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Bridges as optical barriers and population disruptors for the mayfly *Palingenia longicauda*: an overlooked threat to freshwater biodiversity?

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Abstract Freshwater biodiversity is declining faster than marine or terrestrial diversity, yet its drivers are much less known. Although dams were shown to negatively affect river habitats, fragmentation by bridges has received less attention and is not as well understood. We tested whether and how bridges present barriers to aquatic insects by studying mass swarmings of *Palingenia longicauda* mayflies on river Tisza (NE-Hungary). Behavioural observations showed that upon approaching the bridge, upstream-flying mayflies typically turned back and 86% of them never crossed the bridge. Lack of physical contact showed that the bridge was an optical, rather than a mechanical barrier for the polarotactic mayflies. Imaging polarimetry showed that the bridge disrupted the horizontally polarizing channel guiding the flight of mayflies above the river. Energy loss, demonstrated by calorimetry, and time constraints forced females to lay eggs only downstream from the bridge. Counts of larval skins shed by swarming individuals showed nearly 2 to 1 female per male downstream from the bridge, while sex ratio above the bridge was slightly male-biased. We suggest that the surplus of parthenogenetic females, that produce only female larvae, downstream from the bridge may have led to the observed sex-ratio bias since the construction of the bridge (1942). Our results demonstrate that bridges can be optical barriers for aquatic insects and can cause population-level impacts, such as biased sex ratios, in natural populations. Sex ratio biases due to bridges may decrease effective population size and genetic variability, which may have contributed to the recent extinction of this species from most of Europe.

Keywords Aquatic insects · Dispersal · Ephemeroptera: Palingeniidae · Polarization vision · Polarotaxis · Energy use in insect flight · Sex ratio

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Introduction

Freshwater biodiversity is declining at rates much faster than those of marine and terrestrial diversity (Dudgeon et al. 2006, World Wildlife Fund 2008). One of the main reasons for global biodiversity loss is the fragmentation of originally contiguous natural habitats. Although habitat fragmentation, a leading cause for global biodiversity loss, predominantly endangers terrestrial biotas, populations inhabiting flowing waters can also become fragmented due to dams and bridges (Petts 1984; Brittain and Saltveit 1989; Zwick 1992; Dynesius and Nilsson 1994; Lengyel 1998; Ligon et al. 1995). In contrast, fragmentation due to bridges has received little attention to date, possibly because bridges represent less substantial physical barriers than dams do.

However, bridges can also represent barriers, for example, by disrupting natural dispersal processes in aquatic insects. Many insects identify their habitats by means of the horizontal polarization of water-reflected light (Schwind 1991; Horváth and Varjú 2004; Kriska et al. 2007). A wide array of artificial objects, such as plastic surfaces, cars of dark and red colours, glass buildings, oil lakes and solar panels, may even act as polarized ecological traps for such insects (Horváth and Zeil 1996; Horváth et al. 1998, 2010; Kriska et al. 1998, 2006; Bernáth et al. 2008; Malik et al. 2008), a phenomenon termed recently as "polarized light pollution" (Horváth et al. 2009). In contrast, there is only sporadic information about whether and how bridges modify the natural optical environment of rivers, and how these changes may influence the river biota. Ladócsy (1930), for example, has reported that during a mass swarming after a rain many female long-tailed mayflies *P. longicauda* landed and oviposited on the wet asphalt road running on a bridge crossing river Tisza in Hungary, instead of laying their eggs into the river. By modifying the natural optical environment of rivers, bridges can disrupt polarized light patterns and may thus influence habitat selection and dispersal processes in aquatic insects. This hypothesis has received little attention thus far.

The long-tailed mayfly is the largest (up to 10 cm body length) and most spectacular of the 10 species of the family Palingeniidae in the Palearctic. It is also the oldest known mayfly species, first mentioned with certainty by Clutius (1634). The species was once widespread in meandering middle-sections of European rivers, but has disappeared from most rivers in the twentieth century. For example, the species disappeared from the Loire in 1922, from the Rhein in 1952 and from the Danube in 1974 (Andrikovics and Turcsányi 2001). *P. longicauda* is now confined to the catchments of rivers Tisza and Rába in Hungary and possibly the Dnepr in the Ukraine (Sartori et al. 1995;

Andrikovics and Turcsányi 2001; Lengyel et al. 2004). Due to its remarkably rapid extinction in most European countries and high vulnerability in its remaining range, the species has been added recently to the Convention on the Conservation of European Wildlife and Natural Habitats ("Bern Convention") (Sartori and Landolt 1998; Barber-James et al. 2008).

Here we provide evidence that bridges can represent optical barriers to *P. longicauda* mayflies, which can also have population-level consequences such as biased population sex ratios. Our results demonstrate how artificial structures such as bridges can hinder the dispersal of aquatic insects by changing individual behaviour and how these individual responses can scale up to have implications at the population level, which may directly influence population persistence and species conservation.

Materials and methods

Natural history of *P. longicauda* swarming

Larvae of *P. longicauda* live in U-shaped burrows in clay riverbanks for 3 years (Andrikovics and Turcsányi 2001; Lengyel et al. 2004; Málnás et al. 2005). The emergence and mating of adult imagos ('swarming') occurs in the evening hours over 3–5 days in June or July once every year. Male nymphs emerge first and moult into subimagos on the water surface, then they fly to the river bank, where they moult into imagos (Brodskiy 1973; Andrikovics and Turcsányi 2001). The male imagos then fly back above the river, where they fly horizontally at a height of 5–50 cm above the water surface along a zigzag trajectory in search of females. Female larvae emerge at this time, moult into subimagos and mate as subimagos with males on the water surface. After mating, masses of females fly up to 3–4 km upstream 5–15 m above the river midline. This "compensation flight" (Russev 1959) presumably serves to compensate for the river flow so that eggs reach the site where females emerged and for the larval drift that occurs during the aquatic life. At the end of the compensation flight, females lay their eggs into water. Some females (up to 50%, Andrikovics and Turcsányi 2001) do not copulate with males, and their eggs develop parthenogenetically. Because the male is the heterogametic sex in mayflies (Soldán and Putz 2000), unfertilized eggs develop into female larvae. Parthenogenesis may be particularly relevant for population persistence in small populations because synchrony in the emergence of the adults will be less important for reproduction (Gillies and Knowles 1990; Salas and Dudgeon 1999), although synchrony in parthenogenetic mayflies does occur (Sweeney and Vannote 1982). Neither males nor females feed after the emergence

from the water, therefore their energy content and body size at hatching are important in their flight capabilities.

Field experiments by Kriska et al. (2007) suggest that *P. longicauda* has water-searching and water-following flights. In the former, mayflies fly up to heights of 15–30 m in search of horizontally polarized light signals. This flight can be observed only if the mayflies are captured and released on the river bank. *P. longicauda* shows positive polarotaxis (Kriska et al. 2007), similarly to other mayfly species (Schwind 1995; Kriska et al. 1998; Turcsányi et al. 2009) and aquatic insects in general (Schwind 1991; Wildermuth 1998, 2007; Bernáth et al. 2002; Horváth and Varjú 2004; Csabai et al. 2006; Wehner and Labhart 2006; Horváth et al. 2008; Lerner et al. 2008; Kriska et al. 2009). Mayflies receive the polarized light signals reflected from the water surface by their ventral polarization-sensitive eye region (Kriska et al. 2007) and identify water by exactly or nearly horizontally polarized light (with angles of polarization $80° < \alpha < 100°$ clockwise from the vertical) with medium degrees of polarization ($15° < d < 60°$) (Kriska et al. 2009). When mayflies approach surfaces reflecting weakly polarized or vertically polarized light, such as water surfaces shaded by the riverbank vegetation, they suddenly turn back towards the river midline (Kriska et al. 2007).

Documentation of mayfly swarming

We documented mayfly swarming on river Tisza at the bridge between Kisar and Tivadar villages (48°03′N, 22°31′E) on 25 June 2009 (Fig. 1A, B). We recorded the flight of mass-swarming *P. longicauda* on video sequences on both sides of the bridge (Online Resource, videos 1–6). We estimated the number of individuals by counting mayflies on digital photographs (resolution 10 MP) taken once every minute between 18:45 and 19:34 h simultaneously on both sides of the bridge ($n = 100$ photographs, Fig. 1C, E). During the observed swarming, the weather was warm and calm, but after 19:45 a thunderstorm came through and wetted the road surface.

Imaging polarimetry

We measured the reflection-polarization characteristics of river Tisza and the bridge by imaging polarimetry in the red (650 ± 40 nm = wavelength of maximal sensitivity $\pm$ half
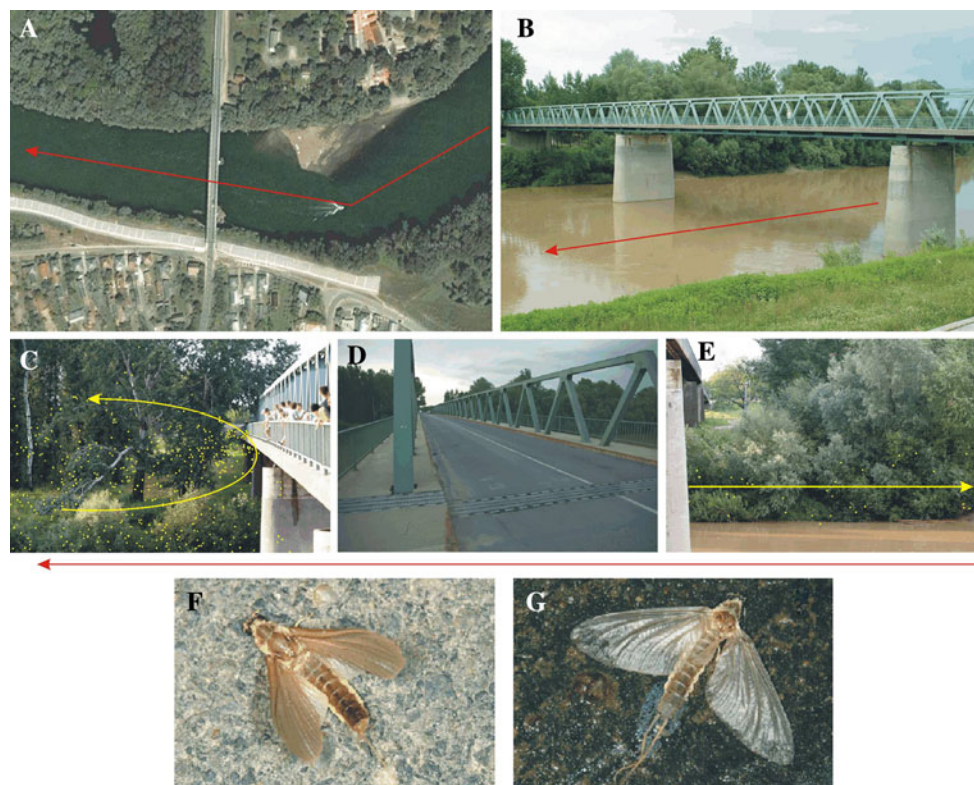

Fig. 1 A Aerial photograph of the bridge over river Tisza at the village Tivadar (http://maps.google.com/maps). **B** The bridge photographed from the left bank of Tisza. *Red arrows* show flow direction. **C** Mayflies (marked by *yellow dots* for visibility) at the downstream side of the bridge. The *yellow arrow* shows the typical turning-back flight. **D** The asphalt road on the bridge. **E** Mayflies (*yellow dots*) at the upstream side of the bridge. The *straight arrow* shows the typical horizontal flight. **F**, **G** Egg-laying *P. longicauda* on the dry **F** and wet **G** asphalt road of the bridge. (Color figure online)
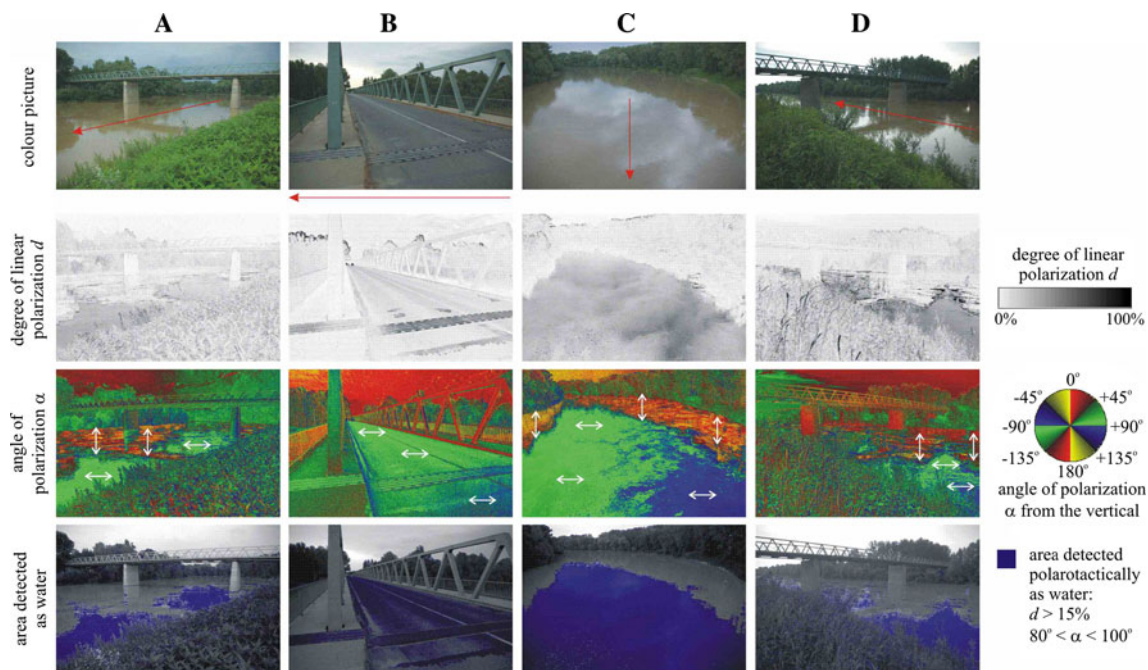
Fig. 2 Colour pictures (row 1), patterns of the degree d (row 2) and angle α (clockwise from the *vertical*) of linear polarization (row 3), and areas detected polarotactically as water ($d > 15\%$ and $80° < \alpha < 100°$, row 4) of the bridge environment measured by imaging polarimetry in the *blue* (450 ± 40 nm) part of the spectrum. Patterns were similar in the *red* and *green* spectral ranges. The angle

of elevation of the optical axis of the polarimeter was $-15°$ from the horizontal. *Red arrows* (row 1) show flow direction and *white arrows* (row 3) show the local directions of polarization of light reflected by water or asphalt. **A** Downstream side of the bridge. **B** Asphalt road on the bridge. **C** River Tisza upstream from the bridge. **D** Upstream side of the bridge. (Color figure online)

bandwidth of the CCD detectors of the polarimeter), green (550 ± 40 nm) and blue (450 ± 40 nm) parts of the spectrum (Figs. 2, 3) on 25 June 2009. Methods for imaging polarimetry have been described in detail elsewhere (Horváth and Varjú 1997, 2004). Water is sensed as such by polarotactic mayfies if (1) the degree of linear polarization d of water-reflected light is higher than a threshold d^*, and (2) the deviation $\Delta\alpha = |90° - \alpha|$ of the angle of polarization α from the horizontal is smaller than a threshold $\Delta\alpha^*$ (Horváth and Varjú 2004). Both thresholds d^* and $\Delta\alpha^*$ may depend on species. As examples, in the fourth row of Figs. 2 and 3 we used $d^* = 15\%$ and $\Delta\alpha^* = 10°$. The threshold d^* is unknown for *P. longicauda*, but for other mayfly species it is close to 15% (Kriska et al. 2009). The use of different values did not influence our conclusions qualitatively.

Calorimetric measurements

We used calorimetry to determine the energy content of swarming mayfly females to evaluate whether the repeated turning back from the bridge causes increased energy loss in females. Females were collected by hand-held nets in two different phases of swarming at three locations. We collected females in early swarming at the bridge ($n = 5$ specimens) and 1 km downstream from the bridge ($n = 13$), and females in late swarming 1 km downstream

from the bridge ($n = 17$). All females captured had emerged more than 1 km downstream from the bridge as they were already in compensation flight when captured. The specimens were stored at $-25°C$ until analysis. In the laboratory, specimens were measured under a microscope (pronotum length, femur length, distance between the eye and the middle suture of the head) and their egg content was classified as either full, medium or empty. Specimens were then dried at 75°C for 24 h until mass constancy, and their dry mass was measured (accuracy 0.1 mg). We used an oxygen-bomb calorimeter (Parr 1341, Moline, IL, USA) to determine the energy content of specimens (Prill et al. 2008). Because the minimum sample to be used with the calorimeter was 0.15 g, we grouped specimens so that their total dry mass was above this level and obtained one calorie value for groups of 2–6 specimens. Specimens with similar dry body mass were grouped together to maximise the number of groups for measurement. In order to estimate the energy content of individual specimens, we divided the total calorie value for a group proportionally to the dry mass of each specimen.

Collection of mayfly exuviae

We collected exuviae (shed nymphal skins) floating on the water surface on 9 days of swarming in 2006, 2007 and

Fig. 3 Reflection-polarization characteristics of river Tisza upstream from the bridge, measured in the *red* (650 ± 40 nm), *green* (550 ± 40 nm) and *blue* (450 ± 40 nm) parts of the spectrum. (Color figure online)
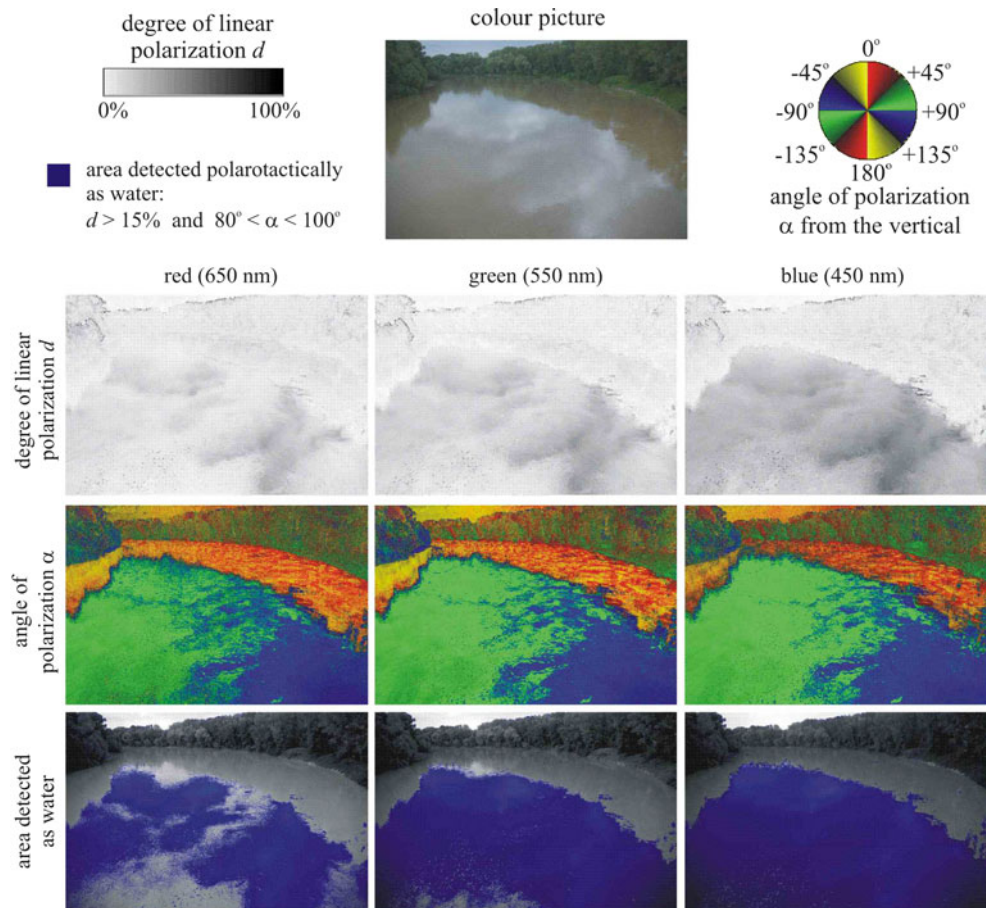
2009 using a sampler consisting of a plastic frame (30 × 30 cm) attached between two parallel wooden boards so that the bottom half of the frame was in water (Fig. S1). A net (mesh size 0.5 mm) was attached to the plastic frame to collect exuviae. We placed and kept the sampler in the driftline from the riverbank using wooden poles and ropes. Almost all exuviae arriving downstream from upper sections floated in the driftline, therefore, our chances to correctly estimate the number of mayflies that hatched upriver were high. We installed one surface sampler just above the bridge to sample the upstream section and another identical sampler 3,800 m downstream from the bridge to sample the downstream section. We determined these locations to ensure that the samplers collected only from the river sections intended to be studied. First, we calculated the river length from where samplers collected exuviae based on river flow velocity measurements. For the upstream sampler, the length of the river from where samplers collected exuviae ranged between 1980 to 2310 m, and for the down-stream sampler, it ranged between 1836 to 2142 m in the 3 years. For the location of the downstream sampler, it was also important to consider that eggs laid at the bridge reach the bottom of the river some distance downstream from the bridge due to drift by

the water flow. Landolt et al.'s (1997) calculations of the drift distance of eggs laid in the river at the Nagyar site (4 km upstream from the studied bridge) showed that the eggs reach the riverbed at a distance of about 1,350 m from the egg-laying location. Since the flow velocity of the river does not differ substantially between the Nagyar site (1.2 m/s) and our study site (1.1 m/s), the eggs laid by females just downstream from the bridge were likely to reach the bottom in the section sampled by the downstream sampler, which was located 3,800 m downstream from the bridge.

The samplers collected exuviae during the entire swarming for 3 days in 2006. On all other days, the sampler was kept in the water only for half of the duration of swarming (in four 10-min periods followed by 10-min non-collecting periods) to measure the temporal dynamics of swarming for other studies. We did not account for the non-collecting periods to avoid biases in estimating the number of swarming individuals, therefore, the numbers presented are minimum estimates. We determined the time periods of collecting exuviae by considering flow velocity measurements to make sure that the samplers collected only from the river sections intended to be studied. The collected samples were sorted in the laboratory and individual

exuviae were sexed based on criteria in Andrikovics and Turcsányi (2001).

Statistical analyses

We used χ^2-tests with Yates' correction for 2×2 contingency tables to analyze sex ratios and one-way ANOVA and General Linear Mixed-Effects (GLME) models in statistical analyses to test differences in energy content among females. The random factor in GLME analyses was the group in which a specimen was measured. Post-hoc testing was based on contrasts. All tests were computed using SPSS 17.0 for Windows.

Results

Mayfly behaviour at the bridge

On 25 June 2009, the swarming of *P. longicauda* mayflies began at 17:30 h, when male subimagos emerged from water. The first female subimagos appeared above the river Tisza at 18:00 h, after which the number of male and female mayflies continuously increased, resulting in a spectacular mass swarming. At 18:30 the females began their upstream compensation flight at a height of 5–15 m above the river midline. Swarming finished suddenly at 19:45 at an approaching thunderstorm, and there were no mayflies flying during or after the storm. Females approaching the bridge displayed four behaviour types (Fig. 1A, B):

(1) Most females approaching the bridge body or its vertical pillars to within 0.5–2 m turned back. After flying downstream about 50–100 m, they turned back again and joined the mayflies flying upstream towards the bridge (Fig. 1C, video 1–3 in Online Resource). Mayflies neither touched, nor landed on the bridge or its pillars, suggesting that the bridge was an optical, rather than a mechanical, barrier.

(2) Some females, especially those flying lower than 8 m, continued to fly upstream below the bridge between the two pillars (Fig. 1E, video 4).

(3) Some females showed upwards water searching flight upon reaching the bridge. When they flew high enough (c. 20 m), they flew over the bridge and continued their compensation flight (video 5).

(4) Some females landed and laid eggs on the dry asphalt road surface of the bridge (Fig. 1F). Females landed and oviposited on both dry and wet asphalt (video 6; the road became wet at the end of swarming when it began raining).
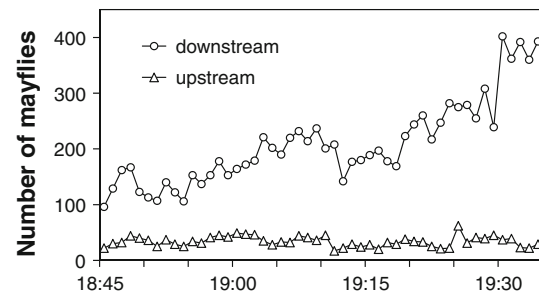

Fig. 4 Number of flying mayflies (*Palingenia longicauda*) counted on photographs taken at the downstream and upstream side of the bridge above the river Tisza on 25 June 2009 once every minute between 18:45 and 19:34 ($n = 50$ photos on each side, t test for unequal variances, $t_{48} = 16.035$, $p < 0.0001$). Mayfly flight ended abruptly at 19:45 at a thunderstorm

During the entire swarming, there were significantly more mayflies on the downstream side (Fig. 1C) than on the upstream side (Fig. 1E) of the bridge (Fig. 4). The majority (86%, total $n = 10\ 449$) of mayflies accumulated at the downstream side of the bridge, and only a minority (14%) continued their upstream flight (Fig. 4). The number of mayflies increased on the downstream side continuously, and particularly so after 19:20, whereas numbers on the upstream side remained relatively constant throughout the entire period (Fig. 4).

Imaging polarimetry of the bridge and the river

Water surfaces reflecting skylight or sunlight were moderately polarized ($30\% < d < 50\%$, row 2 in Figs. 2, 3). Such surfaces always reflect horizontally polarized light (row 3 in Figs. 2, 3) and are sensed by mayflies as water (row 4 in Figs. 2, 3). These two effects create a "polarization channel" (Fig. 3), which is narrower than the width of the river and elicits the water-following flight of males and the compensation flight of females (Kriska et al. 2007). In contrast, the water surface in the shadow of riparian vegetation reflects vertically polarized light (row 3 in Figs. 2, 3) with low degrees of polarization ($0\% \leq d < 10\%$, row 2 in Figs. 2, 3).

The grey concrete horizontal body, vertical pillars and green metal bars of the bridge reflected practically unpolarized light ($d \approx 0\%$, row 2 in Figs. 2, 3). The water surface in the shadow of the bridge also emitted unpolarized light (row 2 in Fig. 2A). The surfaces emitting unpolarized light interrupted the polarization channel guiding the mayflies, probably confusing their light perception and disrupting their compensation flight. Because mayflies turn back if their ventral polarization-sensitive eye region perceives weakly and non-horizontally polarized light (e.g. when they approach the river bank), the depolarizing or non-horizontally polarizing surfaces on and

under the bridge probably elicited a similar reaction of turning back (Fig. 1C).

Mayflies could have easily avoided the fence and metal grid of the bridge. Some females that did so laid eggs on the asphalt road surface on the bridge, which reflected horizontally polarized light with moderate degrees of polarization ($30\% < d < 50\%$, Fig. 2B). A few mayflies flying near the water surface could fly further upstream below the bridge (Fig. 1E). However, most females flew much higher (5–15 m) above the river during compensation flight.

Energy content of females at and below the bridge

The energy content of females varied significantly by the time and location they were collected (Fig. 5A; $F_{2,32} = 8.565$, $p = 0.001$). The energy content of females collected at the bridge was only 68% of that of females collected at the same time 1 km downstream from the bridge ($t_{32} = 2.177$, $p = 0.037$), although there was no difference in dry body mass between the groups (Fig. 5B; $F_{2,32} = 1.368$, $p = 0.269$). There were no differences among the three groups or between the two early-swarming groups in either

Fig. 5 Mean (SD) energy content **A** and dry body mass **B** of *Palingenia longicauda* females collected early during swarming downstream from the bridge, early during swarming at the bridge, and late during swarming downstream from the bridge, respectively. Sample sizes (number of specimens) from left to right: $n = 13, 5, 17$. *Different letters* indicate statistically significant differences among groups based on contrasts ($p < 0.05$)

body size variable (pronotum length: $F_{2,32} = 0.214$, $p = 0.808$; femur length: $F_{2,32} = 2.308$, $p = 0.116$; eye-suture distance: $F_{2,32} = 0.529$, $p = 0.594$), or egg content (Online Resource). When these data were analysed by GLME models, the effect of the random factor (grouping for measurements) was significant for both energy content ($t_{30} = 2.83$, $p = 0.009$) and dry body mass ($t_{31} = 3.94$, $p = 0.0004$), which can be explained by our allocation of specimens with similar dry body mass into groups. In both cases, however, the effect of time/location of collection remained significant (energy content: $F_{2,30} = 11.047$, $p = 0.0003$; dry body mass: $F_{2,31} = 8.014$, $p = 0.0015$), and egg content did not influence these results (Online Resource). These results suggested that females at the bridge were energetically exhausted compared to females on their upstream way to the bridge.

Sex ratio upstream and downstream from bridge

We collected exuviae from 17 637 individuals (Table 1). For all days combined ($n = 9$), the proportion of females was significantly higher than that expected based on unity (1:1) in the downstream section (Table 1, Yates $\chi^2 = 679.157$, $p < 0.0001$), while sex ratio did not differ from unity for the upstream section from the bridge (Yates $\chi^2 = 0.609$, $p = 0.435$). The proportion of females ranged from 56 to 85% in the downstream section (mean 64%, SD 8.4%), suggesting that sex ratio bias approached 2:1 females to males downstream from the bridge. In the upstream section, more males swarmed on all but 1 day (12 July 2006, Table 1) and the proportion of males ranged between 21 and 72% (mean 53%, SD 18.6%; without 12 July 2006: mean 57%, SD 14.4%).
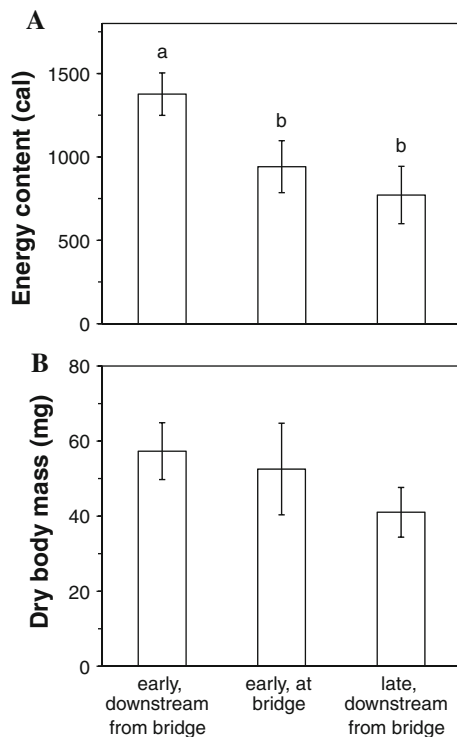
Table 1 Number of male and female *Palingenia longicauda* exuviae (shed nymphal skins) collected from river sections upstream and downstream from the studied bridge

Year	Date	Upstream		Downstream	
		Males	Females	Males	Females
2006	10 July[a]	312	180	362	616
	11 July[a]	1086	770	1413	1986
	12 July[a]	412	1596	535	2920
	13 July	375	143	359	516
2007	19 June	6	16	25	40
	20 June	477	250	479	621
	21 June	18	15	4	7
2009	27 June	592	400	375	604
	28 June	0	0	39	88
	Total	3278	3370	3591	7398

[a] Sampling was conducted during the entire swarming period. On all other days, sampling was conducted during half of the swarming period

Discussion

This study provides four key results. First, our observations on mayfly behaviour demonstrated that the bridge functions as an optical barrier for female mayflies during their upstream compensation flight. Second, polarimetric measurements suggested that the bridge environment presented an unpolarized barrier to most females as if the bridge had been an impenetrable physical barrier. Our data show that 86% of the females had their polarotactic perception confused by the bridge and did not cross the bridge. Third, our measurement of the energy content of females suggested that these females may be energetically exhausted relative to females that have not yet reached the bridge. The exhaustion of females at the bridge probably causes them to lay their eggs in the river section just downstream from the bridge. Finally, the accumulation of females, especially of those reproducing parthenogenetically, may be related to female-biased sex ratios downstream from the bridge and male-biased sex ratios upstream from the bridge.

Our results demonstrated the existence of a narrow polarization channel with horizontally polarized light with a moderate degree of polarization (the reasons for this optical phenomenon are discussed in Online Resource), which helps female mayflies to stay above the river during compensation flight. Our observations show that the bridge and its optical environment disrupts this channel and confuses mayflies, which turn back and fly downstream or try again and again at the bridge. Similar effects were observed in previous field experiments, in which both female and male *P. longicauda* flying above horizontal shiny plastic sheets turned back at the edge of horizontally polarizing plastic surfaces (Kriska et al. 2007). Although our photo and video documentation was conducted on 1 day only, the compensation flight and the turning back of female mayflies occurred the same way each day we witnessed it ($n = 21$ days since 2002; K.M. and S.L., unpublished data), and the polarimetric properties of the bridge and its surroundings were unlikely to change among days or years. Therefore, we believe that our results and conclusions are robust to the actual time of the recordings. Although some mayflies may be attracted to (wet or dry) pavement under streetlights during nighttime, our previous observations also suggested that *P. longicauda* are not active at night and that their swarming ends at sunset. Thus, the role of artificial lights in influencing flight behaviour was not studied here.

The repeated turning back of most females at the bridge resulted in that the majority of females remained at the downstream side of the bridge. The number of mayflies increased continuously at the downstream but not the upstream side of the bridge, resulting in an accumulation of females downstream. Our data showed that these

females may also be energetically exhausted relative to the females still in compensation flight downstream from the bridge. Although the approaching females still had to spend energy to fly to the bridge, the difference in energy content between the two groups was too large (32%) and unlikely to be explained by flight costs alone. A previous study of mayflies near the village of Nagyar (ca. 4 km upstream from the bridge) showed that the majority of the females flew 4 km in compensation flight before laying eggs (Landolt et al. 1997). If females lose 32% of their energy content by flying 1 km, they would not be able to fly more than 3 km. Therefore, the estimated 32% decrease in energy content probably indicates increased energy loss at the bridge. Although much of the energy content of female mayflies stored in the abdomen arise from eggs and thus, measurements using only the head and the thorax may have provided more accurate estimates of energy content, differences in egg content were not likely to explain the differences observed (Online Resource). Similarly, although a measurement of different body components, particularly of proteins and lipids, may have provided more precise estimates, previous evaluations suggested that the protein content is less important than lipid content in evaluating energy reserves available for flight in mayflies (Sartori et al. 1992). Moreover, it appears unlikely that protein content varied among females at certain stages of their flight, therefore, our measurements may reflect real differences in energy content (mostly based on lipids minus the energy stored in eggs, see above) among females.

The inability to cross the bridge, decreased energy levels and time constraints possibly forced females to lay eggs just downstream from the bridge. Before the bridge between Kisar and Tivadar was built (1942), females were presumably evenly distributed along the studied two sections. Our observations show that the bridge may have disrupted this even distribution by presenting a barrier for most females. More importantly, the bridge may be a selective barrier for parthenogenetic and sexually reproducing females. Parthenogenetic females do not participate in mating, which is energy-consuming for females as it involves much chasing by males, likely have higher energy reserves before compensation flight, and probably can fly longer distances than females participating in mating. The disruptive effect was thus likely to be larger on parthenogenetic females, which could have dispersed farther upstream in the absence of the bridge, than on sexually reproducing females.

The accumulation of egg-laying females may be related to the surplus of female exuviae at swarming downstream from the bridge. Estimates suggest that up to 50% of the females do not copulate and lay unfertilized eggs (Andrikovics and Turcsányi 2001), from which only females develop (Soldán and Putz 2000). Although only 52% of the

unfertilized eggs start parthenogenetic development (Landolt et al. 1997), any surplus female offspring from parthenogenetic eggs may initiate a sex ratio bias. Even though the surplus of parthenogenetic females may have been slight initially, the small differences may have accumulated in the downstream section since 1942. Furthermore, successively fewer females could copulate with males in subsequent generations, increasing the frequency of parthenogenetic females even more. Our observations and data support the hypothesis that these two effects may have led to the large sex-ratio differences found downstream from the bridge.

Another expected consequence of the construction of the bridge is that there will be a shortage of females upstream from it. Our data on the sex distribution of exuviae collected upstream from the bridge correspond to this hypothesis as there were more males swarming in this section on 8 of the 9 days studied. Even on the day when more females swarmed than males (12 July 2006), the proportion of males was still higher in the upstream (20%) than in the downstream section (15%) (Table 1).

Whether the observed patterns occur at other bridges is yet to be known. Preliminary observations by us and others, and literature data, however, suggest that the behavioural responses of mayflies to bridges is quite similar along river Tisza (personal observations by MK, LP, SL at bridges between Cigánd and Dombrád, between Tokaj and Rakamaz; personal communication from J. Hamar for a bridge near Szolnok; Ladócsy 1930 for a bridge near Szeged). Therefore, it appears likely that the patterns found here exist at other bridges as well.

In conclusion, our study demonstrates that bridges can represent optical barriers to *P. longicauda* mayflies during their compensation flight. The accumulation of females due to the bridge may result in a disruption of natural dispersal processes along the river length, and may lead to deviations in population sex ratios. Our study, therefore, provides a rare example when individual behavioral responses to man-made structures can be linked to population-level consequences. A conservation implication of these results is that biased sex ratios can decrease effective population size by limiting the number of either sex participating in reproduction. This effect may be particularly important in species that depend on the synchronous presence of large numbers of individuals for successful reproduction, such as *P. longicauda*. Furthermore, the increasing frequency of parthenogenetic females may lead to a decrease in genetic variability in the population. Our study, therefore, raises the possibility that not only chemical pollution, but also polarized light pollution (Horváth et al. 2009, 2010) due to bridges as well as possibly other artificial structures may have contributed to the rapid collapse of *P. longicauda* populations in the former European range of this species.

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