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Flight of mayflies towards horizontally polarised and unpolarised light

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Light in the optical environment is not just coloured, but it is polarised. The visual system of many insect species is polarisation sensitive which allows them to use the polarisation pattern of skylight for navigation purposes. There is also increasing evidence that mayflies and other aquatic and semiaquatic insects are able to detect water bodies on the base of the water-reflected horizontally polarised light using it as an environmental cue to detect suitable habitats. In a pilot experiment we constructed and tested light trap pairs emitting unpolarised and horizontally linearly polarised light with the same intensity and spectrum to record potential polarisation-sensitive species from various insect orders. Aquatic insects are assumed to use horizontally polarised light for habitat detection. Thus, we expected that these species will be captured in significantly greater numbers by the trap emitting horizontally polarised light than by the trap emitting unpolarised light. Trappings were carried out at each night from April to October during 2001 and 2002 at two sites in Hungary. One of the trapping sites was at the edge of the flood basin of a river with some smaller ponds. The other trapping place situated in a protected sand dune area scattered with ephemeral alkali lakes. The same mayfly species were collected by the traps emitting polarised and unpolarised light. From the recorded five species four species were caught in greater numbers and more frequently by the trap emitting horizontally polarised light. *Caenis horaria* and *Cloeon dipterum* mayflies were attracted significantly stronger to the polarised trap, thus they are likely to use positive polarotaxis in habitat detection. *Ephoron virgo* and *Caenis macrura* also showed a tendency for polarotaxis, but they were represented with too low numbers of individuals in the samples to produce significant differences.

Keywords: Ephemeroptera; polarotaxis; horizontally polarised light; light trap

Introduction

Light in the optical environment is not only coloured, but also polarised in many cases (Figure 1a). Although the human photoreceptors are not polarisation-sensitive, the eye of many insect species allows them to use the polarisation pattern of skylight for orientation purposes. Their compound eyes contain specialised dorsal rim ommatidia that analyse the polarisation characteristics of skylight (Horváth and Varjú 2004). Many aquatic insects living in or near water bodies of various types are

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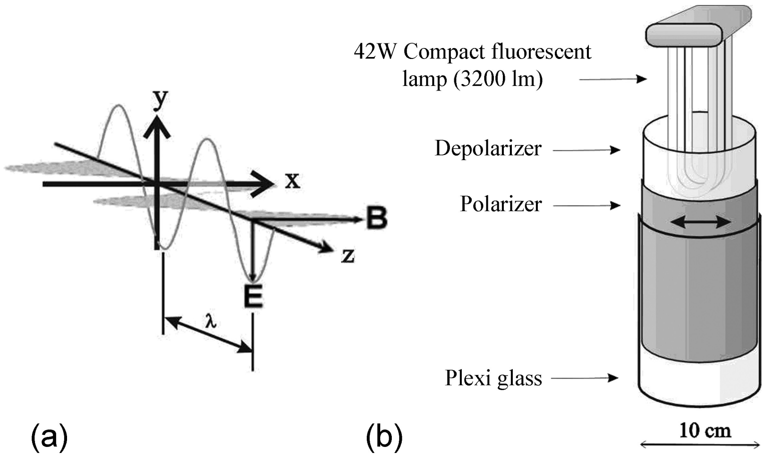

Figure 1. (a) Light is a transversal electromagnetic wave, which means oscillation of electric and magnetic vectors (E and B, respectively) perpendicular to each other and to the direction of propagation. Wavelength is referred as colour, while the orientation of the E vectors is referred as polarisation. Electric vectors in unpolarised light can be oriented to any direction with equal possibility in a coordinate system perpendicular to the direction of propagation. Light with e-vectors more or less arranged to a direction in the above coordinate system is referred as linearly polarised light and can be characterised by (i) the degree of arrangement – degree of polarisation, and (ii) the direction towards which vectors are arranged – direction of polarisation. The degree of polarisation is 0% in the case of unpolarised light, while it is 100% in the case of totally polarised light. (b) Arrangement of the polarised light source. In all traps identical compact fluorescent lamps were used as light sources. The linearly polarised light was produced by a polariser lapped around the lamps within a protecting plexi-glass cylinder. Between the lamp and polariser a transparent depolariser sheet was applied to guarantee the same intensity and spectral composition of light that was emitted by the control trap. To emit unpolarised light the polariser sheet was placed around the lamp within the depolariser sheet inside the plexi-glass cylinder. Depolariser sheets were installed within the polariser in the horizontally polarised-light-emitting trap to identically reduce the intensity of transmitting light.

able to perceive the horizontally polarised light reflected from water surfaces, and use it to detect suitable habitats, as documented by Schwind (1991, 1995) in his multiple-choice field experiments. These insects have ventral polarisation-sensitive eye regions which allow them to find water surfaces by polarotaxis.

For this reason water-seeking aquatic insects are attracted en masse to water or shiny black artificial surfaces (e.g. waste oil lakes, asphalt roads, car roofs, plastic sheets, black gravestones, or glass panes) which reflect horizontally polarised light (Horváth and Zeil 1996; Horváth et al. 1998, 2007; Kriska et al. 1998, 2006, 2008; Bernáth, Szedenics, Molnár, Kriska and Horváth 2001; Wildermuth and Horváth 2005; Horváth and Kriska 2008; Malik, Hegedüs, Kriska and Horváth 2008). However, there are only a limited number of insect species associated with water or moist substrata that are proved to possess polarisation-based habitat detection. Most of them belong to the Heteroptera, Odonata, Diptera, Trichoptera, Plecoptera, Ephemeroptera or Coleoptera (Bernáth et al. 2001; Horváth and Varjú 2004; Szentkirályi, Bernáth, Kádár and Retezár 2005; Csabai, Boda, Bernáth, Kriska and Horváth 2006).

In their choice experiments in Hungary, Kriska et al. (1998, 2007) and Bernáth et al. (2001) documented the polarotactic behaviour of the mayfly species

Baetis rhodani (Pictet, 1843–1845); *Cloeon dipterum* (Linnaeus, 1761); *Ecdyonurus venosus* (Fabricius, 1775); *Epeorus sylvicola* (Pictet, 1865); *Ephemera danica* Müller, 1764; *Habroleptoides confusa* Sartori & Jacob, 1986; *Rhithrogena semicolorata* (Curtis, 1834); and *Palingenia longicauda* (Olivier, 1791). They found that horizontal shiny black surfaces used in these field experiments to mimic water bodies reflected a very attractive supernormal optical stimulus for swarming mayflies due to the degree of polarisation being higher than that of light reflected from water surfaces. Above these artificial surfaces the swarming mayflies showed behavioural elements of reproductive activity (e.g. mating, egg-laying) normally seen with water bodies.

Since light trapping is a mass collection method, traps emitting horizontally polarised light should be efficient instruments to identify further water-associated insect species which are candidates to possess positive polarotaxis. In spite of the frequent use of light trapping for insect monitoring, only two cases were found in the literature documenting the use of traps emitting horizontally polarised light (Kovarov and Monchadskiy 1963; Danthanarayana and Dashper 1986). However, in these experiments mayflies were not recorded.

In a two-year pilot experiment we constructed and tested two light trap pairs emitting unpolarised (control) and horizontally polarised (treatment) light to collect potential polarisation-sensitive species in various insect orders (Szentkirályi et al. 2005). In this experiment, among other taxa the order Ephemeroptera was also represented by more species in samples of two light-trapping localities.

The aims of our study were (1) to record mayfly species flying preferably towards horizontally polarised light sources; (2) to assign mayfly species that are possibly positively polarotactic, i.e. they are attracted in significantly greater numbers to the horizontally polarised light source than to the unpolarised one, and (3) to describe any differences in assemblage structure and seasonal flight characteristics of mayflies attributable to a possible polarisation sensitivity.

Materials and methods

Trapping sites and habitat types

Two trapping sites were selected in the Great Plain lowland of Hungary. Light traps were set up in a dry habitat near Fülöpháza scattered with alkaline lakes (Figures 2a–c), and near River Maros (Figures 2d–f). We wished to collect as many insect species (characterising both types of surroundings with diverse life history) as possible.

At the first trapping site (site 1) near Fülöpháza (46.87°N, 19.42°E), the light traps were set up on the ridge of a longer sand dune within a nature reserve area of the Kiskunság National Park. The characteristic vegetation type on the top and upper slope of the sand hills was opened sandy dry grassland (association: *Festucetum vaginatae*), while on the lower slopes and valleys between dunes closed grassland was the typical cover (Figures 2a–c). Other habitats in the vicinity of the trap site were smaller abandoned house gardens, some arable fields, and weedy stands. The nearest waterside of an alkaline lake, from which the collected mayflies could emerge and fly to traps, was about 3 km away from the trapping site.

The traps of the second trapping site (site 2) near Maroslele (46.23°N, 20.37°E) operated on the middle of the slope of a 6 m high flood-preventing bank at the edge of a riparian forest alongside the River Maros (Figures 2d–f). The bank was covered

Figure 2. Trapping sites and habitat types. (a–c) Site 1 near Fülöpháza, Kiskunság National Park; (a) Jermy-type light trap modified with three baffles and settled on the ridge of a sand dune, (b) operating experimental light trap pair emitting horizontally polarised and unpolarised light on the sand dune, (c) the vegetation type around one of the light traps on top of a sand hill was dry sandy grassland (*Festucetum vaginatae*) scattered with some juniper bushes; (d–f) site 2 near Maroslele, River Maros; (d) Jermy-type light trap set up on the slope of the river dam, (e) the dam with a typical spring flood of River Maros at the light trapping site, (f) after floods the remaining ponds in the riparian forest of the River Maros are also excellent standing water habitats for certain mayfly species.

by natural grassy vegetation, mowed twice a year. The mixed forest consisted of old growth poplar, willow, and oak stands. It belonged to the nature reserve areas of the Körös-Maros National Park. Smaller ponds containing seasonal water were scattered among the understorey vegetation patches. The riverside was about 0.5 km from the traps. The distance between the traps and the forest edge was approximately 10 m, and the light source of the traps was at the height of the lower crown level of the forest.

The type of light trap

In both trapping sites we applied Jermy-type light traps (Figures 2a, d). The light source was 2 m above the ground. A metallic roof with a 120 cm diameter was framed above the bulb, and a metallic collection funnel was installed to protect the bulb and the sample against rain. The diameter of the funnel was 40 cm. Three baffles were arranged around the light sources of the traps used in site 1 (Figure 2a) in order to increase the trapping efficiency for insects associated with a dry sandy habitat. Other characteristics were the same in both trap pairs.

The light source was a compact fluorescent lamp (Philips PL-T 42W/830/4p). The same type of bulb was used as a light source in both trap pairs. A cylindrical polariser sheet (KÄSEMANN B + W^{TM} P-W64) around the lamp in a plexi-glass cylinder (Figure 1b) produced horizontally polarised light. Depolariser sheets composed of white milky tracing paper were placed between the lamp and the polariser or the polariser and the plexi-glass cylinder to allow the traps to emit linearly polarised or unpolarised light with the same intensity and spectral composition.

A large plastic bag attached to the funnel with wire frame was used as a killing jar (Figure 2a). The killing agent was chloroform. The construction and all characteristics (apart from light polarisation) of the trap pair were identical.

Characteristics of trappings

Trappings were run in two seasons, in years 2001 and 2002 at site 1 and in 2001 at site 2. Collection was carried out from 1 April to the end of October at each night from dusk to sunrise. The light traps were switched on and off automatically. In both sites, the traps were set up 80 m apart and there were no bushes or trees between them (Figure 2b). In the spring of 2002, after the first year of operation, the polarised light-emitting and unpolarised light-emitting light sources were transposed in the trap pair at site 1.

Data processing and statistical analyses

We were unable to set up more trap pairs per site, because of the difficulties of managing and the time consumed by the identification of the huge insect materials, thus there was no replication. This fact restricted the eligible statistical evaluations. In the first step of species level analyses, catches were taken into account as if each mayfly specimen would fly towards one of the light source types in an independent choice experiment. A Yates-corrected Chi-square test was used to detect polarisation preferences: the recorded distribution of total catches between the two light source types was compared with the equal (50:50%) sharing expected in case of equal attractivities of polarised and unpolarised light. Non-parametric Wilcoxon matched pairs test was used to find possible differences between daily catches of polarised and unpolarised light sources over the season (see Table 3). Standard weekly catches were

Table 1. Total yearly numbers of males and females of mayfly species collected by light traps equipped with light sources emitting horizontally polarised (POL) and unpolarised (UNPOL) light in 2001 and 2002.

	Maroslele, 2001				Fülöpháza, 2001				Fülöpháza, 2002			
	Males		Females		Males		Females		Males		Females	
Species	POL	UNPOL	POL	UNPOL	POL	UNPOL	POL	UNPOL	POL	UNPOL	POL	UNPOL
Caenis horaria (Linnaeus, 1758)	29>	8	129>	66	207>	0	566>	7	46>	5	125>	24
Caenis macrura Stephens, 1835	6>	1	1	0	–	–	–	–	–	–	–	–
Cloeon dipterum (Linnaeus, 1761)	36	28	17>	3	1	2	0	4	1	1	0	0
Ephoron virgo (Olivier, 1791)	0	0	10>	2	–	–	–	–	–	–	–	–
Heptagenia flava Rostock, 1877	0	0	1	1	–	–	–	–	–	–	–	–
Number of species	3	3	5	4	2	1	1	2	2	2	1	1

Maroslele, Fülöpháza: names of the trapping sites; sign > means that the POL trap collected at least three times the number of individuals than the UNPOL trap.

calculated for each species to characterise the seasonal flight. The degree of synchrony between seasonal activity patterns provided by the polarised light-emitting and unpolarised-light-emitting light traps was measured by the cross-correlation function (CCF) as a time series analytical method. If the traps collected only a few individuals, the CCF was not applicable (see Table 2). The STATISTICA program-package was used for all tests (StatSoft, 2005).

Results

Mayfly imagines and subimagines (both males and females) flew towards both light sources, but as expected, they flew in significantly greater numbers to the horizontally polarised light-emitting source than to the unpolarised light-emitting source. Over the two seasons the unpolarised and polarised light-emitting traps attracted the same two species at site 1, and five species in 2001 at site 2, showing that the trap pair sampled identical local assemblages with similar species composition within 80 m distance (Table 1). Among the five species recorded (Table 1), only two species were found in both sites and with greater abundances: *Caenis horaria* (Linnaeus, 1758) and *Cloeon dipterum* (Linnaeus, 1761). The abundance of *Caenis* and *Cloeon* species (associated mainly with standing waters) showed that this habitat type was characteristic of both sites. The mayflies *Ephoron virgo* (Olivier, 1791) and *Heptagenia flava* Rostock, 1877 known as running water inhabitants were captured only at the site near the river. However, their females were caught only in low numbers because of the 500 m distance from the riverside.

From the five recorded mayfly species four were caught in greater numbers and more frequently by the horizontally polarised light-emitting trap (Table 1). In most cases the capture rates were at least twice as high for the polarised light source than the unpolarised source. The males of both *Caenis* spp. expressed greater catching rates (3.6–200) in the polarised trap than females (2.0–81). Among the recorded mayfly species both sexes of *Caenis horaria*, but only females of *Cloeon dipterum*, were captured in highly significantly greater numbers to the polarised trap than to the unpolarised trap at both sites, according to the Chi-squared test (Table 2) and Wilcoxon matched pairs test (Table 3). This latter statistic shows that the higher

Table 2. Results of Chi-square test and cross-correlation function for frequency distribution of the numbers of individuals of mayfly species collected by the light traps equipped with light sources emitting horizontally polarised and unpolarised light.

	Maroslele, 2001		Fülöpháza, 2001		Fülöpháza, 2002	
Species	χ^2	r_0	χ^2	r_0	χ^2	r_0
Caenis horaria, males	4.58*	+0.595*	134.7***	+0.182	17.2***	+0.992***
Caenis horaria, females	9.42**		354.6***		36.1***	
Caenis macrura	1.16	–	–	–	–	–
Cloeon dipterum, males	0.28	+0.624**	–	–	–	–
Cloeon dipterum, females	4.10*		–	–	–	–
Ephoron virgo, females	1.69	–	–	–	–	–
Heptagenia flava	–	–	–	–	–	–

Trapping sites: Maroslele, Fülöpháza; χ^2: value of Chi-square test (Yates-corrected, df = 1); r_0: value of cross-correlation function without lag; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Table 3. Statistical result of Wilcoxon matched pairs test used for characterising differences between daily mayfly catches produced by trap pairs emitting horizontally polarised or unpolarised light.

Species	Site	Year	Statistic			
			n	*T*	*Z*	*P*
Caenis horaria	Fülöpháza	2001	42	11.5	5.282	**0.0001**
		2002	18	33	2.059	**0.039**
	Maroslele	2001	56	146.5	2.582	**0.010**
Caenis macrura	Maroslele	2001	46	3	1.572	0.116
Cloeon dipterum, males	Maroslele	2001	32	206	0.823	0.410
Cloeon dipterum, females	Maroslele	2001	17	24	2.485	**0.012**
Ephoron virgo, females	Maroslele	2001	48	1.5	1.278	0.201

Abbreviations: *n*, number of paired catches; *T*, the smaller of the sum of ranks for positive or negative differences, *Z*, value of *z*-test, *P*, probability level (bold indicates significant differences).

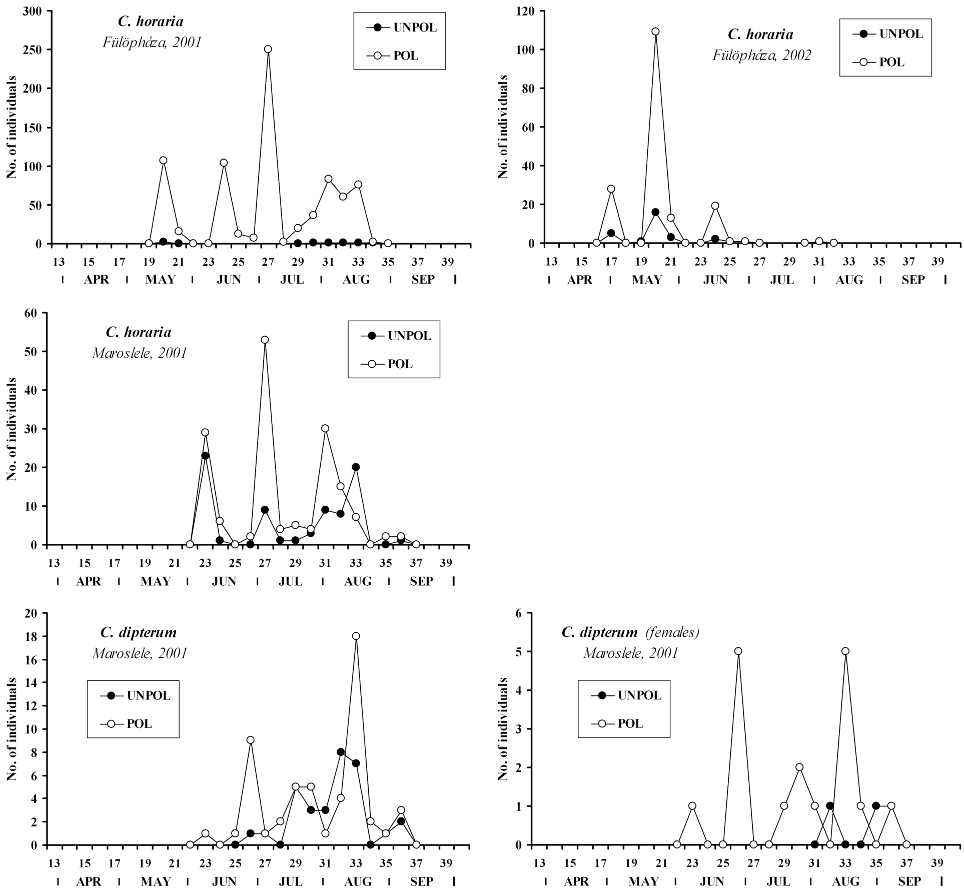

Figure 3. Flight activity patterns of the most abundant mayfly species (*Caenis horaria* and *Cloeon dipterum*) based on standard weekly catches by light traps equipped with horizontally polarised or unpolarised light source (numbers on *x*-axis: numbering of weeks taken from 1 January; POL: horizontally polarised light-emitting trap, UNPOL: unpolarised light-emitting trap).

attractivity of horizontally polarised light was consistent over the flight activity period because of the daily comparisons, thus these mayflies are candidates for using polarisation cues when detecting their aquatic habitats. *Caenis macrura* Stephens, 1835 and *Ephoron virgo* also showed a tendency to fly towards horizontally polarised light, but they were represented with too low numbers of individuals in samples to produce significant differences.

The recorded seasonal flight activities of mayfly species are shown in Figure 3. There are significant synchronies: no temporal shifts were detected by the time series analytical method (CCF) between the seasonal activity patterns of *Caenis horaria* and *Cloeon dipterum* recorded by the two traps (see the significant, positive r_0 values of CCF in Table 2).

The graphs show consistently greater weekly catches of the polarised and unpolarised traps for *Caenis horaria* and *Cloeon dipterum*.

Conclusions

The experienced structural characteristics (number of species, species composition and dominance distribution) of mayfly assemblages recorded by our trappings were not influenced by the polarisation characteristics of the emitted light.

The horizontally polarised light-emitting trap did not record any change of shift in the seasonal flight patterns compared to the unpolarised one.

According to the results of our light trappings, *Caenis horaria* and *Cloeon dipterum* were significantly more attracted to horizontally polarised light, which may refer to polarotactic water detection.

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