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ORIGINAL ARTICLE

Population structure and habitat connectivity of *Phengaris teleius* (Lepidoptera: Lycaenidae) in southern Slovenia: Implications for conservation and landscape management

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Abstract. We studied the vulnerable Scarce Large Blue butterfly (*Phengaris teleius*) population in loosely connected wet grasslands in southern Slovenia. Our goal was to understand the distribution and demographic traits of this population within the Seasonal Lakes of Pivka Nature Park (KP PPJ). From 2021 to 2022, we mapped suitable habitat patches in KP PPJ with the larval host plant *Sanguisorba officinalis* and host ant *Myrmica* spp. Out of eight identified potential patches, five were inhabited for at least one year. Our dispersal model, using an inverse-power function, indicated that all but one suitable patch could be connected by migrations of at least 1% of either sex, suggesting a metapopulation structure. The clustered habitat distribution (nearest neighbour index = 0.14) highlights the role of each patch as a "stepping stone" within the system. We also assessed demographic parameters in a subset of three patches: Klenik, Kalec, and Zagorje. In 2021, mark-release-recapture estimated the populations in two inhabited patches (Klenik, Kalec) at 197 males (95% CI: 159–265) and 315 females (95% CI: 205–525), showing a female-biased sex ratio (males : females = 1 : 1.6). We recorded a relatively long average life span (4.4 and 8.4 days for males and females, respectively) and a weak protandry, however about three-quarters of the population was present simultaneously, mainly in the largest patch (Klenik: > 85%). Recorded demographic traits (female-biased sex ratio, long lifespan, simultaneous peaks of both sexes, and high dispersal) support the persistence of this metapopulation. However, conservation efforts and a denser network of suitable patches are crucial to maintain its viability at this range's southern edge.

INTRODUCTION

Recent habitat loss and fragmentation in increasingly divided landscapes have disrupted gene flow among local populations for many species (Hanski & Gaggiotti, 2004; Crawford & Keygobadi, 2018; Nowicki et al., 2019; Kajzer-Bonk et al., 2024; Sánchez-Sastre et al., 2025). This disruption, along with reduced population sizes and increased genetic drift within isolated populations, negatively impacts their long-term survival (Thomas, 1995; Hanski, 1997), heightening the risk of species extinction (Frankham, 2003).

Species with large populations and high dispersal ability are better adapted to withstand primarily human-induced changes in increasingly fragmented landscapes, as they can maintain connections between local populations (Louy et al., 2007). Estimated population size (including effective population size: Ridley, 2009) alongside dispersal ability and habitat patch suitability, is a crucial indicator for predicting a species' long-term population survival (Hare et al., 2011). Effective population size estimates are essential for understanding a population's viability and adaptive potential, particularly in small populations (Hare et al., 2011). The "50/500" rule suggests a minimum population of 50 individuals is needed to prevent extinction from inbreeding depression in the short term, while 500 individuals are needed for long-term survival (Perez Pereira et al., 2022). However, this rule is generalised and not applicable to all species (Traill et al., 2010; Brook et al., 2011; Flather et al., 2011a, b). Nevertheless, effective population size remains highly relevant in conservation practice, serving as one of the IUCN's criteria for threatened categories (IUCN, 2012). Habitat specialists with specific requirements face a higher risk of (local) extinction (e.g. Labandeira et al., 2002), as they can utilise only a limited portion of available habitats within a given area (*Phengaris teleius*: Nowicki et al., 2005a).

Butterflies (Lepidoptera), including the umbrella species *Phengaris teleius* (Bergsträsser, 1779), are excellent indicators of environmental change. Their well-studied ecology, strong species-specific relationships with larval host

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plants, and essential roles in ecosystems as pollinators, parasite hosts, and prey make them valuable for monitoring (Čelik, 2007; Thomas, 2005). As an umbrella species, the conservation of *Phengaris teleius* can help protect other species that share its habitat (Batary et al., 2007, Skórka et al., 2007). The separation of developmental stages in holometabolous insects like butterflies – where larvae and adults occupy distinct ecological niches – has significant evolutionary and ecological advantages. This life cycle structure allows species to exploit a broader range of habitats, reduce intraspecific competition, and escape natural enemies and unfavourable seasonal conditions (Thomas, 2002). For example, larvae may be restricted to specific host plants, while adults can use more varied nectar sources and dispersal habitats. This stage-specific habitat use increases overall ecological flexibility and contributes to diversification. From a conservation perspective, it highlights the need to protect not only the adult foraging and dispersal habitat, but also the more restricted and specialised habitats required by the larval stages (Hayes, 2015). Highly specialised species like *Phengaris teleius* (Lycaenidae), which depend on both a specific larval host plant (*Sanguisorba officinalis*) and a specific host ant genus (*Myrmica* spp.), are especially vulnerable, as their life cycle can only be completed where both host organisms coexist (Thomas et al., 1989; Elmes et al., 1998; Witek et al., 2010). The species is listed under the EU Habitats Directive 92/43/EEC (Annex II and IV) and classified as Near Threatened on the IUCN Red List. It is legally protected in Slovenia (Uradni list RS 2004) and is included on the Slovenian Red List of butterflies, as it is in many European countries (Uradni list RS 2002).

In *Phengaris teleius*, females lay eggs on the host plant, where larvae feed until the fourth instar, then drop to the ground to be collected by host ants (Thomas et al., 1989; Wynhoff, 2001; Thomas & Settele, 2004). How females detect host ants (*Myrmica* spp.), which forage within 2 m of their nests, has been debated (Wynhoff, 2001). Recent studies revealed that myrmecophilous butterflies in some cases rely on plant volatile compounds emitted by host plants when in proximity to *Myrmica* ant nests. These plant volatiles serve as indirect indicators of ant presence, guiding the butterflies to suitable oviposition sites (Casacci et al., 2019). However, the larvae camouflage themselves chemically to be accepted by the ants, who carry them to their nest (Wynhoff, 2001). There, the larvae feed on ant larvae until winter, when they hibernate, resuming predation in spring (Thomas et al., 1989). Upon emerging the following summer, the adults must quickly leave the nest, as they lose their chemical protection and are seen as intruders (Wynhoff, 2001). In stable and suitable habitats, *P. teleius* can reach high population densities (Wynhoff, 1999; Nowicki et al., 2005a, c), yet disturbances can cause rapid local extinctions (Gao et al., 2016). Major threats include habitat degradation from agricultural intensification (drainage, fertilisation, ploughing, multiple mowing), infrastructure development, abandonment of traditional land use, and overgrowth (Rebeušek, 2006). In Europe, *P. teleius* is locally distributed from France and northern Italy through central Europe, reaching as far north as Latvia, with a broader range across Asia to northern China and Japan (Newland et al., 2020). In Slovenia, it is more common in the east (Verovnik et al., 2012). Over the last 25 years, the range in Slovenia has declined by at least 15%, with increasing isolation between local populations (Verovnik et al., 2012; Zakšek et al., 2018). Southern Slovenia represents the species' southern range edge, where populations may live at the limits of their ecological tolerance and adapt to local conditions (Moritz, 1994, 2002, Crandall et al., 2000, Martin et al., 2021; Sunde et al., 2023). These marginal populations are often at high risk of local extinction due to the increasing temperatures at the southern range limits, which reduce habitat suitability, making them less likely to expand but rather to retreat in the face of changing climate conditions (cf. Popović et al., 2017).

As part of a two-year study (2021–2022) on the distribution and population demography of *P. teleius* in a loosely connected system of potential habitat patches in southwestern Slovenia, we aimed to determine whether the system supports a metapopulation structure for this species, meaning that it is essential that some individuals can migrate between habitat patches (Burgmann & Fox, 2003; Popović et al., 2014). The study's objectives are: (1) to assess the connectivity of nearby habitat patches through estimates of *P. teleius* dispersal ability, (2) to confirm the presence of host ants (*Myrmica* spp.) and the host plant (*S. officinalis*) at each patch inhabited by *P. teleius*, (3) to investigate variations in host plant density across habitat patches, (4) to evaluate demographic parameters (flight period duration, local population size/density, and sex ratio), and (5) to analyse the behaviour of *P. teleius*. These data will inform assessments of population resilience and the long-term survival prospects of this species in the studied area.

MATERIAL AND METHODS

Study sites

The selected study system (Fig. 1) is located within The Seasonal Lakes of Pivka Nature Park (KP PPJ: Mulec et al., 2005) in the Pivka basin of southwestern Slovenia. This park encompasses a system of 17 intermittent lakes (Jugovic & Jeklar, 2021), where wet meadows develop during the dry late spring to summer months (Glasnović & Surina, 2021). These areas support nectaring and host plants (*Sanguisorba officinalis*) as well as the larval host ants (*Myrmica* spp.) crucial for *P. teleius* (Thomas, 1984; Sielezniew & Stankiewicz, 2013). The presence of both species in close proximity (ant nests within 2 m of host plants) is essential for *P. teleius* to complete its life cycle (Wynhoff, 2001). Although mowing in these meadows is often low-intensity and dependent on flood conditions, it may still adversely affect *P. teleius* populations, particularly when *S. officinalis* is cut along with surrounding vegetation during the larval epigean stage, before the larvae have entered nearby ant nests (e.g. Zakšek et al., 2022).

To identify suitable study sites for the mark–release–recapture (MRR) study, we initially considered four habitat patches within KP PPJ. These were suggested by the park authorities based on recent records of *P. teleius*. Of these, three were selected as MRR study sites. The fourth site was excluded, as it had been freshly mown at the beginning of the survey. In parallel with the MRR study, we conducted broader field surveys in 2021 and 2022 to identify additional potential localities for *P. teleius*. These included additional three sites selected based on informal observations and personal communications with local entomologists, as well as one locality identified semi-incidentally during wider butterfly surveys in the area (resulting in eight surveyed patches in total). None of these four additional patches were considered for the MRR study, but they were included in later assessments of habitat quality and host plant availability.

Field work

The three selected habitat patches, located near the settlements of Klenik, Kalec, and Zagorje, were surveyed to estimate demographic parameters of *P. teleius* using a MRR approach. The study was carried out over 13 sampling occasions between 9 July to 15 August, mostly at two- to three-day intervals (exceptionally up to five days), always in clear weather and ambient temperatures above 20°C. Each field day lasted from 9 a.m. to 5 p.m. Field effort was scaled to patch size: Klenik (5.49 ha), approx. 4–4.5 h, Zagorje (1.76 ha) approx. 1.5 h; Kalec (2.83 ha), approx. 2–2.5 h. Each site was surveyed by the same two researchers using a zig-zag walking pattern with 5-m spacing between adjacent transects, ensuring full coverage by both observers at every sampling habitat patch. Captured butterflies were marked individually with a permanent marker on the ventral side of the left hindwing and released unharmed at the capture site. Before capture, individual behaviour (e.g. resting, flying, feeding, mating, oviposition) was noted. For each butterfly, sex, exact location (Garmin Oregon 200, ± 5 m accuracy), and time of capture (date and hour) were recorded. Notably, although all three patches were surveyed throughout the flight period, one of them (Zagorje) remained unoccupied by *P. teleius* in 2021.

At each of the three MRR patches, transect counts of *S. officinalis* were conducted along seven transect lines, each 1 m wide: Klenik (T1 – 535 m, T2 – 200 m, T3 – 200 m), Kalec (T4 – 225 m), and Zagorje (T5 – 120 m, T6 – 120 m, T7 – 80 m). The number and length of transects were determined by the size and shape of each patch (Fig. 2). Counts at T1–T6 were conducted on nine occasions between 12 July and 3 August, while T7 was surveyed three times between 29 July and 3 August. On the same dates, a total count of host plants was carried out across each MRR patch. To facilitate counting in the two largest patches (Klenik and Kalec), each was divided into three subplots. However, at three occasions in Klenik (July 29 and 31, and August 3), a completely accurate count was not possible, resulting in underestimates of plant numbers for those dates.

During the 2021 and 2022 flying seasons, additional fieldwork was conducted to identify further potentially occupied patches across KP PPJ and to confirm the presence of *P. teleius* on all of the localities provided by the park authorities. These efforts relied on (1) the known presence of *S. officinalis*; and (2) active field searches. For each potential habitat patch, the maximum number of flowering host plants was assessed and categorised as follows: 1–10 plants, 11–50 plants, 51–100 plants, 101–500 plants, 501–1000 plants, and ≥ 1001 plants. For categories under 1000 plants, direct counts were performed. To confirm the presence of the larval host ant (*Myrmica* spp.), five pitfall traps were set overnight (31 August – 1 September 2021) at each investigated locality, ideally within 2 m of *S. officinalis* plants. An exception was one site where pitfall trapping was not conducted, as the presence of *Myrmica* ants had already been confirmed through a previous study documenting the occurrence of another myrmecophilous lycaenid, *Phengaris alcon* (Jugovic et al., 2023).

During each visit the extent of mowed area for each of these sites was recorded, and additionally on two visits after the flying season, on 31st August and 1st September. We observed whether mowing occurred shortly before the visit (i.e. after the preceding visit), or the area was mown before that and flowering *S. officinalis* was already present (i.e. recovered area, see Popović et al., 2017).

Data analysis

Host plant densities were calculated per 100 m length for each of the seven transects (based on transect counts) and per ha for each MRR patch (based on total host plant counts) during most visits to the MRR habitat patches. The density was compared among the three MRR patches (Klenik, Kalec, Zagorje) using a non-parametric Kruskall-Wallis test, followed by Dunn's test for pairwise comparisons between each patch in SPSS 20.0. Statistical significance was set at $p < 0.05$.

The minimum convex polygon (Burgmann & Fox, 2003) encompassing all potential habitat patches, with all interior angles less than 180 degrees, was drawn using ESRI ArcGIS version 10.7. Its size was measured as an estimator of home range; however, for species with metapopulation structure, this may overestimate the actual usable area since it includes a large portion of inhospitable matrix (Nowicki et al., 2014).

Distances between pairs of all investigated potential habitat patches – measured as the smallest possible Euclidean (edge-to-edge) distances between the nearest points of each patch – were calculated using ESRI ArcGIS version 10.7. To assess the spatial distribution of the patches, the nearest neighbour index (R_n) was calculated using the formula:

$$R_n = \frac{D_{(obs)}}{0.5 * \sqrt{(a/n)}}$$

where "$D_{(obs)}$" represents the mean distance to the nearest neighbour, "a" is the area of the patches, and "n" is the number of patches. An index value of 1 indicates a random distribution, while values below and above 1 indicate clustered and dispersed distributions, respectively (Clark & Evans, 1954).

To assess whether local populations occupying individual habitat patches are interconnected, we calculated the cumulative proportions of marked individuals for each sex, based on their exact capture locations, moving across multiple 30-m distance classes. These data were fitted to an inverse-power function (IPF), where probability (P) of moving a certain distance (D) is given by $P = CD^{-n}$, with C and n representing two scaling constants (Hill et al., 1996; Jugovic et al., 2017, 2018). This method was chosen because it effectively predicts rare long-distance movements (Baguette et al., 2000; Baguette, 2003; Weyer & Schmitt, 2013; Pennekamp et al., 2014) and is independent of marking effort (Fric & Konvicka, 2007; Pennekamp et al., 2014). Connectivity thresholds of 3% or at least 1% of the population were selected, as these levels have been reported to sufficiently support a metapopulation structure (Zimmermann et al., 2011). Nevertheless, movement rates were also estimated using a negative exponential function (NEF) for comparison.

Using MARK 10.1 software (White & Burnham, 1999), we modelled and estimated demographic parameters (survival rates, capture probabilities, rates of population entry, and overall population size) based on capture histories for each capture occasion. Data were available for two inhabited patches in 2021 (Klenik and Kalec), which were merged prior to analysis for a comprehensive assessment of the entire population system. A two-step procedure (Čelik, 2012) in MARK was employed to select the best models fitting the data. In the first step, we examined changes in survival (φ) and capture probability (p) using the Cormack-Jolly-Seber (CJS) module, exploring models with various parameter configurations. In total, eight variations were considered: a constant parameter equal for both sexes (.), a constant but different for each sex (g), time-variable parameters [undefined variability in time: (t); linear variability in time: (tlin)], and combinations in additive [(g+t), (g+tlin)] or interactive [(g*t), (g*tlin)] forms. This approach yielded 64 different models using various parameter combinations. We identified the most suitable models based on the lowest Akaike Information Criterion (AIC) score corrected for small sample sizes (AICc), considering models with a ΔAICc < 3 as the most favourable (Burnham & Anderson, 2002).

In the second step, we constructed similarly parametrised models within the POPAN module (Schwarz & Arnason, 1996). In addition to survival and capture probabilities, the POPAN module included an additional parameter for probability of entry into the population (b), enabling the modelling of butterfly hatching rates and subsequent population growth throughout the flight season, as well as the total population size (N) over the season. Additionally, the module provided estimates of derived parameters, including daily population sizes (Ni). To enhance our analysis, we incorporated three additional combinations for the entrance parameter in the POPAN module: ($tlin+tlin^2$), ($g+tlin+tlin^2$), and ($g*[tlin+tlin^2]$).

Within the CJS module, we also employed the median c-hat approach to estimate data overdispersion (c-hat parameter). Since the c-hat value was 1, no further correction for overdispersion was necessary.

Effective population size (N_e) was estimated based on the population size estimates for males and females using the following formula (Harmon & Braude, 2010), which accounts for deviations from an equal operational sex ratio (1 : 1):

$$N_e \approx \frac{4*N_m*N_f}{N_m+N_f}$$

where N_m and N_f represent the estimates (with 95% confidence intervals) of male and female population sizes, respectively.

We employed the χ^2-test of homogeneity to determine whether the ratios of captures and recaptures differed between males and females across successive marking occasions for the entire system, accepting a significance level of $p < 0.05$. The sex ratio was calculated from population size estimates derived from the best-fitting POPAN model for males and females at two levels: (i) for the entire system and (ii) for the two individual habitat patches (Klenik and Kalec). These population size estimates were also used to calculate population densities, defined as the maximum daily number of individuals per ha (N_i max / area) on days when females and males were most abundant, as well as total population densities (N / area) for both sexes.

We estimated average life expectancy using the method proposed by Nowicki et al. (2005c), calculated as $e_{avg} = (1 - \varphi)^{-1} - 0.5$, where φ represents the daily survival probability as defined above. This approach is particularly suitable for species such as butterflies, which typically emerge in cohorts early in the morning (Nowicki et al., 2005c). For this calculation, we used the estimate of daily survival probability derived from the first model with a constant survival, differing between males and females [φ(g) p(g) b(t) N(g)].

We employed a χ^2-test of association to investigate potential differences in behaviour between males and females (Jugovic et al., 2018). In cases where expected frequencies were less than 1, we used likelihood ratio (LR) statistics instead. We compared the frequency distributions of various behaviours (excluding oviposition, which occurs only in females) between the sexes, accepting a difference at $p < 0.05$.

RESULTS

Presence of the larval host ant (*Myrmica* sp.) and the presence and abundance of the host plant (*Sanguisorba officinalis*)

Overall, eight habitat patches scattered throughout the Park were identified as at least potentially suitable for *P. teleius* (Table 1, Fig. 1). The larval host plant *Sanguisorba officinalis* was recorded in all patches, while the host ant *Myrmica* sp. was found in all but one (Petelinjsko jezero). Additionally, occupancy by *P. teleius* was confirmed in five of the patches during at least one of the two years of investigation. The minimum convex polygon encompassing these potentially suitable habitat patches covers an area of 2130 ha, with a nearest neighbour index of 0.14, indicating a clustered distribution (Fig. 1). The area occupied by *P. teleius* (i.e. patches occupied in 2021 and/or 2022) spans 64.8 ha, representing nearly half of the potentially suitable habitats (Table 1) but only a small fraction (3%) of the minimum convex polygon area.

Only two (Klenik and Kalec) out of the three habitat patches identified for the MRR study were occupied in 2021, while *P. teleius* was confirmed in the third patch (Zagorje) only a year later, in 2022. Densities of flowering *S. officinalis* along the transect lines did not differ significantly between the three patches (Kruskal-Wallis test, $\chi^2 = 0.859$, df = 2, p = 0.651, N = 57), however the total densities per ha significantly differed among the three MRR patches (Kruskal-Wallis test, $\chi^2 = 12.269$, df = 2, p = 0.002, N = 27), primarily due to the difference between Klenik

Table 1. A list of investigated potential habitat patches for *P. teleius* in the Seasonal Lakes of Pivka Nature Park (KP PPJ) in 2021–2022 includes data on the presence of the host plant *Sanguisorba officinalis* (with an estimate of the maximum class number of host plants in brackets), the host ant (*Myrmica* sp.) and *P. teleius*.

Habitat patch	Surface (ha)	*Myrmica* sp.	*S. officinalis*		*P. teleius*
Petelinjsko jezero	49.80	n.a.	+*	(1–10)	–
Gradec	0.37	+	+	(11–50)	+
Klenik – MRR and extended area	59.08 (MRR: 5.49)	+	+	(≥1001)	+
Veliko Drskovško jezero	17.21	+	+*	(1–10)	–
Zagorje – MRR and along Pivka	3.24 (MRR: 1.76)	+	+	(101–500)	+
Malo Zagorsko jezero	4.31	+	+	(11–50)**	–
Kalec – MRR	2.83 (MRR: 2.83)	+	+	(101–500)	+
Pivka Spring	0.77	+	+	(11–50)	+
Area with *P. teleius* (% of total)	64.81 (47.1 %)				

* Presence of *S. officinalis* was reported by P. Glasnović (unpubl. data); n.a. – not assessed (nevertheless, another myrmecophilous species that also use *Myrmica* sp. as host ant, *Phengaris alcon* has been recorded there in 2022; Jugovic et al., 2023); ** estimate in 2024 (August 6; Jugovic, pers. obs.): 501–1000.

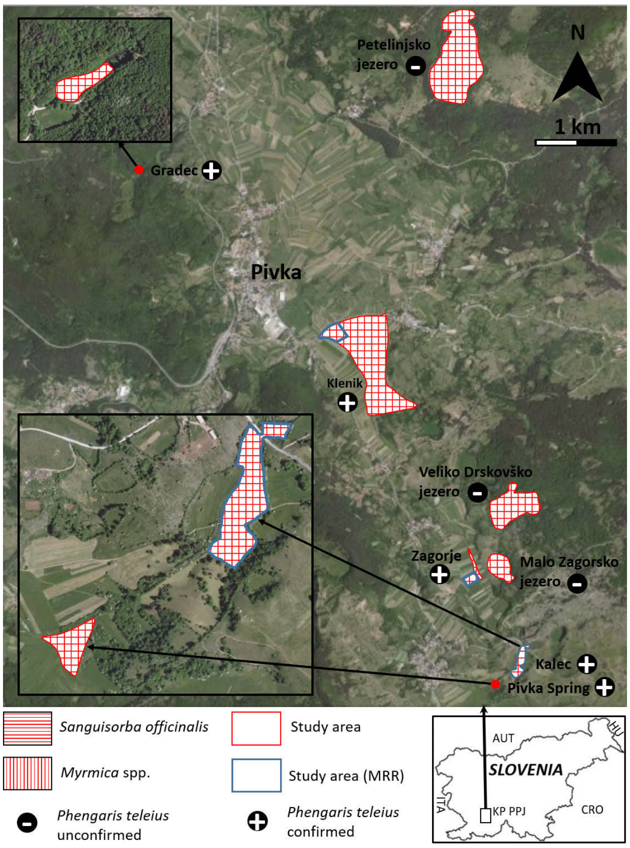

Fig. 1. The geographic positions of eight potential habitat patches (Gradec, Petelinjsko jezero, Klenik, Veliko Drskovško jezero, Malo Zagorsko jezero, Zagorje, Kalec, Pivka Spring) surveyed for the presence of *S. officinalis*, *Myrmica* ants and *Phengaris teleius* in the Seasonal Lakes of Pivka Nature Park (KP PPJ) in 2021–2022. Three patches (Gradec, Kalec, Pivka Spring) are enlarged in the insets due to their small sizes. The Mark-Release-Recapture (MRR) study in 2021 was conducted in parts of patches Klenik and Zagorje (the part excluded from MRR is named "along Pivka" in the text) and in the entire patch Kalec.

with higher and both other patches with lower densities (Fig. S1).

Mowing in 2021

At the three MRR patches, some areas were freshly mown shortly before the beginning of the study (Fig. 2). A large portion of the largest MRR area at Klenik had been mown earlier and had already recovered, as *S. officinalis* was flowering at the beginning of the study. Additionally, some parts of the MRR area were mown during the study, while others were mown afterward (i.e., in the second half of August, Fig. 2).

Movements

No movements of individuals between the three MRR habitat patches (Klenik, Kalec, Zagorje) were recorded. The inverse-power function for males ($P = 0.0043D^{-1,447}$, $R^2 = 0.9435$) and for females ($P = 0.028D^{-0,973}$, $R^2 = 0.8563$) fitted well to the recorded data and indicates that at least 3% of the male and female populations can reach distances of 250 m and 900 m, respectively (the corresponding distances for 1% of the male and female populations are 550 m and 2250 m). These distances exceed the distances between the southernmost neighbouring patches in the surveyed system. Moreover, except for the remote habitat patch Gradec, each potential habitat patch is connected to one to five other patches (including uninhabited patches) or to one to four patches that were inhabited in 2021–2022 (Table 2, see also Table S1).

Demographic parameters

In the 2021 MRR study, spanning 13 marking occasions from July 9 to August 15, butterflies were marked between July 14 and August 10 (Table S2). A total of 191 butterflies were marked (107 males and 84 females; Table S2), with 42 individuals (29 males and 13 females) recaptured,

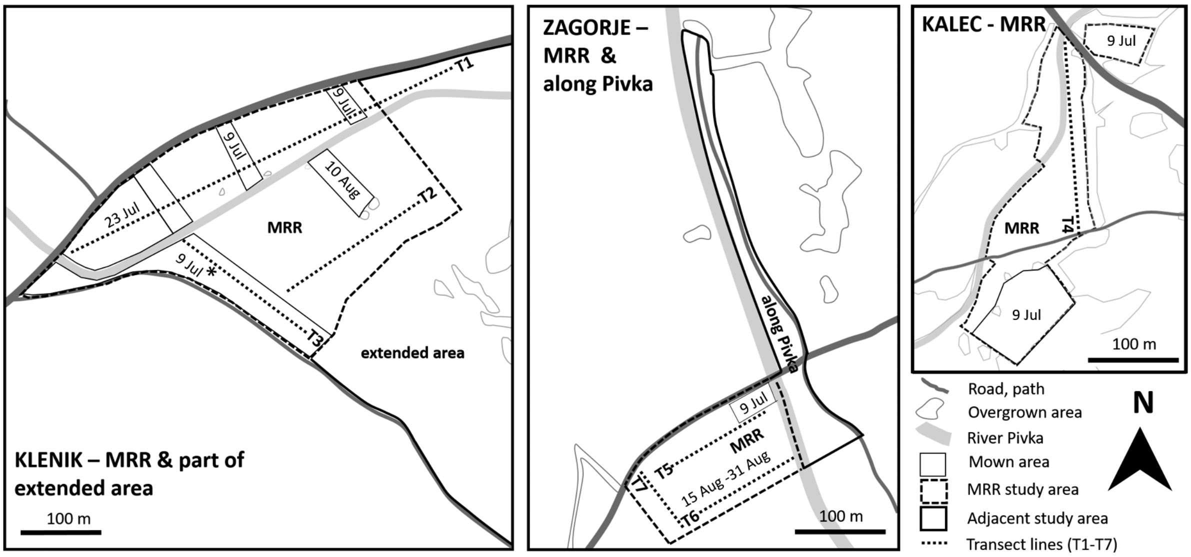

Fig. 2. Mowing at the three MRR patches for the study of demographic parameters for *P. teleius* in the Seasonal Lakes of Pivka Nature Park (KP PPJ) in 2021. The dates in brackets indicate the visit when mowing was recorded in certain areas or provide an estimated timeframe for the mowing event. An asterisk denotes that on 9 July, flowering *S. officinalis* was already present, indicating that mowing occurred some time prior to this observation. Note that in Klenik, the study area extended beyond the MRR patch to the east and south (extended area), while in Zagorje, only the section to the west of Pivka river represented the MRR patch (excluding the area along Pivka) (see Fig. 1 for exact geographic position of the three patches).

yielding a recapture rate of 22.8% (Table S3). The majority of butterflies (83.8%) were marked at Klenik (91 males, 69 females), while the remaining individuals were marked at Kalec (16 males, 15 females). No *P. teleius* individuals were recorded at the third MRR patch, Zagorje, in 2021.

For the analysis, the initial two successful marking occasions (14 and 16 July 2021), during which only four individuals were captured in total and with no captures recorded at one site on one occasion, were combined, resulting in nine analysed sampling occasions (Table S2). For the entire system, only one model showed a ΔQAICc < 2 (with second and third best models with 2 < ΔQAICc < 3), identifying it as the best fit, and this model was subsequently applied to subsystems, with each inhabited patch analysed individually (Table S4). At Klenik, this model ranked as the third-best with a ΔQAICc < 2, while at Kalec, it had a higher ΔQAICc value of 20.9. Nonetheless, in both patches, population size estimates were consistent with those derived from the best models for each subsystem individually (Table S4). For the overall system, the population size was estimated at 197 males (95% CI: 159–265) and 315 females (95% CI: 205–525) according to the best model. When the same model was applied separately to each habitat patch, Klenik's estimated population was 175 males (95% CI: 138–243) and 279 females (95% CI: 166–527), while Kalec had an estimated population of 23 males (95% CI: 18–42) and 36 females (95% CI: 21–89). This suggests that Klenik, the larger of the two patches, supports over 85% of the *P. teleius* population within the KP PPJ system (Table S4). The effective population size for the entire MRR system, based on male and female population size estimates, was calculated at 484 individuals (95% CI: 358–704) for 2021.

The best model for the entire system predicted time-dependent survival (φ) with an additive interaction with sex, indicating higher survival for females compared to males, with survival rates decreasing toward the end of the flight period (Fig. 3). Capture probability (p) was constant but varied by sex, with values of 0.337 (95% CI: 0.215–0.485) for males and 0.099 (95% CI: 0.053–0.178) for females. The recruitment rate (b) was estimated to be the same for both sexes but varied over time, reaching its highest value in the middle of the flight period (July 26–29: 0.562, 95%

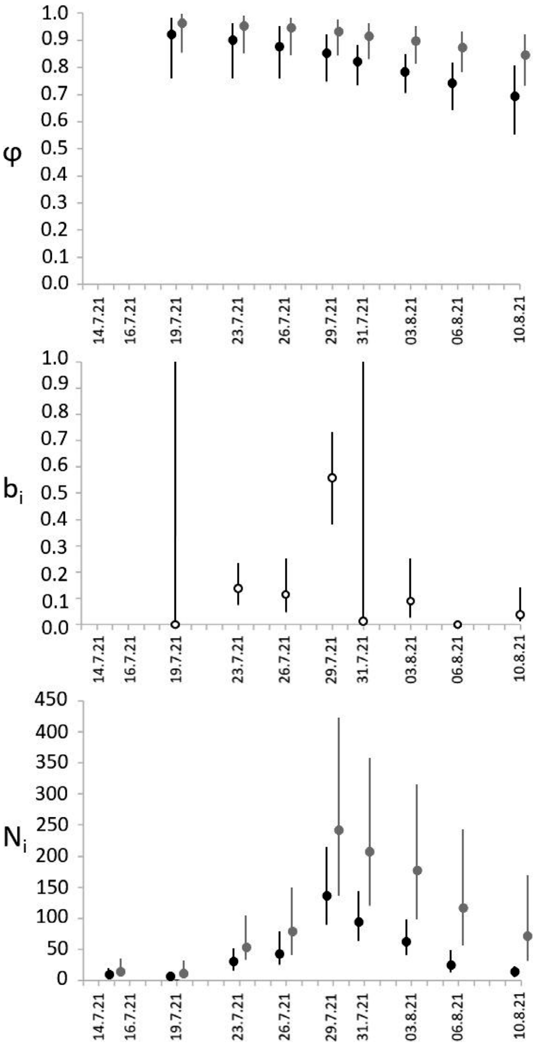

Fig. 3. Estimates and 95% confidence intervals for survival (φ), daily population entry (b_i) and daily population sizes (N_i) of *P. teleius* males (black circles) and females (grey circles) in a system of habitat patches in the Seasonal Lakes of Pivka Nature Park (KP PPJ: Klenik and Kalec) in 2021. For the daily population entry (b_i) data for males and females are equal (empty circles). The data were obtained from predictions of the best POPAN model. For the analysis, the first two successful marking occasions (July 14 and 16, 2021) which involved only four captured individuals, were pooled together and presented as data for July 15, 2021. While daily population estimates (N_i) are shown for each marking occasion, interval parameters (φ, b_i) are presented for the final day of each interval.

Table 2. The smallest geographic distances (in km) between pairs of potential habitat patches for *P. teleius* in the Seasonal Lakes of Pivka Nature Park (KP PPJ) are shown in the upper right corner, along with the percentage of movement capacity for given distances for males (♂) and females (♀) in the lower left corner. The data were derived from the IPF function, based on movements recorded during the MRR study. N – number of all (inhabited only in brackets) patches connected (≥ 1% of either sex) with a given patch. See also Table S1.

	Petelinjsko j.	Gradec	Klenik[a]	V. Drskovško j.	Zagorje[b]	M. Zagorsko j.	Kalec	Pivka Spring
Petelinjsko j.	0	3.35	2.80	4.74	5.44	5.61	6.74	7.12
Gradec		0	3.55	5.92	6.07	6.34	7.46	7.60
Klenik[a]	♀		0	1.13	1.72	2.05	2.93	3.27
V. Drskovško j.			♀♀	0	0.40	0.42	1.50	2.00
Zagorje[b]			♀	♂♀♀♀♀♀♀	0	0.16	0.95	1.20
M. Zagorsko j.			♀	♂♀♀♀♀♀♀	♂♂♂♂♂♂♀	0	0.85	1.26
Kalec			♀	♀	♀♀	♀♀♀	0	0.45
Pivka Spring				♀	♀♀	♀♀	♂♀♀♀♀♀♀	0
N	1 (1)	0 (0)	5 (2)	5 (4)	5 (3)	5 (4)	5 (3)	4 (2)

[a] MRR and extended area, [b] MRR area and along Pivka river; movement ability of males (♂) and females (♀): empty cell < 1%, one symbol ≥ 1%, two symbols ≥ 2%, three symbols ≥ 3% etc.

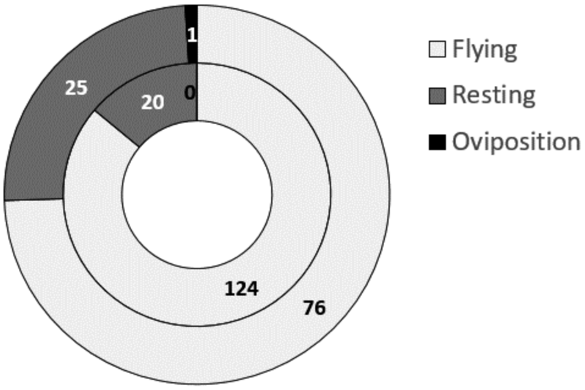

Fig. 4. Behaviour of *P. teleius* (numbers represent frequency of records) in a system of wet meadows in the Seasonal Lakes of Pivka Nature Park (KP PPJ) observed during the MRR study in 2021 (males in the inner circle, females in the outer circle).

CI: 0.381–0.728; Fig. 3). Daily population sizes (N_i) suggested protandry, although peak daily population size for both sexes occurred on the same day (July 29, 2021), with estimates of 138 males (95% CI: 88–215) and 242 females (95% CI: 139–421; Fig. 3). On this peak day, nearly three-quarters of all butterflies in the system were present. In the latter half of the season, the daily population size declined more gradually for females compared to males (Fig. 3).

The ratio between captures of males and females was male-biased (males : females = 1.27 : 1) and varied over time (χ^2-test, LR = 23.71, df = 9, p = 0.005; estimate based on captured animals across each of the ten successful marking occasions). The sex ratio derived from population estimates for the entire system was female-biased, with a ratio of males:females = 1 : 1.60, and this pattern was similar at the largest of the two investigated patches (Klenik; 1 : 1.59). At Kalec, the ratio was also strongly female-biased (1 : 1.57).

In the 2021 MRR study system, total and maximum daily densities were higher for females than males, with densities 3–4 times larger in Klenik than in Kalec (Table S5). In 2022, the presence of *P. teleius* was confirmed in an expanded area of Klenik and also in the MRR patch at Zagorje, where it was not recorded the previous year. During a single visit to Kalec in 2022 (July 19), the presence of *P. teleius* was confirmed again, with 32 individuals observed, more than the 31 individuals recorded during the entire 2021 MRR season at the same patch. Additionally, in 2022, a new inhabited patch was discovered at Pivka Spring, with 12 individuals recorded on July 19, and two individuals were observed at a newly discovered patch near Gradec on July 22 (Table S5).

Based on the average survival rates (males: 0.794; females: 0.887), the average lifetime expectancy was estimated to be 4.4 days for males and 8.4 days for females. The maximum lifespan recorded for males was four days longer than for females (longest living male: July, 16–29; longest living female: July, 26–August, 6), lasting 13 days.

Three behaviours were observed: flying, resting, and a single oviposition by a female. No mating (copula) or feeding was recorded. The frequency of flying and resting differed significantly between sexes (LR = 4.604, df = 1, p = 0.032). Females were resting more (SR = 1.5), while males rested less (SR = –1.3) than statistically expected (Fig. 4). In females, all (100%) of the resting individuals were on *Sanguisorba officinalis*, while in males, four-fifths (80%) rested on *S. officinalis*, and the remaining individuals rested on different grasses (Poaceae).

DISCUSSION

We investigated the demography of *P. teleius* at the southern edge of its distribution to assess whether this species exhibits a metapopulation structure in the study system, where most neighbouring habitat patches are within the species' dispersal ability. The suitability of all investigated habitat patches within the studied network was verified by the presence of the larval host ant species (*Myrmica* sp.), the presence of another *Phengaris* species (*P. alcon*) that shares the same host ant genus (cf. Jugovic et al., 2023), and the presence and abundance of the larval host plant (*Sanguisorba officinalis*). Suitable patches where the butterfly's presence was not confirmed during the survey remain important, as they may be sporadically inhabited or serve as stepping-stone habitats between permanently occupied patches (e.g., Klenik, Kalec). As suggested by Zimmerman et al. (2011), the estimated dispersal distances for 1% of the male population (550 m) and female population (2250 m) ensure connectivity within the metapopulation. In our study, all habitat patches – except Gradec – were estimated to be interconnected by at least 1% of the male and/or female population, based on movement probabilities predicted by IPF model. The presence of *P. teleius* in the isolated patch at Gradec suggests either a denser network of suitable habitats in the metapopulation than recorded during our study, or that this patch belongs to another, not yet discovered, nearby population. In Serbia, Popović et al. (2014) hypothesised a maximal migration distance of 5 km for *P. teleius* (see also Bizenhöfer & Settele, 2000; Stettmer et al., 2001), although the longest recorded flight distance was 1.9 km (Popović et al., 2017). In our study, as well as in research by Popović & Nowicki (2023), females exhibited higher average dispersal abilities than males; however, Popović et al. (2022) also found that males tend to have longer flight times than females. This finding is consistent with our results, where males were recorded in flight 86% of the time, compared to 75% for females (Fig. 4). This difference is relevant as it suggests that males may have greater dispersal capabilities, which could play a key role in population connectivity and the overall structure of the metapopulation. Although no flights between patches were recorded in our study, their connectivity is further supported by the favourable, level landscape and the open mosaic of agricultural land throughout the study area, with the exception of the Gradec patch, which is located within a forested matrix. For all other patches, we can reasonably assume they belong to the same metapopulation, as Nowicki et al. (2014) suggested that inhospitable forest matrix induces strong selection against dispersal in *P. teleius*, leading to a reduced emigration rate. Moreover, the absence of butterflies in some patches in 2021 and patch recolonisation in the consecutive year indicates functional metapopulation structure. Nevertheless, further studies on this are needed as *Phengaris* butterflies tend to have relatively stable populations within the patches (Kajzer-Bonk & Nowicki, 2022). Habitat loss and fragmentation however can negatively impact these populations and force them to seek for new habitats (Nowicki et al., 2014). Popović & Nowicki (2023) found that butterflies from larger unmown patches exhibit the highest dispersal abilities; however, mowing increases butterfly dispersal probability by forcing individuals to find suitable habitats. This aligns with the hypothesis of Nowicki et al. (2005a), which posits that the mobility of *Phengaris* butterflies may differ significantly across various environmental settings (see also Nowicki et al., 2014). Mowing within the habitat patches of KP PPJ occurred in a mosaic pattern and was not simultaneous across all areas, making it unlikely to have had a significant impact on the butterfly populations under study (cf. Jugovic et al., 2013).

Given the large area and low nearest-neighbour index value, the clusters of habitat patches in KP PPJ are loosely connected. Nevertheless, the effective conservation of all suitable habitat patches is crucial (Wynhoff, 1999), and the establishment of additional corridors and/or new suitable patches would be beneficial. Rare but long dispersals over inhospitable matrix, as reported for *P. teleius* by Nowicki et al. (2014), likely assist the species in maintaining a metapopulation structure even in landscapes with loosely connected habitat patches (e.g., the Gradec patch in our study system). Nevertheless, it is possible that the migration distances in our case were underestimated, and the connectivity is better than estimated (e.g. Van Dyck & Baguette, 2005).

Separate analyses of habitat patches Klenik and Kalec clearly showed that in our metapopulation system, one patch (Klenik) supports the vast majority of the studied population. Since the MRR protocol was conducted only in a small portion of the Klenik habitat patch, we can assume the population is even larger when considering the extended area. Both host species (*Myrmica* sp., *Sanguisorba officinalis*) are present there, and the density of *S. officinalis* is high. The central geographic position of Klenik within the investigated system likely allows it to serve as the main source of individuals for other smaller patches. Adult butterflies persisted in the system for a relatively short period, less than a month (29 days). In nearby systems (approximately 7 km south near Rečica in 2014 and 2016, and to the north near Nanoščica in 2019), the season lasted about 8–10 weeks (Verovnik et al., 2015; Zakšek et al., 2016; Jugovic et al., in prep.). The relatively short flight period and simultaneous appearance of males and females (with about three-quarters of the total population estimated to be present on the day of highest density), along with only weakly expressed protandry in our study system, may help the local metapopulation persist. This is because the chance for mating is increased, and the risk of virgin female mortality is reduced (Zonnenveld & Metz, 1991). Furthermore, in a system where one patch (in our case Klenik) supports the majority of the metapopulation, a homogenous habitat (levelled open landscape) may contribute to the synchronisation of the appearance of both sexes.

Although males were more prone to capture than females in our study, the estimated sex ratio was female-biased. Although it cannot be excluded that this estimate may be erroneous due to the less intensive field study (but see Nowicki et al., 2005c), the result is likely linked to behavioural differences, which are well documented in many butterfly species, including *Phengaris*. For example, Kallioniemi (2014) found that differences in mobility between sexes influenced dispersal, with males typically being more mobile than females. Interestingly, although females were observed to move less than males, they were more likely to cross habitat boundaries, highlighting sex-dependent behavioural differences that affect dispersal (Kallioniemi, 2014). In the case of *Phengaris*, as reported from our study, these differences suggest that males may contribute more to population connectivity through greater mobility, while females, with their more localised movements, may be more restricted by habitat boundaries. As reported from our study, females tend to rest more, as they are larger and heavier due to egg development, while males are more conspicuous and easier to catch due to their pronounced flying and patrolling behaviour (e.g. Jugovic et al., 2017, 2018). A female-biased sex ratio, as observed in our study, is uncommon in *P. teleius*, but has been recorded previously (e.g. Nowicki et al., 2005a). Since neither polyandry nor polygyny occurs in butterflies, the sex ratio is expected to approach 1 : 1 over successive generations. Deviation from a balanced sex ratio may indicate population instability (e.g. Kallioniemi, 2014) however, in our case, the higher number of females may have a positive effect, as one male can mate with multiple females (Zonnenveld & Metz, 1991), potentially enhancing population size in the next generation.

The estimated average lifetime expectancy of *P. teleius* in our study area was relatively long (males: 4.4 days, females: 8.4 days), being equal or surpassing the lifespan recorded in other European populations (2–5 days: Slovenia – Malačič, 2005; Poland – Nowicki et al., 2005b, c; Serbia – Popović et al., 2017). Despite following the scheme recommendations for non-intensive monitoring of butterflies on the field (Nowicki et al., 2005c), this could be the result of estimate error. Nevertheless, the longest recorded lifespan (13 days in our study) is similar to that observed in other European populations (15 days: Popović et al., 2017; 6–18 days: Nowicki et al., 2005a).

In summary, the ability for long-distance movements, the synchronised appearance of males and females with a female-biased sex ratio, the presence of one large, centrally positioned habitat patch supporting the majority of the metapopulation, a relatively long life expectancy, and an effective population size close to 500 (noting that our estimate was based on only a portion of the entire metapopulation) all suggest that this metapopulation, at the edge of the species' European distribution, is currently resilient and may persist into the future, provided habitat quality does not deteriorate.

Implications for conservation

Since our study provides the first detailed insight into the population structure of *Phengaris teleius* within the Seasonal Lakes of Pivka Nature Park (KP PPJ), it is still too early to assess its long-term stability. This would require monitoring population size or its temporal variability over a longer period, typically at least eight years (Caughley, 1994; Morris et al., 1999; Lundberg et al., 2000; Nowicki et al., 2005a; Keith et al., 2015). Nevertheless, several characteristics observed in this system suggest a potentially viable population: (1) a relatively short flight period with synchronised appearance of males and females that increases mating opportunities, (2) a comparatively long average lifespan expectancy, (3) high estimated connectivity among habitat patches according to the IPF model, and (4) the presence of a single large, high-quality habitat patch (Klenik) that appears to support the majority of individuals. These factors together may enhance mate encounter rates and reproductive success, contributing positively to overall population size and stability.

At the same time, recognizing that this fragmented population operates as a metapopulation adds an important dimension to its conservation. It highlights that long-term persistence does not depend solely on the quality of individual habitat patches, but also on their spatial configuration and functional connectivity. In such systems, local extinctions can be natural and frequent, but they are offset by recolonisation from nearby patches – provided that the landscape remains sufficiently connected. This means that even small or temporarily unoccupied habitat patches can serve as crucial stepping stones, facilitating dispersal and buffering against local declines. Conversely, degradation or mismanagement of key sites – especially the largest source patch – could have disproportionately negative consequences across the entire system.

Fortunately, current management in KP PPJ already supports the basic requirements of a functioning metapopulation. Infrequent mowing, timed to avoid the larval feeding period, ensures that host plants and developing caterpillars are retained each year (see Popović & Nowicki, 2023). Additionally, practices detrimental to habitat quality – such as mulching, fertilisation, and intensive grazing – are strictly avoided. However, maintaining and strengthening this system will require long-term, landscape-level conservation planning. This includes protecting existing patches, restoring degraded habitats, and improving connectivity through the creation of additional stepping-stone sites. Such efforts will depend on sustained collaboration with landowners and farmers, adequate financial support for targeted agri-environmental measures, and potentially land acquisition. Ultimately, effective conservation of *P. teleius* will rely not only on protecting current populations but also on preserving the processes – like dispersal and recolonisation – that enable them to persist as a dynamic, interconnected metapopulation. This includes targeted efforts to maintain suitable habitat conditions and ensure the availability of key host species – both *Sanguisorba officinalis* and *Myrmica* ants (see Elmes et al., 1998; Seifert, 2018) – which are critical to the species' complex life cycle and long-term viability.

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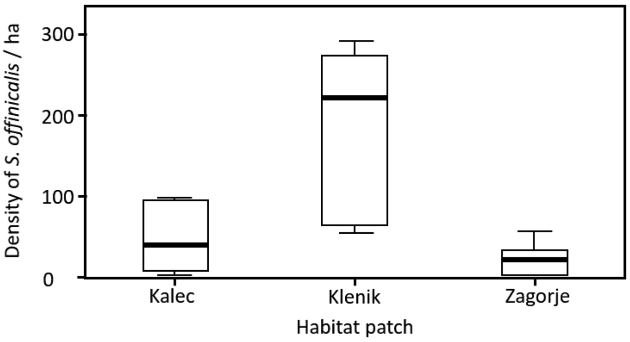

Fig. S1. Densities calculated from total count per ha of *S. officinalis* at three MRR patches of *P. teleius* in the Seasonal Lakes of Pivka Nature Park (KP PPJ) in 2021.

Table S1. Estimated percentage (negative exponent function model: NEF, and inverse power function model: IPF) of *Phengaris teleius* males (♂♂) and females (♀♀) moving certain distances. The percentage closest to 1% for each model × distance combination (in 50-m increments) is highlighted in bold.

Distance (km)	NEF		IPF	
	♂♂ (R^2 = 0.998)	♀♀ (R^2 = 0.931)	♂♂ (R^2 = 0.944)	♀♀ (R^2 = 0.856)
0.05	38.223	53.295	32.814	51.649
0.1	14.104	32.802	12.036	26.312
0.15	5.204	20.189	6.694	17.735
0.16	4.263	18.321	6.097	16.655
0.2	1.920	12.426	4.414	13.405
0.25	**0.709**	7.648	3.196	10.789
0.3	0.261	4.707	2.455	9.035
0.35	0.096	2.897	1.964	7.776
0.4	0.036	1.783	1.619	6.829
0.45	0.013	**1.097**	1.365	6.090
0.5	0.005	0.675	1.172	5.496
0.55	0.002	0.416	**1.021**	5.009
0.6	0.001	0.256	0.900	4.603
…				
2.85	$2{,}16*10^{-23}$	$8{,}37*10^{-11}$	0.095	**1.011**

Table S2. Summary for *P. teleius* males and females caught across three habitat patches in the Seasonal Lakes of Pivka Nature Park (KP PPJ) in 2021. For the analysis in MARK, the initial two successful marking occasions (July 14 and 16, 2021), which captured only four individuals, were combined, yielding nine analysed sampling occasions.

Sampling occassion	1	2	3	4	5	6	7	8	9	10	11	12	13
Date	9.07.	12.07.	14.07.	16.07.	19.07.	23.07.	26.07.	29.07.	31.07.	3.08.	6.08.	10.08.	15.08.
Interval (days)	3	2	2	3	4	3	3	2	3	3	4	5	
No. of males	0	0	3	1	0	9	14	50	37	18	7	5	0
No. of females	0	0	0	0	3	7	6	27	15	17	14	8	0
Total no.	0	0	3	1	3	16	20	77	52	35	21	13	0

Table S3. Summary of data on the numbers of male and female *P. teleius* individuals captured across three habitat patches in the Seasonal Lakes of Pivka Nature Park (KP PPJ) in 2021.

	No. of marked individuals	No. of recaptured individuals	% of recaptured individuals	No. of all captures	No. of recaptures	% of recaptures
Males	107	29	27.1	144	40	27.8
Females	84	13	15.5	97	15	15.5
Total	191	42	22.0	241	55	22.8

Table S4. The best supported POPAN models, selected using the Akaike Information Criterion (AIC; ΔAICc < 3; # = ranking order), for the entire MRR system in the Seasonal Lakes of Pivka Nature Park (KP PPJ; Klenik + Kalec, best model for pooled data shown in bold), as well as for the two subsystems (Klenik and Kalec). Shown are the number of parameters (Np) and the estimated population sizes (N) of males (♂) and females (♀), with 95% confidence intervals (CI).

System	#	Model	Δ AICc	Np	$N_{♂}$ (95% CI)	$N_{♀}$ (95% CI)
Klenik + Kalec	**1st**	$\boldsymbol{\varphi_{(g+tlin)}\ p_{(g)}\ b_{(t)}\ N_{(g)}}$	0	15	197 (159–265)	315 (205–525)
	2nd	$\varphi_{(g+tlin)}\ p_{(g)}\ b_{(g+t)}\ N_{(g)}$	2.1358	16	197 (158–265)	315 (204–526)
	3rd	$\varphi_{(g)}\ p_{(g)}\ b_{(t)}\ N_{(g)}$	2.6254	14	200 (159–271)	319 (207–534)
Klenik	1st	$\varphi_{(g)}\ p_{(g)}\ b_{(t)}\ N_{(g)}$	0	14	170 (132–242)	289 (172–539)
	2nd	$\varphi_{(.)}\ p_{(g)}\ b_{(t)}\ N_{(g)}$	0.0277	13	170 (133–240)	278 (167–530)
	3rd	$\boldsymbol{\varphi_{(g+tlin)}\ p_{(g)}\ b_{(t)}\ N_{(g)}}$	1.5711	15	175 (138–243)	279 (166–527)
	4th	$\varphi_{(tlin)}\ p_{(g)}\ b_{(t)}\ N_{(g)}$	1.8369	14	169 (132–240)	270 (158–523)
	5th	$\varphi_{(.)}\ p_{(g)}\ b_{(g+t)}\ N_{(g)}$	1.8703	14	175 (138–243)	279 (166–527)
Kalec	1st	$\varphi_{(g+tlin)}\ p_{(g)}\ b_{(tlin+tlin^2)}\ N_{(g)}$	0	10	23 (18–42)	36 (21–89)
	2nd	$\varphi_{(g+tlin)}\ p_{(g)}\ b_{(g+tlin+tlin^2)}\ N_{(g)}$	2.7147	11	24 (18–44)	34 (20–82)
	51st	$\boldsymbol{\varphi_{(g+tlin)}\ p_{(g)}\ b_{(t)}\ N_{(g)}}$	20.9026	15	23 (18–42)	36 (21–89)

Table S5. Population density parameters (individuals per ha with 95% CI in brackets: maximal daily density, total density) for *P. teleius* at two habitat patches in the Seasonal Lakes of Pivka Nature Park (KP PPJ) in 2021. Data were derived from the best model for pooled data. In all cases, maximal daily density was recorded on July 29, 2021. Data on numbers of captured butterflies shown 2021 and inventory in 2022.

	Habitat patch	Sex	Maximal daily density	Total density	No. of captured animals
MRR 2021	Klenik	Males	20.9 (14.8–29.9)	31.9 (25.1–44.3)	91
		Females	34.4 (18.0–65.2)	50.8 (30.2–96.0)	69
	Kalec	Males	7.2 (4.3–12.1)	8.1 (6.4–14.8)	16
		Females	12.2 (5.7–27.5)	12.7 (7.4–31.4)	15
	Zagorje	Males	0	0	0
		Females	0	0	0
	Klenik + Kalec	Males	16.6 (10.6–25.8)	23.7 (19.1–31.9)	107
		Females	29.1 (16.7–50.6)	37.9 (20.0–63.3)	84
Inventory 2022	Petelinjsko jezero				0 (July, 19, 21, 22)
	Gradec				2 (July, 22)
	Klenik – MRR and extended area				> 50 (July, 21)
	Veliko Drskovško jezero				0 (July, 19, 21, 22)
	Zagorje – MRR and extended area				4 (July, 19)
	Malo Zagorsko jezero				0 (July, 19, 21, 22)*
	Kalec				32 (July, 19)
	Pivka Spring				12 (July, 19)

* No butterflies were recorded also on August 6, 2024 (Jugovic, unpubl. data).