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CHAPTER 6

Cloud cover amplifies the sleep-suppressing effect of artificial light at night in geese

Sjoerd J. van Hasselt, Roelof A. Hut, Giancarlo Allocca, Alexei L. Vyssotski, Theunis Piersma, Niels C. Rattenborg, Peter Meerlo

Abstract

In modern society the night sky is lit up not only by the moon but also by artificial light devices. Both of these light sources can have a major impact on wildlife physiology and behaviour. For example, a number of bird species were found to sleep several hours less under full moon compared to new moon and a similar sleep-suppressing effect has been reported for artificial light at night (ALAN). Cloud cover at night can modulate the light levels perceived by wildlife, yet, in opposite directions for ALAN and moon. While clouds will block moon light, it may reflect and amplify ALAN levels and increases the night glow in urbanized areas. As a consequence, cloud cover may also modulate the sleep-suppressing effects of moon and ALAN in different directions. In this study we therefore measured sleep in barnacle geese (*Branta leucopsis*) under semi-natural conditions in relation to moon phase, ALAN and cloud cover. Our analysis shows that, during new moon nights stronger cloud cover was indeed associated with increased ALAN levels at our study site. In contrast, light levels during full moon nights were fairly constant, presumably because of moonlight on clear nights or because of reflected artificial light on cloudy nights. Importantly, cloud cover caused an estimated 24.8% reduction in the amount of night-time NREM sleep from nights with medium to full cloud cover, particularly during new moon when sleep was unaffected by moon light. In conclusion, our findings suggest that cloud cover can, in a rather dramatic way, amplify the immediate effects of ALAN on wildlife. Sleep appears to be highly sensitive to ALAN and may therefore be a good indicator of its biological effects.

Introduction

Much of what we know about the regulation of sleep is based on research under tightly controlled laboratory conditions in model organisms such as rats and mice. Studies under natural or semi-natural conditions in non-model organisms will be necessary for understanding the evolution and function of sleep (Helm *et al.*, 2017; Rattenborg *et al.*, 2017). Recent studies in birds indicate that sleep time and distribution is highly sensitive to environmental factors such as light level at night (Aulsebrook *et al.*, 2020a; Aulsebrook *et al.*, 2020b).

Two important sources of light at night are the moon and artificial, man-made light devices. Several studies show that moon light can have pronounced effects on behaviour and physiology of wildlife (Milsom, Rochard and Poole, 1990; Clarke, Chopko and Mackessy, 1996; Cajochen *et al.*, 2013; Reinberg, Smolensky and Touitou, 2016; Portugal *et al.*, 2019; Aulsebrook *et al.*, 2020a). For example, European starlings (*Sturnus vulgaris*) and barnacle geese (*Branta leucopsis*) sleep 2 h less during full moon compared to new moon (van Hasselt *et al.*, 2020b; van Hasselt *et al.*, 2020c).

Artificial light at night (ALAN), common in urbanized areas, may affect wildlife in similar ways (Gaston, Visser and Hölker, 2015). Low levels of ALAN can induce night-time activity and disturbs resting behaviour and sleep (Silva *et al.*, 2014; Ouyang *et al.*, 2017; Silva, Diez-Méndez and Kempenaers, 2017; Aulsebrook *et al.*, 2018; Spoelstra *et al.*, 2018).

Thus, moon light and ALAN may have similar sleep-suppressing effects in wildlife. However, these effects may be modulated by cloud cover and this could be quite different for moon and ALAN. It is known that cloud cover, in dark unpolluted areas darkens the sky by blocking moon and starlight (Jechow, Hölker and Kyba, 2019). Hence, one may expect that cloud cover reduces the moon's sleep-suppressing effect because with heavier clouds the geese perceive less moon light. On the other hand, in light polluted areas, clouds can amplify ALAN levels by reflecting light back down from the clouds (Kyba *et al.*, 2011). Consequently, ALAN might have a stronger sleep-suppressing effect on cloudy nights.

We recently recorded sleep-wake patterns in barnacle geese under semi-natural conditions in both winter and summer (van Hasselt *et al.*, 2020c). In the present paper we analysed sleep in relation to moon phase and ALAN levels, and addressed the question of how these effects are modulated by cloud cover.

Methods

Animals and housing

For this study we used thirteen barnacle geese (*Branta leucopsis*) (8 males and 5 females; 7 - 12 years old). Prior to the study, the birds were kept in a fenced meadow (68 m × 60 m) with a water pond (25 m × 15 m) at the facilities of our institute in Groningen. The geese were fully habituated to the presence of humans. Flight feathers were clipped to prevent them from flying away. One week before the start of the sleep recordings, animals were transferred in groups of 5 to separate outdoor aviaries (5 m × 4 m). In these aviaries the geese remained exposed to outdoor light, ambient temperatures and weather conditions. The area of the research facilities where the recordings took place has multiple artificial light sources, including street lights and sky glow from the city of Groningen, which could modulate the light levels perceived by the geese. All animals had *ad libitum* access to food and water (food item numbers 615220 and 384020; Kasper Faunafood, Woerden, The Netherlands). All procedures were approved by the national Central Authority for Scientific Procedures on Animals (CCD) and the Institutional Animal Welfare Body (IvD, University of Groningen, The Netherlands).

Surgery

The surgical procedures for implantation of EEG and EMG electrodes were done as described earlier (van Hasselt *et al.*, 2020c). Prior to the surgery, the animals received meloxicam as an analgesic (0.5 mg/kg, 0.17 ml subcutaneously) and diazepam to reduce stress (2 mg/kg; 0.68 ml subcutaneously). The surgeries were performed under isoflurane anaesthesia (1.5 – 2%). Five holes were drilled (0.5 mm in diameter) after carefully exposing the crania and the EEG electrodes were inserted to the level of the dura mater. We inserted two frontal electrodes, one per hemisphere covering the hyperpallium (4 mm lateral of the midline). Three more electrodes were inserted 83 mm caudally from the frontal electrodes: an EEG reference electrode (4 mm left lateral of the midline), an electromyogram (EMG) reference electrode (on the midline) and a ground electrode (4 mm right lateral of the midline). The electrodes consisted of gold-plated, round-tipped pins (0.5 mm diameter, BKL Electronic 10120538, Lüdenscheid, Germany). For measuring EMG, two flexible wires were inserted subcutaneously on the neck muscle (PlasticsOne, Ranoke, VA, USA). All electrodes were soldered to a connector (BKL Electronic 10120302, Lüdenscheid, Germany) that was fixed to the skull using Paladur dental cement (Heraeus Kulzer, Hanau, Germany).

A 0.6 mm screw was drilled into the skull to serve as an anchor point for the dental cement-covered implant. The animals could recover for at least two weeks after the surgery before moving them to the recording aviaries.

Sleep recordings

All animals were equipped with a datalogger (Neurologger 2A; Evolocus, Tarrytown, NY, USA) for recording and storing the EEG and EMG signals, as well as head movements by an on-board accelerometer (LIS302DLH; STMicroelectronics Geneva, Switzerland). The data were recorded with a sample frequency of 100 Hz and stored on an on-board memory chip. The device could record for approximately 15 days on a 3.6 V battery (LS 14250; Saft, Levallois-Perret, France).

Sleep-wake patterns in the geese were recorded for periods up to 15 days in winter (February) and summer (June). During these recording periods, the birds were subjected to two different durations of sleep deprivations (4 and 8 hours starting from sunset), as reported in another paper (Van Hasselt et al., 2020c). The sleep deprivation days and subsequent recovery days were excluded from the current analysis. In between the recording sessions in winter and summer, the geese were returned to the larger outdoor meadow.

Data analyses

All recordings were scored with an automated scoring program using machine learning algorithms with input from a human scorer who was unaware of animal identity and time of recording (Somnivore Pty. Ltd., Parkville, VIC, Australia; Allocca et al., 2019). The program used all electrophysiological channels (EEG + EMG + accelerometer) to determine the vigilance state per 4 sec epochs. The recordings were scored for wakefulness (W), rapid-eye-movement (REM) sleep, and non-rapid-eye-movement (NREM) sleep. An epoch was scored as wakefulness when the EEG signal showed low-amplitude and high frequency activity together with high EMG and accelerometer activity. REM sleep was scored when the EEG signal was similar to that of wakefulness but EMG activity was low, and the accelerometer showed either no head movements or signs of head drops. An epoch was scored as NREM sleep when the EEG amplitude was at least twice that of wakefulness, the EMG signal was low, and the accelerometer showed no activity. The automated scoring program has been validated with various species including pigeons and yielded an accuracy for wake of 0.96 ± 0.006 ; NREM 0.97 ± 0.01 ; REM 0.86 ± 0.02 as compared with a human scorer (Allocca et al., 2019). We have also done an additional validation in our geese based

on 4 of the 24-h winter recordings, which yielded correlations between program and human scorer of 0.98 ± 0.01 for Wake, 0.97 ± 0.01 for NREM sleep and 0.84 ± 0.04 for REM sleep (van Hasselt *et al.*, 2020c).

We acquired data on the solar and lunar cycle specifically for our study site from <https://www.timeanddate.com>. In our analyses we used day and night-time. Day was defined as hours from sunrise to sunset and night was defined as hours from sunset to sunrise, for both we excluded hours containing nautical twilight. Data on cloud cover and rainfall were used from a nearby weather station in Eelde, the Netherlands ($53^{\circ}08'07.7''\text{N}$ $6^{\circ}34'12.0''\text{E}$, <https://www.knmi.nl/nederland-nu/klimatologie/uurgegevens>). To analyse the relationship between cloud cover and light levels, we used previously collected data from a light spectrometer located on the roof of a building adjacent to the site where our EEG recordings took place (Woelders *et al.*, 2018). We also used data from <https://www.lightpollutionmap.info> to get detailed information on the average amount of light pollution at our study site and other locations (see table 1) (Falchi *et al.*, 2016). As the sleep-wake recordings used for the current analysis were not specifically aimed at assessing effects of light at night and cloud cover, we did not have a full range of cloud cover for each moon phase and season. All available data on night-time sleep for different cloud covers during different moon phases and seasons are summarized in figure S1.

Statistics

Data were analysed in R by modelling the data according to a linear mixed effect model by taking animal ID as a random effect using the lme4 package (R Development Core Team 3.0.1., 2013; Bates *et al.*, 2015). From this package the BootMer function was used for bootstrapping to make model predictions by running 10000 simulations to acquire more reliable prediction estimates with 95% confidence intervals (CI) (Buckland, Davison and Hinkley, 1998; Morris, 2002). Statistical differences between groups were tested with a posthoc Tukey HSD test using the lsmeans package (Lenth, 2016). Data and text in figures are expressed as mean \pm SEM.

Results

Figure 1A shows the pattern of NREM sleep across the 24-h cycle during full moon and new moon in both winter and summer. In winter, when most sleep takes place during the long nights, full moon was associated with a reduction in NREM sleep

particularly during the night-time (linear mixed effect model, $p = 0.006$, Figure 1B). In summer, when nights are shorter and sleep is more spread out over the 24h cycle, this was not the case (linear mixed effect model, n.s., Figure 1B). During both winter and summer, full moon was associated with a near-significant reduction of NREM sleep during the day ($p = 0.052$, $p = 0.053$ respectively).

To assess levels of moonlight and ALAN in relation to cloud cover, we analysed an earlier reported dataset containing light spectrum measurements at our study site (Woelders *et al.*, 2018). This analysis showed an increase in photons over a broad-spectrum range during new moon nights with overcast compared to clear nights (Figure 2A, left panel, linear model, $p < 0.0001$). During full moon, this was only the case between 550 and 650 nm (Figure 2A, right panel, linear model, $p < 0.001$). The relative contribution to the light spectrum that can be attributed to ALAN has a peak around 600 nm and is significantly higher during new moon nights compared to full moon (Figure S1A, linear model, $p < 0.001$). Furthermore, during new moon nights, the number of photons increased when cloud cover increased (Figure 2B, left panel, linear model, $p = 0.002$), whereas during nights with full moon there is no significant relationship with cloud cover (Figure 2B, right panel). This analysis shows that light levels were lowest during a moonless night with few clouds. However, during a moonless night with maximum cloud cover, artificial light reflecting back from the clouds reached the same level as a full moon. During full moon, light levels were independent of cloud cover presumably because the light levels measured could either be moon light (when there were no clouds) or ALAN (reflecting back from the clouds).

The ALAN levels we observed during moonless cloudy nights at our study site on the edge of the city of Groningen are comparable to the levels seen in rural areas and natural habitats where wild populations of barnacle geese live (e.g., Stad aan 't Haringsvliet, Table 1), but far less than levels of light pollution seen in major cities (e.g., Amsterdam, Table 1).

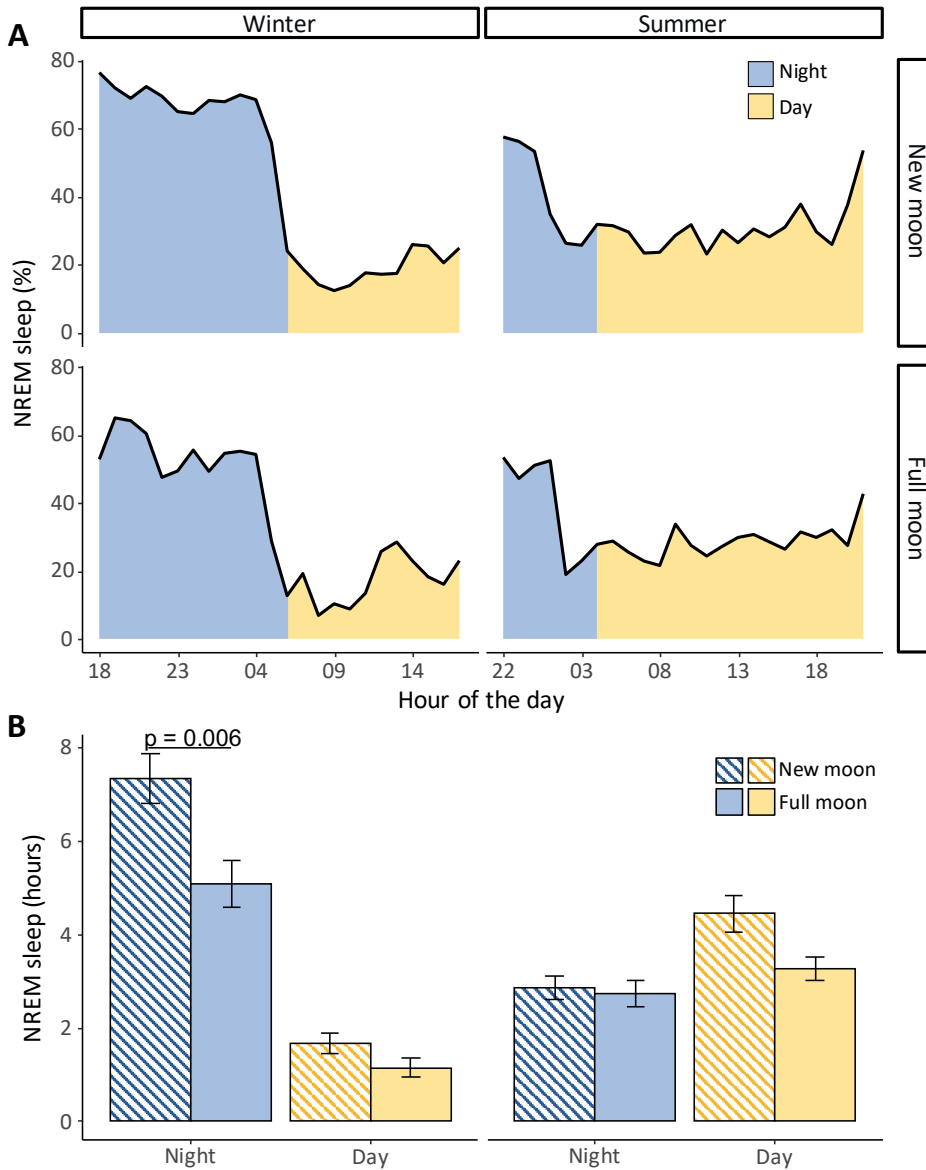


Figure 1. (A) NREM sleep patterns across the day in winter (left) and summer (right) during new moon (top) and full moon (bottom), averaged for all individuals. (B) The effect of moon phase on NREM sleep during the night and day in both winter and summer. During the winter, full moon was associated with a significant reduction of NREM sleep during the night (linear mixed effect model, $p = 0.006$). In both seasons, full moon was associated with a trend towards reduced NREM sleep during daytime (linear mixed effect model, winter: $p = 0.052$; summer: $p = 0.053$).

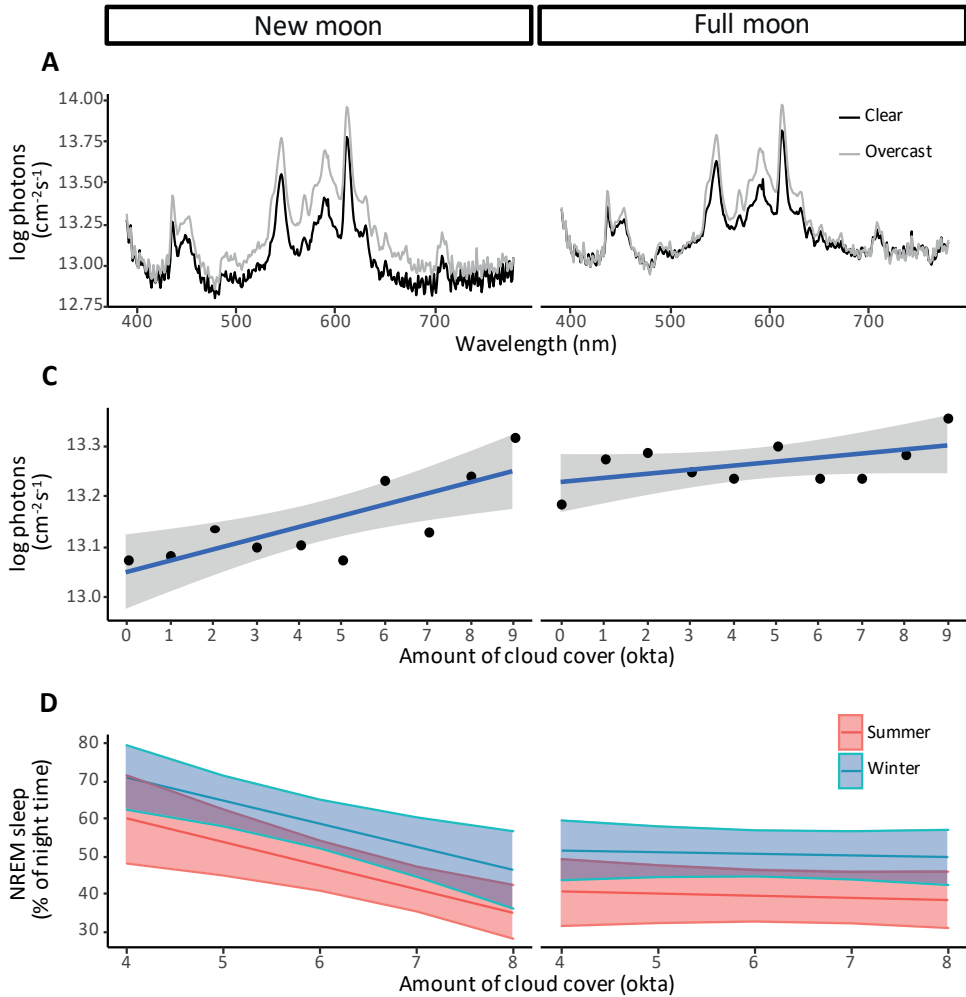


Figure 2. (A) Light power spectra during nights with new moon (left panel) and full moon (right panel) during clear nights (black line) and nights with maximal cloud cover (grey line). (B) During nights with new moon, the number of photons increase with cloud cover (linear model model, $p = 0.002$). There is no significant effect of cloud cover on the number of photons during nights with full moon. (C) Predicted values of the linear mixed effect model based on bootstrapping. The shaded area around the line denotes predicted confidence intervals. The effect of new moon (left) and full moon (right) on the nightly hours of NREM sleep in relation to cloud cover for winter (blue) and summer (red). Barnacle geese sleep significantly less when cloud cover increases during nights with new moon (linear mixed effect model, $p < 0.001$). Cloud cover has no effect on NREM sleep during full moon nights.

The analysis of night-time sleep in relation to moon phase and cloud cover shows an association between cloud cover and sleep time during new moon (linear mixed effect model, $p < 0.001$, Figure 2C) but not during full moon (linear mixed effect model, $p = 0.79$, Figure 2C). During nights with new moon, the amount of NREM sleep significantly decreased by 6.2% (CI = 2.9, 9.5) per okta increase of cloud cover, independent of season (Figure 2C, Figure S1). See table S1 for the predicted model output using bootstrapping. Across the cloud cover range in our data set, this would mean a decrease in the overall amount of night-time NREM sleep for winter from 71.3% (CI = 62.8, 79.7) during nights with medium cloud cover (okta 4) to 46.4% (CI = 36.2, 56.6) during nights with full cloud cover (okta 8). For summer this was from 60.1% (CI = 48.4, 71.5) to 35.3% (CI = 28.2, 42.3).

Besides ALAN, one other possible explanation for the finding of reduced sleep time during cloudy nights might be rainfall. We therefore extracted data on night-time rainfall from the same nearby weather station and added this to the model. In this model the amount of night-time NREM sleep time was explained by season ($p = 0.002$) and by the interaction between moon phase and cloud cover ($p = 0.004$), but not by rainfall (overall effect of rainfall: $p = 0.82$; moon phase \times rainfall interaction: $p = 0.53$).

Discussion

The analysis of night-time light levels and cloud cover at our study site showed a clear relationship with higher light levels during nights with stronger cloud cover. However, this was only true during new moon, not during full moon. During new moon nights stronger cloud cover presumably resulted in stronger reflection of artificial light. Instead, the light levels during full moon nights were fairly constant, either because of moonlight on clear nights or by the reflection of artificial light on cloudy nights. Since ALAN is known to have a major impact on wildlife physiology and behaviour, our finding implies that these effects may be exacerbated by cloud cover. Indeed, an intriguing outcome of our analysis was the strong relationship between cloud cover and sleep time, particularly during new moon when sleep was unaffected by moon light. Our data show that geese slept less when cloud coverage became stronger. In fact, across the range of cloud cover in our dataset from about 4 to 8 okta, i.e., from nights with medium to strong cloud cover, this would mean a 24.8% reduction of night-time NREM sleep.

Table 1. Measures of ALAN at different locations

Location	GPS	SQM ^a	Brightness ^b	Artif. Bright ^c	Bortle ^d
Schiermonnikoog	53.47857, 6.16093	21.76	0.214	42.6	3
Zernike	53.24262, 6.53795	19.91	1.17	998	5
Stad aan 't Haringvliet	51.73445, 4.25338	19.87	1.22	1040	5
Groningen	53.21800, 6.56647	19.12	2.43	2260	6
Amsterdam	52.37303, 4.89800	18.19	5.72	5550	8 - 9
New York	40.72702, -73.99393	17.4	11.9	11700	8 - 9

Levels of ALAN at different locations and habitats as quantified with different measures and scales.

^a Sky brightness (SQM) is an estimate to quantify skyglow in mag./arc sec², where 22 is the darkest sky and < 17.5 is the most illuminated sky (de Miguel *et al.*, 2017).

^b The brightness scale is a measure of how much millicandela is present per square meter where 1 mcd/m² is considered an unilluminated night sky.

^c The artificial brightness is a more sensitive light measurements measured in microcandela per square meter where 1 μ cd/m² is the absolute threshold of human vision (Hood and Finkelstein, 1986).

^d The Bortle scale is a nine-level classification system for night-time brightness from 1 – 9, darkest sky is 1 and most polluted sky is 9 (Bortle, 2001).

According to all 4 scales, our study site at Zernike on the edge of the city of Groningen has average levels of ALAN, comparable to Stad aan 't Haringvliet, a natural habitat where wild populations of geese live. Schiermonnikoog is an island north of the Dutch coast with low levels of light pollution. Much higher levels of light pollution are found in major cities such as Amsterdam or New York. Data are acquired from: <https://lightpollutionmap.info> (Falchi *et al.*, 2016).

A limitation of our study was that we did not measure and manipulate ALAN levels during the sleep recordings. The correlations with cloud cover and sleep time thus need to be examined in more detail in future studies. It is important to keep in mind that our study site was only an average light-polluted area (Table 1). Previous research shows that ALAN levels in cities can be as much as 4 times higher than light levels from the full moon (Kyba *et al.*, 2011). Consequently, in areas with stronger light pollution there might be an even stronger effect on sleep. The notion that ALAN can disrupt or suppress night-time sleep is in agreement with other studies, but none of these studies considered the modulating effect of cloud cover (Erren and Reiter, 2009; Czeisler, 2013; Silva *et al.*, 2014; Stevens and Zhu, 2015; Ouyang *et al.*, 2017; Silva, Diez-Méndez and Kempnaers, 2017; Aulsebrook *et al.*, 2018; Spoelstra *et al.*, 2018; Aulsebrook *et al.*, 2020b; Batra *et al.*, 2020).

The lower amount of NREM sleep during new moon nights with heavy cloud cover,

presumably mediated by ALAN reflecting of the clouds, were similar to the levels of NREM sleep during full moon nights. This suggests that at our study site ALAN had a similar sleep suppressing effect as full moon. Indeed, the full moon significantly reduced NREM sleep in barnacle geese by 2 h, independent of season (van Hasselt *et al.*, 2020c). This finding is in line with recent research reporting that barnacle geese are more active and show an increased body temperature and heart rate on full moon nights (Portugal *et al.*, 2019). Also in other bird species, it has been reported that light from the moon directly suppresses sleep and increases night-time activity (Milsom, Rochard and Poole, 1990; Pinet *et al.*, 2011; York, Young and Radford, 2014; van Hasselt *et al.*, 2020b).

The physiological mechanisms underlying the sleep-suppressing effect of moon light and artificial light might be diverse, but could include a suppression of melatonin. Indeed, it is commonly known that artificial light decreases melatonin levels, also in avian species (Dominoni *et al.*, 2013; Kernbach *et al.*, 2020). Since melatonin is often considered to be a sleep-promoting factor, this may partly explain the sleep-reducing effect of moon light and ALAN. However, light may also have direct effects on sleep that are not mediated by the suppression of melatonin (Rattenborg *et al.*, 2005), for example, through activation of the wake-promoting hypocretin/orexin system (Lin *et al.*, 1999; Hara *et al.*, 2001). This system is highly active when voluntary behaviour occurs such as grooming, eating and exploratory behaviour (Mileykovskiy, Kiyashchenko and Siegel, 2005). Recent studies reported that light-induced neuronal activation is in part mediated by the hypocretin/orexin system (McGregor *et al.*, 2011; Adidharma, Leach and Yan, 2012).

The reduction of sleep with higher levels of ALAN may have both positive and negative consequences. On the one hand, it might be beneficial for species to actively use nights with increased light levels for foraging while still being able to see and avoid predators. This is supported by a study in peahens (*Pavo cristatus*) showing that ALAN caused an increase in vigilance behaviour to avoid predators (Yorzinski *et al.*, 2015). Such effects of ALAN might then add up to effects of moonlight that have been reported. For example, earlier work has shown that barnacle geese spend much more time on the foraging grounds during moonlit nights than during the dark phases of the moon (Ebbinge, Canters and Drent, 1975). Also, in one study on brent geese (*Branta bernicla*) nocturnal feeding was positively correlated with moonlight and was a major part of their energy intake (Tinkler, Montgomery and Elwood, 2009).

On the other hand, the suppression of sleep may be an important mediator of the potential negative health consequences of artificial light at night, for example by affecting immune function. Indeed, it is well known that sleep plays an important role

in regulating both the innate and adaptive immune system (Irwin, 2002; Irwin and Opp, 2017). Studies in house sparrows showed that exposure to ALAN suppressed the immune system which then increased mortality rate caused by the West Nile virus (Kernbach *et al.*, 2020). Also, in another songbird species a strong correlation was reported between the exposure to ALAN and the prevalence of malaria infection (Ouyang *et al.*, 2017). Such an effect of ALAN on immune function might thus be mediated by ALAN-induced sleep disturbance.

In conclusion, our study shows that sleep in geese is highly sensitive to light, both natural and artificial. Sleep is suppressed under full moon, and the effects of artificial light may be as strong as the effects of moon light. Moreover, our findings suggest that cloud cover can amplify the immediate effects of ALAN on wildlife. Sleep appears to be a highly sensitive read-out for the consequences of ALAN, and may therefore serve as an important indicator for future studies. Finally, given the importance of sleep as a recovery process that is crucial for health, the findings may also indicate that effects of ALAN on fitness of animals in the wild may be directly mediated by disturbance of sleep.

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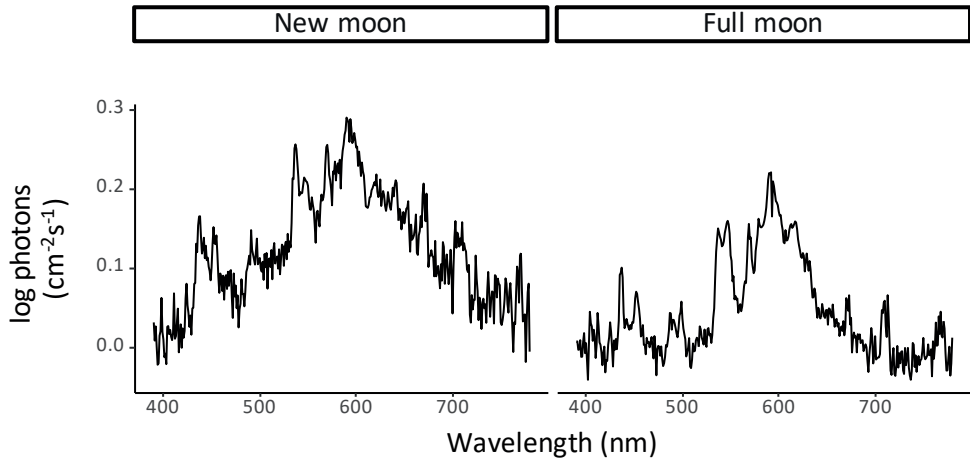


Figure S1. (A) Light spectrum of ALAN reflected by the clouds and perceived by the geese that is derived from the difference between the spectra during clear nights and overcast nights, plotted per moon phase. The relative contribution to the light spectrum that can be attributed to ALAN has a peak around 600 nm and is significantly higher during new moon nights compared to full moon (Figure S1, linear model, $p < 0.001$).

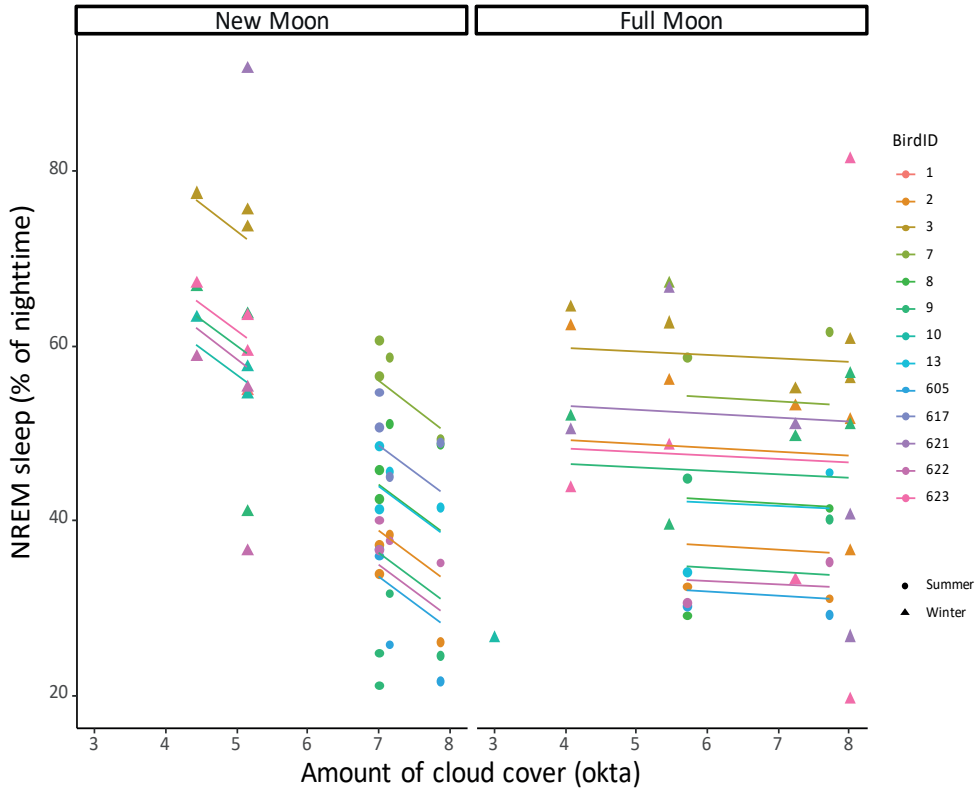


Figure S2. Data based on the model output presented in figure 3C. Cloud cover decreases significantly nightly NREM sleep independent of season (circle: summer; triangles: winter; linear mixed effect model, $p < 0.001$). During full moon, nightly hours of NREM sleep are unaffected by cloud cover increase.

Table S1. Model output

Random Effects	Variance	Standard deviation
BirdID	62.75	7.92
Residual	65.7	9.78
Fixed Effects	Predicted Fit	95% (CI)
(Intercept)	84.83	60.17, 109.50
Full Moon	-42.62	-66.76, -18.33
Cloud cover	-6.20	-9.64, -2.67
Winter	11.18	3.81, 18.52
Full Moon × Cloud cover	5.76	1.85, 9.61

Linear mixed effect model output that was presented in Figure 2C. The model output was obtained by running 10000 simulations using a bootstrapping method.

