How can asphalt roads extend the range of *in situ* polarized light pollution? A complex ecological trap of *Ephemera danica* and a possible remedy

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Abstract

When an artificial surface (e.g. an asphalt road) reflects strongly and horizontally polarized light as water bodies do in the nature, polarotactic aquatic insects, like the creek-dwelling *Ephemera danica* mayflies easily become deceived. After swarming above the creek surface, *E. danica* females begin their upstream compensatory flight and can be deflected at bridges with an asphalt road and continue their flight above the road surface. Thus, the water-mimicking optical signal of the road may deceive water-seeking polarotactic mayflies and lead them to distant, polarized-light-polluting surfaces, which elicit anomalous oviposition. On an asphalt road crossing a creek, we deployed polarizing insect traps at different distances from the bridge. The traps captured *E. danica* mayflies and their catch numbers indicated that these mayflies originated from the direction of the bridge, proving that they followed the track of the road. Our results suggests that distant polarized-light-polluting objects along an asphalt road can trap mayflies emerging from a creek crossing the road. The combination of an asphalt road and a man-made *in situ* (local) polarizing surface forms a complex ecological trap, being capable of luring aquatic insects from greater distances. To eliminate the oviposition of dangered polarotactic aquatic insects emerging from a creek onto the asphalt road crossing the creek, we suggest to deploy strongly and horizontally polarizing water-filled black trays along the edge of the road during the swarming period. Thus, the eggs of the deceived insects can be moved back to the creek in order to assist the conservation of the offspring-generation.

Key words: *Ephemera danica*, mayfly, water detection, polarotaxis, polarized light pollution

Introduction

Polarized light pollution is a phenomenon when behavioral patterns displayed by polarization-sensitive animals are altered due to strongly and horizontally polarized light reflected from artificial surfaces (Horváth et al. 2009). Schwind (1983, 1989, 1991) was the first who showed that several species of aquatic insects are able to recognize water surfaces by means of the horizontal polarization of water-reflected light, and in water detection, under natural illumination, this polarization is the primary cue rather than the spectrum (intensity and colour) of the water-reflected light. Their daily flight activity is greatly affected by the daily change of complex polarization patterns formed by the back-scattering and reflection of direct sunlight and skylight from waters, determined primarily by the solar elevation angle and the albedo of water bodies (Schwind and Horváth 1993; Bernáth et al. 2002). The consequence of this is that aquatic insects fly and seek waters mainly in the morning, early afternoon and evening governed by their so-called 'polarization sun-dial' (Csabai et al. 2006).

Even in the presence of water bodies, aquatic insects frequently choose certain artificial surfaces for egg-laying which reflect strongly and horizontally polarized light but are utterly inappropriate habitats. Observations of striking insect devastations around such objects and artificial surfaces had an important role in the scientific recognition of ecological traps (Horváth and Zeil 1996; Kokko and Sutherland 2001; Schlaepfer et al. 2002; Wildermuth and Horváth 2005; Robertson and Hutto 2006). Ecological traps are behavioral phenomena where individuals of a population follow an earlier adaptive behavioral pattern after a rapid environmental change and choose inappropriate habitats leading to the reduction or extinction of the population (Hale and Swearer 2016). Polarized light pollution (Horváth et al. 2009) is a special form of ecological traps threatening aquatic insects. Typical polarized-light-polluting sources are asphalt roads (Kriska et al. 1998), black horizontal plastic sheets used in the agriculture (Bernáth et al. 2008), glass surfaces of greenhouses and buildings (Malik et al. 2008), red or dark car bodies (Kriska et al. 2006) and solar panels (Horváth et al. 2010). The light reflected from these surfaces is usually strongly and horizontally polarized. When an aquatic insect has to choose between such an artificial surface and a water surface, it selects the former, because the strong and horizontal polarization of reflected light is a supernormal optical stimulus for polarotactic insects (Horváth et al. 2009). These human-made objects appeared extremely fast relative to the time period of existence of the affected aquatic insect species, thus their old, earlier reliable and successful water seeking strategy fails in an environment polluted with artificial, horizontally polarizing objects.

Kriska et al. (2006, 2009) and Horváth et al. (2010) observed that from locations where a polarized-light-polluting asphalt road runs close to the emergence site of *Ephemera danica* [Müller, 1764] mayflies, the females often fly long distances over the road as they naturally do when they perform their upstream compensatory flight above the creek. Our hypothesis is that the relatively weak (i.e. with low degree of polarization) horizontally polarized signal of the asphalt road does not elicit anomalous oviposition *per se*, but elicits water-following behavior of *E. danica*. Thus, besides deflecting the females, the asphalt road may guide them to distant, strongly and horizontally polarizing surfaces that can trigger oviposition. In this work, we present the results of field experiment in which we tested this hypothesis. We also tested whether the deflected females can be attracted to shiny black or red test surfaces placed on the edge of the road, and based on our results, we suggest a simple method for saving the egg-batches to be laid on the dry asphalt.

Materials and Methods

We studied the reproductive behavior of *Ephemera danica* mayflies in field experiments in the evenings from 18:00 to 21:00 h (UTC + 2 h) near the village Dömörkapu (47° 40’ N, 19° 03’ E) between 2014 and 2016. The experiments were conducted in the North Hungarian Mountains at the Bükkös creek, where an asphalt road runs near the creek over several kilometers and crosses the water several times on small bridges (Fig. 1A,B). We have observed in the last two decades, that from May to July around dusk, *E. danica* females fly above the road and follow its track.

Trapping experiment 1

In the first trapping experiment we tested our hypothesis that the weakly and horizontally polarized light reflected from the asphalt surface may deflect *E. danica* females and lead them to remote artificial strongly and horizontally polarizing small artificial surfaces, thus the range of local (*in situ*) sources of distant polarized-light-polluting objects becomes considerably extended. On 11 days between 22 May and 6 June 2014, during the swarming period of *E. danica* we placed three traps on the asphalt road at different distances from the western bridgehead with 20 m gaps between the neighbouring traps (Fig. 1C,D). According to our assumption, we expected the deflected mayflies at the western side of the bridge, because that direction matches better the compensatory flight course of the females (Fig. 1D). Each trap was composed of four rectangular (75 cm × 75 cm) black plastic trays (Fig. 2D-F) filled with common, transparent, slightly yellowish salad oil. They reflected strongly ($d \leq 90$ %) and horizontally ($85° < \alpha < 95°$) polarized light at the Brewster angle ($\theta_{Brewster} = \arctan n = 56.3°$ from the vertical calculated for the refractive index $n = 1.5$ of salad oil). The distance of the closest trap from the bridgehead was 20 m. *E. danica* specimens captured by the traps were counted and preserved in glass vials filled with 50 % ethanol.

Trapping experiment 2

In the first trapping experiment the traps were deployed from only one, the western head of the bridge, thus we still did not know if *E. danica* females performed anomalous compensatory flight along the road in both directions, or not. However, during trapping experiment 1 we also observed deflected females at the eastern side of the bridge, which gave us the inspiration to perform trapping experiment 2. Between 2 and 5 June 2016 we conducted the second trapping experiment by deploying 3-3 shiny black plastic surfaces (1 m × 1 m) (Fig. 2G-I) with 20 m gaps between them on both sides of the bridge. On both sides the first test surface was positioned at the bridgehead (Fig. 1D). Each test surface triplet was monitored by a human observer and the landing events were counted as well as the egg-clutches when the swarming ended. A typical behavior of the landed mayflies was expressing short (t < 1 s) 'jumps' by taking off and landing immediately (Supplementary Video S1). Because the two observer persons did not have the capacity to register the number of these jumps on all test surfaces, one landing event was considered as the union of the first landing, the jumps and the act of flying away. The reflection-polarization characteristics of the test surfaces were practically identical to that of the oil-filled traps of trapping experiment 1.

Color experiment

Here we tested our hypothesis that *E. danica* mayflies are equally attracted to horizontally polarizing black and red surfaces. In general, the darker and shinier a surface, in a given spectral range, the higher is the degree of polarization d of reflected light (Horváth and Varjú 2004). Our aim was to test if the eggs of deflected mayflies can be saved with mayfly-luring water-filled trays. At the same time it is important to make the trays conspicuous for the car drivers as the trays must be set on the edge of the road. The fact that the majority of aquatic insects lack red-sensitive photoreceptors (Horváth 2014) gave us the inspiration to test if horizontal shiny red surfaces are similarly attractive to water-seeking polarotactic mayflies as black ones. To compare the attractiveness of red and black surfaces to mayflies, we used square shaped (1 m × 1 m) metal plates covered by shiny red and shiny black paintwork. The plates, being optically identical to a shiny black or shiny red car body, were positioned 2 m from each other on the road 5 m from the bridge, and their positions were swapped every 10 minutes. During the swarming period of *E. danica* the plates were photographed in every 5 minutes so that the number of landed and closely approaching mayflies could be determined from the photos. Altogether 96 photographs were taken on 7 days between 14 May and 14 June 2014.

Imaging polarimetry

The reflection-polarization characteristics of the asphalt road, oil-filled traps, shiny black plastic surfaces and the water-filled trays were measured in the red (650 nm), green (550 nm) and blue (450 nm) spectral ranges by imaging polarimetry, the method of which is described by Horváth and Varjú (2004). The reflection-polarization characteristics of the shiny black and red metal surfaces used in the color experiment are described by Kriska et al. (2006).

Statistical analysis

For the comparison of the total numbers of mayfly catches, landings and egg-batches in a given experiment, we applied $\chi 2$ tests of homogeneity with the use of the R statistical package v3.0.2 (R Core Team 2013).

Results

The swarming of *E. danica* mayflies began between 19:00 and 20:00 h (UTC + 2 h) in the immediate vicinity of the creek. Their swarms consisted of a few hundred specimens and they often touched the water surface for several seconds. When the females started their upward compensatory flight 1-3 m above the creek, they appeared above the asphalt road near the bridge, and flew 500-1000 meters alongside the road.

In trapping experiment 1, the flying mayflies were noticeably attracted to the oil-filled trays and only a few specimens ignored them and continued their anomalous compensatory flight above the road. According to Table 1, the higher the distance of a given trap was from the bridge, the lower was its catch number. The traps positioned 20, 40 and 60 m away from the bridge caught 93, 30 and 5 mayflies, respectively (Table 1, Fig. 1D). This result shows significant heterogeneity ($\chi^2 = 96.39$, df = 2, $p < 0.0001$). It is also clear that 124/128 ≈ 97 % of the trapped *E. danica* specimens were females.

In trapping experiment 2, the sex of the landed mayflies could not be determined, but the outcome of trapping experiment 1 showed that the deflected specimens were practically females. The numbers of landings and ovipositions on the shiny black test surfaces are shown in Table 2. Landing numbers were significantly different as a function of the distance from the bridge (western side: $\chi^2 = 17.37$, df = 2, $p < 0.0002$; eastern side: $\chi^2 = 17.89$, df = 2, $p < 0.0002$). However, in the case of egg laying on the test surfaces, only the eastern side showed significant heterogeneity (western side: $\chi^2 = 4.19$, df = 2, $p = 0.12$; eastern side: $\chi^2 = 10.9$, df = 2, $p < 0.05$). The frequency of landings and ovipositions were higher in the vicinity of the bridge on both, western and eastern sides (Table 2, Fig 1D). During the first part of the swarming, mainly only landings were registered, while oviposition events occurred in the second half (Supplementary Video S1).

On the photographs taken in the color experiment we counted 46 and 60 mayflies on the shiny black and red test surface, respectively. We found no significant difference ($\chi^2 = 1.8491$, df = 1, $p = 0.1739$) between these numbers, thus the attractiveness of both test surfaces was practically identical.

According to our imaging polarimetric measurements (Fig. 2), all the liquid-filled trays, horizontal shiny black test surfaces and the asphalt road reflected horizontally polarized light, but the degree of polarization was significantly higher in the case of the traps and test surfaces relative to the asphalt ($d_{plastic} = 80.1\ \% \pm 7.5\ \%$, $d_{tray} = 89.2\ \% \pm 2.7\ \%$, $d_{asphalt} = 13.2\ \% \pm 7.9\ \%$).

Discussion

The reason for the observed deflection of mayflies could be the continuous, horizontally polarized optical signal of the asphalt road (Fig. 2A-C), which deceives the insects at the bridge, and elicits anomalous compensatory flight above the road. However, the degree of polarization d of light reflected from the asphalt road was below 20 %, it represented a homogenous, wide (width = 3 m), conspicuous track for flying mayflies. The catch numbers of our liquid traps in trapping experiment

1 (Table 1, Fig. 1D) and the landing and oviposition data of trapping experiment 2 (Table 2, Fig. 1D) support the hypothesis that the main source of mayflies is located at the bridge where the asphalt road is extremely close to the creek, and the road represents a deceptive, creek-imitating track for the flying female *E. danica* specimens. Since the swarming of *E. danica* occurs in the immediate vicinity of the creek, only the females are endangered by deflection, because the upward compensatory flight is characteristic only for females. Therefore, it is presumable that the asphalt road crossing the creek is able to deflect female mayflies and leads them to a distant, strongly and horizontally polarizing artificial surface eliciting polarotaxis-driven oviposition. The interpretation can also be reversed: artificial, strongly and horizontally polarizing surfaces, such as parking shiny dark-colored cars, can lure egg-laying mayflies from distant creeks by means of the polarized-light-polluting asphalt road.

In trapping experiment 2, during the first part of swarming the mayflies were just landing and touching the test surfaces and rarely the asphalt. These events may have been attempts to determine the direction of water flow, because the compensatory flight is the primary objective of females before oviposition, but this assumption should be confirmed in the future. As time passed, in the second part of swarming, female mayflies landed on the test surfaces more frequently (Supplementary Video S1), and finally the horizontally polarized stimulus elicited oviposition on the surfaces (Fig. 3C,D). When a deceived female mayfly oviposits onto an artificial polarizing object, the laid eggs dry out and perish quickly. Since female mayflies oviposit only once, and their egg-batch contains 6-8 thousand eggs (Elliott and Humpesch 1980; Wright et al. 1981), the oviposition to an inadequate place obviously has a negative impact on the offspring generation of *E. danica*. Besides *E. danica*, other mayfly species also oviposited onto the test surfaces, such as *Rhithrogena semicolorata* [Curtis, 1834], *Baetis rhodani* [Pictet, 1843], *Epeorus silvicola* [Pictet, 1865], but they did not disturb the experiments and data collection because *E. danica* mayflies are more corpulent than the latter species. Other difference between these species and *E. danica* was that they did not perform compensatory flight (Brodskiy 1973). They presumably compensate the egg drift with upstream migration in the larval stage.

The reason for using different test surface types in trapping experiments 1 and 2 is two-fold: (1) Handling dry plastic surfaces is significantly easier than liquid-filled trays, and (2) applying dry, non-trapping surfaces does not kill the attracted mayfly specimens and keeps the possibility for their survival. Naturally, due to the dry test surface, in trapping experiment 2 pseudoreplication occured, however this was not critical, since the primary goal of this experiment was to find out whether mayfly females continue their anomalous compensatory flight in both directions from the bridge.

In order to demonstrate that the eggs of mayflies deceived by the polarized-light-polluting asphalt road can be collected and transferred back to the creek, in a pilot experiment we set a black plastic tray (75 cm × 75 cm) filled with creek water on the edge of the asphalt road (distance from bridge = 20 m), and observed the behavior of mayflies during their swarming on 18 and 20 May 2014. We observed several female *E. danica* landing and ovipositing in the tray. Supplementary Video S2 shows a female laying her eggs at length into the water of the black tray. Compared to trapping experiment 2, the behavior of *E. danica* females was slightly different in this preliminary test: When mayflies touched the water surface of the tray, they started to lay eggs immediately (Supplementary Video S2). Probably, because the dry, shiny black plastic surfaces in trapping experiment 2 were similar to water only optically and did not provide the other stimuli which are present when the mayflies touch the water. The optical characteristics of the water-filled tray were identical with those of the oil-filled trays used in trapping experiment 1. Our pilot experiment shows that the eggs of deflected mayflies can be collected easily with horizontally polarizing black water-filled trays placed on the road edge. This brings us straight to the possibility to manually move the eggs into the creek, where they should naturally end up.

The result of the color experiment indicates that red and black horizontal shiny, horizontally polarizing surfaces attract *E. danica* females equally. Consequently, not only black, but red water-filled trays can also be used as mayfly traps to preserve the offspring generation of mayflies by collection of their eggs. The red color is more advantageous, because it is much more striking than black. This is an important factor from the aspect of traffic safety.

We had the opportunity to observe the phenomenon of mayfly deflection at another site on 10 June 2015 between 20:45 and 21:15 h (UTC + 2 h) in the vicinity of Ipoly, which is a narrow tributary of the river Danube (48° 03' N, 18° 52' E). From a bridge overarching the Ipoly, after leaving the water surface, numerous *Ephemera vulgata* [Linnaeus, 1758] specimens flew 3-5 m above the road leaving the river behind them. The largest distance from the bridge along the road we could observe flying mayflies was 3200 m (Fig. 4). If a road, which deflected mayflies does not cross the water again within 1-2 km, the survival chance of the mayflies is very low. Small bridges are obviously candidates for sources of mayfly deflection, because the asphalt gets extremely close to the water. It is still unknown, what is the minimal distance between a water course and an asphalt road which can stimulate a female to continue her flight above the road.

Besides *E. danica* and *E. vulgata*, the river-dwelling *Palingenia longicauda* [Olivier, 1791] and *Ephoron virgo* [Olivier, 1791] mayflies also perform compensatory flight after swarming and can also be observed above the asphalt surface of bridges. However, they do not continue their flight above the road, the reason for which might be the significantly narrower surface of the asphalt road relative to the river surface (Málnás et al. 2011). On the other hand, contrary to *P. longicauda*, *E. virgo* mayflies start to swarm at dusk and they begin their compensatory flight in darkness. Hence, if the bridge is equipped with lights, they attract and trap *E. virgo* in mass. Then the mayflies get exhausted and oviposit on the asphalt of the bridge (Kazanci 2013; Száz et al. 2015).

Our experiments and observations showed that the formation of polarization-based ecological traps and the severity of their effects (how effectively they trap polarotactic insects) may be determined by a series of multiple behavioral elements and taxes governed by multiple physical stimuli guiding the individuals to the traps. Thus, small sources of polarized light pollution may act as parts of complex polarization-based ecological traps causing the decay of large masses of aquatic insects. We have drawn attention to an ecological problem threatening egg-laying *E. danica* females and we suggested a simple and reliable method for their conservation. Further researches should quantitatively determine the negative effect of such polarized-light-polluting sites at population level by comparing the number of deflected specimens with that of the whole population. Studying complex optical ecological traps is still in its infancy, thus explorations are momentous which are aiming to study these ecological traps and to develop different methods to reduce their negative effects.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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Tables

Table 1 Numbers of *Ephemera danica* females and males trapped by the three black, oil-filled traps positioned at different distances from the bridge overarching the creek in trapping experiment 1. The distance of the 1st 2nd and 3rd trap from the western bridgehead was 20, 40, 60 meters, respectively

date (2014)	***Ephemera danica* catches**					
	trap 1 (20 m)		**trap 2 (40 m)**		**trap 3 (60 m)**	
	female	male	female	male	female	male
22 May	0	0	0	0	0	0
23 May	6	0	1	0	0	0
25 May	11	0	2	0	0	0
26 May	14	0	6	0	0	1
27 May	18	0	8	0	2	0
29 May	12	0	3	0	0	0
30 May	9	0	2	1	1	0
1 June	13	1	3	0	0	0
2 June	5	1	4	0	0	0
4 June	3	0	0	0	1	0
6 June	0	0	0	0	0	0
sum	**91**	**2**	**29**	**1**	**4**	**1**
total	**93 (72.66%)**		**30 (23.44%)**		**5 (3.90%)**	

Table 2 Numbers of *Ephemera danica* specimens landed on the shiny black plastic surfaces in trapping experiment 2. The numbers of egg-clutches layed on the test surfaces are shown in brackets. The distance of the 1st 2nd and 3rd trap from the corresponding bridgehead was 0, 20, 40 meters, respectively

date (2016)	***Ephemera danica* landings (numbers of egg clutches)**					
	western head of the bridge			**eastern head of the bridge**		
	trap 1 (0 m)	**trap 2 (20 m)**	**trap 3 (40 m)**	**trap 1 (0 m)**	**trap 2 (20 m)**	**trap 3 (40 m)**
2 June	1 (0)	3 (0)	3 (0)	0 (0)	0 (0)	0 (0)
3 June	11 (1)	10 (0)	6 (2)	12 (0)	10 (0)	4 (1)
4 June	15 (14)	19 (6)	8 (6)	23 (13)	19 (6)	12 (0)
5 June	21 (1)	22 (1)	2 (1)	16 (0)	9 (0)	0 (0)
sum	**48 (16)**	**54 (7)**	**19 (9)**	**51 (13)**	**38 (6)**	**16 (1)**

Figures with Legend

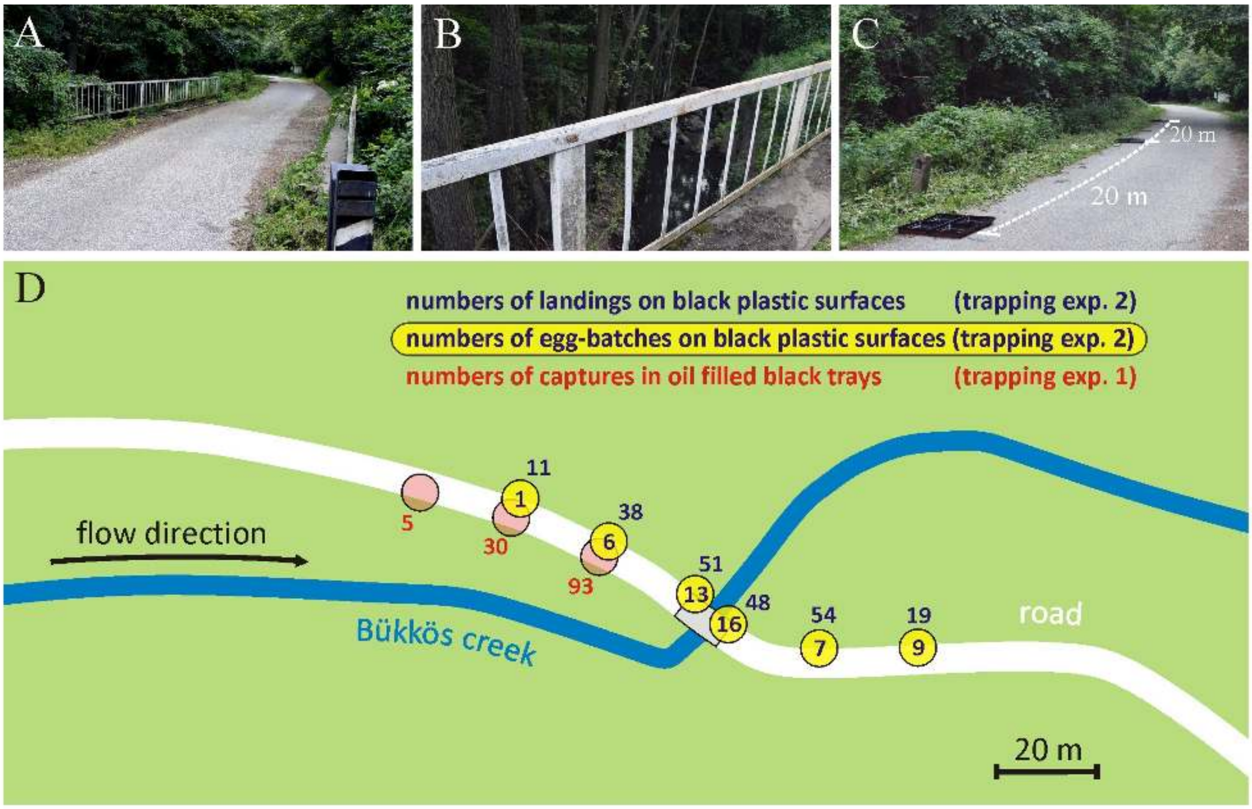

Fig. 1 Site of the field experiments. (A, B) Photographs of the bridge (C) Photograph of the oil-filled black plastic trays used in trapping experiment 1. (D) Arrangement of the oil-filled trays (red circles) and shiny horizontal black surfaces (yellow circles) of trapping experiments 1 and 2. The number of mayfly catches and landings are also shown as well as the number of collected egg-batches

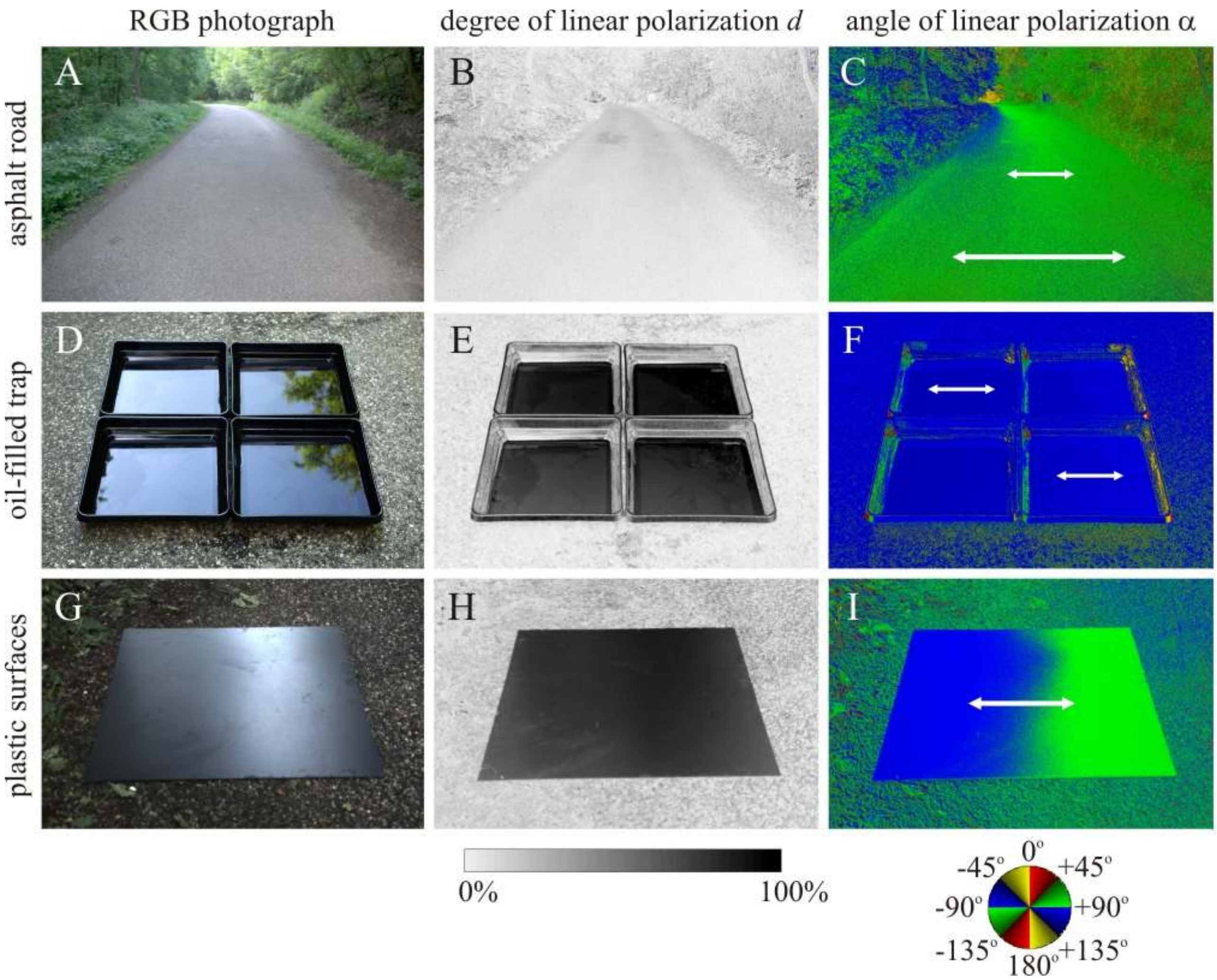

Fig. 2 RGB photographs (A, D, G), patterns of degree of linear polarization *d* (B, E, H), and angle of polarization *α* (C, F, I) for the asphalt road near the western bridgehead, an oil-filled black tray used in trapping experiment 1, and a black test surface used in trapping experiment 2. *d* and *α* were measured by imaging polarimetry in the green (550 nm) spectral range at the time of the swarming of *E. danica*. The arrows represent the angle of polarization of the reflected light

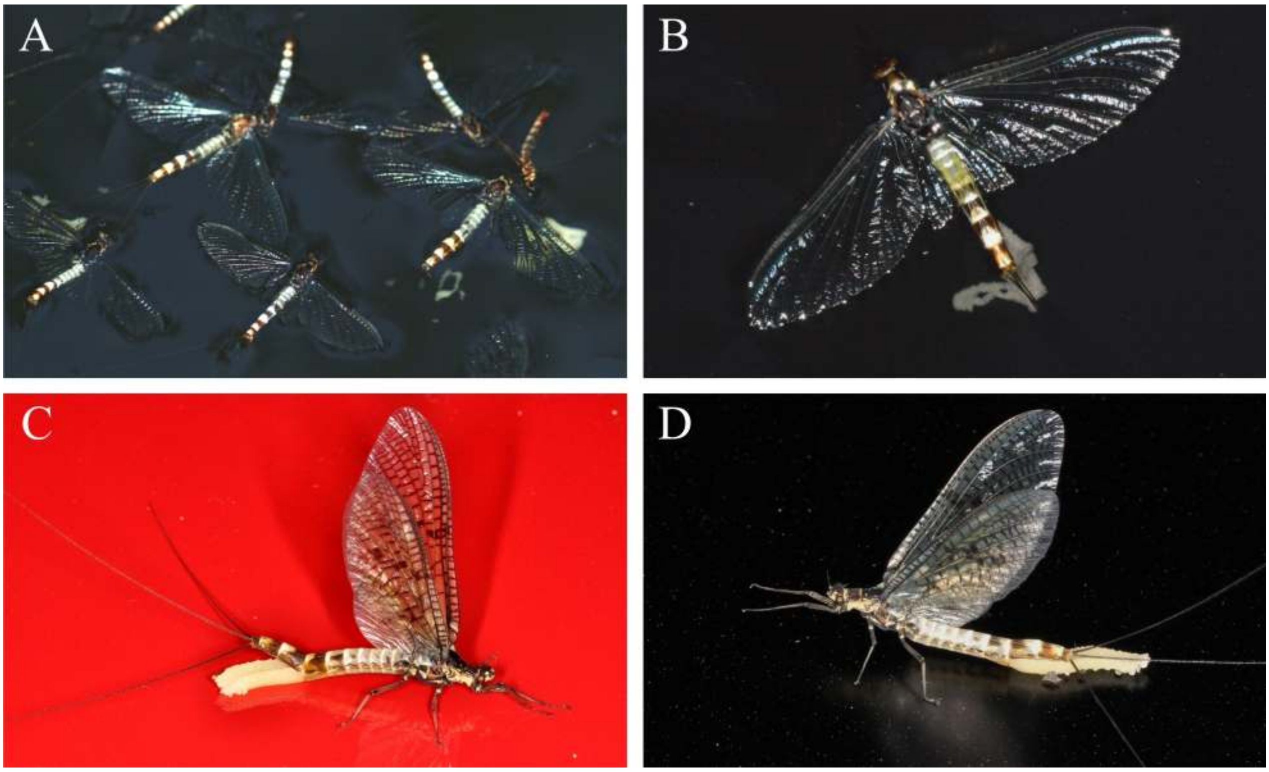

Fig. 3 Photographs of *E. danica* landed onto the traps and test surfaces. (A) Females trapped in the oil-filled traps. (B) *E. danica* female in an oil-filled trap with her egg-batch. (C-D) Egg-laying females on the horizontal, shiny red and black metal (car-body element) surfaces in the color experiment

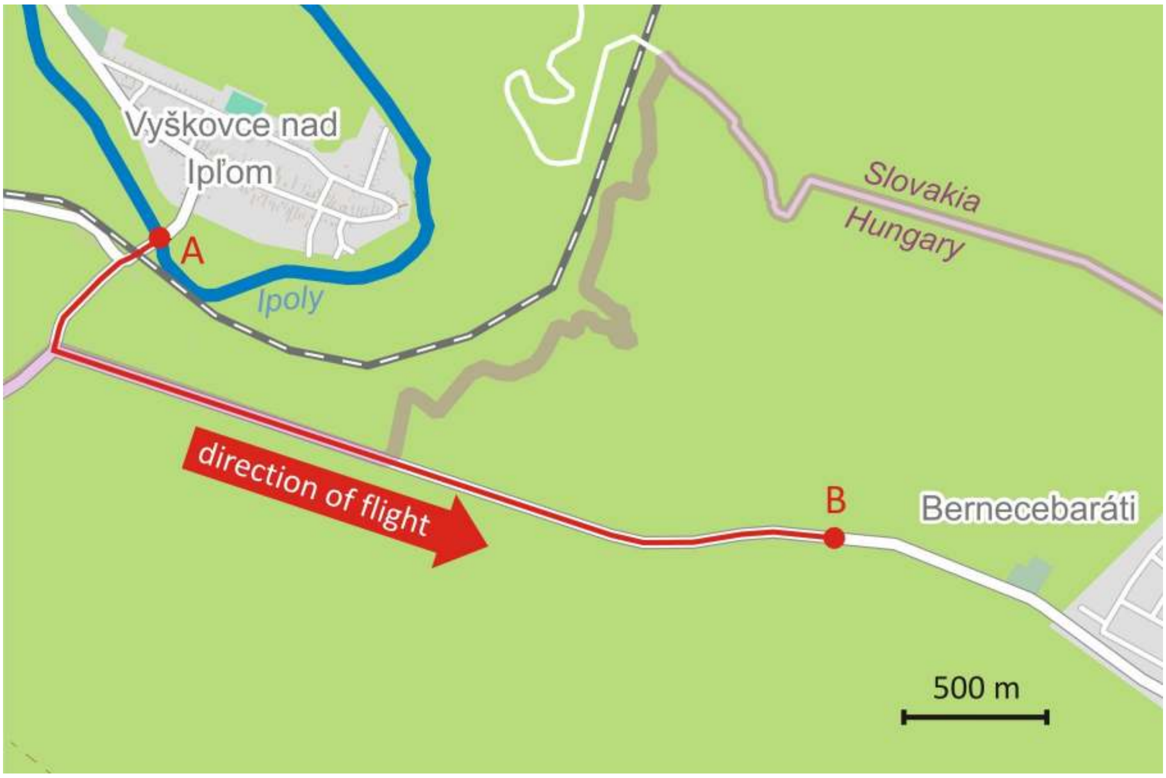

Fig. 4 Map of the area where we observed the deflection of *Ephemera vulgata* mayflies near the river Ipoly. Point A shows the bridge and B is the location where we observed flying specimens furthest from the bridge. The distance between locations A and B is 3200 m