Mapping specific habitat requirements as a base for conservation measures for two strongly endangered burnet moth species in Southern France: Zygaena brizae and Zygaena cynarae (Lepidoptera, Zygaenidae)

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Abstract

Zygaena species are sensitive indicators of ecological changes and habitat destruction. Here, field results on the ecology of two highly endangered species, Zygaena brizae and Zygaena cynarae, from optimal habitats near Col d'Araud in Hautes-Alpes, France are reported. Both species have faced severe population decline in the last decades in Southwest France, probably due to habitat deterioration. During May and June 2022, larvae, imagines and egg batches were mapped in carefully characterized habitat patches. In total, 713 imagines, 67 larvae and 20 egg batches of Zygaena cynarae (which was the most common burnet moth in the area in 2022) and 23 imagines, 84 larvae, 2 pupae and 18 egg batches of Zygaena brizae were observed. While Zygaena brizae occurs both on mesophile and on semi-dry slopes (only north-and west-facing), supported by increasing numbers of the larval food plant (Cirsium tuberosum), larval habitats of Zygaena cynarae are characterized by the common occurrence of the larval food plant (Peucedanum cervaria) and by (optimally) 10-30% tree cover. Most colonized patches are south-facing and flat; open habitat patches with many larval food plants are not inhabited. All inhabited patches are extensively grazed by sheep, goats, cattle or horses-the specifics do not seem to matter. Details of grazing schedule, sunshine duration, slope exposure and other habitat parameters are reported and evaluated to support conservation measures of these burnet moth species. Implications for insect conservation: Our results provide robust data about the specific habitat requirements of these two strongly endangered burnet moth species.

Introduction

Insects play a key role in many ecological processes such as pollination, nutrient cycling, herbivory and detrivory, and they are the source of nutrients for many other animals (Yang and Gratton 2014). Their globally reported decline (Nemésio 2013; Hallmann et al. 2017; Lister and Garcia 2018; van Strien et al. 2019; Sánchez-Bayo and Wyckhuys 2019; Raghavendra et al. 2022; Outhwaite et al. 2022) therefore has a significant impact on ecosystem functioning. For example, a study conducted in the rainforest of Puerto Rico reported arthropod biomass losses ranging from 98% to 78% over a 36-year period (Lister and Garcia 2018). Parallel declines were observed in various arthropod-eating animals such as birds, lizards and frogs. The study indicates that climate change is the main driver for the decline of arthropods in the tropical rainforest and may lead to a collapse of the forest's food web (Lister and Garcia 2018). The loss of insects has also been shown to lead to the decline of insectivorous birds (Li et al. 2020; Hallmann et al. 2014). Furthermore, a model by Smith et al. (2015) suggests that the elimination of pollinators could reduce the supply of fruits by 22.9%, vegetables by 16.3% and nuts and seeds by 22.1%, putting human nutrition and global health at risk and a 50% loss of pollinators would lead to 700,000 additional annual deaths.

The effects of climate change on insects are particularly significant in tropical regions (Seebacher et al. 2015), but insect decline can also be observed in temperate latitudes (Wilson et al. 2007; Essl and Rabitsch 2013). A Citizen Science study from the UK, which counted insect 'splashes' on car number plates, found a 64% decrease in flying insects between 2004 and 2022 (Ball et al. 2022). Hallmann et al.

(2017) found a severe decline of more than 75 % of flying insect biomass over a period of 27 years in different protected areas in Germany.

The reasons for insect decline are manifold. According to Sánchez-Bayo and Wyckhuys (2019), they are, in order of importance:

1) habitat loss and conversion to intensive agriculture and urbanization

2) pollution, mainly by synthetic pesticides and fertilizers

3) biological factors, including pathogens and introduced species

4) climate change.

In addition, the change in forestry from sparse coppice forests (with or without standards) to dark forests and the abandonment of working with clear-cuts in some European countries (e. g., Germany; see Panek, 2020) created habitats no longer suitable for most insects and birds (Kozel et al. 2021; Freese et al. 2006; Hermann and Steiner 2000; Hermann 2021; Straub 2013), and has led to a decrease of non-equilibrium states in habitats, which are one of the prime pre-requisites for a high biodiversity (e. g., Connell, 1978).

While the nature and variety of reasons is mostly un-disputed, the order of importance is still a matter of debate and may be variable for different regions and scales.

No matter what the dominant reason for biodiversity decline in general and insect decline in particular is, the above studies showed that insect decline leads to severe threats for all kinds of food webs and ecosystems. To counteract this unprecedented loss of biodiversity, one must understand the important pre-requisites in terms of habitat quality. Only detailed studies for a large number of species allow for a thorough understanding of complex natural systems and to launch effective programs of nature conservation.

According to the IUCN (2020) Red List, about 20% of all Lepidoptera species are severely threatened and many national "red lists" and new studies show an unprecedented decline. Karbiener and Trusch (2022), for example, could show, that the number of species in the state of Baden-Württemberg, Germany, declined from 1970 to 2020 by 20 to 60% (depending on altitude and location). Also, the number of individuals of a specific species decreased dramatically. The addition of "climate change winners" (i.e., sub-Mediterranean species enlarging their range to the north) by far could not counterbalance the loss by the various reasons above.

Thanks to their rapid response to climate and land use changes, butterflies and burnet moths are especially suited as bioindicators. Tarmann (2009) was able to assess the influence of windblown pesticides from fruit cultivation in the Vinschgau Valley (Alto Adine, Italy) on butterfly and burnet moth communities in dry grasslands (Huemer and Tarmann 2001). Zygaena species proved to be particularly good bioindicators, as they react more quickly to air pollutants than other Lepidoptera (Tarmann 2019).

This was an important reason to conduct the present study on two strongly endangered *Zygaena* species in Southern France.

The Provence-Alpes-Côte d'Azur region (Southern France) hosts 23 of 26 French species of Zygaeninae, including the two species protected by law in France, *Zygaena brizae* and *Zygaena rhadamanthus*. This great diversity is due to a large number of different habitats between sea level and high mountains. *Zygaena cynarae* and *Z. brizae* are among the rarest Zygaeninae in the region with only a handful of populations, most of them being very isolated.

In the Provence-Alpes-Côte d'Azur region, *Zygaena cynarae* is present in four distinct geographical populations, which are all isolated from each other. The first population (subspecies *florianii*, with only one surviving location of formerly about 20) is located in the Massif de la Sainte-Baume (department of Var) between 300 and 700masl. A second metapopulation is present in the hinterland of Cannes and Antibes in the Alpes-Maritimes. Located between 30 and 430masl, it is severely threatened by urbanization. This subspecies *vallettensis* is part of a larger population from the French Alpes-Maritimes to the Italian Riviera and is akin to the *turatii* lineage, with a third population around Sospel (east of the Alpes-Maritimes). The last population flies in the Barronnies, around Éourres (Hautes-Alpes) and Lachau (Drôme) between 800 and 1000masl and is associated with the subspecies *goberti*, described from Grenoble (Isère). All populations of *Z. cynarae* in Southern France and neighbouring Liguria inhabit open mixed woodland and very sparse forests with *Cervaria rivini* (= *Peucedanum cervaria*).

In south-eastern France, *Zygaena brizae* belongs to the subspecies *vesubiana* which forms an isolated subrange from the rest of the global distribution of the species located further to the east (Hungary, Romania, Balkans, Turkey). In this western range, *Zygaena brizae* is confined to some populations in the Provence-Alpes-Côte d'Azur region (Alpes-de-Haute-Provence, Hautes-Alpes and Alpes-Maritimes), in Drôme, in Isère and in Liguria, north-west Italy. In Provence-Alpes-Côte d'Azur, two subpopulations are distinguished: one in the eastern Alpes-Maritimes up to the Ubaye valley, the other one in the Monges up to the Dévoluy and Barronnies, where the species is found in places with *Zygaena cynarae*. Both sub-populations appear to be un-connected, although there were intermediate populations still known in the mid 1900s. *Zygaena brizae* occurs in calcareous biotopes at elevations of 450-2300masl, especially between 800 and 1600masl. The habitats are former pastures and mesophilous forest hem biotope and clearings with various *Cirsium* species.

Both *Zygaena* species are rare and locally threatened by urbanisation, habitat degradation and changes in land use, e. g. the abandonment of grazing and the subsequent bush encroachment. In the regional Red List from 2014, *Zygaena cynarae* is considered "vulnerable", *Zygaena brizae* is "near threatened". Both designations are definitely too low in the light of the recent population decreases, which is the reason, why both are part of the list of species addressed in the regional conservation plan for butterflies and burnet moth species. The present work provides the most detailed account on the specific habitat requirements of these endangered *Zygaena* species in Southern France to date. It was conducted in a

particularly suited area, as it was known from previous work (e.g., Bence & Richaud, 2020), that it comprises a particularly rich Lepidoptera biodiversity including these two threatened species.

Methods

Field work

The investigated area is situated near Col d'Araud and Éourres at the border of the departments Hautes-Alpes and Drôme (Fig. 1). It was pre-mapped in 2021 by S. R. and G. M. and the particular areas of interest were then visited and mapped continuously by I. H. and P. G. between 6.5. and 5.7.2022.

Each of the various biotopes encountered in the area was assigned a EUNIS biotope type based on their plant communities (Table 1; Bissardon and Guibal 2003; Gayet et al. 2018). In addition, factors such as soil moisture, location, slope, exposure, possible presence of water bodies, tree canopy density, open ground proportion, larval host plant density (*P. cervaria* and *C. tuberosum*), old grass stands and the dominant plant species were mapped.

To investigate the preimaginal habitats of the species in question, the study areas Col d'Araud and Éourres were searched for suitable habitats, based on observations of imagines and pre-imaginal stages from previous years as well as literature information on reproductive habitats of *Z. cynarae* and *Z. brizae* (Fartmann and Hermann 2006; Nahirnić and Beshkov 2018; Nahirnić et al. 2019; Hofmann and Tremewan 2020; Bence and Richaud 2020; Lepiforum 2022; Wagner 2022). The boundaries of the different reproduction habitat patches were defined, where the habitat characteristics significantly changed. They are shown in figures 2 and 3 as 'Area mapped in detail'. Each reproduction habitat was numbered: patches 1-7 belong to *Z. cynarae*, patches 8-12 to *Z. brizae*.

Fieldwork was conducted in all patches at least once from 6.5. to 3.7.2022 using Collector for ArcGIS for mobile mapping and direct synchronization of field data to the Esri Cloud. For this purpose, we created a feature class including domains for all species present in the area to facilitate logging of new findings in the field. The larval search took place in the "search area for larvae" (Figs. 2 and 3) from 6.5. to 3.6.2022. In the patches and other promising habitats, the search was carried out multiple times. The field-routine consisted of searching the larval host plants in a previously defined area (approx. 1000-3000 m², two persons) for 50 min (approx. 100 plants per person). For each observation, the location, time, number of individuals as well as a photograph were logged into Collector. Subsequently, after the first copulae had been observed, the search for eggs took place in the period from 29.6. to 3.7.2022. For each species, three promising habitats were identified and searched for eggs, analogous to the larva search. For *Z. cynarae*, the distance of an egg clutch to the nearest woody plant was estimated as another parameter for habitat suitability. The number of eggs per clutch was counted for both species based on the photos taken.

To investigate the density of the larval food plants, *P. cervaria* and *C. tuberosum,* we randomly selected 1 m² plots distributed in our areas of interest, where the host plants were counted and, after 10 repetitions, the overall density was estimated and categorized in ArcGIS Pro for the whole areas.

Trees and the area impacted by shadows as well as bushes (larger than 0.5 m) were mapped to determine canopy/tree density which was estimated by relating these numbers to the total area of each patch and assigned to categories in steps of 10%. The distances of *Z. cynarae* egg clutches to the nearest tree or bush were also categorised in one-metre steps.

In order to compare the *Zygaena* phenology with the phenologies of the various butterfly species of the area, a background "butterfly monitoring survey" was conducted between May 5th and July 4th in the area. In total, 86 butterfly and 19 Zygaenid species (including burnet and forester moths) were observed. The details of this monitoring, however, are beyond the scope of this paper. The imagines of the *Zygaena* species were counted both within the framework of this butterfly monitoring, as well as during specific, targeted inspections.

To obtain information on the intensity and type of grazing, contact was sought to local livestock farmers. The following questions were asked: Where is grazing taking place? Which animal species graze there? How many animals graze there? When do the animals graze there? How long have they been grazing in this way? How was the area previously used? Are there any other known influences on the area, for example by pesticides or herbicides?

GIS-based terrain analysis

Mobile mapping data from Collector were synchronised to ArcGIS Pro from the Esri cloud for statistical analysis and mapping of the respective habitats. For all samples, slope and aspect values were calculated using the World Elevation Terrain service provided as authorative content in the Living Atlas (Esri). Furthermore, solar radiation was calculated for April 15th using the solar radiation analysis tools in ArcGIS Pro. Solar radiation of a specific area is calculated based on methods from the hemispherical viewshed algorithm developed by Rich et al. (1994) and further improved (Rich and Fu 2000; Fu and Rich 2000, 2002; Fu 2000). The tool estimates direct and diffuse radiation as well as duration of direct incoming radiation based on location, digital elevation data and some parameters provided by the user. We integrated radiation over one day using eight zenith divisions, eight azimuth divisions and a "Uniform overcast sky" diffuse model type with 0.3 diffuse proportion and 0.5% transmissivity.

Results

General habitat characterization

The study area is located in the mountainous limestone massifs of the Southern Pre-Alps (Bence and Richaud 2020) and was divided for logistic reasons into three parts, Col d'Araud west, Col d'Araud east and Éourres (Fig. 1). Climatic conditions correspond to those of the winter-humid subtropics, which combine hot dry summers with temperate humid winters (Schultz 2016; Climate-data.org 2022) .

From a geological point of view, the area is in the western part of the Helvetic units of the Southwestern Alps (Veit 2002) and comprise dominantly limestones and some shales. In the sub-areas of Éourres and

Col d'Araud west, only strata of the lower Cretaceous are present, in the north-eastern corner of Col d'Araud east, additionally the highest strata of the Upper Jurassic are found (BRGM 2022). These rocks form a continuous, about E-W striking ridge about 4 km long with a south-facing, very dry slope which is (as other south-facing habitats, e.g., in the valleys) exposed to extreme temperatures up to 40°C in summer. It is mainly dominated by garrigue. The northern slope, on the other hand, is characterised as a dense coniferous forest (Pinus sylvestris). The transition from garrigue to coniferous forest is dominated by sparse to dense Quercus pubescens stands.

The EUNIS habitat types with the largest areal proportion are *Genista cinerea* garrigue (F6.62), supramediterranean *Pinus sylvestris* woodland (G3.49) and western *Quercus pubescens* woodland (G1.711) (Table 1; Bissardon and Guibal 2003; Gayet et al. 2018). The dominant plant species are *Genista cinerea, Pinus sylvestris, Quercus pubescens ssp. pubescens, Buxus sempervirens, Sorbus aria, Lavendula angustifolia* and *Juniperus communis*.

In the Éourres sub-area, a creek flows approximately N-S through dry steep slopes with different exposures. Biotic as well as abiotic factors such as sunshine hours, humidity, vegetation density and species composition vary on a small scale. The most common biotope types here are *Genista cinerea* garrigue (F6.62), Mediterranean mountain meadows (E1.5) and Mediterranean floodplain forest (G1.3). The dominant plant species are *Genista cinerea, Salix purpurea, Salix eleagnos, Thymus sp., Salvia sp., Vicia sp.* and *Lavendula angustifolia*.

The elevation of patches 1-7 of *Z. cynarae* ranges from 775 to 980m, the dip of the slope varies from 0° to 20°. The exposure of five of the *Z. cynarae* patches tends towards the south or southeast. The canopy cover of the *Z. cynarae* patches is 10-20 % for one patch, 20-30 % for four patches, 40-50 % and 80-90 % for one patch each. The proportion of open ground is between 0-20 % in all *Z. cynarae* patches except for patch 6, where it is 60-70 %. On 15.4.2022, the *Z. cynarae* patches had an average of 11.5 hours of sunshine.

Patches 8-12 of *Z. brizae* are situated at altitudes between 880 and 1025m, facing west or north, with dips ranging from 8° to 30°. The canopy cover of *Z. brizae* patches varies between 0 and 60 %. The proportion of open soil usually is 20 to 30 %, only once, 80 to 90 % was observed. The average duration of sunshine of the *Z. brizae* patches on 15.4.2022 was 11 hours. All these data are shown in Table 1.

 Pre-imaginal and imaginal stages of Zygaena cynarae

During the entire study period from 5.5.-4.7.2022, 67 larvae, 713 imagines and 20 egg clutches of *Zygaena cynarae* (Fig. 4) were observed in the Col d'Araud east and west areas (Table 2; Fig. 5). 51 of these larvae and 20 egg clutches were found within the patches mapped in detail (yellow in Fig. 5), the remainder outside these patches. Fig. 6 displays the phenology of the species. Most imagines (n=184) were found on 23.6.2022 in the Col d'Araud east area. In total, 18 copulae were observed between 17.6. and 27.6.2022. The 20 egg clutches contained an average of 32 eggs, with a variation from 7 to 70. Newly hatched L1 larvae in patch 6 were found on 25.6.2022.

Larvae and egg clutches were found in habitats with very variable larval food plant (*P. cervaria*) densities (Figs. 7 and 8): most observations were made in habitats with 5-10 plants/m² (20 larvae and nine egg clutches). Nine larvae and two eggs clutches were found in habitats with higher plant densities of 10-20 plants/m², five larvae and no egg clutches in habitats with more than 20 plants/m², six larvae and four egg clutches in habitats with plant densities of 2-5 plants/m², nine larvae and three egg clutches at 1-2 plants/m² and, finally, two larvae and two egg clutches in habitats with very low plant densities of only 0-1 plant/m².

The correlation between the number of egg clutches or larva observations and the canopy cover in the reproduction habitats is shown in Fig. 9. Most observations (with 31 larvae and 16 egg clutches) were made at 20-30%, 17 larvae and four egg clutches at 10-20% canopy cover. In habitats with 40-50% canopy cover, only one larva and in the only habitat with 80-90% canopy cover, only two larvae were found. No egg clutches were found in any habitat outside of 10-30% canopy cover groups. Interestingly, no egg clutches and no larvae were found in a completely tree- and bushless habitat with very high *P. cervaria* density in patch 3 (see Fig. 7), despite considerable searching efforts.

The distance of the egg clutches to the nearest tree trunk or bush is shown in Fig. 10, the data are visualized on the maps in Fig. 11. Ten of the 20 egg clutches found were only 0-1m away from the nearest tree or bush, another seven were between 1-2m and only one egg clutch each was found at a distance of 2-3, 3-4 and 4-5m. No egg clutch was more than 5m away from the nearest tree or bush.

Pre-imaginal and imaginal stages of Zygaena brizae

A total of 84 larvae, two pupae, 23 imagines and 18 egg clutches of *Zygaena brizae* (Fig. 4) was found in the period between 7.5 to 1.7.2022. As shown in Fig. 12, most of the sites were located in the little river valley west of Éourres (patches 8, 9 and 11, see Figs. 3 and 7; Map A). Some additional larvae and imagines were observed in a small area at Col d'Araud (patch 10; Map B) and a few imagines and larvae southeast of the village of Éourres (patch 12; Map C). The larvae and the egg clutches of *Z. brizae* were found exclusively on *Cirsium tuberosum*.

Table 3 reports the dates and the specific areas where the different developmental stages of *Z. brizae* were found; Fig. 13 illustrates the phenology. Most larvae were found on 21.5.2022 in the area W of Éourres. The number of eggs per clutch varied between three and 17 with an average clutch consisting of 7.5 eggs. Figure 14 shows the correlation between larval hostplant density and pre-imaginal stage observations in the areas mapped in detail. Most pre-imaginal stages of *Z. brizae* were found in areas with a *C. tuberosum* density of 2-5 plants/m².

Grazing regimes

The different grazing strategies in various parts of the study area are shown in Fig. 15. In areas 1-4, a total of 10 cows graze during different periods. Area 1 is grazed from September to November, area 2 from February to March, area 3 from December to January, area 4 is grazed from July to August. About

300 sheep and 10 goats graze area 5 in October and about 400 sheep graze area 6 in July. It is a system of rotating plots that changes every two or three years. Areas 7 and 8 are grazed by 4 to 15 horses in September, from May to September additionally on a daily basis, if horseback riding by tourists is requested. Area 9 is grazed by ten horses from mid-May to mid-June. The exact times of grazing may vary from year to year by one week or ten days.

In areas 1-4, the land has been managed in this way for the past two years. Previously, horses and goats grazed there in similarly small numbers. There is no information on previous grazing strategies for area 5. The about 400 sheep in area 6 have been brought there in July also for the past two years. In previous years, about 60 sheep and 40 goats occupied the area, but the timing and duration could not be figured out. Also, there is no further information for areas 7-9, which are grazed by horses.

Discussion

Uncertainty of estimates

The results of the larvae and egg search do not reflect the population size of *Z. cynarae*, since only a small portion of larvae and eggs can be found, even during a long and tedious search. The search for larvae was complicated by the number of host plants to be examined and the very delicate nature of the feeding marks of *Z. cynarae*.

In contrast, larvae of *Z. brizae* produce very conspicuous feeding marks and, thus, significantly more larvae than imagines could be observed. Hence, population size estimates important for deciding the importance of conservation measures, should be done using larval counts for *Z. brizae,* but imago counts for *Z. cynarae*. However, it was not possible to determine the absolute population size of the two target species, as no mark recapture or removal methods were used. Furthermore, extrapolation from the total number of observed imagines to population size is strongly species-dependent. Multiplier values vary e.g. from below two for *Z. ephialtes*, *Z. loti* and *Z. transalpina* (Gottschalk 2020) to about five for *Z. viciae* (Barbour et al. 2021).

The distance between *Z. cynarae* egg clutches and woody plants was estimated in the field and it is guessed that deviations of up to 50cm are possible. The distribution of larvae and egg clutches in the whole study area may give a distorted picture of the actual distribution in the study area (Hermann 2006). In the case of *Z. cynarae*, more larvae were found in the western area of the Col d'Araud than in the eastern area, although imago observations were higher in the east. This may be related to the roughness of the terrain, as the western area is much more accessible than the eastern area (where more shrubs and bushes hamper the larval search at many places). This made searching for larvae and eggs at Col d'Araud west probably more successful. In the case of *Z. brizae*, further habitats on steep scree slopes, which are difficult to access, may have been overlooked.

Reproduction habitat requirements of Zygaena cynarae

The study shows, that in the Col d'Araud area, a vivid and healthy metapopulation of *Z. cynarae* exists, which is distributed over an area of at least 3 by 1,5km (Figs. 3 and 5). The distribution of both pre-imaginal and imaginal observations indicates that there are at least seven small, inhabited patches each of which is less than 1km away from the next patch.

In agreement with the data of Bence and Richaud (2020), the first imagines flew in mid-June, the flight time peak was in mid/late June (Fig. 6). The number of observations decreased rapidly by the end of June/beginning of July.

The number of observations of imagines can provide an approximate information about the population size (Gottschalk 2020; Collier et al. 2008; Barbour et al. 2021), because the probability that an individual is counted twice on the same day is considered low, as *Z. cynarae* imagines rarely fly (Hofmann and Tremewan 2020; Pellet et al. 2012) and can be easily determined. Based on a "cautious" multiplier value of two (Gottschalk 2020), the minimum population size at Col d'Araud was about 1500 imagines in 2022. Interestingly, despite the high population density at Col d'Araud, not a single individual was found in the Éourres sub-area (which is only 2km away) during regular surveys over a two-month period. This shows that the species is strongly tied to its pre-imaginal habitat and only very slowly disperses, if at all, to more distant habitats. The observations of three imagines more than 500m away from their pre-imaginal habitats (see Fig. 5) along or close to the D24 road indicates, however, that a dispersion of several hundred to probably 1000m is realistic, which is important for this metapopulation.

Both pre-imaginal stages and imagines of *Z. cynarae* were predominantly found in garrigue habitats and in a former orchard (patch 1, Figs. 3 and 5), which is consistent with known habitat characteristics (Nahirnić and Beshkov 2018; Hofmann and Tremewan 2020; Wagner 2022). The best reproduction habitats at Col d'Araud seem to be closer to the forest than to adjacent openland biotopes (see Fig. 7). This implies that trees have a positive effect on habitat suitability, which is also shown in Figs. 9, 10 and 11. This may be related to trees providing shadow in the very hot and dry habitats. However, also the habitat preferences of the larval host plant could influence, in which micro-habitats the larvae are found in a specific patch, as Gils et al. (1975) assigns *P. cervaria* to the thermophilic fringe plant community at the edges of, for example, *Q. pubescens* forests.

A bare ground proportion of 0-10% seems to favour *Z. cynarae*, although the exception of patch 6 indicates that if other habitat characteristics such as host plant density and canopy cover are ideal, the bare ground is of less importance. In patch 6, the host plants on which larvae and egg clutches were found, grew in the shade of large trees or bushes which appears to be more important than host plant density. Only a weak and qualitative relationship exists between *P. cervaria* density and the abundance of pre-imaginal stages. Larvae and egg clutches were rarely found at low abundance of *P. cervaria*. Although the number of larvae increases with plant density up to a certain optimal value (5-10 plants/m^2, see Fig. 8), other factors such as canopy cover or humidity may shift these limits up or down. Obviously, the

minimum number of host plants is related to the number of eggs in an egg clutch (on average 32) which results in a high number of slowly moving larvae in a small space. If the host plant density is too low, many larvae would starve to death. There is no obvious reason for an upper limit of larval host plant density; the only habitat with densities up to 20 plants/m^2 was devoid of trees, which is obviously (probably because of the heat) a knock-out criterion for this patch (see Figs. 7 and 10).

Accordingly, the most important criterion for habitat suitability (in addition to the sheer presence of enough food plants) is the canopy cover. The present study indicates a preferred canopy cover between 10 and 30%, which agrees with the qualitative statements of Hofmann and Tremewan (2020), Nahirnić and Beshkov (2018) and Wagner (2022). *Zygaena cynarae* seems not to colonize habitats without woody plants (see Figs. 7 and 10).

The micro-climatic arguments of trees proposed above are supported by the preferred distance of oviposition sites to the nearest woody plant: 17 of the 20 egg clutches were found at less than 2m from the nearest woody plant which is probably related to the larvae's thermoregulation. Too high or too low larval body temperatures can reduce the feeding rate and, thus, also the development speed, as was shown for various butterfly species (Rawlins and Lederhouse 1981; Bryant et al. 2000; Zografou et al. 2022). Some species can digest more efficiently at higher body temperatures, as long as the temperature remains below the critical limit (Rawlins and Lederhouse 1981). If this also applies to *Z. cynarae*, the possibility of thermoregulation in the habitat is essential and trees and bushes help to manage it.

Five of the seven patches are exposed to the south or southeast, the remaining two patches are flat. Again, this observation of south-facing larval habitats may be related to both the thermophilic properties of the host plant (Gils et al. 1975), or those of the larva (Rawlins and Lederhouse 1981; Bryant et al. 2000; Zografou et al. 2022) or both.

The very extensive grazing of the area is probably one of the decisive factors (if not the most important one) for the high biodiversity at Col d'Araud. The heterogeneous, mosaic-like distribution of the biotopes ensures a variety of ecological niches which in general supports high biodiversity (Habel et al. 2021). Although the precise type of grazing is highly variable in the whole study area, in terms of animals and in terms of the time, when grazing is done, no preference of *Z. cynarae* for any of these grazing strategies is evident - either, because the grazing animals do not eat *P. cervaria* or because the protection strategies of the larvae (dropping from the plant, if it is touched) work. It should be noted, that the type of grazing in the Col d'Araud east and parts of west changed from horses and goats to exclusively cows two years ago. In the period from 1900 to 2019, only 23 observations of the species were reported near Éourres (Bence and Richaud 2020). In 2020, ten observations were made in one day (Artemisiae - Lépidoptères de France 2022). In 2021, the last author could observe about 50 individuals in one day and in 2022, up to 184 individuals were observed in one day. This trend could imply that the population of *Z. cynarae* currently benefits from the change in grazing regime.

Reproduction habitat requirements of Zygaena brizae

Figure 12 shows five reproduction habitats of *Zygaena brizae*, of which patch 8 is the most important one (it is shown in Fig. 7), with a total of 83 pre-imaginal observations. Imagines were observed only in the short period between 16.6. and 30.6.2022, which, however, matches the literature (Bence and Richaud 2020). The period of 14 days between the last larva and the first imago observation also corresponds to the pupation period of 12-14 days known for *Z. brizae* (Hofmann and Tremewan 2020). The pupae were found close to the ground at the base of the leaves of the forage plant. The typical *Z. brizae* method of laying eggs in the tomentum of the host plant (Hofmann and Tremewan 2020) was also observed, but the egg clutch size with an average of 7.5 eggs per clutch is significantly larger than described by Hofmann and Tremewan (2020). This discrepancy may be due to our definition of an egg clutch as the total number of eggs on the underside of a specific leaf, whereas Hofmann and Tremewan (2020) described single or small groups of two to four eggs of which several were found on a single leaf. The time between the first finding of an egg clutch and the first freshly hatched L1 larvae was five days. In Georgia, Hofmann and Tremewan (2020) observed an egg phase of 6-7 days.

It is striking that only 23 imagines, but 103 pre-imaginal stages were observed. This apparent discrepancy is related both to the very conspicuous feeding marks of the larvae, to the larger distribution of eggs than in the case of *Z. cynarae* (the females of *Z. brizae* lay many small clutches, often not far from each other, instead of a few large clutches like *Z. cynarae)* and the small imagines are relatively sedant and remain hidden below flowers, buds or leaves during much of the day, in contrast to many other Zygaena species. *Zygaena brizae*, like most other burnet moths, is strongly bound to its larval habitat and rarely travels long distances (Hofmann and Tremewan 2020; Ebert 1994; Naumann et al. 1999). Zarzycki and Dąbrowski (2014) even described *Z. brizae* as particularly stenotopic.

As can be seen in table 1, patch 8, and thus most of the pre-imaginal findings, are in the EUNIS habitat type of Mediterranean Mountain Meadows. Patches 9, 10 and 11 are in the *Genista cinerea* garrigue and patch 12 is in the western *Quercus pubescens* forests. At first glance, the habitats differ greatly from each other in terms of biotic and abiotic factors, but a closer look reveals that the micro-habitats preferred for oviposition are very similar.

For example, all microhabitats chosen for oviposition show a very high proportion of open soil (between 20 and 30 % in four of the five patches mapped in detail, even 80-90% in the remaining patch 9). In addition, each habitat is on a gentle slope with a dip of 8° to 30°. These observations may be explained by the reproduction strategy of the larvae's food plant *C. tuberosum*, which is a ruderal and pioneer plant, favoured by early succession stages. Interestingly, the ideal habitat in patch 8 is characterized by small landslides and thus offers *C. tuberosum* particularly good growth conditions. The number of pre-imaginal observations is highest in areas with a density of 2-5 *Cirsium tuberosum* plants/m² (Fig. 14).

The density of woody vegetation does not seem to play a role in the reproduction habitats of *Z. brizae*, as long as it is not a closed forest, as detailed mapping revealed values between 0 and 60 % with an average of 0 to 20 % canopy cover. *Zygaena brizae* appears to prefer open to semi-open habitats. Patch 12 has a canopy cover of 50-60 % and it is noteworthy that the imagines moved quite far away from their larval

habitat (compared to the other patches, see Fig. 12), probably to have sufficient access to nectar plants. This suggests that the sub-population of patch 12 is in danger of extinction due to increasing scrub encroachment.

The mean sunshine duration for the habitats of *Z. brizae* are eleven hours per day on April 15th, the scatter is low (10.3-11.7 hours). Interestingly, *Z. brizae* prefers areas with fewer sunshine hours per day compared to *Z. cynarae*. The exposure of the habitats is typically to the north or west (Table 1) and not to the south as in the case of *Z. cynarae*. The north-exposed habitats are cooler and more mesophilic (Nahirnić et al. 2019). Interestingly, the obviously ideal habitat in patch 8 is among the coolest of the five reproduction habitats due to the many trees in the direct neighbourhood. With dips between 8° and 30°, *Z. brizae* habitats cover most dips of the whole area (0°- 38°), but no clear preference was observed.

Z. brizae is, due to its larval food plant *C. tuberosum*, favoured by early successional stages and intermittent, recurrent disturbances. As most habitats lie along a popular hiking trail, also for horseback tours (patches 8, 9 and 11), or at a gate in a pasture (Patch 10), these disturbances are assured. Extensive grazing by horses and cows also contributes to the persistence of these habitats. Increasing scrub encroachment could be problematic in the future, as can be seen in the isolated habitat SE of Éourres (patch 12). This breeding habitat with a canopy density of 50-60 % seems to become less and less favourable as reproduction habitat for *Z. brizae* because of increasing darkness and lack of nectar plants. The small patch at Col d'Araud (patch 10) suggests that there may be additional occurrences of *Zygaena brizae* in the vicinity, also outside the area investigated in the present study. These, too, are likely to be very small-scale, mesophilic habitats that can easily be overlooked.

Declarations

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Compliance with ethical standards

This study was conducted without doing harm to any animal.

Material availability

All data used in the present sudy are part of thie present publication, no data have been deposited anywhere.

Authors' contribution statements

All authors have participated in evaulation of the data and writing the manuscript, the lead was done by I.H., P. v. d. W. and G. M. I. H. and P. v. d. W. did the major part of the field work, S. R. and G. M. a minor part. Technical issues were dealt with by M. B.

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Tables

Tables are available in the Supplementary Files section.

Figures

Figure 1
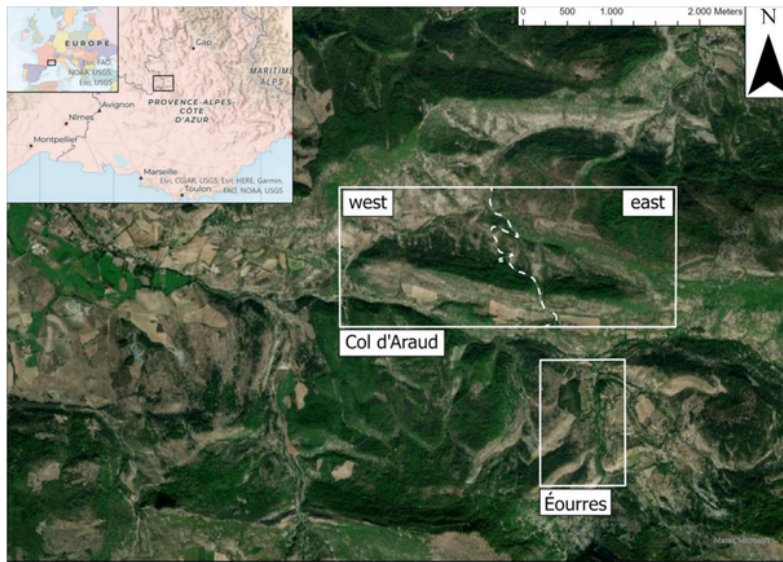
Figure 1

Overview of the area and its position in Europe.
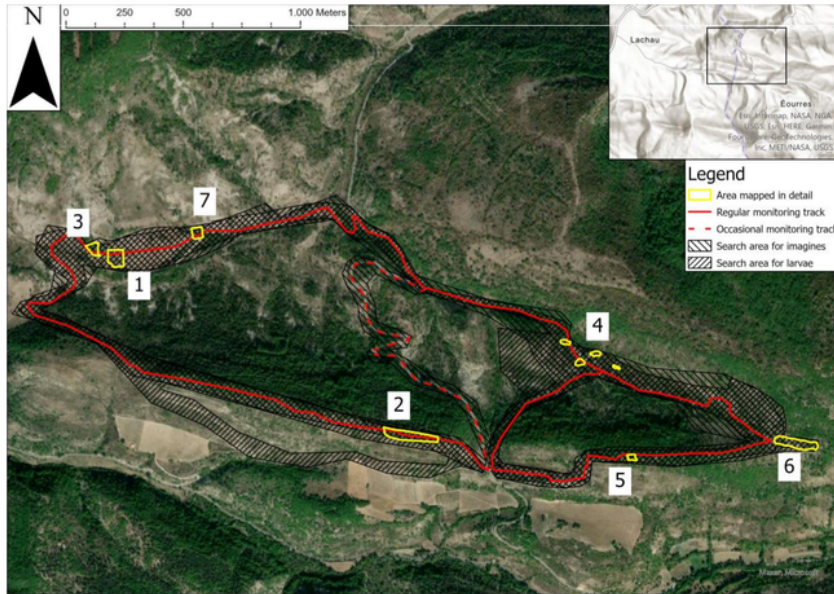
Figure 2

Map of the area around Col d´Araud including the areas investigated in detail for pre-imaginal and imaginal stages (in black hatching), as well as the regular monitoring track (red) and the areas mapped in detail for *Zygaena cynarae* (Patches 1-7, in yellow).

Figure 3
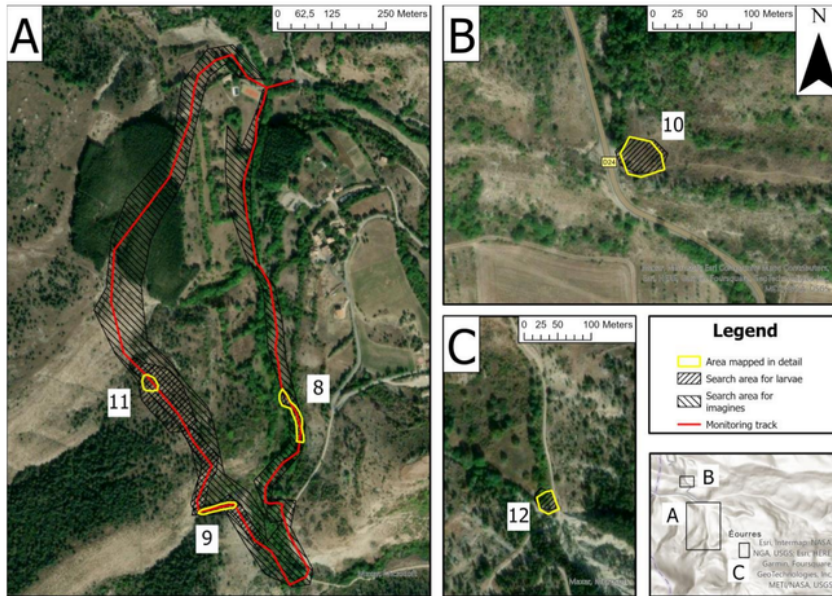
Figure 3

Maps of the sites investigated in detail for *Zygaena brizae*, and their position in the Col d'Araud-Éourres area. The habitats searched for pre-imaginal and imaginal stages in great detail are hatched in black, regular monitoring tracks are shown in red, the patches, where pre-imaginal stages were actually found, are shown in yellow **A** shows the patches 8, 9 and 11 west of Éourres, **B** shows patch 10 at Col d'Araud, and **C** shows patch 12 southeast of the village Èourres.

Figure 4

Zygaena cynarae   Zygaena brizae

Figure 4

Field observations from the area around Col d'Araud and Éourres. A. to D. An egg batch (some blackish eggs are close to hatching), a fully grown larva, typical feeding marks and a copula of *Zygaena cynarae*. E. to H. An egg batch (some blackish eggs are close to hatching), a fully grown larva, typical feeding marks and a copula of *Zygaena brizae*.

Figure 5
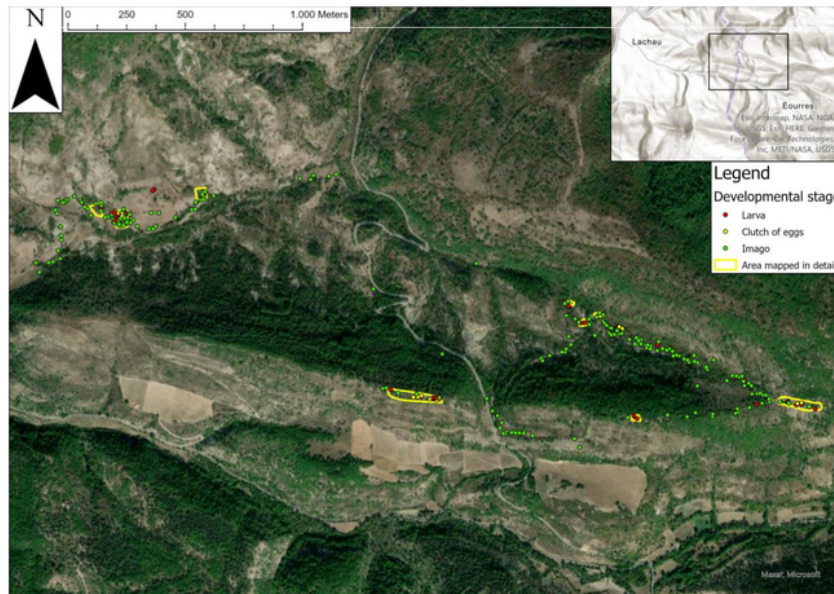
Figure 5

Map of Col d'Araud showing all observed larvae (red dots), imagines (green dots) and egg clutches (yellow dots) of *Z. cynarae*; areas framed in yellow are those mapped in detail for e. g. food plant densities (see below).

Figure 6
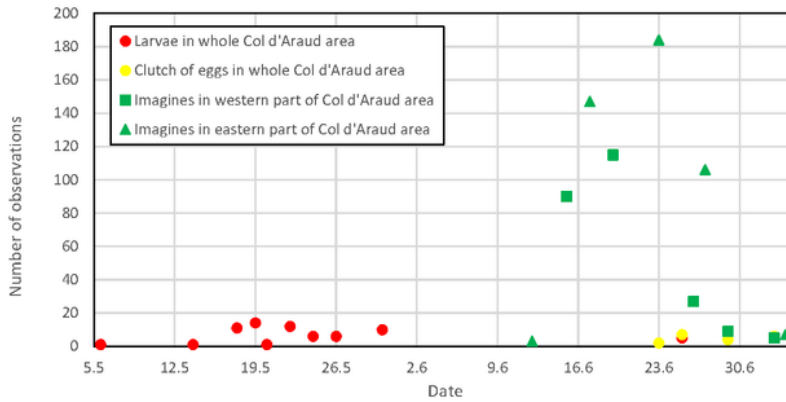
Figure 6

Phenology of *Z. cynarae* in the Col d'Araud east and west areas; red dots represent the number of larvae in the whole Col d'Araud area (n = 67), green squares represent the number of imagines in Col d'Araud west (n = 246), green triangles in Col d'Araud east (n = 447), yellow dots show the number of clutches of eggs in the whole Col d'Araud area (n = 20).

Figure 7

Figure 7

Habitat types in the Col d'Araud-Éourres area. A. Typical semi-open garrigue habitat of *Zygaena cynarae* at Col d'Araud. B. Habitat between patches 1 and 3 with much *P. cervaria*, but without any woody plants - no larvae were observed here. C. and D. Patch 8, the best reproduction habitat of *Zygaena brizae* next to a hiking path west of Éourres. *Cirsium tuberosum* plants ae visible left of the path, as is the open gravel

slope. E. This quite steep slope with much open soil west of Éourres is habitat patch 9, in which a copula, larvae and eggs were found.

Figure 8
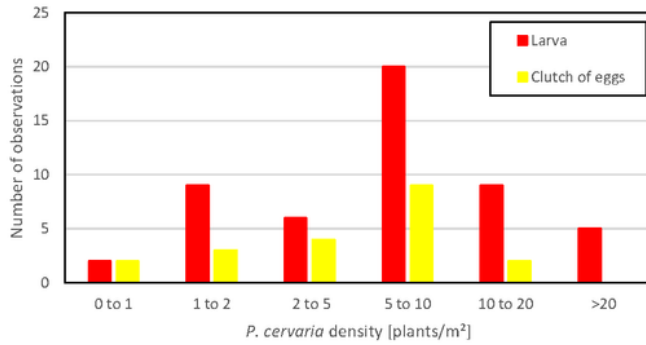
Figure 8

Abundance of caterpillars (n=51) and egg clutches (n=20) of *Z. cynarae* in relation to *P. cervaria* density (see text for details).

Figure 9
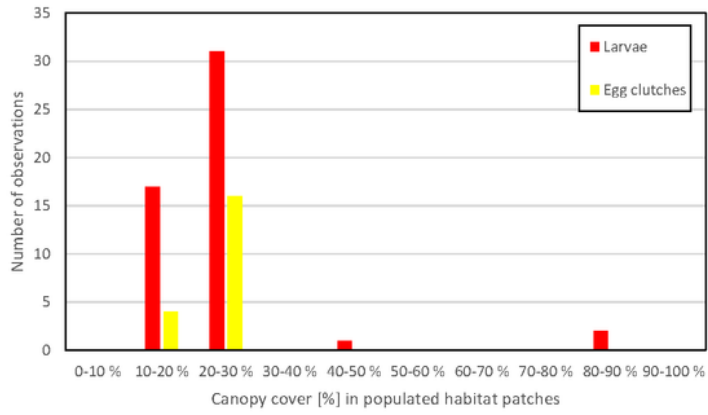
Figure 9

Number of caterpillars (n=51) and egg clutches (n=20) in relation to canopy cover.
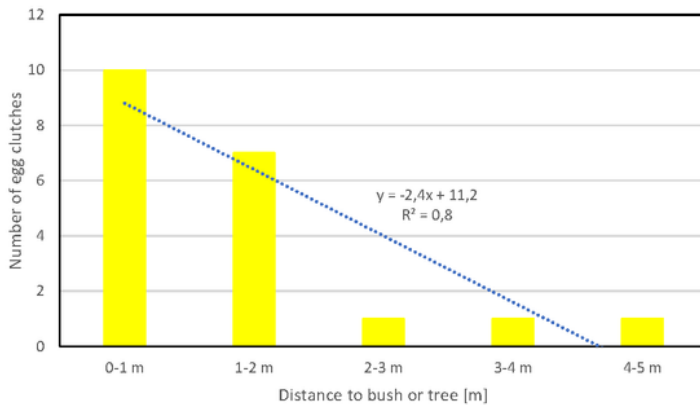
Figure 10

The chart shows "Number of egg clutches" on the y-axis (0 to 12) and "Distance to bush or tree [m]" on the x-axis with categories 0-1 m, 1-2 m, 2-3 m, 3-4 m, 4-5 m. The trend line equation is $y = -2{,}4x + 11{,}2$ and $R^2 = 0{,}8$.

Figure 10

Detailed maps of patches 1 (center) and patch 3 (left). Map A shows canopy cover and proportion of open soil in the areas, Map B shows the different plant densities of P. cervaria [plant/m²]. Both maps show the positions of larvae and egg clutches of Z. cynarae.

Figure 11
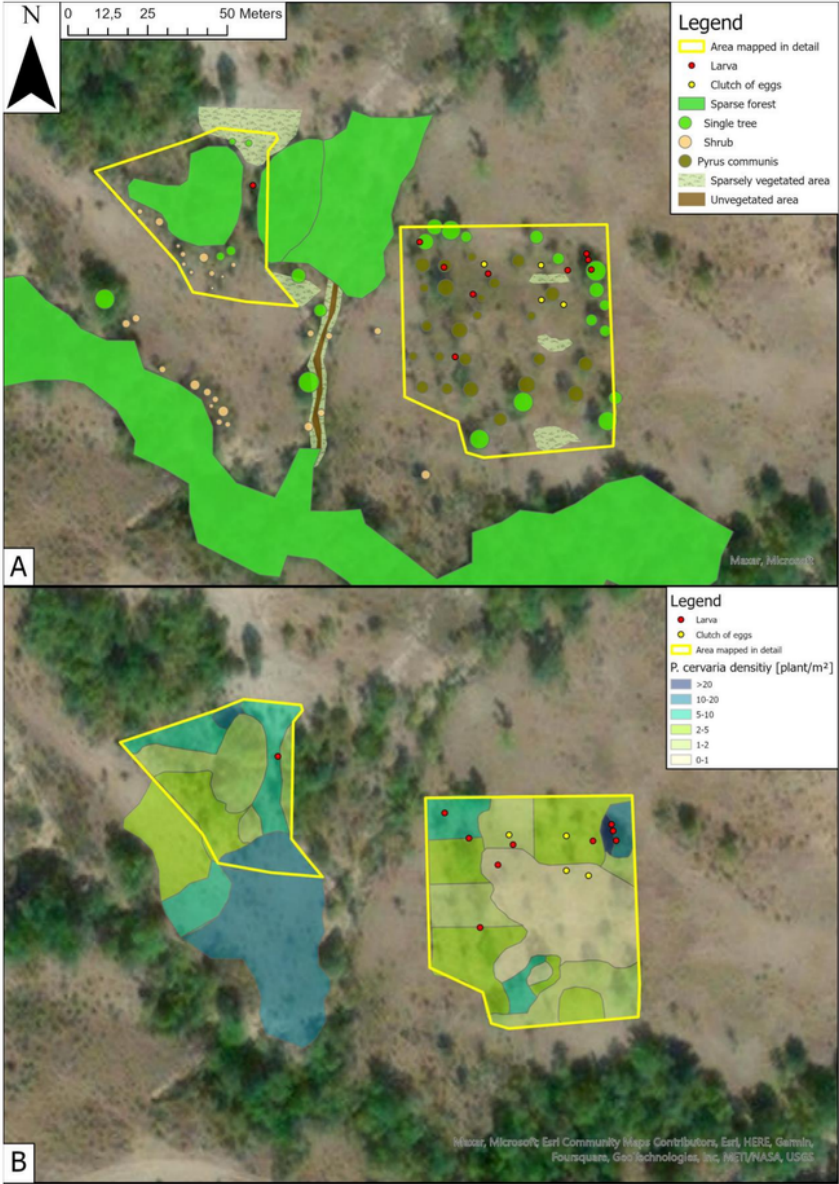
Figure 11

Relation between oviposition site and distance to next bush or tree (n=20).
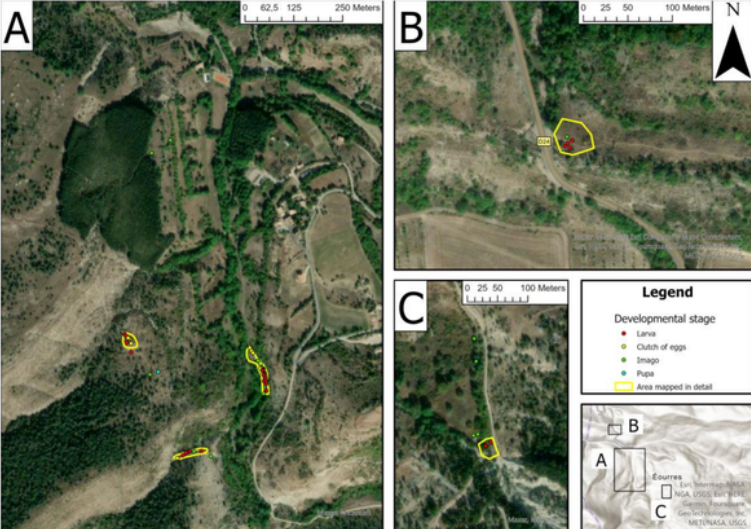
Figure 12

Figure 12

Maps showing all observed larvae (red), imagines (green), pupae (blue) and egg clutches (yellow) of *Z. brizae*.
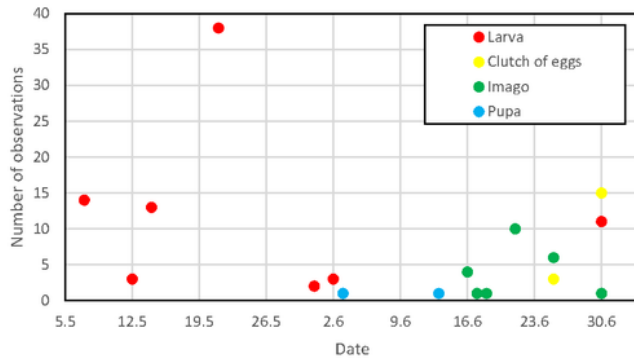
Figure 13

Figure 13

Phenology of *Zygaena brizae*; red dots represent the number of larvae (n = 84), blue dots the number of pupae (n = 2), green dots the number of imagines (n = 23) and yellow dots the number of clutches of eggs (n = 18).
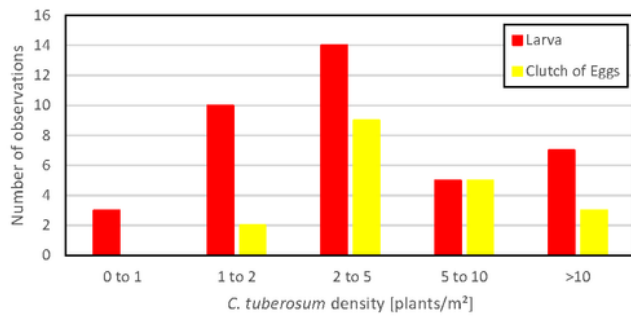
Figure 14

Figure 14

Abundance of larvae (n = 84) and egg clutches (n = 19) of *Z. brizae* in relation to *C. tuberosum* density.
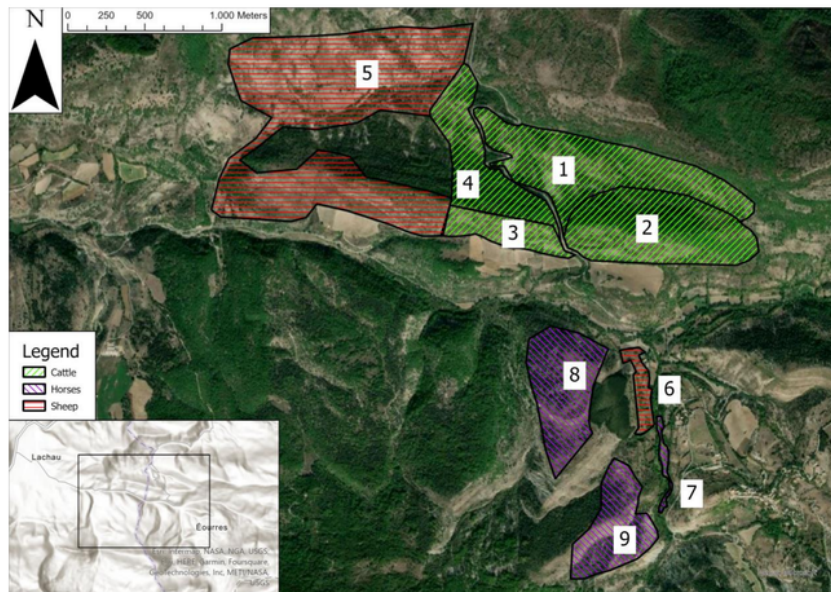
Figure 15

Figure 15

Map displaying the different grazing regimes in the study area; the numbers refer to the patch numbers used in the text.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.pdf
- Table2.pdf
- Table3.pdf