

Testing of UV fluorescence marking with daily recaptures of a low-mobile bush-cricket species

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Academic editor: Laurel B. Symes | Received 1 February 2024 | Accepted 29 April 2024 | Published 28 October 2024

<https://zoobank.org/7F2293AC-33C2-4FCF-8864-488ACE6925FA>

Citation: Varga S, Kenyeres Z (2024) Testing of UV fluorescence marking with daily recaptures of a low-mobile bush-cricket species. Journal of Orthoptera Research 33(2): 281–288. <https://doi.org/10.3897/jor.33.120070>

Abstract

The primary goal of developing new mark–release–recapture (MRR) methods is to make MRR more cost-effective and able to provide more information. To test the effectiveness of UV fluorescence marking, a case study was carried out on an *Isophya costata* population occurring in a mown grassland with a heterogeneous vegetation pattern. The marked individuals were examined daily, from the emergence of imago until the mowing of the habitat.

The 40 marked insects (20 males and 20 females) were recaptured in 206 cases over 16 days (32.2% of the potential maximal recaptures). The finding of males was 26.2% successful, and the finding of females was 38.1% successful. Females released in shorter and sparse vegetation were recaptured more than those released in dense vegetation or than males. Recaptures showed a significant decrease during the study period in almost all groups based on Mann–Kendall trend tests. Detections were made on the upper third of the vertical structure of the vegetation in 88% of cases. In most of the detections (males: 70%, females: 62%), the axis of the insect's body was more likely to be located vertically. The results showed that the chance of detection is significantly reduced when the position of the insect's body is facing the axis of UV illumination. Thus, the visibility of individuals can be greatly increased by marking all sides of the body.

Keywords

Central Europe, Hungary, *Isophya costata*, mown grassland, MRR, vegetation

Introduction

Mark–release–recapture (MRR) is considered the gold standard for estimating population sizes, changes, and lifetime (Southwood 1966, Henderson and Southwood 2016, Schtickzelle et al. 2003). Other common aims of studies using MRR are the determination of movement patterns and dispersal ability (Baguette et al. 1998, Casula and Nichols 2003, Zimmermann et al. 2005, Watts et al. 2017, Heller 2020, Nuhlířková et al. 2023). Both of these goals support population and conservation biology (Bonte et al. 2012, Habel et al. 2018, Kral-O'Brien and Harmon 2021). MRR is

widely used in the study of various invertebrate taxa (Hagler and Jackson 2001), but the focal taxon of MRR studies appears to be butterflies (Zimmermann et al. 2011, Habel et al. 2018, Vlařánek et al. 2018, Henry et al. 2022, Hinneberg et al. 2022). However, many studies have involved dragonflies (Minot et al. 2020, Khelifa et al. 2021) and beetles (Cerrato and Meregalli 2020, Quinby et al. 2021, Bérceš et al. 2022, Kelly et al. 2023). MRR methods with low intensity are also used on other taxa, such as mosquitoes (Epopa et al. 2017, Vavassori et al. 2019), wild bees (Briggs et al. 2022), and ants (Chen and Robinson 2013).

MRR is also used on Orthoptera species. The focal orthopterans are usually large-bodied, slow-moving, flightless species such as wetas (Jamieson et al. 2000, Leisnham et al. 2003, Joyce et al. 2004, McCartney et al. 2006, Watts et al. 2011, 2017) or bush-crickets (Kindvall 1999, Diekotter et al. 2007, Holuřa et al. 2013, Heller 2020, Anselmo 2022, Nuhlířková et al. 2023). Examination of more mobile Orthoptera species is rare (Hein et al. 2003). Locusts are less frequently investigated using MRR, and studies of locusts are usually carried out on flightless species (Matenaar et al. 2014, Bröder et al. 2020).

In the latest developments of MRR methods, the main objective has been to ensure that the technique is non-invasive and cost-effective (Turlure et al. 2017). Non-invasive procedures, such as detection with high-speed video (Khelifa et al. 2021) or photo-matching algorithms (Matthé et al. 2017), are primarily relevant to studies on vertebrate species. The pursuit of more cost-effective methods has put the focus on faster recognition. Traditionally, orthopterans are marked with points on the pronotum or small individually numbered labels on the femur (Gangwere et al. 1964, Buchweitz and Walter 1992, Watts et al. 2017, Anselmo 2022). Use of permanent non-toxic paint markers (e.g., the Edding 780 paint marker; see Matenaar et al. 2014) is sufficient for examining species in open habitats with sparse vegetation, but these markings are unsuitable for effective survey of species occurring in dense, vertically structured vegetation. In such situations, the use of reflective tags significantly increases the efficiency of finding indi-

viduals using a lamp at night (Heller and Helversen 1990, Narisu et al. 1999) but is hindered by dewdrops on leaves reflecting the markings, creating pseudo signatures (Szövényi 1999). Researchers can solve this problem by marking with ink that is visible only under UV lighting (Anselmo 2022). Furthermore, the use of flags or noticeable marks can alter the behavior of the insects in daylight and increase predation rate, suggesting that the best method, in almost every aspect, is the use of fluorescent dye (Faiman et al. 2021) applied by paint marker (Nuhličková et al. 2023).

Despite this knowledge, no intense (e.g., daily) study has been done on the effectiveness of tracking Orthoptera individuals tagged by UV fluorescence markers and the influences of the composition of the insect's habitat (e.g., short, sparse vegetation vs. high, closed grassland). To test the effectiveness of UV fluorescence marking, we carried out a study on an *Isophya costata* Brunner von Wattenwyl, 1878 (Cigliano et al. 2024) population occurring on a mown grassland with heterogeneous vegetation patterns by attempting to locate marked individuals daily from the emergence of imago until mowing (i.e., disappearance of the population). The research questions were as follows: (1) Does search efficiency decrease over time? (2) When the same level of effort is exerted, is there a difference in search efficiency in short and high grassland? (3) How does the behavior of the individual insect affect the efficiency of the MRR method?

Materials and methods

Study area.—The study area (~2 ha) covered by mown grassland belongs to the microregion of Hungary referred to as the Balaton Uplands (Dövényi 2010). The area is dominated by *Arrhenatherum elatius* (L.) P. Beauv. ex J. Presl et C. Presl plant species. Other plant species in the area are characterized by high cover values and include mesophilic dicots such as *Podospermum canum* (C.A. Mey.) Griseb., *Salvia nemorosa* L., *Sanguisorba minor* Scop., *Teucrium chamaedrys* L., *Vicia cracca* L., and *Medicago rigidula* (L.) All. While the plant composition is similar, the structure of the vegetation differs within the study area, with the northern third characterized by shorter (20–30 cm tall on average) and more sparse (overall vegetation cover 80–90%) grassland than the southern two-thirds (height: 50–60 cm, vegetation cover: 100%).

Marking procedure.—From 20–25 May 2023, we noticed an increase in the number of adult *Isophya costata* specimen in the study area. On 27 May 2023, we collected 20 male and 20 female insects for marking. We established two release points within the study area: one in the short, sparse vegetation, which we labeled site N (47.00685°N, 17.95231°E) and another one in the tall, structured grassland, which we labeled site S (47.00579°N, 17.95348°E). To collect as much comparable data as possible, we released groups of insects at these two points. If we released the insects in several different locations (i.e., where we had caught them), the habitat conditions (e.g., vegetation structure, barriers) would have been entirely different, which would not have allowed the evaluation of replicate recapture data.

For fluorescence marking of adult *Isophya costata*, non-toxic, quick-drying, water-resistant Sharpie permanent markers were used: a green marker for insects released in site N and a yellow marker for insects released in site S. The insects were carefully numbered from 0 to 9 on their pronotum (this was done because of the limited surface area; the individuals were renumbered from 1 to 10 for data analysis) and were marked by a line on the back of their abdomen (Fig. 1). On 27 May 2023, 10 marked males and

10 marked females were released at each of the two points. The potential success of detection by UV flashlight was tested immediately after release.

Data collection.—The authors carried out recaptures every day from 28 May 2023 to the day of mowing, 12 June 2023. The marked individuals were searched for with uvBeast V1 (385–395 nm) and uvBeast V3 (385–395 nm) flashlights after dark between 21:30 and 1:00.

Fieldwork involved walking systematically along a transect covering the study area. Turns were made at the edge of the site creating a new line 4 m away from the previous line (Fig. 2); this distance between lines meant the vegetation 1–2 m to the right and left of the searcher was highly visible, ensuring complete survey coverage. Coordinates, marking number (0–9), and sex (M/F) of each recaptured insect were recorded using Trimble Juno 3 GPS-PDA and Garmin GPSMAP 62s devices. All recaptured insects were photographed. During the search, we took great care to ensure that our actions caused minimal disturbance to the insects and little to no trampling damage to the vegetation.

Collection was mostly (11 times/16) carried out under the same weather conditions (calm, dry weather, 15–16 degrees), with conditions differing only on the following days: 5 June: drizzle during the recapture; 6, 7, and 9 June: rain during the day (10 mm, 5 mm, <1 mm, respectively) making the grass moderately wet during the fieldwork; 10 June: some (1–5 mm) rain during the day making the grass extremely wet during fieldwork.

Recapture data were summarized in groups based on sex and location (NM/NF/SM/SF), location (S/N), and total. Data were plotted as box plots, and relationships were examined using t-tests. We calculated the total number of recaptures at the individual level and the total number per day for each group. Changes in temporal data were tested using the Mann–Kendall trend test.

We calculated the recapture success rate based on recaptures divided by the number of potential recaptures (e.g., number of potential recaptures of 10 specimens during 16 days is 160).

To gather impressions of the spatial patterns of the insects' movements, we calculated the distances between the points of detection using geoinformatics. For the calculation of daily movement, we used distances calculated based on the spatial location of each individual detected on a given day and the day before. Distances between the location of detection and the insect's release point were also determined. We calculated the mean±SE of daily movements and the distance of individuals from their release point at detection for all insects, for males and females, and for each release site (N and S). The relationships among the different groups (males, females, and males/females released at N and at S) were examined using t-tests.

We determined potential daily mortality using an indirect method: individuals detected on a given day were considered alive on all previous days. Based on the photographic records, we determined the location (lower, middle, or top third of vegetation) and position (body axis in vertical plane or horizontal plane) of the detected insects (males and females separately). Quantum GIS 3.16.1 (QGIS Development Team 2016) was used for geoinformatics investigations, and Past 3.14 software package (Hammer et al. 2001) was used for statistical analyzes.

Results

Marked individuals were recaptured 206 times over 16 days, representing 32.2% of the potential maximal (pot. max.) recaptures (40×16). Males were successfully recaptured 84 times (26.2% of

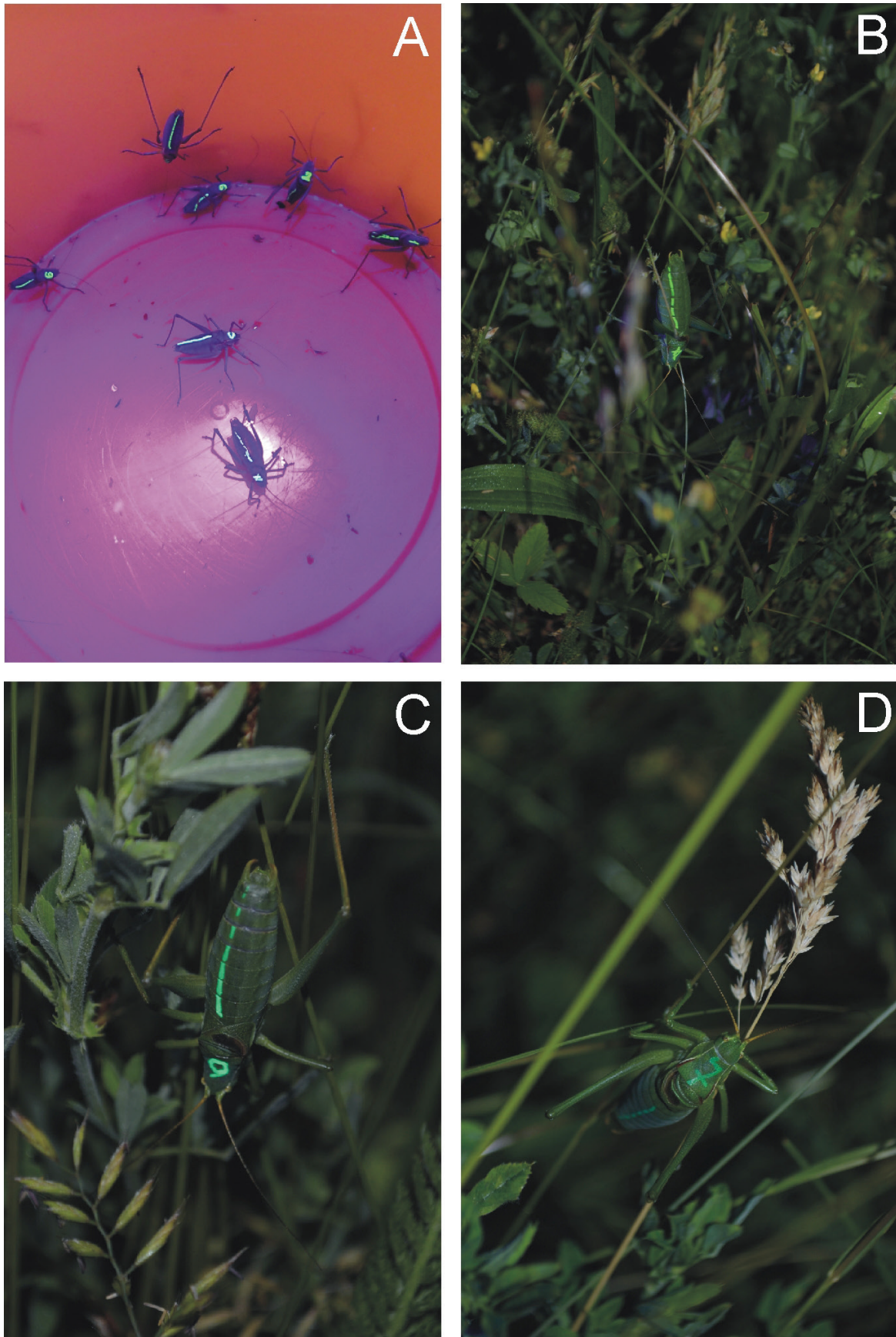


Fig. 1. A. Marked *Isophya costata* in a bucket before release; B–D. Recaptured marked males in different positions in the grassland. Climbing to the upper regions of the vegetation during sunset and night is a typical behavior of the focal species.



Fig. 2. Release points (red dots), transect of the daily examinations (white line), and recaptured males (blue dots) and females (black dots) in the study area.

the pot. max.), and females were successfully recaptured 122 times (38.1% of the pot. max.). Individual *Isophya costata* were recaptured 120 times on site N [males (NM): 46, females (NF): 74] and 86 times on site S [males (SM): 38, females (SF): 48]. The number of recaptures varied widely among individuals. One male released on site N was captured only two times, but some males were recaptured 11 times there. One female released on site N was never recaptured, but another female from the same group was recaptured 13 times. Of those released on site S, the minimum and maximum recaptures were 2 and 8 for females and 1 and 11 for males, respectively.

When the recapture results among sexes and locations were compared, we found that females released on site N were recaptured significantly more than males and females released from S and males released from N (Fig. 3). On site N, males were recaptured at an average of 4.6 times, while females were recaptured at an average of 7.4 times. On site S, the above values were 3.9 for males and 4.8 for females.

In the NF group, six individuals were recaptured at a success rate above 50%; only three individuals were recaptured at a success rate below 25%. In the SF group, seven individuals were recaptured at a success rate between 25% and 50%, with only three individuals recaptured at a success rate below 25%. In the NM group, recapture success was between 25% and 75% and below 25% for five individuals. In the SM group, recapture success was between 25% and 75% for four individuals and below 25% for six individuals (Fig. 4). The percentage distribution of daily recaptures on site N ranged from 0% to 60% for males, with an average of 28.75%, and from 10% to 60% for females, with an average of

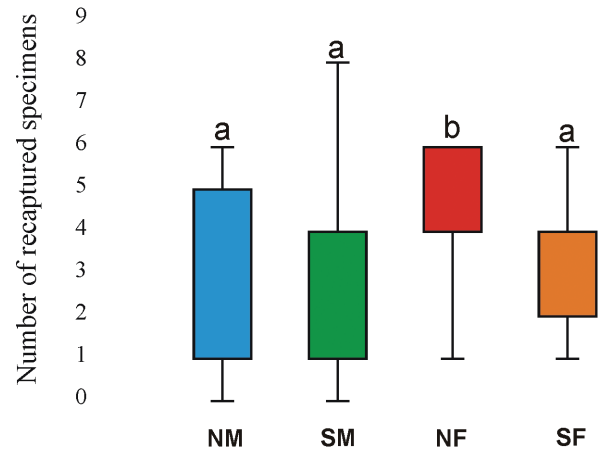


Fig. 3. Females released on site N (NF), characterized by shorter and sparse vegetation, were recaptured significantly more than males released on site N (NM) or males (SM) and females (SF) released on site S.

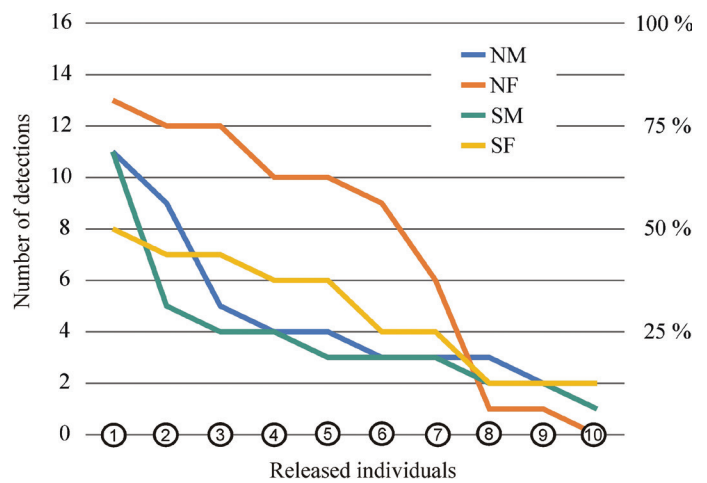


Fig. 4. The individual recapture success rates were highest for females released on site N (NF). In the other groups, recapture success was typically between 25% and 50%.

46.25%. The percentage distribution of daily recaptures on site S ranged from 0% to 80% for males, with an average of 24.38%, and from 10% to 60% for females, with an average of 30%.

Based on Mann-Kendall trend tests, recaptures showed a significant decrease during the study period in all groups (NM: $S = -67, Z = 3.022, p = 0.003$; SM: $S = -76, Z = 3.578, p = 0.001$; SF: $S = -46, Z = 2.091, p = 0.037$), except for females occurring on site N (Fig. 5A), where no statistically significant trend was detected. Analysis of the data determined that the differing weather conditions (i.e., drizzle during recapture or rain during the day) did not affect the success of recaptures.

Based on indirect counting, the number of males alive in site N did not change during the first six days, while 80% of the females marked were alive for at least eight days. At site S, the number of males alive decreased steadily. In contrast, the number of females alive decreased slowly (Fig. 5B). The daily mortality rate overall was ~2% (mean±SE of the potentially dead insects: NM = 0.375 ± 0.154 ; NF = 0.562 ± 0.181 ; SM and SF = 0.5 ± 0.182).

The mean±SE of daily movement and distances from the release points of the individuals were 7.70 ± 0.60 m and

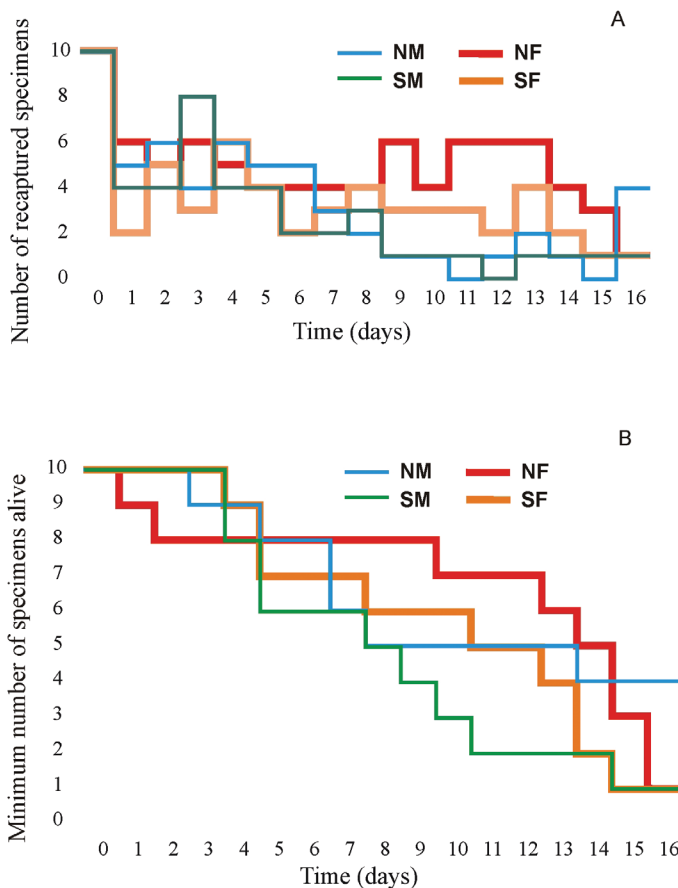


Fig. 5. A. Mann-Kendall trend tests showed a significant decrease in recaptures during the study period in all groups except females released on site N (NF). B. Indirectly counted mortality (an individual recaptured on a given day can be considered as alive on all previous days) was low throughout the study, especially for females, whose detection rate only decreased in the last days of the study, presumably because they went low in the vegetation to lay their eggs and because of the mowing.

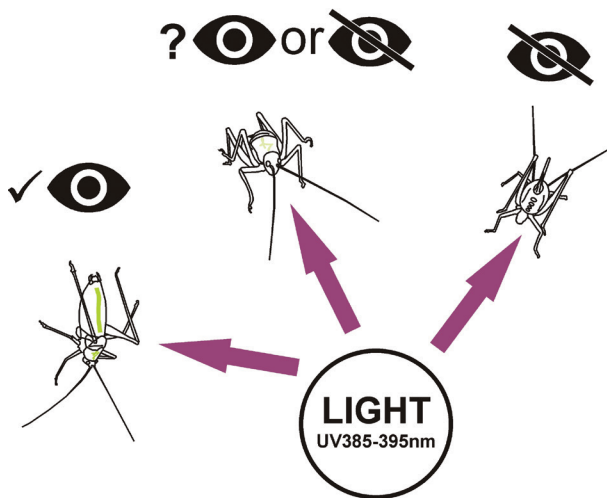


Fig. 6. The likelihood that a marked individual would be detected was 50% if their body was parallel to the axis of UV light illumination and 100% if it was perpendicular to the light's axis. Insects without a mark on the ventral side of the abdomen were invisible to the UV light from the ventral view.

Table 1. Mean±SE of the daily movement intensity and distance of individual insects. Statistically significant differences were detected using t-tests. Significances are indicated as follows: n.s. (not significant) $p \geq 0.05$, * $p < 0.05$, ** $P < 0.001$.

Sex	Daily movement (meters)			Distance from the release points (meters)		
	♂♂	♀♀	<i>p</i>	♂♂	♀♀	<i>p</i>
♂♂ + ♀♀	7.70±0.60			12.06±0.60		
♂♂ + ♀♀	9.80±1.23	6.42±0.58	*	12.50±0.99	11.77±0.74	n.s.
	northern area	southern area	<i>p</i>	northern area	southern area	<i>p</i>
♂♂	11.65±1.77	6.34±0.66	*	15.98±1.48	8.54±0.93	**
♀♀	6.54±0.64	6.16±1.24	n.s.	11.91±1.08	11.54±0.94	n.s.

12.06±0.60 m, respectively. In daily movement, we detected significant differences between males and females and between males released at site N and site S. The distance of males from their release points (site N and site S) was also found to be significant (Table 1).

A small percentage (12%) of the detected individuals were found in the lower parts of the grassland vegetation, but 88% were detected on the upper third of the vertical structure of the vegetation. In most of the detections of males (70%), the axis of the insect's body was more likely to be vertical, and the position of the insect was more likely to be close to horizontal in only 30%; this rate for females was 62% and 38%, respectively. The likelihood of detection was 50% when the insect's body was parallel to the axis of UV light illumination but up to 100% when the insect's body was perpendicular to the light (Fig. 6).

Discussion

Our recapture success rate (32.2% overall, 38.1% for females, and 26.6% for males) was considerably greater than that of other similar investigations. For example, Anselmo et al. (2022) achieved a potential recapture rate of 77% of the large-bodied *Saga pedo* in a small study area of 0.3 ha using 0.8 × 0.8 cm tags attached to the right hind femurs of insects (N=5) and searching over eight study days. In a more similar MRR study of *Isophya beybienkoi*, Nuhlíčková et al. (2023) achieved a 13–16% recapture/potential recapture rate. The lower success rate of that study compared to the present study may have been because the parallel sections of the line transect used in it were 25 m apart, compared to 4 m in the present study. Our results confirm those of Nuhlíčková et al. (2023) in that the overall proportion of male recaptures was lower than that of females.

In the present study, we observed a drastic decrease (~40%) in female detections in the last 2–3 days, especially after mowing. The most significant difference was at site N, where 4 males (40% of the marked individuals) and only one female were recaptured after mowing. The drastic reduction in female recaptures and survival is probably related to oviposition behavior. Based on an analysis of photographs, the first spermatophore appeared on females in site N on 28 May and in site S on 29 May. During the survey, 60% of the females detected in site N and 50% in site S were carrying spermatophores. The drastic decrease in the number of females detected in the last few days suggests that the females had moved under the leaves and began to lay their eggs in the soil, causing higher mortality among females than males, who stayed in the safer upper level of the vegetation during mowing. Therefore, after the mowing, females were likely under the cut plants

while males were on top of the windrows, where they were still observable by UV light even if injured.

We had similar results to Anselmo et al. (2022), but we found UV fluorescence marking to make insects visible 8 m away. Our results agreed with the latter study, but we found that an insect's detection was significantly reduced if their body was parallel to the axis of UV light illumination (Fig. 6).

The results of this study make it clear that the effect of vegetation structure on detection success should not be overlooked. We found that the probability of detection is 40% higher in short (20–30 cm), sparse vegetation than in tall (60–70 cm), dense vegetation. This made observing females and males at site S more challenging.

In many prior MRR studies, detection success rate was seen to decrease drastically over time (Narisu et al. 1999, Hein et al. 2003, Diekotter et al. 2007, Nuhlíčková et al. 2023). We also detected this phenomenon in our study but not uniformly: the trend did not hold for females in site N. This suggests that the increase failure to detect over time was due to predation or insects being hidden in the vegetation rather than because they left the study area or that their markings disappeared (Faiman et al. 2021, Nuhlíčková et al. 2023). This was further confirmed by the spatial data of recaptures, which shows that the marked individuals were no more than 16 m from the release points, with males in the shorter grass of site N being furthest away, while females and males in site S showed much less movement activity.

The potential mortality from trampling appears to be negligible. Based on the previously recorded local density of the species (Kenyeres and Varga 2023) and the footprint size of the two fieldworkers, we calculated that the mortality of individuals of the species from sampling in the first days was at most 5%. Later, this value gradually decreased to less than 1% due to the dispersal of individuals. The results of our prior study (Kenyeres and Varga 2023) revealed regular mowing to be the leading cause of decline in the local *Isophya costata* population. During the current study, 50% of the detected males had been injured by the mowing, which also dramatically reduced the number of detected females.

In conclusion, the answers to our research questions are as follows: (1) the detectability of marked individuals decreased over time, and this should not be considered a methodological error; (2) the probability of detection was 40% higher in short, sparse vegetation than in tall, dense vegetation given the same effort; and (3) the likelihood of detecting a marked individual was 50% if the individual's body was parallel to the axis of UV light illumination but up to 100% if it was in a perpendicular position. Moreover, males were difficult to find because they are smaller and preferred to be in a vertical position rather than horizontal, while finding females was made more difficult when they moved low in the vegetation to lay eggs in the soil.

In future MRR studies of orthopteran species in dense vegetation, marking with UV-ink and searching for marked individuals at night with UV light is recommended. The chance of recapture is increased by marking all sides of the insect, which raises the probability of detecting both vertical and horizontal insects to almost 100% if the insect is in the upper third of the vegetation. Based on the results of our study, the method used seems to be suitable for both the estimation of population sizes and for monitoring the behavior (e.g., mobility, feeding, reproduction, egg laying) of grasshoppers occurring in dense, tall grasslands.

Acknowledgements

The authors would like to express their gratitude to Gábor Takács for his help with geoinformatics issues and to the reviewers for their remarks. We are also grateful to Tony Robillard, Editor-in-Chief of JOR; Laurel B. Symes, Subject Editor of JOR; and Nancy Morris, Editorial Assistant of JOR, for their work on our manuscript.

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