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Scaling-up targets for a threatened butterfly: A method to define Favourable Reference Values

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A B S T R A C T

The accomplishment of a Favourable Conservation Status (FCS) for habitats and species is the Habitats Directive's primary goal (HD, 92/43/EEC). As tools for assessing the measurable parameters of conservation status, the European Commission identified Favourable Reference Values (FRVs), and it described the methodology to set them. However, examples of its application are rare.

We propose a mixed reference-/model-based approach to set the Favourable Reference Population (FRP) and Range (FRR) for a threatened butterfly, *Parnassius apollo*, in the Italian Alps. The approach involves the use of a habitat suitability map obtained via Maxent as a basis for a clumping procedure to identify discrete patches of suitable habitat (clumps), corresponding to potentially viable local (meta)populations. The number and distribution of clumps occupied by the species along geographical gradients are compared to the distribution of all available clumps to define the FRVs.

According to our analyses, 41 clumps are occupied by *P. apollo* in the Italian Alps. Since their distribution reflects clump availability along all geographical gradients, this value can be used to express the FRP, and to subsequently define the FRR as the envelope including the 41 clumps.

Our approach considers several conditions reflecting the species persistence and provides insights for conservation and monitoring. Our objective, transparent method of setting FRVs can be applied to assess FCSs for other threatened species occurring in discrete units, with disjunct populations or local metapopulations.

1. Introduction

The accomplishment of a Favourable Conservation Status (FCS) for habitats and species is one of the main biodiversity goals of the Habitats Directive (HD, EU 4392/EEC), probably the most important tool for biodiversity conservation in Europe (EU - Epstein, 2013). As generally defined in Art. 1 of the HD, to achieve an FCS, the species listed in the HD Annexes (II, IV and V) must maintain themselves on a long-term basis as a viable component of their natural habitats. This implies evaluating different parameters (range, population, habitat for the species, future prospects), all of which are indicators of species status and they must reach favourable values for the FCS objective to be met. Member States (MSs) must promote the FCS of the populations occurring within their territory, contributing to FCS of the species at the European level (Epstein et al., 2015).

Thus, FCS is first of all a legal concept. Nevertheless, it must be understood and applied by scientists, managers and policymakers, and it should be assessed through comparable approaches by the different MSs. The appropriate application of the FCS concept hence requires discussion and clarification on several, controversial aspects related to the concept itself (Epstein et al., 2015) and to the above-mentioned parameters, e.g., whether the FCS should be assessed at the species, population or national level, and how to strictly interpret the long-term viability requirement.

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In this respect, and with particular reference to the population parameter, the adoption of standard methods based on the Minimum Viable Population (MVP, Thomas, 1990; Boyce, 1992; Traill et al., 2007; Frankham et al., 2014; Reed and Mccoy, 2014) has been advocated (Linnell et al., 2008). However, the MVP should only be regarded as a baseline for reaching an FCS, which is achieved with a greater number of individuals than the MVP (DG Environment, 2017).

The HD and the European Commission indeed endorse the idea of positive targets for conservation rather than the adoption of extinction thresholds, which is the most common approach used to compile Red Lists (IUCN). In particular, the EC promotes the adoption of Favourable Reference Values to define positive thresholds both for the population and the range, i.e., for two out of the four parameters (range, population, habitat for the species, future prospects) identified by EU guidelines as tools for assessing FCSs (European Commission, 2006; DG Environment, 2017). For each species, the values of a Favourable Reference Population (FRP, e.g., measured as numbers of individuals) and a Favourable Reference Range (FRR, e.g., area in km^2) should jointly describe a favourable scenario for the species, ensuring its persistence in the long-term period and the maintenance of ecological functionality. In particular, the most recent version of the Commission's own guidelines defines the FRR as the "range within which all significant ecological variations of the species are included for a given biogeographical region and which is sufficiently large to allow the long-term survival of the species". The FRP is "the population in a given biogeographical region considered the minimum necessary to ensure the long-term viability of the species" (DG Environment, 2017). It is essential to state that IUCN has developed an approach aimed to integrate the Red Listing framework by evaluating the level of species recovery and measuring its occupancy, population viability and ecological functionality. This approach has strong similarities with the concept of FRP and considers species life history and habitat characteristics (Akçakaya et al., 2018).

The Favourable Reference Values thus represent a benchmark against which MSs should compare the current range and population values, assessed through the monitoring schemes implemented by competent local agencies. Despite their relevance to assessing whether a species has achieved an FCS, the reviews of MS approaches showed that a few MSs documented the methodology used to set FRVs and that most, if not all, MSs somehow included expert opinion in considering and weighting factors for setting FRVs. The whole process was not necessarily explicit or detailed (McConville and Tucker, 2015; Bijlsma et al., 2018).

Ultimately, the process of identifying target values is compulsory for the assessment of the conservation status, as requested by Art. 1 of the HD. Without a baseline, the FCS definition is critical (Clément, 2014). The Birds Directive (2009/47/EC) in EU and the Endangered Species Act (ESA) in the United States also require the assessment of an FCS. In both cases, the applied approaches are usually pragmatic (Tear et al., 2005).

To achieve a more objective and transparent evaluation of the FCS through FRVs, the Explanatory Notes and Guidelines for Reporting under Article 17 of the Habitats Directive (http://www.reportingdirettivahabitat.it; period 2013–2018 DG Environment, 2017 - hereafter referred to as the Guidelines) delve into and define the general principles that should be considered in the process of setting FRVs. Bijlsma et al. (2018) further detail the whole process of setting FRVs. Such a process requires gathering all the relevant information about the target species to understand its ecological and historical context, and then choosing the best approach to define the FRVs, either reference- or model-based (or a combination of both). Although guidance for selected groups of species and habitats is available, examples of the applied procedure are still lacking.

As a contribution to developing a specific methodology to define applicable FRVs and providing a best-practice example, here we describe how to assess and define FRVs for a butterfly species, *Parnassius apollo* (Linné, 1758) in the Italian Alps.

Defining a robust methodology for invertebrates is particularly valuable since several invertebrates face high extinction risks at local scales. However, monitoring invertebrates and butterflies, in particular, poses crucial problems. There is an apparent difficulty in estimating the number of individuals because (i) sharp annual fluctuations in the population size due to intrinsic biotic features can occur in several species (e.g., Nowicki et al., 2009); (ii) more than one generation can be present, at least in some species. These two features imply a large sampling effort in time and space to correctly assess the number of individuals. Consequently, most of the available information is in the form of presence/pseudo-absence data. With no or limited knowledge on the abundance or the population trends, assessing a reference value for the populations is also difficult because no abundance benchmarks are available for comparison with theoretical values. In contrast, butterfly ecology and biology are generally well known, and methods for assessing their population dynamics are standardized, shared and established, thus making a bulk of robust and reliable data available (Thomas, 2005). Besides, of the whole 29 European HD butterfly species, 16 (55%) occur in Italy, representing the highest proportion recorded for a MS (Bonelli et al., 2018).

Among butterflies, *P. apollo* is an excellent model to assess FRVs as it is a widespread, large-winged butterfly whose biology is well-known; it is easily identified, even by non-specialists, and is a flagship species, primarily for the Alps. Moreover, *P. apollo* is widely distributed, occurring in all major European mountain chains, including those of Southern and Central Spain, the Cantabrian Mountains, the Pyrenees, the French Massif Central, the Balkans, the Pindus and the Alps, up to just over 50°N. It is absent from all Central and Northern Europe, but present in the Scandinavian Peninsula (Kudrna et al., 2011; Kudrna et al., 2015). To the East, *P. apollo* ranges as far as the Altai and the Sayan Mountains of Central Asia.

P. apollo is included in Annex IV of Habitats Directive and Appendix II of Bern Convention. The international commerce of this species is also restricted by the Washington Convention (CITES Appendix 2 and C1 EU).

P. apollo has a Red List status in 23 (15 EU) European countries (Maes et al., 2019). The species is 'near threatened' (NT) both in EU and EU27 (van Swaay et al., 2010); apparently extinct in Latvia and Byelorussia; 'critically endangered' (CR) in Germany (rank 1. Binot et al., 1998); 'endangered' (EN) in Slovakia (Kadlečík, 2014) and Finland (Hyvärinen et al., 2019); 'near threatened' (NT) in Sweden (Gärdenfors, 2010), in Austria (Huemer et al., 1994) and in North Macedonia (Krpač and Darcemont, 2012). In Italy, *P. apollo* is overall a species of 'least concern', mainly due to its widespread occurrence in the Alps (Bonelli et al., 2018).

The aim of our work is to propose and test a procedure to set FRVs for *P. apollo* in Italy. The procedure adheres to the European guidelines, and overall, the methodology is also relevant for other species. This achievement allows calibrating conservation measures and efforts to guarantee the long-term Favourable Conservation Status of a species in a certain MS, as required by the Habitats Directive.

2. Materials and methods

According to the Guidelines (DG Environment, 2017) and Bijlsma et al. (2018), the whole process of setting FRVs can be divided into two steps: (1) gather all the relevant information about the target species to understand its ecological and historical context, and (2) choose the best approach to define the FRVs.

2.1. Information about the target species

Step (1) involves gathering data on the biology and ecology of the species, including past and current distribution, population size, trends, major shifts and pressures acting on the species. This information is essential since FRVs should be set on the basis of robust ecological and biological considerations, using the best available knowledge and scientific expertise (DG Environment, 2017). Thus, we describe the biology and ecology of the species by revising the scientific literature, data provided by national/regional management authorities as well as governmental agencies and for Habitats Directive Reporting. We pay particular attention to studies that provide data on the dispersal ability of individuals, thus shedding light on the spatial scale at which ecological processes affect *P. apollo* populations. The definition of the spatial scale of functioning is crucial. Bijlsma et al. (2018) proposed a classification of species in different population categories related to the behaviour of individuals and features of species groups in terms of spatial requirements and dynamics (e.g., mobility; genetic structure of the population - such as subpopulations, (meta)populations; sedentary *vs.* migratory species). For each category, they proposed an FRV assessment level (national *vs.* supra-national). We use the available literature to classify *P. apollo* into one of the suggested categories and we subsequently define the scale for setting FRVs. As for the spatial scale of application, here we narrow our analyses to the portion of the Alpine biogeographical region corresponding to the Italian Alps, thus excluding the small Apennine area located in Central Italy. According to the species characteristics and consistently with the criteria adopted by Bijlsma et al. (2018), the *P. apollo* alpine (meta)population[1] might be regarded as a single, defined unit of conservation concern. Moreover, the focus on the Italian Alps is motivated by the difference in the *P. apollo* conservation status (Bonelli et al., 2018) and data availability between the Alps and the Apennines.

A 'grey literature' search is also fundamental for the evaluation of the historical perspectives and for the analysis of the species distribution and trends. For this purpose, we mainly consider data gathered for the previous HD reporting (Genovesi et al., 2014), CKmap atlas (Balletto et al., 2007) reporting butterfly Italian distribution (see 2.2.1 for further detail), and local reports on surveys conducted within Protected Areas. Detailed information on *P. apollo* population parameters and size (expressed as the number of individuals) is lacking for the Italian Alps. Only presence data at a scale that varies from localities to 10 × 10 km grid cells are available (see 2.2.1 for details).

2.2. Set Favourable Reference values

Step (2) of the procedure recommended by the Guidelines (DG Environment, 2017) and by Bijlsma et al. (2018) is based on the outcome of the literature search described above and entails the choice between a (i) reference- or a (ii) model-based approach to set FRVs. The Guidelines report the reference-based approaches as those "founded on an indicative historical baseline corresponding to a documented (or perceived by conservation scientists) good condition of a particular species or restoring a proportion of estimated historical losses"; whilst "model-based methods require good knowledge about species ecology and biology, as they are built on biological considerations, such as those used in Population Viability Analysis (PVA) or on other estimates of Minimum Viable Population (MVP) size". MVP was recently revised by Green et al. (2020) and, through a multiplication factor, was adapted to model a population with long-term survival even in dramatic scenarios, like climate change. In some cases, for pragmatic reasons, the number of individuals encompassing the population before the decline has been proposed as a population FRV in the FCS situation (Panjabi et al., 2017).

Model-based methods can also take advantage of habitat suitability analysis (Bijlsma et al., 2018), especially to scale up population targets to the species level (e.g., as in Di Marco et al., 2016). However, according to the Guidelines, the two approaches are not mutually exclusive.

Considering the available data (results of Step (1)), we adopt a mixed reference-/model-based approach for defining the Favourable Reference Population (FRP) and hence the Favourable Reference Range (FRR) for *P. apollo*. In particular, the steps of our approach can be summarized as follows: (i) we model habitat suitability for the species in the Italian Alps using Maxent (Philips *et al.,* 2006–2.2.1); (ii) on the map (Fig. S1) resulting from the Species Distribution Modeling (SDM), we apply a clumping procedure which enables to identify discrete patches of suitable habitat (clumps), each corresponding to a potentially viable local (meta)population (2.2.2); (iii) to scale up the population targets to the level of the Italian Alps, we consider the available knowledge on the past distribution of the species with respect to geographical and ecological conditions (Verity, 1947; Bonelli et al., 2018) and compare the number of available clumps to the number of actually occupied clumps (2.2.3).

2.2.1. Habitat suitability modeling

We investigate habitat suitability in the Italian Alps (48,106 km^2), considering environmental biotic and abiotic variables that are expected to affect the presence of *P. apollo* (Table S1) and relating them to *P. apollo* occurrence data.

We divide the study area into 49,763 grid cells (1 × 1 km) and we select the environmental predictors on the basis of their relevance for *P. apollo* ecological requirements. Mapped variables for the study area are obtained from available online resources as rasters at high spatial resolution (Digital Terrain Model - DTM, 20 m resolution, from www.sinanet.isprambiente.it; bioclimatic data with 30 s resolution, from WorldClim v. 2.0 – www.worldclim.org; land cover description - CUL12[2], 10 m resolution, from http://groupware.sinanet.isprambiente.it/).

We calculate the proportion of coverage in each cell of the grid for six land cover categories (open natural areas including natural pasture, grasslands, sparse or absent vegetation; open semi-natural areas including pasture, crops; anthropogenic areas; ecotones including wooded and shrubby vegetation; forests; wetlands). Six other parameters (coefficient of variation, number of patches, edge length, mean patch size and shape calculated in Grass GIS, GRASS Development Team, 2017, v. 7.2.2) describe open natural areas (see Table S1 for more details on the used GRASS algorithms), which are considered as a key habitat for *P. apollo* populations. Indeed, its food plant *Sedum* spp. grows within open forest glades, lowland meadows and mountain screes (Nakonieczny et al., 2007). The edge length, mean patch size, shape and number of patches are calculated using a toolset for multiscale analysis of landscape structure by applying a moving window of square shape (8 cells) surrounding each pixel. The bioclimatic variables include: seven descriptors of solar radiation, temperature and precipitation at annual scale; the averaged minimum, maximum, and mean temperature between April and May, calculated from monthly data; the averaged mean precipitation between April-May and June-July (Table S1). Regarding topographic predictors, we derive the slope and aspect from DTM in ArcGis 10.6 (ESRI, 2011) and we classify the resulting values into 15° intervals for the slope, and in three classes for aspect (South-East, South-West, other). Then, we calculate the coverage percentages of the topographic predictor classes in each cell. All the variables are re-scaled to the 1-km grid using different rules (Table S1) in ArcGis 10.6.

The presence of *P. apollo* is characterized by different sources: (i) standard butterfly monitoring performed by National/Regional Parks (e.g., Parco del Monviso, Parco del Gran Paradiso, Parco dello Stelvio, Parco Val Grande, Parco delle Dolomiti Bellunesi, Parco Alpi Cozie, and Aree Protette dell'Ossola) under the guideline of the project "Monitoring of Animal Biodiversity in Mountain Ecosystem" (Viterbi et al., 2013)

[1] We report "meta" in brackets since we cannot assess the actual level of interactions and gene flow within the Alpine area, and thus we cannot fully adopt the term metapopulation.

[2] Italian revision of i) Corine Land Cover - High Resolution Layers, Urban Atlas and Riparian Zones - in 2012, ii) mapped records collected by Italian regions, and iii) a national map of land use described in 2012. More information is reported at http://groupware.sinanet.isprambiente.it/uso-copertura-e-consumo-di-suolo/library/copertura-del-suolo/carta-di-copertura-del-suolo

and/or Butterfly Monitoring Scheme Standards; (ii) the Lepidoptera Papilionoidea database updated by Balletto et al. (2007) for the project "CkMap'' that reports the distribution of 10,000 animal species on the behalf of the Italian Ministry of the Environment, Land and Sea (Ruffo and Stoch, 2006). Since its publication, this dataset has been regularly revised by including records of butterfly presence from literature, museum collections and reports. All data receive recent confirmation from iNaturalist. CkMap dataset includes presence data mapped on a 10 × 10 km Universal Transverse Mercator (UTM) grid. However, we used localities' descriptions (e.g., recognizable landmarks or GPS coordinates) noted in this database to scale down the geographical information and work on a more detailed scale (1 × 1 km). The final dataset of *P. apollo* occurrences is conservatively narrowed in time and space, from 1990 to 2017 and altitudes ranging from 600 to 2050 m.

Combining data on environmental predictors and *P. apollo* occurrences, we model habitat suitability using Maxent (Phillips et al., 2006). The modeling procedure compares the environmental features of occurrences and background cells (Molloy et al., 2017). We select the latter (10,000 cells) considering that using a background which accounts for a random selection within a restricted area is recommended to correct for spatial bias in the sampling effort (Fourcade et al., 2014). Hence, we randomly select cells in a restricted background, based on the spatial bias of the species occurrences. Because occurrence data are unequally spread across the Italian regions (NUTS 2, i.e., Italian geographic regions as reported in Table S2), we impose an adjusting coefficient (CF) to correct for the regional differences in survey effort. In each region, we weight the number of background cells on the proportion of occurrence data over the Italian Alps. Following Fourcade et al. (2014) and Phillips (2008), and weighing up the contribution of each administrative region to the default pool of 10,000 background cells, the number of background cells that are sampled in each region (PA_r) is thus defined as:

$$CF_r = \frac{obs_r * N_r}{\sum_{r=1}^{7} N_r}$$

$$PA_r = \frac{CF_r * 10'000}{\sum_{r=1}^{7} CF_r}$$

Here *obs* is the number of cells with presence data for each Italian region *r*, *N* is the total number of cells in each Italian region *r*. The larger the extent and the survey effort, the larger the number of PA_r is (e.g., in Piedmont and Veneto). 10,000 is the default number of background points randomly selected in a study area when modeling in Maxent.

Maxent modeling parameters are defined using the "biomod2" package (Thuiller et al., 2019) in R/RStudio environment (R Core Team, 2019; RStudio Team, 2020). We fit and compare several models with different subsets of predictors. We start with an initial model including all the predictors (Table S1) and we then perform a stepwise process. Each step allows omitting correlated ($r > 0.7$) and/or low contributing variables in order to obtain a more robust model with improved predictive evaluation and a low number of variables (Molloy et al., 2017). The models are set to investigate linear, quadratic and hinge relationships between *P. apollo* presence and environmental predictors using 200 iterations. Model runs are repeated 100 times, whereby presence and background cells are used as response variables and weighted equally. For model calibration, each run randomly selects 80% of the *P. apollo* data, while the remaining 20% is used for model testing.

The predictive performance of the model is evaluated by the area under the Receiver Operating Characteristic curve (ROC; Fielding and Bell, 1997; Lu et al., 2012) and the True Skill Statistics (TSS, Allouche et al., 2006). The AUC (area under the ROC) is used as an evaluation criterion to generate the final ensemble model, setting a quality threshold at 0.7. Finally, we report the percentage of habitat suitability for each grid cell and we identify the suitability threshold that maximizes the TSS of the ensemble model. This threshold is later used to define patches of suitable areas (see 2.2.2).

2.2.2. *Clumping procedure*

On the map resulting from the Species Distribution Modeling (SDM), we apply a clumping procedure that enables to identify discrete patches of suitable habitat. The SDM map is used as an inverted resistance surface, setting thresholds to discriminate between low, intermediate and high resistance values (Rödder et al., 2016). The 5% (5th percentile, Rödder et al., 2016) of records with low suitability are considered geographic barriers (high resistance) for the spatial dispersion of *P. apollo* across patches. Concerning the highly suitable patches (low resistance), we consider the suitability threshold that maximizes the classification accuracy of the distribution model, measured through the True Skill Statistics (TSS, Allouche et al., 2006; see 2.2.1).

We then use the available information on the species dispersal ability (see below, 3.1) to group the low-resistance cells, i.e., to define a group of cells corresponding to potential population clumps. Low resistance cells are considered contiguous if they are within the dispersal range of the species (1–2 km - Brommer and Fred, 2007) even though separated by intermediate resistance habitat. Clumps are considered separated in case of barriers (e.g., at least one high resistance cell) or if there is more than one intermediate resistance cell between them.

Finally, we select only those clumps satisfying the Minimum Area Requirement (MAR) for the species (Baguette and Stevens, 2013). These clumps provide the minimum amount of functional, connected habitat necessary for the population persistence and each clump corresponds to a potentially viable local (meta)population. A clump is thus defined as a spatial population unit, i.e., an area satisfying the requirements of at least one viable (meta)population of the species in terms of habitat availability and connectivity, and clearly separated from other clumps because of the presence of an interposed matrix of unsuitable or high resistance habitat.

We performed the analyses in R environment (R Core Team, 2019), using RStudio (RStudio Team, 2020). The clumping procedure is based on functions from the "raster" package (Hijmans, 2020): 'adjacent', used to identify low resistance contiguous cells, and 'clump', to detect and uniquely label patches of connected cells.

2.2.3. *Scaling-up*

To scale up the population targets to the Italian Alps, we consider that, under favorable conditions, the species distribution should cover the whole range of available ecological conditions and genetic variations of the species itself, as occurred in the past (Bonelli et al., 2018). Therefore, our strategy aims to identify the minimum number of clumps that cover the ecological variation within the species natural range, along latitudinal, longitudinal and altitudinal gradients. For this purpose, we compare the number of predicted available clumps to the number of actually occupied clumps and we qualitatively verify the distribution of the latter with respect to the natural range of the species.

3. Results

3.1. *Information about the target species*

The actual Italian distribution of *P. apollo* ranges from the Alps to the main Apennine massif of Liguria, Tuscany, Latium, Abruzzo, and Aspromonte and Sicily.

Although quantitative historical data on population size is lacking, the species was certainly widely distributed through the Alps in the past.

While we can identify the whole Alpine arc as the species' natural range in the Alpine biogeographical region, several *P. apollo* populations are strongly declining or have disappeared across the Apennines (Bonelli et al., 2018), which are mostly included in the Mediterranean biogeographical region. In the latter, the *P. apollo* conservation status according to the last HD Article 17 reporting is Inadequate (U1), while in the Italian Alpine biogeographical region is Favourable (FV). In the Mediterranean biogeographical region, the species decline has been mostly driven by habitat reduction and fragmentation due to land-use, long-term climatic changes, habitat succession and short-term weather anomalies. In the Alps, *P. apollo* inhabits mountain stony screes and sunny slopes with sparse vegetation from 600 up to 3000 m and is a monovoltine species whose adults are on the wings from June to August, but lower-altitude populations in the Alps could occur in April or persist up to middle September. The flight period in the Apennines is shorter, about 20 days, from mid-July to the beginning of August (Verity, 1947; Balletto et al., in press). Males can be observed several days prior to females. Individuals can be active up to ten hours a day, and their average lifespan ranges from two to four weeks (Lafranchis et al., 2015).

Adults primarily feed early in the morning or late in the afternoon on violet or violet-blue flowers such as those of *Centaurea*, *Cirsium*, *Origanum*, *Scabiosa*, *Eryngium*, *Epilobium*, *Thymus*, *Valeriana* in preference, but also forage on *Narcissus* or yellow Cruciferae (e.g., *Biscutella*), as well as on *Sedum* spp. (Lafranchis et al., 2015).

P. apollo females lay eggs singularly on Crassulaceae, generally on *Sedum* spp. (e.g., primarily *S. album,* but also *S. rupestre, S. montanum,* and *S. acre*) or in the food plant surroundings (Nakonieczny et al., 2007; Balletto et al., in press).

Depending on the abiotic conditions, larval development lasts 3–12 weeks. Usually, larvae overwinter in the egg and hatch in the following spring (Nardelli et al., 1989). From mid-May onwards, pupae can be found under stones or in the litter. The next generation of adults will emerge in two up to seven weeks in case of very cold conditions (Lafranchis et al., 2015).

Field observations suggest that *P. apollo* adults can fly between habitat patches with food plants over distances up to 1840 m (median 260 m). The patch quality is determined by the presence of both adult and larval resources and can drive female movements between areas (Fred et al., 2006). Overall, adults show limited movement capabilities, of about 1–2 km at most, thus *P. apollo* occurs in discrete management units (disjunct populations or local metapopulations) scattered across the species distribution (Brommer and Fred, 1999, 2007).

Genetic variability has been described at individual and population levels. Todisco et al. (2010) have highlighted a strong phylogeographic structure, revealing a number of distinctive mtDNA lineages occurring in different regions or in separate mountain chains. A first lineage inhabits Anatolia, Northern Greece and East-Northern Europe; a second Central and Southern Spain; a third the Alps, the Apennines, the Pyrenees, the Massif Central, Sicily, and the mountains of Peloponnesus. A distinct haplotype, however, occurs in the Madonie mountains of Sicily. The population of the Massif Central is fairly distinct, and those from mainland Iberia share a separate haplotype (Descimon et al., 2001; Nève, 2009).

On the basis of the bibliographic information, we list *P. apollo* under the population category “small species with low mobility with scattered distribution” (S6 category Table 4.1a in Bijlsma et al., 2018), and we set the FRV for the Italian Alps.

3.2. Set Favourable Reference values

3.2.1. Habitat suitability modeling

For Italian Alpine populations, detailed information on population parameters is not available yet, and there is no current estimate of the population size, expressed as the number of individuals. Data on the species are limited to presence-only data (Table S2), characterized by variable precision (from localities to 10 × 10 km grid cells). As a consequence, in the previous reporting under Article 17 of the Habitats Directive (2007–2012), the population size was given as 247 ’10 × 10 km grid cells (grids 10 × 10)’. For the 2013–2018 reporting round, data on the population was expressed as 980 ’number of map 1 × 1 km grid cells (grids1 × 1)’, as recommended (DG Environment, 2017).

However, the population size (980 grid cells) provided for the latter reporting represents a ‘best estimate’, while our presence dataset is based on 344 verified *P. apollo* occurrences (GPS coordinates) gathered by different sources (see 2.2.1) and scattered on 247 cells of 1-km spatial resolution. Background cells are selected accounting for spatial biases (most of data coming from Piedmont and Veneto, no records from Liguria) in presences following Fourcade et al. (2014). We thus randomly select pseudo-absences in a restricted background according to the proportion of presences on the extension of each Italian region.

Starting with the initial model including all (26) explanatory variables (Table S1), through the stepwise process we derive a final model including 13 predictors. The TSS for this model is 0.62, with AUC = 0.88 (Fig. S2). The evaluation coefficients for the selected predictors are reported in Table 1. Our results confirm the key role of open natural areas for the species. On the contrary, anthropogenic factors negatively affect *P. apollo* presence. As for bioclimatic variables, both solar radiation and precipitation appear relevant according to our final model. Indeed, larvae are sun-loving, early spring feeders and show sun-basking behaviour, selecting microhabitats within a specific temperature range (20–28 °C). Temperature also has effects on food consumption, growth, developmental time and adults’ locomotion. The adults prefer warm, sunny days without rainfalls for their normal activity (Descimon et al., 2005; Nakonieczny et al., 2007; Ashton et al., 2009).

3.2.2. Clumping and scaling-up

Based on the True Skill Statistics (TSS, Allouche et al., 2006), the suitability threshold that maximizes the classification accuracy of the distribution model is 34.3%. Using this threshold, we apply the clumping procedure on the species distribution map (Fig. S1), finally identifying 809 potential population clumps (Fig. 1; mean extent = 15.6 km^2, 92.2 st.dev). Out of these clumps, 41 are certainly occupied by the species (mean extent = 192.7 km^2, 361.8 st.dev). Fig. 2 shows the comparison between the number of available clumps to the number of actually occupied along the latitudinal, longitudinal and altitudinal gradients. Occupied clumps are generally well distributed along those gradients. However, the analysis of the longitudinal distribution (Fig. 2b) reveals a gap at about 9°, roughly corresponding to the subdivision between the Western and the Central Alps. The gap emerged from the official distribution maps (Reporting under Article 17 of the Habitats Directive). However, our data shows that at the boundary between the Central and Western Alps suitable clumps are available, albeit in a limited number. Second, the presence of the species is not recorded at the Eastern limit of the range (approx. 13°). Nevertheless, the longitudinal distribution of the occupied clumps shows a bimodal distribution and it seems to reflect quite accurately their availability.

The overlap between the available and occupied clumps also occurs along the latitudinal and altitudinal gradients. No major gaps in the distribution of occupied clumps are detected and records of the species lack only for the extremes of the altitudinal gradient (i.e., at very low or very high altitudes, Fig. 2c) and at Southern latitudes (Fig. 2a).

The distribution of occupied clumps reflects quite accurately their availability, along with all geographical gradients. As a consequence, recalling that a clump is defined as a spatial population unit, the FRP for

Table 1
Final list of variables selected for *P. apollo* Maxent model, with corresponding evaluation coefficients.

Variables	Evaluation coefficient
DTM (m)	0.341
Slope	0.053
Aspect	0.022
Solar Radiation (kJ m^{-2} day^{-1})	0.257
Annual precipitation (BIO12) (mm)	0.193
Seasonal precipitation (BIO15) (mm)	0.14
Averaged mean precipitation (April-May) (mm)	0.245
Averaged mean precipitation (June-July) (mm)	0.195
Open natural areas	0.06
Anthropogenic areas	0.081
Coefficient of variation	0.029
Mean patch size	0.105
Shape of patches	0.05

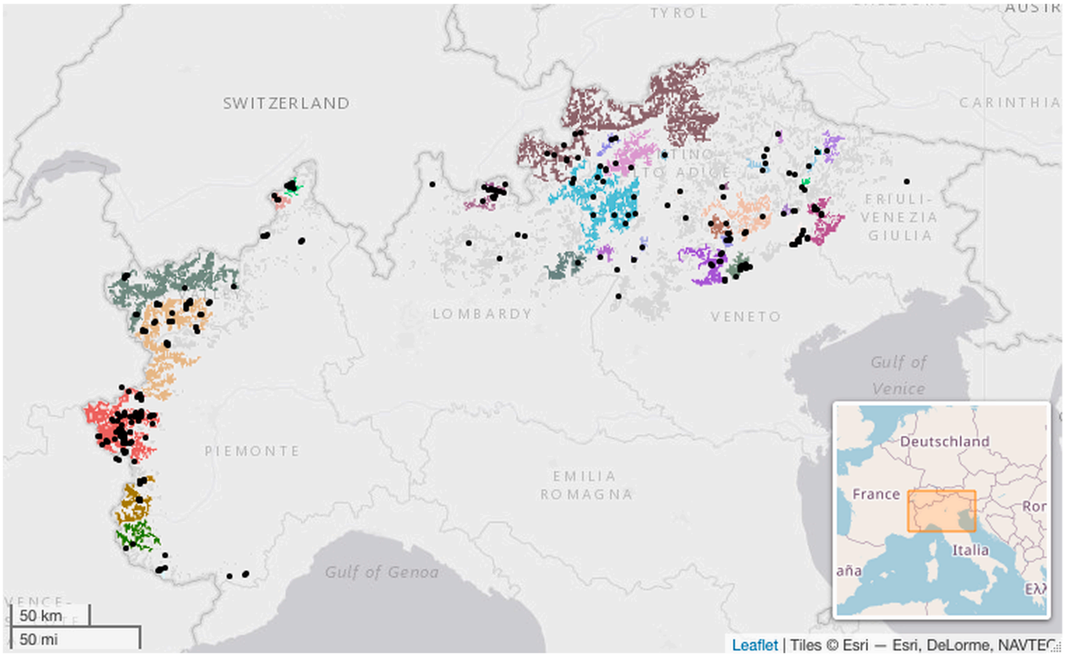

Fig. 1. Population clumps for *P. apollo* in the Italian Alps. Cells within the potential population clumps are in grey. Out of these clumps, the ones certainly occupied by the species are identified by the different colors. Black dots represent *P. apollo* presence data.

P. apollo could be set as 'approximately equal to' the present-day value (i.e., to the present-day number of the grid cells). Subsequently, the FRR can be estimated as the envelope including the 41 occupied clumps, using the procedure outlined in the Guidelines for the definition of the species range (DG Environment, 2017).

4. Discussion

Following the guidelines of the EC and Bijlsma et al. (2018) in particular, our approach to setting FRVs for *P. apollo* relies on both bibliographic information and modeling. Literature data are interpreted to list *P. apollo* under a specific population category and scale up the assessment at the (meta)population level. Modeling is used to investigate habitat suitability, considering environmental biotic and abiotic variables that are expected to affect the presence of *P. apollo*.

For habitat modeling, we use Maxent since it is recognized as a common species distribution modeling (SDM) tool for identifying habitats suitable for a certain species. The quality of results varies according to the quality and appropriateness of the presences, background cells and the explanatory variables selected to feed the model (Molloy et al., 2017). Sampling bias can often occur when data are gathered from different sources into complex SDMs, as in our case study. However, the spatial bias of the sampling effort can be corrected and Maxent remains a valuable tool to extract robust information from non-systematic surveys, opportunistic and presence-only data about the spatial distribution and habitat suitability for pivotal species, such as butterflies (Dennis and Thomas, 2000; Elith et al., 2011; Fourcade et al., 2014).

Regarding the clumping and scaling up procedures, we note that our approach partly follows from considerations reported in Di Marco et al. (2016). Our definition of FRVs for *P. apollo* includes conditions that reflect species persistence, such as the number and location of the populations to be protected. Although we do not explicitly consider genetic data, the identification of these 41 (meta)populations is consistent with the considerations on the strong phylogeographic structure (Todisco et al., 2010) and on the dispersal abilities of the species (Brommer and Fred, 2007). Indeed, the scattered distribution of the 41 clumps should also favor the maintenance of genetic variation at the regional scale.

To set FRVs we exclude the adoption of generalized genetic rules (Frankham et al., 2014), and we rather rely on considerations on the area requirements for the species, selecting only those clumps whose size is larger than the MAR for the species (Baguette and Stevens, 2013). In addition, considering that monitoring the species abundance can be challenging and that the current population unit for the species has been set to 1 × 1 km grid cells, we avoid explicit considerations on the number of individuals. MVP and PVA are popular tools to set conservation targets, and their use has been discussed in the framework of the Habitats Directive and for the definition of FRVs in particular (DG Environment, 2017; Bijlsma et al., 2018; see also Brambilla et al., 2011). Nevertheless, PVA-based MVPs are demanding in terms of data, the results may have a short temporal validity and can be strongly context-dependent (Hilbers et al., 2017). By definition, MVP is the smallest number of individuals required for a population to persist in its natural environment (see Green et al., 2020), but, as a positive target, the FRP should be above an extinction threshold.

Overall, our results are consistent with a qualitative assessment of the status of *P. apollo* in the Italian Alps. Here, the apparent absence or rarity of the species from the NE Alps should be ascribed to data deficiency. However, in the last few years, the number of presence records has increased, thanks to the implementation of projects aiming to record and share data on butterfly observations, such as the Italian Butterfly Monitoring Scheme (https://butterfly-monitoring.net/it).

In general, the species appears well distributed and probably locally abundant. The outcome of our analysis allows us to quantify this latter statement and identify *P. apollo* (meta)populations whose persistence should be ensured in a long-term perspective. In practice, conservation efforts should be aimed at maintaining them in a viable status, and local agencies responsible for the reporting under HD Article 17 could promote specific programs for their monitoring. In Italy, each Region, managing its own Natura 2000 areas directly or through Protected Areas, is responsible for the data collection required to assess Conservation Status.

Our spatial model also identifies other suitable clumps that could host additional populations. In these clumps, the presence of the species and the habitat conditions should be verified. The knowledge on available clumps should promote the set-up of robust conservation policies

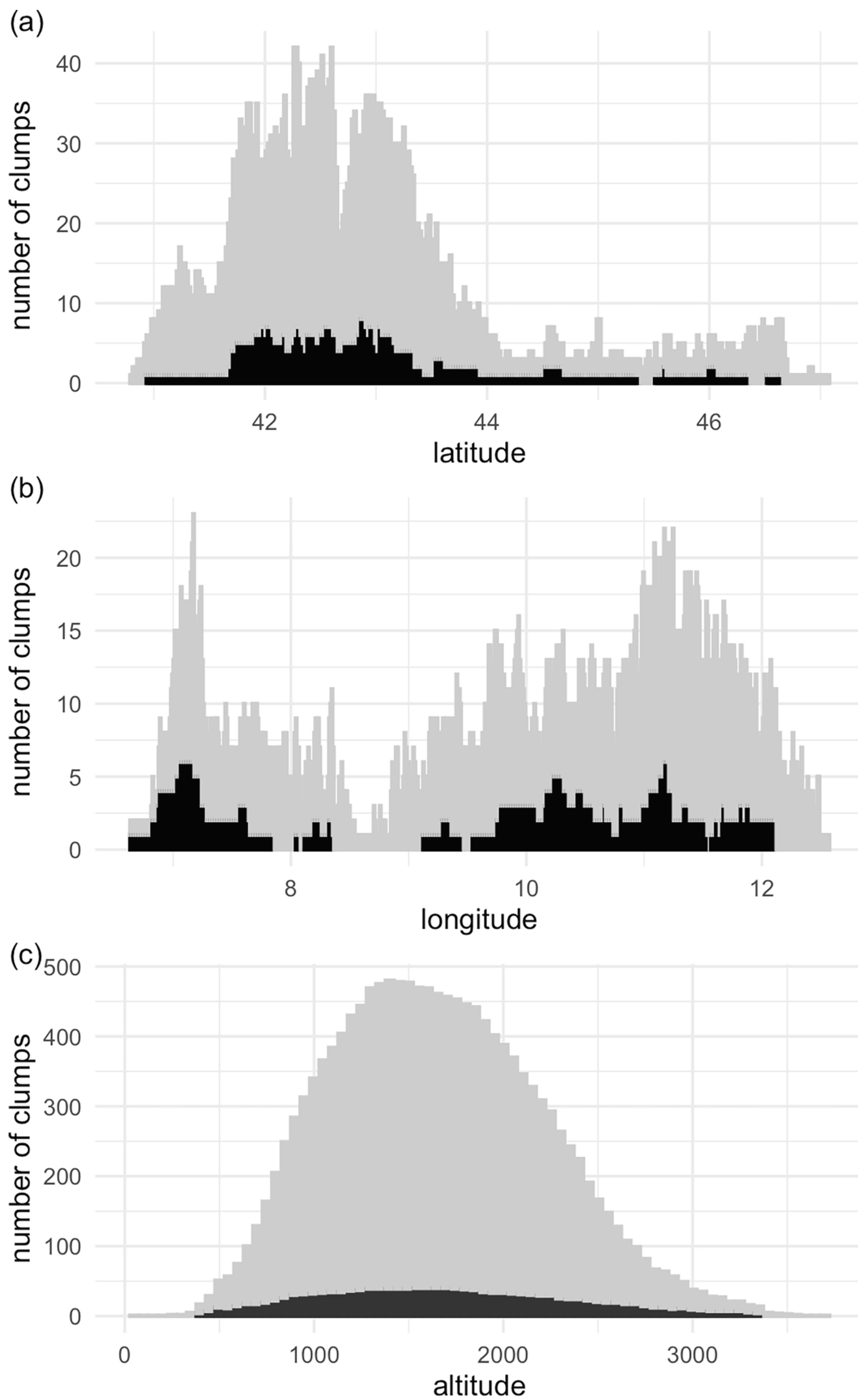

Fig. 2. Latitudinal (a), longitudinal (b) and altitudinal (c) distribution of the available (grey) and occupied (black) clumps in the Italian Alps.

based on the dynamics of large-scale metapopulations and less related to the environmental and demographic stochasticity of the individual populations.

Here, the procedure was limited to *P. apollo* populations of the Italian Alps, but it will be further applied to the Mediterranean biogeographical region. Due to habitat reduction and fragmentation, several *P. apollo* populations are strongly declining or have disappeared across the Apennines and Sicily (Bonelli et al., 2018). Therefore, some relatively isolated peninsular populations should probably be more strictly protected. This applies for instance to the populations flying in Aspromonte (Nikusch, 1996) and Sicily (Madonie), which occur in conditions similar to those described by Napolitano et al. (1990) for the French Massif Central.

The described approach can also be extended to other species. It is suited for animal species that occur in discrete management units, with disjunct populations or local metapopulations, and in particular for those species that fall under the population category "small species with low mobility with scattered distribution", as defined by Bijlsma et al. (2018). For these species, the identification of available and actually occupied clumps and their distribution could be valuable indicators of conservation status. However, the species to which the method can be applied must have a fairly well-known distribution and life cycle. This can be rare in invertebrates, but quite common in protected and charismatic species (Cardoso et al., 2011). In any case, our approach enables to slightly overcome the limit of knowledge on distribution (the so-called Wallacean shortfall). It is resource-based; hence, in part, it fills the lack of information on species abundance and their changes in space and time (the so-called Prestonian shortfall). Thus, our procedure can be used to define FRVs for several Italian butterflies i.e., *Melanargia arge*, *Zerynthia cassandra*, or European species like *Papilio hospiton* and *Argynnis elisa* but also other arthropods as *Rosalia alpina* or *Cerambyx cerdo* among saproxylic coleoptera, or *Cordulegaster trinacriae, Ophiogomphus ceciliae* among odonata or the moth *Proserpinus proserpina*.

For butterflies, the definition of quantitative reference values, supported by the analysis of suitable habitats, could be regarded as an additional step toward improving the conservation status. Italy hosts many HD butterfly species (16 out of 29), more than any other European country. Many extinctions have been observed during the second part of the XX century, particularly in the 1970 s − 90 s (Bonelli et al., 2011). The scenario is more stable in recent years: the latest IUCN assessment underlines a small fraction of species (6%) as threatened with extinction (Bonelli et al., 2018). Being conceptually different from the IUCN assessment of the risk of extinction, the FRVs could now drive towards an additional improvement of the overall conservation status.

However, we should also recall that FRVs are not directly transposable into conservation targets. In addition to the technical aspects, FRVs are, first and foremost, a legal concept. Their interpretation by the MSs must consider the directions of the EC, and it can be eventually challenged by the Court of Justice (Darpö, 2011; Chapron, 2014; Trouwborst, 2014; Darpö and Epstein, 2015). The problem is particularly relevant for the transboundary populations, e.g., for large carnivores, but several species may pose additional issues not addressed in this study (e.g., migratory species, see also Bijlsma et al., 2018).

Even though the Habitats Directive plays a crucial role in the European biodiversity legislation, the scientific discussion about FCS, as one of its cardinal concepts, is still in its infancy (but see Epstein, 2013; Epstein et al., 2015). Following the first directions from the EC, several MSs developed their own approaches to set FRVs (reviewed in McConville and Tucker, 2015; Bijlsma et al., 2018). Nevertheless, the lack of a heated debate about how to interpret the FCS could lead to a depletion of the concept's meaning and its value as a conservation tool. Defining FRVs is part of this discussion process, and the scientific community must be actively involved. Policymakers are responsible for including experts and conservation biologists in the decision process, according to their competencies. On the other hand, scientists must make policymakers aware of what is concretely needed to meet the FCS concept in the broadest sense. By guaranteeing the pragmatism of the approaches, the method described here is a contribution in this sense.

We reckon and highlight that FRVs must go hand in hand with improvements in monitoring scheme programs. In the case of butterflies, obtaining an FRV for each HD species shared among the Butterfly Conservation Europe community would optimize monitoring schemes for each MS and each biogeographical region. In turn, this improvement will ensure the comparison of actual abundances and spatial ranges to the targets expressed by FRVs. For this purpose, simulations of survey designs and efforts should be implemented to identify cost-effective approaches to data collection.

For animal species in general, FRVs will be considered to shape conservation policies, define management plans, identify protection areas when needed, and communicate about all these decisions with stakeholders.

According to the EU Biodiversity Strategy for 2030, it is mandatory for MSs "to ensure that at least 30% of species and habitats not currently in FCS are in that category or show a strong positive trend" (EC 20/05/2020 COM(2020) 380 final). That needs measurable baselines and target objectives identified through structured methods, limiting expert-based evaluations that might introduce subjectivity.

5. Conclusion

According to our analyses, 809 population clumps are available for *P. apollo* in the Italian Alps. Out of these, 41 are certainly occupied by the species.

Despite the difference in absolute numbers, the distribution of occupied clumps accurately reflects their availability, along all gradients. By occupying these 41 clumps located at different longitude, latitude and altitude, the species inhabits various ecological scenarios, and hence it could be less affected by environmental stochasticity. We thus express the Favourable Reference Population (FRP) as the minimum number of 41 clumps, i.e., the present-day number of map 1×1 km grid cells, and the Favourable Reference Range (FRR) as the envelope including these 41 clumps.

In our approach, the outcome of spatial models is interpreted considering the knowledge on the past species distribution and used to scale up targets to the species level. Following Di Marco et al. (2016), we consider several conditions reflecting the species persistence, identifying the number, size (in terms of MAR), and location of the (meta) populations that make up the FRP for *P. apollo*. The scattered distribution of these (meta)populations (i.e., of the clumps hosting them) ensures their representativeness from an ecological and evolutionary perspective and leads to the definition of the FRR for the species.

Our work aims to provide an objective and straightforward method of evaluation, delivering examples of procedures that can be adopted in other biogeographic regions and MSs to determine whether or not the species reaches an FCS. An improved assessment of the conservation status also obtained thanks to the better definition of the FRVs, together with progress in monitoring schemes, will pave the road for long-term species conservation.

CRediT authorship contribution statement

Simona Bonelli: Conceptualization, Methodology, Supervision, Writing – review & editing. **Francesca Barbero:** Investigation, Writing – original draft, Writing – review & editing. **Arianna Zampollo:** Data curation, Writing – review & editing. **Cristiana Cerrato:** Methodology, Writing – review & editing. **Piero Genovesi:** Investigation, Supervision, Writing – review & editing. **Valentina La Morgia:** Conceptualization, Methodology, Supervision, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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