). The cameras were connected to a lap-top computer in a control tent a few metres away. The web-cam data was collected and managed with JaxCam Web Monitoring v3.0 software (Jaxstream Inc., Cupertino, CA). An infra-red light-source was added 1.4 m above the DARK treatment to improve the quality of image capture. This made it possible to record emergence and movement of individuals on the soil surface and mating activity in both treatments.

The experiment ran for fifteen days, from 6 to 21 June (the summer solstice) in 2009. The recording was made each night between 21:30 and 03:30 hrs. During the experiment, sunset changed from 21:49 to 22:03 and sunrise from 03:00 to 02:52. The first seven nights had a period of darkness (lasting from 1 hr 31 min to 50 min), while during the last eight nights, civil twilight by definition prevailed throughout the night.

2.4 Data-set compilation

The web-cam material for each night was reviewed by one individual using a standard media player. As the main response variable, a dichotomic activity variable (0=no earthworm activity, 1=earthworm(s) active on the soil surface) was recorded for each tube in 0.5 hr increments during the night (i.e., 12 observations each night). We counted emergence of any degree, from exposure of the anterior segments to complete emergence from the burrow as surface activity. As a further measure for activity differences, we recorded for each tube the first and last occurrence of surface activity and computed the time when 50% of the cases (tube x session combination) were for the first and last time active. Additionally, we recorded the occurrence of mating in the tubes for each night (0=no matings, 1= mating occurred). As explanatory variables, apart from earthworm origin (FI, UK and US) and light treatment (DARK and AMB), we used air temperature and relative humidity (RH%) (both measured at 2 m height) together with the degree of cloudiness (ceilometer measurements on an eight-level ordinal scale: from 0=bright sky to 8=completely overcast). The measurements of environmental conditions were available for the 0.5 hr periods from the Jokioinen Observatory of the Finnish Meteorological Institute situated 1.2 km from the experimental site.

It has been suggested that the lunar cycle could affect *L. terrestris* activity (Michiels et al., 2001). This could represent an endogenous lunar activity cycle not related to illumination (Ralph, 1957), but it is also conceivable that in some conditions, bright moonlight could evoke a behavioural response in dew-worms. During our experiment, there was a full moon on 7 June and then a waning phase until the end of the experiment. We did not account for the phase of moon as we considered that in the high illumination levels of the experimental period the effect of moonlight was negligible.

During conjugation, *L. terrestris* are less sensitive to light than at other times and they can continue copulating on the soil surface for 1-3 hours after sunrise (Darwin, 1881; our own previous observations and those made during this study). Therefore, we excluded from the analyses of nightly activity all those tubes where a mating occurred during a particular night. Further, web-cam material for one night (12-13 June) was lost because of a technical problem, so the data set consisted of 14 nights in total.

2.5 Statistical analyses

The nightly variation of activity was first analyzed by modelling mean activity in the tubes with a mixed model using light treatment, earthworm origin, mean air temperature and mean relative air humidity as fixed explanatory variables. The model included all interactions with earthworm origin. "Tube" was used as a random variable in the model (six tubes for each origin). The means for each night were calculated from the twelve observations recorded at 0.5 hr intervals. The effects of temperature and humidity on activity was further studied by dividing the nights into three temperature classes (cold ($<5^{\circ}\text{C}$), intermediate ($5 - 9^{\circ}\text{C}$) and warm ($>9^{\circ}\text{C}$)) and two air humidity classes (dry ($<90\%$) and moist ($>90\%$)). Categorical, instead of continuous variables were used in the statistical analyses because weather during many nights was quite similar, the relationship between weather variables and studied responses were not linear (N was too small for non-linear modelling) and weather data was incomplete. The classification of both variables was done so that N was sufficiently high in each class for the analyses.

Normal distribution of the response variable was assumed and the validity of the assumption checked by graphical methods (box-plots and scatter plots of residuals and fitted values). Means and standard errors were estimated by the REML-estimation method. Pair-wise comparisons were undertaken using t-type tests.

The value of the cloudiness data was limited for modelling because of small variation (ten of the fourteen nights were completely or mostly overcast (Fig. 3b)) and close co-variation of cloudiness with temperature (bright nights were cold (Fig. 3a,b)). The effects of temperature and cloudiness were found to be largely congruent, and since it was not possible to separate their effects we limited the analysis to temperature, which improved its efficiency.

To compare the patterns of overnight activity of the three earthworm origins, each night was divided into three successive two-hour periods and the variation of the original dichotomic activity observations was modelled with the same explanatory variables as above. The response variable was assumed to follow a binomial distribution and a logit-link function was used in the analysis. Data for the three periods were analysed separately. The results were expressed as odds ratios (OR; value 1 indicating no difference) and their 95% confidence intervals (95% CI). The confidence intervals were related to the *P*-values such that the odds ratios were not statistically significant if the confidence interval contained 1. The start and end times of activity were analyzed using regression analysis of survival data based on the Cox proportional hazards model. Analysis of mating frequency was also undertaken and used a binomial model with the occurrence of mating in a tube (0 vs. 1 for each tube per night) as a response variable, and the light treatment, earthworm origin, and their interaction as fixed explanatory variables and tube as a random effect.

Mass of worms at the end of the experiment was analyzed using a model including origin, treatment and their interaction effects, as well as mass at the start of the experiment as a covariate. The mean mass of worms in a tube was used as the response variable.

Statistical analyses used PROC MIXED (normal data), PROC GLIMMIX (binomial data) and SAS PHREG (Cox model) of SAS 9.3 statistical software (SAS Institute Inc., 2011).

3. Results

3.1 *Environmental conditions*

The weather varied considerably during the experiment. At the Jokioinen Observatory, mean air temperature during the recording sessions ranged from +1.6°C at the start of the experiment to +17.4°C (Fig. 3) with an overall mean of +8.8°C. Mean relative air humidity was 89.7% (min: 76.4%, max: 96.8%) (Fig. 3a). Most of the nights were cloudy with an overall median cloudiness of seven on the eight-point scale. Three nights were exceptionally bright with a median cloudiness of zero and these nights also tended to be cold (Fig. 3a,b). Despite the large air temperature fluctuation, soil temperature in the control tube remained stable, varying from +12.5°C to +13.6°C with a median of +13.1°C. Soil water content in the control tube was 21-23%, thus remaining close to the initial value.

According to the measurements taken at the end of each recording session, illumination level in the (covered) DARK treatment was near zero lux (min and median: 0 lux, max: 1.0 lux), while above the AMB treatment, the median value was 407 lux (min: 100 lux, max: 940 lux). The median illumination level outside the shelter was 635 lux while above the AMB experimental tubes it was 560 lux on the final six mornings of the experiment. The shelter thus caused some shading and experimental light levels did not exactly correspond with natural conditions. The median relative humidity was slightly higher in DARK (86.5%) than in AMB (84.3%) while the corresponding difference in air temperature was small (+9.2°C vs. +9.1°C). The shelter probably also contributed to small differences in air temperatures measured at the end of the recording session in the AMB treatment and at Jokioinen Observatory. The values correlated closely ($R_s = 0.946$, $P < 0.001$), but the temperature in the shelter averaged a little over one degree Celsius higher than at the Observatory (median (min-max): 9.2 (0.5-18.0)°C and 8.5 (-1.0-17.5) °C, respectively).

3.2 *Earthworm recovery*

At the end of the experiment, all UK and US worms were recovered from the tubes. Of the FI worms, one AMB individual died and disintegrated. Additionally, one FI individual in DARK managed to escape from its tube and entered an adjacent FI DARK tube. Although the number of individuals was thus unbalanced during at least part of the experiment, we did not censor the data from any of the tubes as there was no mixing of origins.

The UK worms had the greatest initial mean fresh weight (6.07 g, sd=1.19), FI were intermediate (5.12 g, sd=1.19), and US worms were the smallest (4.62 g, sd=0.90; N=18 for all origins). In the statistical analysis of the final weights, the effect of covariate was significant ($P < 0.01$), so that if the mean weight in a tube at the beginning of the experiment was 1.0 g larger than in another tube, at the end of the experiment the difference was the same

1.0 g (se=0.25). There was no significant treatment-by-origin interaction ($P = 0.58$), although the final weights of FIN worms were greater in AMB than in DARK (5.8 g vs. 5.4 g) while the opposite was the case for UK (4.6 g vs. 4.7 g) and US (4.8 g vs. 5.0 g). However, none of those differences were statistically significant. The mean final weight was the same in the treatments (DARK: 5.1 g (se=0.17); AMB: 5.1 g, (se=0.15)). There were differences between the origins ($P = 0.03$), FIN dew-worms (5.6 g, (se=0.21)) were significantly larger than UK (4.7 g (se=0.27)) or US (4.9 (se=0.24)) worms at the end of the experiment.

3.3 Overall activity

The general activity pattern in the two light treatments clearly differed (Fig. 4a-c). In DARK, activity began almost immediately after the tubes were covered and remained high before displaying a slight decline towards the end of the recording period for FI and UK earthworms. In AMB, activity increased gradually, peaked close to midnight and then declined steadily towards sunrise. The activity level in AMB remained lower than in DARK during all time intervals.

The difference in overall activity between DARK and AMB was statistically significant ($P < 0.001$). The mean proportion of tubes with surface activity for DARK (0.95) was more than twice that for AMB (0.46). There was also statistically significant ($P = 0.05$) interaction between light treatment and earthworm origin. In DARK, the origins did not differ in activity ($P = 0.92$), but under AMB, origin had a highly significant effect ($P < 0.01$) on activity. In AMB, the mean proportion of active tubes for the three origins was 0.57 (FI), 0.33 (UK) and 0.48 (US). With the lowest activity, UK differed significantly from the others, while FI and US did not differ from each other ($P = 0.17$). In AMB, air temperature significantly affected activity ($P < 0.001$), an increase of one degree raising the proportion of active tubes by 0.04 units (se=0.004). There was a similar effect in DARK ($P = 0.05$), but the increase was only 0.01 (se=0.004).

Whether the nights were classified as cold (N=2), intermediate (N=6) or warm (N=6), there was no significant treatment-by-origin interaction effect on activity ($P = 0.78$). Likewise, the difference among origins did not change depending on temperature class ($P = 0.46$). The treatments differed in activity, however, depending on temperature class ($P < 0.01$). In all three temperature classes, activity in DARK was higher than under AMB ($P < 0.001$), but on warm nights the difference between mean activity in DARK (0.98) and AMB (0.56) was smaller than on cold (0.82 vs. 0.18) or intermediate (0.96 vs. 0.46) nights.

To study the effect of relative humidity on activity, the nights were divided into dry (N=4) and moist (N=10). The treatment-by-origin interaction did not differ for the two classes ($P = 0.52$), neither did the difference in activity of earthworm origins ($P = 0.94$). There was evidence for a differing effect of humidity on activity in the treatments ($P < 0.01$). In DARK there was no difference between humidity classes ($P = 0.98$), but in AMB the difference was close to significant (0.08), with greater mean activity during moist nights (0.50) than on dry nights (0.37).

3.4 Activity during different phases of the night

During the first two hours of observation, in AMB there were significant differences in activity relating to earthworm origin ($P = 0.03$). Using FI as the reference point, the activity of UK was significantly lower (OR=0.13, 95%CI=0.03-0.49; as Odds Ratio is less than 1, the probability of activity occurring is lower). The corresponding activity difference between FI and US was not statistically significant, although relatively large (OR=0.40, 95%CI=0.11-1.49). In AMB, air temperature had a significant influence on activity ($P < 0.001$) without a significant interaction with earthworm origin (the increasing effect on activity of one degree of Celsius: OR=1.34, 95%CI=1.23-1.45). In DARK, there was no corresponding activity difference among earthworm origins ($P = 0.20$) and temperature had no discernible influence on activity ($P = 0.83$). The pattern for the first instance of activity agreed with the above (Table 1): under AMB, FI dew-worms started their activity significantly earlier than those from UK and US ($P < 0.05$), while in DARK the origins did not differ ($P = 0.14$).

During the mid two hours of observation, no significant treatment-by-origin interaction was found ($P = 0.33$) and origins did not differ in activity ($P = 0.83$). Using FI as the reference point, the activity was slightly lower in UK (OR=0.91, 95%CI=0.14-6.00) and US worms (OR=0.65, 95%, CI=0.13-3.26). Overall, the activity in DARK was much higher than under AMB light (OR=38.65, 95%CI=8.27-169.24) and temperature affected the activity ($P < 0.01$; the increasing effect on activity of one degree of Celsius: OR=1.40, 95%CI=1.21-1.62).

During the final two hours, the treatment-by-origin interaction was nearly significant ($P = 0.09$) and under AMB there was an indication of differences among the origins ($P = 0.08$). Using FI as the reference point, UK differed with lower activity (OR=0.38, 95%CI=0.15-0.94) while difference with US was not significant (OR=0.88, 95%CI=0.38-2.07). Temperature had an influence on overall activity ($P < 0.001$; the increasing effect of one degree of Celsius: OR=1.28, 95%CI=1.16-1.40). In DARK, there was no difference in the activity of the origins ($P = 0.21$) while temperature affected the activity ($P < 0.01$, OR: 1.39, 95%CI 1.14-1.68). Under DARK the activity continued until the end of the experiment for all origins (Table 1). In AMB the activity ended earlier, FI dew-worms continuing their activity slightly, but not significantly longer than the other origins ($P = 0.15$).

3.5 Mating frequency

In total, 20 matings were observed in the DARK treatment and 7 in AMB. The difference between the light treatments was significant ($P = 0.03$): using AMB as the control, OR was 3.11 (95%CI=1.11-8.68) indicating higher mating activity in DARK. There was no evidence for differences among earthworm origins in the number of matings ($P = 0.49$), nor for treatment-by-origin interaction ($P = 0.86$). Because of the low number of observations, however, the comparisons of origins were not strong statistically. For the same reason, it was not possible to model the effects of temperature and moisture on mating activity.

4. Discussion

4.1 White nights as an activity constraint

The high illumination level in boreal midsummer night strongly limits the activity of *L. terrestris* on the soil surface as our overall measure under ambient light was less than half of that in darkness. The clear peak of activity close to midnight under ambient light further

underlined the limiting effect of light on surface activity. In the darkened treatment, the dew-worms became highly active immediately, remained so throughout the night, and there was no evidence for differences in this pattern among dew-worm origins. This shows that the dew-worms were mainly responding to the prevailing light environment and that activity rhythms as a consequence of their different longitudinal origins did not decisively control their soil surface activity.

The temperature and humidity conditions, despite their notable variation, remained mainly within suitable limits for all three dew-worm origins during the experiment, as we found no evidence for a differing response to these variables among the groups. In a previous study under temperate, humid field conditions - and presumably at higher average night temperatures than in our case - *L. terrestris* emergence was negatively associated with temperature and positively related to relative humidity (Macdonald, 1983). A positive correlation between surface activity and air humidity was also recorded by Valckx et al. (2010) in the laboratory when the distance covered by dew-worms during foraging bouts was measured in relation to air humidity variation. Our observations of elevated dew-worm activity under high relative humidity were thus in line with previous findings. Although direct rainfall effects were excluded by our experimental design, the air humidity effect likely mirrors the known positive association of *L. terrestris* surface movement with recent rainfall (Macdonald, 1980; Mather and Christensen, 1988). Minimum air temperatures close to zero and the relatively low average temperature probably explain the positive correlation of activity to temperature since the optimal temperature for dew-worm growth and reproduction has been observed to be close to +15°C (Butt, 1991; Daniel et al., 1996). That activity difference between the light level treatments was highest on colder nights was most likely an experimental artifact caused by the slight warming effect of the covering cloth used to provide darkness.

The mating frequency dataset was small, but sufficient to demonstrate that white night conditions limit mating of dew-worms. One explanation for this may relate to the pre-mating behaviour of this species. Preceding mating, *L. terrestris* go through a courtship sequence on the soil surface that involves burrow visiting (Nuutinen and Butt, 1997) and evaluation of partner's size (Michiels et al., 2001). The length of this sequence varies, but it can take up to 90 min (Nuutinen and Butt, 1997). According to our own observations, during the pre-mating sequence dew-worms are sensitive to light and it is possible that the period of low illumination under ambient light was often not long enough to allow the successful completion of the pre-mating phase.

When comparing our results to more natural conditions the following need to be noted. Firstly, the light conditions in the darkened treatment were not designed to exactly mimic any natural night-darkness reference. Therefore, it is difficult to say precisely to what degree the results exaggerate or underestimate the differences between white night conditions and natural night darkness. Nevertheless, as 0.001 lux is equivalent to the illumination provided by star light (e.g. Rich and Longcore, 2006, p. 6) and since our illumination level measurements in the darkened treatment varied between < 0.001 and 1 lux, the dark conditions may have satisfactorily simulated natural night darkness. Secondly, our ambient light treatment corresponded with a special situation where there was no major shading caused by vegetation, although the glass roof shelter did slightly reduce the light level. Our ambient conditions thus corresponded, for instance, to early June in spring cereal fields in Jokioinen where newly sprouted vegetation has yet to provide significant shading. When compared to a forest where

the vegetation induces shading and notable light extinction (e.g. Canham et al., 1994), our ambient treatment must have exaggerated the impact of illumination.

4.2 Geographical differentiation of light response

Of the three geographical dew-worm origins, Finnish worms were the most active under ambient light and were the first to emerge in the evening and the last to withdraw belowground in the morning. While the question of geographical variation in behaviour is an intricate matter, from its correct detection to the interpretation of the reasons behind it (Foster and Endler, 1999), our findings thus support the idea that northernmost populations would be best adapted to high levels of midsummer illumination. However, the activity difference between Finnish and UK earthworms was clearest and statistically discernible, but the difference between Finnish and the southernmost population (US) was not. If activity differences among populations are associated with the historical adaptation to the conditions at the latitudes of their origin, the clear difference between Finnish and UK populations could relate to the potential presence at their respective latitudes for thousands of years since the post-glacial dispersal and settlement. In North America, *L. terrestris* were introduced by European settlers (Tiunov et al., 2006) and the US dew-worm population of the study, originating from unknown latitude(s) in Europe, has had at most a few hundred years exposure to the local conditions. A study from the 1920s reported that *L. terrestris* “has become widely distributed over Ohio in the last ten years” (Olson, 1928) suggesting that settlement at the specific sampling site in Coshocton may be relatively recent.

It is also possible that the differences we observed are not due to any adaptive, genetic differences among the populations, but represent shifting responses that would have gradually waned if the experiment had lasted longer. Hess (1924) demonstrated that the response of *L. terrestris* to light depends on previous exposure, such that exposure to high intensity increases tolerance, at least in the short term. A thorough genetic investigation would be needed to distinguish between genetic adaptation and a conditioned response.

Equally noteworthy, with the observed behavioural differences among the populations, is the fact that UK and US populations, not previously exposed to white nights, were active under these conditions. They thus displayed notable behavioural flexibility and potential for adjusting behaviour according to prevailing conditions. One explanation for not being deterred by high illumination levels could be that, irrespective of the latitudinal origin, dew-worms may be selected for activity in twilight conditions to maximize the time available for foraging, mating and dispersal. Dew-worms are known to remain close to the soil surface in the morning and in the evening (Hess, 1925; Macdonald, 1983) and, while this behaviour may expose them to predators, the benefits could out-weigh the risks. One is also tempted to speculate, whether the known positive reaction to low light intensities in *L. terrestris* (Hess, 1924) could have a behavioural ecology function and relate to early tracking of approaching darkness.

4.3 Implications

Our experiment was designed to investigate the impact of high night illumination level on *L. terrestris* activity on the soil surface and it did not address possible implications for

population ecology or the ecosystem engineering role of this species. Various implications are conceivable and warrant further investigation.

The notable activity differences under the light treatments did not result in statistically discernible changes in the overall mean weight of adult individuals. Neither did we observe significant patterns in the weight changes between the origins under ambient light. Considering the short duration of the experiment, the usage of mature individuals and the measurement of individual mass with gut contents, the study setting was undoubtedly not ideal for detecting consequences on dew-worm growth. It is also possible that the individuals obtained a significant proportion of their nourishment from below-ground organic matter as *L. terrestris* are fully able to feed on organic material mixed into the soil (e.g. Butt 1991). In a more relevant set-up, the inhibition of surface activity by high illumination could well diminish the growth rate of dew-worms and, together with the observed reduction of mating rate, negatively affect population growth. It was previously shown in a laboratory experiment that time spent on the soil surface by *L. terrestris* individuals was positively associated with growth (Field and Michiels, 2006). This was likely due to the positive association of feeding rate and growth rates since individual size correlated positively with midden size. Increased size may, in turn, provide fitness benefits because large dew-worm adults have a relatively high number of sexual partners available due to the special features of dew-worm mating behaviour (Michiels et al., 2001).

It has been estimated that through their nocturnal foraging on soil-surface litter, a dew-worm population alone is capable of burying the annual litter fall in a temperate deciduous forest in a single season (Satchell, 1967). In arable soils, high dew-worm populations can have an equally strong impact on crop residue incorporation (Subler and Kirsch, 1998). Moreover, bioturbation by *L. terrestris* not only affects decomposition, nutrient cycling and soil formation, but also various biological interactions, such as the dynamics of pathogenic fungi associated with the plant residues (Wolfarth et al., 2011). In the boreal zone, these processes may be substantially restricted due to the constraints set by high night illumination during the summer. Likewise, the opportunities for active dispersal of *L. terrestris*, which occurs by movement over the soil surface, are likely to be diminished. The importance of all these restrictions is emphasized as white nights coincide with the period of suitable climatic conditions for dew-worm surface activity. Sub-freezing night temperatures that terminate *L. terrestris* surface activity, usually last from November to April in Jokioinen. Therefore, the period between autumn and spring equinoxes (22-23 Sep and 19-21 March) with short days and long dark periods, cannot effectively compensate for limited summer activity. This pattern may, however, be changing with the increasingly warm winter months predicted by climate change scenarios for northern Scandinavia (Jylhä et al., 2009). For instance, during the exceptionally warm early winter of 2013-14, which may represent the future weather during that time of year, *L. terrestris* were observed to actively forage throughout the night at the surface of unfrozen garden soil still in early January (own observations).

Finally, contemplating the known importance of *L. terrestris* on soil profile development (Nielsen and Hole, 1964), it is perhaps not too outrageous to consider the latitudinal variation in light and day-length as a further potential “factor of soil formation” *sensu* Jenny (1941). It is also interesting to consider how artificial night lighting (Longcore and Rich, 2004) affects dew-worm populations and their related soil processes in the urban soils where this species often abounds.

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Table 1. The time instance from the start of the experiment when 50% of the cases (experimental tube x session -combinations) were active for the first (start time) and for the last time (end time). Within the columns, the values denoted with the same letter do not differ significantly at 0.05 level. For abbreviations, see Fig. 4.

Origin	Activity start time (min)		Activity end time (min)	
	AMB	DARK	AMB	DARK
FI	54 a	5 a	257 a	360 a
UK	112 b	17 a	234 a	360 a
US	71 b	12 a	244 a	360 a

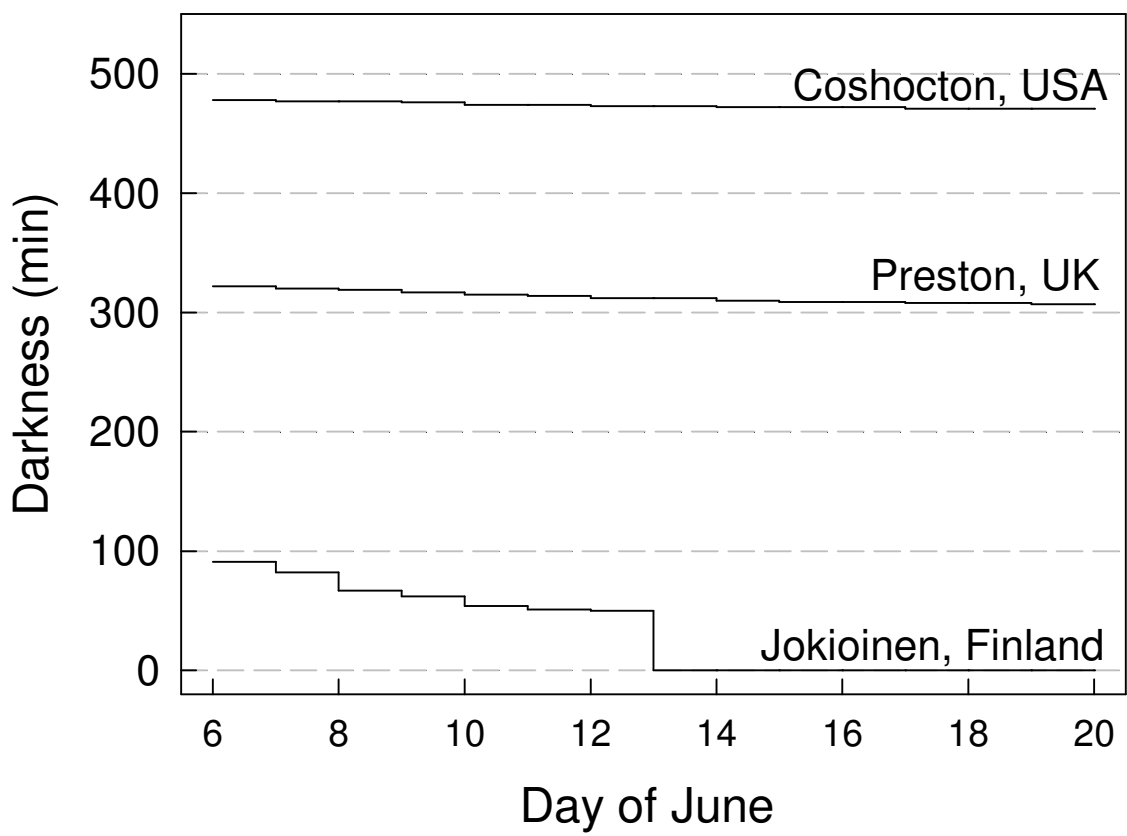
Figure legends

Figure 1. Variation of nightly dark period (in minutes) during the experimental period in June 2009 over the three locations where the experimental *L. terrestris* were collected. The dark period shown represents the time between sunset and sunrise from which the civil twilight periods have been subtracted. The step down to zero dark period length in Jokioinen at 13 June results from the boundary value set for the Sun's position below the horizon in civil twilight definition (see text). U.S. Naval Observatory's astronomical data services were applied in the calculations (U.S. Naval Observatory 2013).

Figure 2. Plan view of the experimental set-up. Three tube replicates, each with three dew-worms of a particular origin, were placed randomly in Dark and Ambient treatments. For further details, see the text.

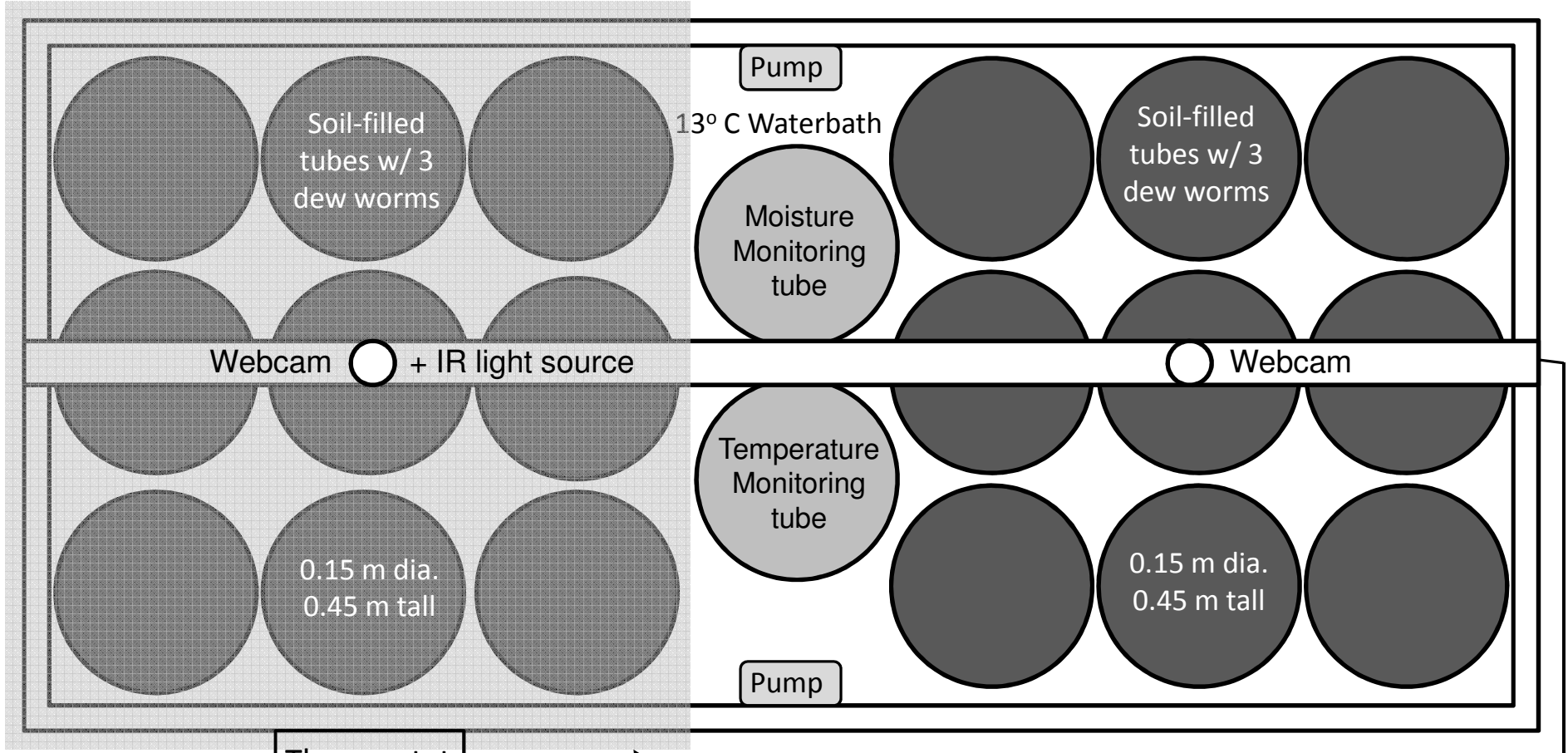
Figure 3. Weather conditions during the study. (a) Mean temperature (black circles, solid line) and air humidity (open circles, broken line). (b) Median cloudiness index. X-axis break is due to one night with missing behavioural observations. All daily values are based on the measurements for the twelve 0.5 hr periods between 21:30 and 03:30 at 1.2 km distance from the experimental site. Data: Finnish Meteorological Institute.

Figure 4. Proportion of experimental units (tubes) with surface activity for the three geographical origins of *L. terrestris*. (a) FI: Jokioinen, Finland; (b) UK: Preston, UK; (c) US: Coshocton, USA). Left panel: darkened treatment (DARK). Right panel: ambient light conditions (AMB).



DARK

AMBIENT



Webcam + IR light source

Webcam

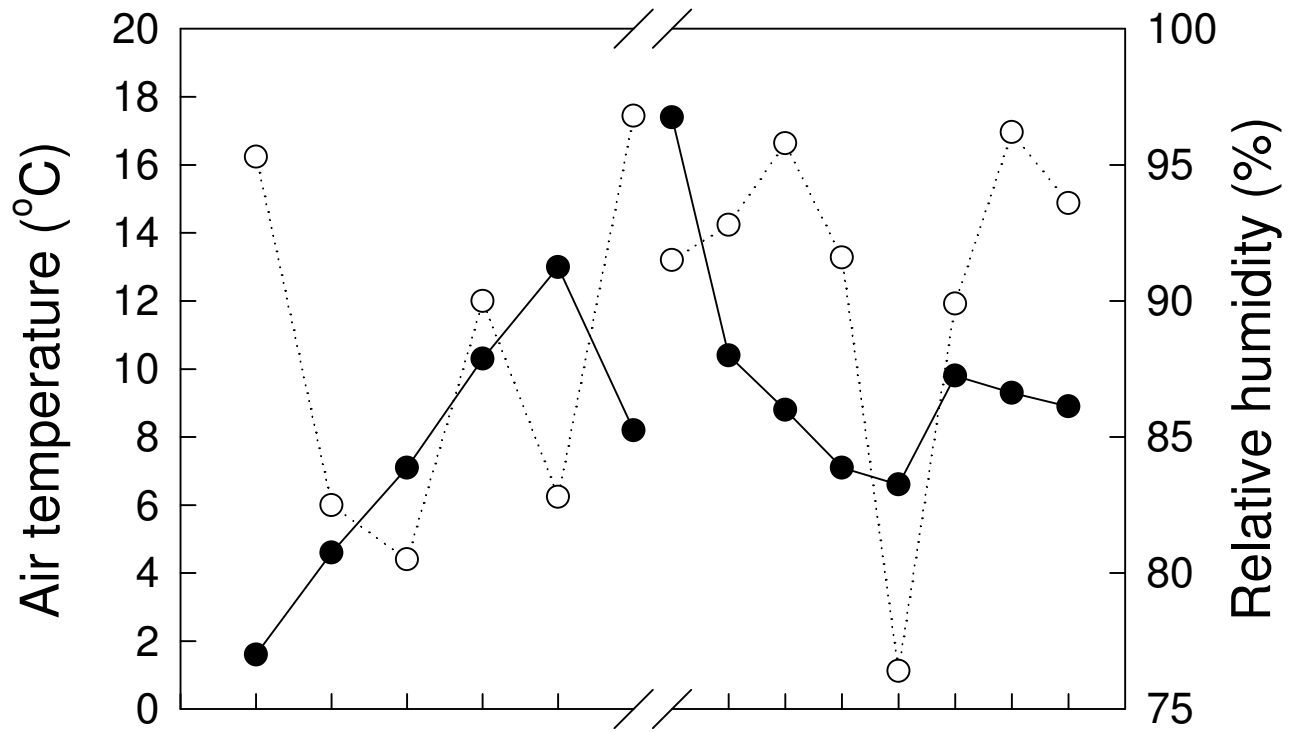
Thermostat

Opaque Cloth

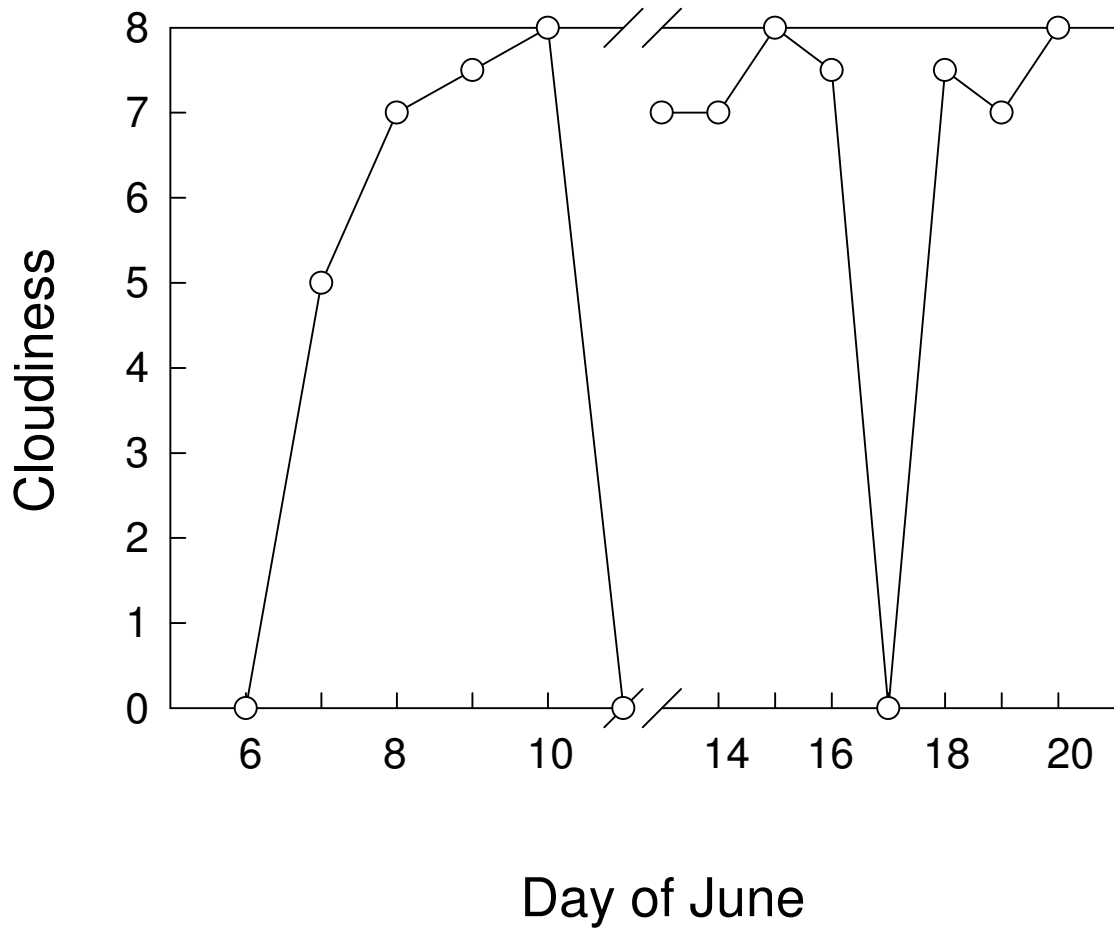
Webcams mounted 1.5 m above tubes



a.



b.

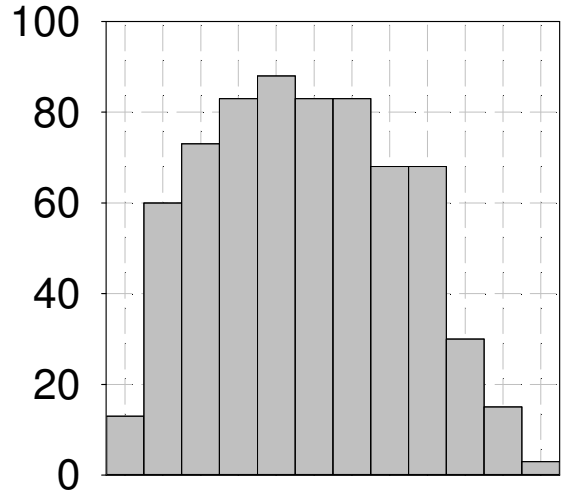
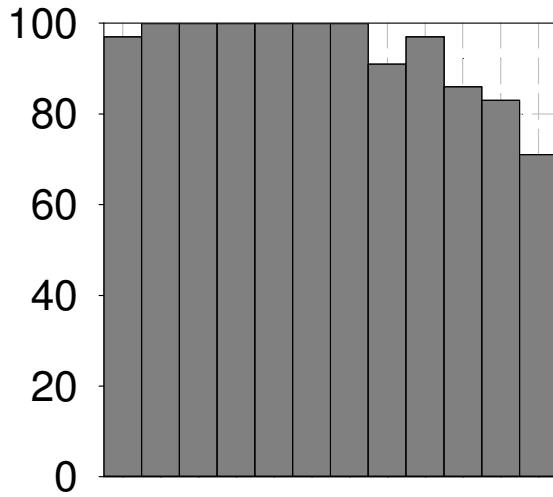


DARK

AMB

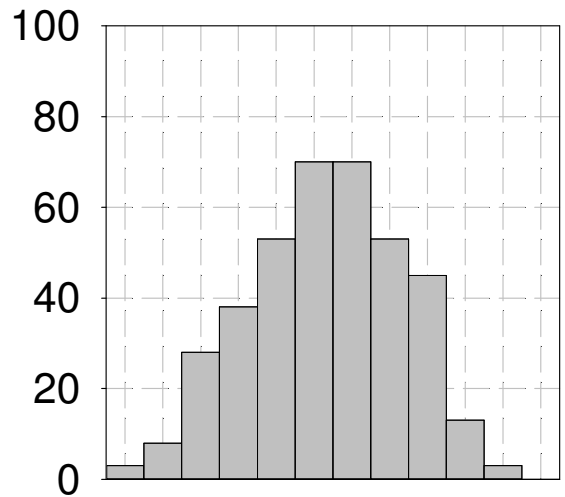
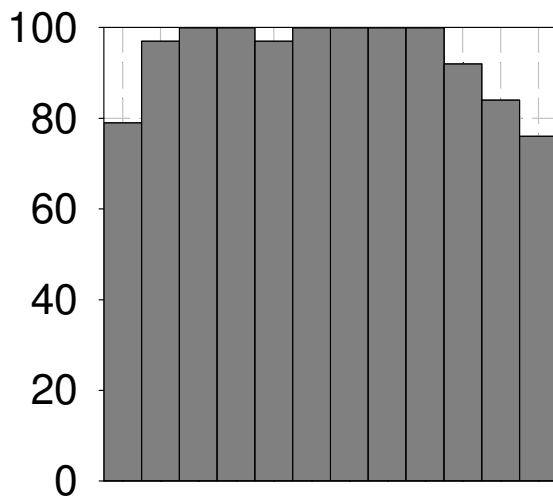
a.

FI



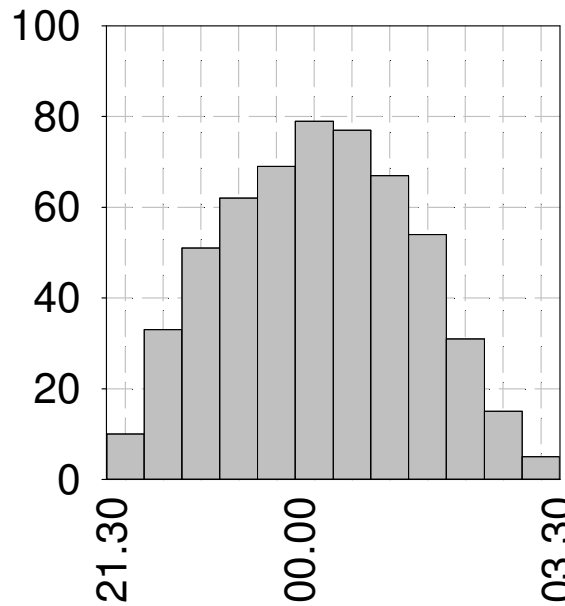
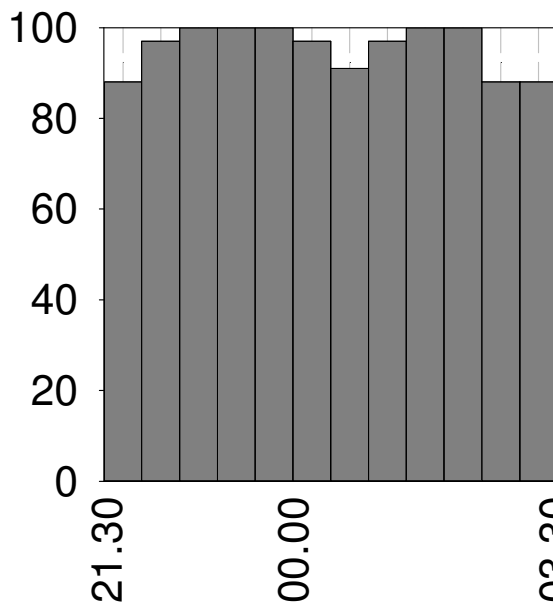
b.

UK



c.

US



Tubes with surface activity (%)

Time of night