Estimation of current population status of the Alcon large blue *Phengaris alcon* (Denis & Schiffermüller, 1775) (Lepidoptera: Lycaenidae) in Bela krajina (SE Slovenia) based on egg counts

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Abstract. An estimate of population size of the endangered Alcon large blue (*Phengaris alcon*) in Bela krajina (SE Slovenia) was performed based on egg counts. The presence of eggs has been confirmed in all seven currently known populations of the larval host plant *Gentiana pneumonanthe* in the area. Owing to the short distances between the populations it is probable that the species forms a single metapopulation with an estimated population size of about 190 individuals. One local extinction is documented on a site where *P. alcon* was present in 2008. We propose conservation measures to preserve the existence of *P. alcon* in Bela krajina.

Key words: *Phengaris alcon*, *Gentiana pneumonanthe*, Papilionoidea, Bela krajina, Slovenia, oviposition, population size, species conservation


Ključne besede: *Phengaris alcon*, *Gentiana pneumonanthe*, Papilionoidea, Bela krajina, Slovenija, ovipozicija, velikost populacije, ohranitev vrste
Introduction

Butterflies of the genus Phengaris (Lepidoptera: Lycaenidae) in Slovenia are represented by four species: *Phengaris arion* (Linnaeus, 1758), *P. teleius* (Bergsträsser, 1779), *P. nausithous* (Bergsträsser, 1779) and *P. alcon* (Denis & Schiffermüller, 1775) (Verovnik et al. 2012). Species from the genus *Phengaris* have been intensively studied due to their interesting and unique life cycle (Als et al. 2002, Mihoci et al. 2007, Czekes et al. 2014). The availability of both specific host plants and specific *Myrmica* ant hosts is essential for the survival of *Phengaris* butterflies species (Nowicki et al. 2005).

Caterpillars spend their early larval stages feeding inside the flower buds of their host plants. After reaching their fourth instar, the larva falls to the ground, waiting for a foraging *Myrmica* ant to adopt it and take it into the nest (Thomas et al. 2010, Czekes et al. 2014). In ant colonies they either prey on ant brood in the case of predatory species (*P. arion, P. nausithous* and *P. teleius*) or are fed by nurse ants in the case of the cuckoo species (*P. alcon*) (Nowicki et al. 2005, Czekes et al. 2014). After spending at least 10–11 months in the ants’ nest, gaining about 98% of their final biomass, they pupate during late spring (Als et al. 2002, Czekes et al. 2014).

The genus *Phengaris* is thought to have evolved in the steppes of central Asia (Sibatani et al. 1994, Als et al. 2002). The European *Phengaris* species were pre-adapted to survive and disperse in traditional European agricultural landscapes (Als et al. 2002). Today, all the *Phengaris* species in Europe are threatened and are declining due to habitat fragmentation, habitat loss, habitat degradation and changes in agricultural management (Als et al. 2002, Maes et al. 2004). As a result, all *Phenagris* species were included in the red lists of most European countries (Czekes et al. 2014). In Slovenia, all four species from the genus *Phengaris* are on the Red List of Slovenian Butterflies (Ur. l. RS 2002) and are also protected at the national level with the Decree on protected wild animal species (Ur. l. RS 2004a). Two species (*P. teleius* and *P. nausithous*) are listed on Annex II and Annex IV of The EU Habitats Directive 92/43/EEC, while *P. arion* is listed in Annex IV. In Slovenia, *P. alcon* is listed as ‘endangered’ (EN) species and is one of the fastest declining butterflies in Slovenia (Verovnik et al. 2012). It has disappeared from large parts of its range, becoming possibly extinct in the Koroška region and having only a handful of isolated populations left in the Štajerska, Gorenjska and Bela krajina regions (Verovnik et al. 2012).

*P. alcon* has two ecotypes based on larval host plant and habitat type (*f. alcon* uses *G. pneumonanthe* and *f. rebeli* uses *G. cruciata*). Here, we focused on *f. alcon* which lives on humid grasslands, but its populations are very local and rare (Verovnik et al. 2012, Rebešek 2006). The only regions where it can be locally abundant are in the karst poljes – flat depressions in the Dinarides, the edges of the Ljubljansko barje south of Ljubljana, and in the eastern part of Gorčko (Verovnik 2000, Verovnik et al. 2012). The distribution of *P. alcon* in Bela krajina (study area) is well known (Verovnik & Škvarč 2002).
The aim of our study was to assess the population status of *P. alcon* in Bela krajina (SE Slovenia). We counted the eggs of *P. alcon* and the genets of *G. pneumonanthe* at locations with known presence of *P. alcon* or *G. pneumonanthe* from the literature. In addition, localities with suitable habitat were checked for possible new findings of larval host plant (*G. pneumonanthe*) or *P. alcon*. The results of the study are important for the conservation of *P. alcon* in Bela krajina.

**Materials and methods**

**Study area**

Our study was conducted in the central part of Bela krajina (SE Slovenia) around the village of Dragatuš with existing old data on occurrence of *P. alcon* or *G. pneumonanthe*. The central part is a flat karst plain (150–200 m a.s.l.) bordered to the north by the Gorjanci Hills, westwards by the karst plateau Kočevski rog, while in the south and towards the east it borders to the Kolpa River, which also forms the border with Croatia.

Bela krajina is a region of temperate continental (sub-Pannonian) climate with annual precipitation of 1200–1300 mm. The types of soil are a reflection of an overwhelming carbonate bedrock. The karst landscape of Bela krajina is characterized by scarce superficial water flows and an intense underground water connection. The Lahinja is the largest river in Bela krajina and flows into the Kolpa (Štangelj & Ivanovič 2013). The Lahinja meanders through the plain forming wetlands that are partly conserved, for example Lahinjske luge and Nerajske luge which are complex wetlands consisting of wet meadows, marshes and reed beds. Of the few existing tributaries, it is worth mentioning the Podturščica, which is app. 3.5 km long, mainly regulated stream. The only part left with meanders are the first 500 m near the spring. Until the beginning of the 20th century the landscape of Bela krajina was agricultural. After mass human emigration in the 20th century and changes in economic activities, the landscape has become heterogeneous with a substantial proportion of land being overgrown with forest (Paušič & Čarni 2012). Compared with the 20th century, the agriculture is today intensive and present in the flat lowlands. The streams were mainly regulated and the agricultural land drained in the 1980s.

The study area is partly protected by law. Lahinjske luge and Nerajske luge are two natural reserves within Lahinja Regional Park (Ur. l. RS 1998). The Lahinja and Podturščica rivers with the pertaining wet meadows are listed as valuable natural features (Ur. l. RS 2004b).
Field work

Field work was carried out on 28. and 29. 7. 2015. Flight period of *P. alcon* in Slovenia begins in June and lasts until early September; however, it depends on the region and on the season. At our study sites we assumed that the flying period was at its end, although we still saw some egg-laying females (at three locations). The egg count is the standard method for assessing the abundance of *Phengaris alcon* (Maes et al. 2004, Van Swaay et al. 2012). The white eggs are very conspicuous on the green flower buds of *G. pneumonanthe*; and most of the (empty) egg shells remain on the host plant until about two weeks after the flight season (Maes et al. 2004), because the larvae do not eat them after eclosion (Ebert & Rennwald 1993, as cited in Verovnik 2002).

Potential habitat patches for *P. alcon* and *G. pneumonanthe* were determined by existing data from Verovnik & Škvarč (2002), data in the database of the Centre for cartography of fauna and flora (15.7.2015; unpublished data of A. Škvarč, M. Govedič, B. Frajman, V. Zakšek and R. Verovnik) and data of locations of *G. pneumonanthe* mentioned by Ivanovič (1983) and Dražumerič (1992). All these locations were subsequently checked for *P. alcon* and *G. pneumonanthe*, including the wet meadows around Podturščica where data for the host plant exist but are spatially inaccurate. The locations were first examined for *G. pneumonanthe*, for which we inspected the meadows and the forest edge (Fig. 1). At locations with small populations of *G. pneumonanthe* we counted all the genets and the number of eggs on each genet. At locations with bigger populations we divided the meadow into different plots according to clear difference in density of genets or a human factor (one part of the meadow being mowed). Afterwards we delimited the habitat patches according to our field survey in ArcGIS (ver 10.2.2, ESRI 2014). In two of our study sites, where *G. pneumonanthe* was abundant (Lahinjske luge and Obrh), we estimated the population of genets and eggs using quadrat method. We randomly threw a stick in the meadow where we placed a 5×5 m² quadrat. Eggs and genets were then counted in ten randomly selected quadrats at Lahinjske luge and five at Obrh. Because the data of eggs and genets per quadrats were not normally distributed we log transformed the data to calculate the confidence intervals. The total number of genets on sites where their populations were small was probably underestimated, because some genets did not flower and therefore were very difficult to spot among the grass. However, we consider the number of such genets to be relatively small as we thoroughly inspected the meadows. To get an estimate of population size of imagos (EPS) we assumed that: (1) a female lays on average 80 eggs (Maes et al. 2004, Mouquet et al. 2005), (2) the sex ratio in the population is 1:1. The EPS was calculated by:

$$\text{EPS} = \frac{\text{no. eggs} \times 2}{80}$$

In case of fewer than 40 eggs the number was rounded to 1 individual.

Maes et al. (2004) determined the maximum local movement distance based on mark-release-recapture as 500 m. They treated flight areas separated by more than 500 m as different populations. Maes et al. (2004) also determined the maximum observed colonization capacity as 2000 m, based on distances from newly colonized habitat patches to the previously existing ones. Thus we defined distinct populations as habitat patches > 500 m apart. Conversely we treated disjoint habitat patches of < 500 m as one population.
Results

In our field study we investigated a total of 35.7 ha potentially suitable habitat in Bela krajina (Fig. 1). Figure 1 shows the current distribution of *P. alcon* and *G. pneumonanthe* based on our study. We recorded 7 populations of *P. alcon* within the studied area. The discoveries of *P. alcon* at the locations Dragatuš and Podlog (habitat patches 6 and 2 in Fig. 1, respectively) are new for the area. *P. alcon* disappeared from the meadow »F« at the location of Nerajske luge (Fig. 3). The adult *P. alcon* on meadow »8« (Fig. 1) found in 2001 (Verovnik & Škvarč 2002) was probably a dispersing individual, as no *G. pneumonanthe* were recorded at the location (neither in 2001 or 2015).

Figure 1. Current distribution of *P. alcon* and its larval host plant *G. pneumonanthe* in the study area in Bela krajina. Location numbers correspond to: 1 – Lahinjske luge, 2 – Podlog, 3 – Nerajske luge, 4 – Podturniščica, 5 – Obrh, 6 – Dragatuš, 7 – Sela and 8 – Pašnik pri Obrhu.

The lowest number of eggs was at Sela, which held 5 eggs (Tab. 1), and the highest at Lahinjske luge with an estimated number of 4,032 eggs (532–4593, p=0.05). The largest number of eggs present on one genet was 289 at the location Podlog.

**Table 1.** Estimation of the population size of *P. alcon* in Bela krajina. With information on the number of the habitat patch corresponding to Fig. 1 (No), area of habitat patch hosting *P. alcon* eggs (A), the number of *G. pneumonanthe* in habitat patch (#GP), the number of eggs (#eggs), estimated population size of imagos based on number of eggs (EPS), density of *G. pneumonanthe* per hectare (GP/ha), presence of adult butterflies at the habitat patch during egg count (Adults; Y = present, N = not present), existence of old data for presence (G = *G. pneumonanthe*, A = *P. alcon*) and the year of the old data. The numbers which are represented with confidence intervals were obtained by quadrat sampling. Otherwise the census on the habitat patch was complete.

<table>
<thead>
<tr>
<th>No</th>
<th>Location</th>
<th>A (m²)</th>
<th>#GP</th>
<th>#eggs</th>
<th>EPS</th>
<th>GP/ha</th>
<th>Adults</th>
<th>Old data</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Lahinjske luge</td>
<td>5929</td>
<td>6735</td>
<td>4032</td>
<td>101</td>
<td>11360</td>
<td>Y</td>
<td>G, A</td>
<td>2001</td>
</tr>
<tr>
<td></td>
<td>(1112-7143)³</td>
<td>(532-4593)³</td>
<td>(13-115)³</td>
<td>(1877-12048)³</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Podlog</td>
<td>1037</td>
<td>405</td>
<td>2654</td>
<td>66</td>
<td>3909</td>
<td>Y</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>3.</td>
<td>Nerajške luge</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Patch »D«⁴</td>
<td>4143</td>
<td>18</td>
<td>514</td>
<td>13</td>
<td>777</td>
<td>Y</td>
<td>G, A</td>
<td>2008</td>
</tr>
<tr>
<td></td>
<td>Patch »E«⁴</td>
<td>49533</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>4</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Podturnščica</td>
<td>627</td>
<td>29</td>
<td>172</td>
<td>5</td>
<td>462</td>
<td>N</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Patch »D«⁴</td>
<td>3396</td>
<td>2853</td>
<td>120</td>
<td>3</td>
<td>8402</td>
<td>Y</td>
<td>G</td>
<td>2008</td>
</tr>
<tr>
<td></td>
<td>Patch »E«⁴</td>
<td>20397</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>N</td>
<td>G, A</td>
<td>2001</td>
</tr>
<tr>
<td>6.</td>
<td>Dragatuš</td>
<td>3784</td>
<td>18</td>
<td>5</td>
<td>1</td>
<td>48</td>
<td>N</td>
<td>G, A</td>
<td>2001</td>
</tr>
<tr>
<td>8.</td>
<td>Pašnik pri Obhr</td>
<td>6140</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>N</td>
<td>A</td>
<td>2001</td>
</tr>
</tbody>
</table>

³ position of patches shown in Fig. 3
⁴ two different methods were used at this location, see Fig. 2
⁵ the mown part of the meadow was not counted

The two locations where we used different methods to estimate the population of eggs and genets are shown in Figs. 2 and 3. The patch »E« (Fig. 3) had 18 genets, but without eggs. The patch »D« (Fig. 3), on the other hand, hosted a population of 322 genets with 514 eggs. The patch »F« (Fig. 3) hosted a population of genets with eggs in 2001 (Verovnik & Škvarč 2002) but is now locally extinct.
Figure 2. Orthophoto of the location Obrh with polygons delineating different parts of the meadow. Mowing status (on 29. 7. 2015) and method used, A: mown, *G. pneumonanthe* present but not counted; B: not mown, quadrat sampling; C: not mown, total census of eggs and host plant.


Figure 3. Orthophoto of the location Nerajske luge with polygons delineating different parts of the meadow. Mowing status (on 29.7. 2015) and method used, D: not mown, total census of eggs and host plant; E: mown, 18 genets with no eggs; F mown, no genets, locally extinct.


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Discussion

*P. alcon* often occurs in small and isolated, but stable habitat patches, supported by a few hectares of suitable land (Nowicki et al. 2005). This might also be the case in Bela krajina. If we strictly follow the 500 m parameter for determining different populations, we have seven separate populations of *P. alcon* in Bela krajina (Lahinjske luge, Nerajske luge, Obrh, Dragatuš, Podlog, Podturnščica and Sela). However, because the distances between adjacent populations are never more than the colonization capacity of 2000 m, we can expect occasional migrations between populations. This implies a classic metapopulation (sensu Hanski 1999) of *P. alcon* in Bela krajina. In its classic form (Hanski 1999), metapopulations experience frequent extinctions and colonisation events. In Bela krajina four populations (Obrh, Dragatuš, Podturnščica and Sela) have less than 5 EPS and relatively small *G. pneumonanthe* population (<110, except Obrh). This makes them highly vulnerable to extinction but also to re-colonization from bigger adjacent populations. *P. alcon* densities predominantly depend on density of *G. pneumonanthe* (Nowicki 2007) and Obrh is the only location in our study that stands out in this view (Tab. 1). The population of *G. pneumonanthe* at Obrh is extensive with more than 2853 genets (without counting the mown half of the meadow, see Fig. 2) but the EPS is only 3. Although we have certainly missed some eggs using the quadrat method on the patch »B« (Fig. 3) we could expect a bigger population of *P. alcon*. The location was known for *G. pneumonanthe* but not *P. alcon* in 2008 (Zakšek, CKFF database), although eggs could had been overlooked. The meadows were certainly mown in the last years which might account for the low local population. Even during our study, half of the suitable habitat with *G. pneumonanthe* was mown (Fig. 2). Early mowing (June and July) is not detrimental to *G. pneumonanthe* but makes it impossible for the larvae of *P. alcon* to survive or eliminates stems suitable for egg laying. It will depend on the mowing regime in the years to come whether this population might increase or go extinct. Local extinction occurred in 2001 at location Nerajske luge habitat patch »F« (Fig. 3) where no *G. pneumonanthe* has been found in 2015. The cause might be excessive mowing and fertilization (Oostermeijer 1994). It is interesting that the small and isolated location Sela hosted 5 eggs and 18 genets (2015), which is very similar to 6 eggs and 20 genets in 2001 (Verovnik & Škvarč 2002). *G. pneumonanthe* can reach a high age of more than 30 years (Oostermeijer 1994), so it is possible these 18 plants are the same as in 2001. It is doubtful that this is a self-sustained population, and it probably depends on frequent recolonizations from the nearby bigger populations.

We can determine three main populations for *P. alcon* in Bela krajina: Lahinjske luge, Podlog and Nerajske luge with 101, 66 and 13 EPS, respectively. We estimate that Obrh is also an important local population based on arguments already stated and a high potential for increase of the population (which presumably depends on the mowing regime).
Implications for conservation

The current populations of *G. pneumonanthe* in Bela krajina are small and localized and thus prone to extinctions. All of them host eggs, implying that the host plant is the main limiting factor for *P. alcon*. The summed number of imagos in the whole metapopulation is 190. Nowicki (2007) surveyed a population of slightly more than 500 individuals of *P. alcon* in Poland by the method of egg count and the population remained stable over the course of two years. The metapopulation in Bela krajina might thus be stable even though the number of individuals seems low. Monitoring in the next years would show the trend for *P. alcon* in Bela krajina and discoveries of new habitat patches with *P. alcon* are also possible.

At the time of our field work some patches with *G. pneumonanthe* were already mown. The biggest patch already mown was at Obrh (Fig. 1). We assume that the frequent mowing at this patch might be the cause for the low number of individuals of *P. alcon*. Although mowing is used to promote grassland *G. pneumonanthe* populations (Oostermeijer et al. 1994), it must be done in late September when *P. alcon* larvae have already left *G. pneumonanthe* (Mouquet et al. 2005). Mowing in July and August should be avoided. Another unfavourable management practice is applying fertilizers, since this leads to eutrophication which deteriorates the living conditions for the *G. pneumonanthe* and should be avoided in meadows where the plant grows.

All the core populations except Lahinjske luge are part of valuable natural features (Ur. I. RS2004bc).

Povzetek

Močvirška forma sviščevega mravljiščarja (*Phengaris alcon f. alcon*) je ena najbolj ogroženih vrst metuljev v Sloveniji in je že izginila iz več območij prvotne razširjenosti (Verovnik 2012). Na Rdečem seznamu metuljev (Lepidoptera) je v kategoriji prizadeta vrsta (E). Življenjski prostor so mokrotni travniki, kjer upravljavajo močvirski svišč (*Gentiana pneumonanthe*). To so pri nas oligotrofni mokrotni travniki (Rebeušek 2006).


Skupno smo pregledali 35,7 ha površin primernih habitatov (mokrotni travniki, gozdni rob, travniki v zaraščanju itd.) v Beli krajini. Zabeležili smo 7 populacij sviščevega mravljiščarja. Ker razdalje med sosednjimi populacijami metulja nikoli niso bile večje od 2000 m, so migracije med habitatnimi krpami možne. Sklepamo lahko, da gre za obstoj ene metapopulacije (sensu Hanski 1999). Največje lokalne
populacije vrste so bile v Lahinjskih lugah, Podlogu in Nerajskih lugah s 101, 66 in 13 ocenjenimi osebki (EPS), v tem vrstnem redu.


V naslednjih letih bo pokazalo, kakšna prihodnost čaka sviščega močvirska vrsta v Beli krajini.


Vse največje populacije razen Lahinjskih lug so v območju naravnih vrednot (Ur. l. RS2004b).

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