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SHORT COMMUNICATION

Positive polarotaxis in a mayfly that never leaves the water surface: polarotactic water detection in *Palingenia longicauda* (Ephemeroptera)

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Abstract Tisza mayflies, *Palingenia longicauda* (Olivier 1791), swarm exclusively over the river Tisza (from which the name of the mayfly was derived). This river is bordered by a high vertical wall of trees and bushes, which hinder *P. longicauda* to move away horizontally from the water. During swarming, Tisza mayflies fly immediately above the river in such a way that their cerci touch the water frequently or sweep its surface. This continuous close connection with water and the vertical wall of the shore and riparian vegetation result in that Tisza mayflies never leave the water surface; consequently, they need not search for water. Several Ephemeroptera species move away far from water and return to it guided by the horizontal polarization of water-reflected light. To reveal whether also *P. longicauda* is or is not polarotactic, we performed a field experiment during the very short swarming period of Tisza mayflies. We show here that also *P. longicauda* has positive polarotaxis, which, however, can be observed only under unnatural conditions, when the animals are displaced from the water and then released above artificial test surfaces. *P. longicauda* is the first species in which polarotactic water detection is demonstrated albeit it never leaves the water surface, and thus, a polarotactic water detection seems unnecessary for it. The polarotactic behaviour of Tisza mayflies explains the earlier observation that these insects swarm above wet asphalt roads running next to river Tisza.

Keywords *Palingenia longicauda* · Tisza mayfly · Ephemeroptera · Water detection · Positive polarotaxis · Polarization vision

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Introduction

Mayflies (Ephemeroptera) develop as larvae in water for 0.5–3 years. After emergence, they swarm and copulate mid-air, then lay eggs into the water. Earlier, Kriska et al. (1998) showed that the mayfly species *Ephemera danica* (Müll.), *Ecdyonurus venosus* (Fabr.), *Epeorus silvicola* (Etn.), *Baetis rhodani* (Pict.), *Rhithrogena semicolorata* (Curt.) and *Haproleptoides confusa* (Hag.) detect water by means of the horizontal polarization of light reflected from the water surface (Gál et al. 2001) and then move towards it thus showing positive polarotaxis. These mayflies have been frequently observed to swarm above dry asphalt roads and to oviposit onto the dry horizontal asphalt surface. The reason for this strange behaviour is that near sunset, when the mentioned mayflies swarm, the degree of linear polarization of light reflected from asphalt is high (often higher than that of light reflected from water surfaces), and the direction of polarization of asphalt-reflected light is approximately horizontal. Thus, at sunset, the highly and horizontally polarizing asphalt surface can be more attractive than the water surface to water-seeking polarotactic mayflies.

According to Brodskiy (1973), on the basis of their swarming site, mayflies can be sorted into three groups: (1) species swarming immediately over water and never moving away horizontally from the water surface; (2) species swarming over the littoral but maintaining visual contact with the water; (3) species swarming far (maximum of about 500–1,000 m) from the water without visual contact with its surface. The six mayfly species studied by Kriska et al. (1998) belong to groups 2 and 3. As among Ephemeroptera, polarotactic water detection has been demonstrated until now only in these mayfly species, the following question arises: Do mayflies belonging to group 1 also detect water by the horizontal polarization of water-reflected light?

The Tisza mayfly, *Palingenia longicauda* (Olivier 1791), is a typical representative of group 1. It swarms exclusively over the surface of the river Tisza (from which the name of the mayfly was derived; Andrikovics et al. 1992; Andrikovics and Turcsányi 2001). Tisza is characterized by a steep, often vertical sandy–clayey shore with a dense growth of bushes and trees (Fig. 1a), and thus, the open water surface is bordered by a high vertical wall of vegetation (Fig. 1b), which hinders *P. longicauda* to move away horizontally from the water. On the other hand, during swarming, Tisza mayflies fly immediately above the river (Fig. 1c,d) in such a way that their cerci touch the water frequently or sweep its surface. This continuous close connection with the water surface and the vertical wall of the shore and riparian vegetation result in that Tisza mayflies never move away horizontally from the water; consequently, they need not search for water.

As mentioned above, several Ephemeroptera species move away far from water and return to it guided by the horizontal polarization of water-reflected light. One could imagine that also Tisza mayflies use the polarization of water-reflected light to recognize the water and stay over it rather than drift to shore. However, they fly so near the water (Fig. 1c,d) that they can recognize it by their tactile

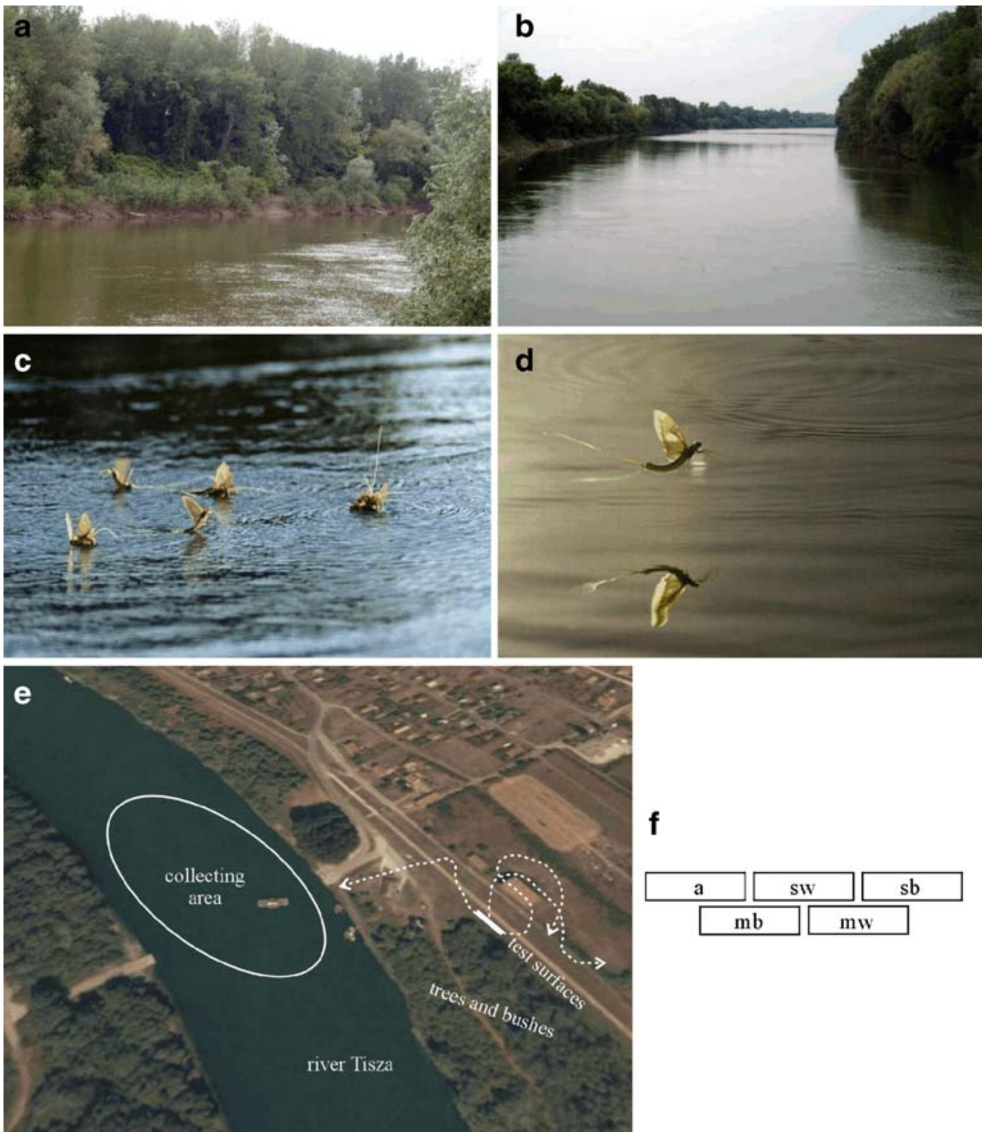

Fig. 1 **a** A typical section of the steep sandy–clayey shore with a dense growth of bushes and trees of river Tisza. **b** The open water is bordered by a high vertical wall of vegetation. **c**, **d** *P. longicauda* mayflies swarm immediately above the river surface (photographs taken by Sandor Zsila). **e** Aerial photograph (source: Google Earth—Imagery ©2005 Digital Globe) about the section of river Tisza where our field experiment was performed with *P. longicauda* mayflies. The *ellipse* represents the river area where the mayflies were collected. The *white rectangle* shows the position of the test surfaces on the shore, from which the water surface was not visible due to trees and bushes. *Dotted curves* represent three typical paths of mayflies leaving the plastic sheets. **f** Arrangement of the test surfaces; *a* aluminium foil, *mb* matt black cloth, *mw* matt white cloth, *sw* shiny white plastic sheet, *sb* shiny black plastic sheet

organs and hygroreceptors of their cerci and wings (Fink and Andrikovics 1997) as well as by the intensity of light reflected from the water surface. Consequently, they might not need an extra sensitivity to polarization for water recognition. Furthermore, Tisza mayflies need not possess polarizaton vision to stay over water because they never drift to shore due to the mentioned wall of the riparian vegetation (Fig. 1a,b). Although such a drift could be induced by the wind, *P. longicauda* never swarm/fly under windy conditions. Hence, it is not obvious that also Tisza mayflies need and thus indeed have positive polarotaxis. The aim of this work was to test whether *P. longicauda* is or is not polarotactic.

Materials and methods

Our field experiment was performed on 27 and 30 June 2005 on the shore of river Tisza (Fig. 1e) at the Hungarian villages Tiszaroff (47°23′N, 20°26′E) and Tiszabura (47°26′N, 20°27′E) between 1800 and 2100 hours (local summer time=UTC+2). During the experiment, every evening, an intense swarming of *P. longicauda* mayflies took place. The angular distance of the sun from the horizon ranged between +23.7° and −3.6° at the time of the experiment.

Sixty individuals of swarming male and female *P. longicauda* were caught over Tisza by hand nets from a motor boat (Fig. 1e). The insects were kept in soft paper boxes (10×10×20 cm). They were transported to the shore and released one after the other at a height of 10 cm above the test surfaces laid onto the ground, from which the river was not visible due to bushes and trees. Five different colourless horizontal test surfaces with dimensions of 2×10 m were applied: (1) shiny black plastic sheet, (2) shiny white plastic sheet, (3) aluminium foil, (4) matt black cloth and (5) matt white cloth. The test surfaces were arranged, as shown in Fig. 1f, on the shore between the dam and the riparian vegetation occluding the river surface. Our earlier field experiments with mayflies demonstrated that horizontal, shiny, black plastic sheets with a size as small as 1×1 m were highly attractive to the species *E. danica*, *E. venosus*, *E. silvicola*, *B. rhodani*, *R. semicolorata* and *H. confusa*, in which positive polarotaxis has been proven (Kriska et al. 1998). Thus, the size of 2×10 m of the test surfaces used in our experiment performed with *P. longicauda* seemed appropriate and large enough to elicit positive polarotaxis.

The behaviour of the released mayflies above the test surfaces was observed visually and recorded by a video camera. Before the experiment, the test surfaces were kept as folded-up, 50×50 cm, flat rectangular packages. Thus, after their unfolding, a latticed pattern composed of 50×50 cm square cells was visible on them. These cells helped us to follow the trajectories of the Tisza mayflies flying above the test surfaces. One of us held a paper sheet with a reduced copy of the cell pattern and drew manually the observed trajectory of a given mayfly (Fig. 2e).

The polarization characteristics of the test surfaces and the surface of river Tisza were measured by videopolarimetry in the red (650±40 nm = wavelength of maximal sensitivity ± half bandwidth of the charge-coupled device detectors of the polarimeter), green (550±40 nm) and blue (450±40 nm) parts of the spectrum during the field experiment under a clear sky at sunset. The optical axis of the polarimeter had an angle of −20° from the horizontal. The method of videopolarimetry was described in detail by Horváth and Varjú (1997). The field of view of our polarimeter was 40° (vertical)×50° (horizontal), and thus, the polarization characteristics of the Brewster zone (from which light with the highest degree of linear polarization p is reflected) could also be measured. For polarotactic insects flying over the water or test surfaces, the light reflected from the Brewster zone is the most relevant (Schwind 1985, 1991, 1995). Although p of light reflected from the test surfaces depends on the viewing angle of the mayflies, this is irrelevant because they could see the most highly polarizing Brewster zone.

The path lengths of the mayflies observed over the white and black plastic sheets (Table 1) were compared by paired t test with the use of the computer program Statistica 6.1.

Results

The flight of male *P. longicauda* imagoes began at about 1700 hours (local summer time = UTC+2). Courting males often flew in large swarms at a maximum height of 10–50 cm above the river. Female imagoes appeared at about 1800 hours. The flight of the adults (Fig. 1c,d) was characterized by approximately straight or zigzagged trajectories. Mating took place mid-air, although copulating pairs often fell into the water. At 1900 hours, imagoes swarmed by hundreds. After copulation, the females flew upstream at a maximum height of 20–25 m in course of a few-kilometers-long compensation flight. Finally, the exhausted females landed on the river, laid their eggs into the water and died soon. At about 2000 hours, all mayflies disappeared.

The delicate *P. longicauda* imagoes are extremely vulnerable; that is why only 16 male individuals of the 60 imagoes caught were able to fly when released from the boxes. Their behaviour could be assigned to two main types of reactions:

(A) Nine mayflies (from 16) left the plastic sheets immediately, raising continuously to a ceiling of about 30 m, sweeping around in large loops. These mayflies departed towards different directions, many

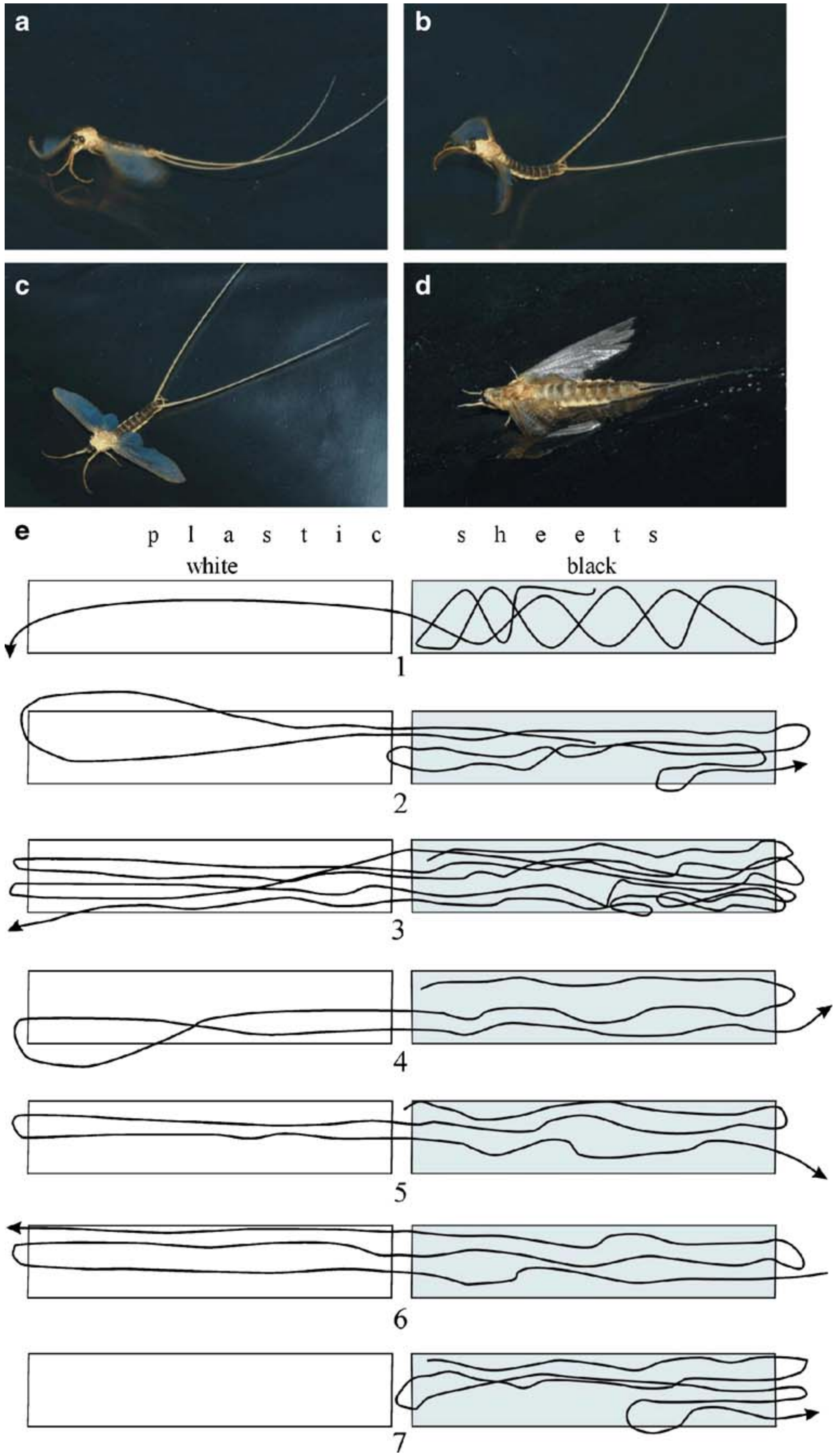

Fig. 2 **a**, **b** Male *P. longicauda* mayflies flying immediately above the horizontal shiny black plastic sheet. **c** A male *P. longicauda* settling down onto the black plastic sheet. **d** A female *P. longicauda* laying eggs onto the black plastic sheet. **e** Flight trajectories of seven (1–7) male *P. longicauda* mayflies released in our field experiment and flying immediately above the shiny black and white plastic sheets laid onto the shore of river Tisza, from which the river surface was not visible. The other three test surfaces (matt black cloth, matt white cloth, aluminium foil), positioned in the immediate vicinity of the plastic sheets (see Fig. 1f), are not displayed because they did not influence the behaviour of the mayflies

Table 1 Path length L (meter) of the flight trajectories of the seven (1–7) male *P. longicauda* mayflies over the white and black plastic sheets shown in Fig. 2e

Individual in Fig. 2e	Path length L (m)	
	White plastic	Black plastic
1	11.4	34.8
2	22.6	42.7
3	49.3	74.0
4	23.3	32.9
5	20.6	33.5
6	30.2	33.1
7	0.0	41.8
Average±standard deviation	22.5±15.3	41.8±14.8

of them away from the river but headed immediately towards it when they sighted the water surface (Fig. 1e). Some of them became prey of swallows (*Hirundo rustica*), which stayed in the vicinity.

(B) Seven mayflies (from 16) stayed over the test surfaces for short periods following the plastic sheets like water surfaces (Fig. 2). These insects flew along nearly straight or zigzag paths above the plastic surfaces at an altitude of 40–80 cm, rising occasionally, but never higher than 120 cm. The individuals stayed above the plastics only until they kept low altitude (i.e. saw the plastic surface under sufficiently wide angles). They followed the plastic sheets and turned back several times at the edges. The shiny aluminium foil, matt black cloth and matt white cloth did not influence the flying imagoes. However, none of the individuals stayed over the plastic sheets longer than 2 min; all of them raised and left the plastics, changing to the type A reaction in the end. All flying individuals left the plastic surfaces when raised above 120 cm.

Figure 2e shows the paths of individuals performing type B reaction over the plastic sheets.

– Mayfly 1 (Fig. 2e1) was released at the middle of the black plastic. It flew above the black plastic slowly along a zigzag path for about 60 s. Finally, it flew straight over the white plastic and left it rising quickly. Soon, sighting the river, it took a straight course towards the coast and joined the swarming mayflies over the river.
– Mayfly 2 (Fig. 2e2) was released at the middle of the black plastic. It flew above the white plastic in an elongated, straight loop, and after turning back at the edge of the white plastic, it flew above the black plastic where it remained for about 40 s flying along a nearly straight path with some zigzagging until leaving the black plastic.
– Mayfly 3 (Fig. 2e3) was released near to the neighbouring border of the black and white plastics. It started to fly towards the black plastic. It flew above both plastics for about 80 s along the long axes of the test surfaces. It flew quite straight above the white plastic but zigzagged and looped above the black one.
– Mayflies 4, 5 and 6 (Fig. 2e4,e5,e6) reacted quite similarly to the plastics. Mayflies 4 and 5 were released near to the neighbouring border of the black and white plastics, and they started to fly towards the black plastic. All three mayflies left the plastic sheets after flying along the long axes of the test surfaces heading straight above the white plastic, but zigzagging above the black one.
– Mayfly 7 (Fig. 2e7) was released near to the neighbouring border of the black and white plastics. It started to fly towards the black plastic. It slowly flew exclusively above the black plastic for about 20 s and not higher than about 40 cm, turning back when reaching the edges. Finally, it flew away, rising high above the ground.

Table 1 contains the path lengths L of the flight trajectories of the seven individuals of *P. longicauda* over the white and black plastic sheets shown in Fig. 2e. As the mayflies flew over the plastics with a nearly constant speed, the time spent above a given test surface is proportional to L, which can be considered as an appropriate measure of the attractiveness of the plastic surface to *P. longicauda*. In average, the path length of the Tisza mayflies was 41.8±14.8 m over the black plastic sheet, which is about 1.9 times as large as that (22.5±15.3 m) over the white plastic. From this significant difference (paired t test: $t=-4.045$; $df=6$; $p<0.007$), we conclude that *P. longicauda* preferred the black plastic against the white one.

Depending on the direction of view with respect to the horizontal, the black plastic was highly ($p\leq74\%$) and horizontally polarizing, whereas the white plastic was weakly ($p<20\%$) and horizontally polarizing. The matt black and white cloths were practically non-polarizing ($p\approx0\%$), whereas the aluminium foil reflected the light from the environment with random direction of polarization. As only the shiny plastic sheets influenced the flight of the Tisza mayflies, and the black plastic sheet was preferred against the white one, we conclude that *P. longicauda* has a positive polarotaxis; that is, it detects water by means of the horizontal polarization of reflected light.

Discussion

In our field experiment, we showed that *P. longicauda* prefers the highly and horizontally polarizing shiny black plastic sheet against the four other weakly and not always horizontally polarizing colourless test surfaces used. This finding can be explained only by positive polarotaxis elicited by horizontally polarized light. (1) The avoidance of the matt black and white cloths shows that surfaces reflecting unpolarized light with low or high intensity are unattractive. (2) The avoidance of the aluminium foil demonstrates that surfaces reflecting weakly linearly polarized light with very high intensity and non-horizontal direction of polarization are also unattractive. (3) The behaviour of the Tisza mayfly over the shiny white plastic sheet shows that surfaces reflecting weakly and horizontally polarized light with higher intensity are only moderately attractive if there is a highly and horizontally polarizing surface (the shiny black plastic sheet) in the immediate vicinity. Hence, *P. longicauda* is the first species in which polarotactic water detection was demonstrated albeit it never leaves horizontally the water surface, and thus, a polarotactic water detection seems unnecessary for it. This demonstrates that polarization-based water detection is an acient, conservative ability among Ephemeroptera.

Over the plastic sheets, we observed two different flying behaviours of *P. longicauda*, which are called as (1) "water-seeking flight" and (2) "water-following flight". The former reaction could only be interpreted as if the mayflies sought for water, and the latter reaction was the very same as the flight over real water surfaces. During the water-seeking flight, Tisza mayflies rose up to a maximum height of 15–30 m while sought highly and horizontally polarizing areas triggering the water-following flight, which was characterized by low flight height (10–50 cm) and approximately straight or zigzagged trajectory above the black plastic surface like over real water surfaces. All the seven observed male Tisza mayflies changed from water-following flight to water-seeking flight when they passed the borders of the plastic sheets. The water-seeking flight may allow individuals emerging accidentally in fresh backwaters and narrow branches to join the swarm above the main river branch. Under natural conditions, we never observed water-seeking flight of Tisza mayflies because they never leave horizontally the water surface.

Over the black plastic sheet, the behaviour of the seven male *P. longicauda* characterized by low altitude, nearly straight and zigzag paths (Fig. 2e) was very similar to that of the creek-inhabitant mayfly species *E. danica*, *E. venosus*, *E. silvicola*, *B. rhodani*, *R. semicolorata* and *H. confusa* above black plastic sheets (Kriska et al. 1998). The latter mayflies, living in bodies of water with a darker appearance, were attracted exclusively to the highly and horizontally polarizing black plastic sheet among the test surfaces. Contrary to these mayflies, five of seven Tisza mayflies flew also over the weakly ($p<20\%$) and horizontally polarizing shiny white plastic sheet. Note that the water of Tisza is milky and relatively bright during the swarming period of *P. longicauda* due to the bright suspending particles of the alluvial deposit (that is why this river is called in Hungary as "blond Tisza"). Because the brighter a water surface, the lower the degree of linear polarization p of reflected light (Horváth and Varjú 2003), the river Tisza reflects moderately ($p<30\%$) or even weakly ($p<20\%$) polarized light in the visible part of the spectrum. Certain water bodies (e.g. eutrophic yellowish–greenish waters), appearing coloured or colourless but bright for the human eye, can reflect highly polarized light in the ultraviolet (UV) range of the spectrum with lower degrees of linear polarization in the visible range (Schwind 1995). Using an imaging polarimeter, which was sensitive also to UV light (Bernáth et al. 2002), we established (unpublished data) that contrary to some natural bright water bodies, the colourless test surfaces used in our field experiment had very similar reflection–polarization characteristics both in the UV and visible parts of the spectrum. Thus, the shiny white plastic sheet was weakly polarizing not only in the visible, but also in the UV spectral range. Therefore, the behaviour of *P. longicauda* over the weakly and horizontally polarizing white plastic sheet may demonstrate that its positive polarotaxis is adapted to the weak polarizing ability and the bright appearance of river Tisza. Nevertheless, it cannot be excluded that the polarization sensitivity of *P. longicauda* might operate in the UV, and river Tisza might reflect highly polarized light in the UV. However, zigzagging of *P. longicauda* was observed only above the highly and horizontally polarizing black plastic sheet. Above Tisza, both straight and zigzag flight paths immediately above the water were observed.

As Tisza mayflies flew straight along the white plastic sheet (Fig. 2e), one might argue that the animals were better oriented over the white plastic than the black one. However, this would be an erroneous conclusion because straight flight occurred also above the black plastic (Fig. 2e). Both types of the natural flight pattern, the straight and the zigzag paths, were observed only over the black plastic. This shows that the behaviour of *P. longicauda* was more natural over the black plastic than over the white one. One could also argue that the Tisza mayflies could better orient over the black plastic because there they presented both components of their natural flight pattern: The zigzag flight requires a better and more sophisticated orientation mechanism due to the sudden, frequent and well-regulated periodical (sinusoidal) turns.

The plastic sheets with an area of 20 m^2 in our experiment were just large enough to elicit the water-following flight in swarming *P. longicauda* mayflies if they were unable to see the river. As males lost their interest towards the plastic sheets when flying higher than 120 cm, our test surfaces surely were not large enough to elicit a continuous water-following flight. Handling test surfaces with larger areas would have been impossible in such a field experiment. Much smaller (minimum of 1 m^2) horizontally polarizing test surfaces were able to attract polarotactic mayflies swarming above creeks (Kriska et al. 1998). Tisza mayflies obviously look for larger water surfaces to swarm above.

Ladócsy (1930, p. 29) reported about an anomalous swarming of Tisza mayflies above a wet asphalt surface:

> We can note as a basic principle that *Palingeniae* always fly above the river; their guide is the glittering water surface. This thesis is strongly supported by an observation of Professor Gelei, that Tisza mayflies left the river in great numbers after rain for the mirroring asphalt surface of the pedestrian in [the Hungarian city of] Szeged and formed a dense swarm in the street. [...] He observed their mating flight in July of 1928 at the corner of Lajos Tisza Boulevard and Rudolph Embankment. In his opinion the glittering asphalt surface deceived them, so they flew there to lay eggs.

On the basis of our present study, it is clear that *P. longicauda* was deceived by the horizontal polarization of light reflected from the wet asphalt near sunset. In Szeged, where Ladócsy (1930) observed this deception of *P. longicauda*, the asphalt road began/begins at the edge of river Tisza flowing between vertical concrete walls. Thus, when the asphalt road is wet, it appears as the direct continuation of the shiny water surface. Tisza mayflies, swarming near sunset and never leaving the water surface, can perceive the horizontally polarized light reflected from the wet asphalt (the degree of linear polarization of which can be even higher than that of light reflected from the water surface) and can be attracted to the asphalt surface.

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