Correlation of the environmental light spectral composition and the types of behaviour in owlfly *Ascalaphus macaronius*

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Methods. The spectral composition of environmental light was measured with AVS-USB2000 Miniature Fiber Optic Spectrometer (Avantes™, Netherlands). The environmental light was measured in two ways: as the spectral composition of the light coming from different parts of the sky (sky spectra) and as the spectral composition of the light coming from the general direction of the sun (sunlight spectra). The end of the optical fibre of the spectrometer was pointed towards a chosen point in the sky using a goniometer stage mounted on a flat board, which could be precisely oriented in the north-south direction and kept levelled. To measure the composition of the light from a specific point in the sky (first mode) we used a quartz collimating lens (COL-UV/VIS), while we used a difusor - cosine corrector (CC-3-UV) to measure the sunlight spectra (second mode). In the latter the goniometer angle was adjusted approximately every 1-2 minutes to keep the face of difusor adjusted orthogonal to the sun. The sky spectra were acquired either in 45° increments along the azimuth and 30° increments in elevation or in 10° increments along the azimuth and 10° increments in elevation. The sunlight spectra were acquired continuously every 30s for 1h or more. We also acquired spectra using the collimating lens directly from the zenith every 30s for 30-60min. Spectra of the sky were collected at various times during the day. Sunlight spectra were collected three times a day: after sunrise, around noon and before and during sunset.

The spectra were acquired using Avantes SpectraWin 4.2 software package and subsequently read into Matlab 5.3 (Mathworks Inc., USA) using a purpose written function. Since we did not possess a UV/VIS calibration light source we resorted to approximate calculations of the photon fluxes. We used a function that took into account the spectral sensitivity of the detector array, grating efficiency and the diameter of the optical fibre. In this way we obtained a lookup table of correction values for all the detector array elements with which we multiplied all the measured values. The absolute irradiance was then calculated taking into account also the integration time used.

We limited our analysis to comparing energy fluxes in the near UV (300-400 nm) and mid-visual (500-600 nm) ranges. We did this by integrating the values over the above mentioned spectral ranges. Simultaneously with spectral measurements we also recorded the behaviour of the owlflies as well as the relative humidity and air temperature. The behaviour was described according to presence or absence of one of the following activities: flying, flying due to disturbance, turning behaviour and opening and closing of the wings. Air temperature was measured with thermometers positioned in the shade 40 cm (just below the tips of grass stalks) and 80 cm from the ground while humidity was measured in the shade at 80 cm above ground.

Results. Owlflies massively land on the grass when clouds obscure the sun and again take off when the sun is no longer shaded by clouds. This type of behaviour has been known for many years and yet its cause remains unsolved. The aim of this study was to try to indicate which part of the light reaching owlflies may be responsible for the changes in their behaviour. Considering strong UV sensitivity of the Ascalaphus dorsofrontal eye the working hypothesis was that the changes in UV pattern of the sky may be responsible for the changes in behaviour.

To reduce the number of parameters we only limited ourselves to comparing the UV and visual parts of the spectrum. This is also the way all the results are presented. Figure 1 shows the map of relative illumination of the sky in UV compared to visual part of the spectrum on two different days. On the first day the sky was almost clear with only few clouds while on the second day the sky was almost entirely covered by clouds of varying thickness. The comparison of UV to visual (VIS) pattern of the sky in both cases indicates that the illumination of the sky is much more uniform in UV than in visual region. This is especially well seen in the first case

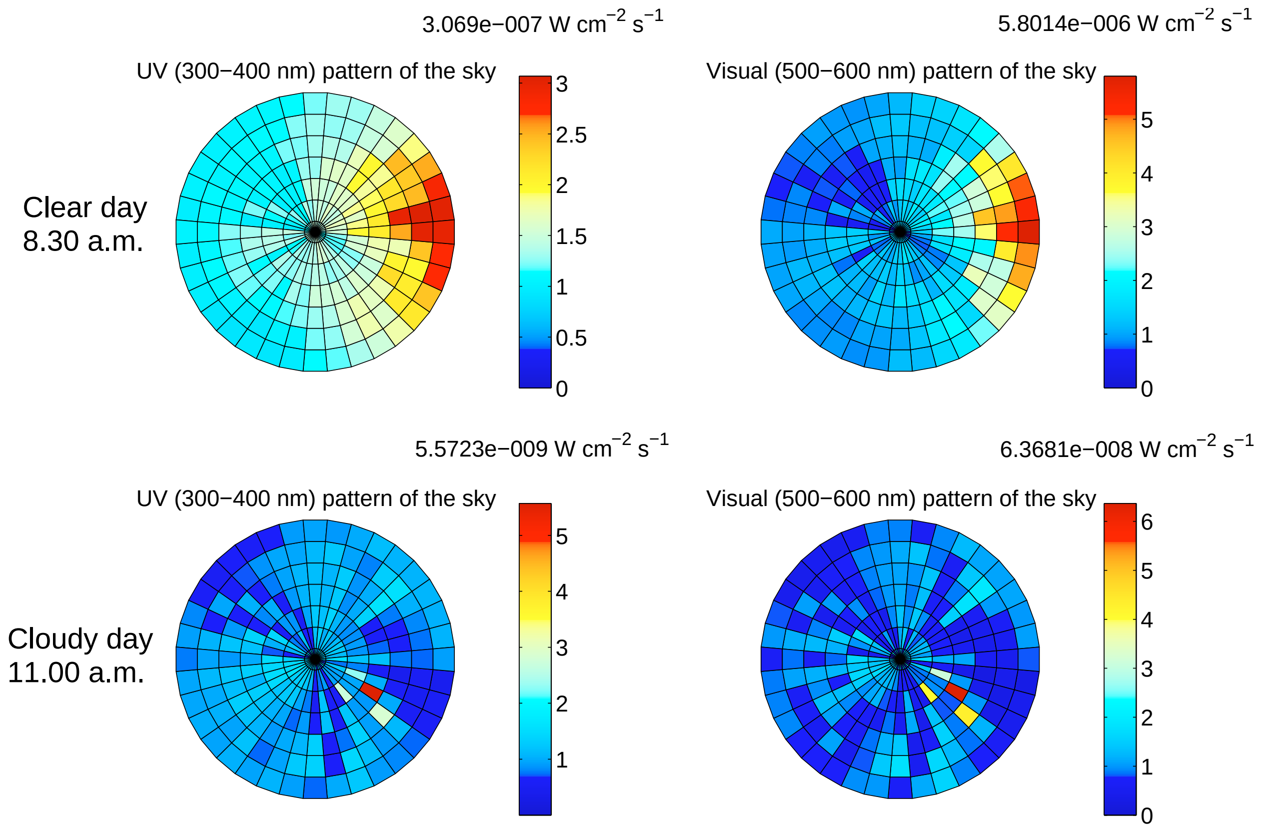

Figure 1:
Comparison of intensity maps done at 10° resolution on 5 / 7 / 2001 (clear day) and 3 / 7 / 2001 (cloudy day).

with large portions of blue (cloud free) sky. Statistical analysis of the dispersion of intensity values for all measured sky illumination maps gives the variation coefficient of 0.31 ± 0.09 for UV and 0.72 ± 0.25 for VIS or the relative interquartile range of 0.29 ± 0.08 for UV and 0.70 ± 0.22 for VIS (all values in mean ± sd; n=8). An example is shown on figure 2 - a sequence of relative illumination maps recorded over more than two hours from 8:20am till 10:33am with varying cloud cover and their respective variation coefficients and relative interquartile ranges. Considering these data it can be argued that in UV the sky presents a much more uniform background than in the visual part of the spectrum. This can also be seen on figure 3. It shows an example of a record that was taken in the morning around sunrise, when the sky was relatively clear. The collimator lens was pointed directly towards the zenith. As the sun was rising there was a gradual increase in the general illumination of the sky in both UV and VIS regions. A couple of white passing clouds directly overhead produced a strong signal increase in the visual part of the spectrum while the relative change in the UV part wasn't very big. During that experiment the sun was obscured most of the time by passing clouds. However, these events are not observable on the record.

The sunlight spectra were made using a CC-3-UV cosine corrector. This gave the value, which was dominated by the sun but did also comprise the contribution of the nearby sky. However, since the sun is a much more powerful source of light than the sky we can claim this is the sunlight spectrum. Figures 4 and 5 show typical recordings from the morning and evening sessions. The indentations on the recorded illumination time course are due to clouds obscuring the sun. These are very often correlated to changes in Ascalaphus behaviour.

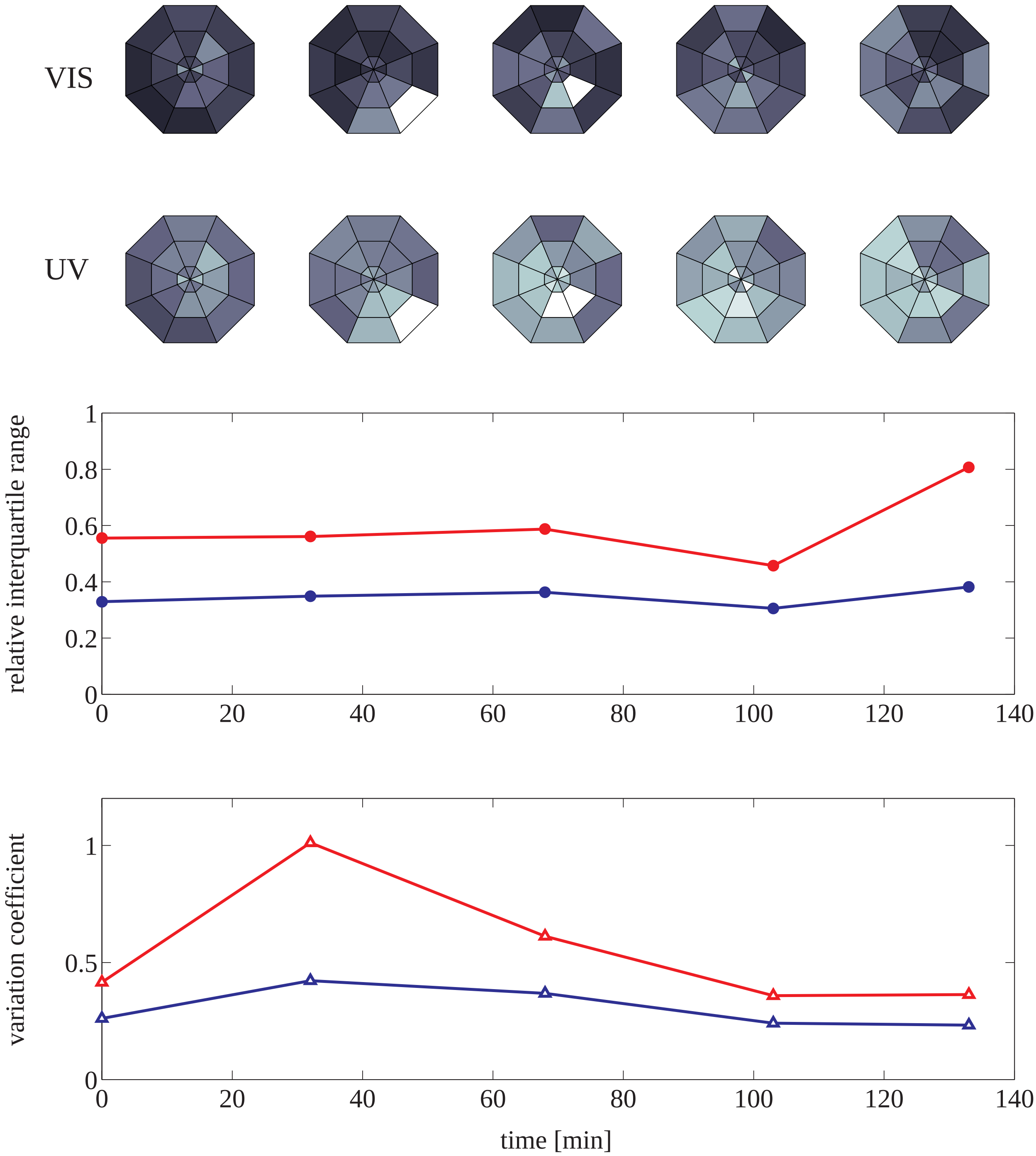

Figure 2:
A sequence of intensity maps done at 45°x30° resolution on a cloudy day (3 / 7 / 2001, starting at 8.20 a.m. Upper two rows show the sequence of intensity maps (left to right) while the lower two panels show the comparison of relative interquaritle range ((Q3-Q1)/Me) and variation coefficient (sd/m). The red lines are for the visual maps while the blue ones are for the UV maps.

The behaviour of owlflies was classified into one of the following types:

- spontaneous flying
- flying due to disturbance (the disturbance was an approaching experimenter)
- turning behaviour (hiding of owlflies behind grass stalks when approached)
- wings open
- wings closed

In the morning Ascalaphi were oriented on grass stalks with closed wings exposed to the rising sun (the sun was shining at approx. right angle on the ventral surface of the wings). As the sun was rising they started opening their wings one by one and hiding behind grass stalks (turning) if approached. When the light intensity fell (a cloud obscured the sun) they closed their wings and opened them again when the sun was shining again. Then followed a

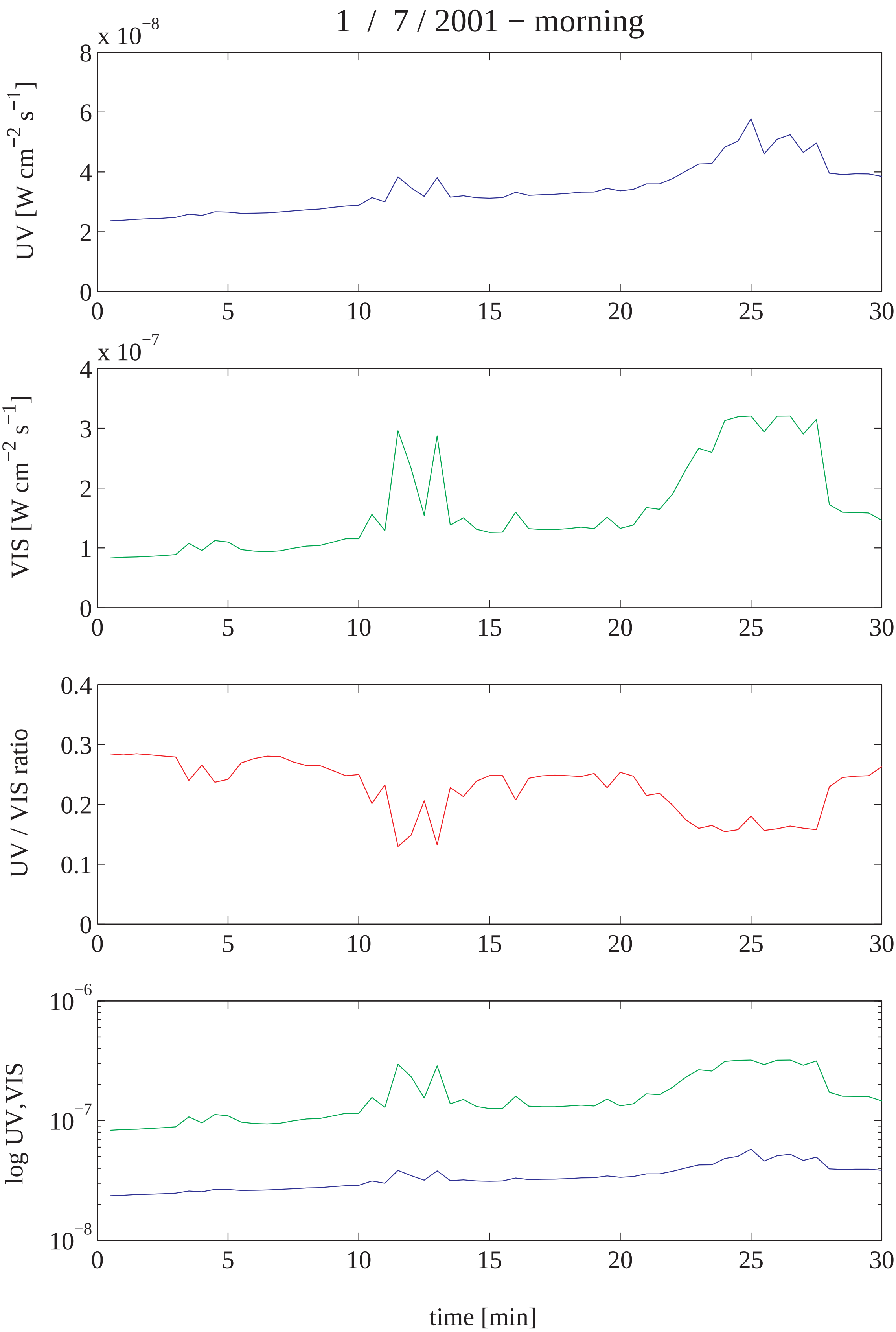

Figure 3:
Time course of the changes in the light intensity measured from the zenith using a collimator lens. The increases in the light intensity visible especially in the visual part of the spectrum come from clouds passing directly overhead (*).

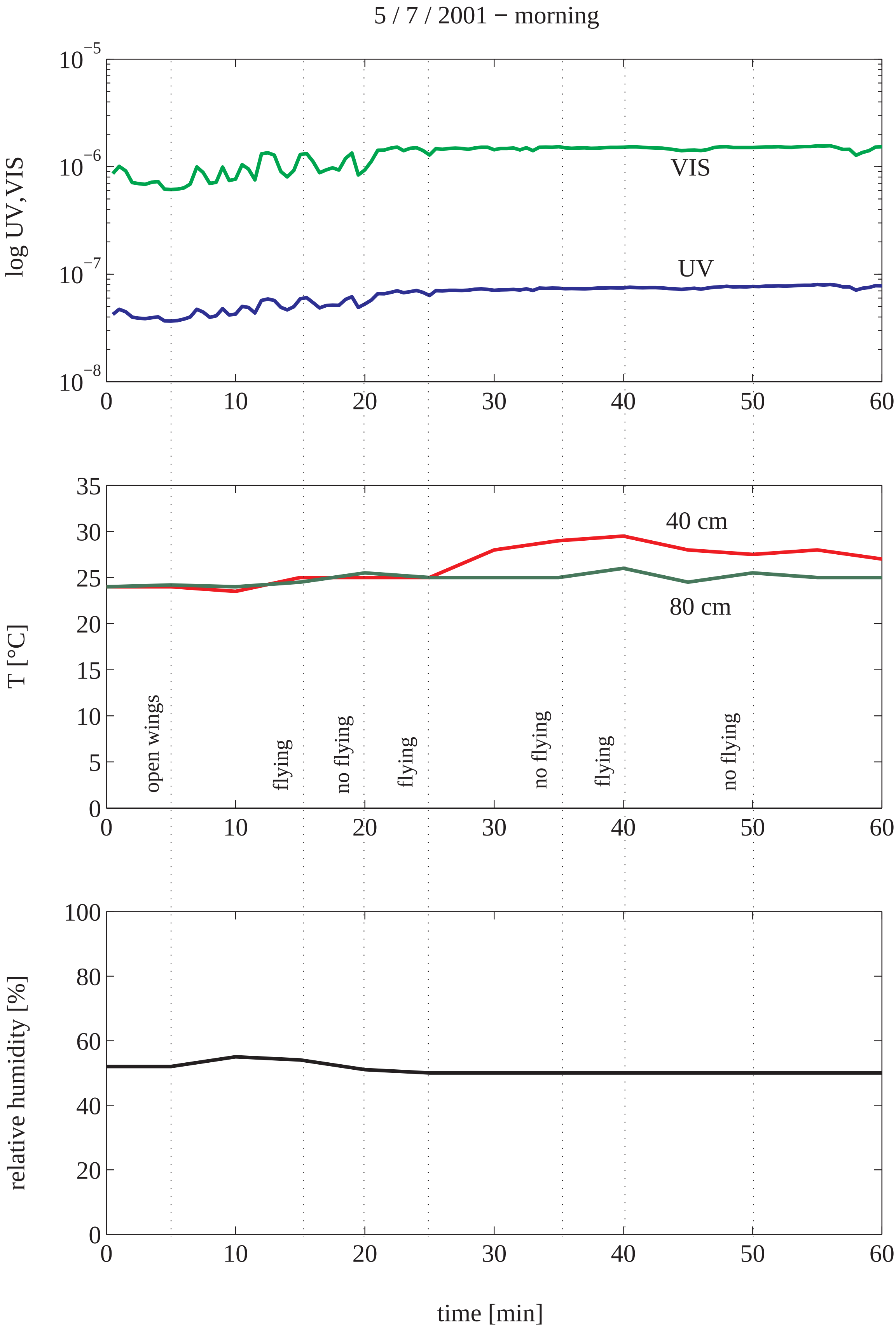

Figure 4:
Time course of the changes in direct solar illumination in the morning (8:48 a.m. - 9:48 a.m.) mreasured with the cosine corrector. The lower two panels show the correaltion with temperature, humidity and the types of behaviour.

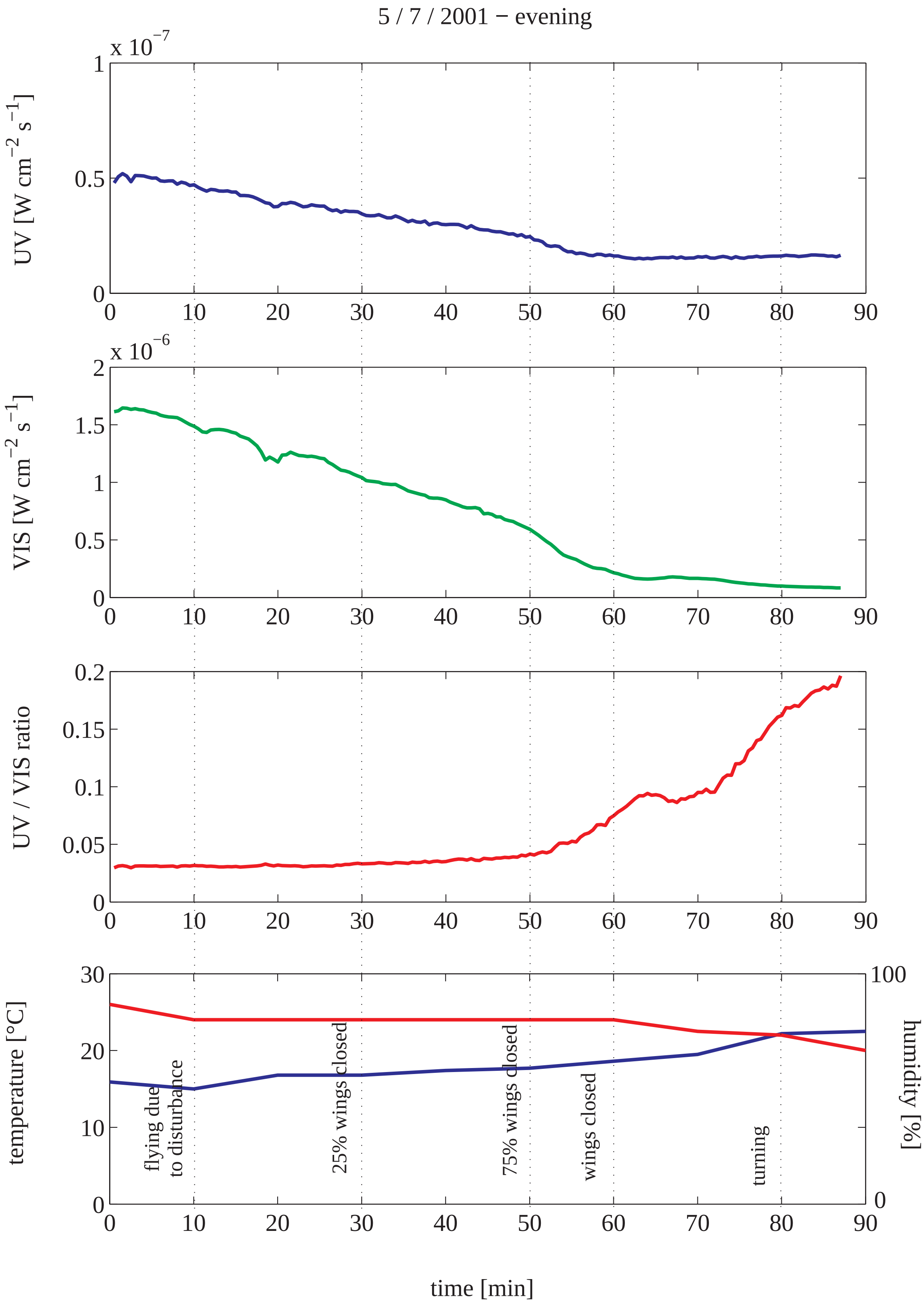

Figure 5:
Time course of the changes in direct solar illumination in the evening around sunset (18:46 - 20:15) mreasured with the cosine corrector. The lowest panel shows the correaltion with temperature at 80 cm above ground (red line), humidity (blue line) and the types of behaviour. The sky was clear and we can see a dramatic change in the ratio between UV and visual light after sunset (around 50-60 min) in the favour of UV.

longer period when all had their wings open, exposed to the sun but did not fly (not shown). Only after a couple of hours did they start to fly but sat down immediately when the sun was obscured by the clouds and did not fly at all on a very cloudy day (not shown). In the evening the sequence of events was the reverse of the morning one: spontaneous flying in the sun › flying due to disturbance › closing wings › turning due to disturbance with wings closed (figures 5).

Simultaneously we were measuring the air temperature and humidity. During observation times there was in general no major change in air humidity or in air temperature at 80 cm above ground apart from the sunrise (not shown) and sunset (figure 5), when there were pronounced changes in air temperature and corresponding humidity. Apart from these times owlflies' behaviour was not correlated to air temperatures (80 cm) and humidity. We observed a raise in the air temperature due to insolation only within the range of grass height (40 cm).

Discussion. In all the parts of the day three observations prevailed:

1. The sky was much more uniformly lit in the UV than in the visual region. Even the changes due to transition of the clouds produced much more subtle changes in its illumination than in visual region. Also the transition of clouds in front of the sun had no effect whatsoever on the spectral composition of the light coming from the sky.
2. We observed that the owlflies descended even when on a relatively clear sky the clouds obscured only the sun. Equally the inverse was true as well, the Ascalaphi started to fly when the sky was mainly cloudy but they themselves were exposed to direct sunlight. Regarding the first observation we can argue that changes in UV light coming from the sky are not the primary stimulus that changes owlfies' behaviour.
3. The spectral composition of the sunlight especially at sunset showed that the decline in the visual part of the spectrum was much sharper than in the UV part and that the changes in behaviour were correlated much more to the visual part of the spectrum than to the UV part.

Contrary to the original hypothesis that there may be visual clues to the owlflies' behaviour it appears that considering a predominantly UV sensitive dorsofrontal eye of the Ascalaphus this is most likely not the case. A more plausible explanation appears to be that the visual and near IR part of the spectrum, which are readily converted to heat, play a more prominent role. This is especially true considering the very high and narrow temperature optimum of Ascalphi, which has been demonstrated in laboratory experiments (30-40° C), as well as very steep temperature dependence of every aspect of eye functioning below 30° C that has been observed (Belušič and Pirih, unpublished data). A further indication that this may be the case is the fact that owlflies' activity is much tighter correlated to direct insolation than to any changes in the colour of the sky.