# Mayflies are least attracted to vertical polarization: a polarotactic reaction helping to avoid unsuitable habitats

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Changes performed on the basis of the comments of Referee 1 are marked by orange

Changes performed on the basis of the comments of Referee 4 are marked by blue

Changes performed on the basis of the comments of Referee 5 are marked by violet

## HIGHLIGHTS

- *Ephoron virgo* and *Caenis robusta* mayflies are attracted less to vertically polarized than to unpolarized light.
- Both species were attracted more to horizontally polarized than to unpolarized light.
- This polarotactic behaviour helps mayflies to avoid unsuitable habitats.
- The attractiveness of mayflies to differently polarized light depends on intensity and species.
- The mirror image of riparian vegetation reflects weakly and non-horizontally polarized light.
- These may facilitate the stability of mayfly swarming above water surfaces.

**Abstract**

Like other aquatic insects, mayflies are positively polarotactic and locate water surfaces by means of the horizontal polarization of water-reflected light. However, may vertically polarized light also have implications for the swarming behaviour of mayflies? To answer this question, we studied in four field experiments the behavioural responses of *Ephoron virgo* and *Caenis robusta* mayflies to lamps emitting horizontally and vertically polarized and unpolarized light. In both species, unpolarized light induces positive phototaxis, horizontally polarized light elicits positive photo- and polarotaxis, horizontally polarized light is much more attractive than unpolarized light, and vertically polarized light is the least attractive if the stimulus intensities and spectra are the same. Vertically polarized light was the most attractive for *C. robusta* if its intensity was about two and five times higher than that of the unpolarized and horizontally polarized stimuli, respectively. We suggest that the mayfly behaviour observed in our experiments may facilitate the stability of swarming above water surfaces. Beside the open water surface reflecting horizontally polarized light, the shadow and mirror image of riparian vegetation at the edge of the water surface reflect weakly and non-horizontally (mainly vertically) polarized light. Due to their positive polarotaxis, flying mayflies remain continuously above the water surface, because they keep away from the unpolarized or non-horizontally polarizing edge regions (water surface and coast line) of water bodies. We also discuss how our findings can explain the regulation of mayfly colonization.

*Keywords*: Mayfly, *Ephoron virgo*, *Caenis robusta*, Polarotaxis, Water surface, Reflection polarization

## 1. Introduction

Since the pioneering work of Schwind (1983, 1995) and the extended successive research (reviewed by Horváth and Varjú 2004; Horváth 2014) it has been known that aquatic insects have positive polarotaxis, that is, they are attracted to horizontally polarized light, because they find their aquatic habitats by means of the horizontal polarization of light reflected from the water surface. Mayflies, as typical aquatic insects, are positively polarotactic as well, because they also find water by means of the horizontally polarized water-reflected light (Kriska *et al.* 1998, 2007, 2009). In the case of mayfly species swarming immediately above the water surface, such as *Ephoron virgo* [Olivier 1791] (Fig. 1) and *Palingenia longicauda* [Olivier 1791], their positive polarotaxis is partly responsible for keeping them above water during their whole flying activity (Málnás *et al*. 2011; Horváth 2014; Száz *et al.* 2015), while other mayflies may leave the water bodies up to a distance of 1 km (Brodskiy 1973). In the latter case, positive polarotaxis guides the females back to water to oviposit.

In this work we study the behavioural responses of *Ephoron virgo* and *Caenis robusta* [Eaton 1884] mayflies to lamps emitting horizontally and vertically polarized and unpolarized light of the same spectrum. We selected these species for our experiments, because they belong to two different mayfly (Ephemeroptera) families (*E. virgo*: Polymitarcidae, *C. robusta*: Caenidae) and inhabit different habitats. The larvae of *E. virgo* develop only in rivers (Kazanci 2013), while the larvae of *C. robusta* occur in slow-flowing streams, still waters and rivers (Langford and Bray 1969; Bradbeer and Savage 1980; Kovács 2006). There are similarities between their behaviours: They start to swarm after sunset (Bradbeer and Savage 1980; Száz *et al*. 2015) and do not leave the vicinity of the water surface (Brodskiy 1973). At the beginning of swarming, the male subimagos of *E. virgo* emerging from exuviae land on the riverbank, moult to imagos, and then fly back to the river surface (Ibanez *et al*. 1991). The male imagos fly rapidly in a straight line at a height of 2.5-5.0 cm directly above the water surface and mate with females. A typical event in the swarming behaviour of these mayflies occurs when they are approaching the bank, and before reaching it they suddenly reverse their direction of flight and fly back to the river mid-line, in order to keep their position above the water surface during swarming (Száz *et al*. 2015). At the beginning, females fly above the water surface together with males (Fig. 1), where they copulate. After copulation, the females increase their altitude and begin their upstream-directed compensatory flight, which ends in oviposition onto the water surface (Kazanci 2013). The swarming behaviour of *E. virgo* is also typical for *P. longicauda* inhabiting rivers (Kriska *et al*. 2007; Málnás *et al*. 2011). *Caenis robusta* mayflies swarm above the water surface, where they form groups including several hundred individuals comprising both males and females. In these congregations, the number of males is 4-6 times greater than that of females (Brodskiy 1973).

Málnás *et al*. (2011) showed an example where mayflies were influenced by an artificial object: the upstream-directed compensatory flight of *P. longicauda* females was interrupted by a bridge and its mirror image and shadow on the river surface. The latter formed an optical barrier displaying a weakly and vertically polarized reflection-polarization signal. Therefore, the continuous highly and horizontally polarized signal of the river surface, guiding the flight of mayflies above water, was broken up by the vertically polarized mirror image and shadow of the bridge crossing the river. Imaging polarimetric measurements of Horváth and Varjú (1997), Bernáth *et al*. (2002, 2008) and Málnás *et al*. (2011) and the reflection-polarization patterns presented here show that a weakly and non-horizontally (mainly vertically) polarized area is also formed along the riverside where the mirror image and shadow of the riparian vegetation are observable on the water surface. May this weakly and non-horizontally polarized signal keep flying mayflies away from the edge regions of water bodies and keep them above the open water surface? If yes, this would be an additional behaviour that could control the stability of mayfly swarming above the water surface, beside the well-known positive polarotaxis induced by horizontal polarization. In field experiments we tested this possibility.

## 2. Materials and methods

### *2.1. Experiment 1*

We observed the mass swarming of *E. virgo* (Fig. 1) in Tahitótfalu (47° 75’ N, 19° 08’ E, Hungary) every evening (from 21:00 to 23:00 h = local summer time = GMT + 2 h) between 15 August and 2 September 2013 at a bridge overarching the river Danube. On 23, 24, 27 and 28 August 2013 between 21:00 and 23:00 h (GMT + 2 h) we performed field experiments to examine the attractiveness of light sources with three different polarization characteristics (unpolarized, horizontally polarized, vertically polarized with the same intensity and spectrum) to *E. virgo* mayflies (Fig. 1D). We fixed a LED torch (UltraFire C8 Cree XM-L T6 Light Emitting Diode) on a tripod on the bank of river Danube 110 m from the bridge, and pointed the light beam at 45° relative to the river flow direction towards the swarm of mayflies. Thereafter numerous *E. virgo* females stopped their compensatory flight and jammed around the torchlight. A filter was mounted in front of the torch lens. The filter was a stack of linearly polarizing sheet (XP42-18 from ITOS, Mainz, Germany) and white tracing paper. This filter could be rotated to ensure any direction of the transmission axis of the polarizer relative to the vertical. According to our imaging polarimetric measurements (see below), the tracing paper completely depolarized (degree of polarization $d$ = 0 %) the torchlight, while the polarizer made it totally linearly polarized ($d$ = 100 %). If the tracing paper faced the torchlight and the polarizer was outside, the transmitted light first became totally depolarized ($d$ = 0 %), then totally polarized ($d$ = 100 %) with direction of polarization determined by the transmission axis. If the polarizer faced the torchlight and the tracing paper was outside, the transmitted light first became totally polarized ($d$ = 100 %), then totally depolarized ($d$ = 0 %). Because of the same stacked structure of the filter in both cases, the intensity and spectrum of the filter-transmitted torchlight were the same for the unpolarized and totally polarized state. This filter method was the same as used in our earlier field experiments with *E. virgo* (Száz *et al*. 2015).

In the first, second and third parts of experiment 1, the torchlight was horizontally polarized, unpolarized and vertically polarized, respectively. The polarization characteristics of torchlight (Fig. 2A-C) were measured with an imaging polarimeter (based on a Nikon D3200 digital camera and a linear polarizer of TIANYA CPL 62 mm) in the red (650±40 nm wavelength of maximal sensitivity ± half bandwidth of the CCD detectors of the polarimeter), green (550±40 nm) and blue (450±40 nm) parts of the spectrum. The method of imaging polarimetry has been described in detail by Horváth and Varjú (1997, 2004).

During each session of this experiment, we took 7 photographs of the light beam with a given state of polarization to quantitatively assess the mayfly responses. After a photograph had been taken, we switched off the torch for five seconds, then after switching it on again, we waited another five seconds before taking the next photograph. Thus, the mayflies from the swarm in the river mid-line could reach the beam. We repeated this 10-second procedure before taking each photograph. After switching off the torch, the torchlight-attracted individuals rejoined the main mid-line swarm and flew torward the unpolarizing bridge-lamps staying 50 m apart. During the 5 seconds when the torch was turned off, the mayflies flew about 20 m upstream, thus they could not still perceive the beam of torchlight pointing at 45° relative to the river flow direction (Fig. 2D). Thus, we photographed expectedly new individuals each time and therefore minimized pseudo-replication. After taking the 7-photograph session at a given state of polarization of the light beam, we changed the polarization state and repeated the whole session. During the four days of experiment 1 these three sessions were continuously repeated with cyclic permutation of the polarization state of torchlight.

The altogether 966 photographs were evaluated with our custom-developed software (AlgoNet, http://www.estrato.hu/algonet) with an algorithm described in Száz *et al*. (2015) to count the mayflies attracted by the beam of torchlight of a given state of polarization. For counting the torchlit *E. virgo* mayflies in front of the dark night background, we first applied a Gaussian filter on the original image with a 31 × 31 pixels window and a 20 × 20 pixels width at half maximum to filter video noise. Next, we applied another Gaussian filter on the original image with a 100 × 100 pixels window and a 61 × 61 pixels width at half maximum to filter occasionally occurring halos around mayflies that flew close to the camera lens and the flash light. Then, we subtracted the second filtered image from the first one. In this image the background became solid black, the mayflies appeared with a blurred edge, and no noise was observable. These images were then converted to grayscale. In the grayscale images every pixel was substituted with black, if the relative pixel intensity was below the 7 % of the maximum intensity of the camera sensor, and with white otherwise. In the next step of evaluation, we applied an identical mask in every image that occluded the lamp and the bridge structure at the lower edge of the photos. This occluded area was painted black. Blobs (concentrations of white pixels) were then identified the same way as in Száz *et al*. (2015), with the exception that blobs close to each other were merged if their distance was less than 50 pixels. Finally, the number of blobs were counted, and this number was considered as the number of mayflies forming the sparse swarms. This method was validated in 25 photos in which the mayflies were also counted visually.

### *2.2. Experiment 2*

We observed the mass swarming of *E. virgo* in Rábahídvég (47° 03’ N, 16° 44’ E, Hungary) every evening from 20:30 to 21:30 h (= GMT + 2 h) between 4 and 6 August 2015 at a bridge overarching the river Rába. On 4 and 6 August 2015 between 20:30 and 21:30 h (GMT + 2 h) we performed field experiments to examine the attractiveness of light sources with the same spectrum but with three different polarization characteristics and with different light intensities to *E. virgo* mayflies. We fixed a LED torch (UltraFire C8 Cree XM-L T6 Light Emitting Diode) on a tripod on the bank of river Rába 30 m far from the bridge, and pointed the light beam at 45° relative to the river flow direction towards the mayfly swarm. In the first part of experiment 2, the torchlight produced with a linearly polarizing sheet (XP42-18 from ITOS, Mainz, Germany) was vertically polarized with a relative intensity of $I_{rel}$ = 100 %. In the second part of experiment 2 the torchlight produced with a neutral density filter (K&F Concept ND4 52 mm) was unpolarized with $I_{rel}$ = 68 %. In the third part of this experiment, the torchlight produced with a linearly polarizing sheet and a neutral density filter was horizontally polarized with $I_{rel}$ = 29 %. The polarization characteristics of torchlight were measured as in experiment 1.

During each session of experiment 2, we took 10 photographs of the light beam with a given state of polarization and intensity to quantitatively assess the mayfly responses, with the same photographic method as in experiment 1. The altogether 360 photographs were evaluated with AlgoNet (see above).

Experiment 2 was conducted at a location (Rábahídvég) being different from that (Tahitótfalu) of experiment 1, because in 2015 we wanted to perform two different choice experiments with *Ephoron virgo*, however, earlier we have experienced that at a given site only one experiment can be successfully performed due to the changing weather during the short (maximum 2 weeks) swarming period of this species.

*2.3. Experiment 3*

We conducted a light trap experiment with twilight-active *C. robusta* mayflies in the territory of the Old Turján in Ócsa Protected Landscape Area (47° 28’ N, 19° 26’ E, Hungary; Keresztessy *et al.*, 2013) on 22 July 2014 from 19:00 to 23:00 h (GMT + 2 h) in order to examine the attraction of this species to light sources with different states of linear polarization. We used three light traps having the same design (Fig. 3), light intensity and spectral characteristics, but with different states of polarization: horizontally polarized, unpolarized and vertically polarized (Fig. 2E-J). The light source consisted of a modified common portable lamp, in which a 8 W fluorescent tube (F8T5) was placed in a glass tube (diameter = 28 mm). The inner wall of the glass tube was covered with a stack of linearly polarizing sheet (XP42-18 from ITOS, Mainz, Germany) and depolarizing white tracing paper (Fig. 3A). In the unpolarized light source, the polarizer was closer to the long axis of the tube than the depolarizer (i.e. the depolarizer was outside and the polarizer inside). In the case of the horizontally and vertically polarized light sources, the depolarizer was closer to the tube axis than the polarizer (that is, the depolarizer was inside and the polarizer outside) and the transmission axis of the polarizer was horizontal and vertical, respectively (Fig. 3A). The three light traps were laid on the ground 10 m apart from each other along a straight line parallel to the edge of a lake at a distance of 2 m (Supplementary Fig. S1). Their order was randomized every 30 minutes in order to eliminate site-specific bias. The traps were clearly visible from the open water surface due to the lack of lakeside vegetation. Any differences in the light intensities of reflected light were wiped out by the dense ripples continuously forming on the water surface. The attracted mayflies were deflected by three white vertical plastic sheets (Fig. 3B) and fell into a killing jar containing chloroform. The collected mayflies were identified later in the laboratory. The reflection-polarization characteristics of the light traps (Fig. 2E-J) were measured by imaging polarimetry (Horváth and Varjú 1997, 2004).

*2.4. Experiment 4*

On 10 August 2015 from 19:00 to 23:00 h (GMT + 2 h) we conducted a light trap experiment with *C. robusta* in the site of experiment 3, in order to examine the attraction of this species to light sources with different states of linear polarization and different intensities (in experiment 3, the light intensity and spectral characteristics of the differently polarized stimuli were the same). We used the light traps of experiment 3, but with different intensities and polarizations. The first light trap with a linearly polarizing sheet (XP42-18 from ITOS, Mainz, Germany) was vertically polarized with a relative intensity of $I_{rel}$ = 100 %. The second light trap was unpolarized with $I_{rel}$ = 50 %, and the third light trap emitted horizontally polarized with $I_{rel}$ = 22 %. The reduction of light intensity was achieved by placing white paper layers around the fluorescent tube. The mayflies were attracted, trapped and identified as in experiment 3.

The motivations for using very different setups in experiments 1, 2 and 3, 4 were the following: (i) In experiments 1 and 2, only *E. virgo* participated, because during their mass swarming at night above the river Danube other similarly large insects practically do not occur. This situation favoured photographing the individuals attracted to the torchlight beam with different states of polarization, since only the bright blobs should have been counted in photographs. In this case, the light trap technique, killing the attracted and trapped insects, could not have been used, because *E. virgo* mayflies are strictly protected in Hungary. (ii) In experiments 3 and 4, during the swarming of *C. robusta*, also countless other similar-sized aquatic insects (e.g., Chironomidae, Ceratopogonidae) swarmed. Thus, the torchlight technique was out of question, because in the photographs numerous different insect species would have occurred, which could not have been differentiated from each other. Furthermore, the site of experiments 3 and 4 was at a lake where the lack of upstream-directed compensatory flight would have caused strong pseudo-replication in a torchlight experiment. Since in Hungary *C. robusta* is not a protected species, like many other species swarming simultaneously, classical light trapping was an ideal and adequate method to characterize the attractiveness of differently polarized light with the same intensity and spectrum to *C. robusta*.

### *2.5. Statistics*

For statistical analyses we applied $\chi^2$-tests with the use of the R statistical package. In the case of pairwise comparisons, Bonferroni correction was performed (Zar 2010). The number of *Ephoron virgo* mayflies registered in experiments 1 and 2 changed drastically due to the altering dynamics influenced by the changing weather. Thus, it would not make sense to calculate the medians and quartiles of daily totals. On the other hand, in experiments 3 and 4 *Caenis robusta* mayflies could be captured only during one day. In this case medians and quartiles cannot be calculated, either. Consequently, in order to compare the results of the statistical analyis, we used $\chi^2$ test for comparisons between the numbers of both mayfly species attracted to the light sources and captured by the light traps emitting differently polarized light in experiments 1-4. Using ANOVA, we tested the statistical significance of differences in experiments 1 and 2, but the results did not change compared to those performed with $\chi^2$ test.

## 3. Results

Figure 2A-C shows the colour photograph and the polarization characteristics of the linearly polarizing and depolarizing filters used in experiment 1 in the blue (450 nm) part of the spectrum. Figure 2E-J shows the patterns of the degree of linear polarization $d$ (Fig. 2E,F,G), and the angle of polarization $\alpha$ (clockwise from the vertical, Fig. 2H,I,J) of the three different light traps used in experiment 3 emitting horizontally polarized ($d$ = 97.4 ± 2.6 %, $\alpha$ = 92.3° ± 0.6°), vertically polarized ($d$ = 98.0 ± 2.0 %, $\alpha$ = 0.1° ± 1.9°) and practically unpolarized ($d$ = 7.7 ± 7.6 %, $\alpha$ = 71.3° ± 34.7°) light measured by imaging polarimetry in the blue (450 nm) part of the spectrum. Although the reflection from the white vertical deflecting plastic sheets of the light traps slightly altered the polarization characteristics of emitted light, the angle of polarization of light reflected from these sheets was the same as that of the emitted light.

The male/female ratio of *E. virgo* could not have been estimated from the photographs taken in experiments 1 and 2, since sex cannot be determined in these photos. However, since the photographs were taken during the upstream-directed compensatory flight, the majority of the photographically registered mayflies might have been females. In experiments 1 and 2, numerous *E. virgo* mayflies gathered in the beam of our torch emitting differently polarized light. When the torchlight was turned off, the small swarms attracted by torchlight broke up immediately and these mayflies rejoined their main swarm performing compensatory flight above the river. On the 966 photographs taken during the four days of experiment 1 about 108000 mayfly individuals were identified by our image processing software. Depending on the swarming day, (i) the horizontally polarized light attracted 5.6-11.9 times more mayflies than the vertically polarized light, (ii) the unpolarized light attracted 1.3-2.7 times more individuals than the vertically polarized, and (iii) the horizontally polarized light was 4.0-5.6 times more attractive than the unpolarized light (Table 1, Fig. 4). Considering the total numbers of attracted mayflies, (a) the horizontally polarized light was 79450/10115 = 7.9 times more attractive than the vertically polarized light, (b) the unpolarized light attracted 18447/10115 = 1.8 times more *E. virgo* than the vertically polarized light, and (c) the horizontally polarized light was 79450/18447 = 4.3 times more attractive than the unpolarized light (Table 1, Fig. 4). According to Supplementary Table S1, these differences are statistically significant.

In experiment 2, *E. virgo* mayflies preferred the horizontally polarized stimulus, the intensity of which was about half and one third of that of the unpolarized and vertically polarized one, respectively (Table 1). Here, the choice of *E. virgo* was governed by positive polarotaxis (attraction to horizontal polarization) which overwhelmed positive phototaxis (attraction to more intense light).

In experiment 3, 3452 *C. robusta* individuals were identified, from which we selected 100 mayflies and determined their sex. Interestingly, all 100 individuals were males. Thus, the vast majority of all the trapped *C. robusta* might have been males. The reason for this could be that in the swarms of *C. robusta* much more males occurred than females (Brodskiy 1973), and/or only the males fly away from the water surface up to the bank (note that our traps were placed on the edge of the lake from which *C. robusta* emerged). We found that (a) the horizontally polarized light was 2419/135 = 17.9 times more attractive to *C. robusta* mayflies than the vertically polarized light, (b) the unpolarized light attracted 898/135 = 6.7 times more *C. robusta* than the vertically polarized light, and (c) the horizontally polarized light was 2419/898 = 2.7 times more attractive than the unpolarized light (Table 1, Fig. 4). These differences are also statistically significant (Supplementary Table S1).

From the results of experiments 1 and 3 we conclude that (1) unpolarized light induces positive phototaxis in *E. virgo* and *C. robusta*, (2) horizontally polarized light elicits positive photo- and polarotaxis in these mayflies, and (3) vertically polarized light is the least attractive for both species if the stimulus intensities are the same.

In the 360 photographs taken during experiment 2, about 38000 *E. virgo* individuals were identified by our image processing software. Depending on the swarming day, (i) the horizontally polarized light attracted 1.4-1.7 times more mayflies than the vertically polarized light, (ii) the unpolarized light attracted 1.2-1.5 times more individuals than the vertically polarized, and (iii) the horizontally polarized light was 1.2 times more attractive than the unpolarized light (Table 1, Fig. 5). According to Supplementary Table S1, these differences are statistically significant. Considering the total numbers of attracted mayflies, (a) the horizontally polarized light was 15503/9371 = 1.7 times more attractive than the vertically polarized light, (b) the unpolarized light attracted 13373/9371 = 1.4 times more *E. virgo* than the vertically polarized light, and (c) the horizontally polarized light was 15503/13373 = 1.2 times more attractive than the unpolarized light (Table 1, Fig. 5). From experiment 2 we conclude that vertically polarized light is still the least attractive for *E. virgo*, even if its intensity is the highest.

We identified 289 *C. robusta* individuals in experiment 4. We found that (a) the vertically polarized light was 207/4 = 51.8 times more attractive to *C. robusta* than the horizontally polarized light, (b) the vertically polarized light attracted 207/78 = 2.7 times more mayflies than the unpolarized light, and (c) the unpolarized light was 78/4 = 19.5 times more attractive than the horizontally polarized light (Table 1, Fig. 5). These differences are statistically significant (Supplementary Table S1). From experiment 4 we conclude that vertically polarized light is the most attractive for *C. robusta* if its intensity is about two and five times higher than that of the unpolarized and horizontally polarized stimuli, respectively.

Figure 6 shows two examples for the reflection-polarization characteristics of an edge region of water bodies reflecting non-horizontally polarized light from the mirror image of riparian vegetation under a clear sky 1 hour prior to sunset. The sky-mirroring part of the water surface reflects horizontally polarized light (with angles of polarization $\alpha \approx 90°$ from the vertical) with high degrees of polarization ($d > 35$ %). On the other hand, the vegetation-mirroring parts of the water surface reflect horizontally and non-horizontally (obliquely and vertically) polarized light with moderate and low degrees of polarization ($d$ < 35 %). Since the threshold $d$* of polarization sensitivity in polarotactic aquatic insects (dragonflies: $d$* ≈ 10-20%, mayflies: $d$* ≈ 30%, tabanid flies: $d$* ≈ 30%; Kriska *et al.* 2009), it is pertinent to suppose that the moderately/weakly polarized light from the mirror image of vegetation (Fig. 6) can also be perceived by the investigated mayflies.

## 4. Discussion

Mayflies, like many other water-seeking insects, actively move toward the source of horizontally polarized light being associated with water (Schwind 1995; Horváth and Varjú 2004; Horváth 2014). In daylight, they do not react to unpolarized ambient light: they are neither attracted to, nor repelled by such light. For terrestrial insects (e.g. migrating desert locusts *Schistocerca gregaria*, Shashar *et al*. 2005), it can be important to detect water by means of the horizontal polarization of reflected light to avoid water, since they may perish if they crash into water. On the other hand, in this work we showed that two mayfly species are less attracted to vertically polarized light than to unpolarized light and much less attracted than to horizontal polarization, assuming equal intensity among stimuli. The important adaptive consequence of this is that mayflies turn back from areas of the water surface from which light with vertical, or more generally, non-horizontal polarization is reflected, which refers to the shoreline being unsuitable for them. A special consequence of this behaviour is that mayflies turn back from a bridge, from the mirror image and shadow of which vertically polarized light is reflected. This latter behaviour can be disadvantageous for the mayfly population as showed by Málnás *et al*. (2011).

A crucial aspect and interpretation of the unattractiveness to vertically polarized light in the night-swarming *E. virgo* and the twilight-active *C. robusta* mayflies is that the shadow and mirror image of the riparian vegetation seen on the surface of water bodies at their edge are moderately/weakly ($d$ < 35 %) and non-horizontally polarized (Fig. 6, see also Figs. 3-6 in Horváth and Varjú 1997; Figs. 4-6 in Bernáth *et al*. 2002; Fig. 4 in Bernáth *et al*. 2008; and Figs. 2-3 in Málnás *et al*. 2011). This polarization feature arises from the following: The reflection-polarization characteristics of the water surface are determined by two light components: (1) light reflected from the water surface, and (2) light originating from below the water surface. Component 1 is usually horizontally polarized because of reflection polarization of sunlight/moonlight and skylight from the air-water interface (Horváth 1995), while component 2 is always vertically polarized due to the refraction polarization of subsurface light at the water-air interface (Horváth and Varjú 1995). If component 1 or 2 dominates, the net polarization of water-returned light is horizontally or vertically polarized, respectively. If both components have similar intensities, the net degree of polarization $d$ is low. From optically open water surfaces skylight and sunlight/moonlight can freely be reflected, and the direction of polarization of the water surface is generally horizontal (Horváth 2014). At the mirror image of the riparian vegetation, only the dim light originating from the leaves is reflected instead of the much brighter skylight and sunlight/moonlight. Thus, component 1 is usually overwhelmed by component 2, especially for brighter water bodies, from which much light is backscattered by the bright, suspended particles. Consequently, the mirror image of vegetation is vertically polarized. Similar is the case for the shadowed areas of the water surface, where the contribution of direct sunlight/moonlight is decreased. The phenomena of mirroring and shadowing are frequently associated, because the riparian vegetation often casts shadow on its own mirror image. Since the polarization characteristics of water surfaces are independent of light intensity, all the above-mentioned polarization patterns of open waters and riparian reflections are valid for both sunlit and moonlit conditions. The only difference is that direct moonlight and scattered moonlight originating from the sky are much dimmer than direct sunlight and scattered sunlight from the sky (Gál *et al*. 2001; Barta *et al*. 2014).

Although male and female mayfly eyes differ considerably (Gupta *et al*. 2000), the sex of mayfly individuals in our photographs could not be determined. Since the compensatory flight is performed only by females, we logically assumed that predominantly female *E. virgo* were involved in our experiment. Our light traps caught only male *C. robusta* specimens, thus we cannot suppose that the results of our experiment are valid for both sexes.

Kriska *et al*. (2007) laid horizontal test surfaces (matt black and white canvas, shiny black and white plastic sheets, aluminium foil) on the grassy ground on the bank of river Tisza and studied the flight behaviour of *Palingenia longicauda* mayflies released above these test surfaces prior to sunset. These mayflies turned back at the edge of the shiny black and white plastic sheets that reflected horizontally polarized light with properly high degrees of polarization. In this experiment, the horizontally polarizing plastic sheets were surrounded by weakly and non-horizontally (obliquely or vertically) polarizing sunlit grass. Hence, in this artificially set up scenario the spatial sequence of optical stimuli (strongly and horizontally polarized plastic-reflected light followed by weakly and non-horizontally polarized grass-reflected light) mimicked the natural situation of water surfaces (strongly and horizontally polarized light reflected from the open water surface followed by weakly and non-horizontally polarized light reflected from the edge of the water surface). In both situations, the flying mayflies turned back at the edge of the horizontally polarizing surface area of river and plastic sheets due to their unattractiveness to vertically polarized light shown in this work.

Málnás *et al*. (2011) proposed that the optical barrier caused by the weakly and vertically polarized shadow and mirror image of the riparian vegetation at the edge of a river might play a crucial role in keeping swarming *P. longicauda* mayflies above the river mid-line. In the opinion of Málnás *et al*. (2011), mayflies avoid surface regions without a horizontally polarized light signal, and their polarimetric measurements have demonstrated the vertical polarization of the shadow and the mirror image of riparian vegetation at river edges. Our results presented here show that *E. virgo* and *C. robusta* mayflies prefer horizontally polarized and unpolarized light against vertically polarized light. We propose that this behaviour has the following adaptive value in mayfly swarming: As mayflies fly toward the riverside and detect its optical signal characterized by the low degrees of polarization and vertical direction of polarization, they turn back, and thus remain above the water surface during their swarming. Horizontal, darker muddy areas can often be found at the edge of flowing and still waters, which are areas usually reflecting horizontally polarized light, so they imitate water surfaces for positively polarotactic aquatic insects. If the vertically polarized shadow and mirror image of riparian vegetation did not occur at the edge of the water surface and would not act as a visual barrier, then female mayflies could land and lay their eggs onto the muddy ground, which is an inappropriate substrate for the development of mayfly larvae.

The practically totally polarized light with $d \approx 100$ % used in our experiments 1-4 is obviously higher than the species-dependent threshold $d^*$ of polarization sensitivity in mayflies determined by Kriska *et al*. (2009), who obtained the following $d^*$-values for four creek-inhabiting mayfly species: *Baetis rhodani* (32 % $\leq d^* \leq$ 55 %), *Ephemera danica*, *Epeorus silvicola*, *Rhithrogena semicolorata* (55 % $\leq d^* \leq$ 92 %). The value of $d^*$ is not known for the two mayfly species (*E. virgo*, *C. robusta*) investigated in this work. The suggested explanation of the adaptive value of the reaction of *E. virgo*, *C. robusta* to vertical polarization, of course, assumes that the weak vertical polarization of light present at the edge of water bodies is sensed ($d^* < d$) by these mayflies.

Száz *et al*. (2015) found that the weakly and vertically polarized shadow and mirror image of a bridge overarching a river also create an optical barrier for female *E. virgo* mayflies, which suspend their compensatory flight and jam at bridges. However, beside the vertically polarized shadow and mirror image of the bridge, the night-swarming *E. virgo* mayflies are also influenced by and attracted to the unpolarizing bridge-lamps due to their positive phototaxis. Thus, they fly to the bridge-lamps, and after getting exhausted, they land on the horizontally polarizing asphalt road below the lamps and oviposit onto the asphalt surface (Kazanci 2013; Száz *et al*. 2015). Although the compensatory flight of female *P. longicauda* mayflies is also interrupted by the vertically polarized shadow and mirror image of bridges, this species swarms in daylight, and is not influenced by bridge-lamps. Thus, *P. longicauda* females lay their eggs always on the water surface (Málnás *et al*. 2011).

Researchers attributed an important role to the compensatory flight in the colonization by mayflies and stoneflies (Spieth 1940; Tercedor and Ortega 1991). As a result of the upstream-directed compensatory flight above the river mid-line, female mayflies fly up to several kilometers, and reaching tributaries flowing into the main stream of the river they can colonize new river habitats. This phenomenon can explain the rapid spread of *E. virgo* experienced in Europe in the last decade (Ibanez *et al*. 1991; Száz *et al*. 2015). In this colonization the polarotaxis of river-inhabiting mayflies can play an adaptive role: Numerous smaller channels and backwaters flow into the rivers as well, which might be unsuitable habitats for the development of river-dwelling mayfly larvae. The width of these channels and backwaters is much smaller than that of the main stream, thus their whole (or almost whole) surface is covered by the weakly and non-horizontally polarized mirror image and shadow of riparian vegetation (Fig. 6). Such non-horizontally polarized water surfaces may prevent flying mayflies from penetrating in these small river branches, which could be inappropriate habitats. If the mouths of these smaller watercourses are wider, mayflies may fly above them, but as they become narrower, the mayflies turn and fly back above the main stream (personal observation of G. H. and G. K.).

In experiment 4, *C. robusta* practically did not fly to the least intense horizontally polarized light. In experiment 3, using differently polarized light stimuli with the same intensity and spectrum, we proved that this species can perceive polarization and has positive polarotaxis elicited by horizontal polarization. Since in experiment 4 the intensity of the horizontally polarized stimulus was about 1/5 of that of the vertically polarized stimulus, it is most probable that the horizontally polarized light was too weak, and thus simply phototaxis took over the control of reactions of *C. robusta* from polarotaxis. Hence, the critical light intensities at which polarotaxis takes over reaction control from phototaxis may be different in *E. virgo* and *C. robusta*.

It is conceivable that some potential (semi-)aquatic insect species are attracted to vertically or obliquely polarized light. The simple reason could be that these species would preferentially live at the shore region of lakes, wanting to avoid open waters that are more dangerous for them. Discovering such a species would be unprecedented, because as of now only insects attracted to or specifically not attracted by horizontally polarized light are known. For the former there are plenty examples, namely, aquatic insects (reviewed in Horváth 2014). For the latter one can mention the desert locus, *Schistocerca gregaria,* which is not attracted to horizontally polarized light, so that it can avoid large water bodies (Shashar *et al*. 2005).

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# Table

**Table 1.** Total numbers of *Ephoron virgo* and *Caenis robusta* mayflies attracted by the light sources and captured by the light traps emitting light with different intensities and polarization characteristics in experiments 1-4. $I_{rel}$: relative light intensity. ±: standard error.

| **emitted light** | **numbers of *Ephoron virgo* (experiment 1, sum of four replicates)** | **numbers of *Caenis robusta* (experiment 3)** |
|---|---|---|
| **horizontally polarized, $I_{rel}$ = 100 %** | 79450 | 2419 |
| **vertically polarized, $I_{rel}$ = 100 %** | 10115 | 135 |
| **unpolarized, $I_{rel}$ = 100 %** | 18447 | 898 |

| **emitted light** | **numbers of *Ephoron virgo* (experiment 2, sum of two replicates)** | **emitted light** | **numbers of *Caenis robusta* (experiment 4)** |
|---|---|---|---|
| **horizontally polarized, $I_{rel}$ = 29 %** | 15503 | **horizontally polarized, $I_{rel}$ = 21.6 %** | 4 |
| **unpolarized, $I_{rel}$ = 68,2 %** | 13373 | **unpolarized, $I_{rel}$ = 49.5 %** | 78 |
| **vertically polarized, $I_{rel}$ = 100 %** | 9371 | **vertically polarized, $I_{rel}$ = 100 %** | 207 |

# Figures with Legends

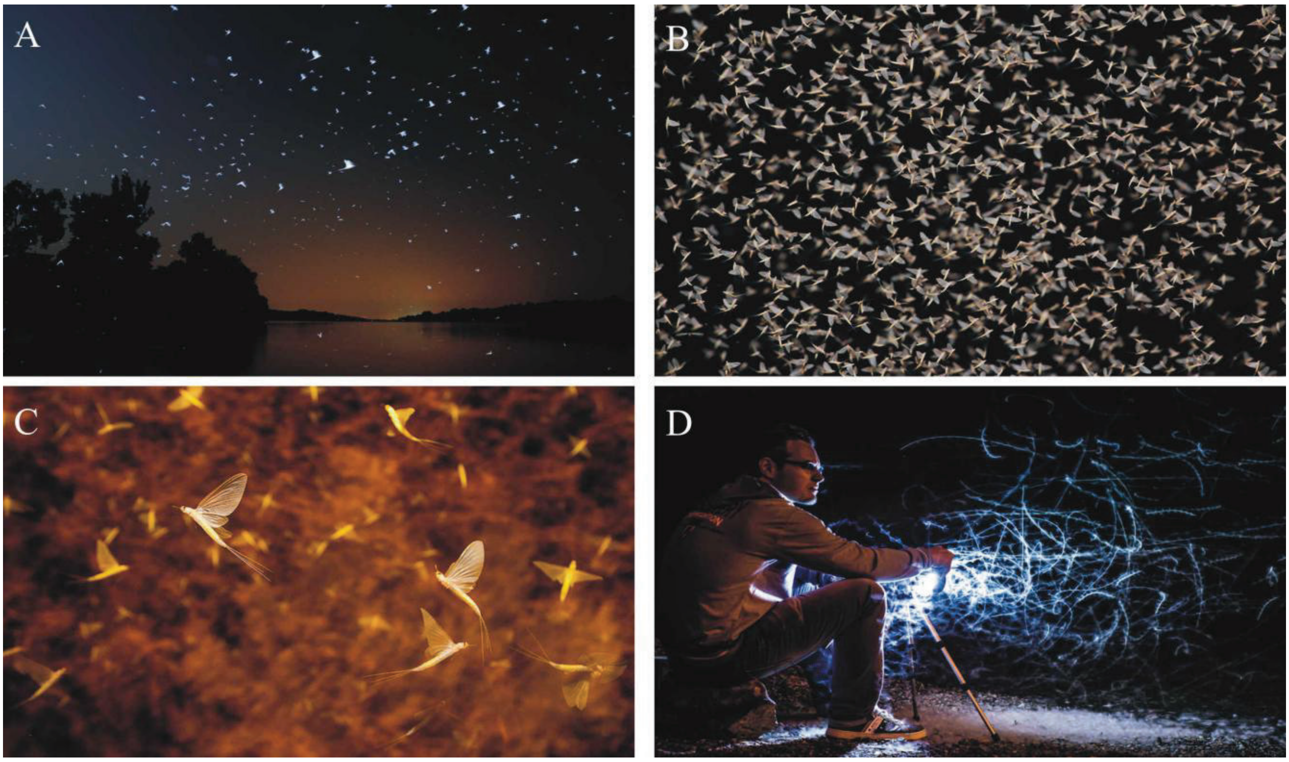


**Figure 1**: (A, B, C) *Ephoron virgo* mayflies swarming above the river Danube at Tahitótfalu, Hungary. (D) *Ephoron virgo* mayflies attracted to horizontally polarized light. Photographs taken by (A, B) Imre Potyó, (C) Dániel Soós, (D) István Sidó.

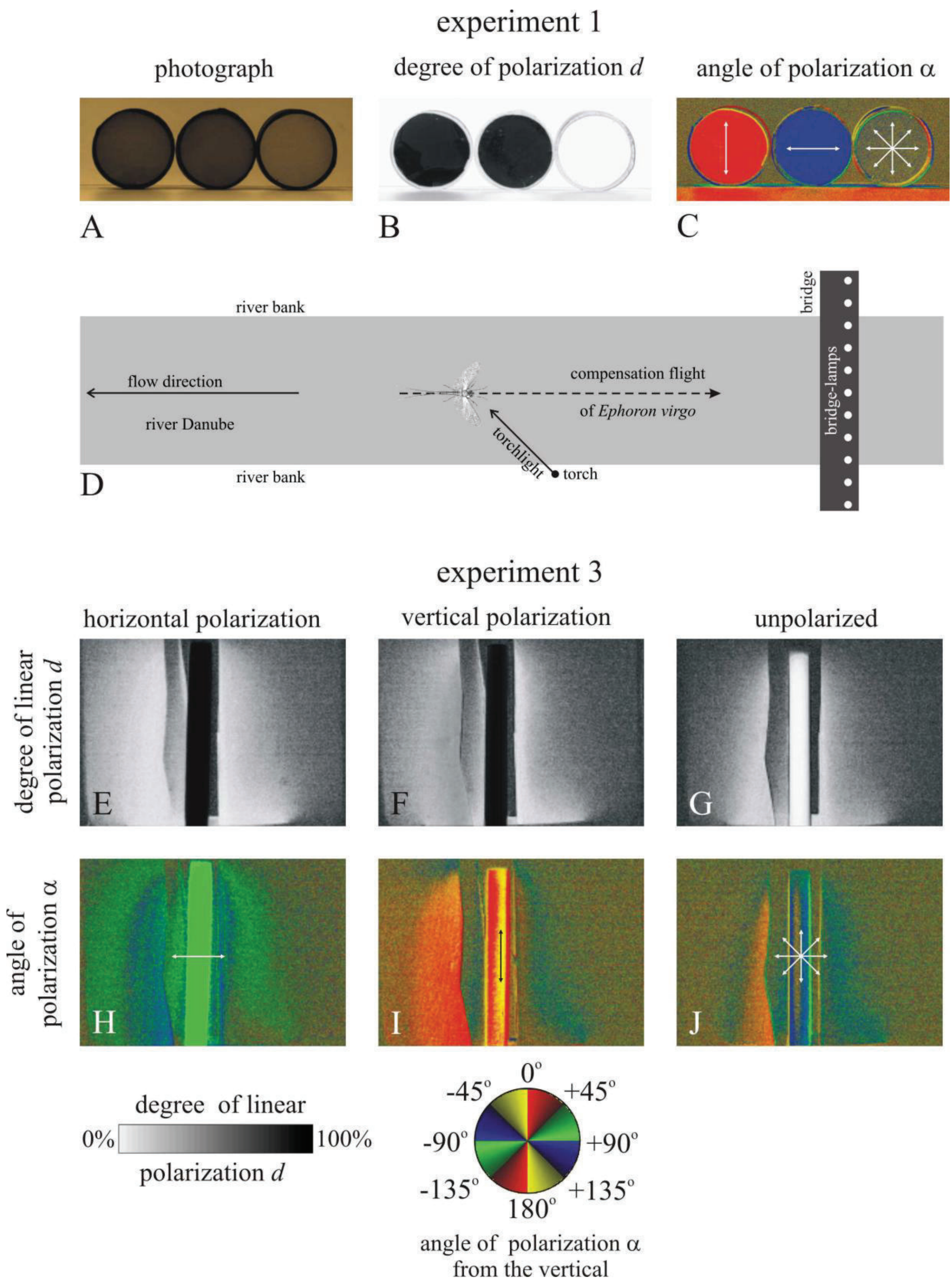


**Figure 2**: Patterns of the degree of linear polarization *d* and the angle of polarization α (clockwise from the vertical) of light sources used in field experiment 1 (A-C) and experiment 3 (E-J) measured with imaging polarimetry in the blue (450 nm) part of the spectrum. (A-C) Experiment 1: colour photograph (A), pattern of *d* (B) and α (C) of the linearly polarizing and depolarizing filters. (D) Setup of experiment 1. (E-J) Experiment 3: patterns of *d* and α of the light traps emitting horizontally polarized (E, H), vertically polarized (F, I) and unpolarized (G, J) light. The optical axis of the polarimeter was horizontal. Double-headed arrows show the local direction of polarization.

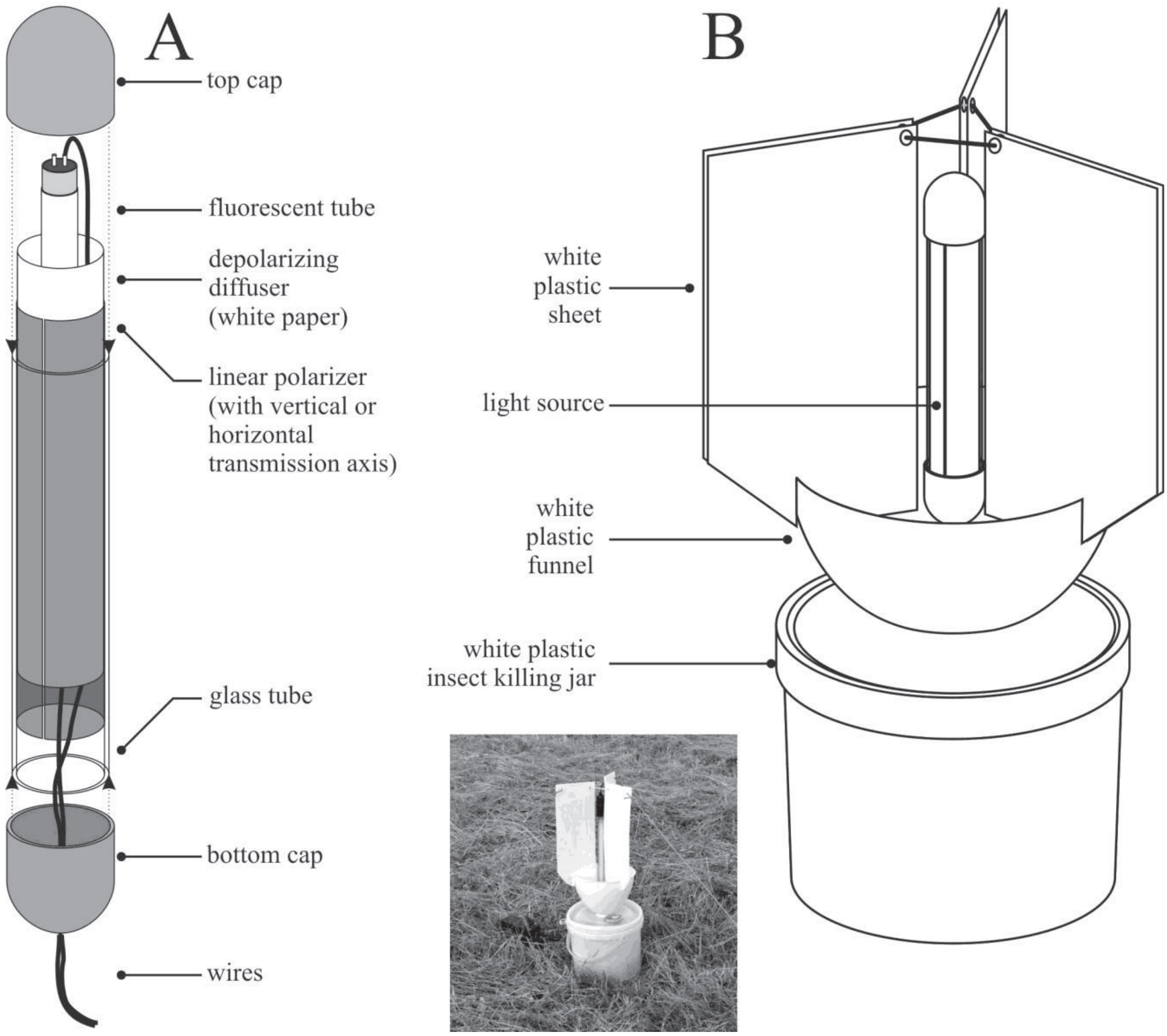


**Figure 3**: (A) Structure of the light source of the traps used in experiment 3. In the case of the unpolarized light source the order of the polarizer and the deoplarizing diffuser paper was reversed. (B) Structure of the light traps used in experiment 3. The inset is the photograph of a light trap.

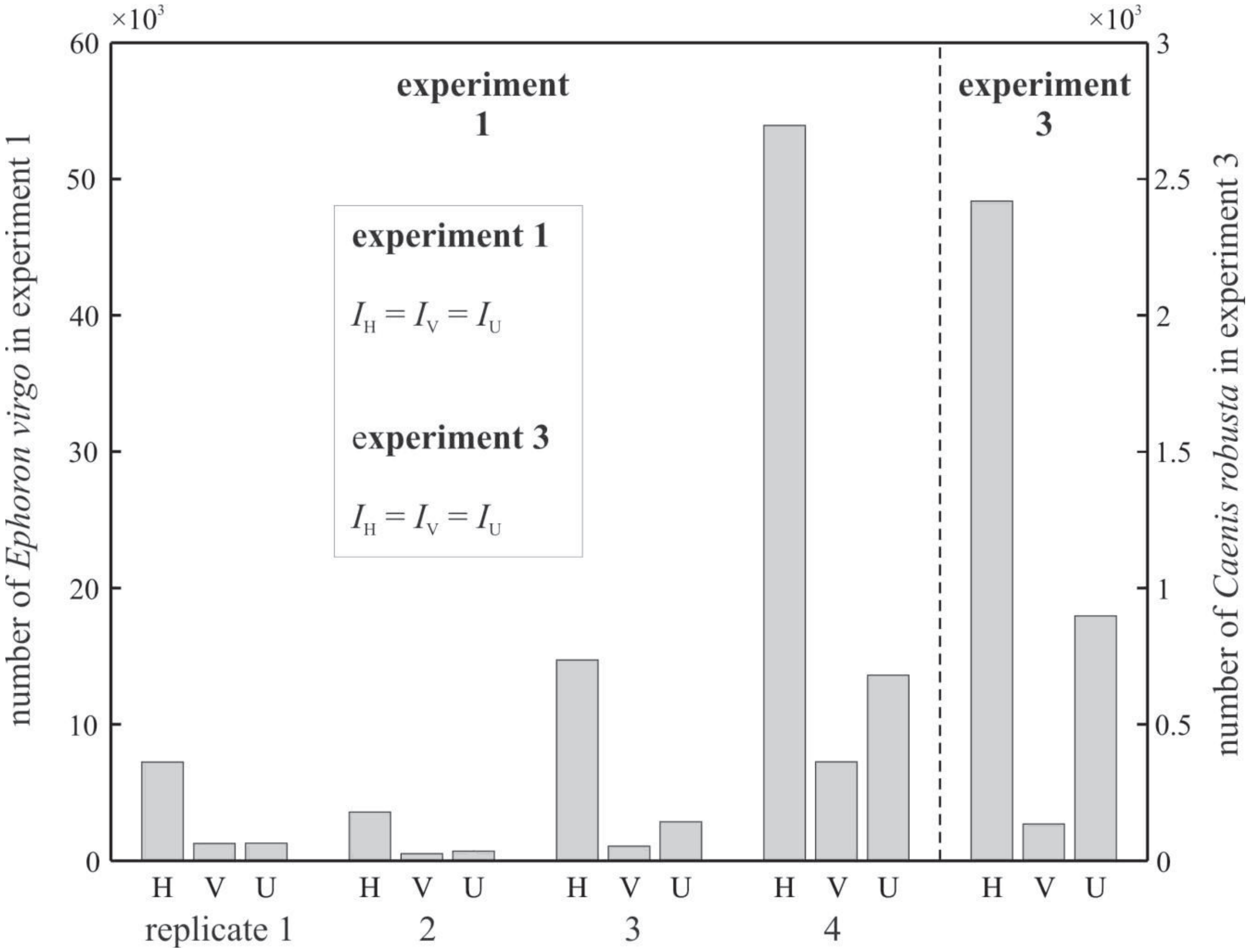


**Figure 4**: Total numbers of *Ephoron virgo* and *Caenis robusta* mayflies attracted to the light sources and captured by the light traps in experiments 1 and 3 (Table 1). H: horizontally polarized light, V: vertically polarized light, U: unpolarized light, *I*: light intensity. Numbers of mayflies included and details of statistical analyses are available in Table 1 and Supplementary Table S1.

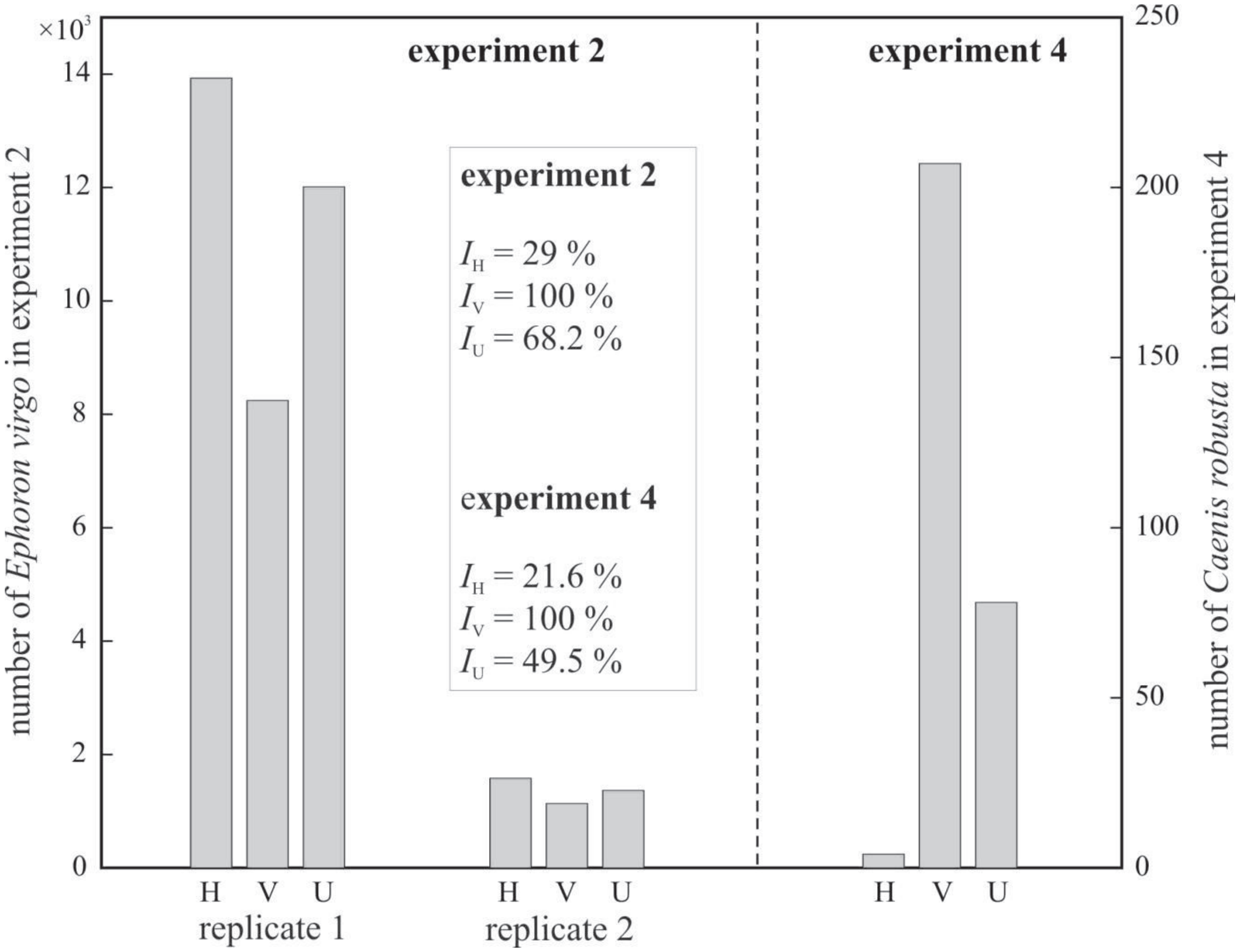


**Figure 5**: Total numbers of *Ephoron virgo* and *Caenis robusta* mayflies attracted to the light sources and captured by the light traps in experiments 2 and 4 (Table 1). H: horizontally polarized light, V: vertically polarized light, U: unpolarized light, *I*: relative light intensity. Numbers of mayflies included and details of statistical analyses are available in Table 1 and Supplementary Table S1.

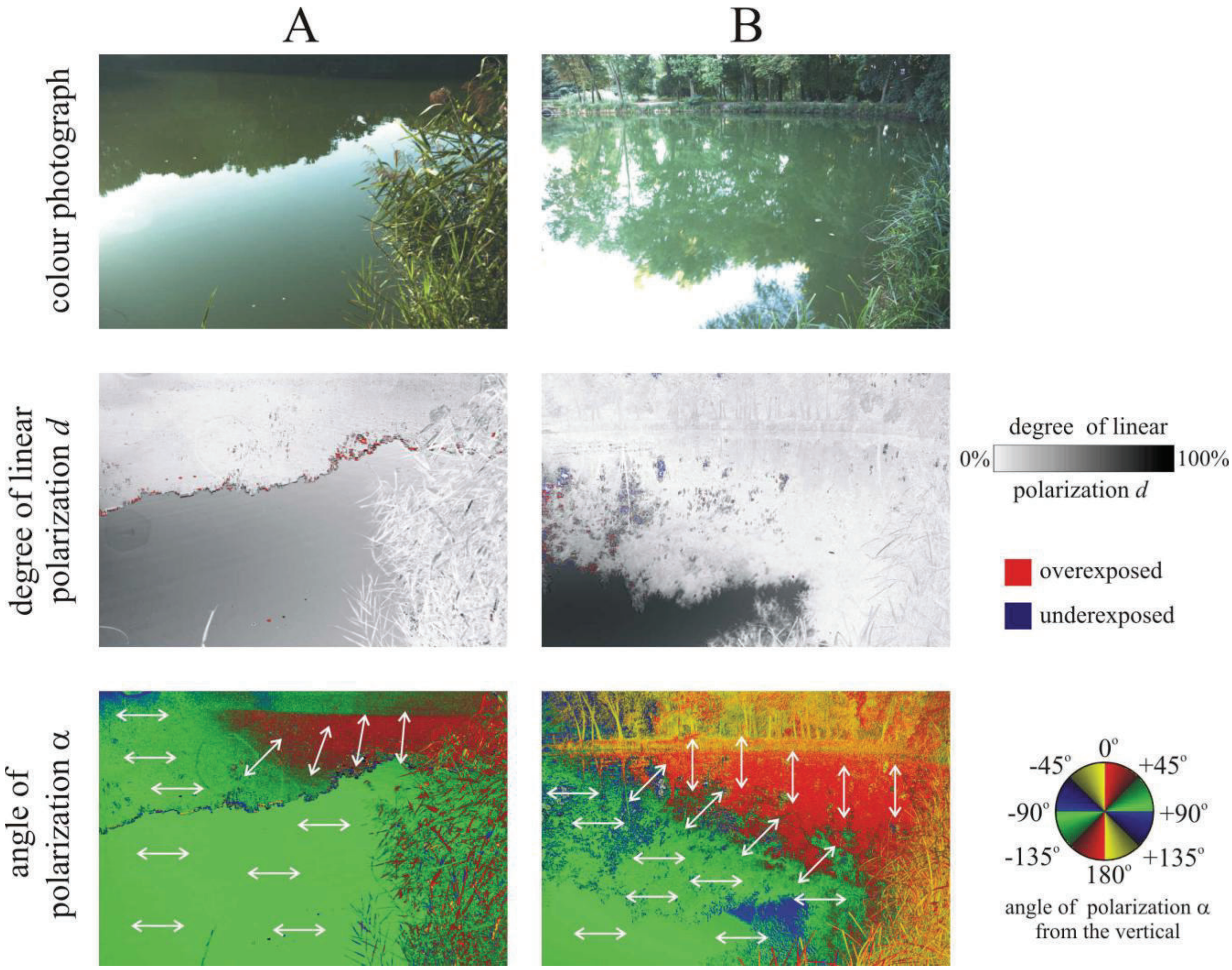


**Figure 6**: Examples for two different edge regions of the same water body reflecting non-horizontally polarized light from the mirror image of riparian vegetation. (A) Reflection-polarization patterns of a water surface under a clear sky 1 hour prior to sunset near the village Vác, Hungary measured with imaging polarimetry in the (450 nm) part of the spectrum. The polarimeter was pointed nearly towards the antisolar meridian, and its optical axis was tilted at –25° from the horizontal. Double-headed arrows display the local direction of polarization of the water surface. (B) The polarimeter was pointed towards the antisolar meridian, and its optical axis was tilted at –37° (Brewster angle) from the horizontal. Both polarimetric measurements were taken practically at the same time.

**Supplementary Materials**