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Populations studies on two endemic *taxa* of southwestern Sardinia: *Dianthus morisianus* Vals. (Caryophyllaceae) and *Anchusa littorea* Moris (Boraginaceae)

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“Arrivato dove desiderava, cominciò a piantare la sua asta di ferro in terra. Faceva così un buco nel quale depositava una ghianda, dopo di che turava di nuovo il buco. Piantava querce. Gli domandai se quella terra gli apparteneva. Mi rispose di no. Sapeva di chi era? Non lo sapeva. Non gli interessava conoscerne i proprietari. Piantò così le cento ghiande con estrema cura. Dopo il pranzo di mezzogiorno, ricominciò a scegliere le ghiande. Misi, credo, sufficiente insistenza nelle mie domande, perché mi rispose. Da tre anni piantava alberi in quella solitudine. Ne aveva piantati centomila. Di centomila, ne erano spuntati ventimila. Di quei ventimila, contava di perderne ancora la metà. Restavano diecimila querce che sarebbero cresciute in quel posto dove prima non c'era nulla.”

L'uomo che piantava gli alberi" Jean Giono

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CHAPTER 1

INTRODUCTION

The environment is a complicated dynamic system, with many interacting component (Tolba 1992). Biological diversity, or biodiversity, refers to the variety of life forms at all levels of organization, from the molecular to the landscape level; in the broad sense, it is the number, abundance, composition, spatial distribution, and interactions of genotypes, populations, species, functional types and traits, and landscape units in a given system (Heywood & Iriondo 2003). Biodiversity is generated and maintained in natural ecosystems, where organisms encounter a wide variety of living conditions and chance events that shape their evolution in unique ways (Daily et al. 1997). Out of convenience or necessity, it is usually quantified in terms of numbers of species, and this perspective has greatly influenced conservation goals (Daily et al. 1997).

Biodiversity is essential for several reasons: it increases the ability of ecosystems to respond to climate change, preserves wealth of important resources fundamental in humans and allows communities to be more resilient against catastrophic events (McGrady-Steed et al. 1997; Naem & Li 1997; Olden et al. 2005). In the broad sense, it also influences ecosystem services, that is, the benefits provided by ecosystems to humans, that contribute to making human life both possible and worth living (Mace et al. 2005). Examples of these services are pollination and seed dispersal of useful plants, regulation of climatic conditions suitable to humans and the animals and plants they consider important, the control of agricultural pests and diseases, and the regulation of human health (Diaz et al. 2006). Also, by affecting ecosystem processes such as biomass production by plants, nutrient and water cycling, and soil formation and retention, biodiversity indirectly supports the production of food, fiber, potable water, shelter, and medicines (Diaz et al. 2006). The evidence available indicates that it is functional composition (the identity, abundance, and range of species traits) that appears to cause the effects of biodiversity on many ecosystem services. At least among species within the same trophic level (e.g., plants), rare species are likely to have small effects at any given point in time. Thus, in natural systems, if we are to preserve the services that ecosystems provide to humans, we should focus on preserving or restoring their biotic integrity in terms of species composition, relative abundance, functional organization, and species numbers (whether inherently species poor or species-rich), rather than on simply maximizing the number of species present (Diaz et al. 2006).

The links between biodiversity and ecosystem services have been gaining increasing attention in the scientific literature of the past few years.(Mace et al. 2005). The past years have seen a remarkable growth in concern for wildlife and the environment, with an increased appreciation of the links between the state of ecosystems and the state of humankind (servirebbe un rif). There is consensus in the scientific community that the current massive degradation of habitat and extinction of many of the Earth's biota is unprecedented and is taking place on a catastrophically short timescale (Novacek & Cleland 2001).

Human actions are causing a biodiversity crisis, with species extinction rating up to 1000 times higher than background (Brooks et al. 2006; Pimm et al. 1995); therefore, based on these estimates, the scenarios involving the extinction of about 30% of all species by the middle of the twenty-first century, are realistic (Erwin 1993; Lawton & May 1995; Pimm et al. 1995; Wilson 1992). Major threats to ecosystems and biodiversity are habitat loss and fragmentation, overexploitation, pollution, invasion of alien species, global climate change (IUCN 2003) and disruption of community structures (Novacek & Cleland 2001).

Many analysts have concluded that achieving sustainable and equitable human development will require, among other measures, taking a more effective approach to managing human impacts on the biosphere (Groombridge & Jenkins 2002). This was reinforced by the 1992 Unit Nations Conference on Environment and Development in Rio de Janeiro (Brazil), at which the Convention on Biological Diversity (CBD) was opened for signature. CBD has been the first initiative for the biodiversity conservation on a global scale and it has established guidelines to elaboration of general strategies for animals, plants and habitats preservation (Williams et al. 2003). It provides the global mechanism to ensure the conservation and sustainable use of biodiversity for the present and future generations. Many conservation and management initiatives worldwide have arisen from efforts to meet the objectives framed by the CBD text. For this reason, at the present, the provisions of the CBD have been implemented by promulgation of the Habitats Directive (92/43/EEC) by the European Union, which represents a fundamental step for the biodiversity conservation in Europe.

Convention on Biological Diversity defined *in situ* and *ex situ* conservation as two distinct approaches to the protection of wild species (Williams et al. 2003; Pritchard et al. 2011). *In situ* conservation, with reference to the protection of species in their natural surroundings, derives primarily from scientific considerations concerning the conservation benefits that accrue from the protection of integrated habitats and ecosystems (Pritchard et al. 2011). *In situ* conservation has been designated, expressly, as the legal and institutional priority. The CBD and other global instruments and funding strategies address a range of practices relating to *in situ* measures for conservation and relegate *ex situ* approaches to a subordinated supply role (CBD 1992: Articles 8 and 9; Pritchard et al. 2011). In addition, other initiatives for the protection and preservation biodiversity has been taken, including the Global Strategy for Plant Conservation (GSPC), and the European Strategy for Plant Conservation (ESPC), both issued in 2002 (Pritchard et al. 2011). The Global Strategy for Plant Conservation (GSPC 2008) and the European Plant Conservation Strategy (Planta Europa 2008) aim to halt the continuing loss of plant diversity and, as part of this, the development of conservation strategies is an issue that needs to be urgently addressed at the national level (GSPC 2008; Sharrock & Jones 2009). The GSPC is a global strategic plan which has as main objectives the study focused on understanding the diversity of plant species and their status in the global and regional levels, through the assessment of their conservation status. It also proposes to preserve diversity through the protection of plant *taxa* and areas in which plants live, considering especially *taxa* threatened with extinction (GSPC 2008). GSPC is constituted by sixteen plant conservation targets. Still, many of the original targets set for 2010 were not achieved. Then, a project was launched in 2009 to put together a consolidated update to the GSPC, and revise the target dates for the first time. In particular, the "Target 7"which refers to "*In Situ* Plant Conservation" supposed for 2010as following: : 60 per cent of the world's threatened species conserved *in situ* and a revised Target for 2020: at least 75 per cent of known threatened plant species conserved *in situ.* About the change: the increase in percentage of the world's threatened plants to be protected by *in situ* means reflects an overall desire to have achieved significantly more progress by 2020 (GSPC 2008).

The ESPC, in Europe, adopted by the Council of Europe and by Planta Europa (www.plantaeuropa.org), represents the European contribution to the implementation of the GSPC. The ESPC recommends to complete an *ex situ* conservation for the 80% of species which could have declined by 2010 and also to start effective conservation measures *in situ*. The European Strategy, revised at the end of 2007, has adopted new objectives to be achieved in the period 2008-2014. Among the main programs for the plant conservation, the "Important Plant Areas (IPAs)" project is a program with a means of identifying and protecting the most important sites for wild plant and habitats in Europe. IPAs are intended to be areas of great botanical importance for threatened species, habitats and plant diversity in general, that can be identified, protected and managed as sites (Anderson 2002; Palmer & Smart 2001). However, the IPA programme is intended to build on this approach to identify areas that are appropriate for a site-based approach to conservation and in Italy such areas have just been identified (Blasi et al. 2011).

A very important role for international conservation is covered by the International Union for Conservation of Nature (IUCN), an international organization founded in 1948; it is a Union of Nations, government departments, non-political organizations, to promote scientifically based actions for the conservation of nature and the natural resources on which all living things depend. Since 1966 the IUCN had started the long and difficult cataloging endangered plant species and for many years, its concern with rare and endangered plants and animals has been expressed through the work of the Species Survival Commission (SSC). This cataloging only appeared in 1970 with the first publication of Red Data Book designation, followed in 1978 by a new expanded edition. They represented the first lists of threatened species worldwide.

The IUCN Red List of Threatened Species (henceforth 'Red List'), elaborated by the Species Survival Commission (SSC), highlights species that are at the greatest risk of extinction (IUCN 2001) and promotes their conservation by 'concentrating minds on true priorities' (Collar 1996). It have increasingly been adopted as the gold standard for information on the conservation status of species (e.g. Grammont de & Cuarón 2006; Rodrigues et al. 2006; Hoffman et al. 2008). The IUCN method is the most common methods used in the world (Grammont de & Cuaròn 2006) and assign a specific *taxa* to risk categories based on quantitative or semi-quantitative criteria regarding the species distribution, size, populations evolution and number of mature individuals, which can ensure the survival of the species (IUCN 2001, 2003, Mace et al. 2008). However, application of IUCN criteria at the local level is often problematic because criteria are primarily designed for application at the global level (Gardenfors 2001; IUCN 2001; Mace & Lande 1991). For these reasons, seems to be more appropriate to adapt criteria IUCN to local conditions (Miller 2005; Miller et al. 2007), especially when their application is based on areas with limited extension and with many endemic species like island and hotspots of biodiversity. At the national level, the first Italian red data book, including vascular plants, bryophytes and lichens, was published in 1992 (Conti et al. 1992), successively followed by revisions for vascular plants and bryophytes, at national and regional level (Conti et al. 1997). During the last years, the Italian Botanical Society promoted a new and comprehensive Red List of the Italian Flora, based on the more recent IUCN criteria and categories (Rossi & Gentili 2008).

To efficiently manage rare and threatened species, it is important to understand their population dynamics and identify the current threats acting on them (Oostermeijer et al. 1996).

Long-term demographic surveys are needed to obtain accurate information on species life-history and identify biotic or abiotic factors that affect population dynamics (Fieberg & Ellner 2001; Fréville et al. 2004; Pfeifer et al. 2006; Waite & Hutchings 1991). Thus, demographic monitoring and understanding the natural history of rare plants are then crucial for both population management and conservation (e.g. Adams et al. 2005; Lehtilä et al. 2006). Specifically, survival and reproduction patterns are a prerequisite in order to predict future growth or decline of populations and to help in the selection of appropriate management strategies for species conservation. Such data can lead to the development of effective conservation plans for rare species (Pino & de Roa 2007).

The Mediterranean Basin is a key area for the conservation of plant diversity (Médail & Quézel 1999; Myers et al. 2000; Mittermayer et al. 2004). Two of the most important and well-known features of the Mediterranean flora in this context are the high rates of overall and regional endemism and the elevated species richness (Debussche & Thompson 2003; Thompson 2005). The Mediterranean flora is also characterized by a high frequency of disjunct distributions of closely related species (Debussche & Thompson 2003; Thompson 2005). In this area, islands and islets constitute the major plant biodiversity centers (Médail & Quezel 1999), mainly due to the narrow distribution of most of their flora (Rosselló et al. 2009). Conservation studies represent a crucial issue in the Mediterranean biome because this area, which represents only 2% of the world’s surface, houses 20% of the world’s total floristic richness (Medail & Quezel 1999). In fact, the Mediterranean basin, with 11.8 endemic plants per 100 km^2, has been recognized as one of the priority regions for conservation in Europe and identified as one of the 34 most important “biodiversity hotspots” of the planet (Mittermeier et al. 2004). To better assess plant conservation priorities in this area 10 different hotspots characterized by high species richness were defined (Medail & Quezel 1997, 1999). More recently, Vela and Benhouhou (2007) individuated a new hotspot named ‘Kabylias-Numidia-Kroumiria’ and suggested to consider also the Dalmatian coast and archipelagos (Croatia), based on preliminary results on endemic plant richness (Nikolic et al. 2008). In this geographical context, Corsica and Sardinia islands are the relevant biodiversity hotspots, in terms of floristic richness and endemism rate (Médail & Quézel 1997; Thompson 2005). These islands have several floristic affinities, even if this Tertiary isolation contributed to the differentiation of neoendemics that are specific to each area and constitute the Tyrrhenian Islands hotspot (Médail & Quézel 1997).

Sardinia is the second largest island in the Mediterranean Sea (after Sicily) and it is situated in the Western Mediterranean basin. Its isolation and high geological diversity have created a wide range of habitats, with high levels of endemism, especially on its mountain massifs, where there are conditions of ecological insularity (Médail & Quézel 1997). The Sardinian flora consists of 2,408 *taxa* including 2,295 species (Conti et al. 2005), 347 of which are endemics and, in particular, 168 being exclusive Sardinian endemics (Bacchetta et al. 2012). About a hundred species endemic to Sardinia (Conti et al. 1992, 1997) have been recognized as threatened; furthermore, five exclusive endemics [i.e. *Aquilegia barbaricina* Arrigoni *et* E. Nardi, *A. nuragica* Arrigoni *et* E. Nardi, *Lamyropsis microcephala* (Moris) Dittrich *et* Greuter, *Polygala sinisica* Arrigoni and *Ribes sardoum* Martelli] have been included by the IUCN/SSC - Mediterranean Island Plant Specialist Group in the "Top 50 Mediterranean Island Plants" to be urgently conserved (de Montmollin & Strahm 2005).

When working in such species-rich areas, "priority lists" should be created in order to identify the target species for conservation measures, as the conservation of biodiversity occurs via the implementation of policy with only limited resources (Balmford et al. 2005; Possingham & Wilson 2005; Wilson et al. 2006). Elaboration of lists is a fundamental step in order to enable studies of conservation biology and, in particular, on population analysis and reproductive biology which enabling to assess the conservation status of species and the possibility of long-term survival populations, allowing the management and protection strategies. Some attempts to set conservation priorities have been carried out at a regional level. In particular, Domínguez Lozano et al. (2003) found that an overall pattern in conservation practice of threatened Iberian plants (including the Balearic Islands) seems to be defined by their ecological specificity, geographical rarity and rate of threat. Jiménez-Alfaro et al. (2010), based on the results achieved in a study focused on the Cantabrian Range (Spain), suggest that different point-scoring procedures might have high impact on the application of priority lists for selecting conservation targets. Gauthier et al. (2010), comparing three rarity-associated criteria for rare plants of the Languedoc-Roussillon region (France) with the aim of establishing regional-level priorities, identified the "regional responsibility" (i.e. highest scores associated to species whose distribution is endemic to the study area) as the first order priority at local level.

In Sardinia a priority list of exclusive endemic species was created by integrating the three kinds of lists reported in Grammont de & Cuarón (2006): (1) lists based on the degree of biological threat, (2) conservation lists and (3) international and national protection catalogues. The conservation priority ranking allowed the identification of the ten most threatened species (Table 1) and an integrated conservation approach was activated on them; in particular, their populations have been characterized and long term conservation measures were carried out by seed collecting and storing at the Sardinian Germplasm Bank (BG-SAR). Furthermore, ecological (Bacchetta et al. 2011; Fenu et al. 2010, 2011a, in press), germination ecophysiology (Cogoni et al. 2012; Mattana et al. 2009, 2010a, b, 2011) as well as population genetic studies (Garrido et al. in press) have been activated for them. All these species are catalogued in the official IUCN Red List (IUCN 2010) with the exception of *Anchusa littorea* Moris, *Dianthus morisianus* Vals., *Astragalus maritimus* Moris and *Astragalus verrucosus* Moris, for which IUCN categories have been proposed by different authors (see Fenu & Bacchetta 2008; Fenu et al. 2010; Bacchetta et al. 2011). All these species are considered or have been proposed to be considered under the Critically Endangered (CR) category (Table 1).

Table 1 - The ten most threatened exclusive endemic species of Sardinia following Bacchetta et al. (2012).

N	***Taxon***	**Family**	**IUCN Category**	**92/43/CEE Directive**
1	*Ribes sardoum* Martelli	Grossulariaceae	CR B1ab(v)+2ab(v) (IUCN, 2010)	P
2	*Polygala sinisica* Arrigoni	Polygalaceae	CR B1ab(ii)+2ab(ii) (IUCN, 2010)	
3	*Lamyropsis microcephala* (Moris) Dittrich *et* Greuter	Asteraceae	CR B1ab(iii)+2ab(iii) (IUCN, 2010; Fenu & al., 2011a)	P
4	*Anchusa littorea* Moris	Boraginaceae	CR B1ab(i,ii,iii,iv,v)+2ab(i,ii,iii,iv,v) (Fenu & Bacchetta, 2008)	
5	*Centranthus amazonum* Fridl. *et* A. Raynal	Valerianaceae	CR B1ab(iii,iv)+2ab(iii,iv); D (IUCN, 2010)	NP
6	*Aquilegia nuragica* Arrigoni *et* E. Nardi	Ranunculaceae	CR B1ab(v)+2ab(v); D (IUCN, 2010)	
7	*Dianthus morisianus* Vals.	Caryophyllaceae	CR B1ab(i,ii,iii) + 2b(i,ii,iii) (Fenu & al., 2010)	
8	*Aquilegia barbaricina* Arrigoni *et* E. Nardi	Ranunculaceae	CR B1ab(ii,iv)+2ab(ii,iv); D (IUCN, 2010)	
9	*Astragalus maritimus* Moris	Fabaceae	CR (Bacchetta & al., 2011c)	P
10	*Astragalus verrucosus* Moris	Fabaceae	CR B1ab(i,ii,iii) (Bacchetta & al., 2011c)	P

Among the top ten of most endangered plants, *Anchusa littorea* and *Dianthus morisianus* were chosen, on the basis of high conservation priority, and ecological and biogeografical criteria. These are two psammophilous species which are present in a coastal dune system of SW Sardinia and they have not still been investigated unlike to the other *taxa* of the top ten list on which population studies were made.

Aims of this work are:

- ✓ To investigate the conservation status of this species (chapters 2 and 6);
- ✓ To individuate and quantify the main threats acting on this population (chapters 2, 3 and 6);

- ✓ To analyse some critical stage of their life-cycle (chapters 4 and 8);
- ✓ To evaluate the phenological pattern (chapters 5 and 7);
- ✓ To propose adequate conservation measures (chapters 2, 6 and 9).

Geographical, biogeographical and climatic context

The southwestern Sardinia is one of the scientifically most interesting coastal areas of Sardinia (Arisci et al. 2003). The coastal karstic region of SW Sardinia forms part of the Cambrian Iglesiente massif, which has been intensively exploited for lead and zinc in the past (Arisci et al. 2003). The Palaeozoic rocks in the area have been described by Pillola (1989) and Bechstadt and Boni (1996). From a stratigraphic point of view the Cambrian succession in SW Sardinia is divided in three major groups: Nebida, Gonnesa and Iglesias. The Nebida Group (Lower Cambrian) is composed of a delta and coastal sediments and is divided into two formations: the Matoppa (sandstones and shales) and Punta Manna (oolithic limestones and calcareous sandstones followed by sandstones with carbonatic fossiliferous lenses and strata;Arisci et al. 2003). The Gonnesa Group (Lower-Middle Cambrian) is characterized by typically carbonatic deep-sea sediments and is divided into two formations according to its trilobite contents: the Santa Barbara Formation (mainly dolomitized rocks) and the San Giovanni Formation (intensely karstified limestones (Arisci et al. 2003). In these carbonatic rocks we find most of the Mississippi Valley type ore deposits and their oxidized equivalents with economically important concentrations of lead and zinc minerals, which have been mined (Arisci et al. 2003). The Iglesias Group (Middle Cambrian-Lower Ordovician) is divided into two formations: the Campo Pisano Formation, composed of nodular limestones, followed by a thick succession of shales of the Cabitza Formation (Arisci et al. 2003). After a long period of continentality and an important tectonic phase (Fase Sarda) the sea returned to occupy this area with the deposition of the Ordovician conglomerates (Puddinga), followed by Silurian and Devonian sediments (Arisci et al. 2003). After the Hercynian orogenesis a long continental period started in the region (Carboniferous-Middle Trias), only shortly interrupted by new transgressions in Middle-Triassic and in Paleocene-Eocene times (Arisci et al. 2003). The prevailing structures in the coastal area of Capo Pecora-Nebida are folds directed N-S that involve both Ordovician and Cambrian rocks and the great anticline of Canalgrande-Punta Sa Gloria with an E-W direction (Civita et al. 1983).

To the south along the coast the Portixeddu locality is situated. This locality has been known since 1880 for its fossils (brachiopods, bryozoans, cystoids, crinoids, gasteropods, bivalves, trilobites, cornulites, conularids and corals). However, because of the incompleteness of the series and its structural complexity Portixeddu was not chosen as the type locality of the formation (Bechstadt & Boni 1996). The dune system of Portixeddu extends over an area of ca. 4 km^2 south of the village and is composed of at least three generations of aeolian deposits dating from the Middle Pleistocene to the Holocene (Arisci et al. 1999; Cesaraccio et al. 1986). From a morphological point of view longitudinal, parabolic and transversal dunes can be distinguished. Since more than 40 years this dune field has been stabilized through the plantation of many trees and shrubs (Arisci et al. 1999).

Is Arenas dune systems is one of the most important and well-preserved coastal system of Sardinia, which spread to ca. 5 Km inland. Geologically, the area mainly consists of Holocene sandstones and Aeolian sands forms which present irregular heights ranging from 10 to 80-90 m (Annino et al. 2000).

The dune areas are rich in endemic plant species (Bartolo et al. 1992; Meyer 1995) and Portixeddu dune is one of the few places of Sardinia in which *Pinus pinea* L. and *Quercus coccifera* L. grow naturally (Mossa 1990).

Available climate data from the nearest weather station (Montevecchio and Fluminimaggiore weather stations for Is Arenas and Portixeddu dune systems, respectively) indicates a typical Mediterranean annual pattern of temperature and precipitation with a durable dry summer. Bioclimatically this area is classified as Oceanic Pluviseasonal Mediterranean (MPO), with upper thermomediterranean thermotype and lower subhumid ombrotype (Bacchetta et al. 2008).

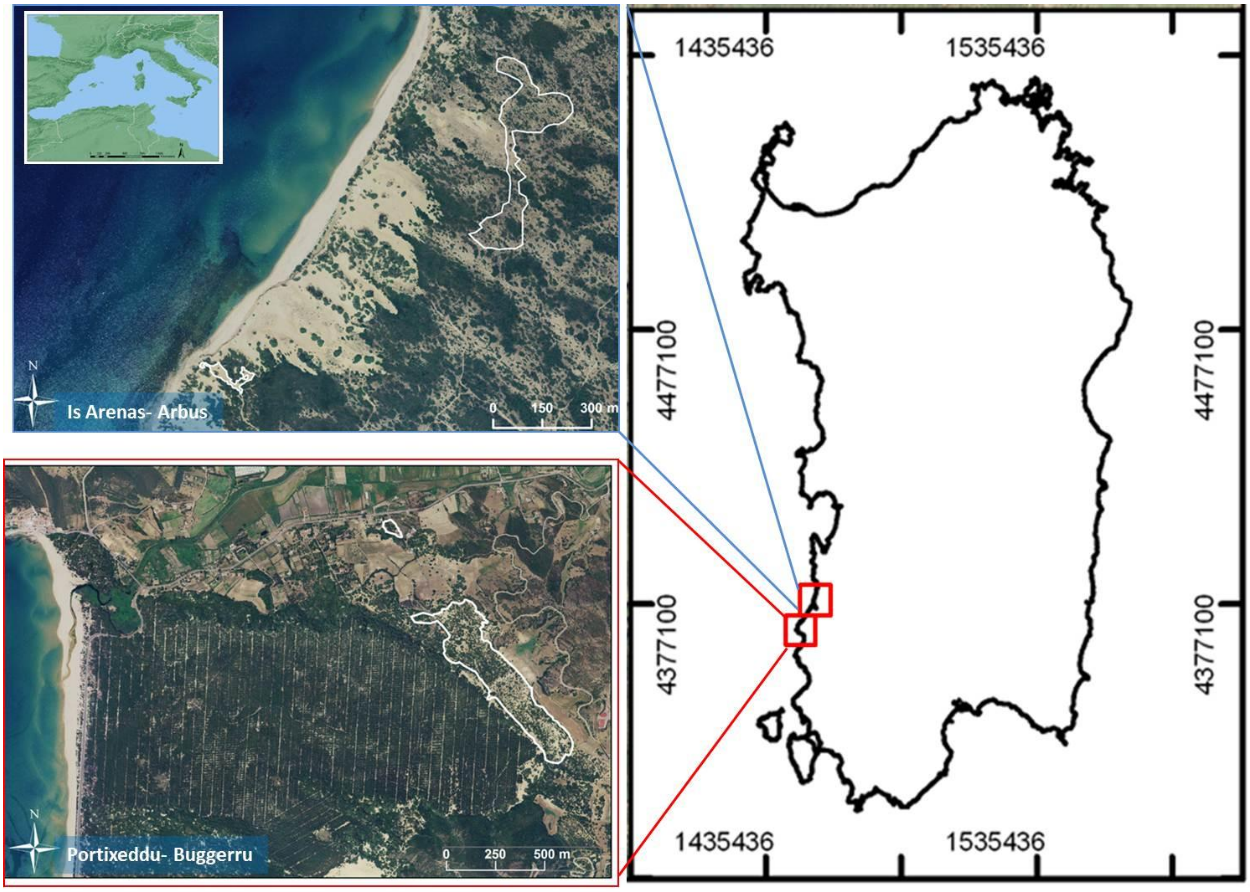

Figure 1 - Study areas in the SW Sardinia

Study species

Anchusa littorea Moris

The *Boraginales*, with c. 130 genera and 2300 species (Gottschling et al. 2001; Langström & Chase 2002; Mabberley 2000), represent an ideal model system with which to test the spatial and temporal origins of species endemic to either or oceanic islands (Mansion et al. 2009).

According to Flora Europaea (Chater 1972), *Boraginaceae* family is characterized by herbs or dwarf shrubs, often hispid. The leaves are alternate, exstipulate and simple. The flowers usually are in scorpioid cymes, usually actinomorphic. The calyx is 5-toothed or-lobed. Corolla is 5-lobed, cylindrical, campanulate, hypocrateriforme or rotate, usually with a distinct tube and limb; tube often with 5 scales, invaginations, or tufts or lines of hairs inside, sometimes with an annulus at the base. The family has 5 stamens, inserted on the corolla and alternating with the lobes. Ovary is superior, 2- or 4- locular; style is usually simple, arising from between the 4 lobes of the ovary (gynobasic), rarely terminal. Fruits of 2 or 4 nutles (rare 1 or 3 by abortion).

Within the family *Boraginaceae*, *Anchusa* L. is a moderate-sized genus, characterized by annual, biennial or perennial herbs, rarely woody at the base (Chater 1972). The flowers are in axillary and terminal cymes, bracteate usually throughout. Calyx lobed from 1/3 to almost to the base. Corolla purple, blue, yellow or white, with cylindrical, straight or curve tube and rotate to campanulate limb divided into equal or unequal lobes, with 5 ovate or oblong, papillose or hairy scales in throat. Stamens included or slightly exserted, variously inserted. The style is included and the stigma is capitate. Nutles ovoid to reniform or hemispherical, erect or oblique, reticulate or rugose, usually more or loss tuberculate, wih a thickened collar-like ring at the base.

Genus *Anchusa* L., with c. 30 species occurring in the Mediterranean Basin and the Middle East, and seven *taxa* restricted to either the coastal (*A. crispa* Viv. ssp. *crispa*, *Anchusa crispa* ssp. *maritima* (Vals.) Selvi & Bigazzi, *Anchusa littorea* Moris, *Anchusa sardoa* (Illario) Selvi & Bigazzi) or mountainous (*Anchusa formosa* Selvi, Bigazzi & Bacchetta, *Anchusa capelli* Moris, *Anchusa montelisana* Angius, Pontecorvo & Selvi) habitats of Sardinia and Corsica (Bacchetta et al. 2008). However, despite some previous taxonomic contributions (Selvi 1998; Selvi & Bigazzi 1998; Valsecchi 1976), these narrow-ranged endemics are still poorly known in terms of both phylogenetic relationships and conservation status (Bacchetta et al. 2008). Although hypotheses of an *in situ* common origin were already proposed on morphological grounds (Selvi & Bigazzi 1998), it was only with a molecular phylogenetic study based on nrITS DNA sequences (Bacchetta et al. 2008; Coppi et al. 2008) that this group emerged as a monophyletic clade possibly sister to the Mediterranean species *A. undulata* L. In a previous analysis based on chloroplast markers (Hilger et al. 2004), however, the two Sardinian endemics *A. capellii* and *A. formosa* resulted more closely related to the south African species *A. capensis* Thunb. Lack of karyotype variation and stable diploid condition in all the Corso-Sardinian *taxa* suggested a model of *in situ*, homoploid speciation triggered by paleogeographical events of range fragmentation and driven by adaptive radiation in coastal dune systems. Based on a recent systematic revision and survey of the conservation status of the whole group on Sardinia (Bacchetta et al. 2008), all seven endemics turned out to fit into the portrait of the rare and endangered species with fewer than five populations and 5.000 individuals (Holsinger & Gottlieb 1991). The main characteristics of the Sardinian *Anchusa* endemics are described in the Table 2.

Table 2 - Number of populations (n.p.), estimated number of fertile individuals (n.ind.), main factors of threat, current IUCN category at the national level, category proposed of the Sardinian *Anchusa* endemics (Bacchetta et al. 2008).

Taxa	**n.p**	**n. ind**	**Main threat**	**National IUCN**	**Proposed IUCN category**
A. capellii	1	1000	Grazing, natural events	CR	VU B2a; D1+2
A crispa ssp. *crispa*	3	2100	Habitat loss by human activity, alien species	EN	CR B1ab(iv)c(iv)+2ab(iv)c(iv)
A. crispa ssp. *maritima*	5	6000	Habitat loss by human activity, alien species	EN	VU B1ab(iii)+2ab(iii); D2
A. formosa	2	2150	Natural events	__	VU B2ac(iv); D2
A. littorea	1	350	Human activities, alien species	CR	CR B1ab(i−v)+2ab(i−v)
A. montelinasana	1	200	Grazing, natural events	__	EN D
A. sardoa	1	1500	Human activities, tourism	__	CR B2ab(iii)

Specifically to the species studied in this thesis, *Anchusa littorea* Moris is an annual plant. It has hispid-setose for dense, tubercle-based trichomes and shorter hairs. Stems decumbent to suberect, branched from base, 4–15 cm. It has lower leaves 3–6 × 0.4–0.8 cm, narrowly oblanceolate and tapering into a short petiole, with repand dentate margins; cauline leaves almost linear, smaller and sessile. Cymes with small flowers distanced at the axyl of cauline leaves, often also in the lower part of the stems just above ground-level, on pedicels 2–3mmlong deflexed in fruit. The calyx is tubulose, 3.5 mm, divided to 1/2–2/3 into narrowly triangular lobes, up to 5mmand spherical-urceolate in fruit. The corolla with tube 4 mm long and limb 4–5 mm diam., is light blue or white, rotate with rounded lobes. Anthers 1.3 mm, not overlapping scales. The style slightly is longer than the calyx. Mericarps light greybrown, small, 1.5–2×0.5–1 mm, with a lateral beak and a thin basal annulus, with finely tuberculate surface. Flowering occurs during March–May while fruiting occurs during March–June. The reproductive biology of this species is almost unknown. Although some insects' activity has been observed in the single known population, autonomous self-pollination is likely to be the main reproductive system. Seed dispersal is mainly performed by wind and ants. *A. littorea* is endemic to southwest Sardinia (Bacchetta et al. 2008; Valsecchi 1980) and, according to past records, this species was distributed in several coastal dune systems of the Island (S'Ena Arrubia, Terralba, Marina di Arbus, Piscinas, Is Arenas, Sant'Antioco at Calasetta bay and San Pietro). In 2005 *A. littorea* was considered extinct in the wild since it was not found during the field investigation for over 25 years and for this reason the "EX" IUCN category has been proposed for this species (Bacchetta & Pontecorvo 2005). Afterwards, Bacchetta et al. (2008) rediscovered a small population of *A. littorea* at Is Arenas locality (Arbus, SW Sardinia).

Figure 2 - Habitat where *A. littorea* grows. In the box a detail of this plant species.

The area is legally closed to the public and included in the Site of Communitarian Interest 'Piscinas-Rio Scivu' (ITB040071). In spite of this, the site is frequented by local people for recreation purposes and is crossed by a footpath, which is the continuation of a service road. The strong human impact on coastal habitat in Sardinia, combined with the lower genetic variation and the natural instability of sand dune ecosystems (Coppi et al. 2008), could determine a higher risk of extinction for *A. littorea*, confirming the critically endangered category proposed for this species (Fenu & Bacchetta 2008). On the other hand, the remarkable rarity and fugacity of this species may also be associated with its peculiar biological and ecological features. In fact, this is the only member of the group that shows a terophytic habit and a strongly abbreviated life cycle. A typical trait of its 'ephemeral' like biology is the unique capacity to produce flowers and fruits shortly after seed germination, when plants are still in an apparently juvenile state. The very small mericarpids are produced in spring contemporarily to flowers, even though selfing, and then immediately released in the mobile sands of dunes exposed to sea-wind. They are easily moved with sand by wind and can remain latent for several years to germinate only when edaphic humidity and position in terms of underground depth, distance from the sea and type of surrounding vegetation are in an optimal combination.

Dianthus morisianus Vals.

The *Caryophyllaceae* family belongs to the order of *Caryophyllales* (Schweingruber et al. 2007). The family is botanically characterized and their position in the phylogenetic system of angiosperms is explained by Judd et al. (2002) and Sitte et al. (2002). The *Caryophyllaceae* family includes 86 genera and 2200 species (distributed mainly in temperate regions of the Northern hemisphere) in the subfamilies *Paronychioideae*, *Alsinoideae* and *Caryophylloideae* (Bittrich 1993). Most of the species are herbaceous; three of the genera are large shrubs or small trees, Sanctambrosia, endemic to San Ambrosio Island, Chile, and the Hawaiian endemics, Alsinidendron and Schiedea (Bittrich 1993). Until now, only the sporadic presence of growth rings in the xylem and phloem has been used for defining the family *Caryophyllaceae* (Judd et al. 2002). Numerous molecular biological studies have concentrated on phylogenetic relationships within core *Caryophyllales*; however, a systematic molecular biological intra-familial classification does not exist (Judd et al. 2002). Anatomical differences in the xylem between most genera and species are not very well known, while the bark characteristics are virtually unknown. According with Flora Europaea (Tutin 1964), *Caryophillaceae* family is characterized by leaves which are usually opposite and decussate, more rarely alternate or verticillate, simple, entire with or without scarious stipules. The flowers are actinomorphic, usually hermaphrodite, often in bracteates dichasia. Sepals 4-5, free, or fused and often united by scarious strips of tissue (commissures) alternating with the calyx-teeth. Petals (0) 4-5, free. Stamens, usually 8-10, are odiplostemonous. Ovary is superior, unilocular at least above, with 1 to numerous campyotropus ovules on a basal or-free central placenta; stigmas (1) 2-5. Fruit usually are capsule, dehiscing with teeth equaling the styles in number or twice as many; more rarely fruit a berry or achene.

Belonging to the family *Caryophyllaceae*, *Dianthus* L. genus represents one of the most diverse plant groups in Europe (Valente et al. 2010). *Dianthus* is distributed throughout Eurasia and Africa (approx. 300 species), but is almost exclusively a temperate *taxon*, with the exception of six tropical African representatives (Valente et al. 2010). Over 100 species of carnations occur in Europe (more than 70 endemic), raising the question of how and when such remarkable diversity arose (Valente et al. 2010). *Dianthus* is a taxonomically difficult clade (Tutin & Walters 1993) characterized by large numbers of endemic species with small geographically restricted ranges, suggesting that diversity has originated only recently (Balao et al. 2010; Valente et al. 2010). According with Flora Europaea (Tutin 1964), the genus is usually characterized by perennial herbs or small shrubs, often with linear, parallel-veaned leaves. It has flowers solitary or in heads surrounded by bracts. The Epicalyx-scales 2-many, is usually appressed to calyx which is tubular, 5-toothed, without scarious commissures. It has 5 petals, long-clawed, entire, dentate or laciniate but not deeply bifid; coronal scales is absent. The stamens are 10, and the styles are 2. It has capsule dehiscing with 4 teeth and carpophore often present. Male-sterile plants of a number of species occurred sporadically and add to the difficulties to identification, as such plants are often dwarf with flower smaller in all their parts than normal and sometimes with a reduced number of epicalyx-scales. Late flowers particularly if borne on lateral branches produced by damaged main stems, may also be abnormal. Species which normally have capitate inflorescences, frequently produce solitary flowers in these circumstances. It is therefore unwise to attempt to identify plants flowering outside their normal season. The shape and measurements of the calyx refer to the calyx at flowering time. The diameter of the stem is measured just below a node. Bracts occur in species with capitate inflorescences, and should be distinguished from epicalyx-scales; they subtend more than one flower. Most of the species are more or less interfertile but, since they are usually geographically or ecologically isolated, hybrids are rather local. They do, however, seem to occur in most localities where two or more species, grow together. The pattern of morphological resemblances within the genus is exceptionally reticulate, so that any linear arrangement of species is more than usually artificial, and division into subgenera, sections, is of little practical value. The most satisfactory scheme so far publisher is that given by Schischkin in Komarov (1936), but this includes only a quarter of the European species, which are placed in 12 groups, but further study of the problem is desirable.

Within the genus *Dianthus*, the *D. sylvestris* Wulfen group can be considered as one of the most complex (Bacchetta et al. 2010). This group is morphologically characterized by woody stocks, shortly branched, usually with dense terminal leaf rosettes, linear and acute leaves, 1-15-flowered stems with well-developed pedicels arranged in loose cymes, epicalyx scales 2-10 (rarely up to 20), glabrous, abruptly contracted into a short mucro, 3-4 times shorter than calyx, glabrous petals with limb cuneate to cuneate-rhombic, denticulate at apex. According to the literature, several *taxa* belonging to this group have been described at specific or subspecific level, though their real taxonomical value is often doubtful. With regard to the Italian territory, several *taxa* belonging to the *D. sylvestris* group have been recorded, even if authors disagree about their taxonomical treatment. In particular, several species of this group have been described from Sicily, such as *D. arrosti* C. Presl, *D. siculus* C. Presl, *D. graminifolius* C. Presl, *D. gasparrinii* Guss., *D. paniculatus* Lojac., *D. contractus* Jan *ex* Lojac. and *D. minae* Mazzola et al. (Giardina et al. 2007; Gussone 1843; Lojacono 1889, 1909; Mazzola et al. 2004; Presl 1822, 1826); many other have been described from central-southern Italy, like *D. longicaulis* Ten., *D. virgatus* Pasquale, *D. tarentinus* Lacaita, *D. garganicus* (Ten.) Brullo, *D. japygicus* Bianco *et* Brullo (Brullo 1988; Groves 1887; Lacaita 1911; Pasquale 1864; Tenore 1819, 1830, 1831), and finally the species from Sardinia are *D. cyathophorus* Moris, *D. morisianus* Vals. and *D. sardous* Bacch. *et al*. (Angiolini et al. 2005; Moris 1852, 1853; Valsecchi 1985). In addition to such local species, many widespread *taxa* have been recorded from many Italian localities; *D. sylvestris* Wulfen, *D. caryophyllus* L., *D. virgineus* Gren. & Godron, *D. tergestinus* (Reichenb.) Kerner, *D. nodosus* Tausch and *D. brachycalyx* Huet *ex* Nyman (Moris 1837; Bertoloni 1839; Reichenbach 1844; Nyman 1878; Cesati et al. 1884; Caruel 1892; Arcangeli 1894; Fiori & Paoletti 1898; Fiori 1924; Pignatti 1982; Greuter et al. 1984; Tutin &Walters 1993).

In Sardinia a total of eight species, belong to the *D. sylvestris* group, were recorded (Table 3): *Dianthus sardous* Bacchetta, Brullo, Casti & Giusso; *Dianthus genargenteus* Bacchetta, Brullo, Casti &Giusso; *Dianthus ichnusae* subsp. *inchnusae* Bacchetta, Brullo, Casti & Giusso; *Dianthus ichnusae* subsp. *toddei* Bacch., Brullo, Casti & Giusso; *Dianthus insularis* Bacch., Brullo, Casti & Giusso; *Dianthus morisianus* Vals; *Dianthus oliastrae* Bacch., Brullo, Casti & Giusso e *Dianthus cyathophorus* Moris (Bacchetta et al. 2010).

Table 3 - Main threat and IUCN category proposed by Bacchetta et al. (2008) for the Sardinian *Dianthus* belonging to the *D. sylvestris* group.

Taxa	Main threat	Proposed IUCN category
Dianthus sardous	Fires, overgrazing	NT
Dianthus genargenteus	Low number of individuals, small area of occupancy	EN
Dianthus ichnusae subsp. ichnusae	Small population size	EN
Dianthus ichnusae subsp. toddei	Small population size	EN
Dianthus insularis	Wide distribution and localization on less disturbed habitats	LC
Dianthus oliastrae	Wide distribution and localization on less disturbed habitats	NT
Dianthus cyathophorus	Wide distribution and localization on less disturbed habitats	LC
Dianthus morisianus	Small population size	CR

The species that requires an immediate and continuous monitoring of conservation is *Dianthus morisianus* Vals. because it is critically endangered (Bilz et al 2011; Fenu et al 2010, 2011b). *D. morisianus* is a perennial suffrutex 30-50(60) cm tall, characterized by numerous woody stocks loosely branched with branches 0.5-3.0 cm long, 1.5- 2.0 mm wide, acute at apex; it has erect stems, 20-45 cm long, with 4-6 internodes and basal rosette with thin and linear leaves, 1-15 cm long. The stems bear terminal multi-flowered heads (normally, 2-18 flowers/head); the calix (25-30 mm long, 4.5-5.5 mm in diameter) is characterized by lanceolate teeth, membranaceous on the margin, acute, overlapping below, 5.5-7.0 mm long; the colour of the corolla is normally pink (Bacchetta et al. 2010) and petals are 35-40 mm long. Anther 4.5 mm long. Ovary 7.5 mm long. Style and stigma 14 mm long. Capsule cylindrical included in the calyx. Small and flat seeds can germinate with high percentages in a wide range of conditions, with a maximum germination rate at 15°C (Cogoni et al. 2012). The flowering season lasts from early May to last June, whereas ripe fruits can be found in June and July (Fenu et al. 2010). *D. morisianus* is a psammophilous chamaephyte which grows on stabilized dunes in contact with micro-forests of *Juniperus* spp. [*J. oxycedrus macrocarpa* Sibth and *J. phoenicea* L. subsp. *turbinata* (Guss.) Nyman] and *Quercus calliprinos* Webb.

Figure 3 - Habitat where *D. morisianus* grows. In the box a detail of this plant species.

The only natural population of *D. morisianus* is located in Portixeddu (Buggerru, S-W Sardinia, N 40° 14’ E 09° 25’) at an altitude of 10-55 m a.s.l. (Bacchetta et al. 2010). Available climate data (from Fluminimaggiore weather station at 45 m a.s.l.) indicates a typical Mediterranean annual pattern of temperature and precipitation, with a long dry summer. *D. morisianus* is listed in the National Red List as endangered (Conti et al. 1992) and in Regional Red List as vulnerable (Conti et al. 1997); more recently, it has been inserted in the European threatened plant list (Sharrock & Jones 2009) and for this species has been proposed the Critically Endangered IUCN category (Bilz et al 2011; Fenu et al 2010, 2011b). However, biology and ecology of this species are still little investigated.

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CHAPTER 2

CONSERVATION STATUS OF THE MEDITERRANEAN COASTAL PLANT SPECIES: THE CASE OF *ANCHUSA LITTOREA* MORIS (BORAGINACEAE)

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Introduction

Monitoring of plant populations is one of the core activities of conservation biology that can be particularly useful for conserving species with scattered populations. Conservation of threatened species requires efforts dedicated to monitoring of population performance (e.g., Green et al. 2005; Hellawell 1991; Lughadha et al. 2005; Plattner et al. 2004; Yoccoz et al. 2001); this activity creates challenges in terms of designing efficient strategies (Balmford et al. 2003; Elzinga et al. 1998; Gerber et al. 1999; Moreno Saiz et al. 2003; Philippi et al. 2001) and analysis of resulting data (Brigham & Schwartz 2003; Morris & Doak 2002). Gathering long-term demographic data is considered essential for appropriate management action in support of the conservation of threatened plants (Byers & Meagher 1997; Lande 1988; Schemske et al. 1994). Schemske et al. (1994) suggest that, to implement species recovery, it is necessary to know the population growth rate, the most important life history stages influencing it, and why differences in critical life stages occur among populations and years.

Monitoring data are used to identify species in decline or at risk of extinction, to track the spread of invasive species (Marsh & Trenham 2008), and to assess whether specific management strategies work (Field et al. 2007; Marsh & Trenham 2008). Species extinction often follows extended periods of population decline (Lande et al. 2003). Demographic monitoring and understanding the natural history of rare plants are then crucial for population management and conservation (Adams et al. 2005; Lehtilä et al. 2006; Massey & Whitson 1980).

The knowledge of population dynamics and the processes that determine the size population is critical to the understanding of a wide range of ecological phenomena such as abundance and rarity, plant distributions, the dynamics of diseases, competition and the structure and dynamics of communities (Crawley 1990; Silvertown & Lovett Doust 1993). Demographic analyses and models of plant populations have studied dynamics at the level of the individual population in both annual (Leiss & Müller-Schärer 2011; Watkinson & Harper 1978) and perennial species (Schemske et al. 1994). Each population is, of course, part of a larger system of local populations which is in turn part of the total population of a species (Hanski & Simberlo 1997). To understand the population dynamics of a species in the widest sense entails understanding and linking its dynamics at all these different levels.

The Mediterranean basin, with 11.8 endemic plants per 100 km^2, has been recognized as one of the 34 most important biodiversity hotspots on the planet (Mittermeier et al. 2004). Sardinia is the second-largest island in the Mediterranean Sea (after Sicily) situated in the West Mediterranean basin and its flora consists of 2,408 *taxa* including 2,295 species (Conti et al. 2005), with 168 being exclusive Sardinian endemics (Bacchetta et al. 2012). The Island represents the major center of diversity and endemism for the genus *Anchusa* L. in the Mediterranean region, with seven allopatric endemic *taxa*, three of them occurring in coastal habitats (Bacchetta et al. 2008). However, despite some previous taxonomic contributions (Valsecchi 1976; Selvi 1998; Selvi & Bigazzi 1998), these endemics are still poorly investigated and recently ecological studies (i.e. focused on *Anchusa crispa* Viv. byQuilichini, 2001), taxonomic and phylogenetic (Bacchetta et al. 2008) and genetic analysis (Coppi et al. 2008; Quilichini & Debussche 2000; Quilichini et al. 2004) were carried out.

Among these *taxa*, *Anchusa littorea* Moris is a narrow endemic species growing on coastal dune systems of southwest Sardinia. In the past times, this species was distributed in several dune systems of the Island: S’Ena Arrubia, Terralba, Marina di Arbus, Piscinas, Is Arenas, Sant’Antioco at Calasetta bay and San Pietro at Spalmatore (Bacchetta et al. 2008; Valsecchi 1980), but, in 2005 this species was considered extinct since it was not found during the field investigation for over 25 years (Bacchetta & Pontecorvo 2005). Afterwards, Bacchetta et al. (2008) rediscovered a small population of *A. littorea* at Is Arenas locality (Arbus, SW Sardinia). The small population size of *A. littorea* increase its extinction risk, due to environmental, demographic, or genetic factors (e.g., Reed & Frankham 2003).

The aim of this work is to analyze the population size and dynamics of *A. littorea* in order to produce and implement conservation measures; in particular we address the following specific objectives: (1) to describe the population in term of ecological requirement and size population (2) to investigate population structure and vital rates, and (3) to assess the conservation status of this threatened plant in Sardinia.

Materials and methods

Study species

A. littorea is a short-lived herb, with a small basal rosette, hispid-setose for dense and shorter hairs. Stems are decumbent or suberect, 4-15 cm long. Leaves are linear and present a repand-dentate margins. Each plant develops several cymes, with small tubular flowers. Corolla is light blue or white, rotate with rounded lobes. Mericarps are small, light grey-brown, with finely tuberculate surface. The flowering period is from March to May and fruiting season, overlapped, is from April to July (Valsecchi 1980; Bacchetta et al. 2008). The reproductive biology of this species is still poorly known, but nevertheless the main reproductive system seems to be autonomous self-pollination, although some insects' activity has been observed in the single known population (Bacchetta et al. 2008).

A. littorea growing in ephemeral psammophilous plant communities constituted principally by therophytes, such as the endemics *Linaria flava* (Poiret) Desf. subsp. *sardoa* (Sommier) Arrigoni, *Phleum sardoum* (Hackel) Hackel and *Silene nummica* Vals. (Bacchetta et al. 2008; Fenu et al. submitted).

The only known population is located in the coastal dunes of Is Arenas (SW Sardinia), which spread to ca. 5 Km inland, is one of the most important and well-preserved sand system in Sardinia. Geologically, this area mainly consists of Holocenic sandstones and Aeolian sands forms, which present irregular heights ranging from 10 to 80-90 m (Annino et al. 2000).

Available climatic data from the nearest weather station (Montevecchio, ca. 12 Km) indicates a typical Mediterranean annual pattern of temperature and precipitation with a long dry season (4-6 months).

Field monitoring

The distribution of *A. littorea* was verified by field surveys during five years in several coastal localities, for which herbarium specimens (*Herbarium* CAG) and/or published data were available (Valsecchi 1980; Bacchetta et al. 2008; Fenu & Bacchetta 2008). When a locality was confirmed or discovered, the following analyses were undertaken. The geographical limits of localities were mapped, with a global positioning system, and areas estimated, using Quantum GIS Version 1.7.3 (QGIS 2011) to detect any annual changes in occupied area. For each locality we noted the altitudinal range, slope, aspect, substratum, vegetation type and habitat type according to the Italian Interpretation Manual of European Habitat Directive (Biondi et al. 2009).

The threats to *A. littorea* population were determined from field observations and categorized following the IUCN threats classification scheme (www.iucnredlist.org/technical-documents/classification-schemes/threats-classification-scheme-ver3).

From 2007 to 2011 the population of *A. littorea* was monitored; data collection began in 2007, considering the historical known locality (Bacchetta et al. 2008) and then, in 2009, the analysis was extended to the new discovered locality, representing them as a two distinct groups, on the basis of their geographical distribution (hereafter ANC1 and ANC2 for the historical and the new group, respectively).

The population was monitored on a monthly basis, from March to July, during the same time (around the 10th of each month) in 2007-2008, while from 2009 surveys was started in February, considering the time of *A. littorea* seedling establishment. Samplings were carrying out by placing randomly permanent plots of 1 m x 1 m (12 and 13 plots for ANC1 and ANC2, respectively) where the plant was found. The corners of the plots were marked by metal tubes (30 cm height) driven into the sandy soil so that plots could be relocated later. Within the plots, all plants were counted, marked with a wooden stakes and measured. All new seedlings that appeared inside the plots were added. During each monitoring the following parameters were measured in all plants: height and maximum diameter of each plant were measured using a digital calliper (ALPA IP65 Topcal 150 PW) and the number of leaves was counted. Furthermore, the number of flowers and fruits presents was counted.

Plant survival was recorded every month and a plant was considered to have survived the following month if still present with at least one remaining fresh leaf.

Data analysis

From 2007 to 2011, the annual values of plant densities were plotted on charts. Exploratory analyses were first carried out in the form of a boxplots to examine the differences in the distribution of density for months and years in overall population, ANC1 and ANC2. The Mann–Whitney U inferential statistical test was applied to evaluate significant differences between the two sample medians of the ANC1 and ANC2 plots for plant density.

In order to select the size variable which will be used for population structuring, morphological and reproductive parameters were analyzed considering the values recorded in March and April, because in this period the population showed the highest number of plants. In order to verify whether a single variable was a good predictor describing the plant size, we calculated the Pearson correlation among all variables (see Appendices). Then, among the groups of variables, “total leaves” was selected because this parameter can be detected more easily without causing damage to the mapped individuals.

Preliminary analysis carried out showed that the Pearson correlation coefficients between categorized measures, based on a sample of 802 plants for overall population, had a significant correlation (*p-value*< 0.001; Table 1).

The same patterns were observed for ANC1 (N = 248 plants) and ANC2 (N = 554 plants; Table 1). Based on the number of leaves, and considering also the reproductive parameters per plant three size classes are considered: class 1: plants with 0-4 leaves; class 2: plants with 5-10 leaves; class 3: plants with>11 leaves. Population structure was displayed in histograms categorized by parameters; then, variations in size classes and reproduction over time were investigated.

Transitions probability were calculated, in order to investigate the monthly vital rates (in particular stasis, growth, mortality and retrogression) in overall population and, separately, for ANC1 and ANC2. Transition probabilities were calculated as the number of plants developing from stage *i* to stage *j* in one month divided by number of plants in stage *i* in the previous month (Schleuning & Matthies 2009).

Differences in growth and mortality rates among classes were tested first using the Kruskal-Wallis test and then, considering classes in couples, the Mann–Whitney U inferential test were performed in order to test differences in vital rates between two classes. Differences in vital rates between ANC1 and ANC2 were tested by the Mann–Whitney U inferential statistical test.

All these analyses were performed with Statistica 8.0 (StatSoft, Inc, Tulsa, Oklahoma, USA) software.

Table 1 - Pearson correlation value, and significance of the total number of leaves (referred to March) with morphological and reproductive variables measured in the overall population (N = 802 plants) ANC1 (N =248) and ANC2 (N = 554). Abbreviations: H.=height; D.=diameter; Fl.=flower; Fr.=fruits; Re=reproduction].

		H. March	**H. April**	**H. May**	**D. March**	**D. April**	**D. May**	**Fl. March**	**Fl. April**	**Fl. May**	**Fr. March**	**Fr. April**	**Fr. May**	**Re. March**	**Re. April**	**Re. May**
No. leaves	**OVERALL POPULATION**															
	r	0.574	0.393	0.116	0.581	0.667	0.332	0.636	0.524	0.405	0.3056	0.284	0.553	0.557	0.306	0.597
	t	19.826	12.110	3.325	20.209	25.332	9.955	23.356	17.436	12.537	9.063	8.395	18.812	18.987	9.096	21.093
	p-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
No. leaves	**ANC1**															
	r	0.412	0.523	0.445	0.727	0.631	0.103	0.553	0.484	0.328	0.125	0.216	0.450	0.413	0.540	0.504
	t	7.100	9.617	7.791	16.599	12.750	1.622	10.412	8.664	5.454	1.977	3.468	7.910	7.115	10.073	9.143
	p-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	> 0.05	< 0.001	< 0.001	< 0.001	< 0.05	< 0.01	< 0.001	< 0.001	< 0.001	< 0.001
No. leaves	**ANC2**															
	r	0.568	0.309	0.071	0.527	0.647	0.669	0.631	0.533	0.408	0.293	0.251	0.539	0.548	0.233	0.585
	t	16.200	7.638	1.675	14.573	19.933	21.171	19.110	14.805	10.497	7.190	6.085	15.050	15.372	5.625	16.968
	p-value	< 0.001	< 0.001	> 0.05	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Conservation status assessment

A grid of 2 x 2 km was used for assessing 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; IUCN 2001), according the National protocol adopted in Italy (Gargano 2011). EOO was assessed following the IUCN guidelines (2011). The conservation status was assessed following the IUCN criteria (2001).

Results

A. littorea population consists of two groups distant ca. 700 m, with an area of ca. 3800 (ANC1) and 50000 (ANC2) m^2 respectively (Figure 1).

Figure 1 - Groups of *A. littorea* population at “Is Arenas” locality (Arbus, SW-Sardinia).

The population grows on stabilized dune, at variable altitudes of 11-46 m, and on slopes with slightest incline and varied aspect (Table 2).

Table 2 - Ecological data of *A. littorea* population.

	ANC1	**ANC2**
Altitudinal range (m a.s.l.).	9–45 m.	11–46 m.
Mean slope (°)	0–5°	5–10°
Prevalent aspect	N-NW	SW
Lithology	Metamorphytes	Metamorphytes
Substratum	Holocenic aeolian sand	Holocenic aeolian sand
Vegetation type	*Malcolmietalia*	*Malcolmietalia*
Habitat Natura 2000	2230	2230

Associated *taxa* are the endemics *Linaria flava* (Poir.) Desf. subsp. *sardoa* (Sommier) A. Terracc. and *Phleum sardoum* (Hack.) Hack. In addition, *Senecio transiens* (Rouy) Jeanm., *Malcolmia ramosissima* (Desf.) Gennari, *Polycarpon tetraphyllum* (L.) L. subsp. *diphyllum* (Cav.) O. Bolòs *et* Font Quer, *Brassica tournefortii* Gouan showed an high frequency. These species constitute a ephemeral herbaceous coenosis ofthe Habitats of community interest "Mediterraneo-Atlantic dune malcolmia communities" (code 2230). The vegetation covers are always low, ranging from 30 to 60% with an average of 45%; average height of plant community is very low, ranging from 3 to 15 cm. The plant community occurs primarily on low angle slopes and in a north-western aspect.

From 2007 to 2011 a total of 583 plants were monitored in ANC1 while 931 plants were monitored in ANC2 in the last three years. *A. littorea* density (overall population) registered the highest values in March and the lowest in June/July. The yearly assessment showed an important increase in the total number of plant monitored in 2011 compared to the two previous years (Table 3). Plant density was significant lower in ANC1 than in ANC2 (*p-value* < 0.001 by Mann-Whitney U test), but a similar yearly trend were observed in the two groups of plants. In ANC1 the yearly assessment showed the highest values of 15.00 (± 18.8) plants m^{-2} (in March 2008) and the lowest values occurred in June and July, when all plants died. In March considerable variation is showed, with the highest values of 15.00 ± 18.8 (2008) and the lowest of 7.00± 6.48 (2009). An important increase has been observed in 2011, compared to the two previous years, in the number of plant monitored (Table 3). In ANC2 the yearly assessment showed the highest values of 32.30 (± 15.01) plants m^{-2} (in March 2011) and the lowest values, like for ANC1, occurred in June and July. Both in ANC1 and in ANC2 the lowest values of density are showed in 2009 with a progressive increase from 2009 to 2011(Table 3).

The boxplots showed that there were considerable differences among the data distributions for ANC1 and ANC2: the population medians appear to be different in plant density (Figure 2).

Table 3 - Total plants monitored inside plots and monthly density.

	Overall population		ANC1		ANC2	
	N. plants	Density plants m^{-2}	N. plants	Density plants m^{-2}	N. plants	Density plants m^{-2}
March 2007	120	10.91 ± 8.4	120	10.91 ± 8.4		
April 2007	120	10.91 ± 8.7	120	10.91 ± 8.7		
May 2007	97	8.82 ± 6.7	97	8.82 ± 6.7		
June 2007	17	1.54 ± 1.5	17	1.54 ± 1.5		
July 2007	0	0	0	0		
March 2008	165	15.00 ± 18.8	165	15.00 ± 18.8		
April 2008	158	14.36 ± 17.3	158	14.36 ± 17.3		
May 2008	107	9.73 ± 12.3	107	9.73 ± 12.3		
June 2008	43	3.91 ± 6.2	43	3.91 ± 6.2		
July 2008	0	0	0	0		
February 2009	63	3.15 ± 5.47	63	6.30 ± 6.41	0	0
March 2009	269	13.4 ± 11.68	71	7.00 ± 6.48	198	19.80 ± 12.46
April 2009	258	12.8 ± 11.50	67	6.60 ± 6.52	191	19.10 ± 12.14
May 2009	148	7.35 ± 7.15	28	2.80 ± 3.26	120	12.00 ± 6.99
June 2009	19	1.35 ± 2.58	0		19	1.90 ± 1.85
July 2009	1	0.05 ± 0.22	0		1	0.10 ± 0.32
February 2010	0	0	0	0	0	0
March 2010	374	16.21 ± 13.96	96	9.60 ± 13.24	278	21.38 ± 12.57
April 2010	348	15.13 ± 13.12	94	9.40 ± 13.06	254	19.54 ± 11.80
May 2010	285	12.08 ± 10.28	76	7.60 ± 10.77	209	16.07 ± 8.68
June 2010	62	2.17 ± 3.36	0		62	4.76 ± 4.14
July 2010	3	0.13 ± 0.45	0		3	0.23 ± 0.59
February 2011	533	23.22 ± 18.62	121	12.10 ± 15.86	412	31.76 ± 16.27
March 2011	549	23.87 ± 18.22	129	12.90 ± 16.56	420	32.30 ± 15.01
April 2011	444	19.30 ± 15.48	103	10.30 ± 13.36	341	26.23 ± 13.63
May 2011	285	12.39 ± 12.67	43	4.30 ± 6.73	242	18.61 ± 12.80
June 2011	24	1.04 ± 2.92	1	0.10 ± 0.32	23	1.76 ± 3.76
July 2011	0		0	0	0	0

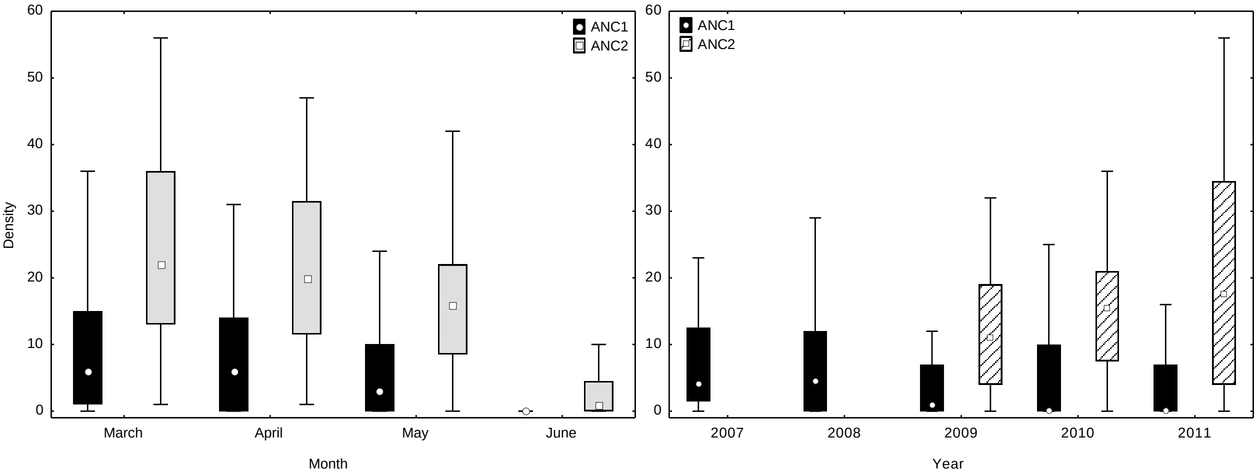

Figure 2 - Boxplots of plants density for months (March-June) and years (2007-2011) in overall population and in ANC1 and ANC2.

Based on density values calculated in these years, the overall size population should be estimated in ca. 854000 plants, varying from a minimum of 586,958 (2007) to a maximum of 1,284,206 (2011). The two nuclei showed different size: ANC1 should be estimated with a much smaller number of plants in relation to ANC2 (ca. 41,400 plants versus 1,224,000 plants) varying from 26,600 (2009) to 57,000 (2008) for ANC1 and from 990,000 (2009) to 1,615,000 (2011) for ANC2.

Population structure and dynamic

The population structure in the overall population consists primarily of plants included in class 2 (52%) followed by class 3 (31%) and class 1 (17%). The same pattern occurs in ANC2 with a highest number of plants in class 2 (55%) followed by Class 3 (23%) and class 1 (22%); ANC1 showed the highest number of plants in class 2 (59%) but, in this case, the Class 1 (31%) counted more plant than class 3 (10%; Figure 3).

Considering the vital rates in the overall population, *stasis* showed high values compared to the other phases, at the beginning of the annual cycle; this trend is shown in all size classes (Figure 4). Afterwards, in April-May period, stasis remains the predominant phase only for individuals of class 3 (Figure 4.). *Growth* rate is always low and decreased during the season and *retrogression* showed low values in all period. *Mortality* showed values increasing during the season, with highest values in May-July. Mortality rate increase with plant size (class1 > class 2 > class 3; Table 4; Figure 4). In the March-May period, class 1 showed always statistically significant highest values than class 2 and 3 (*p-value* < 0.05 and *p-value* < 0.001 by Mann-Whitney U test, respectively). Also, class 2 highlighted significant highest values than class 3 (*p-value* < 0.05 by Mann-Whitney U test; Figure 5). No differences among classes were found in the transition between May and June (*p-value* > 0.05 by Mann-Whitney U test).

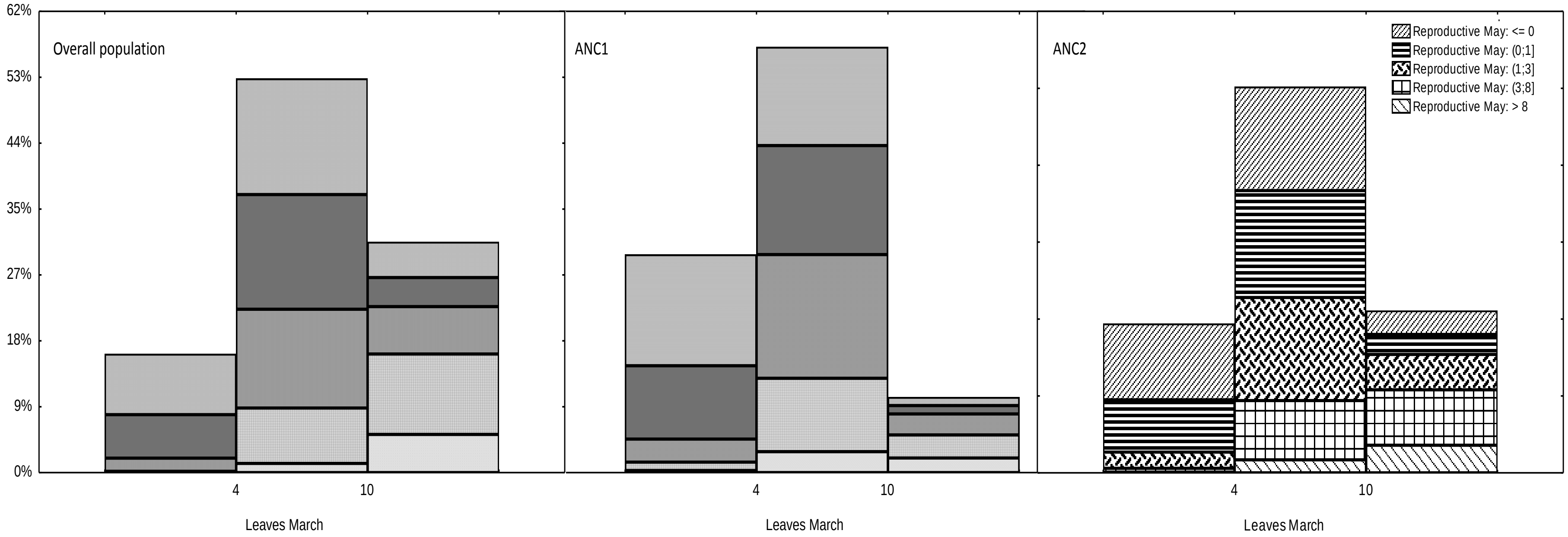

Figure 3 - Size population structure for overall population, ANC1 and ANC1 respectively.

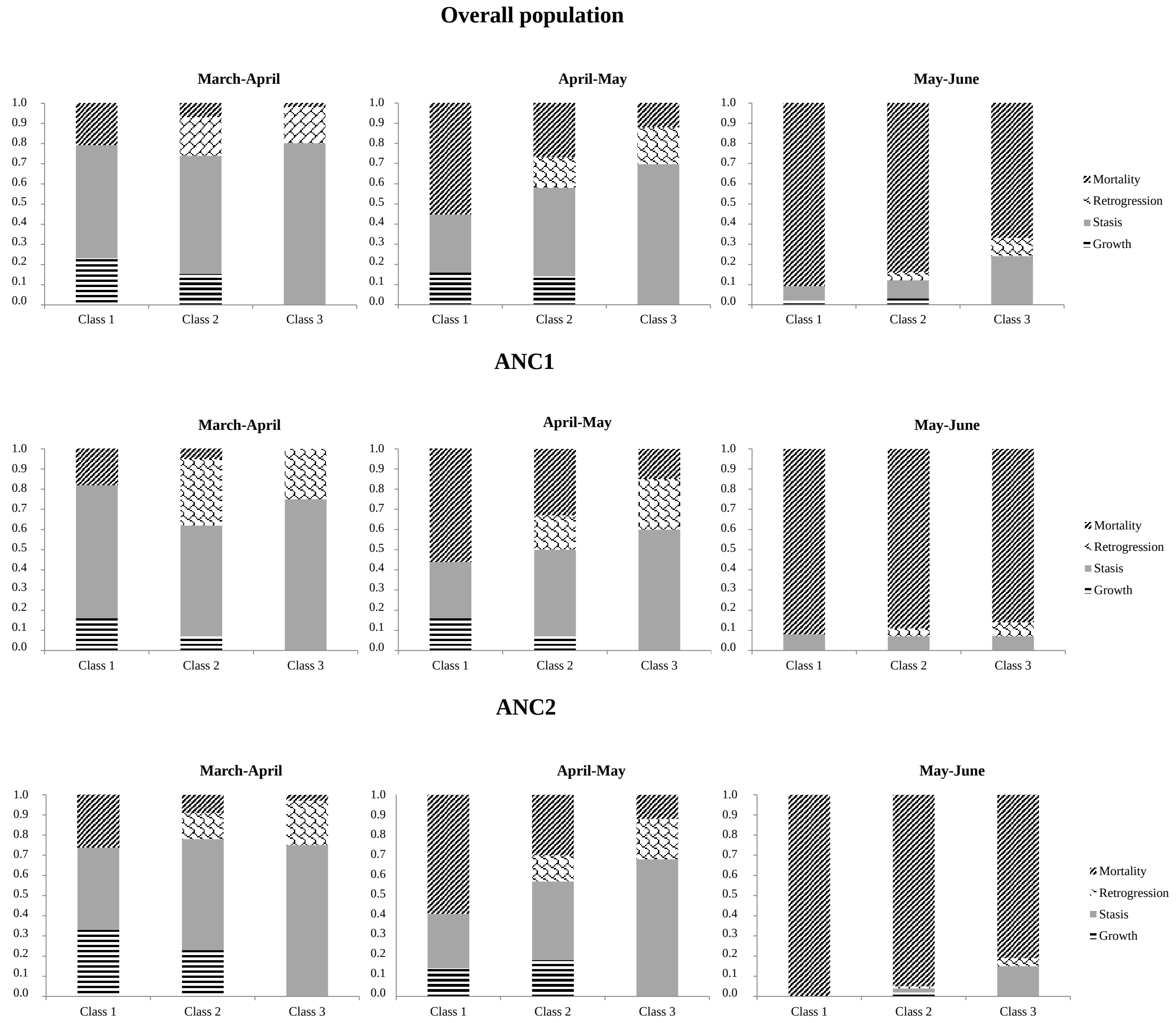

Figure 4 – Cumulative trend of vital rates of the *A. littorea* population from March to June in overall population, ANC1 and ANC2.

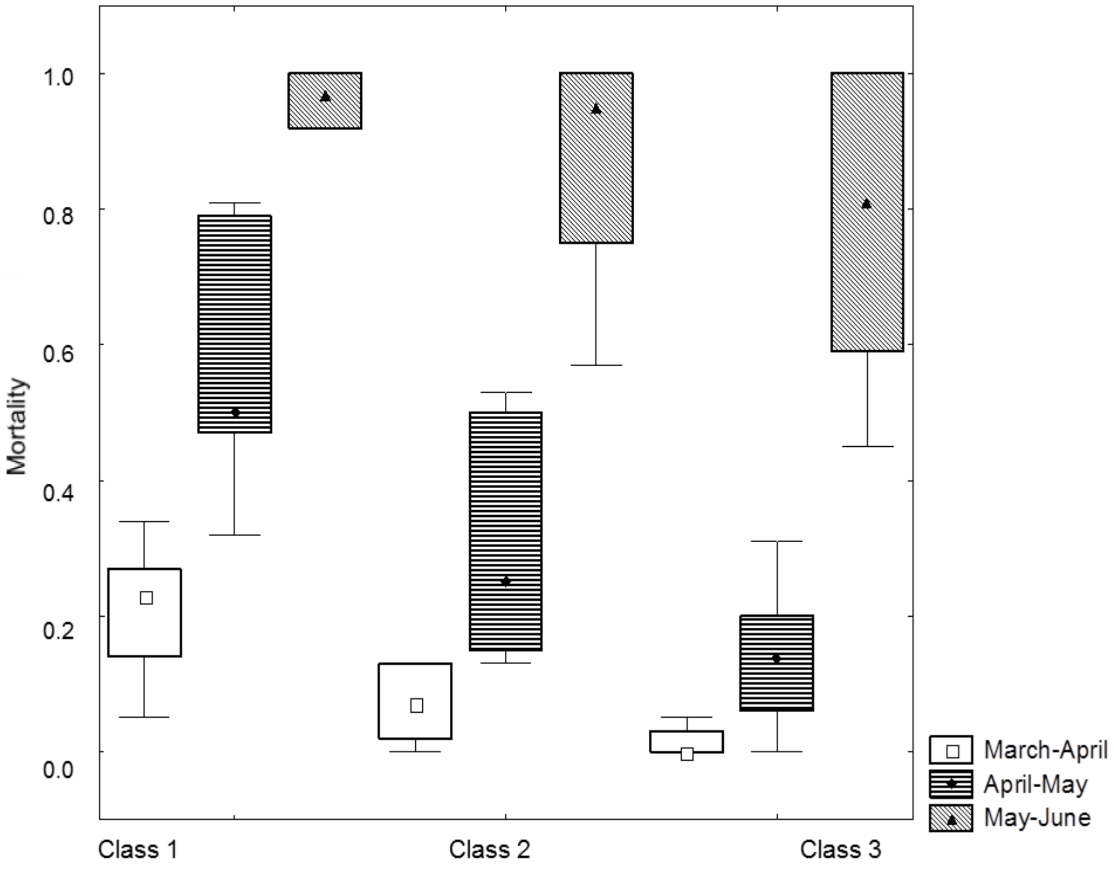

Figure 5 - Mortality rate during the season for classes analyzed

Table 4 - Transition probabilities of *A. littorea* from month *t* (time, columns) to month *t + 1* (rows).

	OVERALL POPULATION				***ANC1***				***ANC2***			
	t+1											
t	***Class 1***	***Class 2***	***Class 3***	***Dead***	***Class 1***	***Class 2***	***Class 3***	***Dead***	***Class 1***	***Class 2***	***Class 3***	***Dead***
March-April												
Class 1	0.56±0.12	0.22±0.08	0.01±0.01	0.21±0.08	0.66±0.07	0.16±0.05	0.00	0.18±0.10	0.41±0.09	0.31±0.14	0.02±0.01	0.26±0.08
Class 2	0.19±0.04	0.58±0.11	0.15±0.08	0.07±0.04	0.33±0.12	0.55±0.15	0.07±0.05	0.05±0.06	0.13±0.05	0.55±0.06	0.23±0.03	0.09±0.03
Class 3	0.03±0.03	0.15±0.12	0.80±0.15	0.02±0.02	0.06±0.08	0.19±0.22	0.75±0.29	0.00	0.03±0.03	0.19±0.07	0.75±0.08	0.03±0.02
April-May												
Class 1	0.29±0.11	0.14±0.06	0.02±0.03	0.56±0.17	0.28±0.09	0.15±0.14	0.01±0.03	0.56±0.20	0.27±0.14	0.12±0.09	0.02±0.03	0.59±0.19
Class 2	0.15±0.06	0.44±0.11	0.14±0.05	0.27±0.15	0.17±0.08	0.43±0.20	0.07±0.06	0.33±0.22	0.13±0.08	0.39±0.06	0.18±0.01	0.30±0.14
Class 3	0.03±0.02	0.15±0.05	0.69±0.10	0.12±0.05	0.05±0.10	0.20±0.08	0.60±0.24	0.15±0.13	0.04±0.02	0.16±0.04	0.68±0.08	0.12±0.05
May-June												
Class 1	0.07±0.13	0.02±0.02	0.00	0.90±0.13	0.08±0.13	0.00	0.00	0.92±0.14	0.00	0.00	0.00	1.00±0.04
Class 2	0.04±0.07	0.09±0.12	0.03±0.01	0.84±0.19	0.04±0.08	0.07±0.13	0.01±0.01	0.89±0.22	0.00	0.02±0.07	0.02±0.03	0.95±0.11
Class 3	0.00	0.09±0.13	0.24±0.07	0.67±0.16	0.00	0.07±0.14	0.07±0.14	0.86±0.27	0.00	0.04±0.02	0.15±0.09	0.81±0.11
June-July												
Class 1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Class 2	0.00	0.00	0.00	1.00±0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00±0.00
Class 3	0.00	0.00	0.03±0.04	0.97±0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.04±0.03	0.96±0.03

ANC1 and ANC2 showed the same pattern of overall population for all vital rates. Growth rates are always highest in ANC2 than ANC1 and the differences are always statistically significant (*p-value* < 0.01 by Mann-Whitney U test; Figure 6).

No differences were found between ANC1 and ANC2 in monthly *mortality* rate (*p-value* > 0.05 by Mann-Whitney U test; Figure 7).

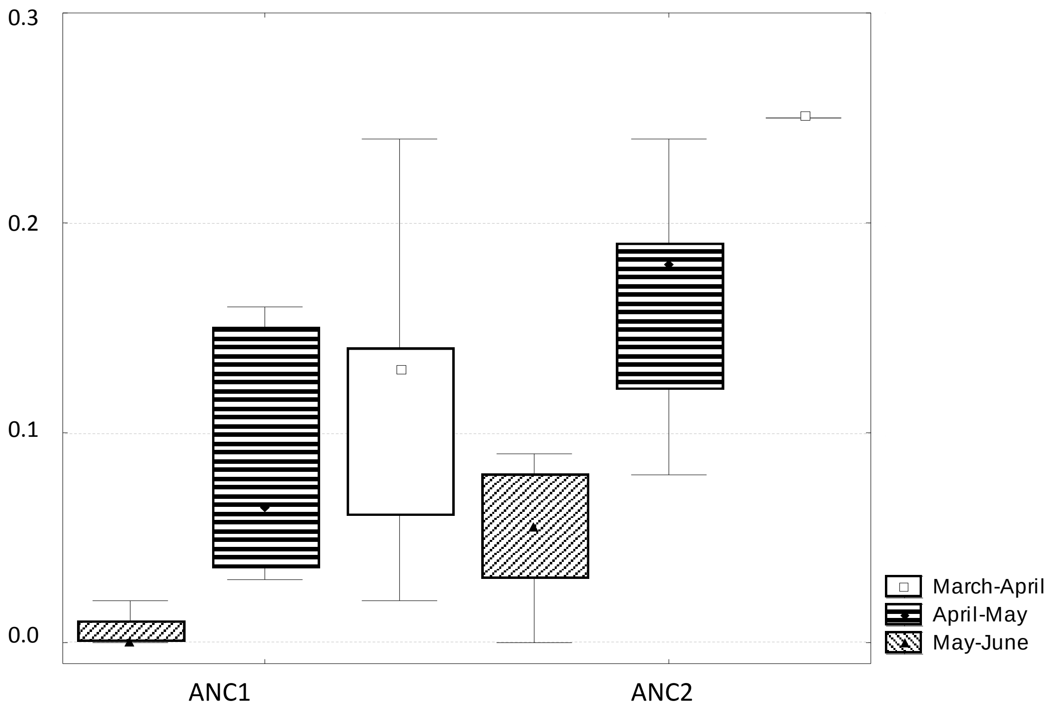

Figure 6 - Comparison in monthly growth rate between ANC1 and ANC2.

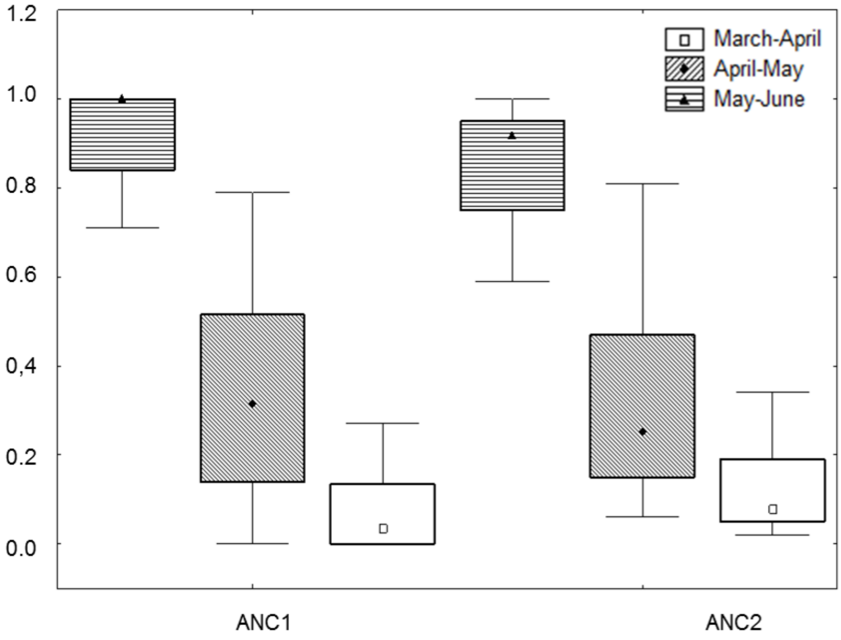

Figure 7 - Comparison in monthly mortality rate between ANC1 and ANC2.

Conservation status assessment

The EOO of the whole population was estimated in 11.07 ha, while the AOO was 4 km^2. According to IUCN Guidelines this population must be considered as one location.

The main threats observed are tourism/recreation (Threat 1.4.3), human trampling (Threat 10.6), poor recruitment/reproduction/regeneration (Threat 9.2) and low plant densities (Threat 9.5).

Based on the EOO, AOO, number of locations (1), the strong fluctuation in size population and the estimated population decline, we confirm the Critically Endangered category for *Anchusa littorea* based on criterion B [CR = B1ab (i, ii, iii, v) + 2ab(i, ii, iii, v) c (II, IV)].

Discussion

Although *A. littorea* was previously found across the west coast of Sardinia (Bacchetta et al. 2008; Selvi & Bigazzi 1998; Valsecchi 1980), it is now confirmed that it only occurs in the sand dune system of Is Arenas. This psammophilous habitat in which it occurs, is dry for most part of the year, but during the first six months of the year a window of opportunity exists for germination, growth and reproduction (see chapter5). In this highly dynamic habitat (Maun 2009), *Anchusa littorea* showed considerable spatiotemporal variation.

The research demonstred that local population size varied in the dune system of Is Arenas and, according to Freckleton and Watkinson (2002), we are able to consider ANC1 and ANC2 as metapopulation, because processes of colonization and extinction/recolonization occurred. While the role of local demography in metapopulation systems remains poorly investigated (Freckleton & Watkinson 2002), it has been demonstrated that metapopulation modeling provides a simple description of the spatiotemporal dynamics in annual plants (Dornier et al. 2011) as demonstrated by the discovery of ANC2.

The spatiotemporal distribution of this plant results from a balance between the colonization of new sites and extinction of old sites in a suitable and discrete habitat network (Dornier et al. 2011). Thus, on a large scale, the picture that emerges is of one in which there is relatively high turnover of metapopulations. According to Hegazy et al. (2010) conserving species in their natural settings, their own habitat, is the key to ensuring their long-term survival; this is very important from a conservation standpoint because the patches which actually have not been colonized by *A. littorea,* may be suitable areas for its colonization or recolononization over time.

For this reason, as a precautionary measure, any conservation strategy for this species should include the entire dune system of Is Arenas. On the other stand, the results indicate that *A. littorea* population has fluctuated greatly during the 5 years of monitoring, showing significant change in population size, which increased from ANC1 (small metapopulation, discovered in 2005) to ANC2 (large metapopulation, discovered in 2009). Fluctuations in the in the population size should be due to climatic factors and density-dependent interactions (Symonides et al. 1986, Thrall et al. 1989).

In addition, fluctuations are mainly due to the inherent instability of the population dynamics (Silvertown 1991; Rees & Crawley 1989, 1991), in particular the therophytic populations are those with largest fluctuations (Silvertown 1991).

Population analyses highlighted that *A. littorea* is an annual plant, with a short-live annual cycle. The dynamics of *A. littorea* population was characterized by an increase in numbers of seedlings from February to March and by the death in May and June of the older, reproductive plants, having reached the end of their life span. Seedlings developed rapidly reaching seed set already within 1-2 months. The decreasing number of new seedlings from April onwards, together with the advancing plant development and subsequent gaining in size, resulted in an increase of the plant hierarchy in May.

The plants in the research area showed a low mortality rate in large plants and high survival in established seedlings and small plants during the first months of the life cycle (i.e. with survivorships typically exceeding 60%). Thus, mortality rates in plants reproducing, i.e. having flower buds, were low. Although environmental stochasticity, whether reflected in growth, mortality, or reproductive status causes decline of population with a high extinction risk (Menges 1992) the high survivorship of large plants could be important for population stability, ensuring that a large population of reproductive is maintained, until replacement occurs with recruitment.

Despite demographic monitoring showed low growth rates, *A. littorea* population showed a regular adult class distribution. This feature may be considered a direct result of the stability in the reproductive phenology of the species. Also, the low values of retrogression from the reproductive class to previous classes, confirmed a stable population structure.

The analysis of the soil seed bank (see Chapter 5) showed an extremely low number of seeds per sample and a lack of a soil seed bank is assumed. The fruits-to-seeds could be considered the most critical transitions stage in the *A. littorea* life cycle; considering that a short-lived plant in such an unpredictable environment, is likely to depend on a long-lived seed bank for population persistence (Brown & Venable 1986; Meyer et al. 2006), the lack of a soil seed bank for *A. littorea* can pose a strong threat to the population persistence. In fact the long-lived soil seed bank is essential to population persistence in a stochastically varying environment, but even seed bank persistence cannot prevent extinction under the scenario of no year-to-year variation (Meyer et al. 2006). Moreover, the lack of a long-term PSB can have important implications for the population dynamics of an annual species, even if the effect is not easy to detect immediately; indeed, soil seed bank reduces the risk of extinction in particularly bad years (i.e. Quintana-Ascencio et al. 2003; Houlè et al. 2001) and increases the effective population size, as detected both in perennial (Dolan et al. 2008) and in annual plants (Nunney 2002).

The major threats to *A. littorea* are tourism and recreational activities and associated human trampling. Tourism and recreational activities are the main threat to ecosystems in the Mediterranean area (Allen 2001) and typically lead to habitat fragmentation (Gibbs 2001).

The site of Is Arenas is frequented by local people for recreation purposes and is crossed by a footpath, which is the continuation of a service road. The analysis highlights that this factor consistently reduced the density of this species (see chapter 3). Thus, human disturbance contributes to the limitation of the habitat available for this species (Fenu et al. submitted), which is at the brink of extinction due to the reduced ecological range, the small and fluctuating population size, together with the lack of *ex situ* collections. For this reason, tourism activities and trampling should be regulated in all dune systems of Is Arenas. Similarly, trampling is also the most serious threat for other coastal endemic species of the genus *Anchusa*; Quilichini and Debussche (2000) report for *Anchusa crispa* that the strong tourist pressure on coastal dune during the summer season, in particular trampling by human, is the most serious threat for the species' survival.

A. littorea could be considered a neglected plant species and its conservation status has been changed during the last years based on studies carried out: in 2005 it was considered extinct in the wild since it was not found for over 25 years (Bacchetta & Pontecorvo 2005) and afterwards, Bacchetta et al. (2008) and Fenu & Bacchetta (2008) proposed the critically endangered category (CR). The new data archived in this study allow to enlarged the previously reported AOO (0.30 ha; Fenu & Bacchetta 2008) and to reassess the conservation status of *A. littorea*, confirming the Critically Endangered classification of this species at global level.

This research made it possible to understand the exact population ecology of *A. littorea* and the presence of two metapopulations. Their moving through time and space across the entire dune system, suggest that the population size and dynamic of this species is extremely difficult to predict. It can be assumed that the "core population" (i.e. site with a highest plant density and a peak of reproductive output; see chapter 5) moving inside the dune system over the years, according to the "colonization and extinction model", proposed for others annual plants (Dornier et al. 2011).

However, estimating the demographic components of a species' life history can be difficult as survival, growth and reproduction in plant populations can vary considerably from site to site and from year to year (Damman & Cain 1998). It is important to acquire knowledge on the population numbers and geographical range of species and their rates of change so that conservation managers can focus on those at greatest risk (Giam et al. 2011). For this reason, more prolonged and detailed monitoring of the present work is needed in order to provide a more precise range of time for dynamic species. A systematic census of the species is also recommended in order to clarify the role of evolutionary factors in determining dynamic of endemic plant and in order to propose conservation measures.

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Appendices

Table 1A - Pearson's correlation coefficient between dimensional variables of March, April and May (2007-2011) on the plants of overall population (in bold the significant correlation values).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
H March (1)	1	**.506**	**.198**	**.442**	**.477**	**.232**	**.574**	**.542**	**.535**	**.457**	**.403**	**.298**	**.223**	**.103**	**.426**	**.402**	**.096**	**.456**
H April (2)		1	**.680**	**.496**	**.566**	**.344**	**.005**	**.412**	**.382**	**.425**	**.473**	**.464**	**.297**	**.546**	**.176**	**.224**	**.653**	**.498**
H May (3)			1	**.729**	**.665**	**.571**	**.139**	**.523**	**.503**	**.536**	**.427**	**.563**	**.270**	**.393**	.339	.396	**.522**	.690
Diam. March (4)				1	**.632**	**.637**	**.151**	**.444**	**.526**	**.586**	**.380**	**.518**	**.383**	**.216**	**.284**	**.492**	**.373**	**.615**
Diam. April (5)					1	.854	**.123**	**.726**	**.712**	**.713**	**.691**	**.488**	**.421**	**.298**	**.390**	**.506**	**.612**	**.664**
Diam. May (6)						1	**.147**	**.630**	**.759**	**.679**	**.505**	**.417**	**.386**	**.099**	**.432**	**.549**	**.367**	**.635**
Leaves March (7)							1	**.102**	**.104**	**.134**	**.059**	**.100**	**.080**	**.006**	**.088**	**.133**	**.030**	**.142**
Leaves April (8)								1	**.742**	**.687**	**.553**	**.483**	**.328**	**.125**	**.215**	**.450**	**.413**	**.540**
Leaves May (9)									1	**.788**	**.550**	**.407**	**.381**	**.102**	**.310**	**.600**	**.396**	**.544**
Flowers March (10)										1	**.577**	**.427**	**.456**	**.192**	**.279**	**.610**	**.473**	**.538**
Flowers April (11)											1	**.355**	**.416**	**.226**	**.094**	**.303**	**.747**	**.353**
Flowers May (12)												1	**.306**	**.202**	**.116**	**.290**	**.348**	**.730**
Fruits March (13)													1	**.055**	**.066**	**.241**	**.284**	**.294**
Fruits April (14)														1	**.058**	**.122**	**.816**	**.204**
Fruits May (15)															1	**.230**	**.095**	**.593**
Reproductive March (16)																1	**.263**	**.393**
Reproductive April (17)																	1	**.348**
Reproductive May (18)																		1

Table 2A - Pearson's correlation coefficient between dimensional variables of March, April and May (2007-2011) on the plants of ANC1 metapopulation (in red the significant correlation values).

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
H March (1)	1	**.680**	**.496**	**.566**	**.344**	.005	**.412**	**.382**	**.425**	**.473**	**.464**	**.297**	**.546**	**.176**	**.224**	**.653**	**.498**	**.308**
H April (2)		1	**.729**	**.665**	**.571**	**.139**	**.522**	**.503**	**.536**	**.427**	**.563**	**.270**	**.393**	**.339**	**.396**	**.522**	**.690**	**.435**
H May (3)			1	**.632**	**.637**	**.151**	**.444**	**.526**	**.586**	**.380**	**.518**	**.383**	**.216**	**.284**	**.492**	**.373**	**.615**	**.561**
Diam. March (4)				1	**.854**	.123	**.726**	**.712**	**.713**	**.691**	**.488**	**.421**	**.298**	**.390**	**.506**	**.612**	**.664**	**.588**
Diam. April (5)					1	**.147**	**.630**	**.759**	**.679**	**.505**	**.417**	**.386**	.099	**.432**	**.549**	**.367**	**.635**	**.608**
Diam. May (6)						1	.102	.104	**.134**	.059	.100	.080	-.01	.088	**.133**	.030	**.142**	**.141**
Leaves March (7)							1	**.742**	**.687**	**.553**	**.483**	**.328**	**.125**	**.215**	**.450**	**.413**	**.540**	**.503**
Leaves April (8)								1	**.788**	**.550**	**.407**	**.381**	.102	**.310**	**.600**	**.396**	**.544**	**.647**
Leaves May (9)									1	**.577**	**.427**	**.456**	**.192**	**.279**	**.610**	**.473**	**.538**	**.687**
Flowers March (10)										1	**.355**	**.416**	**.226**	.094	**.303**	**.747**	**.353**	**.422**
Flowers April (11)											1	**.306**	**.202**	.116	**.290**	**.348**	**.730**	**.365**
Flowers May (12)												1	.055	.066	**.241**	**.284**	**.294**	**.622**
Fruits March (13)													1	.058	.122	**.816**	**.204**	.122
Fruits April (14)														1	**.230**	.095	**.593**	**.214**
Fruits May (15)															1	**.263**	**.393**	**.909**
Reproductive March (16)																1	**.348**	**.334**
Reproductive April (17)																	**1**	.443
Reproductive May (18)																		**1**

Table 3A - Pearson's correlation coefficient between dimensional variables of March, April and May (2009-2011) on the plants of ANC2 population (in red the significant correlation values).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
H March (1)	**1**																	
H April (2)		**1**	**.103**	**.234**	**.333**	**.321**	**.309**	**.302**	**.350**	**.249**	**.199**	**.161**	**.0868**	**.148**	**.321**	**.197**	**.149**	**.323**
H May (3)			1	.044	**.086**	**.146**	.071	.058	**.139**	.079	.043	**.132**	.0020	.008	**.085**	.043	-.007	**.113**
Diam. March (4)				1	**.639**	**.566**	**.527**	**.536**	**.514**	**.461**	**.424**	**.268**	**.3145**	**.249**	**.435**	**.465**	**.258**	**.453**
Diam. April (5)					1	**.778**	**.646**	**.715**	**.691**	**.493**	**.516**	**.362**	**.3361**	**.272**	**.616**	**.498**	**.286**	**.636**
Diam. May (6)						1	**.669**	**.681**	**.847**	**.494**	**.516**	**.501**	**.2540**	**.132**	**.673**	**.445**	**.139**	**.728**
Leaves March (7)							1	**.780**	**.742**	**.631**	**.533**	**.407**	**.2926**	**.250**	**.539**	**.547**	**.232**	**.585**
Leaves April (8)								1	**.707**	**.640**	**.555**	**.387**	**.3022**	**.324**	**.577**	**.558**	**.336**	**.611**
Leaves May (9)									1	**.585**	**.532**	**.506**	**.2960**	**.217**	**.689**	**.524**	**.215**	**.744**
Flowers March (10)										1	**.377**	**.344**	**.3495**	**.364**	**.454**	**.793**	**.321**	**.493**
Flowers April (11)											1	**.339**	**.1805**	**.119**	**.397**	**.331**	**.254**	**.443**
Flowers May (12)												1	.0523	.080	**.339**	**.229**	.072	**.598**
Fruits March (13)													1	**.205**	**.207**	**.847**	**.289**	**.193**
Fruits April (14)														1	**.202**	**.339**	**.858**	**.197**
Fruits May (15)															1	**.391**	**.201**	**.957**
Reproductive March (16)																1	**.368**	**.407**
Reproductive April (17)																	1	**.194**
Reproductive May (18)																		1

CHAPTER 3

THE IMPACT OF HUMAN TRAMPLING ON *ANCHUSA LITTOREA* MORIS (BORAGINACEAE), A COASTAL THREATENED MEDITERRANEAN PLANT

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Introduction

The accelerating destruction of natural habitats and consumption of natural resources by rapidly expanding human populations has caused huge impacts to ecosystems across the globe. In particular, the coastal area is subject to a continuous population growth (Martinez et al. 2007; Vitousek et al. 1997), which is one of the main driving forces of human-induced pressures that affect sandy habitats (McLachlan & Brown 2006).

Intense coastal development has resulted in widespread modification of sandy ecosystems (Coombes et al. 2008; Nordstrom 2000); in this ecosystems, human changes began at least two centuries ago (Nordstrom 2000) and are predicted to intensify over the next few decades (Brown et al. 2008). Evidence for ecological change in coastal ecosystems, which are exposed to human pressures at scales and intensities unmatched in history, is accumulating worldwide (Brown & McLachlan 2002; Coombes et al. 2008; Defeo et al. 2009).

Coastal sandy habitats are extremely sensitive to all forms of disturbance, including those connected with recreational tourism (Kutiel et al. 1999; Pickering & Hill 2007; Schierding et al. 2011; Schlacher et al. 2008a), because these environments are highly dynamic and continually change in response to interactions between wind, waves and sediments (Brown & McLachlan 2002). Increased tourism in recent years has had severe effects on coastal ecosystems contributing to their destruction and reduction of natural and recreational values (Andersen 1995; Lemauviel & Rozé 2003).

Impacts caused by touristic and recreational activities are emerging as significant environmental issues (Schlacher et al. 2008b) and trampling and other recreation linked human activities are rated by among the most severe factors impacting sandy shores (Brown & McLachlan 2002; Schierding et al. 2011). Trampling, which often affects ecosystems of high conservation value (Andrés-Abellán et al. 2006; Bowles & Maun 1982; Liddle & Greig-Smith

1975), is an integral part of the problems of conservation management of natural areas (i.e. Gallet & Rozé 2001, 2002; Kutiel et al. 1999; Rossi et al. 2009).

Trampling studies have mostly examined the characteristics of vegetation and soils that have been walked over, and compared these to untrampled areas, to quantify human impacts (i.e. Lemauviel & Rozé 2003; Rickard et al. 1994; Rossi et al. 2006, 2009). Several studies have analyzed the effects of human activities on dune ecosystems or the effects of human trampling (i.e. Hylgaard 1980; Hylgaard & Liddle 1981; Kelly et al. 2003; Kerbiriou et al. 2008; Lemauviel & Rozé 2003; Schierding et al. 2011) and the impacts caused by off-road vehicles (i.e. Groom et al. 2007; Rickard et al. 1994; Schlacher et al. 2008a). Moderate to high human trampling intensity on coastal sand dunes decreases plant diversity, cover, and productivity, whilst soil compaction increases (Andersen 1995; Kutiel et al. 2000; Liddle & Greig-Smith 1975). The impact of trampling also varies between habitats, notably shifting and semi-stabilized dunes may recover from damage more rapidly than stabilized sand dunes (Kutiel et al. 1999).

The problem of the human alterations of the coastal habitats is very widespread throughout the Mediterranean basin. These ecosystems, classified as Habitat of Community value (Directive 92/43/EEC), have been severely fragmented or destroyed primarily as a result of urbanization, industrialization, and tourism activities (EEA 1999). As a consequence, Mediterranean sandy coasts are highly modified by human impacts, so as to be considered among the most endangered environments in Europe (i.e. Carboni et al. 2009; van der Meulen & Salman 1996).

However, only a few studies have focused on the effects of human trampling on Mediterranean sandy coastal ecosystems (Comor et al. 2008; Kutiel et al. 1999, 2000). In particular, as far as we know, no quantitative studies have been developed on the effect of human trampling on threatened plant growing on coastal sandy dune; moreover long-period data sets describing the dynamics on threatened plant population related to the magnitude of human impacts are scarce. Although for threatened plants the impact of tourism is particularly severe as these species are already at risk of extinction, the impact of tourism on rare flora areas has not been generally recognized as a specific type of threat (Pickering & Hill 2007).

This paper describes the results of a long-term experiment on the effect of human trampling on *Anchusa littorea* Moris (*Boraginaceae*), an endangered endemic plant living on coastal dune in Sardinia. Sardinia is a major centre of diversity and endemism for *Anchusa* L. genus in the Mediterranean area, with seven allopatric endemic *taxa*, four of them occurring in coastal habitats of the island (Bacchetta et al. 2008). Psammophilous *taxa* of this genus [*A. littorea*, *A. sardoa* (Illario) Selvi & Bigazzi, *A. crispa* Viv. subsp. *crispa* and *A. crispa* Viv. subsp.

maritima (Vals.) Selvi & Bigazzi], which are facing an increasing anthropic pressure on the coastal ecosystems, are considered in a precarious conservation status (Bacchetta et al. 2008; Coppi et al. 2008).

Quilichini & Debussche (2000) and Farris & Filigheddu (2008) report, for *Anchusa crispa* and *A. sardoa* respectively, that the strong tourist pressure on coastal dune during summer, in particular trampling by human, is the most serious threat for the species' survival. Instead, long-term data sets describing the natural population dynamics of *A. littorea* was lacking and the biology and ecology of this species is poorly investigated.

A. littorea is endemic to southwest Sardinia (Bacchetta et al. 2008; Valsecchi 1980) and, according to past records, this species was distributed in several coastal dune systems of the Island (S'Ena Arrubia, Terralba, Marina di Arbus, Piscinas, Is Arenas, Sant'Antioco at Calasetta bay and San Pietro). In 2005 *A. littorea* was considered extinct in the wild since it was not found during the field investigation for over 25 years and for this reason the "EX" IUCN category has been proposed for this species (Bacchetta & Pontecorvo 2005). Afterwards, Bacchetta et al. (2008) casually rediscovered a small population of *A. littorea* at Is Arenas locality (Arbus, SW Sardinia). This population consisted of only c. 350 plants over a surface of ca. 2,800 m^2 located within the penal colony of Is Arenas (Figure 1). Nevertheless, the site is frequented for recreation purposes by local people and it is crossed by a footpath; for this reason, human trampling has been suggested as an important threat for this population (Bacchetta et al. 2008). As a consequence we carried out a long-term study for assessing the human trampling on this threatened species.

Human trampling can be studied in two ways, from a conservation viewpoint or as management tool, thus the aims of this study were: (1) to evaluate the effects of trampling on *A. littorea*, and (2) to develop management recommendations for the conservation of the species.

Materials and methods

Study species and site

A. littorea is a short-lived herb, with a small basal rosette, hispid-setose for dense and shorter hairs. Stems are decumbent or suberect, long 4-15 cm. Leaves are linear and present a repand-dentate margins. Each plant develops one or several cymes, with small tubular flowers. Corolla is light blue or white, rotate with rounded lobes. Mericarps are small, light grey-brown, with finely tuberculate testa. The flowering period is from March to May and partially overlaps with the fruiting season, which is from April to July (Bacchetta et al. 2008; Valsecchi 1980).

A. littorea grows in ephemeral herbaceous vegetation on dune systems of siliceous sand, in the discontinuities of *Juniperus macrocarpa* Sibth. micro-forest. The associated *taxa* are principally therophytes, such as the endemics *Linaria flava* (Poiret) Desf. subsp. *sardoa* (Sommier) Arrigoni, *Phleum sardoum* (Hackel) Hackel and *Silene nummica* Vals. and others rare annual plants, such as *Malcolmia ramosissima* (Desf.) Thell., *Brassica tournefortii* Goun and *Polycarpon alsinifolius* (Biv.) DC. (Bacchetta et al. 2008).

The population site (Figure 1) is located in the Is Arenas dune systems, one of the most important and well-preserved coastal system of Sardinia, which spread to ca. 5 Km inland. Geologically, the area mainly consists of Holocene sandstones and Aeolian sands forms which present irregular heights ranging from 10 to 80-90 m (Annino et al. 2000).

Available climate data from the nearest weather station (Montevecchio, ca. 12 Km) indicates a typical Mediterranean annual pattern of temperature and precipitation with a durable dry summer. Bioclimatically this area is classified as Oceanic Pluviseasonal Mediterranean (MPO), with upper thermomediterranean thermotype and lower subhumid ombrotype (Bacchetta et al. 2008).

This area has been protected as a Site of Communitarian Interest, according to the European Habitat Directive 92/43/EEC; nevertheless, the site is frequently visited by tourists through a path accessing the beach, which crosses the population of *A. littorea*.

Data sampling

From 2007 to 2011 the population of A. littorea was monitored, on a monthly basis from March to July during the same time (around the 10th of each month) by placing randomly twelve permanent plots of 1 m x 1 m where the plant was found; the corners of the plots were marked by aluminium tubes (30 cm height) inserted into the sandy soil. Six plots were placed in the trampled area crossed by the footpath and six plots in the untrampled area, where the impact was limited. In March 2007, the A. littorea population consisted of 371 individuals, with 131 reproductive plants, over a surface of c. 2,800 m2.

Trampled and untrampled plots were located in close proximity to each other, in similar environmental conditions and sand properties as well as similar geographical position (see appendix). We therefore assumed that any differences between plots were a result of the intensity of human trampling.

Figure 1 - Area of *A. littorea* population at "Is Arenas" locality (Arbus, SW-Sardinia). In the map the footpath that crosses the area of population is clearly be seen.

Within the plots all plants were counted, marked with a wooden stakes and measured monthly (size and reproductive status). All new seedlings that appeared inside the plots were also counted, measured and mapped. The height and the maximum diameter of each plant were measured using a digital calliper (ALPA IP65 Topcal 150 PW) and the number of leaves was counted. The reproductive capacity per plant was obtained by counting the number of flowers and fruits per plant. Plant survival was recorded every month and a plant was considered to have survived the following month if still present with at least one remaining fresh leaf.

Statistical analysis

From 2007 to 2011, the annual values of plant densities, the size (height, diameter and number of leaves) and reproductive (number of flowers and fruits) variables were plotted on charts both for the trampled and untrampled plots. Exploratory data analyses were first carried out in the form of a boxplots to examine the differences among the distribution of the variables of interest for the trampled and untrampled plots.

To verify whether a single variable was a good predictor describing the plant size, we calculated the Pearson correlation value among morphological and reproductive variables.

Analysis of variance (One-way ANOVA) was applied for the total number of leaves per plant and per month, after verifying that the ANOVA assumptions were accomplished (homogeneity of variance was verified by Bartlett *Chi*-square test and normality distribution by Shapiro-Wilk test). Subsequently *post hoc* Fisher's least significant difference test (LSD) was carried out.

The mean number of flowers and fruits per reproductive plant was calculated as a ratio between total number of flowers or fruits / total number of reproductive plants, considering the data archived in April and May (months with the maximum values for number of flowers and fruits), respectively. The mean number of fruits per plot was calculates as ratio between the total number of fruits counted in all reproductive plants / number of plots; the same procedure was employed for determine the same values in trampled and untrampled plots, utilizing the data for the two categories. For each plot, fruit production was determined by multiplying the average fruit number per plant by the number of fully developed plants in the plot.

The Mann–Whitney U inferential statistical test was applied to evaluate significant differences between the two sample medians of the trampled and untrampled plots for each variables considered. The same test was also applied to evaluate significant differences between the two sample medians of the trampled and untrampled plots for survival rates per plot.

Finally, a logistic regression analysis a type of generalized linear model (Hosmer & Stanley 2000), was performed in order to identify which of the observed characteristics (densities, size and reproductive variables) best differentiate trampled from untrampled plants, and subsequently a model was fitted to the data in order to predict the likelihood that a plant has been trampled or not as a function of the significant variables resulting from the analysis. A logistic regression analysis was performed between each variable and the dichotomous dependent variable with 0 associated to trampled plots and 1 associated to untrampled plots. The data set for this analysis consist of 30 observations on trampled plots and 30 observations on untrampled plots, for each variable analyzed.

All these analyses were performed with Statistica 8.0 (StatSoft, Inc, Tulsa, Oklahoma, USA) software.

Results

Plant density

During 5-years a total of 583 plants were monitored within the plots, 16.30% of them in the trampled plots and 83.70% in the untrampled ones. The yearly assessment of *A. littorea* density (overall population) showed considerable variation in March, with highest values of 14 and 11 plants m^{-2} (in 2008 and 2011, respectively) and lowest of 6 and 8 plants m^{-2} (in 2009 and 2010, respectively). An important increase has been observed in 2011, compared to the two previous years, in the number of plant monitored. This variation is present in both trampled and untrampled plots (Figure 2). In March, plant density was significant lower (*p-value* < 0.001 by Mann-Whitney U test; see appendix for detailed results) in trampled plots than in untrampled ones and a different trend were observed in the two situations. Plant density in trampled plots showed a progressive decrease from 2009 to 2011 with the complete disappearance of the plants in the last 2 years, while the density in the untrampled plots showed annual fluctuations with an important increase in density in the last two years (Figure 2). The same trend was confirmed in the next surveys in April (*p*-value<0.001 by Mann-Whitney U test; see appendix for detailed results).

The boxplot show considerable difference in data distributions for the trampled and untrampled plots: the population medians appear to be different in plant density (Figure 3a).

Plant size and reproductive treats

Size variables showed considerable annual variation in both trampled and untrampled plots. Pearson correlation analyses highlighted a high correlation of the variables *number of leaves* measured in March with other morphological and reproductive parameters (r > 0.4) and these correlations were statistically extremely significant (*p-value* < 0.001; Table 1).

Table 1 - Pearson correlation of the total number of leaves counted in March with morphological and reproductive variables measured in this study.

N. leaves March (N = 583)	Height March	Height April	Diameter March	Diameter April	Flower March	Flower April	Fruit April	Fruit May
r	0.442	0.486	0.737	0.672	0.545	0.526	0.411	0.463
t	11.865	12.764	26.260	20.829	15.661	14.179	10.314	9.644
***p*-value**	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

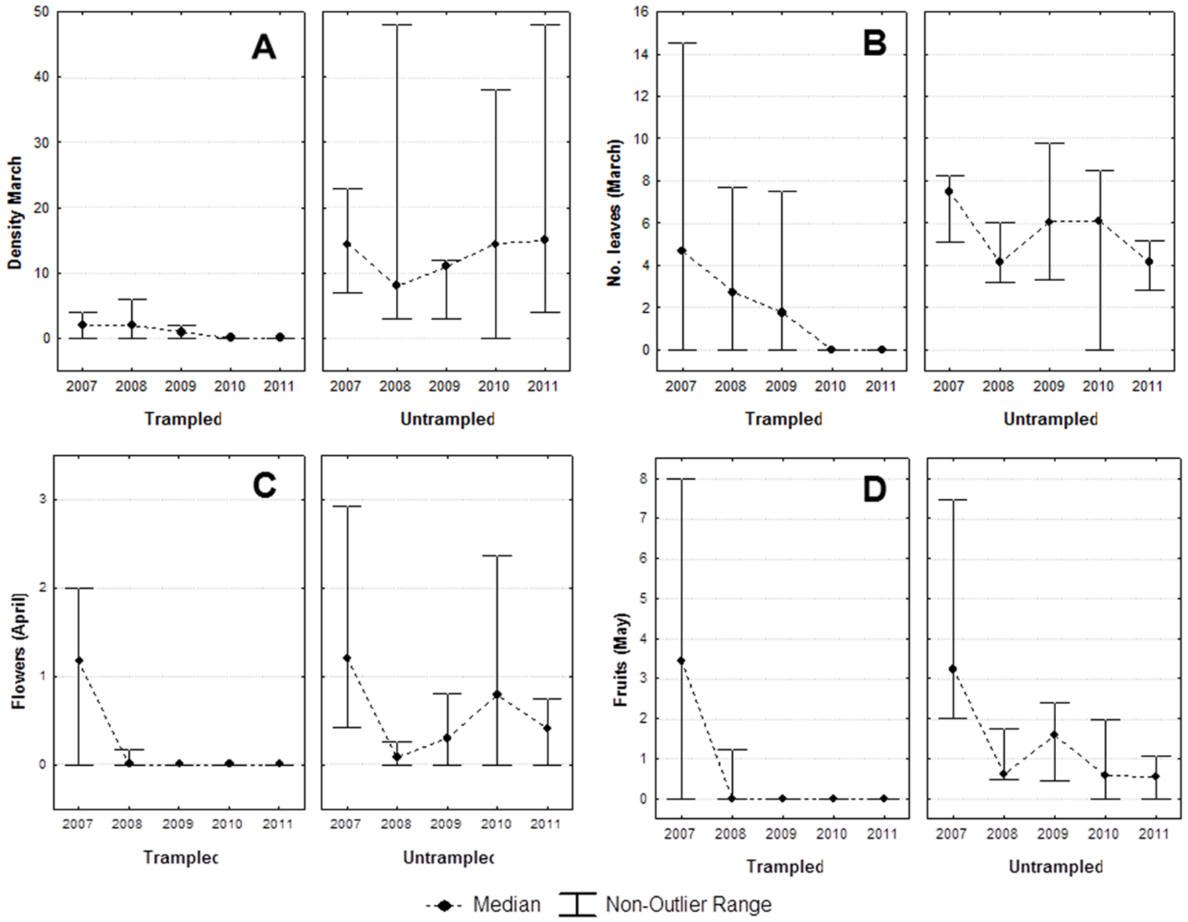

Figure 2 – Plant density (a) and total number of leaves (b) in March, flowers (c) and fruits (d) in April and May respectively in trampled and untrampled plots over the five years. Circles indicate the median values for trampled and untrampled plots.

A. littorea is a short-lived plant and this study indicated that has an annual cycle, which starts in March and finish in June, rarely in July, with a complete time of 3-4 months, depending on the data of emerging seedling. The number of leaves per plant show a high correlation with the plant age (linear regression: age (n. month) = 1.2448+0.4476*no. of leaves; r-squared = 0.108101; r = 0.328787; *p-value* < 0.001) and it results a good predictor of the plant age. The mean number of leaves (± standard error) is 5.64±0.16 after 1 month, 6.11±0.21 after 2 months and 7.03±0.35 after 3 months, with differences among months were statistically significant (*p-value* < 0.01 by One-way ANOVA, followed by *post hoc* Fisher's LSD test).

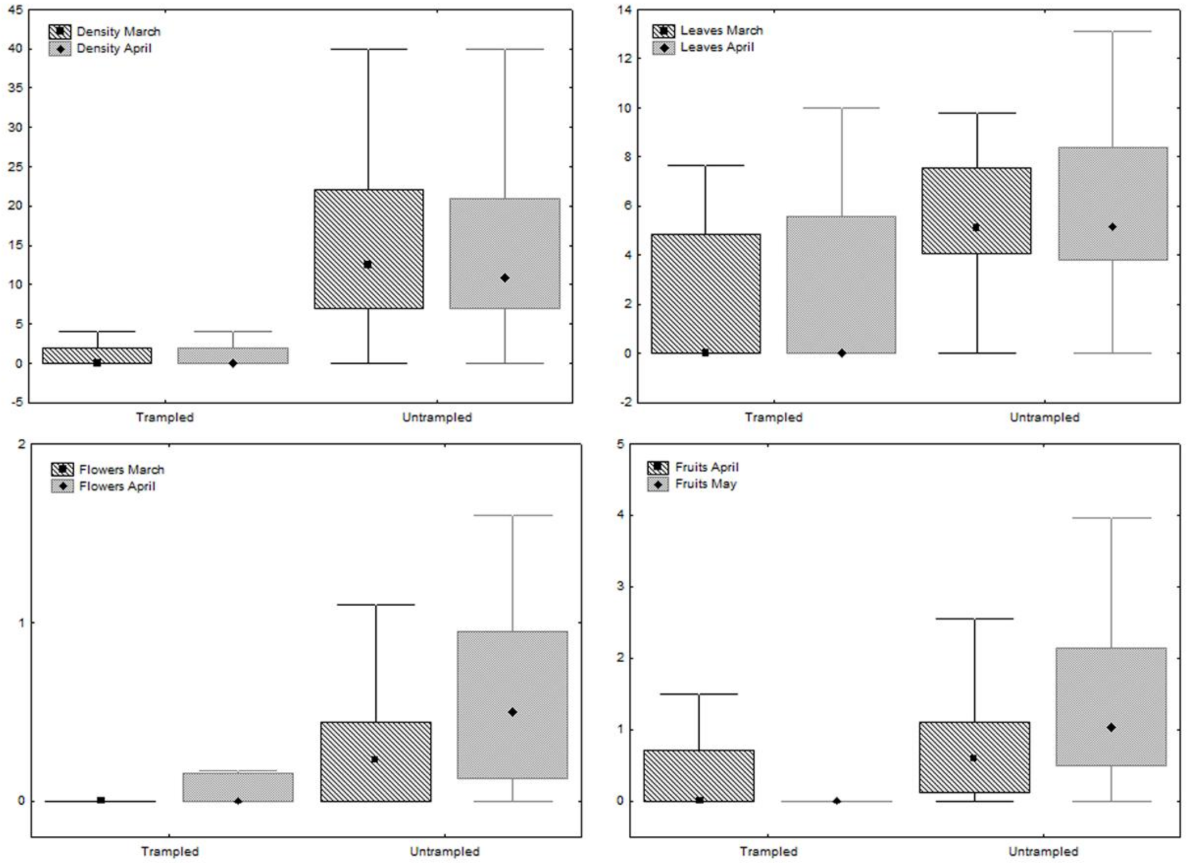

Figure 3 – Plant density, total number of leaves in March, flowers in April and fruits in May in trampled and untrampled plots from 2007 to 2011.

Cumulative monthly assessment of number of leaves highlighted a comparable plant growth between trampled and untrampled plots from March to May, without significant differences between trampled and untrampled plots (*p-value* > 0.05 by One-way ANOVA for March, April and May; Figure 4). In June, with an increase of human frequentation, the number of leaves per plot is remarkably different, with greater values in trampled plots (*p-value* < 0.01 by One-way ANOVA, followed by *post hoc* Fisher's LSD test; Figure 4).

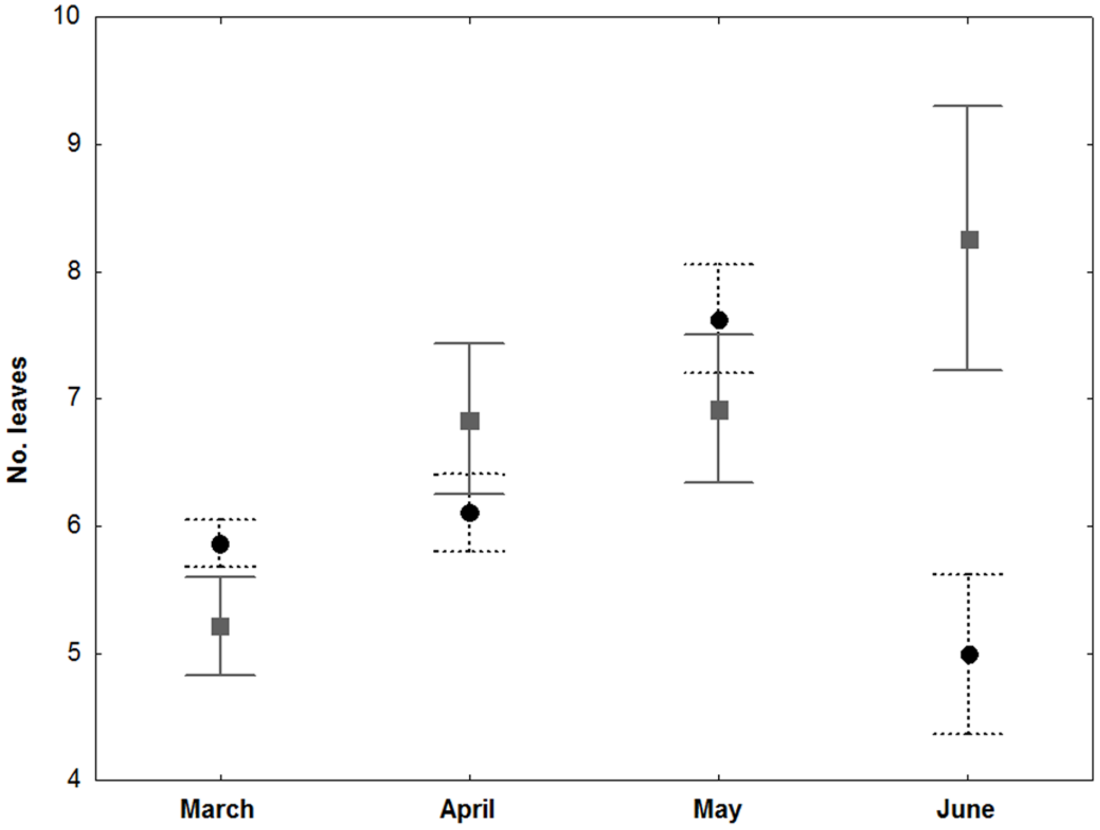

Figure 4 – Cumulative monthly growth in plant size (considering the variable "number of leaves" per plant) in trampled (●) and untrampled (■) plots.

In the disturbed plots, the yearly assessment of the size variable selected (number of leaves) per plot showed a constant reduction over the time (from zero in the last two years), similar trend with previous observed for plant density, while in the undisturbed ones this variable showed considerable difference but didn't show a progressive decline (*p-value* < 0.001 by Mann-Whitney U test; see appendix for detailed results; Figures 2 and 3).

The peak of flowering season was recorded in April, while the peak of fruiting season was found in May (Figure 5). Cumulative number of reproductive plant, compared to the total number of monitored plants, is 364 (62.44%), with lower values in 2008 and 2010 (48.50 and 44.19%, respectively) and higher in 2007, 2009 and 2011 (82.50, 83.10 and 70.83%, respectively). In the trampled plots a total of 63 reproductive plants was found (66.32%), with values ranging from 57.14% (2011) to 74.07% (2007); no plants became reproductive in 2010. On the other hand, in the untrampled plots a total of 301 reproductive plants was found (61.68%), with lower values in

2008 and 2011 (40.71 and 43.44%) and higher in 2007, 2009 and 2010 (84.95, 84.61 and 71.58%, respectively).

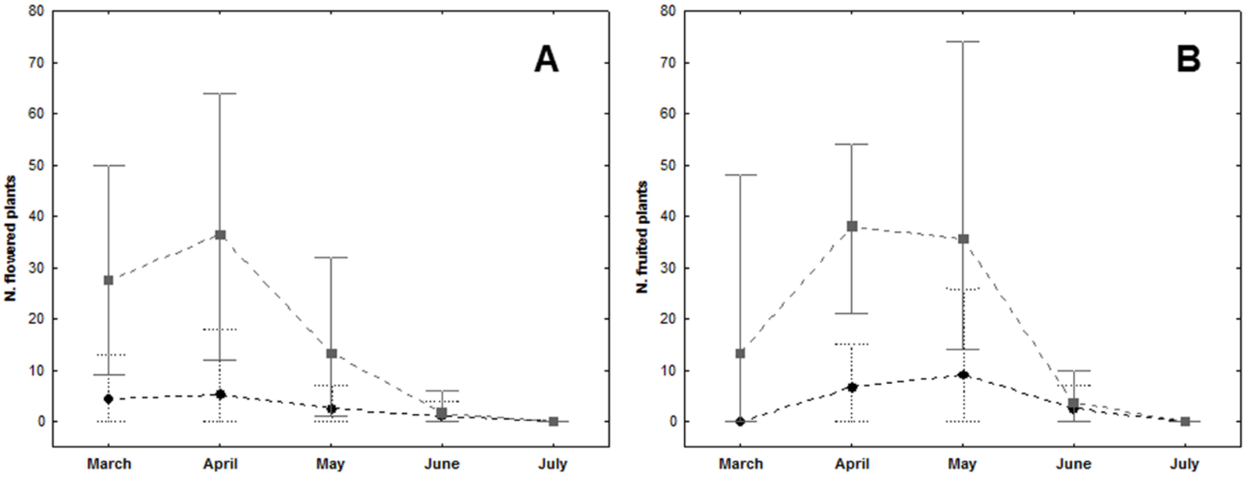

Figure 5 – Cumulative monthly flowered (A) and fruiting (B) plant of *Anchusa littorea* in the trampled (●) and untrampled (■) plots. The bars represent the minimum and the maximum values in each month.

Yearly assessment of flowering and fruiting season, in April and May respectively, showed the same pattern for trampled and untrampled plots. In 2007, flowers and fruits production was similar in trampled and untrampled plots. However in the trampled plots it decreases in 2008 and reaches zero in 2009-2011, while in the untrampled ones flowers and fruits production was throughout different to zero.

Considering the cumulative fruits production (amount of all fruits counted each year from April to June) for trampled and untrampled plots, the final percentage of fruits produced in the trampled plots was only the 25.25% of the total (ranging from 47.48% to zero in the 2008 and 2010, respectively) compared the 74.75% archived in the untrampled ones (ranging from 52.52 to 100% in the 2008 and 2010, respectively; Figure 6).

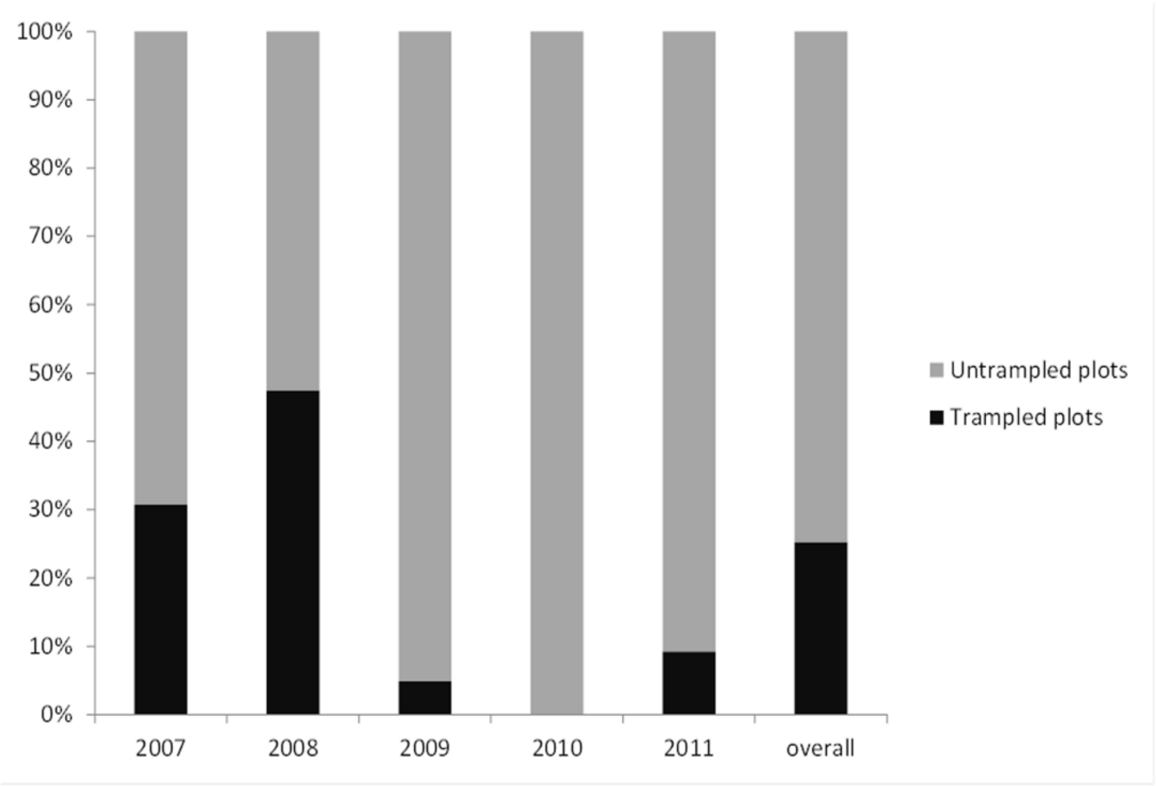

Figure 6 – Cumulative percentage of fruits production in trampled and untrampled plots over the 5-years of study at the population level.

At plant level (Table 2), the percentage of flowered plants was 39.99±26.78% of the total plant monitored, with values ranging from a minimum of 30.04±30.47 to a maximum of 40.22±26.71% in trampled and untrampled plant, respectively. The cumulative percentage of fruiting plants was 59.64±20.84% of the total plant, with values ranging from 45.96±43.41 to 57.89±19.75% in trampled and untrampled plant, respectively. The mean number of flowers per plant was 1.71±0.38, with a mean of 1.02±1.31 and 1.68±0.33 in trampled and untrampled plant respectively, while the mean number of fruit was 2.61±1.50 ranging from a minimum of 2.23±3.29 to a maximum of 2.46±1.23 in trampled and untrampled plant respectively. The mean annual values are reported in Table 2.

The boxplots showed that there were considerable differences among the data distributions for the trampled and untrampled plots: the population medians appear to be different in the observed morphological and reproductive variables (Figure 3b-d).

Table 2 – Reproductive parameters per plant measured over the 5-years in the *A. littorea* population.

	Overall			Trampled plots		Untrampled plots	
	No. of plant	reproductive plants (%)	Mean per plant	reproductive plants (%)	Mean per plant	reproductive plants (%)	Mean per plant
Flowers							
April 2007	120	68.33	2.13	66.67	2.72	68.87	1.97
April 2008	155	10.98	1.41	10.34	1.40	11.12	1.42
April 2009	67	20.89	1.64	0	---	22.23	1.64
April 2010	94	68.08	2.06	0	---	68.08	2.06
April 2011	101	31.68	1.28	42.86	1.00	30.85	1.31
Fruits							
May 2007	97	94.85	5.16	94.74	7.94	98.87	4.48
May 2008	109	53.21	1.62	68.42	1.73	45.07	1.53
May 2009	20	50.00	2.64	0	---	50.00	2.64
May 2010	76	51.32	2.15	0	---	52.00	2.15
May 2011	43	48.84	1.48	66.67	1.50	47.50	1.47

Plant Survival

The survival probability of plants between March and April (30 days, season with low human frequentation of the beach) was lower in the trampled plots (0.36±0.45) than in the untrampled ones (0.88±0.21) with significant differences between the two categories (*p-value* < 0.001 by Mann-Whitney U test; see appendix for detailed results). In the trampled plots the survival decreased over the years, with lower values in 2009 (0.33±0.41) and 2010-2011 (0.17±0.41), showing a similar pattern with the others variables considered. On the contrary, the survival values were similar in the untrampled plots over the years.

This trend is confirmed considering the survival probability between April and May (30 days, season in which human disturbance increase): low values were recorded in the trampled plots (0.20±0.36) and highest in the untrampled ones (0.62±0.30). Differences between areas are extremely significant (*p-value* < 0.001 by Mann-Whitney U test; see appendix for detailed results).

Considering the cumulative period monitored (March-May, 60 days), the survival present low values in the trampled plots (0.20 ± 0.35) and highest in the untrampled ones (0.57±0.29), with significant differences between the two samples (*p-value* < 0.001 by Mann-Whitney U test; see appendix for detailed results).

Logistic regression

The resulting logistic regression curves for all 6 variables (density, height, diameter, number of leaves, number of flowers, and number of fruits) are shown in figure 7. Each logistic regression S-shaped curve represents the probability of a plant belonging to category untrampled as a function of the respective independent variable. The measured data appear either at the top (probability equal 1) or bottom (probability equal 0) of the graph. Given a measure of the independent variable, the logistic regression predict the plant to belong to the untrampled category if the predicted probability value is larger than 0.5. Although there is a general high overlap of the distributions of all independent variables for the untrampled and trampled categories, we can observe that for the variable *density,* the logistic curve fits the data quite well and thus shows the greatest discriminating power between the two categories. The variable *number of flowers* on the other hand shows the worst fit which makes this the less single predictive variable.

Table 3A shows the statistics for a logistic regression model fit that include all the independent variables. Column 2 shows the maximum likelihood estimators (MLE) of the logistic regression coefficients for each independent variable. Column 4 shows the corresponding Wald statistics, a statistic commonly used in logistic regression analysis to assess significance. As can be observed in the table, the only statistically significant coefficient, at the 0.05 level, correspond to the variable *density*.

We then proceeded to fit to the data a simple logistic regression model with only *density* as independent variable. A summary of the resulting statistics is shown in table 3B.

Column 2 contains the MLE for the coefficients of the simple logistic regression model, from which we obtain the following expression for the probability of a plot being untrampled given a specific value of the density:

$$P\left(\text{plot untrampled}\right) = \frac{\exp\left(-1.73 + 0.36 * density\right)}{1 + \exp\left(-1.73 + 0.36 * density\right)}$$

The sign of the MLE for the density coefficient implies that a plot with a high density has a higher probability of being in the untrampled category. Thus, when $P_{(\text{plot untrampled})} > 0.5$ the plot is classified as untrampled, and when $P_{(\text{plot untrampled})} < 0.5$ the plot is classified as trampled. The corresponding logistic regression curve is shown in figure 7.

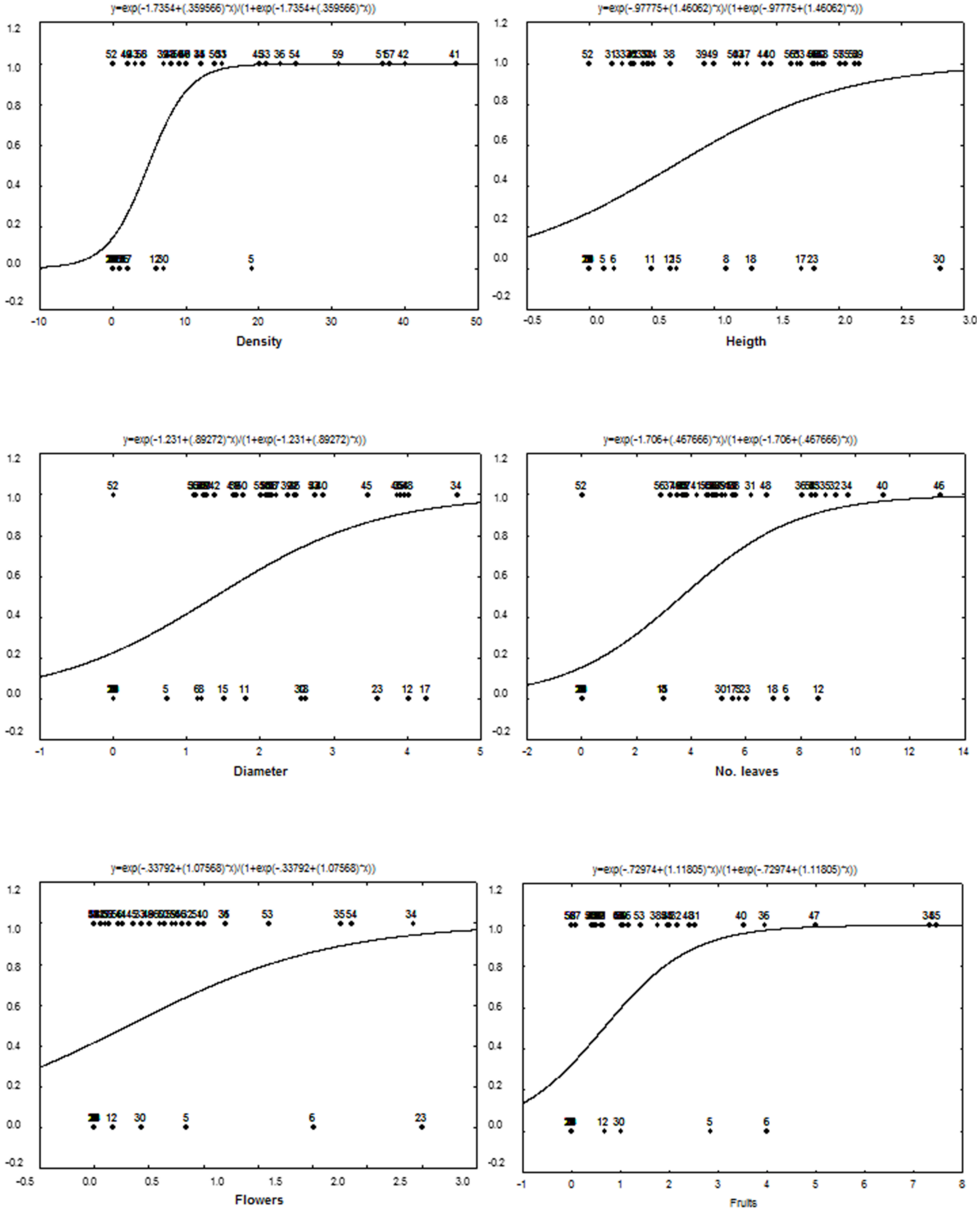

Figure 7 - Single variable logistic regression curves representing, respectively, the probability of a plot to be damaged by trampling as a function of density, height, diameter, number of leaves, number of flowers, and number of fruits. The sample data are represented as observations at either the top or bottom of the graph.

Further analysis indicates that the best model with two independent variables is the one with *density* and *number of leaves*. A logistic regression model with *density* and *number of leaves* as independent variables was then fitted to the data. A summary of the resulting statistics is shown in table 3C.

Table 3 - Results of Logistic regression (Binomial distribution, Link-Logit function): for all variables model (A), for single variable model (B) and for two variables model (C). In bold values statistically significant.

Effect	**Coefficients estimates**	**Standard error**	**Wald statistic**	***p*-level**
A				
Intercept	2.55762	0.802879	10.14779	**0.001445**
Density	-0.22105	0.102437	4.65675	**0.030932**
Height	-1.34701	0.796661	2.85888	0.090871
Diameter	0.34668	0.675473	0.26342	0.607779
No. Leaves	-0.12859	0.303166	0.17991	0.671449
Flowers	1.12799	1.236956	0.83158	0.361817
Fruits	-0.73751	0.581196	1.61023	0.204460
B				
Intercept	1.735405	0.516314	11.29728	**0.000776**
Density	-0.359566	0.098134	13.42513	**0.000248**
C				
Intercept	2.203326	0.677184	10.58629	**0.001139**
Density	-0.277052	0.106951	6.71041	**0.009585**
No. Leaves	-0.192927	0.141105	1.86939	0.171545

Note that according to the Wald test for the null hypothesis that a regression coefficients is equal to zero, the coefficient estimate for *density* (-0.27) is significant beyond the 0.05 level but the coefficient estimate for *number of leaves* (-0.19) is not. Nevertheless, from the fitted model we obtain the following expression for the probability of a plot being trampled given a specific value of the *density* and *number of leaves*:

$$P\left(\text{plot untrampled}\right) = \frac{\exp\left(-2.20 + 0.28 * density + 0.19 * Leaves\right)}{1 + \exp\left(-2.20 + 0.28 * density + 0.19 * Leaves\right)}$$

Again, the sign of the MLE for the regression coefficients implies that a plot with a high density and a large number of leaves has a higher probability of being in the untrampled category. The corresponding logistic regression curve is shown in figure 8. The logistic curves show a high probability of damage caused by trampling associated to decreasing *density* and *number of leaves*.

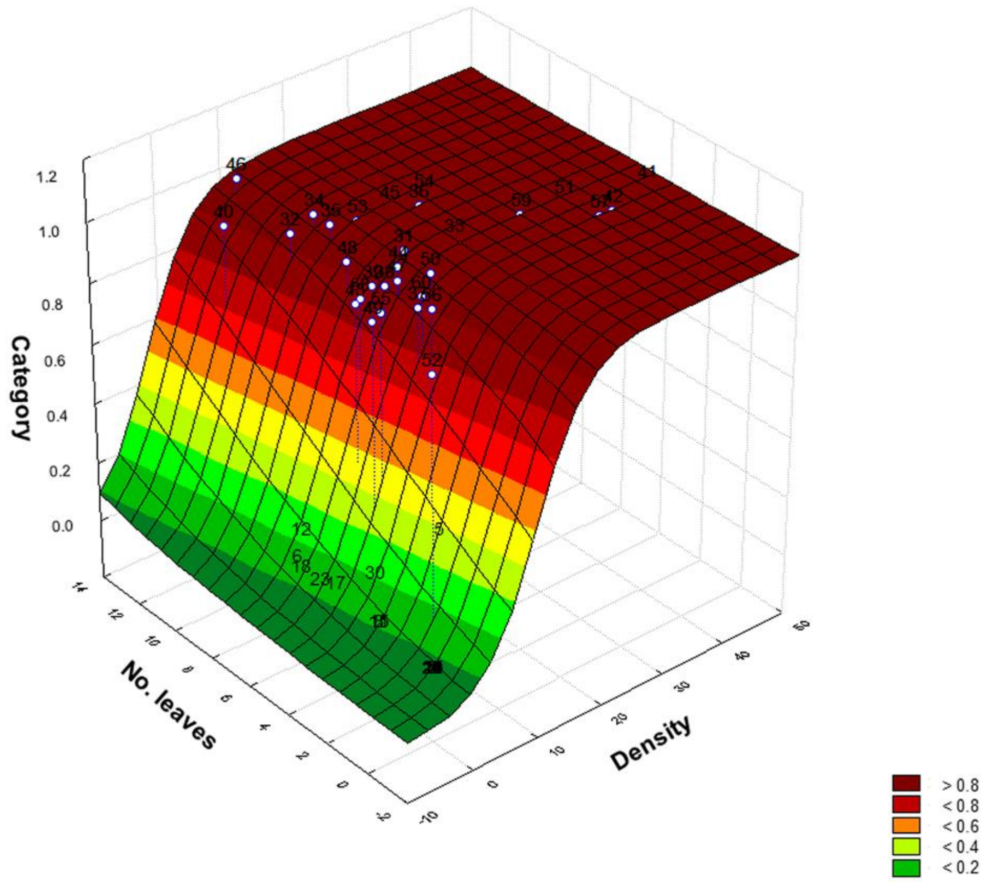

Figure 8 - Logistic regression curve representing the probability of a plot to be damaged by trampling as a function of density and number of leaves. The sample data are represented as observations at either the top or bottom of the graph.

Discussion

Tolerance of species to human trampling varies, sometimes markedly; some annual species are very sensitive to trampling, while others seem to be tolerant or even to benefit from trampling (Yu et al. 2008). In their study on Mediterranean coastal systems, Yu et al. (2009) found that undue trampling leads to a major reduction in species diversity because of the disappearance of some intolerant species. Similarly rare and threatened species seem to have different responses depending on the trampling intensity. Kerbiriou et al. (2008) found that many rare species are able to tolerate a low intensity of trampling and disappear when submitted to greater trampling intensities, while others species appear to be favored by a low and medium trampling level that create a suitable ecological conditions for these species.

Results of 5 years of annual monitoring of *A. littorea* corroborate a several impact from human pressure on this population. The human disturbance consistently reduced the abundance of this species; the overall decline of this threatened species in heavily trampled areas relative to untrampled areas indicates that even the relatively low levels of human trampling that occur in this habitat can dramatically affect plant population.

Human trampling has been suggested as an important threat for other coastal (Farris & Filigheddu 2008; Quiliquini & Debussche 2000) and mountain (Fenu et al. 2011) threatened endemic species in Sardinia, but no specific quantitative study was made before. For the first time in this study is demonstrated that human trampling represents a serious threat to an endemic psammophilous plant in the Mediterranean coastal habitat.

The strong human impact on coastal habitat in Sardinia, combined with the lower genetic variation and the natural instability of sand dune ecosystems (Coppi et al. 2008), could determine a higher risk of extinction for *A. littorea*, confirming the critically endangered category proposed for this species (Fenu & Bacchetta 2008). Our results is consistent with previous analyses conducted on the other two endemic species (*A. crispa* and *A. sardoa*) inhabiting the coastal habitats of Sardinia and Corsica (Farris & Filigheddu 2008; Quiliquini & Debussche 2000) which indicated a recent and strong tourist pressure during summer as the most serious danger for the species' survival. Historical distribution data (Bacchetta et al. 2008; Valsecchi 1980) demonstrate that several populations of *A. littorea* have disappeared from coastal stretches which have been dramatically altered by human actions after the last decades (Bacchetta et al. 2008).

Reductions in density, plant size and reproductive output were observed in the trampled stand in relation to the untrampled ones, as previously reported for other plants in different ecosystems (e.g. Rossi et al. 2006, 2009).

Although many studies have pointed out that the herbaceous plants, and particularly annuals, are more resistant than other life forms to recreational stresses (Kuss 1986; Kutiel et al. 1999), a several impact of human trampling has been detected for *A. littorea*.

Previous studies suggested that plant populations may fluctuate due to their intrinsically unstable dynamics and of all life history strategies annuals show the greatest fluctuations (Gonzales-Andujar & Hughes 2000; Symonides et al. 1986). The five-year analysis showed that *A. littorea* density (but the same pattern was observed for size and reproductive variables) fluctuated yearly, with decline and positive growth periods in both trampled and untrampled plots. We observed strong cyclic variation in population density over time, as expected for annual plants, and this behaviour might be related to the succession of favourable and unfavourable climatic conditions through the years (i.e. Debussche et al. 2004; Rossi et al. 2009), in particular in Mediterranean semiarid environment characterized by high year-to-year precipitation variation. However, a different pattern is confirmed in the untrampled plots, the continue decline of density and final disappearance of plant should be related at the human trampling more than a climatic variability, considering also the small spatial scale of this study. Indeed has been demonstrated that small-scale disturbance play an important role in determining the abundance of suitable micro-sites for establishment (Arany et al. 2005; Cogoni et al. in press) and the number of recruits is a function of both seed production and micro-site availability (Eriksson & Ehrlén 1992).

Quiliquini & Debussche (2000) in their study on *A. crispa* indicate that the human disturbance affects ant populations acting as dispersers, and change the disturbance regime which drives the depth of the seeds in the soil and the fate of the seed bank. Similarly Meyer et al. (2006) conclude that the abrupt decline in population size for *Lepidium papilliferum* (Henderson) Nelson & McBride, an ephemeral desert plant, following the catastrophic trampling event are likely to result from a combination of deep burial of seed and increased germinant mortality. The same process could be suggested for *A. littorea*, considering the habitat similarity and seed morphologies. In disturbed areas a large proportion of seeds can be dispersed by humans (Amrein et al. 2005) and in a case of threatened species may end up in unsuitable areas, considering their ecological requirements (i.e. Cogoni et al. in press). Moreover, considering that soil seed banks play an important role in annual plant populations by buffering populations against temporal variation (Clauss & Venable 2000) and by avoiding the demographic effects of reproductive failure (Evans & Cabin 1995), human disturbance of which drives the depth of the seeds in the soil and the fate of the seed bank could pose a further threat to the population persistence.

The number of leaves for plant represent a good predictor for defines the size classes in annual plant, such as *A. littorea*: number of leaves increase progressively during the months both in trampled and untrampled stands and this appear strong correlated with the plant age. The highest number of leaves detected in the trampled plots compared to the untrampled ones at the beginning of the summer, is clearly related to the increase in human frequentation. The larger plant sizes could be related to the greater distance between individuals (low density), resulted by the human pressure. Moreover, in arid ecosystems sand movement increases the amount of nutrients, as well as the availability of moisture (Knevel & Lubke 2004; Moreno-Casasola 1986). Dune species tend to respond to sand burial, resulted of human trampling, by increasing above-ground in size components such as height or number of leaves (Seliskar 1994; Zhang & Maun 1992). The two of the key processes activated by burial are the elongation of stems and the production of new leaves and the resources used to make these structures may be acquired de novo, derive from shifts in allocation and increased remobilization of stored resources (Brown 1997; Gilbert & Ripley 2008; Zhang & Maun 1992).

Physical damage on plant was rarely observed in the field and this absence of the physical damage is consistent with previous studies indicating that species with a basal rosette present a greater resistance to trampling (Cole 1987; Liddle & Greig-Smith 1975), as the graminoids, which are already known as trampling-resistant species (Cole 1995).

The percentage of plants that become adult reproductive population varies annually, as achieved for other annual plants (Verdú & Traveset 2005; Weekley et al. 2007). Although the percentage of reproductive plants and the reproduction values per plant did not vary significantly between individual growing in trampled and untrampled plots, the cumulative contributions at population level in fruits (and consequently seed output) is an important parameter in population dynamic, especially in an annual plant where the number of individuals of a year depends primarily on the seeds produced the previous years (Klemow & Raynal 1983).

However, significant inter-annual variations in fruit and seed output could place the populations at a high risk of extinction, as a result of stochastic environmental fluctuations (Houle et al. 2001). A reduction in reproductive traits was also observed for a trampled stand of peripheral isolated population of *Salix herbacea* L. (Rossi et al. 2006), *Senecio incanus* L. (Rossi et al. 2009) and significantly alter the performance and sexual reproduction of *Anemone nemorosa* L. (Rusterholz et al. 2009) in other ecosystems.

Whilst regeneration of vegetation cover and richness occurs outside the peak tourist and frequentation season, as detected in another coastal dune systems in Sardinia (Fenu et al. in press), habitats often do not completely recover from the impacts of a single season and so trampling damage may accumulate over a number of years (Coombes et al. 2008). Consequently, a relatively small increase in use could have significant impacts on coastal environments. Furthermore, although regeneration rates of vegetation cover can be high, species richness shows slower recovery. Therefore, although environmental degradation resulting from trampling may not always be visible as habitats are often able to maintain a relatively high level of vegetation cover, losses in species diversity may occur (Coombes et al. 2008). As a consequence, according to Maschinski et al. (1997), long-term data set is needed to observe the true effects of trampling. As reported for *A. littorea*, where in the first two years of study no consistent differences were observed between trampled and untrampled plots, the species may respond slowly to human trampling, i.e. cover may decrease for a long period of time after the trampling event (Cole 1995). Moreover long-term effects of human trampling are more pronounced than short-term effects (i.e. Kissling et al. 2009), because long-lasting trampling has both direct and indirect effects (Amrein et al. 2005; Cole 1987; Roovers et al. 2004). A careful evaluation of the effect of human disturbance, possibly using existing long-term monitoring data, could be very valuable in managing for *A. littorea* population persistence. These aspects are important for the conservation of the species if we consider that the arid ecosystems (i.e. coastal sandy ones), are particularly sensitive to the increase in environmental variability, as provided by models of global climate change (Holmgren et al. 2006).

Conservation remarks

Because there is a strong social and economical pressure for developing tourism in coastal areas, zero perturbation is generally not an acceptable goal. Conservation biologists should better assess the rates of diversity loss and habitat alteration, and evaluate a sustainable level of perturbation, or propose compensatory management that will ensure a suitable and a sustainable habitat conservation state (Kerbiriou et al. 2008). Thus the results of this study have direct implications for managers attempting to remove (or control) visitor pressure in the *A. littorea* population area and to minimize human trampling impacts.

Our research confirmed that *A. littorea* population are highly susceptible to trampling damage, and consequently, active initiatives of *in situ* conservation are advocated here to protect the unique remnant population in the dune system of Is Arenas. Tourist flow should be redirected and confined to others areas in order to promote the natural expansion of *A. littorea* in its original habitat. A possible integrated strategy for the conservation and management of the species consists of combining *in situ* and *ex situ* measures. We suggest the establishment of *in situ* measures mainly addressing the protection of habitat from further human intervention: protecting the habitat by prohibiting and limiting human disturbance is an essential prerequisite of such measures. Although previous studies have shown that the only limitation on the trampling cannot be considered a measure alone can reverse the downward trend of a population, but such action must still be accompanied by other management measures (i.e. Gross et al. 1998; Maschiski et al. 1997), the exclusion of trampling from the population area for its *in situ* conservation is the first step by excluding access to the population or by fencing the population. In parallel, *ex situ* conservation measures, such as seed bank conservation and/or plant propagation (i.e. tissue culture if seeds are limited and difficult to collect or germinate) for reintroduction purposes are essential measures and activities which should be taken in order to ensure the long term conservation of the species.

In conclusion, the touristic / visitor management plans for protected and sensitive areas need to take explicit account of threatened plant species, considering getting to restrict human access to these sensitive areas. Moreover, further research is needed to examine in detail the role of the human disturbance and the mechanisms by which human activities can affect populations of threatened plants. Managers of protected areas should utilize scientific investigations, such as long-term monitoring, to balance visitor use and environmental protection and promote additional studies aimed to quantify and assess the environmental impact of recreational activities on sensitive areas.

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Appendix

Table 1A - Mean values of geographical and pedological parameters measured in the plots. The mean as calculated considering the plot attributes divided by category; for the aspect the extreme values as reported.

	Trampled plots	Untrampled plots
Altitude (m a.s.l.)	29.5±3.6	31.2±1.9
Aspect	SW-NW	SW-NW
Slope (°)	0 – 5	0 – 10
Vegetation type	Ephemeral	Ephemeral
pH	7.7±0.29	7.6±0.34
Conductivity (µS cm^{-1})	116±31.2	101±23.7
Calcimetry (%)	7.5±4.95	7.1±4.77
Organic matter (g kg^{-1})	2.1±0.1	2.1±0.1
Organic C (g kg^{-1})	1±0.1	1±0.1
Mean grain size (mm)	0.30±0.66	0.30±0.69

Table 2A - Mann-Whitney U Test results: there are significant differences for all variables and survival probability in trampled and untrampled plots at p<0.001. Valid N = 30 for trampled and untrampled plots.

	U	*p*-value
Density		
Density March	85.5	0.000000
Density April	86.5	0.000000
Size variable		
No. leaves March	196.0	0.000145
No. leaves April	226.0	0.000787
Reproductive parameters		
No. flowers March	266.0	0.002886
No. flowers April	220.5	0.000372
No. fruits April	252.5	0.002541
No. fruits May	189.0	0.000057
Survival rate		
Survival March-April (30 days)	203.5	0.000141
Survival April-May (30 days)	164.5	0.000013
Survival March-May (60 days)	176.0	0.000028

CHAPTER 4

SEEDLING EMERGENCE AND SURVIVAL OF *ANCHUSA LITTOREA* MORIS (BORAGINACEAE), AN ENDANGERED MEDITERRANEAN PSAMMOPHYLOUS SPECIES

Donatella Cogoni, Giuseppe Fenu & Gianluigi Bacchetta

Introduction

Seed germination and seedling establishment are considered major bottlenecks in the plant life history, often limiting population dynamics and expansion more than seed production per se (Körner 2003; Clark et al. 2007). Seedling constitutes a critical stage in the life cycle of several plant species that relies on sexual reproduction for the persistence of its populations (Grubb 1977; Harper 1977) and the time at which this process occurs often determines subsequent plant performance and success (Weiner 1988). Patterns of seedling recruitment have persistent effects on population and community structures (Yu et al. 2009) as it determines the number of individuals entering the next generation, which has important consequences for the demography and evolutionary ecology of the species (Kitajima & Fenner 2000). Temporal patterns in seedling recruitment are often reliant on physical factors such as light, temperature, soil moisture (Herrera et al. 1994; Russell & Schupp 1998), strong winds and herbivory (Moles & Westoby 2004; Padilla et al. 2009). Among these, light availability and temperature are the most critical environmental factors for plant recruitment (Arrieta & Suarez 2005; Messaoud & Houle 2006) because excessive light and extreme temperatures may damage seedlings (Bainbridge 1994), while soil temperature and moisture also limit seed germination and recruitment success (Herrera et al. 1994).

The timing or phenology of seedling emergence can strongly influence plant fitness (Mercer et al. 2011). Timing of seedlings emergence, due to genetic (Rees 1994; Finch-Savage & Leubner-Metzger 2006; Mercer et al. 2006; Leger et al. 2009) or environmental factors that vary across a species' range, over time, and among microsites (Kalisz 1986), resource availability (Clauss & Venable 2000) or seed density (Ellner 1986), is one of most important and well-studies factor (Howell 1981). Timing of emergence has important consequences for subsequent survival and fitness in competitive situations both between and within species (Smith et al. 2000; Rees

1997a, 1997b; Rice & Dyer 2001; Verdù & Traveset 2005). In fact, even within a population, should be often considerable variation in the time of seedling emergence, which can be spread over several weeks or even months (Verdú & Traveset 2005). Therefore early emergence is likely to be advantageous at least for growth and reproduction, and possibly for survival (Turkington et al. 2005). Presumably, early emergents are better able to deal with environmental stress because of their greater size (Turkington et al. 2005; Verdu & Traveset 2005). Because timing of emergence influences survival, growth, competitive ability and ultimately plant fitness, it is expected to be a crucial trait in the shaping of plant life histories, especially in annual plants, where such effects are likely to persist for a greater proportion of the life cycle than in longer-lived plants (Turkington et al. 2005). In particular it has been demonstrated that variability in establishment could cause differences in fitness (Kalisz 1986; Cabrales-Vargas 1991; Philippi 1993) and, therefore, may promote life-history trade-offs among fitness components (Kalisz 1986).

Seedling establishment and survival is considered one of the most critical stages of the life cycle in Mediterranean and arid ecosystems (Escudero et al. 1999; Rey & Alcántara 2000). In these habitats, for spatial patterns of seedling recruitment, such as biotic factors like seed dispersal and seed survival can play a key roles (Russell & Schupp 1998; Rey & Alcantara 2000) and competition and facilitation (Callaway & Walker 1997) as well as on abiotic factors (e.g. stochastic rainfall patterns; Turner 1990). The strong environmental control on the early stages of plant life implies that those stages would be particularly sensitive to environmental changes (Shevtsova et al. 2009). Mediterranean coastal dunes have been recognized as stressful habitats, with plants experiencing several abiotic limitations, such as low nutrients, lack of moisture, salt spray, high temperatures and erosion as well as sand burial (Maun 2009; Fenu et al. 2012).

Mediterranean regions are therefore suitable systems to explore the consequences of spatio-temporal variations in recruitment patterns, and on the adaptive strategies of the flora to mitigate such variation (Braza & García 2011) and the larger size reached by early emerging seedlings may entail in arid and these environments where species must survive drought periods (Escudero et al. 1999). Despite all the studies previously cited, a clear directional selection for earlier emergence (earlier emergents surviving better and reproducing more than later ones) was found (Howell 1981; Narita 1998); others studies have found opposite results (Lacey 1982). Also, early-emerged seedlings may also have a higher risk of mortality due to seasonal hazards (pathogens, predation, desiccation; e.g., Marks & Prince 1981, Jones & Sharitz 1989; Rice 1990) typical in Mediterranean environment.

The aims of this study was (i) to observe if there was a spatial variability of recruitment components among years (ii) to analyze if early seedling emergence increases plant survival, growth and fitness and (iii) to test if there is an effect of habitat on seedling emergence and survival of the Mediterranean annual species *Anchusa littorea* Moris (*Boraginaceae*).

Materials and methods

Study species and site

Anchusa littorea Moris (*Boraginaceae*) is an annual plant characterized by cauline leaves almost linear, smaller and sessile. The corolla, light blue or white, has a tube 4 mm long and a limb 4–5 mm large. Mericarps are light greybrown, small, 1.5–2×0.5–1 mm, with a lateral beak and a thin basal annulus, with finely tuberculate surface. Flowering of *A. littorea* occurs during March–May and fruiting occurs during March–June (Bacchetta et al. 2008).

A. littorea is a psammophilous species growing in mobile dune systems of siliceous sand, in the discontinuities of *Juniperus macrocarpa* Sibth. micro-forest. The species is known in the past for several localities along the coastal dune ecosystems in SW Sardinia (S'Ena Arrubia, Terralba, Marina di Arbus, Piscinas, Is Arenas, Sant'Antioco at Calasetta bay and San Pietro at Spalmatore) but currently restricted to the Is Arenas dune system (Bacchetta et al. 2008). Actually, the only known population is located in the Is Arenas's dune systems. The vegetation type is characterized by many therophytes such as *Linaria flava* (Poiret) Desf. ssp. *sardoa* (Sommier) Arrigoni, *Phleum sardoum* (Hackel) Hackel, *Malcolmia ramosissima* (Desf.) Thell., *Tuberaria praecox* Groser., *Polycarpopon alsinifolius* (Biv.) DC., *Silene nummica* Vals. (Bacchetta et al. 2008).

Available climate data from the nearest weather station (Montevecchio, ca. 12 Km) indicates a typical Mediterranean annual pattern of temperature and precipitation with a durable dry summer. Bioclimatically this area is classified as Oceanic Pluviseasonal Mediterranean (MPO), with upper thermomediterranean thermotype and lower subhumid ombrotype (Bacchetta et al. 2008).

Data collection

Over a four years (2008-2011) the *A. littorea* population was monthly monitored. Data collection were carried out from February to July, around the 10th day of each month, by monitoring 25 permanent plots of 1 x 1 m, randomly placed where the plant was found. In each plot all seedlings (plants with 1-4 leaves according to the structure population analyses; see Chapter 2), marked with toothpicks, from their emergence until their death are sampled. All seedlings were counted and survival, vegetative traits (number of leaf fall, leaf flush and mature leaf), and reproductive status (floral buds, anthesis flowers, immature and mature fruit) was recorded.

Within its population area, *A. littorea* was found in two different habitats with different environmental conditions: (i) open areas: characterized by annual vegetation, frequently affected by natural elements like wind and salt, or altered by factors such as recurrent human or animal-induced disturbance; (ii) surrounded areas: protected by shrub or forest vegetation, where the intensity of environmental factors is low. Of the 25 overall plots identified, 12 are in open areas and 13 in closed areas.

Data analysis

The four-year observations were treated separately. All the new seedlings, which emerged during each month, were considered as members of the same cohort, considering a cohort as a group of plants which experience the same event within the same time interval. The size classes identified, were then used to calculate transition probabilities between developmental stages (see Chapter 2 for more details), considering transitions as the number of plants developing in 1 month from stage class *i* to stage class *j* divided by the number of plants in stage class *i* in the previous month. More specifically, transition probabilities were calculated to assess demographic variation within population and, in particular, to determine growth and mortality of the seedlings of a same cohort.

It was evaluated how the timing of seedling emergence affects survival and reproduction. Plant survival, in relation to emergence, was analyzed considering the amount of individuals which survived until the following month. Correlations between seedling emergence and reproductive status were analyzed by calculating the time between the emergence and the appearance of flowers.

The Mann–Whitney U inferential statistical test was applied to evaluate significant differences between the two sample medians of cohorts for emergence and survival rates.

One-way analysis of variance (ANOVA), followed by *post hoc* Fisher’s LSD test, on the number of fruit production per cohort was carried out. The same test was also applied to evaluate significant differences between open and closed plots for density rates per year. In order to test differences in the emergence and survival of plants in the two habitats, the Mann–Whitney U inferential statistical test was performed.

All statistical procedures were performed with STATISTICA version 8.0.

Results

Seedling emergence and growth

The number of seedling monitored during this study ranged from a minimum of 53 plants (2009) to a maximum of 313 plants (2011) and its constitute a four different cohorts (February, March, April and May; Table 1).

Although emergence occurred over the entire growing season (the reproductive season of *A. littorea* starts in February/March and ends in last June, see chapter 5), it was not evenly distributed during the year. The maximum emergence has been achieved in first two cohorts (February and March), with a number of plants increased from February to March cohort and, subsequently, decreased gradually over time (Table 1). The lowest values were recorded in April and May and difference statistically significant between the amount of seedlings in first two cohorts and those in late two cohorts, were found (*p-value* <0.05 by *Mann-Whitney U* test).

In February cohort, seedling emergence gradually increased from 2008 to 2010, but a strong increase from 2010 to 2011 was observed (Table 1). March cohort highlighted a similar level of emergence in 2010-2011; the lowest value was found in 2009 while the highest one has been archived in 2008 with much higher values than February cohort. April and May cohorts always registered low emergence values (Table 1).

Table 1 - Patterns in seedling emergence from 2008 to 2011.

	February	**March**	**April**	**May**	**Overall**
2008	11	92	9	4	**116**
2009	25	23	4	0	**52**
2010	43	42	1	0	**86**
2011	271	40	1	0	**312**
Overall	**350**	**197**	**15**	**4**	

In general, transition probabilities in *A. littorea* showed a similar trend among years; the maximum growth rate, was archived in March-April, in plants which passed from class 1 to class 2 (0.14, 0.29 and 0.12 for 2008, 2009 and 2010, respectively); unlike, in 2011 it was archived in

February (0.28;Table 2). In plants of “post March”, the growth rate (from class 1 to class 2) recorded values < 0.1. No plants which passed from class 2 to class 3 was found in any months (Table 2).

Mortality rate decreased increasing timing of emergence. In February there wasn’t seedling mortality, except in 2011 (0.10), and in March was generally low. The pattern of mortality increased substantially from April (Table 2).

Table 2 - Transition probabilities of *A. littorea* size classes from March to May in 2008-2011.

		Class 1	**Class 2**	**Class 3**	**Dead**	**New**
2008	Feb-March	1.00	0.00	0.00	0.00	11.00
	March-April	0.73	0.14	0.00	0.14	92.00
	April-May	0.48	0.08	0.00	0.44	9.00
	May-June	0.20	0.03	0.00	0.75	4.00
2009	Feb-March	1.00	0.00	0.00	0.00	25.00
	March-April	0.50	0.29	0.04	0.17	23.00
	April-May	0.23	0.02	0.00	0.75	4.00
	May-June	0.00	0.00	0.00	1.00	0.00
2010	Feb-March	1.00	0.00	0.00	0.00	43.00
	March-April	0.73	0.12	0.02	0.13	42.00
	April-May	0.47	0.09	0.03	0.39	1.00
	May-June	0.48	0.09	0.01	0.40	0.00
2011	Feb-March	0.62	0.28	0.00	0.10	271.
	March-April	0.58	0.12	0.01	0.29	40.00
	April-May	0.43	0.09	0.02	0.45	1.00
	May-June	0.01	0.01	0.01	0.97	0.00

Seedling survival

Patterns of survival differed significantly between early (February and March) and late (April and May) cohorts: early cohorts showed high survival rates after three months of life, while late cohorts showed high mortality just 30 days after emergency (Figure 1).

Survival rate showed a gradually decrease with increase timing of emergence; after 30 days the great majority of seedlings in first three cohorts survived (98.00±5.00%, 75.00±16.00%, 67.00±39.00% for February, March and April cohort, respectively), whereas May cohort showed the lowest percentage of survival (6.00±13.00%). February cohort showed statistical differences in survival rate with March, April and May cohorts, with this difference being statistically significant (*p-value* < 0.05 by *Mann-Whitney U* test for all comparisons). In March cohort difference statistically significant was found only with May cohort (*p-value* < 0.05 by *Mann-Whitney U* test). Any statistical difference was showed between April and May cohort (*p-value* > 0.05 by *Mann-Whitney U* test; Figure 1).

After 60 days the seedling survival was 87.00±15.00% and 34.00±11.00% for February and March cohort, respectively The *Mann-Whitney U* test highlighted a significant difference (*p-value* < 0.05) while no seedlings survived in April and May cohort (Figure 1).

February cohort, after 90 days, showed seedling survival still more than 50.00 % (51.00±27.00%), and showed difference statistically significant with March cohort (*p-value* < 0.05 by *Mann-Whitney U* test). March cohort showed low values (3.00±6.00%) and no difference statistically significant with April and May cohorts were found (*p-value* > 0.05 by *Mann-Whitney U* test; Figure 1).

After 120 days only seedlings of February cohort were found (10.00±13.00%) and after 150 days all seedlings were died, as expected for an annual plant (Figure 1). Then, the life span of the early cohorts could arrive to 180 days, whereas in the middle and late emergence it arrived to 90 and 30 days, respectively (Figure 1).

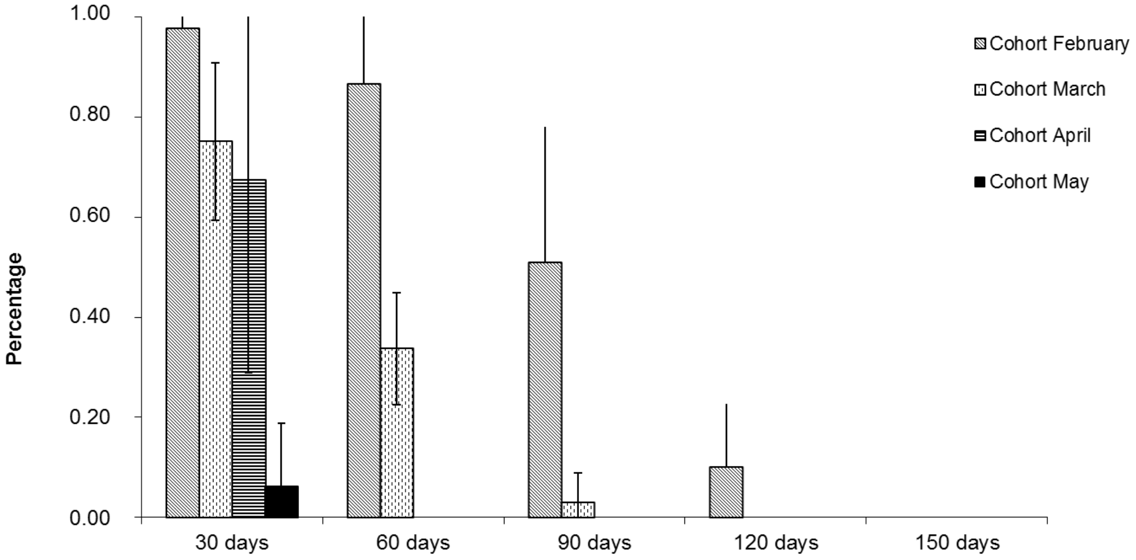

Figure 1 - The mean percentage of seedling survival for 2008-2011.

Reproductive traits

Throughout the years, the results showed similar patterns in reproductive life stages, but different among cohorts (Table 3). Seedlings which emerged in early cohorts showed a wider rate of reproduction than those which emerged in later cohorts. A percentage > 50 (91, 64, 79, 52 % for

2008, 2009, 2010 and 2011, respectively) of seedlings marked in February have reached reproductive stage unlike the of seedlings marked in next cohorts (Table 3).

Table 3 – Number and percentage of flowering, fruiting and total reproductive seedlings of A. littorea by cohort and year.

		February		**March**		**Post March**	
		No.	**%**	No.	**%**	No.	**%**
2008	**New seedlings**	11		92		13	
	Flowering	4	36	6	6	2	15
	Fruiting	7	63	23	25	2	15
	Reproductive	10	91	37	40	4	31
2009	**New seedlings**		25		23		4
	Flowering	2	8	5	22	0	0
	Fruiting	15	60	13	56	1	25
	Reproductive	16	64	13	56	1	25
2010	**New seedlings**		43		42		1
	Flowering	21	49	12	28	0	0
	Fruiting	27	63	14	33	0	0
	Reproductive	34	79	19	45	0	0
2011	**New seedlings**		271		40		1
	Flowering	65	24	2	5	1	100
	Fruiting	114	42	7	17	1	10
	Reproductive	141	52	9	22	1	100

In 2008, in February and March cohorts, the fruit production was 0.73 and 0.39 per plant respectively, compared to 0.11 and 0.25 of April and May cohort. In 2009 were found 1.12, 2.38 and 0.25 fruits per individual for February, March and April cohorts, respectively, while no fruits were found in May cohort. In 2010, February cohort showed the highest average fruit production (1.51 fruit per individual) followed by March cohort (0.57); no fruits was found in April and May cohort. In 2011 February cohort registered 1.42 fruit production per plant; a value of 5.00 and 0.33 fruit per plant was found in March and April cohort¸ no fruits was found in May cohort.

No statistical differences in average fruit per plant have been observed between February and March cohorts (*p-value* > 0.05 by One-way ANOVA), while a significant effect was highlighted (*p-value* < 0.05 by One-way ANOVA) among first two cohorts and last two cohorts.

Habitat effect

The cumulative number of seedlings recorded ranged from 28 (2009) to 146 (2011) and from 25 (2009) to 166 (2011) for open and closed habitats, respectively (Table 4). Statistically significant differences in average emergence between the two habitats were obtained (*p-value* < 0.05 by Mann-Whitney U test). Patterns of emergence varied over years (Table 4) both in open than in closed plots. *A. littorea* seedling was more abundant in closed areas in 2008, 2010 and 2011, but exhibited an opposite pattern in 2009. The highest seedling emergence was found in 2011 and the lowest value was registered in 2009 both in open than in closed areas (Table 4).

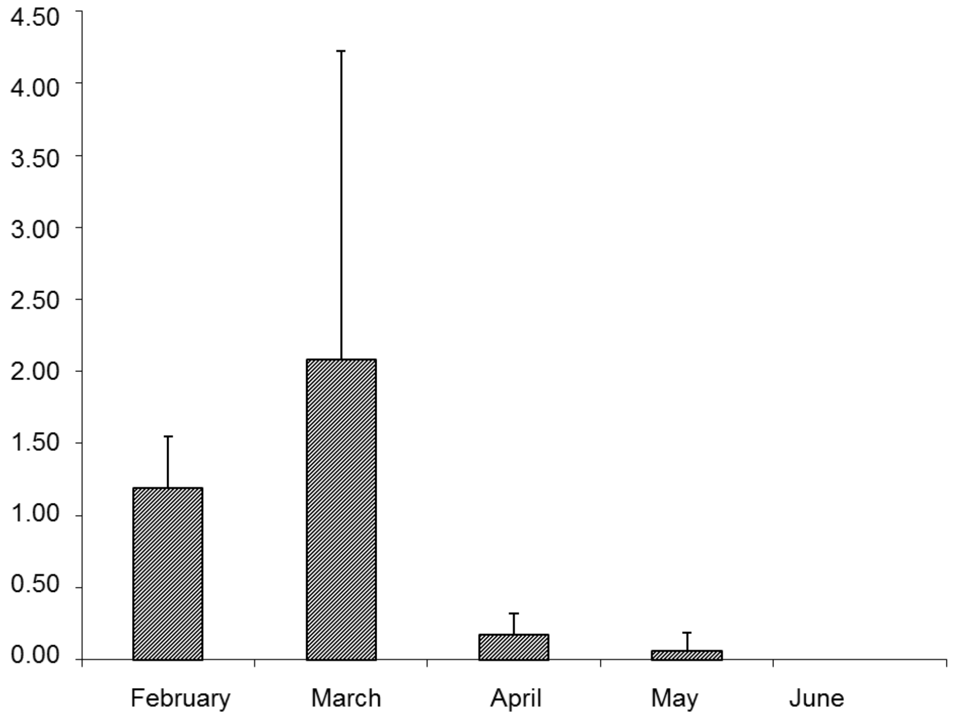

Figure 2 - Average of fruit per cohort.

Table 4 - Trends in seedling emergence from 2008 to 2011 in open and closed areas.

		February	March	April	May	Overall
Open areas	**2008**	4	29	0	0	**33**
	2009	15	10	3	0	**28**
	2010	24	16	1	0	**41**
	2011	122	23	1	0	**146**
	Overall	165	78	5	0	
Closed areas	**2008**	7	63	9	4	**83**
	2009	10	14	1	0	**25**
	2010	19	26	0	0	**45**
	2011	149	17	0	0	**166**
	Overall	185	120	10	4	

Seedling emergence trend was similar in open and closed plot, and both areas recruited >80% of seedlings in two early cohorts (February and March). Number of seedlings emerged decreased gradually over time from March cohort to last cohort. No seedlings in the last two cohorts of open areas were found, whereas in closed areas a low emergence was found (Figure 3).

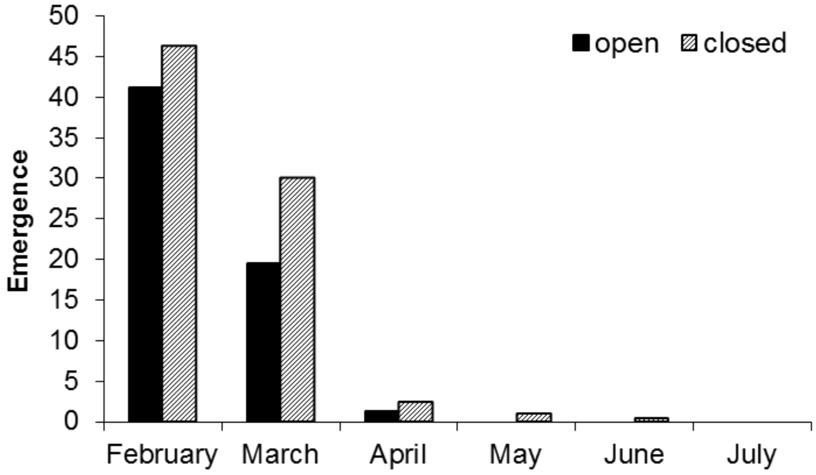

Figure 3 - Mean number of seedlings emerging by month and by habitat.

Survival rate, in relation to the timing of emergence, showed similar values in open and closed areas and no statistically-significant differences in average survival between the two habitats were obtained (*p-value* > 0.05 by *Mann-Whitney U* test; Table 6)

Table 5 - Comparison of mean (±SD) survival rates for open and closed plots.

Cohort	Habitat	30 days	60 days	90 days	120 days	150 days
February	**open**	0.97±0.06	0.85±0.16	0.57±0.34	0.12±0.13	0.00±0.00
	closed	0.99±0.04	0.86±0.16	0.46±0.24	0.07±0.14	0.00±0.00
March	**open**	0.70±0.26	0.43±0.22	0.04±0.09	0.00±0.00	----
	closed	0.80±0.08	0.30±0.15	0.04±0.05	0.00±0.00	----
April	**open**	0.50±0.58	0.00±0.00	----	----	----
	closed	0.36±0.47	0.00±0.00	----	----	----
May	**open**	0.00±0.00	----	----	----	----
	closed	0.06±0.13	0.00±0.00	----	----	----

Closed and open areas showed a similar pattern and average density showed low values and only in 2011 a significant increase has been observed (Figure 5).

Cumulative monthly assessment of density highlighted a significant difference among 2011 and other years years (*p-value* < 0.01 by One-way ANOVA, followed by *post hoc* Fisher's LSD test).

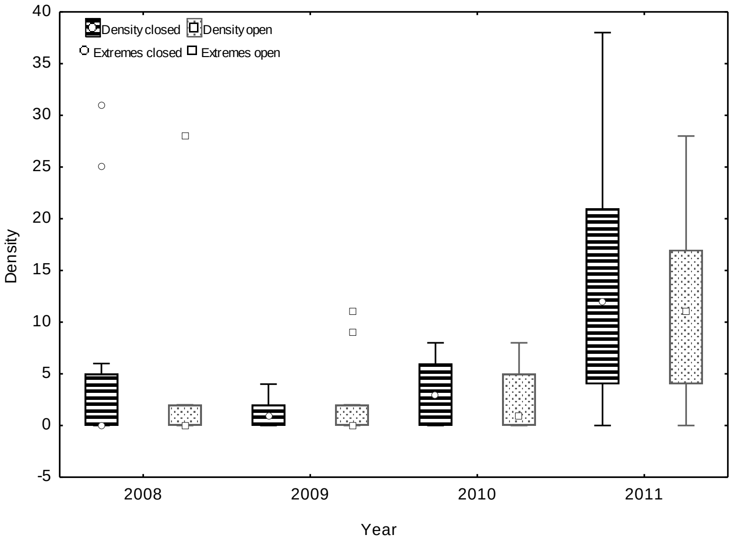

Figure 4 - Density emergence from 2008 to 2011 in open and closed areas.

Discussion

Seedling survival rates may also vary among cohorts that emerge at different times in the annual cycle and the time at which a seedling emerges can determine its future success as a plant (Verdú & Traveset 2005).

Despite the large number of studies that have examined the effect of emergence time on different components of plant fitness (survival, growth, and/or fecundity), the potential evolutionary response to selection on seedling emergence date is still poorly studied.

Early-emerging seedlings may have an advantage because of (1) greater availability of resources, (2) protection from disease or predators, or (3) an indirect relationship between emergence time and seed size (Weekley et al. 2007; Abe et al. 2008).

Based on the results of the research, an early emergence patterns in the annual plant *A. littorea* was identified. Although seedling emergence occurred over the entire growing season, emergence decreased gradually from the end of winter and ended in spring. As already shown in other studies of Mediterranean ecosystem (Lavorel & Lebreton 1992; Lavorel et al. 1993), this pattern could be considered an adaptation to a typical Mediterranean climate, characterized by uneven rainfall throughout the year (Yu et al. 2009). The study area is characterized by rain mainly falls in the wet season from October to March, and the absence of rainfall events for a 5-

month period (from May to September) during that dry time, like most annual plants, could enable the species to pass the unfavourable season as seeds (Yu et al. 2009).

The advantage of early emergence is that plants have time to establish, flower, and finally bear fruits, i.e., complete their life cycle and produce new propagules. However, scattering germination over a four-six months, an evolutionary adaptation to a long-term unfavourable and unpredictable environment, increases the possibility of renewal of generations, especially for short-lived plants (Yu et al. 2009).

Many studies have detected that effects on plant emergence were finally translated to the adult recruitment stage (Suding & Goldberg 1999; Rebollo et al. 2001) and other studies have shown that earlier emergence results in greater biomass of individual plants (Howell 1981; Biere 1991; Stratton 1992).

Such results agree with these predictions: earlier emerging seedlings tended to grow better than later emerging seedlings and the majority of earlier emerging individuals went from seedling to adult stages (> class 2). This suggests that this could have a strong effect on their contribution to the population growth rate.

Early emergence are favored over later not only in growth but also on fecundity influencing the reproductive capacity and the number of fruits produced (Verdú & Traveset 2005) while far fewer studies have demonstrated an advantage for late emergents (Baskin & Baskin 1972; Lacey 1982). Such as result would be expected based simply on time available for growth, as well as any additional competitive advantages due to resource preemption because of an earlier start to growth (Turkington et al. 2005) a correlation between emergence time and success reproductive. *A. littorea* seedlings, as in the case of seedling growth, showed a positive effect of emergence time on fecundity, early seedlings were more fecund than later ones.

The average fruiting from the first two cohorts was an order of magnitude higher than later ones and it is a function of time emerged seedling because seedling emerged earlier had a better percent survival to flowering and to fruiting than seedlings emerging later (furthermore, as soon as they are born more fruits and consequently seeds. Verdú and Traveset (2005) found that the survival benefit of early emergence was greater in perennials than in annuals species. According to Charnov & Schaffer (1973) and Roff (1992), annual life forms would be favoured in environments where the probability for a seed to become a flowering plant within one season is greater than the probability for an adult to survive to another flowering season, whereas perennials would be favoured when the contrary occurs. Other studied showed that seedling emergence and survival, as well as the advantages of an early or late emergence, have been found to be highly variable within species in space and time (Battaglia 1996; Ibáñez & Schupp 2001; Gómez-Aparicio et al. 2005) and showed that in the coastal population emerging late could not be considered disadvantageous under a long-term perspective, given that the differential success of early and late-emerged seedlings in a given year disappeared (Braza & García, 2011). The presence of higher survival rate in early cohorts of *A. littorea* seedlings isn't consistent with these conclusions.

This survival pattern of *A. littorea* seedlings may be an adaptation versus seasonal hazards such as drought. Rainfall patterns determine one of the main resources affecting seedling survival in arid and Mediterranean regions (Escudero et al. 1999; Traveset et al. 2003). Hence, hence, drought is one of the main causes of seedling mortality in Mediterranean ecosystems (Manzaneda et al. 2005; Garrido et al. 2007; Giménez-Benavides et al. 2007; Rodríguez-Pérez & Traveset 2007). In *A. littorea* seedling, plants emerging in the later cohorts mustn't be able to survive up to dry conditions while seedlings emerging early through the dry season would avoid some of the driest conditions. In fact, relatively high mortality rates of seedlings later cohorts were observed, suggesting in late spring and in summer the site was very dry, probably causing further plant stress and inhibition of germination (Leiss & Müller-Schärer 2001). As strategies to cope with temporal unpredictability are expected to be of high importance because strong temporal variation in vital rates like recruitment has important consequences for life-history evolution and population dynamics (Tuljapurkar 1989; Boyce et al. 2006).

Short-lived plants like *A. littorea* are expected to evolve risk-spreading strategies because reproduction occurs only once in their lifetime. Seedling recruitment rates vary in time and space (Oostermeijer et al. 1996; League & Veblen 2006) in response to seasonal weather patterns and local micro-habitat conditions (Weekley et al. 2007) A microhabitats affect the temporal distribution patterns of seedling emergence has been demonstrated; Yu et al. (2009) found that shrub understoreys delayed germination, whereas the trails advanced the timing of seedling germination. Many studied highlighted that the lag in the onset of germination in shrub understoreys could be due to the negative effect of shrub canopies, preventing input of sunlight to the soil, leading to lower temperatures on the soil surface and a darker micro-environment (Shumway 2000; El-Bana et al. 2002). Thicket canopies also intercept rainfall, especially during the first effective rainfall event (Tiëlbörger & Kadmon 2000), leading to drier soil at the beginning of the growing season. In contrast, the reasons for the advance of germination on the trails are the absence of a canopy and the sparse litter cover which lead to intense irradiance and high temperatures on and within the sandy soil surface (El-Bana et al. 2002). On the contrary, in many studies is highlighted that the light restriction treatment was the only resource factor that affected emergence of annual plants(Luzuriaga Arantzazu & Escudero 2008). According to this model microhabitats could affect the temporal pattern of emergence in *A. littorea* because seedlings in closed area showed a higher emergence in comparison to those in the open areas and statistically significant, thus, the seedling emergence could be dependent of the micro-habitat condition (open vs. closed areas).

Seedlings emerging below shrubs may experience different probabilities of survival and/or reproduction than conspecific seedlings germinating in the open areas (Sarig et al. 1994; Tielbörger & Kadmon 1995) but our findings suggest that percent of seedling survival were statistically non-significant between open and closed areas, thus, it is irrespective of the micro-habitat condition.

In summary, we can conclude that early emergence can enhance the fitness of plants due to immediate (survival and growth) and/or delayed (fecundity) effects. This aspect on life cycle of *A. littorea* biology must be carefully considered in order to understand the population dynamics and the reproductive biology of this endangered annual plant.

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CHAPTER 5

SYNCHRONY, SEED SET AND RELATIONSHIPS AMONG PHENOPHASES OF ENDANGERED COASTAL MEDITERRANEAN PLANT

Donatella Cogoni, Giuseppe Fenu & Gianluigi Bacchetta

Introduction

Narrow endemics plants are susceptible to extinction for a variety of reasons, such as habitat destruction, biotic interactions and genetic collapse (Schemske et al. 1994). In order to understand the mechanisms which could affect rare species persistence or extinction, more studies must be conducted on reproductive biology, considering its effects on demography and population genetics has important consequences for the viability of rare plant populations (Evans et al. 2003).

Flowering and fruiting phenology may have an important influence on reproductive success of plant in several ways (Augspurger 1981; Marquis 1988). The timing of flowering can strongly influence the reproductive success of a plant for example: within plants which flower too young may not have adequate resources stored to mature fruits, within populations (asynchronously flowering plants may not find mates), among species (plants flowering at the “wrong” time might not be visited by pollinators, or may be disproportionately affected by seed predators), or abiotic factors (plants flowering too late in the season may be killed by climatic factor before they can mature fruits) (McIntosh 2002). Thus, flowering phenology can affect the ecology of a plant at multiple levels, including individual plant reproductive success, interactions of the plant with other organisms, plant population dynamics, and ecosystem functioning (e.g., the plant-pollinator landscape; Bronstein 1995). Additionally, the phenology of vegetative stages is important as cycles of leaf flush and leaf fall are intimately related to processes such as growth, plant water status and gas exchange (Reich 1995). The timing of biological events (especially in regions with a marked seasonality such as Mediterranean basin) is strongly controlled by climate (Kington 1974; Leith 1974) and consequently, plant phenology could be related to climatic seasonality and environmental variability (Schlichting 1986; Mahall et al. 2010). In arid and semiarid habitat, like Mediterranean sandy coastal ecosystems, plant species are affected by the important shifts that abiotic and biotic factors show throughout the year (Mooney et al. 1974;

Kummerow 1983; Bosch et al. 1997; Cros et al. 1997). Summer drought appears to be the most limiting environmental factor for plant reproduction (Pettersson 1994). In fact, the characteristic of Mediterranean climates is that high-temperature peaks coincide with the lowest rainfall levels along the year (Di Castri et al. 1981). Given the role that some of these factors may play as selective pressures on flowering times (Rathcke & Lacey 1985; Pettersson 1994; Shitaka & Hirose 1998), and considering that in the Mediterranean regions, most plants flower during a few months, and the blooming is concentrated in spring (Mooney et al. 1974; Kummerow 1983; Bosch et al. 1997), an extended flowering season in Mediterranean environments appears to be an exception to the rule (Picó & Retana 2000). However, the fact that an extended flowering season is rare in Mediterranean environments does not mean that it is an ineffective flowering pattern for ensuring successful reproduction (Picó & Retana 2000). Bawa (1983) lists, as possible advantages of an extended flowering and fruiting period, the reduction of the risk of reproductive failure, the possibility of mating with more individuals of the population, a better control over relative investment in flowers and fruits, and the avoidance of seed predators.

Phenological adjustments to deal with the Mediterranean summer drought have been previously studied (Fotelli 2000; Kummerow et al. 1981; Kyparissis et al. 1997; Spano et al. 2003). Highly seasonal climates stimulate high within-population phenological synchrony (Montserrat-Martí et al. 2004). In this way, species from less seasonal climates or those evolved under more stable climates, should be more asynchronous. Instead, basic phenological issues such as phenological synchrony within populations or how suitable are different periods of the year for a wide array of phenophases, are poorly addressed (Milla et al. 2010). The level of overlap between phenophases seems to be relevant for the adaptation of plants to Mediterranean climate (Milla et al. 2010) and the high degree of flowering synchrony in the species also may be related with attracting pollinators or simply due to the fact that plants live in very homogeneous habitats as regards ecological conditions (Thompson 1980). Strong flowering synchrony implies that each plant can exchange genes with most plants of the population, increasing the genetic diversity of the same. In fact, in exogamous species with asynchronous flowering phenology, those individuals highly asynchronous with respect to the population mode show remarkable reduction in reproductive fitness (Augspurger 1981). In parallel to the effects of flowering moment and duration, flowering synchrony had a strong effect on the number of both viable and unviable seeds, so more synchronous plants are more successful in terms of seed production and, therefore, might be naturally selected.

The aims of this study were (1) describe flowering and fruiting phenology of *Anchusa littorea* (2) determine the seed output for this species, (3) investigate the ability to create a soil seed bank, and finally (4) to assess how synchronously different phenophases occur in this psammophilous endemic species.

Materials and methods

Study species and study site

A. littorea is a short-lived herb, with a small basal rosette, hispid-setose for dense and shorter hairs. Stems are decumbent or suberect, 4-15 cm long. Leaves are linear and present a repand-dentate margins. Each plant develops several cymes, with small tubular flowers. Corolla is light blue or white, rotate with rounded lobes. Mericarps are small, light grey-brown, with finely tuberculate surface. The flowering period is from March to May and fruiting season, overlapped, is from April to July (Valsecchi 1980; Bacchetta et al. 2008). The reproductive biology of this species is still poorly known, but nevertheless the main reproductive system seems to be autonomous self-pollination, although some insects' activity has been observed in the single known population (Bacchetta et al. 2008).

A. littorea is a narrow endemic species growing in ephemeral psammophilous plant communities constituted principally by therophytes [i.e. endemics *Linaria flava* (Poiret) Desf. subsp. *sardoa* (Sommier) Arrigoni, *Phleum sardoum* (Hackel) Hackel and *Silene nummica* Vals.] (Bacchetta et al. 2008; Fenu et al. submitted). *A. littorea* population consist of two metapopulations distant ca. 700 m, with an area of ca. 3800 and 50000 m^2 respectively.

The population area is located in the coastal dune systems of Is Arenas (SW Sardinia; Figure 1), which spread to ca. 5 Km inland, is one of the most important and well-preserved sand system in Sardinia. Geologically, the area mainly consists of Holocenic sandstones and Aeolian sands forms, which present irregular heights ranging from 10 to 80-90 m (Annino et al. 2000).

Available climatic data from the nearest weather station (Montevecchio, ca. 12 Km) indicates a typical Mediterranean annual pattern of temperature and precipitation with a long dry season (4-6 months).

Figure 1 - Area of distribution of *A. littorea* at "Is Arenas" (Arbus municipality, SW-Sardinia).

Data sampling

Over a four years (2008-2011) the *A. littorea* population was monthly monitored; specifically, data collection began in 2008, in the historical locality (Bacchetta et al. 2008) and then, in 2009, monitoring activities included the new discovered locality, considering them as a separate group of plant and representing two metapopulations on the basis of their geographical distribution (hereafter ANC1 and ANC2 for the historical and the new discovered metapopulations, respectively).

Data collection were carried out from February to July, around the 10th day of each month, by monitoring 25 permanent plots of 1 x 1 m randomly placed where the plant was found (12 and 13 plots for ANC1 and ANC2, respectively). Within the plots all plants were counted, marked with a wooden toothpick and measured; all new seedlings that appeared inside the plots were counted, mapped and measured. During each sampling was recorded vegetative (number of leaf fall, leaf flush and mature leaf) and reproductive traits (floral buds, anthesis flowers, immature and mature fruit). In 120 plant, randomly chosen, the corolla diameter of all flowers present (n=160) were measured using a digital calliper (ALPA IP65 topcal 150 PW).

Seed output

The average number of fruits per plant was determined as a ratio between the total number of fruits counted / the total number of plants monitored; in order to calculate seed output, each year in the peak season, 30 fruits were collected when ripe outside plots and seeds were also extracted. The average number of seeds per fruit was multiplied per the average number of fruits per reproductive plant, in order to get a prediction of reproductive capacity for plant and for the total population.

To analyze the soil seed bank, a total of 43 superficial sand samples (ca. 500 g of sediment) were taken tri-monthly near to the plots. The samples were taken to the laboratory and, when the sand was dry (after ca. 30 days), they were sieved and then, seed extracts were analyzed with a stereoscope.

Statistical analysis

Phenological patterns and trends of single phenophase were analyzed monthly to evaluate seasonal variations in flowering, fruiting and fruit-set. Percentage values were calculated for each vegetative and reproductive traits (Figure 2).

Pearson correlation analyses between number of leaves (representing a good predictor for the age and size class in *A. littorea*, see Fenu et al. submitted) and the corolla diameter was performed, in order to verify if there's correlation between plant size and flower size.

One-way analysis of variance (ANOVA) was carried out on fruit production between ANC1 and ANC2 and among years; subsequently, *post hoc* Tukey Honestly Significant Difference test (HDS) was conducted to asses significantly differences between groups and among years.

In order to describe phenological differences within metapopulation and similarity among metapopulations, a diversity (Shannon–Wiener diversity index) and similarity index (Morisita–Horn similarity index) was used, respectively. For each individual a grade combination was attributed and different analyses were performed separately for the vegetative and reproductive phases.

Each observation was then categorized on a dichotomous dependent variable with 0 (absence of the characteristic) or 1 (presence of the characteristic). For each group of phenological phases, plants were characterized by the combination of assessed grades. For example: a plant evaluated as 0-0-0 for phenological phases would show grade 0 for bud flower, grade 0 for anthesis flower and grade 0 for withered flowers (the grades totaling 100%).

Shannon–Wiener diversity index was used to estimate diversity of phenological behavior within metapopulations (Goulart et al. 2005). Higher values for this index indicate higher phenological diversity, meaning lower phenological synchrony. According to Magurran (1988), the index is calculated following this formula:

$$\mathbf{H' = -\Sigma}\ \boldsymbol{p_i}\ \mathbf{ln}\ (\boldsymbol{p_i})$$

Frequencies of different phenological states (given by the grade combination) were used instead of frequencies of different species in a community. Accordingly, p_i was considered as the proportion of individuals found in the ${}_i$th combined grade, with $p_i = n_i/N$; ni being the number of individuals showing the phenological status i, and N the total number of individuals in the population. Populations with greater numbers of phenological states show higher values of this index, and less phenological synchrony (Goulart et al. 2005).

Finally, similarity between ANC1 and ANC2 was evaluated monthly by the Morisita–Horn similarity index (Magurran 1988); it was used to estimate synchrony between ANC1 and ANC2 (Goulart et al. 2005), with higher values indicating higher phenological synchrony:

$$\mathbf{MH = [2\Sigma\ n_{\mathit{Ai}}n_{\mathit{Bi}})]/(da+db)N_AN_B}$$

where $da = (Pn_{Ai}\ 2)/N_A\ 2$, $db = (Pn_{Bi}\ 2)/N_B\ 2$, N_A is the total number of individuals in ANC1, N_B the total number of individuals in ANC2, n_{Ai} is the number of individuals in the *ith* combined grade in A, and n_{Bi} is the number of individuals in the *ith* combined grade in B.

Results

Phenological pattern

From a minimum of 184 to a maximum of 583 cumulative numbers of plants of *A. littorea* were annually monitored (2008=184; 2009=278; 2010=377; 2011=583). Begin leaf flushing of *A. littorea* corresponds to the beginning of the reproductive cycle (Figure 3) and leaves fall became pronounced with increasing of the dry season. Leaf fall peaked between June and August, when the majority of observed plants were dead (Figure 3-4).

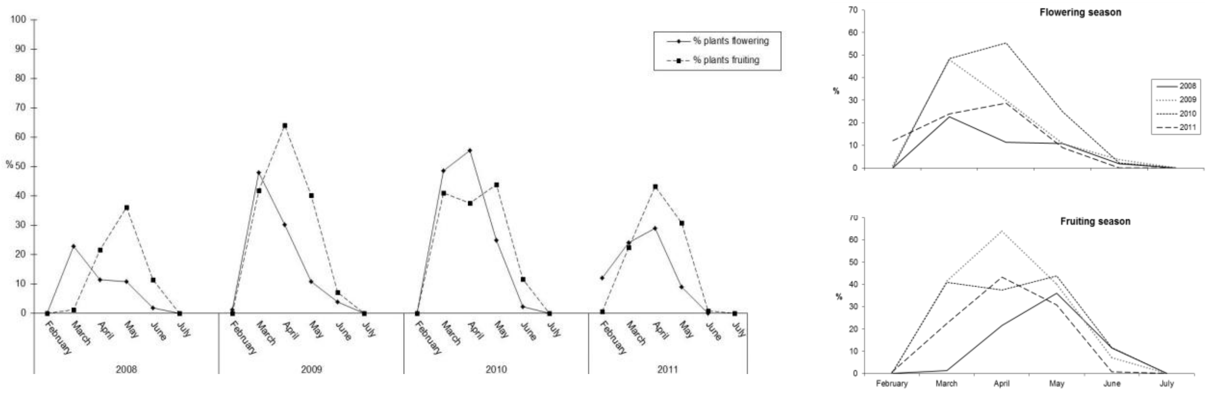

Figure 2 - Monthly patterns of flowering and fruiting seasons in *A. littorea* over four years (2008-2011).

The reproductive season of *A. littorea* starts in February/March and ends in last June, when all plant are dead; the mean flowering duration is 90.00±2.58 days, ranging from 87 to 93 days. The mean fruiting duration is 98.75±17.02 days, with a range from 87 to 124 days (Figure 2).

Flowering and fruiting time exhibited almost a complete overlap. The flowering peak was observed both in March (2008 and 2009) and in April (2010 and 2011), with 22.75, 47.96, 55.35 and 28.78 % of flowered plants for 2008, 2009, 2010 and 2011, respectively (Figure 2). The fruiting peak was recorded between April and May, with 35.93, 63.94, 43.85 and 43.17% of fruiting plants for 2008, 2009, 2010 and 2011, respectively (Figure 2).

The mean value of corolla diameter was 5.54±1.00 mm; the number of leaves per plant (and, consequently, plant size) show a statistically significant negative correlation with the corolla diameter (linear regression: number of leaves = 20.0941-1.372 x corolla diameter; $r^2 = 0.036$; r = -0.19; *p-value* < 0.05; Figure 5).

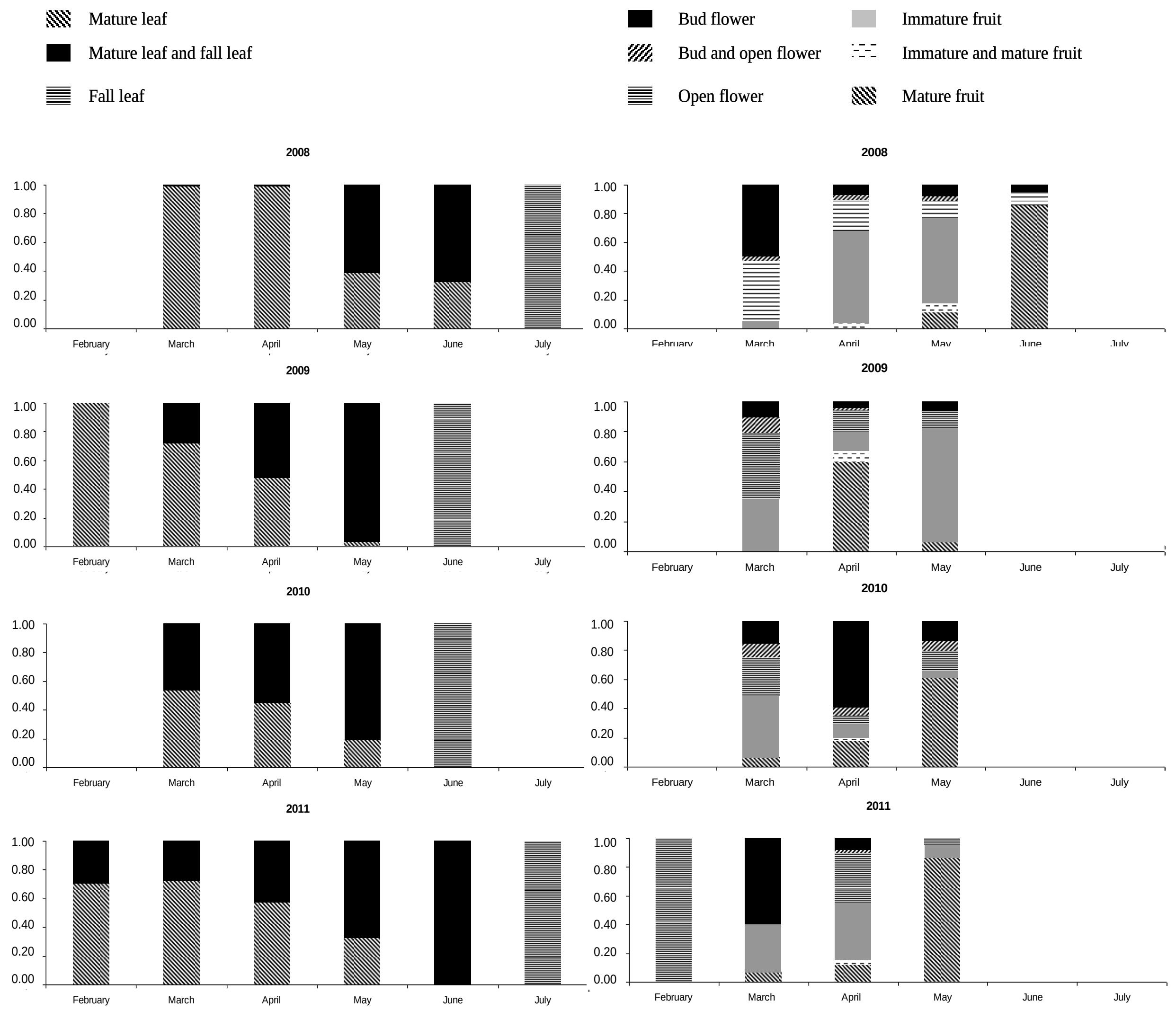

Figure 3 - Monthly percentages of vegetative and reproductive phenologies found in individuals of *A. littorea* from ANC1. Note that for reproductive data, in each month, only individuals that showed reproductive characteristics were considered.

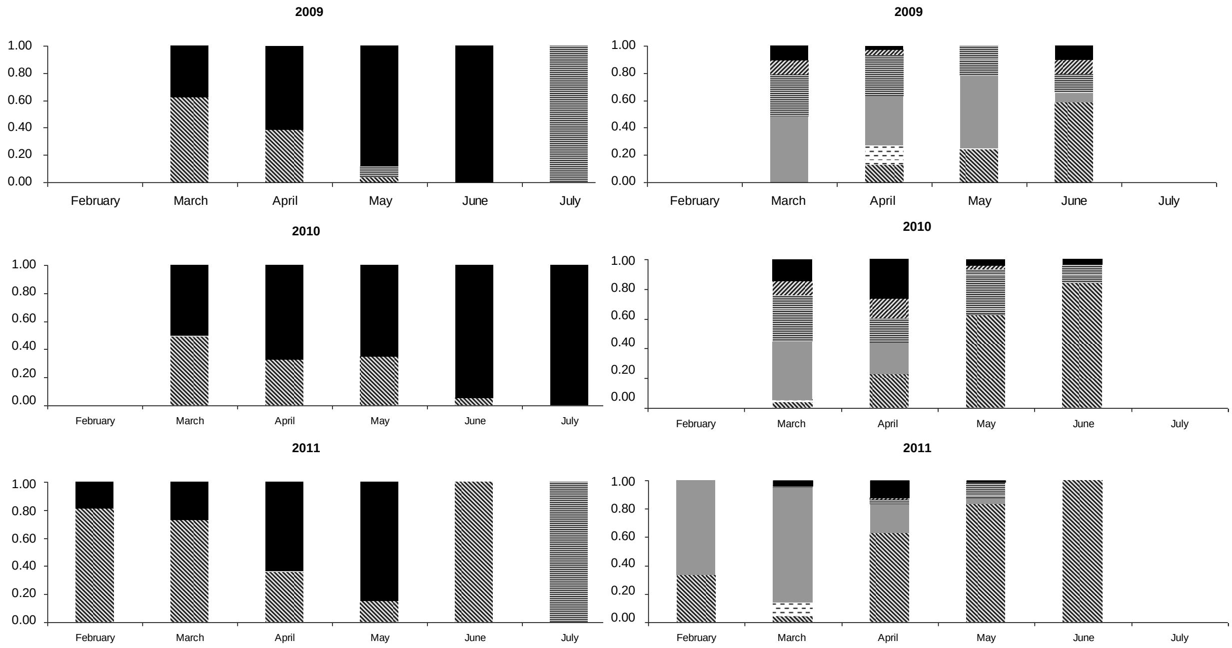

Figure 4 - Monthly percentages of vegetative and reproductive phenologies found in individuals of *A. littorea* from ANC2. Note that for reproductive data, in each month, only individuals that showed reproductive characteristics were considered.

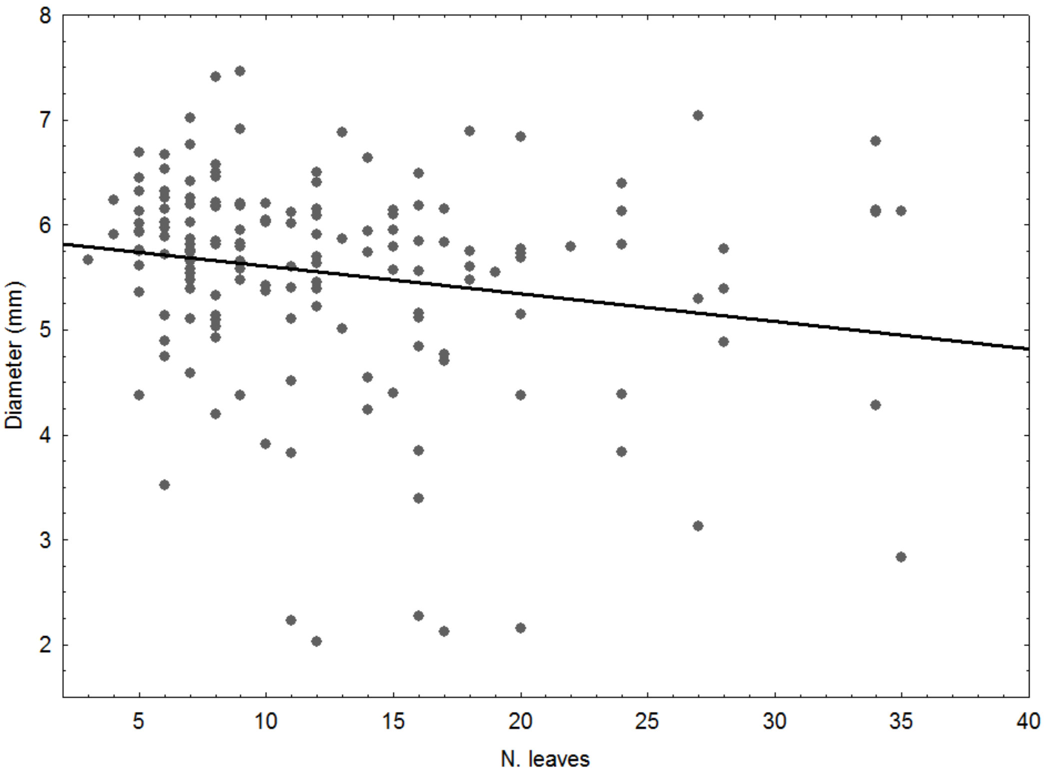

Figure 5 - Negative correlation between corolla diameter and plant size (represented by number of leaves, see Fenu et al. submitted). No. leaves = 20.0941-1.372* corolla diameter; $r^2 = 0.036$; r = -0.19; *p-value* < 0.05.

Seed output

Mean number of fruits produced per plant showed statistically significant variation between ANC1 and ANC2, with mean values of 1.74±0.81 and 2.78±1.54 fruits per plant for ANC1 and ANC2, respectively ($P<0.001$ by one-way ANOVA followed by *post hoc* Tukey HSD test).

Differences in fruits per plant among years were observed between 2008-2010, 2009-2010 and 2010-2011 ($P<0.001$ by one-way ANOVA followed by *post hoc* Tukey HSD test), while the test did not shown any statistical differences between 2008-2009, 2008-2011 and 2009-2011.

The cumulative number of fruits in the overall population of *A. littorea* oscillating from 63 (2008) to 963 (2010) and the lowest mean of fruits per reproductive plant is found in 2008 (2.00±1.15) (Table 1). ANC1 exhibited minimum and maximum number of fruits per plant in 2011 and 2010, respectively, but the highest mean of fruits per plant occurred in 2009 (2.27±1.18). ANC2 showed lowest number of fruits per plant in 2009 and highest one in 2010; the lowest mean values of fruits per reproductive was in 2011 (2.24±1.65; Table 1).

Table 1 - Seed output in the *A. littorea* population; the fruit and seed output for ANC1 and ANC2 over the study period were also reported.

	Year	No. fruits counted	No. seeds estimate	Mean fruits (±sd) per reproductive plant	Mean seeds per reproductive plant
POPULATION	2008	63	252	2.00±1.15	6.00
	2009	703	2812	2.52±1.74	7.56
	2010	963	3852	3.85±3.46	11.55
	2011	710	2840	2.10±1.57	6.30
overall		2439	9756		
ANC1	2008	63	189	2.00±1.15	6.00
	2009	103	309	2.27±1.18	6.81
	2010	131	393	2.15±1.22	6.45
	2011	52	156	1.43±0.75	4.29
overall		349	1047		
ANC2	2008				
	2009	600	1800	2.65±1.92	7.95
	2010	832	2496	4.38±3.75	13.14
	2011	658	1974	2.24±1.65	6.72
overall		2090	6270		

In each fruit, one of the four total seeds present is always abortive. Number of seeds followed the same trend of the number of fruits both for ANC1 and ANC2. In the total population, the number of seeds varied from 252 (2008) with values of seeds per plant of 6.00, to 852 (2010) with mean values per plant of 11.55 (Table 1).

The analysis of the soil seed bank showed an extremely low number of seeds per sample (2.83×10^{-4} seeds per cm^3) with a mean number of 0.5±0.5 seeds per sample. Analysis with stereoscope showed that all seeds retrieved into the sand sampled were empty.

Synchrony between metapopulations

Floral buds and anthesis flowers are observed in March at the same time. After flowering peak, immature fruits were observed in ANC1 and ANC2. Mature fruits are observed immediately after flowering peak and June-July was considered the end of the seed dispersal season.

ANC1 and ANC2 showed similar monthly diversity levels for flowering and similar levels of monthly diversity were found for fruiting (Table 2).

Within metapopulations (Table 2), reproductive monthly phases, from February to June, was marked by intermediate levels of diversity with H' index flowering ranging from 0.04 (February 2011) to 1.21 (March 2010) for ANC1 and from 0.00 (June 2011) to 1.31 (April 2010) for ANC2. H' index fruiting ranging from 0.00 (February 2011) to 1.36 (April 2009) for ANC1 and from 0.04 (February 2011) to 1.57 (May 2009) for ANC2.

Table 2 - Shannon–Wiener diversity index reproductive phenology of *A. littorea* individuals within ANC1 and ANC2 respectively (data collected from February 2008 to June 2011).

Year	Month	H' flowering		H' fruiting	
		ANC1	ANC2	ANC1	ANC2
2008	February	-	-	-	-
	March	0.714	-	0.065	-
	April	0.469	-	0.603	-
	May	0.630	-	1.055	-
	June	0.330	-	1.016	-
2009	February	-	-	-	-
	March	0.883	1.215	0.406	0.692
	April	0.762	0.855	1.362	1.308
	May	0.409	0.733	1.021	1.577
	June	-	1.265	-	0.753
2010	February	-	-	-	-
	March	1.218	1.200	1.081	0.884
	April	0.995	1.310	0.945	1.289
	May	0.996	1.099	1.329	1.354
	June	-	0.519	-	0.972
2011	February	0.048	0.582	0.000	0.048
	March	0.253	0.940	0.209	0.748
	April	0.403	1.274	1.114	1.561
	May	0.110	0.792	1.217	1.322
	June	-	0.000	-	0.462

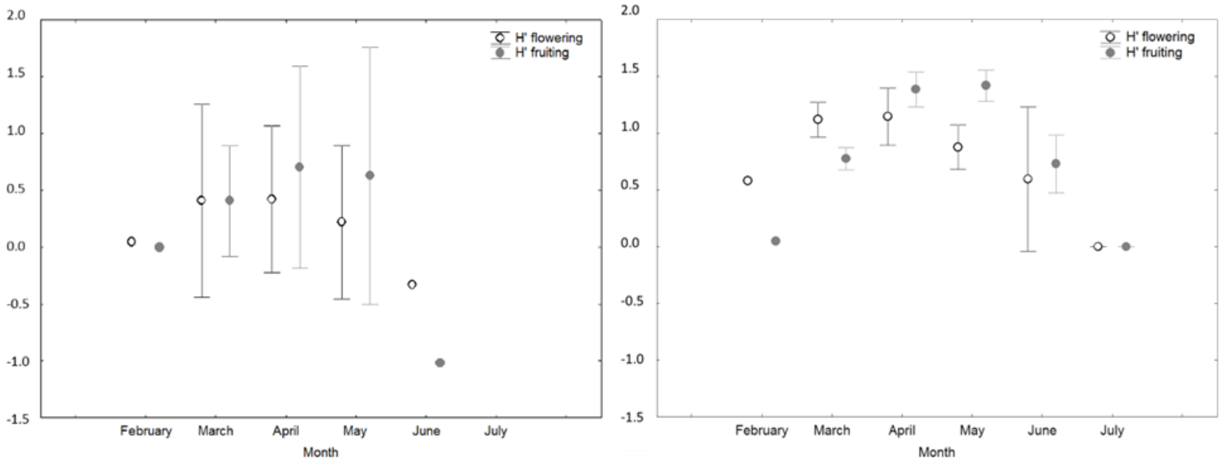

Figure 6 - Monthly variation in the mean of phenological index of diversity within metapopulations (Shannon–Wiener index) of *A. littorea* for ANC1 and ANC2, respectively. Bars indicate ± s.d.

Diversity index, within years, was marked by similar monthly trend in flowering and fruiting season, both for ANC1 and ANC2. For flowering, the highest and lowest levels of diversity were observed in first and last months of the year, while for fruiting was observed the opposite trend. In 2009 H’ highest value is shown in the last month (Table 2; Figure 6).

A similarity between ANC1 and ANC2 was found (Table 3). Years exhibited similar values of similarity in reproductive phenology with a trend showing the maximum values of similarity in the last months of the year (Table 3). Index didn’t show great monthly variation in each year with high levels of similarity (values > 0.5). Values ranged from 0.83 (April 2010) from 1.00 (June 2011) and from 0.50 (April 2009) to 1.00 (February 2011) for flowering and fruiting, respectively; April 2009 showed the lowest values of similarity among years (Table 3; Figure 7).

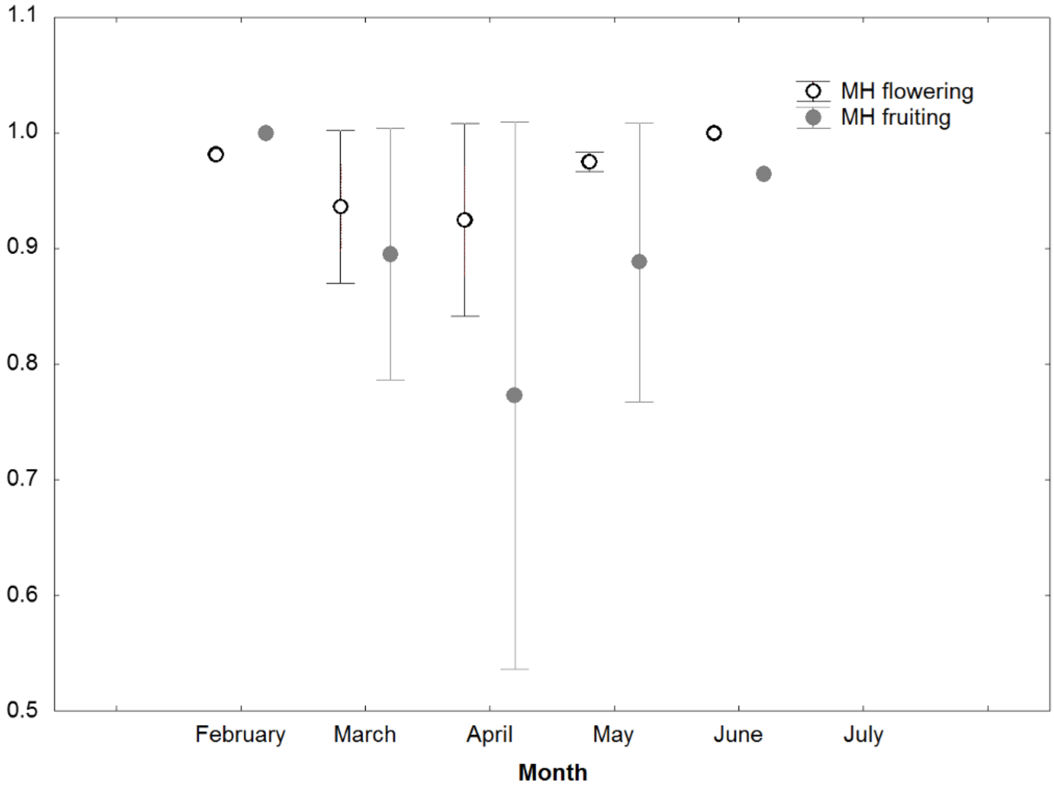

Figure 7 - Monthly variation in the mean of phenological index of similarity between ANC1 and ANC2 (Morisita–Horn index) of *A. littorea*. Bars indicate ± s.d.

Table 3 - Morisita–Horn index similarity index (MH) for reproductive phenology of *A. littorea* between ANC1 and ANC2 (data collected from February 2009 to June 2011).

Year	Month	MH flowering	MH fruiting
2009	February	-	-
	March	0.865	0.771
	April	0.951	0.507
	May	0.983	0.750
	June	-	-
2010	February		
	March	0.994	0.976
	April	0.832	0.962
	May	0.966	0.974
	June	-	-
2011	February	0.981	1.000
	March	0.950	0.939
	April	0.992	0.850
	May	0.976	0.940
	June	1.000	0.965

Discussion

Opportunistic and fixed phonological responses may represent contrasting strategies for optimizing fitness in temporally varying environments; while both strategies can be very important for ensuring reproductive success, these results suggest that local adaptation to temporal resource variation may reflect a balance between flexible and inflexible phenological responses such that highly constrained populations may lack the ability to take advantage of seasonal resource pulses (Dyer et al. in press). For annual plants, one consequence of selection in seasonally variable environments is that opportunistic growth is a strategy for ensuring reproductive success (Dyer et al. in press). In our study, A. littorea show an opportunistic responses depends on the relative variability of resources such as Mediterranean coastal dune.

Long term field phenological monitoring demonstrate that *A. littorea* must be considered a short-lived herb, with an annual cycle (5-6 months), which starts in February and finish in June (rarely in July), depending on the data of seedling emergence, as reported in previous observations (Bacchetta et al. 2008; Fenu et al. submitted). Like for other annual endemic species, such as *Linaria flava* and *Phleum sardoum*, which grow in the same coastal sandy habitat, the vegetative season begins in the late winter-early spring (February–March), with new seedlings which could perhaps appearance from seeds germinating in the previous years considering the lacking of the persistent soil seed bank (PSB *sensu* Thompson et al. 1993) detected in this study. Despite the vegetative period was similar between ANC1 and ANC2 of *A. littorea,* the beginning of leaf fall represented the main differences: in ANC1, plants started to be fall earlier and had a life cycle shorter than in ANC2.

A. littorea presented a flowering and fruiting season totally overlapped and it showed a long reproductive period lasting 4 to 5 months: it began in late winter and finished in early summer, with an optimum ranging from March to April for flowering peak and from June to July for fruiting ones. The similar phenological trends reported for *A. littorea* are consistent with previous field observations carried out on other coastal psammophilous *taxa* of this genus in Sardinia and Corsica [*A. crispa* Viv. ssp. *crispa, A. crispa* Viv. ssp. *maritima* (Vals.) Selvi & Bigazzi and *A. sardoa* (Illario) Selvi & Bigazzi; Bacchetta et al. 2008]. This pattern may should be considered as an adaptive advantage in stressed habitats, where there is high ecological variability in time and space (high temporal unpredictability, particularly in the amount and seasonal pattern of precipitation, alternating dry and rainy years; Braza & Garcìa 2011), like Mediterranean coastal dune, and where water stress would restrict the reproductive success of later flowering plants (Johnson 1992; Copete et al. 2008). In fact, for annual plants, opportunistic phenological responses optimize fitness in habitats with temporally and/or spatially variable resource availability (Alpert & Simms 2002; Dyer et al. in press). Several phenological traits, such as the length of the flowering season can be limited by unfavorable weather conditions (de Jong et al. 1992; Boaz et al. 1994; Rose et al. 1998). Plant species with extended flowering seasons can be less seriously affected by the effects of this environmental unpredictability than species with concentrated flowering seasons (Picó & Retana 2000). In fact, environmental factors such as light and water availability can affect fruit production, both directly because of their effects on resources available for fruit maturation, and indirectly because of their effects on flower production and on abundance of pollinators and seed predators (Ågren et al. 2008). A demonstration of this, the pattern of fructification in 2010 shows a double fruiting peak probably relating to climatic fluctuations. The extended blooming period can increase the individual's chance of having a large number of mates both as pollen donor and recipient (Torres et al. 2002) and it reduces the risk of reproductive failure (Bawa, 1973*)*. Also, for other species the same phenological pattern was been predictable and it has been suggested that it may be an adaptive response to attract pollinators that usually visit other species (Thompson 1980; Torres et al. 2002).

Smaller flowers have been shown to be associated with greater plants, suggesting that an increasing in plant size determine a lowest investment in reproductive structure production for this species. Increased in size components in *A. littorea*, such as number of leaves, is clearly related to sand burial (Fenu at al. submitted). This is an important factor for the reproductive biology of *Boraginaceae*, one of the numerous entomophilous angiosperm groups: in several species of *Anchusa* L., the genera *Apis, Halictus, Tetralonia* are the main pollinators, but Lepidoptera, such as *Maniola* and *Lasiommata*, and long-tongued Diptera, such as *Bombylius,* have also been reported as frequent visitors (Bacchetta et al. 2008). Flower size, as well as other flower and inflorescence traits, are important components of pollinator foraging choice but may also be cues for attracting enemies (Ehrlén et al. 2002; Cariveau et al. 2004; Ashman & Penet 2007). However, at date, no evidence for a clear correlation between floral morphology and pollen vectors has been found for this group (Nepi et al. 2010).

Number of seeds per plant showed high annual variability, with years in which low seed production is recorded, as detected for several annual plants (Meyer et al. 2006). ANC2 produces a greater number of seeds per plant than ANC1 and this may be related to the amount of plants in each group. Indeed, a negative relationship has been reported between a narrow population size (or a low population density) and seed production, with the latter being reduced in small, isolated populations because of both increased inbreeding and reduced number of compatible mates

(Campbell & Husband 2007; Vergeer et al. 2003; Jacquemyn et al. 2002). The presence of aborted seed per fruit should be explained with local adverse climatic conditions especially for endemic. This reproductive losses affected mostly endemic species and the populations located near the sea due to local adverse climatic conditions (Boieiro et al. 2010).

The results of this study suggest that *A. littorea* does not create a PSB. Although the mechanisms which form a PSB are well-known, these have been developed mainly for species of agronomic interest (Ellstrand & Elam 1993; Bakker et al. 1996), while there are few studies on rare or endangered species. The value of soil seed bank detected in *A. littorea* is poor when compared to values obtained for other annual plant (500 seeds m^2; Bakker et al. 1996). The value of the seeds density in the soil was similar to values recorded for a congeneric *A. crispa* in Corsica (Quilichini & Debussche 2000). However, the 31% of *A. crispa* seeds were viable while all seeds of *A. littorea* empty or dead. These results are discordant with previous studies indicating that in coastal Mediterranean semi-arid ecosystems, and especially on sand dunes, the annual plant have a PSB (Yu et al. 2003, 2008).

Moreover, considering that a short-lived plant species in such an unpredictable environment is likely to depend on a long-lived seed bank for population persistence (Brown & Venable 1986; Meyer et al. 2006), the lack of a soil seed bank for *A. littorea* could pose a strong threat to the population persistence. This explanation is supported by the fact that the long-lived seed bank was essential to population persistence in a stochastically varying environment, but even seed bank persistence could not prevent extinction under the scenario of no year-to-year variation (Meyer et al. 2006). Moreover, the lack of a long-term PSB could have important implications for the population dynamics of an annual species, even if the effect is not easy to detect immediately; indeed, soil seed bank reduces the risk of extinction in particularly bad years (i.e. Quintana-Ascencio et al. 2003; Houlè et al. 2001) and increases the effective population size, as detected both in perennial (Dolan et al. 2008) and in annual plants (Nunney 2002).

In the dry season (in months when only flowers withered were present), plants showed no diversity within population in flowering, and hence similarity reached its maximum value. In fact, the differences among individuals are in relation with life reproductive cycle, by which in first months of year, plants have both flower buds, anthesis flowers, withered flowers and, in some case, also immature fruits; this pattern decreases with the end of the cycle, meaning it was the most synchronous period.

Fructification presents an opposite trend because plants at the beginning of the life cycle showed only immature fruits which become mature fruits and fruits with dispersal seeds at the end of the reproductive season.

Reproductive synchrony was high between ANC1 and ANC2. Rathcke and Lacey (1985) suggested that there should be some advantages in partial synchrony of flowering among individuals and populations, as it promotes cross-pollination among distant individuals, thus enhancing genetic diversity, and it also helps to avoid competition for pollinators. The high degree of flowering and fruiting synchrony in the species also may be related with attracting pollinators or simply due to the fact that plants live in very homogeneous habitats as regards ecological conditions (Thompson 1980). This could be an advantage for small population because strong flowering synchrony implies that each plant can exchange genes with most plants of the population, increasing the genetic diversity of the same (Martínez et al. 2011).

However, in some years of our study, has been highlighted a strong asynchronous in last months, both in fruiting and in flowering variables. An explanation could be that in the last months of sampling there was a low number of plants and these had, to each other, different phenological stages. Seghieri and Simier (2002) observed greater individual variability in phenology under less favourable condition, as can be seen the end of life cycle which correspond to the dry season. These authors hypothesized that asynchrony among individuals may reflect population flexibility, as the adaptation to a variety of conditions should contribute to population maintenance and expansion, certainly a hard especially in a stressful environment (Goulart et al. 2005), like sand coastal dune. However, a number of adaptive interpretations have been given for flowering asynchrony, for example; as an evolutionary response to intra-specific competition for pollinators; for promoting inter-plant pollinator movement; the advantage of increasing mate availability; for dispersion of seed predators; variation in intensity and timing of seed predation and dispersal; or differential selection in different years depending upon environmental factors (Zimmerman 1980; Bawa 1983; Primack 1985; Rathcke & Lacey 1985). It is possible that within-population flowering asynchrony is not determined by extrinsic factors, instead, it may be largely due to intrinsic factors such as relaxed selection on natural genetic variability and/or environmental heterogeneity (Ollerton & Lack 1992). More research is required to unravel the underlying causes influencing this phenological feature More research is required to unravel the underlying causes influencing this phenological feature.

The discovery of ANC2 in a site where this species was not found in previous years, suggested that *A. littorea* population might be adapted to large shift in the space, inhabiting similar habitats. Our results highlighted that a significant migration have occurred for *A. littorea* population. Moreover, we found that mean number of fruits per plant is highest in ANC2 than in ANC1 (2.77 and 1.74, respectively) and this could indicated that the “population core” (meaning the point with the maximum productivity and vitality) has moved in the space from ANC1, the only one presented in the area until 2008, to ANC2. This also suggested that this temporal and spatial process could determine that the “population core” might move inside the dune system over the years. In conclusion, this study provides new data in terms of phenological pattern and seed output, improving our knowledge on reproductive ecology of these endemic species.

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CHAPTER 6

LIFE HISTORY AND DEMOGRAPHIC FEATURES OF *DIANTHUS MORISIANUS* VALS., A THREATENED COASTAL SPECIES

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Introduction

To efficiently manage of the rare and threatened species is important to understand their population dynamics and identify the current threats acting on them (Oostermeijer et al. 1996). Long-term demographic surveys are needed to obtain accurate information on species life-history and identify biotic or abiotic factors that affect population dynamics (Fieberg & Ellner 2001; Fréville et al. 2004; Pfeifer et al. 2006). However, for rare and endangered species, the number of long-term studies, especially on plants, is still low while crucially needed for their conservation (Fréville et al. 2004; Jacquemyn et al. 2007).

In Sardinia about a hundred of endemic species have been recognized as threatened (Conti et al. 1992, 1997) and five of these have been included by the IUCN/SSC in the “Top 50 Mediterranean Island Plants” to be urgently conserved (Montmollin de & Strahm 2005). Despite this rich biodiversity and the threats to these species, few biological conservation studies have been carried out on threatened endemic species of Sardinia (Fenu & Mattana 2011).

Dianthus L. is one of the most diverse plant genus in Europe, characterized by a large numbers of endemic species (more than 70 *taxa*) with restricted geographically or ecologically ranges, suggesting that diversity has originated only recently (Valente et al. 2010). Within this genus, the *D. sylvestris* Wulfen group can be considered as one of the most complex, and it is still not well investigated (Bacchetta et al. 2010). In Sardinia 8 endemic *Dianthus* have been recorded, 4 of them narrow distributed; among these *Dianthus morisianus* Vals. has been considered one of the most threatened plants of the island (Bacchetta et al. 2012).

D. morisianus occurs in only one population and it is the only psammophilous species belonging to this group; it grows in a high specialized habitat such as the coastal dune of SW Sardinia (Valsecchi 1985; Bacchetta et al. 2010; Fenu et al. 2010), in a area highly fragmented.

Because of the destruction and fragmentation of habitats, many species today occur mainly in small and isolated populations, which for a number of reasons are expected to face a high risk of extinction (Matthies et al. 2004). In small patches habitat quality may deteriorate (Oostermeijer et al. 1994) and modeling studies suggest that small populations will be particularly vulnerable to the effects of demographic, environmental and genetic stochasticity (Goodman 1987; Menges 1991). While demographic stochasticity is only a threat to very small populations, environmental stochasticity has been identified as the most important factor threatening extinction to fragmented populations (Lande 1993; Menges 1998; Holsinger 2000). Fragmented landscapes influence movement and dispersal of organisms, rates of gene flow, and invasion by exotic competitors, among many other factors (Heywood & Iriondo 2003).

Habitat fragmentation by human has been altering Mediterranean habitats for several thousand of years and it may be an important factor influencing spatial population structure in Mediterranean plant species (Thompson 1999). The negative effects of fragmentation on reproduction and on the performance of offspring should affect the dynamics and survival of populations of short-lived species relatively quickly, because population persistence depends on frequent recruitment (Matthies et al. 2004). In contrast, in long-lived plants the negative consequences of reduced population size and increased isolation may not become visible for a long time, because established plants often have low mortality (Oostermeijer et al. 1994b; Colling et al. 2002).

With respect to the conservation of biodiversity the most important question is what combined effect the various negative effects of reduced population size have on the persistence of populations (Matthies et al. 2004). It has been suggested that populations reduced below a certain threshold number of individuals may enter a so called extinction vortex, i.e. a downward spiral of ever decreasing population size and plant fitness that may drive a population to extinction (Gilpin & Soulé 1986; Lamont et al. 1993). However, while there is some direct empirical evidence from animal studies for the negative effects of small population size on the survival of local populations (Berger 1990), little such evidence exists for plants (Matthies et al. 2004). Empirical studies require long-term data on the dynamics and survival of populations which are rarely available for plants (Matthies et al. 2004). Previous studies have therefore used substitutes for population size like site area (Ouborg 1993) or mean cover (Fischer & Stöcklin 1997). Furthermore, the precise evaluation of the conservation status of a particular species is a necessary condition in order to successfully prevent its extinction (Vischi et al. 2004). An important tool for this purpose is the determination of the degree of threat (or alternatively the expectation of survival) of *taxa* to which a special significance is attributed. Endemic species seem to be, prima facie, more exposed to threats, and therefore the biological features of endemic *taxa* have been the subject of preferential attention by conservationists (Vischi et al. 2004). One way of evaluating the degree of risk of a given *taxon* is to assign it to a standardized category of threat.

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 is the categories and criteria of the IUCN Red List of Threatened Species (IUCN 2001; Grammont de & Cuarón 2006; Rodrigues et al. 2006; Hoffman et al. 2008)

In this work, an analysis of the distribution, population sizes and threats faced of *D. morisianus* in southwestern Sardinia was undertaken. In particular, the aims of this work were: (1) to establish the current area of distribution (2) to evaluate the size and structure of the population, and (3) to determine the status of *D. morisianus* following the IUCN methodology (2001).

Materials and methods

Study species

D. morisianus is a perennial suffrutex 30-50(60) cm tall, characterized by numerous woody stocks loosely branched with branches. The fruit is a cylindrical capsule included in the calyx and they have small and flat seeds which can germinate with high percentages in a wide range of conditions, with a maximum germination rate at 15°C (Cogoni et al. 2012). The flowering season lasts from early May to last June, whereas ripe fruits can be found in June and July (Fenu et al. 2010).

Vegetative sprouting, probably related to woody stocks loosely branched (Bacchetta et al. 2010), has been observed in the natural population, thus field analysis was conducted at ramet level, considering ramet each “visual unit” clearly separated by others (Garcia et al. 2002). *D. morisianus* is a psammophilous species which grows on stabilized dunes in contact with micro-forests of *Juniperus* spp. [*J. macrocarpa* Sibth. and *J. phoenicea* L. subsp. *turbinata* (Guss.) Nyman] and *Quercus calliprinos* Webb. The only natural population of *D. morisianus* is located in Portixeddu (Buggerru, SW Sardinia; Bacchetta et al. 2010; Fenu et al. 2010). Available climate data (from Fluminimaggiore weather station at 45 m a.s.l.) indicates a typical Mediterranean seasonal pattern of temperature and precipitation, with a long dry summer (Cogoni et al. 2012).

D. morisianus is listed in the National Red List as endangered (Conti et al. 1992) and in Regional Red List as vulnerable (Conti et al. 1997); more recently for this species has been proposed the Critically Endangered IUCN category (Fenu et al. 2010) and for this reason it has been inserted in the Global Red List (Fenu et al. 2011) and in the European threatened plant list (Bilz et al. 2011).

Field monitoring

The wider area of only one known population of *D. morisianus* was surveyed; detailed mapping was done using a GPS device and occasionally a tape measure, once a year during the peak of the flowering season of the species. The geographical limits of localities were analysed and areas estimated, using Quantum GIS Version 1.7.3 (QGIS 2011), to detect any annual changes in area occupied. For species locality, altitudinal range, slope, aspect lithology, substratum In addition habitat type according to the European Habitat Directive (DIR 92/43/EEC) was reported following the Italian Interpretation Manual (Biondi et al. 2009).

The threats to *D. morisianus* were determined from field observations and categorized following the IUCN threats classification scheme (www.iucnredlist.org/technical-documents/classification-schemes/threats-classification-scheme-ver3).

The study was carried out over three years (2009-2011). Thirteen permanent plots of 1 x 1 m were established where the plant was found and the population were monitored on a monthly basis from January to July during the same time (around the 20th of each month). The corners of the plots were marked by metal tubes (30 cm height) driven into the sandy soil so that plots could be relocated later. Within the plots all ramets were counted, marked with a wooden stakes and measured monthly in order to analyse their morphological size and reproductive status. The height, the maximum and the minimum diameter of the basal rosette and the height of reproductive stem were measured on each ramet using a digital calliper (ALPA IP65 Topcal 150 PW). For each ramet the number of reproductive stems bearing flowers, as well as the number of fruit were also counted. During each sampling, number of flowers (bud, anthesis and withered flowers) and fruits (immature and mature fruits and fruits with dispersed seeds) were counted. The reproductive capacity (reproduction) per ramet was obtained by counting the number of flowers and fruits per ramet. Survival was recorded every month and a ramet was considered to have survived the following month if still present with at least one remaining fresh leaf.

Data analysis

Monthly values of ramet densities from January to July (the vegetative period of the plant) were calculated, and annual values of ramet densities were plotted on charts for June, which represents the month with maximum values of ramets growth (in term of volume). Annual density was tested by non-parametric Kruskal-Wallis test. Exploratory data analyses were first carried out in the form of a boxplots to examine the differences in the distribution of density monthly and yearly.

In order to select size variable which will be used for population structure of *D. morisianus*, morphological and reproductive parameters were analysed in June. In addition reproduction value, defined as the sum of flowers and fruits counted in the same surveys, was considered. In order to verify whether a single variable was a good predictor describing the plant size, we calculated the Pearson correlation among all variables (see appendices). The Pearson correlation coefficients between categorized measures, based on a sample of 195 ramets showed a significant correlation (p-value< 0.001), except "No. flowers" that showed no significant correlation (p-value> 0.05; Table 1). Then, among the groups of variables, "ramet volume" was selected, because this parameter can be detected more easily without causing damage to the mapped individuals. Ramet volume (V_r) was calculated using parameter "basal rosette height" [h_i (cm)], and the parameter "maximum and minimum diameter" [d_M and d_m (cm)], according to the following formula:

$V_r = [\pi*(d_M/2)*(d_m/2)] *h_i$ [cm^3]

Table 1 - Pearson correlation value of the volume (in June) with morphological and reproductive variables (N = 195 ramets). [Abbreviations: H.=heigh; D.Max= maximum diameter; D.Min= minimum diameter; H.S= Stem height; Fl.=No. flowers; Fr.=No. fruits; Re=No. reproductive structures (flowers and fruits)].

		H.	D. Max	D. Min	H.S	Fl.	Fr.	Re.
No. leaves	***r***	0.777	0.732	0.569	0.265	-0.050	0.288	0.209
	t	17.153	14.934	9.618	3.821	-0.701	4.172	2.968
	p-value	0.000	0.000	0.000	0.000	0.484	0.000	0.003

Considering that flowering and fruiting are overlapped (see chapter 5), the ramet volume was correlated with reproduction values, defined as the sum of flowers and fruits counted in the same surveys, in order to define the size classes for *D. morisianus* population. Based on the ramet volume, three size classes were considered: class 1 (ramets from 0 to 75 cm^3), class 2 (ramets from 75 to 500 cm^3) and class 3 (ramets with more than 500 cm^3).

Population structure was displayed in categorized histograms and it was investigated over time. The variation in ramet sizes categories within population was expressed using the Gini coefficient (Weiner & Solbrig 1984). The Gini coefficient has a minimum of zero and a theoretical maximum of one. If all ramets in a population are of the same size, the coefficient is at the minimum. An increasing coefficient indicates increasing size inequality, which might be used as an indicator of competition effects. The Gini coefficient was computed as Dixon et al. (1987):

$$G = \frac{1}{2\bar{x}n\,(n-1)} \sum_{i=1}^{n} (2i - n - 1)\, x_i$$

Transitions probability was calculated, in order to investigate the yearly vital rates (in particular stasis, growth, mortality and retrogression). Transition probabilities were calculated as the number of ramets developing from stage *i* to stage *j* in 1 year divided by number of ramets in stage *i* in the previous year (Schleuning & Matthies 2009). All these analyses were performed with Statistica 8.0 (StatSoft, Inc, Tulsa, Oklahoma, USA) software.

Conservation status assessment

A grid of 2 x 2 km was used for assessing 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; IUCN 2001), according the National protocol adopted in Italy (Gargano 2011). EOO was assessed following the IUCN guidelines (2011). The conservation status was assessed following the IUCN criteria (2001).

Results

Population ecology

The only one population covering ca. 17.5 ha and it consists of two separated areas with different sizes (Figure 1). The main area covers ca. 17.00 ha while the second one, located at ca 250 m as the crow flies, occupied a surface of ca. 0.4 ha (Figure 1).

D. morisianus grows exclusively on stabilized dunal fields at altitudes above 10-55 m a.s.l. The vegetation covers showed an average of 45% (ranging from 30 to 80%) with an height of plant community ranging from 20 to 80 cm. The plant community occurring on low angle slopes and in a north-western aspect. The plant community is found in inner dunes, partially stabilized by forest vegetation, but subject to a constant sand burial. The more frequent *taxa* recorded with *D. morisianus* were *Cistus salviifolius* L., *Cistus creticus* L. subsp. *eriocephalus* (Viv.) Greuter *et* Burdet, *Osyris alba* L., *Silene beguinotii* Vals. *Lavandula stoechas* L. subsp. *stoechas, Juniperus macrocarpa* Sibth. and *Juniperus phoenicea* L. subsp. *turbinata* (Guss.) Nyman.

Figure 1 - *D. morisianus* at "Portixeddu" locality (Buggerru, SW-Sardinia).

Population size, expressed as the total number of mature ramet in the population monitored, apparently didn't exhibit annual fluctuation and the total local extent did not change (Table 2). However many years of monitoring are need to better understaind the present of fluctuation in this population.Annual density increased from 2009 to 2010, whereas in 2011 it showed similar average values with 2010 (Figure 2). However, no statistically significant differences was found among years (*p-value* > 0.05 by Kruskal-Wallis test).

Based on density values calculated in these years, the size population should be estimated in ca. 1,197,380 ramets (2009) and it increases at ca. 2,111,600 ramets in 2011.

Table 2 - Total ramets inside plots and monthly density

	No. ramets	Density (ramets m^{-2})
March 2009	47	6.71 ± 3.77
April 2009	183	14.00 ± 7.02
May 2009	183	14.00 ± 6.65
June 2009	89	6.85 ± 4.72
July 2009	47	3.62 ± 3.15
January 2010	178	13.69 ± 7.77
February 2010	174	13.38 ± 7.68
March 2010	167	12.85 ± 7.36
April 2010	166	12.77 ± 7.35
May 2010	161	12.38 ± 7.50
June 2010	156	12.00 ± 7.07
July 2010	83	6.38 ± 5.35
January 2011	178	13.69 ± 9.5
February 2011	178	13.69 ± 9.45
March 2011	203	15.62 ± 11.27
April 2011	199	15.38 ± 10.81
May 2011	191	14.69 ± 11.13
June 2011	157	12.08 ± 8.98
July 2011	93	7.15 ± 6.76

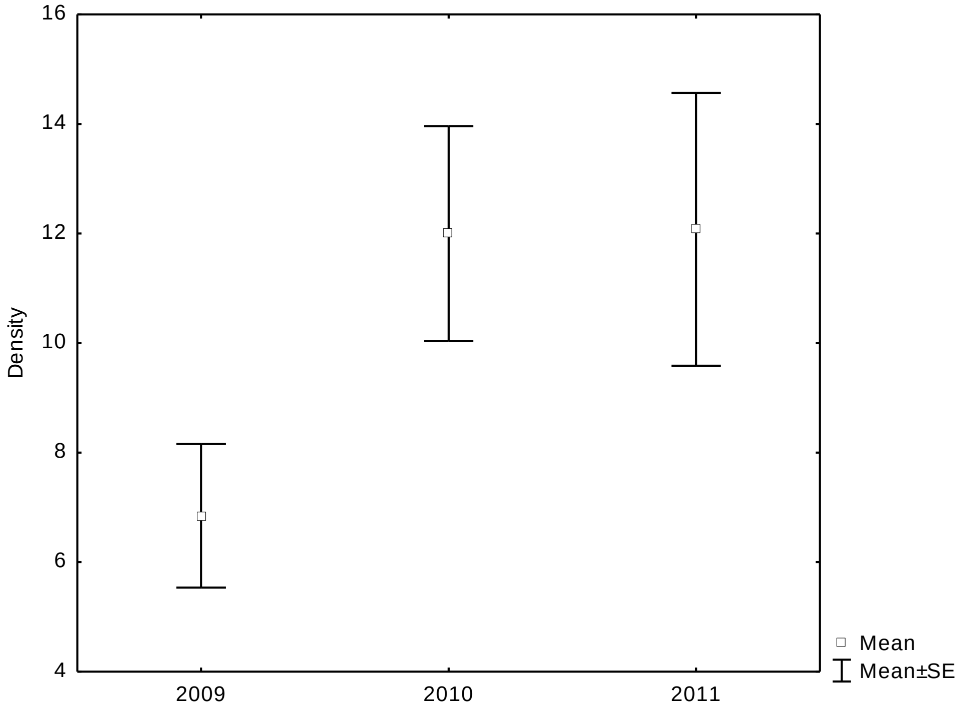

Figure 2 - Boxplots of ramet density in June within the plots among years. Bars represent standard error

Population dynamic

The population consists primarily of ramets included in class 2 (47%) followed by class 3 (43%) and class 1 (10%; Figure 3).

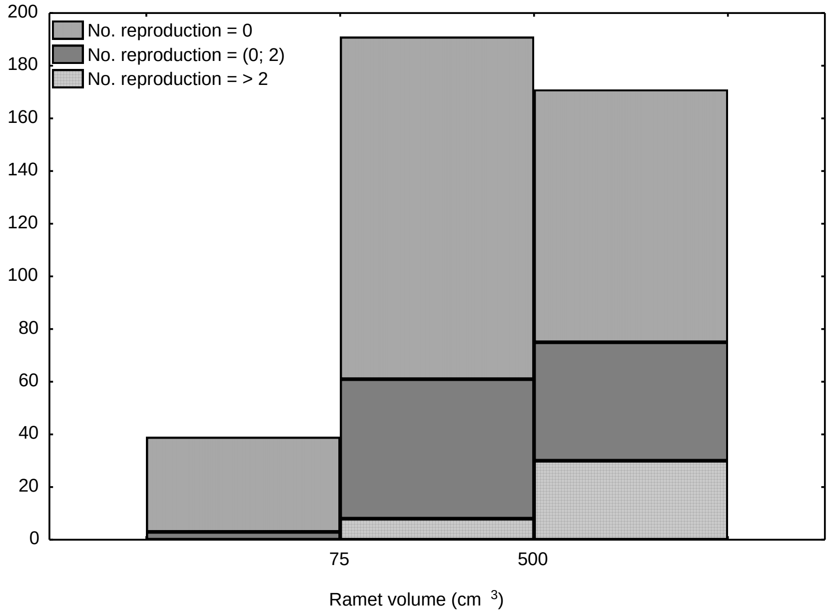

Figure 3 - Population structure: number of *reproduction* per size classes

Ramets of the class 1 gradually increased over three years; ramets of class 2 significantly increased from 2009 to 2010 and the number remained constant in 2011 while ramets of class 3 increased from 2009 to 2010 and decreases in the last year (Figure 4).

The *D. morisianus* had a relatively narrow size hierarchy of individuals during the whole period of study as indicated by mean values of the Gini coefficient lower than 0.3 (mean Gini value = 0.269). During the whole period of observation the Gini coefficient changed only slightly and remained around this value (Figure 4).

Considering the vital rates, *stasis* showed high values in ramets of classes 2 and 3 compared to the ramets of class 1). The *growth* rate has the highest values in ramets of class 1 and lowest in Class 2. *Mortality* rate decreased with increasing ramets size. *Retrogression* rate appeared only in the classes 2 and 3 (Figure 5; Table 3).

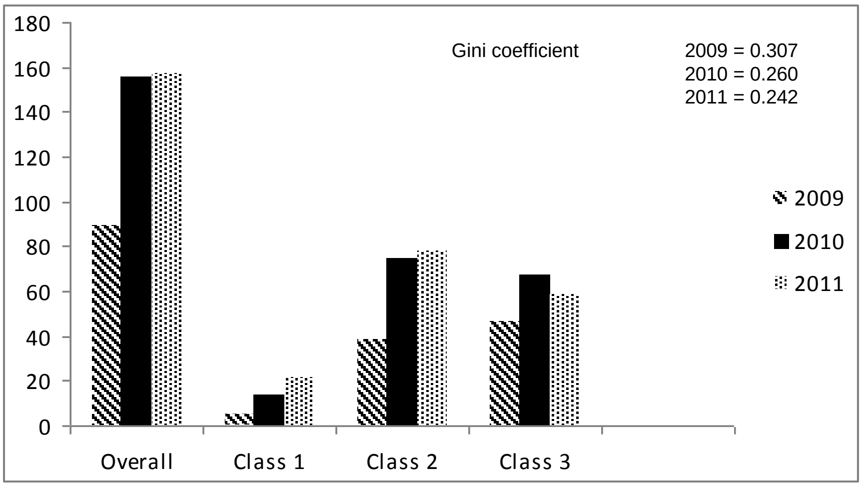

Figure 4 - Population structure: number of ramets of overall population and per size classes.

Table 3 - Mean Transition probabilities (and ± SD) of *D. morisianus* in the period 2009-2011.

	t+1			
t	**Class 1**	**Class 2**	**Class 3**	**Dead**
Class 1	0.179 ± 0.253	0.371 ± 0.323	0.171 ± 0.040	0.279 ± 0.111
Class 2	0.020 ± 0.009	0.450 ± 0.033	0.291 ± 0.185	0.239 ± 0.227
Class 3	0.044 ± 0.001	0.317 ± 0.079	0.536 ± 0.103	0.103 ± 0.023

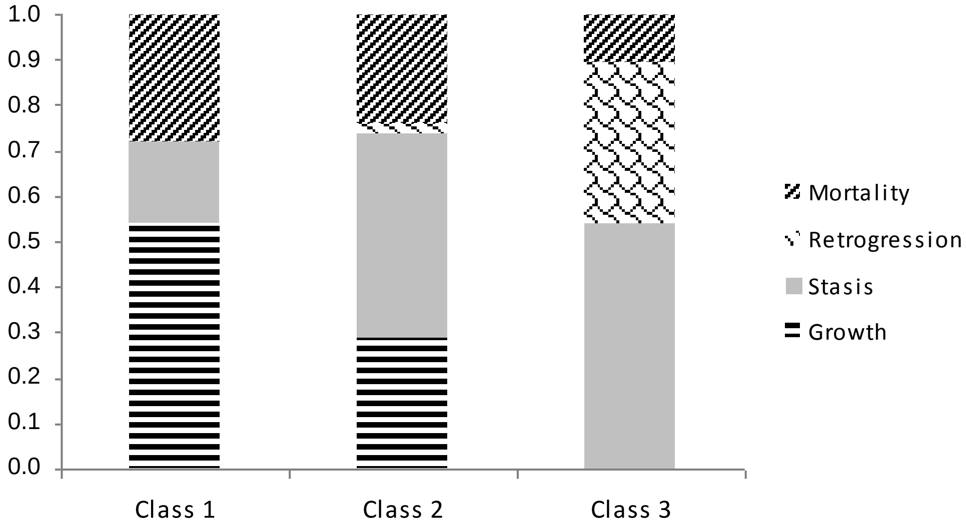

Figure 5 - Cumulative trend of vital rates of the *D. morisianus* population from 2009 to 2011

Conservation status assessment

During the monitoring, the EOO of the whole population was ca. 24 ha and the AOO, based on a 2 x 2 km grid was 4 km^2. According to IUCN Guidelines this population must be considered as one location. The main threats observed are: recreation/tourism (Threat code 10.1), agriculture (Threat code 1.1), wood plantations (Threat code 1.1.2) and poor recruitment/reproduction/regeneration (Threat code 9.2). Considering the cumulative effects of these threats, we able to estimate a continuing decline in habitat quality. Based on the EOO, AOO, number of locations and estimated population decline, we confirm the Critically Endangered category for *D. morisianus* based on criterion B (CR = B1ab (i, ii, iii, v) + 2ab(i, ii, iii, v).

Discussion

According to previous studies on plants growing under adverse environmental conditions (Laberge et al. 2000; Picó & Riba 2002), *D. morisianus* apparently showed a stable population size and didn't show fluctuations. This may be an advantage because for small population, like *D. morisianus*, fluctuations signify the increased possibility for a drastic decline and lack of recovery (Hanski 1999). This species appears extremely specialized, and no opportunity for expansion on large distances as detected for others high specialized plant species (García 2003). In nature there is a continuum of habitat types, from resource-poor habitats that support little or no plant growth, to resource-rich habitats that can potentially support rapid plant growth (Coley et al. 1985).

editerranean coastal dunes have been recognized as stressful habitats, with plants experiencing several abiotic limitations, such as low nutrients, lack of moisture, salt spray, high temperatures and erosion as well as sand burial (Maun 2009; Fenu et al. 2012). Considering the strong effects of abiotic limitations, *D. morisianus* have shown to be able to take advantage of narrow windows of favorable conditions (see chapter 7). Field data confirmed that, exceeded the critical phase of emergence (Cogoni et al. 2012), plants showed high survival and growth rates and use all available resources to grow and to pass to the following classes. In this context, for the long term persistent of population, an high survival adults rates and a long life cycle of individuals is need; and the results obtained in this study confirm this trend. The longevity of individuals represents an important feature for the population survival especially in highly selective habitat, like arid dune systems, where large periods with adverse conditions are common and limit the recruitment rate. Therefore, longevity is a strategy that allows to mitigate the variability and unpredictability of the environment (Garcia et al. 2008).

In ramets of class 2 and 3 mortality decreased and stasis is the predominant phase assuming that the structure remains stable. This is consistent with Harper (1977) which demonstrated that the survival rate of smaller size is the critical stage of the life cycle of several plants, while the largest ones of are better adapted to environmental variability. Moreover, as the number of seedlings is low, and considering from previous studies on the species that seedling emergence seems to be the most critical phase of this species (Cogoni et al. 2012), it is assumed that although the population is stable, it has not a high turnover.

Although no general decline in population sizes was found, the higher extinction rate of small populations could be due to a low turnover in reproductive plants; moreover, stochastic processes (at environmental, climatic or genetic level) are at least partly responsible for the deterministic decline of the number of individuals in small populations and the increased extinction risk (Matthies et al. 2004). Another important factor concerns the capacity of reproductive plants to ensure the long-term viability of a population and these processes strongly affects reproductive success of the species (Fischer et al. 2000; Frankham et al. 2002; Kéry et al. 2000).

Habitat fragmentation, due of the expansion of agriculture (wood plantation in particular), infrastructure for tourism and the construction of roads which determine an high frequency of human at the locations, pose a severe threat for *D. morisianus* persistence. Indeed, fragmentation fragmentation and deterioration of the habitat are the probable cause of *D. morisianus* decline. Habitat fragmentation increases extinction risk for rare species (Holsinger 2000; Matthies et al. 2004; Schleuning & Matthies 2009), interferes with distribution, fitness and seedling recruitment (Lienert 2004; Kolb & Diekmann 2005; Benito et al. 2009; Vere de et al. 2009), reduces the number of breeding individuals and gene flow (Dudash & Fenster 2000) and pollination efficiency (Duncan et al. 2004). Although many plant populations are naturally isolated and small, populations of numerous plant species have become more isolated and further decreased in size due to the recent anthropogenic fragmentation of habitats, small populations are predicted to face the negative genetic consequences of increased inbreeding and reduced genetic variation caused by genetic drift, founder effects and accumulation of deleterious mutations (Lynch et al. 1995; Young et al.1996).

In fact, the distribution area of the plant, expressed in IUCN terms as EOO and AOO, did not change significantly in the monitoring period. The new data archived in this study allow to restrict the previously reported EOO (0.65 Km^2; Fenu et al 2010). Because the reduction in area population, *D. morisianus* appears to be more threatened and data obtained have allowed to reassess the conservation status of *D. morisianus*, confirming the Critically Endangered categorization of this species at global level, previously proposed (Fenu et al. 2010, 2011, Bilz et al. 2011)

The present work was based on a three year study, more prolonged and detailed monitoring is needed in order to provide a more precise informations of population and to identify the critical aspects that affect the survival of the species and to guide the proposal and implementation of sound conservation measures.

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Appendix

Table 1A - Pearson's correlation coefficient between dimensional variables of June (2009-2011) (in bold the significant correlation values). [Abbreviations: H.=heigh; D.Max=diameter maximum; D.Min=diameter minimum; H.S= Stem height; Fl.=No. flowers; Fr.=No. fruits; Re=No. reproductive structures].

	H.	**D. Max**	**Volume**	**D. Min.**	**H.S**	**Fl.**	**Fr.**	**Re.**
H.	1.00	**0.25**	**0.78**	**0.30**	0.08	0.00	0.11	0.09
D. Max		1.00	**0.73**	**0.69**	**0.35**	-0.01	**0.32**	**0.26**
Volume			1.00	**0.57**	**0.27**	-0.05	**0.29**	**0.21**
D. Min.				1.00	**0.17**	0.09	**0.31**	**0.32**
H.S					1.00	**0.32**	**0.42**	**0.55**
Fl.						1.00	**-0.10**	**0.55**
Fr.							1.00	**0.77**
Re.								1.00

CHAPTER 7

PHENOLOGICAL PATTERNS, SEED OUTPUT AND PRE-DISPERSAL SEED PREDATION OF *DIANTHUS MORISIANUS* VALS. (CARYOPHYLLACEAE)

Donatella Cogoni, Giuseppe Fenu & Gianluigi Bacchetta

Introduction

Through its effects on demography and population genetics, reproductive biology has important consequences for the viability of rare plant populations (Evans et al. 2003). Information on the reproductive biology is crucial for predicting their survival capacity and developing the appropriate measures for conservation of endangered plants (Schemske et al. 1994; Menges 1991; Affre et al. 1995).

From anthesis until seed germination the reproductive potential of a plant species diminishes progressively as a consequence of the losses imposed by a variety of factors (Boieiro et al. 2010).

Seed production is the outcome of a complex process involving a several factors including pollinator activity (Wilcock & Neiland 2002; Ashman et al. 2004; Knight et al. 2005), seed predation (Krupnick et al. 1999; Mothershead & Marquis 2000) and resource availability (Medrano et al. 2000; Holland et al. 2004); also herbivory can greatly influence some reproductive traits of plant and thereby reduces plant fitness (Kelly & Dyer 2002; Lavergne et al. 2005).

Flower formation sets an upper limit to fruit production, but in many species only a small proportion of flowers develop into mature fruits (Sutherland 1986). Flower and fruit production can be influenced by interactions with both the abiotic and biotic environment; flower production is often correlated with plant size, which generally increases with resource availability and decreases with population density (e.g. Herrera 1993).

Flowering phenology is affected by many environmental factors, among which temperature and photoperiod, which are reliable signals of seasons, are probably the best studied (Elzinga et al. 2007). Environmental factors affect fruit production directly because of their effects on resources available for fruit maturation, but also indirectly because of their effects on flower production and on abundance of pollinators and seed predators.

In animal-pollinated plants, flowering phenology is a likely target of natural selection by both pollinators and seed herbivores (Brody 1997) because insect and flower abundance vary seasonally. Consequently, plants should have higher fitness when flowering coincides with pollinator but not seed herbivore abundance (Parachnowitsch & Caruso 2008); higher pre-dispersal seed predation to early flowers is also common (e.g. Elzinga et al. 2007). Generally the early cohort had more fruits damaged than the late cohort, and within each cohort there was higher damage on plants that began flowering earlier (Elzinga et al. 2007). Plants flowering at the extremes of the season may, however, have fewer mates, and therefore less available pollen in their environment than at peak flowering (Elzinga et al. 2007), but they may receive relatively more visits because there are also fewer plants competing for pollinators (Collin & Shykoff 2010). Despite the plurality of causes governing the reproductive success, seed predation has reported as a major form of seed mortality (Janzen 1971; Crawley 2000) with seeds being consumed both before and after the dispersal phase (Boieiro et al. 2010).

Animal-pollinated species must be synchronous with their pollinators but unfortunately, plants attract not only mutualist pollen and seed dispersers but also enemies and may suffer from florivory and seed predation (Bopp & Gottsberger 2004). Consequently, plants should have higher fitness when flowering coincides with pollinator but not seed herbivore abundance (Parachnowitsch & Caruso 2008).

Although plants gain mating partners by flowering synchronously with conspecifics, flowering synchrony may enhance plant visibility to antagonists; thus individuals may avoid their enemies by flowering earlier or later than other plants (i.e., escape in time; Augspurger 1981; English-Loeb & Karban 1992). However, this is not possible if pollinators are also antagonists (e.g., pollinating adults with herbivore larvae). Many pollinating insects use flowers as oviposition sites, a situation defined as ‘‘nursery pollination systems’’ (Dufaÿ & Anstett 2003; Kephart et al. 2006), therefore, flowering plants face a dilemma due to the necessity of attracting pollinators that may also be antagonists (Collin & Shykoff 2010).

Moth pollinators that act as seed predators occur in multiple caryophyllaceous genera (e.g. *Silene*, *Dianthus,* etc) and pre-dispersal seed predation by *Hadena* species, described as “parasitic-pollinators”, has demonstrated for several species since females also use the flowers as oviposition sites (e.g., Pettersson 1991, 1994; Biere & Honders 1996; Kolb et al. 2007; Collin et al. 2002; Collin & Shykoff 2010).

Dianthus L. (*Caryophyllaceae*), a genus of > 300 species centred in the Mediterranean Basin (Balao et al. 2010), is characterized by large numbers of endemic species (more than 70 *taxa*) with restricted geographical or ecological range, due to exceptionally high rates of diversification in Mediterranean area (Valente et al. 2010). Unlike the majority of plants adapted to summer drought, *Dianthus* is almost exclusively a summer-flowering genus (Thompson 2005); thus, in the Mediterranean basin, the flowering period of *Dianthus* is conspicuously out of synchronization with the remaining plants, which blossom en masse in spring (Thompson 2005).

One intrinsic characteristic of Mediterranean ecosystems is the high climate variability between seasons and between years (Lionello et al. 2006). Mediterranean habitats show high ecological variability in space, considering the several different types of habitats, and time with high seasonal unpredictability, particularly in the pattern of precipitation, alternating dry and rainy years (Braza & Garcìa 2011). Accordingly flowering period may vary annually, and onset of flowering may be strongly affected by climatic variability (Price & Waser 1998; Makrodimos et al. 2008).

D. morisianus Vals., belonging to the *D. sylvestris* Wulfen complex (Bacchetta et al. 2010), is the only Mediterranean species, with only one small population, growing on sandy coastal dune of SW Sardinia (Valsecchi 1985; Bacchetta et al. 2010; Fenu et al. 2010). Mediterranean coastal dunes have been recognized as stressful habitats, with plants experiencing several abiotic limitations, such as low nutrients, lack of moisture, salt spray, high temperatures and erosion as well as sand burial (Maun 2009; Fenu et al. in press). In addition, the geographical isolation (and the narrow ecological range) of this species, combined with the asynchronous phenology than other typical coastal Mediterranean plants and the potential relationships with animal (i.e. scarcity/abundance of pollinators/seed predators), make this population a very interesting study case.

The aim of this study was to investigate some reproductive traits of *D. morisianus* and, in particular, to analyse the phenological pattern, seed set, and pre-dispersal seed predation in this endemic species growing on Mediterranean coastal dune.

Materials and methods

Study species

D. morisianus is a perennial suffrutex, characterized by numerous woody stocks and erect stems, 20-45 cm long, and by basal rosette with thin and linear leaves, 1-15 cm long. Reproductive stems bear terminal multi-flowered heads (normally, 2-18 flowers/head); the calix is characterized by lanceolate teeth, membranaceous on the margin; the colour of the corolla is normally pink (Bacchetta et al. 2010). Small and flat seeds can germinate with high percentages in a wide range of conditions, with a maximum germination rate at 15°C (Cogoni et al. 2012).

Vegetative sprouting, probably related to woody stocks loosely branched (Bacchetta et al. 2010), has been observed in the natural population, thus field analysis was conducted at ramet level, considering ramet each "visual unit" clearly separated by others (Garcia et al. 2002). *D. morisianus* is a psammophilous species which grows on stabilized dunes in contact with micro-forests of *Juniperus* spp. [*Juniperus macrocarpa* Sibth. and *J. phoenicea* L. subsp. *turbinata* (Guss.) Nyman] and *Quercus calliprinos* Webb. The only natural population of *D. morisianus* is located in Portixeddu (Buggerru, SW Sardinia) at an altitude of 10-55 m a.s.l. (Bacchetta et al. 2010; Fenu et al. 2010). Available climate data (from Fluminimaggiore weather station at 45 m a.s.l.) indicates a typical Mediterranean seasonal pattern of temperature and precipitation, with a long dry summer (Cogoni et al. 2012).

Data collection

The study was carried out over three years (2009-2011). Each year, 13 permanent plots 1 m^2, randomly placed in the area where *D. morisianus* is found, were analysed by mapping and counting all ramets. In each plot all ramets were marked and monitored monthly in order to analyse their phenological patterns, from onset flowering to seed dispersal.

The height, the maximum and the minimum diameter of the basal rosette and the reproductive stem height were measured on each ramet using a digital calliper (ALPA IP65 Topcal 150 PW)., During each sampling, number of flowers (bud, anthesis and withered flowers) and fruits (immature and mature fruits and fruits with dispersed seeds) were counted during the reproductive season. For each ramet the number of reproductive stems bearing flowers, as well as the number of fruit for each stem were also counted. In addition all reproductive stems damage by grazing was scored.

The number of seeds per fruit and per ramets was calculated by checking 150 fruits, in each year, in the month when the maximum number of ripe fruits were detected; seed output was estimated by multiplying the mean number of seeds/stem by the mean number of stems/ramet.

To evaluate the effect of pre-dispersal predation on the fruit, two fruit of each ramet monitored randomly collected was cleaned and analysed in laboratory. One-way analysis of variance (ANOVA), followed by *post hoc* Tukey Honestly Significant Difference test (HDS), was carried out on the number of flowers, fruit production, and seed output.

Data analyses

We calculated individual ramet volume (V_r) using individual basal rosette height h_i [cm], the maximum and minimum diameter d_M and d_m [cm], according to the following formula:

$V_r = [\pi*(d_M/2)*(d_m/2)] *h_i$ [cm^3]

Then, the ramet volume was correlated with reproduction values, defined as the sum of flowers and fruits counted in the same surveys, and the Pearson correlation value between variables were calculated.

Results

Phenological patterns

The reproductive season of *D. morisianus* starts in May/June and the mean flowering duration was 42.00±21.21 days, with a range from 27 to 57 days (Figure 1). The flowering peak for *D. morisianus* is archived in May for all years (36.61, 25.47 and 36.65 for 2009, 2010 and 2011, respectively) with percentages of flowering ramets always less than 40% (Figure 1; Table 1). The flowering season exhibited overlaps of the various phenological phases. Flowers per plant showed significant variation between 2009 and 2010 (one-way ANOVA: $F = 3.677$, $P<0.025$; $P<0.05$ by *post hoc* Tukey HSD test) while between 2009-2011 and 2010-1011 they did not show statistical differences.

The fruiting season is also quite short: fruits are produced ca. 1 month after flowering and the fruiting peak occurs in June or July (42.69, 31.32 and 30.11% for 2009, 2010 and 2011, respectively). The mean fruiting duration was 25.50±3.54 days, with a range from 23 to 28 days (Figure 1).

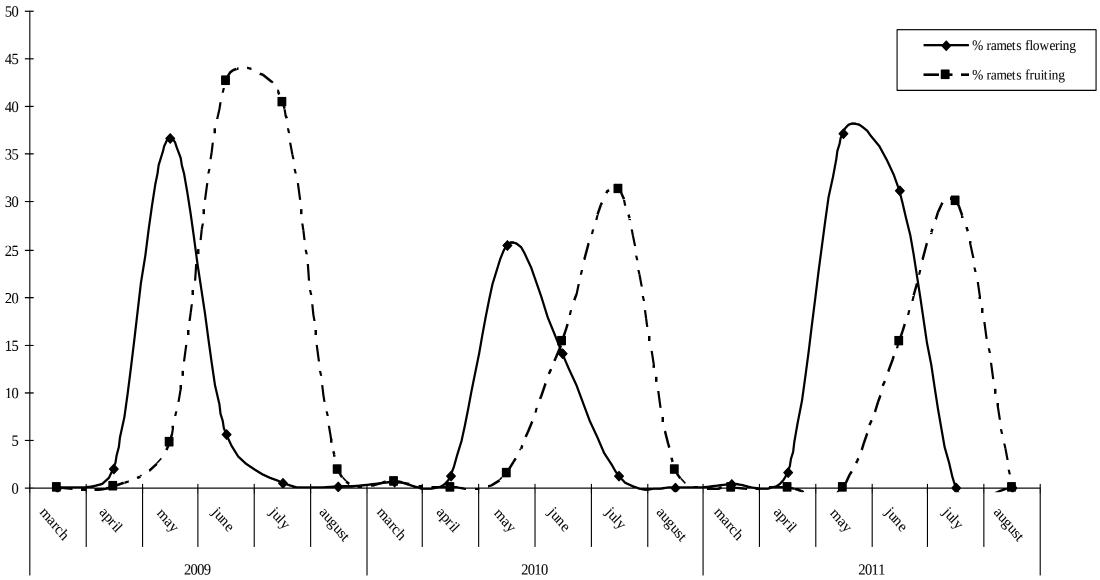

Figure 1 - Monthly trend of flowering and fruiting seasons of *D. morisianus* over three years (2009-2011).

Table 1 - Reproductive traits of *D. morisianus* over three years of study (2009-2011) in the peak month.

	2009	2010	2011
Total monitored ramets	89	83	93
Ramets with stems	62	41	63
Ramets with stems (%)	70	49	68
Mean stems per ramet	2.87	2.32	2.44
Stems flowering (%)	58.99	48.42	43.51
Stems damaged (%)	41.01	51.58	56.49
Mean fruits per ramet	3.98	2.49	2.60
Mean seeds per fruit (±SD)	22.37±40.07	31.95±110.52	27.46±19.02
Mean seeds per ramet	89.08	79.45	71.39
Pre-dispersal fruit predation (%)	20.67	0.56	15.18

Seed output

A total of 79, 51 and 86 reproductive ramets were monitored for 2009, 2010 and 2011, respectively. The highest percentage of ramets with stems was recorded in 2009 (69, 49 and 67% for 2009, 2010 and 2011, respectively) and the total number of stems varied from a minimum of 95 in 2010 to a maximum of 178 in 2009. The mean number of stems per reproductive ramet is 1.44±2.04 and varied from a minimum of 0 to a maximum of 15 (2009). The mean length stem was 13.91±15.96, 21.86±5.53 and 24.87±6.49 cm for 2009, 2010 and 2011, respectively.

Differences in number of flowering stems were statistically significant between 2009-2010 and 2010-2011 (*p-value* < 0.05 by one-way ANOVA, followed by Tukey's tests) while no differences were found between 2010-2011 (P>0.05).

The first year had significantly lower values of grazed stems than other years. On the contrary, 2011 had a significantly greater percentage of grazed stems (Table 1). The number of grazed stems showed statistically significant differences between 2009 and 2010 (*p-value* < 0.05 by one-way ANOVA, followed by Tukey's tests), while no differences were found between 2009-2011 and 2010-2011. Fruit set didn't show significant differences (P>0.05 by one-way ANOVA).

Seed production is quite variable, but in general, all years each ramet produced a large number of seeds. The maximum mean number of seeds per fruit was recorded in July 2010 and the minimum in June 2009 (Table 1). Seed production per fruit didn't show significant variation (P>0.05 by one-way ANOVA). The highest percentage of pre-dispersal fruit predation was found in 2009 (20.67%), while in 2010 only a small percentage of fruits (0.56%) are infected by animal (Table 1).

The number of reproduction per ramet show a positive correlation, statistically significant, with the ramet volume (linear regression: Reproduction = -0.0960022715 + 0.0027918079*ramet biovolume; r-squared = 0.4967; r = 0.7048; *p-value* < 0.0001; Figure 2).

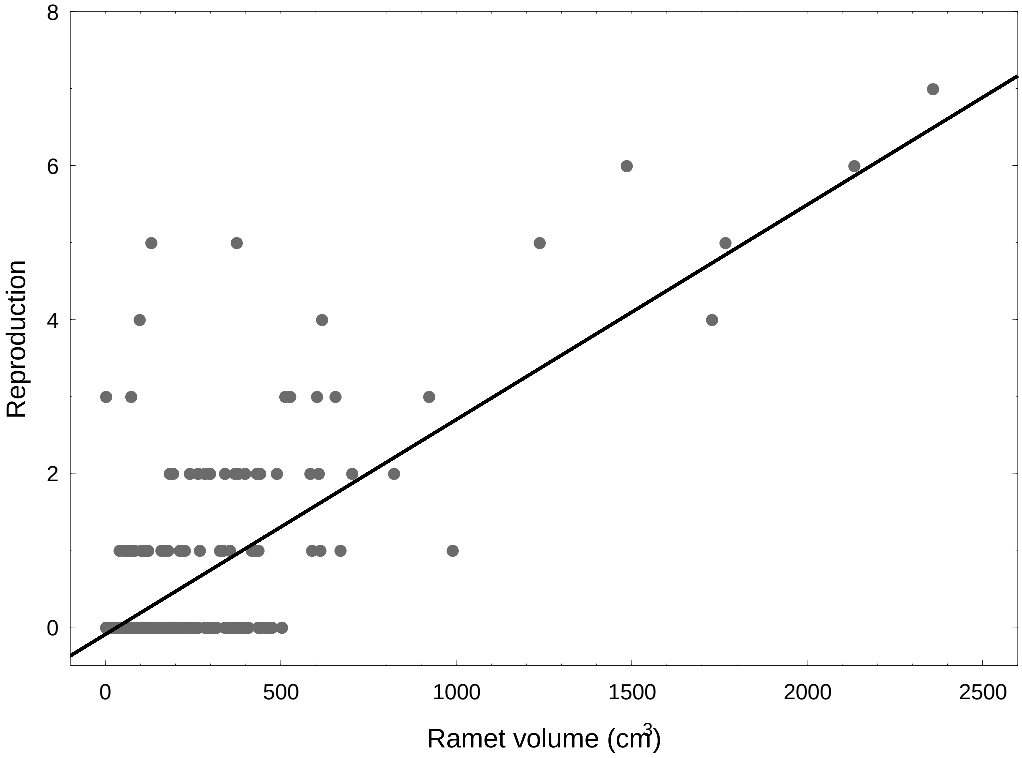

Figure 2 - Correlation between ramet volume and reproduction per ramet (Reproduction = -0.0960022715 + 0.0027918079 x ramet biovolume; r-squared = 0.4967; r = 0.7048; *p-value* < 0.0001).

Discussion

The results on reproductive traits of *D. morisianus* confirm the descriptive data already reported in previous studies, with flowering season lasting from early May to late June (Valsecchi 1985; Bacchetta et al. 2010; Fenu et al. 2010), and ripe fruits which can be found in June and July (Fenu et al. 2010).

Our results highlight that in *D. morisianus* population there was a massive bloom, concentrated in a narrow period of time, sowing a high degree of synchrony within the population; this pattern should be explained considering both environmental factors and pollinators abundance. In our case, the first factors seem to be prevalent and this does not seem to support the hypothesis that the possession of such an unusual phenology in a context of predictable summer drought may have influenced diversification processes in *Dianthus* species by triggering strong local divergence of floral characters in response to pollinators, which are rare in the summer (Valente et al. 2010).

Flowering phenology is affected by many environmental factors, connected to the climatic variability seasonal; accurate detection of such environmental cues and the resulting plastic response of plants enable flowering to occur when climatic conditions are most suitable for reproduction (Elzinga et al. 2007). Environmental factors may thus affect fruit production directly because of their effects on resources available for fruit maturation, but also indirectly because of their effects on flower production and on abundance of pollinators and seed predators (Ollerton 1996).

Variation in environmental factors might also contribute to phenological variation in populations, but these phenotypic plastic responses themselves might also be genetically modulated to some extent (Elzinga et al. 2007). It follows that the type, direction and intensity of selection on flowering time may differ between years, populations and species (Ollerton & Lack 1998). Climatic variation might modify selection on flowering phenology as growing seasons expand or contract (Franks et al. 2007). Climatic variability is pronounced in the Mediterranean coastal habitats and particularly in arid sandy dune. *D. morisianus* is adapted to severe limitation like summer drought and low resources availability and this may be considered as a disadvantage for the phenological responses of the species. The short length of flowering and fruiting seasons could be both the cause of the lower reproductive values, compared with a wider window of non-stressed environment and a highly level of adaptive strategy for this high selective habitat. In arid and semiarid Mediterranean sandy coastal dunes, such as, stress would restrict the reproductive success (Bisigato & Bertiller 2004) and in this environment unpredictability, several species present an extended flowering seasons, as previously detected for others wider distributed Mediterranean species (Picó & Retana 2000). In fact, as the fruit and seed-set of individual plants is correlated with the phenological stages, an extended blooming period could increase the individual's chance of having a large number of reproductive ramets (Bawa 1973) reducing the risk of having a low number of stems and, consequently, a low number of fruits and seeds. On the contrary, *D. morisianus* is adapted, due this restricted favourable conditions, to use all available resources to produce flowers and fruits; this pattern is similar with other *Dianthus* species, that show a short flowering season, occurring over only 6 weeks (Collin et al. 2002).

Climate variability act also on abundance and identity of seed pollinators and predators (Ollerton 1996 and references therein) and this might modify biotic interactions if the phenology of interacting species shifts unevenly with climate (Elzinga et al. 2007). The role of biotic interactions in shaping plant phenological patterns has long been a controversial issue. For instance, a high degree of synchrony within a population (such as our case) has been considered adaptive, through promotion of outcrossing and/or satiation of seed predators (Janzen 1976; Augspurger 1981) though it might equally be the result of a recent population bottleneck reducing genetic variability. A number of adaptive interpretations have been given for flowering asynchrony, for example; as an evolutionary response to intraspecific competition for pollinators; for promoting inter-plant pollinator movement; the advantage of increasing mate availability; for dispersion of seed predators; variation in intensity and timing of seed predation and dispersal; or differential selection in different years depending upon environmental factors (Zimmerman 1980; Frankie & Haber 1983; Primack 1985; Rathcke & Lacey 1985). Alternatively, within-population flowering asynchrony could be due to relaxed selection on natural genetic variability and/or environmental heterogeneity (Ollerton & Lack 1992).

Highly synchronous and resembled flowering phenology was been reported for other *Dianthus* species (Collin et al. 2002) and could be considered an adaptive strategy; plants that bloom at peak population flowering may thus escape enemies through predator satiation (Janzen 1971). This pattern contrasts with the general model of extended phenology whereby the frequency distribution for the date of first flowering is approximately normal, and flowering times are not spatially aggregated, early and late bloomers should have fewer potential mates in their immediate neighborhood than should plants blooming near the modal date. In general, asynchronous flowering might blur fine-scale isolation by distance between potential mates, and so reduce biparental inbreeding (Elzinga et al. 2007).

Antagonistic pre-dispersal seed predators are particularly likely to exert selection on floral traits for two reasons. First, the larvae of many pre-dispersal seed herbivores develop by consuming seeds and other reproductive tissues (e.g. Westerbergh & Westerbergh 2001). Consequently, their effects on plant fitness are direct, and if damage covaries with floral traits, then pre-dispersal seed herbivores will alter the relative fitness of preferred phenotypes and exert selection on those traits. Second, herbivores that oviposit onto flowers of outcrossing, animal-pollinated plants depend upon pollinators to provision their larvae (Strauss & Irwin 2004). Therefore, herbivores should oviposit on flowers that are attractive to pollinators because they are most likely to set fruit (Parachnowitsch & Caruso 2008).

Two nocturnal moth genera (*Hadena*, Noctuidae; *Perizoma*, Geometridae) interact with several caryophyllaceous genera in diverse ways, which suggest that these systems are capable of shifting between antagonism and mutualism (Collin et al. 2002; Dufaÿ & Anstett 2003; Westerbergh 2004; Martinell et al. 2010). In this system, the moths are simultaneously effective seed predators and pollinators (described as “parasitic-pollinators”), with wide variation in the abundances of interacting species, in the costs exacted by larval feeding and in the ecological contexts influencing selection (Pettersson 1991; Westerbergh 2004).

In particular, nocturnal moths of the genus *Hadena*, are the main pollinators (though not exclusively) and predators of *Dianthus* (Kepart 2006). Pre-dispersal seed predation by *Hadena* might nonetheless exert selection on flowering phenology in *Dianthus*, as has been suggested for several species (e.g., Pettersson 1991, 1994; Biere & Honders 1996; reviewed in Kolb et al. 2007). Consequently, plants should have higher fitness when flowering coincides with pollinator but not seed herbivore abundance (Parachnowitsch & Caruso 2008), in a balance of forces imposed by mutualists (pollinators and seed dispersers) or antagonists, such as floral pathogens and pre-dispersal seed predators (Elzinga et al. 2007).

In *D. morisianus* population prevail a mutualistic system (in particular pollination) than antagonistic one; in fact the percentage of pre-dispersal seed predation constitute a small portion of total production. In addition, the annual differences in seed output are probably related to the number of pollinator visits; in fact in years when there was a higher percentage of parasitism an increase in the mean number of seeds per fruit was also observed. This suggests that, when pollinators were scarce in the population site, a reduction in seed output should be expected in the *D. morisianus* population. On the other and, our results suggest that pre-dispersal seed predation could not be the main driver of fruit production and plant fitness in *D. morisianus.* Thus pre-dispersal seed predation can not be considered a serious threat affecting the reproductive success in *D. morisianus*.

Plant size is usually considered a predominantly environmentally influenced characteristic, determined by plant age and growing conditions (Waller 1988). It is normally closely correlated with total flower production and the largest plants in a population are usually the most fecund (Weiner & Thomas 1986; Herrera 1993). Plant size was an important influence on individual reproductive success of *D. morisianus:* larger plants produced more flowers and fruits and seem to be suffered a lower proportion of seed predation than smaller plants. Large plants certainly seem to be at a reproductive advantage and there is a link between plant size and flowering time (Ollerton & Lack 1998), but the overall relative effects of heritability and environmental conditions are at date little investigated.

Another important factor which affects reproductive success is the plant damage due to livestock grazing. In previous studies has been assessed that this factor decrease vegetative growth and reproductive success, in terms of seeds or fruit production (Mutikainen & Delph 1996). In *D. morisianus* population, reproductive stems were more damaged than the basal rosette, more protected by the vegetation and, in particular, reproductive stems with ripe fruits are mainly damaged than flowering one. Despite grazing intensity on reproductive stems being vary yearly (i.e. > 55% in 2011), no difference in the number of fruits output at population level are detected and a considerable number of germinable seeds per plant was recorded each year (Cogoni et al. 2012), indicating that *D. morisianus* should be considered not as a seed-limited species (*sensu* Eriksson & Ehrlén 1992), but as a micro-sites limited species, considering its ecological requirements.

In conclusion, this study highlights the predominance of environmental limitation (in particular climatic variability) affecting reproductive success in *D. morisianus* population, when biotic interaction plays a positive effect in reproductive process. Considering the strong effects of climate change on plant phenology (Gordo & Sanz 2010), *D. morisianus* can be considered extremely sensitive to the slightest changes in climate variation, because of this narrow window of favorable conditions. As previous reported by Bloch et al. (2006) for a small population of *D. carthusianorum* and Martinell et al. (2010) for *Silene sennenii*, an endangered Mediterranean plant, the conservation of the mutualism between *D. morisianus* and its pollinators is crucial to preserve extant population and to guarantee long-term survival of the species.

This study is the first attempt to investigate the reproductive biology of *D. morisianus* in order to identify the critical aspects that affect their reproductive success and to guide implementation of conservation measures. However more detailed analysis is needed in order to assess (1) the role and importance of pollination in the reproductive process, (2) losses in fitness as a result of the presence of seed predators (i.e. more accurate estimates of the number of seeds remaining in capsules and consumed by moth larvae), (3) the effect of the small size population on fitness, (4) the effect of habitat fragmentation and (5) the effective impact of grazing on fruit and seed set in the *D. morisianus* population.

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Appendix

Table 1A - Monthly number of ramets in each phenophase: the average value of flowers/fruits per individual ± standard deviation are reported in brackets.

	Ramets flowered	**Ramets with flowers bud**	**Ramets with flowers at anthesis**	**Ramets with withered flowers**	**Ramets fruited**	**Ramets with immature fruits**	**Ramets with mature fruits**	**Ramets with dispersed seeds**
2009								
May N=183	67 (1.08±2.00)	67 (1.07±2.00)	1 (0.01±0.07)	0	0	0	0	0
June N=89	5 (0.07±0.37)	1 (0.02±0)	4 (0.04±0.20)	1 (0.01±0.10)	37 (0.96±1.80)	35 (1.00±1.25)	2 (0.16±1.39)	1 (0.01±0.11)
July N=47	0	0	0	0	19 (0.80±1.20)	19 (0.80±1.21)	0	0
2010								
March N=167	1 (0.01±0.15)	1 (0.01±0.15)	0	0	0	0	0	0
April N= 166	2 (0.01±0.17)	2 (0.01±0.17)	0	0	0	0	0	0
May N=161	41 (0.57±1.45)	41 (0.57±1.45)	0	0	0	0	0	0
June N=156	22 (0.20±0.56)	8 (0.05±0.22)	3 (0.01±0.13)	14 (0.13±0.45)	24 (0.25±0.72)	24 (0.25±0.71)	0	1 0
July N=83	1 (0.02±0.20)	0	1 (0.02±0.20)	0.00	26 (0.46±0.99)	3 (0.07±0.53)	16 (0.21±0.50)	11 (0.18±0.58)
2011								
April N=199	1 (0.01±0.21)	1 (0.02±0.21)	0	0	0	0	0	0
May N=191	70 (0.91±1.70)	70 (0.90±1.70)	1 (0.01±0.07)	0	0	0	0	0
June N=157	49 (0.62±1.27)	19 (0.16±0.58)	9 (0.08±0.33)	41 (0.38±0.84)	24 (0.33±1.04)	23 (0.32±1.03)	0	1 (0.01±0.08)
July N=93	0	0	0	0	28 (0.59±1.44)	2 (0.06±0.57)	25 (0.43±1.00)	5 (0.10±0.55)

CHAPTER 8

FROM SEED TO SEEDLING, A CRITICAL TRANSITIONAL STAGE FOR THE MEDITERRANEAN PSAMMOPHILOUS SPECIES *DIANTHUS MORISIANUS* (CARYOPHYLLACEAE)

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Introduction

The sand coastal dunes are highly variable ecosystems, because of shifting substrate, burial by sand, bare areas among plants, porous nature of sands and little or no organic matter, especially during the early stages of dune development (Maun 2009). In the coastal strands and foredunes there are many risks that limit the transformation of a seed to a seedling owing to the spatial and temporal variation in the substrate (Maun 1994). The micro-environmental variability mediated by wind and wave action created rather harsh and uncertain conditions for seed germination, emergence of seedlings and their establishment (Maun 1994).

The transition from seed to seedling is a high-risk period in the life cycle of most plants (Harper 1977). Consequently, mechanisms that minimize the risk to this transition will be under strong selection pressure (Meyer et al. 1997), and natural selection should favour seed germination patterns that increase the probability of successful seedling establishment. When a species is restricted in distribution to a peculiar part of an environmental gradient, its seed germination characteristics are likely to be adapted to that particular set of conditions (Daws et al. 2002).

Under a Mediterranean climate, characterized by a highly seasonal alternation of favourable and unfavourable conditions, plant growth and reproduction must occur in a window of favourable conditions that may vary in length and in which environmental cues and constrains play a central role (Thanos et al. 1995; Doussi & Thanos 2002; Gresta et al. 2010). The optimal germination temperature for Mediterranean species is typically within the range 5-15°C and these species are also characterized by having a low germination rate and being negatively affected by prolonged chilling (Thanos et al. 1989; Skordilis & Thanos 1995; Doussi & Thanos 2002). In particular, a surface avoiding mechanism of seedling establishment based on photoinhibition was detected in seeds of many Mediterranean psammophilous species such as *Glaucium flavum* Crantz

(Thanos et al. 1989), *Allium staticiforme* Sibth. & Sm., *Brassica tournefortii* Gouan, *Cakile maritima* Scop. subsp. *maritima* and *Otanthus maritimus* (L.) Hoffmanns. & Link subsp. *maritimus* (Thanos et al. 1991). However, seeds of *Matthiola tricuspidata* (L.) R. Br., another photoinhibited widespread annual plant of Mediterranean sand dunes, did not conform with the Mediterranean germination physiology, showing a very wide range of temperatures and a high germination rate (Thanos et al. 1994). Rapid germination has been suggested to be an adaptation for rapid establishment before the soil drying in unpredictable environments (Daws et al. 2002).

However, low germination rate and a narrow range of cool temperatures are considered an advantageous ecological adaptation to the unpredictable rainfall pattern of species living under "typical" Mediterranean climate conditions, by limiting germination to winter and thereby maximising the length of the growing season before the onset of summer drought (Thanos et al. 1995).

Seedlings are the most vulnerable stage of the life cycle of plants, being subject to a several of abiotic and biotic constrains that may affect their emergence, survival and establishment (Harper 1977; Moles & Westoby 2004; Leck et al. 2008). Seedling establishment is an especially critical phase in the life cycle of plants inhabiting dry environments (Yang et al. 2010). Studies on the survival and establishment of seedlings in coastal sand dunes suggest that nutrient deficiency, lack of moisture, sand accretion, salt spray and predation are probably the most important limiting factors and seedling recruitment of dune species coincides with periods of high moisture availability and occurs in years with high well distributed rainfall (Maun 1994). Seed burial depth is an important factor regulating seed germination and seedling emergence as seed germination is directly related to seed size and the depth at which seeds were buried (Bond et al. 1999; Ren et al. 2002).

The ability to form a persistent soil seed bank is crucial to the survival of many rare or declining species (Keddy & Reznicek 1982; Rowell et al. 1982; Quilichini & Debussche 2000; Eckstein et al. 2006) by conferring a degree of resilience in the face of modern and intensive land use (Thompson et al. 1993) and protecting populations from local extinction when above-ground vegetation is removed (Arroyo et al. 2006). Moreover, this ability was correlated to seed size and shape, with persistent seeds being generally smaller and more rounded than transient seeds (Thompson & Grime 1979; Thompson 1987; Thompson et al. 1993; Funes et al. 1999). Seed mass and seed longevity are negatively correlated (Rees 1997) and persistent seeds are typically smaller and more rounded than seeds forming a transient seed bank (Thompson et al. 1993; Cerabolini et al. 2003). However, Yu et al. (2007) found that, in a Mediterranean coastal dune ecosystem, species with bigger seeds tend to have persistent soil seed bank than those with smaller seeds. Therefore, these authors concluded that the relationships between seed mass, shape and persistence may be habitat-specific and affected by the spatial scale of flora investigation and suggested that significant differences in climate may determine diverse seed persistence patterns.

Dianthus morisianus Vals. (*Caryophyllaceae*) is a psammophilous chamaephyte which grows on stabilized dunes in contact with micro-forests of *Juniperus* spp. [*J. oxycedrus* L. subsp. *macrocarpa* (Sibth. & Sm.) Neilr. and *J. phoenicea* L. subsp. *turbinata* (Guss.) Nyman] and *Quercus calliprinos* Webb, only in a small area near Buggerru (Southwest Sardinia) (Bacchetta et al. 2010). *D. morisianus* is listed in the National Red List as endangered (Conti et al. 1992) and in Regional Red List as vulnerable (Conti et al. 1997); more recently, it has been inserted in the European threatened plant list (Sharrock & Jones 2009) and for this species has been proposed the Critically Endangered IUCN category (Bacchetta & Pontecorvo 2005; Fenu et al. 2010). However, biology and ecology of this species are still little investigated.

In this study, reproductive traits such as (i) seed germination requirements, (ii) seedling emergence and (iii) seed persistence in the soil of this species were investigated, with the main aim to better understand the transitional phases from seed to seedling in a Mediterranean narrow endemic species, growing in a high specialized habitat such as the Mediterranean coastal dune ecosystem.

Materials and methods

Study species

Dianthus morisianus Vals. is a perennial suffrutex, characterized by numerous woody stocks and erect stems, 20-45 cm long, and by basal rosette with thin and linear leaves, 1-15 cm long. The stems bear terminal multi-flowered heads (normally, 2-18 flowers/head); the calix is characterized by lanceolate teeth, membranaceous on the margin; the colour of the corolla is normally pink (Bacchetta et al. 2010). The flowering season lasts from early May to last June, whereas ripe fruits can be found in June and July (Fenu et al. 2010).

Seedlot details

Ripen seeds were collected in 2006, 2008 and 2009, from ca. 100 individuals of the natural population in Portixeddu (Buggerru, South Western Sardinia, N 40° 14’ E 09° 25’) at an altitude of 10-55 m a.s.l. in the fixed dune system. Available climatic data for the population site are reported in Table 1. Seeds collected in 2006 and 2008 were stored at the Sardinian Germplasm Bank (BG-SAR), where they were placed in a dry room at 15°C and 15% relative humidity (r.h.) and then stored at +5°C and -25°C, respectively. Seeds collected in 2009 where kept at laboratory conditions (ca. 20°C and 45-50%) till the start of germination experiments in November 2009. The mean seed masses (± 1 standard deviation, SD) of the three seed lots, calculated by weighing 10 replicates of 20 seeds each, were 1.28 ± 0.07, 1.29 ± 0.03 and 1.19 ± 0.08 mg for 2006, 2008 and 2009 collections, respectively.

Table 1 – Monthly averages of temperatures (°C) and rainfall (mm) for the population site (Data from Regione Autonoma della Sardegna 1982-2005). The month of seed collection for each seed lot is also indicated.

	Jan	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Year**
Rainfall (mm)	92.40	77.90	63.00	65.70	39.00	18.50	2.40	10.70	37.20	88.30	117.50	120.5	733.00
T max (°C)	14.70	15.30	17.20	19.10	24.10	27.70	30.80	31.50	28.00	24.30	19.00	15.50	22.30
T min (°C)	5.40	5.60	6.80	8.60	11.60	14.40	16.50	17.10	15.30	12.70	9.00	6.40	10.80
T mean (°C)	10.00	10.30	12.00	13.90	17.80	20.90	23.60	24.30	21.60	21.70	13.80	10.70	16.7
Seed collection							08/09		06				

Germination tests

Three replicates of 25 seeds per treatment were sown on the surface of 1% water agar, to provide solid, non-sterile medium for germination, in 60 mm plastic Petri dishes and incubated at both constant (5, 10, 15, 20 and 25°C) and alternating temperatures (25/10°C). In all treatments, seeds were exposed to irradiance for 12 h per day. In the alternating temperature regime the 12 h light period coincided with the elevated temperature period. Germination was considered to have occurred at the time of visible radicle emergence. Germination was scored daily for 45 days and germinated seeds removed. To verify the effect of light on germination, a preliminary test was carried out by sowing three extra replicates at 15°C in the dark, achieved by wrapping dishes in aluminium foil. Seeds in this experiment were only scored once, at the end of the test, to avoid any exposure to irradiance. At the end of the germination tests, a cut-test was carried out to determine the viability of the remaining seeds and the final germination percentage calculated on the basis of the total number of filled seeds as the mean of the three replicates ± 1 SD.

Seedling emergence

Three replicates of 25 seeds each were sown at different burial depths: 0, 1, 2, 5 and 10 cm, in 15 square pots filled with sand, within a greenhouse where the air temperature during all the experiment was 16.5 ± 0.7°C, with a photoperiod of 12 h of irradiance per day. Pots were watered daily. During 45 days the emerged seedlings (i.e. the emergence of cotyledons on the sand surface) were scored daily and removed. At the end of the experiment, the sand on the pots was examined and non-germinated intact seeds and split empty seed coats were recorded for each replicate. Non-emerged seedlings were determined by subtracting the number of emerged seedlings to the number of empty seed coats for each replicate. Any remaining, intact non-germinated seeds were sown immediately at 15°C in the light on the surface of 1% water agar in 60 mm plastic Petri dishes to check their viability and germination capacity.

Experimental seed burial

Experimental seed burials were carried out in July 2009 at the time of natural seed dispersal at the site of the original population, following the protocol of Arroyo et al. (2004), modified as reported below. Sets of 3 replicates containing 10 seeds each were introduced into fine grain nylon mesh envelopes which were placed in plastic nets. The envelopes were filled with sieved local soil and buried so that the seed envelopes were at a depth of 5, 10 and 15 cm. After 1 year, the replicates were exhumed. Any remaining, intact, non-germinated seeds were sown immediately at 15°C in the light on the surface of 1% water agar in 60 mm plastic Petri dishes to check their viability and germination capacity.

Data analysis

Theoretical cardinal temperatures were evaluated by determining the seed germination rate (GR), defined as the reciprocal of T_{50} (time to reach 50% of the maximum germination in one replicate) for the germination tests carried out at constant temperatures (5-25°C). The data for each seed lot were regressed using a linear model to estimate the base temperature (tb) and the ceiling temperature (tc), by average of *x*-intercept for both sub-optimal and supra-optimal temperature ranges (Ellis et al. 1986). The optimum temperature (to) was calculated as the intercept of sub- and supra-optimal temperature response functions (Hardegree 2006). The mean time to seedling emergence (MTE) was estimated according to the formula: $MTE=\Sigma (ni\times di)/N$, where ni is the number of emerged seedlings at day i, d the incubation period in days and N the total number of emerged seedlings in the treatment. Analysis of variance (One-way and two-ways ANOVA) was applied for final germination and seedling emergence percentages and MTE values, after verifying that the ANOVA assumptions were accomplished (homogeneity of variance, verified by Bartlett test and normal distribution by Shapiro-Wilk test). Subsequently *post hoc* Fisher's least significant difference test (LSD) was carried out. All the statistical analyses were carried out by using R v. 2.11.1 (R Development Core Team 2009).

Results

Seed germination

At the end of the preliminary germination test carried out at 15°C, *D. morisianus* seeds reached high germination percentages both in the light (100%, 97.33 ± 4.62% and 94.67 ± 6.11% for 2006, 2008 and 2009 seed lots, respectively) and in the dark (97.33 ± 2.31%, 94.67 ± 6.11% and 94.67 ± 6.11% for 2006, 2008 and 2009 seed lots, respectively). The two-ways ANOVA highlighted no statistical differences (*p-value* > 0.05) for photoperiod (*p-value* = 0.3107) and seed lot (*p-value* = 0.4160) factors and for their interaction (*p-value* = 0.7375).

When seeds were sown at different temperature regimes in the light, the three seed lots showed the same germination behaviour, achieving their maximum germination at 15°C (100%, 97.33 ± 4.62 % and 94.67 ± 6.11 % for 2006, 2008 and 2009 seed lots, respectively; *p-value* > 0.05 by *post hoc* Fisher's LSD test) and lower values at either colder and warmer temperatures (Figure 1).

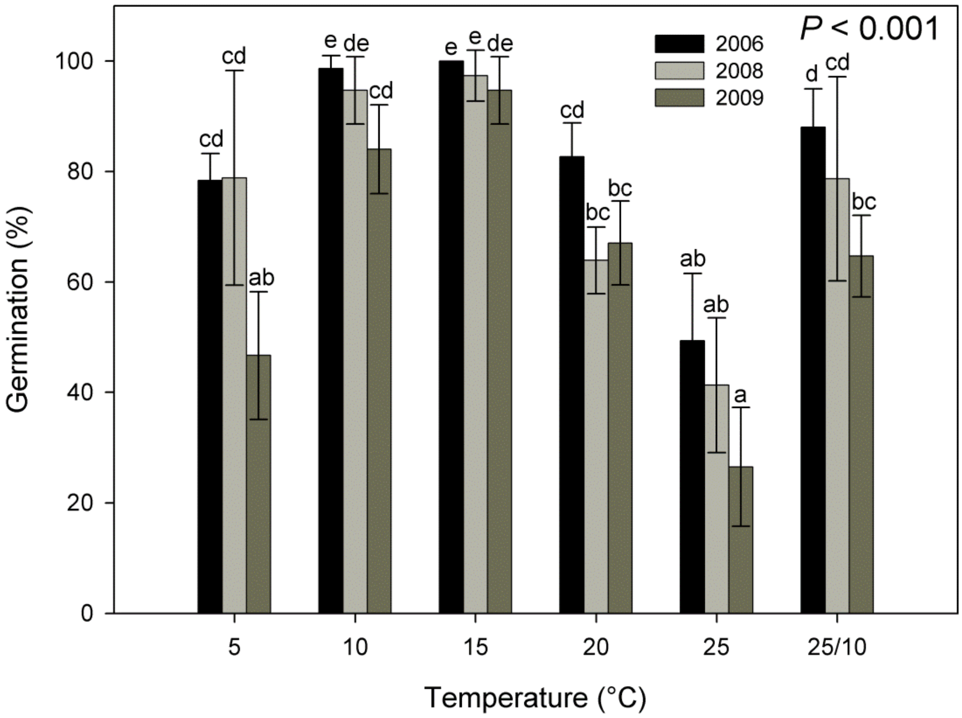

Figure 1 - Final germination percentages at each temperature regime in the light (12 h of irradiance per day) for the three seed lots. *P-value* < 0.001 by One-way ANOVA; bars with the same letters are not significantly different at *p-value* > 0.05 (post hoc Fisher's LSD test). Data are the mean of 3 replicates (± 1 standard deviation).

While the two stored seed lots (2006 and 2008) did not show any statistical differences in their final germination percentages with each others, the 2009 seed lot reached lower germination, respect to the other two seed lots at 5°C and 25/10°C and at 10°C and 20°C, respect to the 2006 seed lot, with this difference being statistically significant (*p-value* < 0.05 by *post hoc* Fisher's LSD test) (Figure 1). At 5°C final germination percentages were 78.33 ± 4.93 %, 78.84 ± 19.43 % and 46.67 ± 11.65 % for 2006, 2008 and 2009 seed lots, respectively, while at 25°C they were less than 50 % (49.33 ± 12.22 %, 41.33 ± 12.22 % and 26.53 ± 10.76 % for 2006, 2008 and 2009 seed lots, respectively; Figure 1). Alternating temperature regime (25/10°C) did not improve final germination for any of the three seed lots (*p-value* < 0.05 by *post hoc* Fisher's LSD test; Figure 1). The two-ways ANOVA highlighted a significant effect (*p-value* < 0.001) for both the seed lot and temperature factors, while no effect was detected for their interaction (*p-value* > 0.05).

The three seed lots showed their maximum germination rate (GR) at 15°C (with values ranging from 0.19 d^{-1} of the 2009 seed lot to 0.25 d^{-1} of 2006 seed lot) and lower values both at colder and warmer temperatures (Figure 2), as highlighted for their final germination percentages (Figure 1).

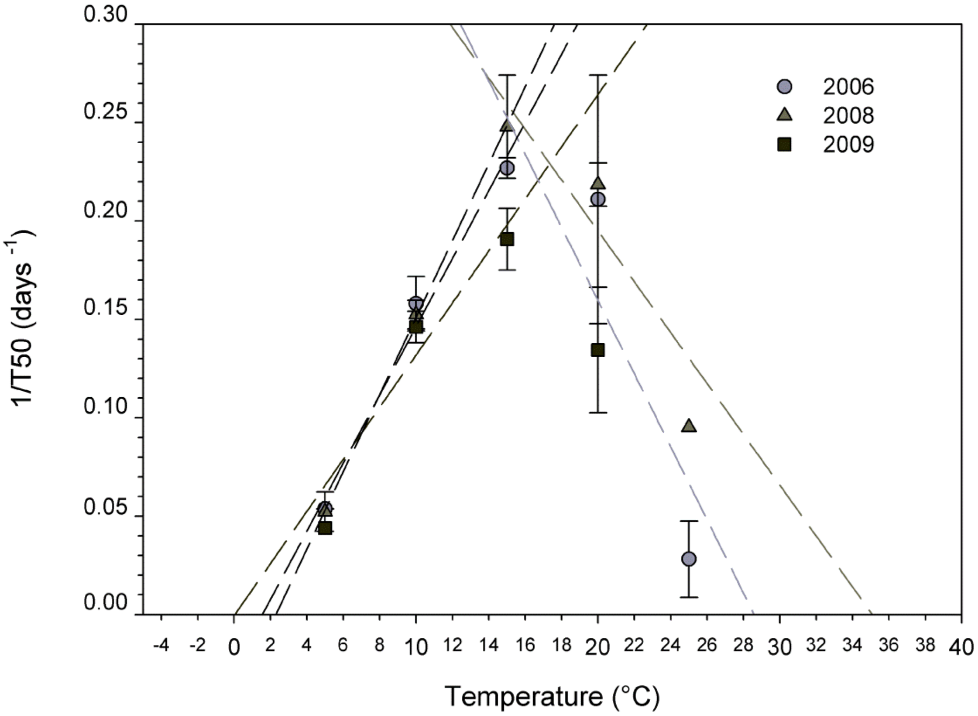

Figure 2 - Germination rates for the three seed lots calculated on the basis of the reciprocal of the T_{50} values. Points correspond to the actual data and solid lines indicate the fitted lines from the linear regressions. Data were the mean of three replicates ± 1 standard deviation.

The 2009 seed lot had a slower germination than the stored seed lots at all the tested temperatures and the low final germination percentage reached by this seed lot at 25°C did not allow calculating the T_{50} for this temperature. The regression equations and the cardinal temperatures calculated for each seed lot and overall are reported in Table 2. The base temperature ranged from ca. 0°C for the 2009 seed lot to 2.29°C for the 2008 seed lot, with a mean of 1.37°C, the ceiling temperature from ca. 28.5°C for the 2006 seed lot to 35.16°C for the 2008 seed lot, with a mean of ca 30°C and the optimal temperature was close to 15.5°C for the 2006 and 2008 seed lots and the average value (Table 2).

Table 2 - Regression equations, r^2 and *P*-values and cardinal temperatures estimated by linear sub- and supra-optimal models for germination rates, calculated for each seed lot and as their mean. tb: base temperature, tc: ceiling temperature and to: optimal temperature.

Seed lot	Function type	Model	r^2	*P*	tb	tc	to
2006	Sub-optimal	$y_1 = -0.0269 + 0.0173x$	0.98	< 0.0001	1.55°C		
	Supra-optimal	$y_2 = 0.5315 - 0.0186x$	0.67	= 0.0137		28.57°C	
		$y_1 = y_2$					15.55°C
2008	Sub-optimal	$y_1 = -0.0448 + 0.0196x$	0.99	< 0.0001	2.29°C		
	Supra-optimal	$y_2 = 0.4536 - 0.0129x$	0.74	= 0.0124		35.16°C	
		$y_1 = y_2$					15.33°C
2009	Sub-optimal	$y_1 = 0.0006 + 0.0132x$	0.89	= 0.0014	-0.04°C		
	Supra-optimal	-	-			-	
overall	Sub-optimal	$y_1 = -0.022 + 0.016x$	0.90	< 0.0001	1.37°C		
	Supra-optimal	$y_2 = 0.504 - 0.017x$	0.60	= 0.0003		29.85°C	
		$y_1 = y_2$					15.87°C

Seedling emergence

No seedlings emerged at the burial depth of 10 cm, few of them (15.28 ± 13.39 %) emerged from 5 cm and their percentage increased significantly (*p-value* < 0.05 by *post hoc* Fisher's LSD test; Figure 3) at depths ranging from 2 cm to soil surface (> 65 %), achieving their maximum at the depth of 1 cm (100%). Non-emerged seedlings, detected by examining the sand on the pots at the end of the experiment, were 90.28 ± 13.39 % and 73.55 ± 20.88 % at 10 and 5 cm of burial, respectively, while all germinated seeds emerged at upper depths (Figure 3). The relationships between soil depths and emerged and non-emerged seedlings were assessed by sigmoidal regression curves (Figure 3).

The mean time of seedling emergence (MTE) ranged from 11.70 ± 1.84 days for 5 cm to 18.54 ± 2.67 days for 0 cm of burial, with these differences being statistically significant (*p-value* < 0.05 by One-way ANOVA; Figure 3). Emergence was as faster as depth of burial decreased, even if this trend was not statistically significant (*p-value* > 0.05 for linear regression analysis).

The germination test carried out at 15°C in the light on the retrieved intact seeds allowed assessing their germination and viability for each burial depth (Figure 3). At the depth of 10 cm 5.56 ± 9.62 % of the seeds were died and 4.29 ± 4.35 % were still viable dormant seeds. At 5 cm of burial 4.17 ± 7.22 % of the seeds germinated after their exhumation and 7.19 ± 2.61 % were viable dormant seeds. At 2 cm the majority of the seeds (5.79 ± 27.35) germinated in the lab, 1.39 ± 2.40 % were still dormant and only 6.85 ± 8.10 % were died. From the sowing on the surface of the sand, none of the retrieved seeds germinated in the lab, with 20.81 ± 16.15 % being died and 10.64 ± 7.63 % viable dormant seeds.

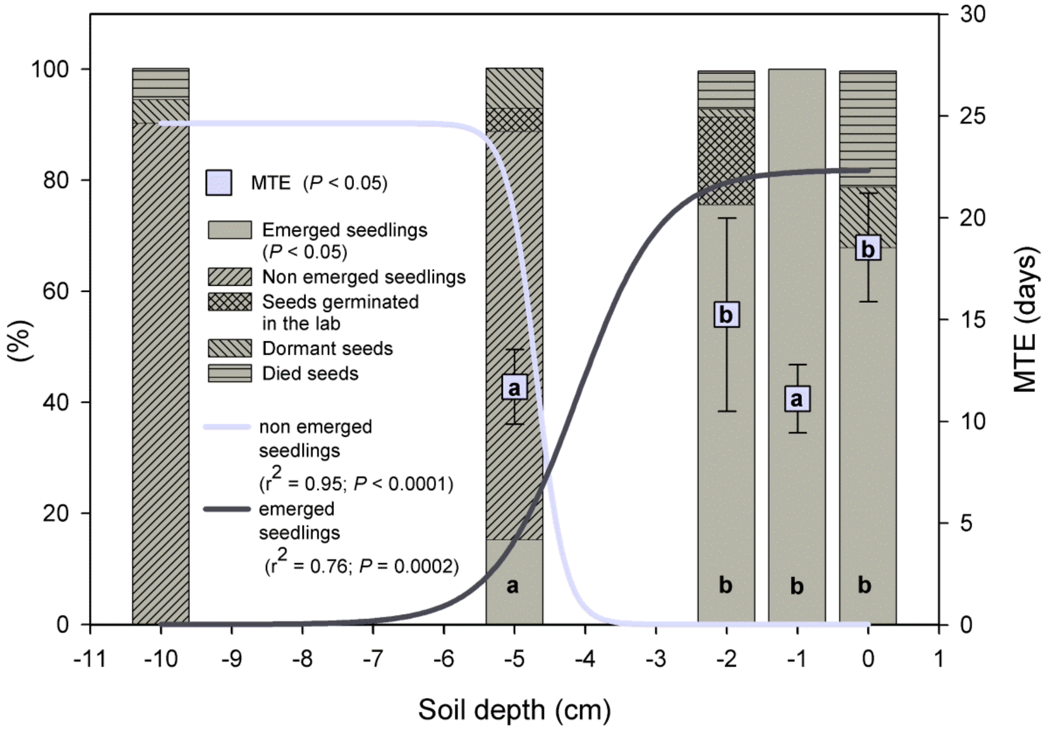

Figure 3 - Seeds and seedlings evaluation categories after sowing at 16.5 ± 0.7°C at different burial depths and the germination test at 15°C in the light carried out on the retrieved intact seeds. Viability of no germinated seeds was assessed by a cut-test. Data are the means of 4 replicates; bars with the same letters are not significantly different at *p-value* > 0.05 (*post hoc* Fisher's LSD test) for emerged seedling percentages. Solid lines indicate the sigmoidal regressions for emerged and non-emerged seedlings. Squares represent the mean time to emergence (MTE) ± 1 SD; squares with the same letters are not significantly different *p-value* > 0.05 (*post hoc* Fisher's LSD test).

Seed persistence in the soil

After one year of burial very few seeds were retrieved intact at all the depths (5, 10 and 15 cm). The great majority of the seeds (73.33 ± 5.77% at 5 cm and 90.00 ± 10.00% at 10 and 15 cm) germinated during the year and empty seed coats were retrieved, showing no statistical differences between depths of burial (*p-value* > 0.05 by One-way ANOVA). All the exhumed remaining intact seeds germinated after sowing immediately in the incubator at 15°C in the light.

Discussion

According to the results achieved in this study, *D. morisianus* seeds are non-dormant (*sensu* Baskin & Baskin 2004), as all seed lots germinated in a wide range of conditions, without any pre-treatment. Maximum germination was achieved at 10-15°C (> 80%), while germination percentages mainly decreasing at temperatures > 15°C, as detected for other Mediterranean species (Thanos et al. 1989, 1995). However, seeds collected in 2006 and 2008 and then stored at 5°C for 3 and at –25°C for 1 year, respectively showed a wider germination range and higher (although not always statistically significant) germination percentages, both at 5°C and > 15°C, respect to the fresh seeds collected in 2009. Moreover, stored seed lots germinated faster at all the tested temperatures than the fresh seed lot, with these differences being higher in the supra-optimal range (> 15°C). A similar effect was detected by Thanos et al. (1995) in seeds of *Satureja thymbra* L., where germination range, final germination percentage and germination rate were positively related to the age of the seed lot. A widening of the temperature range for germination is one of the effects of "dry after-ripening", i.e. a period of usually several months of dry storage of freshly harvested, mature seeds and the rate of dormancy release is usually faster at warmer temperatures (Finch-Savage & Leubner-Metzger 2006). After dispersal in summer, *D. morisianus* seeds experience a dry period (from June to September), characterized by high temperatures associated to soil aridity caused by the lack of rainfall and a low capillarity and water-holding capacity of the sand soil. This period, during which conditions are unsuitable for germination may, however, enhance germination in the subsequent autumn-early spring. From November, mean air temperatures drop to less than 20°C and water availability achieves its maximum as highlighted by the climatic data (Table 1), allowing seeds to germinate.

The maximum germination rate detected for this species (T_{50} ranging from 4 to 5 days at 15°C) was higher respect to other Mediterranean maritime plants such as *Glaucium flavum* (T_{50} of ca. 30 days for untreated seeds at 10°C; Thanos et al. 1989) and *Muscari* spp. (T_{50} ranging from 12 to 16 days at 10°C; Doussi & Thanos 2002). Rapid germination has been suggested to be an adaptation for rapid establishment in advance of soil drying in unpredictable environments (Daws et al. 2002). The fast germination of *D. morisianus* seeds suggested, therefore, that this species is well adapted to a rapid establishment in harsh conditions, as detected by Thanos et al. (1994) for *Matthiola tricuspidata* (maximum germination reached within 3 days at 15 and 20°C), a psammophilous Mediterranean species with a "weedy" opportunistic strategy, and by Mattana et al. (2010) for *Centranthus ruber* L. (T_{50} of 4.5 days at 15°C) a widespread species with a mainly Mediterranean distribution, living in stressed habitats.

The photoinhibition for germination detected by Thanos et al. (1989, 1991) in Mediterranean maritime plants, enable seeds (and eventually seedlings) to avoid germinating (and establishing) at the harsh conditions of the surface (e.g. in the water-stressed, sand or shingle, Mediterranean beaches). However, *D. morisianus* seeds, which achieved high germination percentages both in the light and in dark, did not show this kind of surface avoiding mechanism. This indifference to irradiance and the lack of an alternating temperature regime requirement for germination, suggest that *D. morisianus* seeds are more likely to germinate under the canopy of *Juniperus* spp. and *Quercus calliprinos* micro-forest. In fact, under tree canopy, diurnal temperature alternations are known to be much lower than in the open gaps (Daws et al. 2002). On the other hand, light acts as a depth-sensing mechanism, thereby avoiding possible fatal germination of small seeds buried too depth in the soil (Grime et al. 1981; Milberg et al. 2000). Small seeds are more likely to require light to germinate, as found in several studies both in tropical (seed mass < 0.7 ± 0.4 mg for neotropical pioneer species; Pearson et al. 2002) and temperate climates (seed mass < ca. 1.5 mg for temperate forest herbs; Jankowska-Blaszczuk & Daws 2007). The light indifference for germination detected for *D. morisianus* seeds is consistent with the mean seed mass of ca. 1.2 mg, allowing buried seeds to germinate far from the arid sand surface. Bond et al. (1999) found that there is an allometric correlation between the maximum depth at which seedling emergence and seed mass [maximum depth (mm): 27.3 x seed weight$^{0.334}$ (mg)]. According to this model, *D. morisianus* seedlings should not be able to emerge from depths > 2.9 cm. Laboratory experiments showed that seedlings belonging to the seeds placed on the soil surface established poorly, while the optimal seedling emergence depth of this species was between 1 and 2 cm of burial. However, few seedlings (ca. 15%) emerged from a depth of 5 cm and therefore deeper than the theoretical maximum depth of ca. 3 cm. This emergence pattern may be an adaptation to the spatial and temporal variation of the incoherent sand substrate, that can exhumate seeds buried too close to the soil surface.

Thompson et al. (1993) found a relationship between seed shape and mass and seed longevity in the soil and concluded that small and nearly spherical seeds, with a seed mass < 3 mg, tend to form persistent soil seed banks. *D. morisianus* seeds, with a mean size of ca. 1.2 mg should be persistent, but the results of the seed burial experiments showed that *D. morisianus* is unable to form a persistent soil seed bank. Even though that *D. morisianus* seeds are below the threshold individuated by Thompson et al. (1993), they have a flattened shape, so they may have a higher variance of seed dimensions to form a persistent soil seed bank, as confirmed by the findings of Cerabolini et al. (2003) who detected a transient soil seed bank for other three species of *Dianthus* genus (*D. monspessulanus* L., *D. seguierii* Vill. and *D. sylvestris* Wulfen). Zhao et al. (2011) in a study of seed persistence in a sand soil of Northern China, found that seed mass and shape were significantly and negatively correlated to persistence in the soil, but no obvious threshold of seed mass and seed shape was detected as established by Thompson et al. (1993) to distinguish persistent from transient species. Moreover, Yu et al. (2007) found that, in a Mediterranean coastal sand dune ecosystem, species with bigger seeds tend to have persistent soil seed bank than those with smaller seeds. Many species of dune systems and of the foredunes in particular, create a transient soil seed bank as they grow in habitats with recurrent sand movement, high wind velocities and full sunlight, being, therefore too varied to create a permanent soil seed bank (Planisek & Pippen 1984; Ehrenfeld 1990; Maun 2009). The lack of a permanent soil seed bank detected for *D. morisianus* seems to confirm this pattern also for species of coastal backdunes.

Among the phases of the transitional stage from seed dispersal to seedling establishment analyzed in this study for *D. morisianus*, seedling emergence seems to be the most critical for the long-term persistence of this species. While seeds can germinate with high percentages in a wide range of conditions, they should germinated at the narrow optimal depth of 1-2 cm, avoiding at the same time the arid soil surface and too deep burials. In a dynamic environment such as the coastal backdunes where this plant grows, a very few seeds per year can match these conditions allowing seedling establishment and their subsequent recruitment. The lack of a persistent soil seed bank detected for this species highlights that the fate of the seeds that do not emerge from the soil in the spring after dispersal is to die before the next favourable growing season.

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CHAPTER 9

THE EFFECTIVENESS OF PLANT CONSERVATION MEASURES: THE REINTRODUCTION OF *DIANTHUS MORISIANUS*

Donatella Cogoni, Giuseppe Fenu, Erica Concas & Gianluigi Bacchetta

Endemic species have a high conservation priority, because they are exclusive to a region and are often geographically restricted and ecologically infrequent (Hernández & Gómez-Hinostrosa 2011) and any unfavorable change may cause its rapid extinction (Callmander et al. 2005). In species-rich areas, such as Sardinia, exclusive endemics deserve conservation priority, since the disappearance of local populations determine the whole extinction of these species.

Plants reintroduction, a general term that describes the controlled placement of plant material into a natural or managed ecological area (Godefroid et al. 2011), is still a relatively recent science and a potentially important restoration tool for biodiversity conservation. The basic of reintroductions is establishing new or augmenting existing populations in order to increase a species' survival prospects (Pavlik 1996) and then reintroduction has been encouraged as an extinction prevention strategy for plant species (Falk et al. 1996; IUCN 1998). The ability of reintroduction to contribute to endangered species recovery is significant, and enhanced when it is part of larger, integrated strategies that encompass *in situ* and *ex situ* activities (Albrecht et al. 2011).

Dianthus L. (*Caryophyllaceae*) is one of the most diverse genus in Europe, characterized by large numbers of endemic *taxa* (more than 50% of them are narrow endemic); in particular, rates of *Dianthus* diversification have been exceptionally high in Mediterranean area, where geographic speciation model prevails (Valente et al. 2010). In Sardinia 8 endemic *Dianthus* have been recorded, 4 of them narrow distributed; among these *Dianthus morisianus* Vals., with only one population growing on the coastal dune of Buggerru (SW Sardinia) has been considered one of the most threatened plants of the island (Bacchetta et al. 2012). *Dianthus morisianus,* a perennial plant, is the only member of this genus growing on Mediterranean sandy dunes, in ecological contact with junipers micro-forests (Bacchetta et al. 2010; Fenu et al. 2011). The extent of the its population, assessed by field surveys, consists of ca. 17,50 ha, divided into two nucleus of which the core cover ca. 17 ha (Figure 1). In the last decades the natural habitat of *D. morisianus* was strongly modified by human activities, determining habitat loss and fragmentation. The small size population, the low number of mature plant and the limited seedling recruitment were a serious threats making *Dianthus morisianus* prone to extinction. As a consequence, it is listed as critically endangered in the European (Bilz et al. 2011) and in the Global Red List (Fenu et al. 2011).

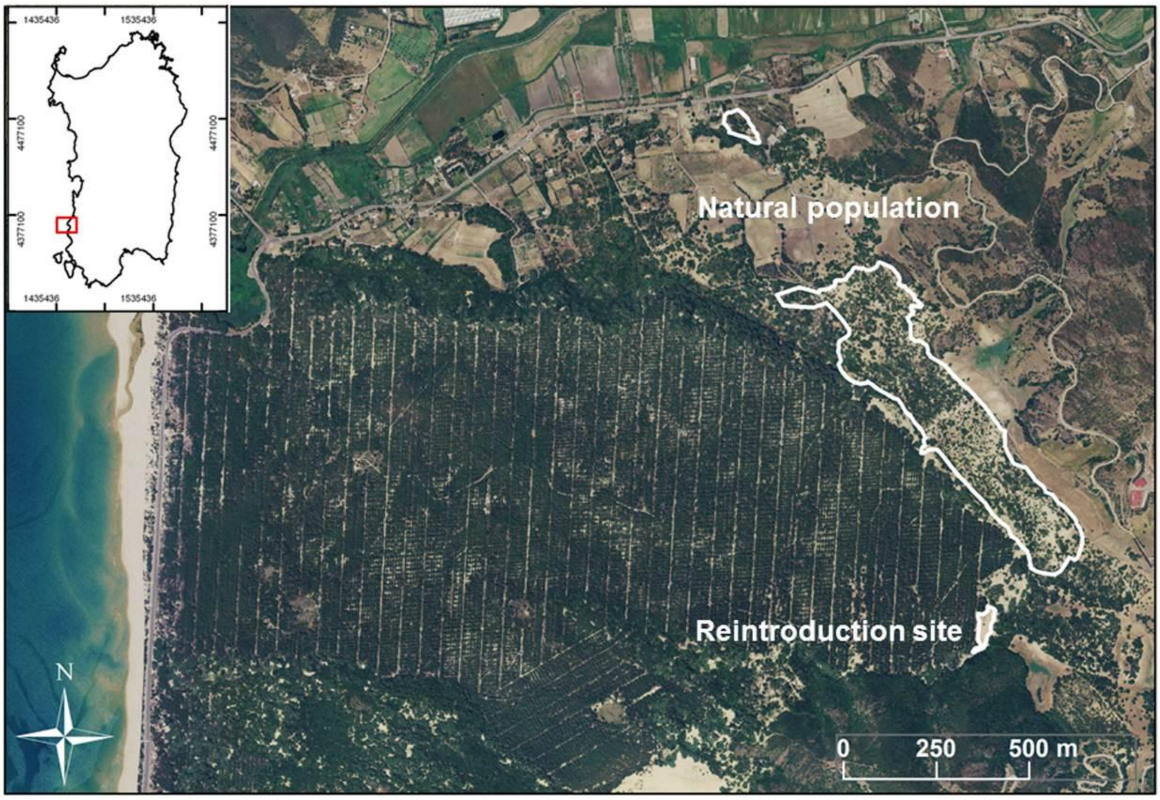

Figure 1 – The two nucleus of wild population and the experimental site of reintroduction of the threatened *Dianthus morisianus* located in the Portixeddu dunal systems (Buggerru, SW Sardinia).

Considering the "regional responsibility" criterion (*sensu* Gauthier et al. 2010) and the "priority list" of the most threatened exclusive endemic plants in Sardinian (Bacchetta et al. 2012), *D. morisianus* has been included in an integrated conservation project, funded by the "Regione Autonoma della Sardegna", that comprise *in situ* and *ex situ* activities; experimental project, such as construction of protective fences (Fenu et al. 2012) and reintroductions, are also included in the project.

Knowledge of the species' ecology and reproductive biology is essential prior to reintroduction (Falk et al. 1996; IUCN 1998), thus in order to understand the habitat requirements, we first examined carnation's ecology and in particular the relationships whit the canopy cover (with increasing tree density and canopy closure, the species presence decreases). Therefore, we asked if human disturbance negatively affects carnation emergence and establishment. Based on these observations, a suitable area was selected, at ca. 150 m as the crow flies of the natural population (Figure 1), included in a site managed by Public Administration (EFS – "Ente Foreste della Sardegna"), protected by human disturbance and animal grazing.

Even though reintroduction success increased by using material originating from multiple populations (Vergeer et al. 2005), when working with a narrowly distributed species that is impossible; we uses transplants germinated from wild-collected seeds in different years, in order to include a sufficient genetic diversity. Fruits collecting were carried out in the wild population in 2008 and 2009 by sampling 50 mature plants; seeds were processed separately at Sardinian Germplasm Bank (BG-SAR). Cogoni et al. (2012) demonstrated that this species seedling emergence and establishment were the most critical stage for *D. morisianus*, then we scheduled a reintroduction program based on juvenile plants. In laboratory, 200 seeds (100 per collection) were sown and incubated at the optimal temperature (Cogoni et al. 2012). After germination, all seedlings produced (92 and 94 for the first and second sowing, respectively) were placed in pots with a substratum composed by sand collected in the natural population site. Growing process of a plant relies on adaptation to environmental conditions, thus multiplication should involve the hardening of plants for future planting into natural habitats, decreasing the stress of the planting out process and increasing survival (Aguraiuja 2011); accordingly, no horticultural precautions (i.e. addition of organic matter, fertilizers, etc.) were adopted. Plants were cultivated at the Botanic Gardens of Cagliari University for 12 and 10 months for transplants germinated in 2008 and 2009, respectively. In November 2010, all survived plant (113, with 50 and 63 for the first and the second sowing, respectively) were reintroduced in 9 groups near each other, located in the same habitats were *D. morisianus* grow, and these were monitored monthly.

A key qualitative measure of the ultimate fate of reintroductions is the survival rates (Godefroid et al. 2011) and the ability of transplants to flower and set fruit (Menges 2008). Moreover, recruitment of new individuals is a critical measure of reintroduction success (IUCN 1998; Godefroid et al. 2011). Although survival, flowering and fruiting rates are generally low (Godefroid et al. 2011), the survival rates obtained in this projects is extremely high and the majority of plant living after one year (Table 1). The flowering and fruiting rates confirm the good outcome of this project: ca. the 40% of plant result reproductive producing a mean number of fruit per plant highest to the mean value calculated in the natural population (2.60; Cogoni et al. data unpublished; Table 1). The high number of fruits per plant and new seedlings (Table 1) were important metric for measuring the success of a reintroduction (Godefroid et al. 2011). Our study confirm the importance of finding the most appropriate micro-habitat, prerequisites unique to each species, was a key in the reintroduction program (see Menges 2008; Reckinger et al. 2010); in fact, if you can find the suitable habitat any management (i.e. watering, reducing the competition with other species, maladaptation, etc.) will not be needed (Aguraiuja 2011).

Table 1 - General summary of reintroduction results obtained after 18 months in the 9 different nucleus, consisting of two plant generations randomly assembled. All activities were carried out in collaboration whit local and regional Public Institutions (Ente Foreste della Sardegna).

Group	No. plant reintroduced	No. dead plant	Mortality rate	flowered plant (%)	fruited plant (%)	Mean fruits per plant	New seedlings*
1	12	1	0.083	33.33	25.00	2.33±2.31	3
2	10	0	0	40.00	40.00	4.00±2.45	7
3	9	0	0	66.66	66.66	4.50±1.52	9
4	15	0	0	46.66	46.66	3.14±1.95	25
5	15	0	0	40.00	33.33	3.60±2.07	1
6	6	0	0	33.33	33.33	1.50±0.71	0
7	20	2	0.100	25.00	25.00	3.20±2.77	0
8	16	0	0	56.25	56.25	5.78±3.27	47
9	10	1	0.100	40.00	30.00	2.67±1.15	0
Overall	**113**	**4**	**0.035**	**41.59**	**38.94**	**3.84±2.48**	**92**

* surveyed in 21.01.2012.

A positive relationship between the number of reintroduced plants and their survival has been confirmed and using larger plants generally improved the success of reintroductions and reduced the mortality rate (Reckinger et al. 2010; Godefroid et al. 2011). Demographic and genetic theories both predict that the persistence time of a population increases with its initial size (Robert et al. 2007), and demographic stochasticity is determinant in populations with fewer than 50 plants (Menges 1991). Knowledge on the species biology, in particular the critical stage of their life-cycle, is crucial in orienting the choices of how to plan for the plant reintroduction (Maunder et al. 2004). Furthermore, *ex situ* expertise in plant multiplication and in cultivation procedures, emulating natural conditions and with minimal maintenance, were important steps in a reintroduction program (Hardwick et al. 2011; Aguraiuja 2011).

Although many years and long-term monitoring of survival and establishment to evaluate the success of reintroductions are needed (Godefroid et al. 2011), this project shows positive results for all indicators proposed in previous studies (Menges 2008; Godefroid et al. 2011).

Reintroduction is generally recognized as a relatively high-risk and high-cost activity (Gorbunov et al. 2008). However, this project is an example of a low-cost reintroduction through voluntary involvement of researchers, public authorities and local stakeholders, and without intensive site management. Finally, this study confirmed the importance of an integrated conservation approach which combined in the same project field analysis, *ex situ* studies (i.e. research on the seed biology) and the expertise of a number of stakeholders involved in biodiversity conservation.

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CHAPTER 10

CONCLUSION

In this study the population size, plant density and the distribution area of the two threatened coastal plants has been analyzed during three years. The answers to the main questions outlined in the general introduction can be summarized in the following points:

A. littorea is an annual plant, with a short-live annual cycle. Its population consist of two distinct metapopulation, distant ca. 700 m each other. In the high dynamic habitat in which it grows, it showed considerable spatio-temporal variation, resulting from a balance between the colonization of new sites and extinction process of old sites with the possibility of an high turnover of metapopulations. Also a important fluctuation in number of plants has been observed. Their moving through time and space across the entire dune system, suggest that the population size and dynamic of this species is extremely difficult to predict and that the “core population” moving inside the dune system over the years.

A. littorea presented a flowering and fruiting season totally overlapped and it showed a long reproductive period. This pattern may should be considered as an adaptive advantage in their stressed habitat, where there is high ecological variability in time and space. In fact, plant with extended flowering seasons can be less seriously affected by the effects of this environmental unpredictability than species with concentrated flowering seasons. The extended blooming period can increase the individual’s chance of having a large number of mates both as pollen donor and recipient and it reduces the risk of reproductive failure. High level of reproductive synchrony between metapopulations was found and this should be an advantage because it could promote cross-pollination among distant individuals, thus enhancing genetic diversity, and it also helps to avoid competition for pollinators. Number of seeds per plant showed high annual variability, with years in which low seed production is recorded. Our results reveal that *A. littorea* does not create a persistent soil seed bank (PSB)and this pose a strong threat to the population persistence. In fact the long-lived soil seed bank is essential to population persistence in a stochastically varying environment and the lack of a long-term PSB can have important implications for the population dynamics of an annual species; indeed, soil seed bank reduces the risk of extinction in particularly bad years and increases the effective population size.

An early emergence annual pattern in *A. littorea* seedlings was identified. The advantage of early emergence is that plants have time to establish, flower, and finally bear fruits, i.e., complete their life cycle and produce new propagules. Our result showed that earlier emerging seedlings grow better than later emerging seedlings and the majority of earlier emerging individuals went from seedling to adult stages. This suggests that this could have a strong effect on their contribution to the population growth rate.

A micro-habitats affect in the temporal distribution patterns of seedling emergence has been demonstrated in *A. littorea*, whereas seedlings survival were statistically non-significant between the two micro-habitat.

Five years analysis of *A. littorea* corroborates a several impact from human pressure (in term of human trampling) on this population. The human disturbance represents the main threat and consistently reduced the abundance of this species*;* the overall decline of this threatened indicates that human trampling that occur in this habitat can dramatically affect plant population. The percentage of reproductive plants varies annually and the mean number of fruits per plant did not vary significantly between trampled and untrampled areas. However, at population level, the cumulative contributions in number of fruits (and consequently seed output) vary significantly and the probability of population persistence depends primarily on the seeds produced by the plants present in untrampled areas.

Dianthus morisianus population structure result stable over time and didn’t show fluctuations in population size. Plants showed high survival and growth rates and use all available resources to grow and to pass to the following classes. The longevity of individuals represents an important feature for the population survival especially in highly selective habitat, like arid dune systems, where large periods with adverse conditions are common and limit the recruitment rate. No general decline in population sizes was found, however, the results of our study demonstrate that habitat losses plays a crucial role in the process of population decline of this endangered species. Habitat fragmentation, due of the expansion of agriculture (wood plantation in particular), infrastructure for tourism and the construction of roads which determine an high frequency of human at the locations, pose a severe threat for *D. morisianus* persistence.

Our results highlight that in *D. morisianus* population there was a massive bloom, concentrated in a narrow period of time, sowing a high degree of synchrony within the population. *D. morisianus* is adapted to severe limitation like summer drought and low resources availability and this may be considered as a disadvantage for the phenological responses of the species. On the contrary, *D. morisianus* is adapted, due this restricted favourable condition, to use all available resources to produce flowers and fruits. An important factor which could affect reproductive success of *D. morisianus* is plant size*:* larger plants produced more flowers and fruits and seem to be suffered a lower proportion of seed predation than smaller plants. Another important factor which could affect reproductive success is the plant damage due to livestock grazing. In *D. morisianus* population, reproductive stems were more damaged than the basal rosette, more protected by the vegetation and, in particular, reproductive stems with ripe fruits are mainly damaged than flowering one. Despite grazing intensity on reproductive stems being vary yearly no difference in the number of fruits output at population level are detected and a considerable number of germinable seeds per plant was recorded each year, indicating that *D. morisianus* should be considered, as a micro-sites limited species, considering its ecological requirements and not as a seed-limited species.

Among the phases of the transitional stage from seed dispersal to seedling establishment analysed in this study for *D. morisianus*, seedling emergence seems to be the most critical for the long-term persistence of this species. While seeds can germinate with high percentages in a wide range of conditions (with a maximum germination achieved at 10-15°C), they should germinated at the narrow optimal depth of 1-2 cm, avoiding at the same time the arid soil surface and too deep burials. In a dynamic environment such as the coastal backdunes where this plant grows, a very few seeds per year can match these conditions allowing seedling establishment and their subsequent recruitment. The lack of a persistent soil seed bank was detected for this species; this highlights that the fate of the seeds that do not emerge from the soil in the spring after dispersal is to die before the next favourable growing season.

The new data archived in this study allowed to define the main threats acting on these threatened plants and to confirm the Critically Endangered IUCN category for both species. Both species are projected to be subject to the continuous decline if conditions remain constant, although the extent and cause of the decline differ between species.

The conservation of *A. littorea* population needs a program based on site protection (extended to the whole dunal system of Is Arenas); in particular this strategy, which should provide the limitation of human disturbance (trampling), is the key to ensuring the long-term population survival.

In addition, in order to actively manage populations, a reintroduction project, as the *D. morisianus'*s reintroduction, is an example of the possibilities for linking *ex situ* conservation activities with in situ conservation goals. The success of this action suggests one possibility for restoring the declining natural populations and it may be essential to maintain them.

In conclusion, the investigation of each biology aspect about these psammphilous endangered plants yielded the information needed in order to identify the critical aspects that affect the survival and to guide the proposal and implementation of conservation measures.

The present work was based on a three year study and a more prolonged and detailed monitoring is needed in order to provide a more precise informations of populations and to identify the critical aspects that affect the survival of these species. In particular some plant traits (i.e. reproductive biology, interaction between pollinations and predations, population genetics etc.) should be investigate to complete of these threatened endemic species an ensoure their persistence in the wild.

APPENDIX 1 – Scheda Informatore Botanico Italiano 42(2): 601-603

Dianthus morisianus Vals.

G. FENU, D. COGONI, E. MATTANA, G. BACCHETTA.

Nomenclatura:

Specie: *Dianthus morisianus* Vals.
Sinonimi: *D. siculus* J. Presl *&* C. Presl subsp. *morisianus* (Vals.) Arrigoni
Famiglia: *Caryophyllaceae*
Nome comune: garofano del Moris

Descrizione. Pianta perenne debolmente legnosa alla base, con radice fittonante, lassamente cespitoso-suffrutticosa, (20)30-50(75) cm alta. Foglie basali erette o eretto-patenti, inserite lungo i rami legnosi, lunghe da 2,5 a 15 cm e larghe 1,5-2 mm, uninervie, acute all'apice, con margine serrulato, guaina fogliare aperta, incisa e larga 3 mm. Foglie caulinari gradatamente ridotte, lunghe 1-15 cm, erette o eretto patenti. Scapi fiorali 20-45(65) cm lunghi, eretti, con 4-6 internodi, ramificati fin dai primi nodi, con rami alterni e opposti. Infiorescenza (1)2-5(10) flora, a cima dicotomo-ramosa con internodi gradatamente raccorciatesi. Epicalice formato da 4(6) squame appressate, con mucrone di 2-3,5 mm di lunghezza; quelle più interne subarrotondate, lunghe 7-8 mm e larghe 5-5,5 mm; quelle esterne ellittiche o, raramente, ellittico-lanceolate, lunghe 6-6,5 mm e larghe 2,5-3,5 mm. Calice cilindrico, 25-30 mm lungo, 4,5-5,5 mm in diametro, striato superiormente; denti lanceolati, acuti, 5,5-7 mm lunghi, striati, membranacei al margine. Petali di colore variabile da bianco-rosato a rosa intenso, lunghi 35-40 mm, con unghia bianca lunga 23-26 mm, lembo rosa cuneato-arrotondato, 10-15 x 8-11 mm, con 6-8 denti irregolari, 0,3-1 mm lunghi. Antere lineari-ellittiche lunghe 4,5 mm. Ovario lungo 7,5 mm; stilo e stigma lunghi 14 mm. Capsula cilindrico-oblunga di 15-20 mm, brevemente stipitata e inclusa nel calice fruttifero. Semi neri di 4 x 3 mm, obovati, con rare e sottili strie raggiate (VALSECCHI, 1985; BACCHETTA *et al.*, 2009).

Biologia. *Dianthus morisianus* è una camefita suffruticosa che fiorisce da maggio a giugno e fruttifica tra giugno e luglio. Il periodo di riposo vegetativo, caratterizzato da completa perdita delle foglie, inizia subito dopo la fruttificazione e si protrae sino all'autunno inoltrato; alla ripresa vegetativa, con l'accrescimento dei vecchi fusti, si ha la produzione di nuovi getti, al cui apice si sviluppano gli scapi fioriferi l'anno successivo.
L'unità di dispersione è costituita da semi piatti e leggeri che non presentano adattamenti a particolari tipi di dispersione, cadendo nel suolo a maturità della capsula. I semi di *D. morisianus* non richiedono particolari necessità per la germinazione e non presentano fenomeni di dormienza. Come la maggior parte dei semi delle piante d'ambiente tipicamente mediterraneo, mostrano una preferenza per le basse temperature, germinando con percentuali elevate (> 80%) al di sotto dei 20°C e raggiungendo il 100% di germinazione a 15°C. Le prove sperimentali realizzate a 15, 20 e 25°C hanno evidenziato, infatti, una progressiva riduzione della percentuale finale e della velocità di germinazione all'aumentare della temperatura (MATTANA, 2009). Pertanto la moltiplicazione per seme potrebbe rappresentare un'efficace strategia di conservazione.
La specie presenta numero cromosomico 2n = 30 (VILLA, 1991).

Ecologia. Specie psammofila che vegeta in cenosi arbustive e/o ai margini delle boscaglie a ginepro e quercia di Palestina. La specie si ritrova sulle dune interne stabilizzate o parzialmente stabilizzate di origine eolica, che affiorano lontano dalla linea di costa e presentano un elevato contenuto in sostanza organica.
Dal punto di vista bioclimatico si rinviene in ambito mediterraneo pluvistagionale oceanico, con termotipo termomediterraneo superiore e ombrotipo secco superiore.
Ancora non esistono analisi di tipo fitosociologico in grado di permettere una definizione delle cenosi a cui partecipa *D. morisianus*.

Distribuzione in Italia.
Regione biogeografica. La popolazione si rinviene nella regione biogeografica Mediterranea, Subregione del Mediterraneo Occidentale, Provincia Italo-Tirrenica, Subprovincia Sarda (Rivas-Martínez, 2007). Studi di carattere biogeografico di dettaglio (BACCHETTA, PONTECORVO, 2005; FENU, BACCHETTA, 2008; ANGIUS, BACCHETTA, 2009) evidenziano che la

popolazione di *D. morisianus* rientra nella superprovincia Italo-Tirrenica, provincia Sardo-Corsa, subprovincia Sarda, settore Sulcitano-Iglesiente, sottosettore Iglesiente, distretto Sud-Occidentale.
Regione amministrativa: la specie è presente esclusivamente in Sardegna.
Numero di stazioni: l'unica popolazione conosciuta è quella del *locus classicus*, coincidente con le aree più interne del campo dunale di Portixeddu (Fluminimaggiore - Buggerru).

Tipo corologico e areale globale. Endemismo esclusivo della Sardegna sud-occidentale.

Minacce. La specie è considerata una tra le più rare dell'endemoflora sarda e, recentemente, è stata inserita tra i 10 *taxa* endemici a maggiore rischio d'estinzione dell'Isola (FENU, 2009; MATTANA, 2009). Le principali minacce che insistono sulla popolazione sono riconducibili allo sfruttamento antropico del territorio; da un lato lo sfruttamento agricolo dell'area (Minaccia 1.1: *Agricolture*), sia per colture e pascolo (principalmente caprino) ma soprattutto per la presenza di un ampio rimboschimento a conifere (Minaccia 1.1.2: *Wood plantations*) che ha modificato l'intero campo dunale; a questo si associa l'utilizzo a scopi turistici (Minaccia 10.1: *Recreation/tourism*), con l'apertura di strade e sentieri, costruzione di abitazioni, calpestio, etc.
Tra le minacce merita segnalare l'elevato rischio di incendio che insiste su tutta l'area (Minaccia 10.5: *Fire*); nell'estate 2009, infatti, un rogo ha interessato il territorio, determinando una sensibile riduzione della popolazione.
Altre minacce che insistono sulla popolazione sono invece legate alla biologia stessa della specie e in particolare il basso tasso di *recruitment* osservato (Minaccia 9.2: *Poor recruitment/reproduction/regeneration*), la bassa densità d'individui (Minaccia 9.5: *Low densities*) e la riduzione della variabilità genetica della popolazione (Minaccia 9.4: *Inbreeding*), che determinano conseguenze sul successo riproduttivo manifestandosi spesso come una riduzione dell'efficienza dei processi d'impollinazione.

Criteri IUCN applicati. L'assegnazione di *D. morisianus* a una categoria di rischio è stata effettuata utilizzando il criterio B.

Criterio B
Sottocriteri
B1-Areale (EOO): 0,65 Km^2.
B2-Superficie occupata (AOO): 4 Km^2.
Superficie occupata effettiva: circa 650.000 m^2.
Opzioni
a) Numero di "location". Si conosce solo una popolazione di dimensioni ridotte, corrispondente al *locus classicus*, ed è presente un'unica *location*.
b) (iii). Declino della qualità dell'habitat. Sulla base delle minacce riscontrate e dei monitoraggi realizzati in questi ultimi anni, è stato possibile osservare un crescente degrado della qualità dell'habitat, legato alle attività agricole e ricreative. Nell'ultimo anno è stato osservato inoltre un aumento del calpestio (sia legato al pascolo che al transito di persone) con apertura di nuovi sentieri nella popolazione; tali pratiche stanno portando alla progressiva frammentazione della popolazione per un crescente degrado della qualità dell'habitat. A questo si deve sommare il costante rischio d'incendio che, periodicamente, determina modificazioni e riduzione dell'habitat disponibile per la specie.
Inoltre, la presenza all'interno del campo dunale di coltivi, con lavorazioni meccaniche del terreno e aree destinate a pascolo, impediscono alla specie di colonizzare spazi limitrofi ed estendere l'area della popolazione, contribuendo allo stesso tempo alla frammentazione della stessa.
b) (v) Declino del numero d'individui maturi. Sulla base delle osservazioni dirette relative alle difficoltà riproduttive della specie e all'elevata percentuale di frutti parassitati, si può dedurre una riduzione nel tempo del numero di individui maturi, anche a seguito della progressiva alterazione del sito in cui si rinviene la popolazione.

Categoria di rischio.

In base al criterio B, quindi alla distribuzione geografica della specie, alla possibile riduzione, nel breve periodo, di EOO e AOO, del ridotto numero di individui maturi e delle difficoltà riproduttive in natura, *D. morisianus* deve essere considerata gravemente minacciata.
Categoria di rischio: Critically Endangered, CR B1ab(i,ii,iii,v)+2ab(i,ii,iii,v)..

Interazioni con la popolazione globale. La popolazione regionale corrisponde alla popolazione globale.

***Status* alla scala "regionale".** CR B1ab(*i,ii,iii,v*)+2ab(*i,ii,iii,v*);
- status a scala globale: *Not evaluated* (NE).
- precedente attribuzione a livello nazionale: E (CONTI *et al.*, 1992), VU (CONTI *et al.*, 1997; SCOPPOLA, SPAMPINATO, 2005), CR B1 ab(*i,ii,iii*) + 2b(*i,ii,iii*) (BACCHETTA, PONTECORVO, 2005; BACCHETTA *et al.*, 2009).

Strategie/Azioni di conservazione e normativa. Il campo dunale di Portixeddu ricade all'interno del S.I.C. "Is Compinxius-Campo dunale di Buggerru – Portixeddu" (Codice ITB042247), ma attualmente non esistono azioni di conservazione e tutela specifiche per *D. morisianus*. La specie inoltre non è inserita in nessun catalogo di protezione vigente in ambito locale, regionale o internazionale.

A partire dal 2007 è stato avviato dal CCB un programma di monitoraggio e studio delle popolazioni *in situ,* finalizzato all'individuazione di misure di conservazione adeguate, e alla conservazione *ex situ* del germoplasma presso le strutture della Banca del Germoplasma della Sardegna (BG-SAR).

D. morisianus è stato recentemente incluso nella lista di specie oggetto di studio del progetto PRIN 2007 "Conservazione ex situ e caratterizzazione tassonomica, ecofisiologica e genetica di specie minacciate della flora spontanea italiana" (prot. 2007JNJ7MX_002), finanziato dal MIUR

A partire dal 2009 sono stati avviati studi più specifici di carattere popolazionale, finalizzati allo studio delle dinamiche evolutive della popolazione.

Note. *D. morisianus* Vals., insieme a *D. cyathophorus* Moris e *D. sardous* Bacch., Brullo, Casti *et* Giusso, appartiene al ciclo di *D. sylvestris* Wulfen (BACCHETTA *et al*., 2004) che, all'interno del genere, è uno dei gruppi più complessi e poco studiati.

La specie appare tassonomicamente isolata e morfologicamente ben differenziata dagli altri *taxa* di questo gruppo; la presenza di 4 squame nell'epicalice è comune anche a *D. sardous*, dal quale però si differenzia ampiamente per morfologia e caratteristiche ecologiche (BACCHETTA *et al*., 2009). Recentemente, ARRIGONI (2005) considera *D. morisianus* come una sottospecie di *D. siculus*, mentre BACCHETTA *et al.* (2009), in accordo con VALSECCHI (1985), considerano validamente la specie, così come precedentemente fatto da CONTI *et al.* (2005).

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APPENDIX 2 – Altre pubblicazioni prodotte durante il dottorato di ricerca

1. Articoli scientifici su riviste indicizzate:
    a. Fenu G, **Cogoni D**, Ferrara C, Pinna MS, Bacchetta G. Relationships between coastal sand dune properties and plant communities' distribution: the case of Is Arenas (Sardinia). Plant Biosyst (doi:10.1080/11263504.2012.656727).
2. Articoli scientifici su riviste non indicizzate:
    a. Fenu G, **Cogoni D**, Ferrara C, Pinna MS, Bacchetta G. 2008. Analisi delle dinamiche evolutive su base sedimentologica e geobotanica del cordone dunale di Is Arenas (Oristano - Sardegna Centro-Occidentale). Atti del Convegno Internazionale "Il Monitoraggio Costiero Mediterraneo: problematiche e tecniche di misura": 79-90.
    b. **Cogoni D**, Fenu G, Bacchetta G. 2010. Analisi dello stato di conservazione di Dianthus morisianus Vals., endemita esclusivo dell'ecosistema dunale di Portixeddu (Sardegna sudoccidentale). Atti del Terzo Simposio Internazionale: "Il monitoraggio costiero mediterraneo: problematiche e tecniche di misura", pp. 93-101.
    c. Fenu G, Pinna MS, **Cogoni D**, Bacchetta G. Schede per una lista rossa della flora vascolare e crittogamica italiana: Astragalus tegulensis Bacch. & Brullo. Informatore Botanico Italiano, in stampa.
    d. Fenu G, Picciau R, **Cogoni D**, Bacchetta G. Schede per una lista rossa della flora vascolare e crittogamica italiana: Cephalaria bigazzii Bacch., Brullo & Giusso. Informatore Botanico Italiano, in stampa.
    e. Fenu G, Picciau R, **Cogoni D**, Bacchetta G. Schede per una lista rossa della flora vascolare e crittogamica italiana: Clinopodium sandalioticum (Bacch. & Brullo) Bacch. & Brullo ex. Informatore Botanico Italiano, in stampa.
    f. Fenu G, Sulis E, **Cogoni D**, Bacchett G. Schede per una lista rossa della flora vascolare e crittogamica italiana: Helianthemum caput-felis Boiss. Informatore Botanico Italiano, in stampa.

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