A sunny spot: habitat management through vegetation cuts increases oviposition in abandoned fields in an endemic Mediterranean butterfly

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Abstract. 1. The abandonment of agricultural land and the afforestation of grassland habitats represent major threats for butterflies in European and Mediterranean areas. A crucial goal for Lepidoptera conservation is to maintain and/or restore habitat quality by targeted management. Nevertheless, there are few experimental studies allowing to derive data-driven strategies to protect butterflies of open grasslands in the Mediterranean region.

2. We developed a habitat management strategy for the conservation of the Italian endemic butterfly Zerynthia cassandra by adopting a three-step procedure: (i) characterising which environmental and host plant features influence oviposition on plants; (ii) identifying and testing the effect of a data-driven habitat management intervention; (iii) understanding which micro-habitat features promote Z. cassandra oviposition in restored places to optimise the intervention.

3. Both patch (areas of 1 m radius hosting Aristolochia shoots) and plant features affect oviposition, with the strongest positive effects showed by high irradiation of the patch and plant quality (high number of flowers and leaves). Accordingly, the management consisted in vegetation cuts to increase irradiation, and 2 years of monitoring demonstrated that this procedure significantly increased oviposition (average increase of about 2 eggs per plant) and larval presence. Micro-habitat sampling demonstrated that the maximum oviposition differed between vegetation structures, highlighting the importance of a local fine-tuning before the intervention.

4. We provided a data-driven, effective, and sustainable management strategy to increase habitat suitability and oviposition for an endemic and endangered Mediterranean butterfly. Our framework can drive management strategies for other species with similar ecological requirements and subjected to similar threats.

Key words. Aristolochia, Elba island, habitat management, Lepidoptera, vegetation management, Zerynthia cassandra.

Introduction

Butterflies are a group of great conservation interest because of their important ecological role in the food web as well as for their contribution in pollination (Rader et al., 2016). Due to the wide positive attitude of the general public towards butterflies (Sumner et al., 2018), they are also considered as biodiversity ambassadors with a key role as charismatic flagship and umbrella
species (New, 1997; Oberhauser & Guiney, 2010). In Europe, where 496 species are recorded (Wiemers et al., 2018), butterflies mostly inhabit open environments (grasslands, shrublands, rocky slopes and steppic areas) (Bonelli et al., 2018). This is likely due to historical reasons, as during most of the last million years Europe was dominated by cold and dry climate, determining the occurrence of large areas covered by steppic formations. Under this perspective, the abandonment of agricultural land with the reduction in vegetation management and/or grazing levels and the consequent afforestation of grassland biotas represent a major threat for butterflies in European and Mediterranean areas (van Swaay et al., 2006; Bubová et al., 2015; Bonelli et al., 2018). Indeed, many butterfly species have suffered from the increase of shrub and tree coverage due to the long-term land abandonment and the consequent fragmentation of remaining habitat patches (Bubová et al., 2015).

In Italy, the European butterfly-richest country, a recent red list identified at least 23 species (out of 290) threatened because of the abandonment of the traditional agropastoral activities (Bonelli et al., 2018). The drastic changes in land-use are particularly threatening for species with limited geographical distribution and/or narrow breadth of habitat requirements (Sykes et al., 2019). This combination of features is highly represented in butterflies species which tend to specialise, mostly in the use of larval host plants (Warren et al., 2001; Dennis et al., 2011; Carnicer et al., 2013). The abandonment of agricultural land reforestation call for the urgent development of management practices for agricultural landscapes mostly in protected areas, where the agricultural use of landscape is predicted to decrease (Bignal & McCracken, 1996; Atauri & De Lucio, 2001; Otero et al., 2015). A crucial goal of Lepidoptera conservation is to maintain and/or restore the quality of habitat within open biotas by targeted management of grasslands and shrublands (Merckx et al., 2013; Bubová et al., 2015). Unfortunately, there are still few examples of conservation actions developed on an experimental basis to protect butterflies of open biotopes in the European areas (e.g. Smallidge & Leopold, 1997; Lawson et al., 2014). These previous experiences showed that it is crucial to understand the habitat requirements of the target species to develop data-driven habitat management strategies.

Here, we implement a framework to improve the conservation status of endangered populations of rare and protected butterflies strictly dependent on resources lost with reforestation. We used as a model the Italian endemic butterfly Zerynthia cassandra (Geyer, 1828) (Fig. 1a), a species which has been recently separated from Zerynthia polyxena (Denis & Schiffermüller, 1775) based on morphological and genetic data (Dapporto, 2010; Zinetti et al., 2013). It is a monophagous species (sensu Stephens et al., 2008) which feeds on few plants of the Aristolochia genus L. (Fig. 1b), that represent the crucial resource determining population size and survival (Vovlas et al., 2014; Ghesini et al., 2018; Cini et al., 2019). Z. cassandra is included in the Appendix IV of the Habitats Directive and in the Appendix II of the Bern Convention and shows two insular populations also recognised as distinct subspecies (Bollino & Racheli, 2012): a very large population on Sicily island and a much more reduced population on Elba island. The Elba population has a significant conservation interest. Indeed, the range of distribution on Elba is rather small (among 4 km², Fig. 1c, d), mostly because in the Mediterranean environment the host plant mainly grows close to water streams and in other humid areas (Nardi, 1984). The availability of water on Elba island is limited due to the dry climate and because of the recurrent illegal water abstraction (personal observation). Moreover, the area is frequently subjected to devastating fires which, in the last decades, showed the potential to destroy in a single event all the locations where the host plants and the species live (Supporting Information Fig. S1). Furthermore, the Elba population differs from the Tuscan mainland population in its mitochondrial signature (Zinetti et al., 2013; Dapporto et al., 2017). This suggests that after the detachment of the island from the mainland, which occurred after the last glacial maximum, there has been no gene-flow (Zinetti et al., 2013). If the Elba population was to disappear, it is unlikely that new propagules would colonise Elba from the mainland but, more important, this will imply the loss of the genetically distinct Elba population.

The distribution range of this species on Elba is composed of abandoned agricultural fields and, due to the incessant shrub growth (Supporting Information Fig. S2), there is a foreseeable decrease in the number of host plants available for oviposition and larval development (only Aristolochia lutea Desf. and Aristolochia rotunda L. occur on the island; Nardi, 1984). Indeed, previous studies agreed that Z. cassandra prefers to lay eggs on Aristolochia shoots exposed to a high level of irradiation (Vovlas et al., 2014; Ghesini et al., 2018; Cini et al., 2019). Moreover, shrub growth can reduce larval fitness for several reasons. First instar larvae tend to hide themselves in the flowers (Vovlas et al., 2014) and newly hatched larvae might take advantage on feeding on flowers as their tissues are less thick, as it has been suggested for the congeneric species Zerynthia rumina (Linnaeus, 1758) (Jordano & Gomariz, 1994). Thus, the increase of shrub vegetation coverage and the loss of irradiation likely decrease plant maturation, reducing the number of flowers, which in turn could negatively affect larval development and survival. Moreover, irradiation might benefit larvae as larval development might be faster at warmer temperatures, as observed for many insect species (Taylor, 1981) and confirmed in several other butterfly species (Sherman & Watt, 1973; Scriber & Lederhouse, 1983; Pullin, 1986; Bryant et al., 2002).

In order to develop effective and sustainable habitat management strategies for the conservation of Z. cassandra, we adopted a three-step procedure, which consisted in (i) characterising the environmental and host plant features that influence oviposition; (ii) testing the efficacy of a data-driven habitat management consisting in cuts of shrubs growing over existing host-plants (thus increasing the irradiation); (iii) understanding which micro-habitat features promote Z. cassandra oviposition within managed patches, where cuts have been performed, in order to optimise the intervention measures. This framework has the potential to be extended to other species and populations showing similar requirements and threats.

Materials and methods

The species and the study area

Zerynthia cassandra is an univoltine species and the adults usually fly from March to May. Larvae develop from May to
Fig. 1. (a) an individual of *Z. cassandra* from Elba island; (b) the host plant *A. rotunda*; (c) an aerial photo of Elba island; (d) the enlarged view of the study area (in red) in the West part of the island; (e) habitat and plant features measured to assess habitat requirements of *Z. cassandra*; (f) the management strategy, that is, the cut of vegetation around *Aristolochia* patches (pre-cut: left; post-cut: right); (g) the methodology used to evaluate the micro-habitat features influencing oviposition, mainly focusing on individual plant distance to the vegetation border and orientation. Map data: Google, SIO, NOAA, US Navy, NGA, GEBCO. [Color figure can be viewed at wileyonlinelibrary.com]
June while the chrysalis is the overwintering stage (Camerini et al., 2018). Zerynthia cassandra is a sedentary species, with a very limited flying range (up to a few hundred metres; Celik, 2012; Vovlas et al., 2014), despite longer dispersal events within the range of a few kilometres are possible (e.g. as observed in Z. polyxena in Greece; Slanarova et al., 2015).

Zerynthia cassandra is strictly dependent on its Aristolochia host plants, such as A. rotunda and A. lutea, for oviposition and larval growth (Vovlas et al., 2014; Camerini et al., 2018; Ghesini et al., 2018; Cini et al., 2019), which are herbaceous plants with an underground persistent apparatus and aerial annual stems. Aristolochia rotunda and A. lutea show differences in the micro-habitat preferences, with the former being more common in hill-mountain areas (Nardi, 1984). The two species are present on Elba island where they are both linked to humid micro-habitats where they can coexist (Cini et al., 2019). Previous findings suggest that plant species (A. rotunda vs. A. lutea) did not influence oviposition but has an effect on larval and adult survivorship, with larvae having higher survivorship and adults having a longer lifespan when fed on A. rotunda compared to A. lutea (Cini et al., 2019). Adults feed on several herbaceous nectar sources (Ghesini et al., 2018 and personal observation).

The area considered in this study, encompassing all the occurrence sites for this species on Elba island, extends over 4 km² in the municipality of Campo nell’Elba (Fig. 1d). It represents part of the southern slope of Mount Capanne (1019 m) and its altitude ranges between 250 and 450 m a.s.l. The dominant vegetation is composed of abandoned pastures, Mediterranean shrubs, artificial pine woods and narrow patches of hygrophilous vegetation along seasonal streams.

Understanding the habitat features promoting oviposition in Z. cassandra

In order to understand the habitat features promoting oviposition, we adopted the same sampling scheme as in Cini et al., 2019 (Fig. 1e). This study, through a survey performed on a single year (2017), documented that both plant and environmental features determine the oviposition (number of eggs laid on Aristolochia plants) of Z. cassandra on Elba island. In particular, more eggs were laid in more irradiated patches and on plants with more flowers and leaves (Cini et al., 2019). Here, we extended the sampling in time for a total of 3 years (2017–2019 retaining the data of Cini et al., 2019).

We established permanent patches around Aristolochia shoots represented by an area of 1 m radius and each year we sampled only those patches where Aristolochia shoots were present. We designed the selection of managed patches in order to cut patches that were in proximity with other control patches in the area where both plant species occur. Average distance between any two patches was 1.20 ± 0.72 km (mean ± SD, range: 0.002–2.0 km). All managed patches were never farther than 200 m from a control patch, that is, within the dispersal range of Z. cassandra (Celik, 2012; Vovlas et al., 2014), thus assuring that butterflies could move between patches. As the species is protected by the 92/43/CEE HABITATS DIRECTIVE, by the 56/2000 regional laws and local illegal catches of both adult butterflies and host plants (also with Z. cassandra brood on them) have already occurred in the area, the exact locations of the patches are not provided.

The parameters collected for each patch were: (i) the percentage of vegetation coverage separated into (ia) grass, (ib) shrub and (ic) trees; (ii) a variable describing irradiation, in which we considered the imaginary cylinder above the 1 m radius area of the patch, and estimated the amount of the surface that was not covered by grass, shrubs or trees higher than Aristolochia plants; thus, irradiation category reflects the amount of surface of the patch that can be reached by a ‘vertical’ beam of light above the patch, that is, the proportion of the surface that remains not covered by the vegetation layers above the Aristolochia plants (Fig. 1e): (1) patch completely shady or irradiated for a maximum of a quarter (0–25%); (2) patch half irradiated (25–50%); (3) patch irradiated for three quarters (50–75%); (4) fully irradiated (75–100%); (iii) the number of Aristolochia plants in the patch; (iv) the presence of other patches in the surrounding area of 10 m radius. Besides the parameters of the patch, the following parameters of single host plants have been measured: (i) plant species (A. lutea or A. rotunda); (ii) number of bloomed flowers; (iii) number of leaves; (iv) plant height and (v) number of Z. cassandra eggs laid on the plant. When the number of plants in the patch was higher than 10, we measured a maximum of 10 randomly chosen plants per patch.

The number of plants found per patch was (median, first and third quartile): 15.0, 8.0, 20.0 for 2017; 16.0, 10.0, 30.0 for 2018; 16.0, 8.88, 30.0 for 2019 (Supporting Information Fig. S3). The sampling periods for eggs were: 17–24 April 2017; 19–21 April 2018 and 8–10 April 2019; larval occurrence was measured from 6 to 9 May 2019. Sampling dates were chosen according to the local phenology of the species in order to obtain data when the number of adults was strongly declining, which occurred much earlier in 2019. A comparison of the day of data collection revealed that the median day of sampling was the same between experimental and control patches in 2018 (day of the year = 111), while in 2019 the experimental patches have been sampled 1 day before (day of the year, medians respectively 96 and 97) (Supporting Information Fig. S4). For this reason, we included the day of sampling in the modelling approach [generalised linear mixed-effects model (GLMM), see the Statistical analyses section]. In the first year (2017), seven recorders (AB, AC, FB, GG, LD, LP, RN) have counted the eggs and measured patch and plant characteristics together for 2 days to standardise the measurements. In 2018 and 2019, three other recorders (LPA, SB and EF) have been trained. We included recorder id in the modelling approach (GLMM, see the Statistical analyses section).

In total, we sampled 141 different patches and 2498 plants. Not all the patches were sampled every year, because (i) Aristolochia plants did not emerge in all the patches every year and (ii) some patches were found along the study period and added to the survey. Those patches that were sampled on a single year were only used to assess aim 1, that is, understanding the habitat features promoting oviposition, but not for aim 2, that is, the analysis of the effect of vegetation cut, which clearly requires comparisons among years of sampling (see next section). Overall, for the assessment of aim 1 we used 132 different
patches and 2022 plants over the 3 years (number of patches: 2017: 75; 2018: 65; 2019: 89).

**Habitat management strategy: does vegetation cut increase oviposition?**

According to several studies (Vovlas et al., 2014; Ghesini et al., 2018; Cini et al., 2019) and to the results of this study (aim 1), the number of laid eggs is higher on plants exposed to a high level of irradiation, possibly because warmer places accelerate larval development as effect of increased metabolism. Additionally, increased irradiation can allow a faster growth of host plants, providing a better resource for the larvae. Thus, we designed a habitat management intervention aimed at increasing the irradiation of the *Aristolochia* patches shaded by Mediterranean shrubs, brambles and cane thickets. The action took place in winter, which avoids the risk of unwanted negative effects on the host plants and on the butterflies since in that period *Aristolochia* shoots and *Z. cassandra* adults have not emerged yet. The intervention involved the clearing of small areas of about 3 m\(^2\) (circles of 1 m radius) by cutting shrubs, brambles or canes (Fig. 1f). The reduced area of the cleaning guaranteed that the vegetation in the surrounding areas has not been altered. We carried out the cuts using manual tools: hacksaw and pruning shears. We selected the patches to be cleaned among those showing an irradiation level between 1 and 3 in 2017 and 2018 (Cini et al., 2019). The first action took place in half February 2018 on 17 patches over the 75 measured in 2017. The action was replicated in 2019 by cleaning eight additional patches on 123 measured. Moreover, shrubs in seven of the patches assessed in 2018 have been cut in the second half of February 2019 by unknown people with the manifest aim of cleaning path margins resulting in 32 managed patches in total. In order to protect the cleaned patches exposed to human stomping, we built little fences with stones, canes and ropes during April 2019. Both managed (cleaned \(n = 32\)) and control (not-cleaned) patches were included in the measurements of environmental characteristics, plant features, number of laid eggs and number of larvae as discussed above.

Moreover, at the beginning of May 2019 (6–9 May), another sampling campaign was repeated by recording the number of larvae (all the larval stages were sampled, without information on instar) together with the same plants and patches features used for aim 1. For this, we used in the analysis 114 patches and 1008 plants.

**Fine-tuning the management strategy: how do micro-habitat features affect oviposition?**

The sampling described above was aimed at understanding the efficiency of vegetation cuts in increasing *Z. cassandra* oviposition. However, in that sampling scheme, we considered all the plants belonging to the same patch as having the same environmental characteristics (vegetation coverage and irradiation). This approach potentially overlooked the influence of micro-habitat variations with a special reference to the irradiation received by each single plant mostly due to different distances from the shrubs. For this reason, in April 2019 we carried out a measurement at the micro-habitat level in seven of the managed patches, located in shrubby vegetation (mostly brambles, \(n = 4\)) or inside cane thickets along a seasonal stream (\(n = 3\)). We defined an area of 4 m around the patch. We divided every area into four quadrants using two ropes perpendicular to each other and fixed in the centre, which represented the origin of two axes (Fig. 1g). The y-axis was oriented from North to South. We measured every single *Aristolochia* shoot occurring in the area by recording its position respect to the axis origin (\(x\) and \(y\)), the species, its height, the number of bloomed flowers, the number of leaves, the dimension of the largest leaf and the number of eggs occurring on it. Using the same axis reference, we recorded a series of points with similar distance to each other which identified polygons describing the boundaries of the shrub or cane vegetation surrounding the area. For each plant, we recorded the distances from all the segments composing the vegetation borders, with a maximum of 4 m if the plant was external to the border, and 0.8 m if the plant was internal (i.e. within the vegetation). As a first component determining irradiation, we measured the distance from the nearest segment to the border. We also hypothesised that a different exposure to the sunlight, determined by different orientation of the plants respect to the borders (e.g. North or South), can affect oviposition. For each segment of the vegetation border, we evaluated its orientation as the absolute value of the sine of the angle formed by the segment of the vegetation border with the North–South direction (Supporting Information Fig. S5). We attributed the highest orientation (orn) value of 1 when the plant was South respect to the border of a segment oriented from East to West and −1 when the plant was placed North to a similar segment. Similarly, when shrub border was located exactly to the East and the West orientation orn was scored as 0 (Supporting Information Fig. S5). The value of orientation of each plant had been calculated according to the following formula:

\[
\text{Orientation} = \frac{\sum_{i=1}^{n} \text{orn}_{i}}{\sum_{i=1}^{n} 1/n}
\]

where \(n\) represents the number of segments composing the shrub border, \(d\) is the minimum distance between a given plant and a given segment of the border (in order to weight more the segments which were closer to a given plant), orn is the orientation of the plant respect to the border segment (see above). We attributed the minimum orientation value of −1 for the plants located inside shrubs, since in these cases the plants were completely shady. Finally, we categorised each patch as belonging to one of two vegetation categories (cane thickets, *Arundo* spp., or shrubs, mainly brambles, *Rubus* spp.) as they were characterised by very different heights of the vegetation around them (cane thickets borders higher than 2.5 m, shrub borders lower than 1.5 m).

**Statistical analyses**

All statistical analyses and graphics were generated using R version 3.5.1 (R Development Core Team, 2018). In order to
characterise the environmental and host plant features that influence oviposition, we modelled the number of eggs laid on plants through a GLMM using a negative binomial family and zero-inflated model with the glmmTMB function of the package ‘glmmTMB’ (Brooks et al., 2017). We included the following predictors: the coverage of grass, shrub and trees (all continuous), irradiation (categorical), number of Aristolochia plants (continuous), and the presence of nearby patches (categorical, yes or no) as patch features and species (categorical), number of flowers and number of leaves (both continuous) and plant height (continuous) as plant features. We also included year and day of recording in the year (both categorical). Correlation among predictors was substantially low (Supporting Information Table S1), and thus we used all predictors in the analyses. Patch and recorder were included as random factors. In case of a significant effect of the three levels of years and the four levels of irradiation, we calculated pairwise comparisons on results of the model by using Tukey tests as implemented in the ‘emmeans’ function of the ‘emmeans’ R package vers. 1.3.0 (Lenth et al., 2018). Since the cases belonging to different factors were unbalanced and we included an interaction term, we calculated P-value by analysis of deviance (type II Wald \( \chi^2 \) tests) obtained with the ‘Anova’ function of the ‘car’ R package. These analyses were applied only on control patches. We then applied the same GLMM to the number of larvae found on plants, with the exception that year was not included as larvae were only sampled in 2019.

In order to test the efficacy of the habitat management, which consisted in cuts of shrubs growing over existing host-plants, we compared changes in eggs laid among years between patches that experienced different changes in irradiation level, by carrying out a glmmTMB (zero inflated negative binomial family) using all patches that have been recorded for at least 2 years. To assess the effect of changes in irradiation on changes in the number of eggs laid, we included in the model year, the type of irradiation change among years (with the following categories: (i): irradiation from 1 to 2; (ii): from 2 to 3; (iii) from 3 to 4; (iv) from 2 to 1; (v) from 3 (and 4 see below) to 2 and (vi) from 4 to 3) and the irradiation level firstly recorded for each patch (instead of the observed irradiation for each year, which would be redundant with the variable irradiation change). Finally, we included an interaction term between year and type of irradiation change. Two patches that passed from irradiation 4 to irradiation 2 have been merged in the larger groups of patches that passed from irradiation 3 to 2. Indeed, based on preliminary results on control patches, there are no differences in eggs laid on patches of irradiation 3 and 4, thus the change is expected to be similar. This model highlighted if, given a certain level of eggs expected based on the original irradiation and year of record, plants from patches that have experienced different kinds of irradiation changes also resulted in a significant change in the number of laid eggs through years compared to plants that did not change irradiation (used as a baseline group, 0). Since the cases belonging to different factors were unbalanced and we included an interaction term, we calculated P-value by an analysis of deviance (type III Wald \( \chi^2 \) tests) obtained with the ‘Anova’ function of the ‘car’ R package.

In all GLMM, we assessed the occurrence of spatial autocorrelation by applying the ‘ Moran.test’ function (and other preliminary functions: ‘nb2listw’ and ‘knearneigh’) of the ‘spdep’ R package vers. 1.1-5 (Bivand et al., 2015) to the residuals of the GLMMs and to the location of each patch.

We also aimed at verifying if egg occurrence and patch manipulation in 2019 affected the number of larvae occurring in the patches. To test this hypothesis, we calculated the mean number of eggs and larvae found in each patch in the two sampling periods (egg sampling and larval sampling) in 2019, respectively. We then applied a quasi-Gamma General Linear Model (GLM) as implemented in the ‘stats’ ver. 3.6.2 R package with assumed variance function that is \( V(\mu) = \mu^2 \), using mean number of larvae as response variable and mean number of laid eggs, the management intervention (binary variable) and their interaction as predictors.

Finally, in order to understand which micro-habitat features promote Z. cassinhera oviposition within managed patches, we analysed the data collected with the micro-habitat level sampling with a generalised additive mixed model (GAMM). We used the ‘gam’ function of the ‘mgcv’ R package vers. 1.8-31 (Wood & Wood, 2015) by including patch identity as random factor, patch category (cane thickets or brambles) as a fixed factor, number of flowers, number of leaves, height of the plant as linear predictors, and orientation and minimum distance from the border as smoothed predictions. We also tested the effect of distance and orientation separated for each patch category (cane thickets vs. brambles).

**Results**

*Understanding the habitat features promoting oviposition in Z. cassinhera*

As expected from previous studies, several variables influenced the number of eggs laid on single Aristolochia shoots with a strong significant effect in the generalised linear mixed model (Table 1, Eggs control patches and Supporting Information Table S2 for GLMM summary table with variable estimates). The different years showed different number of laid eggs and pairwise comparisons (Table 2) revealed that in 2017 we recorded a lower number of eggs compared to the following years which did not differ from each other (Fig. 2b, d). The plants with a higher number of flowers, taller and with more leaves had a significantly higher number of eggs laid on them (Table 1, eggs control patches). The same occurred for the key variable of irradiation. A post hoc test indicated significant differences in the number of eggs laid among all the four degrees of irradiation except for the levels 3 and 4, characterised by a very similar number of laid eggs (Fig. 2a, c; Table 2). Plants that were in patches in proximity of other patches received a higher number of eggs than plants in isolated patches (Table 1). Finally, grass coverage significantly influenced the number of eggs laid, with less eggs laid where grass coverage was higher (Table 1). The following variables did not significantly affect the number of laid eggs: tree coverage, shrub coverage, number of plants within the patch, plant species and the day of data record. The residuals of the GLMM did not show to be significantly spatially autocorrelated (Moran I statistic standard deviate = −2.514, P-value = 0.994).

We tested if the same patch and plant factors influencing the number of eggs laid also influenced larval occurrence and we found moderate support for that. Larval occurrence was influenced by two variables, which entered the GLMM with a
A GLMM including both manipulated and non-manipulated patches revealed a significant interaction between years and changes in irradiation. A summary table comparing each class of change against the baseline of patches that did not change their irradiation showed that the significant interaction was expressed by an increase of number of eggs laid on plants that passed from irradiation 2 to irradiation 3 in both 2018 and 2019 and a significant decrease of eggs laid in patches that passed from irradiation 3 and 4 to irradiation 2 (Table 4, Fig. 4 and Supporting Information Table S4 for type III sum of squares ANOVA table). The residuals of the GLMM did not show to be significantly spatially autocorrelated (Moran I statistic standard deviate = −2.257, P-value = 0.988).

**Habitat management strategy: does vegetation cuts increase oviposition?**

A GLMM including both manipulated and non-manipulated patches revealed a significant interaction between years and changes in irradiation. A summary table comparing each class of change against the baseline of patches that did not change their irradiation showed that the significant interaction was expressed by an increase of number of eggs laid on plants that passed from irradiation 2 to irradiation 3 in both 2018 and 2019 and a significant decrease of eggs laid in patches that passed from irradiation 3 and 4 to irradiation 2 (Table 4, Fig. 4 and Supporting Information Table S4 for type III sum of squares ANOVA table). The residuals of the GLMM did not show to be significantly spatially autocorrelated (Moran I statistic standard deviate = −2.257, P-value = 0.988).

**Table 1.** $\chi^2$ (Chisq) (ANOVA type II) tests on deviance table of generalised linear mixed models based on number of eggs per plant in control patches (eggs control patches), number of larvae per plant in control and managed patches (larvae all patches). The number of larvae and eggs has been explained based on patch features (grass coverage, shrub coverage, tree coverage, irradiation, number of plants, occurrence of nearby patches), plant features (number of flowers, number of leaves, plant height, plant species), year and day of sampling. Patch and recorder identities were included as random factors. The sign of the effect (+: positive, −: negative) is indicated within parentheses. Significant results are reported in bold.

| Variable             | d.f. | Chisq   | P-value | Chisq   | P-value |
|----------------------|------|---------|---------|---------|---------|
| Grass coverage       | 1    | 9.050   | **0.002** (+−) | 1.154   | 0.283   |
| Shrub coverage       | 1    | 1.278   | 0.258   | 4.455   | **0.035** (+) |
| Tree coverage        | 1    | 3.263   | 0.071   | 1.151   | 0.283   |
| Irradiation          | 3    | 38.868  | **<0.001** (+) | 8.290   | **0.040** (+) |
| Number of plants     | 1    | 0.021   | 0.885   | 0.116   | 0.734   |
| Nearby patches       | 1    | 4.041   | **0.044** (+) | 0.895   | 0.344   |
| Plant species        | 1    | 0.070   | 0.792   | 0.581   | 0.446   |
| Number of flowers    | 1    | 23.439  | **<0.001** (+) | 0.287   | 0.592   |
| Number of leaves     | 1    | 8.342   | **0.004** (+) | 3.535   | 0.060   |
| Plant height         | 1    | 4.658   | **0.031** (+) | 0.167   | 0.683   |
| Year                 | 2    | 20.722  | **<0.001** (+) | 4.517   | 0.211   |
| Day                  | 1    | 0.478   | 0.489   | 4.517   | 0.211   |

**Table 2.** Pairwise comparisons (Tukey test) obtained on results of GLMM of the number of laid eggs between the different years and among the four levels of irradiation (1–4) in control patches. Estimates represent differences in estimated marginal means of eggs (as showed in Fig. 2) between the second and the first level of each comparison as reported in the “comparison” column. Significant results are reported in bold.

| Comparison               | Estimate | SE    | z     | P-value |
|--------------------------|----------|-------|-------|---------|
| Year                     |          |       |       |         |
| 2017–2018                | −0.752   | 0.194 | −3.881| **<0.001** |
| 2017–2019                | −0.738   | 0.245 | −3.009| **0.008** |
| 2018–2019                | 0.014    | 0.221 | 0.062 | 0.998   |
| Irradiation level        |          |       |       |         |
| 1–2                      | −1.128   | 0.434 | −2.597| **0.047** |
| 1–3                      | −2.527   | 0.448 | −5.640| **<0.001** |
| 1–4                      | −2.535   | 0.434 | −5.837| **<0.001** |
| 2–3                      | −1.399   | 0.279 | −5.016| **<0.001** |
| 2–4                      | −1.407   | 0.264 | −5.335| **<0.001** |
| 3–4                      | −0.008   | 0.228 | −0.036| 0.999   |

Significant effect (Table 1, Larvae all patches, and Supporting Information Table S3 for GLMM summary table with variable estimates): the irradiation level of the patch (Fig. 3a) and the shrub coverage (the higher the shrub coverage, the more larvae were present). The pairwise comparisons indicated that patches with higher irradiation hosted more larvae, but only the comparison between the lowest and the highest levels of irradiation showed a significant difference (Fig. 3b and Table 3). The residuals of the GLMM did not show to be significantly spatially autocorrelated (Moran I statistic standard deviate = −2.257, P-value = 0.988).
The GLM model of the number of larvae occurring in patches in May against number of eggs observed in April showed that the patches with more eggs also hosted more larvae (Table 5 and Supporting Information Fig. S6). The manipulation of the patches (Table 5) influenced the number of larvae, with more larvae present in the managed patches (Supporting Information Fig. S6).

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Manipulation of the patches did not interact with the number of laid eggs (Table 5).

Fine-tuning the management strategy: how do micro-habitat features affect oviposition?

Micro-habitat sampling meant to understand which small-scale features promote *Zerynthia cassandra* oviposition in restored places revealed a higher number of eggs in patches surrounded by cane thickets, a positive effect for the number of leaves and flowers (Table 6) and a significant effect for minimum distance in both patches surrounded by cane thickets and brambles (Table 6). However, the relationship between number of eggs and minimum distance from the border is linear as indicated by effective degrees of freedom (edf) equal to 1 in patches surrounded by cane thickets (Table 6 and Fig. 5a), while it is bell-shaped in those patches surrounded by brambles (Fig. 5b) as revealed by a much higher edf value (Table 6). The GAMM analysis showed that the number of laid eggs in patches surrounded by brambles reached a maximum around approximately 0.60–0.70 m from the shrub borders (Fig. 5b). The orientation of the plants respect to the vegetation borders did not show any influence on the number of laid eggs in both patches surrounded by cane thickets and brambles (Table 6).

Discussion

In an era of rapid environmental changes, habitat loss is putting at stake thousands of species worldwide (Brooks *et al.*, 2002; Mantyka-pringle *et al.*, 2012). There is the urge to plan economic and sustainable management strategies to cope with resource depletion and guarantee resource availability and survival to threatened species and populations (Myers *et al.*, 2000). Here,
we provided proof that a data-driven habitat management strategy can increase oviposition and larval presence in the iconic and endangered Italian endemism *Z. cassandra*.

As it occurs to many Italian butterflies, a main risk for the population of *Z. cassandra* on Elba island (and in a large fraction of its distribution over Apennines) is the loss of habitat due to vegetation overgrowth as a consequence of the abandonment of non-intensive agricultural managed fields (Bonelli *et al*., 2018). The strategy here presented, increasing the irradiation of areas with *Aristolochia* shoots in order to increase their use as host plants by *Z. cassandra* oviposition, enhanced the quality of the habitat for this butterfly and has therefore a great potential to favour the threatened population on Elba island. Designing and optimising the intervention required three steps: (i) improving the characterisation of the habitat features influencing oviposition; (ii) identifying and testing the effects of the habitat management measure and (iii) understanding the micro-habitat characteristics influencing oviposition within the managed patches.

The three-step framework

First, we evaluated the patch and plant features that influence oviposition by *Z. cassandra* females. On control patches the number of eggs showed fluctuations among years with a higher egg-laying in 2018 and 2019 (Table 1). Moreover, we found that the number of eggs was higher in *Aristolochia* shoots characterised by a greater height, a greater number of leaves and of flowers (Table 1). These results corroborate the oviposition preference of *Z. cassandra* for more mature *Aristolochia* plants (i.e. taller, with more leaves and flowers) which has already been reported in literature (Vovlas *et al*., 2014; Camerini *et al*., 2018, Ghesini *et al*., 2018) and which is likely due to these plants representing a richer food source for the larvae. Additionally, a high number of flowers might be attracting larvae tend to hide in them (Vovlas *et al*., 2014) as well as because newly hatched larvae might take advantage on feeding on flowers as their tissues are less thick. The presence of nearby patches also positively influenced the number of eggs laid, likely as an area rich of *Aristolochia* patches is more visited by females.

The negative effect of grass coverage on the number of eggs laid might be explained by the preference of *Z. cassandra* for laying in marginal patches, as also suggested by the higher number of larvae in marginal, shrub-rich patches, as grass coverage is greater in open habitats and smaller in marginal habitats. Patch irradiation also emerged as a factor affecting oviposition. In our pluriannual survey, the number of eggs laid was higher in shoots growing in highly irradiated patches, with shady areas during the central hours of the day lower than 50% (i.e. the 3–4 irradiation category; Table 1 and Fig. 2a, c). This confirms the preference of this butterfly for sunny spots (Vovlas *et al*., 2014; Camerini *et al*., 2018, Ghesini *et al*., 2018; Cini *et al*., 2019). A
vast array of factors might explain this preference (Dennis, 2020) with a crucial one being that irradiated plants promote a faster development of the larvae, as reported for other butterfly species (Sherman & Watt, 1973; Scriber & Lederhouse, 1983; Pullin, 1986; Bryant et al., 2002; Valimaki & Itamies, 2005). This result suggests that the reforestation of the southern slope of the Mount Capanne, observed in the last decades (Supporting Information Fig. S2), might represent a major threat for the Elba population of Z. cassandra.

Second, based on this evidence, we identified a habitat management strategy that could increase egg-laying. While the number of flowers can hardly be manipulated, the irradiation level of a patch can be easily increased by cutting shrubs on existing patches of Aristolochia plants. We performed this management strategy and 2 months after the management we already observed an increase in the oviposition rate in patches where the irradiation level was increased. In particular, the increment was evident in the managed patches whose irradiation raised from level 2 to 3 (Fig. 3). In these patches the number of eggs laid after the intervention equalled that of control patches having the same irradiation level with an average increase of about 50 laid eggs in each 1 m radius cleaned patch, a number of eggs which is similar to the observed lifetime fecundity of Z. cassandra females (Vovlas et al., 2014). This indicates that after our management, a newly formed patch containing about 25 shoots represented an analogous resource to Z. cassandra as a highly irradiated patch and it could support the entire reproductive output of a female (Vovlas et al., 2014). It is also important to underline that the intervention did not apparently produce any egg sequestration neither an ecological trap. Indeed, during the survey, the eggs laid in the control patches did not decrease (as it was expected if egg sequestration from control patches to managed patches occurred) and overall number of recorded eggs showed a significant increase compared to the first year (contrary to what expected if the cut represented an ecological trap) (Table 1). This suggests that oviposition is not increased in managed patches at the expense of control ones, although future studies should directly rule out this hypothesis. Conversely, we also found that patches that decreased their irradiation for vegetation overgrowth received a significantly lower number of eggs, thus confirming the urgency of the proposed action.

Third, in order to optimise the intervention, it was necessary to understand the micro-habitat features that promote Z. cassandra oviposition within managed patches where vegetation was cut. The occurrence of a higher number of eggs in highly irradiated patches might suggest that oviposition should attain a maximum value at a certain distance from the vegetation border and this should not decrease with increasing distance (i.e. the more irradiated a plant is, the more eggs it will receive). We found mixed evidence for this, depending on the kind of vegetation surrounding the patch, with the depth of the optimal cut being linear to the distance from the border in the case of patches surrounded by (tall) canes, while being bell-shaped [reaching a maximum at around 0.60-0.70 m when surrounded by brambles (thus confirming the previous result)]. This suggests that vegetation height could be a relevant feature to be taken into account when fine-tuning the technique, and especially that the fine-tuning of the cut itself should be performed in each context (Fig. 5 and Table 6). We speculate that the shrub edge represents a sheltered environment from desiccation, meteorological events, stomping by vertebrates and attacks by parasitoids (e.g. Krämer et al., 2012) as also confirmed by the higher number of larvae occurring in more shrubby patches (Table 1). A preference for laying eggs on vegetation edges has been indeed demonstrated in other butterflies like Lopinga achine (Scopoli, 1763) (Bergman, 1999) and in another Parnassinae, Parnassius mnemosyne (Linnaeus, 1758) (Bergström, 2005).

While we documented an increase of oviposition and larval presence on managed patches, a comprehensive evaluation of the effects on the entire population can only be achieved within a longer timeframe and including adult stages. In this perspective, a long-term pollard transect (Pollard, 1977) has been established in the study area, under the Italian Butterfly Monitoring Scheme (https://butterfly-monitoring.net/it).

Table 5. The results of a quasi-gamma GLM explaining the mean number of larvae as a function of the mean number of laid eggs, the management intervention (binary variable: Managed vs Control) and their interaction. Significant results are reported in bold.

|                | Estimate | SE  | t-value | P-value |
|----------------|----------|-----|---------|---------|
| Eggs           | 0.695    | 0.159| 4.388   | 0.000   |
| Management     | 0.855    | 0.407| 2.100   | 0.038   |
| Management * Eggs | -0.377  | 0.211| -1.791  | 0.076   |

Guidelines for a data-driven habitat management through vegetation cuts

Which patches to manage. Our results suggest that an effective and feasible strategy to increase egg-laying of Z. cassandra is to cut vegetation in small patches covered by shrubs. Our results revealed that the greatest effect of the intervention was obtained on patches with low initial irradiation levels which, thanks to the clearing, reach the same egg-laying levels of highly irradiated patches (Table 1 and Fig. 3). We thus suggest performing interventions specifically on those areas where the Aristolochia plants grow shaded by Mediterranean maquis, canes and brambles. Although A. rotunda did not show a higher number of laid eggs compared to A. lutea, a laboratory study showed that a larger fraction of immatures growing on A. rotunda reached the adult stages, and adults also survive longer (Cini et al., 2019). This suggests that a special attention should be given to A. rotunda patches.

When to perform the cut. The cutting intervention has a strong potential to damage Aristolochia shoots. Given the phenoLOGY of these plants, emerging at the end of the winter, the selected patches should be cut during the winter, in order to avoid damaging the plants. This timing also makes the plants immediately available since the increase in oviposition can be immediately detected in the first spring following the winter cuts. Our study did not assess the recovery time of the cut vegetation and its dependence on the initial coverage. Our personal

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observation indicates that in a couple of years a cut of 1 m is already covered again by shrubs. This suggests that the intervention should be repeated every 2 years. However, this aspect would likely depend on the local conditions and thus will need to be locally evaluated.

**How to perform the cut.** Our GAMM analysis (Table 6 and Fig. 5) showed that there is not a single rule to design the cut intervention, as the optimal distance from the border for egg-laying depends on the kind of surrounding vegetation. Notwithstanding the limited number of patches \((n = 7)\) investigated at the micro-habitat level, our results suggested that when the vegetation is high (as in patches surrounded by cane thickets) it likely provides shade even at longer distances and egg-laying increased linearly at least up to three metres. On the contrary, when a patch is surrounded by lower brambles, the highest levels of egg-laying occurred around 0.60–0.70 m from the borders. A possible explanation is that at lower distances there is not enough irradiation, while, at longer distances, the microclimate can be excessively hot and dry and the larvae are less sheltered. The high value of marginal areas is confirmed by the positive correlation between number of larvae and shrub coverage (Table 1). Taken together, these results warn against making wide cuts, as eggs and larvae might benefit from the presence of shrubs. However, since the optimal cut depends on the vegetation and climatic context, the optimal depth of the cut should be assessed in advance in each micro-habitat.

An interesting and successful conservation action for *Z. cassandra* through habitat management has been carried out by Ghesini *et al.* (2019). They created new suitable patches by transplanting *Aristolochia* roots and demonstrated successful colonisation by *Z. cassandra* (Ghesini *et al.*, 2019). Despite being limited in its application by the efforts needed for the transplant and especially by the time required for the spontaneous colonisation by the butterflies, this approach is valuable to extend...
the range of *Z. cassandra* where the host plants do not occur, and it has the potential to increase connectedness among occupied habitats.

Our approach took a different and possibly complementary direction: we aimed at increasing the availability of an already existing local resource. This approach is rather inexpensive and does not require developed skills. Moreover, the small size of the vegetation cuts minimises the impact on the surrounding environment. Consequently, this strategy can be easily implemented in reserve management plans where the host plant already exists and can represent a complementary approach together with more demanding strategies.

**Conclusions and future directions**

We showed that a 3-year research allowed to implement a successful habitat management framework which increased the egg laying and the larval abundance of the endangered *Z. cassandra*. In order to guarantee that this management strategy is a valid conservation measure for this species, future studies should measure and quantify the positive effect on population viability and dynamics.

Since reforestation is among the highest causes of decline for European and Mediterranean butterflies (van Swaay *et al.*, 2006; Bubová *et al.*, 2015; Bonelli *et al.*, 2018), we suppose that our framework can be applied to many butterfly species which possess similar habitat requirements making them threatened by vegetation overgrowth. Congeneric species (such as *Z. rumina* and *Z. polyxena*) are the first candidates, as they share many ecological traits (Jordano & Gomariz, 1994; Celik, 2012; Ochoa-Hueso *et al.*, 2014) and several studies suggested low-vegetation coverage and high irradiation as important factors promoting species abundance (Celik, 2012; Oervoessy *et al.*, 2014). The highly protected species *Parnassius mnemosyne* (Linnaeus, 1758) is another species which might benefit from such an approach. It is a univoltine and monophagous species (sensu Stephens *et al.*, 2008) which feeds on *Corydalis* species, such as *C. cava* (L.) Schweigg. & Körte, *C. solida* (L.) Clairv., and *C. intermedia* (L.) Mérat, which usually grow at forest margins (Konvička & Kuras, 1999; Bergström, 2005; Valimaki & Itämies, 2005; Konvička *et al.*, 2006; Bonelli *et al.*, 2016). In the Mediterranean region, *P. mnemosyne* lives in forest clearings of mountainous areas and preferentially lays eggs on irradiated *C. cava* plants (Bergström, 2005). Unfortunately, several populations are facing a drastic reduction in abundance and habitat loss due to vegetation encroachment is the most likely candidate (Cini *et al.*, 2020), as forest recolonisation could have negative effect on the growth of thermophilic immature stages, oviposition by adult females and also on growth of host plants (Bergström, 2005; Konvička *et al.*, 2006; Cini *et al.*, 2020).

We hope the results here obtained by applying the framework to *Z. cassandra* on Elba island has the potential to promote the application of a similar framework to many other butterfly populations which might be suffering from vegetation encroachment.

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**Conflict of interest**

The authors declare they have no conflict of interest.

**Authors’ contribution**

AC, FB, L. Platania and LD designed the study; AC, FB, L. Platania, AB, GG, SB, EF, RN, L. Pasquali and LD performed the field work; AC, FB, L. Platania and LD performed the analysis; AC, FB, L. Platania and LD wrote the manuscript; AC, FB, L. Platania, AB, GG, SB, EF, RN, L. Pasquali and LD revised and accept the manuscript.

**Data availability statement**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Supporting information**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Appendix S1 Supporting Information.**

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