Biological Conservation 289 (2024) 110410

Contents lists available at ScienceDirect

Biological Conservation

journal homepage: www.elsevier.com/locate/biocon

Conserving localized endemic butterflies through demographic and ecological studies: *Polyommatus humedasae*

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ARTICLE INFO

Keywords:
Conservation measures
Extinction risk
Host plant
Management plan
Mark-release-recapture
Natura 2000

ABSTRACT

1. *Polyommatus humedasae* is an endemic species narrowly distributed in a few sites in the Aosta Valley (NW Italy). Similarly to other alpine butterflies, the survival of the species is closely linked to the conservation of semi-natural grasslands.
2. Despite the species being known since 1976, this is the first comprehensive data collection and analysis on this species, firstly to understand its distribution. At Pont d'Ael, the species core site, we applied the mark-release-recapture (MRR) method to estimate population size and density. To understand the species ecology, we collected data on preimaginal stages (eggs and larvae), host plants, and vegetation composition.
3. The new data on species distribution (with 5 new sites and 2 subpopulations already extinct) permitted to update the extinction risk through IUCN assessment to Critically Endangered (CR). The estimated size (881 individuals) and density (73 N/ha) of the studied population are higher than those of other endemic *Polyommatus* species. Data suggest the species is monophagous on *Onobrychis arenaria* and prefers to lay eggs where the host plant is abundant, in ecotonal areas with some shrub cover (average 8 %), high herbaceous cover (average 68 %) and on plants in better physiological conditions.
4. For species such as *P. humedasae*, which are threaten by woody encroachment, climate change, fires and collection, we propose the application of a management plan that includes: 1) mechanical cut of wood species; 2) small herds on a rotational basis to reduce woody encroachment and maintain shrub patches; 3) dissemination events to highlight the population importance.

1. Introduction

The Earth is currently facing the "sixth extinction wave" similar to the five previous mass extinctions in which species disappeared in a short period of time. However, this is the first major human-caused species extinction, characterized by a high speed, making it the most serious event ever (Ceballos et al., 2015). Insect populations are declining worldwide, especially terrestrial insects (Schuch et al., 2012; Van Klink et al., 2020; Hallmann et al., 2020), including butterflies (Thomas et al., 2004; Forister et al., 2011; Habel et al., 2016). The scenario is even more alarming considering that not all species that are decreasing are fully known, thus underestimating the actual biodiversity loss (Goulson, 2019). Considering that the actual number of living species worldwide is still unknown - one global estimate is from 1 to 6 billion species (Larsen et al., 2017) - we might lose species we do not even know about. Most of the gaps in our knowledge of species are in

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https://doi.org/10.1016/j.biocon.2023.110410
Received 5 October 2023; Received in revised form 27 November 2023; Accepted 1 December 2023
Available online 16 December 2023

specific geographic areas and taxonomic groups, with the largest gap being in invertebrates (Yen and Butcher, 1997; Cardoso et al., 2011; Tyler et al., 2012).

It is crucial to understand the interactions between butterfly species and ecosystems in order to plan appropriate conservation measures. However, the ecological knowledge and scientific research needed for conservation are inconsistent (e.g. Di Marco et al., 2017). Research studies are not evenly distributed among species. For instance, the monarch butterfly *Danaus plexippus* (Linnaeus, 1758; Agrawal, 2019; Grant et al., 2022) or the five European species of the genus *Phengaris* (e.g. Casacci et al., 2019) have received much more attention than other rare and localized ones, such as *Erebia christi* Rätzer 1890 or *Polyommatus ripartii* (Freyer, 1830) (Dincă et al., 2013; Parile et al., 2021). In the Palearctic region, there are 130 endemic assessed Lepidoptera species of which 36 % (47 species) are threatened (VU, EN, CR), while 8.5 % (11 species) are Near Threatened and 17 % (22 species) are Data Deficient (IUCN, 2023). Thus, there is no information about distribution, biology and ecology for 17 % of the endemic species. On the other hand, those species are often quite localized, monophagous and/or univoltine, thus more prone to extinction (IUCN, 2023). In Italy, 28 % of endemic species are endangered (EN), while only *Pseudophilotes barbagiae* De Prins & van der Poorten, 1982 is Data Deficient (Bonelli et al., 2018). For all the Italian 18 endemic species (Balletto et al., 2014) there are no specific ad hoc conservation plans developed to preserve them in long-term scenarios. Some of them are extremely localized, such as *Hipparchia sbordonii* (Kudrna, 1984), *Hipparchia leighebi* (Kudrna, 1986), *P. barbagiae,* and *Polyommatus humedasae* (Toso & Balletto, 1976). The latter two species have at least part of their range within Natura 2000 sites, even if conservation measures are not implemented there to maintain vital populations. Furthermore, we have little information on the ecological needs of these species (Balletto, 1993a; Grill et al., 2002; Casula et al., 2004; De Felici et al., 2005; Dapporto and Dennis, 2008) even if they are often important ESUs due to their phylogeographical history (Menchetti et al., 2021). However, the European Union demands the responsibility of conservation of endemic species to the single countries (Council Directive 92/43/EEC) and, at the regional level, each authority takes action for endemic species in different ways (e.g. Pollo et al., 2022).

Polyommatus humedasae is an endemic species of the Aosta Valley (NW of Italy), described for the first time in 1976. Since then, no studies on its ecology and few genetic studies have been performed (Troiano et al., 1979; Troiano and Giribaldi, 1979; Vila et al., 2010). The species is univoltine and monophagous (Middleton-Welling et al., 2020), and the host plant species has been identified as *Onobrychis montana* DC. (Balletto, 1993a). Species with such strict ecological requirements are known to be very sensitive to habitat changes (Öckinger et al., 2010). The species was classified as endangered (EN) in the most recent assessment, the population trend is decreasing, and collectors and habitat changes, specifically increases in afforestation, have been identified as major threats (Hellmann et al., 1999; Van Swaay et al., 2010; Bonelli et al., 2018). Here, we conducted hierarchical-level 1) species assessment using new and confirmed data, for the first time at the site level; 2) population size estimation of the species core distribution area; and 3) identification of the species' ecological needs at the plot and 4) plant level.

2. Materials and methods

2.1. Study area

The study area of Pont d'Ael is located in the northwestern Italian Alps (WGS84: 45°40′44.7"N; 7°13′13.3″E) at an altitude of about 900 m a.s.l. It is the core area with the largest known population of the target species. The vegetation is characterized by an alternation of open grasslands of the class *Festuco-Brometea* (dominant species: *Bromus erectus* Huds., *Festuca valesiaca* Schleich. ex Gaudin and *Stipa pennata* aggr.) and woody coenoses belonging to the class *Erico-Pinetea* (dominant species: *Pinus sylvestris* L. and *Populus tremula* L.), both related to dry continental conditions. The interconnections between these two vegetation types contribute to the abundance of forest edge communities of the classes *Crataego-Prunetea* and *Trifolio-Geranietea.*

2.2. Study species

Polyommatus humedasae is a univoltine species of the family *Lycaenidae* and endemic to the Italian province of Aosta (Toso and Balletto, 1977). Adults generally fly between July and August. The females oviposit on the inflorescences of plants of the genus *Onobrychis* Mill. (Fabaceae), laying only one egg per inflorescence (Bolognesi, 2000). The larvae develop from July to September, overwintering as larvae (Tolman and Lewington, 1997). The location and time of pupation and the myrmecophily of the species are still theoretical.

The genus *Onobrychis* includes perennial herbaceous plants typically occurring in a variety of habitats including pastures, meadows, forests, forest edges, and calcareous screes from lowland to above 2000 m a.s.l. (Aeschimann et al., 2004; Pignatti et al., 2017). The flowering period in the study area generally ranges from May to July. Balletto (1993a) identified the host plant as *Onobrychis montana*.

2.3. Sampling design

Our research was organized as a hierarchical investigation, that began with a landscape-level definition of species distribution using already known and revised sites (similar to Piccini et al., 2021a), and then reduced the scale to estimate the population size of *P. humedasae* at the site, to characterize the preferences of oviposition at plot and plant levels (similar to Piccini et al., 2022).

2.3.1. Landscape level: IUCN assessment

To determine the risk category of the species by IUCN assessment, we collected information from literature, collections, and citizen science. Only records after the year 2000 were considered. We then confirmed these localities by contacting the person responsible for each record or by personally checking some sites, during the flight period of 2022 and 2023. Moreover, we explored the nearby areas with optimal ecological conditions and host plant presence, trying to investigate homogeneously the area. These records were used to create a distribution map that included all locations where the species occurs. Then, we calculated the extent of occurrence (hereafter EOO) and the area of occupancy (hereafter AOO). Through the present study, additional data were obtained, allowing for revisioning and updating of the previous assessment through the application of the IUCN criteria (IUCN, 2022).

2.3.2. Site level: Mark, Release and Recapture (MRR) method

To estimate the population size, we marked, released and recaptured (MRR) individuals of the target species in the core area of its distribution. We identified the core area as the area with highest presence of target butterflies and its host plants.

Capture events occurred only in suitable weather conditions for 18 days between 30th June and 13th August 2022. We caught each butterfly with an entomological net and marked individuals with a consecutive number on the underside of the left hindwing using a non-toxic fine-tip permanent marker. To limit the damage due to handling specimens, we immediately released them at the same location. For each butterfly, we noted individual number, sex, habitus and GPS position (Garmin. eTrex 20 with a precision of 3 m) and time (date, hour) of the capture/recapture event. We recorded weather conditions on a scale from 0 to 3 for both wind intensity (0 = absent, 1 = weak, 2 = medium, 3 = strong) and cloud cover (0 = no clouds, 1 = few clouds, 2 = many clouds, 3 completely overcast), following Parile et al. (2021).

2.3.3. Plot level: preimaginal census and vegetation surveys

Twenty randomly distributed circular plots with a radius of 1 m were established throughout the study area. Plots were randomly selected into grasslands with some encroached by woody vegetation, with only those containing at least one individual of the host-plant species.

In each plot, we counted **eggs and larvae of *P. humedasae*** (hereafter "*P. humedasae* larvae") and stems and individuals of ***Onobrychis*** spp. on July 27, 2022. Several butterfly species lay their eggs on *Onobrychis* species, hence only eggs found on inflorescences were considered (Bolognesi, 2000). Larvae were identified following the description of Manino et al. (1987).

Within each plot, elevation (m a.s.l.), slope (degrees), and aspect (degrees North) were measured using a portable GPS device, a clinometer, and a compass, respectively. Aspect was then converted into southerness, to avoid problems associated with circular variables.

Vegetation surveys were conducted in the same plots**.** Percent ground cover (1–100 %) of vascular plant species was visually assessed, listing all species present in the plot with at least 1 % cover (Moris et al., 2017) and in each vegetation layer (herbaceous, shrub, and tree). The abundance of each species in the plot was visually estimated as a percentage of the layer. Plant species nomenclature followed Aeschimann et al. (2004). The total herbaceous cover (hereafter "Herbaceous cover") within each plot was visually estimated as a percentage. To characterize the ecological conditions of each plot, the ecological indicators nutrient (N), humus (H), moisture (F) and light (L) (hereafter "N Landolt" "H Landolt" "F Landolt" and "L Landolt") proposed by Landolt et al. (2010) were assigned to each plant species. The index was then applied to each survey by weighting the index by the values of the abundance of each species on the plot.

2.3.4. Host plant level

Eggs and larvae found on each *Onobrychis* plant (hereafter "Larvae on plant") were counted to identify which type of stem was preferred by egg-laying females. Then, the total number of stems per plant (hereafter "Stems"), the average stem height (hereafter "Stem height") and the average number of leaves per plant (hereafter "Leaves") were measured and counted.

2.4. Analyses

The recorded variables were on different scales; thus they were standardized to their *z-scores* at each level and tested for noncorrelation among them. All statistical analyses were carried out within the R environment (v.3.2.1, R Development Core Team, 2017). Each model was fitted using the package 'lme4' (Bates et al., 2015). To evaluate the dispersion of models with Poisson distribution, we used the package 'Dharma' (Hartig, 2019). PCA was performed through the package 'vegan' (Dixon, 2003). Graphs were drawn using the 'visreg' package.

2.4.1. Landscape level: IUCN assessment

The IUCN criteria B were evaluated by calculating the EOO and AOO through the 'redlistr' package (Lee et al., 2019). All the other criteria were revised.

2.4.2. Site level: Mark, release and recapture (MRR) method

POPAN, in the program MARK 8.0 (White and Burnham, 1999), was used to estimate the total population size of *P. humedasae*, which implies the existence of a metapopulation. Thus, we derived the daily survival probability (φ), recruitment rates (probability of entrance; pent), capture probability (p) and estimated the total population size (N). These parameters may be constant (.), dependent on sex (g) or on time (t). This method also provided daily population size (Ni) and daily number of entrances in the population (Bi). The POPAN approach (Schwarz and Arnason, 1996) has already been employed for butterflies, whose population size changes on a daily basis mainly due to recruitment and deaths of adults (e.g. Čelik, 2012; Weyer and Schmitt, 2013; Pennekamp et al., 2014; Jugovic et al., 2017). In MRR studies, the average life span of butterflies emerging discontinuously from pupae is calculated as $(1/(1-\varphi)) - 0.5$, derived from Deevey Jr's (1947) formula for the life expectancy of new-born individuals under the assumption of age-independent survival (Nowicki et al., 2005). The average daily survival (φ) is a weighted mean of the POPAN daily values, weighted by the number of captures on a given day (Čelik, 2012).

We then identified the model having the lowest Akaike's Information Criterion (AIC) as the best-fitting model.

To evaluate the extension of the species core area, we calculated the minimal convex hull using the GPS position of each marked butterfly.

2.4.3. Plot level: preimaginal census and vegetation survey

A hierarchical cluster analysis was performed to classify the vegetation surveys into vegetation communities with similar ecological characteristics. Differences in plant species composition between clusters were evaluated by multivariate analyses of species cover (PCA using "vegan" package). Data were preliminarily transformed according to Hellinger to express species abundances as square-root transformed proportions in each subplot (Legendre and Gallagher, 2001). The transformation was performed using the 'decostan' function in the vegan package. We added indices (N, F, L and H from Landolt et al., 2010; weighted by coverage) as post hoc environmental correlations, passively projected, using the 'envfit' function in the vegan package.

To understand the optimal ecological niche for *Onobrychis* among clusters, we then applied a Generalized Linear Model (GLM) with Poisson distribution using *Onobrychis* plants as the response variable, while cluster classification as a categorical variable was used as the explanatory variable.

The deposition preferences of butterflies on each plot were evaluated modelling the number of larvae with Generalized Linear Models (GLMs) using *Onobrychis* cover (%) and abundance (N), tree, shrub, and herbaceous cover (%), bare soil (%), southness, slope, and L and H indicator values (L and H Landolt) as continuous explanatory variables. Models followed a Poisson distribution of errors, and over/under dispersion of residuals was checked using the Dharma package in R (dispersion = 1.01, p-value = 0.848).

2.4.4. Host plant level

Butterfly deposition preferences as a function of plant factors were evaluated by modelling the sum of larvae per plant with Generalized Linear Mixed Models (GLMM), using stems (calculated as the sum of stems belonging to the plant), leaves, and stem heights (calculated as the mean between stems) as continuous explanatory variables. Plot was added as a random factor. Models followed a Poisson distribution of errors, and over/under dispersion of residuals was checked (dispersion = 1.40, p-value = 0.36).

3. Results

3.1.1. Landscape level: overall assessment of extinction risk

According to IUCN guidelines we calculated the risk of extinction for the species. According to criterion B, the geographical criterion, we considered the species currently present in 12 subpopulations and extinct in two subpopulations (Table 1). Nine historical subpopulations were checked for the presence of the species, but in two of them, both *P. humedasae* and the host plants were not present anymore (Table 1). However, five previously unknown subpopulations were found in the field season 2023. To evaluate the AOO and EOO we considered the 12 subpopulations: seven historical sites where the species is still present and the new five subpopulations (Fig. 1). AOO was estimated at 32 km^2 (8 cells of 2 × 2 km), while EOO at 45.1 km^2 (Fig. 1B). We assume the species was originally present in all 14 subpopulations in Table 1 but it is

Table 1
Polyommatus humedasae subpopulations. New sites have been obscured to prevent the species from being collected.

	Subpopulations	Sites	Elevation	Presence check	Threats	Extinction?	Source
Historical sites of presence	1	Pont d'Ael	800–1000	2022, 2023	Woody plant encroachment.	No	Vila et al., 2010; Sindaco, 2013; Balletto, 1993a; Hellmann et al., 1999
	2	Ozien-Visyes	1000	Checked presence but not found	Woody plant encroachment, management	**Probably extinct due to the high afforestation, few remaining host plants**	Vila et al., 2010
	3	Avise	800	2023	Woody plant encroachment, pesticide usage	The subpopulation has been found at higher elevation than in 2007.	Slot, 2007
	4	Mont Torretta		2022	Woody plant encroachment.	No	Sindaco, 2013
	5	Gressan (Côte de Gargantua nature reserve)		2023 (A. Battisti data), <1 km from the natural reserve	Woody plant encroachment, pesticide usage	No	Sindaco, 2013
	6	Evian	1000	Site not found			Slot, 2007
	7	Ville-Sur-Sarre	1200	Site not found			Slot, 2007
	8	St Nicolas	1200	Checked presence but not found	Mowing and irrigation	**Probably extinct, no remaining host plant**	Slot, 2007
	9	Introd		2023	Woody plant encroachment	No	GBIF
New sites	10	Aosta	1200–1300	2023	Woody plant encroachment.	No	This paper
	11	Aosta	1550–1600	2023	Grazing and habitat degradation	No	This paper
	12	Aosta	1450	2023	Woody plant encroachment.	No	This paper
	13	Aosta	1320	2023	Woody plant encroachment.	No	This paper
	14	Aosta	1000–1300	2023	Woody plant encroachment.	No	This paper

now lost in two of them (14.3 % of the population). Thus, the species population can be considered in decline with a 14 % loss. Moreover, the population is highly fragmented because all subpopulations are generally small and isolated due to ecological barriers such as forests, that has increased in the last 70 years (Fig. S1 in Supplementary material S1), and man-made infrastructures.

Criterion A regards population demography reduction while criterion B is specific to populations with restricted distributions that are also severely fragmented or with few locations (sensu IUCN, 2022) that are continuously declining. Thus, we applied criterion B, specifically criterion B1 of the IUCN assessment (IUCN, 2022). Indeed, EOO (45.1 km^2) is lower than 100 km^2 and (a) subpopulations are highly fragmented and (b) are threatened by continuing decline, observed in (iv) diminishing subpopulations over the years and in (iii) habitat declining due mainly to woody species expansion and agro-pasture abandonment in most of the subpopulations. Thus, we registered a continued decline in the quality of habitat in accordance with Işik, 2011; Orlandi et al., 2016. Criterion B1 a, b (iii, iv) (IUCN, 2022) conditions are fully met (Table 1; Fig. 1). According to our analysis, *P. humedasae* was proposed to move from EN B1ab(iii,v) + B2ab(iii,v) (Van Swaay et al., 2010; Bonelli et al., 2018) to CR B1ab(iii,iv).

3.1.2. Site level: population estimation

The core area of the population is located in Pont d'Ael and it was studied in 2022 using MRR. In total, 341 individuals were marked (males: 196, females: 134), and 127 (37.24 %) of them were recaptured, of which 26 (7.62 %) more than once. Males were recaptured more often than females, respectively, 41.73 % (53 individuals) and 33.07 % (42 individuals). Even if sex ratio might be affected by behavior, catchability and mobility in the field, it was evaluated as 1:1.46 in favor of the males.

The best model indicated that survival probability (φ) was sex-specific and dependent on trend, capture probability (p) was constant, and recruitment rate (pent) was sex- and time-specific (Supplementary material Table S1). The total estimated population size was 881 individuals, 578 ± 57 males and 303 ± 34 females (Supplementary material Table S2). The daily population sizes of females exceeded that of males only in the last third of the flight season (Fig. 2a), while their daily recruitment was lower than that of males throughout the season (Fig. 2b). The minimal convex hull was 12 ha wide and the estimated population density was 73 individuals/ha, and 26 marked individuals/ha. Moreover, we estimated an average lifespan of 6.07 days for males and 7.93 days for females. The mean and maximum number of days between the first and the last captures of the same individual were 6.76 and 38, respectively.

3.1.3. Plot level: ecological preferences

70 plant species were found in the study areas (Table S2 in Supplementary material S1). The only species of the genus *Onobrychis* observed in the study area and used as a host plant by *P. humedasae* is *Onobrychis arenaria* (Kit.) DC. (Fig. 1).

Hierarchical cluster analysis of vegetation surveys revealed four clusters belonging to communities of semi-natural dry grasslands of the class *Festuco-Brometea* (Fig. S2A in Supplementary material S1). Plots belonging to different clusters in PCA Fig. S1b are separated. The PCA shows how the plots are distributed in relation to plant species % cover, driven by Landolt values of light (L: r2 = 0.71, $p = 0.001$***), moisture (F Landolt: r2 = 0.79, p = 0.001***) and nutrients (N: r2 = 0.41, $p = 0.004$***; Fig. S2B and Table S3 in Supplementary material S1).

Onobrychis arenaria individuals showed similar abundance in all clusters (Chi.sq. = 4.536, df = 3, $p = 0.209$; Fig. S3 in Supplementary material S1).

Larvae increased with decreasing light levels (L Landolt: est. = −7.39, Z = -3.64, $p < 0.001$***; Fig. 3a), while positively correlated with *O. arenaria* abundance (evaluated as "*O. arenaria* cover": est. = 0.31, Z = 4.12, p < 0.001***; Fig. 3b; assessed as number of plants "*O. arenaria* plants": est. = 0.13, Z = 2.28, $p = 0.023$*) and with the cover of shrubs (Shrub cover: est. = 0.025, Z = 2.17, $p = 0.030$*; Fig. 3c) and herbaceous plants (Herbaceous cover: est. = 0.045, Z = 2.06, p =

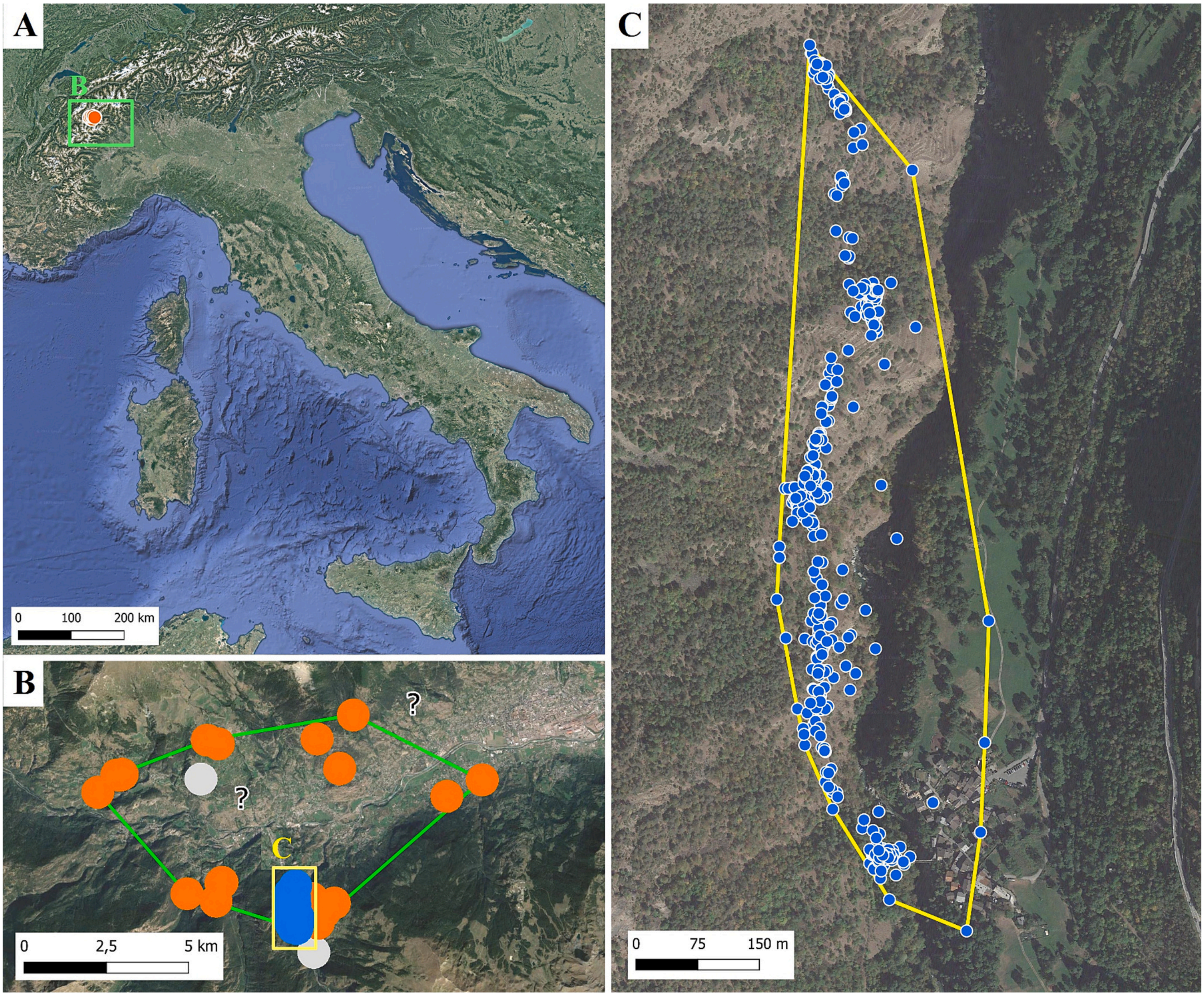

Fig. 1. A) Presence of *Polyommatus humedasae* in Italy*;* B) Distribution of subpopulations with a buffer of 500 m radius around each dot included in the subpopulation: grey areas are for those extinct subpopulations (2 subpopulations), blue for the core area (1 subpopulation), orange for the other subpopulations (9 subpopulations) and question mark for those not found (2 subpopulations) and Extent of Occurrence (green convex hull) in the Aosta Valley and Extent of Occurrence (green convex hull) in the median Aosta Valley; C) Core area (yellow convex hull) of Pont d'Ael and all occurrences as blue dots. Artworks created with QGIS. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

0.039*; Fig. 3d; Table S4 in Supplementary material S1).

3.1.4. Host plant level

Polyommatus humedasae **larvae** preferred taller plants (Height: est. = 0.69, Z = 6.46, $p < 0.001$***; Fig. 4a) with a higher number of stems (Stems: est. = 0.33, Z = 5.18, $p < 0.001$***; Fig. 4b; Table S5 in Supplementary material S1).

4. Discussion

This is the first deep study on the distribution, demography and ecological needs of *Polyommatus humedasae*. Which permit to improve the knowledge of the species. In particular, it allowed us to update the IUCN assessment and to point out conservation measures aiming to protect this species and its habitat, with broader implications on other butterflies having similar ecological needs. *P. humedasae* is now evaluated as Critically Endangered. Even though the core subpopulations are larger than expected, the threats are high and spread in all sites where the species is present. In addition, the ecological needs of the species are strongly linked to host plant abundance (consistent with other monophagous species; Ghidotti et al., 2018; Piccini et al., 2021b) and to ecotonal areas that have higher herbaceous and shrub cover and are less exposed to direct sunlight (Landolt L), likely related to higher-than-average temperatures in 2022 (data Arpa Valle d'Aosta, 2023). Considering that the species is locally threatened by various pressures, conservation measures need to be planned to protect the species in the long-term.

4.1. IUCN assessment

Polyommatus humedasae is an endemic species to Italy, so Global, European and National assessments overlap. Through the 2022 and 2023 fieldwork and based on the updated data reported here, the species will be proposed to be moved to a higher-risk category than the current assessment (EN: Van Swaay et al., 2010; Bonelli et al., 2018).

The species has a restricted range and very limited dispersal possibilities, similar to congeneric species (Parile et al., 2021). The **main direct threat** is grassland abandonment, causing shrubs and trees to

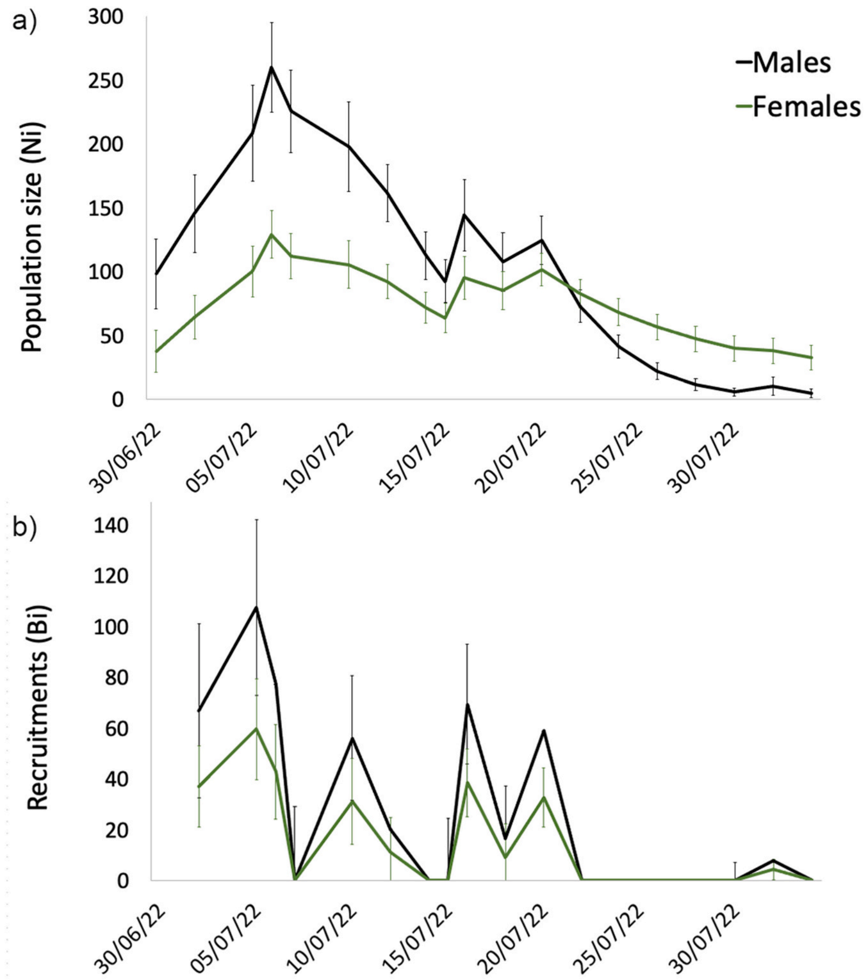

Fig. 2. Estimates of daily population sizes (Ni) and daily recruitments (Bi) of *Polyommatus humedasae* in 2022. The error lines represent the standard errors. Graphs created with excel.

invade the habitat. Due to woody species encroachment, in many sites, the host plants grow only along the roadsides, which if mowed frequently do not allow the butterfly to complete its cycle. Furthermore, the irrigation of naturally xeric grasslands and their frequent mowing causes the disappearance of the host plants. Recently a great fire has marginally affected the core area of the species, but similar events could have occurred in several location in Italy (especially the Mediterranean area; Scandurra et al., 2014) and it might occur again and with greater frequency in the future due also to climate change. It is uncertain whether trade in collected specimens is an additional threat. However, overcollectors might be dangerous in an isolated and localized butterfly species. It is however to be considered in easily accessible sites with a low number of specimens.

Thanks to the validation of recent data through a systematic review of literature, collections, citizen science data, and fieldwork, it has been possible to update the presence or absence of the species at the different sites. Two of the historical records could not be confirmed, because both the host plant and the butterfly are not present anymore. This first allowed the recalculation of its EOO, which is well below 100 km^2 (45.1 km^2), and its AOO (32 km^2). The distribution data indicates the strong fragmentation of the species' area of occupancy: the distance between the two closest *P. humedasae* populations is around 1 km. The dispersal ability of species is low, as in many congeneric species (e.g. Parile et al., 2021); this is also evident when considering the wingspan as a proxy for this trait: the average wingspan is 30.5 mm, well below the average for European and Maghreb butterflies, which is 39.38 mm (Middleton-Welling et al., 2020).

In the mountains of central-southern Europe, semi-natural grasslands are increasingly being abandoned, leading to the overgrowth of grasslands by shrubs and trees (Orlandi et al., 2016). In consequence of this, in the southern European countries, forest cover has greatly increased (70 %) in recent decades, especially in the montane and subalpine belts (Mazzoleni et al., 2004). Open habitats are therefore increasingly fragmented, reducing the dispersal ability of the species they host. The investigated area suffers from the same abandonment from traditional agriculture and pastoral activities, mainly related to management of grasslands and vineyards (Anselmetto et al., 2021). Rare and specialized meadow and pasture species in various taxonomic groups are declining (Hilpold et al., 2018). Due to the decline of its habitat, *P. humedasae* is negatively impacted by this trend. Indeed, forest and shrubs have increased from 1956 to 2022 (see Fig. S1 in Supplementary material S1), thus fragmenting open areas and creating several metapopulations of the species. We have lost 2 subpopulations (currently corresponding to 14.3 %) where there are no host plants and butterflies of the target species. However, considering that metapopulations are composed by several subpopulations that might change over the years, the percentage of loss can vary. Thus, the conditions of IUCN criteria B2 a, b (iii, iv) (IUCN, 2022) are fully met. These results support the uplisting of *P. humedasae* to Critically Endangered (CR) risk category from the previously recognized category Endangered (EN) (Van Swaay et al., 2010; Bonelli et al., 2018).

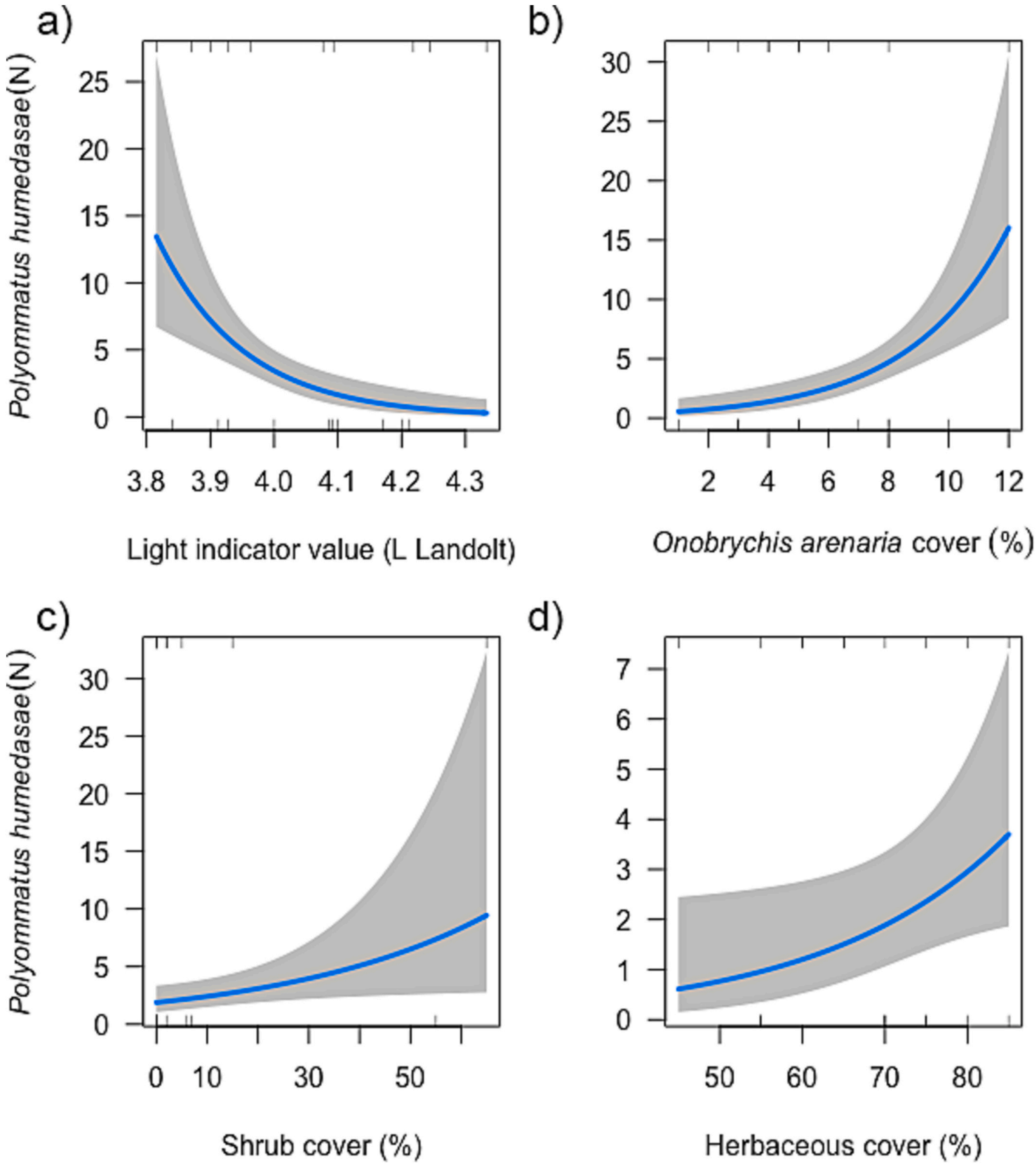

Fig. 3. Plot-level ecological preferences of *Polyommatus humedasae* larvae as a function of a) light indicator value (L Landolt), b) host plant cover (%), c) shrub cover (%) and d) herbaceous cover (%). Lines represent the best-fit models, shadows are the 95 % confidence interval. Graphs were drawn using the 'visreg' package in R.

4.2. Demography

The first adults at the site were recorded on 29 June, and no butterflies were observed after 13 August. In 2022, the flight season started earlier than previously observed (Tolman and Lewington, 1997), likely related to weather conditions. Compared to other studies of rare lycaenid species (Marschalek and Deutschman, 2008; Marschalek and Klein, 2010; Parile et al., 2021), the numbers of captures and recaptures were higher (341 and 127, respectively). The percentage of recaptures (37.24 %) was comparable to other localized populations of *Polyommatus* species (46 %; Parile et al., 2021). The number of marked females was lower than that of males, resulting in a sex ratio of 1.46. The demographic pattern with a higher male catchability and a parabolic trend in daily population size, is similar to other univoltine butterflies (e.

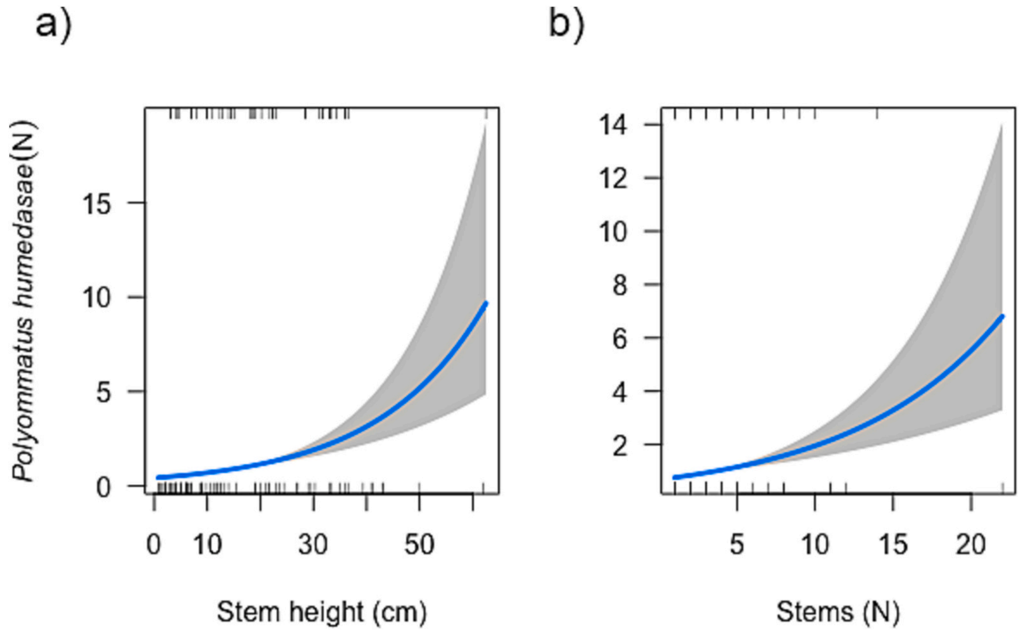

Fig. 4. Plant-level ecological preferences of *Polyommatus humedasae* larvae in relation to a) plant height and b) the number of stems. Lines represent the best-fit models, shadows are 95 % confidence interval. Graphs were drawn using the 'visreg' package in R.

g., Baguette and Schtickzelle, 2003; Fric et al., 2010; Čelik, 2012).

The longest recorded survival was 38 days and the lifespan was estimated to be 6.07 days for males and 7.93 days for females, which was higher than that of *P. ripartii* ESU *P. exuberans* (4.76 days; Parile et al., 2021), *Pseudophilotes bavius hungarica* (Dioszegy, 1913) (2.4–5.4 days; Crişan et al., 2014). Surprisingly, the lifespan was proved to be comparable to that of other univoltine large butterflies, such as *Aporia crataegi* (Linneaus, 1758) (about 7 days; Jugovic et al., 2017) but higher that other species, such as *Zerynthia polyxena* ([Denis and Schiffermüller], 1775) (4.4 days; Čelik, 2012).

The estimated population density was surprisingly high for a lycaenid species (73 estimated individuals/ha), similarly, the observed population density (26 marked individuals/ha) was higher than those previously recorded for the same species (ca. 11/ha: Balletto, 1993a) and for other lycaenids with a dot-like distribution: *P. ripartii* ESU *P. exuberans* (10/ha; Parile et al., 2021), or *P. ripartii* ESU *P. galloi* (6–7/ha; Balletto, 1993b).

4.3. Ecological needs

A major difference from previous studies (Balletto, 1993a) regards the host species. In fact, the only species found in the study area during the surveys was *O. arenaria*. The previously reported *O. montana* (Balletto, 1993a) is doubtful because it can be found locally at lower elevations in nearby areas, but it is mainly linked to higher-altitude calcareous scree slopes. *Onobrychis arenaria* is instead typical of dry steppe grasslands (Aeschimann et al., 2004; Pignatti et al., 2017), the same environments where *P. humedasae* occurs.

Due to climate change, many species have already moved to higher elevations, making the mountain environment crucial for conservation (e.g., Menéndez, 2007). In this context, vegetation composition and structure, and host plant abundance are key variables to identify ecological preferences of target butterflies (e.g. Koch et al., 2015; Piccini et al., 2022). Most European butterflies prefer open areas (Van Swaay et al., 2010), but ecotone habitats could also be important (Piccini et al., 2021b), especially in climate change scenarios where open habitats might become warmer and unsuitable for butterfly species. Indeed, butterfly habits have already been shown to change in relation to altitude, likely due to rising temperatures (Bonelli et al., 2022). It has already been recorded that species microhabitat preferences have changed in regard to climate warming (Hill et al., 2021). Indeed, at lower altitudes (warmer areas) *Parnassius apollo* and *Z. polyxena* larvae prefer shady areas while at higher altitudes (cooler areas) prefer microhabitats with reduced vegetation and higher sunlight exposure (Ashton et al., 2009; Piccini et al., 2022). Thus, those species that present higher plasticity in thermoregulation behavior (Kleckova and Klecka, 2016) and that can switch microhabitat preferences in relation to increasing temperatures might be the best suited to survive climate warming scenarios.

In our study, grassy areas that were partially shaded and rich in shrubs and shade-tolerant plants were the best oviposition sites, with large numbers of larvae. This larval preference could be due to the better host plant quality compared to host plants under a closed canopy or in completely open areas. Extremely high temperatures and low rainfall prevailed in 2022, so the partially shaded plants may have suffered less from the lack of water. Indeed, for future survival of the species in its distribution area, it is relevant to maintain even ecotonal areas that can offer suitable shaded microhabitats especially for future climate change scenarios.

The higher abundance of larvae on host plants with more and taller stems might indicate that they prefer more vigorous plants, probably to use plants that better support the larvae during their growth. Indeed, host plant characteristics may be important variables in larval survival and development (Oervoessy et al., 2014; Piccini et al., 2022).

4.4. Conservation plan

Encroachment of woody plants, climate change, and collection are the major threats to the species in the studied area, which does not have a management plan at the time of publication. However, the core area of the species is located in a Natura 2000 site, where management should be required and desirable. We propose the development and application of management measures that might be applied for several other species subject to the same threats in alpine environment (such as Nippen et al., 2021; Piccini et al., 2022). The plan should include: 1) mechanical removal (cutting or hooping, with subsequent control of any regrowth) of dense encroachment formations of woody and shrubby species such as mowing *Populus tremula*, *Pinus sylvestris*, *Berberis vulgaris* and *Rosa canina* (that might be a measure under P4 action "Restoring, preserving and enhancing ecosystems related to agriculture and forestry" of Rural Development Programme for Valle d'Aosta Region) that should leave some shaded areas of sparse shrubs and trees; 2) grazing by small herds of sheep and/or goats utilizing the biodiversity-friendly rotation (sensu Ravetto Enri et al., 2017) adapting rotation system to avoid damage of preimaginal stages (e.g. in late summer or in autumn). This management is finalized to reduce woody encroachment and maintain scattered shrubs, to benefit host plants with shading, even in the context of climate change; 3) stricter control against collectors (that might be a measure under P4 action); and 4) dissemination events for local authorities and a wide audience that could contribute to the active conservation of the species (that might be a measure of P1 "Fostering knowledge transfer and innovation in agriculture, forestry and rural areas").

Funding

"Regione autonoma Valle d'Aosta - Dipartimento Ambiente, Struttura biodiversità, sostenibilità e aree naturali protette" financially supported all activities. Author S.B. has received research support from it.

CRediT authorship contribution statement

Irene Piccini: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Alessandra Pollo:** Investigation, Project administration, Writing – original draft, Writing – review & editing. **Luca Anselmo:** Investigation, Visualization, Writing – review & editing. **Davide Barberis:** Investigation, Writing – review & editing. **Irene Regaiolo:** Investigation, Writing – review & editing. **Tatjana Čelik:** Formal analysis, Writing – review & editing. **Michele Lonati:** Investigation, Methodology, Writing – review & editing. **Simona Bonelli:** Funding acquisition, Investigation, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Irene Piccini reports article publishing charges was provided by University of Turin. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be available on figshare after acceptance.

Acknowledgments

We are thankful to Andrea Battisti for sharing the confirmation of the subpopulation of Gressan, to Roger Vila to share his information on species historical presence, to Ornella Cerise and Giacomo Marengo for their help to compare 1956 and 2022 maps. Moreover, we are grateful to Beatrice Gammino for her help during fieldwork in 2023. We are grateful also to Regione Valle d'Aosta - Dipartimento Ambiente, Struttura biodiversità, sostenibilità e aree naturali protette that financially supported all activities.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocon.2023.110410.

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