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# Ecological features, populations traits and conservation status of *Helianthemum caput-felis* along its distribution range

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*“Alla fine, conserveremo solo ciò che amiamo,*
*ameremo solo ciò che conosciamo,*
*e conosceremo solo ciò che ci hanno insegnato.”*

*“In the end, we will conserve only what we love,*
*we will love only what we understand,*
*and we will understand only what we are taught.”*

*Baba Dioum*

# SUMMARY

# Introduction

Biodiversity is defined by the Convention on Biological Diversity (CBD) as the "the variability among living organisms from all sources including, *inter alia*, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems" (CBD 1992). The latter ones provide a large number of goods and services that sustain our lives. This biodiversity is essential to our planet, human wellbeing and to the livelihood and cultural integrity of people. Yet biodiversity is currently being lost at unprecedented rates due to human activities (Sala *et al.* 2000; Baillie *et al.* 2004; Perrings 2014).

The major processes now driving species extinction are of anthropogenic origin and result from habitat loss, alteration and fragmentation, overexploitation, pollution, invasion of alien species, global climate change, disruption of community structures and the interactions between these (Sala *et al.* 2000; Novacek and Cleland 2001; IUCN 2003; Thuiller 2007; Mace *et al.* 2008).

Habitat fragmentation caused by human disturbance is currently considered one of the main factors reducing population viability of rare plants (Menges 1991; Schemske *et al.* 1994; Holsinger 2000; Matthies *et al.* 2004; Oostermeijer *et al.* 2003; Thompson 2005; Marrero-Gómez *et al.* 2007; Fenu *et al.* 2013). Habitat fragmentation increases extinction risk for rare species (Holsinger 2000; Matthies *et al.* 2004; Schleuning and Matthies 2009), interferes with distribution, fitness and seedling recruitment (Lienert 2004; Kolb and Diekmann 2005; Benito *et al.* 2009; De Vere *et al.* 2009), and reduces the number of breeding individuals and gene flow (Dudash and Fenster 2000) and pollination efficiency (Duncan *et al.* 2004).

Tourism and recreational activities are the main threat to ecosystems in the Mediterranean area (Allen 2001; Ballantyne and Pickering 2013) and typically lead to habitat fragmentation (Gibbs 2001; Fenu *et al.* 2011). In the Mediterranean Basin in particular, over the last 30 years, coastal ecosystems have suffered a massive reduction of size and quality as a consequence of the disproportionate urban development (Greuter 1995; McLachlan and Brown 2006; Fenu *et al.* 2013; Pinna *et al.* 2015). Reduced range size due to habitat fragmentation may subsequently affect the performance of small plant populations (Kearns *et al.* 1998; Aguilar *et al.* 2006).

In order to contain the species loss, several laws, programs and initiatives for the protection and preservation of biodiversity have been taken. Countries contracting to the Convention on Biological Diversity (CBD 1992) and other international strategies (e.g. GSPC and ESPC) are obliged to monitor biodiversity in order to protect wild fauna and flora.

The Global Strategy for Plant Conservation (GSPC 2008) and the European Plant Conservation Strategy (Planta Europa 2008) aim at halting the continuing loss of plant diversity; as part of this, the development of conservation strategies is an issue that needs to be urgently addressed at national level (GSPC 2008; Sharrock and Jones 2009). GSPC is constituted by 16 plant conservation targets. Still, many of the original targets set for 2010 were not achieved. A project was launched in 2009 to put together a consolidated update to the GSPC and to revise the target dates for the first time (updating to 2011–2020). In particular, the Target 7, which refers to *in situ* plant conservation, supposed that for 2010 the 60 per cent of the world's threatened species should be conserved *in situ*. The same Target was revised for 2020: "at least 75 per cent of known threatened plant species conserved *in situ*". Therefore, the increase in percentage of the world's threatened plants to be protected by *in situ* means reflects an overall desire to achieve significant progress by 2020 (GSPC 2008).

In particular, in Europe, the “Council Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora” (Habitat Directive, hereafter) and the Natura 2000 network are by far the most important conservation efforts implemented to date (Maiorano *et al.* 2007, 2015; Pimm *et al.* 2014; Fenu *et al.* 2015a). These programmes have been proposed as the main strategy to meet the target of halting (or at least significantly reducing) biodiversity loss by 2010 (Balmford *et al.* 2005).

Annex II of Habitat Directive lists the “animal and plant species of community interest whose conservation requires the designation of special areas of conservation”, and although it is not a conservation target list itself, it represents a European list of species for which *in situ* conservation actions should be activated. *In situ* conservation of species means the maintenance or recovery of viable populations of species in their natural surroundings (CBD 2012). The main general aim and long-term goal of *in situ* conservation of target species is to protect, manage and monitor selected populations in their natural habitats. Therefore, species conservation depends critically on identifying the habitats in which they occur and then protecting both the habitat and the species through various kinds of management and/or monitoring. In the case of threatened species, their conservation *in situ* also requires that the threats to them are removed or at least contained; otherwise, the species will continue to decline (Heywood 2014). Hence, active *in situ* conservation measures are needed for policy and threatened species. Some forms of intervention or management, such as the protection and restoration of natural habitats, are the best suggested methods of preserving plant diversity (CBD 1992; Fenu *et al.* 2015a).

The 1982 Bern Convention on the Conservation of European Wildlife and Natural Habitats and the Habitats Directive are therefore the most important environmental regulations at the EU and national levels.

Monitoring and reporting the conservation status of the species listed in the Habitats Directive is mandatory for all EU member states. The EU has also established preferential funds targeted for the conservation of these species and their habitats (i.e., the LIFE Programme; Rossi *et al.* 2016).

To develop a conservation strategy for a species, assessment of conservation status is the first step (Planta Europa 2008) and the now accepted standard for doing this are the categories and criteria of the IUCN Red List of Threatened Species (see www.iucnredlist.org; de Grammont and Cuarón 2006; Rodrigues *et al.* 2006; Hoffman *et al.* 2008; IUCN 2014).

At the global scale, IUCN Red Lists of Threatened Species are considered the best source of information on the conservation status of individuals. The IUCN Red List procedure assesses the relative extinction risk of threatened taxa (Bilz *et al.* 2011; IUCN 2014; Rossi *et al.* 2016).

Using the IUCN Red List criteria to assess the extinction risk of policy taxa is a suitable and rapid method for verifying the effectiveness of national and EU conservation policies (Moreno Saiz *et al.* 2003; de Grammont and Cuarón 2006; Rodrigues *et al.* 2006; Hoffman *et al.* 2008; Rossi *et al.* 2016). Moreover, Red Lists are a valuable tool to focus the attention of policymakers and conservation planners, as well as the general public, on the most threatened species (Rossi *et al.* 2016).

Currently, the IUCN system classifies threatened species into threat categories (critically endangered, endangered, and vulnerable) following qualitative and quantitative criteria based on range and population size, condition and demographic trends (IUCN 2010; Domínguez Lozano *et al.* 2013).

The IUCN Red List is intended to be policy-relevant, but not policy-prescriptive. That is, the IUCN Red List provides the best available information about the conservation status of the listed species, and the relative risk of extinction, often including information on the drivers of that risk, but it is not intended to provide specific recommendations on the appropriate policy response to that information (Baillie *et al.* 2004).

Border populations as well as peripheral isolated plant populations are particularly important from both ecological and genetic points of view (Lesica and Allendorf 1995; Conradt 2001; Holt and Keitt 2005) and require more attention from conservation biologists (Abeli *et al.* 2009). Border populations are usually considered more vulnerable and are more prone to local extinction because of their isolation and restriction to marginal habitats (e.g., Gargano *et al.* 2007; Del Vecchio *et al.* 2012; Villellas *et al.* 2013a; Villellas *et al.* 2013b). Due to their evolutionary significance, the endangered species with small and isolated populations have been key topics of conservation biology studies in the last decade to understand the extinction mechanisms and avoid biodiversity loss (Beissinger 2000; Fréville *et al.* 2007; Abeli *et al.* 2009; Gentili *et al.* 2015).

Consequently, several plant species in the Mediterranean Basin that show outlying populations isolated ecologically and geographically from the rest of their distribution range have been investigated in recent years (e.g., Gargano *et al.* 2007; Del Vecchio *et al.* 2012; Fois *et al.* 2015; Fenu *et al.* 2015b).

Monitoring is needed to improve the biological understanding on which management actions can be based (Nichols and Williams 2006). In fact, the collection of field data related to plant populations is the first step to produce estimates of system status or other variables, which can be compared against model-based predictions for the purpose of learning (MacKenzie 2006; Nichols and Williams 2006). Monitoring can be used to predict the effects of various management practices on population size, condition, stage distribution (i.e. seed production and/or seedlings development) and demographic processes, including survivorship and seedling recruitment (Fenu *et al.* 2015a). All this information is needed to estimate the temporal or spatial variation in population size (in term of number of individuals), density, reproduction traits or demographic rates (as survival, growth and fecundity) of plants populations. Also, monitoring is an important part of *in situ* conservation, not the least during and after management interventions to ascertain how successful these are and how far the population has reached a stable and safe state (Heywood 2015).

Species conservation is closely related to understanding the key factors determining their distribution and abundance. Knowledge of their life-cycle, reproductive traits, flowering and fruiting phenology is essential for identifying limits to population growth and persistence (Bevill and Louda 1999).

To efficiently manage threatened species, it is important to understand their population dynamics and identify the current threats acting on them (Oostermeijer *et al.* 1996); hence determining whether a population is growing or declining is central to conservation biology. Although population trends can be estimated from the densities of individuals, understanding the mechanisms that drive those trends requires the quantification of basic vital rates (growth, survival and fecundity; Metcalf *et al.* 2013). In fact, variation in population growth rates is due to differences in underlying vital rates, such as seedling recruitment, growth, reproduction and death (Buckley *et al.* 2010).

Matrix population models are a common tool in population biology to understand the factors influencing individuals that may contribute to overall population dynamics (Caswell 1989). These models represent the life cycle of individuals; hence, can be used to investigate the dynamics of a population (Jongejans and de Kroon 2012), quantifying all ways (through survival and reproduction) in which plants contribute to the size of the population after one time step. More recently, a new discrete-time structured method was introduced: the integral projection model (IPM, hereafter; Easterling *et al.* 2000), which retains the desirable properties of the matrix projection model, while avoiding entirely the need to group plants into discrete stage classes. The analysis of matrix or integral projection models can also help us to identify which life stages or vital rates most influence population growth rate, information that can be used to focus management and monitoring efforts (Morris and Doak 2002).

The best management solutions to improve the conservation status of threatened species are translocations (population reinforcement, reintroduction and introduction), which aim to enhance population viability, for instance by increasing population size, genetic diversity, or by the representation of specific demographic groups or stages (Godefroid *et al.* 2011). However, many limits remain in the implementation of these conservation actions, such as the high both economic and time costs, the availability of the optimal site, the difficulties on the implementation of these actions on private areas and the high uncertainty of success principally connected to natural stochastic events (Fenu *et al.* 2015c). For this reason, it is often necessary to identify other active measures, such as the fences erection to protect small population or to clone in a local nursery the entire population (e.g. in Sardinia, see Fenu *et al.* 2012; Cogoni *et al.* 2013; Fois *et al.* 2015).

Despite the increase in conservation planning and initiatives in the past few decades, the evidence that habitats are being lost or degraded and species increasingly threatened or even extinguished is beyond doubt, and current trajectories suggest that this will continue unless some innovative approaches are adopted (Heywood 2015).

Conservation strategies represent an important issue in the Mediterranean Basin, because this area, which represents only 2% of the world’s surface, houses 10% of the world’s total floristic richness (e.g. Médail and Quézel 1999; Fenu *et al.* 2015a).

The Mediterranean Basin hosts a flora of around 25–35,000 flowering plants and ferns and has been identified as 1 of 34 biodiversity hotspots of the world (Mittermeier *et al.* 2005), including the area with the highest plant richness in Europe (Bilz *et al.* 2011). In this area it is possible to recognize 10-11 hotspots (Médail and Quézel 1997; Cañadas *et al.* 2014), constituting about 22% (515,000 km$^2$) of the total surface of the Mediterranean Basin and including about 5,500 narrow endemic plants (Médail and Quézel 1999; Thompson 2005).

The high rate of regional endemism is, perhaps, the major characteristic of the Mediterranean flora, with close to 60% of all native taxa being Mediterranean endemics, half of which corresponds to narrow endemic species (Thompson 2005; Fenu *et al.* 2015a). In the Mediterranean Basin, Iberian Peninsula and Balearic Islands host around 7,500 *taxa* (species and subspecies; Castroviejo 2010), followed by Italy with 6,711 *taxa* (Conti *et al.* 2005, 2007).

The Tyrrhenian Islands (Sardinia and Corsica), together with others western Mediterranean Islands (Sicily and Balearic Islands), constitute one of the most important biodiversity hotspots within this region (Blondel and Médail 2009; Cañadas *et al.* 2014). Large Mediterranean islands, due to high long-term climate stability, combine a high richness of plant lineages and species with the persistence of many endemic taxa (Médail and Diadema 2009; Fenu *et al.* 2014).

Despite this rich plant diversity, until a few years ago not many biological conservation studies had been carried out on threatened species of Sardinia; however, the increasing number of papers detected in the last years represents a good signal that more attention will be devoted to threatened endemic species in the near future (Fenu *et al.* 2015a). In particular, in the last 10 years, field work and analysis have been implemented for endemics and policy species in Sardinia. At present, a total of 39 plant species have been periodically surveyed; in particular, 12 policy species, corresponding to the 37.5% of the total, have been monitored (Domínguez Lozano *et al.* 2000; Fenu *et al.* 2015a).

## Study species

Cistaceae is a medium-size family with eight genera and approximately 180 *taxa* distributed in temperate and subtropical regions of the northern hemisphere, and it displays the highest genus and species diversity in the Mediterranean floristic region (Próctor and Heywood 1968; Guzmán and Vargas 2009). Within this family, *Helianthemum* Miller is the most diverse genus, with approximately 100 *taxa* that grow from sea level up to approximately 3000 m in a diverse array of substrates (limestone, dolomite, marl, gypsum, saline and sand-soils), that are concentrated in the western Mediterranean area (Próctor and Heywood 1968).

The genus *Helianthemum* Miller (Cistaceae Jussieu) is a monophyletic group limited to the old World (Arrington and Kubitzki 2003; Guzmán and Vargas 2009; Sorrie 2011). It includes about 110 species and subspecies of small herbs or subshrubs, which are distributed from Macaronesia to central Asia, including Europe and North Africa, and mainly occurring in the West Mediterranean, particularly in the Iberian Peninsula (Greuter *et al.* 1984; López González 1993; Arrington and Kubitzki 2003; Parejo-Farnés *et al.* 2013; Ferrer-Gallego 2015).

Within this genus, *Helianthemum caput-felis* Boiss. (Figure 1) deserves particular attention because it is considered the only extant representative of an ancient lineage (Arrigoni 1971; López-González 1992; Parejo-Farnes *et al.* 2013). *H. caput-felis* is a coastal plant with a narrow fragmented distribution along the south-eastern Iberian Peninsula, Balearic Islands, Sardinia and North Africa (Algeria, Morocco and Melilla) coastlines (Fenu *et al.* 2015b); the widest distribution and the largest populations are located in Spain (López-González 1992; Agulló *et al.* 2011), whereas the presence of this species in Sardinia and northwest Africa is restricted to small areas in unique or reduced places (Quézel and Santa 1963; Arrigoni 1971; Fenu and Bacchetta 2008; Fenu *et al.* 2015b). It is a perennial half shrub tall 35(50) cm. Adults plants produce simple raceme-like cymose inflorescences with 3–10 yellow and hermaphroditic flowers, open at dawn and close at dusk, characterised by a short lifespan (3–4 days; Rodríguez-Pérez 2005). Based on studies carried out in Spain, the flowering period is from late February to late May, and the fruiting season runs from late April to July-August (Rodríguez-Pérez 2005; Agulló *et al.* 2015). Tébar, Gil and Llorens (1997) and Rodríguez-Pérez (2005) reported the allogamous character of this species, being a generalist entomogamous plant. Fruits are trilocular capsules, dehiscent by three valves, that detach at maturity and contain up to 6 (10) seeds with brain-like surface (López-González 1993; Agulló *et al.* 2015). Seeds have a mean maximum diameter (±SD) of 1.45 ± 0.11 mm (Bacchetta *et al.* 2008). Seed germination occurs in autumn, at the onset of the rainy season (Rodríguez-Pérez 2005). *H. caput-felis* shows a low germination, which has been attributed to physical exogenous dormancy (impermeable coat), a widespread trait among the Cistaceae (Thanos *et al.* 1992).

From an ecological point of view, *H. caput-felis* is a thermophilous plant that preferably grows in coastal environments under the direct influence of the sea, mostly on calcareous rocky cliffs (0–200 m a.s.l.) with garrigues or scrublands (Arrigoni 1971; Fenu and Bacchetta 2008; Agulló *et al.* 2011); peculiar populations also grow on different habitats, such as sand dunes (Majorca and Melilla), rocky slopes bordering inland ravines (Melilla; Agulló *et al.* 2011) or, rarely, in open wooded areas (Raynaud 1999).

Plants that display a peculiar distribution and, in particular, species with border and peripheral populations, as *H. caput-felis*, represent interesting targets in ecology, evolutionary biology and genetics (Gargano *et al.* 2007; Eckert *et al.* 2008; Sexton *et al.* 2009; Pouget *et al.* 2013; Fenu *et al.* 2015b).

Moreover, both international organizations (e.g. IUCN, European Council) and the European Strategy for Plant Conservation (Planta Europa 2008), declare that border and isolated populations should be considered an important resource for biodiversity and should therefore be included in conservation programs.

In order to stop the continuous decline of the species (due to the gradual loss of natural habitats in which it is found), knowledge of species ecology, demography, distribution and threats affecting its conservation status represents the first step to develop conservation action plans.

**Figure 1 – *Helianthemum caput-felis* Boiss.**

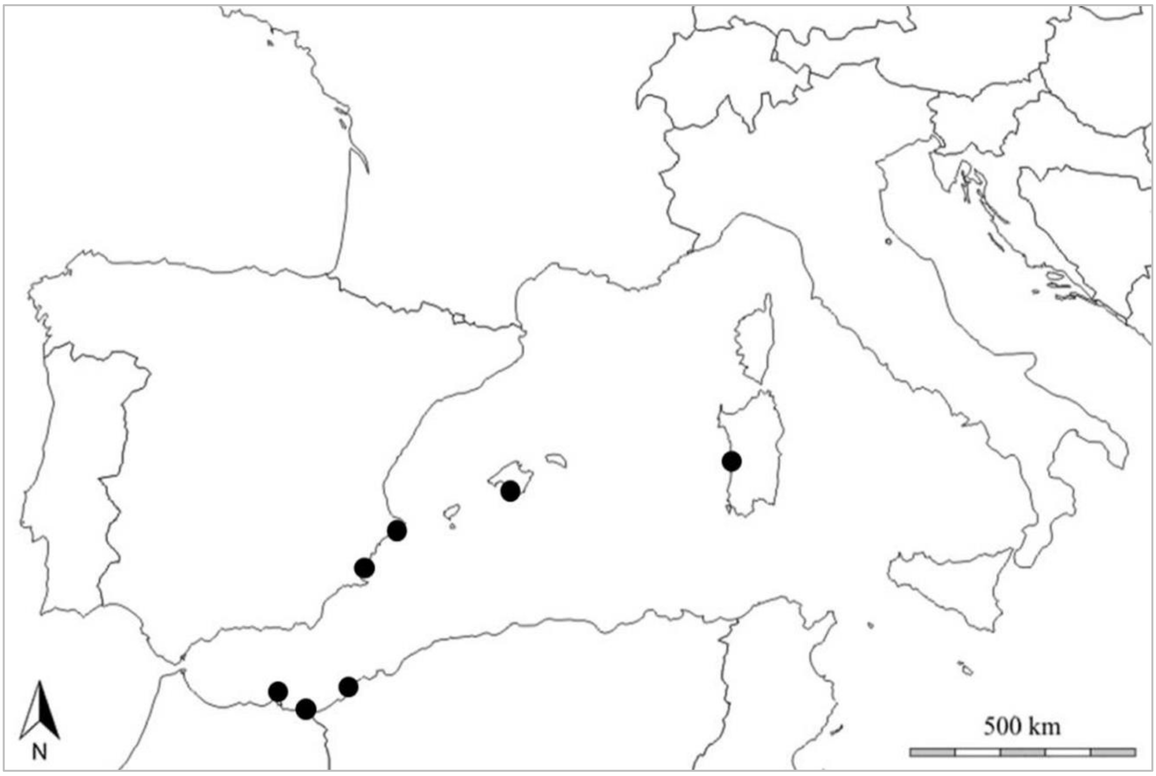

**Figure 2 – Distribution of *H. caput-felis* in the Mediterranean Basin.**

**Figure 3 – Habitats of *H. caput-felis* along its distribution range. a) Capo Mannu (Sardinia, Italy); b) Su Tingiosu (Sardinia, Italy); c) Cabo Roig (Alicante, Spain); d) Moraira (Alicante, Spain); e) Colònia de Sant Jordi (Majorca, Spain); f) Sa Ràpita (Majorca, Spain); g) Taxdirt (Morocco); h) Ras Kebdana (Morocco).**

*Helianthemum caput-felis* is distributed throughout the western Mediterranean Basin (south-eastern Iberian Peninsula, Majorca, Sardinia and northwest Africa) in several fragmented populations (Fenu *et al.* 2015b). From an ecological point of view, it typically grows on coastal environments, under the direct influence of the sea, mostly on calcareous rocky cliffs (0-200 m; Agulló *et al.* 2011).

In Sardinia, which correspond to the eastern range, *H. caput-felis* grows in two main localities (Capo Mannu and Su Tingiosu, central-west part of the Island; Figure 3a and 3b, respectively) that are approximately 3 km apart; small patches are also found in the coastal cliff along the Sinis Peninsula (Arrigoni 1971; Fenu and Bacchetta 2008), at the upper bound of the Gulf of Oristano.

This plant is found in the discontinuities of the *Juniperus* micro-forest and into the maquis, but it mainly grows in the coastal garrigues, where cushion chamaephytes are dominant. *H. caput-felis* is a member of a rupicolous coastal plant community that is rich in narrow endemics, such as *Limonium lausianum* Pignatti and *Polygala sinisica* Arrigoni, as well as other western Mediterranean plants, such as *Viola arborescens* L., *Coris monspeliensis* L. and *Erica multiflora* L. (Fenu *et al.* 2012, 2015b).

*Helianthemum caput-felis*’ Spanish distribution include a central zone, in the Majorcan south coast, and a western zone, in the Valencian Community coast. The Majorcan distribution range is characterized by rocky calcareous cliffs, but also back-dune areas with sand substrate in which grows chamaephytic vegetation. In the eastern Iberian coast, *H. caput-felis* grows in the Alicante Province (Valencian Community), both in the north of Alicante (Figure 3d) and south of it (Figure 3c), at the limit with Murcia Province. These coastal areas are intensely affected by infrastructure construction made during the ’80 Spanish boom, so the areal of the species appears strongly fragmented.

In North Africa the species is located in Melilla (Spain), Morocco (Figure 3g and h) and Algeria’s coasts. In Melilla it grows in rocky slopes bordering inland ravines (Agulló *et al.* 2011).

Among the global distribution range of *H. caput-felis*, six areas were selected in order to carry out demographics and phenological analyses: Capo Mannu and Su Tingiosu (Oristano, Sardinia, Italy), Cabo Roig and Moraira (Alicante, Valencian Community, Spain), Sa Ràpita and Colònia de Sant Jordi (Majorca, Spain).

*Helianthemum caput-felis* is a particularly interesting species because it is protected at European level. In fact, it is listed as Endangered in the European Red List (Bilz *et al.* 2011), included in the Annex I of the Bern Convention (Convention on the Conservation of European Wildlife and Natural Habitats 1979, updated in 2002), in the Washington Convention, and in Annex II of the Habitats Directive (92/43/EEC). At regional level, it is considered Critically Endangered (CR) in Sardinia (Italy; Fenu *et al.* 2015b; Rossi *et al.* 2016) and Endangered (EN) in Spain (Agulló *et al.* 2011). The species is widespread in areas identified as Sites of Community Interest (SCI) according to the Habitats Directive, such as the Sierra de Escalona y Dehesa de Campoamor, Ramblas de las Estacas and Cabo Roig in Spain, and Capo Mannu in Italy. In addition, in the Valencian Community (Spain), Plant Micro-Reserves (PMRs) have been specifically created in limited areas where many species, like *H. caput-felis*, have legal level of protection.

## Aims of this work

The main objective of this Ph.D. thesis was to analyse ecological constraints related to population trends and the conservation status of *Helianthemum caput-felis* Boiss., characterized by a highly fragmented areal isolated from the distribution centre.

In Chapter 1, the distribution range, population size, reproductive traits and the effect of human disturbance of *H. caput-felis* in Sardinia (eastern periphery of its distribution range) were analysed. Moreover, threats affecting the persistence of *H. caput-felis* in Sardinia where investigated, in order to evaluate the conservation status following the IUCN regional protocol.

The study area was expanded in Chapter 2, in which the *H. caput-felis* reproductive traits along the entire European distribution range were analysed. In particular, the Chapter focussed on flowering and fruiting phenology, ecological traits (geomorphology, substrate, slope and human trampling intensity) affecting reproductive outputs (as fruit and seed sets) and the presence of two ecological gradients among the distribution range of the species.

The following phase of work concerned demographic analysis of *H. caput-felis*. Using demographic surveys, matrix and integral projection models, plant performance and population dynamics were investigated both at global level (Chapter 3) than at local scale (Chapter 4), in six localities sampled among the European distribution range in which *H. caput-felis* occurs.

Finally, population dynamics modelling were used in order to know the risk of extinction, and then to assess the global conservation status of *H. caput-felis* populations, together with an accurate analysis of the global area of occupancy of the species, following the IUCN protocol (Chapter 5).

## References

Abeli T., Gentili R., Rossi G., Bedini G. and Foggi B. 2009. Can the IUCN criteria be effectively applied to peripheral isolated plant populations? Biodiversity and Conservation 18: 3877–3890.

Aguilar R., Ashworth L., Galetto L. and Aizen M.A. 2006. Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. Ecology letters 9: 968–980.

Agulló J.C., Juan A., Alonso M.Á. and Crespo M.B. 2010. *Helianthemum caput-felis* Boiss. In: Bañares Á., Blanca G., Güemes J., Moreno J.C. and Ortiz S. eds. 2010. Atlas y Libro Rojo de la Flora Vascular Amenazada de España. Adenda 2010. Dirección General de Medio Natural y Política Forestal (Ministerio de Medio Ambiente, y Medio Rural y Marino), Sociedad Española de Biología de la Conservación de Plantas. Madrid, pp. 76–77.

Agulló J.C., Juan A., Guilló A., Alonso M.Á. and Crespo M.B. 2011. Genetic diversity and phylogeographical assessment of *Helianthemum caput-felis* Boiss. (Cistaceae) based on AFLP markers. Fitosociologia 48: 21–29.

Agulló J.C., Pérez-Bañón C., Crespo M.B. and Juan A. 2015. Puzzling out the reproductive biology of the endangered cat's head rockrose (*Helianthemum caput-felis*, Cistaceae). Flora 217: 75–81.

Allen H.D. 2001. Mediterranean Ecogeography. Pearson Education, Harlow, UK.

Arrigoni P.V. 1971. *Helianthemum caput-felis* Boiss. (2n=24) nuovo reperto per la flora italiana. Webbia 26: 237–243.

Arrington J.M. and Kubitzki K. 2003. Cistaceae. In: Flowering Plants· Dicotyledons, pp. 62–70. Springer Berlin Heidelberg.

Bacchetta G., Grillo O., Mattana E. and Venora G. 2008. Morpho-colorimetric characterization by image analysis to identify diaspores of wild plant species. Flora 203: 669–682.

Baillie J., Hilton-Taylor C., Stuart SN. 2004. IUCN red list of threatened species. A global species assessment. IUCN, Gland, Switzerland.

Ballantyne M. and Pickering C.M. 2013. Tourism and recreation: a common threat to IUCN red-listed vascular plants in Europe. Biodiversity and conservation 22: 3027–3044.

Balmford A., Bennun L., Ten Brink B., Cooper D., Côté I.M., Crane P., Dobson A., Dudley N., Dutton I., Green R.E., Gregory R.D., Harrison J., Kennedy E.T., Kremen C., Leader-Williams N., Lovejoy T.E., Mace G., May R., Mayaux P., Morling P., Phillips J., Redford K., Ricketts T.H., Rodríguez J.P., Sanjayan M., Schei P.J., van Jaarsveld A.S. and Walther B.A. 2005. The convention on biological diversity's 2010 target. Science 307: 43–45.

Beissinger S.R. 2000. Ecological mechanisms of extinction. Proceedings of the National Academy of Sciences 97: 11688–11689.

Benito B.M., Martínez-Ortega M.M., Muñoz L.M., Lorite J. and Peñas J. 2009. Assessing extinction-risk of endangered plants using species distribution models: a case study of habitat depletion caused by the spread of greenhouses. Biodiversity and Conservation 18: 2509–2520.

Bevill R.L. and Louda S.M. 1999. Comparisons of related and common species in the study of plant rarity. Conservation Biology 13: 493–498.

Bilz M., Kell S.P., Maxted N. and Lansdown R.V. 2011. European Red List of Vascular Plants. Publications Office of the European Union, Luxembourg.

Blondel J. and Médail F. 2009. Biodiversity and conservation. In: Woodward J.C. ed. The physical geography of the Mediterranean. Oxford University Press, Oxford, pp. 615–650.

Buckley Y.M., Ramula S., Blomberg S.P., Burns J.H., Crone E.E., Ehrlén J., Knight T.M., Pichancourt J., Quested H. and Wardle G.M. 2010. Causes and consequences of variation in plant population growth rate: a synthesis of matrix population models in a phylogenetic context. Ecology Letters 13: 1182–1197.

Cañadas E.M., Fenu G., Peñas J., Lorite J., Mattana E. and Bacchetta G. 2014. Hotspots within hotspots: Endemic plant richness, environmental drivers, and implications for conservation. Biological Conservation 170: 282–291.

Castroviejo S. 2010. La flora española: una riqueza en biodiversidad de primer orden aún en exploración. El proyecto Flora Ibérica. Documentación Administrativa 278-279: 23-38.

Caswell H. 1989. Analysis of life table response experiments I. Decomposition of effects on population growth rate. Ecological Modelling 46: 221–237.

CBD 1992. Convention on Biological Diversity. Article 2, CBD. http://www.biodiv.org/convention/articles.asp

CBD 2012. Convention on Biological Diversity. Global Strategy for Plant Conservation: 2011-2020. Richmond.

Cogoni D., Fenu G., Concas E. and Bacchetta G. 2013. The effectiveness of plant conservation measures: The *Dianthus morisianus* reintroduction. Oryx 47: 203–206.

Conradt L. 2001. Ecological and evolutionary processes at expanding range margins. Nature 411: 577–581.

Conti F., Abbate G., Alessandrini A. and Blasi C. 2005. An Annotated Check-List of the Italian Vascular Flora. Palombi Editore, Roma.

Conti F., Alessandrini A., Bacchetta G., Banfi E., Barberis G., Bartolucci F., Bernardo L., Bonacquisti S., Bouvet D., Bovio M., Brusa G., Del Guacchio E., Foggi B., Frattini S., Galasso G., Gallo L., Gangale C., Gottschlich G., Grünanger P., Gubellini L., Iiriti G., Lucarini D., Marchetti D., Moraldo B., Peruzzi L., Poldini L., Prosser F., Raffaelli M., Santangelo A., Scassellati E., Scortegagna S., Selvi F., Soldano A., Tinti D., Ubaldi D., Uzunov D. and Vidali M. 2007. Integrazioni alla checklist della flora vascolare italiana. Natura Vicentina 10: 5–74.

De Vere N., Jongejans E., Plowman A. and Williams E. 2009. Population size and habitat quality affect genetic diversity and fitness in the clonal herb *Cirsium dissectum*. Oecologia 159: 59–68.

Del Vecchio S., Giovi E., Izzi F., Abbate G. and Acosta A.T.R. 2012. *Malcolmia littorea*: the isolated Italian population in the European context. Journal for Nature Conservation 20: 357–363.

Domínguez Lozano F., Atkins K.J., Moreno Saiz J.C., Sims A.E. and Dixon K. 2013. The nature of threat category changes in three Mediterranean biodiversity hotspots. Biological conservation 157: 21–30.

Domínguez Lozano F., Galicia Herbada D., Moreno Rivero L., Moreno Saiz J.C. and Sainz Ollero H. 2000. Areas of high floristic endemism in Iberia and the Balearic Islands: an approach to biodiversity conservation using narrow endemics. Belgian Journal of Entomology 2: 171–185.

Dudash M.R. and Fenster C.B. 2000. Inbreeding and outbreeding depression in fragmented populations. In: Young A.G. and Clarke G.M. (eds), Genetics, demography and viability of fragmented populations. Cambridge University Press, pp. 35–53.

Duncan D.H., Nicotra A.B., Wood J.T. and Cunningham S.A. 2004. Plant isolation reduces outcross pollen receipt in a partially self-compatible herb. Journal of Ecology 92: 977–985.

Easterling M.R., Ellner S.P. and Dixon P.M. 2000. Size-specific sensitivity: applying a new structured population model. Ecology 81: 694–708.

Eckert C.G., Samis K.E. and Lougheed S.C. 2008. Genetic variation across species' geographical ranges: the central-marginal hypothesis and beyond. Molecular Ecology 17: 1170–1188.

Fenu G. and Bacchetta G. 2008. La flora vascolare della penisola del Sinis (Sardegna occidentale). Acta Botánica Malacitana 33: 91–124.

Fenu G., Cogoni D., Pinna M.S. and Bacchetta G. 2015a. Threatened Sardinian vascular flora: A synthesis of 10 years of monitoring activities. Plant Biosystems 149: 473–482.

Fenu G., Cogoni D., Sulis E. and Bacchetta G. 2015b. Ecological response to human trampling and conservation status of *Helianthemum caput-felis* (Cistaceae) at the eastern periphery of its range. Botany Letters 162: 191–201.

Fenu G., Cogoni D., Ulian T. and Bacchetta G. 2013. The impact of human trampling on a threatened coastal Mediterranean plant: The case of *Anchusa littorea* Moris (Boraginaceae). Flora 208: 104–110.

Fenu G., Fois M., Cañadas E.M. and Bacchetta G. 2014. Using endemic-plant distribution, geology and geomorphology in biogeography: the case of Sardinia (Mediterranean Basin). Systematics and Biodiversity 12: 181–193.

Fenu G., Fois M., Cogoni D., Porceddu M., Pinna M.S., Cuena Lombraña A., Nebot A., Sulis E., Picciau R., Santo A., Murru V., Orrù M. and Bacchetta G. 2015c. The Aichi Biodiversity Target 12 at regional level: an achievable goal?. Biodiversity 16: 120–135.

Fenu G., Mattana E. and Bacchetta G. 2011. Distribution, status and conservation of a Critically Endangered, extremely narrow endemic: *Lamyropsis microcephala* (Asteraceae) in Sardinia. Oryx 45: 180–186.

Fenu G., Mattana E. and Bacchetta G. 2012. Conservation of endemic insular plants: the genus *Ribes* L. (Grossulariaceae) in Sardinia. Oryx 46: 219–222.

Fenu G., Sulis E., Cogoni D. and Bacchetta G. 2012. Schede per una Lista Rossa della Flora vascolare e crittogamica Italiana: *Helianthemum caput-felis* Boiss.. Informatore Botanico Italiano 44: 233–236.

Ferrer-Gallego P.P. 2015. Remarks on the nomenclatural type of *Cistus origanifolius* Lamarck (Cistaceae). Phytotaxa 233: 191–195.

Fois M., Fenu G., Lombraña A.C., Cogoni D. and Bacchetta G. 2015. A practical method to speed up the discovery of unknown populations using Species Distribution Models. Journal for Nature Conservation 24: 42–48.

Fos S., Laguna E. and Jiménez J. 2014. Plant micro-reserves in the Valencian region (E of Spain): are we achieving the expected results? Passive conservation of relevant vascular plant species. Flora Mediterr 24: 153–162.

Fréville H., McConway K., Dodd M. and Silvertown J. 2007. Prediction of extinction in plants: interaction of extrinsic threats life history traits. Ecology 88: 2662–2672.

Gargano D., Fenu G., Medagli P., Sciandrello S. and Bernardo L. 2007. The status of *Sarcopoterium spinosum* (Rosaceae) at the western periphery of its range: ecological constraints led to conservation concerns. Israel Journal of Plant Sciences 55: 1–13.

Gentili R., Fenu G., Mattana E., Citterio S., De Mattia F. and Bacchetta G. 2015. Conservation genetics of two island endemic *Ribes* spp. (Grossulariaceae) of Sardinia: survival or extinction?. Plant Biology 17: 1085–1094.

Gibbs J.P. 2001. Demography versus habitat fragmentation as determinants of genetic variation in wild populations. Biological Conservation 100: 15–20.

Global Strategy for Plant Conservation (GSPC) 2008. http://www. cbd.int/gspc/

Godefroid S., Rivière S., Waldren S., Boretos N., Eastwood R. and Vanderborght T. 2011. To what extent are threatened european plant species conserved in seed banks? Biological Conservation 144: 1494–1498.

Greuter W., Burdet H.M. and Long G. 1984. Med-Checklist. A critical inventory of vascular plants of the circummediterranean countries 1. Conservatoire et Jardin botaniques de la Ville de Genève and Botanischer Garten and Botanisches Museum, Berlin-Dahlem, 330 pp.

Guzmán B. and Vargas P. 2009. Historical biogeography and character evolution of Cistaceae (Malvales) based on analysis of plastid rbcL and trnL-trnF sequences. Organisms Diversity and Evolution 9: 83–99.

Heywood V.H. 2014. An overview of *in situ* conservation of plant species in the Mediterranean. Flora Mediterranea 24: 5–24.

Heywood V.H. 2015. *In situ* conservation of plant species–an unattainable goal?. Israel Journal of Plant Sciences 1–21.

Hoffmann M., Brooks T.M., da Fonseca G.A., Gascon C., Hawkins A.F.A., James R.E., Langhammer P., Mittermeier R.A., Pilgrim J.D., Rodrigues A.S.L. and Silva J.M.C 2008. Conservation planning and the IUCN Red List. Endangered Species Research 6: 113–125.

Holsinger K.E. 2000. Demography and extinction in small populations. In: Young A.G. and Clarke G.M. (eds), Genetics, demography and viability of fragmented populations. Cambridge University Press, pp. 55–74.

Holt R.D. and Keitt T.H. 2005. Species' borders: a unifying theme in ecology. Oikos 108: 3–6.

IUCN 2003. IUCN Red List of Threatened Species. IUCN, Glands, Switzerland, Cambridge, UK.

IUCN Standards and Petitions Subcommittee 2014. Guidelines for Using the IUCN Red List Categories and Criteria. Version 11. Prepared by the Standards and Petitions Subcommittee. Available at http://www.iucnredlist.org/documents/RedListGuidelines.pdf.

Jongejans E. and de Kroon H. 2012. Matrix models. In: Encyclopedia of Theoretical Ecology (eds. Hastings A. and Gross L.). University of California, pp. 415–423.

Kearns C.A., Inouye D.W. and Waser N.M. 1998. Endangered mutualisms: the conservation of plant–pollinator interactions. Annual review of ecology and systematics 29: 83–112.

Kolb A. and Diekmann M. 2005. Effects of life-history traits on responses of plant species to forest fragmentation. Conservation Biology 19: 929–938.

Laguna E. 1995. Microrreservas de flora: un nuevo modelo de conservación en la Comunidad Valenciana. Quercus 118: 22–26.

Laguna E. 1999. The plant micro-reserves programme in the region of Valencia. Spain. PP.

Laguna E. 2001. The micro-reserves as a tool for conservation of threatened plants in Europe (Vol. 121). Council of Europe.

Lesica P. and Allendorf F.W. 1995. When are peripheral populations valuable for conservation?. Conservation Biology 9: 753–760.

Lienert J. 2004. Habitat fragmentation effects on fitness of plant populations–a review. Journal for nature conservation 12: 53–72.

López González G. 1993. *Helianthemum* Mill. In: Castroviejo S., Aedo C., Cirujano S., Laínz M., Montserrat P., Morales R., Muñoz-Garmendia F., Navarro C., Paiva J. and Soriano C. (Eds.) Flora iberica 3. Real Jardín Botánico, CSIC, Madrid, pp. 365–421.

Mace G.M., Collar N.J., Gaston K.J., Hilton-Taylor C., Akçakaya H.R., Leader-Williams N. and Stuart S.N. 2008. Quantification of extinction risk: IUCN's system for classifying threatened species. Conservation Biology 22: 1424–1442.

MacKenzie D.I. 2006. Occupancy estimation and modeling: inferring patterns and dynamics of species occurrence. Academic Press.

Maiorano L., Amori G., Montemaggiori A., Rondinini C., Santini L., Saura S. and Boitani L. 2015. On how much biodiversity is covered in Europe by national protected areas and by the Natura 2000 network: insights from terrestrial vertebrates. Conservation Biology 29: 986–995.

Maiorano L., Falcucci A., Garton E.O. and Boitani L. 2007. Contribution of the Natura 2000 network to biodiversity conservation in Italy. Conservation Biology 21: 1433–1444.

Marrero-Gómez M.V., Oostermeijer J.G.B., Carqué-Álamo E. and Bañares-Baudet Á. 2007. Population viability of the narrow endemic *Helianthemum juliae* (Cistaceae) in relation to climate variability. Biological conservation 136: 552–562.

Matthies D., Bräuer I., Maibom W. and Tscharntke T. 2004. Population size and the risk of local extinction: empirical evidence from rare plants. Oikos 105: 481–488.

McLachlan A. and Brown A.C. 2006. The Ecology of Sandy Shores (second ed.). Academic Press, Burlington, MA, USA. 373 pp.

Médail F. and Diadema K. 2009. Glacial *refugia* influence plant diversity patterns in the Mediterranean Basin. Journal of Biogeography 36: 1333–1345.

Médail F. and Quézel P. 1997. Hot-spots analysis for conservation of plant biodiversity in the Mediterranean Basin. Annals of the Missouri Botanical Garden 84: 112–127.

Médail F. and Quézel P. 1999. Biodiversity hotspots in the Mediterranean Basin: Setting global conservation priorities. Conservation Biology 13: 1510–1513.

Menges E.S. 1991. The application of minimum viable population theory to plants. In: Falk D.A., Holsinger K.E. (Eds.), Genetics and Conservation of Rare Plants. Oxford University Press, pp. 47–61.

Metcalf C.J.E., McMahon S.M., Salguero-Gómez R. and Jongejans E. 2013. IPMpack: an R package for integral projection models. Methods in Ecology and Evolution 4: 195–200.

Mittermeier R.A., Robles Gil P., Hoffman M., Pilgrim J., Brooks T., Mittermeier C.G., Lamoreux J. and Fonseca G.A.B. 2005. Hotspots revisited: Earth's biologically richest and most endangered terrestrial ecoregions. Chicago, IL, University of Chicago Press.

Moreno Saiz J.C., Domínguez Lozano F. and Sainz Ollero H. 2003. Recent progress in conservation of threatened Spanish vascular flora: a critical review. Biological Conservation 113: 419–431.

Morris W.F. and Doak D.F. 2002. Quantitative conservation biology: The theory and practice of population viability analysis. Sunderland, MA: Sinauer Associates.

Nichols J.D. and Williams B.K. 2006. Monitoring for conservation. Trends in Ecology and Evolution 21: 668–673.

Novacek M.J. and Cleland E.E. 2001. The current biodiversity extinction event: scenarios for mitigation and recovery. Proceedings of the National Academy of Sciences 98: 5466–5470.

Oostermeijer J.G.B., Brugman M.L., De Boer E.R. and Den Nijs H.C. 1996. Temporal and spatial variation in the demography of Gentiana pneumonanthe, a rare perennial herb. Journal of Ecology 84: 153–166.

Oostermeijer J.G.B., Luijten S.H. and den Nijs J.C.M. 2003. Integrating demographic and genetic approaches in plant conservation. Biological Conservation 113: 389–398.

Parejo-Farnés C., Albaladejo R.G., Arroyo J. and Aparicio A. 2013. A phylogenetic hypothesis for *Helianthemum* (Cistaceae) in the Iberian Peninsula. Botanica Complutensis 37: 83–92.

Perrings C. 2014. Our uncommon heritage. Biodiversity Change, Ecosystem Services, and Human Wellbeing. Cambridge University Press.

Pimm S.L., Jenkins C.N., Abell R., Brooks T.M., Gittleman J.L., Joppa L.N., Raven P.H., Robert C.M. and Sexton J.O. 2014. The biodiversity of species and their rates of extinction, distribution, and protection. Science 344: 1246752.

Pinna M.S., Cogoni D., Fenu G. and Bacchetta G. 2015. The conservation status and anthropogenic impacts assessments of Mediterranean coastal dunes. Estuarine, Coastal and Shelf Science 167: 25–31.

Planta Europa 2008. A Sustainable Future for Europe: The European Strategy for Plant Conservation 2008–2014. Plantlife.

Pouget M., Youssef S., Migliore J., Juin M., Médail F. and Baumel A. 2013. Phylogeography sheds light on the central–marginal hypothesis in a Mediterranean narrow endemic plant. Annals of Botany 112: 1409–1420.

Próctor M.C.F. and Heywood V.H. 1968. *Helianthemum* Miller. In: Flora Europaea, edited by Tutin T.G., Heywood H., Burges N.A., Moore D.M., Valentine D.H., Walters S.M. and Webb D.A. 2: 286–292. Cambridge University Press, Cambridge.

Quézel P. and Santa S. 1963. Nouvelle flore de l'Algérie et des régions désertiques méridionales. Volumen 2. Paris: Centre National de la Reherche Scientifique.

Raynaud C. 1999. Cistaceae. In: Flore Pratique du Maroc. Manuel de determination des plantes vasculaires, 1, s. Bot. 36, edited by Fennane M., Ibn Tattou J., Mathez A. and Ouyahya El Oualidi J., pp. 302–326. Travaux Institut Scientifique, Université Mohammed V, Rabat.

Rodrigues A.S., Pilgrim J.D., Lamoreux J.F., Hoffmann M. and Brooks T.M. 2006. The value of the IUCN Red List for conservation. Trends in Ecology and Evolution 21: 71–76.

Rodríguez-Pérez J. 2005. Breeding system, flower visitors and seedling survival of two endangered species of *Helianthemum* (Cistaceae). Annals of Botany 95: 1229–1236.

Rossi G., Montagnani C., Gargano D., Peruzzi L., Abeli T., Ravera S., Cogoni A., Fenu G., Magrini S., Gennai M., Foggi B., Wagensommer R.P., Venturella G., Blasi C., Raimondo F.M. and Orsenigo S. (Eds.) 2013. Lista Rossa della Flora Italiana. 1. Policy Species e altre specie minacciate. Rome. www.iucn.it.

Rossi G., Orsenigo S., Montagnani C., Fenu G., Gargano D., Peruzzi L., Wagensommer R.P., Foggi B., Bacchetta G., Domina G., Conti F., Bartolucci F., Gennai M., Ravera S., Cogoni A., Magrini S., Gentili R., Castello M., Blasi C. and Abeli T. 2016. Is legal protection sufficient to ensure plant conservation? The Italian Red List of Policy species as a case study. Oryx, in press.

Sala O.E˙, Chapin F.S. 3rd, Armesto J.J., Berlow E., Bloomfield J., Dirzo R., Huber-Sanwald E., Huenneke L.F., Jackson R.B., Kinzig A., Leemans R., Lodge D.M., Mooney H.A., Oesterheld M., Poff N.L., Sykes M.T., Walker B.H., Walker M. and Wall D.H. 2000. Global biodiversity scenarios for the Year 2100. Science 287: 1770–1774.

Schemske D.W., Husband B.C., Ruckelshaus M.H., Goodwillie C., Parker I.M. and Bishop J.D. 1994. Evaluating approaches to the conservation of rare and endangered plants. Ecology 75: 584–606.

Schleuning M. and Matthies D. 2009. Habitat change and plant demography: assessing the extinction risk of a formerly common grassland perennial. Conservation Biology 23: 174–183.

Sexton J.P., McIntyre P.J., Angert A.L. and Rice K.J. 2009. Evolution and ecology of species range limits. Annual Review of Ecology Evolution and Systematics 40: 415–436.

Sorrie B.A. 2011. Transfer of North American *Helianthemum* to *Crocanthemum* (Cistaceae): new combinations. Phytologia 93: 270–271.

Tébar F.J., Gil L. and Llorens L. 1997. Reproductive biology of *Helianthemum apenninum* (L.) Mill. and *H. caput-felis* Boiss. (Cistaceae) from Mallorca (Balearic Islands, Spain). Acta Botánica Malacitana 22: 53–63.

Thanos C.A., Georghiou K., Kadis C. and Pantazi C. 1992. Cistaceae: a plant family with hard seeds. Israel Journal of Botany 41: 251–263.

Thompson J.D. 2005. Plant Evolution in the Mediterranean. Oxford University Press, Oxford.

Thuiller W. 2007. Biodiversity: climate change and the ecologist. Nature 448: 550–552.

Villellas J., Ehrlén J., Olesen J.M., Braza R. and García M.B. 2013a. Plant performance in central and northern peripheral populations of the widespread *Plantago coronopus*. Ecography 36: 136–145.

Villellas J., Morris W.F. and García M.B. 2013b. Variation in stochastic demography between and within central and peripheral regions in a widespread short-lived herb. Ecology 94: 1378–1388.

# CHAPTER 1

## Ecological response to human trampling and conservation status of *Helianthemum caput-felis* (Cistaceae) at the eastern periphery of its range

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### Introduction

Several plant species are characterised by a disjunctive distribution in which peripheral populations can be isolated from the main home range. Plants that display a peculiar distribution and, in particular, species with border and peripheral populations represent interesting targets in ecology, evolutionary biology and genetics (Eckert *et al.* 2008; Sexton *et al.* 2009; Pouget *et al.* 2013). In addition, they provide insight into critical phenomena, such as speciation, adaptive radiation, and natural selection (Grant and Antonovics 1978; Holt and Keitt 2005).

Independent of the central/marginal model debate, border populations as well as peripheral isolated plant populations (PIPPs) are particularly important from both ecological and genetic points of view (Lesica and Allendorf 1995; Conradt 2001; Holt and Keitt 2005) and require more attention from conservation biologists (Abeli *et al.* 2009). Border populations are usually considered more vulnerable and are more prone to local extinction due to their isolation and restriction to marginal habitats (e.g. Gargano *et al.* 2007; Del Vecchio *et al.* 2012; Villellas *et al.* 2013a; Villellas *et al.* 2013b). As suggested by international organisations (e.g. IUCN, European Council) and according to *The European Strategy for Plant Conservation* (Planta Europa 2008), border and isolated populations should be considered an important resource for biodiversity and should thus be included in conservation programs. Consequently, several plant species in the Mediterranean Basin that show outlying populations isolated ecologically and geographically from the rest of their distribution range have been investigated in recent years (e.g. Gargano *et al.* 2007; Del Vecchio *et al.* 2012; Fois *et al.* 2015).

Cistaceae is a medium-size family with eight genera and approx. 180 *taxa* distributed in temperate and subtropical regions of the northern hemisphere, and it displays the highest genus and species diversity in the Mediterranean floristic region (Guzman and Vargas 2009). Within this family, *Helianthemum* Miller is the most diverse genus, with approx. 100 *taxa* that grow from sea level up to approx. 3000 metres in a diverse array of substrates (limestone, dolomite, marl, gypsum, saline and sand-soils) that are concentrated in the western Mediterranean area (Próctor and Heywood 1968). Within this genus, *Helianthemum caput-felis* Boiss. deserves particular attention because it is considered the only extant representative of an ancient lineage (Arrigoni 1971; López-González 1992). *H. caput-felis* is a coastal plant distributed throughout the western Mediterranean Basin (SE Iberian Peninsula, Majorca, Sardinia and NW Africa) in several fragmented populations; the widest distribution and the largest populations are located in Spain (Agulló *et al.* 2011; López-González 1992), while the presence of this species in Sardinia and NW Africa is restricted to small areas in unique or reduced places (Arrigoni 1971; Fenu and Bacchetta 2008; Quézel and Santa 1963).

In Sardinia, *H. caput-felis* grows in two main localities (Capo Mannu and Su Tingiosu, central-west part of the island; Figure 1) that are approx. 3 km apart; small patches are also found in the coastal cliff along the Sinis Peninsula (Arrigoni 1971; Fenu and Bacchetta 2008). This plant is found in the discontinuities of the *Juniperus* micro-forest and into the maquis, but it mainly grows in the coastal garrigues, where cushion chamaephytes are dominant. *H. caput-felis* is a member of a rupicolous coastal plant-community that is rich in narrow endemics, such as *Limonium lausianum* Pignatti and *Polygala sinisica* Arrigoni, as well as other western Mediterranean plants, such as *Viola arborescens* L., *Coris monspeliensis* L. and *Erica multiflora* L. (Fenu *et al.* 2012).

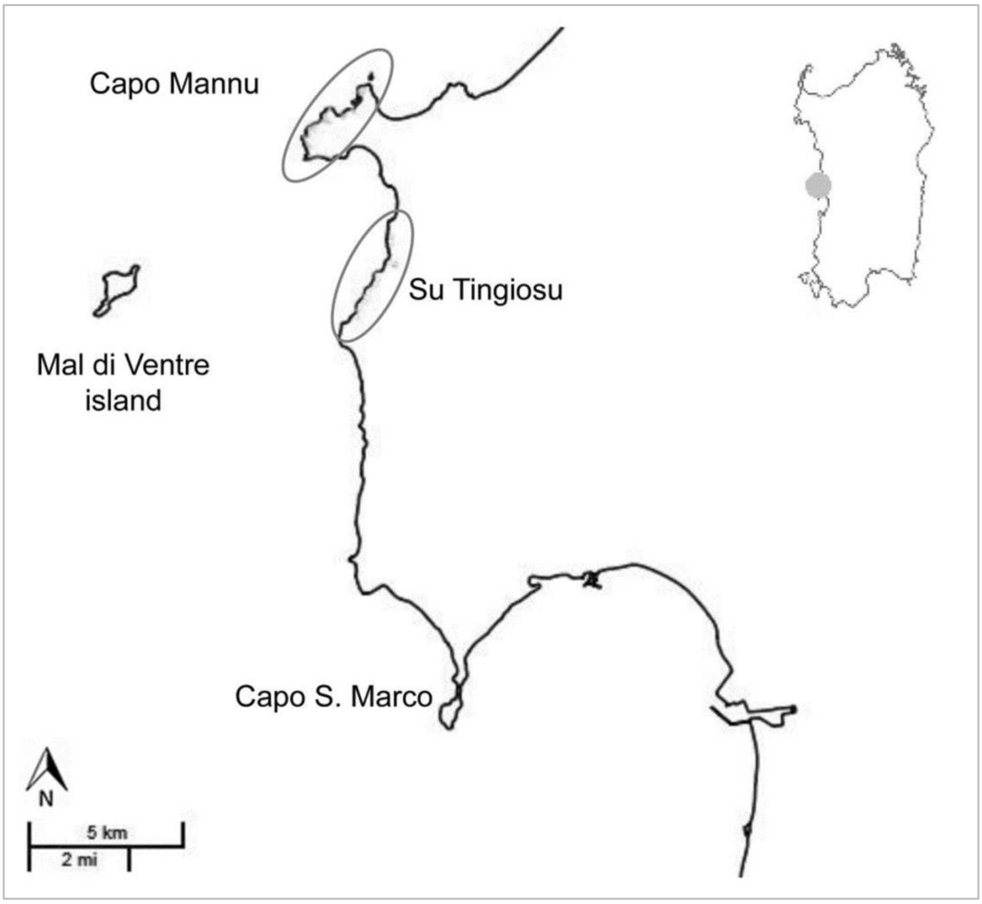

**Figure 1 – Study area in the Sinis Peninsula (CW-Sardinia); the circles indicate the main localities of Capo Mannu and Su Tingiosu where *H. caput-felis* grows.**

*Helianthemum caput-felis* is an emblematic plant from a conservation point of view; it is included in the Berne Convention and Appendix II of the Habitats Directive (European Community Council Directive 92/43/EEC), and it is classified as endangered on the European red list of vascular plants (Bilz *et al.* 2011) according to the IUCN protocol (IUCN 2001). However, to date, no exhaustive reproductive and ecological studies have been conducted on this plant, and in particular on the Sardinian population, and no conservation actions have been implemented for this species.

Mediterranean coastal habitats have been altered by human activity for several thousands of years, and coastal environments are strongly affected by tourism and related infrastructures (Davenport and Davenport 2006). In particular, touristic and recreational activities in coastal areas appear to be common threats to a wide variety of European threatened plants (Bilz *et al.* 2011; Ballantyne and Pickering 2013; Fenu *et al.* 2013; Rossi *et al.* 2016). However, few studies have focused on the effects of human trampling on Mediterranean coastal ecosystems (Comor *et al.* 2008; Kutiel *et al.* 2000; Kerbiriou *et al.* 2008). Although for threatened plants the impact of tourism is particularly severe (Pickering and Hill 2007), to our knowledge, the effect of human trampling on threatened plants growing on coastal areas has yet to be accurately assessed in Mediterranean coastal habitats. In general, tolerance of species to human trampling varies, sometimes markedly. In Mediterranean coastal regions, some plant species (including threatened plants) are very sensitive to trampling, while others seem to be tolerant or even to benefit from trampling (Kerbiriou *et al.* 2008; Yu *et al.* 2009; Fenu *et al.* 2013). Human trampling has been demonstrated to be generally an important threat for threatened plant species in Sardinia (Quilichini and Debussche 2000; Fenu *et al.* 2011, 2013; Rossi *et al.* 2016) and we hypothesize that also the Sardinian population of *H. caput-felis* is particularly affected by human disturbance, as previously reported for the Spanish population (Dominguez Lozano *et al.* 1996).

The main aims of this study were to verify the distribution range and population size of *H. caput-felis* in Sardinia (eastern periphery of its distribution range), to analyse plant size and reproductive traits, to analyse the effect of human disturbance, to identify its principal threats and to assess its conservation status at the regional level following the IUCN protocol.

## Materials and methods

### *Study species*

*Helianthemum caput-felis* is a perennial small shrub that grows to a height of 35(50) cm. Its flowers, which are arranged in inflorescences at the tip of new branches, are yellow and hermaphroditic, open at dawn and close at dusk, and have a short lifespan (3–4 days, Rodríguez-Pérez 2005). Based on studies carried out in Spain, the flowering period is from late February to late May, and the fruiting season runs from late April to July-August (Rodríguez-Pérez 2005). Tébar, Gil and Llorens (1997) and Rodríguez-Pérez (2005) reported the allogamous character of this species, being a generalist entomogamous plant. Fruits are capsules that detach at maturity, and seed germination occurs in autumn, at the onset of the rainy season (Rodríguez-Pérez 2005).

From an ecological point of view, *H. caput-felis* is a thermophilous plant that typically grows in coastal environments under the direct influence of the sea, mostly on calcareous rocky cliffs (0-200 m a.s.l.) with garrigues or scrublands (Arrigoni 1971; Agulló *et al.* 2011; Fenu *et al.* 2012); peculiar populations also grow on different habitats, such as sand dunes (Mallorca and Melilla), rocky slopes bordering inland ravines (Melilla; Agulló *et al.* 2011) or, rarely, in open wooded areas (Raynaud 1999).

### *Data sampling*

The distribution of *H. caput-felis* was verified by field surveys conducted in the localities for which herbarium specimens and/or published data (Arrigoni 1971; Fenu and Bacchetta 2008) were available. The geographical limits of the main localities of Capo Mannu and Su Tingiosu (CM and ST, hereafter) were mapped using a global positioning system, and the areas where the plants were found were estimated using ArcView v. 3.2 (ESRI, Redlands, USA). Localities containing only scattered plants were excluded a priori in this study (Figure 1).

Forty permanent plots of 2 × 1 m (20 per locality) were randomly established (in the area where the plant was found) to estimate the plant densities and extrapolate the population size. Over one year, plants were monitored bimonthly, approximately on the 10th and 20th days of each month. Within the plots, all plants (including seedlings) were counted, marked and measured from March to August, in order to analyse the whole reproductive season of the plant species. During each monitoring, survival, morphological (height, maximum and minimum diameter) and reproductive traits (number of flowers and fruits per plant) were recorded for every plant. Each plant was assigned to one of the following habitats: garrigue, maquis and micro-forest. Similarly, each plant was assigned to a geomorphology and substrate (lowland *versus* slope areas and soil *versus* bedrock, respectively).

Human trampling intensity was visually estimated for each plot and four levels of intensity were considered: absent, low (≤30% of the plot surface), moderate (31–60%), and intense (≥61%); ten plots for each category were considered in this study.

Phenological and reproductive traits were monitored in 378 marked-plants. Phenological phases were considered present when >5% of the plants showed the same pattern and each plant were classified as being reproductive based on the presence of flowers/fruits (censuses were carried out in May). The average number of fruits per plant was determined at the peak of fruiting season (May) from a ratio of the total number of fruits/total number of plants monitored. Seed-set (considered as number of seeds per fruit) was calculated in May by collecting randomly 400 mature fruits from 40 randomly selected plants. Seeds were extracted and counted, and the average number of seeds per fruit was multiplied by the average number of fruits per reproductive plant to predict the mean reproductive capacity of each plant.

### *IUCN assessment at regional level*

Globally, the IUCN Red List procedure is the most widely used protocol for species risk assessment because it facilitates objective, replicable and flexible assessments (e.g. Rodrigues *et al.* 2006; de Grammont and Cuarón 2006; Rossi *et al.* 2016). For IUCN assessment, we evaluated all of the localities where *H. caput-felis* grows (Fenu and Bacchetta 2008). IUCN categories and criteria version 3.1 (2001) were applied according to the procedure for regional assessment (IUCN 2003). According to the New Italian Red List project (Rossi *et al.* 2014), a grid of 2 × 2 km generated by the ESRI® ArcGis™ 9x package and superimposed to a map of Italy was used to assess the area of occupancy (AOO, defined as the area within the extent of occurrence, EOO, that is occupied by a *taxon*, where EOO is defined as the area contained within the shortest continuous imaginary boundary that can be drawn to encompass all the known sites of occurrence of a taxon, excluding cases of vagrancy, *sensu* IUCN 2001, 2014a). All of the parameters required by the IUCN protocol (i.e., EOO, locations, decline rate) and conservation status were assessed following the latest guidelines of the IUCN (2014a).

The major threats to *H. caput-felis* were determined through field observations and categorised following the version 3.2 of IUCN threats classification scheme (IUCN 2014b).

### *Statistical analyses*

Considering that the peak of the reproductive season occurred in May, only the data of this month have been used in the analyses. Morphometric traits collected in the field were used to calculate the volume of each plant (plant size, hereafter). We calculated plant size (V, expressed in $dm^3$) using individual height (h, expressed in dm) and the maximum and minimum diameter ($d_M$ and $d_m$, respectively), according to the following formula:

$$\text{Plant size } [dm^3]\text{: } V = [\pi \times (d_M/2) \times (d_m/2)] \times h$$

The Pearson correlation value was calculated to correlate plant size with reproductive parameter. To evaluate the effect of locality, ecological parameters (geomorphology, substrate and habitat type) and disturbance on plant density, plant size and fruits production, three independent Generalized Linear Models (GLMs) were fitted using a normal function for continuous variables, Poisson error distribution and log as a link function for count data. GLM analyses were performed by a stepwise procedure using JMP 7.0 (SAS, SAS Institute, Cary, NC).

The non-parametric Mann–Whitney U test was performed to verify differences between localities in the mean percentage of seeds per fruit; these analyses were performed using Statistica 8.0 software (Statsoft, USA).

## Results

### *Surface-area and plant density*

The overall surface-area occupied by the *H. caput-felis* populationwas 30.61 hectares (17.87 ha for CM and 12.74 ha for ST); these locations represented almost the entire Sardinian population (99.99%), although a few isolated plants grow in some southern coastal sites along the Sinis Peninsula (data not show).

The estimated mean density was 4.73 ± 2.31 plants $m^{-2}$, and it varied from 4.63 ± 2.25 (CM) to 4.83 ± 2.42 (ST) plants $m^{-2}$; the minimum and maximum density values ranged from 2 to 9.5 plants $m^{-2}$ for CM and from 1 to 8.5 plants $m^{-2}$ for ST (Table 1).

Plant density was higher in bedrock and lowland areas, and the highest density value was observed in garrigue, followed by maquis (Table 2), however the differences among plants growing in different ecological conditions are not statistically significant ($p$-value > 0.05; Table 3a). Our results revealed that only human trampling intensity significantly affected plant density ($p$-value < 0.05; Table 3a): in fact, the lowest plant density was observed in the plot with intense trampling pressure (6.67±4.27 plants $m^{-2}$) compared to undisturbed areas (trampling low and absent, 10.38 ± 4.61 and 10.48 ± 4.96 plants $m^{-2}$, respectively; Figure 2).

**Table 1 – *Helianthemum caput-felis* localities and their population traits: area, mean density, estimated population range, mean plant size, percentage of reproductive plants, mean number of flowers and fruits per plant, percentage of empty fruits and mean number of seeds per fruit.**

| Locality | Area (ha) | Plant density ± SD (plants m$^{-2}$) | Mean no. plants | Population range (max-mix) | Plant size ± SD (dm$^3$) | Adults plants (%) | Flowers per plant (Mean ± SD) | Fruits per plant (Mean ± SD) | Empty fruits (%) | Seeds per fruit (Mean ± SD) |
|---|---|---|---|---|---|---|---|---|---|---|
| Capo Mannu (CM) | 17.87 | 4.63 ± 2.25 | 862230 | 1295400-429100 | 11.21 ± 48.68 | 66.49 | 30.42 ± 48.00 | 36.69 ± 49.43 | 17.5 | 4.32 ± 1.40 |
| Su Tingiosu (ST) | 12.74 | 4.83 ± 2.42 | 589225 | 876250-302200 | 6.11 ± 13.72 | 89.64 | 39.01 ± 44.52 | 78.49 ± 107.15 | 22.5 | 4.27 ± 1.37 |
| Overall population | 30.61 | 4.73 ± 2.31 | 1446320 | 2154000-738600 | 8.61 ± 35.48 | 78.31 | 33.23 ± 46.98 | 58.03 ± 86.47 | 20.0 | 4.29 ± 1.39 |

**Table 2 – *Helianthemum caput-felis* traits at population and plant levels in relation to substrate, geomorphology and habitat in Sardinia.**

| | | **Plant density (plant m$^{-2}$)** | | | **Plant size (plant volume - dm$^3$)** | | | **No. Fruits per reproductive plant** | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Overall | CM | ST | Overall | CM | ST | Overall | CM | ST |
| **SUBSTRATE** | | | | | | | | | | |
| **Bedrock** | Mean ± SD | 4.89 ± 2.41 | 5.17 ± 2.84 | 4.82 ± 2.43 | 3.64 ± 8.10 | 3.44 ± 6.23 | 3.69 ± 8.59 | 68.27 ± 80.26 | 60.61 ± 73.94 | 69.75 ± 81.72 |
| | Min | 1.5 | 2 | 1.5 | 0.0002 | 0.0012 | 0.0002 | 3 | 4 | 3 |
| | Max | 8.5 | 7.5 | 8.5 | 61.28 | 24.46 | 61.28 | 500 | 325 | 500 |
| **Soil** | Mean ± SD | 4.63 ± 2.3 | 4.53 ± 2.23 | 4.83 ± 2.56 | 11.43 ± 43.79 | 12.78 ± 53.17 | 9.05 ± 17.73 | 80.21 ± 98.14 | 55.85 ± 46.81 | 112.48 ± 133.40 |
| | Min | 1 | 2 | 1 | 0.0003 | 0.0003 | 0.009 | 2 | 2 | 2 |
| | Max | 9.5 | 9.5 | 8.5 | 628.32 | 628.32 | 117.76 | 600 | 218 | 600 |
| **GEOMORPHOLOGY** | | | | | | | | | | |
| **Plain** | Mean ± SD | 5.23 ± 2.52 | 5.56 ± 2.26 | 5.03 ± 2.72 | 6.56 ± 15.47 | 5.72 ± 15.87 | 7.12 ± 15.22 | 79.87 ± 102.61 | 44.08 ± 36.43 | 96.43 ± 118.13 |
| | Min | 1 | 2 | 1 | 0.0003 | 0.0003 | 0.002 | 2 | 2 | 2 |
| | Max | 9.5 | 9.5 | 8.5 | 117.76 | 107.35 | 117.76 | 600 | 146 | 600 |
| **Slope** | Mean ± SD | 3.97 ± 1.77 | 3.86 ± 2.04 | 4.20 ± 1.15 | 12.65 ± 57.16 | 17.67 ± 69.39 | 2.49 ± 4.21 | 66.83 ± 62.98 | 69.91 ± 61.22 | 61.86 ± 66.30 |
| | Min | 2 | 2 | 3 | 0.0002 | 0.0003 | 0.0002 | 4 | 4 | 4 |
| | Max | 8.5 | 8.5 | 5.5 | 628.32 | 628.32 | 24.93 | 325 | 325 | 300 |
| **HABITAT** | | | | | | | | | | |
| **Garigues** | Mean ± SD | 4.59 ± 2.18 | 4.35 ± 1.92 | 4.72 ± 2.36 | 5.11 ± 12.26 | 3.08 ± 5.66 | 6.15 ± 14.43 | 82.22 ± 103.07 | 59.12 ± 55.46 | 91.06 ± 115.19 |
| | Min | 1 | 2 | 1 | 0.0002 | 0.0003 | 0.0002 | 2 | 2 | 2 |
| | Max | 8.5 | 7.5 | 8.5 | 117.76 | 31.73 | 117.76 | 600 | 325 | 600 |
| **Maquis** | Mean ± SD | 4.50 ± 2.50 | 3.67 ± 1.53 | 5.75 ± 3.89 | 27.64 ± 94.19 | 50.45 ± 132.19 | 5.82 ± 6.59 | 76.00 ± 62.95 | 77.00 ± 66.96 | 75.24 ± 61.39 |
| | Min | 2 | 2 | 3 | 0.014 | 0.088 | 0.014 | 8 | 8 | 10 |
| | Max | 8.5 | 5 | 8.5 | 628.32 | 628.32 | 133.99 | 217 | 217 | 207 |
| **Micro-forest** | Mean ± SD | 5.43 ± 2.91 | 5.43 ± 2.91 | - | 9.17 ± 18.73 | 9.17 ± 18.73 | - | 46.51 ± 37.10 | 46.51 ± 37.10 | - |
| | Min | 2 | 2 | - | 0.0006 | 0.0006 | - | 2 | 2 | - |
| | Max | 9.5 | 9.5 | - | 107.35 | 107.35 | - | 152 | 152 | - |

**Table 3 – Generalized Linear Models (GLMs) results.**

**a) Effects of locality and ecological parameters on plant density (Observations = 40. Model: - LogLikelihood = 13.80; $\chi^2$ = 27.60; DF = 11; p-value = 0.004).**

| **Source** | $\chi^2$ | **DF** | ***p*-value** |
|---|---|---|---|
| Locality | 0.42 | 1 | 0.515 |
| Site geomorphology | 1.40 | 1 | 0.237 |
| Substrate | 0.20 | 1 | 0.653 |
| Habitat type | 4.99 | 2 | 0.082 |
| Human trampling | 14.44 | 3 | 0.002 |
| Site geomorphology × substrate | 0.01 | 1 | 0.984 |
| Site geomorphology × habitat type | 4.09 | 2 | 0.129 |

**b) Effects of locality and ecological parameters on plant size (plant volume. Observations = 378. Model: - LogLikelihood = 1665332.51; $\chi^2$ = 3330665; DF = 11; p-value < 0.001).**

| **Source** | $\chi^2$ | **DF** | ***p*-value** |
|---|---|---|---|
| Locality | 105189.91 | 1 | < 0.001 |
| Site geomorphology | 122056.28 | 1 | < 0.001 |
| Substrate | 62790.64 | 1 | < 0.001 |
| Habitat type | 186002.73 | 2 | < 0.001 |
| Human trampling | 432806.60 | 3 | < 0.001 |
| Site geomorphology × substrate | 3533.98 | 1 | < 0.001 |
| Site geomorphology × habitat type | 1030061.80 | 2 | < 0.001 |

**c) Effects of locality and ecological parameters on fruit output per plant (Observations = 378. Model: - LogLikelihood = 2696.70; $\chi^2$ = 5393.41; DF = 11; p-value < 0.001).**

| **Source** | $\chi^2$ | **DF** | ***p*-value** |
|---|---|---|---|
| Locality | 1125.31 | 1 | < 0.001 |
| Site geomorphology | 68.21 | 1 | < 0.001 |
| Substrate | 296.44 | 1 | < 0.001 |
| Habitat type | 394.20 | 2 | < 0.001 |
| Human trampling | 1005.00 | 3 | < 0.001 |
| Site geomorphology × substrate | 0.34 | 1 | 0.558 |
| Site geomorphology × habitat type | 201.26 | 2 | < 0.001 |

### *Plant size*

The mean plant size was 8.61 ± 35.48 $dm^3$, ranging from a minimum of $0.20 \times 10^{-3}$ $dm^3$ to a maximum of 628.32 $dm^3$. Plants growing in CM (mean value = 11.21 ± 48.68 $dm^3$) were larger than those growing in ST (mean value = 6.11 ± 13.72 $dm^3$); indeed, a higher percentage of large plants (plant volume > 25.00 $dm^3$) was observed in CM compared to ST (10.3% of the plants measured for CM and 3.1% for ST).

All ecological variables analysed in our study had a statistically significant effect on plant size, as well as the interactions between geomorphology and substrates and between geomorphology and habitat significantly affected plant size (*p*-value < 0.001; Table 3b); in particular, larger plants were found in areas with the following ecological features: soil presence, on the slopes, in the maquis habitat (Table 2) and in areas with intensive human trampling (Figure 2). In contrast, smaller plants were observed in areas without human trampling (5.55 ± 13.08 $dm^3$).

### *Reproductive traits*

Flowering season occurs from March to late May, but isolated flowers were present until mid-June. In CM, the flowering season ranged from March to mid-May, and peak of flowering was recorded in mid-April. The flowering season in ST ranged from early April to mid-May, and peak flowering was recorded in early May. Generally, the fruiting season lasts one month; here, it lasted from April to July, and the peak occurred from mid-May to mid-June in both localities.

The mean percentage of reproductive plants was 76.72%, ranging from 64.86% (CM) to 88.08% (ST). The mean number of flowers per plant was 33.23 ± 46.98, ranging from 1 to 327. Notably, the number of ST flowers per reproductive plant (39.01 ± 44.52) was higher than CM (30.42 ± 48.00).

The mean number of fruits per reproductive plant was 58.03 ± 86.47 and it was higher in ST than in CM (78.49 ± 107.15 and 36.69 ± 49.43, respectively). All ecological variables analysed had a statistically significant effect on the number of fruits per plant, as well as the interactions between geomorphology and habitat significantly affected plant size ($p$-value < 0.001; table 3c); plants displayed a higher mean number of fruits in deep and structured soil and in lowland areas, preferably in the garrigue and maquis habitats (Table 2). The mean number of fruits per plant increased as human trampling intensified, with mean values ranging from 50.33 ± 71.17 (absent human trampling) to 113.18 ± 190.62 (intense human trampling; Figure 2).

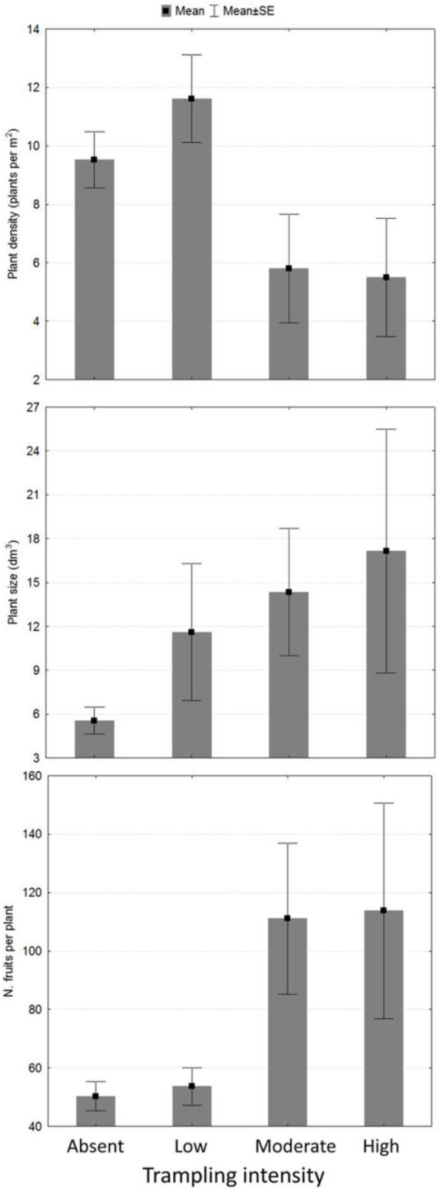

**Figure 2 – Effect of human trampling on plant density, plant size and fitness.**

A positive correlation between number of fruits and plant size was observed ($r = 0.76$, $r^2 = 0.57$ and $p < 0.001$; Figure 3).

Overall, approx. 20% of the fruits were empty, ranging from 17.5 for CM to 22.5% for ST. The mean number of seeds per fruit was 4.29 ± 1.39, ranging from 1 to 7 seeds per fruit; the fruits collected in CM had a higher mean number of seeds per fruit (4.31 ± 1.40) compared to those in ST (4.27 ± 1.37), but this difference was not statistically significant (Mann–Whitney U test, $p > 0.05$).

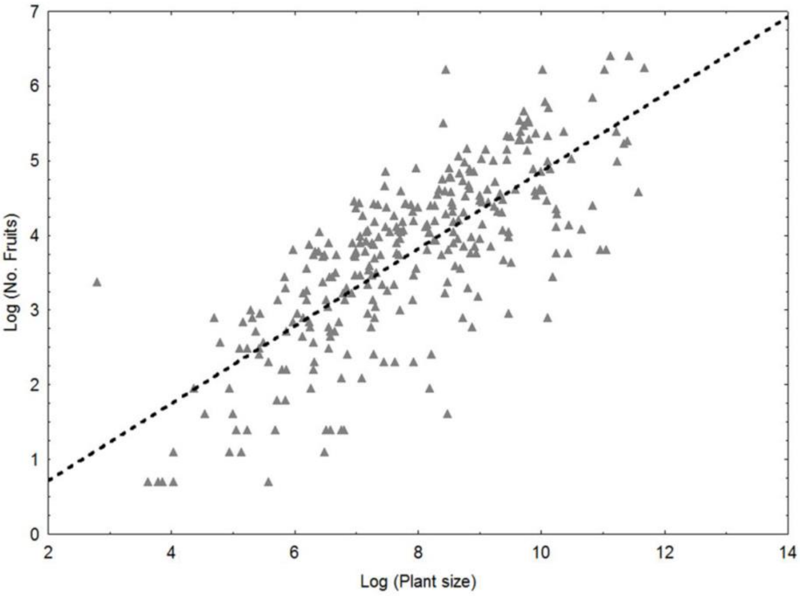

**Figure 3 – Relationships between plant size (plant volume) and reproductive capacity (number of fruits per plant) in *H caput-felis*. The following equation was used: No. of fruits = -0.320 + 0.517 × plant volume; $r^2 = 0.57$; r = 0.76; *p*-value < 0.001.**

*IUCN assessment at regional level*

The same threats were detected in all localities where *H. caput-felis* was found, including areas with scattered plants growing in the southern part of the Sinis Peninsula. The major threat is tourism and other outdoor activities (such as human trampling), followed by the expansion of agricultural/pastoral activities (such as agriculture and wood plantations) and the invasion of exotic plants. All of these pressures result in reduced population size due to habitat loss and fragmentation. Stochastic environmental events (e.g., landslides) pose a significant potential threat. All of these threats could result in the disappearance of small localities with a consequent reduction in EOO and AOO values, as well as in the number of localities. Additionally, continuous, human-induced habitat degradation will continue to occur in a predictable manner in the future.

Based on the EOO (3.99 $km^2$), AOO (16 $km^2$ = 4 cell of 2 × 2 km), decline rate and number of locations (one, *sensu* IUCN 2014a), we propose that *H. caput-felis* should be included on the Red List categorisation of Critically Endangered at the regional level, based on the following formula: B1ab(i,ii,iii,v) + 2ab(i,ii,iii,v).

## Discussion

Our study highlighted important information regarding the distribution pattern, reproductive traits and ecological requirements of *H. caput-felis*, which are relevant issues for developing future conservation measures for this species in Sardinia. To the best of our knowledge, no exhaustive studies have been performed on the central populations of *H. caput-felis*, and the present study is the first investigation which analyse the population traits of this threatened species under natural conditions.

In the Sardinian population, density did not vary between localities and ecological features (geomorphology, substrate, and habitat type), suggesting the absence of strong differences in ecological stress among different local conditions. The actual distribution of *H. caput-felis* in Sardinia is restricted in the two main localities, which constitute “ecological islands” (acting as local refuges) in coastal areas strongly modified by human activity. Thus, *H. caput-felis* could represent a “refuge-model” plant in Sardinia, with a range that simply occupies a reduced fraction of a wider habitat from which it is excluded by intensive human-induced habitat degradation, similar to several endemic species in the Mediterranean basin (Thompson 2005). In this restricted ecological context, *H. caput-felis* represents one of the principal species among coastal vegetation types.

Our data detected a significant effect of topography, landform and habitat type on plant fitness. *H. caput-felis* prefers lowland areas with deep, structured soil due to the amount of water and resources available and morphological soil stability. Moreover, as highlighted by the mean number of fruits per plant, *H. caput-felis* was ecologically optimum in garrigues, whereas it produced few fruits in micro-forests.

Considering previous studies (Tébar, Gil and Llorens 1997; Rodriguez-Pérez 2005), the phenological pattern of *H. caput-felis* is similar in the Sardinian and Balearic populations. Information on the reproductive biology of endangered plants is crucial for predicting their survival capacity and establishing the appropriate measures for their conservation (e.g. Schemske *et al.* 1994; Cogoni *et al.* 2015). However, despite the ecological importance of the Cistaceae in the Mediterranean Basin, few studies have been carried out on this topic for this family (Herrera 1992; Talavera *et al.* 1993; Rodríguez-Pérez 2005; Guzmán *et al.* 2011). Local studies have been carried out on *Helianthemum* species (Tébar *et al.* 1997; Rodriguez-Perez 2005; Aragón and Escudero 2008), but it remains one of the genera for which reproductive biology remains less documented.

The *H. caput-felis* population in Sardinia is mainly composed of reproductive plants (78.31%), while the percentage of saplings and seedlings is relatively low; this result, together with the high number of seeds produced each year, suggests that seedling establishment represent the main critical stage for this plant. However, further studies are needed to understand whether this is related to marginalisation of the population or the lack of suitable micro-sites for plant establishment. In fact, in the case of locally restricted and threatened species, many seeds may end up in unsuitable areas depending on their peculiar ecological requirements (e.g. Cogoni *et al.* 2012). The lowest percentage of reproductive plants was observed in CM (2/3 of the total), suggesting a greater rate of recruitment, whereas in ST, 4/5 of the plants were reproductive. The similar number of empty fruits per plant and number of seeds per fruit between the two localities (lower values in ST) could be related to the impact of agricultural activities that determine habitat fragmentation. However, at the population level, multiplying the number of viable seeds per fruit per the estimated number of reproductive plants per locality by the mean number of fruit per plant gives approx. 75 million viable seeds for CM and 137 for ST; thus, seed production is not a limiting factor for this plant.

Reproductive limitations were not detected for this species (i.e., fruit and seed set, pollination service and seedling survival on natural populations) in previous studies (e.g. Rodríguez-Pérez 2005); considering our findings on the high seed output, the increasing rarity of this species seems to be likely a direct result of the destruction of its natural habitat.

Habitat fragmentation or destruction caused by human disturbance is currently considered one of the main factors responsible for reducing population viability and increasing the extinction risk of rare plants and/or of marginal and small populations (Schemske *et al.* 1994; Holsinger 2000; Schleuning and Matthies 2009). As a consequence, many threatened or rare plants are confined to naturally fragmented habitats or ecological islands that might be separated by large inhospitable areas, as noted in this study.

Among other factors, human trampling is an important threat for threatened endemic species in Sardinia (Quilichini and Debussche 2000; Fenu *et al.* 2011, 2013; Rossi *et al.* 2016). A consistent reduction in reproductive traits was observed in plant populations subjected to human trampling (e.g. Rossi *et al.* 2006; Fenu *et al.* 2013), resulting in a serious threat to the persistence of the population. Accordingly, in the present study, a negative effect of human trampling was observed on plant density. Surprisingly, human trampling enhanced the plant size and the rate of fruit production, suggesting that the reproductive plant of *H. caput-felis* is tolerant to direct damage and probably is benefited from the reduction of inter- and intra-specific competition. However, considering the critical limitation in seedling recruitment, as suggested by the negative effect on plant density, human trampling should be considered a significant threat to the persistence of the Sardinian population.

Red lists highlight the most pressing issues in biodiversity conservation matters, representing taxa that are closer to extinction (Rossi *et al.* 2014, 2016). In this context, Red lists may be policy-relevant for promoting conservation efforts, but they cannot be considered policy-prescriptive (Bilz *et al.* 2011; IUCN 2014a). Very little attention has been given to border populations in the application of the IUCN criteria at the regional level (IUCN 2003; Miller *et al.* 2007) and only recent research assigned some Italian marginal populations of widespread species to high-risk categories (e.g. Gargano *et al.* 2007; Del Vecchio *et al.* 2012; Rossi *et al.* 2016). This study confirms this trend for *H. caput-felis,* and it allows us to raise the risk category at the regional level for this species from Lower Risk (LR; Conti *et al.* 1997) to Critically Endangered (CR). Although criterion B is biased by restricted range and could overestimate the extinction risk, it is strongly supported by the population decline observed in Sardinia. However, to confirm or reject the assumed worse performance and higher vulnerability of border populations, an extensive and integrative approach that compares all population over a wide temporal context is needed; in addition, the effect of population attributes (e.g., population size or structure) or the particular conditions where populations occur (e.g., human trampling intensity analysed in this study) should be taken into account to separate the role of local and positional factors that drive populations (García *et al.* 2009). This approach is essential for all plants at the margin of their distribution range to plan appropriate conservation measures to reduce the extinction risk of these populations.

### *Implication for conservation*

According to the European Strategy for Plant Conservation (Planta Europa 2008), populations at the border of their distribution area, such as *H. caput-felis* in Sardinia, should be considered of high interest, with a need for urgent conservation measures. *In situ* and *ex situ* (e.g., seed conservation in seed banks, cultivation in botanical gardens) conservation efforts should be improved. In particular, greater emphasis should be given to minimising the range of negative impacts, including unsustainable tourism and recreation use (Fenu *et al.* 2011, 2013; Ballantyne and Pickering 2013; Rossi *et al.* 2016). Therefore, touristic and recreational activities should be regulated in known localities, and no new pathways should be opened. A management strategy should exclude trampling in different portions of the population to facilitate the plant recruitment process and population renewal.

In addition, an *ex situ* conservation strategy must be implemented and the seeds collected could be used for future reinforcement or reintroduction of this species in suitable areas, as realised in Spanish regions or following low-cost programs carried out in Sardinia (Cogoni *et al.* 2013). These actions may be extremely important for conservation in a changing climate (Sala *et al.* 2000; Godefroid *et al.* 2011). Moreover, because many threats will affect plant species over the next few decades (e.g. climate change, biological invasions), long-term monitoring programs must be developed to reveal changes in the species conservation status (Balmford *et al.* 2003; Fenu *et al.* 2015), as well as monitoring both the vegetation and the human threat dynamics.

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## References

Abeli T., Gentili R., Rossi G., Bedini G. and Foggi B. 2009. Can the IUCN criteria be effectively applied to peripheral isolated plant populations? Biodiversity and Conservation 18: 3877–3890.

Agulló J.C., Juan A., Guilló A., Alonso M.Á. and Crespo M.B. 2011. Genetic diversity and phylogeographical assessment of *Helianthemum caput-felis* Boiss. (Cistaceae) based on AFLP markers. Fitosociologia 48: 21–29.

Aragón C.F. and Escudero A. 2008. Mating system of *Helianthemum squamatum* (Cistaceae), a gypsophile specialist of semi-arid Mediterranean environments. Botanica Helvetica 118: 129–137.

Arrigoni P.V. 1971. *Helianthemum caput-felis* Boiss. (2n=24) nuovo reperto per la flora italiana. Webbia 26: 237–243.

Ballantyne M. and Pickering C.M. 2013. Tourism and recreation: a common threat to IUCN red-listed vascular plants in Europe. Biodiversity and Conservation 22: 3027–3044.

Balmford A., Green R.E. and Jenkins M. 2003. Measuring the changing state of nature. Trends in Ecology and Evolution 18: 326–330.

Bilz M., Kell S.P., Maxted N. and Lansdown R.V. 2011. European Red List of Vascular Plants. Luxembourg, Publications Office of the European Union.

Cogoni D., Fenu G. and G. Bacchetta. 2015. Reproductive biology of the narrow endemic *Anchusa littorea* Moris (Boraginaceae), an endangered coastal Mediterranean plant. Turkish Journal of Botany 39: 642–652.

Cogoni D., Fenu G., Concas E. and Bacchetta G. 2013. The effectiveness of plant conservation measures: the *Dianthus morisianus* reintroduction. Oryx 47: 203–206.

Cogoni D., Mattana E., Fenu G. and Bacchetta G. 2012. From seed to seedling, a critical stage for the psammophilous species *Dianthus morisianus*. Plant Biosystems 146: 910–917.

Comor V., Orgeas J., Ponel P., Rolando C. and Delettre Y.R. 2008. Impact of anthropogenic disturbances on beetle communities of French Mediterranean coastal dunes. Biodiversity and Conservation 17: 1837–1852.

Conradt L. 2001. Ecological and evolutionary processes at expanding range margins. Nature 411: 577–581.

Conti F., Manzi A. and Pedrotti F. 1997. Liste Rosse Regionali delle Piante d'Italia [Regional Red Lists of Italian Plants]. Camerino: WWF Italia, Società Botanica Italiana.

Davenport J. and Davenport J.L. 2006. The impact of tourism and personal leisure transport on coastal environments: a review. Estuarine, Coastal and Shelf Science 67: 280–292.

de Grammont P.C. and Cuarón A.D. 2006. An evaluation of threatened species categorization systems used on the American continent. Conservation Biology 20: 14–27.

Del Vecchio S., Giovi E., Izzi F., Abbate G. and Acosta A.T.R. 2012. *Malcolmia littorea*: the isolated Italian population in the European context. Journal for Nature Conservation 20: 357–363.

Dominguez Lozano F., Herbada D.G., Rivero L.M., Moreno Saiz J.C. and Ollero H.S. 1996. Threatened plants in Peninsular and Balearic Spain: a report based on the EU Habitats directive. Biological Conservation 76: 123–133.

Eckert C.G., Samis K.E. and Lougheed S.C. 2008. Genetic variation across species' geographical ranges: the central-marginal hypothesis and beyond. Molecular Ecology 17: 1170–1188.

Fenu G, Cogoni D., Pinna M.S. and Bacchetta G. 2015. Threatened Sardinian vascular flora: a synthesis of ten years of monitoring activities. Plant Biosystems 149: 473–482.

Fenu G. and Bacchetta G. 2008. La flora vascolare della penisola del Sinis (Sardegna occidentale). Acta Botánica Malacitana 33: 91–124.

Fenu G., Cogoni D., Ulian T. and Bacchetta G. 2013. The impact of human trampling on a threatened coastal Mediterranean plant: the case of *Anchusa littorea* Moris (Boraginaceae). Flora 208: 104–110.

Fenu G., Mattana E. and Bacchetta G. 2011. Distribution, status and conservation of a critically endangered, extremely narrow endemic: *Lamyropsis microcephala* (Asteraceae) in Sardinia. Oryx 45: 180–186.

Fenu G., Sulis E., Cogoni D. and Bacchetta G. 2012. Schede per una Lista Rossa della Flora vascolare e crittogamica Italiana: *Helianthemum caput-felis* Boiss.. Informatore Botanico Italiano 44: 233–236.

Fois M., Fenu G., Cuena Lombraña A., Cogoni D. and Bacchetta G. 2015. A practical method to speed up the discovery of unknown populations using distribution models. Journal for Nature Conservation 24: 42–48.

García M.B., Goñi D. and Guzmán D. 2009. Living at the edge: local versus positional factors in the long-term population dynamics of an endangered orchid. Conservation Biology 24: 1219–1229.

Gargano D., Fenu G., Medagli P., Sciandrello S. and Bernardo L. 2007. The status of *Sarcopoterium spinosum* (Rosaceae) at the western periphery of its range: ecological constraints led to conservation concerns. Israel Journal of Plant Sciences 55: 1–13.

Godefroid S., Piazza C., Rossi G., Buord S., Stevens A.D., Aguraiuja R., Cowell C., Weekley C.W., Vogg G., Iriondo J.M., Johnson I., Dixon B., Gordon D., Magnanon S., Valentin B., Bjureke K., Koopman R., Vicens M., Virevaire M. and Vanderborght T. 2011. How successful are plant species reintroductions?. Biological Conservation 144: 672–682.

Grant M.C. and Antonovics J. 1978. Biology of ecologically marginal populations of *Anthoxanthum odoratum*. I. Phenetics and dynamics. Evolution 32: 822–838.

Guzmán B. and Vargas P. 2009. Historical biogeography and character evolution of Cistaceae (Malvales) based on analysis of plastid rbcL and trnL-trnF sequences. Organisms Diversity and Evolution 9: 83–99.

Guzmán B., Narbona E. and Vargas P. 2011. Similar reproductive success of the two petal colour polymorphisms of *Cistus ladanifer* (Cistaceae). Plant Biosystems 145: 931–937.

Herrera J. 1992. Flower variation and breeding systems in the Cistaceae. Plant Systematics and Evolution179: 245–255.

Holsinger K.E. 2000. Demography and extinction in small populations. In: Genetics, Demography and Viability of Fragmented Populations, edited by Young A.G. and Clark G.M., 55–74. Cambridge: Cambridge University Press.

Holt R.D. and Keitt T.H. 2005. Species' borders: a unifying theme in ecology. Oikos 108: 3–6.

IUCN 2001. IUCN Red List Categories and Criteria: Version 3.1. IUCN Species Survival Commission. UK Gland, Switzerland and Cambridge: IUCN published.

IUCN 2003. Guidelines for application of IUCN Red List criteria at regional levels version 3.0. IUCN Species Survival Commission. UK Gland, Switzerland and Cambridge: IUCN published.

IUCN 2014a. Guidelines for application of IUCN Red List Criteria at regional levels: version 11. IUCN Species Survival Commission. IUCN, Gland, Switzerland. http://www.iucnredlist.org/documents/reg_guidelines_en.pdf [accessed 20 February 2015].

IUCN 2014b. Threats classification scheme: version 3.2. http://www.iucnredlist.org/technical-documents/classification-schemes/threats-classification-scheme [accessed 20 February 2015]

Kerbiriou C., Leviol I., Jiguet F. and Julliard R. 2008. The impact of human frequentation on coastal vegetation in a biosphere reserve. Journal of Environental Management 88: 715–728.

Kutiel P., Eden E. and Zhevelev Y. 2000. Effect of experimental trampling and off-road motorcycle traffic on soil and vegetation of stabilized coastal dunes, Israel. Environmental Conservation 27: 14–23.

Lesica P. and Allendorf F.W. 1995. When are peripheral populations valuable for conservation?. Conservation Biology 9: 753–760.

López González G. 1992. Apuntes para justificar el tratamiento del género *Helianthemum* Miller, s.l. (Cistaceae), en Flora Iberica. Anales del Jardín Botánico de Madrid 50: 35–63.

Miller R.M., Rodríguez J.P., Aniskowicz-Fowler T., Bambaradeniya C., Boles R., Eaton M.A., Gärdenfors U., Keller V., Molur S., Walker S. and Pollock C. 2007. National threatened species listing based on IUCN criteria and regional guidelines: current status and future perspectives. Conservation Biology 21: 684–696.

Pickering C.M. and Hill W. 2007. Impacts of recreation and tourism on plant biodiversity and vegetation in protected areas in Australia. Journal of Environmental Management 85: 791–800.

Planta Europa 2008. A sustainable future for Europe; the European strategy for plant conservation 2008-2014. Strasbourg: Plantlife International (Salisbury, UK) and the Council of Europe.

Pouget M., Youssef S., Migliore J., Juin M., Médail F. and Baumel A. 2013. Phylogeography sheds light on the central–marginal hypothesis in a Mediterranean narrow endemic plant. Annals of Botany 112: 1409–1420.

Próctor M.C.F. and Heywood V.H. 1968. *Helianthemum* Miller. In: Flora Europaea, edited by Tutin T.G., Heywood H., Burges N.A., Moore D.M., Valentine D.H., Walters S.M. and Webb D.A. 2: 286–292. Cambridge: Cambridge University Press.

Quézel P. and Santa S. 1963. Nouvelle flore de l'Algérie et des régions désertiques méridionales. Volumen 2. Paris: Centre National de la Reherche Scientifique.

Quilichini A. and Debussche M. 2000. Seed dispersal and germination patterns in a rare Mediterranean island endemic (*Anchusa crispa* Viv., Boraginaceae). Acta Oecologica 21: 303–313.

Raynaud C. 1999. Cistaceae. In: Flore Pratique du Maroc. Manuel de determination des plantes vasculaires, 1, s. Bot. 36, edited by Fennane M., Ibn Tattou J., Mathez A. and Ouyahya El Oualidi J., 302–326. Rabat: Travaux Institut Scientifique, Université Mohammed V.

Rodrigues A.S.L., Pilgrim J.D., Lamoreux J.L., Hoffmann M. and Brooks T.M. 2006. The value of the IUCN Red List for conservation. Trends in Ecology and Evolution 21: 71–76.

Rodríguez-Pérez J. 2005. Breeding system, flower visitors and seedling survival of two endangered species of *Helianthemum* (Cistaceae). Annals of Botany 95: 1229–1236.

Rossi G., Montagnani C., Abeli T., Gargano D., Peruzzi L., Fenu G., Magrini S., Gennai M., Foggi B., Wagensommer R.P., Ravera S., Cogoni A., Aleffi M., Alessandrini A., Bacchetta G., Bagella S., Bartolucci F., Bedini G., Bernardo L., Bovio M., Castello M., Conti F., Domina G., Farris E., Gentili R., Gigante D., Peccenini S., Persiani A.M., Poggio L., Prosser F., Santangelo A., Selvaggi A., Villani M.C., Wilhalm T., Zappa E., Zotti M., Tartaglini N., Ardenghi N.M.G., Blasi C., Raimondo F.M., Venturella G., Cogoni D., Puglisi M., Campisi P., Miserere L., Perrino E.V., Strumia S., Iberite M., Lucchese F., Fabrini G. and Orsenigo S. 2014. Are Red Lists really useful for plant conservation? The New Red List of the Italian Flora in the perspective of National Conservation policies. Plant Biosystems 148: 187–190.

Rossi G., Orsenigo S., Montagnani C., Fenu G., Gargano D., Peruzzi L., Wagensommer R.P., Foggi B., Bacchetta G., Domina G., Conti F., Bartolucci F., Gennai M., Ravera S., Cogoni A., Magrini S., Gentili R., Castello M., Blasi C. and Abeli T. 2016. Is legal protection sufficient to ensure plant conservation? The Italian Red List of Policy species as a case study. Oryx in press.

Rossi G., Parolo G., Zonta L.A., Crawford J.A. and Leonardi A. 2006. *Salix herbacea* L. fragmented small population in the N-Apennines (Italy): response to human trampling disturbance. Biodiversity and Conservation 15: 3881–3893.

Sala O.E., Chapin F.S. 3rd, Armesto J.J., Berlow E., Bloomfield J., Dirzo R., Huber-Sanwald E., Huenneke L.F., Jackson R.B., Kinzig A., Leemans R., Lodge D.M., Mooney H.A., Oesterheld M., Poff N.L., Sykes M.T., Walker B.H., Walker M. and Wall D.H. 2000. Global biodiversity scenarios for the year 2100. Science 287: 1770–1774.

Schemske D.W., Husband B.C., Ruckelshaus M.H., Goodwillie C., Parker I.M. and Bishop J.D. 1994. Evaluating approaches to the conservation of rare and endangered plants. Ecology 75: 584–606.

Schleuning M. and Matthies D. 2009. Habitat change and plant demography: assessing the extinction risk of a formerly common grassland perennial. Conservation Biology 23: 174–183.

Sexton J.P., McIntyre P.J., Angert A.L. and Rice K.J. 2009. Evolution and ecology of species range limits. Annual Review of Ecology Evolution and Systematics 40: 415–436.

Talavera S., Gibbs P.E. and Herrera J. 1993. Reproductive biology of *Cistus ladanifer* (Cistaceae). Plant Systematics and Evolution 186: 123–134.

Tébar F.J., Gil L. and Llorens L. 1997. Reproductive biology of *Helianthemum apenninum* (L.) Mill. and *H. caput-felis* Boiss. (Cistaceae) from Mallorca (Balearic Islands, Spain). Acta Botánica Malacitana 22: 53–63.

Thompson J.D. 2005. Plant evolution in the Mediterranean. Oxford: Oxford University Press.

Villellas J., Ehrlén J., Olesen J.M., Braza R. and García M.B. 2013a. Plant performance in central and northern peripheral populations of the widespread *Plantago coronopus*. Ecography 36: 136–145.

Villellas J., Morris W.F. and García M.B. 2013b. Variation in stochastic demography between and within central and peripheral regions in a widespread short-lived herb. Ecology 94: 1378–1388.

Yu S., Bell D. and Kutiel P. 2009. Impact of microhabitats on the heterogeneity of seedling emergence in a Mediterranean coastal sand dunes community. Ecoscience 16: 369–378.

# CHAPTER 2

# Variation of phenological and reproductive patterns in a Mediterranean coastal plant with fragmented distribution

## Introduction

Studies on reproductive biology of endangered plants are crucial for predicting their survival capacity (Evans *et al.* 2003; Zhao *et al.* 2013; Fenu *et al.* 2015; Morellato *et al.* 2016); however, to date, few exhaustive studies have explored the phenological and reproductive patterns of Mediterranean coastal plants (e.g. Estiarte *et al.* 2011; Cogoni *et al.* 2015), in particular those growing in several isolated places.

Several plant species are characterised by a disjunctive distribution in which peripheral populations can be isolated from the main home range (e.g. Thompson 2005; Gargano *et al.* 2007; Pouget *et al.* 2013); plants that display a peculiar distribution and, in particular, species with peripheral populations represent interesting targets in ecology, evolutionary biology and genetics (Eckert *et al.* 2008; Sexton *et al.* 2009; Pouget *et al.* 2013). In addition, they provide insight into critical phenomena, such as speciation, adaptive radiation, and natural selection (Grant and Antonovics 1978; Holt and Keitt 2005; Fenu *et al.* 2015).

Many studies have demonstrated that plant traits (e.g. morphology, breeding system, genetic settings, ecology and demography) vary across the range of a species, which is termed the central/marginal concept (also called ACM or ACH, indicating the abundant centre model or hypothesis, or CPH, centre-periphery hypothesis; e.g. Abeli *et al.* 2014; Pironon *et al.* 2014). This model assumes that the most favourable conditions will be found at the core of a species' range, and thus, the centre will support greater densities, fitness, and genetic diversity than peripheral sites (e.g. Grant and Antonovics 1978; Sagarin and Gaines 2002; Jump and Woodward 2003; Alexander *et al.* 2007; Vaupel and Matthies 2012). However, the findings of several studies diverge from these classical predictions (Sagarin and Gaines 2002; Samis and Eckert 2007; Doak and Morris 2010; Villellas *et al.* 2013a, Villellas *et al.* 2013b) and recent literature reviews raise considerable doubt about the generality of this model in nature (Sagarin and Gaines 2002; Sagarin *et al.* 2006; Eckert *et al.* 2008; Gaston 2009; Sexton *et al.* 2009; Castilla *et al.* 2011). Comparative analyses indicate that the differences between central and peripheral populations can be explained by the specific local environmental conditions in which populations occur (e.g. Samis and Eckert 2007; Villellas *et al.* 2013a, Villellas *et al.* 2013b). In addition, populations at the range margin may or may not be considered marginal from an ecological point of view (Grant and Antonovics 1978; Herrera and Bazaga 2008) because not all ecologically marginal populations are peripherally located and not all geographically peripheral populations are ecologically marginal (Soulé 1973).

Along any ecological gradient, generally a plant species will be most abundant where the conditions allow maximum survival and reproduction and will be progressively less abundant away from this point until a geographic limit is reached (Hengeveld and Haeck 1982; Lawton 1993). However, several studies have shown that some species do no present lower fitness in marginal populations (e.g. Jump and Woodward 2003; Kluth and Bruelheide 2005; Samis and Eckert 2007) suggesting that marginal populations may be locally adapted to sub-optimal conditions at the range edge (Caughley *et al.* 1988; Barton 2001; Abeli *et al.* 2014) and other mechanisms such as dispersal limitation or unstable metapopulation dynamics, rather than departure from ecological optimum, could be also important in limiting geographic distributions of some species (Kawecki and Holt 2002; Holt 2003).Those factors represent exceptions at the ACM hypothesis, which may result from complex patterns of environmental suitability across a species' range (e.g. Abeli *et al.* 2014).

Several studies consider ranges or gradients of latitude, longitude or altitude in order to explain variation in population and reproductive traits (e.g. Angert 2009; Eckhart *et al.* 2011; Villellas *et al.* 2013a). Nevertheless, many others consider a clear dichotomy between geography and environment by focusing rather on central and marginal habitats, regardless of geographic position (Kawecki 2008). As suggested by Abeli et al. (2014), an approach based on the study of population performance along geographical and ecological gradients is more informative than a direct comparison between central and peripheral populations.

In this study, phenological and reproductive patterns of *Helianthemum caput-felis* Boiss. (Cistaceae) were explored in relation to the longitudinal gradient and the ACM model by considering that those two gradients are a good proxies for detect change in plant performance. For this purpose, *H. caput-felis* represent an interesting study case, due to its fragmented distribution: in fact, this plant grows in several fragmented populations throughout the coasts of the western Mediterranean Basin (Fenu *et al.* 2015 and references therein). In addition, this species represents a good study case in order to explore the abundant-center model (ACM) and considering that geographically peripheral populations of *H. caput-felis* will product fewer fruits and seeds *per capita* than central populations. The primary objective of this study is to analyze the geographic pattern of the reproductive output across the distribution range of the species; specifically the aims were: (1) to analyse the flowering and fruiting periods; (2) to study the fruit and seed output per population, including the relationships with local or ecological conditions; (3) to compare the central populations with the marginal ones; (4) to verify the presence of any pattern in fruit and seed outputs in relationship to regions or populations, following a longitudinal gradient.

## Materials and methods

### *Study species*

*Helianthemum caput-felis* Boiss. is a perennial half shrub of 35(50) cm tall. Its flowers, which are arranged in inflorescences at the tip of new branches, are generally yellow and hermaphroditic, open at dawn and close at dusk, and have a short lifespan (3–4 days, Rodríguez-Pérez 2005). Only few local phenological studies were carried out in Majorca and Sardinia, which highlighted a flowering period from late February to late May and a fruiting season from late April to July–August (Rodríguez-Pérez 2005; Fenu *et al.* 2015). Tébar *et al.* (1997) and Rodríguez-Pérez (2005) reported the allogamous character of this species, being a generalist entomogamous plant. More recently, Agulló *et al.* (2015) stated that *H. caput-felis* is a partially self-compatible plant. Fruits are capsules that detach at maturation, and seed germination takes place in autumn, at the onset of the rainy season (Rodríguez-Pérez 2005).

From an ecological point of view, *H. caput-felis* is a termophilous plant that preferably grows in coastal environments under the direct influence of the sea, mostly on calcareous rocky cliffs with garrigues or scrublands; peculiar populations also grow on sand dunes (Majorca), or rocky slopes bordering inland ravines (Fenu *et al.* 2015 and references therein).

*Helianthemum caput-felis* is distributed throughout the coasts of the western Mediterranean Basin (SE Iberian Peninsula, Balearic Islands, Sardinia and NW Africa), in several fragmented populations (Fenu *et al.* 2015 and references therein). Alicante coast's population was considered the centre of *H. caput-felis* distribution range, while Majorcan and Sardinian represented the range's edges.

### *Data collection*

In this study, two assumptions were considered: the term "region" was used to indicate a geographic area along the distribution range of *H. caput-felis* (Alicante, Majorca and Sardinia – ALI, MAJ and SAR hereafter) and the term "population" to indicate a locality geographically isolated by other nucleus/groups of the same plant. Six populations were analysed, equally distributed in the three regions spanning the entire geographic distribution range of the species and corresponding to the eastern, central and western part of its range (Table 1).

Over two years (2013–2014), data sampling was carried out from March to August in a total of 98 plots, randomly placed in the six populations. Overall, a total of 821 plants were fortnightly monitored within the plots (Table 1).

At plot level, during the first survey the altitudinal range, substrate type, geomorphology and human trampling intensity were recorder for each plot. Following previous studies (i.e. Fenu *et al.* 2015), human trampling intensity was visually estimated for each plot; in particular, following the method in Fenu *et al.* (2015), four levels of intensity were considered: absent, low (≤ 30% of the plot surface), moderate (31–60%), and intense (≥ 61%).

At plant level, during each sampling, phenological status and reproductive traits (number of flowers and fruits per plant) were recorded for each plant.

**Table 1 – Principal geographical and ecological traits of *Helianthemum caput-felis* population investigated in this study: region, coordinates, altitudinal range (m), substrate type; in addition the number of plots and the number of plants within the monitored plots were reported.**

| Region (code) | Population (code) | Coordinates U.T.M. (time zone) | Altitude (min-max) | Substrate type | N. plots | N. plants |
|---|---|---|---|---|---|---|
| Alicante (ALI) | Cabo Roig (CR) | 700388.62 m E, 4198390.09 m N (30 S) | 3 - 15 | limestone | 15 | 116 |
| Alicante (ALI) | Moraira (MO) | 250226.01 m E, 4285845.34 m N (31 S) | 3 - 15 | limestone | 15 | 56 |
| Majorca (MAJ) | Sa Ràpita (SR) | 496897.00 m E, 4356780.00 m N (31 S) | 0 - 5 | sand and limestone | 15 | 252 |
| Majorca (MAJ) | Colònia de Sant Jordi (SJ) | 500074.00 m E, 4352060.00 m N (31 S) | 0 - 5 | sand and limestone | 13 | 65 |
| Sardinia (SAR) | Capo Mannu (CM) | 447428.00 m E, 4432412.00 m N (32 S) | 5 - 55 | limestone | 20 | 146 |
| Sardinia (SAR) | Su Tingiosu (ST) | 449657.67 m E, 4428538.64 m N (32 S) | 5 - 25 | limestone | 20 | 186 |

In order to estimate the fruit output per region and population, the number of fruits per plant counted at the peak of fruiting season (ranging from May to June) was considered. The average number of fruits per plant was determined from a ratio of the total number of fruits/total number of reproductive plants monitored.

Seed output (number of seeds per fruit) per region and population, was estimated by analysing 10 mature fruits collected from a randomly selected plant from each plot (achieving a total of 980 fruits). Seeds were extracted and counted, and the average number of seeds per fruit was multiplied by the average number of fruits per reproductive plant to predict the mean reproductive capacity of each plant.

### *Data analyses*

In order to carry out reproductive analyses, empty or parasitic fruits were considered for the fruit output, but excluded from the seed output analyses. Seedlings were excluded from all the analysis.

Normality distribution of data were analysed with the Shapiro-Wilk normality test, followed by a non-parametric Levene test for the analysis of variance homogeneity, calculated by R “car” package (Fox and Weisberg 2011). Differences in fruits per plant and seeds per fruit (both in 2013 and 2014) among regions and populations were analysed by non-parametric Kruskal-Wallis test, followed by Nemenyi post-hoc tests with Chi-squared approximation for independent samples to perform multiple comparisons among them (Zar 1999). This analysis was performed by R “PMCMR” package (Pohlert 2014).

The effects of geomorphology (lowland versus slope areas), substrate type (soil, bedrock or sand), distribution area at region and population levels and their relationships on fruit and seed output were analyzed by fitting Generalized Linear Mixed Models (GLMMs), modelled by the “glmer.nb” and “glmer” functions, included in the R “lme4” package (Bates *et al.* 2014). Moreover, “MASS” and “lmerTest” R packages were used in order to explores the data (Kuznetsova *et al.* 2014).

GLMMs provide a flexible way to model traits which do not satisfy the assumptions of a standard linear modelling, allowing at the same time the distinction between fixed and random factors in the model. Their use in this case is justified by the non-normal distribution of dependent variables under consideration and for the inclusion of random sources of variation, which represent an accurate estimation of the effect of environmental heterogeneity at several relevant scales on the modelled variables (see Littell *et al.* 1996).

Since fruits counts follow a Poisson distribution, a log link function and a negative binomial error distribution have been used in order to account variance larger than the mean (overdispersion). Studies with biological counts generally confirm that the negative binomial does provide the more accurate characterization of the observed variation in field situations, compared to the Poisson theory (Stroup 2015). Empty fruits were treated as a binary scale response (0 = empty, 1 = viable fruit), hence a binomial distribution error with logit-link function was chosen. Poisson error distribution and a log link function were selected in order to analyse the seed output.

Every experimental unit (fruits, empty fruits and seeds per fruit) was hypothesized to be a function of fixed effects, including geomorphology levels (lowland vs. slope), substrate type (bedrock, sand and soil), human trampling intensity (absent, low, moderate and intense) and interactions among these. To select the best model, null models with the other ones were compared through the Akaike Information Criterion value (AIC; Burnham and Anderson 2002).

Models were fit using GLMM by maximum likelihood (Laplace Approximation) with year as the random effect and fixed factors changing according to the specific model. In all models, fixed factor effects were tested with F-tests, and the random factor was tested using the Wald Z-statistic test (Giménez-Benavides and Milla 2012).

All statistical analyses were performed using the R Software (R Core Team 2014). Results were graphically expressed using “ggplot2” package for graphics plot (Wickham 2009).

## Results

### *Phenological pattern*

The reproductive season of *H. caput-felis* showed a general flowering period ranging from March to June and a fruiting period from late April to beginning of August (Figure 1). The flowering peak was always observed in April, except for CR in 2014 (March), while the fruiting peak was recorded in May (2014) or in June (2013; Figure 1).

The mean flowering duration (± SE) was 82.92 ± 2.27 days, ranging from 75.5 to 89.5 days, while the mean fruiting duration was 74.75 ± 2.30 days, with a range from 68.5 to 83.5 days. In 2013, flowering period was longer than 2014 (89.33 and 76.5 days, respectively), while the fruiting period had an opposite trend (71 and 78.5 days in 2013 and 2014, respectively).

In the peak, the mean percentage of flowered plants (± SE) was 68.35 ± 0.63%, whereas the mean percentage of fruited plants was 66.79 ± 0.65 %.

At the region level (Figure 1), in 2013 the mean percentage of flowered plants in ALI populations reached the peak in early April, earlier than MAJ and SAR populations, which reached the peak in late April. In 2014 ALI populations reached the peak in early March, MAJ in early April and SAR in late April, so there was an advance of fifteen days in MAJ and ALI populations respect to 2013, while SAR doesn’t change in time. The mean percentage of flowering plants followed the same pattern across the years; SAR populations had the highest value (81.69% in 2013 and 73.83% in 2014), followed by ALI (64.19% and 66.63% in 2013 and 2014, respectively) and MAJ (59.31% and 65.55% in 2013 and 2014, respectively). Only in the SAR region the percentage of flowering plants was slightly lower in 2014 than 2013.

Considering the mean percentage of fruiting plants, in 2013 all regions reached the peak in early June, while in 2014 the peak was reached in late May (Figure 1). The mean percentage of fruiting plants followed the same pattern across the years (Figure 1); SAR populations had the highest value (84.06% in 2013 and 83.62% in 2014), followed by ALI (62.27% in 2013 and 67.03% in 2014) and MAJ (59.50% in 2013 and 55.18% in 2014). Only in SAR region the percentage of fruiting plants was lower in 2014 than 2013.

At population level, in 2014 CR achieved the flowering peak in late March, earlier than the others, which reached the peak in early April, except ST, which had the peak in late April, like in the 2013 (Figure 2). In both years, ST had the highest value of flowering plants (89.83% and 86.02% in 2013 and 2014, respectively), followed by SJ and CM. The lowest value was found in SR (41.88% and 48.02% in 2013 and 2014, respectively).

Fruiting period showed high percentage of fruiting plants generally between late May and early June, with the exception of SJ population, which reached the peak in early July in 2013, and CR, which achieved the peak in late or early May (2013 and 2014, respectively; Figure 2).

The highest percentage of fruiting plants in 2013 was found in MO (92.68%) and ST (92%), followed by SJ and CM. In 2014, SJ showed the highest value (87.10%), followed by CM and CR. Like flowered plants, lowest percentages of fruiting plants were found in SR (37.61% in 2013 and 36.51% in 2014), but the lowest in 2013 was CR (35.40%).

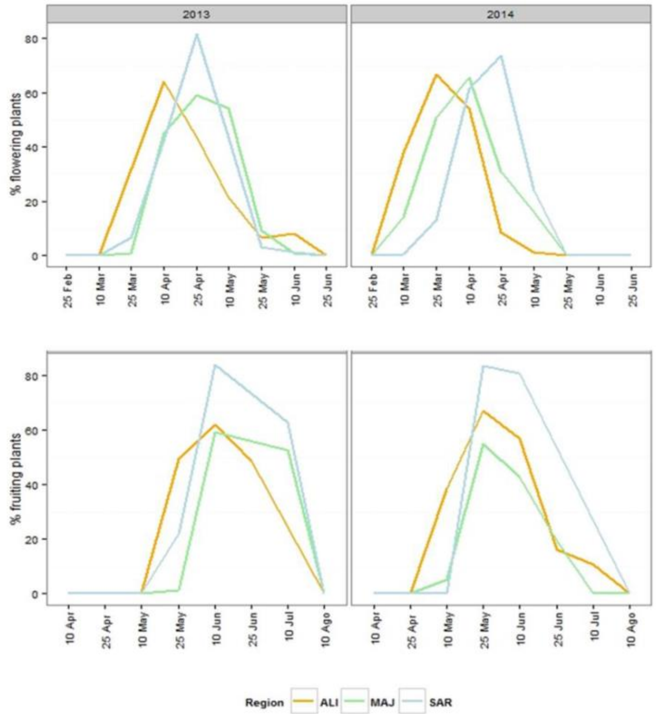

**Figure 1 – Percentage of flowering and fruiting plants per region. In the top row, flowering plants; in the bottom row, fruiting plants. Columns correspond to 2013 and 2014 years.**

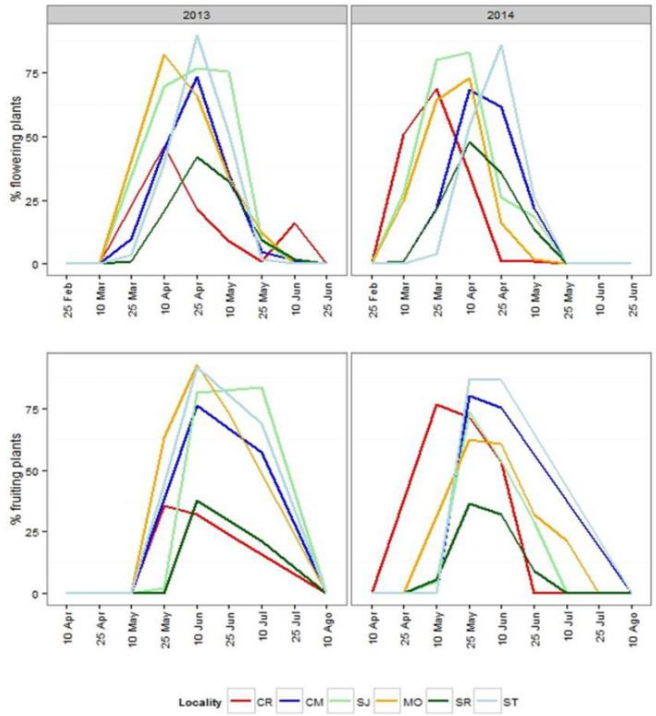

**Figure 2 – Percentage of flowering and fruiting plants per locality. In the top row, flowering plants; in the bottom row, fruiting plants. Columns correspond to 2013 and 2014 years.**

*Fruit output per plant*

In general, more fruits are produced in 2014 (Figure 3). In 2013, the highest average number of fruits per plant was found in SAR (69.70 ± 4.82 fruits per plant), followed by ALI (30.68 ± 6.57 fruits per plant), while the lowest values were found in MAJ (9.15 ± 2.03). The same pattern was found in 2014, when SAR plants produced 116.00 ± 7.05 fruits per plant, while ALI 58.18 ± 7.55 and MAJ 14.61 ± 1.86 fruits per plant. Post-hoc Nemenyi's tests showed that, in 2013 and 2014 SAR had significantly higher fruits production than the other two regions ($p<0.05$), while not difference was found between ALI and MAJ ($p>0.05$) in 2013, whereas in 2014, fruits production was high in ALI ($p<0.05$; Figure 3).

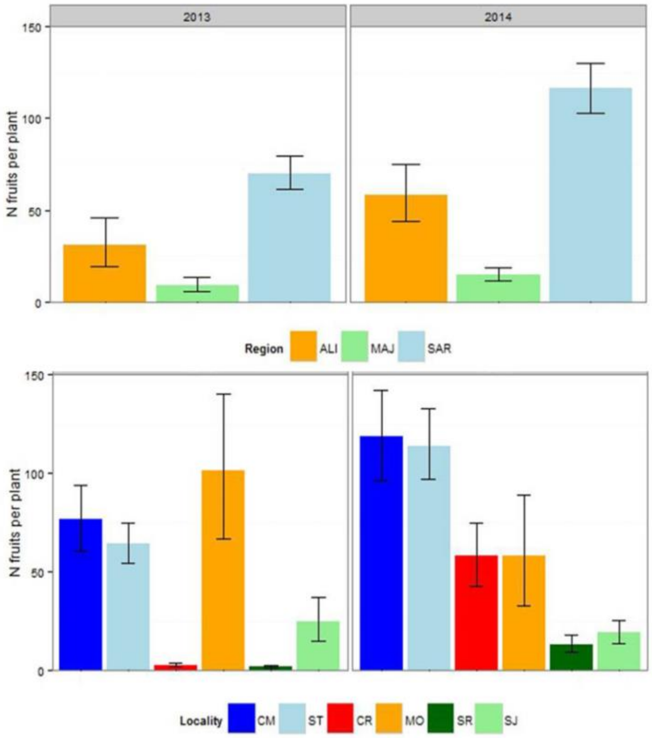

**Figure 3 – Average number of fruits per plant per regions (top row) and localities (bottom row) in 2013 and 2014 (left and right column, respectively), with standard error bars.**

At population level, in 2013 the highest fruit production was observed, in MO (101.33 ± 19.12 fruits per plant), followed by CM (76.40 ± 8.60 fruits per plant) and ST (64.20 ± 5.20 fruits per plant), whereas in 2014 CM showed more fruits per plants (118.98 ± 11.56 fruits per plant; Figure 3), followed by ST (113.75 ± 8.79 fruits per plant). The lowest fruit production was observed in SR (2.09 ± 0.37 and 13.05 ± 2.22 fruits per plant in 2013 and 2014, respectively).

Post-hoc Nemenyi's tests revealed no statistical differences between CM and ST populations ($p>0.05$ in 2013 and 2014), and these Sardinian populations produced more fruits per plant than all the others ones.

The best GLMMs selection for each analysis was reported in Table 1A. Among all the analysed models, the best GLMM that describe fruits production include region, geomorphology and substrate variables (AIC = 11509; $p<0.001$; Table 1A). According to this model, the number of fruits per plant in SAR was statistically higher than ALI and MAJ ($p<0.001$; Table 2; Figure 3). Plants growing on lowland and on sandy substrate generally produced less fruits compared to the plants in slope, on structured soil or on rocky substrate ($p<0.001$; Table 2).

**Table 2 – GLMM output (fruits per plant at regional level): coefficient estimates β, standard errors SE(β), associated Wald's z-score [=β / SE(β)] and significance level *p* for all predictors in the analysis. Random effect (year): 0.057 ± 0.238.**

| Fixed effects | Coef. β | SE(β) | *z* | *p* |
|---|---|---|---|---|
| intercept | 3.257 | 0.201 | 16.205 | < 2e-16*** |
| region MAJ | -0.339 | 0.187 | -1.817 | 0.069 |
| region SAR | 1.052 | 0.143 | 7.382 | 1.56e-13*** |
| geomorphology slope | 0.708 | 0.107 | 6.624 | 3.50e-11*** |
| substrate sand | -1.514 | 0.201 | -7.538 | 4.78e-14*** |
| substrate soil | -0.021 | 0.122 | -0.173 | 0.863 |

At population level, the best selected model includes population, substrate type, geomorphology and human trampling intensity variables (AIC=11442; p<0.05; Table 1A). GLMM's results show that in ST and CM plants produce more fruits than those in the others population (p<0.001; Table 3). On the contrary, plants growing in SR had a significant lower fruit production (p<0.05). Plants produced generally more fruits in slope (p<0.05) and less in sand substrate (p<0.001; Table 3), whereas soil and rocky substrates, as well as trampling intensity, didn't show any significant effect on fruits output.

**Table 3 – GLMM output (fruits per plant at locality level): coefficient estimates β, standard errors SE(β), associated Wald's z-score [=β / SE(β)] and significance level *p* for all predictors in the analysis. Random effect (year): 0.115 ± 0.338.**

| Fixed effects | Coef. β | SE(β) | *z* | *p* |
|---|---|---|---|---|
| intercept | 2.952 | 0.289 | 10.264 | < 2e-16*** |
| population CM | 1.265 | 0.177 | 7.138 | 9.49e-13*** |
| population SJ | 0.667 | 0.241 | 2.774 | 0.006** |
| population MO | 1.173 | 0.214 | 5.490 | 4.03e-08*** |
| population SR | -0.703 | 0.223 | -3.148 | 0.00164** |
| population ST | 1.341 | 0.166 | 8.095 | 5.74e-16*** |
| geomorphology slope | 0.377 | 0.122 | 3.094 | 0.00198** |
| substrate sand | -1.425 | 0.207 | -6.871 | 6.39e-12*** |
| substrate soil | 0.188 | 0.130 | 1.452 | 0.147 |
| trampling low | -0.163 | 0.115 | -1.425 | 0.154 |
| trampling intense | 0.118 | 0.353 | 0.336 | 0.737 |
| trampling moderate | 0.276 | 0.162 | 1.700 | 0.090 |

At region level, ALI showed the highest percentage of empty fruits (29.50% and 44.67% in 2013 and 2014 respectively), followed by MAJ (27.37% and 40.53% in 2013 and 2014, respectively). In SAR, the range varies from 15.75% (2013) to 15.50% (2014). In addition, presence of parasitised fruits was observed in ALI and MAJ regions. The best selected model took into account region, substrate, geomorphology and human trampling intensity variables (AIC=1826; p<0.001; Table 1A). In particular, plants in ALI had more empty fruits than MAJ (p<0.05), while plants in SAR showed the highest number of viable fruits (p<0.001; Table 4). Sand substrate had a negative significant relationship with viable fruits (p<0.05), while soil substrate had a positive relationship (but not significant). At geomorphology level, plants that grew in slope produce more viable fruits than those growing in lowland (p<0.05); low human trampling intensity positively affected the number of viable fruits (p<0.001; Table 4).

**Table 4 – GLMM output (empty fruits per plant at regional level): coefficient estimates β, standard errors SE(β), associated Wald's z-score [=β / SE(β)] and significance level *p* for all predictors in the analysis. Random effect (year): 0.052 ± 0.228.**

| Fixed effects | Coef. β | SE(β) | *z* | *p* |
|---|---|---|---|---|
| intercept | 0.043 | 0.221 | 0.194 | 0.847 |
| region MAJ | 0.434 | 0.201 | 2.152 | 0.0314* |
| region SAR | 1.288 | 0.147 | 8.774 | < 2e-16*** |
| substrate sand | -0.653 | 0.245 | -2.663 | 0.008** |
| substrate soil | 0.031 | 0.141 | 0.219 | 0.827 |
| geomorphology slope | 0.372 | 0.124 | 2.997 | 0.003** |
| trampling low | 0.505 | 0.144 | 3.519 | 0.000433*** |
| trampling intense | 21.135 | 104.512 | 0.202 | 0.839738 |
| trampling moderate | 0.308 | 0.185 | 1.660 | 0.097 |

At population level, in 2013 the highest percentage of empty fruits was observed in SR (48.89%), followed by MO (37%) and CR (22%). A similar pattern was observed in 2014 in MO (50%), followed by SR (42.86%), CR (39.33%) and SJ (39.17%). In both Sardinian populations, the percentage of empty fruits was low (15–17%) and did not vary between years. The best selected model about empty fruits depicted the influence of population, substrate type, geomorphology and human trampling intensity (AIC=1811.6; p<0.001; Table 1A). MO and SR showed a negative relationship with viable fruits and there are more empty fruits than in the others populations (p<0.001; Table 5). On the contrary, CM and ST had the highest quantity of viable fruits (p<0.001). Sandy and soil substrate had a negative relationship with viable fruits number, but the last was not significant (p>0.05). Surprisingly, low and moderate human trampling intensity facilitated the production of viable fruits (p<0.05; Table 5).

**Table 5 – GLMM output (empty fruits per plant at locality level): coefficient estimates β, standard errors SE(β), associated Wald's z-score [=β / SE(β)] and significance level *p* for all predictors in the analysis. Random effect (year): 0.053 ± 0.231.**

| Fixed effects | Coef. β | SE(β) | *z* | *p* |
|---|---|---|---|---|
| intercept | 0.512 | 0.252 | 2.033 | 0.042* |
| population CM | 1.096 | 0.209 | 5.254 | 1.49e-07*** |
| population SJ | 0.295 | 0.243 | 1.212 | 0.226 |
| population MO | -0.679 | 0.202 | -3.366 | 0.000763*** |
| population SR | -0.508 | 0.302 | -1.684 | 0.092 |
| population ST | 0.828 | 0.202 | 4.108 | 4.00e-05*** |
| substrate sand | -0.603 | 0.261 | -2.310 | 0.021* |
| substrate soil | -0.154 | 0.161 | -0.956 | 0.339 |
| geomorphology slope | 0.233 | 0.139 | 1.681 | 0.093 |
| trampling low | 0.463 | 0.146 | 3.166 | 0.002** |
| trampling intense | 17.410 | 28.622 | 0.608 | 0.543 |
| trampling moderate | 0.637 | 0.206 | 3.091 | 0.002** |

### *Seeds output*

Number of seeds per fruit was always higher in SAR (4.21 ± 0.08 and 4.44 ± 0.08 seeds per fruits in 2013 and 2014, respectively; Figure 4).

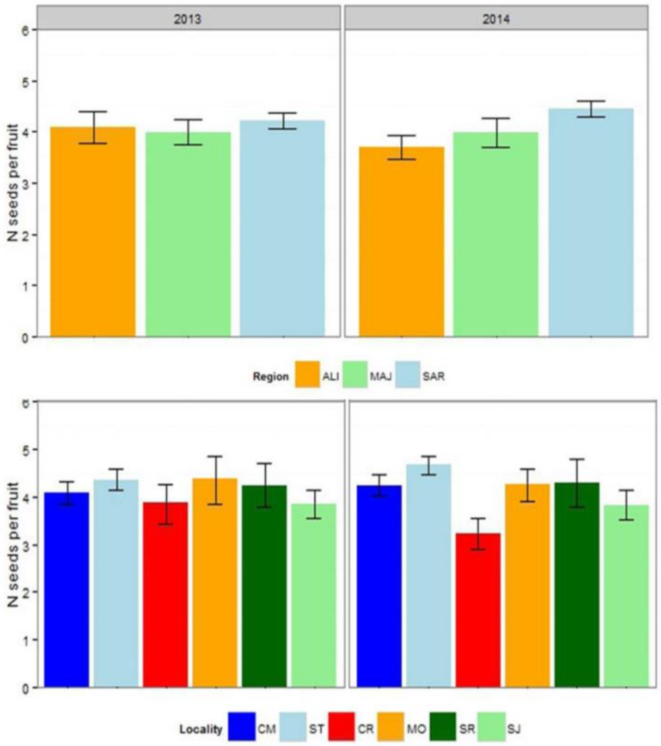

**Figure 4 – Average number of seeds per fruit per regions (top row) and localities (bottom row) in 2013 and 2014 (left and right columns, respectively), with standard error bars.**

The lowest seed output was found in MAJ in 2013 (3.98 ± 0.13 seeds per fruit), and in ALI in 2014 (3.70 ± 0.13 seeds per fruits). Post-hoc Nemenyi's tests revealed that in 2014 only SAR differed from the others, producing more seeds per fruits ($p<0.05$). The best selected model revealed that region, geomorphology and human trampling intensity had a statistical significant effect in seeds production (AIC=4787.9; $p<0.05$; Table 1A). In particular, SAR had the highest mean of seeds per fruit ($p<0.001$), followed by ALI ($p<0.001$). Moderate human trampling intensity enhanced the production of seeds per fruit ($p<0.05$; Table 6).

**Table 6 – GLMM output (seeds per fruit at regional level): coefficient estimates β, standard errors SE(β), associated Wald's z-score [=β / SE(β)] and significance level *p* for all predictors in the analysis. Random effect (year): $8.729^{-16} \pm 2.955^{-08}$.**

| Fixed effects | Coef. β | SE(β) | *z* | *p* |
|---|---|---|---|---|
| intercept | 1.289 | 0.040 | 32.26 | < 2e-16*** |
| region MAJ | 0.002 | 0.045 | 0.04 | 0.964 |
| region SAR | 0.122 | 0.035 | 3.44 | 0.001*** |
| geomorphology slope | 0.056 | 0.029 | 1.89 | 0.059 |
| trampling low | 0.043 | 0.033 | 1.29 | 0.199 |
| trampling intense | 0.199 | 0.104 | 1.92 | 0.056 |
| trampling moderate | 0.101 | 0.043 | 2.37 | 0.018* |

At population level, MO and ST showed the highest seed output in 2013 (4.37 ± 0.26 and 4.36 ± 0.11 seeds per fruit, respectively), while in 2014 ST reached 4.67 ± 0.10 seeds per fruit. The lowest seed output was found in SJ in 2013 (3.85 ± 1.16 seeds per fruit) and in CR in 2014 (3.23 ± 0.16 seeds per fruit), while CM, ST and SR populations showed a higher seed output in 2014 than in 2013. Post-hoc Nemenyi's tests revealed that only in 2014 there were statistical significant differences among populations, which highlighted in particular the lowest seed output for CR and the highest for ST ($p<0.001$ in both cases).

The best selected GLMM revealed that seeds per fruits depended on populations and geomorphology (AIC=4772.8; $p<0.05$; Table 1A). ST had the highest seeds per fruit output, followed by SR, MO, CM and CR ($p<0.001$). Furthermore, plants that grew on slope produced higher value of seeds per fruit ($p<0.05$; Table 7). See Appendix Figures (1A-12A) for details.

**Table 7 – GLMM output (seeds per fruit at locality level): coefficient estimates β, standard errors SE(β), associated Wald's z-score [=β / SE(β)] and significance level *p* for all predictors in the analysis. Random effect (year): 0.**

| Fixed effects | Coef. β | SE(β) | *z* | *p* |
|---|---|---|---|---|
| intercept | 1.218 | 0.045 | 27.264 | < 2e-16*** |
| population CM | 0.164 | 0.049 | 3.364 | 0.0008*** |
| population SJ | 0.070 | 0.058 | 1.219 | 0.223 |
| population MO | 0.187 | 0.058 | 3.206 | 0.001** |
| population SR | 0.233 | 0.069 | 3.384 | 0.0007*** |
| population ST | 0.270 | 0.049 | 5.467 | 4.58e-08*** |
| geomorphology slope | 0.073 | 0.032 | 2.297 | 0.022* |

## Discussion

Studies on reproductive biology of endangered plants are crucial for predicting their survival capacity (Evans *et al.* 2003); plant reproductive success is determined by intrinsic characteristics of the reproductive system of a species, as well as by extrinsic abiotic and biotic factors (Cogoni *et al.* 2012, 2015; Abdala-Roberts *et al.* 2014). In particular, also unmeasured microenvironmental factors (like soil, temperature, etc.) may play a role in affecting phenological variations over space, and more extensive environmental gradients could lead to stronger impacts on spatial patterns of phenology, as suggested by studies in other locations (e.g. Fisher *et al.* 2006; Liang and Schwartz 2009; Zhao *et al.* 2013).

The results of this research on the general flowering and fruiting seasons in *H. caput-felis* are conforming to the previous local studies carried out at Sa Ràpita population (Rodríguez-Pérez 2005; Tébar *et al.* 1995) and in Sardinia (Fenu *et al.* 2015). In general, flowering and fruiting phenology may have an important influence on plant reproductive success in several ways (Augspurger 1981; Elzinga *et al.* 2007). The phenological patterns of *H. caput-felis* were similar among the three analysed geographic regions and among the investigated years, except in ALI region that showed significant inter–annual variability.

The existence of ecological gradients that affect the phenological and reproductive traits among populations was widely analysed. Geographical gradients represent an important issue in plant population ecology studies and several authors investigated plants phenology and reproductive traits along altitudinal and latitudinal gradients (e.g. Giménez-Benavidez *et al.* 2007; Giménez-Benavides and Milla 2012; Abbott and Brennan 2014; De Frenne *et al.* 2011). These studies demonstrate a relationship of those traits with the altitudinal and latitudinal differences. Conversely, longitudinal gradient remains less documented, and no studies were available on this pattern for Mediterranean plants. Plants of *H. caput-felis* growing at the western part of the distribution range (ALI region) advanced the beginning of flowering period respect to the others distribution regions. These differences could reflect a longitudinal gradient; in fact, moving from western to eastern part of the distribution range in the Western Mediterranean Basin, a gradient determined by the following bioclimatic factors can be detected: the aridity decrease from western to eastern localities, while the mean temperatures and the oceanicity increase. In particular, CR population (ALI region) showed higher temperatures (maximum absolute temperature: 43°C; Serra 2005) and aridity (mean annual precipitation: 290 mm; Hijmans *et al.* 2005) with respect to the others *H. caput-felis* populations. This general pattern is consistent with the bioclimatic classification of these territories. In fact, CR population is characterized by Mediterranean xeric-oceanic bioclimate, with low thermomediterranean thermotype and semiarid ombrotype. Conversely, MO, SR and SJ populations are characterised by Mediterranean pluviseasonal-oceanic bioclimate, with upper thermomediterranean thermotype and dry ombrotype. Finally, CM and ST populations (SAR region) showed a Mediterranean pluviseasonal-oceanic bioclimate with upper thermomediterranean thermotype and dry ombrotype (Rivas-Martínez *et al.* 2004). Hence, also bioclimatic data confirm that the mean temperature values decrease when moving from west to east, while the mean rainfall increases.

Generally, climatic factors and climate patterns directly influence the timing, magnitude (productivity), and spatial patterns of vegetation growth cycles, and, for that matter, phenological response (Schwartz 1994; Zhao *et al.* 2013). The correlation among fruits output and seed germination with climatic variability, in particular precipitation, was detected for other congeneric Mediterranean species (i.e. *Helianthemum squamatum* (L.) Dum Cours.; Escudero *et al.* 1999). In addition, juvenile recruitment is generally strongly correlated with climatic variability, and more specifically with precipitation, because water is the principal limiting resource in arid regions (Harrington 1991), as previous highlighted in others *Helianthemum* species (Marrero Gómez *et al.* 2007).

Reproductive output varied widely among populations throughout the geographic range (Yakimowski and Eckert 2007; Giménez-Benavides *et al.* 2007). Plants growing at the eastern edge of the distribution range (SAR region) had a significantly higher fruits per plant and seeds per fruit than the other two regions, while the lowest fruits per plant production were found in MAJ region. No differences were found between CM and ST populations (SAR region), and the higher productive output compared to the others populations should be related to the lower habitat fragmentation observed, as well as the optimal bioclimatic condition (lowest climatic aridity), that could promote the fruits production. Also difference in fruit and seed output among regions and populations can be related to this general pattern and in particular to the aridity gradient, in which summer drought periods increase from a minimum of 4 months in SAR to a maximum of more than 6 months the southern ALI population.

According to the aridity gradient hypothesis, in which an increase of humidity could promote reproductive output, plants growing in arid substrate (sandy habitat – SR population) are the least productive. In addition, dune systems have limited resources available for plants, and only for a limited time during the growing season (e.g. Fenu *et al.* 2012, 2013; Cogoni *et al.* 2013, 2015).

This study highlighted that the optimal ecological condition promoting the fruit and seeds output for *H. caput-felis* are the presence of structured soil in slope areas, according to the previous study carried out in Sardinia (Fenu *et al.* 2015).

Fruit production is typically the most important cost in plant reproduction for most plant species, since it comprises not only seeds but also fruits and many accessory structures related to seed protection and dispersal, and the cost of aborted seeds and empty fruits (Lord and Westoby 2006). A particular reproductive trait observed in ALI region was the presence of a high percentage of empty fruits. The high number of empty fruits per plant in ALI region could be related to the impact of urbanization that determines habitat fragmentation. In fact, habitat fragmentation and degradation may have serious implications for plant populations because isolated and scattered plants may have difficulty attracting pollinator visits, potentially resulting in reduced reproductive performance (total number of flowers, fruits or seeds per plant; e.g. Brys *et al.* 2004; Johnson and Collin 2004; Bruna and Oli 2005; Metcalfe and Kunin 2006). Moreover, the parasitism observed in ALI and MAJ regions, which was detected as an important factor in reducing reproductive output, can reduce the reproductive success of *H. caput-felis*, as detected for other Cistaceae plants (e.g. *Cistus ladanifer* L.; Metcalfe and Kunin 2006). Hence, further researches are needed in order to evaluate the importance of fruits parasitism.

Human trampling has been hypothesized to be generally an important threat for Mediterranean coastal plants (Quilichini and Debussche 2000; Fenu *et al.* 2013, 2015). The results of this study highlighted that moderate intensity trampling promote the production of seeds and viability of fruits, but it was only slightly positively significant in fruit production. However, previous studies considering also population density in Sardinian populations (Fenu *et al.* 2015), underline the negative effect of trampling on plant density, that could be related to the critical limitation in seedling recruitment; so, human trampling should be considered anyway a significant threat to the persistence of the populations. Moreover, trampling and others human-related disturbances appear to have an important detrimental impact on sand dunes, especially in the Mediterranean coasts (Fenu *et al.* 2013; Ciccarelli 2014).

The abundant centre model was not confirmed for *H. caput-felis*. The “central” populations (ALI region) showed lowest reproductive outputs (fruits and seeds) compared to the eastern peripheral populations (SAR region). Although several studies demonstrated a reduced seed production or seed quality at the species’ range margin (Pigott and Huntley 1981; García *et al.* 2000; Jump and Woodward 2003), *H. caput-felis* didn’t follow this pattern, as demonstrated for others plant species (Kluth and Bruelheide 2005; Yakimowski and Eckert 2007). However, the local ecological and historical conditions must be considered; although representing the largest distribution area along the Iberian’s coasts, ALI populations appear strongly fragmented by human disturbance, with the consequent reduction of the suitable habitat. On the contrary, SAR populations are located in more natural sites, without urbanizations and less fragmented respect to the ALI ones. In fact, ACM does not sufficiently explain plant population patterns at range margins and also the population history could be considered in order to understand the effect of marginality (see also Abeli *et al.* 2014). Marginal populations may be locally adapted to sub-optimal conditions at the range edge (Caughley *et al.* 1988; Barton 2001) and other mechanisms, such as dispersal limitation or unstable population dynamics, could be also important in limiting the geographic distribution of some plant species (Kawecki and Holt 2002; Holt 2003).

In conclusion, this study represents the first global analysis of *H. caput-felis* phenological and reproductive traits along the entire European distribution range of this endangered plant species. However, some factors affecting the reproductive systems of this plants remains unknown and further detailed analyses are needed in order to understand the complex interaction governing the populations’ persistence in a fragmented Mediterranean plant.

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## References

Abbott R.J. and Brennan A.C. 2014. Altitudinal gradients, plant hybrid zones and evolutionary novelty. Philosophical Transactions of the Royal Society B: Biological Sciences 369: 20130346.

Abdala-Roberts L., Marrufo-Zapata D., Arceo-Gómez G. and Parra-Tabla V. 2014. Pollen limitation, fruit abortion, and autonomous selfing in three populations of the perennial herb *Ruellia nudiflora*. Plant Species Biology 29: 25–33.

Abeli T., Gentili R., Mondoni A., Orsenigo S. and Rossi G. 2014. Effects of marginality on plant population performance. Journal of Biogeography 41: 239–249.

Agulló J.C., Pérez-Bañón C., Crespo M.B. and Juan A. 2015. Puzzling out the reproductive biology of the endangered cat's head rockrose (*Helianthemum caput-felis*, Cistaceae). Flora 217: 75–81.

Alexander H.M., Price S., Houser R., Finch D. and Tourtellot M. 2007. Is there reduction in disease and pre-dispersal seed predation at the border of a host plant's range? Field and herbarium studies of *Carex blanda*. Journal of Ecology 96: 446–457.

Angert A.L. 2009. Colloquium papers: the niche, limits to species' distributions, and spatiotemporal variation in demography across the elevation ranges of two monkeyflowers. Proceedings of the National Academy of Sciences 106: 19693–19698.

Aragón C.F., Escudero A. and Valladares F. 2008. Stress-induced dynamic adjustments of reproduction differentially affect fitness components of a semi-arid plant. Journal of Ecology 96: 222–229.

Augspurger C.K. 1981. Reproductive synchrony of a tropical shrub: experimental studies on effects of pollinators and seed predators in *Hybanthus prunifolius* (Violaceae). Ecology 62: 775– 788.

Barton N.H. 2001. Adaptation at the edge of a species' range. In: Silvertown J., Antonovics J. (Eds.), Integrating Ecology and Evolution in a Spatial Context. Blackwell Science, Oxford, 365–392.

Bates D., Maechler M., Bolker B. and Walker S. 2014. lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-7 http://CRAN.R-project.org/package=lme4

Brown J.H. 1984. On the relationship between abundance and distribution of species. American Naturalist 124: 255–279.

Bruna E.M. and Oli M.K. 2005. Demographic effects of habitat fragmentation on a tropical herb: life-table response experiments. Ecology 86: 1816–1824.

Brys R., Jacquemyn H., Endels P., Van Rossum F., Hermy M., Triest L., De Bruyn L. and Blust G.D.E. 2004. Reduced reproductive success in small populations of the self-incompatible *Primula vulgaris*. Journal of Ecology 92: 5–14.

Burnham K.P. and Anderson D.R. 2002. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach (2nd ed.), Springer-Verlag.

Castilla A.R., Alonso C. and Herrera C.M. 2011. Exploring local borders of distribution in the shrub *Daphne laureola*: individual and population traits. Acta Oecologica 37: 269–276.

Caughley G., Grice D., Barker R. and Brown B. 1988. The edge of the range. Journal of Animal Ecology 57: 771–778.

Ciccarelli D. 2014. Mediterranean coastal sand dune vegetation: influence of natural and anthropogenic factors. Environmental management 54: 194–204.

Cogoni D., Mattana E., Fenu G. and Bacchetta G. 2012. From seed to seedling, a critical transitional stage for the Mediterranean psammophilous species *Dianthus morisianus* (Caryophyllaceae). Plant Biosystems 146: 910–917.

Cogoni D., Fenu G. and Bacchetta G. 2013. Effects of timing of emergence and microhabitat conditions on the seedling performance of a coastal Mediterranean plant. Ecoscience 20: 131–136.

Cogoni D., Fenu G. and Bacchetta G. 2015. Reproductive biology of the narrow endemic *Anchusa littorea* Moris (Boraginaceae), an endangered coastal Mediterranean plant. Turkish Journal of Botany 39: 642–652.

De Frenne P., Graae B.J., Kolb A., Shevtsova A., Baeten L., Brunet J., Chabrerie O., Cousins S.A.O., Decocq G., Dhondt R., Diekmann M., Gruwez R., Heinken T., Hermy M., Öster M., Saguez R., Stanton S., Tack W., Vanhellemont M. and Verheyen K. 2011. An intraspecific application of the leaf-height-seed ecology strategy scheme to forest herbs along a latitudinal gradient. Ecography 34: 132–140.

Doak D.F. and Morris W.F. 2010. Demographic compensation and tipping points in climate-induced range shifts. Nature 467: 959–962.

Dyer A.R., Hardison J.L. and Rice K.J. 2012. Phenology constrains opportunistic growth response in *Bromus tectorum* L. Plant Ecology 213: 103–112.

Eckert C.G., Samis K.E. and Lougheed S.C. 2008. Genetic variation across species' geographical ranges: The central-marginal hypothesis and beyond. Molecular Ecology 17: 1170–1188.

Eckhart V.M., Geber M.A., Morris W.F., Fabio E.S., Tiffin P. and Moeller D.A. 2011. The geography of demography: longterm demographic studies and species distribution models reveal a species border limited by adaptation. The American Naturalist 178: S26–S43.

Elzinga J.A., Atlan A., Biere A., Gigord L., Weis A.E. and Bernasconi G. 2007. Time after time: flowering phenology and biotic interactions. Trends in Ecology and Evolution 22: 432–439.

Escudero A., Somolinos R.C., Olano J. and Rubio A. 1999. Factors controlling the establishment of *Helianthemum squamatum*, an endemic gypsophile of semi-arid Spain. Journal of Ecology 87: 290–302.

Estiarte M., Puig G. and Penuelas J. 2011. Large delay in flowering in continental versus coastal populations of a Mediterranean shrub, *Globularia alypum*. International Journal of Biometeorology 55: 855–865.

Evans E.K.M., Menges E.S. and Gordon D.R. 2003. Reproductive biology of three sympatric endangered plants endemic to Florida scrub. Biological Conservation 111: 235–246.

Fenu G., Cogoni D., Ferrara C., Pinna M.S. and Bacchetta G. 2012. Relationships between coastal sand dune properties and plant communities' distribution: the case of Is Arenas (Sardinia). Plant Biosystems 146: 586–602.

Fenu G., Cogoni D., Ulian T. and Bacchetta G. 2013. The impact of human trampling on a threatened coastal Mediterranean plant: The case of *Anchusa littorea* Moris (Boraginaceae). Flora 208: 104–110.

Fenu G., Cogoni D., Sulis E. and Bacchetta G. 2015. Ecological response to human trampling and conservation status of *Helianthemum caput-felis* (Cistaceae) at the eastern periphery of its range. Acta Botanica Gallica 162: 191–201.

Fisher J.I., Mustard J.F. and Vadeboncoeur M.A. 2006. Green leaf phenology at Landsat resolution: Scaling from the field to the satellite. Remote sensing of environment 100: 265–279.

Fox J. and Weisberg S. 2011. An R Companion to Applied Regression, Second Edition. Thousand Oaks CA: Sage. URL: http://socserv.socsci.mcmaster.ca/jfox/Books/Companion

Gargano D., Fenu G., Medagli P., Sciandrello S. and Bernardo L. 2007. The status of *Sarcopoterium spinosum* (Rosaceae) at the western periphery of its range:

ecological constraints led to conservation concerns. Israel Journal of Plant Sciences 55: 1–13.

Gaston K.J. 2009. Geographic range limits: achieving synthesis. Proceedings of the Royal Society of London B: Biological Sciences 276: 1395–1406.

Giménez-Benavides L., Escudero A. and Iriondo J.M. 2007. Local adaptation enhances seedling recruitment along an altitudinal gradient in a high mountain Mediterranean plant. Annals of Botany 99: 723–734.

Giménez-Benavidez and Milla 2013. Comparative germination ecology of two altitudinal vicariant *Saxifraga* species endemic to the north of Spain. Plant Biology 15:593–600.

Grant M.C. and Antonovics J. 1978. Biology of ecologically marginal populations of *Anthoxanthum odoratum*. I. Phenetics and dynamics. Evolution 32: 822–838.

Harrington G.N. 1991. Effects of soil moisture on shrub seedling survival in semi-arid grassland. Ecology 72: 1138–1149.

Hengeveld R. and Haeck J. 1982. The distribution of abundance. I. Measurements. Journal of Biogeography 9: 303–316.

Herrera C.M. and Bazaga P. 2008. Adding a third dimension to the edge of a species' range: altitude and genetic structuring in mountainous landscapes. Heredity 100: 275–285.

Hijmans R.J., Cameron S.E., Parra J.L., Jones P.G. and Jarvis A. 2005. Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology 25: 1965–1978.

Holt R.D. 2003. On the evolutionary ecology of species' ranges. Evolutionary Ecology Research 5: 159–178.

Holt R.D. and Keitt T.H. 2005. Species' borders: A unifying theme in ecology. Oikos 108: 3–6.

Johnson S.D. and Collin C.L. 2004. Factors contributing to variation in seed production among remnant populations of the endangered daisy *Gerbera aurantiaca*. Biotropica 36: 148–155.

Jump A.S. and Woodward F.I. 2003. Seed production and population density decline approaching the range-edge of *Cirsium* species. New Phytologist 160: 349–358.

Kawecki T.J. and Holt R.D. 2002. Evolutionary consequences of asymmetrical dispersal rates. American Naturalist 160: 333–347.

Kawecki T.J. 2008. Adaptation to marginal habitats. Annual Review of Ecology Evolution and Systematics 39: 321–342.

Kluth C. and Bruelheide H. 2005. Central and peripheral *Hornungia petraea* populations: patterns and dynamics. Journal of Ecology 93: 584–595.

Kuznetsova A., Brockhoff P.B. and Christensen R.H.B. 2014. lmerTest: Tests in Linear Mixed Effects Models. R package version 2.0-20. http://CRAN.R-project.org/package=lmerTest

Lawton J.H. 1993. Range, population abundance and conservation. Trends in Ecology and Evolution 8: 409–413.

Liang L. and Schwartz M.D. 2009. Landscape Phenology: An Integrative Approach to Seasonal Vegetation Dynamics. Landscape Ecology 24: 465–472.

Littell R.C., Milliken G.A. Stroup W.W. and Wolfinger R.D. 1996. SAS System for Mixed Models. SAS Institute, Cary, North Carolina.

Lord J.M. and Westoby M. 2006. Accessory costs of seed production. Oecologia 150:310–317.

Marrero-Gómez M.V., Oostermeijer J.G.B., Carqué-Álamo E. and Bañares-Baudet Á. 2007. Population viability of the narrow endemic *Helianthemum juliae* (Cistaceae) in relation to climate variability. Biological conservation 136: 552–562.

Maun M.A. 2009. The biology of coastal sand dunes. 1st ed. New York, NY, USA: Oxford University Press Inc.

Metcalfe D.B. and Kunin W.E. 2006. The effects of plant density upon pollination success, reproductive effort and fruit parasitism in *Cistus ladanifer* L. (Cistaceae). Plant Ecology 185: 41–47.

Morellato L.P.C., Alberton B., Alvarado S.T., Borges B., Buisson E., Camargo M.G.G., Cancian L.F., Carstensen D.W., Escobar D.F.E., Leite P.T.P., Mendoza I., Rocha N.M.W.B., Soares N.C., Silva T.S.F., Staggemeier V.G., Streher A.S., Vargas B.C. and Peres C.A. 2016. Linking plant phenology to conservation biology. Biological Conservation 195: 60–72.

Novoplansky A., Cohen D. and Sachs T. 1994. Responses of an annual plant to temporal changes in light environment: an interplay between plasticity and determination. Oikos 69: 437–446.

Pigott C.D. and Huntley J.P. 1981. Factors controlling the distribution of *Tillia cordata* at the northern limits of its geographical range III. Nature and causes of seed sterility. New Phytologist 87: 817–839.

Pironon S., Villellas J., Morris W.F., Doak D.F. and García M.B. 2015. Do geographic, climatic or historical ranges differentiate the performance of central versus peripheral populations? Global Ecology and Biogeography 24: 611–620.

Pohlert T. 2014. The Pairwise Multiple Comparison of Mean Ranks Package (PMCMR). R. package. http://CRAN.R-project.org/package=PMCMR

Pouget M., Youssef S., Migliore J., Juin M., Médail F. and Baumel A. 2013. Phylogeography sheds light on the central–marginal hypothesis in a Mediterranean narrow endemic plant. Annals of Botany 112: 1409–1420.

Quilichini A. and Debussche M. 2000. Seed dispersal and germination patterns in a rare Mediterranean island endemic (*Anchusa crispa* Viv., Boraginaceae). Acta Oecologica 21: 303–313.

R Core Team 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Rivas-Martínez S., Penas A. and Díaz T.E. 2004. Biogeographic map of Europe. Cartographic Service, University of Léon. Spain. http://www.globalbioclimatics.org/form/maps.htm

Rivas-Martínez S. 2007. Mapa de series, geoseries y geopermaseries de vegetación de España. Itinera Geobotanica 17: 5–436.

Rodríguez-Pérez J. 2005. Breeding system, flower visitors and seedling survival of two endangered species of *Helianthemum* (Cistaceae). Annals of Botany 95: 1229–1236.

Sagarin R.D. and Gaines S.D. 2002. The “abundant centre” distribution: to what extent is it a biogeographical rule? Ecology Letters 5: 137–147.

Sagarin R.D., Gaines S.D. and Gaylord B. 2006. Moving beyond assumptions to understand abundance distributions across the ranges of species. Trends in Ecology and Evolution 21: 524–530.

Samis K.E. and Eckert C.G. 2007. Testing the abundant center-model using range-wide demographic surveys of two coastal dune plants. Ecology 88: 1747–1758.

Schwartz M.D. 1994. Monitoring global change with phenology: the case of the spring green wave. International journal of biometeorology 38: 18–22.

Serra Laliga L. 2005. Estudio crítico de la flora vascular de la provincia de Alicante: Aspectos nomenclaturales, biogeográficos y de conservación. Vol. 19. Editorial CSIC–CSIC Press.

Sexton J.P., McIntyre P.J., Angert A.L. and Rice K.J. 2009. Evolution and ecology of species range limits. Annual Review of Ecology Evolution and Systematics 40: 415–436.

Soulé M. 1973. The epistasis cycle: a theory of marginal populations. Annual Review of Ecology Evolution and Systematics 4: 165–187.

Stroup W.W. 2015. Rethinking the Analysis of Non-Normal Data in Plant and Soil Science. Agronomy Journal 107:811–827.

Sultan S.E. and Spencer H.G. 2002. Metapopulation structure favors plasticity over local adaptation. American Naturalist 160: 271–283.

Tébar F.J., Gil L. and Llorens L. 1997. Reproductive biology of *Helianthemum apenninum* (L.) Mill. and *H. caput-felis* Boiss. (Cistaceae) from Mallorca (Balearic Islands, Spain). Acta Botánica Malacitana 22: 53–63.

Thompson J.D. 2005. Plant Evolution in the Mediterranean. Oxford: Oxford University Press.

Vaupel A. and Matthies D. 2012. Abundance, reproduction, and seed predation of an alpine plant decrease from the center toward the range limit. Ecology 93: 2253–2262.

Venables W.N. and Ripley B.D. 2002. Modern Applied Statistics with S. Fourth Edition. Springer, New York.

Villellas J., Ehrlén J., Olesen J.M., Braza R. and García M.B. 2013a. Plant performance in central and northern peripheral populations of the widespread *Plantago coronopus*. Ecography 36: 136–145.

Villellas J., Morris W.F. and García M.B. 2013b. Variation in stochastic demography between and within central and peripheral regions in a widespread short-lived herb. Ecology 94: 1378–1388.

Wickham H. 2009. ggplot2: elegant graphics for data analysis. Springer New York. http://had.co.nz/ggplot2/book

Yakimowski S.B. and Eckert C.G. 2007. Threatened Peripheral Populations in Context: Geographical Variation in Population Frequency and Size and Sexual Reproduction in a Clonal Woody Shrub. Conservation Biology 21: 811–822.

Zar J.H. 1999. Biostatistical Analysis, 4th ed. Prentice-Hall, Upper Saddle River, NJ.

Zhao M., Peng C., Xiang W., Deng X., Tian, D., Zhou X., Yu G., He H. and Zhao Z. 2013. Plant phenological modeling and its application in global climate change research: overview and future challenges. Environmental Reviews 21: 1–14.

# Appendices

**Table 1A – GLMMs selection results on fruit set, empty fruits and seeds per fruits in relation to regions and localities, with AIC statistics test; in bold, the selected model of each selection.**

| Response variable | | | Factors | | AIC | Pr(>Chisq) |
|---|---|---|---|---|---|---|
| | | | fixed | random | | |
| *fruit set* | | | | | | |
| | region | 1 | region | year | 11600 | |
| | | 2 | region + substrate | year | 11552 | 6.899e-12*** |
| | | **3** | **region + substrate + geomorphology** | **year** | **11509** | **1.668e-11*** ** |
| | | 4 | region + substrate + geomorphology + trampling | year | 11508 | 0.08421 |
| | population | 1 | population | year | 11527 | |
| | | 2 | population + substrate | year | 11458 | < 2.2e-16*** |
| | | 3 | population + substrate + geomorphology | year | 11447 | 0.0003246*** |
| | | **4** | **population + substrate + geomorphology + trampling** | **year** | **11442** | **0.0159311*** |
| *empty fruits* | | | | | | |
| | region | 1 | region | year | 1848.8 | |
| | | 2 | region + substrate | year | 1844.1 | 0.0126926* |
| | | 3 | region + substrate + geomorphology | year | 1840.1 | 0.0143794* |
| | | **4** | **region + substrate + geomorphology + trampling** | **year** | **1826.0** | **0.0001628*** ** |
| | population | 1 | population | year | 1825.9 | |
| | | 2 | population + substrate | year | 1826.3 | 0.1630 |
| | | 3 | population + substrate + geomorphology | year | 1826.8 | 0.2301 |
| | | **4** | **population + substrate + geomorphology + trampling** | **year** | **1811.6** | **9.552e-05*** ** |
| *seeds per fruit* | | | | | | |
| | region | 1 | region | year | 4790.0 | |
| | | 2 | region + geomorphology | year | 4790.0 | 0.165994 |
| | | 3 | region + substrate | year | 4793.2 | 1.000000 |
| | | **4** | **region + geomorphology + trampling** | **year** | **4787.9** | **0.009261*** * |
| | population | 1 | population | year | 4776.1 | |
| | | **2** | **population + geomorphology** | **year** | **4772.8** | **0.02158*** |
| | | 3 | population + substrate | year | 4779.7 | 1.00000 |
| | | 4 | population + geomorphology + substrate + trampling | year | 4777.5 | 0.03742* |

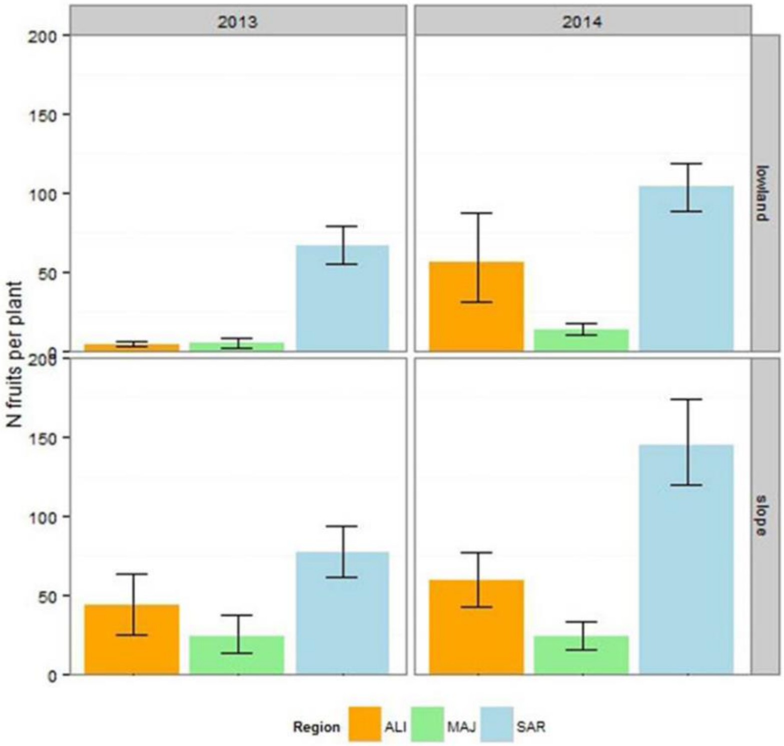


**Figure 1A – Average number of fruits per plant per region, divided for lowland areas (top row) versus slope areas (bottom row) for 2013 and 2014 (left and right columns, respectively), with error bars.**

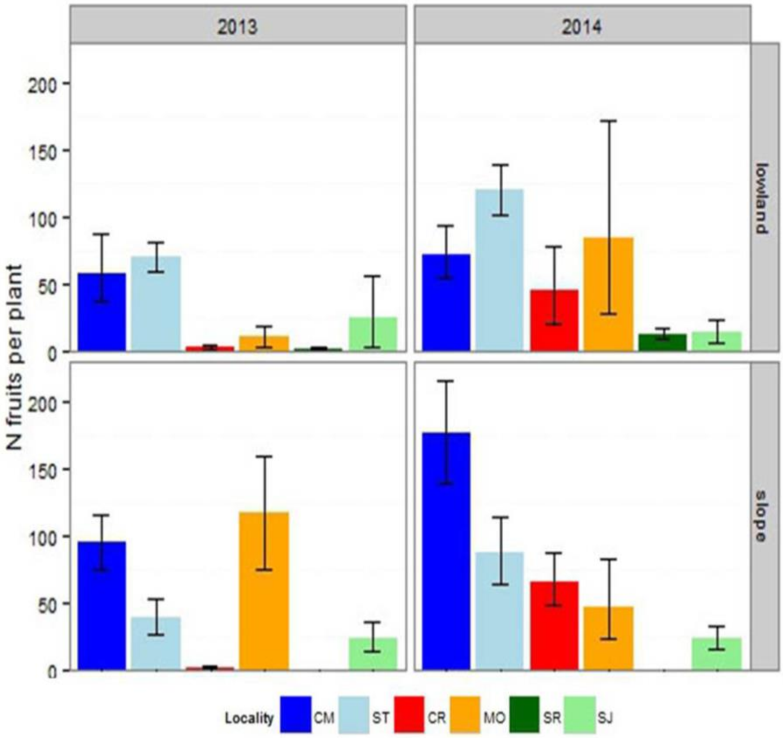


**Figure 2A – Average number of fruits per plant per locality, divided for lowland areas (top row) versus slope areas (bottom row) for 2013 and 2014 (left and right columns, respectively), with error bars.**

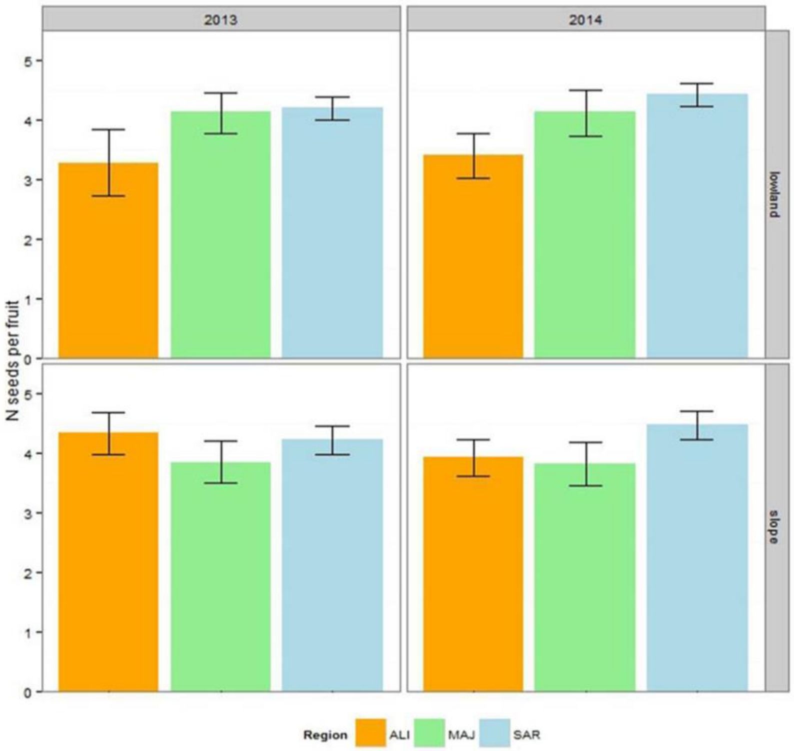


**Figure 3A – Average number of seeds per fruit per region, divided for lowland areas (top row) versus slope areas (bottom row) for 2013 and 2014 (left and right columns, respectively), with error bars.**

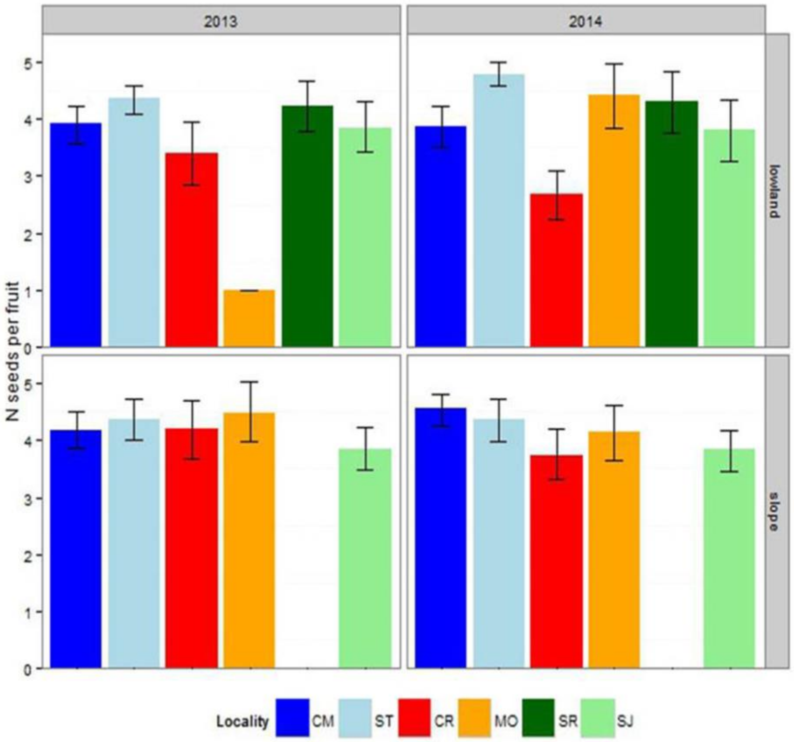


**Figure 4A – Average number of seeds per fruit per locality, divided for lowland areas (top row) versus slope areas (bottom row) for 2013 and 2014 (left and right columns, respectively), with error bars.**

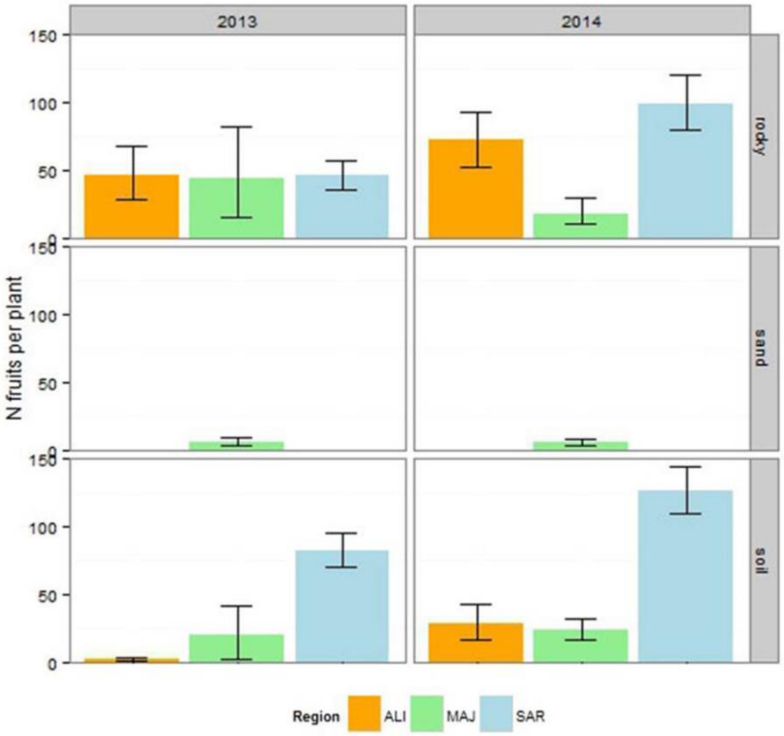


**Figure 5A – Average number of fruits per plant per region, divided for type of substrate: rocky (top row), sand (central row) and soil (bottom row), for 2013 and 2014 (left and right columns, respectively), with error bars.**

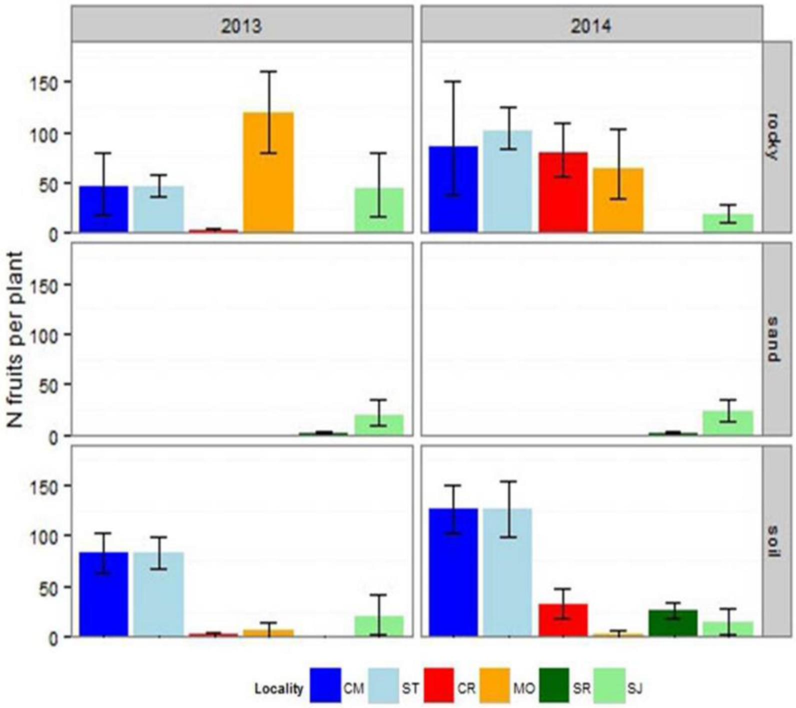


**Figure 6A – Average number of fruits per plant per locality, divided for type of substrate: rocky (top row), sand (central row) and soil (bottom row), for 2013 and 2014 (left and right columns, respectively), with error bars.**

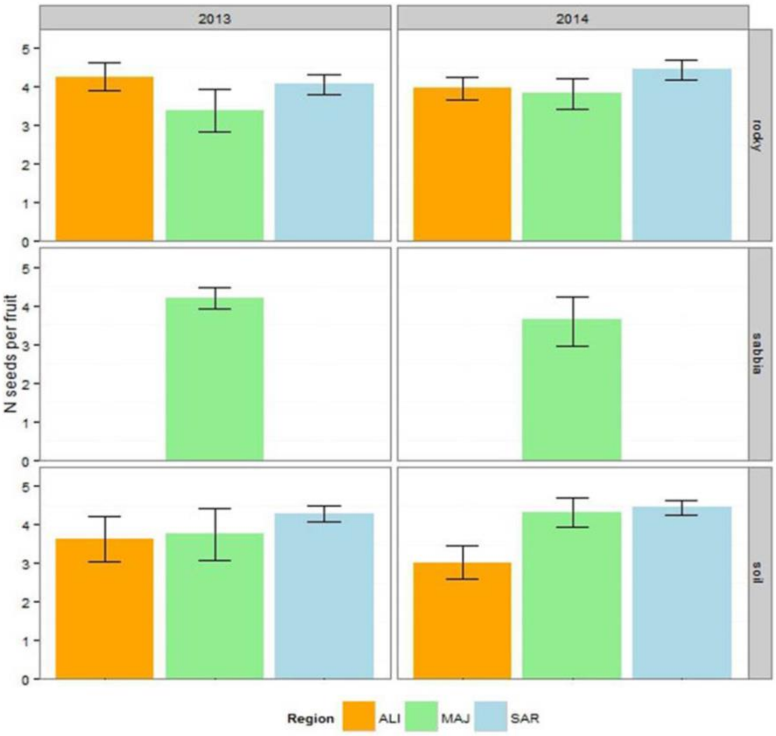


**Figure 7A – Average number of seeds per fruit per region, divided for type of substrate: rocky (top row), sand (central row) and soil (bottom row), for 2013 and 2014 (left and right columns, respectively), with error bars.**

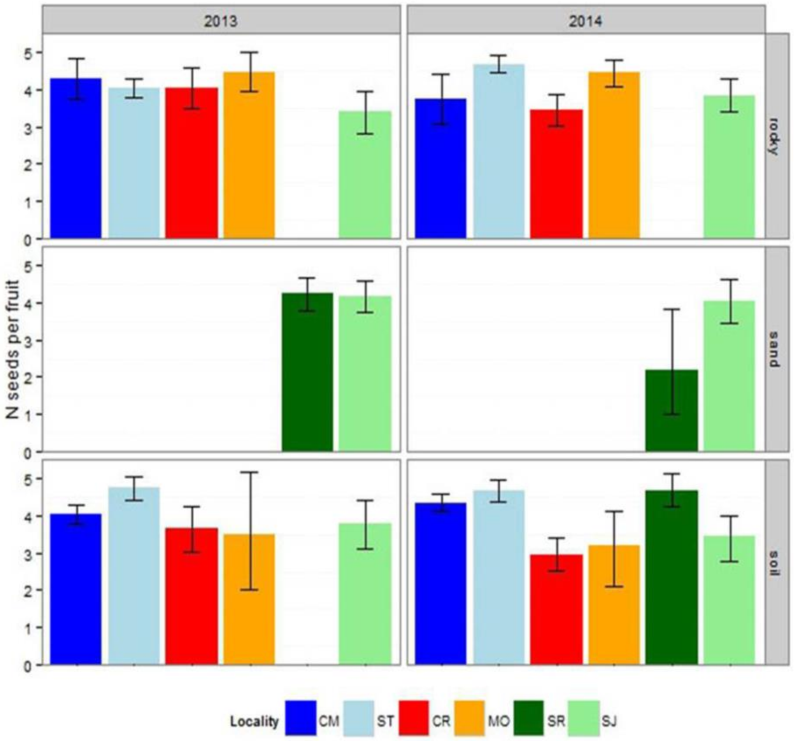


**Figure 8A – Average number of seeds per fruit per locality, divided for type of substrate: rocky (top row), sand (central row) and soil (bottom row), for 2013 and 2014 (left and right columns, respectively), with error bars.**

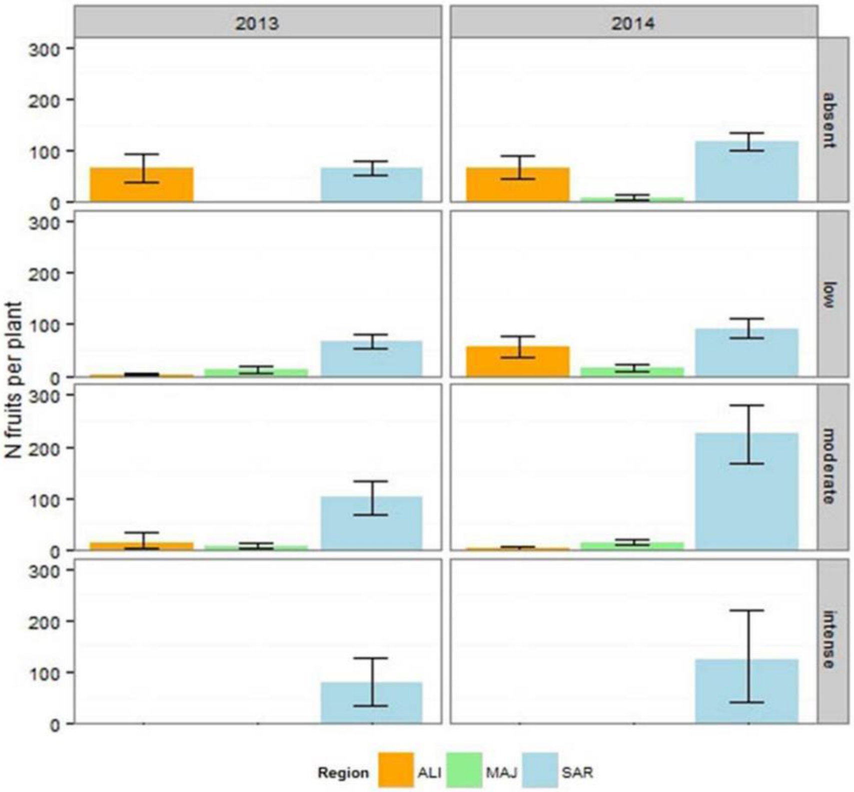


Figure 9A – Average number of fruits per plant per region, divided for trampling intensity: from absent (top row) to intense (bottom row), for 2013 and 2014 (left and right columns, respectively), with error bars.

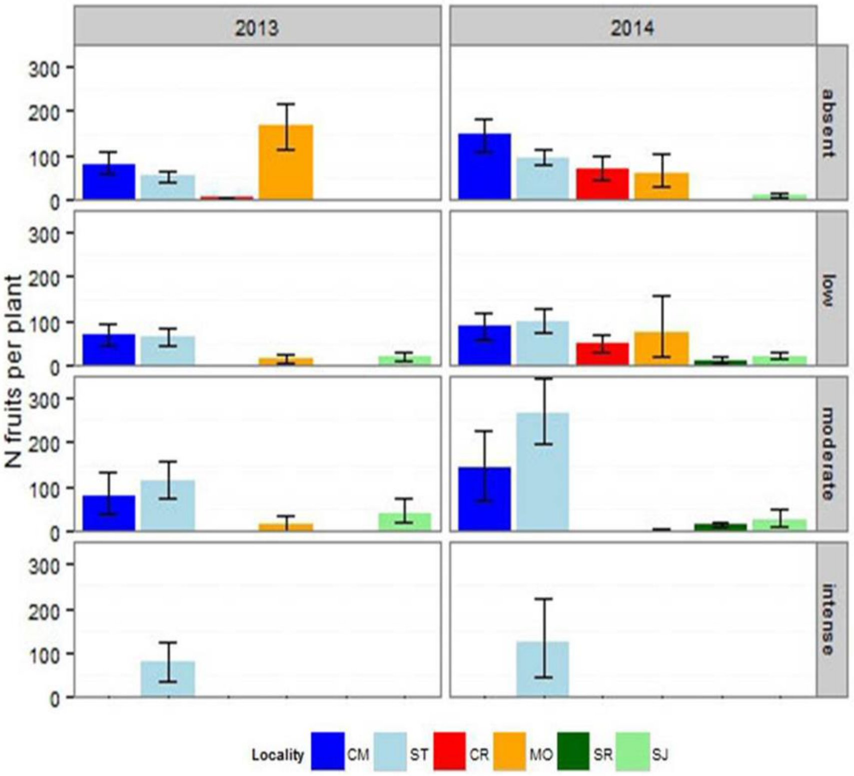


Figure 10A – Average number of fruits per plant per locality, divided for trampling intensity: from absent (top row) to intense (bottom row), for 2013 and 2014 (left and right columns, respectively), with error bars.

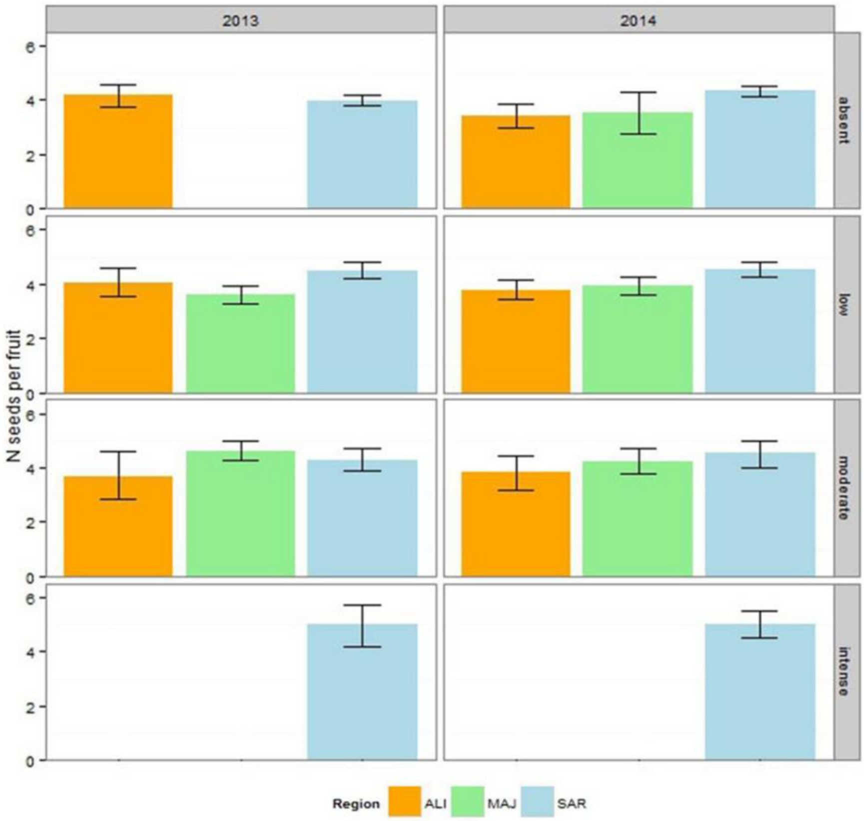


**Figure 11A – Average number of seeds per fruit per region, divided for trampling intensity: from absent (top row) to intense (bottom row), for 2013 and 2014 (left and right columns, respectively), with error bars.**

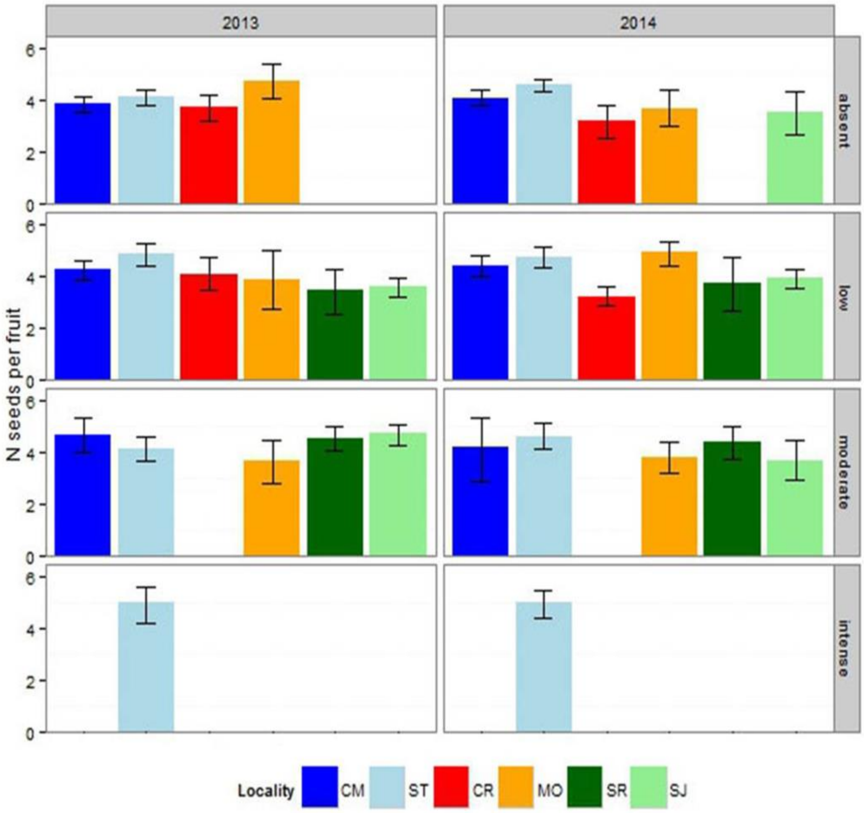


**Figure 12A – Average number of seeds per fruit per locality, divided for trampling intensity: from absent (top row) to intense (bottom row), for 2013 and 2014 (left and right columns, respectively), with error bars.**

# CHAPTER 3

# Evaluation of population dynamics of a perennial Mediterranean coastal plant by different methodological approaches

## Introduction

Population dynamics is the area of science which tries to explain in a simple mechanistic way the time variations of the size and structure of biological populations (Bacaer 2011). Determining whether a population is growing or declining is central to conservation biology, species' range dynamics, invasion biology and biogeography (Metcalf *et al.* 2013). In fact, during the past two decades, population ecologists have repeatedly argued for increased use of quantitative demographic analysis to guide management (Schemske *et al.* 1994; Morris *et al.* 2002; Bakker and Doak 2009; Crone *et al.* 2011); hence, biological demography has witnessed a rapid growth in theory and application (Steiner *et al.* 2012).

In order to manage the conservation of threatened plant species, the contribution of different life history components to the population growth rate must be clearly understood (Oostermeijer *et al.* 1996). Assessing which life history stages are the most critical to population growth is thus needed in order to target those parts of the life cycle when designing management actions (Schemske *et al.* 1994; Heywood and Iriondo 2003). Variation in population growth rates is due to differences in underlying vital rates, such as seedling recruitment, growth, reproduction and death (Buckley *et al.* 2010).

General patterns have been shown within species: good years or sites (i.e. those that have above average population growth rates) are associated with a higher contribution of sexual reproduction, whereas populations in poor years or sites rely more on survival (Oostermeijer *et al.* 1996; Menges and Dolan 1998; Valverde and Silvertown 1998; Jongejans and de Kroon 2005).

Sexual reproduction is crucial for long-term persistence of plant populations. Through sexual seed production, plants benefit from an independent dispersal phase, the opportunity to increase or maintain genetic diversity, and the potential to adapt to new environments (Wilcock and Neiland 2002). However, reproduction is not the only ecological process that determines the growth and persistence of plant populations (e.g. Jules and Rathcke 1999; Lennartsson 2002). Other stages in the life cycle of plants such as seed dispersal and germination or seedling survival and establishment are also important in affecting the demographic dynamics of plant populations (e.g. Santos and Telleria 1997; Benitez-Malvido 1998; Bruna 2003; Aguilar *et al.* 2006).

Population models are widely used in species management studies (Jongejans *et al.* 2008). By the 1990s, matrix population models (MPMs, hereafter; Caswell 1988, 2001) had become one of the most frequently used methods to translate vital rates into population-level outcomes (Caswell 2001; Morris and Doak 2002; Crone *et al.* 2011; Griffith *et al.* 2016). MPMs provided a powerful tool for population biologists to estimate parameters important to population persistence and dynamics by modelling commonly collected demographic data on stage and/or age transitions. These models may result in biases, however, where underlying state variables are continuous (Picard *et al.* 2010; Salguero-Gómez and Plotkin 2010), such as for example, height, weight, biomass (Metcalf *et al.* 2013). MPMs are a common tool in population biology to understand the factors influencing individuals that may contribute to overall population dynamics (Caswell 1989). These models quantify all ways (through survival and reproduction) in which individuals contribute to the size of the population after one time step. Transition matrices contain exactly the same information as life cycle graphs, but then organized in matrix form. Hence, matrix models represent the life cycle of individuals, and can be used to investigate the dynamics of a population (Jongejans and de Kroon 2012).

More recently, a new discrete-time structured method has been introduced: the integral projection model (IPM, hereafter; Easterling *et al.* 2000), which retains the desirable properties of the matrix projection model, while avoiding entirely the need to group plants into discrete stage classes. IPMs describe how a population structured by a continuous individual-level state variable changes in discrete time (Easterling *et al.* 2000). IPMs are defined by a kernel, which represents probability densities of growth between discrete or continuous stages conditional on survival, and the production of offspring (Metcalf *et al.* 2013). It offers tools that can incorporate stage, age and continuous states into similar analysis of population dynamics (Easterling *et al.* 2000; Ellner and Rees 2006).

Essentially, the same tools are available for IPMs as for discrete projection matrices (MPMs). The main difference between an IPM and a MPM is that while in discrete projection matrices the number of classes (i.e., number of stages in the life cycle of the study species) must be defined *a priori*, IPMs impose the discretization of the three-dimensional surface for the purposes of numerical integration. This produces a typically large matrix (e.g., 100 × 100 cells) that is more robust to biases from matrix dimensionality (e.g. Zuidema *et al.* 2010; Salguero-Gómez and Plotkin 2010) and sample size (e.g. Ramula *et al.* 2009) than classical matrix models (Metcalf *et al.* 2014).

Many demographic studies of plant populations have been conducted over the past few decades, including studies on various endangered plant species of international interest (e.g. García 2003; Oostermeijer *et al.* 2003; Pisanu *et al.* 2012; Schwartz *et al.* 2016).

In this study, both MPMs than IPMs are performed for *Helianthemum caput-felis* Boiss., a threatened plant distributed throughout the western Mediterranean Basin (south-eastern Iberian Peninsula, Majorca, Sardinia and northwest Africa) in several coastal fragmented populations (Fenu *et al.* 2015a and references therein). This species deserves particular attention because it is considered the only extant representative of an ancient lineage (Arrigoni 1971; López-González 1992). Moreover, *H. caput-felis* is listed in the Appendices of the Habitat Directive (92/43/EEC) and it is categorised as endangered on the European red list of vascular plants (Bilz *et al.* 2011). As other genera of Cistaceae family, *H. caput-felis* can accumulate long-lived persistent seed banks in the soil through the production of large yields of impermeable-hardcoated seeds (Ferrandis *et al.* 1999). A reserve of dormant seeds in the soil can stabilize population dynamics in response to short-term environmental fluctuations and disturbances (Harper 1977; Cavers 1983; Venable and Brown 1988; Warr *et al.* 1993).

Currently there are no models that assess the population traits of *H. caput-felis,* hence a detailed demographic analysis of this species' population over three years was performed. Populations in a stable environment can be expected to have an asymptotic growth rate near unity, and, in contrast, populations in unstable (abiotic or biotic) environments can be expected to have growth rates different from unity; thus, the aim of this study was to analyse the global population dynamics of *H. caput-felis* along its overall distribution range using MPM, and to verify the population growth rate's output by an IPM. The following questions were addressed: 1) Is the overall *H. caput-felis* population in decline? 2) What vital traits give a more important contribution in the population growth rate?

## Materials and methods

### *Study species and area*

*Helianthemum caput-felis* Boiss. is a half shrub that grows to a height of 35(50) cm. Its flowers are hermaphroditic, and have a short lifespan (3–4 days, Rodríguez-Pérez 2005). Based on studies carried out in Spanish and Sardinian populations, the flowering period is from March to June, and the fruiting season runs from late April to beginning of August (Fenu et al. 2012, 2015a; see Chapter 1 and 2). Fruits are capsules that detach at maturation, and seed germination takes place in autumn, at the onset of the rainy season (Rodríguez-Pérez 2005).

*Helianthemum caput-felis* is a long-lived iteroparous perennial plant, but the information about its exact lifespan is lacking. The life cycle of *H. caput-felis* include a continuous stage, in which growth and reproduce, combined with a discrete stage, with seeds that can enter a permanent soil seed bank or germinate the next spring after dispersion (Figure 1). As other genera of Cistaceae family, *H. caput-felis* can accumulate long-lived persistent seed banks in the soil through the production of large yields of impermeable-hardcoated seeds (Ferrandis *et al.* 1999).

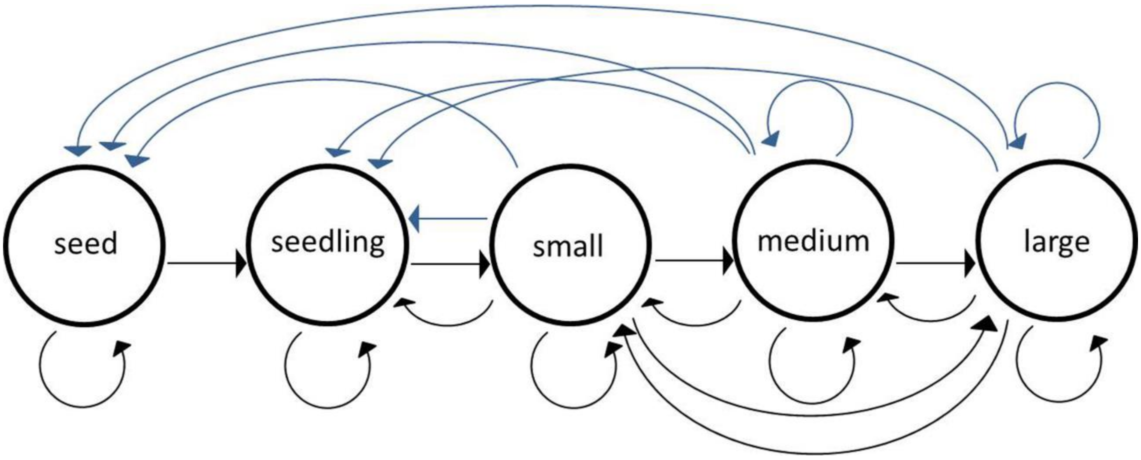


**Figure 1 – Life cycle diagram of *Helianthemum caput-felis*. The black arrows represent survival/growth transitions, while the blue arrows involve production (*via* seeds) of new individuals after one year.**

From an ecological point of view, *H. caput-felis* is a termophilous plant that preferably grows in coastal environments under the direct influence of the sea, mostly on calcareous rocky cliffs (0–200 m a.s.l.) with garrigues or scrublands; peculiar populations also grow on different habitats, such as sand dunes (Majorca), rocky slopes bordering inland ravines or, rarely, in open wooded areas (Fenu *et al.* 2015a and references therein).

### *Data collection*

Plant demographic data was collected in 98 permanent plots of 2 × 1 m, randomly established among the overall distribution range of the species (except NW Africa), which include all ecological situations in which the plant was found. Within these plots, 821 plants were marked, mapped and monitored during the study period. The probabilities of plants remaining in the same class or changing to a different class were calculated from censuses of marked plants in the demographic plots.

**Table 1 – Principal geographical and ecological traits of *Helianthemum caput-felis* populations investigated in this study: region, coordinates, altitudinal range (m), substrate type; in addition the number of plots and the number of plants within the monitored plots were reported.**

| Region | Population (code) | Coordinates U.T.M. (time zone) | Altitudinal range (min-max) | Substrate type | N. plots | N. plants |
|---|---|---|---|---|---|---|
| Alicante | Cabo Roig (CR) | 700388.62 m E,<br>4198390.09 m N<br>(30 S) | 3 - 15 | limestone | 15 | 116 |
| Alicante | Moraira (MO) | 250226.01 m E,<br>4285845.34 m N<br>(31 S) | 3 - 15 | limestone | 15 | 56 |
| Majorca | Sa Ràpita (SR) | 496897.00 m E,<br>4356780.00 m N<br>(31 S) | 0 - 5 | sand and limestone | 15 | 252 |
| Majorca | Colònia de Sant Jordi (SJ) | 500074.00 m E,<br>4352060.00 m N<br>(31 S) | 0 - 5 | sand and limestone | 13 | 65 |
| Sardinia | Capo Mannu (CM) | 447428.00 m E,<br>4432412.00 m N<br>(32 S) | 5 - 55 | limestone | 20 | 146 |
| Sardinia | Su Tingiosu (ST) | 449657.67 m E,<br>4428538.64 m N<br>(32 S) | 5 - 25 | limestone | 20 | 186 |

Over the study period (2013–2015), surveys took place at least three times a year, following Jacquemyn et al. (2010). In early March, all sites were visited a first time for locating all previously mapped individual and mapping new seedlings. During the flowering peak (April-May), when all plants are fully grown, plots were visited a second time to measure each plant (height, minimum and maximum diameter were taken, in order to estimate plant size), count the number of flowers per plant and check for new occasionally individuals, that were added in the data set. During the fruiting peak (latest May-early June) the last survey was made in all sites, to count the number of fruits per plant.

Data analysesIn order to investigate the stability of the *H. caput-felis* global population and to evaluate its population growth rate ($\lambda$), a stage-based MPM for all sampling plants was performed and compared with an IPM (Easterling *et al.* 2000; Ellner and Rees 2006). The modelled asymptotic growth $\lambda$ is an especially important population parameter in matrix or integral projection analysis. In a constant environment, $\lambda > 1$ indicates that the population will eventually increase whereas $\lambda < 1$ indicates that the population will decline to extinction (Ezard *et al.* 2010).

The chosen continuous state variable to evaluate the demographic dynamics of *H. caput-felis* populations was plant size (plant volume). In particular, this parameter was found to be the variable that best explained the vital rates of this species; plant volume (plant size = V, expressed in $cm^3$) was calculated according to the formula in Fenu *et al.* (2015a), in which individual height (h) and the maximum and minimum diameter ($d_M$ and $d_m$, respectively) were multiplied:

$$\text{Plant size } [cm^3]: V = [\pi \times (d_M/2) \times (d_m/2)] \times h$$

Then, according to Rees *et al.* (2014) sizes were log-transformed, because this transformation foot well the data.

As a first step, a matrix model based on the classification of individuals into life stages was constructed (Caswell 2001). Matrix models divide the populations into discrete classes and tracks the contribution of individuals in each class at one census to all classes in the following census (Morris and Doak 2002). This stage classification was devised to characterize the population using a biological approach (Lefkovitch 1965; Werner 1975; Marrero-Gómez *et al.* 2007), which relies on field observations of developmental states. The life cycle of *H. caput-felis* was classified into five developmental stages based on fields' observations. A seed bank class, a seedling class and three adult classes (small, medium and large) were distinguished, based on plant size. Considering that the plant size was positively correlated to the reproductive output (see Chapter 2), all plants were grouped in four classes considering the plant volume only. Hence, totally, five size classes were selected in order to build the matrices: (1) seeds, (2) plants with a volume <5 $cm^3$ (seedling, hereafter), (3) flowering and fruiting plants with a volume of 5.1– 500 $cm^3$ (small, hereafter), (4) adults plants with a volume of 500.1–5000 $cm^3$ (medium, hereafter), and (5) large adults plants (large, hereafter). The seed bank class was calculated by multiplying the proportion of total reproductive output attributable to an individual (the average number of seeds per fruit with the fruit number of each plant; data from Fenu *et al.* 2015a) times the total number of seedlings at the end of the projection interval (Stubben and Milligan 2007). The transitions between the five different life stages observed in the study population are shown in a life-cycle diagram, performed following Wardle (1998; Figure 1), which represent the mean projected matrix.

The basic matrix model is given by:

$$n_{t+1} = \boldsymbol{A} n_t$$

where $n_t$ and $n_{(t+1)}$ are vectors whose elements, $a_{ij}$ are the number of individuals that belong to the *i*th category at time $t$ and $t + 1$, respectively, and $\boldsymbol{A}$ is the non-negative square matrix, whose elements, $a_{ij}$ represent the transitions or contributions from individuals in the *j*th category to the *i*th category after one time step (Caswell 2001). Transition probabilities were obtained by calculating the proportion of individuals in each category experiencing each specific fate from one year to the next (Marrero-Gómez *et al.* 2007).

The survival rate is the expected proportions of plants in class $i$ at the last census that are still alive at the current census. The growth rate is the estimated probability that a surviving plant undergoes a transition from its original class to each of the other potential classes (Morris and Doak 2002).

A transition matrix for each year was generated, adding individual fertility estimates per plant. Then, the annual matrices were created setting the number of time steps for a deterministic model (it=100). A demographic projection matrix for each year was generated, which does a simple deterministic projection of the matrix for each year in order to extract the deterministic λ. To extract the mean deterministic λ of all years, a mean of the three projection matrices for deterministic analysis was calculated, making a random draw with replacement.

Long-term simulations of the fates of *H. caput-felis* population were carried out by incorporating environmental stochasticity into the matrix models. Environmental stochasticity involves chance variation in several external factors such as weather conditions which affects population performance (Pico and Riba 2002). The effect of initial population size on the long-term dynamics of the analysed population was tested in order to calculate the stochastic growth rate ($\lambda_S$, Tuljapurkar *et al.* 2003). The numeric values for the population size (40 seedlings, 186 small, 210 adults and 209 large adults plants) in the first year of monitoring (2013) were used as starting population vector ($n_t$).

The “popbio” package of Stubben and Milligan (2007) in R version 3.1.2 (R Core Team 2014) was used to calculate the finite rate of increase (λ) and the stochastic lambda ($\lambda_S$) of the population *via* Tuljapakar's method (Tuljapurkar 1990). This R package is based upon methods described in Caswell (2001) and Morris and Doak (2002). The bootstrap method (Kalisz and McPeek 1992; Caswell 2001) was used: each annual matrix was randomly resampled with replacement 50,000 times. Therefore, in each sample, the number of plants in each size class equalled the number in the corresponding class in the original data set; then, from the resulting distributions of 50,000 estimates for each matrix, the stochastic growth rate was extracted (the analytic approximation of λ and a percentile 95% confidence interval were computed; Morris and Doak 2005). Bootstrap distributions of population growth rates were calculated using the “boot.transitions” function of the aforementioned package.

The projection matrix itself allows to integrate the contributions of individuals in different states, and the effects of different vital rates, into an overall measure of population growth and viability (Morris and Doak 2002).

Sensitivity and elasticity matrices were calculated for every year transition interval. Sensitivities of a population projection matrix are the direct contributions of each transition to determining λ, while elasticities are sensitivities, weighted by the transition probabilities (Stevens 2009). Elasticity analysis determines the relative contribution of each parameter to the population growth rate, adjusted for the magnitude of each parameter (Adams *et al.* 2005). Also, elasticity represents an analytical tool for decomposing the global population growth rate into the contributions made by the life cycle transitions (de Kroon *et al.* 2000). Damping ratios, which measure the rate of convergence to a stable stage distribution (Caswell 2001) and it is determined by the ratio of the dominant eigenvalue to the second largest eigenvalue (Caswell 2001; Stubben and Milligan 2007), were calculated.

Subsequently, in order to evaluate the MPMs’ outputs, an IPM for each transition year was constructed.

IPMs are similar to MPMs, but differ because populations do not have to be divided into classes but state variables (size, in this case) can be continuous (Easterling *et al.* 2000). IPMs describe how a population structured by a continuous individual-level state variable changes in discrete time (Easterling *et al.* 2000). In the IPM, the state of the population is described by the size distribution *n(y, t).* IPM uses a continuous projection kernel in order to describe the population size distribution by a density function (Easterling *et al.* 2000; Ellner and Rees 2006). In this model, growth, survival and the probability of flowering are described in function of plant size. Constant, linear and quadratic models were fit, and the best fit was selected based on the lowest Akaike Information Criteria (AIC; Dauer and Jongejans 2013). As in the cell of the matrix models, plants in the population could survive, grow, and produce new individuals in each time step (Easterling *et al.* 2000). The IPM of a size structured population is given by:

$$n(y, t+1) \; = \int_{L}^{U} K(y,x) n(x,t) \mathrm{d}x \; = \int_{L}^{U} [P(y,x) + F(y,x)] \, n(x,t) \mathrm{d}x$$

where *n(y, t* + 1) is the size distribution *y* of both established and newly recruited plants in census time *t* + 1, *n(x, t)* the distribution across size of individuals at census time *t*, *L* and *U* are the respective lower and upper size limits modelled in the IPM (Metcalf *et al.*

2013), and these values were set lower and higher than the observed minimum and maximum sizes to avoid unintentional evictions (Williams *et al.* 2012). The kernel (*K*) can be broken down into two sub-kernels (*P* and *F*): the *P* sub-kernel represents transitions attributable to survival and growth, while the *F* sub-kernel describes *per capita* contributions of reproductive individuals given the recruit density function at the next census (Metcalf *et al.* 2013).

The method proposed by Salguero-Gómez (2014) for IPMs for complex life cycles was followed and adapted to the *H. caput-felis*. The survival probability was modelled by logistic regression (binomial error distribution and logit link function), while the growth probability was modelled as a linear regression (Merow *et al.* 2014). Fecundity was the product of two vital rates: the probability of flowering, modelled as a logistic regression (by specifying binomial error distributions and logit link functions in GLM), and the number of fruits in the peak of the year *t* for every monitored plant, modelled as a linear regression (with a Poisson error distribution and a log link function), times the mean number of seedlings in year *t* + 1 per number of fruiting individuals in year *t*, and a probability function of the seedling size distributions. The seedling size distribution in each population and each year was described by a normal distribution with the observed mean and standard deviation.

Survival, growth and fecundity objects that compose the IPM were constructed using the version 2.1 of the “IPMpack” package (Metcalf *et al.* 2014), and matrix were plots with the “fields” package (Nychka *et al.* 2014) in R version 3.1.2 (R Core Team 2014).

Furthermore, other vital rates were not measured for every individual but included in the IPM as constants (i.e. size independent): the mean number of seeds per fruit, the probability of germination and the probability of seedling survival within the year of seed production, the probabilities of seeds entering the seed bank or staying there. These constants form the fecundity object and a discrete stage describes the seed bank. Mean seeds per fruit was calculated by collecting randomly 980 mature fruits from 98 randomly selected plants (one per plot). According to Bruna *et al.* (2014), the theoretical amount of seeds per year was estimated from the multiplication of the total number of fruits (excluding the percentage of empty/aborted fruits) and the mean seeds per fruit. This was also the case of seed germination rate, which has been analysed in laboratory at locality level (Tébar *et al.* 1997), but not documented in the field. In light of this, it was estimated by the proportion of seeds becoming seedlings by counting the number of newly established seedlings during the surveys, dividing this number by the number of seeds produced by all monitored plants in the previous reproductive season (Bruna et al. 2014). This estimation reflects a general low germination probability, as reported in Tébar *et al.* (1997) for the Majorcan population (4%). Seedling survival was estimated from the ratio between seedlings counted in year *t* and seedlings present in *t*+1. Due to the absence of seed bank’s studies regarding this plant species, data about seeds entering and staying in the seed bank were calculated according to Quintana-Ascencio *et al.* (1998). All these estimated data had to be incorporated into fecundity parameter calculation.

The transition of each plant among the continuous and the discrete stage was added in the original database. In the case of *H. caput-felis*, these transitions are:

- continuous stage → discrete stage: individuals with a given volume in year *t* contribute seeds to the seed bank, that is, seeds that were produced, did not germinate, and remain viable;
- discrete stage → discrete stage: prolonged dormancy and survival of seeds in the seed bank;
- discrete stage → continuous stage: germination of seeds from the seed bank to become seedlings of a given above ground volume.

The IPM predicts the population’s asymptotic growth rate (λ), represented by the dominant eigenvalue, with associated eigenvectors and state-dependent sensitivity and elasticity functions (Easterling *et al.* 2000). Dominant right and left eigenvectors *w(x)* and *v(x)* give the stable size distribution and size-specific reproductive value, respectively (Easterling *et al.* 2000). The stable size distribution was extracted and compared with the observed ones, and damping ratios were calculated, following the same procedure in MPM.

The deterministic lambda (λ) was also calculated excluding the seed bank stage ($\lambda_{continuous}$), in order to analyse possible differences with λ.

Moreover, having constructed the IPM kernel, it is then straightforward to calculate other statistics, as the net reproductive rate ($R_0$) and the generation time (T). The net reproductive rate ($R_0$) represents the number of offspring produced on average by an individual over its lifespan (Metcalf *et al.* 2014), or, in other words, the measure of mean lifetime reproductive output, so it represents the global population growth rate per generation (not per unit of time); it is an indicator function for population growth, in which population growth is positive if, and only if, $R_0>1$ (Caswell 2011).

Similarly to the MPMs, sensitivity described the change in λ resulting from a change in demographic parameters (survival or fecundity) affecting only individuals at a particular size (Easterling *et al.* 2000). Elasticity provided details on the size range having the greatest effect on λ (Dauer and Jongejans 2013), and then elasticity values were calculated by dividing the relative increase in λ by the relative increase of the vital rate function (de Kroon *et al.* 2000). Sensitivity and elasticity kernel were calculated from IPMs of both years’ transitions. Moreover, elasticities could be partitioned into contributions from the survival-growth and reproduction components of the kernel (*P* and *F*; Ellner and Rees 2006), and then the percentage of each vital rate contribution were calculated.

The stochastic rate of increase ($\lambda_S$) was also calculated as the geometric mean proportional change in population size over 50,000 model iterations, where one yearly projection kernel was randomly drawn for each iteration.

Knowing λ, it was possible to calculate the generation time (T), which is the time required for the population to increase by a factor of $R_0$ (Coale 1972; Koons *et al.* 2005; Caswell 2001; Williams *et al.* 2011):

$$T = \frac{\log(R_0)}{\log(\lambda)}$$

Generation time measures the typical age at which offspring are produced, and it was calculated both from MPMs then from IPMs. Moreover, from IPM kernel, mean life expectancy or the predicted mean lifespan of an individual of size *z* at time 0 was extracted.

*Life table response experiment analysis (LTRE)*

Life table response experiments (LTREs) are studies that quantify the population-level effect of environmental factors by measuring a complete set of vital rates (a life table) under several conditions (treatments). The terms “experiment” and “treatment” are used loosely to include not only manipulative experiments but also comparative observations under natural conditions (Caswell 1996). A one-way LTRE analysis was performed in order to elucidate the demographic mechanisms underlying differences among years by decomposing differences in λ into the contributions from different demographic variables (Caswell 1989). It was only performed from IPMs, because for large datasets IPMs perform better the data (Ramula *et al.* 2009).

For each year of monitoring the $\mathrm{IPM}_{2013\text{-}2014}$ was arbitrarily defined as the “control”, because the interest of this analysis is to compare the differences in λ from the $\mathrm{IPM}_{2014\text{-}2015}$ in comparison to the IPM “control”, and to know what vital rates change between years. Then, the arithmetic mean of the two IPMs was calculated as a mid-way IPM, which permitted to evaluate the differences in the sensitivities of the IPM kernels to be compared. Finally, the IPM of the differences between the IPM kernels corresponding to the 2013-2014 and 2014-2015 transitions were calculated, and then the parts of the life cycle of the study species that were responsible for the difference in λ between the two transition periods were inspected, by weighting the IPM of the difference between those two IPMs by the sensitivity of the arithmetic mean IPM. LTRE was verified by a cross-check as recommended by Salguero-Gómez (2014).

To interpret the results, kernels’ plots were made with the “image” function of the library “fields” (Nychka *et al.* 2015) of “IPMpack” version 2.1 (Metcalf *et al.* 2014) in R version 3.1.2 (R Core Team 2014).

## Results

The principal outputs of MPMs and IPMs were summarised in Table 2.

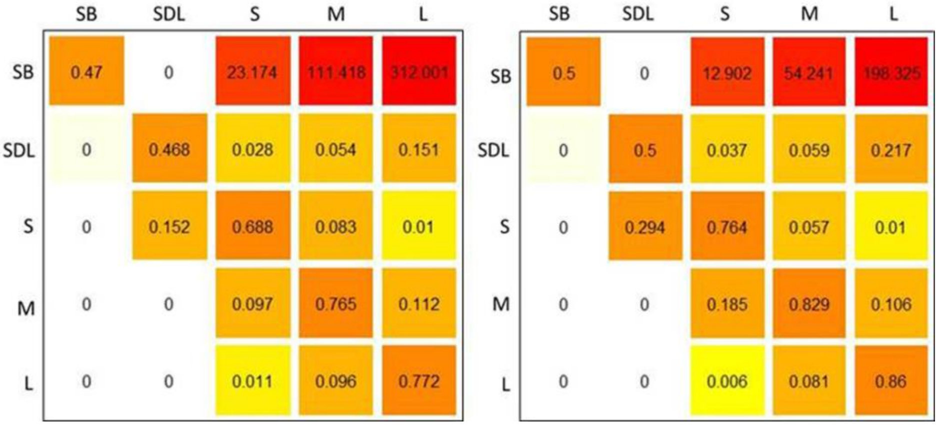
Deterministic population growth rate (λ) extracted from MPMs was higher in the first transition year (1.03) compared to the second (0.92; Table 2). Growth rates calculated considering only the continuous stage (excluding the discrete seed bank stage; $\lambda_{\mathrm{continuous}}$) were slightly lesser from those including the seed bank (λ; Table 2). Stochastic growth rate ($\lambda_S$) did not differ from the deterministic rate of the mean matrix ($\lambda_M$; Table 2).

The two projected matrices (Figure 2) show how large adult plants contribute more than others to fecundity, while the proportion of plants along the diagonal is greater than others.

The mean projected transition matrix was displayed in Figure 3, represented as a life cycle diagram, which represent the mean transitions between the five different life stages observed in the study population.

**Table 2 – Parameters extracted or calculated from matrix population models (MPM) and integral projection models (IPM) of *H. caput-felis* for the two years transitions: projected deterministic population growth rate excluding the seed bank ($\lambda_{continuous}$), projected deterministic population growth rate ($\lambda$) with confidence intervals at 95%, damping ratio ($d_r$), net reproductive rate ($R_0$), generation time (T), deterministic population growth rate of the mean projected matrix ($\lambda_M$), stochastic population growth rate ($\lambda_S$).**

| | MPM | | IPM | |
|---|---|---|---|---|
| | 2013–2014 | 2014–2015 | 2013–2014 | 2014–2015 |
| $\lambda_{continuous}$ | 1.01 | 0.81 | 1.02 | 0.92 |
| $\lambda$ | 1.03 [1.002197 - 1.054032] | 0.92 [0.8914003 - 0.9508634] | 1.08 [1.066577 - 1.106768] | 0.92 [0.9181867 - 0.9258905] |
| $d_r$ | 1.39 | 1.38 | 1.35 | 1.30 |
| $R_0$ | 1.50 | 0.10 | 7.12 | 0.26 |
| T | 32.44 | 24.43 | 14.61 | 17.33 |
| $\lambda_M$ | 0.98 | | 1.01 | |
| $\lambda_S$ | 0.97 [0.9728896 - 0.9755021] | | 1.00 [-] | |



| | SB | SDL | S | M | L |
|---|---|---|---|---|---|
| SB | 0.47 | 0 | 23.174 | 111.418 | 312.001 |
| SDL | 0 | 0.468 | 0.028 | 0.054 | 0.151 |
| S | 0 | 0.152 | 0.688 | 0.083 | 0.01 |
| M | 0 | 0 | 0.097 | 0.765 | 0.112 |
| L | 0 | 0 | 0.011 | 0.096 | 0.772 |

| | SB | SDL | S | M | L |
|---|---|---|---|---|---|
| SB | 0.5 | 0 | 12.902 | 54.241 | 198.325 |
| SDL | 0 | 0.5 | 0.037 | 0.059 | 0.217 |
| S | 0 | 0.294 | 0.764 | 0.057 | 0.01 |
| M | 0 | 0 | 0.185 | 0.829 | 0.106 |
| L | 0 | 0 | 0.006 | 0.081 | 0.86 |

**Figure 2 – Estimated stage class transition matrices (MPMs) of *H. caput-felis* population. On the left, the first year's transition (2013-2014), on the right, the second year's transition (2014-2015). SB: seed bank stage, SDL: seedling, S: small adult, M: medium adult, L: large adult plants.**

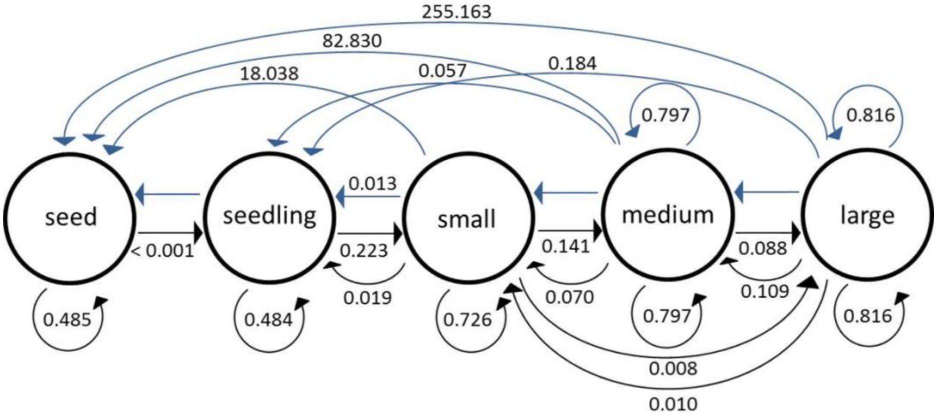
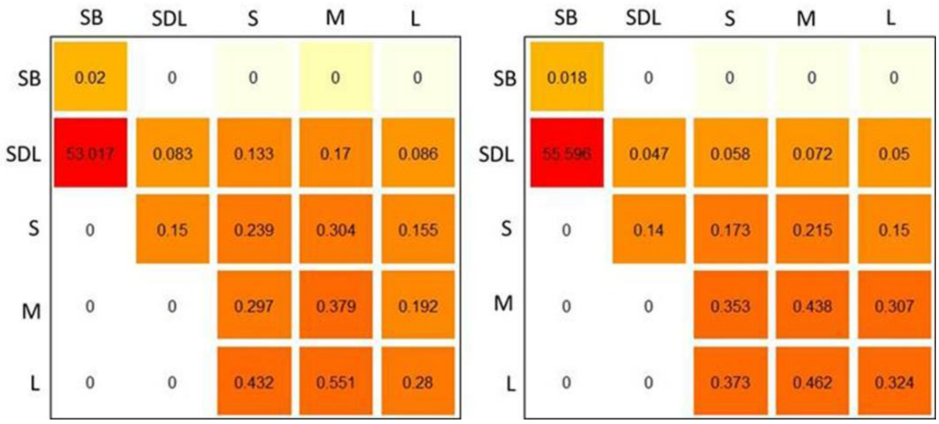


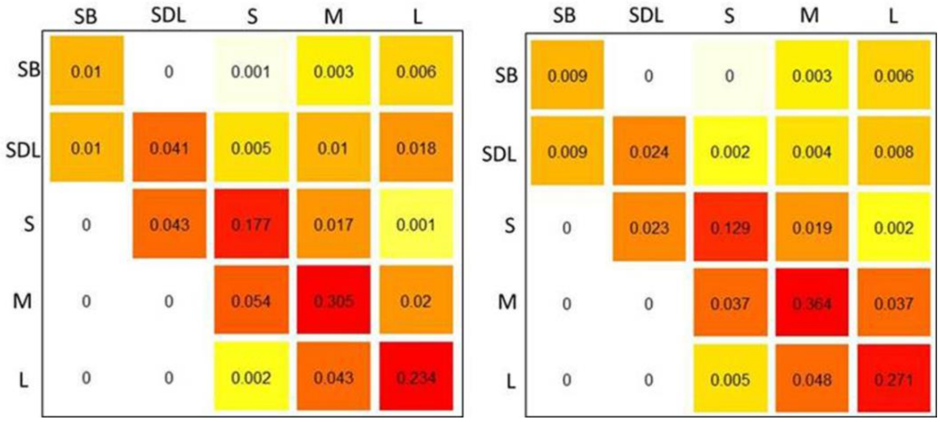
**Figure 3 – Loop analysis of *H. caput-felis*' life cycle. Values correspond to *P* and *F* values of the mean projected matrix. The black arrows represent survival/growth and the accompanying rates give the proportion of individuals that survive and move to a particular class. The blue arrows involve production (*via* seeds) of new individuals after one year. The projected λ of the mean matrix is 0.98, projecting a decrease in population size of 2% per year.**



| | SB | SDL | S | M | L |
|---|---|---|---|---|---|
| SB | 0.02 | 0 | 0 | 0 | 0 |
| SDL | 53.017 | 0.083 | 0.133 | 0.17 | 0.086 |
| S | 0 | 0.15 | 0.239 | 0.304 | 0.155 |
| M | 0 | 0 | 0.297 | 0.379 | 0.192 |
| L | 0 | 0 | 0.432 | 0.551 | 0.28 |

| | SB | SDL | S | M | L |
|---|---|---|---|---|---|
| SB | 0.018 | 0 | 0 | 0 | 0 |
| SDL | 55.596 | 0.047 | 0.058 | 0.072 | 0.05 |
| S | 0 | 0.14 | 0.173 | 0.215 | 0.15 |
| M | 0 | 0 | 0.353 | 0.438 | 0.307 |
| L | 0 | 0 | 0.373 | 0.462 | 0.324 |

**Figure 4 – Sensitivity matrices for the deterministic stage class matrix model of *H. caput-felis* population. On the left, the first year's transition (2013-2014), on the right, the second year's transition (2014-2015). SB: seed bank stage, SDL: seedling, S: small adult, M: medium adult, L: large adult plants.**



| | SB | SDL | S | M | L |
|---|---|---|---|---|---|
| SB | 0.01 | 0 | 0.001 | 0.003 | 0.006 |
| SDL | 0.01 | 0.041 | 0.005 | 0.01 | 0.018 |
| S | 0 | 0.043 | 0.177 | 0.017 | 0.001 |
| M | 0 | 0 | 0.054 | 0.305 | 0.02 |
| L | 0 | 0 | 0.002 | 0.043 | 0.234 |

| | SB | SDL | S | M | L |
|---|---|---|---|---|---|
| SB | 0.009 | 0 | 0 | 0.003 | 0.006 |
| SDL | 0.009 | 0.024 | 0.002 | 0.004 | 0.008 |
| S | 0 | 0.023 | 0.129 | 0.019 | 0.002 |
| M | 0 | 0 | 0.037 | 0.364 | 0.037 |
| L | 0 | 0 | 0.005 | 0.048 | 0.271 |

**Figure 5 – Elasticity matrices for the deterministic stage class matrix model of *H. caput-felis* population. On the left, the first year's transition (2013-2014), on the right, the second year's transition (2014-2015). Values represent the proportional change in λ with a proportional change in each parameter, and are scaled so that elasticities sum to one. SB: seed bank stage, SDL: seedling, S: small adult, M: medium adult, L: large adult plants.**

The sensitivity and elasticity matrices were showed in Figure 4 and 5. The sensitivity analysis indicated that the highest absolute contribution to deterministic population growth rate came from seeds emerging from the seed bank to germinate and become seedlings (Figure 4). On the contrary, the highest elasticity values came from medium adults plants (31% in 2013-2014 and 36% in 2014-2015), followed by adults individuals in stasis (that is, which does not change from one class to another, either growing or shrinking), contributed more than others to elasticity (Figure 5).

The stable size distribution extracted from the projected matrices confirmed the highest values in the seed bank in both years' transitions. However, the damping ratios show a slightly higher value in the first year transition than in the second (1.39 and 1.38, respectively). Therefore, there was a slightly difference between the stable size distribution and the predicted from the MPM models.

The net reproductive rate ($R_0$) was > 1 in 2013-2014 and < 1 in 2014-2015. The mean generation time (T) from MPM was 26.63 years.

In the IPM analyses, vital rates models show that plants volume is correlated with growth, survival, probability of flowering and fruits output (Figure 6). Survival rate increased in correspondence of large adult plants (Figure 6). The size of individuals that survive until the next year (growth) was linear dependent from size in year *t* and show that smaller plants were commonly more likely to grow than larger individuals, which tend to shrink (Figure 6). The probability that plants flower at the first census is strongly related to their size, and plants larger than ca. 400 $cm^3$ (corresponding to log(6) in Figure 6), have an high flowering probability. Among flowered plants, the number of fruits was exponentially dependent on plant’s size, and larger plants produced much more fruits than the smaller ones (Figure 6).

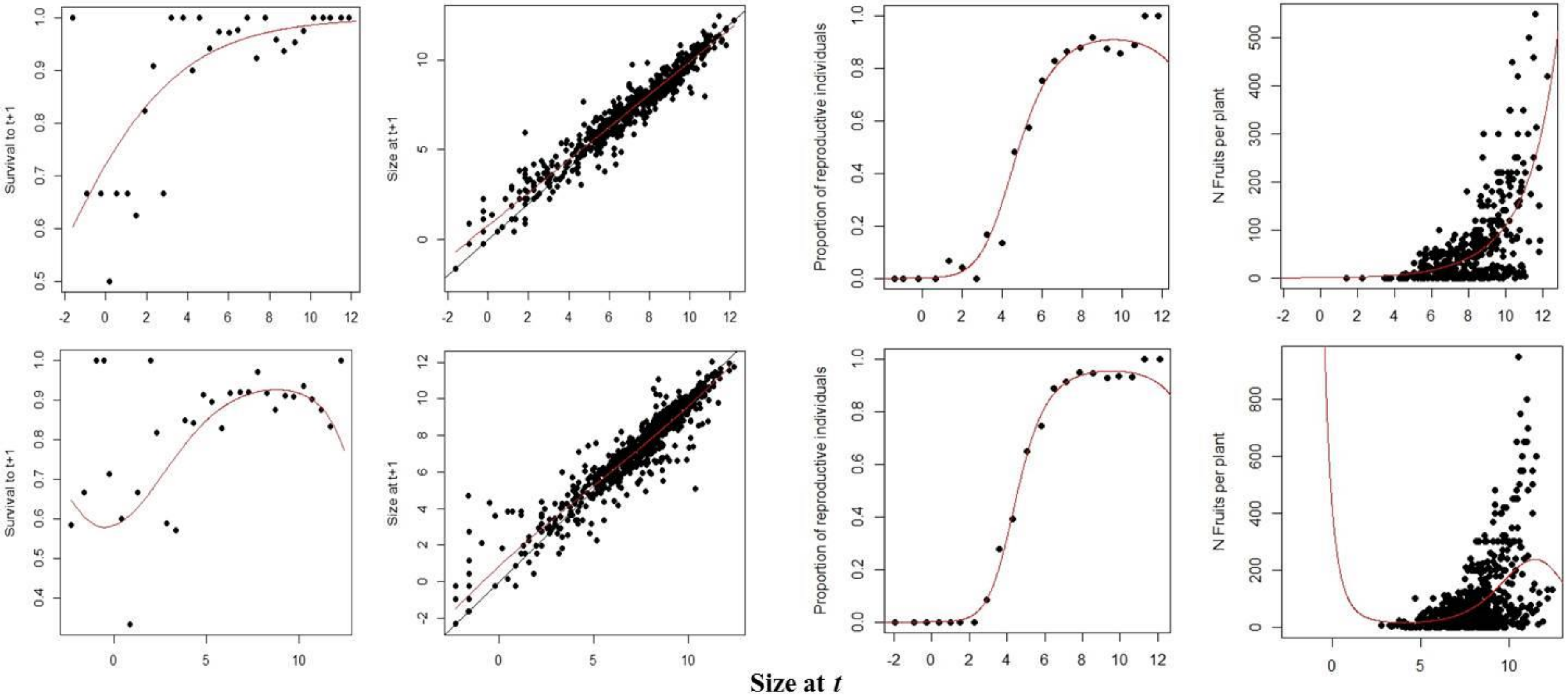


**Figure 6 – Size-dependent survivorship, growth, flowering probability and fruit production of plants during initial (*t*) and subsequent (*t* + 1) surveys. The top line referred to 2013-2014 transition, while the bottom line to 2014-2015 transition. Plant size is showed as log(plant size).**

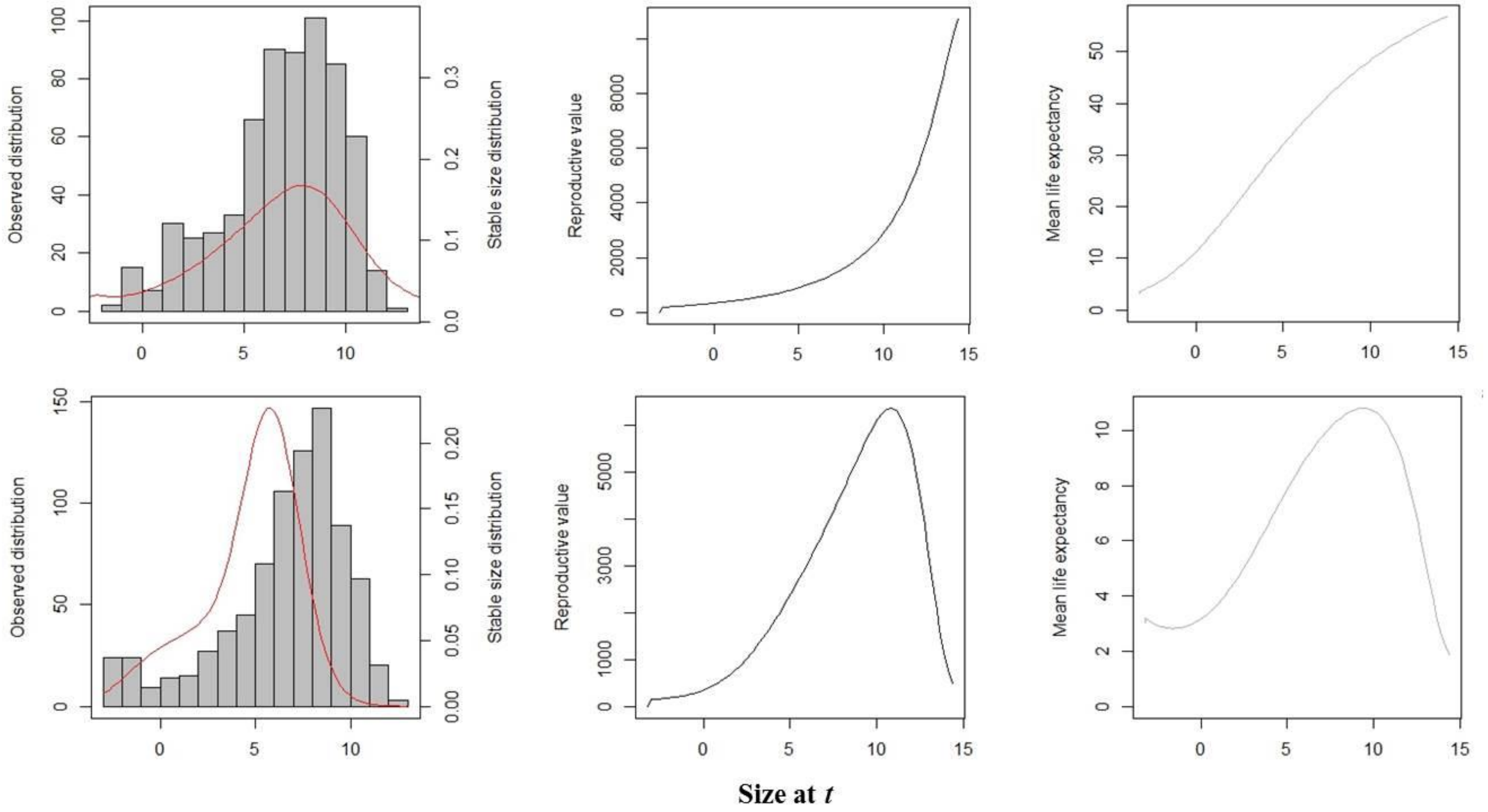


**Figure 7 – Observed versus predicted stable size distribution, reproductive value and mean life expectancy of plants during initial (*t*) and subsequent (*t* + 1) surveys. The top line referred to 2013-2014 transition, while the bottom line to 2014-2015 transition. Plant size is showed as log(plant size).**

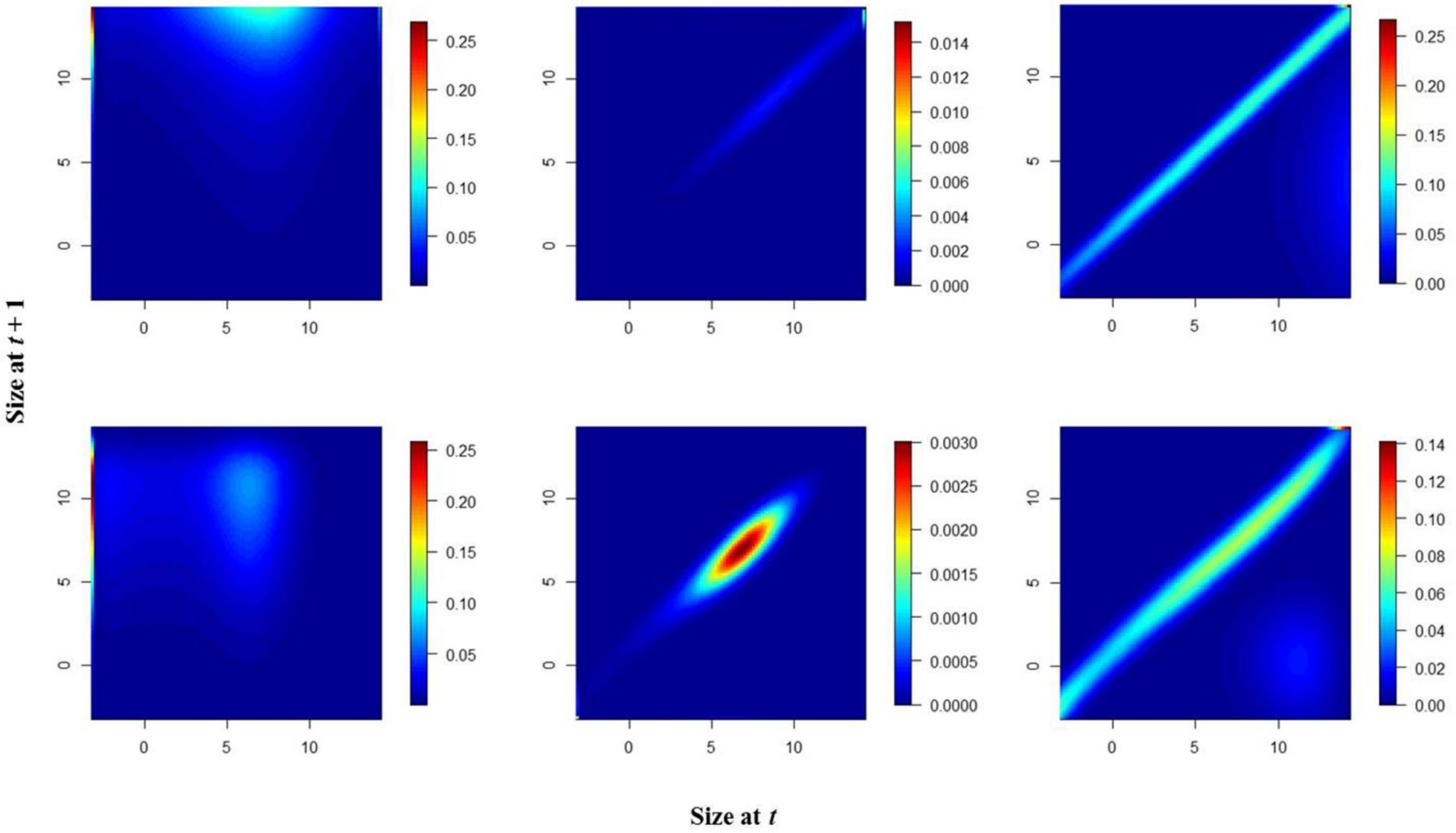


**Figure 8 – Sensitivity, elasticity and IPM kernels of plants during initial (*t*) and subsequent (*t* + 1) surveys. The top line referred to 2013-2014 transition, while the bottom line to 2014-2015 transition. Plant size is showed as log(plant size).**

The predicted stable size distribution (Figure 7) was skewed heavily towards large adults' plants. This indicates that, under deterministic conditions, *H. caput-felis* populationcould include larger numbers of plants with a volume of ca. 400 $cm^3$ and slightly differed from the observed distribution ($d_{r\ 2013\text{-}2014}$ = 1.35 and 1.37, $d_{r\ 2014\text{-}2015}$ = 1.30 and 1.33 for IPM and MPM, respectively; Table 2).

The reproductive value increased with plants' volume, reflecting the fact that larger plants had higher fecundity, survival and larger offsprings, until a maximum of ca. 162,800 $cm^3$ in 2014-2015, after which decrease (Figure 7).

In 2013-2014 mean life expectancy  increased with plant size, while in 2014-2015 the overall highest mean life expectancy (11 years) was found in medium-large plants, with a size of ca. 22,000 $cm^3$ (corresponding to log(10); Figure 7).

Sensitivity kernels showed the highest values in the first column, corresponding to the seeds emerging from the seed bank (Figure 8). Elasticity values were high along the diagonal representing medium-large size individuals, indicating that its contribution to λ is dominated by stasis (Figure 8). The breakdown of IPMs elasticity in his two components revealed that survival/growth component was generally higher than fecundity component in both year transitions (*P* component: 84% and 97% in 2013-2014 and 2014-2015, respectively), even if in 2013-2014 fecundity was higher than 2014-2015 (*F* component: 16% and 3%).

Moreover, the kernel's plots showed the importance of medium-large size individuals (high values near the diagonal central area), indeed were dominated by the survival function, representing individuals who survived the next year without changing much in size (stasis; Figure 8). The slight evidence in the bottom-right region represented the fecundity portion of the kernel. In 2013-2014 the peak was for plants with ca. 1,203,000 $cm^3$ in size (corresponding to log(14) in Figure 8), In the following transition years, the fecundity peak was ca. 59,900 $cm^3$ (corresponding to log(11) in Figure 8), that indicated that individuals of that size contribute most to reproduction. Furthermore, the absence of evidence in the bottom left area, that represent new-borns entering in the small size class, mean that only few seedlings were produced and few of these could survive to the next year; hence there was a high mortality of smaller plants. Offsprings measured 51.78 ± 81.28 $cm^3$ in 2014 and 1.45 ± 11.25 $cm^3$ in 2015.

Note that the kernels don't show the discrete stage (seed bank) for display reasons, but it was included in all the analyses.

The rate of population increase over a generation ($R_0$) extracted from the IPM was higher than 1 in 2013-2014 and less than 1 in 2014-2015. Mean generation time (T) was 15.97 years.

### *Life table response experiment analysis (LTRE)*

Sensitivity of the arithmetic mean IPM kernel included seed bank dynamics; its plots showed the importance of the emergence of seeds from the seed bank to λ (Figure 9a). The sensitivity of the mean IPM to changes in the emergence of seeds from the seed bank was high, meaning that there was a high impact of new-borns in the population growth rate. Nevertheless, the colour graduation of new seeds into the seed bank denoted a low impact on λ. In fact, λ calculated considering only the continuous stage ($\lambda_{continuous}$) does not change from λ including the seed bank dynamics (λ; Table 2).

The seed bank dynamics were then cancelled in the kernel of differences (Figure 9 b and c), and their contribution to the difference between $\lambda_{2013\text{-}2014}$ and $\lambda_{2014\text{-}2015}$ were null; those kernels showed only the continuous stage. Through those plots it was possible to see the differences between the kernels of the two years transitions.

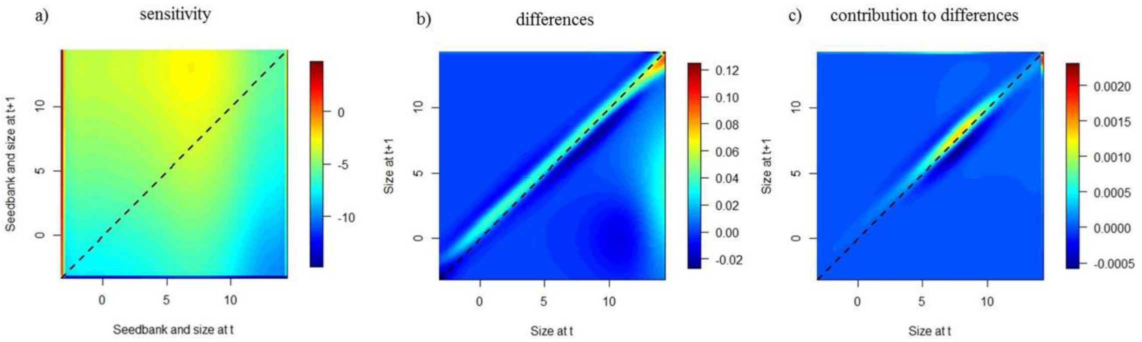


**Figure 9 – Life table response experiment analysis (LTRE). a) sensitivity of the mid-way IPM, including the seed bank stage; b) kernel of differences between the IPMs of the two years transition; c) kernel of the contributions to differences in expression (λ) of plants during initial (*t*) and subsequent (*t* + 1) surveys. Plant size was showed as log(plant size).**

The plot in Figure 9b showed a general difference both in survival/growth than in fecundity. In particular, a greater shrinkage of individuals in the second year of study was displayed, involving both larger and small plants.

Furthermore, a difference in fecundity between years was observed, with a reduction of its impact in 2014-2015, involving individuals of smaller size, respect to the previous year transition. The shrinkage of individuals in the second year of study and the minor fecundity rate could be considered the main responsible of lowest λ.

The contributions to differences in λ (Figure 9c) showed how part of the life cycle of the study species were responsible for the differences in λ between 2013-2014 and 2014-2015. In the latter, the stasis of plants involved a bigger range of size (in other words, there was more variability), and plants of medium-large size tended to shrink (Figure 9c).

## Discussion

Demographic studies of Mediterranean coastal plants are still lacking and only few researchs were performed on threatened plants (e.g. Pisanu *et al.* 2012; Cursach *et al.* 2013). This analysis represents the first demographic investigation for *H. caput-felis*, including plants sampled along a representative part of its Mediterranean distribution. The population showed a general stability in the first year transition, after which a decline from the second to the third study year (2014-2015) was observed; hence, in this study, a "good year" was followed by a "bad year". This trend was also confirmed by the net reproductive rate, which indicated that the rate of population increase over a generation was higher in the first year transition than the second.

Plants survival increased positively with plant volume, and growth of smaller plants was greater than those in larger ones, which tended to shrink, probably because larger individuals typically acquire more resources than smaller ones, which means they have more energy available to spend on growth, reproduction and maintenance (Rees *et al.* 2014). Moreover, fruits production was exponentially dependent on plant's size, meaning that larger plants produced much more fruits than smaller ones, as stated in previous phenological studies for *H. caput-felis* (Fenu *et al.* 2015a; Chapter 2).

The predicted stable size distributions indicated that, under deterministic conditions, *H. caput-felis* population could include larger numbers of individuals with a large volume. In fact, large individuals are the most important for the population growth rate, as showed in the elasticity matrices and kernels. Furthermore, it must be considered that it takes some years with transient dynamics before asymptotic dynamics are reached. In fact, the damping ratio value, which did not differ between IPMs and MPMs, underlined that the dominant stable stage distribution will be reached fairly soon.

Sensitivity and elasticity patterns did not substantially change between the two models. This could mean that the class stage *a priori* chosen for build the matrix model were representative of the population structure. In fact, Easterling *et al.* (2000) demonstrated how sensitivity and elasticity values are inclined to change a lot from a matrix model which does not correctly reflect the plants size in the population, to an integral projection model. Therefore, the importance of the inizial chosed class size boundaries in the matrix model was fundamental in performing demographics plant population models.

The assessments of management option depended on sensitivity and consequent elasticity results. Sensitivity outputs showed how a change in seeds emerging from the seed bank and becoming seedlings will have a much larger effect on the population growth rate of *H. caput-felis*, but, rescaling the sensitivity for the proportional change in vital rates, large-adult plants showed the highest values; hence, the survival-growth transitions were the critical determinant of the population growth rate. In other words, if this transition can be increased, this will result in much greater enhancement of population growth rate than a similar increase in other stages (Morris and Doak 2002).

The highs values in the medium-large adults' plants elasticity meant that a reduction of fecundity is not expected to have large negative effects on population dynamics, in contrast to any reduction of survival and growth. This pattern is typical from those observed in many long-lived perennial plant species (Pfister 1998; Caswell 2001; Fréville *et al.* 2004), as confirmed by the reproductive value and the mean life expectancy outputs, which seem reasonable for a long-lived perennial plant. For instance, survival of adult plants and high longevity represent a typical strategy of several Mediterranean endemic plants (Lavergne *et al.* 2004; Thompson 2005).

The high generation times and the mean long life span predicted from both models are probably a consequence of strong selection in very stressful habitats where growth is slow and colonization infrequent, as highlighted in other Mediterranean long-lived species (García *et al.* 2002). Long life spans may buffer populations against rapid extinction because of a series of "bad" years for reproduction or germination, if extant individuals survive (García *et al.* 2002). Annual seedling recruitment becomes less important in this context, due to the long period each plant has to replace itself (García *et al.* 2002).

The presence of seed banks may enhance population persistence by decreasing the chances of population extinction in "bad" years. However, seed banks may retard population growth rates in "good" years because dormant individuals are delaying reproduction (Kalisz *et al.* 1993). Seed banks can also stabilize population dynamics by damping oscillations in population size to stable equilibria (Kalisz *et al.* 1993). Thus, seed banks should be an important life stage when modelling the persistence of plant populations for conservation purposes (Adams *et al.* 2005).

In this study, environmental variability was added into the demographic analyses to addressed the demographic consequences of seed banks. The LTRE analysis and the absence of significant differences among population growth rates, calculated excluding the seed bank stage, showed how few importance has seeds staying in the seed bank. In contrast, emergence of seeds from the seed bank to becoming seedlings in the following census had a high impact in population growth rates, but recruitment rate was mainly absent or very low. In fact, previous studies suggested how recruitment is a critical stage for this species (Fenu *et al.* 2015a), as demonstrated for several endemic plants along Mediterranean coasts (Cogoni 2012, 2013). Recruitment is particularly critical in stressful habitats such as those found in arid and semiarid climates, where water is the main limiting factor because of both overall water scarcity and its heterogeneity and unpredictability in contrasting spatial and temporal scales (Sher *et al.* 2004; Olano *et al.* 2011), as also detected for other congeneric perennial plant (Escudero *et al.* 1999). Furthermore, the low germination rate of *H. caput-felis* (Tébar *et al.* 1997) attributed to physical exogenous dormancy, a widespread trait among the Cistaceae species (Thanos *et al.* 1992), must be considered.

This pattern was similar to the dynamic of other relict plant populations growing in the Mediterranean Basin, that showed a limited seedling recruitment rate and the survival of mature plants were the main factors for the population persistence (e,g. Pico and Riba 2002; García *et al.* 2002; Copete *et al.* 2009) as also confirmed by elasticity analysis for *H. caput-felis*. Such situations would call for measures that enhance successful sexual reproduction, i.e., stimulation of life cycle parameters that have low, rather than high, elasticities (de Kroon *et al.* 2000).

As a further explanation, for long-lived, iteroparous species, fecundity is generally less important for population growth than survival (Silvertown *et al.* 1993; Franco and Silvertown 2004; Ramula *et al.* 2008), as demonstrated for *H. caput-felis*. The elasticity partition in its two components (survival/growth and fecundity) showed a higher fecundity in 2013-2014 than 2014-2015. This fact can explicate the higher deterministic population growth rate in the first years transition, because a matrix or an integral with a high $\lambda$ will likely have higher elasticity values for fecundity than a matrix for the same species with a low $\lambda$ (Jongejans 2012). The larger sensitivity of population growth rate to changes in growth and survival of adult plants than to changes in reproduction is a main characteristic shares with long-lived plants (Silvertown *et al.* 1993). Moreover, the LTRE indicated that the differences in $\lambda$ for the two years transitions were due primarily to differences in the probability of growing into or remaining in large size ranges; in fact, large plants are most likely to reproduce and produce more fruits (Bruna *et al.* 2014), as highlighted in previous study for this species (Fenu *et al.* 2015a; Salguero-Gómez *et al.* 2015, 2016). This pattern is consistent with the results of a recent study, which analyse vital rates of more than 1,000 plant species (includying species with complex life-history traits), in which retrogressive growth (shrinkage) correlates negatively with reproductive traits, in agreement with the frequent increase in reproductive output with plant size (Harper and White 1974; Salguero-Gómez *et al.* 2016).

The $\lambda$ results varied with the environmental variability; in fact, variation between models in the stochastic population growth rate was observed (MPM = 0.96 and IPM = 1.01). The lower $\lambda_S$ predicted by the MPM, highlighted a slowly decline over time of ca. 4% in the analysed population, maybe due to the difference between the two models. Despite this, *H. caput-felis* population oscillated around the equilibrium under the range of environmental variability simulated in both models; the constancy in $\lambda$ has been previous found in demographic studies as an additional population trait of long-lived perennials plants (Nault and Gagnon 1993; Ehrlén 1995; Damman and Cain 1998; Nantel and Gagnon 1999; Pico and Riba 2002). Temporal variation in environmental factors may be important to population dynamics both in terms of long-term trends and short-term fluctuations (Nicolé *et al.* 2011). Temporal variation is often included in population models in terms of stochastic variation among years. Linking among-year variation in population performance to environmental variation is likely to improve predictions of demographic models in many cases. For example, it enables exploration of the effects of climate change on population viability in a more direct way (cf. Maschinski *et al.* 2006; Jenouvrier *et al.* 2009; Jongejans *et al.* 2010; Jonzén *et al.* 2010; Nicolé *et al.* 2011).

The results of this research were similar to those found in other population dynamics studies of Mediterranean species, as *Centaurea horrida* Bad. (Pisanu *et al.*

2012) and also in congeneric species, as *Helianthemum juliae* Wildpret (Marrero Gómez *et al.* 2007), *Helianthemum polygonoides* Peinado, Mart. Parras, Alcaraz & Espuelas (Copete *et al.* 2009) and *Helianthemum teneriffae* Coss.. Those species showed the importance of survival of mature reproductive plants and life spans comparable with *H. caput-felis*. Common patterns in Mediterranean species were populations whit lambdas closer to the equilibrium, longevity of established individuals and low recruitment rate (García 2008), probably because those populations contained the highest proportion of reproductive plants and lowest frequency of seedlings, a pattern shared with species growing in calcareous rocky habitats (*Petrocoptis pseudoviscosa*; García 2008) or small isolated populations (as *Kosteletzkya pentacarpos*; Pino *et al.* 2007).

Moreover, also for *H. juliae* introducing stochastic variability offered results similar to the deterministic analysis, as demonstrated in this study.

Summarizing, for comparative demography, IPMs may also prove to be very useful because they solve the problem that different matrix studies use a range of matrix dimensions (Jongejans 2012).

Easterling *et al.* (2000) demonstrate that, using a large data set (> 600 individuals), MPMs and IPMs produced identical estimates of population growth rates for a perennial herb. Instead, Ramula *et al.* (2009) show how small demographic data sets (< 300 individuals) are likely to benefit from the use of IPMs because matrix models have the greatest probability of producing biased population estimates for such data sets. Due to the greater precision, IPM were considered more adapted than MPM to monitoring species with restricted range, as *H. caput-felis*. Time and resources for population monitoring are limited (e.g. Lindenmayer and Likens 2010; Fenu *et al.* 2015b), so this greater accuracy represents an advantage regarding the efforts in terms of cost and time of monitoring.

Moreover, further analyses are needed, both at global level, in order to understand transient dynamics, than at local level, to evaluate each local situation, which may shows differences from the global population.

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## References

Adams V.M., Marsh D.M. and Knox J.S. 2005. Importance of the seed bank for population viability and population monitoring in a threatened wetland herb. Biological Conservation 124: 425–436.

Aguilar R., Ashworth L., Galetto L. and Aizen M.A. 2006. Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. Ecology letters 9: 968–980.

Arrigoni P.V. 1971. *Helianthemum caput-felis* Boiss. (2n=24) nuovo reperto per la flora italiana. Webbia 26: 237–243.

Bacaër N. 2011. A short history of mathematical population dynamics. Springer Science & Business Media.

Bakker V.J. and Doak D.F. 2009. Population viability management: ecological standards to guide adaptive management for rare species. Frontiers in Ecology and the Environment 7: 158–165.

Benitez-Malvido J. 1998. Impact of forest fragmentation on seedling abundance in a tropical rain forest. Conservation Biology 12: 380–389.

Bilz M., Kell S.P., Maxted N. and Lansdown R.V. 2011. European Red List of Vascular Plants. Publications Office of the European Union, Luxembourg.

Bruna E.M. 2003. Are plant populations in fragmented habitats recruitment limited? Tests with an Amazonian herb. Ecology 84: 932–947.

Buckley Y.M., Ramula S., Blomberg S.P., Burns J.H., Crone E.E., Ehrlén J., Knight T.M., Pichancourt J., Quested H. and Wardle G.M. 2010. Causes and consequences of variation in plant population growth rate: a synthesis of matrix population models in a phylogenetic context. Ecology Letters 13: 1182–1197.

Caswell H. 1988. Approaching size and age in matrix population models. Size structured Populations: Ecology and Evolution (eds Ebenman B.& Persson L.), 85–105. Springer, New York.

Caswell H. 1989. Analysis of life table response experiments I. Decomposition of effects on population growth rate. Ecological Modelling 46: 221–237.

Caswell H. 2001. Matrix population models: construction, analysis, and interpretation, Sinauer, Sunderland, MA.

Cavers P.B. 1983. Seed demography. Canadian Journal of Botany 61: 3578–3590.

Cogoni D., Mattana E., Fenu G. and Bacchetta G. 2012. From seed to seedling, a critical transitional stage for the Mediterranean psammophilous species *Dianthus morisianus* (Caryophyllaceae). Plant Biosystems 146: 910–917.

Copete M.Á., Ferrandis P., Martínez-Duro E., Herranz J.M., Domínguez F.and Albert M.J. 2009. *Helianthemum polygonoides* Peinado, Mart. Parras, Alcaraz & Espuelas. In: Iriondo J.M., Albert M.J., Giménez Benavides L., Domínguez Lozano F. and Escudero A. (Eds.) 2009. *Poblaciones en Peligro: Viabilidad Demográfica de la Flora Vascular Amenazada de España*. Dirección General de Medio Natural y Política Forestal (Ministerio de Medio Ambiente, y Medio Rural y Marino), Madrid, pp. 93–96.

Crone E.E., Menges E.S., Ellis M.M., Bell T., Bierzychudek P., Ehrlén J., Kaye T.N., Knight T.M., Lesica P., Morris W.F., Oostermeijer G., Quintana-Ascencio P.F., Stanley A., Ticktin T., Valverde T. and Williams J.L. 2011. How do plant ecologists use matrix population models? Ecology Letters 14: 1–8.

Dauer J.T. and Jongejans E. 2013. Elucidating the population dynamics of Japanese Knotweed using Integral Projection Models. PLoS ONE 8:e75181.

de Kroon H., Van Groenendael J. and Ehrlén J. 2000. Elasticities: a review of methods and model limitations. Ecology 81: 607–618.

Easterling M.R., Ellner S.P. and Dixon P.M. 2000. Size-specific sensitivity: applying a new structured population model. Ecology 81: 694–708.

Ellner S.P. and Rees M. 2006. Integral projection models for species with complex demography. The American Naturalist 167: 410–428.

Escudero A., Somolinos R.C., Olano J.M. and Rubio A. 1999. Factors controlling the establishment of *Helianthemum squamatum* (L.) Dum., an endemic gypsophile of semi-arid Spain. Journal of Ecology 87: 290–302.

Ezard H.G., Bullock J.M., Dalgleish H.J., Millon A., Pelletier F. and Ozgul A. 2010. Matrix models for a changeable world: The importance of transient dynamics in population management. Journal of Applied Ecology 47: 515–523.

Fenu G., Cogoni D., Sulis E. and Bacchetta G. 2015a. Ecological response to human trampling and conservation status of *Helianthemum caput-felis* (Cistaceae) at the eastern periphery of its range. Botany Letters 162: 191–201.

Fenu G., Cogoni D., Pinna M.S. and Bacchetta G. 2015b. Threatened Sardinian vascular flora: A synthesis of 10 years of monitoring activities. Plant Biosystems 149: 473–482.

Fenu G., Sulis E., Cogoni D. and Bacchetta G. 2012. Schede per una Lista Rossa della Flora vascolare e crittogamica Italiana: *Helianthemum caput-felis* Boiss.. Informatore Botanico Italiano 44: 233–236.

Ferrandis P., Herranz J.M. and Martínez-Sánchez J.J. 1999. Effect of fire on hard-coated Cistaceae seed banks and its influence on techniques for quantifying seed banks. Plant Ecology 144: 103–114.

García M.B., Guzmán D. and Goñi D. 2002. An evaluation of the status of five threatened plant species in the Pyrenees. Biological Conservation 103:151–161.

García M.B. 2003. Demographic viability of a relict population of the critically endangered plant *Borderea chouardii*. Conservation Biology 17: 1672–1680.

García M.B. 2008. Life history and population size variability in a relict plant. Different routes towards long-term persistence. Diversity and Distributions 14: 106–113.

Griffith A.B., Salguero-Gómez R., Merow C. and McMahon S. 2016. Demography beyond the population. Journal of Ecology 104: 271–280.

Harper J.L. 1977. Population biology of plants. Academic Press, New York, NY, US.

Harper J.L. adn White J. 1974. The demography of plants. Annual review of ecology and systematics 5: 419–463.

Heywood V.H. and Iriondo J.M. 2003. Plant conservation: old problems, new perspectives. Biological Conservation 113: 321–335.

Jacquemyn H., Brys R. and Jongejans E. 2010. Size-dependent flowering and costs of reproduction affect population dynamics in a tuberous perennial woodland orchid. Journal of Ecology 98: 1204–1215.

Jenouvrier S., Caswell H., Barbraud C., Holland M., Strœve J. and Weimerskirch H. 2009. Demographic models and IPCC climate projections predict the decline of an emperor penguin population. Proceeding of the Natural Academy of Sciences of the United States of America 106: 1844–1847.

Jongejans E. and de Kroon H. 2005. Space versus time variation in the population dynamics of three co-occurring perennial herbs. Journal of Ecology 93: 681–692.

Jongejans E. and de Kroon H. 2012. Matrix models. In: Encyclopedia of Theoretical Ecology (eds. Hastings A. and Gross L.). University of California, pp. 415–423.

Jongejans E., de Kroon H., Tuljapurkar S. and Shea K. 2010. Plant populations track rather than buffer climate fluctuations. Ecology Letters 13: 736–743.

Jongejans E., Skarpaas O. and Shea K. 2008. Dispersal, demography and spatial population models for conservation and control management. Perspectives in Plant Ecology, Evolution and Systematics 9: 153–170.

Jonzén N., Pople T., Knape J. and Sköld M. 2010. Stochastic demography and population dynamics in the red kangaroo *Macropus rufus*. Journal of Animal Ecology, 79, 109–116.

Jules, E.S. and Rathcke B.J. 1999. Mechanisms of reduced Trillium recruitment along edges of old-growth forest fragments. Conservation Biology 13: 784–793.

Lefkovitch L.P. 1965. The study of population growth in organisms grouped by stages. Biometrics 21: 1–18.

Lennartsson T. 2002. Extinction thresholds and disrupted plant–pollinator interactions in fragmented plant populations. Ecology 83: 3060–3072.

Lindenmayer D.B. and Likens G.E. 2010. The science and application of ecological monitoring. Biological conservation 143: 1317–1328.

López González G. 1992. Apuntes para justificar el tratamiento del género *Helianthemum* Miller, s.l. (Cistaceae). In Flora Iberica. Anales del Jardín Botánico de Madrid 50: 35–63.

Marrero-Gómez M.V., Oostermeijer J.G.B., Carqué-Álamo E. and Bañares-Baudet Á. 2007. Population viability of the narrow endemic *Helianthemum juliae* (Cistaceae) in relation to climate variability. Biological conservation 136: 552–562.

Maschinski J., Baggs J.E., Quintana-Ascencio P.F. and Menges E.S. 2006. Using population viability analysis to predict the effects of climate change on the extinction risk of an endangered limestone endemic shrub, Arizona cliffrose. Conservation Biology 20: 218–228.

Menges E.S. and Dolan R.W. 1998. Demographic viability of populations of *Silene regia* in midwestern prairies: relationships with fire management, genetic variation, geographic location, population size and isolation. Journal of Ecology 86: 63–78.

Metcalf C.J.E., McMahon S.M., Salguero-Gómez R. and Jongejans E. 2013. IPMpack: an R package for integral projection models. Methods in Ecology and Evolution 4: 195–200.

Metcalf C.J.E., McMahon S.M., Salguero-Gómez R., Jongejans E. and Merow C. 2014. IPMpack: an R package for demographic modeling with Integral Projection Models (v. 2.1).

Morris W.F. and Doak D.F. 2002. Quantitative conservation biology: The theory and practice of population viability analysis. Sunderland, MA: Sinauer Associates.

Morris W.F. and Doak D.F. 2005. How general are the determinants of the stochastic population growth rate across nearby sites? Ecological Monographs 75: 119–137.

Morris W.F., Bloch P.L., Hudgens B.R., Moyle L.C. and Stinchcombe J.R. 2002. Population viability analysis in endangered species recovery plans: past use and future improvements. Ecological Applications, 12: 708–712.

Nicolè F., Dahlgren J.P., Vivat A., Till-Bottraud I. and Ehrlén J. 2011. Interdependent effects of habitat quality and climate on population growth of an endangered plant. Journal of Ecology 99: 1211–1218.

Olano J.M., Eugenio M. and Escudero A. 2011. Site effect is stronger than species identity in driving demographic responses of *Helianthemum* (Cistaceae) shrubs in gypsum environments. American journal of botany 98: 1016–1023.

Ojeda Land E., Mesa Coello R., Marrero M.V., Carqué Álamo E., Giménez Benavides L. and Albert. M.J. 2009. *Helianthemum teneriffae* Coss. In: Iriondo J.M., Albert M.J., Giménez Benavides L., Domínguez Lozano F. and Escudero A. (Eds.) 2009. *Poblaciones en Peligro: Viabilidad Demográfica de la Flora Vascular Amenazada de España*. Dirección General de Medio Natural y Política Forestal (Ministerio de Medio Ambiente, y Medio Rural y Marino), Madrid, pp. 97–99.

Oostermeijer J.G.B., Brugman M.L., de Boer E.R. and den Nijs H.C.M. 1996. Temporal and spatial variation in the demography of *Gentiana pneumonanthe*, a rare perennial herb. Journal of Ecology 84: 153–166.

Oostermeijer J.G.B., Luijten S.H., den Nijs J.C.M., 2003. Integrating demographic and genetic approaches in plant conservation. Biological Conservation113: 389–398.

Picard N., Ouédraogo D. and Bar-Hen A. 2010. Choosing classes for size projection matrix models. Ecological Modelling 221: 2270–2279.

Picó F.X. and Riba M. 2002. Regional-scale demography of *Ramonda myconi*: Remnant population dynamics in a preglacial relict species. Plant Ecology 161: 1–13.

Pino J., Picó F.X. and De Roa E. 2007. Population dynamics of the rare plant *Kosteletzkya pentacarpos* (Malvaceae): a nine-year study. Botanical Journal of the Linnean Society 153: 455–462.

Pisanu S., Farris E., Filigheddu R. and García M.B. 2012. Demographic effects of large, introduced herbivores on a long-lived endemic plant. Plant Ecology 213: 1543–1553.

R Core Team 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Ramula S. 2008. Responses to the timing of damage in an annual herb: fitness components versus population performance. Basic and Applied Ecology 9: 233–242.

Ramula S., Rees M. and Buckley Y.M. 2009. Integral projection models perform better for small demographic data sets than matrix population models: a case study of two perennial herbs. Journal of Applied Ecology 46: 1048–1053.

Rees M., Childs D.Z. and Ellner S.P. 2014. Building integral projection models: a user's guide. Journal of Animal Ecology 83: 528–545.

Rodríguez-Pérez J. 2005. Breeding system, flower visitors and seedling survival of two endangered species of *Helianthemum* (Cistaceae). Annals of Botany 95: 1229–1236.

Salguero-Gómez R. and Plotkin J.B. 2010. Matrix dimensions bias demographic inferences: implications for comparative plant demography. The American Naturalist 176: 710–722.

Salguero-Gómez R., Jones O.R., Archer C.R., Buckley Y.M., Che-Castaldo J., Caswell H., Hodgson D., Scheuerlein A., Conde D.A., Brinks E., de Buhr H., Farack C., Gottschalk F., Hartmann A., Henning A., Hoppe G., Romer G., Runge J., Ruoff T., Wille J., Zeh S., Davison R., Vieregg D., Baudisch A., Altwegg R., Colchero F., Dong M., de Kroon H., Lebreton J.D., Metcalf C.J.E., Neel M.M., Parker I.M., Takada T., Valverde T., Velez-Espino L.A., Wardle G.M., Franco M. and Vaupel J.W. 2015. The COMPADRE Plant Matrix Database: an open online repository for plant demography. Journal of Ecology 103: 202–218.

Salguero-Gómez R., Jones O.R., Jongejans E., Blomberg S.P., Hodgson D J., Mbeau-Ache C., Zuidema P.A., de Kroon H. and Buckley Y.M. 2016. Fast–slow continuum and reproductive strategies structure plant life-history variation worldwide. Proceedings of the National Academy of Sciences 113: 230–235.

Santos T. and Telleria J.L. 1997. Vertebrate predation on Holm Oak, *Quercus ilex*, acorns in a fragmented habitat: effects on seedling recruitment. Forest Ecology and Management 98: 181–187.

Schemske D.W., Husband B.C., Ruckelshaus M.H., Goodwillie C., Parker I.M. and Bishop J.G. 1994. Evaluating approaches to the conservation of rare and endangered plants. Ecology 75: 584–606.

Schwartz L.M., Gibson D.J. and Young B.G. Using integral projection models to compare population dynamics of four closely related species. Population Ecology 1–8.

Sher A.A., Goldberg D.E. and Novoplansky A. 2004. The effect of mean and variance in resource supply on survival of annuals from Mediterranean and desert environments. Oecologia 144: 353–362.

Stevens M.H. 2009. A Primer of Ecology with R. Springer Science & Business Media.

Stubben C. and Milligan B. 2007. Estimating and analyzing demographic models using the popbio package in R. Journal of Statistical Software 22: 1–23.

Tuljapurkar S. 1990. Population Dynamics in Variable Environments. New York: Springer-Verlag.

Tuljapurkar S., Horvitz C.C. and Pascarella J.B. 2003. The many growth rates and elasticities of populations in random environments. The American Naturalist 162: 489–502.

Valverde T. and Silvertown J. 1998. Variation in the demography of a woodland understorey herb (*Primula vulgaris*) along the forest regeneration cycle: projection matrix analysis. Journal of Ecology 86: 545–562.

Venable D.L. and Brown J.S. 1988. The selective interactions of dispersal, dormancy and seed size as adaptations for reducing risks in variable environments. American Naturalist 131: 360–384.

Wardle G.M. 1998. A graph theory approach to demographic loop analysis. Ecology 79: 2539–2549.

Warr S.J., Thompson K. and Kent M. 1993. Seed banks as a neglected area of biogeographic research: a review of literature and sampling techniques. Progress in Physical Geography 17: 329–347.

Werner P.A. 1975. Predictions of fate from rosette size in teasel (*Dipsacus fullonum* L.). Oecologia 20: 197–201.

Wilcock C. and Neiland R. 2002. Pollination failure in plants: why it happens and when it matters. Trends in plant science 7: 270–277.

Zuidema P.A., Jongejans E., Chien P.D., During H.J. and Schieving F. 2010. Integral projection models for trees: a new parameterization method and a validation of model output. Journal of Ecology 98: 345–355.

Kalisz S. and McPeek M.A. 1993. Extinction dynamics, population growth and seed banks. Oecologia 95: 314–320.

# CHAPTER 4

## Assessing the local population dynamics of *Helianthemum caput-felis* along its distribution range

### Introduction

A significant challenge in ecology is to accurately describe the ecological processes that cause changes in the distribution and abundance of organisms. In fact, population ecology seeks to understand the drivers of changes in abundance over time and space, with demography more specifically related to how underlying vital rates (survival, growth, reproduction, etc.) structure populations (Griffith *et al.* 2016).

This is particularly important when significant changes are occurring, such as endangered species that are declining spatially and numerically (Jongejans *et al.* 2011). The monitoring and the research of population dynamics are necessary to assess appropriate management strategies (Silva *et al.* 2015). Monitoring populations is one of the main tools in conservation biology for identifying declining species or species with a high risk of extinction (Marsh and Trenham 2008). Data related to plant populations can be used to predict the effects of various management practices on population size, condition, stage distribution (i.e. seed production and/or seedlings development) and demographic processes, including survivorship and seedling recruitment and, in general, temporal variations in population size and geographic range (Kull et al. 2008; Fenu *et al.* 2015a).

Although the general population trends can be estimated from the densities of individuals, understanding the mechanisms that drive those trends requires the quantification of basic vital rates (growth, survival and fecundity; Metcalf *et al.* 2013). Despite this, long-term sampling programmes are uncommon because of the need to maintain monitoring structures and funding in the long run (Silva *et al.* 2015).

Population size is regulated by several demographic parameters whose alteration may have very different impacts on the population growth rate (Cursach *et al.* 2013). Assessing which life history stages are the most critical to population growth is thus needed in order to target those parts of the life cycle when designing management actions (Schemske *et al.* 1994; Heywood and Iriondo 2003; Kerr *et al.* 2016). Such approach relies on the estimation of survival, growth and fecundity using intensive individual monitoring (Cursach *et al.* 2013). Knowledge about the contributions of different stages of the life cycle to population growth rate enhances our understanding of the life histories of species (de Kroon *et al.* 2000). In fact, elasticities have become a popular tool in conservation biology, because they quantify the relative importance of a matrix element to population growth rate. Therefore, it is generally inferred that management should focus on the demographic parameters with the largest elasticities (e.g. Menges 1990; Doak *et al.* 1994; Heppell *et al.* 1994; Caswell 1996; de Kroon *et al.* 2000). In addition, particularly for endemic and endangered plants, also the difference in population trends at local level must be evaluated in order to understand the mechanisms driving the vital rates in different localities. In fact, previous studies demonstrate as different population of the same species, despite the areal distribution proximity, shows strong local adaptations and a low correlation with the overall population performance (García 2008).

The Integral Projection Model (IPM hereafter) was elaborated by Easterling et al. (2000) in order to analyse individuals which vary continuously in size, and subsequently developed in several ways (e.g. monocarpic plants in Rees and Rose 2002). The model avoids the need to divide data into discrete classes, and does not require any extra biological assumptions (Easterling *et al.* 2000). In fact, one problem with size-based stage classifications is that it can be hard to find clear borders among one size group and the next: the sizes of individuals in a population often follow a continuous distribution, and vital rates mostly show gradual change with size (Jongejans and de Kroon 2012). In particular, for long-lived and slow-growing plants, the choice of the number of size classes is not without consequences: matrix dimension can have profound impact on the projected population growth rate ($\lambda$). Conversely, in IPMs vital rates are continuous functions of size, and these can be copied directly from statistical regression analyses. For comparative demography IPMs may also prove to be very useful because they solve the problem that different matrix studies use a range of matrix dimensions (Jongejans and de Kroon 2012). Moreover, IPMs' characteristic is that it has many properties in common with matrix models; for example, they both allow the calculation of the stable size distribution, the population growth rate, and the sensitivities and elasticities of $\lambda$ (Childs *et al.* 2003). IPM has been particularly advocated for small demographic datasets (less than 300 plants; Ramula *et al.* 2009), and therefore it is very useful for studies of fragmented plant populations.

The comparison of population dynamics under different conditions is one of the most valuable approaches to understand ecological and evolutionary processes (Salguero-Gómez 2014). An useful tool/method designed for such data is the Life Table Response Experiment (LTRE; the term was introduced by Caswell 1989), a study that compares a complete set of vital rates under two or more conditions (Caswell 2010), based on variance decomposition techniques (Jongejans and de Kroon 2012). This approach helps to compare vital rates among different populations or different years of the same populations (Jongejans and de Kroon 2012), and has been applied to IPMs (Williams and Crone 2006; Williams *et al.* 2010; Shou-Li *et al.* 2011; Gonzalez *et al.* 2012; Bassar *et al.* 2013; Yule *et al.* 2013; Salguero-Gómez 2014).

Although IPMs have been successfully applied in plant ecology and evolutionary biology (e.g. Rees and Rose 2002; Williams 2009; Coulson 2012), their use in conservation biology remains still lacking (e.g. Zuidema *et al.* 2010; Nicolè *et al.* 2011; Ferrer-Cervantes *et al.* 2012). Indeed, although much interest has been devoted to the Mediterranean flora (e.g. Médail and Quézel 1997; Nikolic *et al.* 2008; Bacchetta *et al.* 2012; Iliadou *et al.* 2014), demographic studies on endangered plant populations are uncommon (e.g. Pino *et al.* 2007; Pisanu *et al.* 2012; Cursach *et al.* 2013), even more so at local level (Cursach *et al.* 2014).

Therefore, the main aim of this research was to assess the local population dynamics of *H. caput-felis*, a coastal plant of European interest (92/43/EEC), which showed several fragmented distribution along the western Mediterranean Basin. Using demographic analyses, plant performance and population dynamics were investigated in six populations representative of the European distribution of *H. caput-felis*. Specifically, study questions are:

(1) Do the six populations exhibit similar population dynamics?

(2) What demographic parameters contribute to variation in fitness over time within populations?

(3) How do the six populations differ in terms of fecundity, growth, and plant survival?

(4) What stages of the life cycle have the greatest influence on population maintenance and growth?

## Materials and methods

### *Study species*

*Helianthemum caput-felis* Boiss. is a half shrub of 35(50) cm tall. Its flowers are hermaphroditic and have a short lifespan (3–4 days; Rodríguez-Pérez 2005). Based on studies carried out in Spanish and Sardinian populations, the flowering period is from March to June, and the fruiting season runs from late April to beginning of August (see Chapter 1 and 2). Fruits are capsules that detach at maturation, and seed germination takes place in autumn, at the onset of the rainy season (Rodríguez-Pérez 2005).

*Helianthemum caput-felis* is a long-lived iteroparous perennial plant, but the information about its exact lifespan is lacking. The life cycle of *H. caput-felis* (see Chapter 4) include a continuous stage, in which growth and reproduce, combined which is a discrete stage, with seeds that can enter a permanent soil seed bank or germinate the next spring after dispersion. As other genera of Cistaceae family, *H. caput-felis* can accumulate long-lived persistent seed banks in the soil through the production of large yields of impermeable-hardcoated seeds (Ferrandis *et al.* 1999).

*Helianthemum caput-felis* is a coastal plant distributed throughout the western Mediterranean Basin (south-eastern Iberian Peninsula, Majorca, Sardinia and northwest Africa) in several fragmented populations (Fenu *et al.* 2015b and references therein).

From an ecological point of view, *H. caput-felis* is a termophilous plant that preferably grows in coastal environments under the direct influence of the sea, mostly on calcareous rocky cliffs (0–200 m a.s.l.) with garrigues or scrublands; peculiar populations also grow on different habitats, such as sand dunes (Majorca), rocky slopes bordering inland ravines (Melilla) or, rarely, in open wooded areas (Fenu *et al.* 2015b and references therein).

### *Data collection*

Data were directly collected over three years (2013–2015) from plants belonging to six populations within the distribution range of *H. caput-felis*: two located in Sardinia (Italy), at the eastern margin of its distribution range (Capo Mannu and Su Tingiosu – CM and ST hereafter), two in Majorca's island, in the center of its range (Sa Ràpita and Colònia de Sant Jordi – SR and SJ hereafter) and two in Alicante's coasts, at the western part of its distribution (Cabo Roig and Moraira – CR and MO hereafter; Table 1). Overall, those populations encompass a large part of the *H. caput-felis* geographic distribution.

Plant demographic data were collected in 98 permanent plots of 2 × 1 m, randomly established in the six selected populations (in the area where the plant was found); across the six populations a total of 821 plants were marked, mapped and monitored during the study (Table 1).

Over the study period, surveys took place at least three times a year, following Jacquemyn *et al.* (2010). In early March, all sites were visited a first time for locating all previously mapped individual and mapping new seedlings. During the flowering peak (April-May), when all plants are fully grown, sites were visited a second time, in order to measure individuals (height, minimum and maximum diameter were taken), count the number of flowers per plant and check for new occasional individuals, that were added in the data set. During the fruiting peak (latest May-early June) the third survey was carried out in all populations, to count the number of fruits per plant.

**Table 1 – Principal geographical and ecological traits of *Helianthemum caput-felis* populations investigated in this study: region, coordinates, altitudinal range (m), substrate type; in addition the number of plots and the number of plants within the monitored plots were reported.**

| Region | Population (code) | Coordinates U.T.M. (time zone) | Altitude (min-max) | Substrate type | N. plots | N. plants |
|---|---|---|---|---|---|---|
| Alicante | Cabo Roig (CR) | 700388.62 m E,<br>4198390.09 m N<br>(30 S) | 3 - 15 | limestone | 15 | 116 |
| Alicante | Moraira (MO) | 250226.01 m E,<br>4285845.34 m N<br>(31 S) | 3 - 15 | limestone | 15 | 56 |
| Majorca | Sa Ràpita (SR) | 496897.00 m E,<br>4356780.00 m N<br>(31 S) | 0 - 5 | sand and limestone | 15 | 252 |
| Majorca | Colònia de Sant Jordi (SJ) | 500074.00 m E,<br>4352060.00 m N<br>(31 S) | 0 - 5 | sand and limestone | 13 | 65 |
| Sardinia | Capo Mannu (CM) | 447428.00 m E,<br>4432412.00 m N<br>(32 S) | 5 - 55 | limestone | 20 | 146 |
| Sardinia | Su Tingiosu (ST) | 449657.67 m E,<br>4428538.64 m N<br>(32 S) | 5 - 25 | limestone | 20 | 186 |

### *Data analyses*

An Integral Projection Model (IPM) for each population and year of sampling was performed in order to evaluate the presence of changes in population dynamics among years. IPMs describe how a population structured by a continuous individual-level state variable changes in discrete time (Easterling *et al.* 2000). In the IPM, the state of the population is described by the size distribution *n(y, t)*. Plant size was chosen as the continuous state variable to evaluate the demographic dynamics of *H. caput-felis* in the six populations. In particular, plant volume was found to be the variable that best explained the vital rates of this species (Fenu *et al.* 2015b). Plant size was calculated according to the formula in Fenu *et al.* (2015b) and then, according to Rees *et al.* (2014), plant sizes were log-transformed, because this transformation foot well the data.

Data were separated by site and year in order to elucidated differences between them, if presents.

IPM uses a continuous projection kernel in order to describe the population size distribution by a density function (Easterling *et al.* 2000; Ellner and Rees 2006). In our model, growth, survival and the probability of flowering are described in function of plant size. Constant, linear and quadratic models were fit, and the best fit was selected on the basis of the lowest Akaike Information Criteria (AIC; Dauer and Jongejans 2013).

Plants in the population could survive, grow, and produce new individuals in each time step (Easterling *et al.* 2000). The IPM of a size structured population is given by:

$$n(y, t+1) = \int_L^U K(y,x)n(x,t)\mathrm{d}x = \int_L^U [P(y,x) + F(y,x)]\, n(x,t)\mathrm{d}x$$

where *n(y, t* + 1) is the size distribution *y* of both established and newly recruited plants in census time *t* + 1, *n(x, t)* the distribution across size of individuals at census time *t*, and *L* and *U* are the respective lower and upper size limits modelled in the IPM (Metcalf *et al.* 2013). The latter ones were set lower and higher than the observed minimum and maximum sizes to avoid unintentional evictions (Williams *et al.* 2012). The kernel (*K*) can be broken down into two sub-kernels (*P* and *F*): the *P* sub-kernel represents transitions attributable to survival and growth, while the *F* sub-kernel describes *per capita* contributions of reproductive individuals given the recruit density function at the next census (Metcalf *et al.* 2013).

Survival, growth and fecundity objects that compose the IPMs were constructed using the version 2.1 of the "IPMpack" package (Metcalf *et al.* 2014), and matrix were draw with the "fields" package (Nychka *et al.* 2014) in R version 3.1.2 (R Core Team 2014).

The survival probability was modelled by logistic regression (binomial error distribution and logit link function), while the growth probability was modelled as a linear regression (Merow *et al.* 2014a). Fecundity was composed by two vital rates: the probability of flowering (fec0), modelled as a logistic regression (by specifying binomial error distributions and logit link functions in generalized linear models), and the number of fruits in the peak of the year *t* for every monitored plant (fec1), modelled as a linear regression (with a Poisson error distribution and a log link function), times the mean number of seedlings in year *t* + 1 per number of fruiting individuals in year *t*, and a probability function of the seedling size distributions. The seedling size distribution in each population and each year was described by a normal distribution with the observed mean and standard deviation.

Furthermore, other vital rates were not measured for every individual but pooled from every population and are therefore included in the IPM as constants (i.e. size independent): the mean number of seeds per fruit (fec2), the probability of germination (fec3) and the probability of seedling survival (fec4) within the year of seed production, the probabilities of seeds entering the seed bank (goSB) or staying there (staySB). These constants form the fecundity object and a discrete stage describes the seed bank. Mean seeds per fruit (fec2) was calculated by collecting randomly 980 mature fruits from 98 randomly selected plants (one per plot). According to Bruna *et al.* (2014), the theoretical amount of seeds per year was estimated from the multiplication of the total number of fruits (excluding the percentage of empty/aborted fruits) and the mean seeds per fruit. This was also the case of seed germination rate (fec3), which has been analysed in laboratory at locality level (Tébar *et al.* 1997), but not documented in the field. In light of this, it was estimated by the proportion of seeds becoming seedlings by counting the number of newly established seedlings during the surveys, dividing this number by the number of seeds produced by all monitored plants in the previous reproductive season (Table 1A; Bruna *et al.* 2014). This estimation reflects a general low germination probability, as reported in Tébar *et al.* (1997) for the Majorcan population (4%). Seedling survival (fec4) was estimated from the ratio between seedlings counted in year *t* and seedlings present in *t*+1. Due to the absence of seed bank's studies regarding this plant species, data about seeds entering and staying in the seed bank were taken according to Quintana-Ascencio *et al.* (1998). All this estimated data had to be incorporated into fecundity parameter calculation.

The transition of each plant among the continuous and the discrete stage was added in the original database. In the case of *H. caput-felis*, these transitions are:

- continuous stage → discrete stage: individuals with a given volume in year *t* contribute seeds to the seed bank, that is, seeds that were produced, did not germinate, and remain viable;

- discrete stage → discrete stage: prolonged dormancy and survival of seeds in the seed bank;
- discrete stage → continuous stage: germination of seeds from the seed bank to become seedlings of a given above ground stem height (Salguero-Gómez 2014).

The IPM predicts a population's asymptotic growth rate ($\lambda$), represented by the dominant eigenvalue, with associated eigenvectors and state-dependent sensitivity and elasticity functions (Easterling *et al.* 2000). Dominant right and left eigenvectors *w(x)* and *v(x)* give the stable size distribution and size-specific reproductive value, respectively (Easterling *et al.* 2000). The stable size distribution was extracted and compared with the observed ones, and damping ratios were calculated. The absence of recruitment (probability of seed germination and seedling survival = 0) in MO and SJ populations cause the impossibility to calculate reproductive values for those sites.

The deterministic lambda ($\lambda$) was also calculated excluding the seed bank stage ($\lambda_{\text{continuous}}$), in order to analyse possible differences with $\lambda$.

Sensitivity describes the change in $\lambda$ resulting from a change in demographic parameters (survival or fecundity) affecting only individuals at a particular size (Easterling *et al.* 2000). Elasticity provided details on the size range having the greatest effect on $\lambda$ (Dauer and Jongejans 2013), and then elasticity values were calculated by dividing the relative increase in $\lambda$ by the relative increase of the vital rate function (de Kroon *et al.* 2000).

Elasticity can be partitioned into contributions from the survival-growth and reproduction components of the kernel (*P* and *F*; Ellner and Rees 2006), than the percentage of each vital rate contribution were calculated.

The stochastic rate of increase ($\lambda_S$) was also calculated as the geometric mean proportional change in population size over 50,000 model iterations, where one yearly projection kernel was randomly drawn for each iteration.

The ratio between the dominant eigenvalue and the second highest eigenvalue of a transition matrix (damping ratio) was also calculated.

Life table response experiments (LTREs) are studies that quantify the population-level effect of environmental factors by measuring a complete set of vital rates (a life table) under several conditions (treatments). The terms "experiment" and "treatment" are used loosely to include not only manipulative experiments but also comparative observations under natural conditions (Caswell 1996b). A one-way LTRE analysis was performed for each population in order to elucidate the demographic mechanisms underlying differences among years by decomposing differences in $\lambda$ into the contributions from different demographic variables (Caswell 1989).

For each population the $\text{IPM}_{2013\text{-}2014}$ was arbitrarily defined as the "control", because the interest of this analysis is to compare the differences in $\lambda$ from the $\text{IPM}_{2014\text{-}2015}$ in comparison to the IPM "control", and to know what vital rates change between years. Then, the arithmetic mean of the two IPMs as a mid-way IPM, which permits to evaluate the differences in the sensitivities of the IPM kernels to be compared, was designating. In MO and SJ populations, the mean IPM plots were inaccurate, because since there are too many zeros in the matrix, R generates "-inf" for those zero entries with the logarithmic function; hence the square root of the values were taken, in order to visualize differences in values.

Finally, the IPM of the differences between the IPM kernels corresponding to the 2013-2014 and 2014-2015 transitions was calculated, and then the parts of the life cycle of the study species that are responsible for the difference in $\lambda$ between the two transition periods were inspected, by weighting the IPM of the difference between those two IPMs by the sensitivity of the arithmetic mean IPM. LTRE was verified by a cross-check as recommended by Salguero-Gómez (2014).

Due to the strong low values of some population (particularly in elasticity), plots were scaled in order to display the main differences.

To interpret the results, kernels' plots were made with the "image" function of the library "fields" (Nychka *et al.* 2014) of "IPMpack" version 2.1 (Metcalf *et al.* 2014) in R version 3.1.2 (R Core Team 2014).

## Results

Plants volume is correlated with survival, growth, flower probability and fruits number in both years of study and for each population (Figure 1–4).

Survival rate generally increased in correspondence of large adult plants (Figure 1). Some exceptions to this pattern were detected, like SR in 2013 and SJ in both years of study. In SR population plants showed lowest survival for little-intermediate volumes, but high survival for lowest and highest volumes, whereas in SJ there was no relationship between survival and size. In 2014, exceptions were represented by CR population, where survival initially decrease and then, in correspondence of high volumes, increased. Also SR population in 2014 have a positive relationship between survival and size until ca. 2,900 $cm^3$ (corresponding to log(8) in Figure 1), after which it decreases.

The sizes of individuals that survive until the following year (growth) is always linear dependent from size in year *t* and generally follow the same pattern among populations, although smaller plants were commonly more likely to grow than larger ones (Figure 2).

The exceptions to this pattern were represented by MO and SJ populations; in the first, there was a linear negative relationship between growth and size, hence plants reduced their size in the following year, whereas in the seconds there was no difference between smaller and large plants growth. Furthermore, growth's plots highlighted the general concentration of large plants compared to smaller.

The probability that plants flowered at the first census was strongly related to their size, with most plants larger than ca. 400 $cm^3$ (corresponding to log(6) in Figure 3) flowering, except in MO.

Among flowered plants, the number of fruits was exponentially dependent on plant's size, and larger plants produced many more fruits than the smaller ones (Figure 4). Fecundity's plots showed that Majorcan and Iberian east-coast populations (SR, SJ, CR and MO) presented a higher number of larger plants which did not produced any fruits compared to Sardinians' (CM and ST). Moreover, a high fruit production was detected in 2014 than 2013 of CM, ST, CR and SR populations (Figure 5).

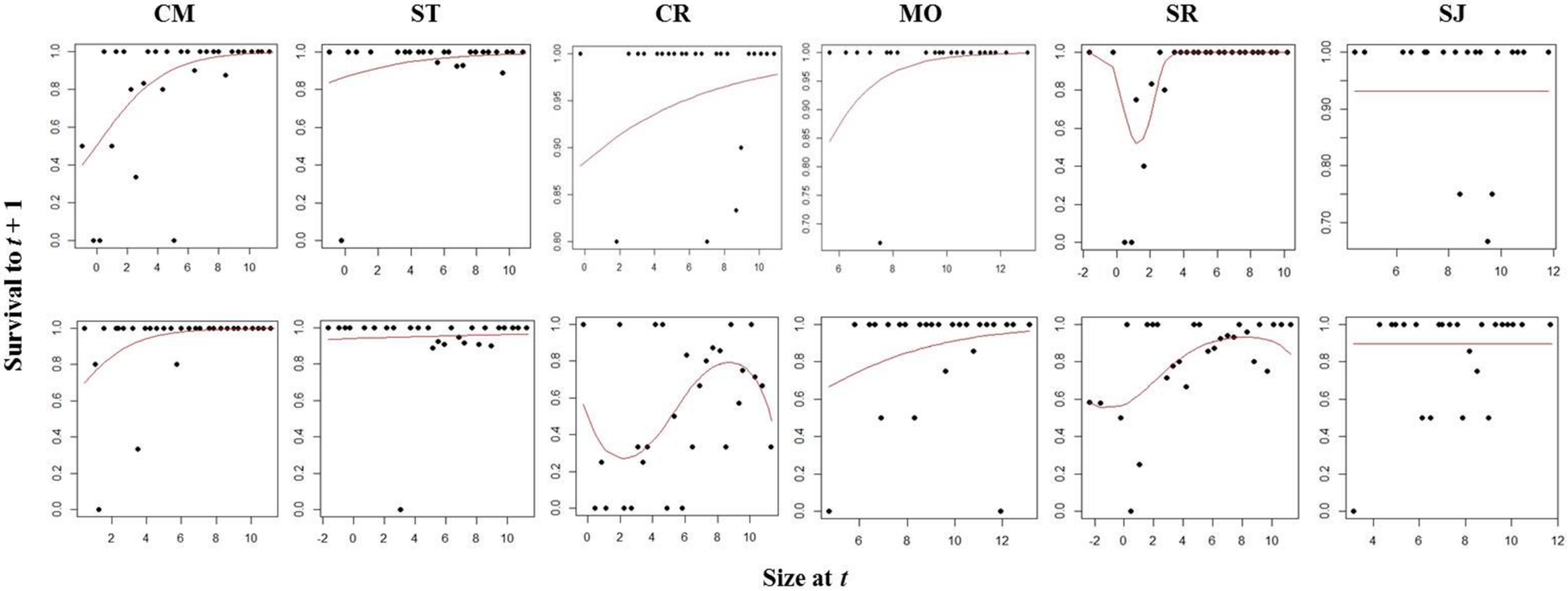

**Figure 1 – Relationship between plant size and survival probability from 2013 to 2014 and from 2014 to 2015 (first and second row, respectively). Columns correspond to the six studied populations (see Table 1). Red lines show the best-fit model prediction. The x-axis represent plant size at *t*; the y-axis represent survival probability of plants at *t* + 1.**

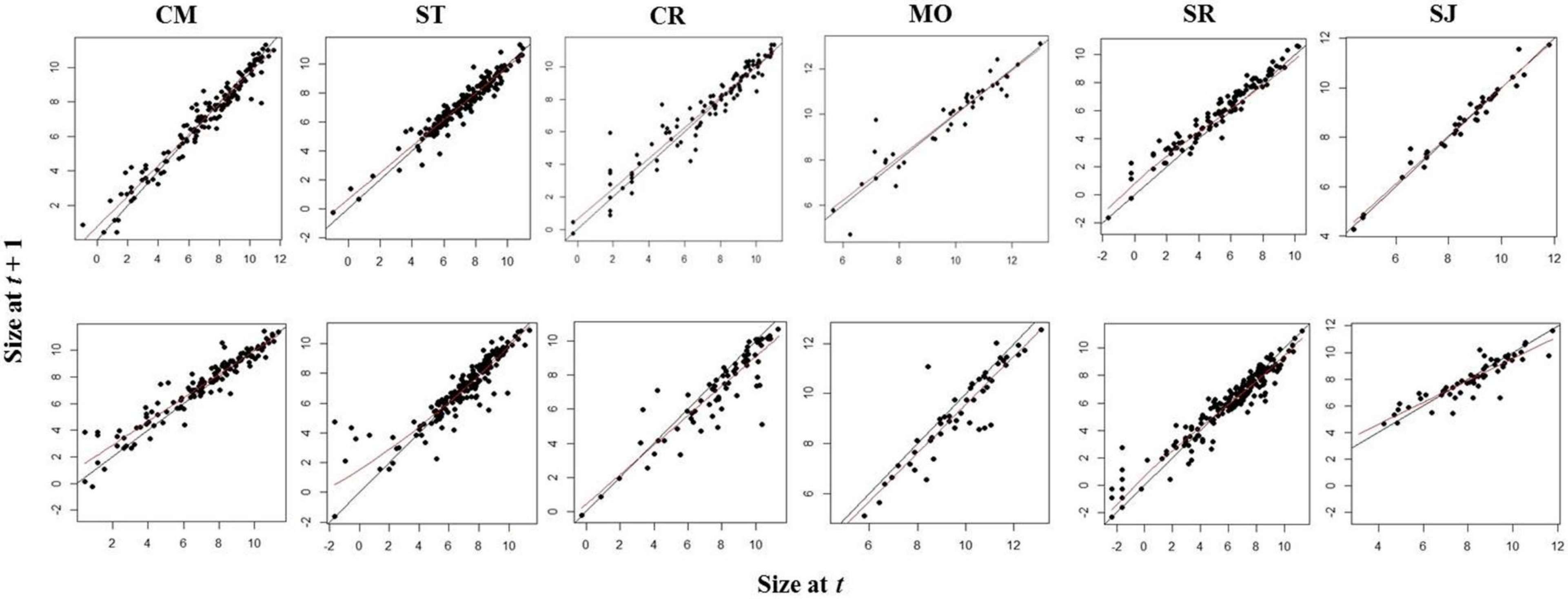

**Figure 2 – Relationship between plant size and growth from 2013 to 2014 and from 2014 to 2015 (first and second row, respectively). Columns correspond to the six studied populations (see Table 1). Red lines show the best-fit model prediction. The x-axis represent plant size at *t*; the y-axis represent plant size at *t* + 1.**

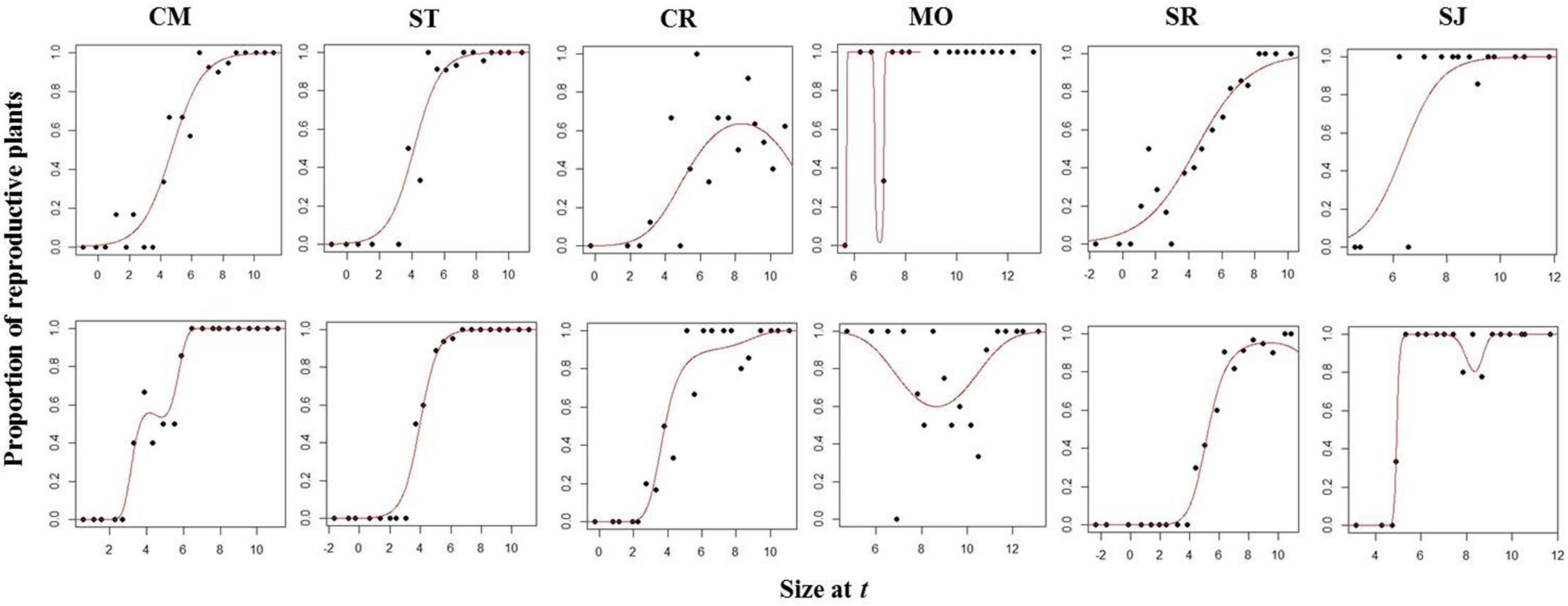

**Figure 3 – Plants's flowering probability from 2013 to 2014 and from 2014 to 2015 (first and second row, respectively). Columns correspond to the six studied populations (see Table 1). Red lines show the best-fit model prediction. The x-axis represent plant size at *t*; the y-axis represent the proportion of reproductive plants at *t* + 1.**

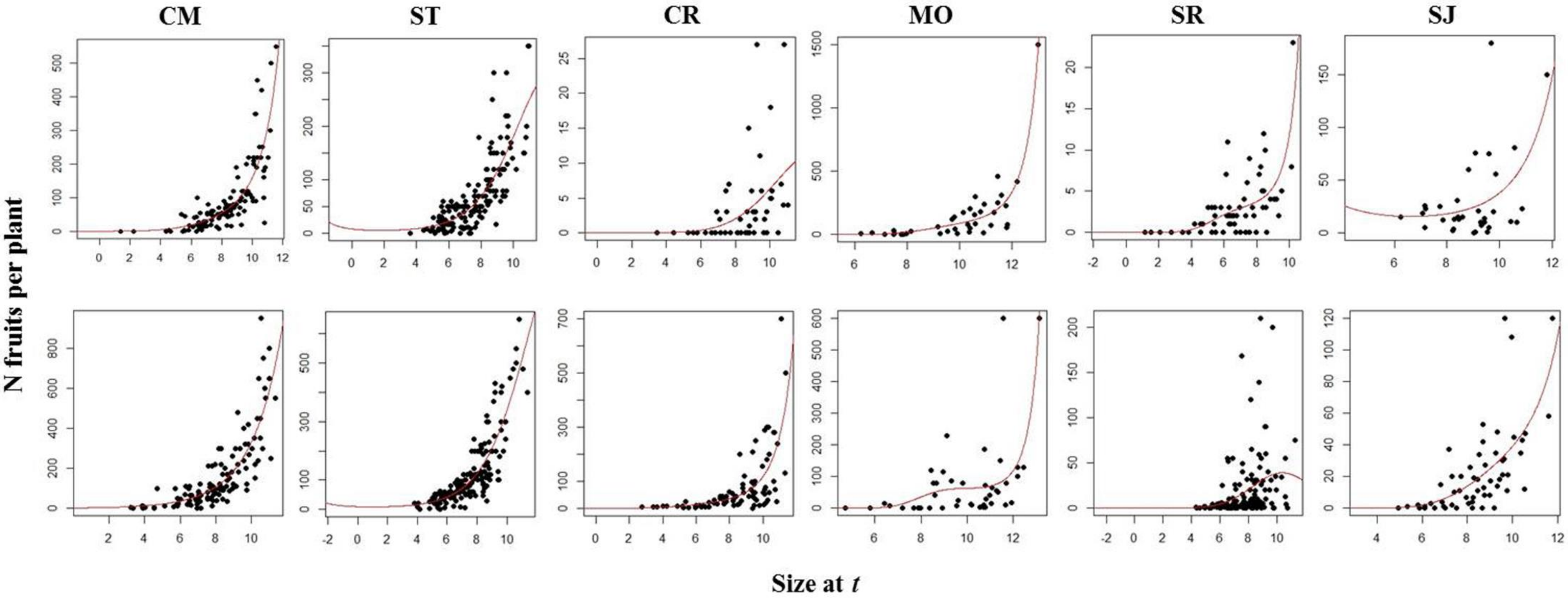

**Figure 4 – Relationship between plant size and fruits production from 2013 to 2014 and from 2014 to 2015 (first and second row, respectively). Columns correspond to the six studied populations (see Table 1). Red lines show the best-fit model prediction. The x-axis represent plant size at *t*; the y-axis represent the number of fruits per reproductive plant.**

**Table 2 – Population growth rate (λ), population growth rate excluding the seed bank ($\lambda_{continuous}$), stochastic population growth rate ($\lambda_S$), elasticity partition (*P* and *F* components) and damping ratio ($d_r$) of the six analysed populations in the two years transitions.**

| Population (code) | λ | | $\lambda_{continuous}$ | | $\lambda_S$ | *P* component | | *F* component | | $d_r$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2013-2014 | 2014-2015 | 2013-2014 | 2014-2015 | | 2013-2014 | 2014-2015 | 2013-2014 | 2014-2015 | 2013-2014 | 2014-2015 |
| Capo Mannu (CM) | 0.98 | 1.06 | 0.98 | 1.06 | 1.03 | 0.98 | 0.94 | 0.02 | 0.06 | 1.23 | 1.23 |
| Su Tingiosu (ST) | 1.01 | 1.01 | 1.01 | 1.00 | 1.01 | 0.97 | 0.97 | 0.03 | 0.03 | 1.15 | 1.18 |
| Cabo Roig (CR) | 1.02 | 0.67 | 1.02 | 0.59 | 0.80 | 0.97 | 1 | 0.03 | 0 | 1.18 | 1.14 |
| Moraira (MO) | 1.65 | 1.61 | 1.65 | 1.61 | 1.63 | 0.43 | 0.39 | 0.57 | 0.61 | 1.71 | 2.14 |
| Sa Ràpita (SR) | 1.13 | 0.97 | 1.13 | 0.96 | 1.03 | 0.91 | 0.93 | 0.09 | 0.07 | 1.17 | 1.42 |
| Colònia de Sant Jordi (SJ) | 0.93 | 1.79 | 0.93 | 1.79 | 1.30 | 1 | 0.44 | 0 | 0.56 | 1.03 | 1.84 |

Asymptotic growth rates (λ) varied widely both between years and populations (from 0.93 in SJ to 1.65 in MO in 2013–2014 and from 0.67 in CR to 1.79 in SJ in 2014–2015; Table 2).

Population growth rates considering only the continuous stage, that is, excluding the seed bank stage ($\lambda_{continuous}$), did not change from the total population growth rate λ, except in CR in 2014, where $\lambda_{continuous}$ had a value 0.08 inferior to λ (Table 2). Stochastic growth rates ($\lambda_S$) varied from 0.80 (CR) to 1.63 (MO).

The predicted stable size distribution was generally skewed heavily towards large adults' plants (Figure 5).

This indicates that, under deterministic conditions, *H. caput-felis* populations could include larger numbers of plants with a volume ranging from ca. 400 $cm^3$ and ca. 60,000 $cm^3$. However, the predicted stable size distributions did not correspond with the observed size histogram distribution in $CR_{2013\text{-}2014}$, $MO_{2013\text{-}2014}$, $MO_{2014\text{-}2015}$ and $SR_{2013\text{-}201,}$ as confirmed by the damping ratio value (Table 2).

The reproductive value increased with plants volume, reflecting the fact that larger plants had higher fecundity, survival and larger offsprings (Figure 6).

Generally, the sensitivity values increased with plant volume (data not shown) and the elasticity values were high along the diagonal representing the survival of medium-large size individuals. The same pattern was detected for all years and populations, except in CR (Figure 7).

The breakdown of elasticity in his two components revealed that *P* component is higher than *F* component in almost all populations, hence, in *H. caput-felis* populations, the survival-growth transitions were the critical determinant of λ (Table 2). Moreover, the contribution of growth to λ was dominated by transitions into the larger plants where reproduction occurs (Figure 7). Only MO (in both years) and SJ (in 2014) populations represented an exception. In those populations, the *F* component was higher than the *P* component, meaning that fecundity contributed more than survival/growth to the projected population growth rate.

The kernel's plots showed the importance of medium-large size's plants (high values near the diagonal central area); indeed they were dominated by the survival function, representing individuals who survive the following year without changing much in size (stasis; Figure 8). Generally, the majority of medium size individuals could reach larger size, as demonstrate by elasticities.

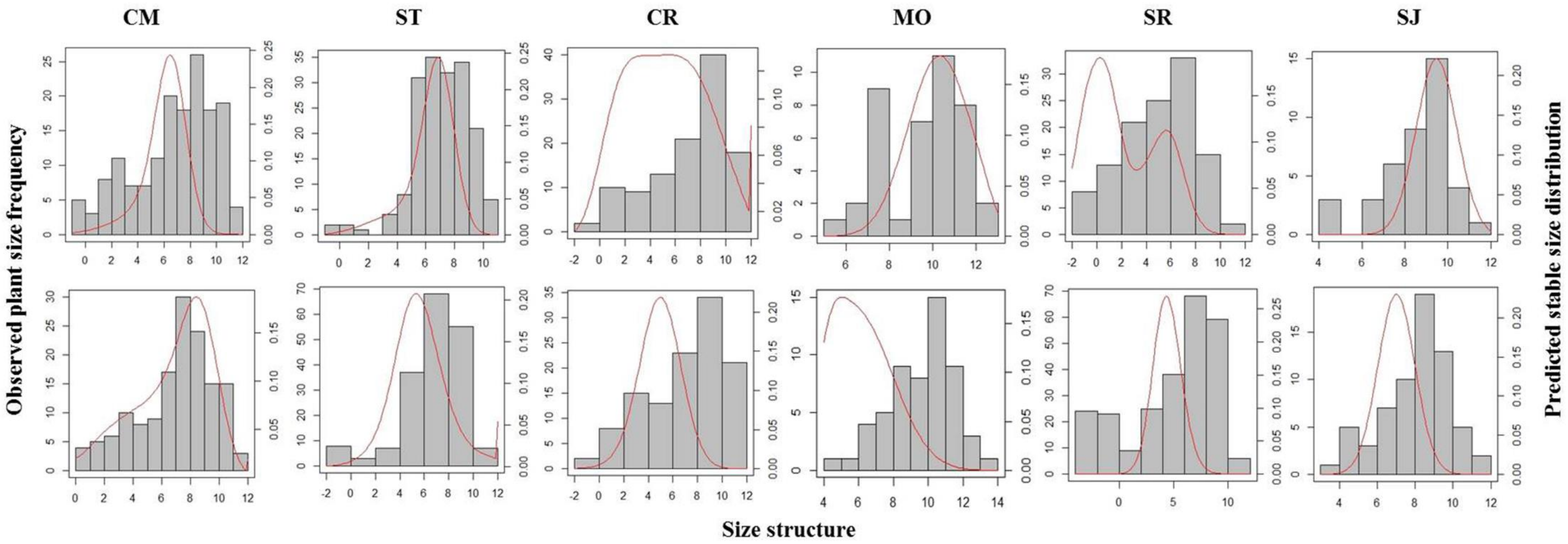

**Figure 5 – Histogram distribution of plant sizes (log of plant volume in cm3) and stable size distribution (red lines) for the IPM fitted from 2013 to 2014 and from 2014 to 2015 (first and second row, respectively). Rows correspond to the six studied populations (see Table 1).**

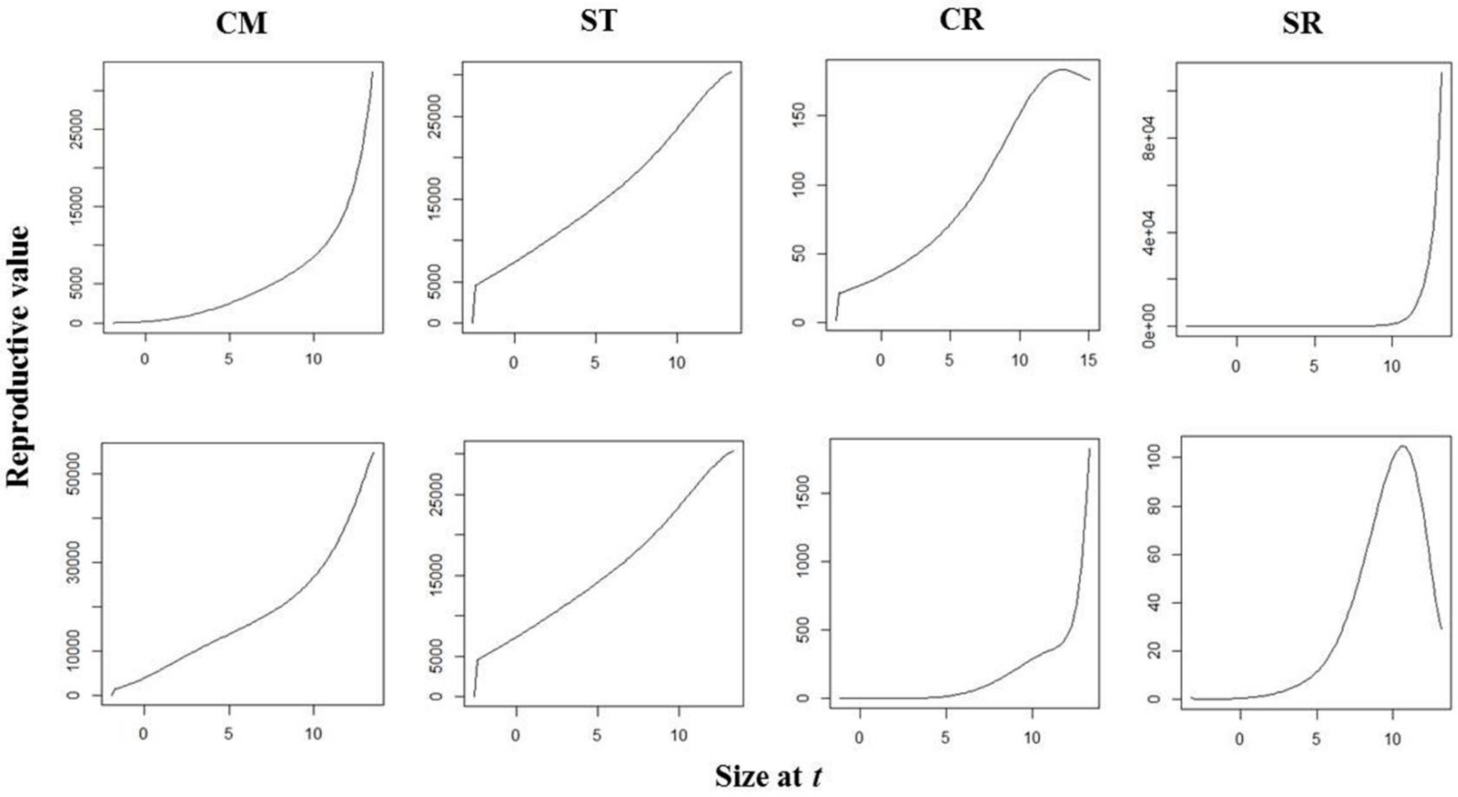

**Figure 6 – Relationship between plant size and reproductive value from 2013 to 2014 and from 2014 to 2015 (first and second row, respectively). Columns correspond to four of the six studied populations (see Table 1). The absence of recruitment (see Table 1A) in MO and SJ cause the impossibility to calculate reproductive values for those sites.**

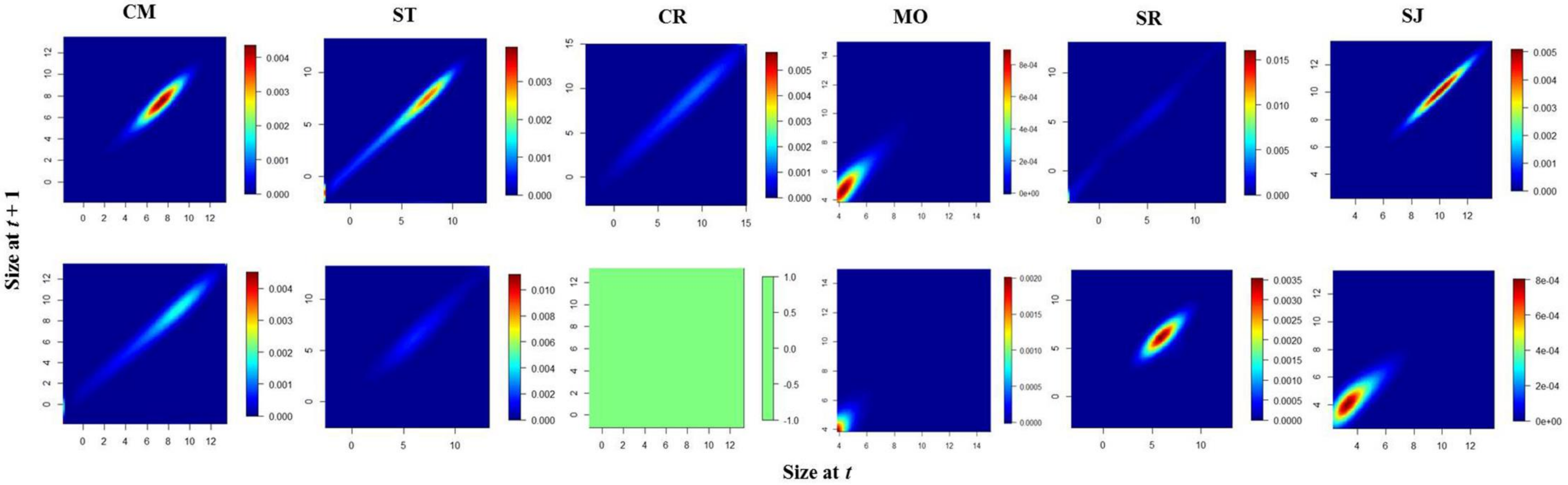

**Figure 7 – Relationship between plant size and elasticity from 2013 to 2014 and from 2014 to 2015 (first and second row, respectively). Columns correspond to the six studied populations (see Table 1). The x-axis represent plant size at *t*; the y-axis represent plant size at *t* + 1.**

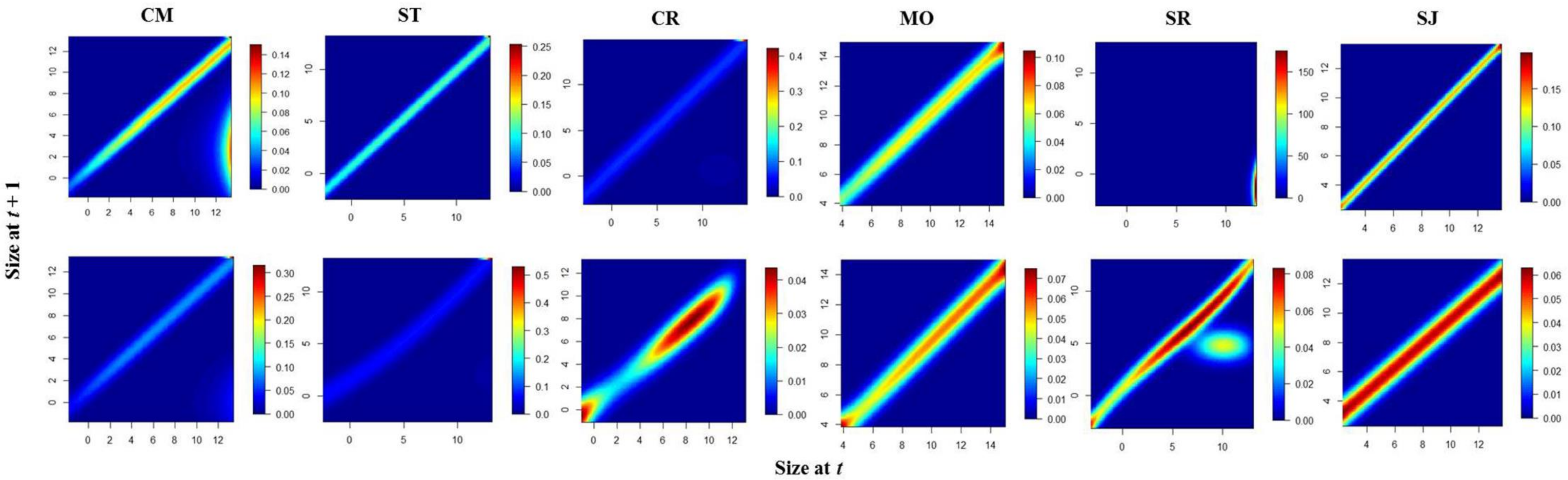

**Figure 8 – IPM kernels from 2013 to 2014 and from 2014 to 2015 (first and second row, respectively). Columns correspond to the six studied populations (see Table 1). The x-axis represent plant size at *t*; the y-axis represent plant size at *t* + 1.**

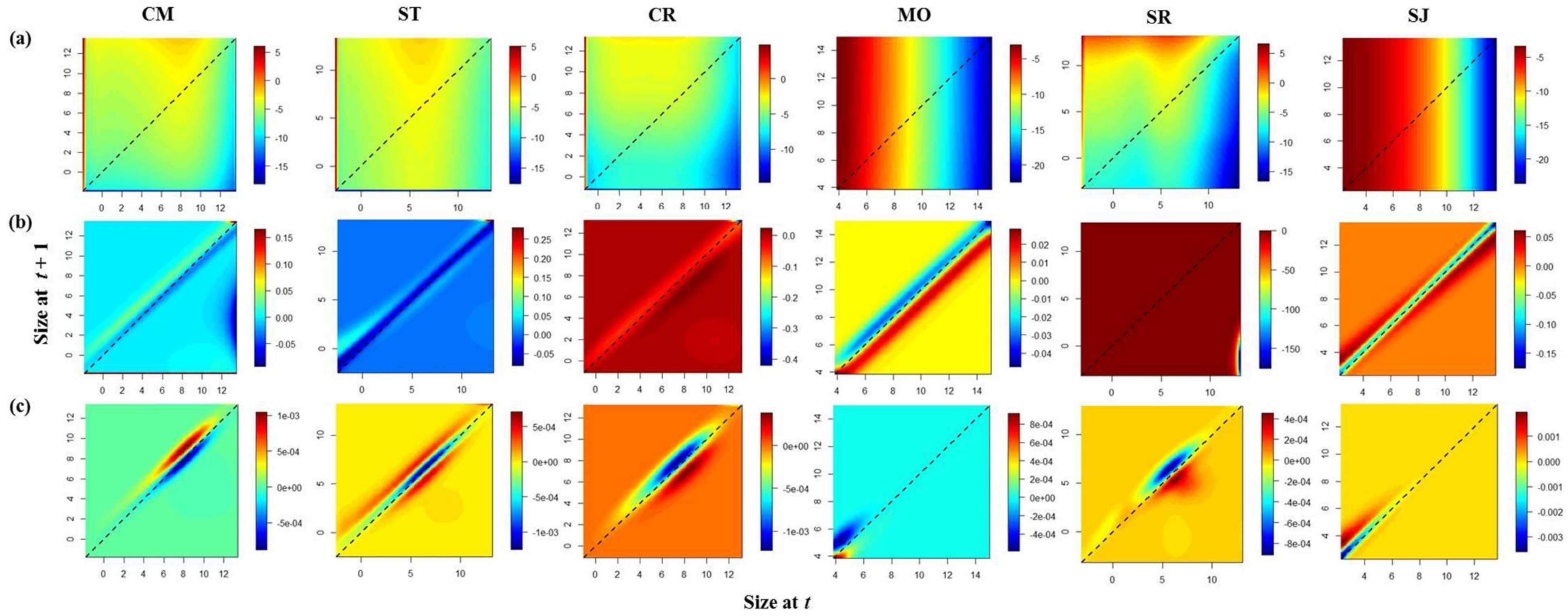

**Figure 9 – LTRE outputs. Columns correspond to the six studied populations (see Table 1). For each populations were represented: (a) Sensitivity of the arithmetic mean IPM kernel, including seed bank dynamics; (b) difference between the kernels 2013-2014 and 2014-2015; (c) contributions to the difference in λ between the two *H. caput-felis* IPMs. The x-axis represent plant size at *t*; the y-axis represent plant size at *t* + 1.**

The slight evidences in the bottom-right region represent the *F* portion of the kernel. The high peak was ca. 22,000 cm$^3$ (corresponding to log(10) in Figure 8), indicating that individuals of this size contributed most to reproduction. The *F* component is difficult to see in the kernels because its values are very low compared to those from *P* component, which dominate the IPMs.

Furthermore, it is important to draw attention to the absence of evidence in the bottom left area, which represents new-borns entering in the small size class. This absence means that only few seedlings were produced and few of this could survive to the following year, hence a high mortality of smaller plants. Note that the kernels don't show the discrete stage (seed bank) for display reasons, but it is included in all the analyses.

Furthermore, results showed that high damping ratios were found for both years in MO population and for SR and SJ populations in 2014-2015 transition (Table 2).

Sensitivity of the arithmetic mean IPM kernel included seed bank dynamics; its plots showed the importance of the emergence of seeds from the seed bank to λ (Figure 9a). The sensitivity of the mean IPM to changes in the emergence of seeds from the seed bank was high, with values that increase with plant size. Nevertheless, the colour graduation of new seeds into the seed bank denotes a low impact on λ. In fact, λ calculated considering only the continuous stage ($\lambda_{continuous}$) does not change from λ including the seed bank dynamics (λ; Table 2).

The seed bank dynamics were then cancelled in the kernel of differences (Figure 9b and 9c), and their contribution to the difference between $\lambda_{2013\text{-}2014}$ and $\lambda_{2014\text{-}2015}$ were null; those kernels show only the continuous stage. Through those plots it is possible to see the differences between the kernels of the two years transitions. Populations whose $\lambda_{2014\text{-}2015}$ was lower than $\lambda_{2013\text{-}2014}$ (CR, MO and SR) displayed a general difference both in survival/growth than in fecundity (Figure 9b). In particular, in the two Alicante's populations (CR and MO) a greater shrinkage of individuals in the second year of study was displayed, strongly emphasized in MO population, where the shrinkage involved both larger and small plants (whereas, in CR, only medium-large individuals shrink). Furthermore, a difference in fecundity between years in CR population was observed, with a total absence in elasticity of 2014. The absence of fecundity in the IPM was observed in both years in MO populations, in $SJ_{2013\text{-}2014}$ and in $CR_{2014\text{-}2015}$ populations. Clearly, the shrinkage of individuals in the second year of study of these two Iberian populations could be considered the main responsible of lowest λ. SR population represents an exception, because differences between years are due to the high fecundity of large plants in the first year.

In Sardinian populations a similar trend was observed, described by slight changes in fecundity of large plants and by a more emphasized growth of both smaller and large individuals, especially in CM population, which λ increases slightly in the second year of study (whereas ST stayed at equilibrium: λ = 1.01 in both years).

In addition to CM, SJ was another population whose $\lambda_{2014\text{-}2015}$ were higher than $\lambda_{2013\text{-}2014}$ ; differences among years are due to a change between stasis of all size individuals to a general growth in size involving a higher range of sizes, more marked in smaller plants, with shrinkage only in large individuals.

The contributions to differences in λ (Figure 9c) show how part of the life cycle of the study species are responsible for the differences in λ between 2013-2014 and 2014-2015. In Sardinian populations the higher $\lambda_{2014\text{-}2015}$ of CM is due to a high growth of medium and large individuals (while in the precedent year transition there was more shrinkage of plants of the same size). Instead, ST population present demographic equilibrium among year transition, but in 2014-2015 the stasis situation of plants involves a bigger range of size (Figure 9c). Conversely, in CR and MO there was a high shrinkage (of medium-large plants and of smaller plants, respectively), which have a stronger impact on $\lambda$ with respect to the growth of individuals in the past year transition. The same occur in SR population, where the highest shrinkage of medium size plants contributed most to the lowest $\lambda_{2014\text{-}2015}$. Instead, in SJ population the higher $\lambda_{2014\text{-}2015}$ is due to a strong growth of smallest plants with respect to the past year transition.

## Discussion

Population performance can be assessed using demographic models that result in an estimate of the long-term population growth rate (Ramula 2014). In fact, demographic census is a powerful tool for detecting and diagnosing decline of plants populations (Menges 1990; Keith 2002). Long-lived plant populations may be locally adapted and respond differentially to the same overall weather (García 2008).

*Helianthemum caput-felis* showed vital rates and population dynamics that varying among populations: some populations growth, some seems at equilibrium and some decline, showing high variability among them, depending from the zone of origin and environmental or stochastic factors.

Generally, the four basic demographic functions (i.e. growth, survival, probability of flowering and fruits output) were size dependent, and highlight that survival increase with size, smaller plants are generally more likely to grow than larger individuals and larger plants have higher fecundity, as already detected in a previous study at locality level on Sardinian populations (Fenu *et al.* 2015b).

Growth's pattern reflects that larger individuals typically acquire more resources than smaller conspecifics, which means they have more energy available to spend on growth, reproduction and maintenance (Rees *et al.* 2014). This occurs because, when individuals are small, maintenance costs increase slowly with size relative to acquisition, resulting in a positive relationship between size and absolute growth rate (Rees *et al.* 2014). Later in life, when individuals are large, maintenance costs increase more rapidly with size relative to acquisition, leading to a negative relationship between size and growth (Rees *et al.* 2014).

Similar patterns were found in others studies on the populations and dynamics of cliff-dwelling plants (Silva *et al.* 2015). Coastal plants are characterised by high local persistence (Lavergne *et al.* 2004; Thompson 2005), extraordinarily long-lived lifespan (Larson *et al.* 2000; García *et al.* 2002), and unusually stable population sizes (Morris and Doak 1998; Picó and Riba 2002; García 2003).

At regional level, Sardinian populations seems to have greater equilibrium ($\lambda$ closer to 1) than Spanish populations, as demonstrated by the correspondence between $\lambda$ and $\lambda_S$ for ST population and a slight increase in CM population over the years, even if with values always near 1 (see Table 1). This is probably due to the reached stable structure of population, mainly composed from large individuals, which produce more fruits per plant, then more seeds per fruit (Fenu *et al.* 2015b). Moreover, these results are in agreement with those obtained in Chapter 2, which underlines that Sardinian populations produce less empty fruits per plant compared to Spanish populations.

*Helianthemum caput-felis* populations dynamics seem to be in agreement with those founds in another Mediterranean coastal species, the narrow endemic *Centaurea horrida* Bad. (Asteraceae), a perennial sea-cliff plant restricted to the North of Sardinia and belonging to Habitat Directive (43/92/EEC). As discovered for this species through demographic analyses (Pisanu *et al.* 2012), populations of *H. caput-felis* are dominated by adults, and this, together with the slow growth of the plants (frequency of stasis), suggests that this species behaves as a typical long-lived Mediterranean plant: low colonization ability (Colas *et al.* 1997) and high local persistence (Lavergne *et al.* 2004; Thompson 2005; Pisanu *et al.* 2012). This pattern fortifies the hypothesis that *H. caput-*

*felis* in Sardinia is restricted in two main localities, which constitute “ecological islands” (acting as local refuges) in coastal areas strongly modified by human activity (Fenu *et al.* 2015b); over time they have provided stable ecological conditions and thus have enabled the persistence of narrow endemic species (Albert *et al.* 2001).

Conversely, eastern Iberian and Majorcan populations have a higher variability of population growth rates, both among different localities in the same year and different years of the same population. The higher λ of $MO_{2013\text{-}2014}$, $MO_{2014\text{-}2015}$ and $SJ_{2014\text{-}2015}$ populations were strongly influenced by the fecundity parameters that compose the elasticity matrix. In fact, there is a systematic variation in elasticity values of life cycle stages with population growth rate (de Kroon *et al.* 2000). Changing vital rate functions related to sexual reproduction (flower probability, fruits production and seed germination) had the largest impact on λ (Jacquemin *et al.* 2010). Therefore, a matrix with a high λ will likely have higher elasticity values for reproduction elements than a matrix for the same species with a low λ (Jongejans and de Kroon 2012). The larger sensitivity of population growth rate to changes in growth and survival of adult plants than to changes in reproduction is a property shares with many long-lived plants (Silvertown *et al.* 1993). In fact, for long-lived, iteroparous species, fecundity is generally less important for population growth than survival (Silvertown *et al.* 1993; Franco and Silvertown 2004; Ramula *et al.* 2008; Ramula 2014).

Furthermore, MO population shows a uniform shrinkage of individuals, probably due to a more intense fragmentation of the population.

The predicted stable size distributions of *H. caput-felis* populations were generally skewed heavily towards large adults plants. This indicates that, under deterministic conditions, *H. caput-felis* populations could include larger numbers of individuals with a large volume, as Sardinian population. Nevertheless, the predicted stable size distribution do not match with the observed size histogram distribution in CR, MO, $SR_{2013\text{-}2014}$ and in $SJ_{2014\text{-}2015}$ populations, fact further corroborated by the highs damping ratio, which meaning that the dominant stable stage distribution is reached fairly soon.

It has long been recognized that transient population dynamics greatly differ from asymptotic dynamics (Koons *et al.* 2006; Caswell 2007). Although asymptotic perturbation analyses are widely used in demographic studies (Caswell 2001), recently there is an increase of interest in transient rather than asymptotic dynamics, and transient dynamics are more relevant for what happens in real populations that are never in stable state due to stochastic dynamics or disturbances (Jongejans and de Kroon 2012), hence further analyses are needed.

In two extreme cases total elasticity was made up almost entirely of contributions from stasis transitions: $SJ_{2013\text{-}2014}$ and $CR_{2014\text{-}2015}$ populations (Table 2). In those populations fecundity completely fails (*F* component of elasticity = zero). At low values of λ delay mechanisms such as self-loops (stasis) and retrogression prevail at the expense of reproductive loops, which become important only at the higher population growth rates. These patterns imply that caution should be taken to base management on elasticities of a matrix of a single population at a single time, especially if a population is declining (Silvertown *et al.* 1996). In perennial plants, remnant populations with very little recruitment may only persist by the survival of some mature individuals (Eriksson 1996). Such situations would call for measures that enhance successful sexual reproduction, i.e. stimulation of life cycle parameters that have low, rather than high, elasticities (de Kroon *et al.* 2000).

Moreover, in semiarid and arid climates (as in the case of CR population) characterized by a high environmental stochasticity, perennial shrubs usually rely on their longevity to persist over time (García and Zamora 2003), because the effort invested in new plant establishment seems to be very high and the allocation of energy to stress tolerance mechanisms is supposed to increase survival but to reduce energy for reproduction (Escós *et al.* 2000; Aragón *et al.* 2009). Hence, the different population dynamics observed among the analysed localities of *H. caput-felis* should be correlated to particular microclimate and ecological conditions, i.e. the high aridity of CR and the sand substrate of SR (Chapter 2), which affects the relationship among plant size and vital rates.

The absence of recruitment is validated by the LTRE analysis. Contributions to the difference between $\lambda_{2013\text{-}2014}$ and $\lambda_{2014\text{-}2015}$ highlights that the difference in shrinkage is the leading cause of the difference in $\lambda$ in Spanish populations. As demonstrated in a recent study analysing vital rates of more than 1,000 plant species (including species with complex life-history traits), retrogressive growth (shrinkage) correlates negatively with reproductive traits, in agreement with the frequent increase in reproductive output with plant size (Salguero-Gómez *et al.* 2016).

Considering only the continuous stage, that is, excluding the seed bank stage ($\lambda_{\text{continuous}}$), population growth rates do not change from the total population growth rate $\lambda$, except in $\text{CR}_{2014\text{-}2015}$ population, where $\lambda$ continuous had a value inferior to $\lambda$.

Iteroparous perennial plants optimise the conservative strategy, investing in vegetative tissues at the expense of reproduction (Wiegand *et al.* 1995; Wiegand *et al.* 2004). If water availability is limited, perennials could fail to survive their first stressful period (Sánchez and Peco 2007); hence seed availability and seedling establishment are critical for persistence in arid environments. Seed availability limitations are most likely to occur at low conspecific densities where suitable sites for germination are plentiful (Eriksson and Ehrlen 1992). At high conspecific densities, seedling completion in suitable microsites may lead to populations becoming limited by establishment (Maron and Gardner 2000), that seems to be the critic stage of this species (Fenu *et al.* 2015b). Furthermore, it must be taken into account the low germination of *H. caput-felis* (Tébar *et al.* 1997), attributed to physical exogenous dormancy, a widespread trait among the Cistaceae (Thanos *et al.* 1992).

Lastly, *H. caput-felis*' populations have variable demography behaviour, close to the equilibrium where the stable size distribution is reached (Sardinian populations), while in a slight decline in populations where fecundity is the most important vital rate for the population growth rate.

Finally, this study confirm the general pattern shows within species, under which $\lambda$ over 1 are associated with a higher contribution of sexual reproduction, whereas populations with low $\lambda$ rely more on survival (Oostermeijer *et al.* 1996; Menges and Dolan 1998; Valverde and Silvertown 1998; Jongejans and de Kroon 2005). *H. caput-felis* populations in demographic equilibrium, as the Sardinians', could be locally adapted to ecological and microclimate conditions.

Furthermore, it must be taken into account that it takes some years with transient dynamics before asymptotic dynamics are reached (Jongejans and de Kroon 2012), hence further analyses are clearly needed, in order to reflect a more realistic situation.

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## References

Albert M.J., Escudero A. and Iriondo J.M. 2001. Female reproductive success of narrow endemic *Erodium paularense* in contrasting microhabitats. Ecology 82: 1734–1747.

Aragón C.F., Méndez M. and Escudero A. 2009. Survival costs of reproduction in a short-lived perennial plant: live hard, die young. American Journal of Botany 96: 904–911.

Arrigoni P.V. 1971. *Helianthemum caput-felis* Boiss. (2n=24) nuovo reperto per la flora italiana. Webbia 26: 237–243.

Bacchetta G., Fenu G. and Mattana E. 2012. A checklist of the exclusive vascular flora of Sardinia with priority rankings for conservation. Anales del Jardín Botánico de Madrid 69: 81–89.

Bassar R.D., Lopez-Sepulcre A., Reznick D.N. and Travis J. 2013. Experimental evidence for density-dependent regulation and selection on Trinidadian Guppy life histories. The American Naturalist 181: 25–38.

Bruna E.M., Izzo T.J., Inouye B.D. and Vasconcelos H.L. 2014. Effect of mutualist partner identity on plant demography. Ecology 95: 3237–3243.

Cardona M.A. and Contandriopoulos J. 1979. Endemism and evolution in the islands of the Western Mediterranean. In: Bramwell D. (Ed.), Plants and Islands. Academic Press, London, 133–169.

Caswell H. 1989. Analysis of life table response experiments I. Decomposition of effects on population growth rate. Ecological Modelling 46: 221–237.

Caswell H. 1996a. Second derivatives of population growth rate: calculation and applications. Ecology 77: 870–879.

Caswell H. 1996b. Analysis of life table response experiments II. Alternative parameterizations for size- and stage-structured models. Ecological Modelling 88: 73–82.

Caswell H. 2001. Matrix population models: construction, analysis, and interpretation. Sinauer, Sunderland, MA.

Caswell H. 2007. Sensitivity analysis of transient population dynamics. Ecology Letters 10: 1–15.

Caswell H. 2010. Life table response experiment analysis of the stochastic growth rate. Journal of Ecology 98: 324–333.

Cavers P.B. 1983. Seed demography. Canadian Journal of Botany 61: 3578–3590.

Childs D.Z., Rees M., Rose K.E., Grubb P.J. and Ellner S.P. 2003. Evolution of complex flowering strategies: an age–and size–structured integral projection model. Proceedings of the Royal Society of London B: Biological Sciences 270: 1829–1838.

Colas B., Olivieri I. and Riba M. 1997. *Centaurea corymbosa*, a cliff-dwelling species tottering on the brink of extinction: a demographic and genetic study. Proceedings of the National Academy of Sciences 94: 3471–3476.

Coulson T. 2012. Integral projections models, their construction and use in posing hypotheses in ecology. Oikos 121: 1337–1350.

Crouse D.T., Crowder L.B. and Caswell H. 1987. A stage-based population model for loggerhead sea turtles and implications for conservation. Ecology 68: 1412–1423.

Cursach J. and Rita J. 2014. *Naufraga balearica*, a threatened narrow endemism of the Balearic Islands (western Mediterranean basin): assessing the population dynamics of two subpopulations. Plant Species Biology 29: 192–201.

Cursach J., Besnard A., Rita J., Fréville H. 2013. Demographic variation and conservation of the narrow endemic plant *Ranunculus weyleri*. Acta Oecologica 53: 102–109.

Dauer J.T. and Jongejans E. 2013. Elucidating the Population Dynamics of Japanese Knotweed Using Integral Projection Models. PLoS ONE 8:e75181.

de Kroon H., van Groenendael J. and Ehrlén J. 2000. Elasticities: a review of methods and model limitations. Ecology 81: 607–618.

del Cacho M., Saura-Mas S., Estiarte M., Peñuelas J. and Lloret F. 2012. Effect of experimentally induced climate change on the seed bank of a Mediterranean shrubland. Journal of Vegetation Science 23: 280–291.

Doak D.F., Kareiva P. and Klepetka B. 1994. Modeling population viability for the desert tortoise in the Western Mojave desert. Ecological Applications 4:446–460.

Doak D.F. 1992. Lifetime impacts of herbivory for a perennial plant. Ecology 73: 2086–2099.

Easterling M.R., Ellner S.P. and Dixon P.M. 2000. Size-specific sensitivity: applying a new structured population model. Ecology 81: 694–708.

Ellner S.P. and Rees M. 2006. Integral projection models for species with complex demography. The American Naturalist 167: 410–428.

Eriksson O. 1996. Regional dynamics of plants: a review of evidence for remnant, source-sink and metapopulations. Oikos 77: 248–258.

Eriksson O. and Ehrlen J. 1992. Seed and microsite limitation of recruitment in plant populations. Oecologia 91: 360–364.

Escós J., Alados C.L., Pugnaire F.J., Puigdefábregas J. and Emlen J. 2000. Stress resistance strategy in an arid land shrub: Interactions between developmental instability and fractal dimension. Journal of Arid Environments 45: 325–336.

Fenu G., Cogoni D., Pinna M.S. and Bacchetta G. 2015a. Threatened Sardinian vascular flora: A synthesis of 10 years of monitoring activities. Plant Biosystems 149: 473–482.

Fenu G., Cogoni D., Sulis E. and Bacchetta G. 2015b. Ecological response to human trampling and conservation status of *Helianthemum caput-felis* (Cistaceae) at the eastern periphery of its range. Botany Letters 162: 191–201.

Ferrandis P., Herranz J.M. and Martínez-Sánchez J.J. 1999. Effect of fire on hard-coated Cistaceae seed banks and its influence on techniques for quantifying seed banks. Plant Ecology 144: 103–114.

Ferrer-Cervantes M.E., Méndez-González M.E., Quintana-Ascencio P.F., Dorantes A., Dzib G. and Durán R. 2012. Population dynamics of the cactus *Mammillaria gaumeri*: an integral projection model approach. Population Ecology 54: 321–334.

Franco M. and Silvertown J. 2004. A comparative demography of plants based upon elasticities of vital rates. Ecology 85: 531–538.

García D. and Zamora R. 2003. Persistence, multiple demographic strategies and conservation in long-lived Mediterranean plants. Journal of Vegetation Science 14: 921–926.

García M.B. 2003. Demographic viability of a relict population of the critically endangered plant *Borderea chouardii*. Conservation Biology 17: 1672–1680.

García M.B. 2008. Life history and population size variability in a relict plant. Different routes towards long-term persistence. Diversity and Distributions 14: 106–113.

García M.B., Guzmán, D. and Goni D. 2002. An evaluation of the status of five threatened plant species in the Pyrenees. Biological Conservation 103: 151–161.

González E.J., Rees M. and Martorell C. 2012. Identifying the demographic processes relevant for species conservation in human-impacted areas: does the model matter? Oecologia 171: 347–356.

Griffith A.B., Salguero-Gómez R., Merow C. and McMahon S. 2016. Demography beyond the population. Journal of Ecology 104: 271–280.

Harper J.L. 1977. Population biology of plants. Academic Press, New York, NY, US.

Heppell S.S., Walters J.R. and Crowder L.B. 1994. Evaluating management alternatives for red-cockaded woodpeckers: a modeling approach. Journal of Wildlife Management 58: 479–487.

Heywood V.H. and Iriondo J.M. 2003. Plant conservation: old problems, new perspectives. Biological conservation 113: 321–335.

Iliadou E., Kallimanis A.S., Dimopoulos P. and Panitsa M. 2014. Comparing the two Greek archipelagos plant species diversity and endemism patterns highlight the importance of isolation and precipitation as biodiversity drivers. Journal of Biological Research-Thessaloniki 21: 16.

Jacquemyn H., Brys R. and Jongejans E. 2010. Size-dependent flowering and costs of reproduction affect population dynamics in a tuberous perennial woodland orchid. Journal of Ecology 98: 1204–1215.

Jacquemyn H., Brys R., Davison R., Tuljapurkar S. and Jongejans E. 2012. Stochastic LTRE analysis of the effects of herbivory on the population dynamics of a perennial grassland herb. Oikos 121: 211–218.

Jongejans E. and de Kroon H. 2005. Space versus time variation in the population dynamics of three co-occurring perennial herbs. Journal of Ecology 93: 681–692.

Jongejans E. and de Kroon H. 2012. Matrix models. In: Encyclopedia of Theoretical Ecology (eds. Hastings A. and Gross L.). University of California, pp. 415–423.

Jongejans E., Shea K., Skarpaas O., Kelly D. and Ellner S.P. 2011. Importance of individual and environmental variation for invasive species spread: a spatial integral projection model. Ecology 92: 86–97.

Keith D.A. 2002. Population dynamics of an endangered heathland shrub, *Epacris stuartii* (Epacridaceae): recruitment, establishment and survival. Austral Ecology 27: 67–76.

Kerr N.Z., Baxter P.W., Salguero-Gómez R., Wardle G.M. and Buckley Y.M. 2016. Prioritizing management actions for invasive populations using cost, efficacy, demography and expert opinion for 14 plant species world-wide. Journal of Applied Ecology 53: 305–316.

Koons D.N., Rockwell R.F. and Grand J.B. 2006. Population momentum: implications for wildlife management. Journal of Wildlife Management 70: 19–26.

Kull T., Sammul M., Kull K., Lanno K., Tali K., Gruber B., Schmeller D. and Henle K. 2008. Necessity and reality of monitoring threatened European vascular plants. Biodiversity and Conservation 17: 3383–3402.

Larson D.W., Matthes U., Gerrath J.A., Larson N.W.K., Gerrath J.M., Nekola J.C., Walker G.L, Porembski S. and Charlton A. 2000. Evidence for the widespread occurrence of ancient forests on cliffs. Journal of Biogeography 27: 319–331.

Lavergne S., Thompson J.D., Garnier E. and Debussche M. 2004. The biology and ecology of narrow endemic and widespread plants: A comparative study of trait variation in 20 congeneric pairs. Oikos 107: 505–518.

Li S.-L., Yu F.-H., Werger M.J., Dong M. and Zuidema P.A. 2011. Habitat-specific demography across dune fixation stages in a semi-arid sandland: understanding the expansion, stabilization and decline of a dominant shrub. Journal of Ecology 99: 610–620.

López González G. 1992. Apuntes para justificar el tratamiento del género *Helianthemum* Miller, s.l. (Cistaceae), en Flora Iberica. Anales del Jardín Botánico de Madrid 50: 35–63.

Maron J.L. and Gardner S.N. 2000. Consumer pressure, seed versus safe-site limitation, and plant population dynamics. Oecologia 124: 260–269.

Marsh D.M. and Trenham P.C. 2008. Current trends in plant and animal population monitoring. Conservation Biology 22: 647–655.

Médail F. and Quézel P. 1997. Hot-spots analysis for conservation of plant biodiversity in the Mediterranean Basin. Annals of the Missouri Botanical Garden 84: 112–127.

Médail F. and Quézel P. 1999. Biodiversity hotspots in the Mediterranean Basin: setting global conservation priorities. Conservation Biology 13: 1510–1513.

Médail P. and Diadema K. 2009. Glacial refugia influence plant diversity patterns in the Mediterranean Basin. Journal of Biogeography 36: 1333–1345.

Menges E. 1990. Population viability analysis for an endangered plant. Conservation Biology 4: 52–62.

Merow C., Latimer A.M., Wilson A.M., McMahon S.M., Rebelo A.G. and Silander J.A. Jr 2014b. On using integral projection models to generate demographically driven predictions of species' distributions: development and validation using sparse data. Ecography 37: 1167–1183.

Metcalf C.J.E., McMahon S.M., Salguero-Gómez R. and Jongejans E. 2013. IPMpack: an R package for integral projection models. Methods in Ecology and Evolution 4: 195–200.

Metcalf C.J.E., McMahon S.M., Salguero-Gómez R., Jongejans E. and Merow C. 2014. IPMpack: an R package for demographic modeling with Integral Projection Models (v. 2.1).

Morris W.F. and Doak D.F. 1998. Life history of the long-lived gynodioecious cushion plant *Silene acaulis* (Caryophyllaceae), inferred from size-based population projection matrices. American Journal of Botany 85: 784–784.

Morris W.F. and Doak D.F. 2002. Quantitative conservation biology: The theory and practice of population viability analysis. Sunderland, MA: Sinauer Associates.

Nicolè F., Dahlgren J.P., Vivat A., Till-Bottraud I. and Ehrlén J. 2011. Interdependent effects of habitat quality and climate on population growth of an endangered plant. Journal of Ecology 99: 1211–1218.

Nikolić T., Antonić O., Alegro A.L., Dobrović I., Bogdanović S., Liber Z. and Rešetnik I. 2008. Plant species diversity of Adriatic islands: An introductory survey. Plant Biosystems 142: 435–445.

Nychka D., Furrer R. and Sain S. 2014. fields: Tools for spatial data. R package version 7.1. http://CRAN.R-project.org/package=fields

Obeso J.R. 2002. The cost of reproduction in plants. New Phytologist 155: 321–348.

Picó F.X. and Riba M. 2002. Regional-scale demography of *Ramonda myconi*: Remnant population dynamics in a preglacial relict species. Plant Ecology 161: 1–13.

Pino J., Picó F.X. and De Roa E. 2007. Population dynamics of the rare plant *Kosteletzkya pentacarpos* (Malvaceae): a nine-year study. Botanical Journal of the Linnean Society 153: 455–462.

Pisanu S., Farris E., Filigheddu R. and García M.B. 2012. Demographic effects of large, introduced herbivores on a long-lived endemic plant. Plant Ecology 213: 1543–1553.

Quintana-Ascencio P.F., Dolan R.W. and Menges E.S. 1998. *Hypericum cumulicola* demography in unoccupied and occupied Florida scrub patches with different time-since-fire. Journal of Ecology 86: 640–651.

R Core Team 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Ramula S. 2008. Responses to the timing of damage in an annual herb: fitness components versus population performance. Basic and Applied Ecology 9: 233–242.

Ramula S. 2014. Linking vital rates to invasiveness of a perennial herb. Oecologia 174: 1255–1264.

Ramula S., Rees M. and Buckley Y.M. 2009. Integral projection models perform better for small demographic data sets than matrix population models: a case study of two perennial herbs. Journal of Applied Ecology 46: 1048–1053.

Rees M. and Rose K.E. 2002. Evolution of flowering strategies in *Oenothera glazioviana*: an integral projection model approach. Proceedings of the Royal Society B: Biological Sciences 269: 1509–1515.

Rees M., Childs D.Z. and Ellner S.P. 2014. Building integral projection models: a user's guide. Journal of Animal Ecology 83: 528–545.

Rodríguez-Pérez J. 2005. Breeding system, flower visitors and seedling survival of two endangered species of *Helianthemum* (Cistaceae). Annals of Botany 95: 1229–1236.

Salguero-Gómez R. 2014. Appendix C: IPMs for complex life cycles. In: Merow C., Dahlgren J.P., Metcalf C.J.E., Childs D.Z., Evans M.E.K., Jongejans E., Record S., Rees M., Salguero-Gómez R. and. McMahon S.M. 2014. Appendices Advancing population ecology with integral projection models: a practical guide. Methods in Ecology and Evolution 5: 99–110.

Salguero-Gómez R., Jones O.R., Jongejans E., Blomberg S.P., Hodgson D J., Mbeau-Ache C., Zuidema P.A., de Kroon H. and Buckley Y.M. 2016. Fast–slow continuum and reproductive strategies structure plant life-history variation worldwide. Proceedings of the National Academy of Sciences 113: 230–235.

Sánchez A.M. and Peco B. 2007. Lack of recruitment in *Lavandula stoechas* subsp. *pedunculata*: a case of safe-site limitation. Acta Oecologica 31: 32–39.

Schemske D.W., Husband B.C., Ruckelshaus M.H., Goodwillie C., Parker I.M. and Bishop J.G. 1994. Evaluating approaches to the conservation of rare and endangered plants. Ecology 75: 584–606.

Silva J.L., Mejías J.A. and García M.B. 2015. Demographic vulnerability in cliff-dwelling Sonchus species endemic to the western Mediterranean. Basic and Applied Ecology 16: 316–324.

Silvertown J., Franco M. and Menges E. 1996. Interpretation of elasticity matrices as an aid to the management of plant populations for conservation. Conservation Biology 10: 591–597.

Silvertown J., Franco M., Pisanty I. and Mendoza A. 1993. Comparative plant demography-relative importance of life-cycle components to the finite rate of increase in woody and herbaceous perennials. Journal of Ecology 81: 465–476.

Tébar F.J., Gil L. and Llorens L. 1997. Reproductive biology of *Helianthemum apenninum* (L.) Mill. and *H. caput-felis* Boiss. (Cistaceae) from Mallorca (Balearic Islands, Spain). Acta Botánica Malacitana 22: 53–63.

Thanos C.A., Georghiou K., Kadis C. and Pantazi C. 1992. Cistaceae: a plant family with hard seeds. Israel Journal of Botany 41: 251–263.

Thompson J.D. 2005. Plant Evolution in the Mediterranean. USA: Oxford University Press.

Venable D.L. and Brown J.S. 1988. The selective interactions of dispersal, dormancy and seed size as adaptations for reducing risks in variable environments. American Naturalist 131: 360–384.

Warr S.J., Thompson K. and Kent M. 1993. Seed banks as a neglected area of biogeographic research: a review of literature and sampling techniques. Progress in Physical Geography 17: 329–347.

Wiegand K., Jeltsch F. and Ward D. 2004. Minimum recruitment frequency in plants with episodic recruitment. Oecología 141: 363–372.

Wiegand T., Milton S.J. and Wiessel C. 1995. A simulation model for a shrub ecosystem in the semiarid Karoo, South Africa. Ecology 76: 203–2229.

Williams J.L. 2009. Flowering life-history strategies differ between the native and introduced ranges of a monocarpic perennial. American Naturalist 174: 660–672.

Williams J.L. and Crone E.E. 2006. The impact of invasive grasses on the population growth of *Anemone patens*, a long-lived native forb. Ecology 87: 3200–3208.

Williams J.L., Auge H. and Maron J.L. 2010. Testing hypotheses for exotic plant success: parallel experiments in the native and introduced ranges. Ecology 91: 1355–1366.

Williams J.L., Miller T.E. and Ellner S.P. 2012. Avoiding unintentional eviction from integral projection models. Ecology 93: 2008–2014.

Yule K.M., Miller T.E.X. and Rudgers J.A. 2013. Costs, benefits, and loss of vertically transmitted symbionts affect host population dynamics. Oikos 122: 1512–1520.

Zuidema P.A., Jongejans E., Chien P.D., During H.J. and Schieving F. 2010. Integral projection models for trees: a new parameterization method and a validation of model output. Journal of Ecology 98: 345–355.

# Appendix

**Table 1A – Reproductive values of *H. caput-felis* calculated in order to estimate the IPM's parameters (fec2, fec3 and fec4).**

**2013**

| population code | % aborted | N. counted fruits | N. aborted | N. viable fruits | mean seeds per fruit (fec2) | estimated seed bank | seedlings | establishment (fec3) | seedling survival (fec4) |
|---|---|---|---|---|---|---|---|---|---|
| **CM** | 15.5 | 10698 | 1658.19 | 9039.81 | 4.07 | 36792.03 | 21 | 0.000570776 | 0.67 |
| **ST** | 16 | 11011 | 1761.76 | 9249.24 | 4.36 | 40326.69 | 5 | 0.000123987 | 0.8 |
| **CR** | 22 | 181 | 39.82 | 141.18 | 3.86 | 544.95 | 12 | 0.022020175 | 0.83 |
| **MO** | 37 | 5551 | 2053.87 | 3497.13 | 4.37 | 15282.46 | 0 | 0 | 0 |
| **SR** | 48.89 | 146 | 71.38 | 74.62 | 4.24 | 316.39 | 25 | 0.079016068 | 0.72 |
| **SJ** | 8 | 1003 | 80.24 | 922.76 | 3.85 | 3552.63 | 0 | 0 | 0 |

**2014**

| population code | % aborted | N. counted fruits | N. aborted | N. viable fruits | mean seeds per fruit (fec2) | estimated seed bank | seedlings | establishment (fec3) | seedling survival (fec4) |
|---|---|---|---|---|---|---|---|---|---|
| **CM** | 14.00 | 19506 | 2730.84 | 16775.16 | 4.24 | 71126.68 | 11 | 0.000154654 | 0.82 |
| **ST** | 17.00 | 20092 | 3415.64 | 16676.36 | 4.67 | 77856.50 | 11 | 0.000141286 | 1 |
| **CR** | 39.33 | 6798 | 2673.65 | 4124.35 | 3.23 | 13321.64 | 10 | 0.000750658 | 0.4 |
| **MO** | 50.00 | 3259 | 1629.50 | 1629.50 | 4.27 | 6957.97 | 0 | 0 | 0 |
| **SR** | 42.86 | 2584 | 1107.50 | 1476.50 | 4.30 | 6348.94 | 57 | 0.008977877 | 0.58 |
| **SJ** | 39.17 | 1254 | 491.19 | 762.81 | 3.82 | 2913.93 | 0 | 0 | 0 |

# CHAPTER 5

## Conservation status at global level of *Helianthemum caput-felis* (Cistaceae), a plant of international interest

### Introduction

Countries contracting the Convention on Biological Diversity (CBD 1992) and other international strategies (e.g., GSPC, EPCS, Planta Europa) are obliged to monitor biodiversity in order to protect wild fauna and flora. In particular, in Europe, the "Council Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora" (Habitat Directive, hereafter) and the Natura 2000 network are by far the most important conservation efforts implemented to date (e.g., Maiorano *et al.* 2007, 2015; Bastian 2013; Pimm *et al.* 2014; Fenu *et al.* 2015a). Annex II of Habitat Directive lists the "animal and plant species of community interest whose conservation requires the designation of special areas of conservation", and although it is not a conservation target list itself, it represents a European list of species for which *in situ* conservation actions should be activated. These conservation measures, such as the protection and restoration of natural habitats, are the best suggested methods of preserving plant diversity (CBD 1992). The importance of *in situ* conservation of endangered plant species has been highlighted also by the Target 7 of the GSPC for 2020, which scheduled that at least 75% of known threatened plant species should be conserved *in situ* (e.g., GSPC 2008; Fenu *et al.* 2015c; Heywood 2015).

Monitoring and reporting the conservation status of species listed in the Habitat Directive is mandatory for all of the signatory EU Member States (European Commission 1992; Rossi *et al.* 2015; Fenu *et al.* 2015a, 2015c). Regardless those international regulatory requirements, an increasing number of species face risk of extinction because they occur in very specific or highly fragmented areas, have a reduced number of populations or individuals, or are declining (World Conservation Union 1994; Pimm 1995; Novacek and Cleland 2001). The persistence of a population depends on a numerical equilibrium over time, whatever the schedules of fecundity, recruitment and survival, and the integration of such parameters in simulation models allows exploration of their relative importance (Schemske *et al.* 1994; Caswell 2001; Morris and Doak 2002; García 2003; Adler *et al.* 2014).

Quantifying the demographic behaviour of a species is a key element for understanding its present status and to explore possible scenarios of conservation or management (González *et al.* 2016). Demographic data are necessary to analyse species' vital rates, of which the most strongly correlated with changes in population growth rate are often the object of management actions to increase census population size (Reed *et al.* 2009; Johnson *et al.* 2010; Andrello *et al.* 2012). Viable populations are those that have a suitably low chance of going extinct before a specified future time (Morris and Doak 2002). Population viability analysis (PVA) is a modelling tool that estimates the future size and risk of extinction for populations (Brook *et al.* 1997, 2000). PVA works by using life history or population growth rate data to parameterize a population model that is then used to project dynamics and estimate future population size and structure (Ludwig 1999; Akçakaya 2000; Brigham and Thomson 2003; Rueda-Cediel *et al.* 2015). However, in real-world populations, vital rates are unlikely to remain constant over time, so adding stochasticity (both demographic and environmental) into the population models allows more realistic projections; this can be achieved by giving to the vital rate values that at each time step are taken from distribution based on mean rate values and process variance (Morris and Doak 2002; Mills 2007). Small but stable populations may be at lower risk than larger populations subjected to strong sources of variability, so a better approach is to perform analyses of population dynamics that can assess the stochastic population growth rate (Morris and Doak 2002).

To develop a conservation strategy for a species, assessment of conservation status is the first step (Planta Europa 2008; Fenu *et al.* 2015c; Rossi *et al.* 2015). Identifying the key traits linked to high extinction risk is critical for predicting future declines and provides an opportunity for implementing preemptive conservation measures (Cardillo *et al.* 2006). The most widely known quantitative system of classifying imperilled species is the International Union for Conservation of Nature's (IUCN) Red List Categories and Criteria (IUCN 2001), which includes various categories and time horizons (in both years and generation time) in its categorization system (Mace *et al.* 2008; Fenu *et al.* 2011, 2012; Rossi *et al.* 2014, 2015). In addition, the IUCN also considers quantifiable determinants of extinction risk (i.e., population trends, population size, and geographic range). In a recent evaluation of endangered species categorization systems to determine which had the most desirable characteristics, the IUCN categorization system ranked highest (de Grammont and Cuarón 2006).

Geographic range size, and how it changes through time, is one of the fundamental ecological and evolutionary characteristics of a species (Gaston 2003). Perhaps most immediately, however, range size is a strong predictor of extinction risk (Gaston and Fuller 2009).

The IUCN Red Lists provide reliable and rapid tools to evaluate the extinction risk of species (Moreno *et al.* 2003); IUCN criteria are clearly defined, scientifically sound, adaptable from global to local scale and based on quantitative data, which makes the Red Lists the most used assessment system all over the world (de Grammont and Cuarón 2006; Rodrigues *et al.* 2006; Hoffman *et al.* 2008; Rossi *et al.* 2015).

Although the evaluation of the conservation status of species listed in the Habitats Directive is mandatory for EU member states (Dimopoulos *et al.* 2005; Iliadou *et al.* 2014; Rossi *et al.* 2015; Fenu *et al.* 2015c), only in the last decade the efforts to assess species conservation status have increased, both at global, regional and local levels (e.g., Gargano *et al.* 2007; Fenu *et al.* 2011, 2012, 2015c; Rossi *et al.* 2015).

The Mediterranean Basin hosts a flora of around 25–35,000 flowering plants and ferns (Fenu *et al.* 2015a) and has been identified as 1 of 34 biodiversity hotspots of the world (Mittermeier *et al.* 2005). The Mediterranean flora shows a high rate of regional endemism (close to 60%) of all native *taxa* being Mediterranean endemics, half of which correspond to narrow endemic species (Thompson 2005; Fenu *et al.* 2015a). In this region, rocky habitats shelter many endemic and endangered plant (Domínguez Lozano *et al.* 1996; Médail and Verlaque 1997; Cañadas *et al.* 2014; Fenu *et al.* 2014) for which demographic data is lacking (e.g., García 2003).

In addition, several Mediterranean plants show a fragmented distribution, in particular those related to coastal environment. Due to its highly fragmented distribution, border populations are usually considered more vulnerable and are more prone to local extinction because of their isolation and restriction to marginal habitats (e.g. Gargano *et al.* 2007; Del Vecchio *et al.* 2012; Villellas *et al.* 2013a, 2013b). In recent years, several plants species with peripheral and isolated populations (PIPPs) in the Mediterranean Basin that show outlying populations isolated ecologically and geographically from the rest of their distribution range have been investigated (e.g., Gargano *et al.* 2007; Del Vecchio *et al.* 2012; Fenu *et al.* 2015b). Due to their ecological ad genetic importance, as suggested by international organisations (IUCN, European Council), PIPPs should be considered as an important resource for biodiversity and thus included in conservation actions (Abeli *et al.* 2009).

*Helianthemum caput-felis* is a coastal species with peripheral and isolated populations, distributed throughout the western Mediterranean Basin (south-eastern Iberian Peninsula, Majorca, Sardinia and northwest Africa) in several fragmented populations (Fenu *et al.* 2015b and reference therein).

*Helianthemum caput-felis* deserves particular attention because it is considered the only extant representative of an ancient lineage (Arrigoni 1971; López-González 1992; Fenu *et al.* 2015b). This species is particulary interesting because it is protected by European and regional (Spanish and Italian) regulations. In fact, it is included in the Bern Convention (Convention on the Conservation of European Wildlife and Natural Habitats), and in the Habitats Directive; in addition, *H. caput-felis* is listed as Endangered in the European Red List (Bilz *et al.* 2011) and, at regional level, is considered Critically Endangered (CR) in Italy (Fenu *et al.* 2015b; Rossi *et al.* 2015) and Endangered (EN) in Spain (Bañares *et al.* 2010).

Until now, the evaluation of the *H. caput-felis*’ conservation status carried out using IUCN parameters was only based on geographical information. For example, Marcer *et al.* (2013) estimate the EOO and AOO, on the basis of available bibliographic and *Herbaria* records; the results indicate a distribution thresholds compatible with Endangered and Vulnerable categories, respectively. However, although local and partial studies have been carried out (e.g., Fenu *et al.* 2015b; Agulló *et al.* 2010; Rossi *et al.* 2015), no data at global level is actually available for this priority interest species in Europe.

The main aim of this study was to detect the comprehensive assessment of *H. caput-felis* at global scale using distributive data and population dynamics models. Particularly, the objectives of this study were: 1) to determine species distributions by analyse the populations, 2) to quantify population sizes through censuses and to calculate population trends, 3) to identify the main threat to its persistence in natural habitats, 4) to assess the global status of *H. caput-felis*, and 5) to propose possible conservation measures in order to preserve the persistence of those populations or reduce the threats impact.

## Materials and methods

### *Study species*

*Helianthemum caput-felis* Boiss. is a long-lived half shrub tall 35(50) cm. Its flowers are hermaphroditic, and have a short lifespan (3–4 days, Rodríguez-Pérez 2005). Based on studies carried out on some Spanish and Sardinian populations, the flowering period is from March to June, and the fruiting season runs from late April to beginning of August (Chapter 1). Fruits are capsules that detach at maturation, and seed germination takes place in autumn, at the onset of the rainy season (Rodríguez-Pérez 2005; Fenu *et al.* 2015b and references therein; Chapter 1).

The life cycle of *H. caput-felis* includes a continuous stage, in which it grows and reproduces, combined with a discrete stage, with seeds that can enter a permanent soil seed bank or germinate the next spring after dispersion (Chapter 3).

From an ecological point of view, *H. caput-felis* is a termophilous plant that preferably grows in coastal garrigues or scrublands under the direct influence of the sea, mostly on calcareous rocky cliffs (0-200 m a.s.l.); peculiar populations also grow on different habitats, such as sand dunes (Majorca), rocky slopes bordering inland ravines

(Melilla) or, rarely, in open wooded areas (Fenu *et al.* 2015b and references therein; Chapter 1).

### *Data collection*

The geographic distribution of *H. caput-felis* was verified over three years (2012–2015) by numerous field surveys in the localities for which herbarium specimens and bibliographic references were available (Arrigoni 1971; Fenu and Bacchetta 2008; Agulló *et al.* 2010; Fenu *et al.* 2012b). In particular, a preliminary herbarium analysis was carried out in the main museum in the W-Mediterranean Basin (CAG, SS, SASSA, VAL, HJBS, SEV, GDA, MGC, BC, MA, MPU, P, AL).

A limit of 1 km as crow flies was used to consider two (or more) localities as geographically separated. In addition, all sites showing suitable ecological conditions along the Mediterranean coast of Sardinia, Balearic Islands (Majorca), Mediterranean Spanish and Moroccan coast were surveyed; only for Algeria’s coast fields surveys were not carried out, due to the problematic political and social situation.

For each locality, where the plants were found, the geographic limits were mapped using a global positioning system, and several ecological traits (e.g. coordinate, altitudinal range, slope, aspect and habitat type according to the Habitats Directive) were noted.

The major threats to *H. caput-felis* were determined through field observations and categorised following the IUCN Threats Classification Scheme (version 3.2; IUCN 2012).

The demographic trend was based on results of the data presented in Chapter 3. Moreover, data taken from six localities were chosen in order to perform more detailed demographic surveys: Capo Mannu and Su Tingiosu in Sardinia (Italy), Cabo Roig and Moraira in the west Iberian coast, and Sa Ràpita and Colònia de Sant Jordi in south Majorcan coast. In each studied locality, demographic data at plant level were collected.

The selected localities included all ecological situations in which the plant grown. Demographic analyses started in 2013, considering a total of 645 plants, and finished in 2015, year in which 745 plants were surveyed (see Chapter 3 and 4 for details).

All studied plants were counted, marked and measured, in order to recorder survival, morphological (height, maximum and minimum diameter) and reproductive traits (number of flowers and fruits per plant) for each plant, including seedlings.

Over the study period, surveys took place at least thrice yearly, following Jacquemyn *et al.* (2010). In early March, all sites were visited a first time for locating all previously mapped individuals and mapping new seedlings. During the flowering peak (April-May), when all plants are fully grown, site were visited a second time, in order to measure individuals (height, minimum and maximum diameter were taken), count the number of flowers per plant and check for new occasional individuals, that were added in the data set. During the fruiting peak (latest May-early June) the third survey was carried out in all populations, to count the number of fruits per plant. The current total population size of *H. caput-felis* was considered large enough to expect that demographic stochasticity would not play an important role (García 2003).

### *Data analyses*

The areas and perimeters of the surveyed localities where the plants were found were calculated using ArcView v. 3.2 (ESRI, Redlands, CA, USA). The distance between each locality and all the others was calculated in order to obtain the mean distance among localities; in this analysis, all available localities (including Herbaria and bibliographic data) were considered.

For population and demographic analyses, only confirmed localities were analysed.

Plant density was obtained during each monitoring using a variable number of homogeneously distributed sampling plots (2 × 1 m), in which we recorded the number of individuals in order to estimate population size.

The population size was analysed considering only the localities confirmed by this research (through field trips, in which the plants' number or density were determined). Conversely, in the localities confirmed only by bibliography, this research could neither confirm nor exclude the presence/absence of the study species.

The population size (including only mature individuals) was considered as $d \times A \times p$, where $d$ is an estimate of population density, $A$ is an estimate of area, and $p$ is an estimate of the proportion of individuals that are mature (*sensu* IUCN 2014), and it was estimated in each investigated locality.

In order to understand the frequency classes of population size and the omogeneity level of distribution whitin the localities, four classes were *a priori* defined: 1) 1–100, 2) 101–2000, 3) 2001–5000 and 4) >5000 plants per locality.

### *Extinction risk*

In order to characterize the population dynamics of *H. caput-felis*, a size-structured matrix model was constructed (Caswell 2001), from which demographic vital rates, their relative importance and the quasi-extinction risk were calculated. Although integral projection models (IPMs) better perform data of species with restricted range and small demographic data sets (< 300 individuals; Ramula *et al.* 2009; Chapter 4), the R package "IPMpack" actually does not permit to calculate the quasi-extinction risk probability. For this reason, matrix projection models (MPMs) were performed, using the R package "popbio" (Stubben and Milligan 2007), the outputs of which are therefore comparable to IPM's outputs (Chapter 3).

Population viability and the stochastic rate of increase ($\lambda_s$) were calculated both for the overall population and for the six selected populations, separately, due to the observed wide range of the size population. Size stage based matrices, the principal tool for assessing the viability of structure populations (populations in which individuals differ in their contributions to population growth), were used (Chapter 3). They divides the population into discrete classes and tracks the contribution of individuals in each class at one census to all classes in the following census (Morris and Doak 2002). Lefkovitch matrices based on the classification of individuals into life stages were used (Caswell 2001).

The life cycle of *H. caput-felis* was classified into four developmental stages based on fields' observations. Considering that the plant size was positively correlated to the reproductive output (see Chapter 1), all plants were grouped in classes considering the plant volume only, which was the variable that best explained the vital rates of this species (Fenu *et al.* 2015b; Chapter 3). The four size classes were: (1) plants with a volume <5 $cm^3$ (seedling, hereafter), (2) flowering and fruiting plants with a volume of 5.1–500 $cm^3$ (small, hereafter), (3) adults plants with a volume of 500.1–5000 $cm^3$ (medium, hereafter), and (4) large adults plants (large, hereafter). The seed bank stage was omitted from those analyses, due to the low impact in the population growth rate founded in previous studies (Chapter 3).

An idealized case in which the population size changes according to:

$$N_{t+1} = \lambda_t N_t$$

where $N_{t+1}$ is the population size in year $t$ and $\lambda_t$ is the finite rate of increase, were analysed (Morris and Doak 2002).

Stochastic population growth rates ($\lambda_s$) were calculated with Tuljapurkar's approximation (Tuljapurkar 1982) for realized population growth in the presence of independently and identically distributed environmental fluctuations (Crone *et al.* 2013), which takes into account how stochastic variation in the matrix elements affects the long-term stochastic growth rate (Caswell 2001). It can be more accurate in cases where there is covariation between matrix elements within the same year but may not be as accurate when there is a high level of temporal variation (Morris and Doak 2002). Stochastic population growth rates ($\lambda_s$) were calculated by randomly selecting one of three annual matrices with equal probability for each of 1000 model iterations, and taking the geometric mean of the obtained annual growth rates (Caswell 2001; Morris and Doak 2002). Than, to estimate sampling errors in $\lambda_s$, resampled the data, a bootstrap vector of the same sample size was generated, and $\lambda$ and bias-corrected 95% confidence intervals (CI) were calculated (Schleuning and Matthies 2009). Stochastic lambdas provide a more conservative risk assessment than those estimated from mean matrices for species in fluctuating environments (Menges 2000).

Damping ratio (ratio between the dominant eigenvalue and the second highest eigenvalue) of a transition matrix (Caswell 2001), which represents a measure of the speed with which the population converges to stability, was also calculated; this parameter was proportional to generation time (T; Franco and Silvertown 2004; Ezard *et al.* 2010), that represents the time required for the population to increase by a factor of $R_0$ (Coale 1972; Koons *et al.* 2005; Caswell 2001; Williams *et al.* 2011; IUCN 2014). The net reproductive rate ($R_0$), which is the number of offsprings produced on average by an individual over its lifespan (Metcalf *et al.* 2014), or, in other words, the measure of mean lifetime reproductive output, was also extracted from the matrices, to calculate T. It represents the global population growth rate per generation (not per time unit), and it is an indicator function for population growth, in which population growth is positive if, and only if, $R_0>1$ (Caswell 2011). Generation time (T) values for the global population of *H. caput-felis* were calculated both with IPM than with MPM methods (see Chapter 3), in order to carry out a most accurate extinction risk analysis.

Extinction probabilities ($P_e$) were calculated for 100 years as the proportion of 500 model iterations where the quasi-extinction threshold was reached (Van der Meer 2014). Matrices were selected at random with replacement (each matrix had an equal probability of selection; Morris and Doak 2002). A quasi-extinction threshold of 20 individuals was *a priori* designated to help minimize demographic stochasticity associated with small population size (Morris and Doak 2002). The number of surveyed plants (i.e., global population in this study) in 2013 was used as the population vector (*sensu* Morris et al. 1999) and consisted of 40 seedlings, 186 juveniles, 210 small adults, and 209 large adults.

Furthermore, in order to assess the quasi-extinction risk, the $\lambda_s$ were imposed by multiplying the estimated global population growth rate with four populations' size classes, in order to create different quasi-extinction scenarios in relation to the population sizes, and to understand which population size presented a decline.

Moreover, the same method was performed in order to calculate the extinction risk of the six selected localities, representative of the high eterogeneity of size population classes and different habitat types.

Demographic analyses and the estimation of quasi-extinction risk were performed using the "popbio" package (Stubben and Milligan 2007), based upon methods described in Caswell (2001) and Morris and Doak (2002), in R version 3.1.2 (R Core Team 2014).

### *IUCN assessment*

For the risk assessment, IUCN categories and criteria version 3.1 (IUCN 2012b) and the most recent guidelines for its application (IUCN 2014) were applied. In particular, three IUCN criteria (A, B and E) were used. The A criterion was used to evaluate the size population reduction, in particular considering the reduction decline in AOO or EOO. The actual geographic range size (B criterion) was calculated both with the EOO than the AOO (B1 and B2, respectively). The E criterion is based on quantitative analysis of extinction risk in the wild (e.g., Population Viability Analysis; IUCN 2012a).

Both the EOO and AOO analyses (AOO, defined as the area within the extent of occurrence, EOO, that is occupied by a taxon, where EOO is defined as the area contained within the shortest continuous imaginary boundary that can be drawn to encompass all the known sites of occurrence of a taxon, excluding cases of vagrancy; IUCN 2001) were based on a geo-database (including all literature/herbarium records as well as new records from our field surveys) illustrating current global species distribution. In estimating EOO, firstly a minimum convex polygon (also called convex hull), including all the sites of occurrence at a given time, were drew. It is the smallest polygon in which no internal angle exceeds 180 degrees and which contains all sites. Then, a large portion of obviously unsuitable areas were excluded, by deriving the correspondent α-hull through the following steps: a Delauney triangulation (*sensu* Burgman and Fox 2003) was created by drawing lines joining the points, constrained so that no lines intersected between points; then, the lengths of all of the lines were measured, and the average line length was calculated; then, all lines that are longer than a mulitple ($\alpha = 2$) of the average line length were deleted, and, finally, the areas of habitat were calculated by summing the areas of all remaining triangles (Burgman and Fox 2003; Gargano *et al.* 2007).

According to the IUCN suggestion, a grid of 2 × 2 km generated by the ESRI® ArcGis™ 9.2 package and superimposed onto a map of the West Mediterranean Basin was *ad hoc* created to assess the AOO. All of the parameters required by the IUCN protocol (i.e., EOO, locations and decline rate) and conservation status were assessed following the latest guidelines of the IUCN (2014).

Population trends (i.e., decline, equilibrium or growth of a population) of *H. caput-felis* population were calculatedat global and locality levels basing on the PVA abovementioned.

In order to evaluate the conservation status of *H. caput-felis* following the A criterion, the difference in time of AOO and EOO were analysed, while the actual EOO, combined with the population trends, was used to follow the B criterion.

Moreover, the PVA and the estimated quasi–extinction risks were used to follow the E criterion. Than, as suggested in the IUCN’s guidelines (2014), all three global assessments (10 years or three generations, 20 years or five generations and 100 years) for *taxa* with a generation length less than 20 years, such as *H. caput-felis*, are needed. Hence, the generation time (T), considered *a priori* equivalent to the “generation length” definition in IUCN (2014), was multiplied and related with the probability of extinction ($P_e$), in order to applicate the criterion E.

## Results

A total of 50 localities for *H. caput-felis* along the Mediterranean coastal environments were reported. The presence of *H. caput-felis* was confirmed in 43 localities, 33 of which confirmed in the field, while the remaining 10 confirmed by bibliography or Herbaria speciemens (Table 1).

Field surveys in Cala Conta (Ibiza) and Maioris (Majorca) were unsuccessful; hence, in those localties, the species was considered as “not retrieved”, but not “extinct”, due to the precautionary approach. In two localities (Santa Pola in Spain and Seu in Sardinia) this species was certainly extinct in the wild. The three Algerian localities still remain uncertain, due to the old age of the Herbarium specimens found in Oran museum (1918–1930; Brahim Zeineb 2012; Battandier and Trabut 1893).

The species grows in three localities in Italy, 31 in Spain (seven in Majorca, eight in north Alicante’s coast and 14 in south Alicante’s coast, two in Melilla), nine in Morocco and three documented in the Algerian coast (Table 1 and Figure 1).

**Table 1 – Investigated localities within the distribution range of *H. caput-felis*. Plants number were counted (superscript C) or estimated (superscript E). N.D.: not determined data.**

| Country, Region | Locality (Municipality) | Area (ha) | Altitudinal range (m, min-max) | Plant density (plants/m2) ± SD | No. plants | Seedlings presence | Situation | Source |
|---|---|---|---|---|---|---|---|---|
| Italy, Sardinia | Is Arutas (Cabras) | 0.001 | 5 - 15 | N.D. | $24^{C}$ | N.D. | confirmed | this research |
| Italy, Sardinia | Su Tingiosu – Porto Suedda (Cabras) | 12.743 | 5 - 25 | 4.83 ± 2.42 | $615491.73^{E}$ | yes | confirmed | this research |
| Italy, Sardinia | Seu (Cabras) |  | 18 |  |  |  | extinct | this research |
| Italy, Sardinia | Capo Mannu (San Vero Milis) | 17.868 | 5 - 55 | 4.63 ± 2.25 | $827306.92^{E}$ | yes | confirmed | this research |
| Spain, Majorca | Punta es Bauç (Santanyí) |  | 8 - 10 | N.D. | $20^{C}$ | N.D. | confirmed | this research |
| Spain, Majorca | Colònia de Sant Jordi - Playa del Puerto (Ses Salines) | 5.533 | 3 - 8 | 1.58 ± 0.74 | $87422.98^{E}$ | no | confirmed | this research |
| Spain, Majorca | Colònia de Sant Jordi - Es Trenc (Ses Salines) | 0.493 | 2 - 4 | 3 ± 1.41 | $14784^{E}$ | yes | confirmed | this research |
| Spain, Majorca | Sa Ràpita - backdune (Campos) | 10.495 | 2 | 7.8 ± 5.57 | $818594.4^{E}$ | yes | confirmed | this research |
| Spain, Majorca | Sa Ràpita - nautic club (Campos) | 0.416 | 3 - 6 | 9.6 ± 1.85 | $39945.6^{E}$ | yes | confirmed | this research |
| Spain, Majorca | Cap Blanc (Llucmajor) | 101.359 | 90 - 110 | 2.55 ± 1.14 | $2584646.85^{E}$ | yes | confirmed | this research |
| Spain, Majorca | Cala Pi |  | 15- 20 | N.D. | $50^{E}$ | N.D. | confirmed | this research |
| Spain, Majorca | Maioris (Llucmajor) |  |  |  |  |  | not retrieved | this research |
| Spain, Ibiza | Cala Conta (San José) |  |  |  |  |  | not retrieved | this research |
| Spain, Alicante | Cap d'Or (Teulada) | 0.21 | 40 - 50 | N.D. | $20^{E}$ | N.D. | confirmed | this research |
| Spain, Alicante | Cala del Portitxolet (Teulada) | 1.038 | 9 - 14 | 1.41 ± 0.86 | $14630.16^{E}$ | no | confirmed | this research |
| Spain, Alicante | L'Andragó – Les Playetes (Teulada) | 0.166 | 6 - 12 | 1.6 ± 0.54 | $2662.4^{E}$ | no | confirmed | this research |
| Spain, Alicante | Cala els Pinets, Cala Lobella, Cala Advogat (Benissa) | 2.203 | 10 - 20 | 2.9 ± 0.74 | $63895.7^{E}$ | yes | confirmed | this research |
| Spain, Alicante | Cala Fustera (Benissa) | 0.182 | 7 - 11 | 1.2 ± 0.57 | $2180.4^{E}$ | no | confirmed | this research |
| Spain, Alicante | Cala de les Bassetes (Benissa) | 0.869 | 6 - 20 | 2.8 ± 1.04 | $24337.6^{E}$ | yes | confirmed | this research |
| Spain, Alicante | Cala de la Calalga (Calpe) | 0.129 | 6 - 10 | 1.5 ± 0.79 | $1941^{E}$ | yes | confirmed | this research |
| Spain, Alicante | Calpe (Calpe) | 0.034 | 5 - 6 | 3 ± 1.37 | $1008^{E}$ | yes | confirmed | this research |
| Spain, Alicante | Santa Pola (Santa Pola) |  |  |  |  |  | extinct | this research |
| Spain, Alicante | Cabo Cervera (Torrevieja) | 1.676 | 5 - 14 | 1.1 ± 0.65 | $18438.53^{E}$ | no | confirmed | this research |
| Spain, Alicante | Torrevieja (Torrevieja) | 0.039 | 2 - 3 | 1.4 ± 0.82 | $546^{E}$ | no | confirmed | this research |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Spain, Alicante | Cala Mosca and Punta Prima (Orihuela) | 0.155 | 13 | N.D. | N.D. | N.D. | confirmed | Padilla *et al.* 2013 |
| Spain, Alicante | Rambla de las Estacas (Orihuela) | 0.195 | 8 - 10 | N.D. | $1^{C}$ | N.D. | confirmed | Molina *et al.* 2011 |
| Spain, Alicante | Cala de las Estacas (Orihuela) | N.D. | 0 - 3 | N.D. | N.D. | N.D. | confirmed | Molina *et al.* 2011 |
| Spain, Alicante | Barranco de la Cala del Capitan (Orihuela) | 0.167 | 15 - 20 | N.D. | N.D. | N.D. | confirmed | Molina *et al.* 2014 |
| Spain, Alicante | Casa de Los Leoncios (Orihuela) | 0.381 | 15 - 20 | N.D. | N.D. | N.D. | confirmed | Molina *et al.* 2014 |
| Spain, Alicante | Cala Mosca - Playa Flamenca (Orihuela) | 9.723 | 1 - 9 | 2 ± 0 | $194461.4^{E}$ | yes | confirmed | this research |
| Spain, Alicante | Cabo Roig (Orihuela) | 0.706 | 3 - 7 | 3.5 ± 1.67 | $24703^{E}$ | no | confirmed | this research |
| Spain, Alicante | Punta de la Glea (Orihuela) | 4.179 | 5 - 17 | 3.61 ± 3.53 | $150865.51^{E}$ | yes | confirmed | this research |
| Spain, Alicante | Dehesa de Campoamor (Orihuela) | 0.017 | 5 - 10 | 1.5 | $261^{E}$ | no | confirmed | this research |
| Spain, Alicante | Punta de la Horadada (Pilar de la Horadada) | N.D. | 0 - 5 | N.D. | N.D. | N.D. | confirmed | Molina *et al.* 2014 |
| Spain, Alicante | Mil Palmeras (Pilar de la Horadada) | 0.178 | 3 - 7 | 7.66 ± 1.53 | $13596.5^{E}$ | no | confirmed | this research |
| Spain, Alicante | Rio Mar (Pilar de la Horadada) | 0.377 | 0 - 2 | 3.66 ± 1.53 | $13812.84^{E}$ | no | confirmed | this research |
| Spain, Nador | Barranco del Quemadero (Melilla) | 36.908 | 110 | 2.4 ± 1.56 | $885784.8^{E}$ | yes | confirmed | this research |
| Spain, Nador | Barranco del Nano (Melilla) | 24.630 | 99 | 3.2 ± 1.04 | $365040^{E}$ | yes | confirmed | this research |
| Morocco, Nador | near Beni Chiker (Beni Chiker) | N.D. | N.D. | N.D. | N.D. | N.D. | confirmed | this research |
| Morocco, Nador | Taxdirt - Cabo de Tres Forcas (Beni Chiker) | 115.639 | 100 - 210 | 3.2 ± 1.56 | $506958.35^{E}$ | yes | confirmed | this research |
| Morocco, Nador | near Beni Sidel (Beni Sidel) | N.D. | 200 | N.D. | $30^{C}$ | N.D. | confirmed | this research |
| Morocco, Nador | road to Cap de Trois Fourches (Cap de Trois Fourches) | N.D. | N.D. | N.D. | N.D. | N.D. | confirmed | SEV *Herbarium* |
| Morocco, Nador | Douar Ighzar -n- Yamrabthan (Douar Ighzar -n- Yamrabthan) | N.D. | N.D. | N.D. | N.D. | N.D. | confirmed | SEV *Herbarium* |
| Morocco, Nador | Ras El Má (Ras Kebdana) | 4.211 | 50 | 2.3 ± 1.20 | $96855.3^{E}$ | yes | confirmed | this research |
| Morocco, Nador | Ras El Má (Ras Kebdana) | 3.686 | 52 | 2.3 ± 1.20 | $84771.1^{E}$ | yes | confirmed | this research |
| Morocco, Nador | coast between Azzanene and Sidi Messaous (Sidi Messaoud) | N.D. | N.D. | N.D. | N.D. | N.D. | confirmed | SEV *Herbarium* |
| Morocco, Nador | Târia n Tît (Charrana) | N.D. | N.D. | N.D. | N.D. | N.D. | confirmed | SEV *Herbarium* |
| Algeria, Oran | Cabo Lindlés (Aïn El Turk) | N.D. | 90 | N.D. | N.D. | N.D. | undetermined | Taieb Brahim Zeineb (2012), MA *Herbarium* |
| Algeria, Oran | Cap Falcon (Aïn El Turk) | N.D. | 50 | N.D. | N.D. | N.D. | undetermined | Battandier and Trabut (1893) |
| Algeria, Oran | Aïn El Turk (Aïn El Turk) | N.D. | 75 - 80 | N.D. | N.D. | N.D. | undetermined | Taieb Brahim Zeineb (2012), Battandier and Trabut (1893) |

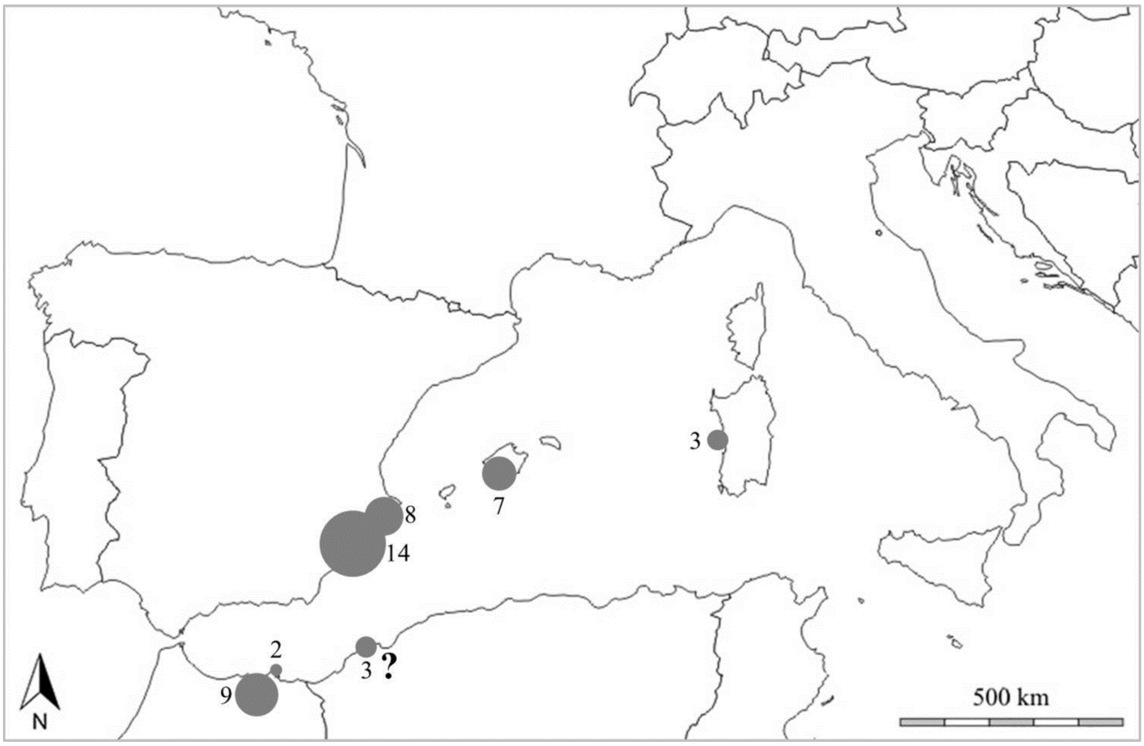

**Figure 1 – *H. caput-felis*' distribution map. The circle size is proportionally to the number of confirmed locations.**

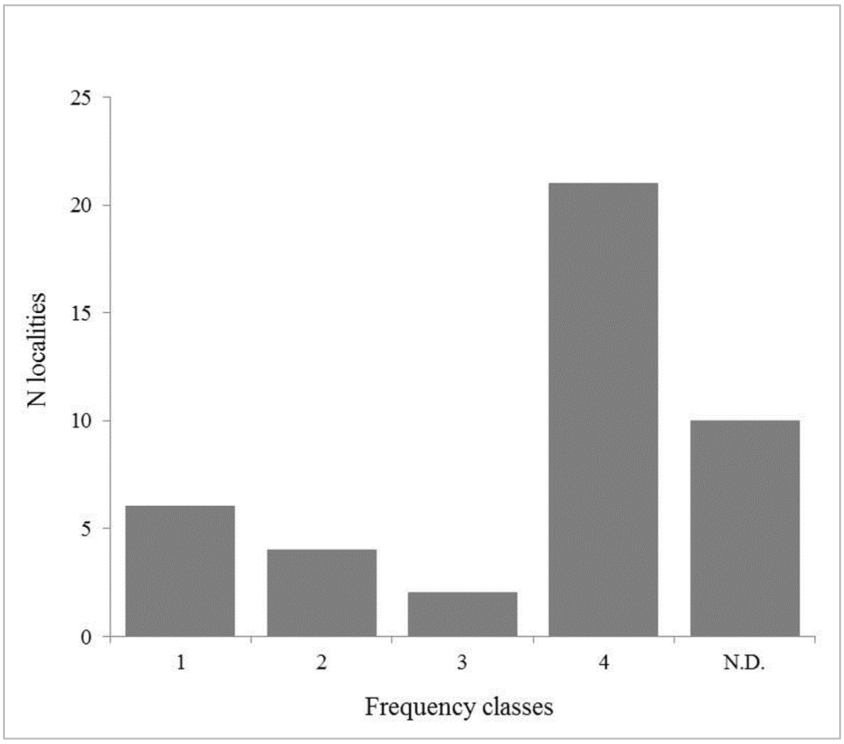

**Figure 2 – Frequency of four size population's classes within the global confirmed *H. caput-felis* distribution. Class 1: ≤ 100 plants; class 2: 101 – 2000 plants; class 3: 2001 – 5000 plants and class 4: > 5000 plants. N.D.: localities whitout data (not confirmed or confirmed only in bibliography; see Table 1 for details). Extinct, not retrieved and uncertain localities were excluded from this analysis.**

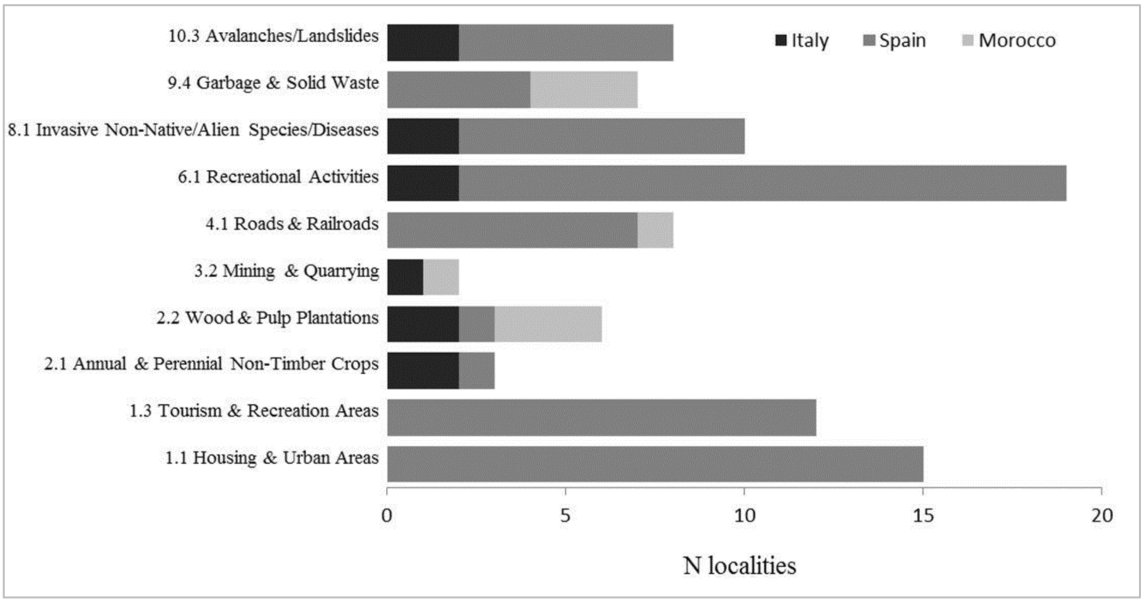

**Figure 3 – Threats frequency for *H. caput-felis* along its distribution range following the IUCN Threat Classification Scheme (IUCN 2012); different colors highlight the number of localities affected by a single threat in Italy, Spain and Morocco.**

*Helianthemum caput-felis* distribution was centred in the westernmost part of the Mediterranean Basin, and, in particular, along the eastern Iberian coasts. This plant reached the eastern limit of distribution in Su Tingiosu (Sardinia, Italy), the northern in Capo Mannu (Sardinia, Italy), the western in Beni Chiker and the southern in Beni Sidel (Morocco; Figure 1). The innermost locality where *H. caput-felis* grew is near Beni Sidel, in Morocco (3.44 km far from the coast line).

The mean distance among localities was 392.28 km, while the distances between each locality and the others were showed in Table 1A.

The altitudinal range of *H. caput-felis* varied from the sea level to a maximum of 210 m a.s.l. (Taxdirt, Morocco), in population generally located on variable slope of 0–45°. The localities surface was also highly variable, ranging from 10 $m^2$ to 115.64 ha (Is Arutas in Sardinia and Taxdirt in Morocco, respectively), while Cap Blanc (Majorca, Spain) represents the second biggest locality (101.36 ha).

The mean plant density was 3.19 ± 2.13 plants/$m^2$, and it varied from 1.10 ± 0.65 plants/$m^2$ (Cabo Cervera, south Alicante) to 9.6 ± 1.85 plants/$m^2$ (Sa Ràpita, Majorca).

According to the classification adopted in this study, the estimate size population varied considerably among localities (Figure 2), from one to ca. 2,6 million plants (Rambla de las Estacas in sout Alicante and Cap Blanc in Majorca, respectively). The majority of localities (63.64%) fall into the fourth class (> 5000 plants), followed by localities into the first class (18.18%; ≤ 101 plants); only the 6.06% of localities fall into the third class (2001–5000 plants; Figure 2).

The populations' structure was mainly characterised by adult plants (reproductive plants), and seedlings were observed only in the 63% of the total of investigated localities. No correlation between seedlings presence (and abundance) and population size was observed (Table 1).

At global level, the main threats affecting the persistence of *H. caput-felis* localities were recreational activities (code 6.1), observed in 19 localities (57.58% of the total), followed by housing and urban areas (code 1.1, 45.45%) and tourism and recreation areas (code 1.3, 36.36%; Figure 3). Invasive alien species (code 8.1), such as *Carpobrotus* sp., *Agave* sp., *Acacia* sp., and *Ricinus communis* L., roads (code 4.1) and landslides (code 10.3) represented also important treaths, being present in 30.30% and 24.24% of the localities (Figure 3 and 4).

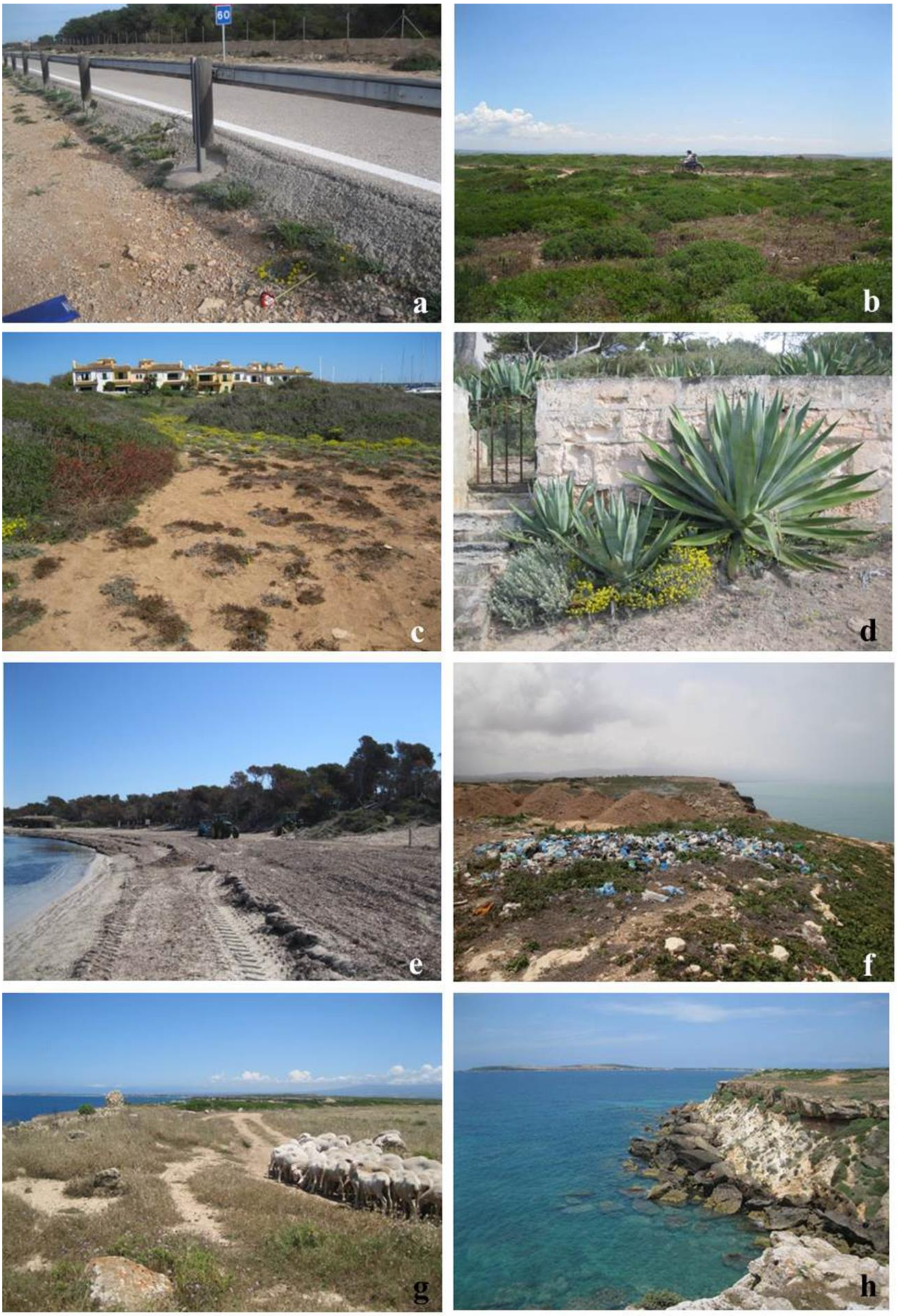

**Figure 4 – Threats affecting the persistence of *H. caput-felis* along its distribution range; a) road in Cap Blanc (Majorca, Spain); b) a biker in Su Tingiosu (Sardinia, Italy); c) houses in Sa Ràpita (Majorca, Spain); d) houses and invasive species in Colònia de Sant Jordi (Majorca, Spain); e) bulldozers removing *Posidonia oceanica* banquettes removal in Colònia de Sant Jordi (Majorca, Spain); f) garbage in Ras el Ma (Ras Kebdana, Morocco); g) grazing in Su Tingiosu (Sardinia, Italy); h) landslides in Su Tingiosu (Sardinia, Italy).**

At regional level, the Spanish localities were more exposed to threats than the other regions. In Sardinia, stochastic events (i.e. landslides) and human–relacted factors (i.e. invasive species, recreational activities, wood plantation and agriculture) represented the main threats to the persistence of *H. caput-felis* localities, while in Spain the main threats were related to recreational activities. Morocco' localities showed a different situation from the European one, as the garbage and wood plantations represented the main pressure (Figure 3 and 4).

The presence of the parasitic plant *Cytinus hypocistis* (L.) L. could be considered a negative factor for *H. caput-felis*, as well as the high presence of empty fruits, in particular in Spanish populations.

### *Global population demography*

Demographic parameter of *H. caput-felis* were summarised in Table 2. The stochastic population growth rate of *H. caput-felis* was 0.97, which highlighted an overall population decline rate of 3% (Table 2). The analysis, at population level, showed stochastic populations growth rates which varied from 0.77 (CR) to 1.01 (CM and SR; Table 2).

The probability of reaching a quasi-extinction threshold (20 individuals) based on 500 iterations of population growth over 100 years generate the simulated cumulative distribution functions showed in Figure 5. From a starting population size of 645 plants, the probability of hitting a threshold density of 20 individuals reaches a value of 0.1 after only ca. 75 years, a value of 0.5 after ca. 90 years and the 0.85 after ca. 100 years; according to this simulation, the 100% probability of quasi-extinction risk will be reached in 130 years.

**Table 2 – Parameters extracted from matrix population models (MPMs) of *H. caput-felis* for the two years transitions: projected deterministic population growth rate (λ), deterministic population growth rate of the mean projected matrix ($\lambda_M$), stochastic population growth rate ($\lambda_S$) with confidence intervals at 95%, damping ratio ($d_r$) and probability of extinction ($P_e$) of six localities: Capo Mannu (CM), Su Tingiosu (ST), Cabo Roig (CR), Moraira (MO), Sa Ràpita (SR), Colònia de Sant Jordi (SJ) and the overall population (GLOBAL).**

| | **λ** | | **$\lambda_M$** | **$\lambda_S$** | **$d_r$** | | **$P_e$ (time)** |
|---|---|---|---|---|---|---|---|
| | 2013-2014 | 2014-2015 | | | 2013-2014 | 2014-2015 | |
| **CM** | 0.97 | 1.05 | 1.01 | 1.01<br>[1.010 - 1.010] | 1.29 | 1.45 | - |
| **ST** | 0.99 | 1.00 | 1.00 | 1.00<br>[1.001 - 1.002] | 1.37 | 1.39 | - |
| **CR** | 1.00 | 0.60 | 0.79 | 0.77<br>[0.767 - 0.771] | 1.05 | 1.16 | 100<br>(in 16 years) |
| **MO** | 1.00 | 0.86 | 0.87 | 0.93<br>[0.933 - 0.934] | 1.33 | 1.11 | 100<br>(in 10 years) |
| **SR** | 1.08 | 0.94 | 1.02 | 1.01<br>[1.013 - 1.013] | 1.28 | 1.26 | - |
| **SJ** | 1.00 | 1.00 | 0.96 | 1<br>[1-1] | 1.10 | 1.14 | 100<br>(in 13 years) |
| **GLOBAL** | 1.03<br>[1.002 - 1.054] | 0.92<br>[0.891 - 0.951] | 0.98 | 0.97<br>[0.973 - 0.976] | 1.37 | 1.33 | 85<br>(in 100 years) |

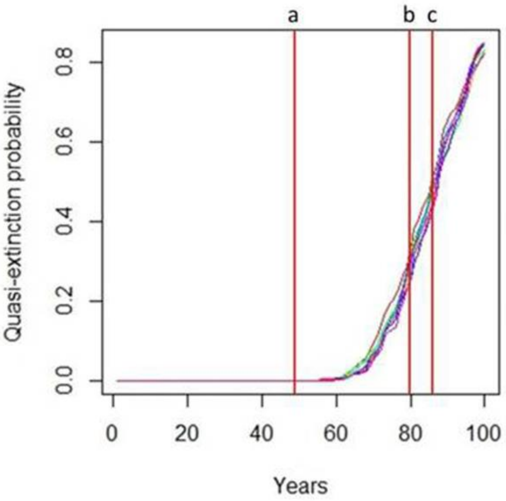

**Figure 5 – Simulated cumulative distribution functions for the time to reach a quasi-extinction threshold of 20 individuals for the global monitored population of *H. caput-felis*, starting at 645 individuals. a) three generations, b) five generations, c) three generation basing on generation time calculated with MPM.**

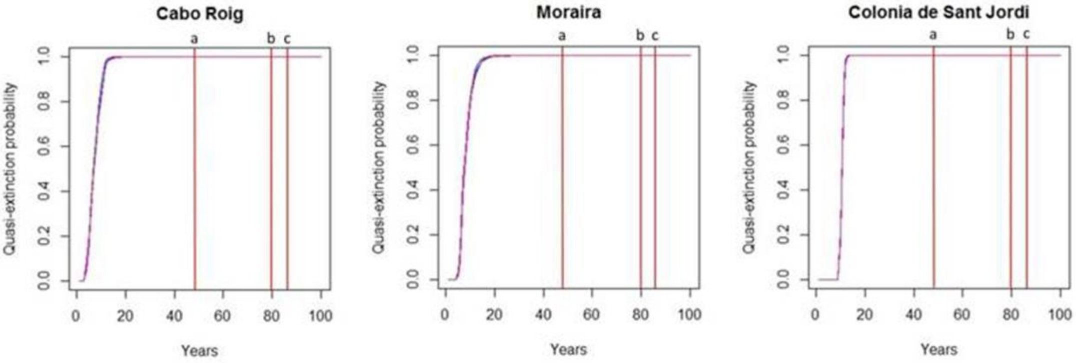

**Figure 6 – Simulated cumulative distribution functions for the time to reach a quasi-extinction threshold of 20 individuals for three *H. caput-felis*' populations: Cabo Roig (CR), Moraira (MO) and Colònia de Sant Jordi (SJ). a) three generations, b) five generations, c) three generations based on generation time calculated with MPM.**

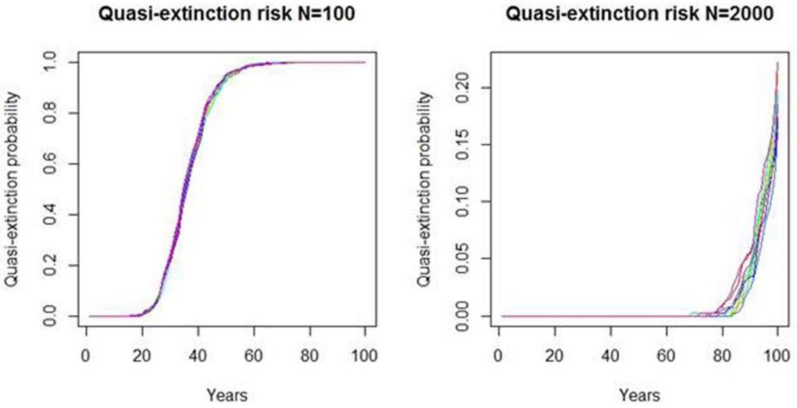

**Figure 7 – Simulated cumulative distribution functions for the time to reach a quasi-extinction threshold of 20 individuals for two scenarios of *H. caput-felis*' populations (100 and 2000 individuals, respectively).**

*Helianthemum caput-felis*' generation time (T) extracted from the global IPM was 14.61 years in 2013–2014 and 17.33 years in 2014–2015, that covers a mean period of time of 15.97 years. Consequently, three generations for this plant last 47.91 years, which correspond to none extinction risk (see line *a* in Figure 5), and five generations (79.85 years) correspond to an extinction probability of ca. 30%. The generation time extracted from the MPM was 32.44 years in 2013–2014 and 24.43 years in 2014–2015. Considering the MPM mean generation time (28.44 years), three generations involve a period of 85.31 years, which correspond to a risk of quasi-extinction of ca. 35% (see line *c* in Figure 5).

At local level, only Cabo Roig (CR) and Moraira (MO) show a stochastic lambda < 1, but Colònia de Sant Jordi (SJ) too face risk of extinction, due to the absence of seedlings (Table 2). In particular, the quasi-extinction risk analyses showed that CR, MO and SJ will be extinct in 16, 10 and 13 years, respectively (Figure 6 and Table 2).

The four quasi extinction risk scenario's models calculated for the four size population classes showed that one class (N < 100 individuals) achieved the 100% quasi–risk extinction probability before 100 years (67 years, Figure 7). The class two (101–2000 individuals) achieved the quasi–extinction probability of 20% in 100 years (and the 100% in 160 years, Figure 7). Only in the classes three and four (2001 – 5000 and N > 5000 plants, respectively) sites faced no high risk of extinction according to this models; in fact, localities in class three and four will not reach populations sizes of 20 or fewer individuals in 100 years. The third class (2001–5000 plants) achieved the 100% probability of extinction in ca. 180 years (data not showed).

The extinction probability according to the four scenario models of each locality where *H. caput-felis* was confirmed was reported in Table 3.

The models' outputs showed a general trend which highlights that, of 33 confirmed localities, six will be extinct in 10 years, while 27 will persist more than 20 years; finally, for 10 localities it was not possible to estimate the extinction risk with this method, due to the absence of data about the size populations. No locality fell into the temporal limit of 20 years.

**Table 3 – Probability of extinction of *H. caput-felis* localities, based on the four scenarios outputs of population size classes (1) 1–100, 2) 101–2000, 3) 2001–5000 and 4) >5000 individuals per locality. Cross symbols indicate extinction, while tick symbols indicate localities which will survive over the correspondent time threshold.**

| Country, Region | Locality (Municipality) | Situation | No. plants | Class | Years | | |
|---|---|---|---|---|---|---|---|
| | | | | | ≤10 | ≤20 | > 20 |
| Italy, Sardinia | Is Arutas (Cabras) | confirmed | 24$^{C}$ | 1 | ✗ | | |
| Italy, Sardinia | Su Tingiosu – Porto Suedda (Cabras) | confirmed | 615491.73$^{E}$ | 4 | | | ✓ |
| Italy, Sardinia | Capo Mannu (San Vero Milis) | confirmed | 827306.92$^{E}$ | 4 | | | ✓ |
| Spain, Majorca | Punta es Bauç (Santanyí) | confirmed | 20$^{C}$ | 1 | ✗ | | |
| Spain, Majorca | Colònia de Sant Jordi - Playa del Puerto (Ses Salines) | confirmed | 87422.98$^{E}$ | 4 | | | ✓ |
| Spain, Majorca | Colònia de Sant Jordi - Es Trenc (Ses Salines) | confirmed | 14784$^{E}$ | 4 | | | ✓ |
| Spain, Majorca | Sa Ràpita - backdune (Campos) | confirmed | 818594.4$^{E}$ | 4 | | | ✓ |
| Spain, Majorca | Sa Ràpita - nautic club (Campos) | confirmed | 39945.6$^{E}$ | 4 | | | ✓ |
| Spain, Majorca | Cap Blanc (Llucmajor) | confirmed | 2584646.85$^{E}$ | 4 | | | ✓ |
| Spain, Majorca | Cala Pi | confirmed | 50$^{E}$ | 1 | ✗ | | |
| Spain, Alicante | Cap d'Or (Teulada) | confirmed | 20$^{E}$ | 1 | ✗ | | |
| Spain, Alicante | Cala del Portitxolet (Teulada) | confirmed | 14630.16$^{E}$ | 4 | | | ✓ |
| Spain, Alicante | L'Andragó – Les Playetes (Teulada) | confirmed | 2662.4$^{E}$ | 3 | | | ✓ |
| Spain, Alicante | Cala els Pinets, Cala Lobella, Cala Advogat (Benissa) | confirmed | 63895.7$^{E}$ | 4 | | | ✓ |
| Spain, Alicante | Cala Fustera (Benissa) | confirmed | 2180.4$^{E}$ | 3 | | | ✓ |
| Spain, Alicante | Cala de les Bassetes (Benissa) | confirmed | 24337.6$^{E}$ | 4 | | | ✓ |
| Spain, Alicante | Cala de la Calalga (Calpe) | confirmed | 1941$^{E}$ | 2 | | | ✓ |
| Spain, Alicante | Calpe (Calpe) | confirmed | 1008$^{E}$ | 2 | | | ✓ |
| Spain, Alicante | Cabo Cervera (Torrevieja) | confirmed | 18438.53$^{E}$ | 4 | | | ✓ |
| Spain, Alicante | Torrevieja (Torrevieja) | confirmed | 546$^{E}$ | 2 | | | ✓ |
| Spain, Alicante | Rambla de las Estacas (Orihuela) | confirmed | 1$^{C}$ | 1 | ✗ | | |
| Spain, Alicante | Cala Mosca - Playa Flamenca (Orihuela) | confirmed | 194461.4$^{E}$ | 4 | | | ✓ |
| Spain, Alicante | Cabo Roig (Orihuela) | confirmed | 24703$^{E}$ | 4 | | | ✓ |
| Spain, Alicante | Punta de la Glea (Orihuela) | confirmed | 150865.51$^{E}$ | 4 | | | ✓ |
| Spain, Alicante | Dehesa de Campoamor (Orihuela) | confirmed | 261$^{E}$ | 2 | | | ✓ |
| Spain, Alicante | Mil Palmeras (Pilar de la Horadada) | confirmed | 13596.5$^{E}$ | 4 | | | ✓ |
| Spain, Alicante | Rio Mar (Pilar de la Horadada) | confirmed | 13812.84$^{E}$ | 4 | | | ✓ |
| Spain, Nador | Barranco del Quemadero (Melilla) | confirmed | 885784.8$^{E}$ | 4 | | | ✓ |
| Spain, Nador | Barranco del Nano (Melilla) | confirmed | 365040$^{E}$ | 4 | | | ✓ |
| Morocco, Nador | Taxdirt - Cabo de Tres Forcas (Beni Chiker) | confirmed | 506958.35$^{E}$ | 4 | | | ✓ |

| | | | | | | |
|---|---|---|---|---|---|---|
| Morocco, Nador | near Beni Sidel (Beni Sidel) | confirmed | 30<sup>C</sup> | 1 | ✗ | |
| Morocco, Nador | Ras El Má (Ras Kebdana) | confirmed | 96855.3<sup>E</sup> | 4 | | ✓ |
| Morocco, Nador | Ras El Má (Ras Kebdana) | confirmed | 84771.1<sup>E</sup> | 4 | | ✓ |
| Spain, Majorca | Maioris (Llucmajor) | not retrieved | | ? | | |
| Spain, Ibiza | Cala Conta (San José) | not retrieved | | ? | | |
| Spain, Alicante | Cala Mosca and Punta Prima (Orihuela) | confirmed | N.D. | ? | | |
| Spain, Alicante | Cala de las Estacas (Orihuela) | confirmed | N.D. | ? | | |
| Spain, Alicante | Barranco de la Cala del Capitan (Orihuela) | confirmed | N.D. | ? | | |
| Spain, Alicante | Casa de Los Leoncios (Orihuela) | confirmed | N.D. | ? | | |
| Spain, Alicante | Punta de la Horadada (Pilar de la Horadada) | confirmed | N.D. | ? | | |
| Morocco, Nador | near Beni Chiker (Beni Chiker) | confirmed | N.D. | ? | | |
| Morocco, Nador | road to Cap de Trois Fourches (Cap de Trois Fourches) | confirmed | N.D. | ? | | |
| Morocco, Nador | Douar Ighzar -n- Yamrabthan (Douar Ighzar -n- Yamrabthan) | confirmed | N.D. | ? | | |
| Morocco, Nador | coast between Azzanene and Sidi Messaous (Sidi Messaoud) | confirmed | N.D. | ? | | |
| Morocco, Nador | Târia n Tît (Charrana) | confirmed | N.D. | ? | | |
| Algeria, Oran | Cabo Lindlés (Aïn El Turk) | uncertain | | ? | | |
| Algeria, Oran | Cap Falcon (Aïn El Turk) | uncertain | | ? | | |
| Algeria, Oran | Aïn El Turk (Aïn El Turk) | uncertain | | ? | | |

*Conservation status assessment*

The extent of occurrence of *H. caput-felis* was 100682.36 km$^2$ (Figure 8), which does not fall into any threatened categories, according to the B criterion. However, this species falls into the Endangered category, according to the formula B2ab (ii,iii,iv,v), basing on the AOO calculated (42 cells = 168 km$^2$), the fragmented distribution and the calculated/observed continuous decline rate in AOO, habitat quality, number of localities, and number of mature plants (decline rate = 3%).

During recent decades few extinctions (Santa Pola and Seu) have been recorded in the *H. caput-felis* populations. However, the reduction in geographical range (EOO) was negligible, because these two localities were located into the convex polygon or next to the edge. Based on the number of occupied cells in a grid of 2 × 2 km, the AOO decrease amounted to 2 cells (8 km$^2$), which correspond to a decline rate of 4.55 % in the last 50 years. Those estimates do not reach the minimum threshold imposed by the A criterion, and, according to the A2c subcriterion, the species results as not threatened (NT).

Based on IPM generation time (T), five generation lasts 79.85 years, that represent the threshold of a probability of extinction of ca. 30% (line *b* in Figure 5), and, consequently, this species must be included into the Endangered category (EN). In addition, three generations for *H. caput-felis*, calculated by using the generation time (T) extracted by the MPM, involve a period of 85.31 years, which correspond to quasi-extinction risk of ca. 35% (line *c* in Figure 5); also in this case, these parameter allowed to attribute the Endangered (EN) category to the study species.

Based on these results and applying the precautionary approach recommend by the IUCN, the *H. caput-felis* must be considered Endangered (EN) at global level, according to the formula B2ab (ii,iii,iv,v) + E.

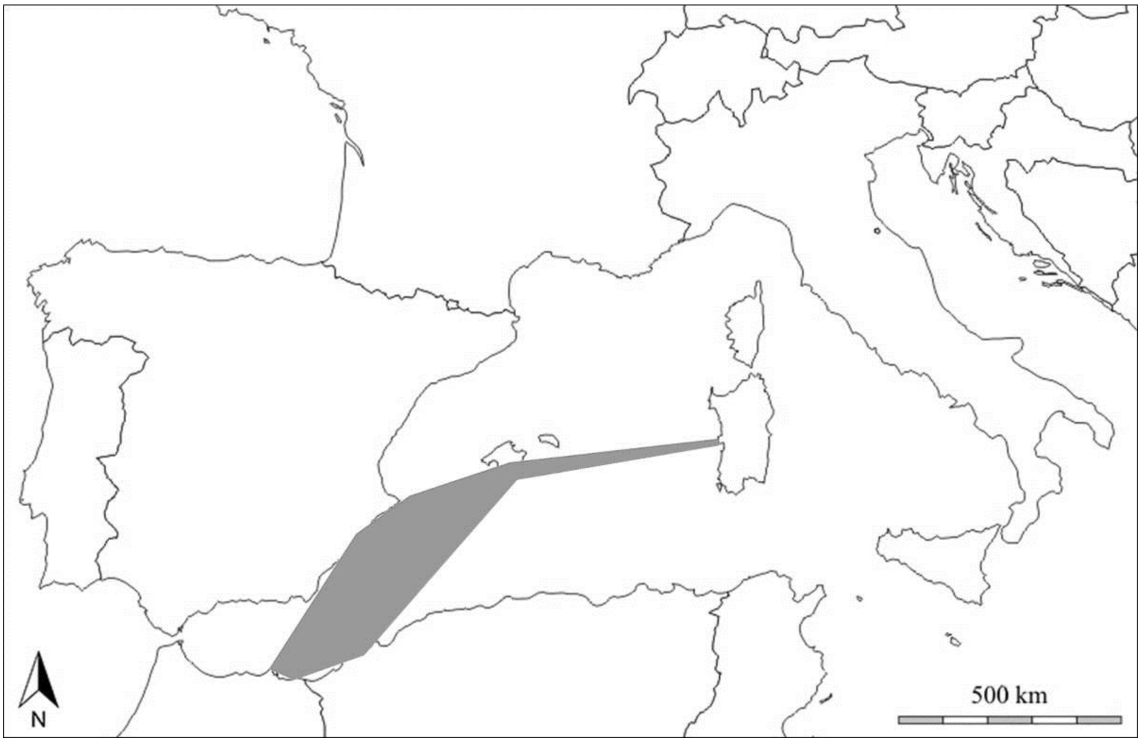

**Figure 8 – Actual extent of occurrence (EOO) of *H. caput-felis* (α-hull = 100682.36 km$^2$).**

## Discussion

Despite countries contracting to the Convention on Biological Diversity (CBD 1992) and other international laws (e.g., Habitat Directive) are obliged to monitoring biodiversity in order to protect wild fauna and flora, few in–depth studies have focused on the conservation status' analysis at global level. In addition, for species distributed in different countries, global analyses are often more complicated.

To our knowledge, no exhaustive studies have been performed at global level on *H. caput-felis*, and the present study represents the first attempt to analyse the distribution and the population dynamics, as well as to assess at global level of the conservation status of this threatened species.

Althought Red Lists were recognised as the best method allowing to identify the species risk status, the assessments of several plant species of high conservation interest are still lacking. The assessment procedure of threatened species was mainly based on the criterion B, related to the plant distribution; only in few cases, when reliable data on population trends or population size were available, other criteria were applied (e.g., Fenu *et al.* 2015c; Rossi *et al.* 2016). Distributional data are often the most diffuse (Gärdenfors 2000; Hartley and Kunin 2003; Moreno 2008), at least in some areas, and in many cases applying the criterion B is the only possibility to assess a *taxon* under the IUCN categorization system (e.g., Abeli *et al.* 2009). However, as suggested by Abeli *et al.* (2009), the criterion B should be used to evaluate PIPPs only when the decline is really demonstrable and not just suspected.

In this study, several IUCN criteria and quantitative analyses were mixed in order to characterise the conservation status. The A criterion clearly underestimates the status of *H. caput-felis*, undervaluing the calculated decline rate. Converserly, the B and E criteria offer a more realistic status of this species, according to the specific demographic studies (Chapter 3 and 4). Moreover, although the B criterion is less accurate than E, in this case the two assessment are congruents. Nevertheless, in many cases, and in particular for PIPPs, the E criterion indicates a lower risk category than the other ones (Abeli *et al.* 2009). When the short-term risk of extinction is low, but the current population size is small, or only short-term data are available, growth rate may be a more important indicator of potential future problems than extinction probability (Morris and Doak 2002). In fact, at local level, the high quasi-extinction probability calculated for Cabo Roig, Moraira and Colónia de Sant Jordi underline how more detailed analyses (as PVA) are needed, also in population with a high number of individuals. Despite this, assessments at restricted geographic scale are discouraged by IUCN (2012a), and, for PIPPs, is important to consider the whole population independently from the administrative regions or districts in which they occurs (Abeli *et al.* 2009).

This research indicate that *H. caput-felis* is an endangered plant at global level, supporting the previous regional assessments that highlighted a critical status in Spain and Italy (Agulló *et al.* 2010; Fenu *et al.* 2012b; Rossi *et al.* 2016; Chapter 1). This assessment is strongly supported by the quantitative analysis. Matrix population models are a potentially powerful tool for assessing population status and extinction risk, as well as effects of past or future changes in management or in the environment (Crone *et al.* 2011). Those models are an increasingly influential tool for diagnosis of the persistence probability of threatened species (Milner-Gulland and Rowcliffe 2007).

Under natural conditions, both demographic and environmental stochasticity have been claimed as important factors in determining viability of species with limited distributions and/or small population sizes.

*Helianthemum caput-felis* suffers a continuing decline in AOO, quality of its habitat, number of localities and number of reproductive plants. This decline rate is mainly due to the habitat loss, and the expansion of infrastructures, associated with an extinction probability in ca. 130 years. In fact, in the last decades, two localities have disappeared (Santa Pola, Aguilella *et al.* 2010 and Agulló *et al.* 2010;  Seu) and one decreased up to have survived only one individual (Rambla de las Estacas, Giménez-Font 2008; Molina *et al.* 2011).

Tourism and recreational activities, followed by housing and urban areas, promoting habitat fragmentation, are the main threat to *H. caput-felis*, as well as several ecosystems in the Mediterranean area (e.g. Dominguez Lozano 1996; Allen 2001; Gibbs 2001; Fenu *et al.* 2011). Habitat fragmentation increases extinction risk for endangered species (Holsinger 2000; Matthies *et al.* 2004; Schleuning and Matthies 2009), interfering with distribution, fitness and seedling recruitment (Lienert 2004; Kolb and Diekmann 2005; Benito *et al.* 2009; De Vere *et al.* 2009). Despite this, more small-size populations in eastern Iberian coasts grew within urbanised areas, which have experienced strong urban development linked to tourism since the 1970s, with the consequent reduction of the habitat of the species (Zaragozí *et al.* 2012).

Demographic analyses strongly support the conservation status category for *H. caput-felis*; these analyses reflect that the global population growth rate showed a slight declining trend. In addition, the analyses of vital rates indicate that the persistence of *H. caput-felis* localities was mainly related to the adults survival. Despite the seedling presence in the majority of visited localities, previous studies (Chapter 3 and 4) highlight how seedlings have a high mortality probability; in fact, only few of these reach survive to the next year, hence seedling establishment seems to be the critic stage of this species, confirming Fenu *et al.* (2015b). Those information on survival strategies of threatened species are critical for optimizing and determining the success of *in situ* and *ex situ* conservation efforts (Fenu *et al.* 2011).

### *Implications for conservation and management*

Monitoring endangered plants, as *H. caput-felis*, faces several difficulties, as the need to maintain a sustained effort of monitoring across years, in order to ensure the collection of relevant monitoring time series, and the need to obtain precise monitoring data which allow the detection of significant changes across space and time in biodiversity (Fenu *et al.* 2015a). These needs come into conflict with the usually limited amount of available financial and human resources (Schmeller *et al.* 2009; Fenu *et al.* 2015a). In addition, plants with a complex distribution, such as *H. caput-felis*, present further problems: presence in  four countries, in different geographic and ecological situations (i.e. two island, remote sites), as well as private lands, entaile a significant effort, both in economic resources and in required time. Despite this, long-lived plants, with slow life histories, present a demanding challenge for plant conservation biologists, because low natural recruitment and mortality make detection of decline difficult without strong commitment to collection of demographic data (Schwartz *et al.* 2000; Schwartz 2003; García 2003; Marrero-Gómez *et al.* 2007; Dominguez *et al.* 2011).

Hence, the results of this study could be useful for assessing the status of the species and identifying effective conservation measures. The quantification of quasi-extinction probability highlights how *H. caput-felis* global population faces a substantial risk of extinction over the short-medium time, if no additional management actions will be carry out. In other words, this finding indicates that the passive current conservation measures (e.g., SCI inclusion or protected areas) are inadeguate to guarantee the persistence of the studies species.

Actually, some populations are protected by Site of Community Importance (SCI), both in Italy and Spain. In Majorca, all populations are located in protected areas, making its local conservation status more favourable (Rodríguez-Pérez 2005), even if the high impact of recreational activities in the coasts still remains a problem. In particular, Morocco and Algerian populations do not present any legal protection, and therefore it is difficult to implement active conservation measures.

Effective conservation measures could be focussed on protecting the single localities where *H. caput-felis* grows, in order to avoid further species decline. In this contest, conservation efforts should be primarily aimed at protecting established individuals (mature plants) against disturbances, as suggested for other long-lived species (e.g., Garcia and Zamora 2003). An effective measure at small spatial scale was the establishment of Plants Micro-Reserves (PMRs), as experimented in Spain (Padilla and Ramón 1997; Laguna *et al.* 2004; Zaragozí *et al.* 2012).

Other successful experiences, that could be positively replicated in other territorial context, were carried out in Spain. For example, in Majorca (Ses Covetes), 68 unauthorized flats, built during the ’80 Spanish boom, were destroyed in 2013, in order to promote an important habitat restoration plan, followed by the invasive plant’s eradication and, subsequently, the reintroduction of *H. caput-felis* and others autochthonous species. Similarly, in Les Bassetes (Benissa, Alicante province), a habitat restoration was promoted, after a destruction in the ’70 and ’80 due to intensive urbanisation processes. Also in a suitable area near to Barranco del Nano (Melilla), *H. caput-felis* reintroductions and reinforcements are periodically done. These represent effective examples, comparable to others low-cost project carried out in Sardinia for other coastal endangered plants (Cogoni *et al.* 2013; Fenu *et al.* 2015), which could be replicate for the main threatened localities, in order to enhance population viability (e.g., by increasing population size, genetic diversity, or by the representation of specific demographic groups or stages; Godefroid *et al.* 2011; Fenu *et al.* 2015c).

In addition, an *ex situ* conservation strategy was implemented, and the seeds collected could be used for future reinforcement or reintroduction of this species in suitable Sardinian areas (Fenu *et al.* 2015c).

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## References

Abeli T., Gentili R., Rossi G., Bedini G. and Foggi B. 2009. Can the IUCN criteria be effectively applied to peripheral isolated plant populations?. Biodiversity and Conservation 18: 3877–3890.

Adler P.B., Salguero-Gómez R., Compagnoni A., Hsu J.S., Ray-Mukherjee J., Mbeau-Ache C. and Franco M. 2014. Functional traits explain variation in plant life history strategies. Proceedings of the National Academy of Sciences 111: 740–745.

Aguilella A., Fos S. and Laguna E. (Eds.) 2010. Catálogo Valenciano de Especies de Flora Amenazadas. Colección Biodiversidad 18. Conselleria de Medi Ambient, Aigua, Urbanisme i Habitatge, Generalitat Valenciana. Valencia.

Agulló J.C., Juan A., Alonso M.Á. and Crespo M.B. 2010. *Helianthemum caput-felis* Boiss. In: Bañares Á., Blanca G., Güemes J., Moreno J.C. and Ortiz S. eds. 2010. Atlas y Libro Rojo de la Flora Vascular Amenazada de España. Adenda 2010. Dirección General de Medio Natural y Política Forestal (Ministerio de Medio Ambiente, y Medio Rural y Marino) - Sociedad Española de Biología de la Conservación de Plantas. Madrid, pp. 76–77.

Akçakaya H.R. 2000. Population viability analyses with demographically and spatially structured models. Ecological Bulletins 48: 23–38.

Allen H.D. 2001. Mediterranean Ecogeography. Pearson Education, Harlow, UK.

Andrello M., Nicolè F., Till-Bottraud I. and Gaggiotti O.E. 2012. Effect of stage-specific vital rates on population growth rates and effective population sizes in an endangered iteroparous plant. Conservation Biology 26: 208–217.

Arrigoni P.V. 1971. *Helianthemum caput-felis* Boiss. (2n=24) nuovo reperto per la flora italiana. Webbia 26: 237–243.

Bacchetta G., Farris E. and Pontecorvo C. 2012. A new method to set conservation priorities in biodiversity hotspots. Plant Biosystems 146: 638–648.

Bañares Á., Blanca G., Güemes J., Moreno J.C. and Ortiz S. eds. 2010. Atlas y Libro Rojo de la Flora Vascular Amenazada de España. Adenda 2010. Dirección General de Medio Natural y Política Forestal (Ministerio de Medio Ambiente, y Medio Rural y Marino) - Sociedad Española de Biología de la Conservación de Plantas. Madrid, 170 pp.

Bañares Á., Blanca G., Güemes J., Moreno J.C. and Ortiz S. eds. 2004. Atlas y Libro Rojo de la Flora Vascular Amenazada de España. Dirección General de Conservación de la Naturaleza. Madrid, 1.069 pp.

Bastian O. 2013. The role of biodiversity in supporting ecosystem services in Natura 2000 sites. Ecological Indicators 24: 12–22.

Battandier J.A., Trabut L., Petit P.C.M., Debray F. and Flagey C. 1893. Flore de l'Algérie: contenant la description de toutes les plantes signalées jusqu'à ce jour comme spontanées en Algérie et catalogue des plantes du Maroc (Vol. 1). Adolphe Jourdan.

Benito B.M., Martínez-Ortega M.M., Muñoz L.M., Lorite J. and Peñas J. 2009. Assessing extinction-risk of endangered plants using species distribution models: a case study of habitat depletion caused by the spread of greenhouses. Biodiversity and Conservation 18: 2509–2520.

Bilz M., Kell S.P., Maxted N. and Lansdown R.V. 2011. European Red List of Vascular Plants. Luxembourg: Publications Office of the European Union.

Brigham C.A. and Thomson D.M. 2003. Approaches to modeling population viability in plants: an overview. In: Population viability in plants. Springer Berlin Heidelberg, pp. 145–171.

Brook B.W., Lim L., Harden R. and Frankham R. 1997. Does population viability analysis software predict the behaviour of real populations? A retrospective study on the Lord Howe Island woodhen *Tricholimnas sylvestris* (Sclater). Biological Conservation 82: 119–128.

Brook B.W., O'Grady J.J., Chapman A.P., Burgman M.A., Akçakaya H.R. and Frankham R. 2000. Predictive accuracy of population viability analysis in conservation biology. Nature 404: 385–387.

Bullock J.M., Pywell R.F. and Coulson-Phillips S.J. 2008. Managing plant population spread: prediction and analysis using a simple model. Ecological Applications 18: 945–953.

Burgman M.A., Fox J.C. 2003. Bias in species range estimates from minimum convex polygons: implications for conservation and options for improved planning. Animal Conservation 6: 19–28.

Cañadas E.M., Fenu G., Peñas J., Lorite J., Mattana E. and Bacchetta G. 2014. Hotspots within hotspots: endemic plant richness, environmental drivers, and implications for conservation. Biological Conservation 170: 282–291.

Cardillo M., Mace G., Gittleman J.L. and Purvis A. 2006. Latent extinction risk and the future battlegrounds of mammal conservation. Proceedings of the National Academy of Sciences of the United States of America 103: 4157–4161.

Caswell H. 2001. Matrix population models: construction, analysis, and interpretation. 2nd edition. Sinauer Associates, Sunderland, Massachusetts.

Caswell H. 2011. Beyond $R_0$: demographic models for variability of lifetime reproductive output. PloS ONE 6: e20809.

CBD 1992. Convention on Biological Diversity. 5 June 1992, Rio de Janeiro (Brasil).

Coale A.J. 1972. The growth and structure of human populations: A mathematical approach. Princeton University Press, Princeton, NJ, USA.

Cogoni D., Fenu G., Concas E. and Bacchetta G. 2013. The effectiveness of plant conservation measures: The *Dianthus morisianus* reintroduction. Oryx 47: 203–206.

Colas B., Olivieri I. and Riba M. 1997. *Centaurea corymbosa*, a cliff-dwelling species tottering on the brink of extinction: a demographic and genetic study. Proceedings of the National Academy of Sciences 94: 3471–3476.

Crone E.E., Ellis M.M., Morris W.F., Stanley A., Bell T., Bierzychudek P., Ehrlén J., Kaye T.N., Knight T.M., Lesica P., Oostermeijer G., Quintana-Ascencio P.F., Ticktin, Valverde T., Williams J., Doak D.F., Ganesan R., McEachern K., Thorpe A.S. and Menges E.S. 2013. Ability of matrix models to explain the past and predict the future of plant populations. Conservation Biology 27: 968–978.

Crone E.E., Menges E.S., Ellis M.M., Bell T., Bierzychudek P., Ehrlén J., Kaye T.N., Knight T.M., Lesica P., Morris W.F., Oostermeijer G., Quintana-Ascencio P.F., Stanley A., Ticktin T., Valverde T. and Williams J.L. 2011. How do plant ecologists use matrix population models? Ecology Letters 14: 1–8.

de Grammont P.C. and Cuarón A.D. 2006. An evaluation of threatened species categorization systems used on the American continent. Conservation Biology 20: 14–27.

De Vere N., Jongejans E., Plowman A. and Williams E. 2009. Population size and habitat quality affect genetic diversity and fitness in the clonal herb Cirsium dissectum. Oecologia 159: 59–68.

Del Vecchio S., Giovi E., Izzi C.F., Abbate G. and Acosta A.T. 2012. *Malcolmia littorea*: the isolated Italian population in the European context. Journal for Nature Conservation 20: 357–363.

Dimopoulos P., Bergmeier E. and Fischer F. 2005. Monitoring and conservation status assessment of habitat types in Greece: fundamentals and exemolary cases. Annali di botanica 5: 9–22.

Domínguez F., Galicia D., Moreno L., Moreno J.C. and Sainz H. 1996. Threatened plants in peninsular and Balearic Spain: a report based on the EU Habitats Directive. Biological Conservation 76: 123–133.

Domínguez F., Moreno J.C. and Schwartz M.W. 2011. Demographic modeling and monitoring cycle in a long-lived endangered shrub. Journal for Nature Conservation 19: 330–338.

Dudash M.R. and Fenster C.B. 2000. Inbreeding and outbreeding depression in fragmented populations. In: Genetics, Demography and Viability of Fragmented

Populations (eds A.G. Young and G.M. Clarke), pp. 35–53. Cambridge University Press, Cambridge, UK.

Duncan D.H., Nicotra A.B., Wood J.T. and Cunningham S.A. 2004. Plant isolation reduces outcross pollen receipt in a partially self-compatible herb. Journal of Ecology 92: 977–985.

Ezard H.G., Bullock J.M., Dalgleish H.J., Millon A., Pelletier F. and Ozgul A. 2010. Matrix models for a changeable world: The importance of transient dynamics in population management. Journal of Applied Ecology 47: 515–523.

Fenu G. and Bacchetta G. 2008. La flora vascolare della penisola del Sinis (Sardegna occidentale) [The vascular flora of the peninsula del Sinis (Western Sardinia)]. Acta Botánica Malacitana 33: 91–124.

Fenu G. and Mattana E. 2011. Conservation studies on threatened endemic plants of the Mediterranean area: a literature overview for Sardinia. Fitosociologia 481: 5–12.

Fenu G., Cogoni D., Pinna M.S. and Bacchetta G. 2015a. Threatened Sardinian vascular flora: A synthesis of 10 years of monitoring activities. Plant Biosystems 149(3): 473–482.

Fenu G., Cogoni D., Sulis E. and Bacchetta G. 2015b. Ecological response to human trampling and conservation status of *Helianthemum caput-felis* (Cistaceae) at the eastern periphery of its range. Botany Letters 162: 191–201.

Fenu G., Fois M., Cañadas E.M. and Bacchetta G. 2014. Using endemic-plant distribution, geology and geomorphology in biogeography: the case of Sardinia (Mediterranean Basin). Systematics and Biodiversity 12: 181–193.

Fenu G., Fois M., Cogoni D., Porceddu M., Pinna M.S., Cuena Lombraña A., Nebot A., Sulis E., Picciau R., Santo A., Murru V., Orrù M. and Bacchetta G. 2015c. The Aichi Biodiversity Target 12 at regional level: an achievable goal?. Biodiversity 16: 120–135.

Fenu G., Mattana E. and Bacchetta G. 2011. Distribution, status and conservation of a Critically Endangered, extremely narrow endemic: *Lamyropsis microcephala* (Asteraceae) in Sardinia. Oryx 45: 180–186.

Fenu G., Mattana E. and Bacchetta G. 2012. Conservation of endemic insular plants: the genus *Ribes* L. (Grossulariaceae) in Sardinia. Oryx 46: 219–222.

Fenu G., Sulis E., Cogoni D. and Bacchetta G. 2012b. Schede per una Lista Rossa della Flora vascolare e crittogamica Italiana: *Helianthemum caput-felis* Boiss. Informatore Botanico Italiano 44: 233–236.

Franco M. and Silvertown J. 2004. A comparative demography of plants based upon elasticities of vital rates. Ecology 85: 531–538.

García D. and Zamora R. 2003. Persistence, multiple demographic strategies and conservation in long-lived Mediterranean plants. Journal of Vegetation Science 14: 921–926.

García M.B. 2003. Demographic viability of a relict population of the critically endangered plant *Borderea chouardii*. Conservation Biology 17: 1672–1680.

García M.B., Guzmán D. and Goni D. 2002. An evaluation of the status of five threatened plant species in the Pyrenees. Biological Conservation 103: 151–161.

Gargano 2008. La procedura IUCN, generalità. In: Rossi G., Gentili R.F., Abeli T., Foggi B., Raimondo F.M. and Blasi C. (eds.) 2008. Flora da conservare. Iniziativa per l’implementazione in Italia delle categorie e dei criteri IUCN (2001) per la redazione di nuove Liste Rosse. Informatore Botanico Italiano 40: 25–34.

Gargano D., Fenu G., Medagli P., Sciandrello S. and Bernardo L. 2007. The status of *Sarcopoterium spinosum* (Rosaceae) at the western periphery of its range: Ecological constraints lead to conservation concerns. Israel Journal of Plant Sciences 55: 1–13.

Gaston K.J. 2003. The structure and dynamics of geographic ranges. Oxford University Press, Oxford, United Kingdom.

Gaston K.J. and Fuller R.A. 2009. The sizes of species' geographic ranges. Journal of Applied Ecology 46: 1–9.

Gibbs J.P. 2001. Demography versus habitat fragmentation as determinants of genetic variation in wild populations. Biological Conservation 100: 15–20.

Giménez-Font P., Sánchez Pardo Á., Padilla Blanco A. and Marco Molina J.A. 2008. Integración de una cartografía corológica a escala de detalle mediante GPS en el proceso urbanizador: *Helianthemum caput-felis* Boiss. en el litoral sur de Alicante (España). XI Coloquio Ibérico de Geografía. La perspectiva geográfica ante los retos de la sociedad y el medioambiente en el contexto ibérico. Alcalá de Henares 1–15.

Global Strategy for Plant Conservation (GSPC) 2008. https://www.cbd.int/gspc/

Godefroid S., Rivière S., Waldren S., Boretos N., Eastwood R. and Vanderborght T. 2011. To what extent are threatened european plant species conserved in seed banks? Biological Conservation 144: 1494–1498.

Gómez-Campo C. and Malato-Beliz J. 1985. The Iberian Peninsula. In: Gómez-Campo C., Plant conservation in the Mediterranean area. Dr W. Junk Publishers, Dordrecht, The Netherlands, pp. 47–70.

González E.J., Martorell C. and Bolker B.M. 2016. Inverse estimation of integral projection model parameters using time series of population-level data. Methods in Ecology and Evolution 7: 147–156.

Goodman D. 1987. The demography of chance extinction. In: Soule M.E., editor. Viable populations for conservation. Cambridge University Press, Cambridge, United Kingdom, pp. 11–34.

Guzmán B. and Vargas P. 2009. Historical biogeography and character evolution of Cistaceae (Malvales) based on analysis of plastid rbcL and trnL-trnF sequences. Organisms Diversity and Evolution 9: 83–99.

Heppell S.S., Crowder L.B. and Crouse D.T. 1996. Models to evaluate headstarting as a management tool for long-lived turtles. Ecological Applications 6: 556–565.

Heywood V.H. 2015. In situ conservation of plant species–an unattainable goal?. Israel Journal of Plant Sciences 1–21.

Hoffmann M., Brooks T.M., da Fonseca G.A., Gascon C., Hawkins A.F.A., James R.E., Langhammer P., Mittermeier R.A., Pilgrim J.D., Rodrigues A.S.L. and Silva J.M.C 2008. Conservation planning and the IUCN Red List. Endangered Species Research 6: 113–125.

Holsinger K.E. 2000. Demography and extinction in small populations. In: Genetics, Demography and Viability of Fragmented Populations (eds A.G. Young and G.M. Clark), Cambridge University Press, Cambridge, UK, pp. 55–74.

Horvitz C. and Schemske D.W. 1995. Spatiotemporal variation in demographic transtitions of a tropical understory herb: projection matrix analysis. Ecological Monographs 65: 155–192.

Iliadou E., Panitsa M., Raus T. and Dimopoulos P. 2014. Flora and factors affecting species diversity in protected "Natura 2000" sites of the Ionian area: the Echinades islet group (Greece). Willdenowia 44: 121–136.

IUCN 2001. IUCN Red List Categories and Criteria version 3.1. Gland, Switzerland and Cambridge, U.K., IUCN Species Survival Commission. Available at www.iucnredlist.org/technical-documents/categories-and-criteria

IUCN 2012. Threats Classification Scheme (Version 3.2). Available at http://www.iucnredlist.org/technical-documents/classification-schemes/threats-classification-scheme

IUCN Standards and Petitions Subcommittee 2014. Guidelines for Using the IUCN Red List Categories and Criteria. Version 11. Prepared by the Standards and Petitions Subcommittee. Available at http://www.iucnredlist.org/documents/RedListGuidelines.pdf.

Jacquemyn H., Brys R. and Jongejans E. 2010. Size-dependent flowering and costs of reproduction affect population dynamics in a tuberous perennial woodland orchid. Journal of Ecology 98: 1204–1215.

Jermy T. and Szentesi Á. 2003. Evolutionary aspects of host plant specialisation—a study on bruchids (Coleoptera: Bruchidae). Oikos 101: 196–204.

Johnson H.E., Mills L.S., Stephenson T.R. and Wehausen J.D. 2010. Population-specific vital rate contributions influence management of an endangered ungulate. Ecological Applications 20: 1753–1765.

Kolb A. and Diekmann M. 2005. Effects of life-history traits on responses of plant species to forest fragmentation. Conservation Biology 19: 929–938.

Koons D.N., Grand J.B., Zinner B. and Rockwell R.E. 2005. Transient population dynamics: relations to life history and initial population state. Ecological Modelling 185: 283–297.

Laguna E., Deltoro V.I., Pérez-Botella J., Pérez-Rovira P., Serra L., Olivares A. and Fabregat C. 2004. The role of small reserves in plant conservation in a region of high diversity in eastern Spain. Biological Conservation 119: 421–426.

Larson D.W., Matthes U., Gerrath J.A., Larson N.W.K., Gerrath J.M., Nekola J.C., Walker G.L, Porembski S. and Charlton A. 2000. Evidence for the widespread occurrence of ancient forests on cliffs. Journal of Biogeography 27: 319–331.

Lavergne S., Thompson J.D., Garnier E. and Debussche M. 2004. The biology and ecology of narrow endemic and widespread plants: A comparative study of trait variation in 20 congeneric pairs. Oikos 107: 505–518.

Lesica P. and Allendorf F.W. 1995. When are peripheral populations valuable for conservation? Conservation Biology 9: 753–760.

Lienert J. 2004. Habitat fragmentation effects on fitness of plant populations—a review. Journal for Nature Conservation 12: 53–72.

López González G. 1992. Apuntes para justificar el tratamiento del género *Helianthemum* Miller, s.l. (Cistaceae), en Flora Iberica [Notes to explain the treatment of the genus *Helianthemum* Miller, s.l. (Cistaceae), on Flora Iberica]. Anales del Jardín Botánico de Madrid 50: 35–63.

Ludwig D. 1999. Is it meaningful to estimate a probability of extinction? Ecology 80: 298–310.

Mace G.M. and Lande R. 1991. Assessing extinction threats: toward a re-evaluation of IUCN threatened species categories. Conservation Biology 5: 148–157.

Mace G.M., Collar N.J., Gaston K.J., Hilton-Taylor C., Akçakaya H.R., Leader-Williams N. and Stuart S.N. 2008. Quantification of extinction risk: IUCN's system for classifying threatened species. Conservation Biology 22: 1424–1442.

Maiorano L., Amori G., Montemaggiori A., Rondinini C., Santini L., Saura S. and Boitani L. 2015. On how much biodiversity is covered in Europe by national protected areas and by the Natura 2000 network: insights from terrestrial vertebrates. Conservation Biology 29: 986–995.

Maiorano L., Falcucci A., Garton E.O. and Boitani L. 2007. Contribution of the Natura 2000 network to biodiversity conservation in Italy. Conservation Biology 21: 1433–1444.

Marcer A., Sáez L., Molowny-Horas R., Pons X. and Pino J. 2013. Using species distribution modelling to disentangle realised versus potential distributions for rare species conservation. Biological conservation 166: 221–230.

Marco Molina J.A., Giménez Font P., Padilla Blanco A. and Sánchez Pardo Á. 2011. Crecimiento urbano y conservación de flora amenazada: aplicaciones cartográficas en el caso de *Helianthemum caput-felis* Boiss. Serie Geográfica 17: 125–139.

Marco Molina J.A., Sánchez Pardo Á., Padilla Blanco A. and Giménez Font P. 2014. Identificación de impactos sobre flora rara, endémica o amenazada mediante el análisis de secuencias espacio-temporales. In: XVI Congreso Nacional de Tecnologías de Información Geográfica.

Marrero-Gómez M.V., Oostermeijer J.G.B., Carqué-Álamo E. and Bañares-Baudet Á. 2007. Population viability of the narrow endemic *Helianthemum juliae* (Cistaceae) in relation to climate variability. Biological conservation 136: 552–562.

Matthies D., Bräuer I., Maibom W. and Tscharntke T. 2004. Population size and the risk of local extinction: empirical evidence from rare plants. Oikos 105: 481–488.

Médail F. and Verlaque R. 1997. Ecological characteristics and rarity of endemic plants from Southeast France and Corsica: implications for biodiversity conservation. Biological Conservation 80: 269–281.

Menges E.S. 2000. Population viability analyses in plants: challenges and opportunities. Trends in Ecology and Evolution 15: 51–56.

Metcalf J., McMahon S.M., Salguero-Gómez R., Jongejans E., and Merow C. 2014. IPMpack: an R package for demographic modeling with Integral Projection Models (v. 2.1).

Mills L.S. 2007. Conservation of wildlife populations: demography, genetics, and management. Blackwell Publishing, Malden, Massachusetts.

Milner-Gulland E.J. and Rowcliffe J.M. 2007. Conservation and Sustainable Use. Oxford University Press, Oxford.

Mittermeier R.A., Robles Gil P., Hoffman M., Pilgrim J., Brooks T., Mittermeier C.G., Lamoreux J. and Fonseca G.A.B. 2005. Hotspots revisited: Earth’s biologically richest and most endangered terrestrial ecoregions. Chicago, IL, University of Chicago Press.

Moreno Saiz J.C. coordinator 2008. Lista Roja 2008 de la flora vascular española. Dirección General de Medio Natural y Política Forestal (Ministerio de Medio Ambiente, y Medio Rural y Marino, y Sociedad Española de Biología de la Conservación de Plantas), Madrid, 86 pp.

Moreno Saiz J.C., Domínguez Lozano F. and Sainz Ollero H. 2003. Recent progress in conservation of threatened Spanish vascular flora: a critical review. Biological Conservation 113: 419–431.

Moreno Saiz J.C., Domínguez Lozano F., Marrero Gómez M. and Bañares Baudet Á. 2015. Application of the Red List Index for conservation assessment of Spanish vascular plants. Conservation Biology 29: 910–919.

Morris W.F. and Doak D.F. 1998. Life history of the long-lived gynodioecious cushion plant *Silene acaulis* (Caryophyllaceae), inferred from size-based population projection matrices. American Journal of Botany 85: 784–793.

Morris W.F. and Doak D.F. 2002. Quantitative conservation biology: The theory and practice of population viability analysis. Sunderland, MA: Sinauer Associates.

Novacek M.J. and Cleland E.E. 2001. The current biodiversity extinction event: scenarios for mitigation and recovery. Proceedings of the National Academy of Sciences 98: 5466–5470.

Or K. and Ward D. 2007. Maternal effects on the life histories of bruchid beetles infesting *Acacia raddiana* in the Negev desert, Israel. Entomologia experimentalis et applicata 122: 165–170.

Padilla A. and Ramón A. 1997. Planeamiento ambiental a escala de detalle: Microrreservas de flora en la Comunidad Valenciana. Investigaciones Geográficas 17: 117–128.

Picó F.X. and Riba M. 2002. Regional-scale demography of *Ramonda myconi*: Remnant population dynamics in a preglacial relict species. Plant Ecology 161: 1–13.

Pimm S.L., Jenkins C.N., Abell R., Brooks T.M., Gittleman J.L., Joppa L.N., Raven P.H., Robert C.M. and Sexton J.O. 2014. The biodiversity of species and their rates of extinction, distribution, and protection. Science 344: 1246752.

Pimm S.L., Russel G.J., Gittleman J.L. and Brooks T.M. 1995. The Future of Biodiversity. Science 269: 347–350.

Pisanu S., Farris E., Filigheddu R. and García M.B. 2012. Demographic effects of large, introduced herbivores on a long-lived endemic plant. Plant Ecology 213: 1543–1553.

Planta Europa 2008. A Sustainable Future for Europe: The European Strategy for Plant Conservation 2008–2014. Plantlife.

Possingham H.P., Andelman S.J., Burgman M.A., Medellín R.A., Master L.L., Keith D.A. 2002. Limits to the use of threatened species lists. Trends in Ecology and Evolution 17: 503–507.

Próctor M.C.F. and Heywood V.H. 1968. *Helianthemum* Miller. In: Flora Europaea, edited by T.G. Tutin, H. Heywood, N.A. Burges, D.M. Moore, D.H. Valentine, S.M. Walters and D.A. Webb Cambridge: Cambridge University Press 2: 286–292.

R Core Team 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Ramula S., Rees M. and Buckley Y.M. 2009. Integral projection models perform better for small demographic data sets than matrix population models: a case study of two perennial herbs. Journal of Applied Ecology 46: 1048–1053.

Randrianasolo A., Miller J.S. and Consiglio T.K. 2002. Application of IUCN criteria and Red List categories to species of five Anacardiaceae genera in Madagascar. Biodiversity and Conservation 11:1289–1300.

Reed J.M., Fefferman N. and Averill-Murray R.C. 2009. Vital rate sensitivity analysis as a tool for assessing management actions for the desert tortoise. Biological Conservation 142: 2710–2717.

Richter-Dyn N. and Goel N.S. 1972. On the extinction of a colonising species. Theoretical Population Biology 3: 406–433.

Rodrigues A.S., Pilgrim J.D., Lamoreux J.F., Hoffmann M. and Brooks T.M. 2006. The value of the IUCN Red List for conservation. Trends in Ecology and Evolution 21: 71–76.

Rodríguez-Pérez J. 2005. Breeding system, flower visitors and seedling survival of two endangered species of *Helianthemum* (Cistaceae). Annals of Botany 95: 1229–1236.

Rossi G., Orsenigo S., Montagnani C., Fenu G., Gargano D., Peruzzi L., Wagensommer R.P., Foggi B., Bacchetta G., Domina G., Conti F., Bartolucci F., Gennai M., Ravera S., Cogoni A., Magrini S., Gentili R., Castello M., Blasi C. and Abeli T. 2016. Is legal protection sufficient to ensure plant conservation? The Italian Red List of Policy species as a case study. Oryx in press.

Schemske D.W., Husband B.C., Ruckelshaus M.H., Goodwillie C., Parker I.M. and Bishop J.D. 1994. Evaluating approaches to the conservation of rare and endangered plants. Ecology 75: 584–606.

Schleuning M. and Matthies D. 2009. Habitat change and plant demography: assessing the extinction risk of a formerly common grassland perennial. Conservation Biology 23: 174–183.

Schmeller D.S., Henry P.-Y., Julliard R., Gruber B., Clobert J., Dziock F., Lengyel S., Nowicki P., Déri E., Budrys E., Kull T., Tali K., Bauch B., Settele J., Van Swaay C., Kobler A., Babij V., Papastergiadou E. and Henle K. 2009. Advantages of volunteer-based biodiversity monitoring in Europe. Conservation Biology 23: 307–316.

Schwartz M.W. 2003. Assessing population viability in long-lived plants. In: Brigham C.A. and Schwartz M.W. (Eds.), Population viability in plants . Springer Berlin Heidelberg, pp. 239–266.

Schwartz M.W., Hermann S.M. and van Mantgem P.J. 2000. Estimating the magnitude of decline of the Florida torreya (*Torreya taxifolia* Arn.). Biological conservation 95: 77–84.

Silva J.L., Mejías J.A. and García M.B. 2015. Demographic vulnerability in cliff-dwelling Sonchus species endemic to the western Mediterranean. Basic and Applied Ecology 16: 316–324.

Stubben C. and Milligan B. 2007. Estimating and analyzing demographic models using the popbio package in R. Journal of Statistical Software 22: 1–23.

Taieb Brahim Zeineb 2012. L'Herbier du Musée d'Oran: Inventaire Et Taxonomie. Ph.D. dissertation, Universitè d'Oran, Algery.

Tavsanoglu C. 2010. Seed production and fruit parasitism in *Cistus salviifolius* L. (Cistaceae) along a post-fire successional gradient. Journal of Animal and Veterinary Advances 9: 1120–1127.

Thompson J.D. 2005. Plant Evolution in the Mediterranean. Oxford: Oxford University Press.

Tuljapurkar S.D. 1982. Population dynamics in variable environments. II. Correlated environments, sensitivity analysis and dynamics. Theoretical Population Biology 21: 114–140.

van der Meer S., Dahlgren J.P., Mildén M. and Ehrlén J. 2014. Differential effects of abandonment on the demography of the grassland perennial *Succisa pratensis*. Population Ecology 56: 151–160.

Villellas J., Ehrlén J., Olesen J.M., Braza R. and García M.B. 2013a. Plant performance in central and northern peripheral populations of the widespread *Plantago coronopus*. Ecography 36: 136–145.

Villellas J., Morris W.F. and García M.B. 2013b. Variation in stochastic demography between and within central and peripheral regions in a widespread short-lived herb. Ecology 94: 1378–1388.

Vischi N., Natale E. and Villamil C. 2004. Six endemic plant species from central Argentina: an evaluation of their conservation status. Biodiversity and Conservation 13: 997–1008.

Williams J.L., Ellis M.M., Bricker M.C., Brodie J.F. and Parsons E.W. 2011. Distance to stable stage distribution in plant populations and implications for near-term population projections. Journal of ecology 99: 1171–1178.

Zaragozí B., Giménez P., Navarro J.T., Dong P. and Ramón A. 2012. Development of free and opensource GIS software for cartographic generalisation and occupancy area calculations. Ecological Informatics 8: 48–54.

## Main conclusions

This research produced important new information regarding the distribution pattern, reproductive traits, demography and ecological requirements of *H. caput-felis*, which are relevant issues for developing future conservation measures for this species. In particular, this research helped to identify the key traits linked to extinction risk, which are critical for predicting future decline, and to evaluate the conservation status of the species at global level.

The distribution area of *H. caput-felis* in Sardinia consists of two distinct localities, approximately 3 km apart. In this region, the species preferably grows in lowland areas with deep structured soil, due to the amount of water and resources available and morphological stability. *H. caput-felis* shows the highest reproductive output in garrigues, habitat that represents its ecologically optimum. The high seed production indicates that this is not a limiting factor for this plant, in contrast to seedling establishment, which represent its main critical stage. Surprisingly, in Sardinia, human trampling enhanced the plant size and the rate of fruits production, suggesting that reproductive plants of *H. caput-felis* are tolerant to direct damage, and probably benefit from the reduction of inter-specific and intra-specific competition (Chapter 1).

An extensive and integrative approach that compares the phenology and reproductive output along the entire European distribution range of this species over a two year temporal context was adopted. The results indicate the phenology and the importance of abiotic factors, as geomorphology, substrate, slope and human trampling intensity, on reproductive outputs of this species. The general phenological pattern is similar among Sardinian and Majorcan regions, whereas, in the eastern Iberian populations (the western part of the distribution range), the southern population (Cabo Roig) showed a higher inter-annual variability and an early flowering period. Those differences could be related to a longitudinal aridity gradient for this plant: the aridity decreases from western to eastern localities while the mean temperatures and the oceanicity increase. Plants growing at the eastern edge of the distribution range (Sardinia) produce more viable fruits, fruits per plant and seeds per fruit than the other two regions, while plants growing in arid substrate (as in the sandy habitat of Sa Ràpita, Majorca) are the least productive, confirming that sand substrate is negatively correlated with fruit production and viable fruits, as well as lowland geomorphology. Hence, these analyses underline that the Abundant Centre Model assumptions, by which reproductive traits should be higher at the centre of species distribution, is not confirmed for *H. caput-felis*, maybe because the central populations, represented by the largest distribution area along the Iberian's coasts, appear strongly fragmented mainly by human disturbance (Chapter 2).

Demographic traits of *H. caput-felis* population were analysed, by using demographic surveys, integrated by Matrix and Integral Projection Models (MPM and IPM, respectively). Plant performance and population dynamics were investigated in plants sampled along the comprehensive and representative part of the European distribution range in which the species occurs. The global population showed a general stability in the first year transition (2013–2014), after which it declined (2014–2015). This trend was probably due to the higher shrinkage of plants in the last year of study, that correlates negatively with reproductive traits, in agreement with the frequent increase in reproductive outputs with plant size. Despite this decline, stochastic population growth rate highlighted as *H. caput-felis* population oscillated around the equilibrium. Elasticity analysis indicated the higher importance of large individuals and the determinant role of the survival-growth transitions for the population growth rate. Moreover, plants reached high generation times and mean long life span, which, with the limited seedling recruitment rate and the presence of a consistent seed bank, reflect patterns that are typical both of many long-lived plants and of calcareous rupicolous Mediterranean species. IPM methods were found to be more accurate than MPM to monitoring species with restricted range, as *H. caput-felis* (Chapter 3).

A more detailed demography investigation was carried out at local level. The pattern of typical long-lived Mediterranean plants, underlined in Chapter 3, was confirmed also at local level; in fact, populations were mainly composed from large individuals, characterized by slow growth (frequency of stasis). *H. caput-felis* showed vital rates and population dynamics varying among populations: the Sardinians' seems to have greater equilibrium ($\lambda$ closer to 1) than Spanish populations. The high population growth rates of Moraira (north eastern Iberian coast) and Colònia de Sant Jordi (south Majorcan coast) were strongly influenced by the fecundity parameters. In fact, for long-lived, iteroparous species, fecundity is generally less important for population growth than survival; hence, for these populations, results should be considered with caution. Plants growing in Moraira show a uniform shrinkage of individuals, probably due to a more intense fragmentation of the population. This study confirms the general pattern within the species: populations with $\lambda$ over 1 are associated with a higher contribution of sexual reproduction, whereas populations with low $\lambda$ rely more on survival. Moreover, the different population dynamics observed among the analysed localities of *H. caput-felis* should be correlated to particular microclimate and ecological conditions, i.e. the high aridity of Cabo Roig and the sand substrate of Sa Ràpita (Chapter 2), which probably affected the relationship among size and vital rates. On the contrary, *H. caput-felis* populations in demographic equilibrium, as the Sardinians', could be locally adapted to ecological and microclimate conditions (Chapter 4).

Finally, population dynamics models were applied to analyse the extinction risk and then assess the global conservation status of *H. caput-felis*, together with an accurate analysis of the global area of occupancy of the species, following the IUCN protocol. This research highlighted that *H. caput-felis* should be considered an Endangered (EN) plant at global level, according to the formula B2ab (ii,iii,iv,v) + E. *H. caput-felis* suffered a continuing decline in AOO, quality of its habitat, number of localities and number of reproductive plants. This decline rate was mainly due to the habitat loss and the expansion of infrastructures detected in the last decades. Hence, effective future conservation measures could be focussed on protecting the single localities where *H. caput-felis* grows, in order to avoid further species decline. In this contest, conservation efforts primarily aimed to protect established individuals (mature plants) against disturbances, as suggested for other long-lived species (Chapter 5).

# Appendix – Altre pubblicazioni prodotte durante il dottorato di ricerca

*Articoli scientifici su riviste non indicizzate*:
**Sulis E.**, Fenu G., Cogoni D. and Bacchetta G. 2014. *Helianthemum morisianum* Bertol. Informatore Botanico Italiano 46: 93–152.

*Articoli scientifici su riviste indicizzate*:
Fenu G., Fois M., Cogoni D., Porceddu M., Pinna M.S., Cuena Lombraña A., Nebot A., **Sulis E.**, Picciau R., Santo A., Murru V., Orrù M. and Bacchetta G. 2015. The Aichi Biodiversity Target 12 at regional level: an achievable goal?. Biodiversity 16: 120–135.

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